TRANSFORMATION OF POTATO WITH MYB4 TRANSCRIPTION FACTOR AND EVALUATION OF ABIOTIC STRESS TOLERANCE AND GENE EXPRESSION PROFILES IN TRANSGENIC PLANTS

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ABSTRACT

TRANSFORMATION OF POTATO WITH MYB4 TRANSCRIPTION FACTOR AND EVALUATION OF ABIOTIC STRESS TOLERANCE AND GENE EXPRESSION PROFILES IN TRANSGENIC PLANTS

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Potato (*Solanum tuberosum* L. cv. Kennebec) was transformed via *Agrobacterium tumefaciens* (EHA105) harbouring two different binary vectors containing *Oryza sativa myb4* gene, which encodes MYB4 transcription factor; under the control of CaMV35S promoter or cold inducible COR15a promoter. The transgenic plants were not growth retarded and there was no significant difference ($p<0.05$) in their tuber yield compared to wild-type plants. Wild-type and transgenic plants were subjected to abiotic stresses to compare their stress tolerances. There was no significant difference in boron, freezing and drought tolerances of wild-type and transgenic lines. Two of the transgenic lines were more salt tolerant than wild-type with respect to growth parameters. Transcriptomes of wild-type and these two lines, one expressing *myb4* under the control of 35S promoter and the other COR15a promoter, were analyzed to elucidate the *myb4*-regulated processes and downstream target genes in potato. Differentially regulated genes in transgenic lines showed that *myb4* controls a large and complex transcriptional network associated with diverse cellular processes, primarily defense and rescue, metabolism and development. Genes involved in sucrose synthesis, some peroxidases and CBF3 transcription factor were

up-regulated in transgenic plants upon exposure to freezing stress. This suggested that *myb4* may configure freezing response in potato primarily by oxidative stress defence mechanisms, osmotic adjustment or activation of CBF3 regulated genes that may confer cold tolerance. Despite up-regulation of these stress related genes, transgenic potato was not more drought or freezing tolerant compared to WT under the tested conditions. Further experiments should be conducted to better elucidate the involvement of these genes in regulation of stress response in transgenic potato expressing *myb4*.

Keywords: Potato, MYB4, *Agrobacterium*-mediated gene transfer, abiotic stress, microarray analysis

ÖZ

PATATESİN MYB4 TRANSKRİPSİYON FAKTÖRÜ İLE TRANSFORMASYONU VE TRANSGENİK BİTKİLERDE ABİYOTİK STRES TOLERANSI VE GEN İFADE PROFİLLERİNİN İNCELENMESİ

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Kennebec çeşidi patates (*Solanum tuberosum* L.), MYB4 transkripsiyon faktörünü kodlayan *Oryza sativa myb4* genini CaMV35S promotoru kontrolünde ve COR15a promotoru kontrolünde taşıyan iki farklı plazmidi barındıran *Agrobacterium tumefaciens* (EHA105) aracılığıyla transforme edilmiştir. Transgenik bitkilerde herhangi bir büyüme geriliğine rastlanmamış ve bu bitkiler yabani tip bitkilerle karşılaştırıldıklarında yumru verimi açısından anlamlı bir farklılık ($P<0.05$) görülmemiştir. Yabani tip ve gen aktarılmış olan bitkiler çeşitli abiyotik streslere tabi tutularak stres toleransları karşılaştırılmıştır. Bu bitkiler arasında bor, donma ve kuraklık toleransları bakımından herhangi bir farklılık olmadığı görülmüştür. Büyüme parametreleri açısından değerlendirildiklerinde transgenik hatlardan ikisinin tuz toleransının yabani tip bitkilere göre daha yüksek olduğu tespit edilmiştir. Biri *myb4*'ü 35S promotorunun kontrolünde, diğeri ise COR15a promotorunun kontrolünde ifade eden bu iki hat ve yabani tip bitkilerin transkriptomları, *myb4*'ün kontrol ettiği prosesleri ve hedef genleri açığa çıkarmak amacıyla analiz edilmiştir. Transgenik hatlarda farklı ifade edilen genler, *myb4*'ün savunma, metabolizma ve

büyüme gibi farklı hücresel prosesleri etkilemek suretiyle büyük ve karmaşık bir transkripsiyon ağını kontrol ettiğini göstermiştir. Transgenik bitkiler donma stresine tabi tutulduklarında sukroz sentezini düzenleyen bazı genler, bazı peroksidazlar ve CBF3 transkripsiyon faktörünün ifadesi artmıştır. Bu durum *myb4*'ün, patatesin düşük sıcaklığa karşı oluşturduğu tepkiyi oksidatif stres savunma mekanizmaları, ozmotik denge ya da CBF3 aracılığı ile soğuğa karşı direnç sağlayan bazı genleri aktive etmek suretiyle kontrol ettiğine işaret etmektedir. Stresle ilişkili olan bu genlerin ifadesinin artmasına rağmen, yapılan deneyler sonucunda transgenik bitkilerin kuraklık ve donma toleranslarının yabani tip bitkilerden daha yüksek olmadığı görülmüştür. *myb4*'ü idafe eden transgenik bitkilerin daha farklı deney koşullarında incelenmesiyle bu genlerin patatesin stres toleransındaki rolü daha iyi anlaşılabilir.

Anahtar kelimeler: Patates, MYB4, *Agrobacterium*-aracılığıyla gen transferi, abiyotik stres, mikroarray analizi

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to my family

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LIST OF ABBREVIATIONS

ABA	Abscisic acid
Hsp	Heatshock protein
LEA	Late embryogenesis abundant
ROS	Reactive oxygen species
SOD	Superoxide dismutase
RD	Responsive to dehydration
KIN	Cold induced
COR	Cold responsive
AP2/ERF	Apetala 2/ ethylene responsive factor
HD-ZIP	Homeodomain leucine zipper
MYC	Myelocytomatosis
ABRE	ABA-responsive element
ABF	ABRE binding factor
AREB	ABA responsive element binding protein
DREB	Drought responsive element binding
NAC	NAM, ATAF1, 2 and CUC
DRE	Drought-responsive element
CRT	C-RepeaT
SUMO	Small Ubiquitin-like MOdifier
TF	Transcription factor
HTH	Helix-turn-helix
PM	Perfect match
MM	Mismatch
ZR	Zeatin riboside
NAA	Naphthalene acetic acid
GA	Gibberellic acid
CTAB	Hexadecyltrimethylammonium bromide
DEPC	Diethylpyrocarbonate

DIG	Digoxigenin
SDS	Sodium dodecyl sulfate
DMSO	Dimethyl sulfoxide
RMA	Robust Multiarray Analysis
TFGD	Tomato Functional Genomics Database
BLAST	Basic Local Alignment Search Tool

CHAPTER 1

INTRODUCTION

1.1 Potato

The potato (*Solanum tuberosum*) is the world's fourth most important food crop after wheat, maize and rice with an annual world production of 325.6 million metric tonnes (FAOSTAT, 2008).

Potato plant is a perennial herb belonging to the family *Solanaceae*. Potatoes produce flowers that can either be self-pollinated or cross-pollinated, to produce fruits and true seed. The potato tuber is the swollen end of an underground stem called a stolon. Potatoes can be grown from the botanical seeds or propagated vegetatively by planting pieces of tubers (Bradshaw & Ramsay, 2009).

Potatoes are among the most efficient sources of energy and other nutrients including vitamins and minerals. Table 1.1 shows the chemical composition of potatoes (Li *et al.*, 2006; Storey, 2007). New cultivars of potatoes with better yield, disease resistance, and desirable end-use are being developed with the help of breeding techniques. Since cultivated potato is a tetraploid it cannot easily be crossed with many wild potatoes, which are mostly diploids. This limits inter-specific crosses and favours potato breeding within the cultivated species. But this is not a serious limitation because the true seeds exhibit immense genetic variation. Following the rational development of genetic engineering, many genetically modified varieties of potatoes have also been produced via biotechnological approaches (Bradshaw & Ramsay, 2009).

Table 1.1 Chemical composition of potatoes on a fresh-weight basis

Component	Content
Dry matter	15–28%
Starch	12.6–18.2%
Glucose	0.01–0.6%
Fructose	0.01–0.6%
Sucrose	0.13–0.68%
Dietary fiber	1–2%
Lipid (fat)	0.075–0.2%
Protein	0.6–2.1%
Asparagines (free)	110–529 mg/100 g
Glutamine (free)	23–409 mg/100 g
Proline (free)	2–209 mg/100 g
Other amino acids (free)	0.2–117 mg/100 g
Polyphenols	123–441 mg/100 g
Carotenoids	0.05–2 mg/100 g
Tocopherols	Up to 0.3 mg/100 g
Thiamin B1	0.02–0.2 mg/100 g
Riboflavin	0.01–0.07 mg/100 g
Vitamin B6	0.13–0.44 mg/100 g
Vitamin C	8–54 mg/100 g
Vitamin E	~0.1 mg/100 g
Folic acid	0.01–0.03 mg/100 g
Nitrogen (total)	0.2–0.4%
Potassium	280–564 mg/100 g
Phosphorus	30–60 mg/100 g
Calcium	5–18 mg/100 g
Magnesium	14–18 mg/100 g
Iron	0.4–1.6 mg/100 g
Zinc	~0.3 mg/100 g
Glycoalkaloids	< 20 mg/100 g

1.2 Environmental Stress Factors Limiting Plant Growth

Environmental stresses represent the most limiting factors for agricultural productivity. Biotic and abiotic stress conditions severely limit plant growth and cause great reductions in annual crop yield. Table 1.2 represents the major stress factors contributing crop losses. Although there is a great contribution of biotic factors to crop yield and productivity only affect of abiotic factors will be focused in this chapter (Mahajan & Tuteja, 2005).

Abiotic stress is reported to reduce average yields for most major crop plants by more than 50% worldwide (Bray *et al.*, 2000). Rapid changes in environment even reduce the survival rates of plants subjected to stress. As amply discussed by scientists, accumulation of greenhouse gases causes elevated UV radiation levels to reach the ground and also result in changes of extreme temperatures. Another threat for crops is the intense use of fertilizers and artificial irrigation in agriculture. These practices have increased the salinity of the soils in many areas of the world. The serious salinization of soils is expected to affect more than 50% of all arable lands by the year 2050 (Hirt & Shinozaki, 2004; Wang *et al.*, 2003). These rapid environmental changes makes drought, temperature and salinity stresses the major stress factors influencing agricultural yield and productivity.

Table 1.2 Various abiotic and biotic stress factors affecting plant growth.

Abiotic stresses	***Biotic stresses***
1. Cold 2. Heat 3. Salinity 4. Drought 5. Excess water 6. Radiations (high intensity of visible light and ultra-violet) 7. Chemicals and pollutants 8. Oxidative stress (reactive oxygen species, ozone) 9. Wind 10. Nutrient deprivation in soil	1. Pathogens 2. Insects 3. Herbivores 4. Rodents

Water comprises about 90% of the fresh weight of herbaceous plants and it is the most important constituent of a plant. If the water status of a plant is insufficient, the plants experience water deficit, also described as drought. Not only lack of water but also abiotic stresses like low temperature and salinity cause water deficit (Hirt & Shinozaki, 2004; Wood, 2005). Exposure to drought or salt stress triggers many

common reactions as they ultimately result in dehydration of the cell and osmotic imbalance. They also cause formation of reactive oxygen species which affects cellular structures and metabolism negatively. Removal of water from the membrane disrupts the integrity and the selectivity of the membrane which in turn results in loss of activity of enzymes that are primarily membrane based. Besides the membrane damage it may lead to reduced activity of proteins and the proteins may even undergo complete denaturation when dehydrated (Bray, 1997; Mahajan & Tuteja, 2005). Plant responses to salt and drought overlap except for the ionic component. Decrease in hormonal processes like increased levels of abscisic acid or reduction in photosynthesis rate are some of the similar metabolic processes. Elevated intracellular concentrations of sodium and chloride ions are another problem that plants face during salinity stress (Bartels & Sunkar, 2005).

Water constitute a great proportion of potato tubers. Low soil moisture at the dry areas decreases the yield in potato especially during tuberization stages and it also affects development of the organs. Potato is not a tolerant crop plant to high levels of salt. Therefore potato production at marginal lands such as dry areas is severely limited by high levels of salt in the field and irrigated water (Harris, 1978).

Saline soils are characterized by high concentrations of soluble salts. Sodium chloride is the most soluble and abundant salt in soil. In saline soils the concentration of NaCl exceeds 40 mM generating an osmotic pressure of approximately 0.2 MPa. Plants differ greatly in their tolerances to salt. Rice (*Oryza sativa*) is known to be the most sensitive and barley (*Hordeum vulgare*) is the most tolerant among cereals (Munns & Tester, 2008). Negative effects of salt stress on plant cells are **(i)** nutritional defects because of decreased uptake of phosphorus, potassium, nitrate and calcium **(ii)** ion cytotoxicity mainly due to Na^+, Cl^- plus SO_4^- and **(iii)** osmotic stress. Sodium ions compete with potassium ions in biochemical reactions which is detrimental to cellular processes. There are three main tolerance mechanisms of salt tolerance for plants. The first is cellular homeostasis through ion homeostasis and

osmotic adjustment. The second is growth regulation and the last one is stress damage control and repair or detoxification (Chinnusamy & Zhu, 2004).

Another abiotic stress factor limiting plant growth is cold stress which includes chilling (<20 ˚C) and/or freezing (<0 ˚C) temperatures. Plants vary in their temperature requirement that the optimum range for each plant' proper growth and development is different. Cold acclimation is a process by which plants increase their freezing tolerance upon prior exposure to low non-freezing temperatures. During cold acclimation expression of a large number of stress responsive genes controlling production of proteins and metabolites which protect integrity of cellular structures and functions from freezing damage is altered. Crop plants such as rice, maize, soybean, cotton, potato and tomato, are chilling sensitive and they cannot cold acclimate (Chinnusamy *et al.,* 2007).

Exposure of plants to subzero temperatures results in extracellular ice formation, inhibition of water uptake, and cellular dehydration. Therefore, freezing tolerance is strongly correlated with tolerance to dehydration (caused by e.g. drought or high salinity). The major detrimental effect of freezing is the ice formation rather than low temperatures. Ice formation in plants begins in the apoplastic space where the solute concentration is lower. The unfrozen cytoplasmic water migrates from the cell cytosol to the apoplast. This leads to enlargement of existing ice crystals and causes a mechanical strain on the plasma membrane and the cell wall which in turn leads to cell rupture. Freeze induced dehydration can cause denaturation of proteins and disruption of macromolecular complexes. A common denominator in several stresses, including low temperature is the production of reactive oxygen species, which can generate damage to different macromolecules in the cells (Hirt & Shinozaki, 2004; Mahajan & Tuteja, 2005).

The potato buds are especially sensitive to alterations in temperature at early growing stages. Therefore both low and high temperatures may limit growth and sometimes a severe damage may lead to death of potato plants. The highlands like Andes and hill

sides of East Africa and Central Asia, where potato is grown, are much more affected by the drastic changes in the night temperature. Besides the early growth stages, frost and freezing are also important over the growing period of potato (Watanabe, 2002).

1.3 Plant Responses to Abiotic Stress

Abiotic stresses cause morphological, physiological, biochemical and molecular changes that severely limit plant growth and productivity. Elucidating the mechanisms underlying plant cells' tolerance to abiotic stress is a vital prerequisite for improving agricultural and horticultural crop productivity and growth under limited water concentrations. Figure 1.1 schematically represents the complex plant responses to abiotic stress (Wang, *et al.*, 2003).

The molecular control mechanism of abiotic stress tolerance is based on the expression of specific stress-related genes. These genes are classified into three major categories: **(i)** genes that function directly in the protection of membranes and proteins (e.g. heatshock proteins (Hsps), late embryogenesis abundant (LEA) proteins, osmoprotectants); **(ii)** genes involved in water and ion uptake and transport (e.g. aquaporins and ion transporters); **(iii)** genes involved in signaling cascades and in transcriptional control (e.g. MAP kinases, phospholipases, and transcriptional factors such as CBF/DREB and ABF/ ABAE families) (Wang, *et al.*, 2003).

1.3.1 Genes Functioning Directly in the Protection of Membranes and Proteins

Under abiotic stress conditions, plants should adapt to stress conditions and evolve specific tolerance mechanisms to keep growth and productivity. One of the ways of plant modification for enhanced tolerance is over-expression of genes functioning directly in the protection of membranes and proteins. Genes encoding for heatshock proteins (Hsps), late embryogenesis abundant (LEA) proteins, osmoprotectants, and free-radical scavengers fall into this group.

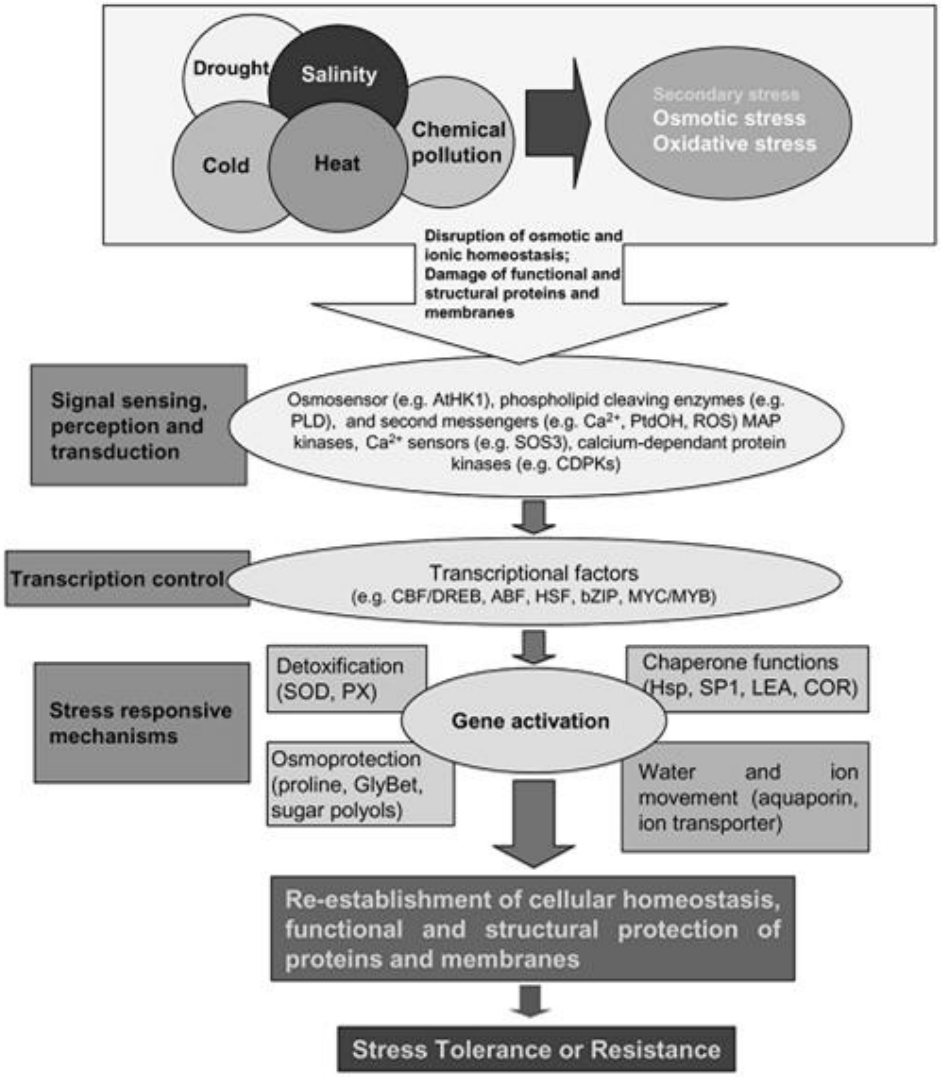

Figure 1.1 Plant responses to abiotic stresses.

There are many plants that respond to stress by accumulating low molecular weight organic compounds known as compatible solutes or osmolytes. These compounds protect plants from stress by (1) osmotic adjustment (2) detoxification of free radicals and (3) stabilization of 3D structure of proteins. Plants keep their osmotic balance by decreasing their osmotic potential via accumulation of compatible solutes. The increase in the solute concentration in the cell triggers movement of water into the leaf resulting in increase in leaf turgor. Compatible solutes may be classified into three groups: amino acids (e.g. proline), quaternary amines (e.g. glycine betaine) and

polyol/sugars (e.g. mannitol, trehalose). During drought and cellular dehydration these compounds help the cells to keep their hydrated state. The hydroxyl group of sugar alcohols substitutes the OH group of water and that helps in maintaining the hydrophilic interaction with membrane lipids and proteins. This protects the structural integrity of the cell membrane. Since these compounds help in osmotic adjustment they are also known an osmoprotectants (Chaves & Oliveira, 2004; Mahajan & Tuteja, 2005; Wang, *et al.*, 2003).

Proline is synthesized from glutamate via glutamic-γ-semialdehyde (GSA) and Δ^1-pyrroline-5-carboxylate (P5C). Conversion of glutamate to P5C is catalyzed by P5C synthase (P5CS). Then P5C reductase (P5CR) converts P5C to proline. In the reverse reaction, proline is metabolized to glutamate in a feed-back manner, via P5C and GSA. This reaction is cataylzed by proline dehydrogenase (ProDH) and P5C dehydrogenase (P5CDH). Hmida-Sayari *et al.* (2005) attempted to increase salt tolerance in potato, and transferred a P5CS cDNA from *Arabidopsis thaliana* to potato plants. It was observed that proline production was greater in transgenic plants when compared to control plants. Accumulation of proline improved tolerance of transgenic potato plants to salinity. The transformation did not reduce yield or tuber weight in the transgenic plants when compared to the non-transgenic ones.

Trehalose, a non-reducing disaccharide of glucose commonly found in bacteria, fungi, insects and some plant species, is known to stabilize membranes and macromolecules during drought. Over-expression of trehalose increases protection of PS II against photooxidation and thus enhances photosynthetic activity. Yeo *et al.* (2000) introduced trehalose- 6-phosphate synthetase gene (*TPS1*) of *Saccharomyces cerevisiae* to potato under the control of 35S promoter. Some of the trehalose-accumulating transgenic plants exhibited growth retardation or aberrant root development in tissue culture tubes. However the plants recovered when grown in soil mixture. The TPS1 transgenic plants were found to be more resistant to drought when compared to non-transgenic control plants.

Hsps and LEA proteins accumulate upon exposure to water, salinity and extreme temperature stresses. Dysfunction of enzymes and proteins is a major problem that accompanies abiotic stress. Many stress-responsive proteins, especially Hsps, act as molecular chaperones and protect proteins and membranes and also assist protein refolding. The small heat-shock proteins (sHsps) are the most common group of Hsps in plants. Some sHsps are reported to stabilize or reactivate inactivated enzymes (Mahajan & Tuteja, 2005; Wang, *et al.*, 2003).

LEA proteins accumulate in seeds during the maturation phase when seeds are developing desiccation tolerance. These proteins may also be expressed in vegetative tissue upon exposure to abiotic stress. Because of their extreme hydrophilic nature, LEA proteins have been predicted to play various roles such as maintenance of protein or membrane structure, sequestration of ions, binding of water, and operation as molecular chaperons. LEA proteins are heat stable that they do not coagulate upon boiling and in most cases the relative expression of these proteins are transcriptionally regulated and responsive to ABA (Bray, 1997; Wang, *et al.*, 2003). Some LEA-like proteins were overexpressed in plants to elucidate their functions. Transgenic rice plants overexpressing a barley LEA gene, *HVA1*, was shown to be more tolerant to cold and salt stress compared to WT (Xu *et al.*, 1996). Overexpression of transcription factors regulating the LEA-like genes were also reported to improve tolerance to various abiotic stresses (Jaglo-Ottosen *et al.*, 1998).

Formation of reactive oxygen species such as hydrogen peroxide, hydroxyl radicals and superoxide anions is common to salt, freezing and drought stresses. ROS are produced during normal cellular activities such as photorespiration and β-oxidation of fatty acids, but their concentration increase by exposure to biotic or abiotic stresses. These free radicals damage membranes, proteins and nucleic acids and lead to oxidative stress especially in the mitochondria and chloroplasts. Some plants scavenge reactive oxygen species via the antioxidative enzymes such as superoxide dismutases (SODs), peroxidases, catalases and glutathione reductases (Bray, 1997; Holmberg & Bülow, 1998; Wang, *et al.*, 2003). Tang *et al.* (2006) expressed the

genes of Cu/Zn superoxide dismutase and ascorbate peroxidase in potato chloroplasts under the control of *SWPA2* which is an oxidative stress inducible promoter. Transgenic plants showed increased tolerance to oxidative stress. The damage under stress conditions was much less in transgenic plants compared to non-transgenic plants. When plants were subjected to high temperatures (42 °C for 20 h) the decrease in photosynthetic activity of transgenic plants was 6%, whereas it was 29% for the control plants. These results indicated that manipulation of the antioxidative mechanism in potato may increase tolerance to multiple abiotic stresses.

1.3.2 Genes Involved in Water and Ion Uptake and Transport

Salinity, osmotic stress and ion toxicity especially Na^+ and Cl^- may impair intracellular ionic homeostasis which is vital for a living cell. Ion transporters on the membrane selectively transport ions and help in maintaining the concentration of toxic ions below a threshold level and also accumulation of essential ions. High concentrations of K^+ and low concentrations of Na^+ is maintained in the cytosol via active transport (H^+/ATPases) or secondary transport (channels and co-transporters). The Na^+/H^+ antiporters catalyze the exchange of Na^+ for H^+ across membranes and regulate cytoplasmic pH, sodium levels and cell turgor. Intracellular K^+ and Na^+ homeostasis is critical for enzymatic activities and also for keeping membrane potential. Elevated levels of Na^+ ions may reduce the rate of photosynthesis and lead to production of reactive oxygen species. Potassium ions are important for regulation of metabolism, growth and adaptation to stress (Mahajan *et al.*, 2008; Zhu, 2003).

Gain or loss of function studies in plants showed that the role of ion transporters is crucial in ion homeostasis. Overexpression of *NHX1* gene encoding for vacuolar Na^+/H^+ antiporter has increased salt tolerance in *Arabidopsis*, tomato (Zhang & Blumwald, 2001), and *Brassica (Zhang et al., 2001)* plants.

Aquaporins, located on the cell membrane, facilitate water transport by forming water-permeable complexes and they are involved in regulating cellular water status in response to water deficits. Besides water they may also transport other small

molecules such as solutes and ions and they display cytosolic pH-dependent gating. In case of cytosol acidosis, pH-dependent gating co-ordinatedly inhibit plasma membrane aquaporins. This justifies the limited ability of roots to absorb water under anoxic conditions during flooding (Chaves & Oliveira, 2004). The tobacco aquaporin NtAQP1 was shown to act as a CO_2 membrane-transport-facilitating protein (Uehlein *et al.*, 2003). Overexpression of NtAQP1 in tobacco affected photosynthesis by increasing membrane permeability for CO_2 and water, and increasing leaf growth. The rate of photosynthesis was increased by 36% in the transgenic plants under ambient CO_2 (380 ppm) and by 81% at elevated CO_2 (810 ppm). The stomatal conductance was also increased which was suggested to be involved in the elevated rate of photosynthesis.

1.3.3 Genes Involved In Signaling Cascades and in Transcriptional Control

The cellular response to an environmental stress factor starts with perception of the stress. The particular signal molecule which acts in this step may or may not be specific to a certain stress. This environmental cue results in a signal transduction cascade leading to widespread changes in cellular metabolism. These changes include activation of the expression of thousands of genes (Hazen *et al.*, 2003).

The stress is first perceived by the receptors located on the membrane of the plant cells. The signal is then transmitted downstream which result in generation of second messengers such as calcium, reactive oxygen species and inositol phosphates (Figure 1.2 A). These second messengers may further increase the intracellular calcium level. Ca^{2+} sensors, calcium binding proteins, sense the alteration in cytosolic Ca^{2+} level. These sensors in turn bind to their respective interacting partners and then start a phosphorylation cascade. The targets of these molecules are stress responsive genes or the transcription factors regulating expression of these stress responsive genes (Mahajan & Tuteja, 2005).

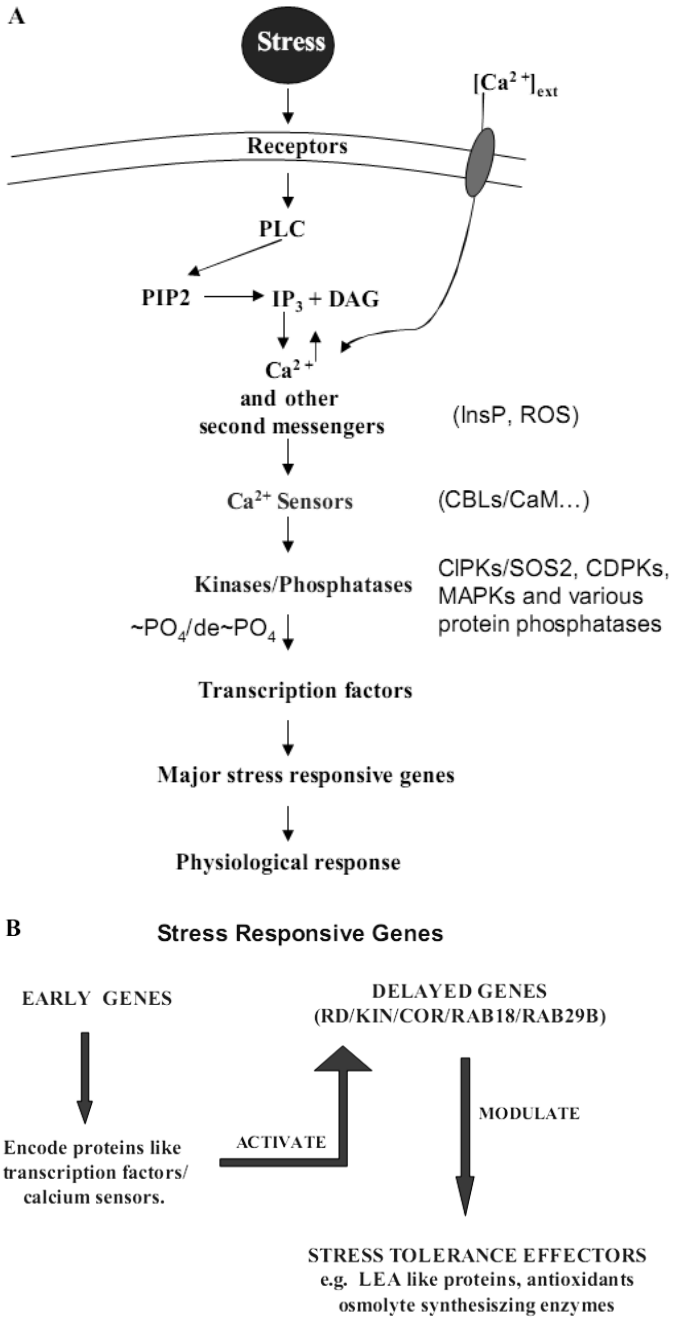

Figure 1.2 (A) Generic signal transduction pathway and (B) early and late genes involved in abiotic stress signaling.

Stress induced changes in gene expression may affect synthesis of hormones like ABA, salicylic acid and ethylene. Functions of ABA in plants are (1) inducing seed dormancy and delaying its germination (2) maturation of embryo (3) promoting

stomatal closure (4) activation of stress responsive genes. ABA is involved in activation of genes involved in osmotic adjustment, ion compartmentation and regulation of shoot versus root growth during stress adaptation. Expression pattern of some genes regulated by cold, drought, high salt or ABA application overlap. Some genes that respond to dehydration and cold stresses are also induced by exogenous ABA application to plants. However some genes responsive to dehydration and cold do not respond to exogenous ABA application. Therefore function of ABA in cold-response is not clear. This indicates that there are ABA-independent and ABA-dependent signal transduction cascades regulating the expression of stress responsive genes (Agarwal & Jha, 2010; Yamaguchi-Shinozaki & Shinozaki, 2006).

Besides the plant hormones that act in stress signaling there are some other molecules known as accessory molecules which are involved in modification or assembly of signaling molecules. Enzymes for myristoylation, methylation, ubiquitination and glycosylation are examples of protein modifiers (Mahajan & Tuteja, 2005).

The genes expressed under abiotic stress conditions can be grouped as early and late induced genes (Figure 1.2 B). Early genes are generally expressed transiently and in a short time by the perception of stress signal. The signaling components for some transcription factors are already primed and they do not require synthesis of new proteins. Therefore these transcription factors are regarded as early genes. The late induced genes which are downstream of early genes are activated more slowly. Late induced genes such as those responsive to dehydration or cold, encode and modulate the proteins required for synthesis of protective compounds such as osmolytes, late embryogenesis abundant proteins or antioxidants (Mahajan & Tuteja, 2005).

The molecular mechanisms regulating gene expression involved in abiotic stress responses have been studied by analyzing the *cis*- and *trans*-acting elements that function in ABA-dependent and ABA-independent gene expression during the stresses in *Arabidopsis* (Yamaguchi-Shinozaki & Shinozaki, 2006). The

transcriptional regulation of cold, salt and drought stresses is described in Figure 1.3 (Hirayama & Shinozaki, 2010).

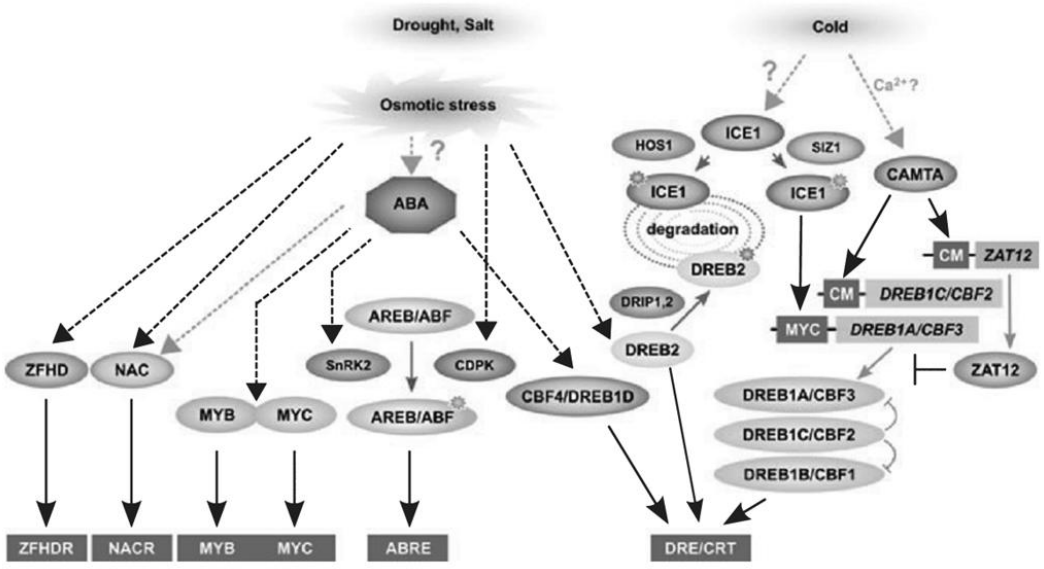

Figure 1.3 Transcriptional regulation of drought, salinity and cold stress responses. Elliptical objects indicate functional proteins and gray boxes indicate cis-elements. Solid lines show direct links and dotted lines show indirect links.

Transcription factors, regulatory sequences and some genes involved in abiotic stresses are well characterized. Transcription factors bind to *cis*-acting elements in the promoters of certain abiotic stress responsive genes and thus regulate the expression of downstream genes resulting in abiotic stress tolerance. *Cis*-acting elements and corresponding binding proteins with different DNA binding domains have been identified in *Arabidopsis thaliana*. Some of these DNA binding domains are basic leucine zipper, AP2/ERF (apetala 2/ ethylene responsive factor), MYB (myeloblastosis), HD-ZIP (homeodomain leucine zipper), MYC (myelocytomatosis) and different classes of zinc finger domains (Shinozaki & Yamaguchi-Shinozaki, 2000). The molecular mechanisms for ABA-dependent and ABA-independent pathways are not entirely clear. There are some differences in transcriptional

activation but they also interact to regulate the gene expression upon exposure to abiotic stress (Agarwal & Jha, 2010).

ABFs (ABRE binding factor)/AREBs (ABA responsive element binding protein), DREB2 (drought responsive element binding), MYC/MYB and NAC (NAM, ATAF1, 2 and CUC) transcription factors involved in ABA-dependent and ABA-independent pathways are activated by dehydration and salt stress. Cold stress regulates an ABA-independent pathway through CBF/DREB1 transcription factors. Transcription factors control gene expression under stress conditions and they directly or indirectly regulate certain genes associated with stress tolerance in plants. Therefore overexpression of certain transcription factors can increase stress tolerance in plants (Agarwal & Jha, 2010).

The promoter region of a drought-, cold- and high salinity-responsive gene contains ABRE (ABA-responsive element) and DRE (drought-responsive element)/CRT (C-RepeaT) *cis*-acting elements. DREBs induce certain genes that confer abiotic stress tolerance in plants. There are two subclasses of ERF family transcription factors, DREB1/CBF and DREB2. DREB1 transcription factors are induced by cold and DREB2 are induced by dehydration. There are many DREB genes isolated and characterized in plants (Agarwal & Jha, 2010). Kasuga *et al.* (1999) overexpressed *DREB1a* under the control of 35S promoter in Arabidopsis plants. *DREB1a* over-expression activated stress responsive genes such as *cor, P5CS, erd* and *rd29* under normal growing conditions and increased freezing, salinity and drought tolerance. Constitutive expression of *DREB1a* led to growth retardation under normal growing conditions. The same research group observed minimal effects on plant growth when they expressed the same gene under the control of *rd29A*, a stress inducible promoter. *DREB2* genes may activate the genes involved in drought stress tolerance. Liu *et al.* (1998) overexpressed DREB2 in Arabidopsis plants. However, they did not observe a stress tolerance which indicates post-translational modification of DREB2 proteins. Sakuma *et al.* (2006) transformed Arabidopsis with an active form of DREB2 and they showed trans-activation of stress-inducible genes and improvement

of drought tolerance in transgenic plants. The DREB2 protein was shown to be activated by osmotic stress via post-translational modification in the early stages of stress.

Recent research indicate that post-transcriptional and post-translational modifications are important in regulation of abiotic stress response (Figure 1.4) (Mazzucotelli *et al.*, 2008).

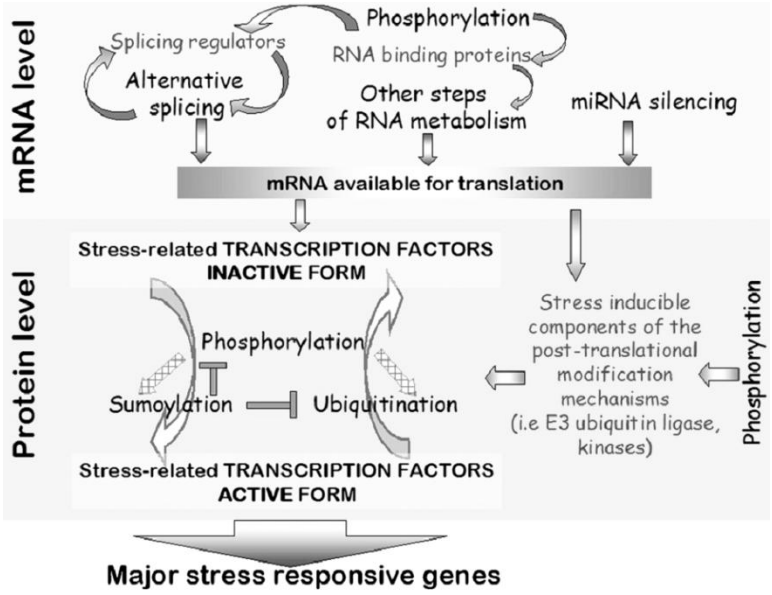

Figure 1.4 Model describing the cross-talk between post-transcriptional (mRNA level) and post-translational (protein level) regulations of plant responses to abiotic stress. Grating arrows indicate connections identified in animals but not reported in plants yet.

These regulation mechanisms rapidly and finely modulate the amount and activity of pre-existing transcripts and proteins, respectively. One of those mechanisms is alternative splicing which generates different transcrips and thus proteins. RNA regulated silencing is another alternative mechanism controlling the amount of specific transcripts by their degradation. After the translation various molecules can transiently or constitutively bind to proteins and modify their sub-cellular localization, activity and half-life. Phosphorylation, ubiquitination and somoylation

are emerging post-translational regulatory mechanisms in eukaryotes. Phosphorylation of transcription factors regulates their activity by affecting their conformation, localization, activity and stability of target proteins. Ubiquitin which controls degradation of target proteins plays an important role in stress induced signalling and response mechanisms. In contrast to ubiquitination, sumoylation regulates the activity of target proteins. SUMO (Small Ubiquitin-like MOdifier) peptides covalently conjugate to protein substrates and alter their function by changing their conformation, masking and/or adding interaction surfaces. Sumoylation may affect sub-cellular re-localization, change enzymatic activity and protect from ubiquitin-directed degradation. Post-transcriptional and post-translational regulations affect transcription factors and other regulatory elements of the stress signalling and result in activation or repression of their activities. This regulates appropriate temporal and spatial expression of downstream genes and provides adaptive responses (Hirayama & Shinozaki, 2010; Mazzucotelli, *et al.*, 2008).

1.3.3.1 MYB Transcription Factors in Plants

In plants MYB transcription factors (TFs) family is one of the most abundant classes of transcription factors. The first MYB protein was identified in the aleurone of maize (*Zea mays*) kernels. *ZmC1* is encoded by COLORED1 (C1) locus and it is responsible for synthesis of anthocyanins. Publication of Arabidopsis genome sequence provided comprehensive information about plant *MYB* genes and their functions in plants. These transcription factors are involved in diverse biochemical and physiological processes such as regulation of secondary metabolism, meristem formation, cell morphogenesis and floral and seed development. They are also involved in certain defense and stress responses and in hormone signaling (Du *et al.*, 2009; Dubos *et al.*, 2010). MYB domain which is the DNA-binding domain is highly conserved in MYB transcription factors. MYB domain consists of up to four imperfect repeats. Each of these repeats is about 52 amino acids and form three α-helices. The second and third helices of each repeat form a helix-turn-helix (HTH)

structure. Three regularly spaced tryptophan residues in the HTH form a hydrophobic core in the 3D structure. The third helix is essential for DNA recognition (Dubos, *et al.*, 2010).

MYB proteins can be classified into four subfamilies, MYB-1R, R2R3-MYB, MYB-3R, and MYB-4R, depending on the number of repeats in the MYB domain. MYB-1R is involved in regulation of circadian clock and telomeric DNA-binding protein. MYB-3R participates in formation of B-type cyclin. The R2R3-MYB subfamily is the largest and functionally most diverse represented with more than 100 members. They are involved in regulating anthocyanin biosynthesis, response to gibberellic acid signal and also determining cell shape and formation of different plant organs. They are related to the pathogenesis and also involved in response to dehydration and salicylic acid in Arabidopsis. Most of the MYB transcription factors are positive regulators of transcription but there are also some negative regulators (Chen *et al.*, 2005; Dubos, *et al.*, 2010).

Table 1.3 represents roles and functions of some R2R3-MYB transcription factors identified in Arabidopsis. More than half of the data about the roles of MYB transcription factors in plants comes from extensive research on Arabidopsis and have been identified within the past three years (Dubos, *et al.*, 2010 and references therein).

Research on plants other than Arabidopsis show that MYB transcription factors have similar roles in different species. Liao et al. (2008) identified 156 *GmMYB* genes from soybean plants and found that 43 genes were differentially regulated by exogeneous ABA application, drought, salt and/or cold stress. Overexpression of *GmMYB76*, *GmMYB92*, and *GmMYB177* differentially regulated some downstream genes and increased basal salt tolerance in transgenic Arabidopsis plants. It was shown that the transgenic lines exhibited reduced sensitivity to ABA treatment.

Ma *et al.* (2009) showed involvement of *OsMYB3R-2* both in stress and developmental processes in rice (*Oryza sativa*). *OsMYB3R-2* expression was induced by cold treatment and the cold tolerance of transgenic plants overexpressing *OsMYB3R-2* was higher when compared to non-transgenic control plants. Overexpression of *OsMYB3R-2* also increased transcript levels of several G2/M phase specific genes such as *OsCycB1;1*. This shows that *OsMYB3R-2* also regulates the progress of the cell cycle during chilling via increasing expression of *OsCycB1;1*.

Table 1.3 Roles and functions of some R2R3-MYB transcription factors identified in Arabidopsis.

MYB code	Function	Role (regulation of)
AtMYB058	Metabolism	Phenylpropanoid pathway / Lignin biosynthesis
AtMYB004	Metabolism	Phenylpropanoid pathway / Sinapate ester biosynthesis
AtMYB011	Metabolism	Phenylpropanoid pathway / Flavonol biosynthesis
AtMYB028	Metabolism	Glucosinolate biosynthesis / Aliphatic pool
AtMYB005	Metabolism	Mucilage biosynthesis
AtMYB046	Metabolism	Cell wall thickening (fibers and vessels)
AtMYB030	Defense	Abiotic stress response / HR response, SA-mediated (VLC-lipid metabolism)
AtMYB072	Defense	Biotic stress response/Pathogens (induced systemic resistance)
AtMYB041	Defense	Abiotic stress response / Osmotic, ABA-mediated
AtMYB033	Defense	Abiotic stress response / ABA sensitivity
AtMYB060	Defense	Biotic stress response / Drought, ABA-mediated (stomatal closure)
AtMYB037	Development	Axillary meristem regulation / Lateral organ formation (shoot branching, GA-mediated)
AtMYB033	Development	Stamen development / Anther development (tapetum)
AtMYB077	Development	Growth regulation, auxin-mediated
AtMYB023	Differentiation	Cell fate / Root hair patterning
AtMYB106	Differentiation	Cell fate / Trichome branching

Peel *et al.* (2009) identified Legume Anthocyanin Production 1 (*LAP1*) gene from *Medicago truncatula*. *LAP1* overexpression in transgenic alfalfa, white clover or *M. truncatula* increased accumulation of anthocyanin pigments comprising multiple glycosidic conjugates of cyanidin.

Deluc *et al.* (2006) cloned and characterized *VvMYB5a*, a cDNA isolated from a grape L. cv. Cabernet Sauvignon berry library. Overexpression of this gene in tobacco (*Nicotiana tabacum*) affected the expression of structural genes regulating synthesis of phenylpropanoid, and metabolism of anthocyanins, flavonols, lignins and tannins.

Plett *et al*. (2010) identified a mutant line with increased foliar trichome density during screening of activation-tagged *Populus tremula* x *Populus alba* 717-1B4 trees. This phenotype was attributed to activation tagging and increased expression of the gene encoding *PtaMYB186*. Mis-expression of this gene also affected pest resistance and growth rate, indicating that *PtaMYB186* might also be useful for improvement of biotic stress tolerance and growth.

Bomal *et al.* (2008) investigated the involvement of *PtMYB1* and *PtMYB8* genes from *Pinus taeda* L., in secondary cell wall formation and phenylpropanoid metabolism. These genes were overexpressed in *Picea glauca* and enhanced lignin deposition was determined in the transgenic plants. Overexpression of these MYB transcription factors led to up-regulation of some genes involved in phenylpropanoid metabolism and in synthesis of lignin monomers.

The research listed above shows that MYB transcription factors from different plant species have similar roles and functions as those identified in Arabidopsis.

1.3.3.2 MYB4 Transcription Factor

AtMyb4 identified in *Arabidopsis thaliana,* was shown to regulate accumulation of sinapoylmalate which is a molecule involved in protecting plants against UV. *AtMyb4* represses transcription of the gene encoding for the phenylpropanoid enzyme cinnamate 4-hydroxylase (C4H) (Hemm *et al.*, 2001; Jin *et al.*, 2000). This was the first data to show the role of a MYB transcription factor as a transcriptional

repressor. In an Arabidopsis mutant which could not express *myb4, C4H* mRNA was more abundant and it accumulated elevated levels of UV sunscreens.

Vannini *et al.* (2004) demonstrated by transient expression that rice (*Oryza sativa*) *OsMyb4* trans-activated the cold inducible promoters, PAL2, ScD9, SAD and COR15a in rice. Overexpression of *Myb4* in transgenic Arabidopsis showed an increased cold and freezing tolerance compared to non-transgenic wild type plants.

In another report the involvement of *Osmyb4* in drought tolerance in transgenic Arabidopsis plants was demonstrated. *Osmyb4*-overexpressing plants accumulated higher amounts of compatible solutes (glucose, sucrose, fructose, glycine betaine, proline and sinapoyl malate) compared to the wild type, both under normal and stress conditions. Accumulation of compatible solutes improved the drought tolerance in transgenic plants (Mattana *et al.*, 2005).

Tomato plants overexpressing *Osmyb4* showed an increased tolerance to drought stress like Arabidopsis. The transgenic plants was much more tolerant to virus disesases however, they were not more cold tolerant than the wild type. This data supports the idea that *Osmyb4* activity depends on the genomic background of the host (Vannini *et al.*, 2007).

Ectopic expression of *Osmyb4* in apple (*Malus pumila* Mill.) improved drought and cold tolerance and also affected accumulation of metabolites involved in abiotic stress response (Pasquali *et al.*, 2008).

Overexpression of *Osmyb4* in *Osteospermum ecklonis*, an ornamental and perennial plant, increased the freezing and cold tolerance in the transgenic plants. It also affected accumulation of osmoprotectants such as soluble sugars and proline (Laura *et al.*, 2010).

1.3.3.3 Application of Microarray Technology to the Analysis of Expression Profiles in Response to Abiotic Stress

Microarray is a DNA chip-based technology that arrays oligonucleotides or cDNA sequences on a glass surface at a density >1000 genes/cm^2. The arrayed sequences are called probes. The probes are hybridized to targets which are either fluorescently labeled cRNA or cDNA samples. This hybridization enables direct and large-scale comparative analysis of gene expression profiles. The fluorescently labeled targets hybridize to their complementary probes on the array. The intensity of the fluorescence coming from hybridized probe and target enables quantification of gene expression. The intensity of the signal may represent level of the transcript for RNA samples or the sequence similarity of probes and targets (Clarke & Zhu, 2006).

There are two main types of microarrays, the oligonucleotide microarray and cDNA microarray. The best known oligonucleotide microarray is the Affymetrix GeneChip®. Oligonucleotide microarrays are often more expensive but they offer several advantages. Custom design of the probes reduces the risk of human errors. The sequences of the probes are highly specific that the risk of non-specific binding with unintended or similar sequences is reduced. By this way the accuracy of the technique is ensured and non-specific hybridizations are avoided. It is also a very sensitive technique that even low level expressions are detected (Hazen, *et al.*, 2003).

Affymetrix GeneChip® probes are composed of 25 oligonucleotide bases, designed with regard to sequence uniqueness and composition. Eukaryotic probe sequences are designed mainly from the 3' end of the mRNA molecules which is less susceptible to degradation. Eleven to twenty probe sets are used for the detection of each transcript of interest, and each probe set consists of a 25-mer perfect match (PM) probe and a 25-mer mismatch (MM) probe, which only differ in the middle (13th) base. The fluorescence intensity caused by non-specific or semi-specific hybridization to MM probes is then subtracted from the PM probe signal. The use of multiple probe sets per transcript and MM probes comprise the two levels of probe

redundancy, which is defined as the use of multiple oligonucleotides of different sequences to bind to the same transcript. These two levels of redundancy greatly reduce background noise caused by non-specific and semi-specific binding (Lipshutz *et al.*, 1999).

Plant growth and productivity is severely affected by certain abiotic stresses. Plants respond and adapt to these stress factors by changing expression of thousands of genes. A plant' response to abiotic stresses may be understood by monitoring its transcriptome in a spatial and temporal manner. Microarray analysis using cDNAs or oligonucleotides is a valuable tool for analyzing transcriptome of plants under stress. It is important to analyze genes involved in stress response to display the molecular mechanisms involved in stress tolerance and to improve the stress tolerance of crops by gene manipulation (Shinozaki & Yamaguchi-Shinozaki, 2007; Sreenivasulu *et al.*, 2007). Determination of complete genome sequences of Arabidopsis, *Oryza sativa* spp. *japonica* cv. Nipponbare, and other plants enabled a genome-wide gene expression profiling. Microarray technology can be used to determine downstream targets of transcription factors involved in stress response and to determine the *cis*-acting elements. This helps in combining the expression data with the genomic sequence data (Hirayama & Shinozaki, 2010).

Microarray technology was reported to be used in analysis of expression profiles in response to abiotic stresses such as drought, cold and salinity stresses. Seki *et al.* (2002) analyzed the transcriptome of Arabidopsis upon exposure to drought, low temperature and salinity stresses, using a cDNA microarray containing ≈7000 independent cDNA probes. They have identified more than fivefold increase in 53, 277 and 194 genes upon exposure to cold, drought and high-salinity treatments respectively when compared to the control genes. 22 of the stress-inducible genes were differentially regulated after all three stresses. They have found that differentially regulated 40 transcription factor genes constituted 11% of all stress inducible genes identified.

Chen *et al.* (2002) analyzed expression profiles obtained from microarray experiments for elucidating functions of known or putative Arabidopsis transcription factors. Expression levels of 402 transcription factors were analyzed using the samples collected from plants at different developmental stages and under various stress conditions. The genes coding for these transcription factors were classified depending on their expression upon exposure to various stresses. The importance of these transcription factors were displayed in mutants with reduced or abolished functions.

Rensink et *al.* (2005) subjected potato seedlings to cold (4 °C), heat (35 °C) or salt (100mM NaCl) stress to identify the gene expression profiling. Root or leaf samples were collected 3, 9 and 27h after stress exposure and spotted on a ~12,000 clone potato cDNA microarray to scan the expression profiles. 3.314 clones were differentially regulated in response to at least one of the stress factors. Ten most up- and down-regulated genes were determined to be coding for molecular chaperons, late embryogenesis abundant proteins, heat-shock proteins and genes encoding for enzymes. These gene products were involved in abiotic stress responses in Arabidopsis and rice which indicates a similar response pathway in potato.

1.4 Aim of the Study

Environmental stresses especially abiotic stress greatly affects plant growth and productivity. Therefore any conventional or biotechnological approach that may reduce the crop loss due to abiotic stresses is of great value. Transferring transcription factors to plants is one of the recent approaches to enhance tolerance of crop plants to stress factors.

Previously *myb4* was overexpressed in Arabidopsis, tomato, apple and *Osteospermum ecklonis* to determine the role of this transcription factor in abiotic stress tolerance. This study was conducted to explore the potential of *myb4* gene to enhance tolerance towards abiotic stresses in potato. Therefore the first objective of

this thesis is generation of transgenic potato plants expressing *myb4*. The next step is comparison of abiotic stress tolerances of WT and transgenic plants upon exposure to certain stress factors.

Another objective of this dissertation is to explore influence of *myb4* expression on gene expression profiles in potato. Microarray is a powerful tool that enables global expression profiles of plants subjected to abiotic stresses. Comparing microarray data of WT and transgenic plants may show the differentially regulated genes in transgenics. This information may help in determining downstream genes and biological processes regulated by *myb4* in potato. To the best of our knowledge, this is the first study to analyze the transcriptome of transgenic potato expressing *myb4.*

CHAPTER 2

MATERIALS AND METHODS

2.1. Materials

2.1.1 Bacterial Strain

Agrobacterium tumefaciens strain EHA105 was used for plant transformation.

2.1.2 Plasmids

Two different plasmids, pSA-MYB4 (carrying CaMV35S promoter and Nos terminator, Figure 2.1) and pCOR15-MYB4 (carrying COR15a promoter and Nos terminator, Figure 2.2), carrying *myb4* gene in their multiple cloning sites were used as binary vectors for plant transformation. 1202 bp mRNA coding for MYB4 transcription factor (Appendix A) was isolated from three days old coleoptiles of *Oryza sativa* Japonica Group. *Agrobacterium tumefaciens* strain EHA105 carrying these plasmids were kindly provided by Dr. Ming-Tsair Chan from Agricultural Biotechnology Research Center, Academia Sinica, Taiwan.

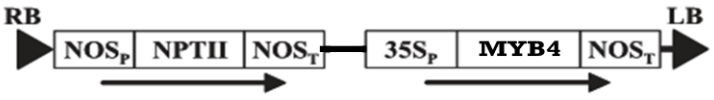

Figure 2.1 T-DNA region of pSA-MYB4.

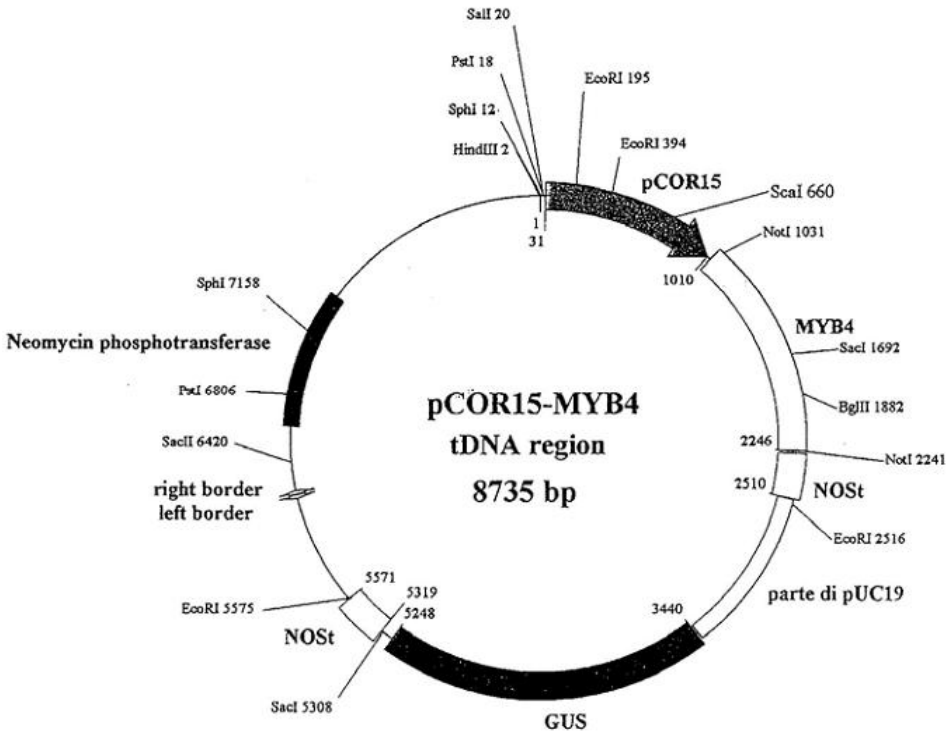

Figure 2.2 Map of pCOR15-MYB4.

2.1.3 Bacterial Growth Medium

YEP medium (Appendix B) supplemented with 100mg/L kanamycin was used for growth of *A. tumefaciens*. Cultures were grown with aeration (180 rpm) at 28 °C. For further storage, medium containing 1.5 % bacterial agar and 100mg/L kanamycin was cultured and kept at 4 °C for several weeks. The bacteria carrying the specific plasmids were also kept at -80 °C in 20 % glycerol for long term storage.

2.1.4 Plant Material and Tissue Culture Media

The potato cultivar Kennebec was used in the experiments. It was obtained from Natural Seed Stock Center in Taiwan.

All of the media that were used throughout the study were MS (M0222) based media (Murashige & Skoog, 1962). After addition of appropriate hormones, pH was adjusted to 5.7-5.8 with NaOH and autoclaving was done at 121 °C for 20 min. The composition and the purpose of the media are given in Table 2.1.

Table 2.1 The compositions and purpose of plant tissue culture media

Media	Composition	Purpose of Use
1/2T	M0222 + 30 g/L sucrose	Diluting *Agrobacterium* during infection
HH	M0222 + 30 g/L sucrose + 10 mg/L NAA + 10 mg/L ZR + 3g/L phytagel	Pre-treatment of leaf strips
LSR-1	M0222 + 30 g/L sucrose + 0.2 mg/L NAA + 2 mg/L ZR + 0.02 mg/L GA3 + 3g/L phytagel	Co-cultivation of Agrobacterium treated leaf strips, callus induction medium for leaf strips (100 mg/L Kanamycin was added for selection of transformants)
LSR-2	M0222 + 30 g/L sucrose + 2 mg/L ZR + 0.02 mg/L GA3 + 3g/L phytagel	Shoot induction medium for leaf strips (100 mg/L Kanamycin was added for selection of transformants)
CM	M0222 + 30 g/L sucrose + 3g/L phytagel	Rooting medium during micropropagation, induction of root formation on regenerated shoots (100 mg/L Kanamycin was added for selection of transformants)

2.1.5 Other Chemicals and Materials

The chemicals used in the preparation of solutions were all commercially available from Duchefa, Merck, Sigma, Seakem and Amresco. The chemicals used for the

molecular biology studies were from Biolabs, Genomics, Promega, MDBio, Qiagen, Waters, Affymetrix and Roche.

2.2 Methods

2.2.1 Bacterial Growth

EHA105 strain of *A. tumefaciens* was grown in YEP medium at 28 °C. 1.5 % agar was added when solid media was needed. Filter (0.2μm) sterilized antibiotics were added after autoclave for selection.

2.2.2 Preparation of Plant Tissue Culture Media

Plant tissue culture media used in micropropagation studies and for rooting purposes were MS based M0222 medium (Duchefa) including vitamins. Media were solidified with 0.3 % phytagel and pH was adjusted to 5.7-5.8 with 1M NaOH before autoclaving at 121 °C for 20 min. According to the purpose, filter (Whatman, 0.22 μm) sterilized antibiotics (timentin and kanamycin) were added into the media. Compositions and purposes of the media are given in Table 2.1.

2.2.3 Micropropagation

Apical buds and stem segments containing one node, excised from the shoots were placed on the rooting media and were incubated in a growth chamber room with a day length of 16 h at a temperature range of 23-25 °C and 2000-3000 lux light intensity. Plantlets formed from the buds or nodes after 3-4 weeks were used for further experiments. Sub-culturing was performed every month to maintain fresh plantlets for the experiments.

2.2.4 Plant Transformation Studies

Agrobacterium-mediated transformation of potato was performed using leaf strips as plant material (Wenzler *et al.*, 1989).

2.2.4.1 Preparation of Tissue Culture Media

Medium used during transformation, regeneration and selection studies was MS basal salt medium; M0222 (Duchefa). pH was adjusted to 5.7-5.8 with 1 N NaOH and autoclaving was done at 121 °C for 20 min. According to the purpose, appropriate hormones (ZR, NAA and GA_3) were added to the media after they were filter sterilized. The compositions and the purposes of the plant tissue culture media are given in Table 2.1.

2.2.4.2 Transformation

Agrobacterium strain was inoculated in YEP medium supplemented with 100 mg/L kanamycin and was grown at 28 °C overnight with shaking until OD_{595} reached around 1.0. Strips of potato leaves obtained from *in vitro* cultures were used as explants in the transformation experiments. Leaves were removed from 3-4 weeks old shoots and cut into 2-3 mm wide strips. The explants were placed abaxial-side down on Petri plates containing HH medium. They were incubated under dark conditions for 3 days in the tissue culture room. Following this pre-treatment the leaf strips were placed in the 1:30 diluted bacterial culture and co-cultivated in room temperature for 15 minutes shaking 2-3 times. At the end of 15 minutes the explants were blot dry and transferred to Petri plates containing Kanamycin free LSR-1 medium. The explants were incubated in dark for 3 days until a slight bacterial ring developed at the cut-edge surfaces of the explants. Then they were transferred to LSR-1 plates containing 100 mg/L Kanamycin and 300 mg/L Timentin. The explants were kept in this medium until callus formation was seen. After callus formation the explants were transferred to LSR-2 medium for shoot formation. This

medium was also supplemented with 100 mg/L Kanamycin for selection of transformants and 300 mg/L Timentin to eliminate bacterial growth. Both LSR-1 and LSR-2 were refreshed every two weeks. As the shoots reached 1-2 cm height they were transferred to jars containing CM supplemented with 300 mg/L Timentin for shoot elongation and root formation. Kanamycin was removed in this stage to encourage rooting. After the plantlets reached 6-8 leaf stage the apical bud was excised and transferred to jars containing CM supplemented with Kanamycin. Plantlets that were able to form roots in this selective medium were regarded as putative transgenic plants.

2.2.5 Analysis of Transgenic Plants

2.2.5.1 Molecular Analysis

2.2.5.1.1 Plant Genomic DNA Isolation

Genomic DNA was isolated from fresh or frozen leaf tissue using CTAB (hexadecyltrimethylammonium bromide) extraction buffer (Ausubel *et al.*, 2005).

5-6 potato leaves were ground to fine powder with a pestle and mortar using liquid nitrogen. The powder was further ground shortly by the addition of 1 mL pre-warmed (65°C) CTAB extraction buffer (Appendix C) and 10 µL RNase A (10mg/mL) and then transferred to centrifuge tubes. The tubes were incubated in 65°C in a water bath for one hour with occasional shaking. The mixture was centrifuged at 10000 rpm for 10 minutes. After centrifugation the supernetant was transferred to a new tube and an equal volume of chloroform: isoamyl alcohol (24:1) was added. The mixture was mixed by invertion and spinned at 10000 rpm for 10 minutes. The upper phase was mixed with an equal volume of cold isopropanol in a new tube and inverted gently to precipitate DNA. The tubes were kept at -20 °C overnight and DNA was collected by centrifugation at 3000 rpm for 5 minutes. Then the supernetant was removed and the precipitated DNA was washed in 1 mL ethanol

and centrifuged at 3000 rpm for 5 minutes. Finally the precipitate was air dried and dissolved in H_2O. The isolated DNA was run on 1% agarose gel to check the integrity. The purity and concentration was determined via reading the absorbance in nanodrop spectrophotometer (Thermo Scientific NanoDrop 3300 Fluorospectrometer). DNA was stored in -20 °C for further use.

2.2.5.1.2 Total RNA Isolation

Total RNA was isolated from fresh or frozen leaf tissue using TRIzol® reagent (Invitrogen) according to manufacturer's instructions.

5-6 potato leaves was ground to fine powder with a pestle and mortar using liquid nitrogen. The pestle and mortar was pre-cooled by pouring some liquid nitrogen over it. The powder was transferred to a centrifuge tube pre-chilled in liquid nitrogen and put back into liquid nitrogen until grinding of all the samples were finished. Then 1 mL of TRIzol® reagent (Invitrogen) was added to each tube and mixed well by vortex. After an incubation of 5 minutes in room temperature 200 µL chloroform was added to each tube, mixed by vortex and further incubated in room temperature for 2-3 minutes. The tubes were centrifuged for 15 minutes at a speed of 12000g in a centrifuge pre-cooled to 4 °C. The supernetant was transferred to a new tube and an equal volume of isoprapanol was added. It was mixed by inversion and kept in room temperature for 10 minutes. RNA was collected by centrifugation at 4 °C for 10 minutes at 12000g. Then the supernetant was removed and the precipitated RNA was washed in 1 mL ethanol and centrifuged at 4 °C for 5 minutes at 7500 g. Finally the precipitate was air dried and dissolved in DEPC (Diethylpyrocarbonate) treated H_2O. The concentration and purity of RNA was checked via reading the absorbance in nanodrop spectrophotometer (Thermo Scientific NanoDrop 3300 Fluorospectrometer). RNA was stored in -80 °C for further use.

2.2.5.1.3 Agarose Gel Electrophoresis

Agarose gel electrophoresis was performed to separate and visualize DNA and RNA samples or PCR products. According to the size of the sample one or two percent agarose gel was prepared in 0.5% TBE buffer (Appendix D). The gels were run at ~100V for ~30 minutes in 0.5% TBE buffer until the samples were separated appropriately.

2.2.5.1.4 Northern Blot Analysis

Northern blot analysis was performed with total RNA samples of wild type and transgenic potato plants (Sambrook and Russell, 2001).

Electrophoresis of RNA through Agarose Gels Containing Formaldehyde

1% agarose gel was prepared in 1X gel buffer (Appendix E) and 8.4% formaldehyde. First agarose was completely dissolved in 1X gel buffer by microwave and cooled down. Then formaldehyde was added and the gel was cast and allowed to polymerize. Meanwhile the samples were prepared. 10 µg RNA was used for each sample. The samples were mixed with equal volume of sample buffer (Appendix E) and incubated at 68 °C for 10 minutes. After the incubation samples were chilled on ice and loaded immediately. Before the samples were loaded the gel was pre-run 15-20 minutes at 50 V in running buffer (Appendix E). After loading the samples the gel was run at 70 V for about 2 hours. Afterwards the gel was washed twice with distilled water for half an hour to remove formaldehyde.

Northern Transfer of RNA

A platform was established in a tray and covered with a sheet of Whatman 3MM paper wide as the gel but longer so that the ends will drape over the edges of the platform. The paper was soaked with 10X SSC (Appendix E) and the bubbles

between the platform and the paper was removed by the help of a plastic rod. Two other sheets of Whatman 3MM paper exactly the same size as the gel was placed in the same way. Afterwards the gel was placed on the support in an inverted position. The top of the gel was wetted with 10X SSC and the positively charged nylon membrane (Amersham) was placed after being soaked in 10X SSC. Two pieces of 3MM paper cut in exactly the same size as the gel was placed on top of the membrane. Any air bubbles were removed with the plastic rod. A stack of paper towels (5-8 cm high) was placed on top of the 3MM papers. The tray was half filled with 10X SSC buffer and covered with Saran Wrap to prevent loss of buffer via evaporation. A glass plate and a 500g weight were put on the stack. Upward transfer of RNA was allowed to occur overnight. The capillary transfer system was dismantled the next day. The membrane was placed on a piece of clean paper towel and UV cross-linked. To assess the efficiency of transfer the membrane was stained with blot stain blue (Sigma) and the nucleic acids were visualized. The membrane was first treated with 10% acetic acid for 5 minutes on a shaker. Then acetic acid was removed and the membrane was briefly rinsed in distilled water. Afterwards the membrane was stained with blot stain blue for just enough time to visualize the rRNAs (approximately 3-5 minutes). Before a photo of the membrane was taken the membrane was washed with distilled water several times to destain the background. Afterwards the blot stain blue was removed by washing the membrane twice in dH_2O for half an hour. The membrane may directly be used in hybridization after washing. If it is not going to be used immediately in hybridization it is dried and wrapped loosely in aluminum foil and stored in 4 °C until use.

Northern Hybridization

The membrane was incubated in 10 mL of DIG Hybridization buffer (Appendix E) for 1-2 hours at 42 °C in the hybridization oven. DIG (Digoxigenin) labeled double stranded probe was denatured by heating in the boiling water for 5 minutes. Then the probe was chilled on ice immediately. At the end of 1-2 hours of pre-hybridization, denatured probe was added to DIG Hybridization buffer and the incubation was

resumed for 12-16 hours. After hybridization the membrane was removed from the hybridization tube and transferred to a plastic box containing low stringency wash buffer composed of 2X SSC and 0.1% SDS. The box was placed on an orbital shaker and agitated gently for 5 minutes in room temperature. The solution was refreshed and the washing step was repeated once again. Afterwards the low stringency wash buffer was replaced with a pre-warmed (65 °C) high stringency wash buffer composed of 0.1X SSC and 0.1% SDS. The membrane was washed twice with high stringency wash buffer for 15 minutes under agitated conditions. Then the membrane was rinsed in maleic acid buffer (Appendix E) with 0.2% tween 20 in room temperature for 5 minutes. Afterwards the membrane was transferred to a plastic bag and 10 mL of 1% blocking solution (Roche) was added. The bag was sealed and the membrane was treated with the blocking solution in room temperature for 30 minutes on an orbital shaker. At the end of 30 minutes 1µL anti-Digoxigenin-AP solution was added to the blocking solution and the membrane was treated another 30 minutes in this solution. Then the membrane was transferred to a plastic box and rinsed in maleic acid buffer with 0.2 % tween 20 for 15 minutes twice in room temperature. After this washing step the membrane was put into a plastic bag again and 10 mL of CDP-Star solution (Biolabs) was added. The membrane was treated with CDP-Star solution for 15 minutes in room temperature on an orbital shaker. Since the CDP-Star solution is light sensitive the treatment was conducted under dark conditions. After CDP-Star treatment one end of the bag was opened and the excess solution was removed completely. The membrane was exposed to X-ray film or imaged with a CCD imaging system. If the membrane is going to be re-probed it should not be allowed to dry between hybridization and stripping as this may cause the probe to bind to the matrix.

2.2.5.1.5 Southern Blot Analysis

Southern blot analysis was performed with genomic DNA samples of wild type and transgenic potato plants (Sambrook and Russell, 2001).

Digestion of DNA Samples

Prior to loading on agarose gel the DNA samples should be digested using one or more restriction enzymes. In this study *Hind*III (BioLabs) was used as a restriction enzyme which has a single cut site in the T-DNA region. 30 µg genomic DNA was restricted using 200 units of *Hind*III at 37 °C overnight. The following day DNA was mixed with loading dye and loaded on agarose gel.

Electrophoresis of DNA through Agarose Gel

Appropriate amount of 6X loading dye was added to each digest and loaded on 0.8 % ethidium bromide free agarose gel. λ/*Hind*III was loaded as DNA size marker into the first well. The next well was left empty and the samples were loaded side by side. After loading was finished the gel was run in 0.5X TBE (Appendix D) at 20 V overnight. Then the gel was stained with ethidium bromide for 20 min in dark and then it was photographed. A transparent ruler was placed alongside the gel so that the distance that any band of DNA had migrated could be read directly from the photographic image.

Denaturation of DNA

The gel was put in a plastic box and washed twice in dH_2O. The gel was treated with 0.25N HCl for 15 min in room temperature with constant gentle agitation. The HCl solution was removed and the gel was rinsed twice with dH_2O to get rid of residual HCl. Denaturation solution (Appendix D) was added and the gel was agitated in this solution for 15 min. Then it was washed briefly with dH_2O and replaced with neutralization solution (Appendix D). The gel was treated with neutralization solution for 30 min. to neutralize the DNA and gel matrix. Lastly it was rinsed with dH_2O and transferred to membrane.

Transfer of DNA to Nylon Membrane

DNA was transferred to positively charged nylon membrane in the way as it was described for northern blot. The upward capillary transfer was allowed to occur overnight. Then the DNA was immobilized on the membrane by UV irradiation.

Southern Hybridization

Pre-hybridization and hybridization was carried out at 42 °C in the way as it was described for northern blot. CDP-Star solution was used for detection of the signal and then the membrane was exposed to X-ray film or imaged with a CCD imaging system.

DIG Labeling of DNA Probes by the Polymerase Chain Reaction

DIG labeling system relies on incorporation of digoxigenin-labeled deoxyuridine-triphosphate (DIG-dUTP) to the DNA probe during PCR. The DIG-labeled probe is then coupled with the immobilized target DNA in a hybridization step. The amount of attached probe is measured by enzyme-linked chemiluminescent immunoassay using anti-digoxigenin-alkaline phosphatase conjugate. 20µl PCR reactions were set up on ice for amplification and labeling of *nptII*, *myb4* and *ef1α* gene fragments (Table 2.2). PCR was performed using gene specific primers for each gene (Table 2.3).

Control reaction mixtures were prepared for each gene fragment using dNTP mixture instead of DIG DNA labeling mixture. PCR conditions were adjusted according to the primers and the amplified fragment. The PCR conditions for synthesis of *nptII*, *myb4* and *ef1α* probes are given in Table 2.4 and Table 2.5. 3 µl PCR products for both labeled and non-labeled control samples were run on 1% agarose gel. The DIG labeled product should be a bit heavier than the control PCR product lacking DIG. Therefore the labeled probe should have run a shorter distance

on the gel. After confirming the labeling depending on the distance run on agarose gel the remaining 17 µl DIG labeled probe was added to 10 mL of hybridization buffer and was kept in -20 °C until use.

Table 2.2 PCR components used for probe synthesis.

Reagent	**Volume**
10X Taq polymerase reaction buffer	2 µl
10X DIG DNA labeling mix.(Roche)	2 µl
10 µM forward primer	1 µl
10 µM reverse primer	1 µl
DNA template (plasmid DNA)	1 ng
Taq DNA polymerase (5 unit/µl)	0.5 µl
dH_2O	Volume completed to 20 µL

Table 2.3 Primer sequences for *nptII*, *myb4* and *ef1α* genes.

Gene	**Direction**	**Primer sequence**
myb4	Forward	CGAGAAGATGGGGCTCAAG
	Reverse	TCGGCTTCTTGTGCTTCTTGC
nptII	Forward	ATGATTGAACAAGATGGATTGCACG
	Reverse	TCAGAAGAACTCGTCAAGAAGGCGA
ef1α	Forward	ATTGGAAACGGATATGCTCCA
	Reverse	TCCTTAACCTGAACGCCTGTCA

Table 2.4 PCR cycling conditions for synthesis of *nptII* probe.

Step / Segment		**Temperature**	**Duration**	**Cycle**
Initial Denaturation		95°C	5 min	1
Amplification	Denaturation	95°C	30 sec	30
	Annealing	55°C	30 sec	
	Extension	72°C	30 sec	
Final Extension		72°C	10 min	1

Table 2.5 PCR cycling conditions for synthesis of *myb4* and *ef1α* probes.

			myb4		***ef1α***	
Step / Segment		**Temperature**	**Duration**	**Cycle**	**Duration**	**Cycle**
Initial Denaturation		95°C	5 min	1	5 min	1
Amplification	Denaturation	95°C	30 sec	35	30 sec	40
	Annealing	58°C	25 sec		30 sec	
	Extension	72°C	30 sec		30 sec	
Final Extension		72°C	10 min	1	10 min	1

Removal of Probe from Hybridized Membrane

If the membrane is going to be re-probed the current probe should be stripped from the membrane. After visualization the membrane should first be rinsed briefly in distilled water. The membrane used for northern blotting should be washed in pre-warmed stripping buffer (Appendix E) at 80 °C for one hour with a mild agitation. This step should be repeated once with the fresh stripping solution. The membrane used for southern blotting should be rinsed in 0.2M NaOH solution containing 0.1% SDS. After rinsing in this solution for 20 minutes with constant agitation, the solution was exchanged with the fresh one and this step was repeated. After the rinsing steps, the membranes should be thoroughly and briefly rinsed in 2X SSC and allowed to dry. The membranes should be kept in 4 °C until the next hybridization.

2.2.5.2 Growth and Tuberization of Wild Type and Transgenic Plants

After verifying transfer and expression of *myb4* gene via Southern and Northern blot analyses, four lines with the COR15a promoter and three lines with the CaMV35S promoter was selected and used as transgenic lines for further experiments. Wild type and transgenic plantlets grown in rooting medium for one month were transferred to soil and grown in greenhouse. Six plants per line was transferred to pots and were covered with transparent bags during the first two days for acclimatization. The temperature in the greenhouse was ~25 °C and the light source was natural day light. The plants were watered twice a week and grown for four

months. At the end of this period, the tubers of all plants were harvested and tuberization of wild type and transgenic lines was evaluated. The fresh weight of tubers was recorded and the number of tubers per plant was counted.

2.2.5.3 Determination of Sugar, Ascorbic Acid and Anthocyanin Content of Wild Type and Transgenic Plants

2.2.5.3.1 HPLC Determination of Sugar and Ascorbic Acid

The potato plants grown in the green house for four months were harvested and the tubers were used for HPLC determination of sucrose, glucose, fructose and ascorbic acid content of WT and transgenic potato plants. The tubers were peeled and 20 grams of tuber was weighed and stored at -80 °C for determination of sugar content. 15 grams of tissue was frozen and stored for ascorbic acid determination.

For sugar analysis twenty grams of frozen potato tissue were blended with 50 mL ethanol (95%) for 1 min using a blender. Internal standard (1 mL) containing mannitol (80 mg), epicatechin (2.5 mg) and α-aminobutyric acid (40 mg) was added to the resulting mixture. The mixture was filtered through Whatman No 1 paper and the residue was rinsed twice with 50 mL ethanol (80%). The ethanol was evaporated at 40 °C using a rotary evaporator (BÜCHI Rotavapör R-200) and taken to a volume of 15 mL with distilled water. The extract was centrifuged (10000 x g) for 10 min and the supernatant was used for sucrose, glucose and fructose analysis. Five mL of the potato extract were passed through a Waters C_{18} Sep-Pak cartridge (previously activated with methanol and rinsed with water). The eluate was filtered through a 0.45 μm PTFE filter (Waters) and injected into the HPLC. Chromatographic separation was accomplished by a high performance liquid chromatograph (Varian ProStar) equipped with Metacarb 87C (300 x 7.8 mm) A5200 column and Varian 350 Refractive Index Detector. The mobile phase was distilled water run at 0.6 mL/min at 85 °C. Standard curves were prepared with nine concentrations of sucrose (0.02-8 mg/mL), glucose (0.01-4 mg/mL), fructose (0.005-2 mg/mL) and mannitol

(0.005-2 mg/mL) in distilled water. All peaks were identified based on retention times of known standards (RodriguezSaona & Wrolstad, 1997).

For ascorbic acid analysis 15 grams of potato tissue were blended with a solution containing 15 mL 2.5% metaphosphoric acid and 30 mL acetonitrile:0.05M KH_2PO_4 (75:25) for 1 min. The homogenate was filtered through a Whatman No. 1 paper, passed through a C_{18} Sep-Pak cartridge (Waters) and filtered through a 0.45 μm PTFE filter (Waters). Chromatographic separation was accomplished by a high performance liquid chromatograph (Varian ProStar) equipped with Varian Microsorb-MV NH2 (250 x 4.6 mm, 5 μm, R0086700C5) column and Varian ProStar 330 PDA detector. The mobile phase was acetonitrile:0.05M KH_2PO_4 (75:25) with 1 g/L dithiothreitol (Sigma), run isocratically at a flow rate of 1 mL/min. The effluent was monitored at 254 nm and the spectra were recorded for all peaks. An ascorbic acid standard curve was prepared using solutions containing 0.00312–0.25 mg/mL ascorbic acid (RodriguezSaona & Wrolstad, 1997).

2.2.5.3.2 Determination of Anthocyanin

Anthocyanin analysis was conducted using leaf discs collected from fully expanded third and fourth leaves of WT and transgenic lines (Gould *et al.*, 2000). Three leaf disc collected from each of three independent plants per line were agitated gently in the dark for 24 hours at 4 °C in 1 mL 3 M HCl : H_2O : MeOH (1:3:16, by vol.). Anthocyanin levels were estimated from the methanolic extracts as A_{530}–0.24 A_{653}. The anthocyanins in this solution absorbed maximally at 530 nm; subtraction of 0.24 A_{653} compensated for the small overlap in absorbance at 530 nm by the chlorophylls.

2.2.5.4 Stress Treatment of Wild Type and Transgenic Plants

Plantlets grown in rooting medium until 5-6 leaves stage were used for stress treatments. Wild type and transgenic plants were subjected to freezing, salt, boron and drought stresses to compare their tolerances. In order to induce the cold

inducible COR15a promoter, the lines carrying the *myb4* gene under the control of this promoter were kept in 4 °C for 2 days before the stress treatments. Not only the plants with COR15a promoter but all the lines were kept in 4 °C for 2 days to keep conditions the same for all the plants. After 2 days of promoter induction in 4 °C 8 plantlets were selected for each treatment. The apical buds of the plantlets were removed and the remaining shoot was cut into four pieces each carrying a single node with a leaf. 32 single nodal segments for each treatment were put on the medium in the order as shown in Figure 2.3.

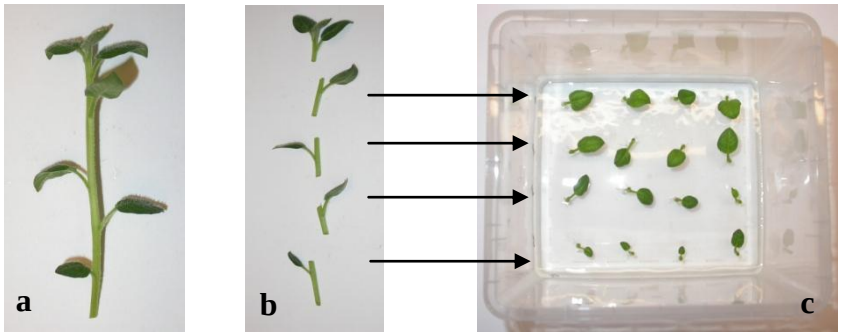

Figure 2.3 Plantlets used for abiotic stress treatments (a) plantlet grown until five-six leaves stage (b) shoot cut into single nodal segments (c) single nodal segments put onto medium.

As the responses of plantlets upon exposure to abiotic stresses may differ according to their age, plantlets grown until similar growth stage was selected for all applications. After removal of apical buds the remaining 4 nodal segments were placed on the medium in an order and the number of nodal segments from each position was always 8 for each stress treatment.

2.2.5.4.1 NaCl Treatment

Plantlets that were grown in rooting medium until five-six leaves stage were used for NaCl treatment. Firstly, the concentration of NaCl that limits growth of wild type was determined. For this purpose wild type plantlets were subcultured and placed

onto rooting medium containing 0 mM, 50 mM, 100 mM and 150 mM NaCl. The minimum concentration limiting growth of plantlets was used for further bioassays. 32 plantlets per line were grown in NaCl containing rooting medium for one month. At the end of this time the plantlets were removed from the medium and their shoot length, root length and fresh weights were recorded. Then the shoots and roots were kept in 80 °C for 24h and the dry weights were determined.

2.2.5.4.2 Boron Toxicity

Plantlets that were grown in rooting medium until five-six leaves stage were used for boron toxicity assays. Wild type plantlets were subcultured and placed onto rooting medium containing 0 mM, 2 mM, 3 mM and 4 mM boric acid to determine the boric acid concentration limiting growth of plantlets. The mimimum concentration that limits growth of wild type plantlets was used for treatment of transgenic lines. 32 plantlets per line were grown for one month in boric acid containing medium. At the end of one month of growth, the plantlets were removed from the medium and their shoot length, root length and fresh weights were recorded. Then the shoots were kept in 80 °C for 24h and the dry weights were determined.

2.2.5.4.3 Drought Treatment

Plantlets that were grown in rooting medium until five-six leaves stage were used for drought treatment. Stem segments with one node were placed onto perlit wetted with ½ MS containing 5, 10, 15 and 20 % PEG 6000 (polyethylene glycol). The mimimum concentration that limits growth of WT was determined and that concentration was used for further bioassays. 32 plantlets per line were grown for one month in this medium. At the end of one month the plantlets were removed from the medium and their shoot length, root length and fresh weights were recorded. Then the shoots were kept in 80 °C for 24h and the dry weights were determined.

2.2.5.4.4 Freezing Treatment

Plants grown in green house for one month were used for freezing treatment. Prior to freezing treatment plants were grown one week in 4 °C for cold acclimation. The control plants were not cold acclimated and grown for another week in the green house. Because some genes involved in cold stress tolerance are known to follow a circadian rhythm (Sauerbrunn & Schlaich, 2004), all the samples used for freezing treatment were harvested at 10:00 a.m. For each temperature point evaluated, three independent plants and three replicate samples per plant were used. Three leaf discs were collected from fully expanded second and third leaves per sample assayed and placed in test tubes. They were incubated at –1 °C for one hour in a water bath placed in a cooled incubator. Ice nucleation was initiated by adding an ice chip to each tube, samples maintained at –1.5 °C for an additional 1 h, and then the temperature lowered 1 °C/h. Sample tubes were removed at –3, –4, –5 and –6 °C, and slow-thawed overnight at 2 °C. Freezing injury of thawed leaf samples was assessed by determining electrolyte leakage using a conductivity meter. Following conductivity measurements, all samples were frozen at –20 °C for 24 h, thawed at room temperature, and total conductivity determined. In conjunction with one of the trials, replicate leaf disc samples of wild-type and transgenic plants were subjected to the treatment conditions and removed at –4 °C for RNA isolation and gene expression analysis.

2.2.5.5 Microarray Analysis

Microarray analysis was performed to compare the gene expression profiles in WT and transgenic lines. One of the transgenic lines carrying the *myb*4 gene under the control of CaMV 35S promoter, and one of the transgenic lines carrying the *myb*4 gene under the control of COR15a promoter was selected for microarray analysis. The growth parameters of these two lines were better under salt stress conditions when compared to WT. Therefore these lines were selected for microarray analysis. Leaf discs removed from WT, and transgenic lines were subjected to freezing

treatment at –4 °C and then total RNA of the leaf samples were isolated. Leaf discs were also removed from WT and transgenic plants that were grown under normal conditions and not subjected to freezing treatment. These samples were regarded as control samples and used to compare the gene expression profiles under stress and control conditions.

Total RNA isolation was performed using TRIzol® reagent according to the manufacturer's instructions. The integrity of the samples was checked by Agilent 2100 Bioanalyzer and the concentrations were determined using NanoDrop spectrophotometer (Thermo Scientific NanoDrop 3300 Fluorospectrometer). The RNA samples were prepared for hybridization according to the protocols described in Affymetrix GeneChip® 3' IVT Express Kit user manual. A total of 16 hybridizations with RNA from three biological replicates were performed. However number of biological replicates was two for the control samples of the transgenic lines.

The first step in sample preparation was reverse transcription to synthesize first-strand cDNA. 0.5 µg total RNA was used as template and T7 oligo (dT) was used as primer for this reaction. The next step was second-strand cDNA synthesis which converts the single-stranded cDNA into a double-stranded DNA (dsDNA) template for transcription. The reaction employs DNA polymerase and RNase H to simultaneously degrade the RNA and synthesize second-strand cDNA. Multiple copies of aRNA (cRNA is also known as amplified RNA or aRNA) was generated from the double-stranded cDNA templates via *in vitro* transcription. Biotin-congugated nucleotides were incorporated and labeled aRNA was generated in this amplification step. The next step was aRNA purification which removes unincorporated NTPs, salts, enzymes, and inorganic phosphate to improve the stability of the biotin-modified aRNA. After the cleanup, quantification of labeled aRNA was determined by measuring its absorbance at 260 nm. Before hybridized onto the arrays, 7.5 µg of biotin-labeled aRNA was fragmented using fragmentation buffer. The fragmented aRNA samples were run on 2% agarose gel to check the

fragmentation. After verification of fragmentation, 100 µl hybridization coctail consisting of 5 µg fragmented and labeled aRNA, 50 pM control oligonucleotide B2, hybridization controls [bioB (1.5 pM), bioC (5 pM), bioD (25 pM), cre (100 pM)], hybridization mix and 10% DMSO was prepared and loaded on GeneChip® Tomato Genome Array (Affymetrix) which contains 10,209 probe sets.

The arrays were hybridized in Affymetrix Hybridization Oven 640 at 45 °C and 60 rpm for 16h with the hybridization coctail. After hybridization, arrays were washed in Fluidics Station 450 (Affymetrix) and stained with streptavidin-phycoerythrin (Invitrogen) and biotinylated antistreptavidin antibody (Sigma), according to the standard protocol for Affymetrix 169 format tomato array. Arrays were then scanned with GeneChip® Scanner 3000 (Affymetrix). Hybridization, scanning and preliminary analyses with GeneChip® Operating Software 1.4 were performed at METU Central Laboratory.

Data from all hybridizations were analyzed using GeneSpringGX 11.0 (Agilent) software. Expression values, computed from .CEL files, were processed first by Robust Multiarray Analysis (RMA) which is a model of normalization over multiple arrays. Filtering on expression levels and fold changes (≥ 2) were performed for determination of differentially expressed genes. Statistical analyses were done using one-way ANOVA at $P < 0.05$. Fold change of at least 2 and P-value of at most 0.05 was considered as an indication of significantly different gene expression. The significantly different probe sets were annotated using Tomato Functional Genomics Database (TFGD) website. The probe sets were grouped according to the biological processes they are involved in. Number of genes down/up regulated for each biological prosess was determined using the database. Differentially regulated genes in WT and transgenic lines under control and freezing stress conditions were visualized in the context of existing knowledge (pathway) using MapMan 3.5.0 Beta Software. The mapping file was Slyc_AFFY_SGN_BUILD2_070709 (1.1).

2.2.5.6 Real-Time qPCR for the Confirmation of Microarray Data

Real-Time qPCR was performed to confirm microarray results by an independent gene expression profiling method. The first step in confirming array results by real-time qPCR is selection of gene-specific primer pairs. For this purpose primer pairs were designed for nine significantly differentially regulated ($p<0.05$, $FC>2$) probe sets (Table 2.6).

Table 2.6 Primer sequences of the probe sets subjected to validation test by real-time qPCR.

Probe Set ID	Direction	Primer Sequence	Tm (°C)	Amplicon size (bp)
Les.5834.1.S1_at	Forward Reverse	TATGGTGTTTATACATCAGCCC CGTTGCTTTCTACCACATCG	48.8 50.8	126
LesAffx.35136.1.S1_at	Forward Reverse	GAGTTGTTTAATGATTCTCGAAC AAGTTCAAGACACATAAGGGC	47.2 47.8	118
LesAffx.51226.1.A1_at	Forward Reverse	CTATATACGTAGGCGGGAACAG CGTTATGGTTATCTCATACCCC	50.5 49.8	146
Les.3377.2.S1_at	Forward Reverse	TCCGTCTCCTCAACATTTTG GCCACTGGATTCTCTCAAAC	50.0 48.5	128
LesAffx.44474.1.A1_at	Forward Reverse	TTGGGTGTTGCTTTATATGG CGATTTGACAGCAAAAGC	47.9 46.4	129
LesAffx.66410.1.S1_at	Forward Reverse	AAAAGACATACAACCTTGGC AGCTAACCAACTTCTAGGAGAG	46.1 46.3	139
LesAffx.22051.2.S1_at	Forward Reverse	CACTTGCGTATTGTACGAGAAAT CTCCACCATTTGTGGTACATAAG	54.9 53.2	120
LesAffx.54522.1.S1_at	Forward Reverse	TGAAGAGGACGAGTTGTTGC TTACACCACCGGAGACGAC	50.1 50.4	117
LesAffx.69865.1.S1_at	Forward Reverse	TTACGAAGGATCAACTTACAGC AAGATTTTCCGGTGTGCC	48.5 49.6	112

The sequences of probe sets were downloaded from Affymetrix NetAffx database and primers were designed using Vector NTI software. The probe sets on the tomato

chip are synthesized acoording to the sequences in the tomato genome. However in this study the gene expression profiles of potato samples were analysed using tomato microarray. Therefore the sequences of the probe sets downloaded from Affymetrix NetAffx database were blasted to check the sequence similarity in the potato genome. Only the probe sets with a high similarity were selected to ensure binding of the primer sequences to cDNA samples synthesized from WT and transgenic potato samples.

cDNA Synthesis for Real-Time Two-Step qPCR

For quantification of RNA samples, RNA must first be reverse transcribed into cDNA. A portion of the reverse-transcription reaction is then transferred to another tube where real-time qPCR takes place. This entire process is known as real-time two-step qPCR, since reverse transcription and real-time qPCR are carried out in separate tubes.

Real-time qPCR allow accurate quantification of starting amounts of an amplicon. Usually, the amount of product is directly related to the fluorescence of a reporter dye. SYBR Green-based detection method was used for quantification of the selected probe sets. SYBR Green specifically binds double-stranded DNA by intercalating between base pairs, and fluoresces only when bound to dsDNA. However SYBR Green detects any double-stranded DNA non-specifically. Therefore, PCRs using this detection method must generate single, gene-specific amplicons without the co-amplification of non-specific secondary products. Before real-time qPCR anaysis, conventional PCR with specific primers was performed to confirm generation of single gene-specific amplicons. For conventional PCR analysis RNA samples from WT and transgenic lines extracted before and after freezing treatment were pooled and converted to cDNA using QIAGEN QuantiTect Reverse Transcription Kit. 1µL RNA from each sample was pooled and the concentration of pool RNA was determined by reading the absorbance at 260 nm. 1µg pool RNA sample was briefly incubated in gDNA Wipeout Buffer at 42°C for 2 minutes to effectively remove

contaminating genomic DNA. After genomic DNA elimination, the RNA sample was used for reverse transcription. cDNA was synthesized according to the manufacturer's instructions. PCR was performed with the selected primers to check specific amplification of the probe sets (Table 2.7). The PCR conditions for synthesis of probe set fragments are given in Table 2.8.

Table 2.7 PCR components used for conventional PCR.

Reagent	**Concentration**
Forward primer	0.3 µM
Reverse primer	0.3 µM
dNTPs	0.2 mM
10X Taq polymerase reaction buffer	1 X
$MgCl_2$	2 mM
Taq DNA polymerase	1 unit
DMSO	3%
cDNA	50 ng
dH_2O	Volume completed to 20 µL

Table 2.8 Conventional PCR cycling conditions for synthesis of selected probe set fragments.

Step / Segment		**Temperature**	**Duration**	**Cycle**
Initial Denaturation		96°C	3 min	1
Amplification	Denaturation	96°C	20 sec	30
	Annealing	50°C	30 sec	
	Extension	72°C	30 sec	
Final Extension		72°C	5 min	1

Amplified PCR products were run on 2% agarose gel to check generation of single gene-specific amplicons. Three of the PCR products that have a single specific amplicon seperated on agarose gel were selected to be used for real-time qPCR analysis.

Real-Time Quantification of cDNA Targets

Real-time qPCR enables quantification of target nucleic acids using either absolute quantification or relative quantification. The quantity of selected probe sets was determined using relative quantification which determines the ratio between the amount of target and the amount of a control (e.g., an endogenous reference molecule, usually a suitable housekeeping gene). This normalized value was then used to compare differential gene expression in different samples. *ef1α* which was used as an internal control for northern blot analysis was also used as control for real-time qPCR.

Generating Standard Curves

The quantification procedure differs depending on whether the target and the endogenous reference gene are amplified with comparable or different efficiencies. Amplification efficiency of three selected probe sets and *ef1α* (endogenous reference gene) was compared by preparing a dilution series for pool cDNA. For this purpose real-time qPCR was performed with 50 ng, 5 ng, 0.5 ng, 0.05 ng and 0.005 ng pool cDNA samples using primer pairs for three selected probe sets and *ef1α* (Table 2.9). Quantitative real-time PCR was performed using the Corbett Rotor-Gene™ 6000 real-time rotary analyzer. Each PCR run included three no-template control (Adeva, *et al.*) reactions which contained all qPCR components except the template. Amplifications in the NTC reaction indicated DNA contamination in the reaction master mix, or during the pipetting process. qPCR reactions for each dilution was performed with three technical replicates using QuantiTect SYBR Green PCR Kit (Table 2.10). After amplification of each dilution series in real-time qPCR, the C_T (treshold cycle) values obtained were used to construct standard curves for selected probe sets and *ef1α*. C_T values/crossing points of different standard dilutions were plotted against the logarithm of input amount of standard material to generate standard curves.

Table 2.9 Cycling conditions for real-time qPCR with dilution series of pool cDNA for selected probe sets and *ef1α*.

	PCR conditions			
Probe set ID / Gene name	**Step / Segment**	**Temperature**	**Duration**	**Cycle**
LesAffx.35136.1.S1_at Les.3377.2.S1_at	Pre-Incubation	95 °C	15 min	1
	Quantification	94 °C 52 °C 72 °C	15 sec 30 sec 20 sec	40
	Melt Curve	50 – 99 °C	5 sec / 1 °C	–
LesAffx.66410.1.S1_at	Pre-Incubation	95 °C	15 min	1
	Quantification	94 °C 54 °C 72 °C	15 sec 30 sec 20 sec	40
	Melt Curve	50 – 99 °C	5 sec / 1 °C	–
ef1α	Pre-Incubation	95 °C	15 min	1
	Quantification	94 °C 50 °C 72 °C	15 sec 30 sec 30 sec	40
	Melt Curve	50 – 99 °C	5 sec / 1 °C	–

Table 2.10 PCR components used for amplification of selected probe sets and *ef1α* in real-time qPCR using pool cDNA and specific primers.

Reagent	**Concentration**
Forward primer	0.3 µM
Reverse primer	0.3 µM
QuantiTect Master Mix	10 µL
cDNA	0.005-50 ng
dH_2O	Volume completed to 20 µL

The slope of a standard curve provides an indication of the efficiency of the real-time qPCR. The difference in C_T values of target gene and endogenous reference gene is plotted against the logarithm of the template amount. Amplification efficiencies of the target gene and the endogenous reference gene are only comparabe if the slope of the resulting straight line is <0.1. After deciding on comparability of the probe sets and *ef1α*, real-time PCR was performed with cDNA of WT and transgenic lines. For

this purpose 0.5 µg RNA sample of WT and transgenic lines were reverse transcribed using QIAGEN QuantiTect Reverse Transcription Kit. The reaction was performed with two biological replicates and three technical replicates for each sample using 1 µL cDNA, 0.3 µM primers and 10µL QuantiTect Master Mix in a final volume of 20 µL. Two no-template control reactions were included in each PCR run to check DNA contamination.

The amount of target and reference in the samples were calculated using their C_T values and the corresponding standard curve. The amount of target was divided by the amount of reference to calculate the normalized amount of target. The average of replicates was calculated and log transformed (log 2). The log transformed expression values were compared with those obtained by microarray analysis.

2.2.5.7 Statistical Analysis

The significance of difference between mean values was determined by one-way analysis of variance at 95% confidence interval by using SPSS 15.0 software programme. The standard errors of means were calculated by descriptive statistics test at the same programme.

CHAPTER 3

RESULTS AND DISCUSSION

3.1 Transformation

Potato leaf strips were transformed via *Agrobacterium* carrying the plasmid pCOR15-MYB4 or pSA-MYB4. Figure 3.1 shows the basic steps in regeneration of putative transgenic plants via indirect organogenesis.

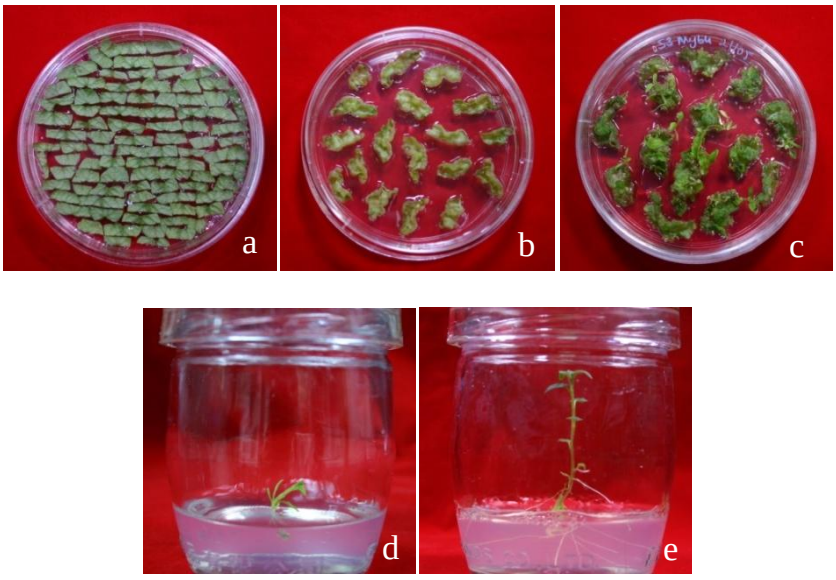

Figure 3.1 Basic steps in regeneration of putative transgenic plants via indirect organogenesis (a) pre-treatment of leaf strips in HH medium (b) callus formation in LSR-1 medium (c) shoot formation in LSR-2 medium (d, e) shoot elongation and rooting in CM medium respectively

Eight sets of transformation was performed with the plasmid carrying the *myb4* gene under the control of COR15a promoter and one set of transformation was performed

with the plasmid carrying the *myb4* gene under the control of CAMV35S promoter. Number of explants used in each set of transformation and number of shoots generated via indirect organogenesis in selective (kanamycin) medium are given in Table 3.1.

Table 3.1 Number of explants used for transformation and number of putative transgenic shoots generated in selective medium.

	pCOR15aMyb4		**pCAMVMyb4**	
Set no	Total number of explants used for transformation	Number of shoots generated in selective medium	Total number of explants used for transformation	Number of shoots generated in selective medium
1	18	9	80	22
2	49	56		
3	52	50		
4	86	56		
5	77	2		
6	82	10		
7	79	7		
8	91	11		
Total	**534**	**201**	**80**	**22**

534 leaf strips were used for transformation with pCOR15aMyb4. 201 shoots were generated via indirect organogenesis in kanamycin containing selective plates. The shoots grown in selective medium were transferred to non-selective rooting medium to encourage rooting. After reaching 5-6 leaves stage, apical meristems of these plantlets were transferred to selective rooting medium to select putative transgenic plantlets. 157 out of 201 shoots grew roots in the selective rooting medium and they were regarded as putative transgenic plantlets. 534 explants were used for transformation and 157 putative transgenic lines were obtained. Therefore potato plants were transformed with pCOR15aMyb4 with a transformation efficiency of 29.4%. 80 leaf strips were used for transformation with pCaMVMyb4. 22 shoots were generated via indirect organogenesis in kanamycin containing selective plates.

These shoots were transferred to non-selective rooting medium and grown until 5-6 leaves stage. The leaves were then collected and used for RNA isolation. Northern blot analysis was performed with these RNA samples and transformation efficiency was calculated according to the number of lines expressing *myb4* gene. The number of shoots generated in selective medium by transformation of potato with pCOR15aMyb4 was 157. Since the number of putative transgenic plants was so high northern blot analysis was not performed for all of these plants. Instead, transformation efficiency with this plasmid was calculated according to the number of plants that can grow roots in selective rooting medium.

3.2. Analysis of Transgenic Plants

3.2.1 Molecular Analyses

Total RNA was isolated from leaf samples of WT and randomly selected 13 putative transgenic lines carrying the *myb4* gene under the control of COR15a promoter. The lines with this promoter were represented with the capital letter 'M' and a number. The concentrations of the RNA samples were determined by reading the absorbance at 260 nm in nanodrop spectrophotometer. The absorbances were also determined at 280 nm and OD_{260}/OD_{280} was calculated to check the purity of the samples (Table 3.2). Ratio of 1.8~2.0 are considered ideal purity. OD_{260}/OD_{280} is ~1.7 in M30 and M31 but the others are in the range of 1.8-2.0. Therefore the purity of the samples was good enough to be used in northern blot analysis.

Total RNA was also isolated from leaf samples of all putative transgenic lines carrying the *myb4* gene under the control of CaMV35S promoter. The lines with this promoter were represented with the capital letter 'S' and a number. The concentrations of the RNA samples are given in Table 3.3. OD_{260}/OD_{280} of all the samples were in the range between 1.8 and 2.0 except S2 and S15. This shows that the RNA samples were pure and there was no contamination.

Table 3.2 RNA concentration of WT and selected putative COR15aMyb4 transgenic lines.

	OD_{260}	OD_{280}	OD_{260} / OD_{280}	RNA conc. (µg/µl)
WT	80.8	39.7	2.03	3.23
M26	77.4	37.9	2.04	3.10
M30	0.36	0.21	1.71	1.45
M31	74.5	44.7	1.67	2.98
M37	88.4	44.1	2.00	3.53
M39	61.9	30.2	2.05	2.48
M42	60.7	29.9	2.03	2.43
M45	81.5	40.3	2.02	3.26
M46	60.4	29.6	2.04	2.42
M48	35.3	17.3	2.04	1.41
M62	79.5	39.5	2.01	3.18
M67	103.4	54.8	1.89	4.14
M82	103.2	54.1	1.91	4.13
M85	44.4	22.3	1.99	1.78

Table 3.3 RNA concentrations of putative CaMVMyb4 transgenic lines.

	OD_{260}	OD_{280}	OD_{260} / OD_{280}	RNA conc. (µg/µl)
S1	93.3	47.2	1.97	3.73
S2	117.7	76.3	1.54	4.71
S3	42.2	21.0	2.01	1.69
S4	85.6	42.8	2.00	3.42
S5	47.5	23.1	2.06	1.90
S6	96.2	49.2	1.95	3.85
S7	96.0	49.0	1.96	3.84
S8	73.3	36.3	2.02	2.93
S9	84.7	42.0	2.02	3.39
S10	77.6	38.6	2.01	3.10
S11	64.5	31.7	2.03	2.58
S12	29.5	15.0	1.97	1.18
S13	95.8	48.8	1.96	3.83
S14	116.7	74.9	1.56	4.67
S15	115.0	70.5	1.63	4.60
S16	84.8	42.4	2.00	3.40
S17	63.0	30.6	2.06	2.52
S18	62.1	31.1	1.99	2.48
S19	34.0	16.6	2.04	1.36
S20	66.8	32.8	2.03	2.67
S21	57.8	28.2	2.05	2.31
S22	40.1	19.6	2.05	1.60

Genomic DNA of WT and putative transgenic lines was isolated to be used in southern blot analysis. Table 3.4 shows the DNA concentration of WT and selected COR15aMyb4 lines. OD_{260}/OD_{280} was determined to check the purity of the samples and the integrity was verified by separation of the samples on 1% agarose gel (Figure 3.2). DNA concentration of selected CaMVMyb4 lines is given in Table 3.5. Figure 3.3 shows the genomic DNAs separated on 1% agarose gel.

Table 3.4 DNA concentration of WT and selected COR15aMyb4 lines.

	OD_{260}	OD_{280}	OD_{260} / OD_{280}	DNA conc. (µg/µl)
WT	47.4	22.9	2.07	2.37
M26	22.9	10.6	2.16	1.14
M31	35.7	16.5	2.16	1.78
M37	48.4	22.8	2.12	2.42
M42	51.3	24.1	2.13	2.56
M46	30.1	14.6	2.06	1.50
M48	23.1	12.2	1.89	1.15
M62	40.0	20.9	1.91	2.00
M67	24.1	11.5	2.09	1.20

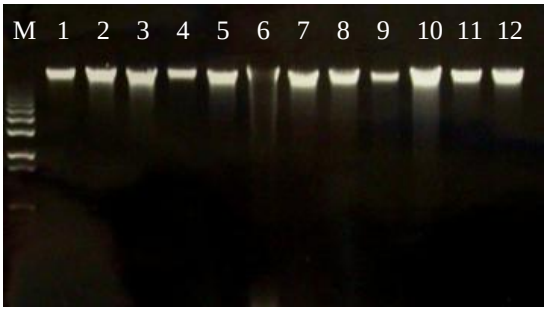

Figure 3.2 Genomic DNA of WT and selected COR15aMyb4 lines separated on 1% agarose gel M:1kb DNA Ladder, 1:WT, 2:M26, 3:M30, 4:M31, 5:M37, 6:M39, 7:M42, 8:M46, 9:M48, 10:M62, 11:M67, 12:M82

DIG labeled *ef1α, myb4* and *nptII* probes and non-labeled control PCR products for each gene were separated on agarose gel to check labeling of the probes (Figure 3.4). DIG labeled probes were heavier than the non-labeled control PCR products. So they

were seen on upper part of the gel when compared to control PCR products and this verified that they were successfully labeled.

Table 3.5 DNA concentrations of selected CaMVMyb4 lines.

	OD_{260}	**OD_{280}**	**OD_{260} / OD_{280}**	**DNA conc. (µg/µl)**
S1	62.1	27.7	2.24	3.10
S2	82.1	36.8	2.23	4.10
S3	79.1	35.8	2.21	3.95

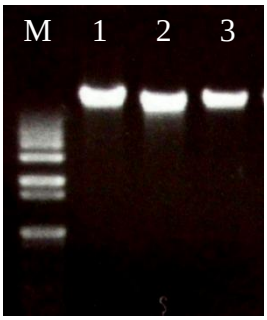

Figure 3.3 Genomic DNA of selected CaMVMyb4 lines separated on 1% agarose gel (M: 1kb DNA Ladder, 1: S1, 2: S2, 3: S3).

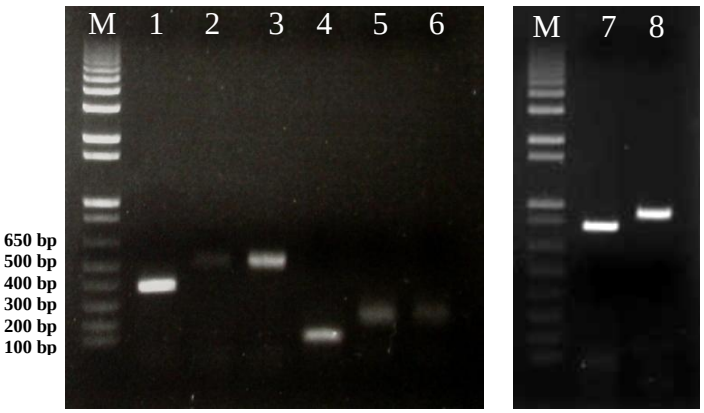

Figure 3.4 DIG labeled *ef1α, myb4* and *nptII* probes and non-labeled control PCR products separated on agarose gel (M: 1kb DNA Ladder, 1: non-labeled *myb4* PCR product, 2&3: DIG labeled *mb4* probe, 4: non-labeled *ef1α* PCR product, 5&6: DIG labeled *ef1α* probe, 7: non-labeled *nptII* PCR product, 8: DIG labeled *nptII* probe).

3.2.1.1 Northern Blot Analysis

Northern blot analysis was performed with randomly selected COR15aMyb4 lines to check the expression of *myb4* in putative transgenic lines grown in selective medium (Figure 3.5).

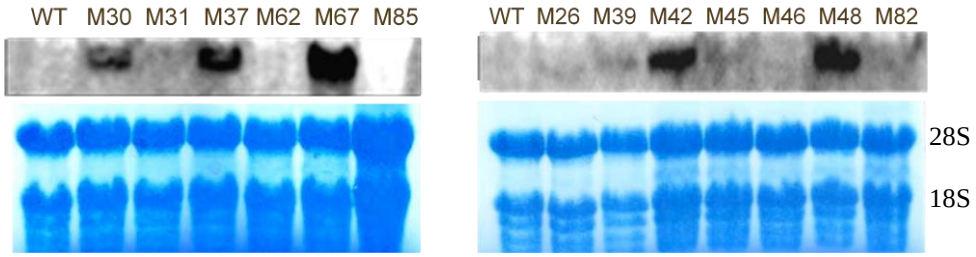

Figure 3.5 Northern blot of COR15aMyb4 lines. The signals seen on the upper panel shows expression of *myb4*. Lower panel represents total RNA transferred to membrane and stained with blot stain blue.

Northern blot analysis was performed with randomly selected 13 lines and high expression of *myb4* was detected in 5 of the COR15aMyb4 lines. The transgenic lines M37, M42, M48 and M67 were selected to be used in further experiments. Expression of *nptII* and *ef1α* genes was also examined (Figure 3.6) in these lines. Agrobacterium strain used for transformation contains *nptII* gene in the T-DNA region. Therefore the transgenic lines are expected to express *nptII* which is not naturally expressed in plant cells. Since expression of *ef1α* is reported to be stable during abiotic stress treatments (Nicot *et al.*, 2005) it used as internal control for northern blot analysis. COR15a is a cold inducible promoter therefore the plants were incubated in 4 °C for 8 hours before RNA isolation to induce the promoter. RNA samples were also isolated before promoter induction to see the expression pattern without promoter induction. The level of expression was higher in the transgenic lines after promoter induction except the line M48. Although *myb4* gene was expressed under the control of a stress inducible promoter there was a low expression without promoter induction indicating that the promoter is leaky.

Northern blot analysis was performed with all CaMVMyb4 lines to check the expression of *myb4* in putative transgenic lines (Figure 3.7).

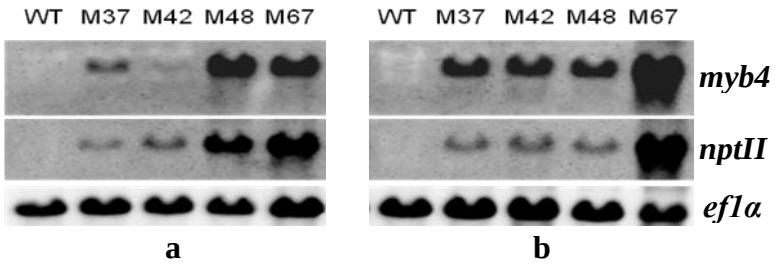

Figure 3.6 Expression of *myb4*, *nptII* and *ef1α* before (a) and after (b) promoter induction in COR15aMyb4 lines.

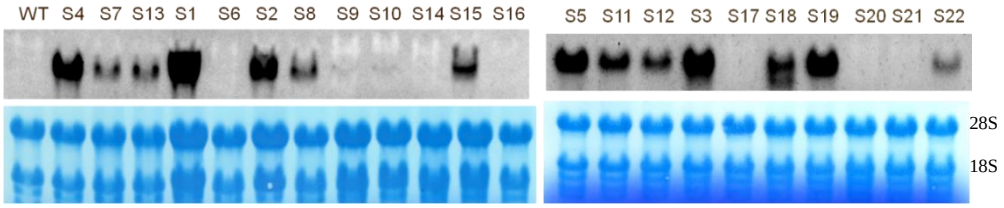

Figure 3.7 Northern blot of CaMVMyb4 lines. The bands seen on the upper lane shows expression of *myb4*. Lower lane represents total RNA transferred to membrane and stained with blot stain blue.

Northern blot analysis showed that 14 out of 22 lines were expressing MYB4 in varying levels. 80 leaf strips were used for transformation with pCaMVMyb4. 14 transgenic lines expressing MYB4 were obtained out of 80 explants. So the transformation efficiency of potato with pCaMVMyb4 was 17.5%. The transgenic lines S1, S2 and S3 highly expressing *myb4* were selected and used in further experiments. Expression of *nptII* and *ef1α* genes were also examined (Figure 3.8) in these lines.

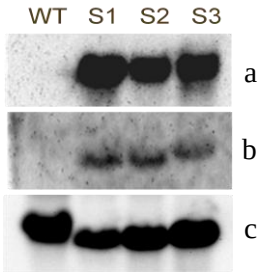

Figure 3.8 Expression of *myb4* (a), *nptII* (b) and *ef1α* (c) in selected CaMVMyb4 lines.

3.2.1.2 Southern Blot Analysis

Prior to Southern blotting 30 µg genomic DNA of WT and transgenic lines were digested with *Hind*III and separated on 0.8% agarose gel. Figure 3.9 and Figure 3.11 (a) shows the digests of Cor15aMyb4 lines and CaMV35SMyb4 lines separated on agarose gel respectively. Digested DNA samples transferred to nylon membrane were hybridized with *nptII* and *myb4* probes. The hybridized probes verified insertion of multi copies of *nptII* and *myb4* in the genome of Cor15aMyb4 lines (Figure 3.10). Southern hybridization of *nptII* probe verified insertion of three copies of *nptII* in S1 and S3 lines and two copies of insertion in S2 line (Figure 3.11 (b)).

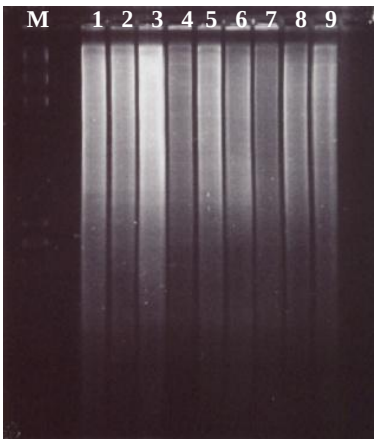

Figure 3.9 Agarose gel electrophoresis of *Hind*III digested genomic DNA of WT and COR15aMyb4 lines M: λ *Hind*III DNA ladder, 1: WT, 2: M37, 3: M42, 4: M48, 5: M67, 6: M46, 7: M62, 8: M31, 9: M26

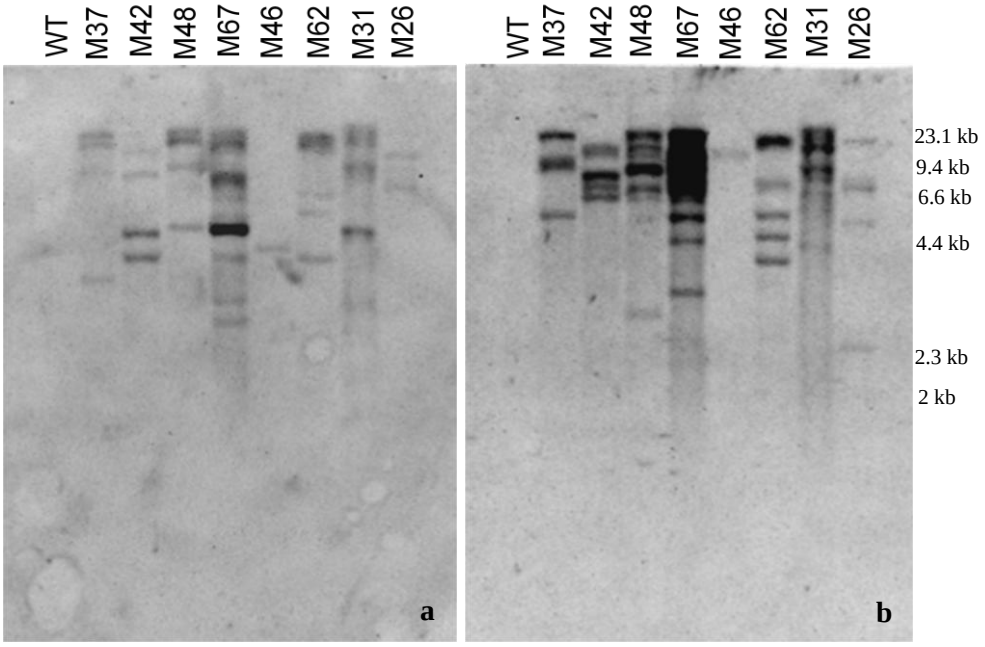

Figure 3.10 Southern blot of selected COR15aMyb4 lines (a) hybridization with *nptII* probe (b) hybridization with *myb4* probe

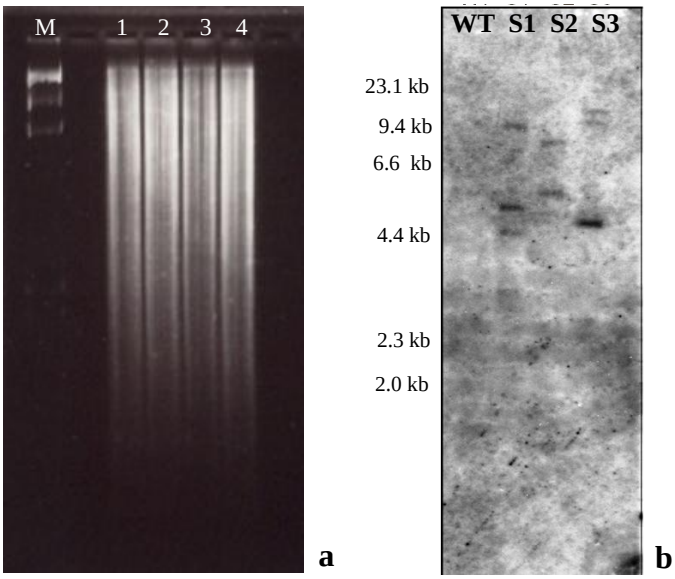

Figure 3.11 Agarose gel electrophoresis and southern hybridization of WT and CaMVMyb4 lines (a) Agarose gel electrophoresis of *Hind*III digested genomic DNA samples (b) Southern hybridization of WT, S1, S2 and S3 lines with *nptII* probe M: λ *Hind*III DNA ladder, 1: WT, 2: S1, 3: S2, 4: S3

3.2.2 Growth and Tuber Formation of Wild Type and Transgenic Plants

Figure 3.12 shows WT and the transgenic lines M37, M42, M48, M67, S1, S2 and S3 grown in greenhouse for 7 weeks. It is cited in literature that overexpression of transcription factors usually lead to growth retardation in transgenic plants (Kasuga, *et al.*, 1999; Liu, *et al.*, 1998). Vannini, *et al.* (2004) reported a dwarf phenotype in transgenic Arabidopsis plants which was depending on the *Osmyb4* level of expression. However the same research group observed no dwarf phenotype in tomato plants overexpressing *Osmyb4* (Vannini, et al., 2007).

After 7 weeks of growth the phenotypes of WT, CaMV35SMyb4 and COR15aMyb4 transgenic potato plants were similar. This shows that expression of MYB4 transcription factor in potato does not cause growth retardation either under the control of CaMV35S constitutive promoter or COR15a cold inducible promoter.

Six plants for WT and each transgenic line were grown for four months in the greenhouse. At the end of this period the plants were harvested and tuber formation potency of each line was evaluated (Figure 3.13). Table 3.6 represents the tuber number, tuber yield and tuber size of WT and transgenic plants.

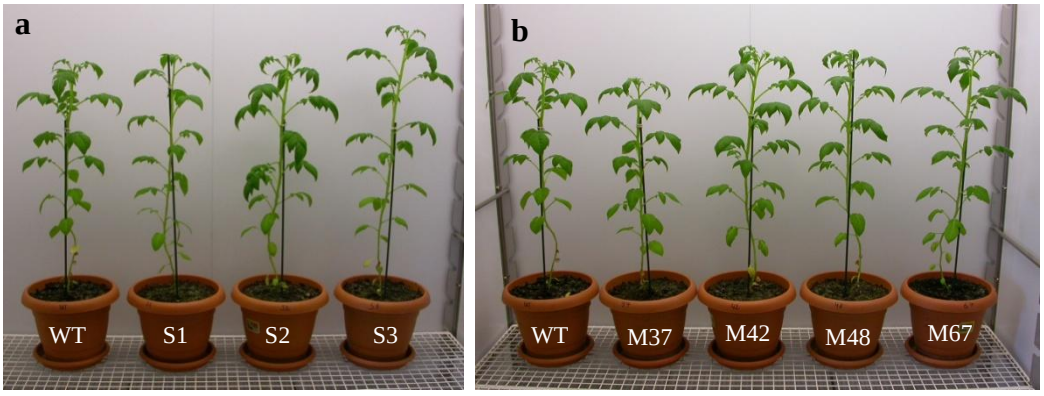

Figure 3.12 Wild-type and transgenic plants grown in greenhouse for 7 weeks. (a) CaMV35SMyb4 lines (b) COR15aMyb4 lines

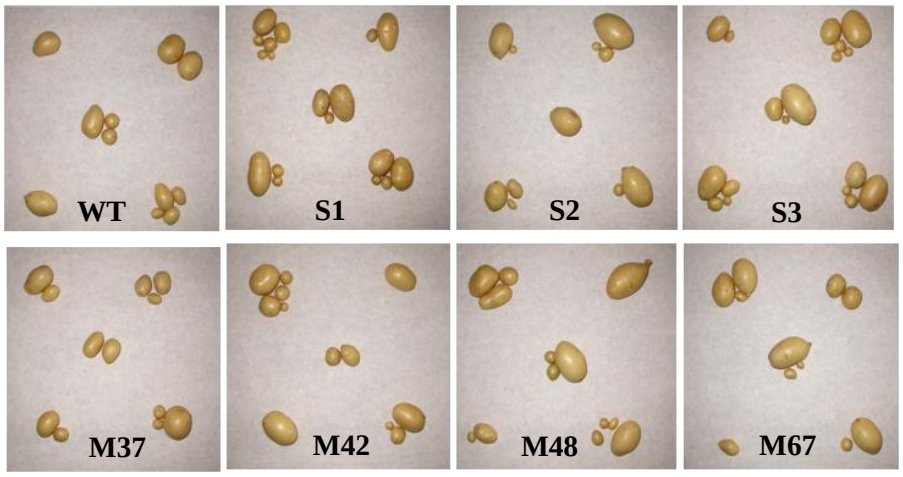

Figure 3.13 Tubers of WT and transgenic lines harvested after four months of growth in greenhouse. Each group of tubers that belong to a line is harvested from an independent plant.

Table 3.6 Tuber number, tuber yield and tuber size of WT and transgenic lines. Tuber number and tuber yield data represent mean values of 6 plants ± SEM.

	Tuber number (no. /plant)	**Tuber yield (g/plant)**	**Mean tuber size (g)**
WT	2.5 ± 1.38	30.75 ± 11.87	12.30
S1	3.7 ± 1.51	35.06 ± 6.27	9.56
S2	2.2 ± 0.75	34.35 ± 12.7	15.86
S3	3.2 ± 1.47	34.89 ± 17.26	11.02
M37	2.4 ±0.55	24.48 ± 7.51	10.20
M42	2.3 ± 1.51	29.17 ± 12.53	12.50
M48	2.3 ± 1.21	36.10 ± 16.23	15.47
M67	2.3 ± 0.82	32.12 ± 16.37	13.76

The tuber number per plant is 2.5 for WT and it is lower in the transgenic lines except S1 and S3. Although the tuber numbers per plant is different they are not significantly different on 5% significance level when compared to WT. The tuber yield per plant is 30.75g for WT and it is higher in the transgenic lines except M37 and M42. Although tuber yield is higher in most of the transgenic lines it is not significantly different on 5% significance level when compared to WT. This shows

that expression of MYB4 transcription factor in potato does not reduce the tuber yield which is the main economic criteria in potato production. There is also no significant difference for mean tuber size.

3.2.3 Determination of Sugar, Ascorbic Acid and Anthocyanin Content of Wild Type and Transgenic Plants

Sucrose, glucose, fructose and ascorbic acid content of WT and transgenic potato tubers harvested after four months of growth in greenhouse under normal conditions were determined via HPLC (Table 3.7). One sample per line was prepared for HPLC analysis and the amount of sugars and ascorbic acid in 100 g tuber was calculated.

Table 3.7 Sucrose, glucose and fructose content of WT and transgenic plants determined using HPLC.

	Sucrose (mg/100g)	**Glucose (mg/100g)**	**Fructose (mg/100g)**	**Ascorbic acid (mg/100g)**
WT	115.5	51.4	10.3	7.17
S1	145.0	48.6	9.3	6.21
S2	145.2	44.6	11.6	8.42
S3	98.7	52.3	11.1	6.63
M37	106.2	13.3	12.6	5.04
M42	112.9	83.8	12.1	10.88
M48	67.5	36.5	11.9	6.14
M67	86.0	44.2	16.2	2.66

Since there was only one sample per line no statistical analysis could be performed to evaluate the difference in content of sugar and ascorbic acid in transgenic plants compared to WT. There is not a sharp difference between the sugar and ascorbic acid contents of CaMVMyb4 and COR15aMyb4 transgenic lines. The content of the sugars and ascorbic acid is lower than WT in some of the transgenic lines and higher in some of the others. Therefore it may not be concluded that expression of MYB4 decreases or increases the content of sugars and ascorbic acid in transgenic potato plants. Potato is a valuable food for human diet due to its carbohydrate and vitamin

content. There is not a great reduction of sugar and ascorbic acid in transgenic potato plants expressing MYB4 transcription factor which shows that transgenic potato tubers are as nutritive as wild type.

Besides the nutrition facts, free sugars and ascorbic acid is also important for abiotic stress tolerance. Accumulation of sugars in the form of sucrose and glucose is a mechanism by which plants acclimate to various stresses. These sugars may serve as osmoprotectants during stress or they can be quickly used for energy production in a recovery period after stress (Bohnert & Jensen, 1996). Many stresses, act at least in part by causing oxidative damage. Ascorbic acid is an antioxidant molecule and in association with other components of the antioxidant system, it protects plants against oxidative damage (Smirnoff, 1996). In this study ascorbic acid and free sugar levels in some transgenic lines were higher when compared to WT. Improvement of sugar or ascorbic acid content by expression of *myb4* in transgenic lines may in part contribute to abiotic stress tolerance in potato. Measuring sugar and ascorbic acid levels in transgenic plants after subjecting to an abiotic stress factor may improve our understanding of the role of these compounds in abiotic stress tolerance. Therefore another experiment may be set to see the effect of an abiotic stress factor on accumulation of these compounds in transgenic potato plants. Sucrose, glucose and fructose concentrations in transgenic Arabidopsis plants overexpressing *Osmyb4* were almost twice the amount of the WT before any stress treatment. Cold and drought stresses induced the accumulation of these sugars also in the WT plants but at lower concentrations with respect to the transgenic lines (Mattana, *et al.*, 2005). In normal growth conditions the concentrations of sugars in transgenic tomato plants expressing *myb4* under the control of 35S promoter was higher with respect to WT and transgenic lines expressing *myb4* under the control of COR15a promoter. In response to water deficit conditions the soluble sugar content increased in all genotypes. However the values of WT were lower than those of transgenic lines in all cases (Vannini, *et al.*, 2007). Increased sugar concentrations before and after stress treatment was also reported in transgenic apple and *Osteospermum ecklonis* overexpressing *myb4* (Laura, *et al.*, 2010; Pasquali, *et al.*, 2008).

In plants, many MYB transcription factors are involved in regulating anthocyanin biosynthesis (Dubos, *et al.*, 2010; Feng *et al.*, 2010; Jin & Martin, 1999). Anthocyanins are water-soluble pigments found in all plant tissues and are responsive to some environmental factors including visible and UVB radiation, water and cold stress. The induction of anthocyanins by low temperatures indicates that they may have a protective function (Chalker-Scott, 1999). McKown *et al.* (1996) showed that Arabidopsis mutants deficient in freezing tolerance could not accumulate anthocyanins indicating a relationship between anthocyanins and freezing tolerance. It is widely known that anthocyanins are induced in autumn and they accumulate in woody plants during cold hardiness and dormancy. There is not a direct link set between drought resistance and anthocyanin content but there is some literature data pointing this direction For example, the 'Pretty Purple' cultivar of pepper is more resistant to drought compared to the green cultivars (Bahler *et al.*, 1991). Resurrection plants extremely tolerant to dehydration, accumulate much more anthocyanins during dehydration compared to normal growth conditions (Sherwin & Farrant, 1998).

In order to examine the affect of *myb4* expression on anthocyanin content in transgenic potato plants, the anthocyanin content was determined in WT and transgenic plants. Three independent plants and three samples from each plant were used for determination of anthocyanin. Figure 3.14 shows the mean values of 9 samples per line. The samples were collected from plants grown for 7 weeks in greenhouse under normal growth conditions. One way ANOVA results showed that there was no significant difference in anthocyanin contents of transgenic lines when compared to WT. This indicates that *myb4* expression does not significantly alter anthocyanin content under normal growth conditions. However determination of anthocyanin content in samples collected after stress application may better show the affect of *myb4* expression on anthocyanin content. Therefore this experiment may be repeated with samples collected from WT and transgenic plants subjected to an abiotic stress factor such as drought or cold.

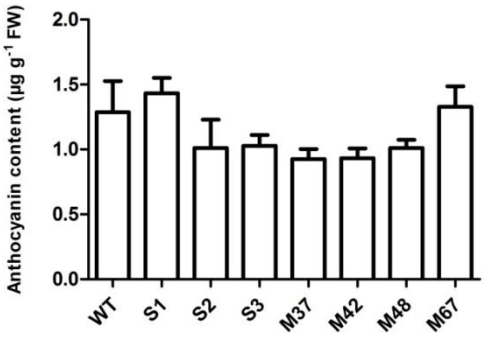

Figure 3.14 Anthocyanin contents of WT and transgenic lines. The bars represent mean values for each line and SEMs of nine samples per line are indicated as error bars.

3.2.4 Stress Treatment of Wild Type and Transgenic Plants

32 independent plants for WT and each transgenic line were evaluated for their responses upon exposure to NaCl, excess boron and drought treatments. Three plants and three leaf discs from each plant were subjected to freezing temperatures. After NaCl, excess boron and drought treatments shoot length, root length, shoot fresh weight, root fresh weight, shoot dry weight and and root dry weights were determined to evaluate the growth of WT and transgenic lines under stress conditions. Ion leakage of WT and transgenic lines were determined after freezing treatment to evaluate the damage generated by freezing temperatures.

3.2.4.1 NaCl Treatment

Wild type plantlets were grown on MS medium containing 0 mM, 50 mM, 100 mM and 150 mM NaCl for one month and 100 mM NaCl was found to be the minimum concentration of salt limiting growth. Growth parameters were determined only for plantlets that could form roots (Figure 3.15) under salt stress conditions. 32 plantlets per line were grown on MS medium with 100 mM NaCl and the growth parameters were recorded (Figure 3.16). Physiological effect of excess salt on growth is given in Appendix F.

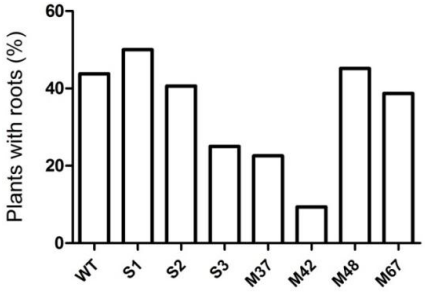

Figure 3.15 % of plants with roots grown on MS medium containing 100 mM NaCl.

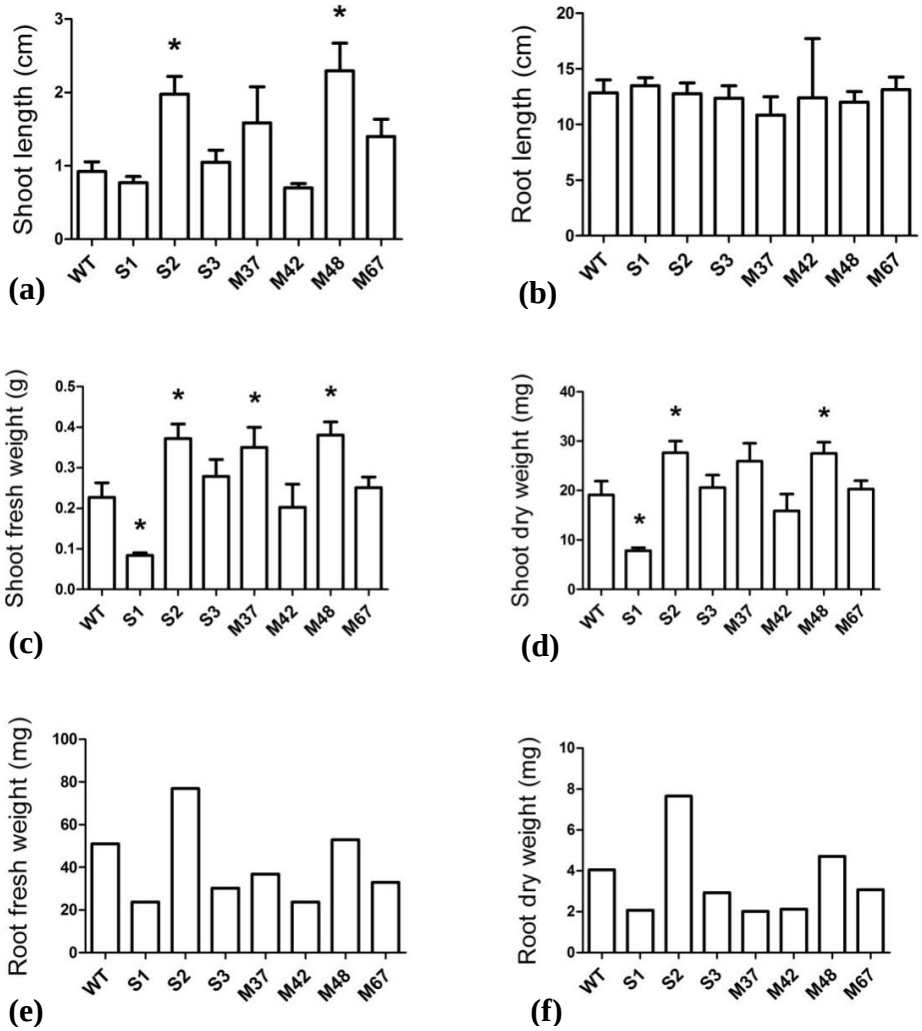

Figure 3.16 The effect of 100 mM NaCl on (a) shoot length, (b) root length, (c) shoot FW, (d) shoot DW, (e) root FW and (f) root DW of WT and transgenic lines. Only plants with roots were evaluated and SEMs of samples are indicated as error bars. * The values are significantly different on 5% significance level compared to WT (FW: fresh weight, DW: dry weight).

High salt stress disrupts homeostasis in water potential and ion distribution. Drastic changes in ion and water homeostasis lead to molecular damage, growth arrest and even death. Therefore resuming growth is important to achieve salt tolerance (Zhu, 2001). Providing a consistent, highly controlled and monitored experimental environment is critical for salinity research since fluctuating environmental conditions confound the exact plant response to salinity. Uniform, regulated in vitro systems provide such an environment that allows successful selection for salt tolerance in plants. Growth parameters together with mineral acquisition are often monitored to explain the mechanisms behind plant response to salinity (Shibli *et al.*, 2007). In this study growth parameters of WT and transgenic plants were monitored to compare their salt tolerances.

The number of plants that can grow on high salt medium was higher in some of the transgenic lines and lower in some of the others (Figure 3.15). There was no significant ($P<0.05$) difference between root lengths of WT and transgenic lines. The shoot length of the transgenic lines S2 and M48 were significantly higher when compared to WT. Shoot fresh weights of S2, M37, M48 and shoot dry weights of S2 and M48 were also significantly higher compared to WT plantlets. The root dry weights and fresh weights of S2 and M48 were also higher than WT. Since the weights of the roots were so low, the root of each plantlet was not weighed separately. The roots of plantlets that belong to same line were collected and weighed together and divided by the number of plantlets with roots to determine the mean weight for each line. Therefore no statistical analysis was performed for fresh weight and dry weight data of the root samples.

The growth parameters for shoot and roots show that there is not a great difference between the characteristics of COR15aMyb4 and CaMV35SMyb4 transgenic lines. Therefore it can be concluded that the type of promoter does not make a great difference in salt tolerance of transgenic plants.

Reduction of growth in response to salinity stress was greater in WT plants compared to the transgenic lines S2 and M48. Reduction in growth with increased salinity could be attributed to induced water deficit and/or ion toxicity associated with excessive uptake particularly of Na^+ and Cl^- and nutritional imbalance as a result of depressed uptake, transport and impaired internal distribution of minerals especially K^+ and Ca^{+2} (Lutts *et al.*, 1996). Higher reduction rates of growth in WT compared to S2 and M48 showed that expression of MYB4 transcription factor may increase salt tolerance in transgenic lines. Previously it was demonstrated that transgenic Arabidopsis plants overexpressing *myb4* was also much more tolerant to salt stress. Following 300 mM NaCl treatment, about 50% of transgenic plants but none of the WT plants were survived (Vannini *et al.*, 2006).

3.2.4.2 Boron Toxicity

Wild type plantlets were grown on MS medium containing 0 mM, 2 mM, 3 mM and 4 mM boric acid for one month and 3 mM boric acid was found to be the minimum concentration of boron limiting growth. The growth parameters were determined only for plantlets that could form roots (Figure 3.17) under toxic boron concentrations. 32 plantlets per line were grown on MS medium with 3 mM boric acid and the growth parameters were recorded (Figure 3.18). Physiological effect of boron toxicity on growth is given in Appendix F.

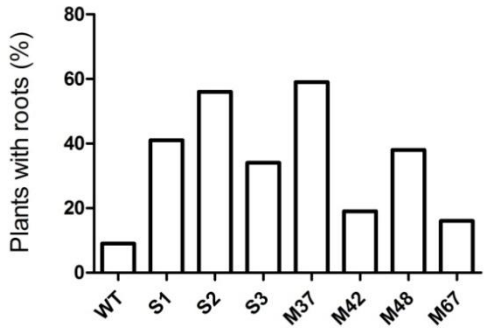

Figure 3.17 % of plants with roots, grown on MS medium containing 3 mM boric acid.

Boron (B) is an essential micronutrient for plant growth. However excess boron in the soil due to low rainfall, irrigation and pollution becomes toxic to plants. Tolerance to B toxicity varies between plant types and between cultivars of the same species. B toxicity leads to inhibition of cell division and elongation, disruption of cell wall development and metabolic disruption by binding to the ribose moieties of NADPH, ATP and NADH (Reid *et al.*, 2004). Therefore growth parameters of plants grown under high boron concentrations may indicate boron toxicity tolerarance.

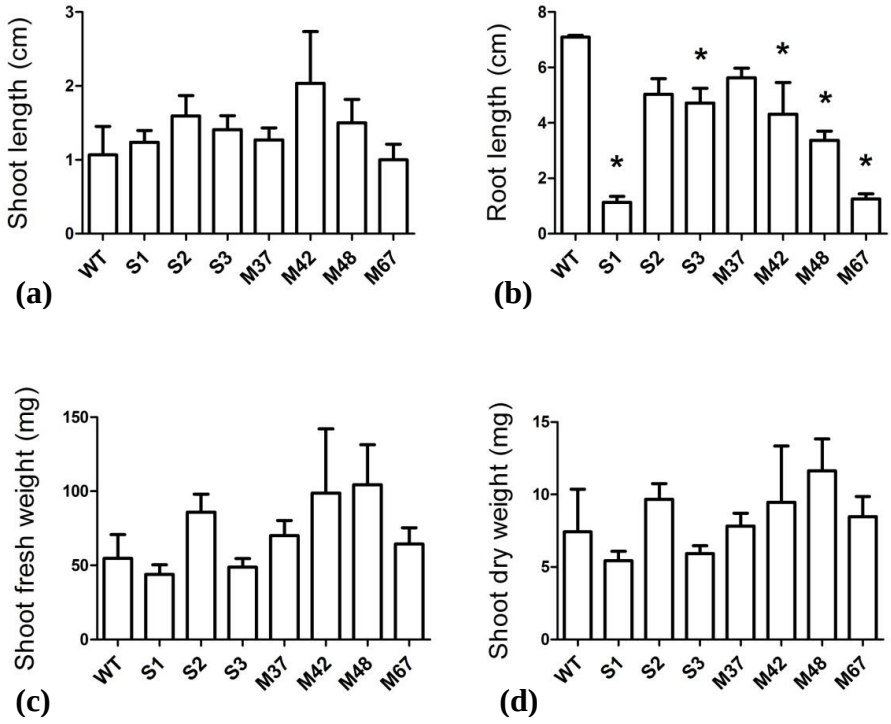

Figure 3.18 The effect of 3 mM boric acid on (a) shoot length, (b) root length, (c) shoot FW and (d) shoot DW of WT and transgenic lines. Only plants with roots were evaluated and SEMs of samples with roots per line are indicated as error bars. * The values are significantly different on 5% significance level compared to WT.

Only 9% of WT plants could grow roots on MS medium containing 3 mM boric acid (Figure 3.17). This ratio was greater in all of the transgenic lines which may reflect a higher B toxicity tolerance in transgenic plants. The root length of the transgenic

lines S1, S3, M42, M48 and M67 were significantly lower ($P<0.05$) when compared to WT. The shoot length, shoot fresh weight and shoot dry weight of some of the transgenic lines were higher when compared to WT. The growth response of each single nodal segment in boric acid containing medium was very different within a line. This resulted in high standard error of means for the measured parameter in each line. Therefore the growth parameters of many transgenic lines are much better than WT but this is not a significant difference due to high SEMs. There was not a significant difference in growth response of COR15aMyb4 and CaMV35SMyb4 transgenic lines to toxic boron concentrations. Therefore type of promoter does not appear to influence the affect of *myb4* expression on tolerance of transgenic plants to boron toxicity.

This is the first data in literature which reveals the effect of B toxicity on transgenic plants expressing *myb4*. Therefore no comparison can be made with data obtained in other species.

3.2.4.3 Drought Treatment

Wild type plantlets were grown on perlit wetted with ½ MS containing 5, 10, 15 and 20 % PEG 6000 for one month and 15% PEG was found to be the minimum concentration of PEG limiting growth. The growth parameters were determined only for plantlets that could form roots (Figure 3.19) under drought stress conditions generated by PEG. 32 plantlets per line were grown on medium with 15% PEG and the growth parameters were recorded (Figure 3.20).

Adaptive strategy of plants to drought stress is drought escape by cutting short their growth duration and avoiding the stress by keeping their high tissue water potential via reducing water loss or improved water uptake, or both. Rapid inhibition of shoot and, to a lesser extent, root growth is the first symptom encountered in drought stress (Akcay *et al.*, 2010; Turner *et al.*, 2007). Therefore the drought tolerance of WT and transgenic potato plants were evaluated with regard to growth parameters.

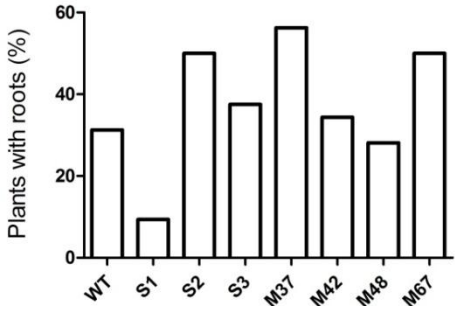

Figure 3.19 % of plants with roots, grown on perlit wetted with ½ MS containing 15% PEG.

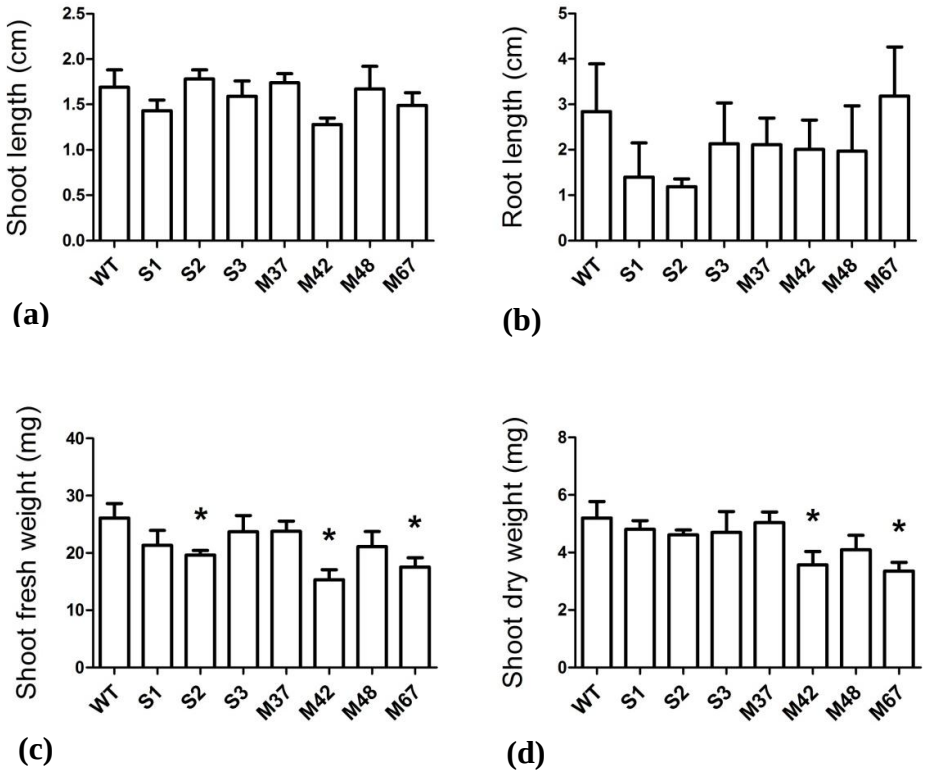

Figure 3.20 The effect of 15% PEG on (a) shoot length, (b) root length, (c) shoot FW and (d) shoot DW of WT and transgenic lines. Only plants with roots were evaluated and SEMs of samples with roots per line are indicated as error bars. * The values are significantly different on 5% significance level when compared to WT.

The shoot length and root length of transgenic lines were not significantly different ($p<0.05$) when compared to WT. The shoot fresh weights of S2, M42 and M67 and

the shoot dry weight of M42 and M67 were significantly lower when compared to WT. Previously Mattana *et al.* (2005) reported increased drought tolerance in transgenic Arabidopsis plants overexpressing *OsMyb4* and Vannini *et al*. (2007) demonstrated that transgenic tomato plants expressing *OsMyb4* were more tolerant to drought compared to WT plants. However in this study drought stress severely limited growth in all of the plants and there was no increased drought tolerance in transgenic potato lines in the tested conditions. The different behavior of transgenic potato plants compared to the response of other species may be due to type of the stress employed. It should also be kept in mind that expression of the same gene may have different effects in different organisms which may be related to host genomic background.

3.2.4.4 Freezing Treatment

Plants grown in green house for one month and cold acclimated one week in 4 °C were subjected to subzero temperatures and the ion leakage was determined at -3, -4, -5 and -6 °C (Figure 3.21).

Change in membrane integrity and fluidity induced by temperature is one of the immediate responses of plants upon exposure to freezing which shows that it may be the site of perception and/or injury (Sung *et al.*, 2003). Electrolyte leakage is widely used as an indicator for membrane damages generated by various stresses in all structures (Parvanova *et al.*, 2004). In this study electrolyte leakage of WT and transgenic lines were measured to compare the damage resulting from freezing temperatures.

The electrolyte leakage was lower in certain time points in WT when compared to transgenic plants indicating a higher freezing tolerance. However this low electrolyte leakage was not maintained for all the temperature points evaluated. Therefore there was no consistent freezing tolerance in WT compared to transgenic plants.

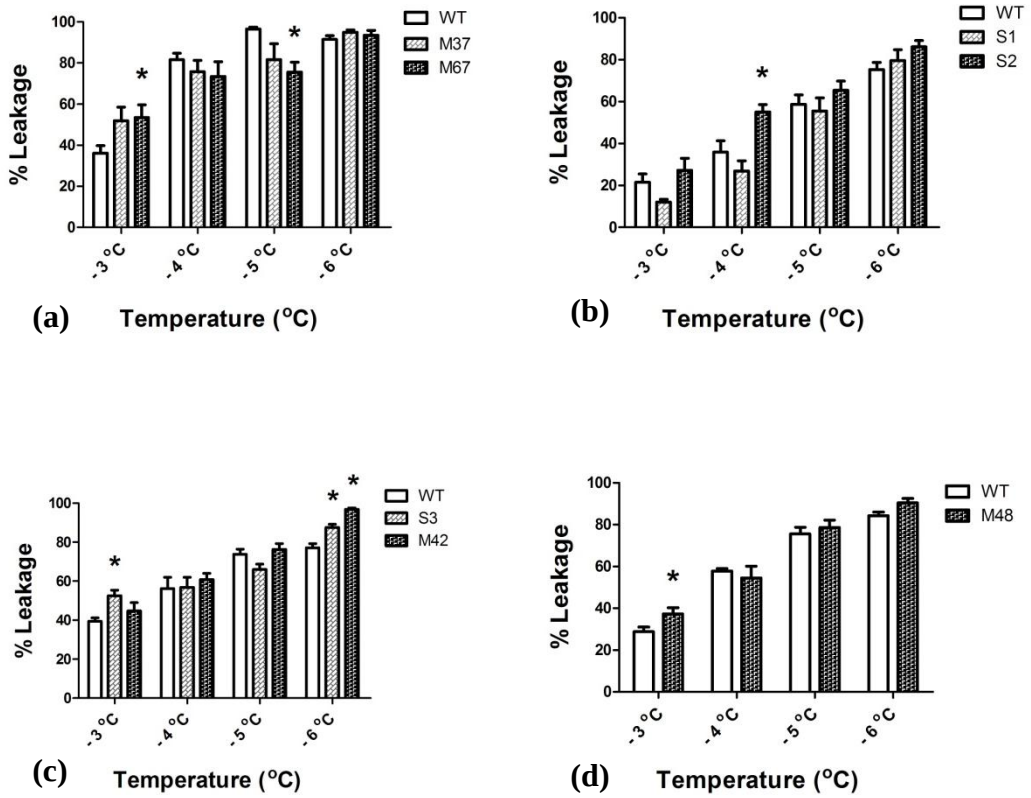

Figure 3.21 Ion leakage (%) of WT and transgenic lines (a-d) subjected to freezing temperatures. SEMs of nine samples per temperature point evaluated are indicated as error bars. * The values are significantly different on 5% significance level compared to WT.

Vannini *et al.* (2004) reported an increased freezing tolerance in transgenic Arabidopsis plants overexpressing *Osmyb4*. Laura *et al.* (2010) showed that constitutive expression of *Osmyb4* improved the cold and freezing tolerance in *Osteospermum ecklonis*. Pasquali *et al.* (2008) assessed the cold tolerance of WT and transgenic apple plants transformed with *Osmyb4*, depending on respiration rates at different temperatures. They demonstrated that *Osmyb4* overexpressing plants had a higher respiratory capacity at lower temperatures than WT plants. The findings of this study showing the effect of *myb4* expression on freezing tolerance of transgenic potato plants conflicts with data obtained in Arabidopsis, *Osteospermum ecklonis* and apple. However findings of Vannini *et al.* (2007) is in accordance with our data which reports that transgenic tomato plants overexpressing *Osmyb4* are not more

cold tolerant than WT. They discuss that different selective pressures in response to different environments colonised by a species may lead to different behaviours upon exposure to stresses. Tomato is a tropical plant and selection pressures could have caused the loss of the target genes in the cold pathway controlled by *myb4*. Another research group also pointed the same direction demonstrating that the tomato CBF regulon involved in freezing tolerance is much smaller in size and potentially less diverse in function compared to Arabidopsis (Zhang *et al.*, 2004). These differences in tomato which prevents improved cold tolerance in *OsMyb4* transgenic plants may be valid for potato as well. Since both potato and tomato belong to *Solanaceae* family the cold pathway genes may have been evolved in the same direction.

3.2.5 Microarray Analysis of Wild Type and Transgenic Potato Plants

Microarray analysis was performed with RNA samples isolated from leaves of plants grown under normal conditions and from leaf discs subjected to -4 °C freezing stress. The samples collected from plants grown under normal conditions (control) were designated by a capital "C", and those collected after freezing stress (after stress) were designated by the capitals "AS". Prior to freezing treatment wild type, S2 (transgenic line with CaMV35S promoter) and M48 (transgenic line with COR15a promoter) lines were grown in green house for one month. Then the plants were cold acclimated at 4 ˚C for one week and then subjected to freezing temperatures. Plants that were neither cold acclimated nor freezing treated were used as control. In order to reduce biological variability and to increase significance of the results statistically, three biological replicates per condition were used. Two replicates were used for S2 and M48 control conditions.

The integrity and quality of total RNA samples were checked using Agilent 2100 Bioanalyzer (Figure 3.22) and the concentrations of the samples were determined using Thermo NanoDrop spectrophotometer (Table 3.8).

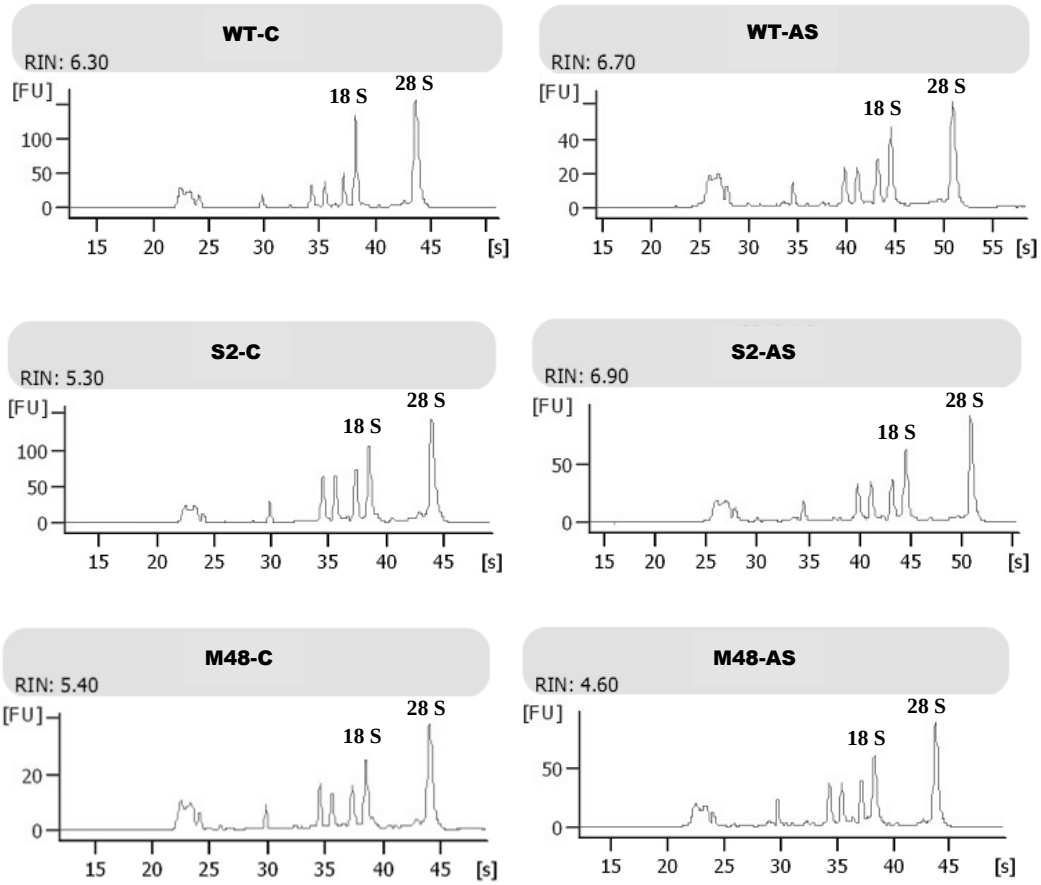

Figure 3.22 Agilent 2100 bioanalyzer electropherograms of RNA samples used for microarray analysis. RNA samples of WT, S2 and M48 were isolated from leaves of plants grown under normal conditions (C) and from leaf discs subjected to -4 °C freezing stress (AS).

The RNA integrity number (RIN) is an algorithm for assigning integrity values to RNA measurements where a value of 10 represents the highest quality RNA. RNA samples with RIN between 5 and 7 can be used for many types of experiments. The RINs of RNA samples (Figure 3.22) used for microarray analysis were between 4.6 and 6.9 which shows that the integrity and quality of the samples were good enough to be used in gene expression profiling. Three replicates were analyzed for each line and each condition but electropherogram of only one RNA sample was shown in Figure 3.22. RINs of the other samples were also in the range between 5 and 7.

Table 3.8 Concentrations of RNA samples used for microarray analysis.

	RNA conc. (µg/µl)
WT-C1	2.31
WT-C2	2.50
WT-C3	2.70
S2-C1	2.30
S2-C2	3.87
M48-C1	1.79
M48-C2	1.94
WT-AS1	1.40
WT-AS2	0.86
WT-AS3	0.55
S2-AS1	2.68
S2-AS2	1.61
S2-AS3	2.00
M48-AS1	1.60
M48-AS2	1.49
M48-AS2	2.03

The next step after checking the quality of the RNA samples was reverse transcription to synthesize first-strand cDNA. The single-stranded cDNA was then converted into a double-stranded DNA (dsDNA) template for transcription.

Multiple copies of aRNA (cRNA is also known as aRNA) was generated from the double-stranded cDNA templates via *in vitro* transcription. Biotin-congugated nucleotides were incorporated and labeled aRNA was generated in this amplification step. After purification of aRNA it was quantified and 7.5 µg of biotin-labeled aRNA was fragmented using fragmentation buffer. The fragmented aRNA samples were analyzed using Agilent 2100 Bioanalyzer to verify fragmentation. Figure 3.23 shows electropherograms of un-fragmented and fragmented purified WT aRNA samples. Un-fragmented aRNA samples have a broader size distribution whereas the fragmented aRNA samples have a narrower size distribution. This reduction in the size distribution shows that purified aRNA samples were fragmented appropriately.

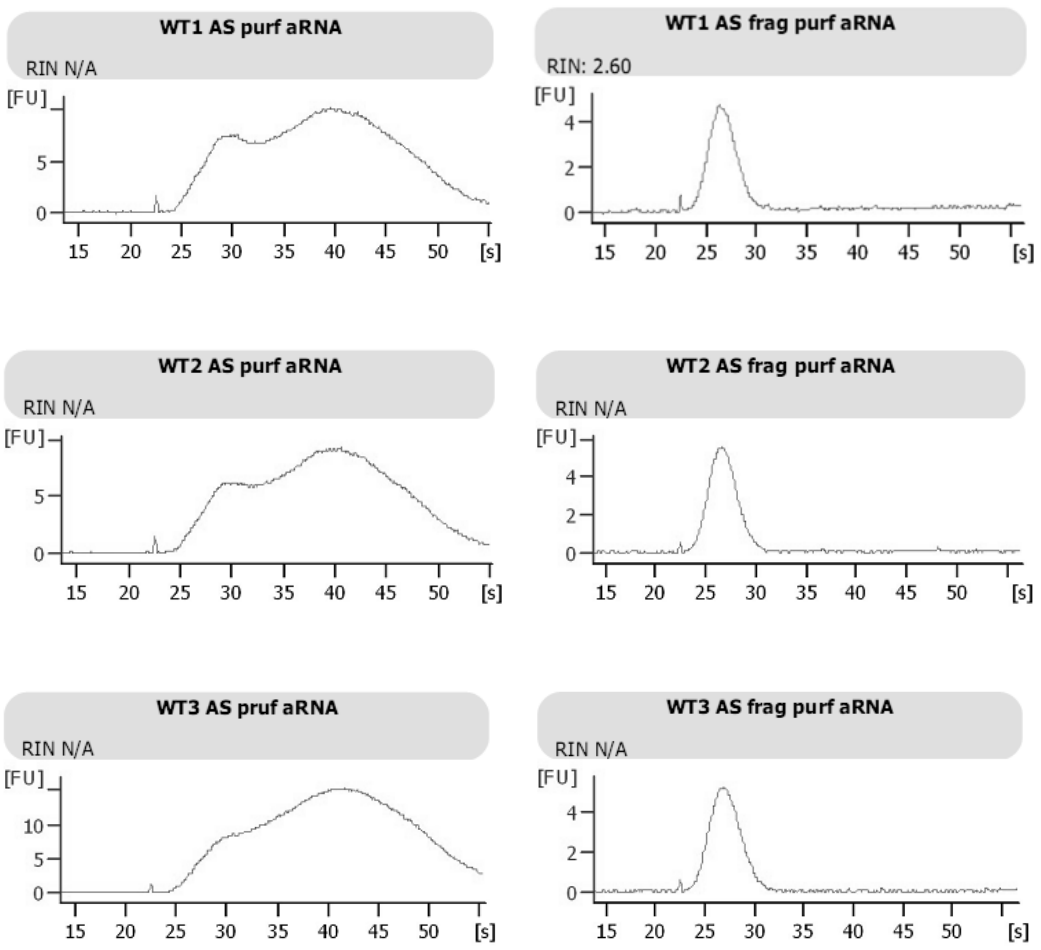

Figure 3.23 Agilent 2100 bioanalyzer electropherograms of aRNA samples from WT RNA isolated after freezing treatment (AS). The electropherograms on the left shows un-fragmented purified aRNA samples and electropherograms on the right shows fragmented purified aRNA samples. WT1, WT2 and WT3 stands for three biological replicates.

The rest of the fragmented aRNA samples were run on 2% agarose gel to check the fragmentation. Using Bioanalyzer instead of agarose gel electrophoresis is a better way of checking the size distribution and fragmentation of aRNA samples. However it is much more costly. Therefore after checking the fragmentation of WT aRNA samples, it was decided that the procedure used for fragmentation works appropriately. The rest of the samples were run on agarose gel and the fragmentation was verified by this way (Figure 3.24).

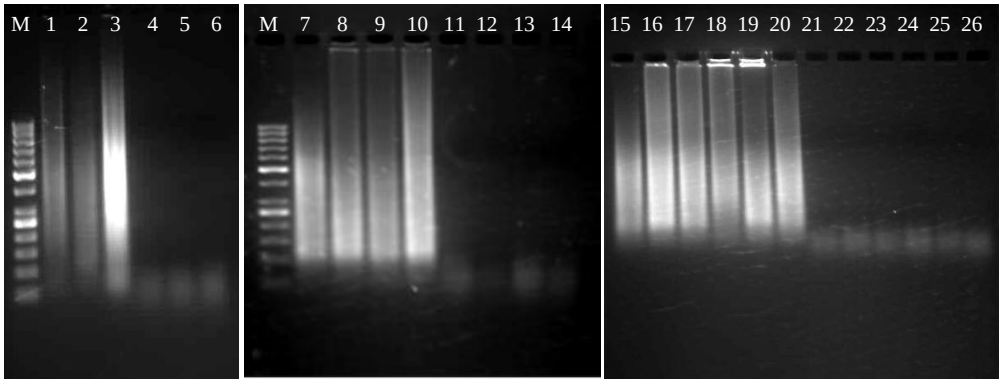

Figure 3.24 Agarose gel electrophoresis of purified and unfragmented or fragmented aRNAs from WT, S2 and M48. M: 1kb DNA ladder, 1-3: un-fragmented WT-C aRNA, 4-6: fragmented WT-C aRNA, 7&8: un-fragmented S2-C aRNA, 9&10: un-fragmented M48-C aRNA, 11&12: fragmented S2-C aRNA, 13&14: fragmented M48-C ARNA, 15-17: un-fragmented S2-AS aRNA, 18-20: un-fragmented M48-AS aRNA, 21-23: fragmented S2-AS aRNA, 24-26: fragmented M48-AS aRNA.

After verification of fragmentation, hybridization coctails were prepared and loaded on Affymetrix GeneChip® Tomato Genome Arrays and hybridized in hybridization oven. Then the arrays were washed, scanned and the transcript abundance profiles in the samples were analyzed. Since Affymetrix Potato Genome Array is not manufactured yet Affymetrix GeneChip® Tomato Genome Array was used for analysis of gene expression profiles in potato. The Solanaceae is a large (>3000 species), diverse dicot family and the genetic relationships between the family members (tobacco, tomato, eggplant, potato and pepper) is well-established. It contains species originated from both the Old (eggplant – India, China) and New World (pepper/potato/tomato – Central and South America). The basic chromosome number in many Solanaceae species is the same (x =12). Although the genome sizes are different (~ 950 Mb, 1800 Mb, 3000 Mb, and 1100 Mb, respectively) and there are some chromosomal rearrangements, the genic content of tomato, potato, pepper, and eggplant remains remarkably similar. The genomes of tomato and potato differ by only five paracentric inversions (Moore *et al.*, 2005). This high similarity in genomes of tomato and potato facilitates utilisation of tomato microarrays for gene expression analysis in potato.

3.2.5.1 Data Analysis

Microarray data analysis was perfomed based on three groups of comparisons. First of all transcriptomes of transgenic plants were compared to wild type under control conditions. The second group was comparison of expression profiles of wild type and transgenics under freezing stress with respect to control conditions. In the last group cold-mediated trascriptomes of transgenic lines were compared with wild type after stress application. All data analysis was performed by GeneSpring GX 10 software. The microarray raw data was normalized using the RMA algorithm (Robust Multiarray Analysis). Significantly expressed probe sets with p-values lower than 0.05 were determined by one way ANOVA. Among significantly expressed probe sets fold change of at least two was considered as differentially expressed probe sets. Figure 3.25 shows Principle Component Analysis (PCA) for WT, S2 and M48 which was generated by using significantly different probe sets lists.

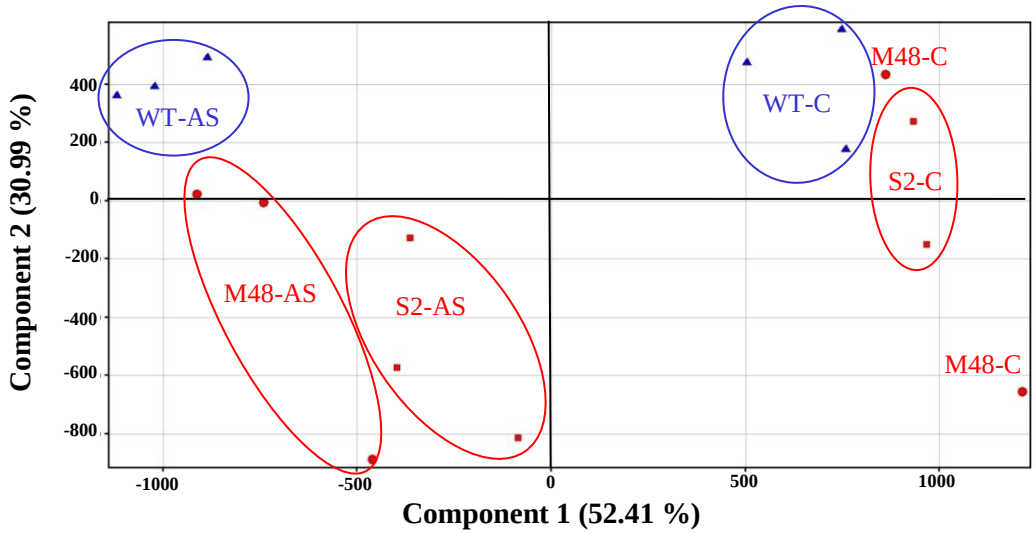

Figure 3.25 Principal Component Analysis plot visualization of the 16 Affymetrix arrays. Hybridizations with RNA from WT, S2 and M48 for control (C) and stress conditions (AS) are shown. Analysis was done with entities from one-way ANOVA.

PCA is a statistical technique that simplifies large data sets and captures common patterns in data of high dimensions and it allows viewing of variation among groups of replicates. In Figure 3.25, the data dimension was reduced to two principal components with X- and Y-axis, respectively representing 52.4% and 31.0% of total variance. Data points of control samples and freezing treated samples were in different areas (x-axis) of the plot. This showed that the stress factor, freezing temperatures, led to a great variation among samples. On the other hand not all the data points of WT and transgenic lines were in separate areas (y-axis) of the plot. Therefore being transgenic or non-transgenic did not generate a variation as much as the stress factor. The proximity of data points of biological replicates is preliminary proof that the plants responds similarly to the same treatment. The data points of biological replicates used in this study were in close proximity except the control samples of M48. Therefore it can be concluded that the responses of biological replicates in control or stress conditions were not so diverse.

3.2.5.2 Effect of *myb4* Expression on Transcriptome of Potato

Microarray data of control samples were analyzed to elucidate *myb4* regulated transcriptome of potato. Significantly different probe set lists ($P<0.05$; $FC\geq2$) were determined for WT and transgenic lines grown under control conditions. Numbers of probe sets found significantly different in transgenic lines compared to WT are given in Table 3.9. Under control conditions the number of up-regulated and down-regulated significantly different probe sets compared to WT was greater in the transgenic line S2. Both S2 and M48 lines are transformed with *myb4*. They only differ in the promoter controlling the expression of MYB4. The higher number of up-regulated and down-regulated probe sets in S2 may be due to the constitutive CaMV35S promoter. The Venn diagram in Figure 3.26 shows that a great proportion of differentially regulated genes in S2 and M48 overlap. Scatter plots of differentially regulated ($P<0.05$; $FC\geq2$) genes under control conditions and their expression values are given in Figure 3.27.

Table 3.9 Number of significantly (P<0.05) different probe sets that changed more than 2-fold in transgenic lines compared to WT under control conditions.

Regulation	S2	M48
up-regulated	203	164
down-regulated	194	148
Total	397	312

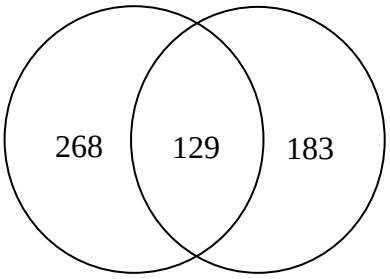

Figure 3.26 Venn diagram showing the overlap of differentially regulated genes in S2 (left) and M48 (right) compared to WT under control conditions.

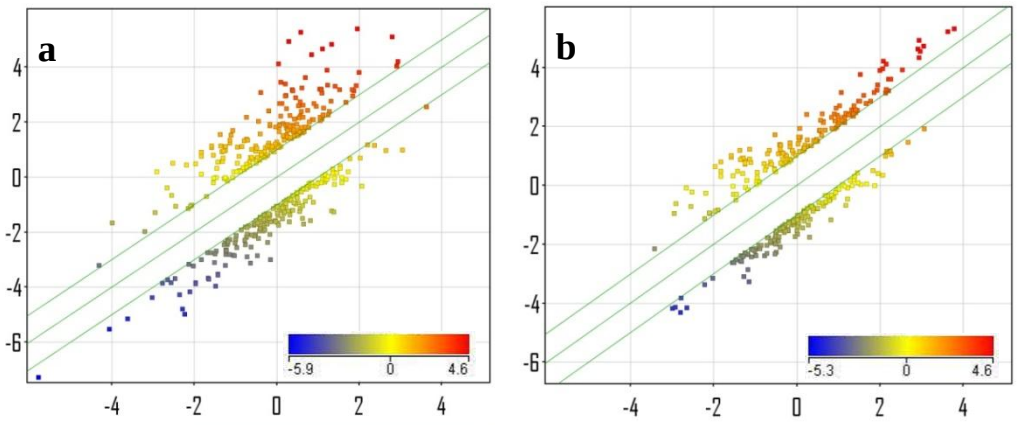

Figure 3.27 Scatter plots of differentially regulated (p<0.05; FC≥2) genes under control conditions and their expression values. Normalized expression values of differentially regulated genes in (a) S2 and (b) M48 compared to WT are displayed. Diagonal lines indicate twofold difference lines. Points above and below the diagonal lines indicate up- and down-regulated genes, respectively.

The annotation and biological processes for significantly different probe set lists ($P<0.05$; $FC\geq2$) were assigned using Tomato Functional Genomics Database (http://ted.bti.cornell.edu/). The up- and down-regulated genes for each line and each condition were assigned by the database to a certain biological process according to Gene Ontology (GO) information in the annotations. Figure 3.28 and Figure 3.29 show up-regulated and down-regulated biological processes in S2 and M48 under control conditions compared to WT respectively.

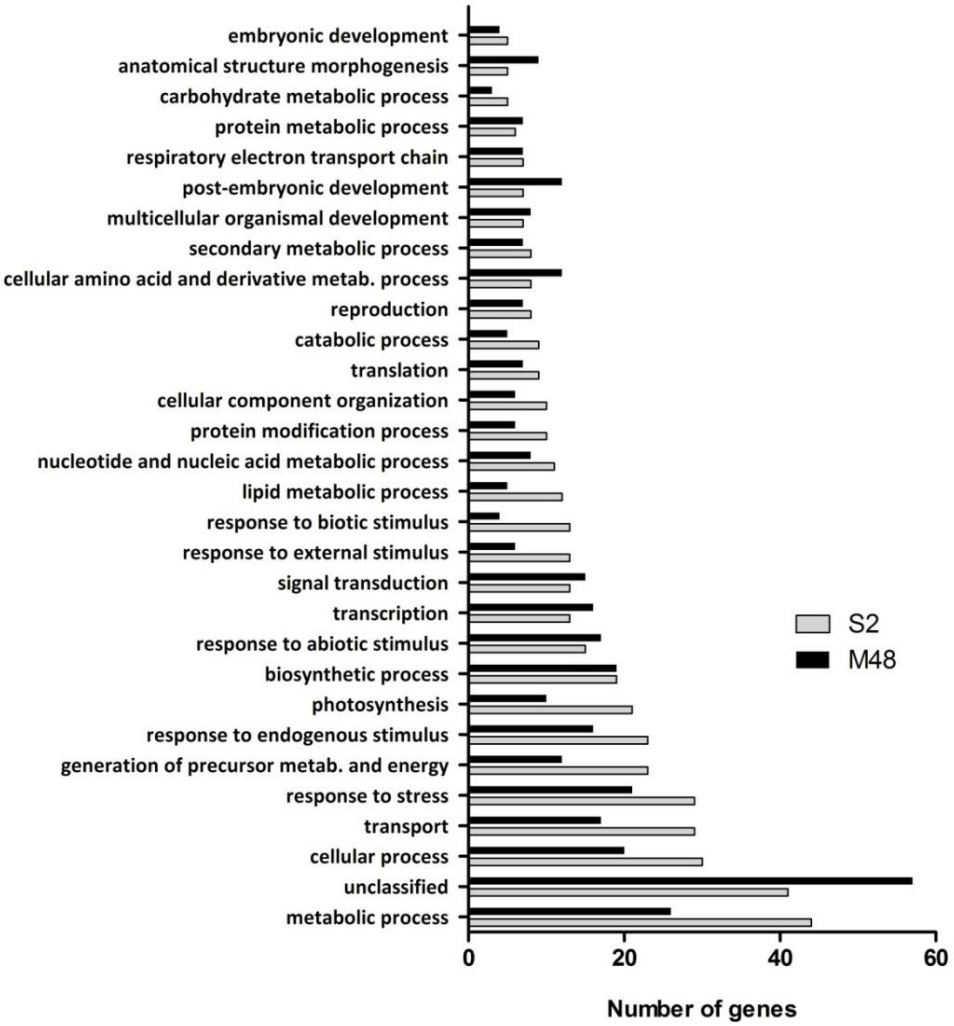

Figure 3.28 Up-regulated biological processes in S2 and M48 under control conditions compared to WT. Significantly different ($P<0.05$) probe sets were used for gene classifications.

In S2, 57% of classified up-regulated genes and 51% of classified up-regulated genes in M48 that were differentially regulated under control conditions fall into the biological processes: metabolic process, cellular process, transport, response to stress, generation of precursor metabolites & energy, response to endogenous stimulus, photosynthesis, biosynthetic process, response to abiotic stimulus, transcription and signal transduction (Figure 3.28). Besides these classified genes there were a high number of unclassified genes both in S2 and M48. The number of genes involved in each biological process was higher in S2 when compared to M48. This shows that stress inducible and constitutive expression of *myb4* affects same processes but 35S promoter alters expression of a higher number of genes.

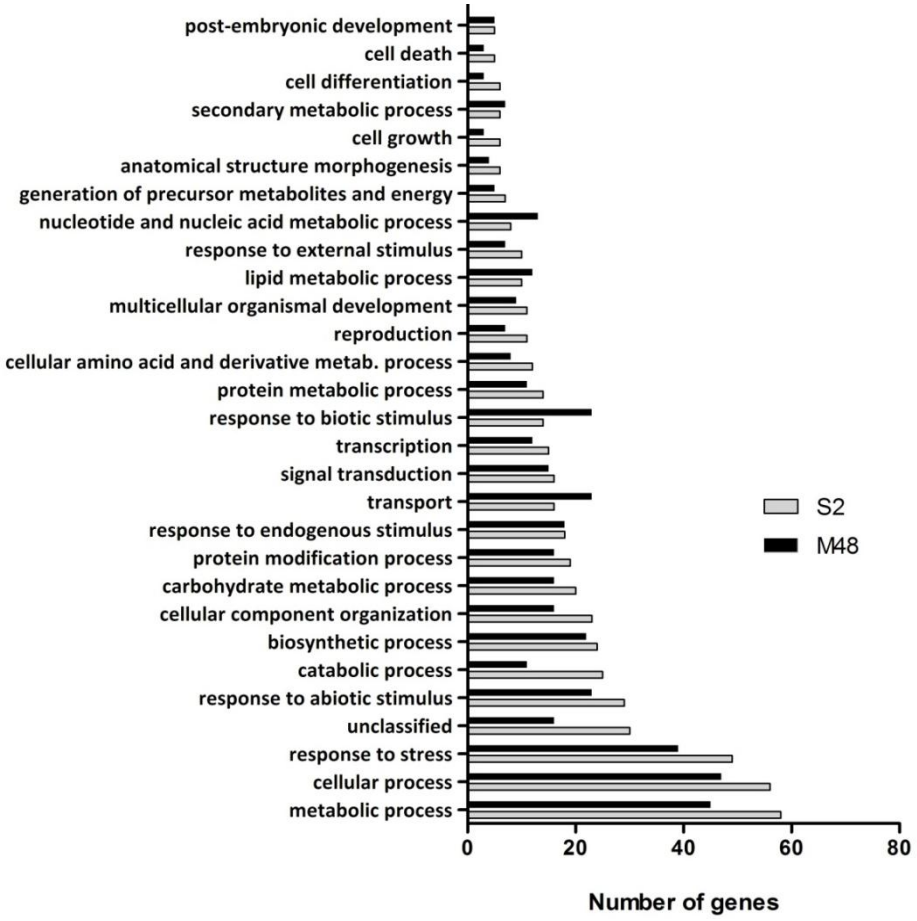

Figure 3.29 Down-regulated biological processes in S2 and M48 under control conditions compared to WT. Significantly different ($P<0.05$) probe sets were used for gene classifications.

57% of classified down-regulated genes in S2 and 54% of classified down-regulated genes in M48 differentially regulated under control conditions fall into the biological processes: metabolic process, cellular process, response to stress, response to abiotic stimulus, catabolic process, biosynthetic process, cellular component organization and carbohydrate metabolic process (Figure 3.29). The number of down-regulated genes involved in each biological process was higher in S2 when compared to M48 which may be due to constitutive expression of *myb4* in S2. The number of both down- and up-regulated genes involved in metabolic processes, cellular processes, response to stress and biosynthetic processes were high. This indicated that these biological processes are the most affected by *myb4* expression under normal growth conditions.

The visualization of significantly different probe sets in the context of existing knowledge (pathway) was performed using MapMan 3.5.0 Beta Software. BINs were generated for each pathway depending on the transcript abundancy. BINs represented with high numbers of transcripts were divided to subBINs according to their functions. BINs and subBINs were generated for biotic and abiotic stress, signalling, metabolism, transcription, translation, transport and development. The genes in the BINs were represented by different colors according to their expression levels. Figure 3.30 and Figure 3.31 represent up/down regulated genes in selected pathways under normal growth conditions in M48 and S2 compared to WT respectively.

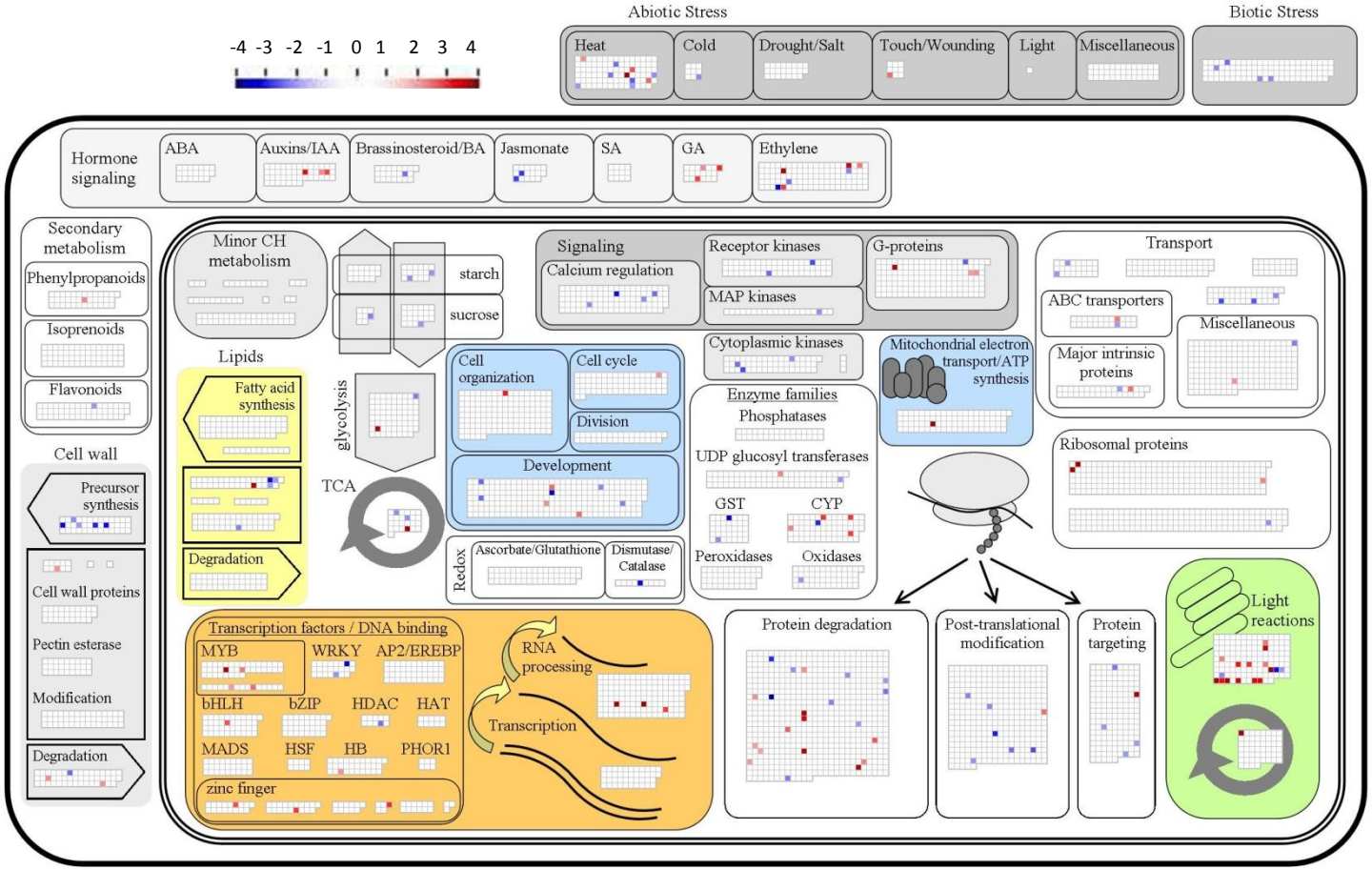

Figure 3.30 Differentially regulated genes in M48 compared to WT in selected pathways under normal growth conditions. The blocks in the figure represent a BIN or a subBIN and each square in a block represents a gene. Down-regulated genes are represented with blue colour and up-regulated genes are represented with red colour.

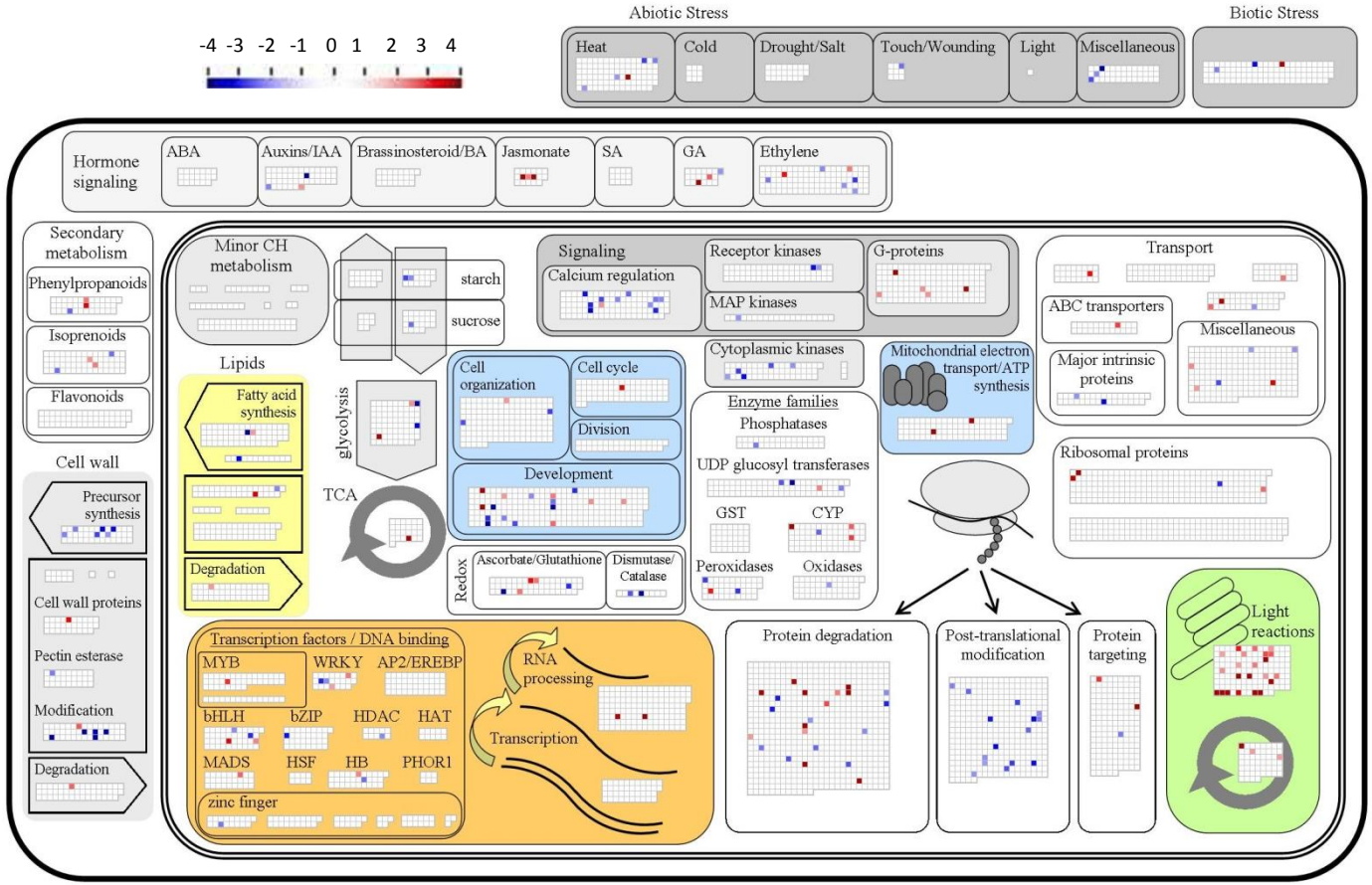

Figure 3.31 Differentially regulated genes in S2 compared to WT in selected pathways under normal growth conditions. The blocks in the figure represent a BIN or a subBIN and each square in a block represents a gene. Down-regulated genes are represented with blue colour and up-regulated genes are represented with red colour.

3.2.5.3 Cold-Mediated Changes in Transcriptomes of WT and Transgenic Potato

Microarray data of freezing treated WT and transgenic lines were compared to their respective controls to elucidate cold-mediated transcriptomes. Number of significantly different probe sets ($P<0.05$) that changed more than 2-fold in WT, S2 and M48 after freezing stress compared to control conditions is given in Table 3.10. The number of up-regulated and down-regulated probe sets in S2 was always lower than those in WT and M48. The total number of differentially regulated probe sets in WT and transgenic lines was between 1410 and 1957. This is a great number which indicates that freezing stress affects many processes both in WT and transgenic lines. Figure 3.32 represents scatter plots of differentially regulated ($P<0.05$; $FC\geq2$) genes after freezing treatment in WT, S2 and M48 compared to control conditions and their expression values.

Table 3.10 Number of significantly ($P<0.05$) different probe sets that changed more than 2-fold in WT, S2 and M48 after freezing stress compared to control conditions.

Regulation	**WT**	**S2**	**M48**
up-regulated	1021	717	975
down-regulated	936	693	977
Total	1957	1410	1952

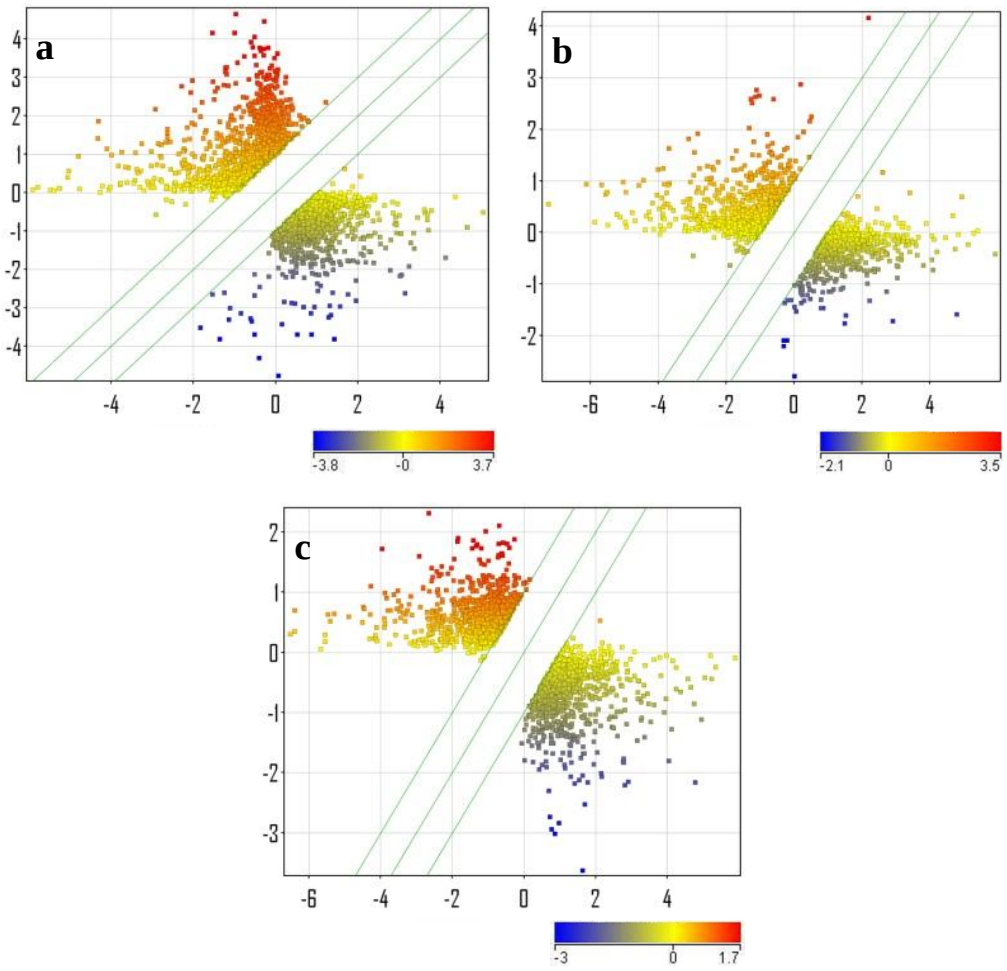

Figure 3.32 Scatter plots of differentially regulated (P<0.05; FC≥2) genes after freezing treatment and their expression values. Normalized expression values of differentially regulated genes upon exposure to freezing in (a) WT, (b) S2 and (c) M48 compared to control conditions are displayed. Diagonal lines indicate twofold difference lines. Points above and below the diagonal lines indicate up- and down-regulated genes, respectively.

Up-regulated and down-regulated biological processes in WT, S2 and M48 upon exposure to freezing temperatures are depicted in Figure 3.33 and Figure 3.34 respectively.

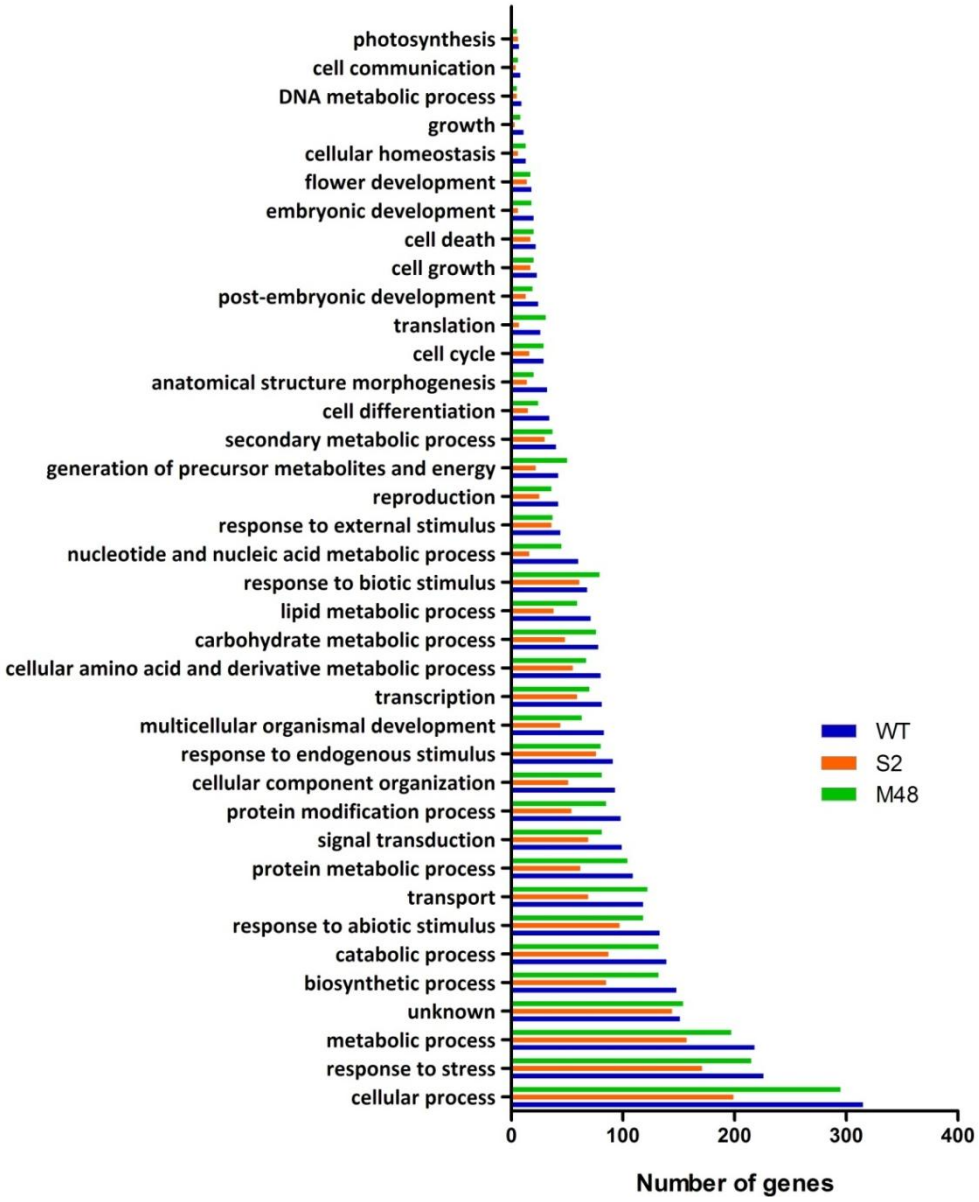

Figure 3.33 Up-regulated biological processes in WT, S2 and M48 upon exposure to freezing compared to control conditions. Significantly different ($P<0.05$) probe sets were used for gene classifications.

Freezing treatment up-regulated a high number of genes in the biological processes: cellular process, response to stress, metabolic process, biosynthetic process, catabolic process, response to abiotic stimulus, transport, protein metabolic process, signal transduction and protein modification process (Figure 3.33). The total numbers of genes involved in these biological processes were so high indicating that freezing

temperature activated many genes in WT and transgenic lines. The number of genes involved in each process in S2 was always lower than those in WT and M48. The number of genes involved in the processes in WT was higher than in M48 for most of the processes. This indicated that freezing stress up-regulated much more genes in WT when compared to transgenic lines.

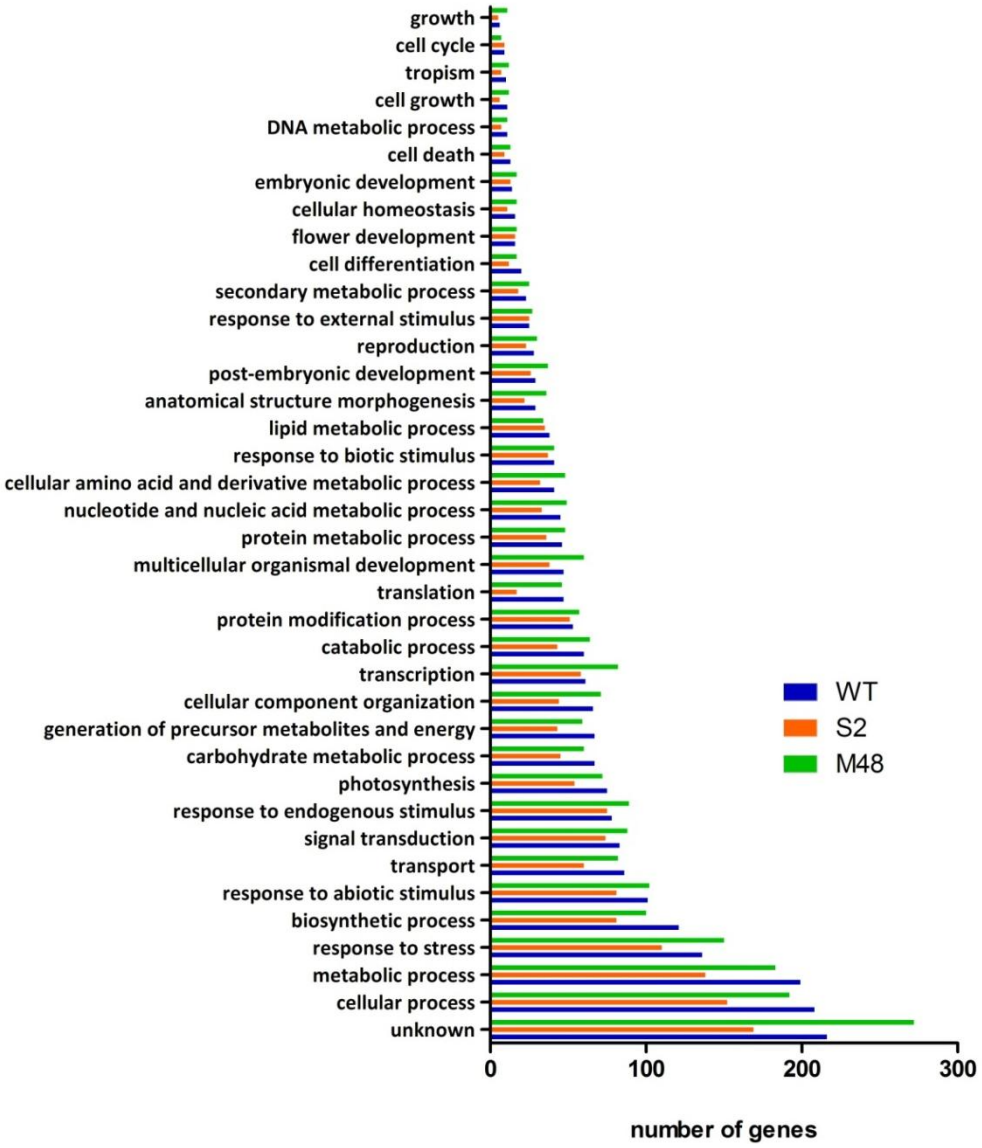

Figure 3.34 Down-regulated biological processes in WT, S2 and M48 upon exposure to freezing compared to control conditions. Significantly different ($P<0.05$) probe sets were used for gene classifications.

Freezing temperatures down-regulated a high number of genes in the biological processes: cellular process, metabolic process, response to stress, biosynthetic process, response to abiotic stimulus, transport, signal transduction, response to endogeneous stimulus and photosynthesis (Figure 3.34). The total number of down-regulated genes involved in each biological process was always lower in S2 when compared to WT and M48. Differentially regulated genes upon exposure to freezing temperatures in WT and the transgenic lines were determined according to their expression values in control conditions. The constitutive expression of *myb4* in S2 may have already down-regulated certain genes which were only down-regulated in WT and M48 after being exposed to freezing temperatures. This may have led to a lower number of down-regulated genes in the transgenic line S2 after freezing treatment. Similar biological processes were affected by expression of *myb4* in S2 and M48. This indicated that stress inducible and constitutive expression of *myb4* has affected same processes.

Figure 3.35, Figure 3.36 and Figure 3.37 represents up/down regulated genes in selected pathways in WT, M48 and S2 upon exposure to freezing temperatures compared to normal growth conditions respectively.

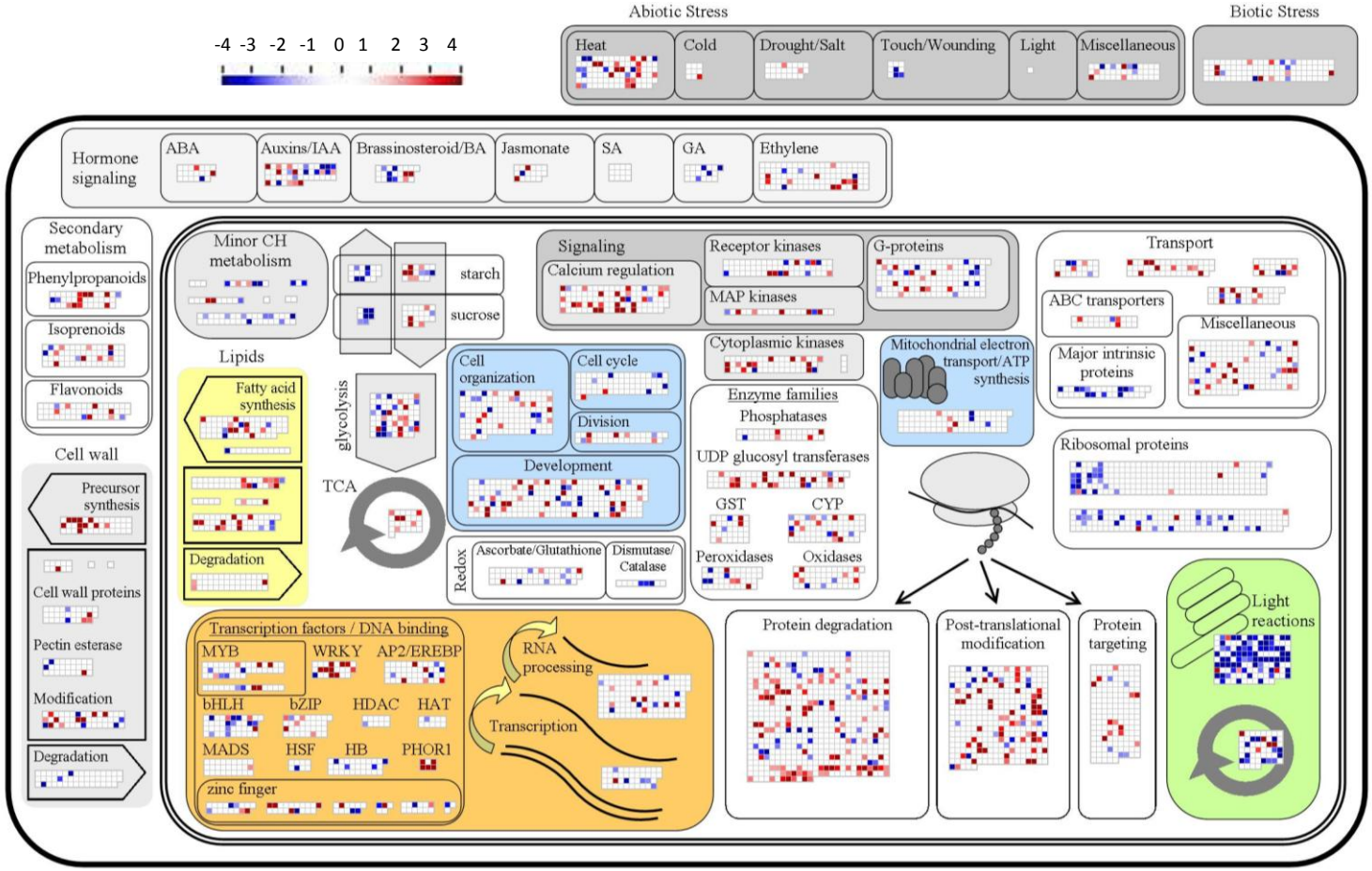

Figure 3.35 Differentially regulated genes in WT in selected pathways upon exposure to freezing temperatures compared to control conditions. The blocks in the figure represent a BIN or a subBIN and each square in a block represents a gene. Down-regulated genes are represented with blue colour and up-regulated genes are represented with red colour.

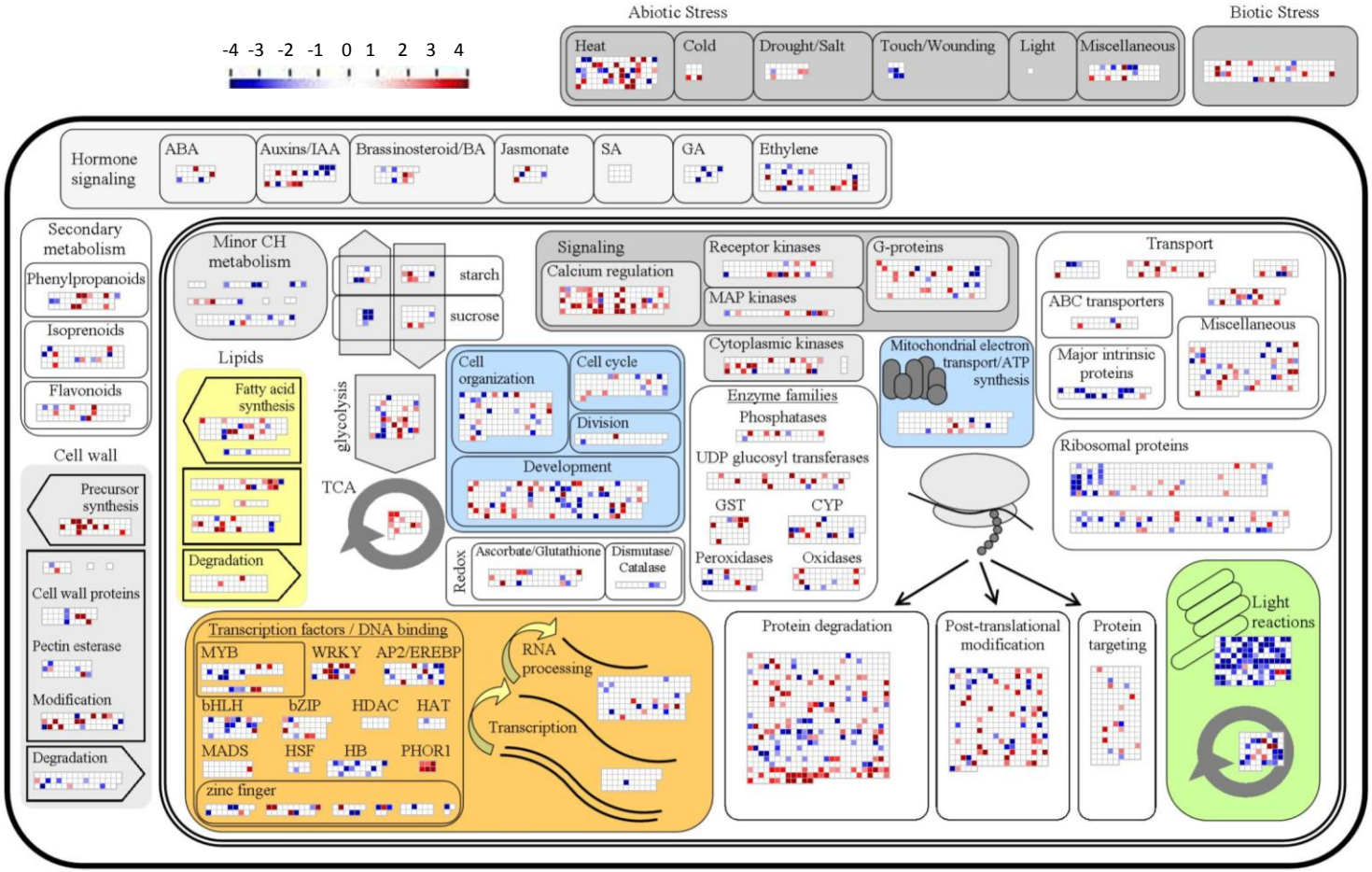

Figure 3.36 Differentially regulated genes in M48 in selected pathways upon exposure to freezing temperatures compared to control conditions. The blocks in the figure represent a BIN or a subBIN and each square in a block represents a gene. Down-regulated genes are represented with blue colour and up-regulated genes are represented with red colour.

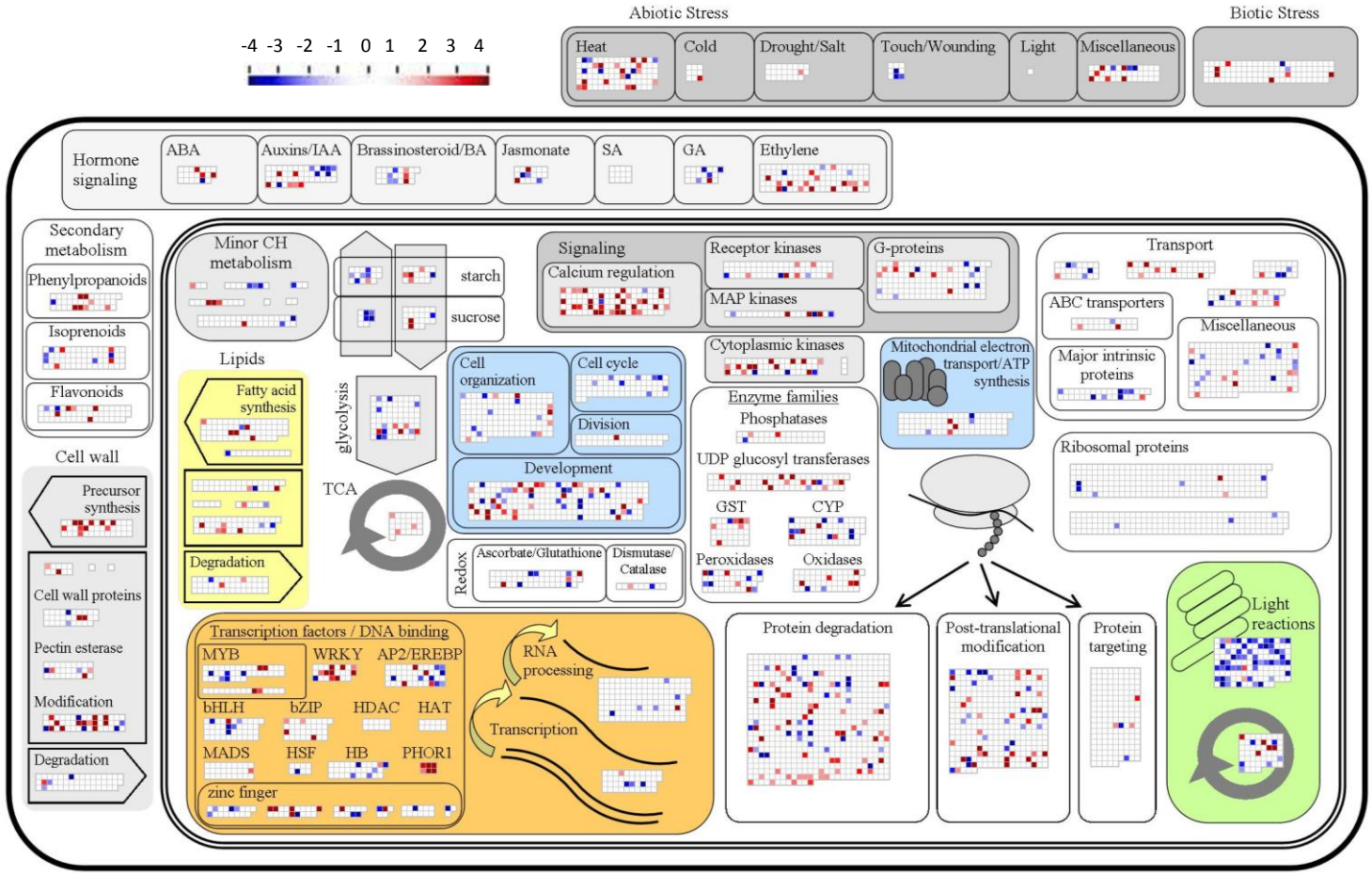

Figure 3.37 Differentially regulated genes in S2 in selected pathways upon exposure to freezing temperatures compared to control conditions. The blocks in the figure represent a BIN or a subBIN and each square in a block represents a gene. Down-regulated genes are represented with blue colour and up-regulated genes are represented with red colour.

MapMan output files were used to generate tables showing differentially regulated cold responsive genes involved in selected metabolic pathways. The complete list of significantly regulated genes ($P<0.05$ and $FC\geq2$) in WT, S2 and M48 upon exposure to freezing stress compared to control conditions was very long. Therefore the list is given in Appendix G and only selected genes involved in certain metabolic processes are given within the text.

Significantly regulated transcripts involved in abiotic and biotic stress responses in WT and transgenic lines upon exposure to freezing temperatures compared to control conditions are displayed in Table 3.11. Genes encoding heat shock family proteins (Hsps) were the group of genes which was represented by the highest number of differentially regulated genes involved in abiotic stress response. Some of the Hsps were up-regulated and some of them were down-regulated in WT and the transgenic potato. Hsps/chaperones may be induced during high temperature stress and in response to other abiotic stresses, such as salinity, water stress and osmotic, oxidative and cold stress (Wang *et al.*, 2004). Microarray analysis of WT and transgenic potato plants in this study verified expression of Hsps by freezing stress. Genes encoding for chitinases and disease resistance proteins were involved in biotic stress responses of WT and transgenic lines. It is interesting that these genes are cold responsive and differentially regulated. Expression of these genes in transgenic plants may be affected by *myb4* expression. However differential regulation of these genes also in WT indicates that either these genes are cold responsive or their expression changes as a result of crosstalk between responses to different stresses. Previously WRKY transcription factors which have been implicated in pathogen responses were shown to be cold responsive in Arabidopsis (Lee *et al.*, 2005). These results suggest that some genes may be involved in plant responses to multiple stresses. Cold responsive activation or repression of some genes may have affected expression of biotic stress response genes in WT and transgenic potato plants. Therefore cold responsive expression of these genes may be an indirect affect of cold exposure.

Table 3.11 Significantly (P<0.05) regulated transcripts involved in abiotic and biotic stress responses upon exposure to freezing stress. Probe set ID, *Arabidopsis thaliana* (AT) locus name and putative annotation of each transcript is listed. Values represent fold changes of transcripts in WT, S2 and M48 after freezing stress compared to normal growth conditions. Negative values show down-regulation and positive values show up-regulation. Only transcripts with FC≥8 in at least one of the transgenic lines or WT are displayed. FC<2 are indicated with -. (FC: Fold Change, HS: Heat Shock, RD: responsive to dehydration)

Probe Set ID	AT locus – Putative Annotation	Fold Change		
		WT AS	S2 AS	M48 AS
ABIOTIC STRESS / HEAT				
les.1931.1.a1_at	AT1G59725 – DNAJ HS protein, putative	-2.45	-9.93	-12.42
les.3160.1.s1_at	AT1G56410 – HSP70T-1, ERD2 (early-RD 2); ATP binding	52.74	2.28	3.81
les.3581.1.s1_at	AT5G52640 – HSP90.1, HS83, HSP81.1, HSP83, HSP81-1 (HS protein 81-1); ATP binding / unfolded protein binding	14.97	43.28	21.22
les.4705.1.s1_at	AT4G21320 – HSA32 (heat-stress-associated 32); catalytic	6.07	12.92	5.72
les.4819.1.s1_at	AT5G02500 – HSP70-1, HSC70, HSC70-1 (HS cognate 70 kDa protein 1); ATP binding	13.28	8.58	22.75
lesaffx.17345.1.s1_at	AT4G13830 – J20 (DNAJ-LIKE 20); HS protein binding	-8.51	-2.54	-12.68
lesaffx.43628.1.s1_at	AT1G80920 – J8 HS protein binding / unfolded protein binding	-5.78	-5.98	-16.94
lesaffx.63687.1.s1_at	AT4G21870 – 26.5 kDa class P-related HS protein (HSP26.5-P)	-12.76	-6.95	-26.13
ABIOTIC STRESS / UNSPECIFIED				
les.4233.1.s1_at	AT3G11930 – universal stress protein (USP) family protein	6.98	12.53	5.63
lesaffx.23349.1.s1_at	AT5G20630 – GLP3A, GLP3B, GLP3 (GERMIN-like protein 3); manganese ion binding / metal ion binding / nutrient reservoir	-25.70	-11.81	-11.95
lesaffx.64062.1.s1_at	AT1G72610 – GLP1 (GERMIN-like protein 1); manganese ion binding / metal ion binding / nutrient reservoir	-9.23	3.95	-2.06
ABIOTIC STRESS / TOUCH/WOUNDING				
les.4910.1.s1_at	AT1G19660 – wound-responsive family protein	-8.09	-4.92	-6.71
BIOTIC STRESS				
les.122.1.s1_at	AT3G12500 – PR3, CHI-B, B-CHI, ATHCHIB (basic chitinase); chitinase	4.40	10.18	13.63
les.3756.1.s1_a_at	AT2G43330 – ATINT1 (inositol transporter 1); carbohydrate transmembrane transporter/ sugar:hydrogen ion symporter	-2.80	-8.94	-
lesaffx.69659.1.s1_at	AT3G54420 – CHITIV, CHIV, ATEP3 (chitinase class IV)	5.60	4.20	9.32

Significantly regulated transcripts involved in transcription and post-transcription in WT and transgenic lines upon exposure to freezing temperatures compared to control conditions are displayed in Table 3.12. Recent researches showed that post-transcriptional regulation is critical for cold acclimation as well as transcriptional regulation. Regulation of gene expression at post-transcriptional level includes pre-mRNA stability, mRNA processing, export from nucleus and translation steps (Chinnusamy, *et al.*, 2007). After freezing treatment there were many differentially regulated genes involved in post-transcription (RNA processing) in WT and transgenic potato (Appendix G). Especially some spliceosome-associated (PSP) family proteins and splicing factors were differentially regulated. In stress conditions alternative splicing may enable synthesis of different proteins from a single gene. In wheat, two COR genes were shown to be regulated by intron retention in their mature mRNAs under cold stress (Barilli *et al.*, 2004). In this study differentially regulated genes involved in splicing may also be important in synthesizing different proteins in response to cold stress.

The transcription factor (TF) families; MYB, WRKY, AP2/EREBP, bHLH (basic helix-loop-helix), bZIP, PHOR1, homeobox and zinc finger were differentially expressed in WT and transgenic potato upon exposure to cold (Table 3.12). Cold stress is known to induce AP2/EREBP family transcription factors such as CBFs that can bind to *cis*-acting elements in the promoters of COR genes and regulate their expression. Transgenic plants ectopically expressing CBFs were shown to induce the expression of COR genes and cold acclimation even at warm temperatures (Stockinger *et al.*, 1997). There was a great up-regulation of CBF3 and CBF4 in WT and transgenic potato verifying cold regulation of these transcription factors. 10 WRKY domain transcription factors were up-regulated by freezing treatment. Recently significant regulation of 16 cold responsive *WRKY* genes was detected in rice (Ramamoorthy *et al.*, 2008). Many genes encoding for MYB transcription factors were differentially expressed in WT and transgenic potato. MYB15 was up-regulated in WT potato which is known to be an upstream transcription factor negatively regulating CBFs. MYB15 gene transcript was up-regulated by cold stress

and resulted in reduced expression of CBF genes in Arabidopsis (Agarwal, *et al.*, 2006). MYB and bHLH proteins often interact with each other to control transcription (Ramsay & Glover, 2005). A total of 10 out of 13 differentially expressed bHLH transcription factors were down-regulated in WT or transgenic potato. Cold induced MYB TFs and down-regulated bHLH TFs were also reported in Arabidopsis. The differential expression of these TFs suggested that the regulation of some cold responsive genes may be achieved by modulating the ratio of these partner factors (Lee, *et al.*, 2005). There were six PHOR1 TFs differentially expressed in WT and transgenic potato. There was a great up-regulation in expression of all of these genes. To the best of our knowledge there is no report on cold regulation of PHOR1 TFs. Therefore further research may be conducted to elucidate the possible involvement of PHOR1 TFs in cold response.

Table 3.12 Significantly (P<0.05) regulated transcripts involved in transcription and post-transcription upon exposure to freezing stress. Probe set ID, *Arabidopsis thaliana* (AT) locus name and putative annotation of each transcript is listed. Values represent fold changes of transcripts in WT, S2 and M48 after freezing stress compared to normal growth conditions. Negative values show down-regulation and positive values show up-regulation. Only transcripts with FC≥6 in at least one of the transgenic lines or WT are displayed. Fold changes less than 2 are indicated with -. (TF: Transcription factor, ZF: zinc finger)

Probe Set ID	AT locus – Putative Annotation	Fold Change		
		WT AS	S2 AS	M48 AS
TRANSCRIPTION				
les.4269.1.a1_at	ATCG00740 – RPOA RNA polymerase alpha subunit	-6.34	-3.65	-4.16
RNA PROCESSING				
les.1762.1.s1_at	AT2G35370 – GDCH (Glycine decarboxylase complex H)	-37.27	-7.76	-10.22
les.2982.2.s1_at	AT1G02840 – ATSRP34, SRP34, SR1 (splicing factor 2); RNA binding	7.17	-	2.41
les.5958.1.s1_at	AT3G44260 – CCR4-NOT transcription complex protein, putative	3.68	11.74	4.22
lesaffx.65616.1.s1_at	AT3G01150 – PTB (polypyrimidine tract-binding) heterogeneous nuclear ribonucleoprotein	7.65	-	3.14
TRANSCRIPTION FACTOR / MYB DOMAIN TF FAMILY				
les.38.1.s1_at	AT5G16600 – MYB43 (myb domain protein 43); DNA binding / TF	7.52	6.56	8.82

Table 3.12 (continued)

Probe Set ID	AT locus – Putative Annotation	Fold Change		
		WT AS	S2 AS	M48 AS
les.5017.1.a1_at	AT3G46130 – MYB48, MYB111 (myb domain protein 111)	-4.01	-4.10	-11.16
les.5017.1.s1_at	AT3G46130 – MYB48, MYB111 (myb domain protein 111)	-	-3.25	-9.92
lesaffx.54522.1.s1_at	AT2G23290 – MYB70 (myb domain protein 70); DNA binding / TF	10.37	-	3.46
les.4923.1.s1_at	AT2G46830 – CCA1 (circadian clock associated 1); TF	13.63	23.80	5.75
TRANSCRIPTION FACTOR / WRKY DOMAIN TF FAMILY				
les.2667.2.s1_at	AT2G38470 – WRKY33 (WRKY DNA-binding protein 33); TF	7.05	7.60	3.71
les.3963.1.s1_at	AT1G29280 – WRKY65 (WRKY DNA-binding protein 65); TF	-6.06	-4.70	-4.13
les.3969.1.s1_at	AT4G31550 – ATWRKY11 (WRKY DNA-binding protein 11); TF	6.39	6.52	7.55
lesaffx.36712.1.s1_at	AT4G23810 – WRKY53 (WRKY DNA-binding protein 53); DNA binding/ protein binding/transcription activator/ TF	17.24	13.11	17.47
lesaffx.43341.1.s1_at	AT3G56400 – WRKY70 (WRKY DNA-binding protein 70); TF	3.74	-	9.58
lesaffx.4793.1.s1_at	AT4G11070 – WRKY41 (WRKY DNA-binding protein 41); TF	5.12	3.02	7.67
lesaffx.735.1.s1_at	AT2G38470 – WRKY33 (WRKY DNA-binding protein 33); TF	8.06	7.99	6.00
lesaffx.9910.1.s1_at	AT1G80840 – WRKY40 (WRKY DNA-binding protein 40); TF	14.86	13.21	7.03
TRANSCRIPTION FACTOR / AP2/EREBP FAMILY				
les.124.1.s1_at	AT4G25480 – CBF3, DREB1, DREB1A (dehydration response element B1A); DNA binding / transcription activator/ TF	21.17	37.62	29.49
lesaffx.12586.1.a1_at	AT3G16280 – DNA binding / TF	-5.48	-3.80	-8.38
lesaffx.58308.1.s1_at	AT5G51990 – CBF4, DREB1D (C-repeat-binding factor 4); DNA binding / transcription activator/ TF	54.37	47.94	36.99
lesaffx.70635.1.s1_at	AT4G34410 – AP2 domain-containing TF, putative	54.22	44.39	37.86
TRANSCRIPTION FACTOR / bHLH (BASIC HELIX-LOOP-HELIX) FAMILY				
les.5638.1.s1_at	AT4G34530 – bHLH family protein	-15.69	-13.36	-32.15
lesaffx.62334.1.s1_at	AT1G26945 – transcription regulator	-2.34	-7.38	-2.91
lesaffx.64675.1.s1_a_at	AT5G57150 – bHLH family protein	6.36	-	5.38
TRANSCRIPTION FACTOR / bZIP TF FAMILY				
les.5129.1.s1_at	AT2G46270 – GBF3 (G-box binding factor 3); TF	8.41	4.72	12.75
TRANSCRIPTION FACTOR / PHOR1				
lesaffx.15878.2.a1_at	AT3G52450 – U-box domain-containing protein	42.04	65.66	69.42

Table 3.12 (continued)

Probe Set ID	AT locus – Putative Annotation	Fold Change		
		WT AS	**S2 AS**	**M48 AS**
lesaffx.15878.2.s1_at	AT3G52450 – U-box domain-containing protein	-	6.56	2.61
lesaffx.50112.1.s1_at	AT5G64660 – U-box domain-containing protein	14.56	10.01	19.72
lesaffx.56802.1.s1_at	AT3G19380 – U-box domain-containing protein	16.87	2.37	3.48
lesaffx.63659.1.s1_at	AT3G18710 – U-box domain-containing protein	31.02	5.56	11.44
TRANSCRIPTION FACTOR / ZINC FINGER TF FAMILY				
lesaffx.71242.2.s1_at	AT1G25440 – ZF (B-box type) family protein	-6.09	-5.27	-6.67
lesaffx.54718.1.s1_at	AT3G54810 – BME3-ZF, BME3 (BLUE MICROPYLAR END3); TF	4.13	7.81	3.54
lesaffx.70821.1.s1_at	AT3G21270 – ADOF2 (AT DOF ZF protein 2); DNA binding / TF	7.24	4.65	5.12
les.5126.1.s1_at	AT1G27730 – ZAT10, STZ (Salt Tolerance ZF); nucleic acid binding / TF / zinc ion binding	38.44	33.66	41.73
les.681.1.a1_at	AT1G66140 – ZFP4 (ZF Protein 4); nucleic acid binding/ zinc ion binding/ TF	-18.98	-11.55	-47.26
lesaffx.22830.1.s1_at	AT3G28210 – PMZ; ZF (AN1-like) family protein; zinc ion binding	7.67	7.49	14.88
lesaffx.64439.1.s1_at	AT2G37430 – ZF (C2H2 type) family protein (ZAT11)	44.18	53.86	107.84
lesaffx.71311.1.s1_at	AT5G59820 – ZAT12, RHL41 (responsive to high light 41); nucleic acid binding / TF / zinc ion binding	11.06	24.94	17.11

The number of genes involved in translation and post-translational modifications was the largest of those significantly regulated in cold-mediated potato transcriprome. 194 genes involved in protein degradation, 93 genes involved in post-translational modifications, 72 genes encoding for ribosomal proteins and 24 genes involved in protein targeting were significantly regulated (Appendix G). Some of these genes are represented in Table 3.13. Many genes encoding for carboxypeptidases and ubiquitin family proteins were up-regulated indicating increased protein degradation upon exposure to freezing in potato. On the other hand there was a down-regulation in some genes encoding for ubiquitin ligases which are known to confer substrate specificity for regulated proteolysis by the ubiquitination. It is well known that

controlled proteolysis of transcriptional regulators has an important role in shaping the cold-responsive transcriptome (Chinnusamy, *et al.*, 2007).

Table 3.13 Significantly (P<0.05) regulated transcripts involved in translation and post-translational modifications upon exposure to freezing stress. Probe set ID, *Arabidopsis thaliana* (AT) locus name and putative annotation of each transcript is listed. Values represent fold changes of transcripts in WT, S2 and M48 after freezing stress compared to normal growth conditions. Negative values show down-regulation and positive values show up-regulation. Only transcripts with FC≥10 in at least one of the transgenic lines or WT are displayed. Fold changes less than 2 are indicated with -. (CAM: calmodulin, LRR: leucine-rich repeat, RD: responsive to dehydration, PK: protein kinase)

Probe Set ID	AT locus – Putative Annotation	Fold Change		
		WT AS	S2 AS	M48 AS
PROTEIN DAGRADATION				
les.15.1.s1_at	AT5G67360 – ARA12; subtilase	-27.87	-13.25	-27.09
les.2294.2.a1_at	AT1G79110 – protein binding / zinc ion binding	-5.20	-15.07	-22.36
les.2960.3.s1_a_at	AT3G10410 – SCPL49 (serine carboxypeptidase-like 49); serine carboxypeptidase	23.06	-	3.05
les.3343.2.s1_at	AT1G15670 – kelch repeat-containing F-box family protein	-3.11	-11.64	-11.02
les.3343.3.s1_at	AT1G15670 – kelch repeat-containing F-box family protein	-9.28	-8.86	-12.68
les.3621.1.s1_at	– wound-induced proteinase inhibitor 1 precursor	-	-12.11	-
les.513.1.s1_at	AT5G67090 – subtilase family protein	-2.88	-20.16	-
les.5183.1.s1_at	AT2G02870 – kelch repeat-containing F-box family protein	10.01	8.88	5.54
lesaffx.23969.1.a1_at	AT3G61460 – BRH1 (brassinosteroid-responsive ring-H2); protein binding / zinc ion binding	-46.68	-32.89	-37.76
lesaffx.56104.1.s1_at	AT2G04240 – XERICO; protein binding / zinc ion binding	-17.05	-23.88	-16.47
lesaffx.62975.1.s1_at	AT5G27420 – ZF (C3HC4-type RING finger) family protein	17.91	5.46	4.95
lesaffx.63935.1.s1_at	AT1G24140 – matrixin family protein	7.32	16.54	15.60
POST TRANSLATIONAL MODIFICATIONS				
les.2027.3.s1_at	AT2G23770 – PK family protein / peptidoglycan-binding LysM domain-containing protein	20.65	9.87	5.37
les.3502.1.s1_at	AT2G05940 – PK, putative	9.92	22.93	15.35
les.3869.1.s1_at	AT1G07160 – protein phosphatase 2C, putative / PP2C, putative	6.56	24.90	10.91

Table 3.13 (continued)

Probe Set ID	AT locus – Putative Annotation	Fold Change		
		WT AS	S2 AS	M48 AS
les.5647.1.s1_at	AT3G08720 – S6K2, ATPK2, PK 19; kinase	14.69	5.13	10.85
lesaffx.26661.1.s1_at	AT5G47070 – PK, putative	5.87	13.83	9.14
lesaffx.36086.1.s1_at	AT5G16480 – tyrosine specific protein phosphatase family protein	-13.00	-14.36	-14.21
RIBOSOMAL PROTEINS				
les.392.1.s1_at	AT5G24490 – 30S ribosomal protein, putative	-10.74	-3.29	-17.74
lesaffx.1195.2.s1_at	AT4G27940 – MSC family protein	9.99	-	2.00
PROTEIN TARGETING				
les.4519.3.s1_at	AT2G34250 – protein transport protein sec61	19.96	-	3.53

Almost all of 93 differentially regulated genes involved in post-translational modifications in potato upon exposure to freezing temperatures were encoding either phosphatases or kinases. Protein kinases and phosphatases catalyze opposing reactions. The shift between phosphorylation and dephosphorylation regulates low temperature signal transduction cascade by phosphorylation of cold-specific proteins which lead to expression of cold specific genes and development of cold tolerance (Monroy *et al.*, 1997). The differential regulation of phosphtases and kinases in potato during freezing stress points out the shift in the equilibrium between phosphorylation and dephosphorylation which is important for directing cold-regulated gene expression.

Freezing treatment induced/repressed a number of genes involved in transport of amino acids, major intrinsic proteins and sugars in potato (Table 3.14). Genes encoding for transporters located on the plasma membrane or mitochondrial membrane were also differentially expressed All of the genes encoding for metabolite transporters at the mitochondrial membrane were up-regulated whereas genes encoding for other transporters were either induced or repressed. Cold responsive expression of genes involved in transport was also reported in Arabidopsis. The microarray data of Arabidopsis supports the notion that proper organellar functions, especially mitochondrial electron transport, are important for

plant cold responses, including cold regulated gene expression (Lee, *et al.*, 2005). Up-regulation of genes encoding for metabolite transporters at the mitochondrial membrane in potato may improve proper functioning of mitochondria upon exposure to freezing stress. Altered hormone, carbohydrate or protein metabolism might have led to differential expression of genes responsible for transport of these metabolites in response to cold in potato.

Table 3.14 Significantly (P<0.05) regulated transcripts involved in transport upon exposure to freezing stress. Probe set ID, *Arabidopsis thaliana* (AT) locus name and putative annotation of each transcript is listed. Values represent fold changes of transcripts in WT, S2 and M48 after freezing stress compared to normal growth conditions. Negative values show down-regulation and positive values show up-regulation. Only transcripts with FC≥7 in at least one of the transgenic lines or WT are displayed. Fold changes less than 2 are indicated with -. (MSC: mitochondrial substrate carrier, ZIFL: zinc induced facilitator-like)

Probe Set ID	AT locus – Putative Annotation	Fold Change		
		WT AS	S2 AS	M48 AS
TRANSPORT				
les.4789.1.s1_at	AT1G53580 – ETHE1/GLX2-3/GLY3 (glyoxalase 2-3); hydroxyacylglutathione hydrolase	-10.29	-4.19	-9.35
lesaffx.16086.1.s1_at	AT1G78820 – curculin-like (mannose-binding) lectin family protein / PAN domain-containing protein	8.38	2.32	2.39
lesaffx.49378.1.s1_at	AT5G13750 – ZIFL1; tetracycline:hydrogen antiporter/ transporter	16.72	-	2.72
lesaffx.52437.1.s1_at	AT5G65980 – auxin efflux carrier family protein	16.99	2.39	7.02
TRANSPORT/ AMINO ACID				
les.58.1.s1_at	AT2G39890 – ATPROT1, ProT1 (proline transporter 1)	-4.71	-12.74	-7.44
lesaffx.35418.1.s1_at	AT2G01170 – amino acid permease family protein	7.97	-	2.61
lesaffx.46368.1.s1_at	AT2G38120 – WAV5, PIR1, MAP1, AUX1 (auxin resistant 1); amino acid transmembrane transporter	-4.12	-3.31	-7.00
TRANSPORT/ ABC TRANSPORTERS				
lesaffx.269.2.s1_at	AT2G47800 – EST3, ATMRP4 (multidrug resistance-associated protein 4)	3.32	3.90	7.50
TRANSPORT/ METABOLITE TRANSPORTERS AT MITOCHONDRIAL MEMBRANE				
les.4779.1.s1_at	AT2G22500 – MSC family protein	44.08	30.57	53.63
les.4912.1.s1_at	AT3G05290 – MSC family protein	16.49	5.38	7.51

Table 3.14 (continued)

Probe Set ID	AT locus – Putative Annotation	Fold Change		
		WT AS	S2 AS	M48 AS
TRANSPORT/ METABOLITE TRANSPORTERS AT THE ENVELOPE MEMBRANE				
lesaffx.34390.1.s1_at	AT3G11320 – organic anion transmembrane transporter	11.46	2.45	4.96
TRANSPORT/ MAJOR INTRINSIC PROTEINS				
les.173.1.s1_at	AT4G35100 – PIP2;7, SIMIP, PIP3 (plasma membrane intrinsic protein 3); water channel	-11.64	-	-23.66
les.5231.1.s1_at	AT4G10380 – NIP5;1/NLM6/NLM8 (NOD26-like intrinsic protein 5;1); boron transporter	-4.89	-7.39	-6.56
TRANSPORT/ SUGAR				
les.3756.1.s1_a_at	AT2G43330 – ATINT1 (inositol transporter 1); carbohydrate transmembrane transporter/ sugar	-2.80	-8.94	-
les.5374.1.s1_at	AT1G79820 – SGB1; carbohydrate transporter/ sugar:hydrogen ion symporter	19.73	17.93	20.19
lesaffx.49378.1.s1_at	AT5G13750 – ZIFL1; tetracycline:hydrogen antiporter/ transporter	16.72	-	2.72
lesaffx.61214.1.s1_at	AT3G01280 – porin, putative	5.32	-	9.05

Perception of low temperature is the initial step in cold signalling cascade. Low temperature leads to alteration in membrane fluidity, nucleic acid and protein conformation and/or metabolite concentration which initiates a signaling cascade. Increase of Ca^{2+} in the cytosol mediated by cold may also be induced via mechano-sensitive or ligand-activated Ca^{2+} channels that are activated by rigidification of the membrane. Calcium signal amplification and phospholipid signaling might be involved in cold-stress signaling after Ca^{2+} increase in the cytosol (Chinnusamy *et al.*, 2006). Calcium-dependent protein kinases (CDPKs), calmodulin (CaM) and calmodulin-like proteins (CMLs) and calcineurin B-like proteins (CBLs) are the three main Ca^{2+} sensors in plants. These proteins bind to Ca^{2+} and participate in calcium-mediated signalling pathway. After binding to calcium these proteins change their conformation so that they can interact with target proteins and regulate their activity. These target proteins initiate a series of events that are involved in regulation of expression of certain genes (Winfield *et al.*, 2010). Genes encoding for CaM, CMLs and CDPKs involved in Ca^{2+} regulation and receptor like cytoplasmic kinases were up-regulated in potato upon exposure to freezing. Genes encoding for

receptor kinases, MAP kinases and G-proteins were also differentially expressed (Table 3.15). The differential expression of these signaling molecules in potato point their potential involvement in signal sensing and transduction mechanisms acting in specific cold related gene cascades.

Table 3.15 Significantly (P<0.05) regulated transcripts involved in signalling upon exposure to freezing stress. Probe set ID, *Arabidopsis thaliana* (AT) locus name and putative annotation of each transcript is listed. Values represent fold changes of transcripts in WT, S2 and M48 after freezing stress compared to normal growth conditions. Negative values show down-regulation and positive values show up-regulation. Only transcripts with FC≥10 in at least one of the transgenic lines or WT are displayed. Fold changes less than 2 are indicated with -. (TF: Transcription Factor, ERF: Ethylene Responsive Element Binding Factor, ZF: zinc finger, CPK: calcium-dependent protein kinase, CAM: calmodulin, LRR: leucine-rich repeat, PK: protein kinase)

Probe Set ID	AT locus – Putative Annotation	Fold Change		
		WT AS	S2 AS	M48 AS
HORMONE SIGNALING / AUXIN				
les.3757.1.s1_at	AT4G27450 – unknown auxin down-regulated protein	-17.06	-	-48.00
les.5177.1.s1_at	AT2G14960 – GH3.1 similar to IAA-amido synthases	25.77	3.35	5.43
lesaffx.15284.1.s1_at	AT1G28330 – DRM1 (dormancy-associated protein 1)	-10.85	-7.66	-23.22
lesaffx.44071.1.s1_at	AT2G44500 – unknown protein	14.29	3.64	4.97
lesaffx.44071.2.s1_at	AT2G44500 – unknown protein	14.17	10.97	14.13
HORMONE SIGNALING / GIBERELLIN				
les.827.1.s1_at	AT3G02885 – GASA5 (GAST1 protein homolog 5) gibberellin-regulated protein 5 (GASA5) / gibberellin-responsive protein 5	-17.66	-6.68	-50.53
les.417.1.s1_at	AT5G59845 – gibberellin-regulated protein	-11.72	-2.98	-5.76
HORMONE SIGNALING / JASMONATE				
les.13.1.s1_at	AT5G42650 – CYP74A, AOS (allene oxide synthase); hydro-lyase/ oxygen binding	33.60	9.97	8.30
les.3632.1.s1_at	AT1G17420 – LOX3 (Lipoxygenase 3); iron ion binding / lipoxygenase/ metal ion binding / oxidoreductase	13.61	5.53	23.35
les.3980.1.s1_at	AT3G45140 – ATLOX2 (Lipoxygenase 2)	-26.81	-41.17	-5.13
HORMONE SIGNALING / ETHYLENE				
les.4233.1.s1_at	AT3G11930 – universal stress protein (USP) family protein	6.98	12.53	5.63
les.4139.1.s1_at	AT5G07580 – member of the ERF subfamily B-3 of ERF/AP2 TF family	-4.09	-5.52	-17.31

Table 3.15 (continued)

Probe Set ID	AT locus – Putative Annotation	Fold Change		
		WT AS	S2 AS	M48 AS
lesaffx.8333.1.s1_at	AT1G05010 – ACO4, EAT1 EFE (Ethylene Forming Enzyme)	-3.05	-10.33	-14.43
SIGNALING / CALCIUM REGULATION				
les.5056.1.s1_a_at	AT5G42380– CML39, CML37; calcium ion binding	61.48	224.21	114.62
les.5056.1.s1_x_at	AT5G42380– CML39, CML37; calcium ion binding	42.11	67.64	53.78
lesaffx.3635.2.a1_at	AT2G26190– CAM-binding family protein	4.02	10.87	3.92
lesaffx.47666.1.s1_at	AT4G34150– C2 domain-containing protein	15.47	4.08	3.63
SIGNALING / G-PROTEINS				
les.2350.1.s1_at	AT4G35750– Rho-GTPase-activating protein-related	-11.28	-5.04	-5.42
les.2350.2.s1_at	AT4G35750– Rho-GTPase-activating protein-related	-10.71	-8.45	-7.80
les.4857.2.s1_at	AT5G45130– AtRab5A, AtRABF2a, Rha1, RHA1, Ras-related protein (RHA1) / small GTP-binding protein	-16.08	-40.37	-25.36
SIGNALING / RECEPTOR LIKE CYTOPLASMIC KINASES				
les.2027.3.s1_at	AT2G23770– PK family protein / peptidoglycan-binding LysM domain-containing protein	20.65	9.87	5.37
les.3502.1.s1_at	AT2G05940– PK, putative	9.92	22.93	15.35
lesaffx.26661.1.s1_at	AT5G47070– PK, putative	5.87	13.83	9.14

Growth and development in plants are regulated by hormones. Growth and development patterns of plants are altered when they are subjected to cold stress. This may regulate altered hormone homeostasis and/or signal transduction under low temperature conditions (Lee, *et al.*, 2005). The growth rate slows down in plants in response to low temperature. Since many metabolites increase in response to low temperature it may not be due to resource limitation. Therefore coordinate changes among hormone responsive transcription factor families and altered expression of enzymes of hormone metabolism point involvement of hormones in this regulation (Hannah *et al.*, 2005). The genes involved in ethylene metabolism were up-regulated and genes involved in auxin and gibberellin metabolisms were down-regulated in potato upon exposure to freezing stress. Genes involved in jasmonate metabolism was also significantly regulated (Table 3.15). A rapid increase in ethylene was

detected in rye plants transferred to cold temperature. When nonacclimated plants were exposed to ethylene, both antifreeze activity and the concentration of apoplastic protein was increased in rye leaves (Yu *et al.*, 2001). Up-regulation of genes involved in ethylene metabolism in potato may also be cold-responsive. The genes involved in auxin metabolism were down-regulated in potato. Down-regulation of auxin-induced genes was also reported in Arabidopsis upon cold treatment (Hannah, *et al.*, 2005).

Members of the large enzyme families; CYP (Cytochrome P450), GST (glutathione S-transferase), oxidases, peroxidases, phosphatases and UDP glucosyl and glucoronyl transferases were significantly regulated in potato upon exposure to freezing stress (Table 3.16).

Cold or chilling stresses cause disruption of cellular homeostasis and the uncoupling of major physiological processes which enhances generation of ROS. These free radicals cause improper cell functioning and they may even lead to cell death (Suzuki & Mittler, 2006). Antioxidative enzymes and antioxidant molecules can scavenge ROS therefore they participate in the cold acclimation process (Tao *et al.*, 1998). Antioxidants such as glutathione and ascorbic acid and ROS-scavenging enzymes such as glutathione peroxidase (GPX), superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX) and peroxiredoxin (PrxR) are involved in removal of ROS generated under stress conditions. In wheat only transcripts for some glutathione transferases and peroxidases were significantly expressed in response to cold. This is similar to our findings which revealed differential expression of 8 transcripts for GSTs and 9 transcripts for peroxidases. There was no significant difference in other antioxidant enzymes. Glutathione transferases may exhibit glutathione peroxide activity and may also play a role in stress-related signal transduction (Dixon *et al.*, 2002).

Table 3.16 Significantly (P<0.05) regulated transcripts involved in large enzyme families upon exposure to freezing stress. Probe set ID, *Arabidopsis thaliana* (AT) locus name and putative annotation of each transcript is listed. Values represent fold changes of transcripts in WT, S2 and M48 after freezing stress compared to normal growth conditions. Negative values show down-regulation and positive values show up-regulation. Only transcripts with FC≥6 in at least one of the transgenic lines or WT are displayed. Fold changes less than 2 are indicated with - (TF: Transcription factor, LHC: light harvesting complex, CYP: Cytochrome P450, GST: Glutathione S-transferase)

Probe Set ID	AT locus – Putative Annotation	Fold Change		
		WT AS	S2 AS	M48 AS
CYTOCHROME P450				
les.1859.3.s1_at	AT3G14690 – CYP72A15 (CYP, family 72, subfamily A, polypeptide 15); oxygen binding	7.65	-	-
les.438.1.s1_at	AT1G69780 – ATHB13; DNA binding / transcription factor	-3.45	-3.03	-6.99
les.4443.1.a1_s_at	AT5G38970 – ATBR6OX, CYP85A1, BR6OX1 (brassinosteroid-6-oxidase); oxygen binding	-2.45	-4.45	-6.17
les.5057.1.s1_at	AT3G61880 – CYP78A9; oxygen binding	-2.06	-4.73	-8.21
lesaffx.22297.1.s1_at	AT2G34500 – CYP710A1 (CYP, family 710, subfamily A, polypeptide 1); C-22 sterol desaturase/ oxygen binding	8.77	2.84	4.57
lesaffx.3698.3.s1_at	AT4G30210 – AR2, ATR2 (P450 reductase 2)	5.11	6.74	4.94
lesaffx.9038.1.s1_at	AT2G32440 – CYP88A4, KAO2 (ent-kaurenoic acid hydroxylase 2); oxygen binding	-2.14	-9.62	-
GLUTATHIONE S-TRANSFERASE				
les.293.1.s1_at	AT2G29420 – GST25, ATGSTU7 (glutathione S-transferase 25)	12.37	9.60	14.76
lesaffx.1959.1.s1_at	AT2G47730 – GST6, ATGSTF5, GSTF8, ATGSTF8 (glutathione S-transferase 8)	3.71	2.33	7.32
OXIDASES				
les.2189.1.s1_at	AT1G76160 – SKS5 (SKU5 Similar 5); copper ion binding / oxidoreductase	2.59	4.19	6.88
lesaffx.21603.1.s1_at	AT5G16990 – NADP-dependent oxidoreductase, putative	-6.97	-9.45	-8.05
lesaffx.44382.1.s1_at	AT1G76160 – SKS5 (SKU5 Similar 5); copper ion binding / oxidoreductase	8.23	-	3.63
PEROXIDASES				
les.2092.1.s1_at	AT4G21960 – PRXR1 (peroxidase 42)	-20.96	-6.30	-18.48
lesaffx.57363.1.s1_at	AT1G14550 – anionic peroxidase, putative	4.14	13.59	4.67
PHOSPHATASES				
lesaffx.44584.1.a1_at	AT5G03080 – phosphatidic acid phosphatase-related / PAP2-related	3.60	3.20	6.56
lesaffx.67373.2.s1_at	AT1G09870 – histidine acid phosphatase family protein	7.92	-	3.04

Table 3.16 (continued)

Probe Set ID	AT locus – Putative Annotation	Fold Change		
		WT AS	S2 AS	M48 AS
UDP GLUCOSYL AND GLUCORONYL TRANSFERASES				
les.1155.1.s1_at	AT4G34120 – LEJ1 (loss of the timing of ET and JA biosynthesis 1) CBS domain-containing protein	4.41	6.91	8.52
les.3777.1.s1_at	AT4G34135 – UGT73B2; UDP-glycosyltransferase	7.33	36.46	10.48
les.842.2.s1_a_at	AT1G01420 – UDP-glucoronosyl/UDP-glucosyl transferase family protein	26.96	-	-
lesaffx.17107.1.a1_at	AT3G01040 – GAUT13 (Galacturonosyltransferase 13)	17.75	4.09	4.65
lesaffx.59059.1.s1_at	AT1G70090 – GATL9, LGT8 (Galacturonosyltransferase-like 9)	19.62	9.71	14.91
lesaffx.66380.1.s1_at	AT4G34131 – UGT73B3 (UDP-glycosyltransferase 73B3); abscisic acid glucosyltransferase	4.61	6.40	-
lesaffx.68107.1.s1_at	AT3G28340 – GATL10 (Galacturonosyltransferase-like 10); polygalacturonate 4-alpha-galacturonosyltransferase	13.07	27.07	30.45

Flavonoids are secondary metabolites with essential functions in higher plants. They are derived from phenylalanine and acetate metabolism and play an important role as antioxidans. These compounds accumulated under stress conditions may absorb UV light and protect cells from photo-oxidative stress (Winkel-Shirley, 2002). They might ptotect photosystem II at low temperatures (Huner *et al.*, 1998). The significant overrepresentation of upregulated genes of secondary metabolism in response to cold was mainly due to the genes involved in flavonoid synthesis pathway in Arabidopsis (Hannah, *et al.*, 2005). The key enzymes in flavonoid biosynthesis, chalcone isomerase and chalcone synthase, were differentially expressed in wheat during cold acclimation (Winfield, *et al.*, 2010). The transcripts involved in flavonoid metabolism was also differentially expressed in potato upon exposure to freezing (Table 3.17). These data point possible involvement of the flavonoid pathway in cold stress response.

Table 3.17 Significantly (P<0.05) regulated transcripts involved in secondary metabolism upon exposure to freezing stress. Probe set ID, *Arabidopsis thaliana* (AT) locus name and putative annotation of each transcript is listed. Values represent fold changes of transcripts in WT, S2 and M48 after freezing stress compared to normal growth conditions. Negative values show down-regulation and positive values show up-regulation. Only transcripts with FC≥8 in at least one of the transgenic lines or WT are displayed. Fold changes less than 2 are indicated with -. (TF: Transcription factor, LRR: leucine-rich repeat)

Probe Set ID	AT locus – Putative Annotation	Fold Change		
		WT AS	S2 AS	M48 AS
SECONDARY METABOLISM / FLAVONOIDS				
les.842.2.s1_a_at	AT1G01420 – UDP-glucoronosyl/UDP-glucosyl transferase family protein	26.96	-	-
lesaffx.31836.1.s1_at	AT1G68540 – oxidoreductase family protein	6.07	4.41	8.95
SECONDARY METABOLISM / PHENYLPROPANOIDS				
les.281.1.s1_at	AT1G51680 – AT4CL1 (4-coumarate-COA ligase 1)	9.69	4.68	5.22
les.281.3.s1_at	AT1G51680 – AT4CL1 (4-coumarate-COA ligase 1)	13.99	-	2.03
les.4271.2.s1_at	AT2G37040 – PAL1 (PHE ammonia lyase 1); phenylalanine ammonia-lyase	8.59	8.67	7.79
SECONDARY METABOLISM / CELL WALL PRECURSOR SYNTHESIS				
les.1852.2.s1_at	AT3G29360 – UDP-glucose 6-dehydrogenase, putative	6.46	4.92	9.45
les.1852.3.s1_at	AT3G29360 – UDP-glucose 6-dehydrogenase, putative	35.78	2.73	3.74
les.2813.1.s1_at	AT1G08200 – AXS2 (UDP-D-Apiose/UDP-D-Xylose Synthase 2)	14.68	2.89	8.69
lesaffx.38740.1.s1_at	AT4G10960 – UGE5 (UDP-D-glucose/UDP-D-galactose 4-epimerase 5); UDP-glucose 4-epimerase/ protein dimerization	19.62	6.08	12.36
SECONDARY METABOLISM / CELL WALL MODIFICATION				
les.210.1.s1_at	AT3G23730 – xyloglucan:xyloglucosyl transferase, putative / xyloglucan endotransglycosylase, putative / endo-xyloglucan transferase, putative	4.08	40.18	6.01
les.276.1.s1_at	AT4G14130 – XTR7 XTR7 (xyloglucan endotransglycosylase 7); hydrolase, acting on glycosyl bonds	18.08	34.79	23.63
les.3537.1.s1_at	AT2G01850 – ATXTH27, EXGT-A3 (endo-xyloglucan transferase A3); hydrolase, acting on glycosyl bonds / xyloglucan:xyloglucosyl transferase	-	12.76	-
les.3590.1.s1_at	AT5G13870 – EXGT-A4 (endoxyloglucan transferase A4); hydrolase, acting on glycosyl bonds	-	30.66	-
les.3697.1.s1_at	AT5G57550 – XTR3 (xyloglucan endotransglycosylase 3); hydrolase, acting on glycosyl bonds	-2.88	-2.56	-8.02

Table 3.17 (continued)

Probe Set ID	AT locus – Putative Annotation	Fold Change		
		WT AS	S2 AS	M48 AS
les.2688.1.s1_at	AT1G20190 – ATHEXP ALPHA 1.14, ATEXPA11 (AT expansin A11)	-11.95	-8.12	-18.22
les.3733.1.s1_at	AT2G40610 – ATEXPA8 (AT EXPANSIN A8)	-46.61	-80.33	-62.38
les.429.1.s1_at	AT4G14130 – XTR7 (xyloglucan endotransglycosylase 7); hydrolase, acting on glycosyl bonds	64.91	98.00	137.54
les.4353.1.s1_at	AT4G25810 – XTH23, XTR6 (xyloglucan endotransglycosylase 6); hydrolase, acting on glycosyl bonds	46.99	134.99	62.27
les.4769.1.s1_at	AT3G45970 – ATEXPL1, ATEXLA1 (expansin-like A1)	5.15	36.01	8.37
les.4530.1.s1_at	AT3G23730 – xyloglucan:xyloglucosyl transferase, / xyloglucan endotransglycosylase, / endo-xyloglucan transferase/ putative	2.20	14.64	4.87
les.4968.1.s1_s_at	AT2G01850 – ATXTH27, EXGT-A3 (endo-xyloglucan transferase A3); hydrolase, acting on glycosyl bonds / xyloglucan:xyloglucosyl transferase	-	9.08	-
lesaffx.4662.1.s1_at	AT1G10550 – XET, XTH33 (xyloglucan:xyloglucosyl transferase 33); hydrolase, acting on glycosyl bonds	10.25	27.39	9.38
SECONDARY METABOLISM / CELL WALL PECTIN ESTERASES				
les.67.1.s1_at	AT3G14310 – ATPME3 (AT pectin methylesterase 3)	-9.56	-7.01	-2.66
SECONDARY METABOLISM / CELL WALL PROTEINS				
les.3320.2.s1_at	AT3G22440 – hydroxyproline-rich glycoprotein family protein	23.77	-	-
les.4368.1.s1_s_at	AT1G62440 – LRX2 (LRR/Extensin 2); protein binding / structural constituent of cell wall	-	12.85	13.49
lesaffx.43685.2.s1_s_at	AT1G62440 – LRX2 (LRR/Extensin 2); protein binding / structural constituent of cell wall	-	14.69	13.13
SECONDARY METABOLISM / CELL WALL DEGRADATION				
les.3991.1.s1_at	AT5G64570 – ATBXL4, XYL4 (beta-xylosidase 4); hydrolase, hydrolyzing O-glycosyl compounds, glycosyl hydrolase family 3 protein	-14.87	-2.08	-12.52
les.5579.1.s1_at	AT4G13710 – pectate lyase family protein	-5.12	-8.45	-

The phenylpropanoid pathway is activated by various stress factors. Phenolic compounds accumulate in plants under low temperature conditions and protect them

aganist frost, cold and pathogens (Peltonen *et al.*, 1997). Phenylalanine ammonia-lyase (PAL) is the enzyme catalyzing the first step in phenylpropanoid pathway. It is well known that genes encoding for PAL are activated and expressed upon exposure to low temperatures (Christie *et al.*, 1994). In Festulolium (*Festuca pratensis* X *Lolium multiflorum*) genotypes tolerant to frost the content of phenolics was significantly lower compared to the susceptible genotypes after cold acclimation whereas PAL activity was significantly higher. In the tolerant genotypes the activity of PAL was not correlated with the level of soluble phenolic compounds. This suggested that soluble phenolic compounds might have been quickly polymerized to lignin. This makes the plants more resistant to mechanical damage generated by ice formation in the intercellular space. Phenolic compounds in tolerant genotypes may also be accumulated in response to other signalling processes but not by cold acclimation (Pociecha *et al.*, 2009). Transcripts involved in phenylproranoid metabolism were also differentially regulated in potato during freezing stress (Table 3.17). Expression of two transcripts encoding for PAL was significantly up-regulated. This may indicate involvement of the phenylpropanoid pathway in cold stress response of potato.

As plants are subjected to low temperatures ice formation begins in the apoplastic space where the solute concentration is lower. The unfrozen cytoplasmic water migrates from the cell cytosol to the apoplast. This leads to enlargement of existing ice crystals and causes a mechanical strain on the plasma membrane and the cell wall which in turn leads to cell rupture (Mahajan & Tuteja, 2005). Therefore differential regulation of genes involved in cell wall precursor synthesis, degradation and modification is expected in response to cold. There was a significant upregulation in the genes encoding for xyloglucan endotransglycosylase in potato upon exposure to freezing (Table 3.17). Xyloglucan (XG) is a primary wall hemicellulose that coats and cross-links cellulose microfibrils. It is assumed that disconnection of XG from the microfibrils or breakage of the cross-links may allow the microfibrils to move apart which in turn enables expansion of the wall. Xyloglucan endotransglycosylase can cut and rejoin XG chains, and it is assumed a key agent in regulation of cell wall

expansion. It is also known to incorporate new XG molecules into the wall (Bourquin *et al.*, 2002). The transcripts for cell wall precursor synthesis were also up-regulated in potato. The alterations in expression pattern of these genes in potato indicate that the damage on the cell wall generated by cold exposure may be tolerated by synthesis of new cell wall precursors and xyloglucan endotransglycosylases.

There was an increase in starch and sucrose degradation and a decrease in their synthesis in potato upon exposure to freezing (Table 3.18). In stress conditions, plants generally redirect assimilates from supporting new growth to the synthesis of soluble sugars and low molecular weight carbohydrates. Thus they avoid carbohydrate deficiency and depletion of cell energy. The low molecular weight compounds are known to act as osmoprotants that increase the tolerance to further abiotic factors (Oufir *et al.*, 2008). Carbohydrates may play an important role in freezing tolerance. A relationship was shown between accumulation of simple sugars (e.g. raffinose, trehalose and sucrose) and increased freezing tolerance. Some studies also showed that various sugars may stabilize the membranes at freezing temperatures (Winfield, *et al.*, 2010). In this study transcrips involved in sucrose and starch synthesis were down-regulated and transcripts for sucrose and starch degradation were up-regulated in potato. This may reflect an expression pattern in which new sucrose molecules are not synthesized but the concentration of simple sugars are enhanced by sucrose and starch breakdown.

Cellular membranes are a primary site of freezing damage, and changes in their composition may better protect cells during cold stress. For instance an increase in the proportion of unsaturated fatty acids might reduce freezing-induced membrane damage. lipases and lysophospholipases (Hannah, *et al.*, 2005). In this study there is a significant overrepresentation of differentially regulated genes of lipid metabolism in potato (Table 3.18). The number of transcripts differentially regulated was 48 upon exposure to freezing stress. The transcripts involved in lipid synthesis were mainly up-regulated with few down-regulated transcripts. On the other hand

transcripts for lipid degradation were all up-regulated. This lipid degradation may reflect the initial damage in response to low temperature. The up- and down-regulation in lipid synthesis and metabolism may indicate their role in maintaining the altered lipid composition seen after cold acclimation.

Table 3.18 Significantly (P<0.05) regulated transcripts involved in carbohydrate (CH) and lipid metabolism upon exposure to freezing stress. Probe set ID, *Arabidopsis thaliana* (AT) locus name and putative annotation of each transcript is listed. Values represent fold changes of transcripts in WT, S2 and M48 after freezing stress compared to normal growth conditions. Negative values show down-regulation and positive values show up-regulation. Only transcripts with FC≥6 and 10 in at least one of the transgenic lines or WT are displayed. Fold changes less than 2 are indicated with - (TF: Transcription factor)

Probe Set ID	AT locus – Putative Annotation	Fold Change		
		WT AS	S2 AS	M48 AS
CH METABOLISM / SUCROSE SYNTHESIS				
les.1617.1.s1_s_at	AT1G43670 – fructose-1,6-bisphosphatase, / D-fructose-1,6-bisphosphate 1-phosphohydrolase, / FBPase, putative	-17.20	-3.88	-7.77
les.1617.2.s1_s_at	AT1G43670 – fructose-1,6-bisphosphatase/ D-fructose-1,6-bisphosphate 1-phosphohydrolase/ FBPase, putative	-22.65	-3.82	-14.62
les.1617.3.a1_s_at	AT1G43670 – fructose-1,6-bisphosphatase / D-fructose-1,6-bisphosphate 1-phosphohydrolase / FBPase, putative	-18.48	-2.52	-13.03
les.4946.1.s1_at	AT1G43670 – fructose-1,6-bisphosphatase / D-fructose-1,6-bisphosphate 1-phosphohydrolase, / FBPase, putative	-23.43	-3.35	-13.99
CH METABOLISM / SUCROSE DEGRADATION				
les.157.1.s1_at	AT3G43190 – SUS4; UDP-glycosyltransferase/ sucrose synthase/ transferase, transferring glycosyl groups	4.11	7.86	3.54
CH METABOLISM / STARCH SYNTHESIS				
les.1310.1.s1_at	AT1G32900 – starch synthase, putative	-5.99	-	-3.97
les.78.1.s1_at	AT5G19220 – APL1, ADG2 (ADPG pyrophosphorylase 2); glucose-1-phosphate adenylyltransferase	-6.40	-3.32	-4.52
CH METABOLISM / STARCH DEGRADATION				
les.1401.2.s1_at	AT5G18670 – BAM9, BMY3 (beta-amylase 9); beta-amylase	7.78	2.57	2.26
les.1401.3.s1_at	AT5G18670 – BAM9, BMY3 (beta-amylase 9); beta-amylase	11.59	-	-
les.2844.1.s1_at	AT3G23920 – BMY7, TR-BAMY, BAM1 (beta-amylase 1); beta-amylase	4.42	4.94	7.11

Table 3.18 (continued)

Probe Set ID	AT locus – Putative Annotation	Fold Change		
		WT AS	S2 AS	M48 AS
LIPID METABOLISM				
les.5293.1.s1_at	AT2G26640 – beta-ketoacyl-CoA synthase	6.62	16.49	8.16
les.2265.1.s1_at	AT2G43710 – FAB2, SSI2 (fatty acid biosynthesis 2); acyl-[acyl-carrier-protein] desaturase	14.99	15.74	15.00
les.3383.1.s1_at	AT3G48990 – AMP-dependent synthetase and ligase family protein	5.81	15.25	2.28
les.4040.1.s1_at	AT2G26640 – beta-ketoacyl-CoA synthase	14.51	4.69	7.78
les.4710.1.s1_at	AT1G48600 – phosphoethanolamine N-methyltransferase 2, putative (NMT2)	-13.87	-21.76	-29.90
les.3710.1.s1_at	AT1G13580 – LAG13 (LAG1 Longevity Assurance Homolog 3)	9.63	17.02	21.85

A high number of genes involved in glycolysis, TCA cycle and mitochondrial electron transfer were differentially regulated in potato upon exposure to freezing (Appendix G). Some of them are displayed in Table 3.19.

Table 3.19 Significantly ($P<0.05$) regulated transcripts involved in energy metabolism (glycolysis, TCA, mitochondrial electron transfer) upon exposure to freezing stress. Probe set ID, *Arabidopsis thaliana* (AT) locus name and putative annotation of each transcript is listed. Values represent fold changes of transcripts in WT, S2 and M48 after freezing stress compared to normal growth conditions. Negative values show down-regulation and positive values show up-regulation. Only transcripts with FC≥6 in at least one of the transgenic lines or WT are displayed. Fold changes less than 2 are indicated with -. (TF: Transcription factor)

Probe Set ID	AT locus – Putative Annotation	Fold Change		
		WT AS	S2 AS	M48 AS
GLYCOLYSIS				
les.2677.1.s1_at	AT3G08590 – 2,3-biphosphoglycerate-independent phosphoglycerate mutase, putative / phosphoglyceromutase, putative	20.45	-	-
les.3129.2.s1_at	AT5G08570 – pyruvate kinase, putative	16.37	-	2.19
les.3242.1.a1_at	AT1G42970 – GAPB (glyceraldehyde-3-phosphate dehydrogenase B subunit)	-22.70	-3.89	-12.67
les.3242.3.s1_at	AT1G42970 – GAPB (glyceraldehyde-3-phosphate dehydrogenase B subunit)	-18.67	-3.89	-7.85
MITOCHONDRIAL ELECTRON TRANSFER				
les.3021.1.s1_at	AT4G10040 – CYTC-2 (cytochrome C-2); electron carrier	-8.21	-6.15	-
lesaffx.33042.1.s1_at	AT5G40382 – cytochrome-c oxidase	-2.96	-	-6.23

Table 3.19 (continued)

Probe Set ID	AT locus – Putative Annotation	Fold Change		
		WT AS	**S2 AS**	**M48 AS**
les.4222.1.s1_at	AT1G32350 – AOX1D (alternative oxidase 1D)	19.01	27.46	32.43
TCA				
lesaffx.23253.1.s1_at	AT2G44350 – CSY4, ATCS (citrate synthase 4)	18.08	-	2.11
lesaffx.23253.2.s1_at	AT2G44350 – CSY4, ATCS (citrate synthase 4)	5.97	-	-

There was a significant down-regulation in energy metabolism however pyruvate kinases involved in glycolysis, enzymes involved in TCA cycle and alternative oxidases involved in mitochondrial electron transfer were up-regulated. A general down-regulation of energy metabolism and reduced growth rate is a common phenomenon to all abiotic stresses.

Abiotic stress may affect CO_2 diffusion, electron transport, PSII efficiency, ribulose-1,5-bisphosphate (RuBP) content, photorespiration, ROS formation and ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) activity during photosynthesis (Saibo *et al.*, 2009). The causes of reduced photosynthetic rate upon exposure to abiotic stresses are not clear yet. There is controversy about the main physiological targets involved in photosynthetic impairment. Under cold, salinity or drought stresses stomatal closure is considered the primary response (Flexas & Medrano, 2002). Photosynthesis maintains a balance between the energy absorbed by photosystems I and II (PSI and PSII) and that consumed by the metabolic reactions of the plant. Therefore it is highly sensitive to changes in environmental conditions. Being independent of temperature, photochemical and physical reactions of PSI and PSII are extremely rapid. On the other hand biochemical reactions are temperature dependent and much slower. The decrease in temperature slows biochemical reactions which results in uncoupling of the two systems. Electrons from PSI are transferred to oxygen which generates ROS (Winfield, *et al.*, 2010). ROS are thought to be involved in signaling cascade under stress conditions. Therefore generation of

light-induced ROS may participate in cold stress perception (Suzuki & Mittler, 2006).

A great number of genes involved in photosynthesis were significantly regulated in potato during freezing stress (Appendix G). Most of 80 transcripts for light reactions and 23 transcripts for Calvin cycle were down-regulated. Some of these transcripts are given in Table 3.20. The down-regulation of photosynthetic genes in response to cold has been previously reported (Ensminger *et al.*, 2006; Kosova *et al.*, 2007). There was a down-regulation especially in NADH and many transcripts encoding for light harvesting complexes or subunits of PSI and PSII in potato after freezing treatment. This shows that the main targets of low temperature was these components in potato.

Table 3.20 Significantly (P<0.05) regulated transcripts involved in photosynthesis upon exposure to freezing stress. Probe Set ID, *Arabidopsis thaliana* (AT) locus name and putative annotation of each transcript is listed. Values represent fold changes of transcripts in WT, S2 and M48 after freezing stress compared to normal growth conditions. Negative values show down-regulation and positive values show up-regulation. Only transcripts with FC≥10 in at least one of the transgenic lines or WT are displayed. Fold changes less than 2 are indicated with -. (TF: Transcription factor, LHC: light harvesting complex)

Probe Set ID	AT locus – Putative Annotation	Fold Change		
		WT AS	S2 AS	M48 AS
PHOTOSYNTHESIS / LIGHT REACTIONS				
les.1603.1.a1_at	AT1G61520 – LHCA3 (Photosystem I LHCgene 3)	-7.10	-4.56	-12.82
les.3016.1.s1_at	AT5G54270 – LHCB3-1, LHCB3 (light-harvesting Chlorophyll Binding Protein 3)	-4.90	-6.17	-11.76
les.3297.1.s1_at	AT3G47470 – CAB4, LHCA4 (Photosystem I LHCgene 4); chlorophyll binding	-15.75	-5.50	-15.09
les.4428.1.s1_a_at	AT1G20340 – DRT112 (DNA-damage-repair/toleration protein 112); copper ion binding / electron carrier	-36.64	-14.43	-13.90
les.4615.1.s1_at	AT2G31040 – ATP synthase protein I – related	-12.60	-7.17	-7.43
lesaffx.35136.1.s1_at	ATCG01070 – NDHE - NADH dehydrogenase ND4L	-28.07	-4.29	-4.23
lesaffx.51226.1.a1_at	ATCG00540 – PETA - cytochrome f apoprotein	-14.66	-2.64	-2.76

Table 3.20 (continued)

Probe Set ID	AT locus – Putative Annotation	Fold Change		
		WT AS	S2 AS	M48 AS
PHOTOSYNTHESIS / CALVIN CYCLE				
les.376.1.s1_at	AT5G38420 – ribulose bisphosphate carboxylase small chain 2B / RuBisCO small subunit 2B (RBCS-2B) (ATS2B)	-22.76	-3.16	-6.94
les.4807.1.s1_at	AT5G13200 – GRAM domain-containing protein / ABA-responsive protein-related	8.95	14.06	8.32
lesaffx.344.2.s1_at	AT1G67090 – RBCS1A; ribulose-bisphosphate carboxylase	4.35	14.56	5.85
lesaffx.344.5.s1_at	AT1G67090 – RBCS1A; ribulose-bisphosphate carboxylase	4.55	11.16	4.58

The transcripts involved in cell division, cell cycle, cell organization and development were differentially regulated in potato during freezing stress (Table 3.21, Appandix F). Temperature and membrane rigidification mediated by cold which affects membrane fluidity is considered as the first step in cold perception. Membrane rigidification may also regulate cold-induced Ca^{2+} increase in the cytosol. Ca^{2+} increase in the cytosol amplifies the calcium and phospholipid signaling which indicates their involvement in cold-stress signalling (Chinnusamy, *et al.,* 2006). Stimulation of cold induced Ca^{2+} influx by disruption of microtubules and actin microfilaments was shown in Tobacco protoplasts (Mazars *et al.*, 1997). Role of actin microfilaments in K^{+} channel activity in stomatal opening was also reported (Hwang *et al.*, 1997). Cytoskeleton re-organization was shown to be an integral component of low temperature signal transduction in alfalafa. It is suggested to serve as a link between membrane rigidification and calcium influx during cold acclimatization (Orvar *et al.*, 2000). The transcripts for microtubule associated proteins, actin related proteins and tubulins were differentially expressed in potato during freezing stress (Appendix G). This alteration in expression pattern may indicate involvement of these cytoskeleton components in potato cold signaling.

Table 3.21 Significantly (P<0.05) regulated transcripts involved in cell division, organization and development upon exposure to freezing stress. Probe set ID, *Arabidopsis thaliana* (AT) locus name and putative annotation of each transcript is listed. Values represent fold changes of transcripts in WT, S2 and M48 after freezing stress compared to normal growth conditions. Negative values show down-regulation and positive values show up-regulation. Only transcripts with FC≥10 in at least one of the transgenic lines or WT are displayed. Fold changes less than 2 are indicated with - (TF: Transcription factor, RD: responsive to dehydration, PK: protein kinase)

Probe Set ID	AT locus – Putative Annotation	Fold Change		
		WT AS	S2 AS	M48 AS
CELL DIVISION				
les.5167.1.s1_at	AT3G55580 – regulator of chromosome condensation (RCC1) family protein	9.13	11.23	12.68
CELL ORGANIZATION				
lesaffx.37916.1.s1_at	AT1G09155 – ATPP2-B15 (Phloem protein 2-B15); carbohydrate binding	10.54	7.94	5.30
DEVELOPMENT				
les.1798.2.a1_at	AT5G20700 – senescence-associated protein-related	14.67	11.87	20.80
les.3070.2.a1_at	AT4G25150 – acid phosphatase, putative	-2.58	-29.27	-
les.3759.1.s1_at	AT5G53560 – ATB5-A (Cytochrome b5 A), late embryogenesis (lea)-like protein	7.16	18.76	7.84
les.4483.1.s1_at	AT1G01720 – ANAC002, ATAF1 (NAC domain containing protein 2); TF	23.46	20.64	10.63
les.4975.1.s1_at	AT4G29270 – acid phosphatase class B family protein	-2.48	-13.05	-
lesaffx.15284.1.s1_at	AT1G28330 – DRM1 (dormancy-associated protein 1)	-10.85	-7.66	-23.22
lesaffx.2597.1.s1_at	AT4G35770 – DIN1 (dark inducible 1) senescence-associated protein (SEN1)	-18.24	-4.63	-31.92
lesaffx.46519.1.s1_at	AT1G21460 – nodulin MtN3 family protein	-7.22	-6.29	-12.22
lesaffx.60966.2.s1_at	AT5G48150 – PAT1 (phytochrome a signal transduction 1); TF	12.22	10.81	16.32
lesaffx.69815.1.s1_at	AT3G14770 – nodulin MtN3 family protein	17.43	-	13.27
lesaffx.70563.1.s1_at	AT5G48150 – PAT1 (phytochrome a signal transduction 1); TF	34.84	17.86	23.35

The expression patterns of genes involved in development were also altered in potato upon exposure to freezing. Many transcripts involved in development were down-regulated which is a phenomenon common to many abiotic stresses. Despite the down-regulation in a high number of transcripts, 9 genes encoding for NAC family transcription factors were up-regulated. Plant-specific NAC family transcription factors are implicated in plant development (Vroemen *et al.*, 2003; Xie *et al.*, 2000).

They are also involved in biotic and abiotic stress responses (Fujita *et al.*, 2004; Tran *et al.*, 2004). Therefore up-regulation of these transcription factors in potato might reflect their function in reprogramming plant development to cope with cold stress.

3.2.5.4 Comparison of Cold-Mediated Transcriptomes of Wild Type and Transgenic Potato

Number of probe sets found significantly different in transgenic lines compared to WT upon exposure to freezing temperatures is given in Table 3.22. The number of up-regulated and down-regulated probe sets was greater in S2 when compared to M48. The number of down-regulated probe sets was much more than up-regulated probe sets in both lines which indicate that freezing stress inhibits many metabolic reactions in the plant.

Table 3.22 Number of significantly ($P<0.05$) different probe sets that changed more than 2-fold in transgenic lines compared to WT after freezing treatment.

Regulation	**S2**	**M48**
up-regulated	450	109
down-regulated	662	431
Total	1112	540

The Venn diagram in Figure 3.38 depicts the overlap of differentially regulated genes in S2 and M48 after freezing stress. 496 of 540 differentially regulated genes in M48 overlap with the differentially regulated genes in S2. Only 44 of the differentially regulated genes do not overlap. The number of genes not overlapping is 616 for S2 line. This indicates that there are many processes or reactions differentially regulated by constitutive expression of MYB4 in S2 line. Scatter plots of differentially regulated ($p<0.05$; $FC\geq2$) genes after freezing treatment and their expression values are given in Figure 3.39.

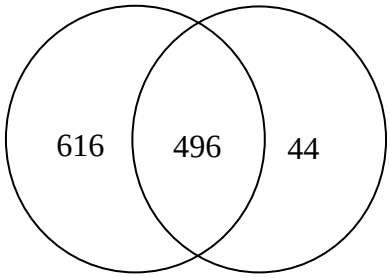

Figure 3.38 Venn diagram showing the overlap of differentially regulated genes in S2 (left) and M48 (right.) compared to WT after freezing stress.

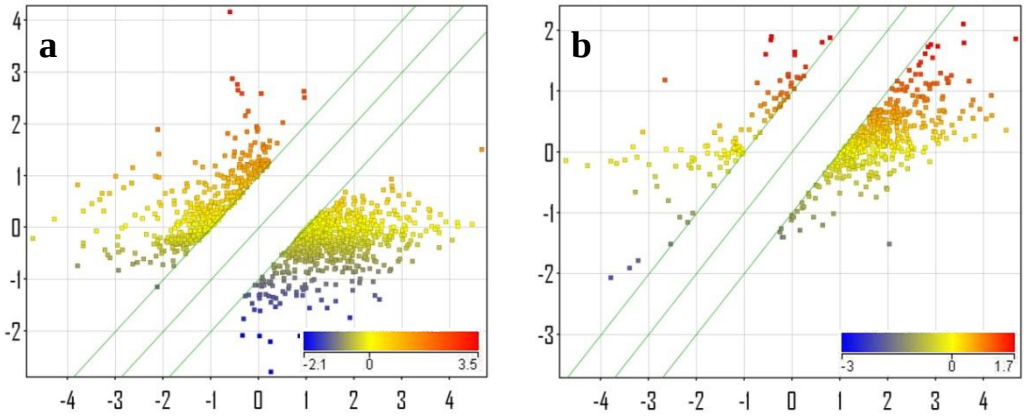

Figure 3.39 Scatter plots of differentially regulated ($p<0.05$; $FC\geq2$) genes after freezing treatment and their expression values. Normalized expression values of differentially regulated genes in (a) S2 and (b) M48 compared to WT are displayed. Diagonal lines indicate twofold difference lines. Points above and below the diagonal lines indicate up- and down-regulated genes, respectively.

49% of up-regulated genes in S2 and 60% of up-regulated genes in M48 upon exposure to freezing temperatures fall into the biological processes: metabolic process, cellular process, biosynthetic process, response to stress, photosynthesis, transport and generation of precursor metabolites and energy (Figure 3.40). The total number of genes involved in each of these processes in S2 is more than two fold of those in M48. This supports the idea that 35S promoter regulating expression of MYB4 in S2 activates many genes involved in these processes upon exposure to freezing temperatures. On the other hand COR15a promoter regulating expression of MYB4 in M48 activates lesser genes involved in these processes.

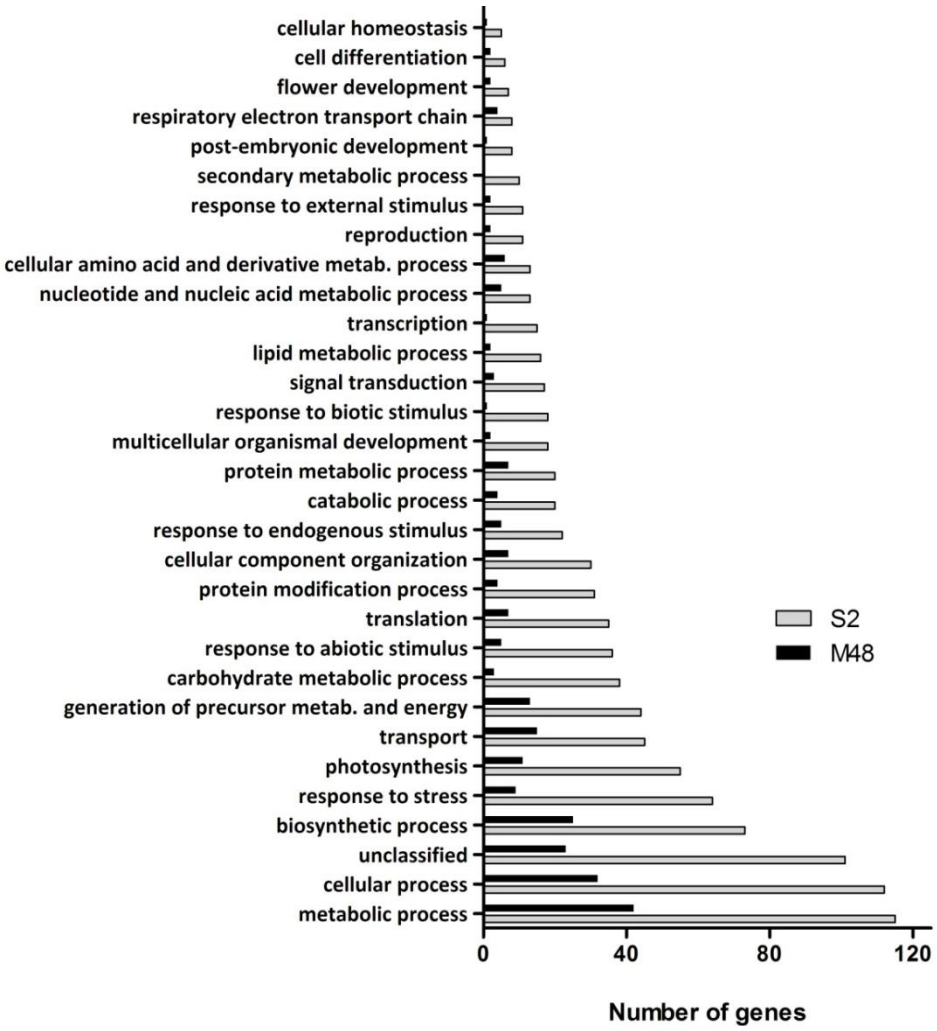

Figure 3.40 Up-regulated biological processes in S2 and M48 after freezing stress compared to WT. Significantly different (P<0.05) probe sets were used for gene classifications.

52% of classified down-regulated genes in S2 and 54% of classified down-regulated genes in M48 upon exposure to freezing stress fall into the biological processes: cellular process, metabolic process, biosynthetic process, response to stress, catabolic process, protein modification process, transport, response to abiotic stimulus and cellular component organization (Figure 3.41). The number of genes involved in each biological process was higher in S2 when compared to M48. This indicated that constitutive expression of *myb4* in S2 line has a greater impact on the cold-mediated transcriptome compared to stress inducible expression. The number of both down- and up-regulated genes involved in metabolic processes, cellular processes, response

to stress, biosynthetic processes and transport were high. This indicated that these biological processes are the most affected by *myb4* expression under freezing stress conditions. MapMan visualization of up/down regulated genes in selected pathways in M48 and S2 compared to WT upon exposure to freezing temperatures is given in Figure 3.42 and Figure 3.43 respectively.

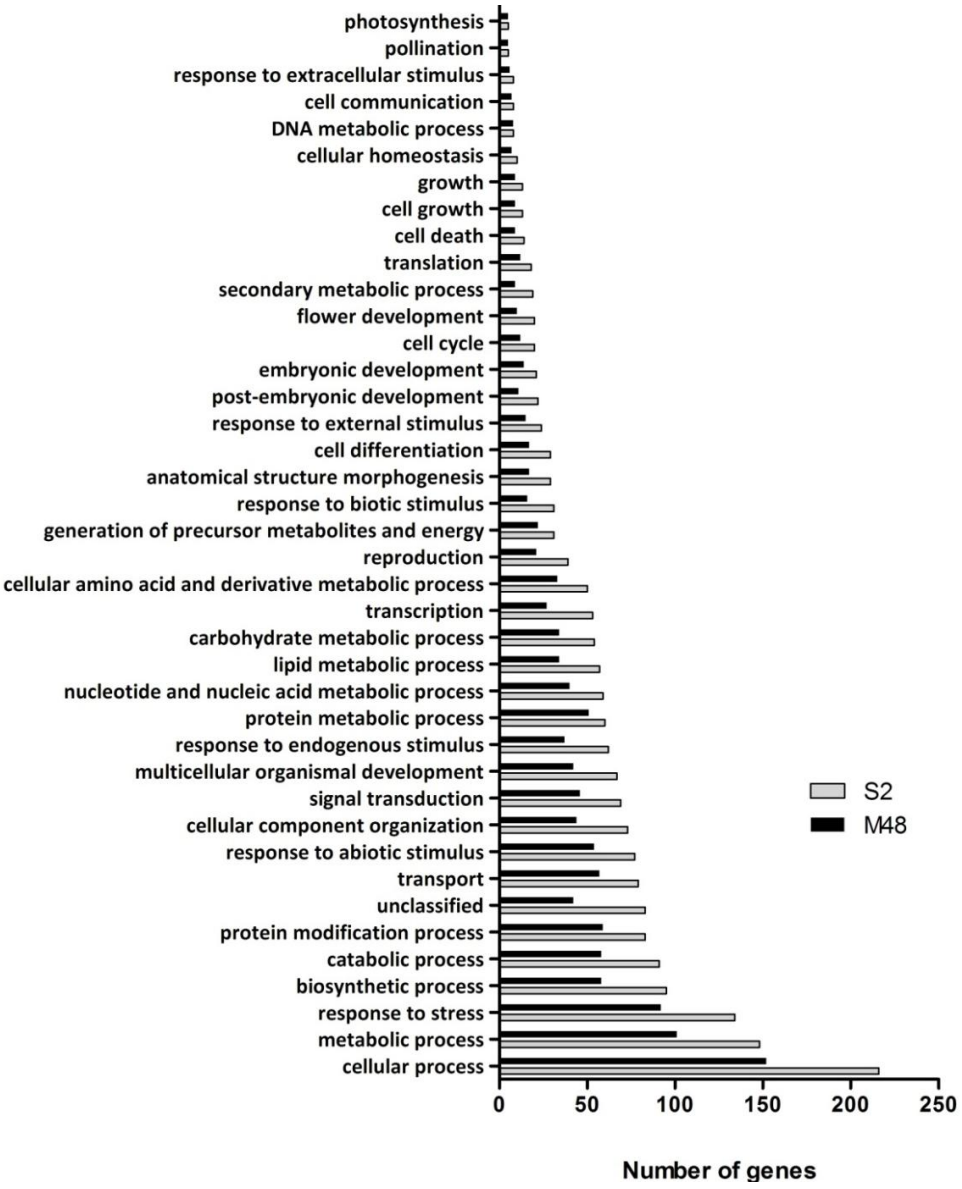

Figure 3.41 Down-regulated biological processes in S2 and M48 after freezing stress compared to WT. Significantly different ($P<0.05$) probe sets were used for gene classifications.

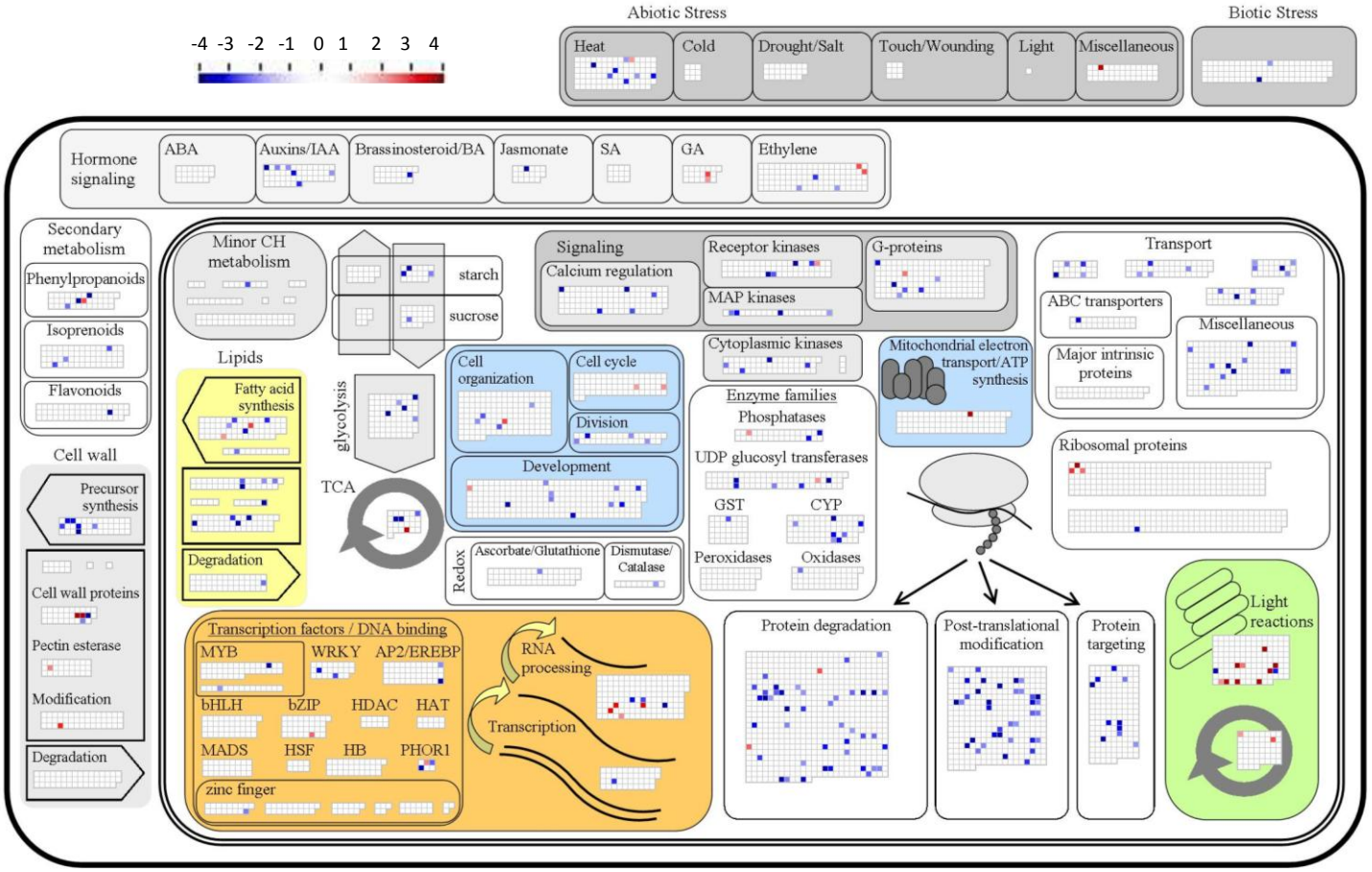

Figure 3.42 Differentially regulated genes in M48 compared to WT in selected pathways after freezing stress. The blocks in the figure represent a BIN or a subBIN and each square in a block represents a gene. Down-regulated genes are represented with blue colour and up-regulated genes are represented with red colour.

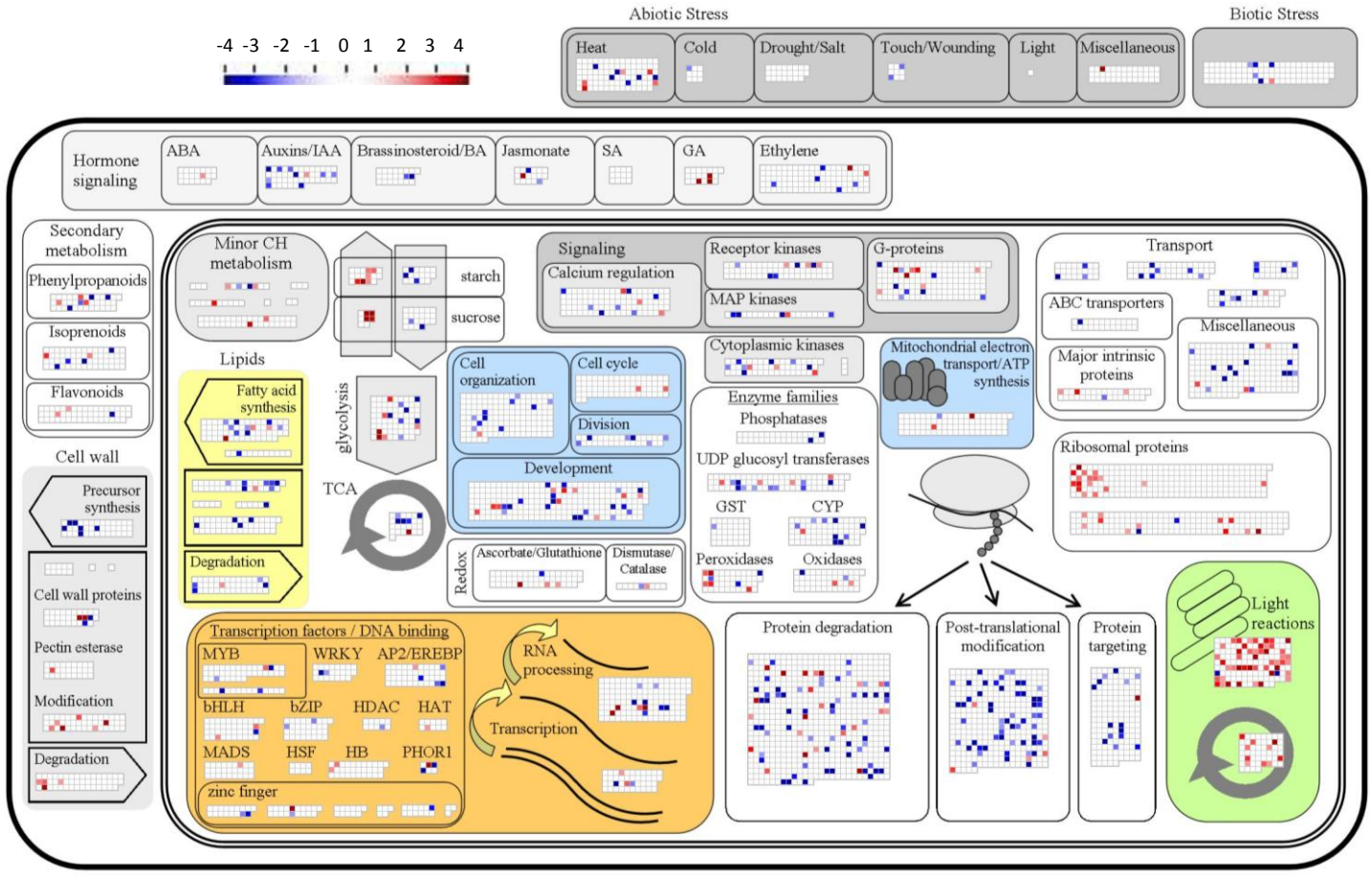

Figure 3.43 Differentially regulated genes in S2 compared to WT in selected pathways after freezing stress. The blocks in the figure represent a BIN or a subBIN and each square in a block represents a gene. Down-regulated genes are represented with blue colour and up-regulated genes are represented with red colour.

Previously in section 3.2.5.2 effect of *myb4* expression on transcriptome of poatato was discussed and figures representing differentially regulated genes involved in certain pathways in transgenic lines compared to wild type were generated using MapMan. These figures were used to select genes regulated upon *myb4* expression in transgenics under control conditions. Selected list of genes were presented together with the differentially regulated genes in transgenic lines compared to wild type in stress conditions. A list of all differentially regulated genes ($P<0.05$ and $FC\geq2$) in control and stress conditions is given in Appendix H.

Some HSPs, especially HSP90, HSP70 and several small HSPs, increase in abundance upon exposure to low temperature. These Hsps are involved in the refolding of denatured proteins and preventing their aggregation and also membrane protection (Renaut *et al.*, 2006; Timperio *et al.*, 2008). Selected differentially regulated genes involved in abiotic and biotic stress responses in transgenic lines in control and stress conditions are depicted in Table 3.23. The number of differentially expressed genes in transgenic potato compared to WT under control conditions was lower than those differentially expressed after freezing stress. In S2 there was one Hsp transcript up-regulated and four Hsps transcripts down-regulated which were involved in heat response under control conditions. The number of up-regulated transcripts was four and down-regulated transcripts were six for M48. The number of differentially expressed transcripts was higher under freezing stress. There were six down-regulated and five up-regulated Hsp transcripts in S2. The number of down-regulated transcripts was seven and number of up-regulated transcripts was one in M48. *myb4* expression in transgenic potato have a broad impact on global expression profiles. Therefore alteration in expression of a gene may be due to a direct effect of *myb4* expression or it may be an indirect effect generated by *myb4* induced other molecules. This may be the reason of up- and down-regulations in Hsps in transgenic potato. Up-regulation of a higher number of Hsps in S2 compared to M48 after freezing stress might be due to constitutive promoter controlling *myb4* expression in S2.

Table 3.23 Significantly (P<0.05) regulated transcripts involved in abiotic and biotic stress responses. Probe set ID, *Arabidopsis thaliana* (AT) locus name and putative annotation of each transcript is listed. Values represent fold changes of transcripts in S2 C and M48 C compared to WT C and in S2 AS and M48 AS compared to WT AS. Negative values show down-regulation and positive values show up-regulation. Only transcripts with FC≥3.5 in at least one of the transgenic lines or WT are displayed. Fold changes less than 2 are indicated with -. (HS: heat shock, LRR: leucine-rich repeat)

Probe Set ID	AT locus – Putative Annotation	Fold Change			
		S2 C	M48 C	S2 AS	M48 AS
ABIOTIC STRESS / HEAT					
les.1931.1.a1_at	AT1G59725 – DNAJ HS protein, putative	4.45	4.31	-	-
les.3160.1.s1_at	AT1G56410 – ERD2/HSP70T-1 (early-RD 2)	-	-	-17.61	-11.76
les.3160.3.s1_at	AT3G12580 – HSP70 (HS protein 70)	-	-2.36	-5.11	-2.63
les.3179.2.s1_at	AT3G44110 – ATJ, ATJ3 (DnaJ homologue 3)	-	-	-4.66	-2.05
lesaffx.44913.1.s1_at	– DNAJ HS N-terminal domain-containing protein	-	-2.15	-4.02	-3.21
lesaffx.45577.1.s1_at	AT5G64360 – DNAJ HS N-terminal domain-containing protein	-	-	-3.54	-3.10
lesaffx.68350.1.s1_at	AT5G23590 – DNAJ HS N-terminal domain-containing protein	-	-	-5.82	-3.23
BIOTIC STRESS					
les.3406.1.s1_at	AT3G12500.1 –basic endochitinase, identical to basic endochitinase precursor	-3.21	-	-3.52	-
les.3756.1.s1_a_at	AT2G43330 – ATINT1 (inositol transporter 1); carbohydrate transmembrane transporter/ sugar:hydrogen ion symporter	3.93	-	-	-
lesaffx.1458.1.s1_at	AT1G75800 – pathogenesis-related thaumatin family protein	-	-	-3.90	-2.07
lesaffx.71532.1.s1_at	AT1G33590 – disease resistance protein-related / LRR protein-related	-	-2.23	-4.69	-5.56

Some of the differentially regulated genes in transgenic potato that were involved in biotic stress responses under control and freezing stress conditions are displayed in Table 3.23. There were five transcripts down-regulated in S2 and two transcripts down-regulated in M48 after freezing stress. Although the number of differentially regulated genes upon exposure to freezing (compared to control conditions) was high, the number of differentially regulated genes compared to WT was low. There are some researches reporting increased disease resistance in Arabidopsis (Vannini,

et al., 2006) and tomato (Vannini, *et al.*, 2007) ectopically expressing *myb4*. However there was no significant up-regulation in the genes involved in biotic stress in transgenic potato compared to WT. This suggests that transgenic potato plants may not be much more disease tolerant than WT potato.

There were four MYB family TFs and five bHLH TFs differentially regulated in transgenic potato compared to WT under control conditions (Table 3.24). Differential expression of these TF families was also reported in Arabidopsis (Vannini, *et al.*, 2006) and in rice (Park *et al.*, 2010) overexpressing *myb4*. There was one MYB family TF up-regulated in S2 and four MYB family TFs up-regulated in M48. *myb4* expression in transgenic lines may have up-regulated expression of these MYB family transcription factors. There were two transcripts up-regulated and two transcripts down-regulated in S2 that encodes bHLH TFs and there was one gene up-regulated in M48. The differential regulation of these bHLH TFs in transgenic potato may be *myb4* dependent. Because MYB TFs are known to interact with bHLH proteins to control transcription (Ramsay & Glover, 2005).

In transgenic rice overexpressing *myb4*, 38 TFs were up-regulated and 15 TFs were down-regulated after chilling treatment (Park, *et al.*, 2010). Some of the up-regulated TFs belong to MYB, ERF, ARF, bHLH, NAC and bZIP TF families. There were differentially expressed transcripts for MYB, bHLH, PHOR and AP2/EREBP TFs in transgenic potato compared to WT under stress conditions. Although WRKY, bZIP, HB (homeobox) and zinc finger TFs were also differentially regulated upon exposure to freezing (compared to control conditions) it was not a significant regulation compared to WT. There were no down-regulated MYB TFs in cold independent transcriptome (control conditions) of S2 however there were 4 down-regulated MYB TFs in cold-dependent transcriptome. There were also 2 down-regulated MYB TFs in M48. This alteration in MYB expression pattern in potato may be regulated indirectly through intermediary TFs acting on specific types of *cis*-elements within certain target genes.

PHOR1 transcription factors were not differentially regulated in potato under control conditions. However they were differentially regulated in the cold-mediated transcriptome. To our knowledge there is no report showing involvement of PHOR1 TFs in the cold-response of plants. Antisense inhibition of *PHOR1* in *S. tuberosum* spp. *andigena* produced a semidwarf phenotype as giberellic acid (GA) deficient plants. Antisense lines also showed reduced GA responsiveness. Conversely transgenic lines overexpressing *PHOR1* showed an enhanced response to GA. GA application induced rapid migration of PHOR1-GFP protein to the nucleus. Thus *PHOR1* appears to be a general component of GA signaling pathways that relocalizes to the nucleus in the presence of GA (Amador *et al.*, 2001). Previously involvement of protein degradation was suggested in GA signaling due to the observation that activation of the GA response is linked with disappearance of the RGA/SLR1 repressor protein from the nucleus (Itoh *et al.*, 2002; Silverstone *et al.*, 2001). This hypothesis raised the question whether U-box arm-repeat protein PHOR1 is involved in ubiquitination of one or more components of the GA signal transduction pathway, to target them for degradation by the proteasome pathway (Monte *et al.*, 2003). Proteasome-mediated protein degradation has been shown to be involved in a variety of plant cellular responses including auxin (Gray *et al.*, 2001), jasmonic acid (Xie *et al.*, 1998), cold response (Lee *et al.*, 2001) and disease resistance pathways (Austin *et al.*, 2002). Monte *et al.* (2003) proposed a hypothetical model of PHOR1 action. They suggest that GA binds to an as yet unidentified GA receptor, it activates second messengers and G proteins and causes PHOR1 to be localized to the nucleus. Then in the nucleus PHOR1, as a single protein or as part of a multiprotein complex, ubiquitinates the repressor RGA/GAI and targets it for degradation by the proteasome system. In the absence of GA, PHOR1 is localized in the cytosol and the repressors RGA/GAI are stable, thus inhibiting the GA response.

In potato transcripts for GAs and G-proteins were differentially expressed after freezing treatment (Table 3.27). Expression of GAs were especially up-regulated in S2 after freezing stress. Since PHOR1 is already known to be a general component of

GA signaling in potato, differential regulation of PHOR1 may be responsible in part for up-regulation of GAs. According to the hypothetical model of PHOR1 action proposed by Monte *et al.* the differential regulation of PHOR1 may have also affected the differential expression of G-proteins.

Table 3.24 Significantly (P<0.05) regulated transcripts involved in transcription and post-transcription. Probe set ID, *Arabidopsis thaliana* (AT) locus name and putative annotation of each transcript is listed. Values represent fold changes of transcripts in S2 C and M48 C compared to WT C and in S2 AS and M48 AS compared to WT AS. Negative values show down-regulation and positive values show up-regulation. Fold changes less than 2 are indicated with -. (TF: transcription factor)

Probe Set ID	AT locus – Putative Annotation	Fold Change			
		S2 C	M48 C	S2 AS	M48 AS
TRANSCRIPTION FACTORS/ MYB					
les.5017.1.a1_at	AT3G46130– AtMYB48, MYB111 (myb domain protein 111)	-	2.37	-	-
les.5017.1.s1_at	AT3G46130– AtMYB48, MYB111 (myb domain protein 111)	2.94	4.55	-	-
lesaffx.40173.1.s1_at	AT2G31180– AtMYB14	-	-	2.52	-
lesaffx.54522.1.s1_at	AT2G23290– AtMYB70	-	-	-10.30	-4.04
lesaffx.62138.1.s1_at	AT5G49620– AtMYB78	-	-	-2.12	-
les.3716.1.s1_at	AT4G39250– DNA binding / TF	-	2.05	-	-
les.4923.1.s1_at	AT2G46830– CCA1 (circadian clock associated 1); TF	-	2.48	-	-
lesaffx.25436.1.s1_at	AT5G47390– myb family TF	-	-	-3.72	-2.10
lesaffx.56221.1.s1_at	AT3G09600– myb family TF	-	-	-2.95	-
TRANSCRIPTION FACTORS/ bHLH					
les.1376.2.a1_at	AT5G41315– MYC6.2, GL3 (GLABRA 3); TF	-	-	2.24	-
les.4085.1.s1_at	AT2G27230– LHW (lonesome highway)	-2.16	-	-	-
les.501.2.a1_at	AT3G57800– basic helix-loop-helix (bHLH) family protein	-	-	2.51	-
les.5638.1.s1_at	AT4G34530– basic helix-loop-helix (bHLH) family protein	-	2.68	-	-
lesaffx.37213.1.s1_at	AT5G46690– BHLH071 (beta hlh protein 71); DNA binding / TF	-2.97	-	-	-
lesaffx.62334.1.s1_at	AT1G26945– transcription regulator	3.45	-	-	-
lesaffx.64675.1.s1_a_at	AT5G57150– basic helix-loop-helix (bHLH) family protein	2.26	-	-3.47	-
TRANSCRIPTION FACTORS/ PHOR1					
lesaffx.15878.2.s1_at	AT3G52450– U-box domain-containing protein	-	-	3.85	2.26

Table 3.24 (continued)

Probe Set ID	AT locus – Putative Annotation	Fold Change			
		S2 C	M48 C	S2 AS	M48 AS
lesaffx.56802.1.s1_at	AT3G19380– U-box domain-containing protein	-	-	-4.22	-3.79
lesaffx.63659.1.s1_at	AT3G18710– U-box domain-containing protein	-	-	-4.95	-2.58
TRANSCRIPTION FACTORS/ AP2/EREBP					
les.124.1.s1_at	AT4G25480– CBF3, DREB1, DREB1A (dehydration response element B1A); DNA binding / transcription activator/ TF	-	-	2.06	-
les.5885.2.s1_at	AT1G78080– RAP2.4 (related to AP2 4); DNA binding / TF	-	-	-6.08	-5.60
les.5885.3.s1_at	AT1G78080– RAP2.4 (related to AP2 4); DNA binding / TF	-	-	-2.36	-2.17
lesaffx.60947.1.s1_at	AT4G36920– FLO2, FL1, AP2 (APETALA 2)	-	-	-2.58	-
lesaffx.64978.1.s1_at	AT5G67190– AP2 domain-containing TF	-	-	-3.23	-
RNA PROCESSING					
les.1762.1.s1_at	AT2G35370– GDCH (Glycine decarboxylase complex H)	-	-	8.36	3.33
les.2914.1.s1_at	AT1G32470– glycine cleavage system H protein. mitochondrial. putative	-	-	2.54	-
les.2940.2.s1_at	AT1G49760– PAB8 (poly(A) binding protein 8); RNA binding / translation initiation factor	5.53	6.17	4.69	3.38
les.2940.2.s1_s_at	AT1G49760– PAB8 (poly(A) binding protein 8); RNA binding / translation initiation factor	3.88	4.42	4.00	3.29
les.2982.1.a1_at	AT1G02840– SRP34, SR1 (splicing factor 2); RNA binding	-	-	-2.98	-
les.2982.2.s1_at	AT1G02840– SRP34, SR1 (splicing factor 2); RNA binding	-	-	-10.82	-4.30
les.5651.1.s1_at	AT2G18510– EMB2444 (embryo defective 2444); RNA binding	-	-	-2.04	-
lesaffx.14782.2.s1_at	AT3G49430– SRP34A (SER/ARG-rich protein 34A)	-	2.83	-	-
lesaffx.31254.1.s1_at	AT5G52040– ATRSP41 (arginine/serine-rich splicing factor 41)	-	-	-3.33	-
lesaffx.44533.1.a1_at	AT4G21660– proline-rich spliceosome-associated (PSP) family protein	-	-	-2.04	-
lesaffx.54261.1.s1_at	AT5G53180– polypyrimidine tract-binding protein / heterogeneous nuclear ribonucleoprotein/ putative	-	-	-5.18	-2.76
lesaffx.59682.1.s1_at	AT2G35120– glycine cleavage system H protein, mitochondrial	-	-	-	2.18
lesaffx.65616.1.s1_at	AT3G01150– PTB (polypyrimidine tract-binding)	-	-	-6.17	-3.42

Further studies are required to demonstrate whether PHOR1 does function as an E3 ubiquitin ligase. If there is such a function of these TFs it is important to define the targets of its ubiquitin ligase activity. Determination of these targets may help in enlightening the cold responsive expression of PHOR1 in potato.

AP2/EREBP TFs were significantly regulated in potato after freezing treatment but not in control conditions. This TF family is very well known to be induced by abiotic stresses such as drought and cold. CBFs the most studied AP2/EREBP TFs could activate the expression of COR genes and induce cold tolerance. Park, et al. (2010) suggested a *myb4* network in transgenic rice which is independent of *DREB/CBF*. Cold-induced expression of *OsMyb4* resulted in the activation of 38 TF but none of them were *DREB/CBF* TFs. In S2 four AP2 domain containing TFs were down-regulated and one AP2 domain containing TF (CBF3) was up-regulated. There were two AP2 domain containing TFs down-regulated in M48. The differential regulation of these TFs show that the *myb4* network is not independent of *DREB/CBF* in potato and that activity of *myb4* may be species dependent.

There were three transcrips differentially regulated in control conditions and 12 transcripts differentially regulated after freezing stress which were involved in RNA processing in at least one of the transgenic lines compared to WT (Appendix H). Most of these transcripts were involved in splicing. It is known that alternative splicing in response to environmental cues enable cells to synthesize different proteins from a single gene. *myb4* expression in transgenic lines may have regulated differential expression of the transcripts involved in splicing which may affect synthesis of different proteins. These proteins may contribute in generation of a different cold response in transgenic potato compared to WT.

Only a few transcripts for ribosomal proteins were differentially regulated in transgenic potato compared to WT under control conditions (Table 3.25, Appendix H). However there were a high number of transcripts up-regulated in S2 compared to WT after freezing treatment. Freezing stress down-regulated many transcripts in WT

but there were few transcripts in S2 that were down-regulated. Therefore there seems to be an up-regulation in transcripts in S2 compared to WT. Most of the down-regulated genes upon exposure to freezing stress encode for structural constituents of ribosomes. For this reason S2 may synthesize much more ribosomes and this may lead to increased protein synthesis. There were a low number of transcripts involved in protein degradation; modification and targeting that were differentially regulated in transgenic lines compared to WT under control conditions (Table 3.25, Appendix H). On the other hand the number of differentially expressed transcripts after freezing stress was very high in transgenic lines compared to WT. The different expression patterns in WT and transgenic potato may be a direct or indirect effect of *myb4* expression in transgenic plants. *myb4* is known to control a large and complex transcriptional network associated with diverse cellular processes, primarily defence and rescue, metabolism and development (Park, *et al.*, 2010). The broad function of *myb4* may be in part responsible for the differentially regulated genes in transgenic potato that are involved in protein degradation, modification and targeting.

Table 3.25 Significantly (P<0.05) regulated transcripts involved in translation and post-translation. Probe set ID, *Arabidopsis thaliana* (AT) locus name and putative annotation of each transcript is listed. Values represent fold changes of transcripts in S2 C and M48 C compared to WT C and in S2 AS and M48 AS compared to WT AS. Negative values show down-regulation and positive values show up-regulation. Only transcripts with FC≥4 in at least one of the transgenic lines or WT are displayed. Fold changes less than 2 are indicated with -. (HS: heat shock)

Probe Set ID	AT locus – Putative Annotation	Fold Change			
		S2 C	M48 C	S2 AS	M48 AS
RIBOSOMAL PROTEINS					
les.4298.1.s1_s_at	ATCG00380– RPS4, Chloroplast encoded ribosomal protein S4	3.78	4.81	3.26	2.95
lesaffx.33796.2.s1_at	ATCG00900– RPS7.1, chloroplast ribosomal protein S7	5.68	4.77	24.78	12.62
lesaffx.1195.2.s1_at	AT4G27940– mitochondrial substrate carrier family protein	-	-	-7.68	-3.54
lesaffx.67298.1.s1_at	AT1G48350– ribosomal protein L18 family protein	-	-	4.93	-
PROTEIN DEGRADATION					
les.1675.1.s1_at	AT4G23630.1– reticulon family protein (RTNLB1)	7.09	-2.14	-	-

Table 3.25 (continued)

Probe Set ID	AT locus – Putative Annotation	Fold Change			
		S2 C	M48 C	S2 AS	M48 AS
les.2574.3.s1_at	AT5G09900.2 – 26S proteasome regulatory subunit, putative (RPN5), p55 protein-like	-	-	-6.42	-3.18
les.2960.2.s1_at	AT3G10410– SCPL49 (serine carboxypeptidase-like 49)	-	-	-5.17	-3.81
les.2960.3.s1_a_at	AT3G10410– SCPL49 (serine carboxypeptidase-like 49)	-	-	-13.99	-9.25
les.3293.2.s1_at	AT5G50920– HSP93-V, DCA1, CLPC (HS protein 93-V); ATP binding / ATPase	-	-	-4.64	-5.22
les.3308.3.s1_at	AT4G05320– UBQ10 (polyubiquitin 10)	-	-	-5.56	-5.38
les.3369.2.s1_at	AT2G30950– FTSH2, VAR2 (variegated 2); ATP-dependent peptidase/ ATPase/ metallopeptidase/ zinc ion binding	-	-	-5.83	-6.39
les.3621.1.s1_at	– wound-induced proteinase inhibitor 1 precursor	10.29	-	-	-
les.3940.2.a1_at	– wound-induced proteinase inhibitor 1 precursor	5.46	-	-	-
les.4287.2.s1_at	AT1G20200.1 – 26S proteasome regulatory subunit S3, putative (RPN3)	-	-	-7.28	-4.04
les.4792.1.s1_at	AT1G51710– UBP6 (ubiquitin-specific protease 6)	-	-2.25	-4.05	-2.37
les.4820.1.s1_x_at	AT3G12490– cysteine protease inhibitor, putative / cystatin, putative	6.83	-	-	-
les.494.2.s1_at	AT1G45000– 26S proteasome regulatory complex subunit p42D	-	-	-7.88	-2.62
les.494.3.s1_at	AT1G45000– 26S proteasome regulatory complex subunit p42D	-	-2.32	-5.27	-2.26
les.5264.1.s1_at	AT5G67360– ARA12; subtilase	-	-	4.93	2.68
les.795.2.s1_at	AT1G29150– RPN6, ATS9 (19S proteosome subunit 9)	-	-	-5.44	-3.36
lesaffx.24556.1.s1_at	AT1G18660– zinc finger (C3HC4-type RING finger) family protein	-	-	-4.09	-3.17
lesaffx.3308.2.s1_at	AT4G19006– 26S proteasome regulatory subunit, putative (RPN9)	-	-	-6.83	-2.22
lesaffx.40158.1.s1_at	AT2G18280– AtTLP2 (tubby like protein 2); phosphoric diester hydrolase/ TF	-2.66	-	-4.66	-2.33
lesaffx.49541.1.s1_at	AT3G09770– zinc finger (C3HC4-type RING finger) family protein	-	-	-4.45	-3.15
lesaffx.51779.1.s1_at	AT1G47710– (ATSERPIN1); cysteine protease inhibitor/ serine-type endopeptidase inhibitor	-3.01	-2.39	-5.84	-2.23
lesaffx.59101.1.s1_at	AT2G19560– proteasome protein-related	-	-	-4.65	-4.12

Table 3.25 (continued)

Probe Set ID	AT locus – Putative Annotation	Fold Change			
		S2 C	M48 C	S2 AS	M48 AS
lesaffx.61619.1.s1_at	AT5G42270– FTSH5, VAR1 (VARIEGATED 1); ATP-dependent peptidase/ ATPase/ metallopeptidase	-	-	-5.01	-3.35
lesaffx.63935.1.s1_at	AT1G24140– matrixin family protein	-3.10	-4.28	-	-2.01
lesaffx.66215.2.s1_at	AT1G44130– nucellin protein. putative	-3.07	-	-5.93	-3.70
lesaffx.68556.1.s1_at	AT4G38630– MCB1, MBP1, RPN10 (regulatory particle non-ATPase 10)	-	-	-11.75	-3.76
POST-TRANSLATIONAL MODIFICATIONS					
les.390.1.s1_at	AT5G14640– PK family protein	-	-	-7.36	-4.32
les.1297.1.s1_at	AT3G21630– CERK1 (chitin elicitor receptor kinase 1); kinase/ receptor signaling protein/ transmembrane receptor PK	-3.19	-2.74	-4.06	-2.90
les.2027.3.s1_at	AT2G23770– PK family protein / peptidoglycan-binding LysM domain-containing protein	-2.70	-	-5.64	-7.01
les.3124.1.s1_at	AT1G17550– HAB2 (Homology to ABI2); protein serine/threonine phosphatase	-	-	-11.77	-6.12
les.2459.1.s1_at	AT5G14640– PK family protein	-	-	-6.27	-4.08
les.2459.2.s1_at	AT5G14640– PK family protein	-	-	-4.11	-2.69
les.3124.3.s1_at	AT1G72770– HAB1 (homology to ABI1)	-	-	-8.67	-3.07
les.3248.2.s1_at	AT4G18710– DWF12, UCU1, BIN2 (brassinosteroid-insensitive 2); kinase	-	-	-6.10	-4.40
les.3377.2.s1_at	AT5G26751– ATSK11 (SHAGGY-related kinase 11); PK	-	-	-15.00	-5.53
les.390.2.s1_at	AT5G14640– PK family protein	-	-	-4.03	-2.45
lesaffx.10444.1.s1_at	AT3G11410– AHG3/PP2CA (protein phosphatase 2CA); protein binding / protein serine/threonine phosphatase	-	-	-4.27	-
lesaffx.11410.1.s1_at	AT3G17510– SnRK3.16, CIPK1 (CBL-interacting protein Kinase 1); kinase	-	-	-7.88	-4.18
lesaffx.33.1.s1_at	AT3G62260– protein phosphatase 2C, putative / PP2C, putative	-	-	-4.56	-2.29
lesaffx.46935.1.s1_at	AT2G33700– protein phosphatase 2C, putative	-	-	-4.07	-2.21
lesaffx.63721.1.s1_at	AT5G42440– PK family protein	-	-	-4.03	-2.93
lesaffx.64984.1.s1_at	AT2G23070– casein kinase II alpha chain, putative	-2.15	-	-6.13	-2.23
lesaffx.65398.3.s1_at	AT3G25800– PR65, PDF1 (65 KDA regulatory subunit of protein phosphatase 2A); protein phosphatase type 2A regulator	-	-	-11.82	-5.26
lesaffx.65524.1.s1_at	AT1G16220– protein phosphatase 2C family protein / PP2C family protein	-	-	-7.29	-6.49

Table 3.25 (continued)

Probe Set ID	AT locus – Putative Annotation	Fold Change			
		S2 C	M48 C	S2 AS	M48 AS
lesaffx.66270.1.s1_at	AT5G01820– SnRK3.15, CIPK14, ATSR1 (serine/threonine PK 1); kinase	-	-	-4.23	-
lesaffx.65817.2.s1_at	AT1G09020– ATSNF4, SNF4 (Sucrose NonFermenting 4)	-	-	-5.21	-3.46
lesaffx.68000.1.s1_at	AT3G05580– serine/threonine protein phosphatase, putative	-3.12	-2.20	-10.09	-3.24
lesaffx.70383.1.s1_at	AT1G16220– protein phosphatase 2C family protein / PP2C family protein	-	-	-5.19	-4.06
lesaffx.70796.1.s1_at	AT5G03470– ATB' ALPHA (PP2A, B' subunit, alpha isoform); protein phosphatase type 2A regulator	-2.52	-	-4.50	-3.57
lesaffx.7177.1.s1_at	AT1G14000– PK family protein / ankyrin repeat family protein	-	-	-8.16	-4.14
PROTEIN TARGETING					
les.2874.3.s1_at	AT4G30600– signal recognition particle receptor alpha subunit family protein	-	-	-5.26	-3.39
les.393.2.s1_at	AT4G32940– GAMMA-VPE, (Vacuolar processing enzyme gamma) cysteine-type endopeptidase	-	-	-6.55	-3.45
les.4519.3.s1_at	AT2G34250– protein transport protein sec61, putative	-	-	-11.55	-8.34
lesaffx.22051.2.s1_at	AT1G51980– mitochondrial processing peptidase alpha subunit, putative	-	-	-9.48	-7.04
lesaffx.35587.1.s1_at	AT4G32940– GAMMA-VPE (Vacuolar processing enzyme gamma) cysteine-type endopeptidase	-2.32	-	-7.73	-3.65
lesaffx.35741.1.s1_at	AT1G09270– importin alpha-1 subunit, putative (IMPA4)	-	-2.35	-4.54	-3.26
lesaffx.51291.1.s1_at	AT4G24880– unnamed protein product	-	-	-4.39	-3.32

There were a low number of transcripts involved in transport that were differentially regulated in transgenic lines compared to WT under control conditions (Table 3.26, Appendix H). On the other hand the number of differentially expressed transcripts after freezing stress was very high in transgenic lines compared to WT. Most of these differentially regulated transcripts were down-regulated in transgenic lines. Most of the down-regulated genes were encoding transporters either for transport of sugars, amino acids or hormones. In this chapter differential regulation of these molecules in

transgenic lines compared to WT upon exposure to freezing was already mentioned. This differential regulation may have led to the great variation in expression of transporters involved in these molecules' transport in transgenic lines.

Table 3.26 Significantly (P<0.05) regulated transcripts involved in transport. Probe set ID, *Arabidopsis thaliana* (AT) locus name and putative annotation of each transcript is listed. Values represent fold changes of transcripts in S2 C and M48 C compared to WT C and in S2 AS and M48 AS compared to WT AS. Negative values show down-regulation and positive values show up-regulation. Only transcripts with FC≥4 in at least one of the transgenic lines or WT are displayed. Fold changes less than 2 are indicated with – (ZIFL: zinc induced facilitator-like).

Probe Set ID	AT locus – Putative Annotation	Fold Change			
		S2 C	M48 C	S2 AS	M48 AS
lesaffx.34390.1.s1_at	AT3G11320– organic anion transmembrane transporter	-	-2.06	-7.65	-4.77
lesaffx.68360.2.s1_at	AT5G15640– mitochondrial substrate carrier family protein	-	-	-4.51	-2.02
lesaffx.35418.1.s1_at	AT2G01170– amino acid permease family protein	-	-	-8.32	-5.85
lesaffx.48830.1.s1_at	AT3G59360– ATUTR6/UTR6 (UDP-galactose transporter 6); nucleotide-sugar transmembrane transporter	-	-	-4.67	-2.58
lesaffx.49378.1.s1_at	AT5G13750– ZIFL1; tetracycline: hydrogen antiporter/ transporter	-	-	-13.17	-5.74
lesaffx.55549.1.s1_at	AT5G13750– ZIFL1; tetracycline: hydrogen antiporter/ transporter	-	-	-4.18	-2.54
les.4264.1.a1_s_at	AT3G22200– HER1, GABA-T, POP2 (pollen-pistil incompatibility 2); 4-aminobutyrate transaminase	-	-	-4.42	-3.05
lesaffx.1490.1.s1_at	AT4G21800– QQT2 (QUATRE-QUART2); ATP binding	-	-	-4.31	-2.62
lesaffx.16086.1.s1_at	AT1G78820– curculin-like (mannose-binding) lectin family protein / PAN domain-containing protein	-	-	-4.45	-4.88
lesaffx.49378.1.s1_at	AT5G13750– ZIFL1; tetracycline: hydrogen antiporter/ transporter	-	-	-13.17	-5.74
lesaffx.52437.1.s1_at	AT5G65980– auxin efflux carrier family protein	-	-	-10.33	-2.74
lesaffx.55549.1.s1_at	AT5G13750– ZIFL1; tetracycline: hydrogen antiporter/ transporter	-	-	-4.18	-2.54
lesaffx.62361.1.s1_at	AT5G19300– unnamed protein	-	-	-4.15	-2.41
lesaffx.70660.1.s1_at	AT3G46450– SEC14 cytosolic factor family protein / phosphoglyceride transfer family protein	-2.01	-	-4.98	-3.85

There were a few number of genes differentially expressed in transgenic lines compared to WT under control conditions that were auxin responsive or involved in auxin synthesis/degradation. The number of differentially regulated genes was higher after freezing treatment (Table 3.27, Appendix H). Lee *et al.* (2005) reported down-regulation of auxin transport and auxin-responsive genes in Arabidopsis upon exposure to cold. This is consistent with our findings in potato. The same research group have suggested that down-regulation of genes involved in auxin metabolism may eventually contribute to reduced plant growth rate under cold stress.

A gene involved in giberellin synthesis/degradation, and two genes that were giberellin regulated/responsive were up-regulated in S2 compared to WT upon exposure to freezing (Table 3.27, Appendix H). There were also two GA regulated/responsive transcripts up-regulated in M48. GAs modulate many responses during plant growth and development, such as seed germination, stem elongation, flower initiation, and seed and fruit development (Swain & Olszewski, 1996). G-proteins, Ca^{2+}, calmodulin and protein kinases are known to be involved in GA signal transduction cascade in cereal aleurone cells (Lovegrove & Hooley, 2000). Involvement of PHOR1 in GA signaling in potato was mentioned previously in this chapter. The differential regulation of these proteins or signaling molecules in transgenic potato may have contributed in differential regulation of giberellin metabolism.

Ca^{2+}, G-proteins, receptor kinases and cytoplasmic kinases which are signaling molecules known to be involved in cold response were differentially regulated in transgenic potato compared to WT. The number of transcripts were higher after freezing stress compared to control conditions. Cold stress induces/represses many genes in plants through the activity of signaling molecules listed above. Since *myb4* is a transcription factor with a broad function, the induced/repressed genes may differ in transgenic lines compared to WT according to activated/repressed downstream target genes. Therefore different genes may function in transgenic lines for a certain

reaction which are regulated through different signaling molecules. Therefore the expression pattern of signalling molecules may differ in WT and transgenic lines.

Table 3.27 Significantly (P<0.05) regulated transcripts involved in signalling. Probe set ID, *Arabidopsis thaliana* (AT) locus name and putative annotation of each transcript is listed. Values represent fold changes of transcripts in S2 C and M48 C compared to WT C and in S2 AS and M48 AS compared to WT AS. Negative values show down-regulation and positive values show up-regulation. Only transcripts with FC≥4 in at least one of the transgenic lines or WT are displayed. Fold changes less than 2 are indicated with -.

Probe Set ID	AT locus – Putative Annotation	Fold Change			
		S2 C	M48 C	S2 AS	M48 AS
HORMONE SIGNALING / AUXINS					
les.3216.1.s1_at	AT1G51760 – JR3, IAR3 (IAA-alanine resistant 3); metallopeptidase	-	-	-7.91	-4.29
les.3757.1.s1_at	– Stem-specific protein TSJT1, putative	-4.12	3.26	2.16	-
lesaffx.44071.1.s1_at	AT2G44500 – similar to unknown protein	-	-	-6.04	-3.33
HORMONE SIGNALING / JASMONATE					
les.13.1.s1_at	AT5G42650 – CYP74A, AOS (allene oxide synthase); hydro-lyase/ oxygen binding	-	-	-3.55	-5.07
les.3478.1.s1_at	AT4G15440 – CYP74B2, HPL1 (hydroperoxide lyase 1); heme binding / iron ion binding / monooxygenase	4.38	-	-	-
les.3980.1.s1_at	AT3G45140 – LOX2 (lipoxygenase 2)	10.95	-2.88	7.13	-
HORMONE SIGNALING / GIBERELLIC ACID					
les.417.1.s1_at	AT5G59845 – gibberellin-regulated family protein	2.60	-	10.20	2.84
les.4766.1.s1_at	AT2G18420 – Gibberellin-regulated GASA/GAST/Snakin family protein	-	-	7.77	2.11
les.64.1.s1_at	AT4G25420 – GA20OX1, AT2301, GA5 (GA requiring 5); gibberellin 20-oxidase/ gibberellin 3-beta-dioxygenase	4.15	2.81	4.84	-
SIGNALING / CALCIUM REGULATION					
les.3334.2.s1_at	AT5G66210 – CPK28 (calcium-dependent PK 28)	-	-	-3.15	-4.01
lesaffx.57054.2.s1_at	AT5G54130 – calcium-binding EF hand family protein	-2.50	-	-6.89	-3.98
SIGNALING / G-PROTEINS					
lesaffx.37222.1.s1_at	AT5G61530 – small G protein family protein / RhoGAP family protein	-	-	-8.59	-3.43
les.4749.1.s1_at	AT2G46710 – rac GTPase activating protein, putative	-	-	5.15	2.40

Table 3.27 (continued)

Probe Set ID	AT locus – Putative Annotation	Fold Change			
		S2 C	M48 C	S2 AS	M48 AS
les.4857.2.s1_at	AT5G45130 – Rab5A, RABF2a, RHA1	5.00	-	-	-
lesaffx.51015.1.s1_at	AT4G21520 – transducin family protein / WD-40 repeat family protein	-	-	-6.21	-2.98
lesaffx.68259.1.s1_at	AT4G34460 – ELK4, AGB1 (GTP binding protein beta 1)	-	-	-9.02	-4.08
SIGNALING / RECEPTOR KINASES					
les.1297.1.s1_at	AT3G21630 – CERK1 (chitin elicitor receptor kinase 1); kinase/ receptor signaling protein/ transmembrane receptor PK	-3.19	-2.74	-4.06	-2.90
lesaffx.16086.1.s1_at	AT1G78820 – curculin-like (mannose-binding) lectin family protein / PAN domain-containing protein	-	-	-4.45	-4.88
lesaffx.40862.1.s1_at	AT4G00300 – fringe-related protein	-	-2.48	-10.92	-6.11
SIGNALING / CYTOPLASMIC KINASES					
les.1297.1.s1_at	AT3G21630 – CERK1 (chitin elicitor receptor kinase 1); kinase/ receptor signaling protein/ transmembrane receptor PK	-3.19	-2.74	-4.06	-2.90
les.2027.3.s1_at	AT2G23770 – PK family protein / peptidoglycan-binding LysM domain-containing protein	-2.70	-	-5.64	-7.01
lesaffx.63721.1.s1_at	AT5G42440 – PK family protein	-	-	-4.03	-2.93

Peroxidases are one of the antioxidative enzyme families that are involved in scavenging ROS generated by cold or other abiotic stresses. There were three peroxidases differentially expressed in S2 compared to WT under control conditions (Table 3.28, Appendix H). The number of differentially regulated genes was eight after freezing treatment. There was no differential expression in M48 either in control conditions or after freezing treatment. This suggests that the activity of peroxidases may be dose dependent in potato. The constitutive expression of *myb4* may have regulated differential expression of peroxidases in S2.

Other antioxidant enzymes such as dehydroascorbate reductase and ascorbate peroxidase were also differentially regulated in S2 under control and freezing stress

Table 3.28 Significantly (P<0.05) regulated transcripts involved in enzymatic processes. Probe set ID, *Arabidopsis thaliana* (AT) locus name and putative annotation of each transcript is listed. Values represent fold changes of transcripts in S2 C and M48 C compared to WT C and in S2 AS and M48 AS compared to WT AS. Negative values show down-regulation and positive values show up-regulation. Only transcripts with FC≥3 in at least one of the transgenic lines or WT are displayed. Fold changes less than 2 are indicated with -.

Probe Set ID	AT locus – Putative Annotation	Fold Change			
		S2 C	M48 C	S2 AS	M48 AS
ENZYME FAMILIES/ PHOSPHATASES					
lesaffx.24391.1.s1_at	AT1G72880– acid phosphatase survival protein SurE, putative	-	-	-4.34	-3.18
lesaffx.67373.2.s1_at	AT1G09870– histidine acid phosphatase family protein	-	-	-7.47	-3.55
ENZYME FAMILIES/ PEROXIDASES					
les.2092.1.s1_at	AT4G21960 – PRXR1 (peroxidase 42)	-	-	3.85	-
les.4492.3.s1_at	AT4G21960 – PRXR1, (peroxidase 42); peroxidase	3.11	-	3.09	-
les.4999.1.s1_at	AT2G37130– peroxidase 21 (PER21) (P21) (PRXR5)	-	-	-4.27	-
lesaffx.32359.1.s1_at	AT4G37530– peroxidase, putative	-2.89	-	-3.29	-
ENZYME FAMILIES/ CYP					
les.1859.3.s1_at	AT3G14690– CYP72A15 (cytochrome P450, family 72, subfamily A, polypeptide 15); oxygen binding	-	-	-4.54	-4.11
les.3094.2.s1_at	AT1G11680– EMB1738, CYP51A2, CYP51,CYP51G1 (cytochrome P450 51); oxygen binding	-	-	-6.00	-3.29
lesaffx.22297.1.s1_at	AT2G34500– CYP710A1 (cytochrome P450, family 710, subfamily A, polypeptide 1); C-22 sterol desaturase/ oxygen binding	-	-	-5.14	-3.33
lesaffx.31317.11.s1_at	AT1G64940– CYP89A6 (cytochrome P450, family 87, subfamily A, polypeptide 6); oxygen binding	-	-	-4.06	-3.26
lesaffx.8720.2.s1_at	AT3G52970– CYP76G1 (cytochrome P450, family 76, subfamily G, polypeptide 1); oxygen binding	-	-	-4.44	-2.10
lesaffx.9038.1.s1_at	AT2G32440– CYP88A4, KAO2 (ent-kaurenoic acid hydroxylase 2); oxygen binding	4.52	-	-	-
ENZYME FAMILIES/ REDOX					
les.1925.1.a1_at	AT1G19570– DHAR1 (dehydroascorbate reductase)	3.26	-	-	-
les.3759.1.s1_at	AT5G53560– B5-A (Cytochrome b5 A)	-4.09	-	-	-
les.5581.1.s1_at	AT4G09010– APX4 (ascorbate peroxidase 4); peroxidase	2.39	-	5.85	-
lesaffx.54488.1.s1_at	AT1G63940– monodehydroascorbate reductase, putative	-	-	-3.33	-2.30

conditions compared to WT. They were not significantly regulated in M48 which may also reflect dose dependent activity of these enzymes. Up-regulation of peroxidases, dehydroascorbate reductase and ascorbate peroxidase point *myb4* regulated antioxidant responses in S2. Transgenic rice overexpressing *myb4* was also shown to have higher peroidase activity compared to WT (Park, *et al.*, 2010). Genes encoding for catalase, glutathione S-transferase and peroxidases were up-regulated in transgenic Arabidopsis overexpressing *myb4 (Vannini, et al., 2006)*. These reports are in line with our findings. This indicates that the expression of these antioxidant enzymes may be *myb4*-regulated.

The transcripts involved in phenylpropanoid metabolism were differentially regulated in S2 compared to WT under control and freezing stress conditions (Figure 3.29). The number of transcripts was higher under stress which may indicate cold-responsive expression of phenypropanoids. The number of transcripts differentially regulated in M48 after freezing stress was lower compared to S2. This suggests that constitutive expression of *myb4* in S2 may be responsible for involvement of a higher number of genes in phenylpropanoid metabolism. Phenylalanine ammonia-lyase (PAL) which is the enzyme catalyzing the very first step in phenylpropanoid pathway was up-regulated in S2 after stress. However 4-coumarate: CoA Ligase 1 (4CL1) which catalyzes another step in phenylpropanoid pathway was down-regulated. m*yb4*-activation of PAL was previously reported in transgenic Arabidopsis overexpressing *myb4*. The gene encoding 4CL1 was not *myb4* regulated in transgenic Arabidopsis plants (Vannini, *et al.*, 2006). This indicates that *myb4* expression may differentially regulate phenylpropanoid pathway in different organisms.

There were only three transcripts in S2 and one transcript in M48 differentially regulated compared to WT under control conditions that were involved in phenylpropanoid metabolism. The expression of PAL involved in the first step of phenylpropanoid pathway was not altered in transgenic lines. The anthocyanin content of WT and transgenic potato grown under control conditions were previously

determined and the results were given in Figure 3.14. According to one way ANOVA results there was no significant difference between anthocyanin contents of WT and transgenic lines. This result is consistent with the microarray data.

Park *et* al. (2010) reported activation of many genes involved in isoprenoid metabolism in transgenic rice overexpressing *myb4*. In potato the genes involved in isoprenoid metabolism was also differentially regulated after freezing in transgenic lines compared to WT. However not all the transcripts were up-regulated. There were no transcripts up-regulated in M48 and only two transcripts up-regulated in S2. There were four genes down-reglated in S2 and three genes in M48. The differential regulation of isoprenoid metabolism in potato and rice point that *myb4* expression may affect isoprenoid metabolism in a different way in these species.

A high number of transcripts which were involved in cell wall precursor synthesis, degradation and modification were differentially regulated in transgenic potato compared to WT in control and stress conditions (Table 3.29). Cell wall precursor synthesis was up-regulated in WT and transgenic lines upon exposure to freezing compared to control conditions. This may reflect the damage on cell walls or the rapid response for repair which has started with synthesis of new cell precursors. The number of up-regulated transcripts was higher in WT. This may indicate much extensive cell wall damage in WT compared to transgenic lines. It may also point a rapid cell wall repair system in WT.

Xyloglucan endotransglycosylase can cut and rejoin XG chains, and it is considered a key agent regulating wall expansion and is believed to be the enzyme responsible for the incorporation of newly synthesized XG into the wall (Bourquin, *et al.*, 2002). There were five transcripts down-regulated in S2 which were encoding xyloglucan endotransglycosylases under control conditions. These transcripts were up-regulated in stress conditions. Up-regulation of this enzyme may allow cell walls to expand in S2 which is especially important in newly synthesized cells. There was no significant up-regulation in xyloglucan endotransglycosylase expression in M48. This suggests

that the activity of this enzyme may be dose dependent. Higher expression of *myb4* under the control of constitutive promoter in S2 may be responsible for differential expression of the genes encoding xyloglucan endotransglycosylases.

In stress conditions, plants generally redirect assimilates from supporting new growth to the synthesis of low molecular weight carbohydrates and soluble sugars. Thus they avoid a deficiency in carbohydrates and avoid running out of cell energy. The low molecular weight compounds are effective osmoprotective substances which increase the tolerance to further abiotic factors (Oufir, *et al.*, 2008). Free sugars in the form of glucose and sucrose help plants acclimate to various stresses. Besides serving as osmoprotectants these sugars can be quickly used for energy production upon return to more favorable conditions. In this study sucrose and starch synthesis were down-regulated and sucrose and starch degradation were up-regulated in WT and transgenic potato by freezing treatment which is a well known phenomenon in plants under stress. Expression of some genes involved in sucrose and starch synthesis were less down-regulated in S2 compared to WT. Therefore expression of these genes are up-regulated in S2 compared to WT. Increased sucrose synthesis in S2 may provide a better protection during freezing stress compared to WT. However increased starch synthesis in S2 under stress condition may lead to energy deprivation. Previously increased sugar concentration upon exposure to cold was also reported in transgenic Arabidopsis (Vannini, *et al.*, 2006) and tomato (Vannini, *et al.*, 2007) plants overexpressing *myb4*.

Expression of two beta-amylases and one alpha-amylase involved in starch degradation was down-regulated in transgenic lines. Down-regulation of these enzymes may lead to reduced free sugar production in transgenic plants compared to WT which means a lower osmoprotection. On the other hand the up-regulation of amylases in WT may also reflect a higher requirement of energy in WT under stress conditions.

According to HPLC results sucrose concentration of WT was lower than S2 and higher than M48 under control conditions (Table 3.7). Microarray data showed that there was not a significant difference in the expression of transcripts involved in sucrose degradation and sucrose synthesis in transgenic lines compared to WT. In order to compare HPLC and microarray data HPLC analysis should be repeated with a higher number of samples.

Table 3.29 Significantly (P<0.05) regulated transcripts involved in metabolic reactions. Probe set ID, *Arabidopsis thaliana* (AT) locus name and putative annotation of each transcript is listed. Values represent fold changes of transcripts in S2 C and M48 C compared to WT C and in S2 AS and M48 AS compared to WT AS. Negative values show down-regulation and positive values show up-regulation. Only transcripts with FC≥4 in at least one of the transgenic lines or WT are displayed. Fold changes less than 2 are indicated with -.

Probe Set ID	AT locus – Putative Annotation	Fold Change			
		S2 C	M48 C	S2 AS	M48 AS
SECONDARY METABOLISM/ PHENYLPROPANOIDS					
les.220.1.s1_at	AT5G01210– transferase family protein	-2.53	-	-5.60	-2.43
les.281.3.s1_at	AT1G51680– 4CL1 (4-coumarate: CoA Ligase 1)	-	-	-7.23	-7.37
les.3741.1.s1_at	AT4G37980– ELI3-1 (elicitor-activated gene 3); binding / catalytic/ oxidoreductase/ zinc ion binding	-	-	-4.59	-
lesaffx.47885.1.s1_at	AT1G20510– OPCL1 (OPC-8:0 CoA ligase1); 4-coumarate-CoA ligase	-	-	-2.59	-4.21
SECONDARY METABOLISM/ ISOPRENOIDS					
les.3544.1.s1_at	AT5G52570– BETA-OHASE 2 (beta-carotene hydroxylase 2)	-	-	-4.04	-
lesaffx.44987.1.s1_at	AT1G78510– SPS1 (solanesyl diphosphate synthase 1)	-2.42	-	-4.29	-2.72
lesaffx.44987.1.s1_at	AT1G78510– SPS1 (solanesyl diphosphate synthase 1)	-2.42	-	-4.29	-2.72
CELL WALL/ CELL WALL PROTEINS					
les.3320.2.s1_at	AT3G22440– hydroxyproline-rich glycoprotein family protein	-	-	-19.51	-11.31
les.4368.1.s1_s_at	AT1G62440– LRX2 (LRR/extensin 2); protein binding / structural constituent of cell wall	-	-	8.56	5.09
lesaffx.43685.2.s1_s_at	AT1G62440– LRX2 (LRR/extensin 2); protein binding / structural constituent of cell wall	-	-	9.28	4.92
CELL WALL/ MODIFICATION					
les.4769.1.s1_at	AT3G45970– EXPL1, HEXP BETA 2.1, ATEXLA1 (expansin-like A1)	-5.57	-	-	-

Table 3.29 (continued)

Probe Set ID	AT locus – Putative Annotation	Fold Change			
		S2 C	M48 C	S2 AS	M48 AS
les.210.1.s1_at	AT3G23730– xyloglucan:xyloglucosyl transferase, putative / xyloglucan endotransglycosylase, putative / endo-xyloglucan transferase, putative	-4.13	-	2.39	-
les.3537.1.s1_at	AT2G01850– XTH27, EXGT-A3 (endo-xyloglucan transferase A3); hydrolase, acting on glycosyl bonds / xyloglucan:xyloglucosyl transferase	-4.45	-	2.06	-
les.3590.1.s1_at	AT5G13870– EXGT-A4 (endoxyloglucan transferase A4); hydrolase. acting on glycosyl bonds	-5.53	-	5.55	-
les.4530.1.s1_at	AT3G23730– xyloglucan: xyloglucosyl transferase, putative / xyloglucan endotransglycosylase, putative / endo-xyloglucan transferase. putative	-	-	5.83	3.05
CELL WALL/ DEGRADATION					
les.3991.1.s1_at	AT5G64570– BXL4, XYL4 (beta-xylosidase 4); hydrolase, hydrolyzing O-glycosyl compounds	-	-	6.92	-
CARBOHYDRATE METABOLISM/ STARCH SYNTHESIS					
les.1310.1.s1_at	AT1G32900– starch synthase, putative	-	-	5.48	-
CARBOHYDRATE METABOLISM/ STARCH DEGRADATION					
les.1401.2.s1_at	AT5G18670– BAM9, BMY3 (beta-amylase 9); beta-amylase	-2.76	-	-8.36	-3.49
les.1401.3.s1_at	AT5G18670– BAM9, BMY3 (beta-amylase 9); beta-amylase	-	-	-11.88	-7.70
CARBOHYDRATE METABOLISM/ SUCROSE SYNTHESIS					
les.1617.2.s1_s_at	AT1G43670– fructose-1,6-bisphosphatase / D-fructose-1,6-bisphosphate 1-phosphohydrolase/ FBPase/ putative	-	-	6.27	-
les.1617.3.a1_s_at	AT1G43670– fructose-1,6-bisphosphatase / D-fructose-1,6-bisphosphate 1-phosphohydrolase/ FBPase/ putative	-	-	10.54	-
les.4946.1.s1_at	AT1G43670– fructose-1,6-bisphosphatase / D-fructose-1,6-bisphosphate 1-phosphohydrolase/ FBPase/ putative	-	-	10.13	-
CARBOHYDRATE METABOLISM/ SUCROSE DEGRADATION					
lesaffx.53904.1.s1_at	AT5G40510– unknown protein	-	-2.08	-4.58	-
LIPIDS/ FATTY ACID SYHTHESIS					
les.3383.1.s1_at	AT3G48990– AMP-dependent synthetase and ligase family protein	-5.51	-	-2.10	-
les.5952.1.s1_at	AT4G25050– ACP4 (acyl carrier protein 4)	-	-	4.10	2.06

Table 3.29 (continued)

Probe Set ID	AT locus – Putative Annotation	Fold Change			
		S2 C	M48 C	S2 AS	M48 AS
lesaffx.36985.1.s1_at	AT5G47720– acetyl-CoA C-acyltransferase, putative / 3-ketoacyl-CoA thiolase, putative	-	-	-4.24	-2.83
lesaffx.55337.2.s1_at	AT1G68530– CER6, G2, POP1, CUT1 (cuticular 1); catalytic	-	-	-5.69	-3.71
GLYCOLYSIS					
les.2677.1.s1_at	AT3G08590– 2,3-biphosphoglycerate-independent phosphoglycerate mutase / phosphoglyceromutase / putative	-	-	-11.63	-11.48
les.2909.1.s1_at	AT1G53310– ATPPC1 (phosphoenolpyruvate carboxylase 1)	-3.47	-	-4.67	-2.17
les.2909.2.s1_at	AT1G53310– ATPPC1 (phosphoenolpyruvate carboxylase 1)	-4.31	-2.31	-12.50	-3.85
les.3129.2.s1_at	AT5G08570– pyruvate kinase, putative	-	-	-13.19	-7.80
les.3242.1.a1_at	AT1G42970– GAPB (glyceraldehyde-3-phosphate dehydrogenase B subunit)	-	-	5.58	-
LIGHT REACTIONS					
les.3031.2.s1_at	AT4G38510– (vacuolar ATP synthase subunit B2); hydrogen ion transporting ATP synthase, rotational mechanism	-	-	-5.83	-4.85
les.3297.1.s1_at	AT3G47470– CAB4, LHCA4 (Photosystem I LHC gene 4); chlorophyll binding	-	-	4.30	-
les.5852.2.s1_at	AT5G08690– ATP synthase beta chain 2, mitochondrial	-	-3.16	-4.44	-3.12
lesaffx.11323.1.s1_at	ATCG00730– PETD, A chloroplast gene encoding subunit IV of the cytochrome b6/f complex	4.37	2.92	4.31	-
lesaffx.29730.2.s1_at	ATMG01190– ATP1, ATPase subunit 1	2.37	-	14.77	10.85
lesaffx.35136.1.s1_at	ATCG01070– NDHE, NADH dehydrogenase ND4L	3.56	3.69	23.32	24.49
lesaffx.44224.1.a1_at	ATCG01100– NDHA, NADH dehydrogenase ND1	11.21	3.45	6.14	4.64
lesaffx.44224.1.s1_at	ATCG01100– NDHA, NADH dehydrogenase ND1	4.14	3.48	2.09	-
lesaffx.44474.1.a1_at	ATCG01050– NDHD, Represents a plastid-encoded subunit of a NAD(P)H dehydrogenase complex	7.70	6.01	6.56	5.20
lesaffx.44474.1.s1_at	ATCG01050– NDHD, Represents a plastid-encoded subunit of a NAD(P)H dehydrogenase complex	5.68	5.25	3.27	-
lesaffx.51226.1.a1_at	ATCG00540– PETA, cytochrome f apoprotein	3.96	3.16	22.04	16.74
lesaffx.66410.1.s1_at	ATCG00280– PSBC, CP43 subunit of the photosystem II reaction center	6.29	3.76	15.90	6.94
lesaffx.70106.2.s1_at	AT1G76450– oxygen-evolving complex-related	2.36	-	4.03	-

Table 3.29 (continued)

Probe Set ID	AT locus – Putative Annotation	Fold Change			
		S2 C	M48 C	S2 AS	M48 AS
lesaffx.70450.1.s1_at	ATCG01080– NDHG, NADH dehydrogenase ND6	4.60	3.20	2.11	2.14
lesaffx.70834.1.s1_at	ATCG00150– ATPI, subunit of ATPase complex CF0	5.29	4.13	15.50	6.21
les.376.1.s1_at	AT5G38420– ribulose bisphosphate carboxylase small chain 2B / RuBisCO small subunit 2B (RBCS-2B) (ATS2B)	-	-	7.00	2.74
lesaffx.70764.1.s1_at	ATCG00490– RBCL, large subunit of RUBISCO	4.89	6.03	3.18	2.05

Many transcrips involved in light reactions were up-regulated in S2 under control conditions and especially after freezing treatment (Table 3.29). The number of transcripts was very low in M48 compared to S2. The up-regulation of transcripts encoding for structural components of LHC (Light Harvesting Commplex) or enzyme complexes involved in photosynthesis may indicate a direct/indirect regulation of these transcripts by *myb4*. This up-regulation may reflect a higher photosynthesis capacity in transgenic potato.

The transcripts involved in development and cell organization was differentially regulated in S2 in control conditions and especially after freezing stress (Table 3.30). The number of transcrips was lower in M48. There were up- and down-regulated genes involved in development but almost all of the genes involved in cell organization were down-regulated. There were six NAC type TFs down-regulated and one NAC type TF up-regulated in transgenic potato upon exposure to freezing. This suggests that these TFs may be *myb4* regulated in potato.

Previously in section 3.2.5.2 it was explained that cytoskeleton re-organization serves as a link between membrane rigidification and calcium influx during cold acclimatization in alfaalfa. Stimulation of cold induced Ca^{2+} influx by disruption of microtubules and actin microfilaments was also shown in Tobacco. The differential regulation of both Ca^{2+} signalling and cell organization indicates that there may be

such a link between signalling components and cytoskeleton components in transgenic potato mediated by *myb4*.

Table 3.30 Significantly (P<0.05) regulated transcripts involved in development. Probe set ID, *Arabidopsis thaliana* (AT) locus name and putative annotation of each transcript is listed. Values represent fold changes of transcripts in S2 C and M48 C compared to WT C and in S2 AS and M48 AS compared to WT AS. Negative values show down-regulation and positive values show up-regulation. Only transcripts with FC≥4 in at least one of the transgenic lines or WT are displayed. Fold changes less than 2 are indicated with -.

Probe Set ID	AT locus – Putative Annotation	Fold Change			
		S2 C	M48 C	S2 AS	M48 AS
DEVELOPMENT					
les.162.1.s1_at	AT3G58040.1 – seven in absentia (SINA) family protein	-	-	-5.74	-2.97
les.162.3.s1_at	AT3G58040– seven in absentia (SINA) family protein	-	-	-4.70	-3.55
les.2569.1.s1_at	AT1G69490– ANAC029, ATNAP, NAP (NAC-like, activated by AP3/PI); TF	-5.01	-	-6.52	-2.34
les.3070.2.a1_at	AT4G25150– acid phosphatase, putative	11.40	-2.50	-	-
les.3759.1.s1_at	AT5G53560– ATB5-A (Cytochrome b5 A)	-4.09	-	-	-
les.3766.1.s1_at	AT1G01470– LSR3, LEA14 (late embryogenesis abundant 14)	-5.12	-	-	-
les.4975.1.s1_at	AT4G29270– acid phosphatase class B family protein	6.40	-2.46	-	-
lesaffx.4509.1.s1_at	AT2G19520– ACG1, MSI4, NFC4, NFC04, FVE	-	-	-6.42	-4.01
lesaffx.64505.2.s1_at	AT1G56010– ANAC021, ANAC022, NAC1; TF	-	2.51	-8.12	-
lesaffx.69815.1.s1_at	AT3G14770– nodulin MtN3 family protein	-	-	-6.96	-2.31

3.2.6 Real-Time PCR for Verification of Microaaray Data

RNA samples were isolated from leaf samples of WT, S2 and M48 which were collected before and after freezing treatment. RNA samples were reverse transcribed and the cDNA was used in quantitative real-time PCR (qPCR). The primers used for qPCR were selected for an optimal GC content of ~50%, a primer length of ~20 bp, and a product size ranging between 112 and 146 bp, so that the product could be

easily amplifiable, and could generate sufficiently detectable fluorescence signal when binding to SYBR Green. The housekeeping potato *ef1α* gene was used as the reference gene for relative quantification.

The fragments of selected probe sets and the reference gene *ef1α* amplified by conventional PCR were run on 2% agarose gel to check generation of single gene-specific amplicons (Figure 3.44). Amplification of some of the fragments generated primer-dimers or non-specific products. Therefore only the PCR products designated by the numbers 2, 4 and 6 that have a single specific amplicon seperated on agarose gel were selected and used for qPCR analysis.

Table 3.31 represents GenBank best blastx hits for the probe sets. Three probe sets selected for qPCR were: LesAffx.35136.1.S1_at, Les.3377.2.S1_at and LesAffx.66410.1.S1_at. The annotations for probe sets are NADH dehydrogenase subunit (LesAffx.35136.1.S1_at), NtK-1-like protein (Les.3377.2.S1_at) and photosystem II 44 kDa protein (LesAffx.66410.1.S1_at).

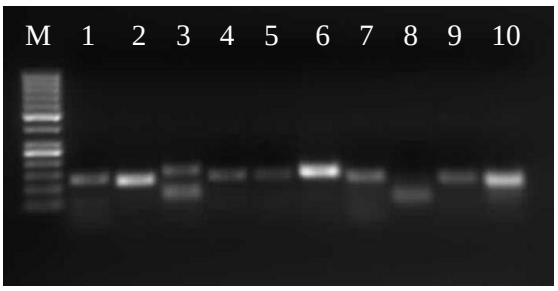

Figure 3.44 PCR amplified fragments of selected probe sets and reference gene *ef1α* separated on 2% agarose gel. M: 50 bp DNA ladder 1: Les.5834.1.S1_at, 2: LesAffx.35136.1.S1_at, 3: LesAffx.51226.1.A1_at, 4: Les.3377.2.S1_at, 5: LesAffx.44474.1.A1_at, 6: LesAffx.66410.1.S1_at, 7: LesAffx.22051.2.S1_at, 8: LesAffx.54522.1.S1_at, 9: LesAffx.69865.1.S1_at, 10: ef1α.

After selection of gene-specific primer pairs that amplify single gene-specific amplicons, qPCR was performed with each primer pair using a dilution series of

cDNA. Standard curves, amplification plots and melting curves were generated for all probe sets and *ef1α* using different standard dilutions. A representative standard curve, amplification plot and melting curve for *ef1α* are given in the figures below. Figure 3.45 shows a representative standard curve that was generated for *ef1α*.

Table 3.31 GenBank best blastx hits for the selected probe sets.

Probe Set ID	**GenBank best blastx hit**
Les.5834.1.S1_at	YP_514837 ATP synthase CF1 alpha chain [Solanum lycopersicum]
LesAffx.35136.1.S1_at	NP_054559 NADH dehydrogenase subunit 4L [Nicotiana tabacum]
LesAffx.51226.1.A1_at	YP_514865 cytochrome f [Solanum lycopersicum]
Les.3377.2.S1_at	ABB87119 NtK-1-like [Solanum tuberosum]
LesAffx.44474.1.A1_at	YP_514903 NADH dehydrogenase subunit 4 [Solanum lycopersicum]
LesAffx.66410.1.S1_at	NP_783228 photosystem II 44 kDa protein [Atropa belladonna]
LesAffx.22051.2.S1_at	P29677 Mitochondrial-processing peptidase subunit alpha, Ubiquinol-cytochrome-c reductase subunit II
LesAffx.54522.1.S1_at	BAC53938 Myb-like protein [Nicotiana tabacum]
LesAffx.69865.1.S1_at	ABB86250 RNA-binding protein AKIP1-like [Solanum tuberosum]

Figure 3.46 represents amplification plot of *ef1α* with different standard dilutions of cDNA. Melting curves were obtained during qPCR by monitoring the fluorescence of dsDNA dyes as the temperature passed through the product denaturation temperature. Figure 3.47 shows the melting curve analysis of amplicons generated by qPCR.

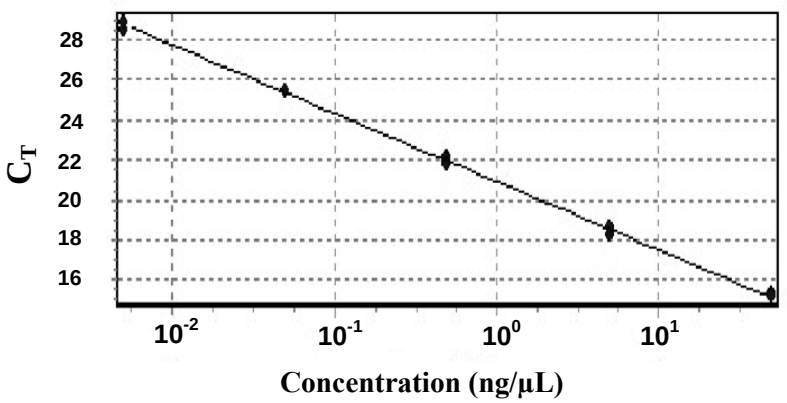

Figure 3.45 A representative standard curve. qPCR was performed with *ef1α* gene-specific primers and C_T values of different standard dilutions were plotted against the input amount of cDNA to generate a standard curve.

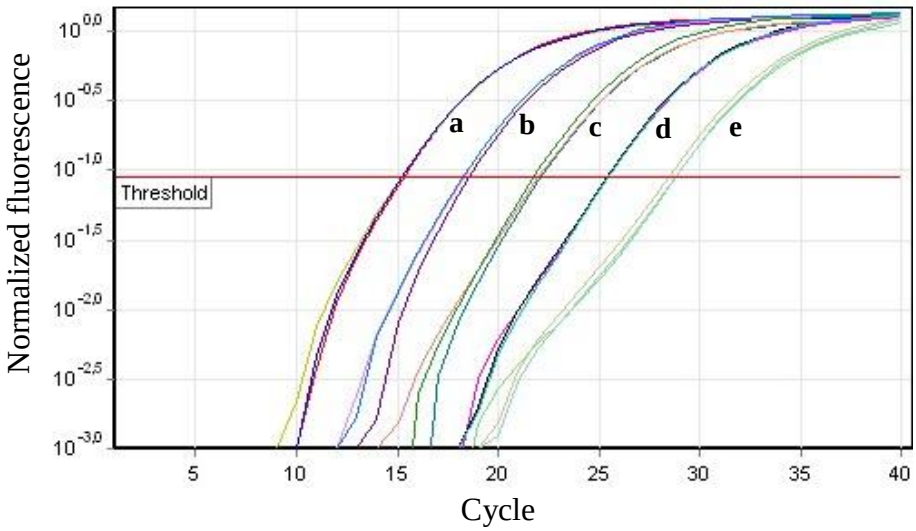

Figure 3.46 Amplification plot of *ef1α* with different standard dilutions of cDNA. Concentration of standard dilutions was a: 50ng/µL, b: 5ng/µL, c: 0.5ng/µL, d: 0.05ng/µL, e: 0.005ng/µL. qPCR was performed with three technical replicates for each dilution series.

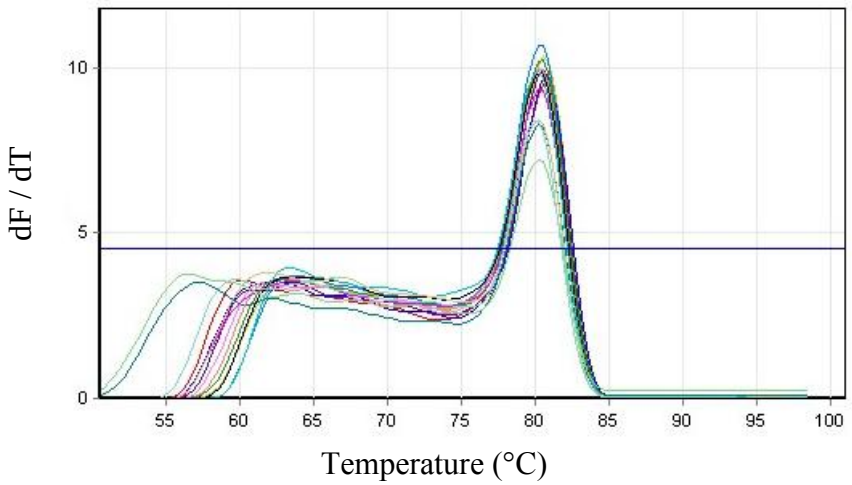

Figure 3.47 A representative melting curve analysis of *ef1α* that was amplified using different standard dilutions (0.005-50 ng/µL) of cDNA. Change in fluorescence over time was plotted against temperature.

Melting curve of a product is dependent on GC content, length and sequence. Therefore PCR products can be distinguished by their melting curves. Melt curve analysis of all probe sets and *ef1α* showed similar melt temperatures, with one narrow peak, indicating amplification of one pure product.

Real-time quantification procedure differs depending on whether the target and the endogenous reference gene are amplified with comparable or different efficiencies. Therefore amplification efficiencies should be determined for target and reference gene. After generation of standard curves for the probe sets and *ef1α*, amplification efficiencies were calculated according to the following equation:

$$E = 10^{(-1/S)} - 1 \quad (S = \text{slope of the standard curve})$$

The amplification efficiency was 0.96976 for *ef1α*, 0.91924 for LesAffx.35136.1.S1_at, 0.97216 for Les.3377.2.S1_at and 0.98978 for LesAffx.66410.1.S1_at. To compare the amplification efficiencies of the probe sets, the C_T values of *ef1α* were subtracted from the C_T values of probe sets. The

difference in C_T values is then plotted against the logarithm of the template amount (Figure 3.48). If the slope of the resulting straight line is <0.1, amplification efficiencies are comparable.

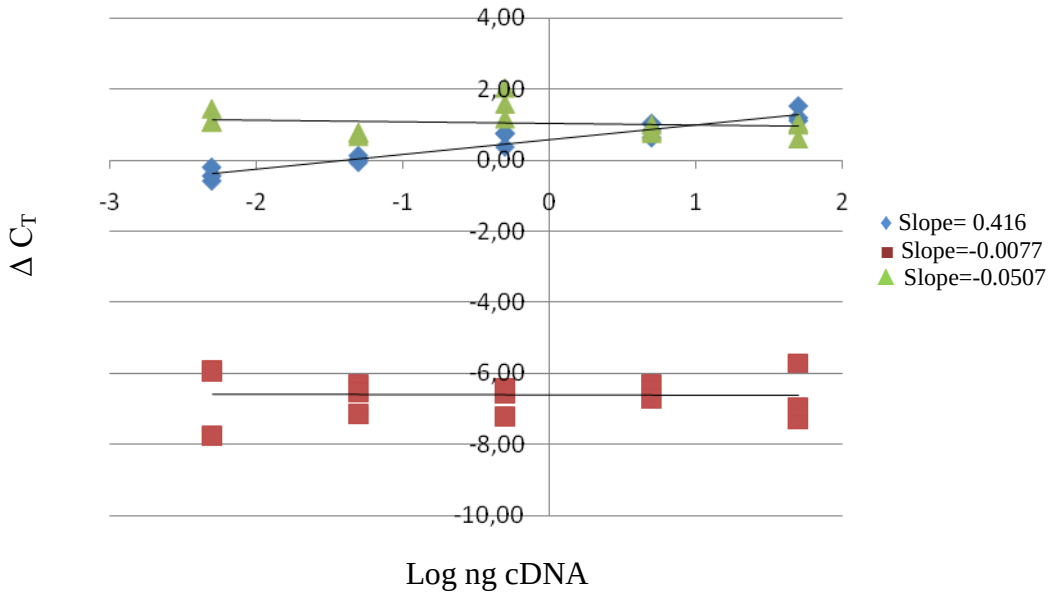

Figure 3.48 Amplification efficiency comparisons of probe sets. ♦LesAffx.35136.1.S1_at, ■Les.3377.2.S1_at**,** ▲LesAffx.66410.1.S1_at. ΔC_T values for three probe sets were generated by subtracting the C_T values of *ef1α* from the C_T values of each probe set and ΔC_T values were plotted against the logarithm of log cDNA amount of different standard dilutions.

Three straight lines in Figure 3.48 represent plot of ΔC_T values for three probe sets which were generated by subtracting the C_T values of *ef1α* from the C_T values of each probe set. The slope of Les.3377.2.S1_at and LesAffx.66410.1.S1_at were <0.1 which showed that the amplification efficiencies of the probe sets and *ef1α* were comparable. However the slope of LesAffx.35136.1.S1_at was greater than 0.1. Therefore seperate standard curves were prepared for the probe sets as well as for the endogenous reference gene by plotting C_T values against the log of cDNA amount. qPCR was performed with the cDNA samples of WT and transgenic lines and the starting amount of probe sets and *ef1α* in the samples were calculated using their C_T

values and the corresponding standard curve. Normalized amount of probe sets was calculated by dividing the amount of probe set by the amount of *ef1α* (average value of three replicates were used for each sample). Normalized amounts of transcripts were log transformed (log2) and compared with the normalized and log transformed (log2) expression data obtained from microarray analysis (Figure 3.49).

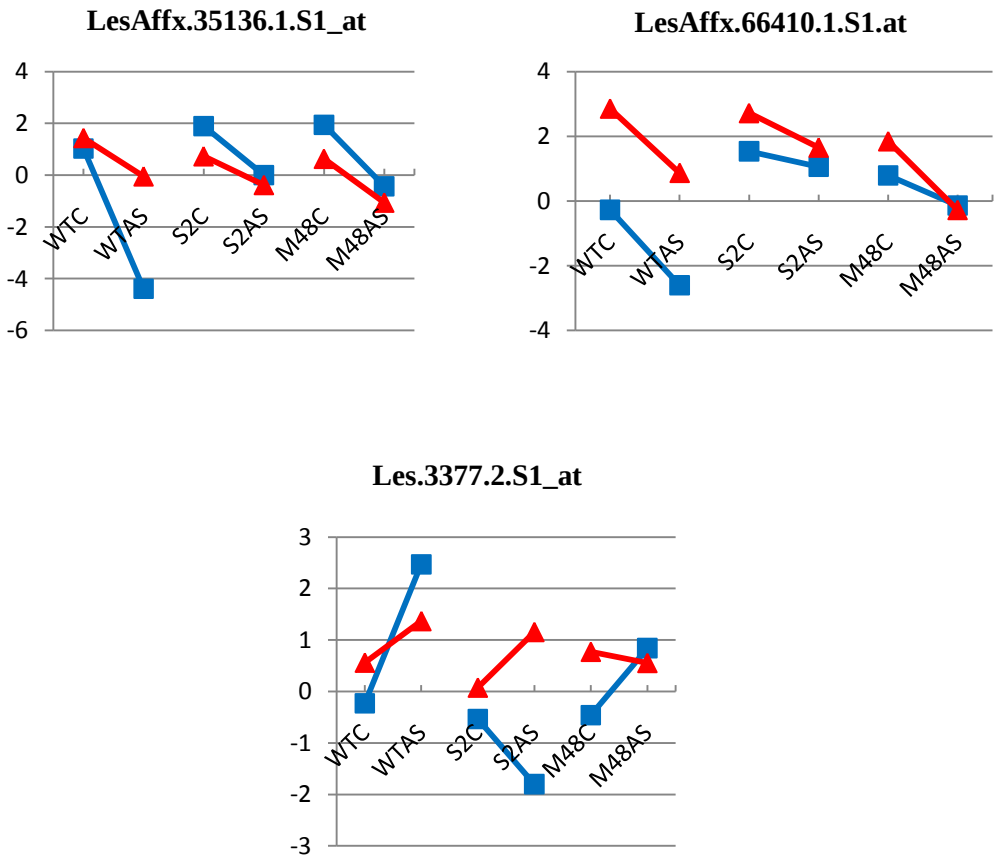

Figure 3.49 Comparison of microarray expression profile of selected probe sets with expression data obtained from real-time qPCR analysis. Blue lines represent expression data from microarray and the red lines represent real-time qPCR data. The microarray data is log base 2 and RMA normalized. The qPCR data is log base 2 and normalized.

qPCR data of LesAffx.35136.1.S1_at and Les.3377.2.S1_at showed correlation with the expression profiles obtained from microarray analysis. However qPCR data of LesAffx.66410.1.S1_at showed correlation only for WT but not for S2 and M48.

CHAPTER 4

CONCLUSIONS

Environmental stresses greatly affect plant growth and productivity. Abiotic stress is reported to reduce average yields for most major crop plants by more than 50% worldwide. Therefore any conventional or biotechnological approach that may reduce the crop loss due to abiotic stresses is of great value. Biotechnological applications such as transferring transcription factors to plants is one of the recent approaches to enhance tolerance of crop plants to stress factors.

Previously *myb4* was isolated from rice and it was shown to increase drought and freezing tolerance in Arabidopsis. It enhanced drought tolerance in tomato and it also led to improved tolerance to cold in apple and *Osteospermum ecklonis*. This study was conducted to explore the potential of *myb4* gene to enhance tolerance towards abiotic stresses in potato. For this purpose transgenic plants expressing *myb4* under the control of cold inducible or constitutive promoters were generated by *Agrobacterium*-mediated gene transfer technique. Transgenic plants were subjected to drought, salt, boron toxicity and freezing stresses. WT and transgenic plants grown under stress conditions were compared with respect to growth parameters. In the tested conditions transgenic plants only showed a better growth under high salt concentrations. Although transgenic plants overexpressing *myb4* showed enhanced tolerance to freezing or drought in other species, transgenic potato plants did not appear to be more drought or freezing tolerant compared to WT in any tested condition. This data indicate that the specificity and the degree of *myb4* activity depend on the host genomic background. No distinct differences were observed in the responses of potato plants expressing *myb4* under the control of different

promoters. This showed that stress inducible and constitutive expression of *myb4* similarly affected abiotic stress tolerance in transgenic potato.

In this study one of the objectives was to explore effect of *myb4* expression on gene expression profiles in transgenic potato plants. To the best of our knowledge, this is the first report to compare the transcriptome of transgenic potato expressing *myb4* under the control of cold inducible and constitutive promoters. Transcriptomes of wild-type and transgenic lines expressing *myb4* under the control of constitutive and stress inducible promoters were analyzed to elucidate the *myb4*-regulated biological processes and downstream targets in potato. Different expression patterns in transgenic lines compared to WT showed that *myb4* controls a large and complex transcriptional network associated with diverse processes such as defense, metabolism and development. Gene expression profiles were altered to a greater extent in S2 compared to M48. Especially the number of up-regulated genes in the cold-mediated transcriptome was higher in S2. This suggested that expression level of *myb4* may affect its impact on downstream targets.

Alteration of gene expression profiles involved in signaling, transport, transcription and translation in cold-mediated transcriptomes of transgenic plants pointed that different molecules may be activated or repressed by *myb4* upon exposure to freezing. Up/down regulation of different signaling molecules may activate different signal transduction pathways in transgenic potato. Up-regulation of genes involved in sucrose synthesis, some peroxidases and CBF3 transcription factor in transgenic plants suggested that *myb4* may configure freezing response in potato primarily by oxidative stress defence mechanisms, osmotic adjustment or activation of CBF3 regulated genes that may confer cold tolerance. Activation/repression of certain transcription factors in transgenic potato may activate/repress different downstream target genes that may contribute to the stress response. Up-regulation of peoxidases, oxidases, catalase and antioxidant molecules such as ascorbate and glutathione pointed that the oxidative defence mechanism is an important component of cold-response in transgenic potato. Increased antioxidant activity in transgenic potato may

have contributed protection of chloroplasts. This protection may partially be responsible for the up-regulated light reactions and carbohydrate synthesis in transgenic lines.

Transcriptome data may be supported by certain physiological or biochemical assays to better elucidate the molecules involved in cold-response of transgenic potato. For this purpose PSII activity in the leaves of WT and transgenic plants may be determined and the level of osmoprotectants such as trehalose, glycine betaine or sucrose may be measured.

Given all the microarray data generated in this study, next goal of research should be detailed investigation of the link between *myb4* and some downstream target genes. Spatial or temporal expression of *myb4* and target genes should be investigated and protein products should be quantified. This will shed more light on the way to developing crop plants more tolerant to environmental stresses like cold and freezing.

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APPPENDIX A

Osmyb4 mRNA SEQUENCE

```
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DEFINITION  O.sativa mRNA for myb factor, 1202 bp.
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ORGANISM    Oryza sativa Japonica Group
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             Poales; Poaceae; BEP clade; Ehrhartoideae; Oryzeae; Oryza.
REFERENCE   1
  AUTHORS   Pandolfi,D., Solinas,G., Valle,G. and Coraggio,I.
  TITLE     The electronic Plant Gene Register
  JOURNAL   Plant Physiol. 114 (2), 747-749 (1997)
   PUBMED   9235602
REFERENCE   2 (bases 1 to 1202)
  AUTHORS   Coraggio,I.
  TITLE     Direct Submission
  JOURNAL   Submitted (21-FEB-1997) I. Coraggio, Istituto
            Biosintesi Vegetali, CNR, Via Bassini 15, 20133, Milano, ITALY
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ORIGIN
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APPPENDIX B

YEP MEDIUM (1 L)

Peptone	10 gr
Yeast extract	10 gr
NaCl	5g
Agar	15 gr (if solid medium is required)

pH is adjusted to 7

APPPENDIX C

CTAB EXTRACTION BUFFER

2% (w/v) CTAB

1.42 M NaCl

20 mM EDTA (pH:8)

100 mM TrisHCl (pH:8)

0.2% (w/v) PVP 40

5 mM Ascorbic Acid

0.02% 2-mercaptoethanol

APPPENDIX D

SOLUTIONS USED FOR SOUTHERN BLOT ANALYSIS

TBE Buffer

89 mM Tris Base

89 mM Borate

2 mM EDTA (pH:8)

Denaturation Solution

1.5 M NaCl

0.5 M NaOH

Neutralisation Solution

1.5 M NaCl

0.5 M Tris Base

pH is adjusted to 8 with concentrated HCl

APPPENDIX E

SOLUTIONS USED FOR NORTHERN BLOT ANALYSIS

40X Gel Buffer

1.6 M Triethanolamine

80 mM Sodium EDTA

pH is adjusted to 7.5 with 85% H_3PO_4

Sample Buffer

40X Gel Buffer	0.25 mL
Formaldehyde	1.65 mL
Formamide	5 mL
Glycerol	1.1 mL
Bromophenol Blue	20 mg

20X SSC

3 M NaCl

300 mM Sodium Citrate

pH is adjusted to 7 with 14N HCl

DIG Hybridisation Buffer

%50 Formamide (v/v)

5X SSC

1X Blocking Reagent (Roche)

0.02% SDS

0.1% N-Lauroylsarcosine

5% dH_2O

Maleic Acid Buffer

0.1 M Maleic Acid

0.15 M NaCl

pH is adjusted to 7.5 with NaOH

Running Buffer

1X Gel Buffer

8.4% Formaldehyde (v/v)

Stripping Buffer

50% deionized formamide

50 mM Tris-HCl (pH: 7.5)

5% SDS

APPPENDIX F

PHYSIOLOGICAL EFFECT OF EXCESS SALT AND BORON ON GROWTH OF WILD-TYPE AND TRANSGENIC PLANTS

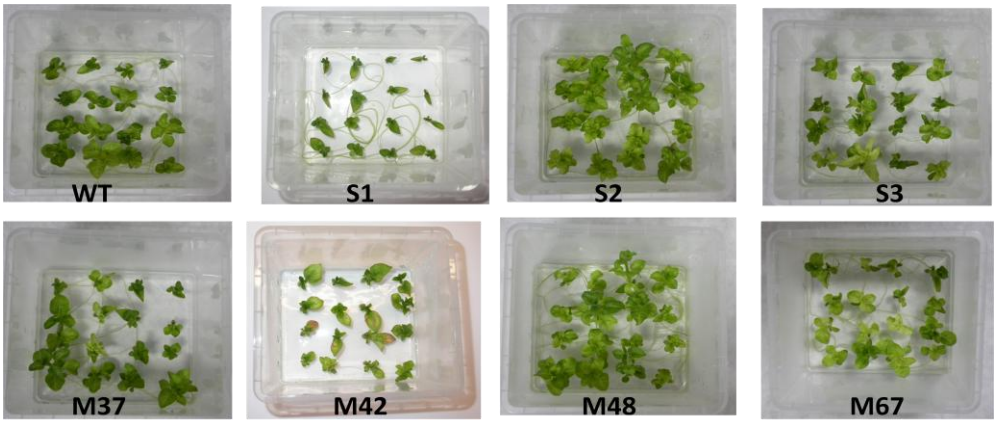

Figure F.1. Physiological effect of 100 mM NaCl on growth of WT and transgenic lines.

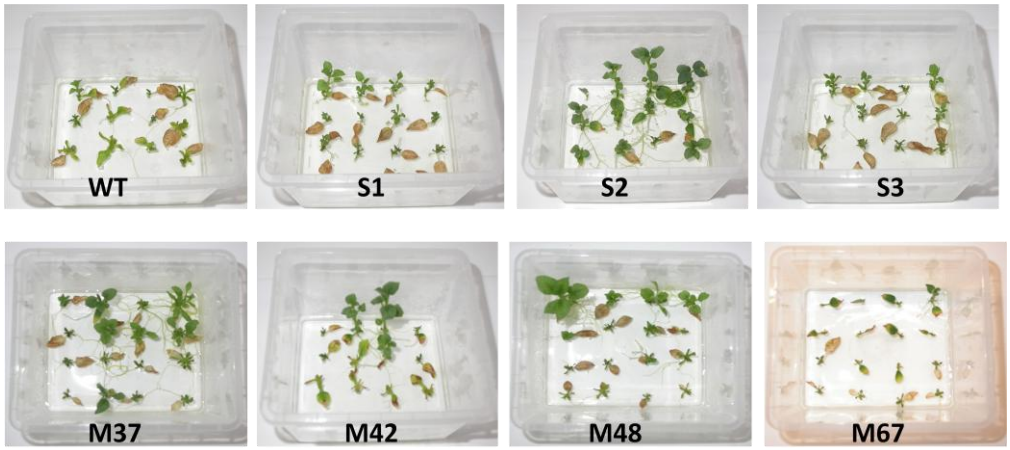

Figure F.2. Physiological effect of 3 mM boric acid on growth of WT and transgenic lines.

APPPENDIX G

DIFFERENTIALLY REGULATED GENES IN WILD-TYPE AND TRANSGENIC PLANTS UPON EXPOSURE TO FREEZING

Table G.1 Significantly (P<0.05) regulated transcripts involved in abiotic and biotic stress responses upon exposure to freezing stress. Probe set ID, *Arabidopsis thaliana* (AT) locus name and putative annotation of each transcript is listed. Values represent fold changes of transcripts in WT, S2 and M48 after freezing stress compared to normal growth conditions. Negative values show down-regulation and positive values show up-regulation. FC<2 are indicated with - (FC: Fold Change, HS: Heat Shock, RD: responsive to dehydration, LRR: leucine-rich repeat).

Probe Set ID	AT locus – Putative Annotation	Fold Change		
		WT AS	S2 AS	M48 AS
ABIOTIC STRESS / HEAT				
les.140.1.s1_at	AT5G28540 – BIP1 ATP binding	2.39	-	7.89
les.1739.1.s1_at	AT2G20560 – DNAJ HS family protein	3.08	2.80	2.67
les.1931.1.a1_at	AT1G59725 – DNAJ HS protein, putative	-2.45	-9.93	-12.42
les.3151.1.s1_at	AT3G44110 – ATJ, ATJ3 (DnaJ homologue 3)	-	-3.59	-2.74
les.3160.1.s1_at	AT1G56410 – HSP70T-1, ERD2 (early-RD 2); ATP binding	52.74	2.28	3.81
les.3160.2.s1_at	AT3G12580 – HSP70 (HS protein 70); ATP binding	2.63	2.07	3.25
les.3160.3.s1_at	AT3G12580 – HSP70 (HS protein 70); ATP binding	6.47	-	5.81
les.3179.2.s1_at	AT3G44110 – ATJ, ATJ3 (DnaJ homologue 3)	3.51	-	2.03
les.321.1.s1_at	AT5G56030 – ERD8, HSP81-2 (early-RD 8); ATP binding	-	-	3.09
les.3237.1.s1_at	AT5G21430 – DNAJ HS N-terminal domain-containing protein	-2.53	-	-3.63
les.3237.2.s1_at	AT5G21430 – DNAJ HS N-terminal domain-containing protein	-2.81	-	-2.28
les.3255.1.s1_at	AT3G14200 – DNAJ HS N-terminal domain-containing protein	-	-	-2.36
les.3581.1.s1_at	AT5G52640 – HSP90.1, HS83, HSP81.1, HSP83, HSP81-1 (HS protein 81-1); ATP binding / unfolded protein binding	14.97	43.28	21.22
les.3677.1.s1_at	AT4G27670 – HSP21 (HS protein 21)	-	3.51	3.32
les.4326.1.s1_at	AT5G03160 – DNAJ HS N-terminal domain-containing protein	-	2.32	4.16
les.4705.1.s1_at	AT4G21320 – HSA32 (heat-stress-associated 32); catalytic	6.07	12.92	5.72
les.4819.1.s1_at	AT5G02500 – HSP70-1, HSC70, HSC70-1 (HS cognate 70 kDa protein 1); ATP binding	13.28	8.58	22.75
les.5771.1.s1_at	AT2G29970 – HS protein-related	-4.39	-2.42	-4.20
les.5776.1.s1_at	AT5G09590 – HSC70-5, mtHSC70-2 (HS protein 70); ATP binding / unfolded protein binding	-	-	2.05
les.5892.1.s1_at	AT3G13310 – DNAJ HS N-terminal domain-containing protein	-2.31	3.09	-
lesaffx.10596.1.s1_at	AT5G59720 – HSP18.2 (HS protein 18.2)	-	5.87	3.37
lesaffx.16848.1.s1_at	AT1G59725 – DNAJ HS protein, putative	-2.51	-3.45	-2.10
lesaffx.17345.1.s1_at	AT4G13830 – J20 (DNAJ-LIKE 20); HS protein binding	-8.51	-2.54	-12.68
lesaffx.38184.1.s1_at	AT4G39150 – DNAJ HS N-terminal domain-containing protein	2.68	2.46	3.69

Table G.1 (continued)

Probe Set ID	AT locus – Putative Annotation	Fold Change		
		WT AS	S2 AS	M48 AS
lesaffx.43628.1.s1_at	AT1G80920 – J8 HS protein binding / unfolded protein binding	-5.78	-5.98	-16.94
lesaffx.44913.1.s1_at	AT5G49060 – DNAJ HS N-terminal domain-containing protein	2.70	-	-
lesaffx.45577.1.s1_at	AT5G64360 – DNAJ HS N-terminal domain-containing protein	2.88	-	-
lesaffx.47187.1.s1_at	AT1G74310 – HSP101, HOT1, ATHSP101(HS protein 101); ATP binding / ATPase	4.76	6.32	3.29
lesaffx.58365.1.s1_at	AT3G08970 – DNAJ HS N-terminal domain-containing protein	2.64	2.15	5.27
lesaffx.56637.1.s1_at	AT5G03030 – DNAJ HS N-terminal domain-containing protein	3.09	4.29	4.45
lesaffx.63687.1.s1_at	AT4G21870 – 26.5 kDa class P-related HS protein (HSP26.5-P)	-12.76	-6.95	-26.13
lesaffx.68350.1.s1_at	AT5G23590 – DNAJ HS N-terminal domain-containing protein	4.01	-	-
lesaffx.69957.1.s1_at	AT1G52560 – 26.5 kDa class I small HS protein-like (HSP26.5-P)	-	2.10	-
lesaffx.70264.1.s1_at	AT5G37670 – 15.7 kDa class I-related small HS protein-like (HSP15.7-CI)	-3.01	-	-3.61
lesaffx.805.1.s1_at	AT3G62600 – DNAJ HS family protein	2.69	2.89	5.66
lesaffx.9815.1.s1_at	AT4G36040 – DNAJ HS N-terminal domain-containing protein (J11)	-	-	-4.78
ABIOTIC STRESS / UNSPECIFIED				
les.4233.1.s1_at	AT3G11930 – universal stress protein (USP) family protein	6.98	12.53	5.63
les.4235.1.s1_at	AT4G08685 – SAH7 / pollen Ole e 1 allergen and extensin family protein	-	3.03	2.74
les.4344.1.s1_at	AT1G01170 – ozone-responsive stress-related protein, putative	-2.00	-	-2.37
les.4896.1.s1_at	AT4G08685 – SAH7 / pollen Ole e 1 allergen and extensin family protein	-2.13	-2.83	-2.98
les.966.1.s1_at	AT1G09560 – GLP5 (GERMIN-like protein 5); manganese ion binding / metal ion binding / nutrient reservoir	2.40	4.32	4.07
lesaffx.16925.1.s1_at	AT2G21620 – RD2	4.42	7.07	3.03
lesaffx.23349.1.s1_at	AT5G20630 – GLP3A, GLP3B, GLP3 (GERMIN-like protein 3); manganese ion binding / metal ion binding / nutrient reservoir	-25.70	-11.81	-11.95
lesaffx.3568.1.s1_a_at	AT1G76180 – dehydrin (ERD14)	2.04	3.16	-
lesaffx.48086.1.s1_at	AT1G70840 – MLP31 (MLP-like protein 31)	-2.11	-2.89	-3.56
lesaffx.64062.1.s1_at	AT1G72610 – GLP1 (GERMIN-like protein 1); manganese ion binding / metal ion binding / nutrient reservoir	-9.23	3.95	-2.06
ABIOTIC STRESS / TOUCH/WOUNDING				
les.4910.1.s1_at	AT1G19660 – wound-responsive family protein	-8.09	-4.92	-6.71
les.5290.1.s1_at	AT5G66050 – similar to wound-responsive family protein	-	-	-2.51
lesaffx.69647.1.s1_at	AT1G75380 – wound-responsive protein-related	-2.89	-2.70	-3.58
lesaffx.69647.2.s1_at	AT1G75380 – wound-responsive protein-related	-3.72	-3.32	-3.52
BIOTIC STRESS				
les.122.1.s1_at	AT3G12500 – PR3, CHI-B, B-CHI, ATHCHIB (basic chitinase); chitinase	4.40	10.18	13.63
les.1842.1.s1_at	AT1G33970 – avirulence-responsive protein, putative / avirulence induced gene protein, putative / AIG protein, putative	-	-	2.20
les.2529.2.s1_at	AT5G17540 – transferase family protein	2.38	-	2.97
les.335.1.s1_at	AT5G51060 – RBOHC, RHD2 (root hair defective 2) respiratory burst oxidase protein C (RbohC) / NADPH oxidase	2.07	2.84	-

Table G.1 (continued)

Probe Set ID	AT locus – Putative Annotation	Fold Change		
		WT AS	S2 AS	M48 AS
les.3506.1.s1_at	AT1G47890 – disease resistance family protein	4.36	5.36	5.96
les.3681.1.s1_at	AT4G04930 – DES-1-LIKE (fatty acid desaturase 1-like); oxidoreductase	-2.11	-	-2.72
les.3714.1.s1_at	AT1G32210 – DAD1 (defender against apoptotic death 1)	-	-	3.00
les.3756.1.s1_a_at	AT2G43330 – INT1 (inositol transporter 1); carbohydrate transmembrane transporter/ sugar:hydrogen ion symporter	-2.80	-8.94	-
les.5035.1.s1_at	AT3G05660 – kinase/ protein binding	-5.22	-	-4.07
les.5208.1.s1_at	AT3G48090 – EDS1 (enhanced disease susceptibility 1); signal transducer/ triacylglycerol lipase	-	3.33	6.23
les.75.1.s1_s_at	AT3G46730 – disease resistance protein (CC-NBS class), putative	-2.23	-	-2.15
lesaffx.1458.1.s1_at	AT1G75800 – pathogenesis-related thaumatin family protein	2.88	-	-
lesaffx.16769.1.s1_at	AT1G65870 – disease resistance-responsive family protein	2.12	5.76	7.74
lesaffx.29252.1.s1_at	AT5G08050 – unnamed protein product	-2.66	-2.04	-2.35
lesaffx.44598.1.s1_at	AT4G37000 – ATRCCR, ACD2 (accelerated cell death 2)	-	-	2.10
lesaffx.50167.1.s1_at	AT5G51700 – RPR2, PBS2 (PPHB Susceptible 2); protein binding / zinc ion binding / disease resistance protein (RAR1)	-	-	3.30
lesaffx.69659.1.s1_at	AT3G54420 – CHITIV, CHIV, ATEP3 (chitinase class IV)	5.60	4.20	9.32
lesaffx.71532.1.s1_at	AT1G33590 – disease resistance protein-related / LRR protein-related	6.16	-	2.47

Table G.2 Significantly (P<0.05) regulated transcripts involved in transcription and post-transcription upon exposure to freezing stress. Probe set ID, *Arabidopsis thaliana* (AT) locus name and putative annotation of each transcript is listed. Values represent fold changes of transcripts in WT, S2 and M48 after freezing stress compared to normal growth conditions. Negative values show down-regulation and positive values show up-regulation. Fold changes less than 2 are indicated with - (TF: Transcription factor, ZF: zinc finger).

Probe Set ID	AT locus – Putative Annotation	Fold Change		
		WT AS	S2 AS	M48 AS
TRANSCRIPTION				
les.1973.1.a1_at	AT3G21350 – RNA polymerase transcriptional regulation mediator-related	-2.30	-	-
les.4269.1.a1_at	ATCG00740 – RPOA RNA polymerase alpha subunit	-6.34	-3.65	-4.16
les.61.1.s1_at	AT1G14790 – ATRDRP1, RDR1 (RNA-dependent RNA polymerase 1); nucleic acid binding	-2.46	-4.45	-
lesaffx.1461.2.s1_at	AT3G10330 – transcription initiation factor IIB-2 / general TF, TFIIB2	-	-2.54	-
lesaffx.43941.1.s1_at	AT1G73820 – Ssu72-like family protein	-	2.14	-
lesaffx.47052.1.s1_at	AT4G14660 – RNA polymerase Rpb7 N-terminal domain-containing protein	-2.40	-	-
lesaffx.63021.1.s1_at	AT3G21350 – RNA polymerase transcriptional regulation mediator-related	-2.19	-	-
lesaffx.66331.1.s1_at	AT5G24120 – SIG5, SIGE (RNA polymerase sigma subunit E); DNA binding / DNA-directed RNA polymerase/ sigma factor/ TF	4.71	-	-
RNA PROCESSING				
les.1762.1.s1_at	AT2G35370 – GDCH (Glycine decarboxylase complex H)	-37.27	-7.76	-10.22
les.2548.3.s1_at	AT1G49760 – PAB8 (POLY(A) binding protein 8); RNA binding / translation initiation factor	2.30	-	-
les.2914.1.s1_at	AT1G32470 – glycine cleavage system H protein, mitochondrial, putative	-5.60	-2.25	-4.43
les.5691.1.s1_at	AT4G15420 – PRLI-interacting factor K	2.05	-	-

Table G.2 (continued)

Probe Set ID	AT locus – Putative Annotation	Fold Change		
		WT AS	S2 AS	M48 AS
les.2982.1.a1_at	AT1G02840 – ATSRP34, SRP34, SR1 (splicing factor 2); RNA binding	3.09	-	2.16
les.2156.1.a1_at	AT2G45620 – nucleotidyltransferase family protein	-	-	-2.19
les.2982.2.s1_at	AT1G02840 – ATSRP34, SRP34, SR1 (splicing factor 2); RNA binding	7.17	-	2.41
les.4240.1.s1_at	AT5G26880 – tRNA/rRNA methyltransferase (SpoU) family protein	-	-	-2.48
les.5651.1.s1_at	AT2G18510 – EMB2444 (embryo defective 2444); RNA binding	2.33	-	-
les.5958.1.s1_at	AT3G44260 – CCR4-NOT transcription complex protein, putative	3.68	11.74	4.22
lesaffx.14782.2.a1_at	AT3G49430 – SRP34A (SER/ARG-Rich Protein 34A) pre-mRNA splicing factor	-2.87	-2.25	-3.16
lesaffx.14782.2.s1_at	AT3G49430 – SRP34A (SER/ARG-Rich Protein 34A) pre-mRNA splicing factor	-	-	-3.14
lesaffx.44533.1.a1_at	AT4G21660 – proline-rich spliceosome-associated (PSP) family protein	2.59	-	-
lesaffx.54261.1.s1_at	AT5G53180 – polypyrimidine tract-binding protein, putative / heterogeneous nuclear ribonucleoprotein, putative	5.24	-	-
lesaffx.59682.1.s1_at	AT2G35120 – glycine cleavage system H protein, mitochondrial, putative	-	-	2.95
lesaffx.59895.1.s1_at	AT1G32470 – glycine cleavage system H protein, mitochondrial, putative	-2.07	-	-2.05
lesaffx.64456.1.s1_at	AT2G47580 – U1A (spliceosomal protein U1A); RNA binding	-	-	2.08
lesaffx.65616.1.s1_at	AT3G01150 – PTB (polypyrimidine tract-binding) heterogeneous nuclear ribonucleoprotein	7.65	-	3.14
lesaffx.67103.2.s1_at	AT3G61860 – RSP31 (arginine/serine-Rich Splicing Factor 31); RNA binding	-3.00	-2.33	-2.46
lesaffx.68956.1.s1_at	AT1G24450 – NFD2 (nuclear fusion defective 2); RNA binding / ribonuclease III family protein	-3.23	-2.71	-2.93
TRANSCRIPTION FACTOR / MYB DOMAIN TF FAMILY				
les.38.1.s1_at	AT5G16600 – MYB43 (myb domain protein 43); DNA binding / TF	7.52	6.56	8.82
les.41.1.s1_at	AT4G38620 – MYB4 (myb domain protein 4); TF	-2.77	-3.54	-4.13
les.4982.1.s1_at	AT3G46130 – MYB48, MYB111 (myb domain protein 111)	-2.50	-2.41	-4.17
les.5017.1.a1_at	AT3G46130 – MYB48, MYB111 (myb domain protein 111)	-4.01	-4.10	-11.16
les.5017.1.s1_at	AT3G46130 – MYB48, MYB111 (myb domain protein 111)	-	-3.25	-9.92
les.5091.1.s1_at	AT3G23250 – Y19, MYB15 (myb domain protein 15); DNA binding	2.49	-	-
lesaffx.40173.1.s1_at	AT2G31180 – MYB14 (myb domain protein 14); DNA binding / TF	-	3.87	-
lesaffx.50750.1.s1_at	AT4G38620 – MYB4 (myb domain protein 4); TF	-2.59	-4.65	-3.22
lesaffx.54522.1.s1_at	AT2G23290 – MYB70 (myb domain protein 70); DNA binding / TF	10.37	-	3.46
lesaffx.62138.1.s1_at	AT5G49620 – MYB78 (myb domain protein 78); DNA binding / TF	2.05	-	-
les.3716.1.s1_at	AT4G39250 – DNA binding / TF	-	-	-3.17
les.3722.1.s1_at	AT5G04760 – myb family TF	-2.87	-	-2.35
les.4923.1.s1_at	AT2G46830 – CCA1 (circadian clock associated 1); TF	13.63	23.80	5.75
les.5724.1.s1_at	AT5G67580 – TRB2, ATTRB2 (telomere repeat binding factor 2); DNA binding / TF	-2.09	-	-2.09
lesaffx.56221.1.s1_at	AT3G09600 – myb family TF	5.40	2.85	3.66

Table G.2 (continued)

Probe Set ID	AT locus – Putative Annotation	Fold Change		
		WT AS	S2 AS	M48 AS
TRANSCRIPTION FACTOR / WRKY DOMAIN TF FAMILY				
les.2667.2.s1_at	AT2G38470 – ATWRKY33 (WRKY DNA-binding protein 33); TF	7.05	7.60	3.71
les.3963.1.a1_at	AT1G29280 – ATWRKY65 (WRKY DNA-binding protein 65); TF	-	-	-3.35
les.3963.1.s1_at	AT1G29280 – ATWRKY65 (WRKY DNA-binding protein 65); TF	-6.06	-4.70	-4.13
les.3964.1.s1_at	AT4G31550 – ATWRKY11 (WRKY DNA-binding protein 11); TF	2.91	4.77	3.77
les.3969.1.s1_at	AT4G31550 – ATWRKY11 (WRKY DNA-binding protein 11); TF	6.39	6.52	7.55
les.5102.1.s1_at	AT3G58710 – ATWRKY69 (WRKY DNA-binding protein 69); TF	-	-	-2.37
les.931.1.a1_at	AT4G24240 – ATWRKY7 (WRKY DNA-binding protein 7); TF	-	2.28	-
lesaffx.21820.1.s1_at	AT2G47260 – ATWRKY23 (WRKY DNA-binding protein 23); TF	4.07	2.45	-
lesaffx.36712.1.s1_at	AT4G23810 – ATWRKY53 (WRKY DNA-binding protein 53); DNA binding / protein binding / transcription activator/ TF	17.24	13.11	17.47
lesaffx.43341.1.s1_at	AT3G56400 – ATWRKY70 (WRKY DNA-binding protein 70); TF	3.74	-	9.58
lesaffx.4793.1.s1_at	AT4G11070 – ATWRKY41 (WRKY DNA-binding protein 41); TF	5.12	3.02	7.67
lesaffx.735.1.s1_at	AT2G38470 – ATWRKY33 (WRKY DNA-binding protein 33); TF	8.06	7.99	6.00
lesaffx.9910.1.s1_at	AT1G80840 – ATWRKY40 (WRKY DNA-binding protein 40); TF	14.86	13.21	7.03
TRANSCRIPTION FACTOR / AP2/EREBP FAMILY				
les.124.1.s1_at	AT4G25480 – CBF3, DREB1, DREB1A (dehydration response element B1A); DNA binding / transcription activator/ TF	21.17	37.62	29.49
les.2646.1.a1_at	AT4G13040 – AP2 domain-containing TF family protein	-	-	-2.34
les.3574.1.s1_at	AT4G27950 – CRF4 (Cytokinin Response Factor 4); DNA binding / TF	-3.75	-5.62	-2.83
les.4102.1.s1_at	AT2G47520 – AP2 domain-containing TF, putative	-	3.06	2.21
les.5287.1.s1_at	AT4G36920 – FLO2, FL1, AP2 (APETALA 2); TF	-2.37	-5.21	-2.45
les.5885.1.a1_at	AT1G78080 – RAP2.4 (related to AP2 4); DNA binding / TF	-2.56	-2.79	-4.79
les.5885.2.s1_at	AT1G78080 – RAP2.4 (related to AP2 4); DNA binding / TF	3.31	-2.05	-3.23
les.5885.3.s1_at	AT1G78080 – RAP2.4 (related to AP2 4); DNA binding / TF	-	-2.43	-4.10
lesaffx.12586.1.a1_at	AT3G16280 – DNA binding / TF	-5.48	-3.80	-8.38
lesaffx.50814.1.s1_at	AT4G36900 – RAP2.10 (related to AP2 10); DNA binding / TF	-	-	-5.31
lesaffx.58308.1.s1_at	AT5G51990 – CBF4, DREB1D (C- repeat-binding factor 4); DNA binding / transcription activator/ TF	54.37	47.94	36.99
lesaffx.60947.1.s1_at	AT4G36920 – FLO2, FL1, AP2 (APETALA 2); TF	-	-2.94	-
lesaffx.64978.1.s1_at	AT5G67190 – AP2 domain-containing TF, putative	-	-2.71	-2.47
lesaffx.70635.1.s1_at	AT4G34410 – AP2 domain-containing TF, putative	54.22	44.39	37.86
lesaffx.71529.1.s1_at	AT5G21960 – AP2 domain-containing TF, putative	2.26	-	-
TRANSCRIPTION FACTOR / bHLH (BASIC HELIX-LOOP-HELIX) FAMILY				
les.174.1.a1_at	AT5G62610 – bHLH family protein	-2.28	-2.54	-3.37
les.1864.1.s1_at	AT1G09530 – PIF3, POC1, PAP3 (phytochrome interacting factor 3); DNA binding / TF / transcription regulator	-3.65	-2.02	-5.74
les.4085.1.s1_at	AT2G27230 – LHW (lonesome highway)	-3.77	-	-2.17

Table G.2 (continued)

Probe Set ID	AT locus – Putative Annotation	Fold Change		
		WT AS	S2 AS	M48 AS
les.501.1.a1_a_at	AT3G57800 – bHLH family protein	-2.12	-	-2.25
les.501.2.a1_at	AT3G57800 – bHLH family protein	-2.85	-	-2.86
les.5171.1.s1_at	AT3G26744 – ICE1 (inducer of CBF expression 1); DNA binding	-4.54	-3.30	-5.29
les.5638.1.s1_at	AT4G34530 – bHLH family protein	-15.69	-13.36	-32.15
lesaffx.33757.1.s1_at	AT3G47640 – bHLH family protein	2.53	3.73	2.46
lesaffx.37213.1.s1_at	AT5G46690 – BHLH071 (beta HLH protein 71); DNA binding / TF	-4.38	-	-3.56
lesaffx.62334.1.s1_at	AT1G26945 – transcription regulator	-2.34	-7.38	-2.91
lesaffx.64675.1.s1_a_at	AT5G57150 – bHLH family protein	6.36	-	5.38
lesaffx.64675.2.a1_at	AT5G57150 – bHLH family protein	2.50	-	2.32
lesaffx.71494.1.s1_at	AT5G54680 – ILR3 (IAA-leucine resistant3); DNA binding / TF	-2.46	-	-
TRANSCRIPTION FACTOR / bZIP TF FAMILY				
les.1446.1.a1_at	AT5G28770 – ATBZIP63, BZO2H3 (AT Basic Leucine Zipper 63); DNA binding / TF	-2.24	-	-3.24
les.145.1.s1_at	AT5G06950 – TGA2, AHBP-1B (bZIP TF HBP-1b homolog)	2.25	-	2.13
les.4296.1.s1_at	AT4G38900 – bZIP protein	2.25	2.06	-
les.5129.1.s1_at	AT2G46270 – GBF3 (G-box binding factor 3); TF	8.41	4.72	12.75
les.5194.1.s1_at	AT1G06070 – bZIP TF, putative (bZIP69)	-	-	-2.09
les.57.1.s1_a_at	AT4G36730 – GBF1 (G-box binding factor 1); TF	2.56	2.25	2.14
les.5916.1.s1_at	AT3G62420 – ATBZIP53 (Basic Region/Leucine Zipper Motif 53); DNA binding / protein heterodimerization/ sequence-specific DNA binding / TF	2.23	5.70	2.82
lesaffx.12573.1.s1_at	AT1G42990 – ATBZIP60 (Basic Region/Leucine Zipper Motif 60); DNA binding / TF	-	-	3.04
TRANSCRIPTION FACTOR / PHOR				
lesaffx.15878.2.a1_at	AT3G52450 – U-box domain-containing protein	42.04	65.66	69.42
lesaffx.15878.2.s1_at	AT3G52450 – U-box domain-containing protein	-	6.56	2.61
lesaffx.50112.1.s1_at	AT5G64660 – U-box domain-containing protein	14.56	10.01	19.72
lesaffx.56802.1.s1_at	AT3G19380 – U-box domain-containing protein	16.87	2.37	3.48
lesaffx.59769.1.s1_at	AT1G49780 – U-box domain-containing protein	3.89	3.61	2.63
lesaffx.63659.1.s1_at	AT3G18710 – U-box domain-containing protein	31.02	5.56	11.44
TRANSCRIPTION FACTOR / HSF (HEAT-SHOCK TF) FAMILY				
les.2876.2.s1_at	AT5G03720 – ATHSFA3 (*Arabidopsis thaliana* HS TF A3); DNA binding / TF	-4.12	-4.98	-2.16
TRANSCRIPTION FACTOR / HB (HOMEOBOX TF) FAMILY				
les.1831.1.s1_at	AT3G01470 – ATHB1, HD-ZIP-1, HAT5 (Homeobox-leucine zipper protein HAT5); TF	-	-	-2.05
les.2372.1.a1_at	AT4G32880 – ATHB8 (homeobox gene 8); DNA binding / TF	-3.16	-2.18	-4.16
les.2858.1.s1_at	AT2G35940 – EDA29, BLH1 (embryo sac development arrest 29)	-	-2.66	-
les.354.1.s1_at	AT5G25220 – KNAT3 (KNOTTED1-like homeobox gene 3)	-	-2.54	-2.06
les.3570.1.s1_at	AT1G62990 – IXR11, KNAT7 (Knotted-like TF); DNA binding / TF	-4.44	-3.69	-4.09
les.3751.1.s1_at	AT2G22430 – ATHB6 (AT homeobox protein 6); TF	-	-	-2.38
les.4315.1.s1_at	AT5G41410 – BEL1; DNA binding / TF	-	-5.48	-3.22
les.4731.1.s1_at	AT5G60690 – IFL, IFL1, REV (REVOLUTA); DNA binding / lipid binding / TF	-4.18	-	-3.03
les.5271.1.s1_s_at	AT3G60390 – HAT3 (homeobox-leucine zipper protein 3); TF	-	-	-2.59

Table G.2 (continued)

Probe Set ID	AT locus – Putative Annotation	Fold Change		
		WT AS	S2 AS	M48 AS
les.5941.1.a1_at	AT1G52150 – ATHB15, CNA, ICU4 (incurvata 4) homeobox-leucine zipper family protein / lipid-binding START domain-containing protein	-	2.30	-
lesaffx.2632.2.s1_at	AT4G37790 – HAT22 (homeobox-leucine zipper protein 22); TF	-2.86	-	-3.85
TRANSCRIPTION FACTOR / ZINC FINGER TF FAMILY				
les.2921.1.s1_at	AT5G24930 – ZF (B-box type) family protein	-2.26	-2.46	-4.47
les.4604.1.s1_at	AT2G47890 – ZF (B-box type) family protein	-	-2.14	-
lesaffx.18330.1.s1_at	AT5G57660 – ZF (B-box type) family protein	-2.05	-2.14	-5.64
lesaffx.25097.1.s1_at	AT5G54470 – ZF (B-box type) family protein	2.30	-	2.56
lesaffx.52572.1.s1_at	AT5G15850 – COL1 (CONSTANS-LIKE 1); TF / zinc ion binding	3.99	-	-
lesaffx.71242.2.s1_at	AT1G25440 – ZF (B-box type) family protein	-6.09	-5.27	-6.67
lesaffx.36944.1.a1_at	AT3G24050 – GATA TF 1 (GATA-1)	-	-	-3.20
lesaffx.36944.1.s1_at	AT3G24050 – GATA TF 1 (GATA-1)	-3.23	-3.21	-3.82
lesaffx.54718.1.s1_at	AT3G54810 – BME3-ZF, BME3 (BLUE MICROPYLAR END3); TF	4.13	7.81	3.54
les.1733.1.a1_at	AT4G00950 – MEE47 (maternal effect embryo arrest 47); TF	-3.94	-3.50	-4.25
lesaffx.14110.2.s1_at	AT3G47500 – CDF3 (Cycling DOF Factor 3); DNA binding / protein binding / TF	-3.46	-5.50	-5.35
lesaffx.70821.1.s1_at	AT3G21270 – ADOF2 (AT DOF ZF protein 2); DNA binding / TF	7.24	4.65	5.12
les.2296.1.a1_at	AT3G50700 – ATIDD2 (AT indeterminate(ID)-domain 2); nucleic acid binding / TF / zinc ion binding	-	-	-2.21
les.5126.1.s1_at	AT1G27730 – ZAT10, STZ (Salt Tolerance ZF); nucleic acid binding / TF / zinc ion binding	38.44	33.66	41.73
les.681.1.a1_at	AT1G66140 – ZFP4 (ZF Protein 4); nucleic acid binding / TF / zinc ion binding	-18.98	-11.55	-47.26
lesaffx.22830.1.s1_at	AT3G28210 – PMZ; ZF (AN1-like) family protein; zinc ion binding	7.67	7.49	14.88
lesaffx.36193.1.s1_at	AT1G27730 – ZAT10, STZ (Salt Tolerance ZF); nucleic acid binding / TF / zinc ion binding	-	5.21	-
lesaffx.64439.1.s1_at	AT2G37430 – ZF (C2H2 type) family protein (ZAT11)	44.18	53.86	107.84
lesaffx.71311.1.s1_at	AT5G59820 – ZAT12, RHL41 (responsive to high light 41); nucleic acid binding / TF / zinc ion binding	11.06	24.94	17.11

Table G.3 Significantly (P<0.05) regulated transcripts involved in translation and post-translational modifications upon exposure to freezing stress. Probe set ID, *Arabidopsis thaliana* (AT) locus name and putative annotation of each transcript is listed. Values represent fold changes of transcripts in WT, S2 and M48 after freezing stress compared to normal growth conditions. Negative values show down-regulation and positive values show up-regulation. Fold changes less than 2 are indicated with - (CAM: calmodulin, LRR: leucine-rich repeat, RD: responsive to dehydration, PK: protein kinase).

Probe Set ID	AT locus – Putative Annotation	Fold Change		
		WT AS	S2 AS	M48 AS
PROTEIN DAGRADATION				
affx-le_ubiquitin_3_at	AT4G05320 – UBQ10 (polyubiquitin 10)	-	2.17	-
affx-le_ubiquitin_5_at	AT5G03240 – UBQ3 (polyubiquitin 3); protein binding	3.71	-	-
les.1132.1.a1_at	AT1G17870 – ATEGY3	-	4.40	-
les.1207.1.a1_s_at	AT2G39050 – hydroxyproline-rich glycoprotein family protein	-	2.22	-

Table G.3 (continued)

Probe Set ID	AT locus – Putative Annotation	Fold Change		
		WT AS	S2 AS	M48 AS
les.1421.1.a1_at	AT1G57790 – F-box family protein	-	-	-2.00
les.1450.1.s1_at	AT1G21760 – FBP7, F-box family protein	-	-2.05	-2.30
les.15.1.s1_at	AT5G67360 – ARA12; subtilase	-27.87	-13.25	-27.09
les.1541.1.a1_at	AT1G01650 – peptidase	-2.07	-	-
les.1569.1.s1_at	AT4G10150 – ZF (C3HC4-type RING finger) family protein	-2.34	-	-2.97
les.1616.2.s1_at	AT2G17190 – ubiquitin family protein	2.66	-	2.46
les.1616.3.s1_at	AT2G17190 – ubiquitin family protein	2.06	2.07	2.18
les.1675.1.s1_at	– wound-induced proteinase inhibitor 2 precursor	-	-8.12	-
les.1700.1.s1_at	AT2G21270 – ubiquitin fusion degradation UFD1 family protein	-	-	2.05
les.1867.2.s1_at	AT1G53750 – RPT1A (regulatory particle triple-A 1A); ATPase	2.39	-	2.95
les.1871.1.s1_at	AT1G76410 – ATL8; protein binding / zinc ion binding	-3.11	-2.81	-3.56
les.1871.2.a1_at	AT1G76410 – ATL8; protein binding / zinc ion binding	-2.33	-2.57	-3.85
les.2026.2.a1_at	AT4G03510 – RMA1 (Ring finger protein); protein binding / ubiquitin-protein ligase/ zinc ion binding	-	-	-2.31
les.2055.1.s1_at	AT5G67250 – VFB4, SKIP2 (SKP1 interacting partner 2); ubiquitin-protein ligase	3.42	3.04	3.02
les.2068.1.a1_at	AT3G45010 – SCPL48 (serine carboxypeptidase-like 48); serine carboxypeptidase	-	2.56	-
les.21.1.s1_at	AT5G67090 – subtilase family protein	-	-	-2.04
les.2129.1.s1_at	AT1G56450 – PBG1 (20S proteasome beta subunit G1); peptidase	-	-	2.72
les.2167.1.s1_at	AT1G56700 – pyrrolidone-carboxylate peptidase family protein	-	-	-2.10
les.2173.1.a1_at	– wound-induced proteinase inhibitor 1 precursor	-	-3.39	-
les.2255.1.s1_at	AT1G21410 – SKP2A; protein binding	-	-	2.45
les.2294.2.a1_a_at	AT1G79110 – protein binding / zinc ion binding	-	-3.55	-2.83
les.2294.2.a1_at	AT1G79110 – protein binding / zinc ion binding	-5.20	-15.07	-22.36
les.2458.1.s1_at	– trypsin inhibitor 1 precursor	-2.79	-2.14	-5.26
les.2574.1.s1_at	AT5G09900 – EMB2107/MSA/RPN5A (embryo defective 2107)	2.08	-	-
les.2574.2.s1_at	AT5G09900 – EMB2107/MSA/RPN5A (embryo defective 2107)	3.27	-	2.13
les.2574.3.s1_at	AT5G09900 – EMB2107/MSA/RPN5A (embryo defective 2107)	5.43	-	2.07
les.2604.1.a1_at	AT5G23340 – protein binding	-	-	-2.87
les.2632.1.a1_at	AT5G42790 – PAF1 (proteasome alpha subunit F1); peptidase	2.61	-	-
les.2689.2.s1_at	AT4G29490 – X-Pro dipeptidase	2.08	-	-
les.2711.1.s1_at	AT2G47110 – UBQ6 (ubiquitin 6); protein binding	-	-	2.07
les.2816.1.s1_at	AT1G53750 – RPT1A (regulatory particle triple-A 1A); ATPase	3.32	2.12	2.24
les.2845.1.s1_at	AT5G61900 – BON1 (BONZAI1); calcium-dependent phospholipid binding	2.15	2.55	2.62
les.2845.2.s1_at	AT5G61900 – BON1 (BONZAI1); calcium-dependent phospholipid binding	3.32	-	2.33
les.2845.3.s1_at	AT5G61900 – BON1 (BONZAI1); calcium-dependent phospholipid binding	2.28	2.58	-

Table G.3 (continued)

Probe Set ID	AT locus – Putative Annotation	Fold Change		
		WT AS	S2 AS	M48 AS
les.2912.1.s1_at	AT1G21720 – PBC1 (20S proteasome beta subunit C1); peptidase	2.37	2.68	3.26
les.2960.2.s1_a_at	AT3G10410 – SCPL49 (serine carboxypeptidase-like 49); serine carboxypeptidase	2.87	-	-
les.2960.2.s1_at	AT3G10410 – SCPL49 (serine carboxypeptidase-like 49); serine carboxypeptidase	7.66	2.83	3.84
les.2960.3.s1_a_at	AT3G10410 – SCPL49 (serine carboxypeptidase-like 49); serine carboxypeptidase	23.06	-	3.05
les.2960.3.s1_at	AT3G10410 – SCPL49 (serine carboxypeptidase-like 49); serine carboxypeptidase	2.91	-	-
les.298.1.s1_at	AT1G04850 – ubiquitin-associated (Lubar & Bahler)/TS-N domain-containing protein	2.28	2.28	2.36
les.3048.2.s1_a_at	AT1G64230 – UBC28; ubiquitin-protein ligase	-	-2.14	-
les.3155.1.s1_at	AT1G64230 – UBC28; ubiquitin-protein ligase	2.45	3.96	2.21
les.3164.1.a1_at	AT5G63160 – BT1 (BTB and TAZ domain protein 1); protein binding / transcription regulator	-	-	-2.86
les.3164.2.s1_at	AT5G63160 – BT1 (BTB and TAZ domain protein 1); protein binding / transcription regulator	-3.82	-9.49	-5.21
les.3228.1.s1_at	AT1G47128 – RD21; cysteine-type peptidase	2.50	-	2.42
les.3228.2.s1_a_at	AT1G47128 – RD21; cysteine-type peptidase	2.36	2.29	2.60
les.3228.2.s1_at	AT1G47128 – RD21; cysteine-type peptidase	2.45	-	2.62
les.3246.1.s1_at	AT2G05840 – PAA2 (20S proteasome subunit PAA2); peptidase	2.10	2.08	2.62
les.3256.1.s1_at	AT3G22630 – PRCGB, PBD1 (proteasome subunit PRGB); peptidase	2.44	2.70	3.20
les.3260.1.s1_at	AT3G46460 – UBC13 (ubiquitin-conjugating enzyme 13); ubiquitin-protein ligase	-	-3.25	-3.11
les.3260.2.s1_at	AT3G46460 – UBC13 (ubiquitin-conjugating enzyme 13); ubiquitin-protein ligase	-	-	-3.96
les.3260.3.a1_at	AT3G46460 – UBC13 (ubiquitin-conjugating enzyme 13); ubiquitin-protein ligase	-	-2.38	-2.47
les.3262.2.s1_at	AT2G46500 – phosphatidylinositol 3- and 4-kinase family protein / ubiquitin family protein	2.00	3.41	2.59
les.3262.3.s1_at	AT2G46500 – phosphatidylinositol 3- and 4-kinase family protein / ubiquitin family protein	2.57	3.57	3.25
les.3266.3.s1_at	AT4G17830 – peptidase M20/M25/M40 family protein	-	-2.39	-
les.3293.2.s1_at	AT5G50920 – HSP93-V, DCA1, CLPC (HS protein 93-V); ATP binding / ATPase	3.78	-	-
les.3293.3.s1_at	AT5G50920 – HSP93-V, DCA1, CLPC (HS protein 93-V); ATP binding / ATPase	3.14	-	-
les.3307.1.s1_at	AT5G15610 – proteasome family protein	2.42	-	-
les.3308.2.s1_at	AT5G62350 – invertase/pectin methylesterase inhibitor family protein / DC 1.2 homolog	4.05	5.48	3.47
les.3308.3.s1_at	AT4G05320 – UBQ10 (polyubiquitin 10)	9.79	-	-
les.3343.2.s1_at	AT1G15670 – kelch repeat-containing F-box family protein	-3.11	-11.64	-11.02
les.3343.3.s1_at	AT1G15670 – kelch repeat-containing F-box family protein	-9.28	-8.86	-12.68
les.3369.2.s1_at	AT2G30950 – FTSH2, VAR2 (variegated 2); ATP-dependent peptidase/ ATPase/ metallopeptidase/ zinc ion binding	6.46	-	-
les.3515.1.s1_at	AT4G12910 – SCPL20 (serine carboxypeptidase-like 20); serine carboxypeptidase	-	-3.21	-
les.3541.1.s1_at	AT4G26840 – SUMO1, SUM1 (small ubiquitin-like modifier 1)	-2.41	-2.03	-2.56
les.3621.1.s1_at	– wound-induced proteinase inhibitor 1 precursor	-	-12.11	-

Table G.3 (continued)

Probe Set ID	AT locus – Putative Annotation	Fold Change		
		WT AS	S2 AS	M48 AS
les.3655.1.s1_at	AT3G05530 – ATS6A.2, RPT5A (regulatory particle triple-A 5A); ATPase/ CAM binding	2.14	-	2.45
les.3824.1.a1_at	AT1G69330 – ZF (C3HC4-type RING finger) family protein	-2.69	-2.00	-4.75
les.3940.2.a1_at	– wound-induced proteinase inhibitor 1 precursor	-	-7.72	-
les.4022.1.s1_at	– proteinase inhibitor type-2 cevi57 precursor	2.39	-	-
les.425.1.s1_at	AT1G73760 – ZF (C3HC4-type RING finger) family protein	-	-	-2.16
les.4287.2.s1_at	AT1G20200 – EMB2719 (embryo defective 2719)	7.13	-	-
les.4289.2.s1_at	AT3G60820 – PBF1 (20S proteasome beta subunit F1); peptidase	-	2.06	3.01
les.433.2.s1_at	AT2G27020 – PAG1 (20S proteasome alpha subunit G1); peptidase	2.01	-	3.24
les.4485.1.s1_at	AT2G39940 – COI1 (coronatine insensitive 1); ubiquitin-protein ligase	-	-	-2.26
les.4556.1.s1_at	AT5G22000 – RHF2A, CIC7E11; protein binding / zinc ion binding	-	-	-2.16
les.4563.1.s1_at	AT1G35340 – ATP-dependent protease La (LON) domain-containing protein	-7.39	-2.45	-5.57
les.4708.1.s1_at	AT2G27350 – OTU-like cysteine protease family protein	-	-	2.11
les.471.1.s1_at	AT4G34980 – SLP2 (subtilisin-like serine protease 2); subtilase	2.25	-	-
les.4712.1.s1_at	AT1G49850 – ZF (C3HC4-type RING finger) family protein	-	4.30	2.56
les.4747.1.s1_at	AT4G37040 – MAP1D (methionine aminopeptidase 1d); metalloexopeptidase	-	-	-2.32
les.4770.1.s1_at	AT5G14420 – RGLG2 (ring domain ligase2)	2.95	3.14	-
les.4792.1.s1_at	AT1G51710 – UBP6 (ubiquitin-specific protease 6)	5.32	-	5.07
les.4810.1.s1_at	AT4G30810 – SCPL29 (serine carboxypeptidase -like 29); serine carboxypeptidase	5.98	3.38	3.07
les.4820.1.s1_x_at	AT3G12490 – cysteine protease inhibitor, putative / cystatin, putative	-	-6.47	-
les.483.1.s1_at	AT3G12490 – cysteine protease inhibitor, putative / cystatin, putative	-	2.88	2.58
les.494.1.a1_at	AT1G45000 – 26S proteasome regulatory complex subunit p42D, putative	2.03	2.10	2.47
les.494.2.s1_at	AT1G45000 – 26S proteasome regulatory complex subunit p42D, putative	7.98	-	4.84
les.494.3.s1_at	AT1G45000 – 26S proteasome regulatory complex subunit p42D, putative	4.51	-	4.63
les.5003.1.s1_at	AT1G19310 – ZF (C3HC4-type RING finger) family protein	-	-	-2.89
les.5025.1.s1_at	AT1G79210 – 20S proteasome alpha subunit B, putative	2.59	2.98	5.58
les.5027.1.s1_at	AT3G47160 – protein binding / zinc ion binding	-5.78	-2.86	-5.19
les.5050.1.s1_at	AT2G46620 – AAA-type ATPase family protein	-2.17	-2.24	-2.52
les.513.1.s1_at	AT5G67090 – subtilase family protein	-2.88	-20.16	-
les.5183.1.s1_at	AT2G02870 – kelch repeat-containing F-box family protein	10.01	8.88	5.54
les.5217.1.s1_at	AT1G49050 – aspartyl protease family protein	-2.19	-	-
les.5240.1.s1_at	AT5G67090 – subtilase family protein	-	2.04	-
les.5264.1.s1_at	AT5G67360 – ARA12; subtilase	-3.50	2.13	-
les.5334.1.s1_at	AT3G02090 – MPPBETA; metalloendopeptidase	2.17	-	2.33
les.5363.1.s1_at	AT1G11910 – aspartyl protease family protein	3.41	-	-

Table G.3 (continued)

Probe Set ID	AT locus – Putative Annotation	Fold Change		
		WT AS	S2 AS	M48 AS
les.5452.1.s1_at	AT3G29270 – similar to ZF (C3HC4-type RING finger) family protein	-	-	-2.21
les.5473.1.s1_at	AT3G02290 – ZF (C3HC4-type RING finger) family protein	-	-	-2.08
les.5478.1.s1_at	AT2G27020 – PAG1 (20S proteasome alpha subunit G1); peptidase	2.64	2.45	4.08
les.5514.1.s1_at	AT2G35000 – ZF (C3HC4-type RING finger) family protein	5.87	2.32	2.95
les.5596.1.s1_at	AT3G14290 – PAE2 (20S proteasome alpha subunit E2); peptidase	2.16	-	2.88
les.5613.1.s1_at	AT3G53970 – proteasome inhibitor-related	-	-	2.63
les.5648.1.s1_at	AT3G54940 – cysteine proteinase, putative	2.42	2.07	3.78
les.5654.1.s1_at	AT3G16720 – ATL2; protein binding / zinc ion binding	6.38	27.28	18.58
les.5691.1.s1_at	AT4G15420 – PRLI-interacting factor K	2.05	-	-
les.5705.1.s1_at	AT3G52030 – F-box family protein / WD-40 repeat family protein	-	-	-2.16
les.5710.1.s1_at	AT1G21780 – BTB/POZ domain-containing protein	-2.55	-	-
les.5711.1.s1_at	AT4G39090 – RD19; cysteine-type peptidase	2.46	-	2.08
les.795.1.s1_at	AT1G29150 – RPN6, ATS9 (19S proteosome subunit 9)	3.09	2.06	3.15
les.795.2.s1_at	AT1G29150 – RPN6, ATS9 (19S proteosome subunit 9)	6.50	-	2.12
les.861.1.a1_at	AT1G32080 – membrane protein, putative	-3.02	-2.37	-3.28
lesaffx.11231.1.s1_at	AT4G33160 – ubiquitin-protein ligase	2.40	2.22	-
lesaffx.12254.1.s1_at	AT2G03890 – phosphatidylinositol 3- and 4-kinase family protein	-2.83	-	-2.94
lesaffx.12254.2.s1_at	AT2G03890 – phosphatidylinositol 3- and 4-kinase family protein	-3.73	-	-3.85
lesaffx.1244.1.s1_at	AT3G22260 – OTU-like cysteine protease family protein	2.28	3.26	3.45
lesaffx.1244.2.a1_at	AT3G22260 – OTU-like cysteine protease family protein	2.38	2.78	2.42
lesaffx.12489.1.s1_at	AT5G42790 – PAF1 (proteasome alpha subunit F1); peptidase	3.04	2.12	5.58
lesaffx.1574.1.s1_at	AT3G61710 – autophagy protein Apg6 family	2.72	-	-
lesaffx.1574.2.s1_at	AT3G61710 – autophagy protein Apg6 family	2.13	-	-
lesaffx.18549.1.s1_at	AT4G30890 – UBP24 (ubiquitin-specific protease 24); ubiquitin-specific protease	-	-	2.12
lesaffx.18906.1.s1_at	AT3G26340 – 20S proteasome beta subunit E, putative	-	-	3.46
lesaffx.18906.2.s1_at	AT3G26340 – 20S proteasome beta subunit E, putative	-	-	2.61
lesaffx.20429.1.s1_at	AT3G22110 – PAC1 (20S proteasome alpha subunit C1); peptidase	2.17	2.18	3.66
lesaffx.21877.1.s1_at	AT5G03240 – UBQ3 (polyubiquitin 3); protein binding	-	4.42	-
lesaffx.22812.2.s1_at	AT3G14250 – protein binding / zinc ion binding	-	-	2.06
lesaffx.23154.1.s1_at	AT5G27430 – signal peptidase subunit family protein	-	-	2.30
lesaffx.23154.2.s1_at	AT5G27430 – signal peptidase subunit family protein	-	-	2.07
lesaffx.23969.1.a1_at	AT3G61460 – BRH1 (brassinosteroid-responsive ring-H2); protein binding / zinc ion binding	-46.68	-32.89	-37.76
lesaffx.23969.1.s1_at	AT3G61460 – BRH1 (brassinosteroid-responsive ring-H2); protein binding / zinc ion binding	-	-	-3.33
lesaffx.24128.1.a1_at	AT4G24210 – SLY1 (SLEEPY1)	-	-	-2.20
lesaffx.24128.1.s1_at	AT4G24210 – SLY1 (SLEEPY1)	-2.19	-	-2.75

Table G.3 (continued)

Probe Set ID	AT locus – Putative Annotation	Fold Change		
		WT AS	S2 AS	M48 AS
lesaffx.24384.1.s1_at	AT1G69330 – ZF (C3HC4-type RING finger) family protein	-2.80	-	-2.17
lesaffx.24556.1.s1_at	AT1G18660 – ZF (C3HC4-type RING finger) family protein	2.30	-	-
lesaffx.2457.1.s1_at	AT1G64520 – RPN12A (regulatory particle non-atpase 12A); peptidase	2.05	2.41	2.98
lesaffx.30842.1.s1_at	AT2G27310 – F-box family protein	4.07	3.11	2.89
lesaffx.31022.1.a1_at	AT4G16563 – aspartyl protease family protein	-	3.75	-
lesaffx.31192.1.s1_at	AT5G47050 – ATP binding / protein binding / shikimate kinase/ zinc ion binding	-	2.64	-
lesaffx.31317.17.s1_at	AT5G05780 – RPN8A, AE3/ATHMOV34 (asymmetric leaves enhancer3)	2.38	2.07	2.98
lesaffx.3308.1.s1_at	AT4G19006 – 26S proteasome regulatory subunit, putative (RPN9)	4.88	-	5.78
lesaffx.3308.2.s1_at	AT4G19006 – 26S proteasome regulatory subunit, putative (RPN9)	7.73	-	5.08
lesaffx.33164.1.a1_at	AT5G57860 – ubiquitin family protein	-	-	-2.15
lesaffx.33164.1.s1_at	AT5G57860 – ubiquitin family protein	-2.34	-	-
lesaffx.33402.1.a1_at	AT1G02170 – MCP1B, AMC1, LOL3 (LSD ONE LIKE 3); caspase/ cysteine-type endopeptidase	3.04	3.36	3.65
lesaffx.39240.1.s1_at	AT1G24440 – protein binding / zinc ion binding	-3.11	-	-2.02
lesaffx.40158.1.s1_at	AT2G18280 – AtTLP2 (TUBBY like protein 2); phosphoric diester hydrolase/ transcription factor	2.13	-	-
lesaffx.44472.1.s1_at	AT4G31300 – PBA1 (20S proteasome beta subunit A 1); peptidase	2.58	2.43	3.65
lesaffx.46734.1.a1_at	AT3G01400 – armadillo/beta-catenin repeat family protein	3.73	-	-
lesaffx.48114.1.a1_at	AT1G32740 – protein binding / zinc ion binding	-	3.62	2.94
lesaffx.48314.2.s1_at	AT3G26730 – ZF (C3HC4-type RING finger) family protein	2.09	-	-
lesaffx.49541.1.s1_at	AT3G09770 – ZF (C3HC4-type RING finger) family protein	-	-2.56	-
lesaffx.49548.1.s1_at	AT1G16470 – PAB1 (proteasome subunit PAB1); peptidase	2.31	2.14	3.35
lesaffx.51779.1.s1_at	AT1G47710 – (ATSERPIN1); cysteine protease inhibitor/ serine-type endopeptidase inhibitor	3.72	-	3.99
lesaffx.51888.1.s1_at	AT3G56740 – ubiquitin-associated (Lubar & Bahler)/TS-N domain-containing protein	-	-	2.21
lesaffx.53232.1.s1_at	AT3G47990 – ZF (C3HC4-type RING finger) family protein	-	-	2.10
lesaffx.54921.1.s1_at	AT3G27430 – PBB1 (20S proteasome beta subunit B 1); peptidase	2.41	-	3.19
lesaffx.5583.1.s1_at	AT3G26085 – CAAX amino terminal protease family protein	-4.99	-3.76	-2.87
lesaffx.56104.1.s1_at	AT2G04240 – XERICO; protein binding / zinc ion binding	-17.05	-23.88	-16.47
lesaffx.57013.1.s1_at	AT5G45360 – F-box family protein	-2.35	-	-
lesaffx.57874.1.s1_at	AT1G71980 – protease-associated ZF (C3HC4-type RING finger) family protein	-	-	-2.64
lesaffx.59101.1.s1_at	AT2G19560 – proteasome protein-related	3.41	-	-
lesaffx.61619.1.s1_at	AT5G42270 – FTSH5, VAR1 (VARIEGATED 1); ATP-dependent peptidase/ ATPase/ metallopeptidase	3.11	-	-
lesaffx.62785.1.s1_at	AT3G13550 – EMB144, COP10, CIN4, FUS9 (FUSCA 9); ubiquitin-protein ligase	-3.66	-	-4.26
lesaffx.62975.1.s1_at	AT5G27420 – ZF (C3HC4-type RING finger) family protein	17.91	5.46	4.95
lesaffx.63263.1.s1_at	AT5G41350 – ZF (C3HC4-type RING finger) family protein	2.32	-	2.62
lesaffx.63367.1.s1_at	AT2G03120 – signal peptide peptidase family protein	2.31	-	4.94

Table G.3 (continued)

Probe Set ID	AT locus – Putative Annotation	Fold Change		
		WT AS	S2 AS	M48 AS
lesaffx.63935.1.s1_at	AT1G24140 – matrixin family protein	7.32	16.54	15.60
lesaffx.64239.1.s1_at	AT1G75460 – ATP-dependent protease La (LON) domain-containing protein	-2.02	-2.90	-
lesaffx.64823.1.s1_at	AT4G02075 – PIT1 (PITCHOUN 1); protein binding / zinc ion binding	-3.82	-	-9.04
lesaffx.65152.1.s1_at	AT3G07990 – SCPL27 (serine carboxypeptidase-like 27); serine carboxypeptidase	-2.20	-2.11	-2.19
lesaffx.65209.1.s1_at	AT1G68070 – ZF (C3HC4-type RING finger) family protein	-	-2.22	-2.69
lesaffx.66215.1.s1_at	AT1G44130 – nucellin protein, putative	3.75	3.12	3.83
lesaffx.66215.2.s1_at	AT1G44130 – nucellin protein, putative	9.93	5.15	4.07
lesaffx.66459.1.s1_at	AT4G33565 – ZF (C3HC4-type RING finger) family protein	2.23	2.09	2.27
lesaffx.67780.1.s1_at	AT5G60160 – aspartyl aminopeptidase, putative	2.22	-	-
lesaffx.67923.1.s1_at	AT1G77480 – nucellin protein, putative	2.34	-	-
lesaffx.68388.1.s1_at	AT3G07990 – SCPL27 | SCPL27 (serine carboxypeptidase-like 27); serine carboxypeptidase	2.48	-	-
lesaffx.68556.1.s1_at	AT4G38630 – MCB1, MBP1, RPN10 (regulatory particle non-ATPASE 10)	8.77	-	4.10
lesaffx.68623.1.s1_at	AT1G27340 – F-box family protein	3.43	-	-
lesaffx.68854.1.s1_at	AT2G22120 – protein binding / zinc ion binding	2.21	-	2.60
lesaffx.69210.1.s1_at	AT2G31980 – cysteine proteinase inhibitor-related	-	-	2.41
lesaffx.70371.1.s1_at	AT1G15100 – RHA2A | RHA2A (RING-H2 finger A2A); protein binding / zinc ion binding	-	-	2.07
lesaffx.70855.1.s1_at	AT5G59550 – ZF (C3HC4-type RING finger) family protein	7.96	6.40	5.24
lesaffx.71026.1.s1_at	AT3G47550 – ZF (C3HC4-type RING finger) family protein	2.02	-	-
lesaffx.71026.2.s1_at	AT3G47550 – ZF (C3HC4-type RING finger) family protein	-	-	2.26
lesaffx.741.1.s1_at	AT1G19310 – ZF (C3HC4-type RING finger) family protein	-2.61	-	-2.29
POST TRANSLATIONAL MODIFICATIONS				
les.1235.1.a1_at	AT1G34750 – protein phosphatase 2C, putative / PP2C, putative	-	2.53	-
les.1281.1.a1_at	AT1G53430 – LRR family protein / PK family protein	-4.25	-5.13	-4.42
les.1297.1.s1_at	AT3G21630 – CERK1 (chitin elicitor receptor kinase 1); kinase/ receptor signaling protein/ transmembrane receptor PK	3.18	2.50	3.01
les.1806.1.s1_at	AT3G09830 – PK, putative	2.40	3.50	3.32
les.1892.1.a1_at	AT5G21170 – 5'-AMP-activated PK beta-2 subunit, putative	-3.90	-2.46	-8.26
les.2027.3.s1_at	AT2G23770 – PK family protein / peptidoglycan-binding LysM domain-containing protein	20.65	9.87	5.37
les.2459.1.s1_at	AT5G14640 – PK family protein	5.79	-	-
les.2459.2.s1_at	AT5G14640 – PK family protein	2.87	-	-
les.2855.1.s1_at	AT4G29810 – MKK2, MK1, ATMKK2 | ATMKK2 (MAP kinase kinase 2)	2.07	-	-
les.2899.1.s1_at	AT5G61640 – PMSR1 (peptidemethionine sulfoxide reductase 1); peptide-methionine-(S)-S-oxide reductase	-	-	2.77
les.3124.1.s1_at	AT1G17550 – HAB2 (Homology to ABI2); protein serine/threonine phosphatase	9.19	-	-
les.3124.3.s1_at	AT1G72770 – HAB1 (homology to ABI1)	4.65	-	2.79
les.322.1.s1_at	AT4G27800 – protein phosphatase 2C PP2C, PPH1 (PPH1)	-3.27	-	-3.66

Table G.3 (continued)

Probe Set ID	AT locus – Putative Annotation	Fold Change		
		WT AS	S2 AS	M48 AS
les.3248.2.s1_at	AT4G18710 – DWF12, UCU1, BIN2 (brassinosteroid-insensitive 2); kinase	4.52	-	-
les.3248.3.s1_at	AT4G18710 – DWF12, UCU1, BIN2 (brassinosteroid-insensitive 2); kinase	2.11	-	-
les.3377.2.s1_at	AT5G26751– SK11 (SHAGGY-related kinase 11); PK	7.05	-	-
les.3403.1.s1_at	AT5G27930 – protein phosphatase 2C, putative / PP2C, putative	3.68	-	2.76
les.3403.2.s1_at	AT5G27930 – protein phosphatase 2C, putative / PP2C, putative	3.26	2.66	4.63
les.3403.3.a1_at	AT5G27930 – protein phosphatase 2C, putative / PP2C, putative	2.52	-	2.41
les.3502.1.s1_at	AT2G05940 – PK, putative	9.92	22.93	15.35
les.3539.1.s1_at	AT3G04530 – PEPCK2, PPCK2 (phosphoenolpyruvate carboxylase kinase 2); kinase	-	-4.95	-2.25
les.3869.1.s1_at	AT1G07160 – protein phosphatase 2C, putative / PP2C, putative	6.56	24.90	10.91
les.390.1.s1_at	AT5G14640 – PK family protein	6.85	-	-
les.390.2.s1_at	AT5G14640 – PK family protein	3.15	-	-
les.4099.1.s1_at	AT1G08650– PPCK1 (phosphoenolpyruvate carboxylase kinase); kinase	-2.64	-2.14	-4.38
les.4262.1.s1_at	AT5G21170 – 5'-AMP-activated PK beta-2 subunit, putative	-3.99	-2.88	-5.39
les.428.1.s1_at	AT5G24430 – CPK, putative / CDPK, putative	2.44	3.61	2.52
les.4526.1.s1_at	AT3G15260 – protein phosphatase 2C, putative / PP2C, putative	-	2.11	-
les.4574.1.s1_at	AT5G44100 – CKL7 (Casein Kinase I-like 7); casein kinase I/ kinase	-2.28	-	-2.81
les.4586.1.s1_at	AT4G25130 – peptide methionine sulfoxide reductase, putative	-2.84	-	-2.87
les.4613.1.s1_at	AT1G09840 – shaggy-related PK kappa / ASK-kappa (ASK10)	-3.45	-2.99	-4.55
les.4684.1.s1_at	AT2G26980 – SnRK3.17, CIPK3 (CBL-interacting PK 3); kinase	4.24	7.70	2.05
les.4777.1.s1_at	AT5G63940 – PK family protein	-	-3.86	-2.39
les.4780.1.s1_at	AT4G33920 – protein phosphatase 2C family protein / PP2C family protein	3.18	2.30	2.88
les.5182.1.s1_at	AT4G13020 – MHK	-2.64	-	-3.22
les.5339.1.s1_at	AT3G27580 – PK7 (serine/threonine-PK 7); kinase	4.71	2.93	2.93
les.5346.1.s1_at	AT1G18040 – CDCKD;3/ CAK2AT/ CDKD1;3 (cyclin-dependent kinase d1;3); kinase/ PK |	-	-	2.01
les.5382.1.s1_at	AT3G13690 – PK family protein	-4.78	-3.11	-4.94
les.5647.1.s1_at	AT3G08720 – S6K2, ATPK2, PK 19; kinase	14.69	5.13	10.85
les.5830.1.s1_at	AT4G33950 – OST1/P44/SNRK2-6/SRK2E (open stomata 1, SNF1-related PK 2.6)	-2.36	-2.15	-2.95
les.5833.1.s1_at	AT1G53570 – MAPKKK3, MAP3KA (Mitogen-activated PK kinase kinase 3); kinas	-	3.22	-
les.5954.1.s1_at	AT1G73500 – MKK9 (MAP kinase kinase 9); kinase	-3.28	-4.21	-7.48
les.896.1.s1_at	AT4G31750 – protein phosphatase 2C, putative / PP2C, putative	2.93	2.54	2.42
les.975.1.s1_at	AT2G02800 – APK2B (PK 2B); kinase	3.13	5.00	3.77
lesaffx.10313.1.a1_at	AT2G17220 – PK, putative	7.15	6.96	5.11
lesaffx.10444.1.s1_at	AT3G11410 – AHG3/ATPP2CA (protein phosphatase 2CA); protein binding / protein serine/threonine phosphatase	2.20	-2.29	-

Table G.3 (continued)

Probe Set ID	AT locus – Putative Annotation	Fold Change		
		WT AS	S2 AS	M48 AS
lesaffx.11410.1.s1_at	AT3G17510 – SnRK3.16, CIPK1 (CBL-interacting PK 1); kinase	3.39	-	-
lesaffx.13824.1.s1_at	AT3G22750 – PK, putative	2.62	-	2.11
lesaffx.16082.1.s1_at	AT3G08760 – SIK; kinase	4.10	2.29	2.39
lesaffx.21461.1.s1_at	AT5G63650 – SNRK2-5/SNRK2.5/SRK2H (SNF1-related PK 2.5); kinase	2.26	-	-
lesaffx.26003.1.a1_at	AT3G08720 – S6K2, ATPK2, ATPK19; kinase	2.93	-	-
lesaffx.26661.1.a1_at	AT5G47070 – PK, putative	-	4.02	-
lesaffx.26661.1.s1_at	AT5G47070 – PK, putative	5.87	13.83	9.14
lesaffx.31185.1.s1_at	AT2G02710 – PAC motif-containing protein	-3.64	-8.73	-7.54
lesaffx.33.1.s1_at	AT3G62260 – protein phosphatase 2C, putative	7.40	3.22	6.40
lesaffx.344.11.s1_at	AT5G50000 – PK, putative	-	-2.14	-
lesaffx.344.12.s1_at	AT1G34750 – protein phosphatase 2C, putative	4.56	2.49	3.74
lesaffx.36086.1.s1_at	AT5G16480 – tyrosine specific protein phosphatase family protein	-13.00	-14.36	-14.21
lesaffx.38907.2.s1_at	AT3G51550 – FER (FERONIA); kinase/ PK	5.05	4.53	6.05
lesaffx.40940.1.s1_at	AT4G38470 – PK family protein	-	-2.85	-3.91
lesaffx.40975.1.s1_at	AT4G14340 – CKL11, CKI1 (casein kinase I); casein kinase I/ kinase	3.65	-	-
lesaffx.41333.2.s1_at	AT2G29380 – protein phosphatase 2C, putative / PP2C, putative	-	-	2.23
lesaffx.41758.1.s1_at	AT4G18700 – SnRK3.9, CIPK12 (SNF1-related PK 3.9); kinase	-	-2.02	-
lesaffx.43162.2.s1_at	AT5G59160 – PPO, TOPP2 (Type one serine/threonine protein phosphatase 2); protein serine/threonine phosphatase	-	-2.15	-
lesaffx.48560.1.s1_at	AT3G62260 – protein phosphatase 2C, putative / PP2C, putative	3.79	2.39	4.21
lesaffx.50307.1.s1_at	AT1G72710 – CKL2; casein kinase I/ kinas	2.94	-	2.33
lesaffx.53506.1.s1_at	AT5G59770 – similar to PAS2 (PASTICCINO 2)	-4.08	-3.25	-3.65
lesaffx.54873.1.s1_at	AT3G45240 – GRIK1 (Geminivirus REP interacting kinase 1); kinase	-	-	2.23
lesaffx.56130.1.s1_at	AT1G25390 – PK family protein	2.51	2.12	2.04
lesaffx.58097.1.s1_at	AT2G07180 – PK, putative	2.59	4.80	2.50
lesaffx.58478.1.s1_at	AT3G02750 – protein phosphatase 2C family protein / PP2C family protein	2.08	-	-
lesaffx.5860.1.a1_at	AT4G28400 – protein phosphatase 2C, putative / PP2C, putative	-	3.92	-
lesaffx.63721.1.s1_at	AT5G42440 – PK family protein	5.90	-	2.45
lesaffx.63980.1.s1_at	AT3G02800 – phosphoprotein phosphatase	-	-	-2.70
lesaffx.64445.1.s1_at	AT1G78230 – protein binding	6.32	5.42	3.58
lesaffx.64831.1.s1_at	AT3G26020 – serine/threonine protein phosphatase 2A (PP2A) regulatory subunit B', putative	2.04	-	-
lesaffx.65398.3.s1_at	AT3G25800 – PR65, PDF1 (65 KDA regulatory subunit of protein phosphatase 2A); protein phosphatase type 2A regulator	7.51	-	-
lesaffx.65524.1.s1_at	AT1G16220 – protein phosphatase 2C family protein / PP2C family protein	6.69	-	-

Table G.3 (continued)

Probe Set ID	AT locus – Putative Annotation	Fold Change		
		WT AS	S2 AS	M48 AS
lesaffx.65695.1.s1_at	AT5G50180 – PK, putative	-4.57	-2.88	-4.97
lesaffx.65817.2.s1_at	AT1G09020 – SNF4 (Sucrose NonFermenting 4)	4.21	-	-
lesaffx.66270.1.s1_at	AT5G01820 – SnRK3.15, CIPK14, ATSR1 (serine/threonine PK 1); kinase	4.18	-	3.36
lesaffx.66523.1.s1_at	AT4G14340 – CKL11, CKI1 (casein kinase I); casein kinase I/ kinase	-2.07	-	-
lesaffx.67629.1.s1_at	AT5G58380 – SIP1, SNRK3.8, PKS2, CIPK10 (CBL-interacting PK 10); kinase	2.25	-	-
lesaffx.67743.1.s1_at	AT2G02710 – PAC motif-containing protein	-2.96	-2.37	-3.59
lesaffx.68000.1.s1_at	AT3G05580 – serine/threonine protein phosphatase, putative	5.61	-	3.81
lesaffx.68419.1.s1_at	AT2G43850 – ankyrin PK, putative (APK1)	-2.06	-	-2.38
lesaffx.68979.1.s1_at	AT3G51630 – ZIK1, WNK5 (WNK kinase 5)	3.20	-	-
lesaffx.69264.1.s1_at	AT5G50200 – NRT3.1, WR3 (wound-responsive 3); nitrate transmembrane transporter	-2.58	-2.92	-3.67
lesaffx.70335.1.s1_at	AT3G57700 – PK, putative	-	2.70	3.43
lesaffx.70383.1.s1_at	AT1G16220 – protein phosphatase 2C family protein / PP2C family protein	3.55	-	-
lesaffx.70568.1.s1_at	AT3G21070 – NADK1 (NAD kinase 1); NAD+ kinase/ NADH kinase/ CAM binding	4.72	-	2.81
lesaffx.70796.1.s1_at	AT5G03470 – ATB' alpha (PP2A, B' subunit, alpha isoform); protein phosphatase type 2A regulator	-	-	-2.12
lesaffx.7177.1.s1_at	AT1G14000 – PK family protein / ankyrin repeat family protein	3.02	-	-
RIBOSOMAL PROTEINS				
les.1323.1.s1_at	AT5G65220 – ribosomal protein L29 family protein	-2.92	-	-2.70
les.1323.2.a1_at	AT5G65220 – ribosomal protein L29 family protein	-2.62	-	-2.77
les.1528.1.a1_s_at	AT1G64510.1– ribosomal protein S6 family protein, similar to plastid ribosomal protein S6 precursor	-2.47	-	-2.88
les.1528.2.s1_at	AT1G64510 – ribosomal protein S6 family protein	-3.24	-	-3.56
les.1816.1.s1_at	AT1G68590 – plastid-specific 30S ribosomal protein 3, putative / PSRP-3, putative	-8.26	-3.92	-6.91
les.241.2.s1_at	AT2G33800 – ribosomal protein S5 family protein	-2.67	-	-2.55
les.2654.1.s1_at	AT5G20180 – ribosomal protein L36 family protein	-2.22	-	-2.08
les.3007.3.a1_at	AT1G78630 – EMB1473 (embryo defective 1473); structural constituent of ribosome	-2.59	-	-2.56
les.3050.1.s1_at	AT4G36130 – 60S ribosomal protein L8 (RPL8C)	2.34	-	2.21
les.3080.3.s1_at	AT4G34670 – 40S ribosomal protein S3A (RPS3aB)	2.13	-	2.90
les.3086.3.s1_a_at	AT3G47370 – 40S ribosomal protein S20 (RPS20B)	-	-	2.14
les.3121.1.s1_at	AT2G34480 – 60S ribosomal protein L18A (RPL18aB)	-	-	2.26
les.3154.1.a1_at	AT1G35680 – 50S ribosomal protein L21, chloroplast / CL21 (RPL21)	-2.37	-	-
les.3154.2.s1_at	AT1G35680 – 50S ribosomal protein L21, chloroplast / CL21 (RPL21)	-2.59	-	-
les.3354.2.s1_at	AT5G06360 – ribosomal protein S8e family protein	-	-	2.09
les.3380.1.s1_at	AT5G39740 – 60S ribosomal protein L5 (RPL5B)	-	-	2.67
les.392.1.s1_at	AT5G24490 – 30S ribosomal protein, putative	-10.74	-3.29	-17.74
les.3927.2.s1_at	AT5G64140 – RPS28 (ribosomal protein S28); structural constituent of ribosome	-	-	2.07

Table G.3 (continued)

Probe Set ID	AT locus – Putative Annotation	Fold Change		
		WT AS	S2 AS	M48 AS
les.3998.1.s1_at	AT1G29070 – ribosomal protein L34 family protein	-2.09	-	-2.28
les.4313.1.s1_at	AT5G02610 – 60S ribosomal protein L35 (RPL35D)	-	2.02	2.25
les.4379.1.s1_at	AT3G27830 – RPL12-A (ribosomal protein L12-A); structural constituent of ribosome	-3.76	-	-3.18
les.4379.2.s1_a_at	AT3G27850 – RPL12-C (ribosomal protein L12-C); structural constituent of ribosome	-4.25	-	-3.20
les.4379.2.s1_at	AT3G27850 – RPL12-C (ribosomal protein L12-C); structural constituent of ribosome	-	-	-2.23
les.4410.1.a1_at	AT1G64510 – ribosomal protein S6 family protein	-2.54	-	-3.47
les.446.1.a1_at	AT1G08845 – structural constituent of ribosome	-2.81	-2.99	-2.59
les.644.1.s1_at	AT2G33450 – 50S ribosomal protein L28, chloroplast (CL28)	-2.63	-	-2.47
lesaffx.19618.1.s1_at	AT3G18760 – ribosomal protein S6 family protein	-2.18	-	-
lesaffx.33796.2.s1_at	ATCG00900 – RPS7, chloroplast ribosomal protein S7, a constituent of the small subunit of the ribosomal complex	-5.43	-	-2.05
lesaffx.46494.1.s1_at	AT3G09200 – 60S acidic ribosomal protein P0 (RPP0B)	2.78	-	3.15
lesaffx.55735.1.s1_at	AT3G15190 – chloroplast 30S ribosomal protein S20, putative	-3.14	-	-4.20
lesaffx.62501.1.s1_at	AT1G18540 – 60S ribosomal protein L6 (RPL6A)	-	-2.25	-2.20
lesaffx.67632.1.a1_at	AT5G13720 – structural constituent of ribosome	-	-	-2.36
lesaffx.67632.1.s1_at	AT5G13720 – structural constituent of ribosome	-2.24	-	-2.21
lesaffx.70421.1.s1_at	AT4G31460 – ribosomal protein L28 family protein	-	-	2.49
lesaffx.71228.1.s1_at	AT5G54600 – 50S ribosomal protein L24, chloroplast (CL24)	-5.40	-2.22	-4.93
lesaffx.71577.1.s1_a_at	– late embryogenis abundant protein 5	4.25	8.07	2.13
les.1885.1.s1_at	AT5G15220 – ribosomal protein L27 family protein	-2.03	-	-
les.2169.1.s1_at	AT5G14320 – 30S ribosomal protein S13, chloroplast (CS13)	-2.97	-	-3.57
les.2169.2.s1_at	AT5G14320 – 30S ribosomal protein S13, chloroplast (CS13)	-3.60	-	-3.59
les.241.1.a1_at	AT2G33800 – ribosomal protein S5 family protein	-2.13	-	-3.21
les.241.2.s1_at	AT2G33800 – ribosomal protein S5 family protein	-2.67	-	-2.55
les.241.3.s1_at	AT2G33800 – ribosomal protein S5 family protein	-2.32	-	-2.14
les.2522.1.s1_at	AT1G07320 – RPL4 (ribosomal protein L4); poly(U) binding / structural constituent of ribosome	-2.47	-	-2.28
les.2743.1.s1_at	AT3G49910 – 60S ribosomal protein L26 (RPL26A)	-	-	2.04
les.2860.1.s1_at	AT1G05190 – EMB2394 (embryo defective 2394); structural constituent of ribosome	-4.04	-	-2.92
les.3007.1.s1_at	AT1G78630 – EMB1473 (embryo defective 1473); structural constituent of ribosome	-2.08	-	-
les.3007.2.s1_at	AT1G78630 – EMB1473 (embryo defective 1473); structural constituent of ribosome	-2.71	-	-2.05
les.3007.3.a1_at	AT1G78630 – EMB1473 (embryo defective 1473); structural constituent of ribosome	-2.59	-	-2.56
les.3139.1.s1_at	AT2G32060 – 40S ribosomal protein S12 (RPS12C)	-	-	2.08
les.3149.2.s1_at	AT3G09630 – 60S ribosomal protein L4/L1 (RPL4A)	3.91	-	3.06
les.3149.3.s1_at	AT3G09630 – 60S ribosomal protein L4/L1 (RPL4A)	-	-	3.30
les.3206.2.s1_a_at	AT1G70600 – 60S ribosomal protein L27A (RPL27aC)	-	-	2.11

Table G.3 (continued)

Probe Set ID	AT locus – Putative Annotation	Fold Change		
		WT AS	S2 AS	M48 AS
les.3306.1.s1_at	AT3G13120 – 30S ribosomal protein S10, chloroplast, putative	-	-	-2.19
les.3399.2.s1_at	AT2G44120 – 60S ribosomal protein L7 (RPL7C)	-	-	2.97
les.3399.3.s1_at	AT2G44120 – 60S ribosomal protein L7 (RPL7C)	-	-	2.43
les.4397.2.a1_at	AT1G18540 – 60S ribosomal protein L6 (RPL6A)	-	-	-2.89
les.4437.1.s1_at	AT4G01310 – ribosomal protein L5 family protein	-3.67	-	-2.32
les.4437.2.s1_at	AT4G01310 – ribosomal protein L5 family protein	-4.34	-	-3.28
les.4437.3.a1_at	AT4G01310 – ribosomal protein L5 family protein	-2.00	-	-2.16
lesaffx.1195.1.s1_at	AT4G27940 – MSC family protein	-3.45	-	-2.52
lesaffx.1195.2.s1_at	AT4G27940 – MSC family protein	9.99	-	2.00
lesaffx.22811.1.s1_at	AT2G43030 – ribosomal protein L3 family protein	-2.55	-	-2.39
lesaffx.26489.1.s1_at	AT3G54210 – ribosomal protein L17 family protein	-3.07	-	-3.28
lesaffx.27123.1.s1_at	AT5G16140 – peptidyl-tRNA hydrolase family protein	-2.18	-	-
lesaffx.31317.2.s1_at	AT3G53870 – 40S ribosomal protein S3 (RPS3B)	-	-	2.51
lesaffx.48130.1.s1_at	AT1G75350 – EMB2184 (embryo defective 2184); structural constituent of ribosome	-6.95	-2.83	-5.35
lesaffx.5957.1.s1_at	AT3G25920 – RPL15 (ribosomal protein L15)	-2.87	-	-
lesaffx.61614.1.s1_at	AT3G23620 – brix domain-containing protein	2.59	-	2.30
lesaffx.62501.1.s1_at	AT1G18540 – 60S ribosomal protein L6 (RPL6A)	-	-2.25	-2.20
lesaffx.64285.1.s1_at	AT1G74970 – TWN3, RPS9 (ribosomal protein S9); structural constituent of ribosome	-2.26	-	-
lesaffx.67298.1.s1_at	AT1G48350 – ribosomal protein L18 family protein	-4.84	-	-3.39
lesaffx.9508.1.s1_at	AT3G63490 – ribosomal protein L1 family protein	-3.88	-2.36	-3.59
PROTEIN TARGETING				
les.2862.2.s1_at	AT1G11890 – SEC22 (secretion 22); transporter	-	-	3.47
les.2874.2.s1_at	AT4G30600 – signal recognition particle receptor alpha subunit family protein	2.59	-	-
les.2874.3.s1_at	AT4G30600 – signal recognition particle receptor alpha subunit family protein	3.59	-	2.03
les.393.2.s1_at	AT4G32940 – GAMMA-VPE (Vacuolar processing enzyme gamma); cysteine-type endopeptidase	3.38	-	-
les.4103.1.s1_at	AT1G29260 – PEX7 (peroxin 7)	-2.23	-	-2.27
les.4519.2.s1_at	AT2G34250 – protein transport protein sec61, putative	2.32	-	2.20
les.4519.3.s1_at	AT2G34250 – protein transport protein sec61, putative	19.96	-	3.53
les.4643.1.s1_at	AT2G43640 – signal recognition particle 14 kDa family protein / SRP14 family protein	-	-	2.02
lesaffx.22051.2.s1_at	AT1G51980 – mitochondrial processing peptidase alpha subunit, putative	6.88	-	-
lesaffx.35587.1.s1_at	AT4G32940 – GAMMA-VPE (Vacuolar processing enzyme gamma); cysteine-type endopeptidase	-	-3.66	-3.23
lesaffx.35741.1.s1_at	AT1G09270 – importin alpha-1 subunit, putative (IMPA4)	2.39	-	-
lesaffx.44463.1.s1_at	AT3G59340 – similar to unknown protein	-2.13	-2.82	-
lesaffx.51420.1.s1_at	AT1G55900 – EMB1860, TIM50 (embryo defective 1860)	-	-	2.62
lesaffx.55863.1.s1_at	AT3G62880 – ATOEP16-4; P-P-bond-hydrolysis-driven protein transmembrane transporter	-2.06	-	-2.22

Table G.3 (continued)

Probe Set ID	AT locus – Putative Annotation	Fold Change		
		WT AS	S2 AS	M48 AS
lesaffx.60265.1.s1_at	AT1G48160 – signal recognition particle 19 kDa protein, putative / SRP19, putative	-	-	2.45
lesaffx.51291.1.s1_at	AT4G24880 – similar to unnamed protein product	3.50	-	-
lesaffx.64328.2.s1_at	AT1G60780 – clathrin adaptor complexes medium subunit family protein	2.94	-	-
lesaffx.64403.2.s1_at	AT3G49870 – ATARLA1C (ADP-ribosylation factor-like A1C); GTP binding	-2.42	-	-2.27
lesaffx.64992.1.a1_at	AT4G05000 – vacuolar protein sorting-associated protein 28 family protein / VPS28 family protein	-	2.19	-
lesaffx.64992.1.s1_at	AT4G05000 – vacuolar protein sorting-associated protein 28 family protein / VPS28 family protein	2.24	2.24	2.66
lesaffx.66676.1.s1_at	AT5G14030 – translocon-associated protein beta (TRAPB) family protein	-	-	2.54
lesaffx.67471.1.s1_at	AT1G71480 – nuclear transport factor 2 (NTF2) family protein	-2.33	-	-2.54
lesaffx.70036.1.s1_at	AT3G20000 – TOM40 (translocase of the outer mitochondrial membrane 40); voltage-gated anion channel	2.67	-	3.32

Table G.4 Significantly (P<0.05) regulated transcripts involved in transport upon exposure to freezing stress. Probe set ID, *Arabidopsis thaliana* (AT) locus name and putative annotation of each transcript is listed. Values represent fold changes of transcripts in WT, S2 and M48 after freezing stress compared to normal growth conditions. Negative values show down-regulation and positive values show up-regulation. Fold changes less than 2 are indicated with - (MSC: mitochondrial substrate carrier, ZIFL: zinc induced facilitator-like).

Probe Set ID	AT locus – Putative Annotation	Fold Change		
		WT AS	S2 AS	M48 AS
TRANSPORT				
les.2156.1.a1_at	AT2G45620 – nucleotidyltransferase family protein	-	-	-2.19
les.270.1.s1_at	AT5G65380 – ripening-responsive protein, putative	4.85	5.07	-
les.2947.1.s1_at	AT1G21065 – similar to unnamed protein produc	-	-	-2.12
les.2947.2.a1_at	AT1G21065 – similar to unnamed protein product	-	-	-2.27
les.3530.1.s1_at	AT5G20960 – AO1, AAO1 (aldehyde oxidase 1)	-2.12	-6.95	-3.45
les.4264.1.a1_s_at	AT3G22200 – HER1, GABA-T, POP2 (pollen-pistil incompatibility 2); 4-aminobutyrate transaminase	2.93	-	-
les.4607.1.s1_at	AT5G01990 – auxin efflux carrier family protein	2.51	2.61	2.56
les.4619.1.s1_at	AT3G07020 – UDP-glucose:sterol glucosyltransferase (UGT80A2)	-	-2.17	-
les.4789.1.s1_at	AT1G53580 – ETHE1/GLX2-3/GLY3 (glyoxalase 2-3); hydroxyacylglutathione hydrolase	-10.29	-4.19	-9.35
les.4838.1.s1_at	AT1G50510 – indigoidine synthase A family protein	-	-	2.46
les.5000.1.s1_at	AT4G26860 – alanine racemase family protein	-2.56	-2.55	-4.27
les.5013.1.s1_at	AT2G20840 – secretory carrier membrane protein (SCAMP) family protein	-	-	2.61
les.5512.1.s1_at	AT2G34460 – flavin reductase-related	-3.72	-	-2.19
les.5533.1.s1_at	AT4G12590 – similar to hypothetical protein	-	-	2.56
les.5539.1.s1_at	AT2G23390 – similar to hypothetical protein	-2.82	-3.04	-4.86
les.5594.1.s1_at	AT4G21210 – unknown protein	-	-2.37	-2.21
les.5624.1.s1_at	AT5G03905 – hesB-like domain-containing protein	-	-	2.98

Table G.4 (continued)

Probe Set ID	AT locus – Putative Annotation	Fold Change		
		WT AS	S2 AS	M48 AS
les.5756.1.s1_at	AT5G54750 – transport protein particle (TRAPP) component Bet3, putative	-	-	2.21
lesaffx.1490.1.s1_at	AT4G21800 – QQT2 (QUATRE-QUART2); ATP binding	2.94	-	-
lesaffx.15299.1.s1_at	AT1G80380 – phosphoribulokinase/uridine kinase-related	-2.09	-2.11	-
lesaffx.16086.1.s1_at	AT1G78820 – curculin-like (mannose-binding) lectin family protein / PAN domain-containing protein	8.38	2.32	2.39
lesaffx.16442.1.a1_at	AT3G21690 – MATE efflux family protein	3.65	2.03	3.71
lesaffx.23791.1.s1_at	AT1G71140 – MATE efflux family protein	-	-2.12	-2.01
lesaffx.31434.1.s1_at	AT3G10530 – transducin family protein / WD-40 repeat family protein	2.96	-	-
lesaffx.32974.1.s1_at	AT4G27585 – band 7 family protein	3.55	-	2.64
lesaffx.37702.2.s1_at	AT5G18660 – PCB2, DVR | DVR (pale-green and chlorophyll B reduced 2); 3,8-divinyl protochlorophyllide a 8-vinyl reductase	-3.12	-2.28	-
lesaffx.37887.1.a1_at	AT1G14345 – oxidoreductase	-	-	-2.02
lesaffx.47024.1.s1_at	AT5G04520 – oxidoreductase/ transition metal ion binding	2.24	-	3.60
lesaffx.49378.1.s1_at	AT5G13750 – ZIFL1; tetracycline:hydrogen antiporter/ transporter	16.72	-	2.72
lesaffx.52437.1.s1_at	AT5G65980 – auxin efflux carrier family protein	16.99	2.39	7.02
lesaffx.55549.1.s1_at	AT5G13750 – ZIFL1; tetracycline:hydrogen antiporter/ transporter	6.80	2.28	4.44
lesaffx.60146.1.s1_at	AT1G34470 – permease-related	4.17	-	3.38
lesaffx.60146.2.s1_at	AT1G34470 – permease-related	3.74	2.56	4.85
lesaffx.62361.1.s1_at	AT5G19300 – similar to unnamed protein product	2.31	-2.10	-
lesaffx.64061.1.s1_at	AT1G34470 – permease-related	2.37	-	-
lesaffx.64104.1.s1_at	AT2G34460 – flavin reductase-related	-2.03	-	-
lesaffx.65023.1.s1_at	AT1G26690 – emp24/gp25L/p24 family protein	-	-	2.13
lesaffx.65724.1.s1_at	AT3G27340 – unnamed protein product	-3.02	-2.09	-3.01
lesaffx.65940.1.s1_at	AT2G44920 – thylakoid lumenal 15 kDa protein, chloroplast	-4.81	-5.73	-6.58
lesaffx.66107.1.s1_at	AT2G21120 – similar to unknown protein	4.21	2.19	2.07
lesaffx.67115.1.s1_at	AT1G09330 – Uncharacterized FAM18-like protein	-	-	2.26
lesaffx.67392.1.s1_at	AT1G12250 – thylakoid lumenal protein-related	-3.51	-2.30	-3.06
lesaffx.69151.1.s1_at	AT3G03620 – MATE efflux family protein	6.20	2.95	5.05
lesaffx.70660.1.s1_at	AT3G46450 – SEC14 cytosolic factor family protein / phosphoglyceride transfer family protein	2.15	-	-
TRANSPORT/ AMINO ACID				
les.4073.1.s1_at	AT1G77380 – AAP3 (amino acid permease 3); amino acid transmembrane transporter	5.16	2.88	3.15
les.58.1.s1_at	AT2G39890 – ATPROT1, ProT1 (proline transporter 1)	-4.71	-12.74	-7.44
les.59.1.s1_at	AT3G55740 – PROT2, (proline transporter 2)	2.80	-	-
lesaffx.35418.1.s1_at	AT2G01170 – amino acid permease family protein	7.97	-	2.61
lesaffx.35418.2.s1_at	AT2G01170 – amino acid permease family protein	3.16	-	-
lesaffx.39098.1.s1_at	AT1G47670 – amino acid transporter family protein	2.70	-	2.06
lesaffx.46368.1.s1_at	AT2G38120 – WAV5, PIR1, MAP1, AUX1 (auxin resistant 1); amino acid transmembrane transporter/ transporter	-4.12	-3.31	-7.00

Table G.4 (continued)

Probe Set ID	AT locus – Putative Annotation	Fold Change		
		WT AS	S2 AS	M48 AS
lesaffx.55216.1.s1_at	AT1G77690 – amino acid permease, putative	-	-2.39	-
TRANSPORT/ ABC TRANSPORTERS				
les.4068.1.a1_at	AT3G54540 – ATGCN4 (general control non-repressible 4)	2.78	-	-
les.4385.2.s1_at	AT5G60790 – ATGCN1 (AT general control non-repressible 1)	3.09	-	-
les.452.1.s1_at	AT5G39040 – TAP2 (transporter associated with antigen processing protein 2); ATPase	-	2.34	3.04
les.490.1.a1_at	AT3G24430 – HCF101 (high-chlorophyll-fluorescence 101); ATP binding	-2.72	-2.08	-2.87
lesaffx.269.2.s1_at	AT2G47800 – EST3, ATMRP4 (multidrug resistance-associated protein 4)	3.32	3.90	7.50
TRANSPORT/ METABOLITE TRANSPORTERS AT THE MITOCHONDRIAL MEMBRANE				
les.1241.1.s1_at	AT1G14685 – BBR/BPC2 (basic pentacysteine 2)	-	-	2.09
les.2069.1.a1_at	AT5G01340 – MSC family protein	2.73	3.62	3.00
les.3691.1.s1_at	AT3G54110 – PUMP1, UCP1; binding / oxidative phosphorylation uncoupler	-	-	2.53
les.4779.1.s1_at	AT2G22500 – MSC family protein	44.08	30.57	53.63
les.4912.1.s1_at	AT3G05290 – MSC family protein	16.49	5.38	7.51
lesaffx.10723.1.s1_at	AT5G42130 – MSC family protein	5.19	-	-
lesaffx.59407.1.s1_at	AT3G53940 – MSC family protein	2.52	-	-
lesaffx.62906.1.s1_at	AT1G79900 – BAC2 (mitochondrial basic amino acid carrier 2); L-ornithine transmembrane transporter/ binding / carnitine:acyl carnitine antiporter	3.80	3.90	2.03
lesaffx.68106.1.s1_at	AT4G15010 – MSC family protein	2.09	-	-
lesaffx.68360.1.s1_at	AT5G15640 – MSC family protein	4.30	2.78	4.95
lesaffx.68360.2.s1_at	AT5G15640 – MSC family protein	5.09	2.01	3.76
TRANSPORT/ METABOLITE TRANSPORTERS AT THE ENVELOPE MEMBRANE				
les.1426.1.s1_at	AT1G12500 – phosphate translocator-related	-2.12	-	-
les.3324.1.s1_at	AT5G46110 – TPT, APE2 (acclimation of photosynthesis to environment)	-3.33	-2.04	-3.75
les.4628.1.s1_at	AT5G33320 – PPT, CUE1 (CAB underexpressed 1); antiporter/ triose-phosphate transmembrane transporter	2.76	-	-
lesaffx.2135.1.s1_at	AT5G42740 – glucose-6-phosphate isomerase, cytosolic (PGIC)	2.23	-	-
lesaffx.2135.3.s1_at	AT5G42740 – glucose-6-phosphate isomerase, cytosolic (PGIC)	-	-	-2.03
lesaffx.2135.4.s1_at	AT5G42740 – glucose-6-phosphate isomerase, cytosolic (PGIC)	-	-2.32	-
lesaffx.34390.1.s1_at	AT3G11320 – organic anion transmembrane transporter	11.46	2.45	4.96
lesaffx.46624.1.s1_at	AT5G17630 – glucose-6-phosphate/phosphate translocator, putative	-	-5.40	-
lesaffx.52329.1.s1_at	AT5G17520 – MEX1, RCP1 (root cap 1)	-5.39	-5.32	-5.93
TRANSPORT/ MAJOR INTRINSIC PROTEINS				
les.173.1.s1_at	AT4G35100 – PIP2;7, SIMIP, PIP3 (plasma membrane intrinsic protein 3); water channel	-11.64	-	-23.66
les.2866.1.a1_at	AT4G11160 – translation initiation factor IF-2, mitochondrial, putative	-3.54	-4.24	-3.96
les.3017.1.a1_at	AT3G16240 – TIP2;1, AQP1, DELTA-TIP (delta tonoplast integral protein); water channel	-2.50	-2.77	-4.56

Table G.4 (continued)

Probe Set ID	AT locus – Putative Annotation	Fold Change		
		WT AS	S2 AS	M48 AS
les.3017.2.s1_at	AT3G16240 – TIP2;1, AQP1, DELTA-TIP (delta tonoplast integral protein); water channel	-2.99	-2.16	-4.97
les.3063.1.s1_at	AT3G21055.1– photosystem II 5 kD protein, putative	-5.08	-2.53	-4.97
les.4276.1.s1_at	AT2G37170 – PIP2B (plasma membrane intrinsic protein 2;2); water channel	-4.43	-	-5.66
les.4953.1.s1_at	AT4G01470 – GAMMA-TIP3/TIP1;3 (tonoplast intrinsic protein 1;3); water channel	-5.86	-2.47	-5.01
les.5231.1.s1_at	AT4G10380 – NIP5;1/NLM6/NLM8 (NOD26-like intrinsic protein 5;1); boron transporter/ water channel	-4.89	-7.39	-6.56
les.5610.1.s1_at	AT1G01620 – PIP1C (plasma membrane intrinsic protein 1;3)	-2.96	-	-3.46
les.5960.1.s1_at	AT4G17340 – DELTA-TIP2/TIP2;2 (tonoplast intrinsic protein 2;2); water channel	-5.74	-4.17	-3.17
lesaffx.53438.1.s1_at	AT3G04090 – SIP1A (small and basic intrinsic protein 1A)	-	2.73	2.24
lesaffx.59952.1.s1_at	AT3G53420 – PIP2A (plasma membrane intrinsic protein 2A)	-2.10	-	-
TRANSPORT/ SUGAR				
les.1784.1.s1_at	AT1G54730 – sugar transporter, putative	-	-5.04	-2.12
les.1942.1.s1_at	AT3G01280 – porin, putative	-	-	2.38
les.3532.1.s1_at	AT2G02860 – SUC3, SUT2 (sucrose transporter 3); carbohydrate transmembrane transporter	-	2.88	3.02
les.3725.1.s1_at	AT1G11260 – STP1 (sugar transporter 1); carbohydrate transmembrane transporter	-	-	-3.15
les.3755.1.a1_at	AT5G59250 – sugar transporter family protein	-2.74	-2.59	-2.46
les.3756.1.s1_a_at	AT2G43330 – ATINT1 (inositol transporter 1); carbohydrate transmembrane transporter/ sugar	-2.80	-8.94	-
les.3973.1.s1_at	AT1G22710 – SUT1, SUC2 (sucrose-proton symporter 2); carbohydrate transmembrane transporter	-	-2.72	-
les.4824.1.s1_at	AT3G01280 – porin, putative	2.58	-	3.65
les.5374.1.s1_at	AT1G79820 – SGB1; carbohydrate transmembrane transporter/ sugar:hydrogen ion symporter	19.73	17.93	20.19
lesaffx.10299.1.a1_at	AT3G18830 – ATPLT5 (polyol transporter 5); carbohydrate transmembrane transporter/ galactose	-	2.31	-
lesaffx.10299.1.s1_at	AT3G18830 – ATPLT5 (polyol transporter 5); carbohydrate transmembrane transporter/	2.22	2.44	2.62
lesaffx.48830.1.s1_at	AT3G59360 – UTR6 (UDP-galactose transporter 6); nucleotide-sugar transmembrane transporter	4.83	-	-
lesaffx.49378.1.s1_at	AT5G13750 – ZIFL1; tetracycline:hydrogen antiporter/ transporter	16.72	-	2.72
lesaffx.55549.1.s1_at	AT5G13750 – ZIFL1; tetracycline:hydrogen antiporter/ transporter	6.80	2.28	4.44
lesaffx.61214.1.s1_at	AT3G01280 – porin, putative	5.32	-	9.05

Table G.5 Significantly (P<0.05) regulated transcripts involved in signalling upon exposure to freezing stress. Probe set ID, *Arabidopsis thaliana* (AT) locus name and putative annotation of each transcript is listed. Values represent fold changes of transcripts in WT, S2 and M48 after freezing stress compared to normal growth conditions. Negative values show down-regulation and positive values show up-regulation. Fold changes less than 2 are indicated with - (TF: Transcription Factor, ERF: Ethylene Responsive Element Binding Factor, ZF: zinc finger, CPK: calcium-dependent protein kinase, CAM: calmodulin, LRR: leucine-rich repeat, PK: protein kinase).

Probe Set ID	AT locus – Putative Annotation	Fold Change		
		WT AS	S2 AS	M48 AS
HORMONE SIGNALING / AUXIN				
les.165.1.s1_at	AT3G22850 –unknown auxin down-regulated protein	-2.22	-	-
les.165.2.s1_at	AT5G43830 –unknown auxin down-regulated protein	-2.08	-	-
les.207.1.s1_at	AT5G19140 – auxin/aluminum-responsive protein, putative	-4.90	-3.01	-4.16
les.2605.1.a1_at	AT5G15740 – unknown protein	2.01	2.43	2.41
les.2668.2.a1_at	AT2G14960 – GH3.1	2.00	-	-
les.3216.1.s1_at	AT1G51760 – JR3, IAR3 (IAA- Alanine Resistant 3); metallopeptidase	4.17	-	-
les.3216.2.s1_at	AT1G51760 – JR3, IAR3 (IAA-Alanine Resistant 3); metallopeptidase	2.30	-	-
les.3216.3.s1_at	AT1G51760 – JR3, IAR3 (IAA- Alanine Resistant 3); metallopeptidase	2.39	-	-
les.3563.1.s1_at	AT4G02980 – ABP, ABP1 (Endoplasmic Reticulum Auxin Binding Protein 1)	-2.31	-	-
les.368.1.s1_at	AT2G46370 – JAR, FIN219, JAR1 (jasmonate resistant 1)	-3.08	-2.49	-5.25
les.3757.1.s1_at	AT4G27450 – unknown auxin down-regulated protein	-17.06	-	-48.00
les.5146.1.s1_at	AT1G75590 – auxin-responsive family protein	-5.50	-3.70	-6.02
les.5177.1.s1_at	AT2G14960 – GH3.1 similar to IAA-amido synthases	25.77	3.35	5.43
lesaffx.15284.1.s1_at	AT1G28330 – DRM1 (dormancy-associated protein 1)	-10.85	-7.66	-23.22
lesaffx.3081.1.s1_at	AT5G35735 – auxin-responsive family protein	8.09	5.66	7.42
lesaffx.44071.1.s1_at	AT2G44500 – unknown protein	14.29	3.64	4.97
lesaffx.44071.2.s1_at	AT2G44500 – unknown protein	14.17	10.97	14.13
lesaffx.55216.1.s1_at	AT1G77690 – amino acid permease, putative	-	-2.39	-
lesaffx.58573.1.s1_a_at	AT1G52630 – unknown protein	2.02	-	-
lesaffx.63209.1.s1_at	AT2G21220 – auxin-responsive protein, putative	-3.51	-3.78	-4.86
lesaffx.64980.1.s1_at	AT5G50760 – auxin-responsive family protein	2.36	2.25	3.48
lesaffx.71035.1.s1_at	AT4G38840 – auxin-responsive protein, putative	-3.67	-7.70	-8.36
HORMONE SIGNALING / ETHYLENE				
les.126.1.s1_at	AT1G05710 – ethylene-responsive protein, putative	-	5.12	2.87
les.132.1.s1_at	AT1G05010 – ACO4, EAT1, EFE (Ethylene Forming Enzyme)	2.28	2.40	-
les.1841.1.s1_at	AT4G11280 – ACS6 (1-Aminocyclopropane-1-Carboxylic Acid (ACC) Synthase 6)	-	3.14	2.12
les.2560.1.s1_at	AT1G05010 – ACO4, EAT1, EFE (Ethylene Forming Enzyme)	3.68	5.22	5.04
les.274.1.s1_at	AT5G47230 – ERF5; DNA binding / transcription activator/ TF	-	2.60	-
les.3041.1.s1_at	AT1G50640 – ERF3; DNA binding / protein binding / TF/ transcription repressor	-	2.64	-

Table G.5 (continued)

Probe Set ID	AT locus – Putative Annotation	Fold Change		
		WT AS	S2 AS	M48 AS
les.35.1.s1_at	AT3G04580 – EIN4 (ethylene insensitive 4)	-	2.50	-
les.3225.2.s1_at	AT1G05010 – ACO4, EAT1, EFE (Ethylene Forming Enzyme)	-	-2.09	-
les.3551.1.s1_at	AT3G24500 – ATMBF1C (multiprotein bridging factor 1C); DNA binding / transcription coactivator/ TF	4.04	7.51	6.29
les.3575.1.s1_at	AT3G23240 – ERF1; DNA binding / transcription activator/ TF	2.41	3.69	5.62
les.36.1.s1_at	AT3G04580 – EIN4 (Ethylene Insensitive 4)	5.68	2.16	4.16
les.3661.1.s1_at	AT1G01480 – AT-ACC2, ACS2 (1-Amino-cyclopropane-1-carboxylate synthase 2)	-	-	-2.91
les.3662.1.s1_at	AT1G01480 – AT-ACC2, ACS2 (1-Amino-cyclopropane-1-carboxylate synthase 2)	5.93	2.65	-
les.3769.1.s1_at	AT4G11280 – ACS6 (1- Aminocyclopropane-1-Carboxylic Acid (ACC) Synthase 6)	3.10	-	-
les.4101.2.s1_a_at	AT2G32970 – unnamed protein product	2.57	2.19	-
les.4139.1.s1_at	AT5G07580 – member of the ERF subfamily B-3 of ERF/AP2 TF family	-4.09	-5.52	-17.31
les.4140.1.s1_at	AT5G47220 – ERF 2; DNA binding / transcription activator/ TF	-	2.34	-2.55
les.4233.1.s1_at	AT3G11930 – universal stress protein (USP) family protein	6.98	12.53	5.63
les.5181.1.s1_at	AT5G51690 – ACS12 (1-Amino-cyclopropane-1-carboxylate synthase 12)	-	-	-2.40
les.5571.1.s1_at	AT5G44210 –ATERF9 (ERF domain protein 9); DNA binding / TF / transcription repressor	3.59	5.39	3.15
les.5601.1.s1_at	AT1G17020 – ATSRG1, SRG1 (senescence-related gene 1); oxidoreductase	-	-	-4.17
lesaffx.29801.1.s1_at	AT5G24530 – oxidoreductase, 2OG-Fe(Agarwal *et al.*) oxygenase family protein	-	-2.07	-
lesaffx.51274.1.s1_at	AT5G47230 –ATERF5 (ERF5); DNA binding / transcription activator/ TF	-	3.69	5.90
lesaffx.63189.1.s1_at	AT1G17020 – ATSRG1, SRG1 (senescence-related gene 1); oxidoreductase	-4.15	-	-4.43
lesaffx.70590.1.s1_at	AT1G61660 – basic helix-loop-helix (bHLH) family protein	3.48	-	2.63
lesaffx.8333.1.s1_at	AT1G05010 – ACO4, EAT1 EFE (Ethylene Forming Enzyme)	-3.05	-10.33	-14.43
lesaffx.9824.1.s1_at	AT2G36690 – oxidoreductase, 2OG-Fe(Agarwal, *et al.*) oxygenase family protein	-2.26	-	-2.88
HORMONE SIGNALING / GIBERELLIN				
les.417.1.s1_at	AT5G59845 – gibberellin-regulated family protein	-11.72	-2.98	-5.76
les.4311.1.s1_at	AT1G14920 – RGA2, GAI (GA insensitive); TF	-3.76	-3.67	-5.93
les.4766.1.s1_at	AT2G18420 – Encodes a Gibberellin-regulated GASA/GAST/Snakin family protein	-	9.26	-
les.64.1.s1_at	AT4G25420 – ATGA20OX1, AT2301, GA5 (GA requiring 5); gibberellin 20-oxidase/ gibberellin 3-beta-dioxygenase	-2.60	-2.23	-5.79
les.827.1.s1_at	AT3G02885 – GASA5 (GAST1 protein homolog 5) gibberellin-regulated protein 5 (GASA5) / gibberellin-responsive protein 5	-17.66	-6.68	-50.53
HORMONE SIGNALING / JASMONATE				
les.13.1.s1_at	AT5G42650 – CYP74A, AOS (allene oxide synthase); hydro-lyase/ oxygen binding	33.60	9.97	8.30
les.3632.1.s1_at	AT1G17420 – LOX3 (Lipoxygenase 3); iron ion binding / lipoxygenase/ metal ion binding / oxidoreductase	13.61	5.53	23.35

Table G.5 (continued)

Probe Set ID	AT locus – Putative Annotation	Fold Change		
		WT AS	S2 AS	M48 AS
les.3980.1.s1_at	AT3G45140 – ATLOX2 (Lipoxygenase 2)	-26.81	-41.17	-5.13
les.3762.1.s1_at	AT2G06050 – OPR3 (OPDA-Reductase 3)	-	-2.68	-
les.3986.1.s1_at	AT5G42650 – CYP74A, AOS (allene oxide synthase); hydro-lyase/ oxygen binding	-	-2.36	-
les.5411.1.s1_at	AT3G07720 – kelch repeat-containing protein	-	-	-2.72
SIGNALING / CALCIUM REGULATION				
les.1672.1.s1_at	AT3G10190– CAM, putative	-	4.67	2.64
les.1989.1.a1_at	AT2G38800– CAM-binding protein-related	-	-2.91	-2.75
les.2231.1.s1_at	AT1G74690– IQD31 (IQ-domain 31); CAM binding	-2.05	-	-2.13
les.2645.2.s1_at	AT3G43810– CAM7; calcium ion binding	-	-	2.94
les.2854.1.s1_at	AT2G41410– CAM, putative	2.90	3.36	5.11
les.3133.2.s1_at	AT3G43810– CAM7; calcium ion binding	-	-	2.28
les.3334.1.s1_at	AT5G66210– CPK28	-	2.44	-
les.3334.2.s1_at	AT5G66210– CPK28	9.03	2.63	2.61
les.3457.1.s1_at	AT4G04720– CPK21; CAM-dependent PK	-	2.24	2.31
les.4149.2.s1_a_at	AT4G38810– calcium-binding EF hand family protein	4.88	3.96	5.40
les.428.1.s1_at	AT5G24430– CPK, putative	2.44	3.61	2.52
les.4548.1.s1_at	AT3G59690– IQD13 (IQ-domain 13); CAM binding	2.21	-	-
les.4651.1.s1_at	AT5G61790– calnexin 1 (CNX1)	-	-	2.63
les.4720.1.s1_at	AT1G35670– CPK11, ATCDPK2 (CPK 2); CAM-dependent PK	-	-	2.10
les.4783.1.s1_at	AT5G37770– CML24, TCH2 (TOUCH 2); calcium ion binding	-	2.30	-
les.4870.1.s1_at	AT4G20780– calcium-binding protein, putative	2.97	3.07	3.21
les.5056.1.s1_a_at	AT5G42380– CML39, CML37; calcium ion binding	61.48	224.21	114.62
les.5056.1.s1_x_at	AT5G42380– CML39, CML37; calcium ion binding	42.11	67.64	53.78
les.5197.1.s1_at	AT5G57580– CAM-binding protein	2.30	4.44	2.13
les.5318.1.s1_at	AT3G52870– CAM-binding family protein	-	3.79	3.89
les.557.1.s1_at	AT3G10190– CAM, putative	-2.81	-2.23	-
les.5939.1.s1_at	AT4G27280– calcium-binding EF hand family protein	3.07	5.75	9.22
les.934.1.s1_at	AT1G18210– calcium-binding protein, putative	3.24	6.80	3.01
lesaffx.11542.1.a1_at	AT1G76650– CML38 calcium-binding EF hand family protein	2.65	5.04	5.47
lesaffx.11542.1.s1_at	AT1G76650– CML38 calcium-binding EF hand family protein	4.11	4.17	3.97
lesaffx.16164.1.s1_at	AT3G29000– calcium-binding EF hand family protein	2.43	5.94	2.28
lesaffx.17339.1.s1_at	AT1G19870– IQD32 (IQ-domain 32); CAM binding	-3.43	-	-
lesaffx.30900.1.s1_at	AT3G57530– CDPK32, CPK32; CAM-dependent PK	2.15	2.18	-
lesaffx.3635.1.s1_at	AT2G26190– CAM-binding family protein	4.04	4.92	3.64
lesaffx.3635.2.a1_at	AT2G26190– CAM-binding family protein	4.02	10.87	3.92
lesaffx.41062.1.s1_s_at	AT3G43810– CAM7; calcium ion binding	-	2.16	2.11

Table G.5 (continued)

Probe Set ID	AT locus – Putative Annotation	Fold Change		
		WT AS	S2 AS	M48 AS
lesaffx.47093.1.s1_at	AT3G43810– CAM7; calcium ion binding	-	2.49	-
lesaffx.47666.1.s1_at	AT4G34150– C2 domain-containing protein	15.47	4.08	3.63
lesaffx.56.5.a1_a_at	AT5G57110– AT-ACA8 (autoinhibited CA2+ -ATPASE, ISOFORM 8); CAM binding	-	-	2.52
lesaffx.57054.2.s1_at	AT5G54130– calcium-binding EF hand family protein	3.51	-	-
lesaffx.57454.1.s1_at	AT1G18530– CAM, putative	-	5.93	2.51
lesaffx.60825.1.s1_at	AT5G28830– calcium-binding EF hand family protein	3.48	5.62	3.88
lesaffx.63947.2.s1_at	AT3G57530– CDPK32, CPK32; CAM-dependent PK	4.69	2.85	2.68
lesaffx.64561.1.s1_at	AT5G62390– ATBAG7 (BCL-2-associated athanogene 7); CAM binding	-2.31	-	-2.82
lesaffx.66814.1.s1_at	AT1G73805– CAM binding	4.74	5.77	8.35
lesaffx.69808.1.s1_at	AT2G26190– CAM-binding family protein	5.11	8.08	5.80
lesaffx.70732.1.s1_at	AT3G50770– CAM-related protein, putative	-	2.44	-
lesaffx.9367.1.s1_at	AT5G49480– ATCP1 (CA^{2+}-binding protein 1); calcium ion binding	4.42	3.49	3.89
SIGNALING / RECEPTOR KINASES				
les.1281.1.a1_at	AT1G53430– LRRfamily protein / PK family protein	-4.25	-5.13	-4.42
les.1297.1.s1_at	AT3G21630– CERK1 (chitin elicitor receptor kinase 1); kinase / receptor signaling protein / transmembrane receptor PK	3.18	2.50	3.01
les.1334.1.a1_at	AT5G38280– PR5K (PR5-like receptor kinase); kinase / transmembrane receptor protein serine/threonine kinase	-3.26	-	-2.12
les.398.1.a1_at	AT5G49760– LRR family protein / PK family protein	-2.76	-2.03	-2.46
les.4500.1.s1_at	AT3G42880– LRR transmembrane PK, putative	-3.30	-3.37	-4.12
les.4673.1.s1_at	AT1G70740– PK family protein	5.49	7.91	7.84
les.5205.1.s1_at	AT2G31880– LRR transmembrane PK, putative	-	2.29	-
les.5333.1.s1_at	AT1G28340– leucine-rich repeat family protein	-2.91	-	-
lesaffx.16086.1.s1_at	AT1G78820– curculin-like (mannose-binding) lectin family protein / PAN domain-containing protein	8.38	2.32	2.39
lesaffx.40862.1.s1_at	AT4G00300– fringe-related protein	8.44	-	3.42
lesaffx.56130.1.s1_at	AT1G25390– PK family protein	2.51	2.12	2.04
lesaffx.59625.1.s1_at	AT5G48540– 33 kDa secretory protein-related	-3.84	-	-
lesaffx.65273.1.s1_at	AT1G16670– PK family protein	3.75	-	2.59
lesaffx.70335.1.s1_at	AT3G57700– PK, putative	-	2.70	3.43
SIGNALING / MAP KINASES				
les.273.1.s1_at	AT3G61490– glycoside hydrolase family 28 protein / polygalacturonase (pectinase) family protein	-	-2.22	-
les.273.2.s1_at	AT3G61490– glycoside hydrolase family 28 protein / polygalacturonase (pectinase) family protein	-	-3.04	-
les.4023.1.s1_at	AT3G14720– ATMPK19 (MAP kinase 19)	-2.36	-	-2.47
les.4316.1.s1_at	AT3G45640– MPK3 (mitogen-activated PK 3)	4.67	5.69	6.33
les.4949.1.s1_at	AT1G10210– MPK1 (mitogen-activated PK 1)	-2.93	-4.33	-2.74
les.5060.1.s1_at	AT4G01370– MPK4 (MAP kinase 4)	2.31	-	-
lesaffx.16424.1.a1_at	AT3G45640– MPK3 (mitogen-activated PK 3)	-	4.63	3.02

Table G.5 (continued)

Probe Set ID	AT locus – Putative Annotation	Fold Change		
		WT AS	S2 AS	M48 AS
lesaffx.16424.1.s1_s_at	AT3G45640– MPK3 (mitogen-activated PK 3)	6.08	5.82	7.87
lesaffx.69648.1.s1_at	AT2G18170– MPK7 (MAP kinase 7)	6.90	-	-
lesaffx.69648.2.s1_at	AT2G18170– MPK7 (MAP kinase 7)	3.80	-	2.01
SIGNALING / G-PROTEINS				
les.176.1.s1_at	AT1G02130– AtRABD2a, ARA5, ATRAB1B (responsive to abscisic acid 1B); GTP binding	3.25	3.77	3.22
les.1954.1.s1_at	AT2G31060– elongation factor family protein	-	-	2.30
les.2350.1.s1_at	AT4G35750– Rho-GTPase-activating protein-related	-11.28	-5.04	-5.42
les.2350.2.s1_at	AT4G35750– Rho-GTPase-activating protein-related	-10.71	-8.45	-7.80
les.240.1.s1_at	AT1G48630– guanine nucleotide-binding family protein / activated PK C receptor / RACK / putative	2.28	2.13	3.51
les.320.1.s1_at	AT2G44610– AtRABH1b, RAB6; GTP binding	2.16	-	-
les.3670.1.s1_at	AT2G26300– GPA1 (G protein alpha subunit 1); signal transducer	2.53	-	2.58
les.396.1.s1_at	AT5G10260– AtRABH1e (Rab GTPase homolog H1e); GTP binding	-2.15	-	-
les.4749.1.s1_at	AT2G46710– rac GTPase activating protein, putative	-3.13	-	-
les.4812.1.s1_at	AT1G50920– GTP-binding protein-related	-	-2.04	-
les.4857.2.s1_at	AT5G45130– AtRab5A, AtRABF2a, Rha1, RHA1, Ras-related protein (RHA1) / small GTP-binding protein	-16.08	-40.37	-25.36
les.4860.1.s1_at	AT4G02080– ATSARA1C, ATSAR2, ASAR1 (secretion-associated RAS super family 2); GTP binding	2.06	2.49	2.90
les.4861.1.s1_at	AT1G07410– AtRABA2b (Rab GTPase homolog A2b); GTP binding	-2.64	-	-4.94
les.5265.1.s1_at	AT5G57960– GTP-binding family protein	-2.29	-	-2.73
les.5316.1.s1_at	AT5G66470– GTP binding / RNA binding	-2.00	-2.12	-
les.5749.1.s1_at	AT3G54190– unknown protein	-	-	-2.11
les.5877.1.s1_at	AT5G47520– AtRABA5a (Arabidopsis Rab GTPase homolog A5a); GTP binding	-3.82	-2.18	-6.62
lesaffx.10175.1.s1_at	AT2G38360– prenylated rab acceptor (PRA1) family protein	-	-	2.52
lesaffx.37222.1.s1_at	AT5G61530– small G protein family protein / RhoGAP family protein	7.33	-	3.00
lesaffx.39914.1.s1_at	AT5G54310– AGD5 (ARF-GAP DOMAIN 5); DNA binding	3.59	-	-
lesaffx.51015.1.s1_at	AT4G21520– transducin family protein / WD-40 repeat family protein	6.39	-	2.31
lesaffx.59893.1.s1_at	AT1G55190– prenylated rab acceptor (PRA1) family protein	-	2.30	-
lesaffx.59893.2.s1_at	AT1G55190– prenylated rab acceptor (PRA1) family protein	-	2.34	-
lesaffx.66384.1.s1_at	AT5G02040– prenylated rab acceptor (PRA1) family protein	8.29	3.14	5.87
lesaffx.67440.1.s1_at	AT4G35860– ATRABB1B, ATGB2, ATRAB2C (GTP-binding 2)	-	-	2.17
lesaffx.67491.1.s1_at	AT4G28950– ARAC7, RAC7, ROP9 (RHO-related protein from plants 9); GTP binding	-3.40	-3.40	-
lesaffx.68801.1.s1_at	AT5G52580– RAB GTPase activator	2.14	-	-
lesaffx.69528.1.s1_at	AT5G60860– AtRABA1f (Rab GTPase homolog A1f); GTP binding	3.62	5.31	6.37

Table G.5 (continued)

Probe Set ID	AT locus – Putative Annotation	Fold Change		
		WT AS	S2 AS	M48 AS
lesaffx.68259.1.s1_at	AT4G34460– ELK4, AGB1 (GTP binding protein beta 1)	4.68	-	-
lesaffx.69931.1.s1_at	AT5G03530– AtRab18B, AtRABC2a, ATRAB ALPHA (Rab GTPase homolog C2a); GTP binding	-3.50	-3.84	-2.84
lesaffx.70078.1.s1_at	AT4G18800– AthSGBP, AtRab11B, AtRABA1d (Rab GTPase homolog A1d); GTP binding	-	-	2.66
SIGNALING / RECEPTOR LIKE CYTOPLASMIC KINASES				
les.1281.1.a1_at	AT1G53430– LRR family protein / PK family protein	-4.25	-5.13	-4.42
les.1297.1.s1_at	AT3G21630– CERK1 (chitin elicitor receptor kinase 1); kinase/ receptor signaling protein	3.18	2.50	3.01
les.1806.1.s1_at	AT3G09830– PK, putative	2.40	3.50	3.32
les.2027.3.s1_at	AT2G23770– PK family protein / peptidoglycan-binding LysM domain-containing protein	20.65	9.87	5.37
les.3502.1.s1_at	AT2G05940– PK, putative	9.92	22.93	15.35
les.4777.1.s1_at	AT5G63940– PK family protein	-	-3.86	-2.39
les.5382.1.s1_at	AT3G13690– PK family protein	-4.78	-3.11	-4.94
les.975.1.s1_at	AT2G02800– APK2B (PK 2B); kinase	3.13	5.00	3.77
lesaffx.10313.1.a1_at	AT2G17220– PK, putative	7.15	6.96	5.11
lesaffx.16082.1.s1_at	AT3G08760– TSIK; kinase	4.10	2.29	2.39
lesaffx.26661.1.a1_at	AT5G47070– PK, putative	-	4.02	-
lesaffx.26661.1.s1_at	AT5G47070– PK, putative	5.87	13.83	9.14
lesaffx.38907.2.s1_at	AT3G51550– FER (FERONIA); kinase	5.05	4.53	6.05
lesaffx.56130.1.s1_at	AT1G25390– PK family protein	2.51	2.12	2.04
lesaffx.58097.1.s1_at	AT2G07180– PK, putative	2.59	4.80	2.50
lesaffx.63721.1.s1_at	AT5G42440– PK family protein	5.90	-	2.45

Table G.6 Significantly (P<0.05) regulated transcripts involved in large enzyme families upon exposure to freezing stress. Probe set ID, *Arabidopsis thaliana* (AT) locus name and putative annotation of each transcript is listed. Values represent fold changes of transcripts in WT, S2 and M48 after freezing stress compared to normal growth conditions. Negative values show down-regulation and positive values show up-regulation. Fold changes less than 2 are indicated with - (TF: Transcription factor, LHC: light harvesting complex, CYP: Cytochrome P450, GST: Glutathione S-transferase).

Probe Set ID	AT locus – Putative Annotation	Fold Change		
		WT AS	S2 AS	M48 AS
CYTOCHROME P450				
les.1859.2.s1_at	AT3G14690 – CYP72A15 (CYP, family 72, subfamily A, polypeptide 15); oxygen binding	2.90	-	-
les.1859.3.s1_at	AT3G14690 – CYP72A15 (CYP, family 72, subfamily A, polypeptide 15); oxygen binding	7.65	-	-
les.2988.1.s1_at	AT2G30490 – ATC4H, C4H, CYP73A5 (cinnamate 4-hydroxylase); trans-cinnamate 4-monooxygenase	3.63	-	-
les.3094.2.s1_at	AT1G11680 – EMB1738, CYP51A2, CYP51, CYP51G1; oxygen binding	4.55	-	-
les.3645.1.s1_at	AT5G38970 – ATBR6OX, CYP85A1, BR6OX1 (brassinosteroid-6-oxidase); oxygen binding	-	-2.72	-2.98
les.438.1.s1_at	AT1G69780 – ATHB13; DNA binding / transcription factor	-3.45	-3.03	-6.99

Table G.6 (continued)

Probe Set ID	AT locus – Putative Annotation	Fold Change		
		WT AS	S2 AS	M48 AS
les.4443.1.a1_s_at	AT5G38970 – ATBR6OX, CYP85A1, BR6OX1 (brassinosteroid-6-oxidase); oxygen binding	-2.45	-4.45	-6.17
les.4880.1.s1_at	AT5G07990 – CYP75B1, D501, TT7 (transparent testa 7); flavonoid 3'-monooxygenase/ oxygen binding	-	-	3.36
les.4915.1.s1_at	AT3G53130 – CYP97C1, LUT1 (lutein deficient 1); oxygen binding	-2.17	-	-
les.4980.1.s1_at	AT4G36220 – CYP84A1, FAH1 (ferulate-5-hydroxylase 1); ferulate 5-hydroxylase	-	-5.45	-2.41
les.5057.1.s1_at	AT3G61880 – CYP78A9; oxygen binding	-2.06	-4.73	-8.21
lesaffx.22297.1.s1_at	AT2G34500 – CYP710A1 (CYP, family 710, subfamily A, polypeptide 1); C-22 sterol desaturase/ oxygen binding	8.77	2.84	4.57
lesaffx.3253.2.s1_at	AT4G19230 – CYP707A1 (CYP, family 707, subfamily A, polypeptide 1); oxygen binding	2.40	5.52	3.00
lesaffx.3698.3.s1_at	AT4G30210 – AR2, ATR2 (P450 reductase 2)	5.11	6.74	4.94
lesaffx.51311.1.s1_at	AT4G37320 – CYP81D5 (CYP, family 81, subfamily D, polypeptide 5); oxygen binding	2.23	-	-
lesaffx.59842.1.s1_at	AT3G50660 – CYP90B1, CLM, SNP2, DWF4 (DWARF 4)	-3.13	-3.88	-5.52
lesaffx.63244.1.s1_at	AT2G45580 – CYP76C3 (CYP, family 76, subfamily C, polypeptide 3); oxygen binding	-	-2.83	-
lesaffx.8720.2.s1_at	AT3G52970 – CYP76G1 (CYP, family 76, subfamily G, polypeptide 1); oxygen binding	2.15	-	-
lesaffx.9038.1.s1_at	AT2G32440 – CYP88A4, KAO2 (ent-kaurenoic acid hydroxylase 2); oxygen binding	-2.14	-9.62	-
lesaffx.9038.3.s1_at	AT4G37400 – CYP81F3 (CYP, family 81, subfamily F, polypeptide 3); oxygen binding	2.92	4.29	5.59
GST				
les.131.1.s1_at	AT3G09270 – ATGSTU8 (AT Glutathione S-transferase (class tau) 8)	-	2.75	2.25
les.293.1.s1_at	AT2G29420 – GST25, ATGSTU7 (glutathione S-transferase 25)	12.37	9.60	14.76
les.3276.3.s1_at	AT1G78380 – GST8, ATGSTU19 (glutathione transferase 8)	-2.30	-	-
les.3735.1.s1_at	AT2G29420 – GST25, ATGSTU7 (glutathione s-transferase 25)	-	2.23	3.69
les.4501.1.s1_at	AT3G09270 – ATGSTU8 (AT Glutathione S-transferase (class tau) 8)	2.10	3.55	4.78
lesaffx.1959.1.s1_at	AT2G47730 – GST6, ATGSTF5, GSTF8, ATGSTF8 (glutathione S-transferase 8)	3.71	2.33	7.32
lesaffx.27206.1.s1_at	AT2G02390 – GST18, ATGSTZ1 (glutathione S-transferase 18)	-3.26	-3.44	-2.43
lesaffx.3002.1.s1_at	AT3G09270 – ATGSTU8 (AT Glutathione S-transferase (class tau) 8)	3.83	2.45	5.36
OXIDASES				
les.1478.1.s1_at	AT1G23740 – oxidoreductase, zinc-binding dehydrogenase family protein	-2.46	-	-2.08
les.2189.1.s1_at	AT1G76160 – SKS5 (SKU5 Similar 5); copper ion binding / oxidoreductase	2.59	4.19	6.88
les.2704.2.s1_at	AT5G37510 – EMB1467 (Embryo Defective 1467); NADH dehydrogenase	2.27	-	-
les.4618.1.s1_at	AT4G15760 – monooxygenase, putative (MO1)	-	3.32	-
les.5468.1.s1_at	AT5G16990 – NADP-dependent oxidoreductase, putative	-2.21	-2.30	-2.11
lesaffx.21603.1.s1_at	AT5G16990 – NADP-dependent oxidoreductase, putative	-6.97	-9.45	-8.05
lesaffx.2934.1.s1_at	AT2G35660 – CTF2A; monooxygenase	3.17	4.91	3.26

Table G.6 (continued)

Probe Set ID	AT locus – Putative Annotation	Fold Change		
		WT AS	S2 AS	M48 AS
lesaffx.44382.1.s1_at	AT1G76160 – SKS5 (SKU5 Similar 5); copper ion binding / oxidoreductase	8.23	-	3.63
lesaffx.4617.1.a1_at	AT4G12420 – SKU5 (skewed 5); copper ion binding	2.88	4.47	3.31
lesaffx.46243.1.s1_at	AT1G65840 – ATPAO4 (polyamine oxidase 4); amine oxidase	-	3.30	-
lesaffx.50540.1.s1_at	AT3G28480 – oxidoreductase, 2OG-Fe(Agarwal, *et al.*) oxygenase family protein	3.32	-	2.43
PEROXIDASES				
les.2092.1.s1_at	AT4G21960 – PRXR1 (peroxidase 42)	-20.96	-6.30	-18.48
les.2832.1.s1_at	AT1G80070 – EMB158, EMB33, EMB177, EMB14, SUS2 (abnormal suspensor 2)	-	4.11	-
les.3198.1.s1_at	AT1G05260 – RCI3A, RCI3 (rare cold inducible gene 3); peroxidase	-	-2.32	-2.04
les.3608.1.s1_at	AT5G06720 – peroxidase, putative	-	-3.17	-
les.4492.2.s1_at	AT1G15820 – CP24, LHCB6 (LHCPSII); chlorophyll binding	-3.89	-2.62	-4.45
les.4492.3.s1_at	AT1G15820 – CP24, LHCB6 (LHCPSII); chlorophyll binding	-4.06	-4.08	-4.44
les.4999.1.s1_at	AT2G37130 – peroxidase 21 (PER21) (P21) (PRXR5)	-	-3.42	-
lesaffx.32359.1.s1_at	AT4G37530 – peroxidase, putative	2.75	2.41	3.21
lesaffx.55638.1.s1_at	AT1G30870 – cationic peroxidase, putative	2.45	-	-
lesaffx.57363.1.s1_at	AT1G14550 – anionic peroxidase, putative	4.14	13.59	4.67
lesaffx.66432.1.s1_at	AT4G37530 – peroxidase, putative	4.33	3.66	3.76
lesaffx.71388.1.s1_at	AT4G33420 – peroxidase, putative	-3.49	-	-2.68
PHOSPHATASES				
les.3614.1.s1_at	AT3G17790 – ATPAP17, PAP17, ATACP5 (acid phosphatase 5); protein serine/threonine phosphatase	-	-	-2.21
les.3743.1.s1_at	AT4G25150 – acid phosphatase, putative	-	2.10	3.70
les.4141.1.s1_at	AT4G25150 – acid phosphatase, putative	-	-	2.70
lesaffx.24391.1.s1_at	AT1G72880 – acid phosphatase survival protein SurE, putative	3.32	-	-
lesaffx.3438.1.a1_at	AT3G02600 – LPP3, ATLPP3 (lipid phosphate phosphatase 3)	-3.66	-3.02	-2.35
lesaffx.44584.1.a1_at	AT5G03080 – phosphatidic acid phosphatase-related / PAP2-related	3.60	3.20	6.56
lesaffx.67373.1.s1_at	AT1G09870 – histidine acid phosphatase family protein	2.10	-	-
lesaffx.67373.2.s1_at	AT1G09870 – histidine acid phosphatase family protein	7.92	-	3.04
UDP GLUCOSYL AND GLUCORONYL TRANSFERASES				
les.1155.1.s1_at	AT4G34120 – LEJ1 (loss of the timing of ET and JA biosynthesis 1) CBS domain-containing protein	4.41	6.91	8.52
les.1238.1.s1_at	AT3G15940 – glycosyl transferase family 1 protein	-2.52	-	-
les.2659.2.a1_at	AT1G61050 – alpha 1,4-glycosyltransferase family protein / glycosyltransferase sugar-binding DXD motif-containing protein	-	-3.10	-
les.2826.1.s1_at	AT1G06780 – GAUT6 (Galacturonosyltransferase 6); polygalacturonate 4-alpha-galacturonosyltransferase	-	2.10	-
les.2826.2.a1_at	AT1G06780 – GAUT6 (Galacturonosyltransferase 6); polygalacturonate 4-alpha-galacturonosyltransferase	-	2.13	-
les.3777.1.s1_at	AT4G34135 – UGT73B2; UDP-glycosyltransferase	7.33	36.46	10.48

Table G.6 (continued)

Probe Set ID	AT locus – Putative Annotation	Fold Change		
		WT AS	S2 AS	M48 AS
les.5538.1.s1_at	AT5G01220 – SQD2 (sulfoquinovosyl diacylglycerol 2); UDP-sulfoquinovose:DAG sulfoquinovosyltransferase	-3.03	-2.00	-2.66
les.5832.1.s1_at	AT2G36750 – UGT72C1 (UDP-glucosyl transferase 72C1); UDP-glycosyltransferase	2.61	3.37	2.50
les.842.1.s1_at	AT1G01420 – UDP-glucoronosyl/UDP-glucosyl transferase family protein	2.08	-	-
les.842.2.s1_a_at	AT1G01420 – UDP-glucoronosyl/UDP-glucosyl transferase family protein	26.96	-	-
les.842.2.s1_at	AT4G01070 – GT72B1; UDP-glucosyltransferase	2.64	-	-
les.938.1.a1_at	AT2G20370 – KAM1, MUR3 (MURUS 3); catalytic/ transferase, transferring glycosyl groups	-	2.23	-
lesaffx.15898.2.s1_at	AT1G05680 – UDP-glucoronosyl/UDP-glucosyl transferase family protein	4.23	-	2.43
lesaffx.17107.1.a1_at	AT3G01040 – GAUT13 (Galacturonosyltransferase 13)	17.75	4.09	4.65
lesaffx.2369.1.s1_at	AT5G61840 – GUT1; catalytic	2.21	-	2.24
lesaffx.2471.1.s1_at	AT4G02500 – ATXT2; UDP-xylosyltransferase, transferring glycosyl groups	4.78	4.48	4.35
lesaffx.31317.8.s1_at	AT1G73740 – glycosyl transferase family 28 protein	2.25	-	-
lesaffx.47218.1.s1_at	AT5G61840 – GUT1; catalytic	3.91	-	-
lesaffx.59059.1.s1_at	AT1G70090 – GATL9, LGT8 (Galacturonosyltransferase-like 9)	19.62	9.71	14.91
lesaffx.59059.2.s1_at	AT1G70090 – GATL9, LGT8 (Galacturonosyltransferase-like 9)	5.92	2.94	3.56
lesaffx.61149.1.s1_at	AT5G49690 – UDP-glucoronosyl/UDP-glucosyl transferase family protein	3.79	-	-
lesaffx.66380.1.s1_at	AT4G34131 – UGT73B3 (UDP- glycosyltransferase 73B3); abscisic acid glucosyltransferase	4.61	6.40	-
lesaffx.6688.1.s1_at	AT4G14090 – UDP-glucoronosyl/UDP-glucosyl transferase family protein	3.48	2.47	-
lesaffx.68107.1.s1_at	AT3G28340 – GATL10 (Galacturonosyltransferase-like 10); polygalacturonate 4-alpha-galacturonosyltransferase	13.07	27.07	30.45
lesaffx.70136.2.s1_at	AT5G54010 – glycosyltransferase family protein	-	-	-2.15
lesaffx.70524.1.s1_at	AT3G21750 – UDP-glucoronosyl/UDP-glucosyl transferase family protein	2.92	-	-

Table G.7 Significantly (P<0.05) regulated transcripts involved in secondary metabolism upon exposure to freezing stress. Probe set ID, *Arabidopsis thaliana* (AT) locus name and putative annotation of each transcript is listed. Values represent fold changes of transcripts in WT, S2 and M48 after freezing stress compared to normal growth conditions. Negative values show down-regulation and positive values show up-regulation. Fold changes less than 2 are indicated with - (TF: Transcription factor, LRR: leucine-rich repeat).

Probe Set ID	AT locus – Putative Annotation	Fold Change		
		WT AS	S2 AS	M48 AS
SECONDARY METABOLISM / FLAVONOIDS				
les.1205.3.s1_at	AT3G29590 – AT5MAT; O-malonyltransferase/ transferase	-	-2.29	-2.53
les.1664.1.s1_at	AT3G55410 – 2-oxoglutarate dehydrogenase E1 component, putative / oxoglutarate decarboxylase, putative / alpha-ketoglutaric dehydrogenase, putative	-	2.12	2.65
les.1968.1.a1_at	AT5G05270 – chalcone-flavanone isomerase family protein	2.48	5.26	-
lesaffx.12150.1.a1_at	AT2G23910 – cinnamoyl-CoA reductase-related	3.79	4.38	4.79

Table G.7 (continued)

Probe Set ID	AT locus – Putative Annotation	Fold Change		
		WT AS	S2 AS	M48 AS
les.2278.1.s1_at	AT3G51240 – TT6, F3H (transparent testa 6); naringenin 3-dioxygenase	-	-	2.91
les.3649.1.s1_at	AT5G13930 – CHS, TT4, ATCHS (chalcone synthase); naringenin-chalcone synthase	-2.25	-3.31	-2.32
les.4979.1.s1_at	AT1G15950 – IRX4, ATCCR1, CCR1 (cinnamoyl coa reductase 1)	-2.78	-	-2.91
les.842.1.s1_at	AT1G01420 – UDP-glucoronosyl/UDP-glucosyl transferase family protein	2.08	-	-
les.842.2.s1_a_at	AT1G01420 – UDP-glucoronosyl/UDP-glucosyl transferase family protein	26.96	-	-
lesaffx.31836.1.s1_at	AT1G68540 – oxidoreductase family protein	6.07	4.41	8.95
lesaffx.40547.1.s1_at	AT4G34540 – isoflavone reductase family protein	-2.30	-	-
lesaffx.68320.1.s1_at	AT5G05270 – chalcone-flavanone isomerase family protein	2.78	6.93	2.62
SECONDARY METABOLISM / ISOPRENOIDS				
les.3123.1.s1_at	AT1G74470 – geranylgeranyl reductase	-5.48	-2.59	-4.78
les.3510.1.s1_at	AT4G15560 – DEF, CLA, DXS, DXPS2, CLA1 (cloroplastos alterados 1) 1-deoxy-D-xylulose 5-phosphate synthase, putative	-2.80	-2.90	-3.50
les.3544.1.s1_at	AT5G52570 – B2, CHY2, BETA-OHASE 2 (beta-carotene hydroxylase 2)	4.76	-	2.31
les.3545.1.s1_at	AT4G25700 – B1, CHY1, BETA-OHASE 1 (beta-hydroxylase 1)	2.08	-	-
les.3644.1.s1_at	AT3G10230 – LYC (lycopene cyclase)	2.70	-	2.11
les.415.1.a1_at	AT5G58770 – dehydrodolichyl diphosphate synthase, putative / DEDOL-PP synthase, putative	-5.77	-3.39	-5.85
les.4438.1.a1_s_at	AT5G17230 – PSY (phytoene synthase)	-	-2.74	-2.03
les.4989.1.s1_at	AT5G12210 – geranylgeranyl transferase type II beta subunit, putative	-	-2.68	-
les.5562.1.s1_at	AT3G63410 – VTE3, APG1 (albino or pale green mutant 1); methyltransferase	-2.12	-	-2.69
les.601.1.s1_at	AT2G26930 – PDE277, ISPE, ATCDPMEK (pigment defective 277); 4-(cytidine 5'-diphospho)-2-C-methyl-D-erythritol kinase	2.50	-	3.35
lesaffx.44987.1.s1_at	AT1G78510 – SPS1 (solanesyl diphosphate synthase 1)	5.31	3.00	3.11
lesaffx.44987.3.s1_at	AT1G78510 – SPS1 (solanesyl diphosphate synthase 1)	2.29	3.26	-
SECONDARY METABOLISM / PHENYLPROPANOIDS				
les.1097.1.a1_at	AT3G21240 – AT4CL2 (4-coumarate-CoA ligase 2)	-	-	2.09
les.220.1.s1_at	AT5G01210 – transferase family protein	2.36	-	-
les.281.1.s1_at	AT1G51680 – AT4CL1 (4-coumarate-COA ligase 1)	9.69	4.68	5.22
les.281.3.s1_at	AT1G51680 – AT4CL1 (4-coumarate-COA ligase 1)	13.99	-	2.03
les.2934.2.s1_at	AT4G37990 – ELI3-2 (elicitor-activated gene 3)	-2.28	-	-2.39
les.3741.1.a1_at	AT4G37980 – ELI3-1 (elicitor-activated gene 3); binding / catalytic/ oxidoreductase/ zinc ion binding	3.77	2.51	2.71
les.3741.1.s1_at	AT4G37980 – ELI3-1 (elicitor-activated gene 3); binding / catalytic/ oxidoreductase/ zinc ion binding	7.18	-	4.88
les.4271.1.s1_at	AT2G37040 – PAL1 (PHE ammonia lyase 1); phenylalanine ammonia-lyase	3.19	4.78	4.19
les.5848.2.s1_at	AT1G07040 – similar to unknown protein	-	2.12	-
les.4271.2.s1_at	AT2G37040 – PAL1 (PHE ammonia lyase 1); phenylalanine ammonia-lyase	8.59	8.67	7.79

Table G.7 (continued)

Probe Set ID	AT locus – Putative Annotation	Fold Change		
		WT AS	S2 AS	M48 AS
les.5068.1.s1_at	AT5G23230 – NIC2 (nicotinamidase 2); catalytic/ nicotinamidase	-2.55	-	-2.50
les.5174.1.s1_at	AT4G05160 – 4-coumarate-CoA ligase, putative / 4-coumaroyl-CoA synthase, putative	-	2.21	-
les.5848.1.a1_at	AT1G65060 – 4CL3 (4-coumarate-CoA ligase 3)	3.09	5.84	4.77
lesaffx.42701.1.s1_at	AT2G22570 – NIC2, NIC1 (nicotinamidase 1); catalytic	-3.78	-3.30	-2.83
lesaffx.47885.1.s1_at	AT1G20510 – OPCL1 (OPC-8:0 COA ligase1); 4-coumarate-CoA ligase	3.39	-	-
CELL WALL PRECURSOR SYNTHESIS				
les.1852.1.a1_at	AT3G29360 – UDP-glucose 6-dehydrogenase, putative	4.65	3.72	5.93
les.1852.2.s1_at	AT3G29360 – UDP-glucose 6-dehydrogenase, putative	6.46	4.92	9.45
les.1852.3.s1_at	AT3G29360 – UDP-glucose 6-dehydrogenase, putative	35.78	2.73	3.74
les.2813.1.s1_at	AT1G08200 – AXS2 (UDP-D-Apiose/UDP-D-Xylose Synthase 2)	14.68	2.89	8.69
les.2813.2.a1_at	AT1G08200 – AXS2 (UDP-D-Apiose/UDP-D-Xylose Synthase 2)	5.79	6.06	7.06
les.4583.1.s1_at	AT4G30440 – GAE1 (UDP-D-glucuronate 4-epimerase 1); UDP-glucuronate 4-epimerase/ catalytic	-	5.41	-
les.963.2.s1_at	AT1G30620 – HSR8, MUR4, UXE1 (MURUS 4)	5.97	3.11	7.21
lesaffx.14736.1.s1_at	AT3G46440 – UXS5 (UDP-Xyl synthase 5); catalytic, NAD-dependent epimerase/dehydratase family protein	2.48	2.73	5.24
lesaffx.31904.2.s1_at	AT1G63000 – UER1, NRS/ER (nucleotide-rhamnose synthase/epimerase-reductase)	-	-	3.22
lesaffx.38740.1.s1_at	AT4G10960 – UGE5 (UDP-D-glucose/UDP-D-galactose 4-epimerase 5); UDP-glucose 4-epimerase/ protein dimerization	19.62	6.08	12.36
lesaffx.62950.1.s1_at	AT4G23920 – UGE2 (UDP-D-glucose/udp-d-galactose 4-epimerase 2); UDP-glucose 4-epimerase/ protein dimerization	6.15	-	-
lesaffx.9043.1.s1_at	AT3G62830 – AUD1, ATUXS2, (UDP-glucuronic acid decarboxylase 2) NAD-dependent epimerase/dehydratase family protein	3.90	-	5.14
CELL WALL MODIFICATION				
les.141.1.s1_at	AT2G37640 – ATHEXP ALPHA 1.9, ATEXPA3 (expansin A3)	3.11	3.25	-
les.210.1.s1_at	AT3G23730 – xyloglucan:xyloglucosyl transferase, putative / xyloglucan endotransglycosylase, putative / endo-xyloglucan transferase, putative	4.08	40.18	6.01
les.2688.1.s1_at	AT1G20190 – ATHEXP ALPHA 1.14, ATEXPA11 (AT expansin A11)	-11.95	-8.12	-18.22
les.276.1.s1_at	AT4G14130 – XTR7 XTR7 (xyloglucan endotransglycosylase 7); hydrolase, acting on glycosyl bonds	18.08	34.79	23.63
les.3537.1.a1_at	AT2G01850 – ATXTH27, EXGT-A3 (endo-xyloglucan transferase A3); hydrolase, acting on glycosyl bonds / xyloglucan:xyloglucosyl transferase	-	2.27	-
les.3537.1.s1_at	AT2G01850 – ATXTH27, EXGT-A3 (endo-xyloglucan transferase A3); hydrolase, acting on glycosyl bonds / xyloglucan:xyloglucosyl transferase	-	12.76	-
les.3590.1.s1_at	AT5G13870 – EXGT-A4 (endoxyloglucan transferase A4); hydrolase, acting on glycosyl bonds	-	30.66	-
les.369.1.s1_at	AT1G26770 – ATEXPA10 (AT expansin A10)	-7.43	-7.15	-7.57
les.3697.1.s1_at	AT5G57550 – XTR3 (xyloglucan endotransglycosylase 3); hydrolase, acting on glycosyl bonds	-2.88	-2.56	-8.02

Table G.7 (continued)

Probe Set ID	AT locus – Putative Annotation	Fold Change		
		WT AS	S2 AS	M48 AS
les.3733.1.s1_at	AT2G40610 – ATEXPA8 (AT EXPANSIN A8)	-46.61	-80.33	-62.38
les.3972.1.s1_at	AT2G39700 – ATEXPA4 (AT EXPANSIN A4)	-3.37	-5.73	-2.19
les.4008.2.s1_at	AT4G03210 – XTH9 (xyloglucan endotransglucosylase/hydrolase 9); hydrolase, acting on glycosyl bonds	2.24	-	2.37
les.429.1.s1_at	AT4G14130 – XTR7 (xyloglucan endotransglycosylase 7); hydrolase, acting on glycosyl bonds	64.91	98.00	137.54
les.4353.1.s1_at	AT4G25810 – XTH23, XTR6 (xyloglucan endotransglycosylase 6); hydrolase, acting on glycosyl bonds	46.99	134.99	62.27
les.4530.1.s1_at	AT3G23730 – xyloglucan:xyloglucosyl transferase, / xyloglucan endotransglycosylase, / endo-xyloglucan transferase/ putative	2.20	14.64	4.87
les.4769.1.s1_at	AT3G45970 – ATEXPL1, ATEXLA1 (expansin-like A1)	5.15	36.01	8.37
les.4968.1.s1_s_at	AT2G01850 – ATXTH27, EXGT-A3 (endo-xyloglucan transferase A3); hydrolase, acting on glycosyl bonds / xyloglucan:xyloglucosyl transferase	-	9.08	-
lesaffx.4662.1.s1_at	AT1G10550 – XET, XTH33 (xyloglucan:xyloglucosyl transferase 33); hydrolase, acting on glycosyl bonds	10.25	27.39	9.38
lesaffx.62989.1.s1_at	AT4G25810 – XTH23, XTR6 (xyloglucan endotransglycosylase 6); hydrolase, acting on glycosyl bonds	-	-	2.25
CELL WALL PECTIN ESTERASES				
les.3122.1.s1_a_at	AT1G11580 – PMEPCRA pectinesterase	-	-2.08	-2.33
les.4754.1.s1_at	AT5G23870 – pectinacetylesterase family protein	-	-	-2.29
les.5233.1.s1_at	AT1G53840 – ATPME1 (pectin methylesterase 1); pectinesterase	-	2.65	2.08
les.5410.1.s1_at	AT5G26670 – pectinacetylesterase, putative	6.80	5.72	4.73
les.67.1.s1_at	AT3G14310 – ATPME3 (AT pectin methylesterase 3)	-9.56	-7.01	-2.66
lesaffx.10497.2.a1_at	AT3G49220 – pectinesterase family protein	-3.89	-	-3.00
lesaffx.42926.2.a1_at	AT2G46930 – pectinacetylesterase, putative	-	2.02	-
CELL WALL PROTEINS				
les.2584.1.a1_at	AT3G19320 – LRR family protein	-3.39	-2.00	-3.62
les.2584.2.s1_a_at	AT3G19320 – LRR family protein	-	-	-2.43
les.2946.2.s1_at	– extensin-like protein	-2.30	-6.28	-2.51
les.3320.2.s1_at	AT3G22440 – hydroxyproline-rich glycoprotein family protein	23.77	-	-
les.3320.3.s1_at	AT3G22440 – hydroxyproline-rich glycoprotein family protein	2.70	-	-
les.4368.1.s1_s_at	AT1G62440 – LRX2 (LRR/Extensin 2); protein binding / structural constituent of cell wall	-	12.85	13.49
les.4739.1.s1_at	AT3G02230 – ATRGP1, RGP1 (reversibly glycosylated polypeptide 1)	2.50	-	4.08
lesaffx.43685.2.s1_s_at	AT1G62440 – LRX2 (LRR/Extensin 2); protein binding / structural constituent of cell wall	-	14.69	13.13
CELL WALL DEGRADATION				
les.2187.1.a1_at	AT5G66460 – (1-4)-beta-mannan endohydrolase, putative	-	-2.32	-3.14
les.2298.2.a1_a_at	AT3G16850 – glycoside hydrolase family 28 protein / polygalacturonase (pectinase) family protein	-	-	-2.78

Table G.7 (continued)

Probe Set ID	AT locus – Putative Annotation	Fold Change		
		WT AS	S2 AS	M48 AS
les.1847.1.a1_at	AT3G47000 – glycosyl hydrolase family 3 protein	-	-	2.16
les.3991.1.s1_at	AT5G64570 – ATBXL4, XYL4 (beta-xylosidase 4); hydrolase, hydrolyzing O-glycosyl compounds, glycosyl hydrolase family 3 protein	-14.87	-2.08	-12.52
les.5081.1.s1_at	AT3G47000 – glycosyl hydrolase family 3 protein	-	2.94	-
les.5495.1.s1_at	AT3G55140 – pectate lyase family protein	-2.93	-	-2.66
les.5579.1.s1_at	AT4G13710 – pectate lyase family protein	-5.12	-8.45	-
les.62.1.s1_at	AT5G27530 – glycoside hydrolase family 28 protein / polygalacturonase (pectinase) family protein	-	-	2.05
lesaffx.59336.1.s1_at	AT1G49320 – BURP domain-containing protein, similarity to RD22 precursor	-	-	-2.02

Table G.8 Significantly (P<0.05) regulated transcripts involved in carbohydrate (CH) and lipid metabolism upon exposure to freezing stress. Probe set ID, *Arabidopsis thaliana* (AT) locus name and putative annotation of each transcript is listed. Values represent fold changes of transcripts in WT, S2 and M48 after freezing stress compared to normal growth conditions. Negative values show down-regulation and positive values show up-regulation. Fold changes less than 2 are indicated with - (TF: Transcription factor).

Probe Set ID	AT locus – Putative Annotation	Fold Change		
		WT AS	S2 AS	M48 AS
CH METABOLISM / SUCROSE SYNTHESIS				
les.1617.1.s1_s_at	AT1G43670 – fructose-1,6-bisphosphatase, / D-fructose-1,6-bisphosphate 1-phosphohydrolase, / FBPase, putative	-17.20	-3.88	-7.77
les.1617.2.s1_s_at	AT1G43670 – fructose-1,6-bisphosphatase/ D-fructose-1,6-bisphosphate 1-phosphohydrolase/ FBPase, putative	-22.65	-3.82	-14.62
les.1617.3.a1_s_at	AT1G43670 – fructose-1,6-bisphosphatase / D-fructose-1,6-bisphosphate 1-phosphohydrolase / FBPase, putative	-18.48	-2.52	-13.03
les.4675.1.s1_at	AT1G43670 – fructose-1,6-bisphosphatase/ D-fructose-1,6-bisphosphate 1-phosphohydrolase/ FBPase, putative	-2.15	-	-
les.4946.1.a1_at	AT1G43670 – fructose-1,6-bisphosphatase / D-fructose-1,6-bisphosphate 1-phosphohydrolase, / FBPase, putative	-2.30	-	-2.15
les.4946.1.s1_at	AT1G43670 – fructose-1,6-bisphosphatase / D-fructose-1,6-bisphosphate 1-phosphohydrolase, / FBPase, putative	-23.43	-3.35	-13.99
CH METABOLISM / SUCROSE DEGRADATION				
les.157.1.s1_at	AT3G43190 – SUS4; UDP-glycosyltransferase/ sucrose synthase/ transferase, transferring glycosyl groups	4.11	7.86	3.54
les.3458.1.s1_at	AT3G52600 – ATCWINV2 (cell wall invertase 2); hydrolase, hydrolyzing O-glycosyl compounds	-2.21	-3.28	-2.56
les.3460.1.s1_at	AT3G52600 – ATCWINV2 (cell wall invertase 2); hydrolase, hydrolyzing O-glycosyl compounds	3.85	3.95	-
les.3639.1.s1_at	AT5G51830 – pfkB-type carbohydrate kinase family protein	2.43	-	-
lesaffx.53904.1.s1_at	AT5G40510 – unknown protein	2.37	-	2.58
CH METABOLISM / STARCH SYNTHESIS				
les.1248.2.s1_a_at	AT2G39770 – VTC1, SOZ1, EMB101, GMP1, CYT1 (Cytokinesis Defective 1); nucleotidyltransferase , gdp-mannose pyrophosphorylase	-	-2.04	-
les.1310.1.s1_at	AT1G32900 – starch synthase, putative	-5.99	-	-3.97
les.4650.1.s1_at	AT2G39770 – VTC1, SOZ1, EMB101, GMP1, CYT1 (Cytokinesis Defective 1); nucleotidyltransferase , gdp-mannose pyrophosphorylase	-2.33	-	-

Table G.8 (continued)

Probe Set ID	AT locus – Putative Annotation	Fold Change		
		WT AS	S2 AS	M48 AS
les.3350.1.s1_at	AT3G01180 – ATSS2 (STARCH SYNTHASE 2); transferase, transferring glycosyl groups	-3.01	-2.73	-
les.4764.1.s1_at	AT2G36390 – BE3, SBE2.1 (starch branching enzyme 2.1); 1,4-alpha-glucan branching enzyme	-	2.24	2.14
les.4890.1.s1_at	AT5G24300 – SSI, ATSS1 (starch synthase I); transferase, transferring glycosyl groups	-5.12	-3.02	-2.75
les.78.1.s1_at	AT5G19220 – APL1, ADG2 (ADPG pyrophosphorylase 2); glucose-1-phosphate adenylyltransferase	-6.40	-3.32	-4.52
les.79.1.s1_a_at	AT1G27680 – APL2 (large subunit of AGP 2) glucose-1-phosphate adenylyltransferase large subunit 2, chloroplast precursor	-	-2.01	-
CH METABOLISM / STARCH DEGRADATION				
les.1401.1.s1_at	AT5G18670 – BAM9, BMY3 (beta-amylase 9); beta-amylase	2.13	2.30	-
les.1401.2.s1_at	AT5G18670 – BAM9, BMY3 (beta-amylase 9); beta-amylase	7.78	2.57	2.26
les.1401.3.s1_at	AT5G18670 – BAM9, BMY3 (beta-amylase 9); beta-amylase	11.59	-	-
les.2844.1.s1_at	AT3G23920 – BMY7, TR-BAMY, BAM1 (beta-amylase 1); beta-amylase	4.42	4.94	7.11
les.3199.1.s1_at	AT3G46970 – ATPHS2, PHS2 (alpha-glucan phosphorylase 2); phosphorylase/ transferase, transferring glycosyl groups	-2.05	-	-
lesaffx.34425.1.s1_at	AT4G25000 – ATAMY1, AMY1 (alpha-amylase-like); alpha-amylase	2.05	-	2.45
lesaffx.44604.1.s1_at	AT5G26570 – ATGWD3, OK1, PWD (Phosphoglucan Water Dikinase)	-2.27	-	-
lesaffx.52329.1.s1_at	AT5G17520 – MEX1, RCP1 (Root Cap 1)	-5.39	-5.32	-5.93
lesaffx.9.1.s1_at	AT1G76130 – ATAMY2, AMY2 (alpha-amylase-like 2); alpha-amylase	2.05	2.47	2.76
LIPID METABOLISM / SYNTHESIS				
les.2265.1.s1_at	AT2G43710 – FAB2, SSI2 (fatty acid biosynthesis 2); acyl-[acyl-carrier-protein] desaturase	14.99	15.74	15.00
les.2747.2.s1_at	AT2G33150 – PED1, KAT2 (peroxisome defective 1); acetyl-CoA C-acyltransferase	-	-	2.08
les.2857.1.s1_at	AT5G17165 – unknown protein	-3.60	-2.65	-3.30
les.3383.1.s1_at	AT3G48990 – AMP-dependent synthetase and ligase family protein	5.81	15.25	2.28
les.4040.1.a1_at	AT2G26640 – beta-ketoacyl-CoA synthase, putative	2.51	-	2.64
les.4040.1.s1_at	AT2G26640 – beta-ketoacyl-CoA synthase, putative	14.51	4.69	7.78
les.5293.1.s1_at	AT2G26640 – beta-ketoacyl-CoA synthase, putative	6.62	16.49	8.16
les.5427.1.s1_at	AT3G16170 – acyl-activating enzyme 13 (AAE13)	-2.70	-	-2.26
les.5841.1.s1_at	AT2G33150 – PED1, KAT2 (peroxisome defective 1); acetyl-CoA C-acyltransferase	3.60	2.56	3.30
les.5952.1.s1_at	AT4G25050 – ACP4 (ACYL carrier protein 4)	-5.40	-	-3.91
les.971.1.a1_at	AT1G68530 – CER6, G2, POP1, CUT1 (CUTICULAR 1); catalytic	-2.39	-	-3.08
lesaffx.10235.1.s1_at	AT1G65880 – BZO1; benzoate-CoA ligase	-	-	2.42
lesaffx.29378.1.s1_at	AT5G46290 – KAS I (3-KETOACYL-ACYL carrier protein synthase I); fatty-acid synthase	-	-	2.76
lesaffx.36985.1.s1_at	AT5G47720 – acetyl-CoA C-acyltransferase, putative / 3-ketoacyl-CoA thiolase, putative	3.68	-	-
lesaffx.55337.2.s1_at	AT1G68530 – CER6, G2, POP1, CUT1 (cuticular 1); catalytic	2.96	-	-

Table G.8 (continued)

Probe Set ID	AT locus – Putative Annotation	Fold Change		
		WT AS	S2 AS	M48 AS
lesaffx.55353.1.s1_at	AT1G08510 – FATB (fatty acyl-ACP thioesterases B); acyl carrier/ acyl-ACP thioesterase	2.01	-	-
les.2415.3.a1_at	AT4G30950 – FADC, SFD4, FAD6 (fatty acid desaturase 6); omega-6 fatty acid desaturase	-	-	-2.71
lesaffx.38304.1.s1_at	AT3G15850 – FADB, JB67, ADS3, FAD5 (fatty acid desaturase 5); oxidoreductase	-6.08	-3.44	-3.53
les.3526.1.s1_a_at	AT2G20900 – diacylglycerol kinase, putative	6.63	7.96	7.11
les.4365.1.s1_at	AT1G32200 – ACT1, ATS1 (acyltransferase 1)	3.05	-	2.19
les.4710.1.s1_at	AT1G48600 – phosphoethanolamine N-methyltransferase 2, putative (NMT2)	-13.87	-21.76	-29.90
les.5034.1.s1_at	AT2G20900 – diacylglycerol kinase, putative	-2.38	-	-5.01
les.5388.1.a1_at	AT2G38670 – PECT1 (Phosphorylethanolamine cytidylyl Transferase 1); ethanolamine-phosphate cytidylyltransferase	3.76	2.28	5.28
lesaffx.6068.1.s1_at	AT2G45150 – phosphatidate cytidylyltransferase family protein	2.66	-	2.27
lesaffx.67256.1.s1_at	AT4G22340 – phosphatidate cytidylyltransferase, putative / CDP-diglyceride synthetase, putative	2.56	-	-
lesaffx.69290.1.s1_at	AT4G38570 – PIS2 (probable CDP-diacylglycerol--inositol 3-phosphatidyltransferase 2); phosphotransferase	2.36	-	5.06
lesaffx.69290.2.s1_at	AT4G38570 – PIS2 (probable cdp-diacylglycerol--inositol 3-phosphatidyltransferase 2); phosphotransferase	2.02	-	2.86
lesaffx.70325.2.s1_at	AT2G18730 – diacylglycerol kinase, putative	2.80	-	2.44
lesaffx.70664.1.s1_at	AT1G53000 – cytidylyltransferase family	-	-	2.55
LIPID METABOLISM / METABOLISM				
les.1510.1.s1_at	AT1G27980 – pyridoxal-dependent decarboxylase family protein	5.40	-	3.32
les.2708.1.s1_at	AT4G27270 – quinone reductase family protein	4.36	2.33	5.50
les.2708.2.s1_at	AT4G27270 – quinone reductase family protein	4.53	2.32	3.31
les.3024.1.a1_at	AT3G55360 – ECR, CER10, ATTSC13, TSC13 (enoyl-COA reductase); 3-oxo-5-alpha-steroid 4-dehydrogenase/ fatty acid elongase/ trans-2-enoyl-CoA reductase	-2.82	-3.43	-3.86
les.3710.1.s1_at	AT1G13580 – LAG13 (LAG1 Longevity Assurance Homolog 3)	9.63	17.02	21.85
les.4491.1.s1_at	AT4G19860 – lecithin:cholesterol acyltransferase family protein / LACT family protein	-2.53	-	-3.10
les.4619.1.s1_at	AT3G07020 – UDP-glucose:sterol glucosyltransferase (UGT80A2)	-	-2.17	-
les.799.1.a1_at	AT3G07020 – UDP-glucose:sterol glucosyltransferase (UGT80A2)	-2.10	-	-
lesaffx.2428.1.s1_at	AT5G23670 – LCB2 (Serine palmitoyltransferase LCB2 (long chain base) subunit gene); serine C-palmitoyltransferase	3.69	-	-
lesaffx.30807.1.s1_at	AT2G46210 – delta-8 sphingolipid desaturase, putative	2.47	2.72	3.58
lesaffx.60450.1.s1_at	AT3G45070 – sulfotransferase family protein	-	-	-2.81
lesaffx.70280.1.s1_at	AT4G34640 – ERG9, SQS1 (squalene synthase 1); farnesyl-diphosphate farnesyltransferase	6.83	-	2.09
lesaffx.71307.1.s1_at	AT5G58800 – quinone reductase family protein	3.67	5.24	4.96
LIPID METABOLISM / DEGRADATION				
les.5631.1.s1_at	AT4G38690 – 1-phosphatidylinositol phosphodiesterase-related	-	2.97	2.66
lesaffx.24167.1.s1_at	AT3G15650 – phospholipase / carboxylesterase family protein	-	2.21	3.73

Table G.8 (continued)

Probe Set ID	AT locus – Putative Annotation	Fold Change		
		WT AS	S2 AS	M48 AS
lesaffx.3244.1.s1_at	AT5G18640 – lipase class 3 family protein	2.19	-	-
lesaffx.3244.2.s1_at	AT5G18640 – lipase class 3 family protein	2.00	-	-
lesaffx.52646.1.s1_at	AT3G45300 – ATIVD, IVD (Isovaleryl-CoA-Dehydrogenase)	4.59	-	-
lesaffx.7472.1.s1_at	AT4G18550 – lipase class 3 family protein	-	-3.12	-

Table G.9 Significantly (P<0.05) regulated transcripts involved in energy metabolism (glycolysis, TCA, mitochondrial electron transfer) upon exposure to freezing stress. Probe set ID, *Arabidopsis thaliana* (AT) locus name and putative annotation of each transcript is listed. Values represent fold changes of transcripts in WT, S2 and M48 after freezing stress compared to normal growth conditions. Negative values show down-regulation and positive values show up-regulation. Fold changes less than 2 are indicated with - (TF: Transcription factor).

Probe Set ID	AT locus – Putative Annotation	Fold Change		
		WT AS	S2 AS	M48 AS
GLYCOLYSIS				
affx-les-gapdh-m_at	AT1G13440 – GAPC-2 glyceraldehyde 3-phosphate dehydrogenase, cytosolic, putative / NAD-dependent glyceraldehyde-3-phosphate dehydrogenase, putative	2.09	-	3.68
les.18.1.s1_at	AT3G14940 – ATPPC3 (phosphoenolpyruvate carboxylase 3)	-	-	2.30
les.19.1.s1_at	AT1G53310 – ATPPC1 (phosphoenolpyruvate carboxylase 1)	-4.17	-	-
les.2323.1.s1_at	AT3G52990 – pyruvate kinase, putative	2.56	-	4.44
les.262.1.s1_at	AT3G04120 – GAPC-1, GAPC (glyceraldehyde-3-phosphate dehydrogenase C subunit)	-	-	2.24
les.2677.1.s1_at	AT3G08590 – 2,3-biphosphoglycerate-independent phosphoglycerate mutase, putative / phosphoglyceromutase, putative	20.45	-	-
les.2677.2.s1_at	AT1G09780 – 2,3-biphosphoglycerate-independent phosphoglycerate mutase, putative / phosphoglyceromutase, putative	-	-	2.24
les.2715.1.s1_at	AT1G23190 – phosphoglucomutase, cytoplasmic, putative / glucose phosphomutase, putative	-2.12	-	-
les.2888.1.s1_at	AT3G26650 – GAPA-1, GAPA (glyceraldehyde 3-phosphate dehydrogenase A subunit)	-5.47	-	-2.94
les.2900.1.s1_at	AT4G04040 – MEE51 (maternal effect embryo arrest 51); diphosphate-fructose-6-phosphate 1-phosphotransferase	-2.24	-2.28	-
les.2909.2.s1_at	AT1G53310 – ATPPC1 (phosphoenolpyruvate carboxylase 1)	2.45	-	-
les.2933.1.s1_at	AT1G42970 – GAPB (glyceraldehyde-3-phosphate dehydrogenase B subunit)	-5.85	-2.78	-3.79
les.303.1.s1_at	AT2G36530 – LOS2 (Low expression of osmotically responsive genes 1); phosphopyruvate hydratase	2.48	2.61	5.20
les.3072.2.s1_at	AT1G12900 – GAPA-2 glyceraldehyde 3-phosphate dehydrogenase, chloroplast, putative / NADP-dependent glyceraldehydephosphate dehydrogenase	-3.91	-3.57	-4.11
les.3072.3.s1_at	AT3G26650 – GAPA-1, GAPA (glyceraldehyde 3-phosphate dehydrogenase A subunit)	-4.30	-2.03	-2.32
les.3129.1.s1_at	AT5G08570 – pyruvate kinase, putative	2.21	2.37	2.39
les.3129.2.s1_at	AT5G08570 – pyruvate kinase, putative	16.37	-	2.19
les.3242.1.a1_at	AT1G42970 – GAPB (glyceraldehyde-3-phosphate dehydrogenase B subunit)	-22.70	-3.89	-12.67

Table G.9 (continued)

Probe Set ID	AT locus – Putative Annotation	Fold Change		
		WT AS	S2 AS	M48 AS
les.3242.3.s1_at	AT1G42970 – GAPB (glyceraldehyde-3-phosphate dehydrogenase B subunit)	-18.67	-3.89	-7.85
les.3932.1.s1_at	AT2G36530 – LOS2 (Low expression of osmotically responsive genes 1); phosphopyruvate hydratase	2.52	-	-
les.4336.3.s1_at	AT1G56190 – phosphoglycerate kinase, putative	-2.82	-	-
les.5649.1.s1_at	AT1G32440 – PKP3 (plastidial pyruvate kinase 3)	5.84	4.85	4.70
les.716.1.s1_at	AT1G12230 – transaldolase, putative	-2.65	-2.62	-2.75
les.720.1.a1_at	AT3G52990 – pyruvate kinase, putative	-2.31	-2.15	-3.71
lesaffx.3379.1.a1_at	AT4G34030 – MCCB (3-methylcrotonyl-coa carboxylase); biotin carboxylase	3.22	3.11	2.91
lesaffx.51723.1.s1_at	AT4G26270 – phosphofructokinase family protein	-	-	2.22
lesaffx.55680.1.s1_at	AT5G64380 – fructose-1,6-bisphosphatase family protein	-3.26	-2.94	-2.46
lesaffx.58242.1.s1_at	AT3G50520 – phosphoglycerate / bisphosphoglycerate mutase family protein	-2.30	-	-
lesaffx.59008.1.s1_at	AT2G34590 – transketolase family protein	2.29	-	2.05
lesaffx.66367.1.s1_at	AT5G56350 – pyruvate kinase, putative	3.35	-	3.25
TCA				
les.1186.1.a1_at	AT4G35260 – IDH1 (isocitrate dehydrogenase 1)	-	-	2.04
les.2307.1.s1_at	AT5G40650 – SDH2-2 (succinate dehydrogenase 2-2)	2.07	2.34	2.53
les.4025.1.s1_at	AT5G23250 – succinyl-CoA ligase (GDP-forming) alpha-chain, mitochondrial, putative	2.33	2.13	3.89
les.4036.1.s1_at	AT2G20420 – succinyl-CoA ligase (GDP-forming) beta-chain, mitochondrial, putative	-	-	2.19
les.5115.1.s1_at	AT4G26910 – 2-oxoacid dehydrogenase family protein	2.45	2.16	3.13
lesaffx.10095.1.a1_at	AT3G25860 – PLE2, LTA2 (Plastid E2 Subunit of Pyruvate Decarboxylase); dihydrolipoyllysine-residue acetyltransferase	-	-	2.68
lesaffx.23253.1.s1_at	AT2G44350 – CSY4, ATCS (citrate synthase 4)	18.08	-	2.11
lesaffx.23253.2.s1_at	AT2G44350 – CSY4, ATCS (citrate synthase 4)	5.97	-	-
lesaffx.3802.1.s1_at	AT3G13930 – dihydrolipoamide S-acetyltransferase, putative	3.16	-	-
lesaffx.3802.2.s1_at	AT3G13930 – dihydrolipoamide S-acetyltransferase, putative	-	-	3.19
MITOCHONDRIAL ELECTRON TRANSFER				
les.2704.2.s1_at	AT5G37510 – EMB1467 (Embryo Defective 1467); NADH dehydrogenase	2.27	-	-
les.2760.1.s1_at	AT5G25450 – ubiquinol-cytochrome C reductase complex 14 kDa protein, putative	-	-	2.10
les.3021.1.s1_at	AT4G10040 – CYTC-2 (cytochrome C-2); electron carrier	-8.21	-6.15	-
les.4222.1.s1_at	AT1G32350 – AOX1D (alternative oxidase 1D)	19.01	27.46	32.43
les.4223.1.s1_at	AT3G22370 – AOX1A (alternative oxidase 1A)	2.55	2.85	2.53
les.4625.1.s1_at	AT1G50940 – ETFALPHA (Electron Transfer Flavoprotein Alpha); FAD binding / electron carrier	2.30	3.76	2.02
les.4785.1.s1_at	AT5G40810 – cytochrome c1, putative	-	-	2.00
les.5029.1.s1_at	AT1G49380 – cytochrome c biogenesis protein family	-3.83	-2.23	-5.01
lesaffx.33042.1.s1_at	AT5G40382 – cytochrome-c oxidase	-2.96	-	-6.23
lesaffx.65209.1.s1_at	AT1G68070 – ZF (C3HC4-type RING finger) family protein	-	-2.22	-2.69

Table G.10 Significantly (P<0.05) regulated transcripts involved in photosynthesis upon exposure to freezing stress. Probe Set ID, *Arabidopsis thaliana* (AT) locus name and putative annotation of each transcript is listed. Values represent fold changes of transcripts in WT, S2 and M48 after freezing stress compared to normal growth conditions. Negative values show down-regulation and positive values show up-regulation. Fold changes less than 2 are indicated with - (TF: Transcription factor, LHC: light harvesting complex).

Probe Set ID	AT locus – Putative Annotation	Fold Change		
		WT AS	S2 AS	M48 AS
PHOTOSYNTHESIS / LIGHT REACTIONS				
les.1260.1.s1_at	AT4G32260 – ATP synthase family	-2.39	-	-2.55
les.1472.1.s1_at	AT4G05180 – PSBQ, PSBQ-2, PSII-Q - (photosystem II subunit Q-2); calcium ion binding	-4.33	-2.49	-4.47
les.1603.1.a1_at	AT1G61520 – LHCA3 (Photosystem I LHCgene 3)	-7.10	-4.56	-12.82
les.2168.1.s1_at	AT5G64040 – PSAN (photosystem I reaction center subunit PSI-N); CAM binding	-5.07	-4.29	-6.56
les.2286.1.s1_at	AT1G45474 – LHCA5 (Photosystem I LHCgene 5)	-5.26	-3.75	-3.00
les.241.2.s1_at	AT2G33800 – ribosomal protein S5 family protein	-2.67	-	-2.55
les.2478.1.s1_at	AT1G29930 – AB140, CAB140, LHCB1.3, CAB1 (Chlorophyll A/B Binding Protein 1)	-4.55	-3.34	-4.70
les.2620.1.s1_at	AT2G30570 – PSBW (photosystem II reaction center W)	-5.79	-3.03	-5.29
les.2620.2.s1_at	AT2G30570 – PSBW (photosystem II reaction center W)	-3.48	-2.63	-3.55
les.2856.1.s1_at	AT4G03280 – PGR1, PETC (photosynthetic electron transfer C)	-2.38	-	-2.47
les.3016.1.s1_at	AT5G54270 – LHCB3-1, LHCB3 (light-harvesting Chlorophyll Binding Protein 3)	-4.90	-6.17	-11.76
les.3029.1.s1_at	AT3G50820 – PSBO2 (photosystem II subunit O-2); oxygen evolving/ poly(U) binding	-2.79	-2.17	-2.90
les.3031.2.s1_at	AT4G38510 – (vacuolar ATP synthase subunit B2); hydrogen ion transporting ATP synthase	4.59	-	-
les.3062.1.s1_at	AT5G01530 – chlorophyll A-B binding protein CP29 (LHCB4)	-4.60	-2.43	-4.59
les.3087.1.a1_at	AT2G26500 – cytochrome b6f complex subunit (petM), putative	-5.32	-2.48	-5.71
les.3087.2.s1_at	AT2G26500 – cytochrome b6f complex subunit (petM), putative	-7.47	-2.57	-5.00
les.3099.1.s1_at	AT4G12800 – PSAL (photosystem I subunit L)	-4.31	-2.09	-5.10
les.3170.1.a1_at	AT3G16140 – PSAH-1 (photosystem I subunit H-1)	-7.09	-4.09	-9.68
les.3170.2.s1_a_at	AT3G16140 – PSAH-1 (photosystem I subunit H-1)	-4.86	-2.26	-4.81
les.3170.2.s1_at	AT3G16140 – PSAH-1 (photosystem I subunit H-1)	-4.73	-2.61	-4.86
les.3217.2.s1_at	AT5G13200 – GRAM domain-containing protein / ABA-responsive protein-related	-3.96	-	-2.82
les.3234.1.a1_at	AT1G60950 – FD2 FED A (ferredoxin 2)	-2.57	-2.15	-
les.324.1.s1_at	AT1G31330 – PSAF (photosystem I subunit F)	-4.32	-2.59	-4.69
les.325.3.a1_a_at	AT1G34000 – OHP2 (one-helix protein 2)	-	-	-2.26
les.3287.1.s1_at	AT5G66190 – LFNR1 (leaf FNR 1); poly(U) binding, ferredoxin--NADP(+) reductase	-2.85	-2.00	-3.04
les.3297.1.s1_at	AT3G47470 – CAB4, LHCA4 (Photosystem I LHCgene 4); chlorophyll binding	-15.75	-5.50	-15.09
les.3419.1.s1_at	AT4G38510 – - (vacuolar ATP synthase subunit B2); hydrogen ion transporting ATP synthase	2.12	-	-
les.3533.1.s1_at	AT2G27510 – ATFD3 (ferredoxin 3); electron carrier	-	2.01	-
les.3775.1.s1_at	AT3G54890 – LHCA1 chlorophyll A-B binding protein / LHCI type I (CAB), identical to chlorophyll A/B-binding protein	-6.56	-3.25	-6.91

Table G.10 (continued)

Probe Set ID	AT locus – Putative Annotation	Fold Change		
		WT AS	S2 AS	M48 AS
les.3929.1.s1_at	AT1G54290 – eukaryotic translation initiation factor SUI1, putative	-	-	-2.07
les.4007.1.s1_at	AT3G50820 – PSBO2 (photosystem II subunit O-2); oxygen evolving/ poly(U) binding	-3.00	-2.37	-3.20
les.4083.1.s1_at	AT3G61470 – LHCA2 (Photosystem I LHCgene 2); chlorophyll binding	-3.37	-	-3.28
les.4228.1.a1_at	AT1G04760 – VAMP726, ATVAMP726 (vesicle-associated membrane protein)	-2.31	-	-2.54
les.4259.1.s1_at	AT1G61520 – LHCA3 (Photosystem I LHCgene 3)	-3.95	-2.49	-4.44
les.4301.1.s1_at	AT1G20340 – DRT112 (DNA-damage-repair/toleration protein 112); copper ion binding / electron carrier	-6.27	-2.85	-4.61
les.4330.1.s1_s_at	AT1G06760 – histone H1, putative	-4.38	-	-
les.4345.1.s1_at	AT1G29930 – AB140, CAB140, LHCB1.3, CAB1 (chlorophyll A/B binding protein 1)	-2.10	-	-2.63
les.4345.2.a1_a_at	AT1G29930 – AB140, CAB140, LHCB1.3, CAB1 (chlorophyll A/B binding protein 1)	-5.19	-3.39	-6.43
les.4345.2.a1_x_at	AT1G29930 – AB140, CAB140, LHCB1.3, CAB1 (chlorophyll A/B binding protein 1)	-5.12	-2.87	-4.68
les.4345.3.s1_x_at	AT2G34420 – LHCB1.5, LHB1B2 (Photosystem II LHCgene 1.5); chlorophyll binding	-5.36	-3.04	-6.90
les.4345.4.a1_at	AT1G29930 – AB140, CAB140, LHCB1.3, CAB1 (chlorophyll A/B binding protein 1)	-3.25	-2.80	-4.92
les.4345.4.a1_x_at	AT1G29930 – AB140, CAB140, LHCB1.3, CAB1 (chlorophyll A/B binding protein 1)	-2.96	-2.83	-3.95
les.4359.1.s1_at	AT1G29920 – AB165, LHCB1.1, CAB2 (Chlorophyll a/b-binding protein 2); chlorophyll binding	-4.39	-3.79	-4.78
les.436.1.a1_at	AT3G08940 – LHCB4.2 (LHCPSII)	2.79	2.28	-
les.4428.1.s1_a_at	AT1G20340 – DRT112 (DNA-damage-repair/toleration protein 112); copper ion binding / electron carrier	-36.64	-14.43	-13.90
les.4492.2.s1_at	AT1G15820 – CP24, LHCB6 (LHCPSII); chlorophyll binding	-3.89	-2.62	-4.45
les.4492.3.s1_at	AT1G15820 – CP24, LHCB6 (LHCPSII); chlorophyll binding	-4.06	-4.08	-4.44
les.4508.1.s1_s_at	AT1G08380 – PSAO (photosystem I subunit O)	-6.03	-2.78	-7.06
les.4508.2.s1_s_at	AT1G08380 – PSAO (photosystem I subunit O)	-6.34	-3.55	-9.85
les.4586.1.s1_at	AT4G25130 – peptide methionine sulfoxide reductase, putative	-2.84	-	-2.87
les.4615.1.s1_at	AT2G31040 – ATP synthase protein I –related	-12.60	-7.17	-7.43
les.482.1.s1_at	AT1G06680 – OEE2, PSII-P, PSBP-1 (oxygen-evolving enhancer protein 2)	-3.26	-	-3.02
les.4867.1.s1_at	AT4G04640 – ATPC1 (ATP synthase gamma chain 1)	-3.23	-	-2.36
les.4995.1.s1_at	AT4G09650 – ATP synthase delta chain, chloroplast, putative / H(+)-transporting two-sector ATPase, delta (OSCP) subunit, putative	-3.04	-	-2.82
les.5157.1.s1_at	AT1G08380 – PSAO (photosystem I subunit O)	-5.73	-3.60	-8.05
les.5738.1.s1_at	AT3G55330 – PPL1 (PSBP-like protein 1); calcium ion binding	-5.40	-2.35	-2.75
les.5852.2.s1_at	AT5G08690 – ATP synthase beta chain 2, mitochondrial	3.45	-	3.50
les.5852.3.s1_at	AT5G08690 – ATP synthase beta chain 2, mitochondrial	-	-	3.19
les.608.1.s1_at	AT4G10340 – LHCB5 (LHCof Photosystem II 5); chlorophyll binding	-2.62	-2.11	-3.43
les.626.1.s1_at	AT2G39470 – PPL2 (PSBP-like protein 2); calcium ion binding	-3.76	-5.50	-5.20

Table G.10 (continued)

Probe Set ID	AT locus – Putative Annotation	Fold Change		
		WT AS	S2 AS	M48 AS
les.626.2.s1_at	AT2G39470 – PPL2 (PSBP-like protein 2); calcium ion binding	-2.34	-	-3.01
les.626.3.s1_at	AT2G39470 – PPL2 (PSBP-like protein 2); calcium ion binding	-4.57	-4.53	-4.09
les.656.2.s1_at	AT3G16250 – erredoxin-related	-5.07	-2.90	-4.18
les.867.1.s1_at	AT4G02770 – PSAD-1 (photosystem I subunit D-1)	-4.23	-3.05	-3.90
les.986.1.s1_at	ATCG00130 – ATPF - ATPase F subunit	-	-	-2.72
lesaffx.11323.1.a1_at	ATCG00730 – PETD - chloroplast gene encoding subunit IV of the cytochrome b6/f complex	-	2.84	2.04
lesaffx.29730.2.s1_at	ATMG01190 – ATP1 - ATPase subunit 1	-6.72	-	-
lesaffx.35136.1.s1_at	ATCG01070 – NDHE - NADH dehydrogenase ND4L	-28.07	-4.29	-4.23
lesaffx.44224.1.a1_at	ATCG01100 – NDHA - NADH dehydrogenase ND1	-4.12	-7.52	-3.06
lesaffx.44224.1.s1_at	ATCG01100 – NDHA - NADH dehydrogenase ND1	-	-2.22	-3.23
lesaffx.44474.1.a1_at	ATCG01050 – NDHD - plastid-encoded subunit of a NAD(P)H dehydrogenase complex	-	-2.28	-2.24
lesaffx.44474.1.s1_at	ATCG01050 – NDHD - plastid-encoded subunit of a NAD(P)H dehydrogenase complex	-	-	-2.58
lesaffx.48402.1.s1_at	AT4G15510 – photosystem II reaction center PsbP family protein	-3.57	-	-4.17
lesaffx.51226.1.a1_at	ATCG00540 – PETA - cytochrome f apoprotein	-14.66	-2.64	-2.76
lesaffx.66410.1.s1_at	ATCG00280 – PSBC-CP43 subunit of the photosystem II reaction center	-3.68	-	-
lesaffx.70106.1.s1_at	AT1G76450 – oxygen-evolving complex-related	-3.43	-2.60	-
lesaffx.70106.2.s1_at	AT1G76450 – oxygen-evolving complex-related	-4.62	-2.71	-2.02
lesaffx.70216.1.s1_at	AT5G58260 – subunit NDH-N of NAD(P)H:plastoquinone dehydrogenase complex	-5.79	-	-3.97
lesaffx.70450.1.s1_at	ATCG01080 – NDHG - NADH dehydrogenase ND6	-3.22	-7.03	-4.81
lesaffx.70834.1.s1_at	ATCG00150 – ATPI - subunit of ATPase complex CF0	-4.42	-	-2.94
PHOTOSYNTHESIS / CALVIN CYCLE				
les.1829.1.s1_at	AT2G01140 – fructose-bisphosphate aldolase, putative	-	-	2.26
les.2024.1.a1_at	AT3G54050 – fructose-1,6-bisphosphatase, putative / D-fructose-1,6-bisphosphate 1-phosphohydrolase, putative / FBPase, putative	-	-	-2.88
les.2024.2.s1_at	AT3G54050 – fructose-1,6-bisphosphatase, putative / D-fructose-1,6-bisphosphate 1-phosphohydrolase, putative / FBPase, putative	-5.86	-3.11	-4.98
les.2959.1.s1_at	AT3G60750 – transketolase, putative	-2.83	-	-2.21
les.3060.1.s1_at	AT4G38970 – fructose-bisphosphate aldolase, putative	-2.70	-	-3.46
les.3174.1.s1_at	AT4G38970 – fructose-bisphosphate aldolase, putative	-6.10	-	-5.77
les.3217.1.s1_s_at	AT5G38410 – ribulose bisphosphate carboxylase small chain 3B / RuBisCO small subunit 3B (RBCS-3B) (ATS3B)	-	-	-2.03
les.3217.2.s1_at	AT5G13200 – GRAM domain-containing protein / ABA-responsive protein-related	-3.96	-	-2.82
les.376.1.s1_at	AT5G38420 – ribulose bisphosphate carboxylase small chain 2B / RuBisCO small subunit 2B (RBCS-2B) (ATS2B)	-22.76	-3.16	-6.94
les.424.1.s1_at	AT1G32060 – PRK (Phosphoribulokinase); ATP binding / phosphoribulokinase/ protein binding	-4.02	-	-3.64
les.4275.1.s1_at	AT4G38970 – fructose-bisphosphate aldolase, putative	-5.69	-	-3.46
les.4493.1.s1_at	AT2G39730 – RCA (RUBISCO ACTIVASE)	-2.59	-	-2.62

Table G.10 (continued)

Probe Set ID	AT locus – Putative Annotation	Fold Change		
		WT AS	S2 AS	M48 AS
les.4616.1.s1_at	AT3G54050 – fructose-1,6-bisphosphatase, putative / D-fructose-1,6-bisphosphate 1-phosphohydrolase, putative / FBPase, putative	-6.85	-2.08	-4.98
les.4617.1.s1_at	AT2G24270 – ALDH11A3 (Aldehyde dehydrogenase 11A3); 3-chloroallyl aldehyde dehydrogenase/ glyceraldehyde-3-phosphate dehydrogenase (NADP+)	2.82	4.26	2.18
les.4807.1.s1_at	AT5G13200 – GRAM domain-containing protein / ABA-responsive protein-related	8.95	14.06	8.32
les.5598.1.s1_at	AT2G36460 – fructose-bisphosphate aldolase, putative	-	-	2.70
lesaffx.16966.1.a1_at	AT1G14030 – ribulose-1,5 bisphosphate carboxylase oxygenase large subunit N-methyltransferase, putative	-2.58	-2.36	-2.21
lesaffx.31052.1.a1_at	AT5G13200 – GRAM domain-containing protein / ABA-responsive protein-related	-4.37	-3.34	-2.84
lesaffx.344.2.s1_at	AT1G67090 – RBCS1A; ribulose-bisphosphate carboxylase	4.35	14.56	5.85
lesaffx.344.5.s1_at	AT1G67090 – RBCS1A; ribulose-bisphosphate carboxylase	4.55	11.16	4.58
lesaffx.50610.1.s1_at	AT4G20130 – PTAC14 (plastid transcriptionally active14)	-	-2.07	-
lesaffx.70764.1.s1_at	ATCG00490 – RBCL - large subunit of RUBISCO	5.65	3.68	-

Table G.11 Significantly (P<0.05) regulated transcripts involved in cell division, organization and development upon exposure to freezing stress. Probe set ID, *Arabidopsis thaliana* (AT) locus name and putative annotation of each transcript is listed. Values represent fold changes of transcripts in WT, S2 and M48 after freezing stress compared to normal growth conditions. Negative values show down-regulation and positive values show up-regulation. Fold changes less than 2 are indicated with - (TF: Transcription factor, RD: responsive to dehydration, PK: protein kinase).

Probe Set ID	AT locus – Putative Annotation	Fold Change		
		WT AS	S2 AS	M48 AS
CELL CYCLE				
les.1030.2.a1_at	AT1G26550 – peptidyl-prolyl cis-trans isomerase PPIC-type family protein	-	-	-2.18
les.107.1.s1_at	AT1G80370 – CYCA2;4 (CYCLIN A2;4); cyclin-dependent PK regulator	-2.25	-2.68	-
les.1748.1.a1_at	AT3G15520 – peptidyl-prolyl cis-trans isomerase TLP38, chloroplast / thylakoid lumen PPIase of 38 kDa / cyclophilin / rotamase	-	-2.41	-2.81
les.3263.1.a1_a_at	AT5G13120 – peptidyl-prolyl cis-trans isomerase cyclophilin-type family protein	-2.01	-2.04	-
les.3520.1.s1_at	AT5G67260 – CYCD3;2 (CYCLIN D3;2); cyclin-dependent PK	-	-3.16	-
les.5428.1.s1_at	AT3G63120 – CYCP1;1 (cyclin p1;1); cyclin-dependent PK	-	-	-2.23
lesaffx.22503.1.s1_at	AT2G36130 – peptidyl-prolyl cis-trans isomerase / cyclophilin / rotamase / putative	-	-	2.49
lesaffx.33777.1.s1_at	AT5G35100 – similar to CYP5 (cyclophilin 5)	-4.49	-2.47	-2.21
lesaffx.33777.2.a1_at	AT5G35100 – similar to CYP5 (cyclophilin 5)	3.36	-	2.42
lesaffx.46550.1.s1_at	AT2G44740 – CYCP4;1 (cyclin p4;1); cyclin-dependent PK	-3.54	-3.52	-6.92
lesaffx.64876.1.s1_at	AT2G29960 – CYP5, CYP19-4, (cyclophilin 5)	-	-	2.38
lesaffx.67887.1.s1_at	AT5G45680 – FK506-binding protein 1 (FKBP13) FKBP-type peptidyl-prolyl cis-trans isomerase 3, chloroplast precursor (Ppiase) (Rotamase)	-6.07	-2.61	-2.71
lesaffx.69699.1.s1_at	AT1G26940 – peptidyl-prolyl cis-trans isomerase cyclophilin-type family protein	-	-	2.53

Table G.11 (continued)

Probe Set ID	AT locus – Putative Annotation	Fold Change		
		WT AS	S2 AS	M48 AS
CELL DIVISION				
les.2998.3.s1_at	AT3G11450 – DNAJ HS N-terminal domain-containing protein / cell division protein-related	2.61	-	-
les.5167.1.s1_at	AT3G55580 – regulator of chromosome condensation (RCC1) family protein	9.13	11.23	12.68
les.5200.1.s1_at	AT5G11580 – UVB-resistance protein-related / regulator of chromosome condensation (RCC1) family protein	-2.21	-	-2.89
lesaffx.25483.1.s1_at	AT5G26640.1 – anaphase promoting complex subunit 11	-2.08	-	-
lesaffx.56618.1.s1_at	AT5G08710 – regulator of chromosome condensation (RCC1) family protein / UVB-resistance protein-related	2.39	-	-
lesaffx.66230.1.s1_at	AT1G66510 – AAR2 protein family	4.32	-	-
lesaffx.66230.2.s1_at	AT1G66510 – AAR2 protein family	2.16	-	-
CELL ORGANIZATION				
les.1267.1.a1_at	AT4G29950 – microtubule-associated protein	-	2.19	-
les.1514.1.s1_at	AT2G35490 – plastid-lipid associated protein PAP, putative	-2.14	-	-2.22
les.2756.2.s1_at	AT5G38530 – tryptophan synthase-related	-	2.27	2.35
les.289.1.s1_at	AT5G43070 – WPP1 (WPP domain protein 1) MFP1 attachment factor, putative	-	-	2.18
les.4394.1.a1_s_at	AT4G14960 – TUA6 (tubulin alpha-6 chain)	-	-	-2.01
les.4424.1.s1_at	AT4G10840 – kinesin light chain-related	-3.19	-2.07	-
les.4424.1.s1_s_at	AT4G10840 – kinesin light chain-related	-2.43	-	-2.26
les.4593.1.s1_at	AT3G24530 – AAA-type ATPase family protein / ankyrin repeat family protein	5.31	6.98	5.33
les.4703.1.s1_at	AT5G12250 – TUB6 (Beta-6 Tubulin)	-3.87	-2.49	-4.16
les.5560.1.s1_at	AT5G23860 – TUB8 (tubulin beta-8)	-	-	2.40
les.5584.1.s1_at	AT3G53000 – ATPP2-A15 (Phloem protein 2-A15); carbohydrate binding	-2.25	-3.11	-
les.5924.1.s1_at	AT5G45110 – NPR3 (NPR1-like protein 3); protein binding	2.29	2.15	-
les.5940.1.s1_at	AT1G64280 – SAI1, NIM1, NPR1 (nonexpresser of PR genes 1); protein binding	2.25	2.03	2.54
les.726.1.s1_at	AT5G60210 – cytoplasmic linker protein-related	-3.68	-	-2.93
lesaffx.15353.1.s1_at	AT3G61060 – ATPP2-A13 F-box family protein / lectin-related, similarity to PP2 lectin polypeptide	-2.70	-	-4.76
lesaffx.1574.1.s1_at	AT3G61710 – autophagy protein Apg6 family	2.72	-	-
lesaffx.1574.2.s1_at	AT3G61710 – autophagy protein Apg6 family	2.13	-	-
lesaffx.24533.1.a1_at	AT5G53400 – nuclear movement family protein	-	2.12	-
lesaffx.35363.1.s1_at	AT3G61060 – ATPP2-A13 F-box family protein / lectin-related, similarity to PP2 lectin polypeptide	2.38	-	-
lesaffx.37916.1.s1_at	AT1G09155 – ATPP2-B15 (Phloem protein 2-B15); carbohydrate binding	10.54	7.94	5.30
lesaffx.50408.1.s1_at	AT4G31340 – myosin heavy chain-related	2.52	-	2.86
lesaffx.51979.1.s1_at	AT2G16700 – ADF5 (actin-depolymerizing factor 5); actin binding	-4.92	-2.52	-5.81
lesaffx.54238.1.s1_at	AT4G08580 – microfibrillar-associated protein-related	3.33	-	2.05
lesaffx.57312.1.s1_at	AT3G04710 – ankyrin repeat family protein	3.08	-	2.57
lesaffx.6012.1.a1_at	AT4G15930 – dynein light chain, putative	-2.31	-	-2.78

Table G.11 (continued)

Probe Set ID	AT locus – Putative Annotation	Fold Change		
		WT AS	S2 AS	M48 AS
lesaffx.6012.1.s1_at	AT4G15930 – dynein light chain, putative	-	-	-3.90
lesaffx.64540.1.s1_at	AT5G19940 – plastid-lipid associated protein PAP-related / fibrillin-related	-3.28	-2.34	-3.26
lesaffx.65646.1.s1_at	AT1G18450 – ARP4, ATARP4 (actin-related protein 4)	2.16	-	-
lesaffx.65646.2.s1_at	AT1G18450 – ARP4, ATARP4 (actin-related protein 4)	3.20	-	-
lesaffx.68447.1.s1_at	AT2G46910 – plastid-lipid associated protein PAP / fibrillin family protein	-3.96	-2.03	-3.52
lesaffx.70593.1.s1_at	AT5G61230 – ankyrin repeat family protein	-	-2.94	-2.46
lesaffx.71150.1.s1_at	AT3G26070 – plastid-lipid associated protein PAP / fibrillin family protein	-4.38	-3.38	-3.64
DEVELOPMENT				
les.1439.1.s1_at	AT4G19185 – integral membrane family protein	-	-	-3.07
les.1439.2.a1_at	AT4G19185 – integral membrane family protein	-	-	-2.35
les.162.1.s1_at	AT3G58040 – seven in absentia (SINA) family protein	3.68	-	2.16
les.162.2.s1_at	AT3G58040 – seven in absentia (SINA) family protein	2.16	-	-
les.162.3.s1_at	AT3G58040 – seven in absentia (SINA) family protein	3.86	-	-
les.1798.2.a1_at	AT5G20700 – senescence-associated protein-related	14.67	11.87	20.80
les.192.1.a1_at	AT1G56220 – dormancy/auxin associated family protein	-4.39	-2.96	-3.14
les.192.2.s1_at	AT1G56220 – dormancy/auxin associated family protein	-8.22	-4.38	-6.34
les.2091.1.s1_at	AT1G69170 – squamosa promoter-binding protein-like 6 (SPL6)	-	-	-2.45
les.23.1.s1_at	AT4G27410 – ANAC072, RD26	2.71	2.92	3.29
les.2333.1.s1_at	AT3G48140 – senescence-associated protein, putative, similar to B12D protein	-2.53	-	-2.91
les.2437.1.s1_at	AT3G47650 – bundle-sheath defective protein 2 family / bsd2 family	-2.51	-2.80	-2.62
les.2717.1.s1_at	AT3G48140 – senescence-associated protein, putative	2.20	2.97	2.49
les.2717.2.a1_at	AT3G48140 – senescence-associated protein, putative	-	2.12	-
les.3065.1.s1_at	AT2G33810 – SPL3 (SQUAMOSA promoter binding protein-like 3); TF	-2.12	-	-
les.3070.2.a1_at	AT4G25150 – acid phosphatase, putative	-2.58	-29.27	-
les.3095.1.s1_at	AT1G01720 – ANAC002, ATAF1 (NAC domain containing protein 2); TF	2.22	-	-
les.3157.1.s1_at	AT3G48740 – nodulin MtN3 family protein	-2.42	-2.82	-3.09
les.3348.1.s1_at	AT5G62200 – embryo-specific protein-related	-	3.05	2.68
les.3743.1.s1_at	AT4G25150 – acid phosphatase, putative	-	2.10	3.70
les.3759.1.s1_at	AT5G53560 – ATB5-A (Cytochrome b5 A), late embryogenesis (lea)-like protein	7.16	18.76	7.84
les.3766.1.s1_at	AT1G01470 – LSR3, LEA14 (late embryogenesis abundant 14)	2.65	7.49	-
les.4141.1.s1_at	AT4G25150 – acid phosphatase, putative	-	-	2.70
les.4373.1.s1_at	AT3G19240 – dem protein-related / defective embryo and meristems protein-related	-	2.73	-
les.4483.1.s1_at	AT1G01720 – ANAC002, ATAF1 (NAC domain containing protein 2); TF	23.46	20.64	10.63
les.4683.1.s1_at	AT2G33430 – plastid developmental protein DAG, putative	-4.46	-2.02	-3.72

Table G.11 (continued)

Probe Set ID	AT locus – Putative Annotation	Fold Change		
		WT AS	S2 AS	M48 AS
les.476.2.s1_at	AT5G14520 – pescadillo-related	3.03	-	2.36
les.4903.1.s1_at	AT5G24520 – TTG, TTG1 (transparent testa glabra 1); nucleotide binding	-	2.54	-
les.4963.1.s1_at	AT3G49530 – ANAC062 (NAC domain containing protein 62); TF	2.05	3.33	2.39
les.4975.1.s1_at	AT4G29270 – acid phosphatase class B family protein	-2.48	-13.05	-
les.4987.1.s1_at	AT3G45600 – TET3 (tetraspanin3) senescence-associated family protein	-	2.12	3.05
les.5128.1.s1_at	AT2G17840 – ERD7 (early-RD 7)	3.84	8.81	3.65
les.5190.1.s1_at	AT5G66770 – scarecrow TF family protein	-6.11	-3.25	-6.82
les.5219.1.s1_at	AT3G49530 – ANAC062 (NAC domain containing protein 62); TF	4.25	8.94	9.73
les.5403.1.s1_at	AT1G07530 – scarecrow-like TF14 (SCL14)	-	2.65	2.15
les.5485.1.s1_at	AT2G23810 – TET8 (tetraspanin8) similar to senescence-associated family protein	-	2.03	2.07
les.5489.1.s1_at	AT3G61790 – seven in absentia (SINA) family protein	-	-	-2.60
les.5572.1.s1_at	AT4G08300 – nodulin MtN21 family protein	-	-2.19	-2.27
les.5626.1.s1_at	AT1G07530 – scarecrow-like TF 14 (SCL14)	2.25	2.75	-
les.5699.1.s1_at	AT2G17040 – ANAC036 (NAC domain containing protein 36); TF	-	3.28	-
les.5728.1.s1_at	AT4G26740 – ATS1 (seed gene 1); calcium ion binding	2.52	-	-
les.5791.1.s1_at	AT5G48150 – PAT1 (phytochrome a signal transduction 1); TF	-5.12	-	-4.90
lesaffx.13113.1.s1_at	AT1G09380 – integral membrane family protein / nodulin MtN21-related	-	-2.05	-
lesaffx.15284.1.s1_at	AT1G28330 – DRM1 (dormancy-associated protein 1)	-10.85	-7.66	-23.22
lesaffx.25827.1.s1_at	AT1G10200 – WLIM1; TF, pollen-specific protein sf3, putative	2.62	2.08	3.20
lesaffx.2597.1.s1_at	AT4G35770 – DIN1 (dark inducible 1) senescence-associated protein (SEN1)	-18.24	-4.63	-31.92
lesaffx.31434.1.s1_at	AT3G10530 – transducin family protein / WD-40 repeat family protein	2.96	-	-
lesaffx.31514.1.s1_at	AT1G75500 – nodulin MtN21 family protein	-	-3.05	-2.97
lesaffx.43329.1.s1_at	AT5G61430 – ANAC100, ATNAC5 (AT NAC domain containing protein 100); TF	4.19	-	-
lesaffx.4509.1.s1_at	AT2G19520 – ACG1, MSI4, NFC4, ATMSI4, FVEWD-40 repeat protein (MSI4)	4.79	-	-
lesaffx.46519.1.s1_at	AT1G21460 – nodulin MtN3 family protein	-7.22	-6.29	-12.22
lesaffx.4763.1.s1_at	AT2G41380 – embryo-abundant protein-related	4.40	5.41	3.61
lesaffx.51271.1.s1_at	AT1G10200 – WLIM1; TF	-6.34	-2.85	-9.95
lesaffx.52241.1.a1_at	AT4G27990 – YGGT family protein	-	-	2.96
lesaffx.54455.1.s1_at	AT1G28560 – SRD2 (shoot redifferentiation defective 2)	-	-	-2.38
lesaffx.56.1.s1_at	AT1G22160 – senescence-associated protein-related, similar to SAG102	-	-	-2.16
lesaffx.57572.1.s1_at	AT4G37050 – PLP4, PLA V (Patatin-like protein 4); nutrient reservoir	9.76	7.92	8.64
lesaffx.58104.1.a1_at	AT5G44120 – ATCRA1, CRU1 (cruciferina); nutrient reservoir	2.03	4.87	3.36
lesaffx.6024.1.a1_at	AT2G44670 – senescence-associated protein-related	-	-2.83	-2.30

Table G.11 (continued)

Probe Set ID	AT locus – Putative Annotation	Fold Change		
		WT AS	**S2 AS**	**M48 AS**
lesaffx.6024.1.s1_at	AT2G44670 – senescence-associated protein-related	-5.44	-7.83	-3.66
lesaffx.60947.1.s1_at	AT4G36920 – FLO2, FL1, AP2 (APETALA 2); TF	-	-2.94	-
lesaffx.60966.2.s1_at	AT5G48150 – PAT1 (phytochrome a signal transduction 1); TF	12.22	10.81	16.32
lesaffx.64505.2.s1_at	AT1G56010 – ANAC021, ANAC022, NAC1 (NAC domain containing protein 21, AT NAC domain containing protein 22); TF	-	-6.84	-6.76
lesaffx.65682.1.a1_at	AT5G50790 – nodulin MtN3 family protein	-	-	-4.31
lesaffx.66359.1.s1_at	AT2G43000 – ANAC042 (AT NAC domain containing protein 42); TF	-	-	2.38
lesaffx.66436.1.s1_at	AT1G70670 – caleosin-related family protein	4.87	-	-
lesaffx.69298.1.s1_at	AT3G12090 – TET6 (TETRASPANIN6) senescence-associated family protein	-2.20	-2.63	-
lesaffx.69815.1.s1_at	AT3G14770 – nodulin MtN3 family protein	17.43	-	13.27
lesaffx.70386.1.s1_at	AT3G55770 – LIM domain-containing protein	-	-2.12	-
lesaffx.70563.1.s1_at	AT5G48150 – PAT1 (phytochrome a signal transduction 1); TF	34.84	17.86	23.35
lesaffx.71577.1.s1_a_at	AT4G02380 – late embryogenesis abundant 3 family protein	4.25	8.07	2.13
lesaffx.71623.1.s1_at	AT4G27410 – ANAC072, RD26	4.63	-	3.00

APPPENDIX H

COLD-INDEPENDENT AND COLD-MEDIATED DIFFERENTIALLY REGULATED GENES IN WILD-TYPE AND TRANSGENIC PLANTS

Table H.1 Significantly (P<0.05) regulated transcripts involved in abiotic and biotic stress responses. Probe set ID, *Arabidopsis thaliana* (AT) locus name and putative annotation of each transcript is listed. Values represent fold changes of transcripts in S2 C and M48 C compared to WT C and in S2 AS and M48 AS compared to WT AS. Negative values show down-regulation and positive values show up-regulation. Fold changes less than 2 are indicated with - (HS: heat shock, LRR: leucine-rich repeat).

Probe Set ID	AT locus – Putative Annotation	Fold Change			
		S2 C	M48 C	S2 AS	M48 AS
ABIOTIC STRESS / HEAT					
les.140.1.s1_at	AT5G28540 – luminal binding protein 1 (BIP1)	-	-2.60	-	-
les.1931.1.a1_at	AT1G59725 – DNAJ HS protein, putative	4.45	4.31	-	-
les.3160.1.s1_at	AT1G56410 – ERD2/HSP70T-1 (early-RD 2)	-	-	-17.61	-11.76
les.3160.3.s1_at	AT3G12580 – HSP70 (HS protein 70)	-	-2.36	-5.11	-2.63
les.3179.2.s1_at	AT3G44110 – ATJ, ATJ3 (DnaJ homologue 3)	-	-	-4.66	-2.05
les.3581.1.s1_at	ATHSP90.1 – ATHS83, HSP81.1, HSP83, (HS protein 81-1)	-2.82	-	-	-
les.4004.1.s1_a_at	AT1G07400 – 17.8 kDa class I HS protein (HSP17.8-CI)	-	-	2.96	-
les.4705.1.s1_at	AT4G21320 – HSA32 (heat-stress-associated 32)	-	-	2.11	-
les.4819.1.s1_at	AT5G02500 – HSP70-1, AT-HSC70-1, HSC70, HSC70-1 (HS cognate 70 kDa protein 1)	-	-2.01	-	-
les.5892.1.s1_at	AT3G13310 – DNAJ HS N-terminal domain-containing protein	-2.07	-	3.44	-
lesaffx.10596.1.s1_at	AT5G59720 – HSP18.2 (HS protein 18.2)	-	-	2.63	-
lesaffx.13063.1.s1_at	AT3G08910 – DNAJ HS protein	-2.05	-	-	-
lesaffx.17345.1.s1_at	AT4G13830 – J20 (DNAJ-LIKE 20); HS protein binding	-2.31	-	-	-
lesaffx.38184.1.s1_at	AT4G39150 – DNAJ HS N-terminal domain-containing protein	-	-2.05	-	-
lesaffx.43628.1.s1_at	AT1G80920 – J8 HS protein binding / unfolded protein binding	-	2.16	-	-
lesaffx.44913.1.s1_at	– DNAJ HS N-terminal domain-containing protein	-	-2.15	-4.02	-3.21
lesaffx.45577.1.s1_at	AT5G64360 – DNAJ HS N-terminal domain-containing protein	-	-	-3.54	-3.10
lesaffx.55071.1.s1_at	AT2G22360 – DNAJ HS family protein	-	-	-	-2.04
lesaffx.56637.1.s1_at	AT5G03030 – DNAJ HS N-terminal domain-containing protein	-	-	-	2.03
lesaffx.58365.1.s1_at	AT3G08970 – DNAJ HS N-terminal domain-containing protein	-	-2.42	-	-

Table H.1 (continued)

Probe Set ID	AT locus – Putative Annotation	Fold Change			
		S2 C	M48 C	S2 AS	M48 AS
lesaffx.63687.1.s1_at	AT4G21870 – 26.5 kDa class P-related HS protein (HSP26.5-P)	-	2.67	-	-
lesaffx.68350.1.s1_at	AT5G23590 – DNAJ HS N-terminal domain-containing protein	-	-	-5.82	-3.23
lesaffx.69957.1.s1_at	AT1G52560 – 26.5 kDa class I small HS protein-like (HSP26.5-P)	-	-	2.56	-
lesaffx.9815.1.s1_at	AT4G36040 – DNAJ HS N-terminal domain-containing protein (J11)	-	2.52	-	-
BIOTIC STRESS					
les.122.1.s1_at	AT3G12500 – PR3, CHI-B, B-CHI, ATHCHIB (basic chitinase)	-2.27	-2.15	-	-
les.2529.2.s1_at	AT5G17540 – transferase family protein	-	-	-2.04	-
les.3406.1.s1_at	AT3G12500.1 –basic endochitinase, identical to basic endochitinase precursor	-3.21	-	-3.52	-
les.3756.1.s1_a_at	AT2G43330 – ATINT1 (inositol transporter 1); carbohydrate transmembrane transporter/ sugar:hydrogen ion symporter	3.93	-	-	-
les.4307.1.s1_at	AT4G11650 – OSM34 (osmotin 34)	-	-	-2.29	-
les.5208.1.s1_at	AT3G48090 – EDS1 (enhanced disease susceptibility 1); signal transducer/ triacylglycerol lipase	-	-2.45	-	-
lesaffx.1458.1.s1_at	AT1G75800 – pathogenesis-related thaumatin family protein	-	-	-3.90	-2.07
lesaffx.16769.1.s1_at	AT1G65870 – disease resistance-responsive family protein	-	-2.24	2.31	-
lesaffx.71532.1.s1_at	AT1G33590 – disease resistance protein-related / LRR protein-related	-	-2.23	-4.69	-5.56

Table H.2 Significantly (P<0.05) regulated transcripts involved in transcription and post-transcription. Probe set ID, *Arabidopsis thaliana* (AT) locus name and putative annotation of each transcript is listed. Values represent fold changes of transcripts in S2 C and M48 C compared to WT C and in S2 AS and M48 AS compared to WT AS. Negative values show down-regulation and positive values show up-regulation. Fold changes less than 2 are indicated with - (TF: transcription factor).

Probe Set ID	AT locus – Putative Annotation	Fold Change			
		S2 C	M48 C	S2 AS	M48 AS
TRANSCRIPTION FACTORS/ MYB					
les.5017.1.a1_at	AT3G46130– AtMYB48, MYB111 (myb domain protein 111)	-	2.37	-	-
les.5017.1.s1_at	AT3G46130– AtMYB48, MYB111 (myb domain protein 111)	2.94	4.55	-	-
lesaffx.40173.1.s1_at	AT2G31180– AtMYB14	-	-	2.52	-
lesaffx.54522.1.s1_at	AT2G23290– AtMYB70	-	-	-10.30	-4.04
lesaffx.62138.1.s1_at	AT5G49620– AtMYB78	-	-	-2.12	-
les.3716.1.s1_at	AT4G39250– DNA binding / TF	-	2.05	-	-
les.4923.1.s1_at	AT2G46830– CCA1 (circadian clock associated 1); TF	-	2.48	-	-
lesaffx.25436.1.s1_at	AT5G47390– myb family TF	-	-	-3.72	-2.10
lesaffx.56221.1.s1_at	AT3G09600– myb family TF	-	-	-2.95	-

Table H.2 (continued)

Probe Set ID	AT locus – Putative Annotation	Fold Change			
		S2 C	M48 C	S2 AS	M48 AS
TRANSCRIPTION FACTORS/ bHLH					
les.1376.2.a1_at	AT5G41315– MYC6.2, GL3 (GLABRA 3); TF	-	-	2.24	-
les.4085.1.s1_at	AT2G27230– LHW (lonesome highway)	-2.16	-	-	-
les.501.2.a1_at	AT3G57800– basic helix-loop-helix (bHLH) family protein	-	-	2.51	-
les.5638.1.s1_at	AT4G34530– basic helix-loop-helix (bHLH) family protein	-	2.68	-	-
lesaffx.37213.1.s1_at	AT5G46690– BHLH071 (beta hlh protein 71); DNA binding / TF	-2.97	-	-	-
lesaffx.62334.1.s1_at	AT1G26945– transcription regulator	3.45	-	-	-
lesaffx.64675.1.s1_a_at	AT5G57150– basic helix-loop-helix (bHLH) family protein	2.26	-	-3.47	-
TRANSCRIPTION FACTORS/ PHOR1					
lesaffx.15878.2.s1_at	AT3G52450– U-box domain-containing protein	-	-	3.85	2.26
lesaffx.56802.1.s1_at	AT3G19380– U-box domain-containing protein	-	-	-4.22	-3.79
lesaffx.63659.1.s1_at	AT3G18710– U-box domain-containing protein	-	-	-4.95	-2.58
TRANSCRIPTION FACTORS/ AP2/EREBP					
les.124.1.s1_at	AT4G25480– CBF3, DREB1, DREB1A (dehydration response element B1A); DNA binding / transcription activator/ TF	-	-	2.06	-
les.5885.2.s1_at	AT1G78080– RAP2.4 (related to AP2 4); DNA binding / TF	-	-	-6.08	-5.60
les.5885.3.s1_at	AT1G78080– RAP2.4 (related to AP2 4); DNA binding / TF	-	-	-2.36	-2.17
lesaffx.60947.1.s1_at	AT4G36920– FLO2, FL1, AP2 (APETALA 2)	-	-	-2.58	-
lesaffx.64978.1.s1_at	AT5G67190– AP2 domain-containing TF	-	-	-3.23	-
RNA PROCESSING					
les.1762.1.s1_at	AT2G35370– GDCH (Glycine decarboxylase complex H)	-	-	8.36	3.33
les.2914.1.s1_at	AT1G32470– glycine cleavage system H protein. mitochondrial. putative	-	-	2.54	-
les.2940.2.s1_at	AT1G49760– PAB8 (poly(A) binding protein 8); RNA binding / translation initiation factor	5.53	6.17	4.69	3.38
les.2940.2.s1_s_at	AT1G49760– PAB8 (poly(A) binding protein 8); RNA binding / translation initiation factor	3.88	4.42	4.00	3.29
les.2982.1.a1_at	AT1G02840– SRP34, SR1 (splicing factor 2); RNA binding	-	-	-2.98	-
les.2982.2.s1_at	AT1G02840– SRP34, SR1 (splicing factor 2); RNA binding	-	-	-10.82	-4.30
les.5651.1.s1_at	AT2G18510– EMB2444 (embryo defective 2444); RNA binding	-	-	-2.04	-
lesaffx.14782.2.s1_at	AT3G49430– SRP34A (SER/ARG-rich protein 34A)	-	2.83	-	-
lesaffx.31254.1.s1_at	AT5G52040– ATRSP41 (arginine/serine-rich splicing factor 41)	-	-	-3.33	-
lesaffx.44533.1.a1_at	AT4G21660– proline-rich spliceosome-associated (PSP) family protein	-	-	-2.04	-
lesaffx.54261.1.s1_at	AT5G53180– polypyrimidine tract-binding protein. putative / heterogeneous nuclear ribonucleoprotein, putative	-	-	-5.18	-2.76
lesaffx.59682.1.s1_at	AT2G35120– glycine cleavage system H protein, mitochondrial, putative	-	-	-	2.18
lesaffx.65616.1.s1_at	AT3G01150– PTB (polypyrimidine tract-binding)	-	-	-6.17	-3.42

Table H.3 Significantly (P<0.05) regulated transcripts involved in translation and post-translation. Probe set ID, *Arabidopsis thaliana* (AT) locus name and putative annotation of each transcript is listed. Values represent fold changes of transcripts in S2 C and M48 C compared to WT C and in S2 AS and M48 AS compared to WT AS. Negative values show down-regulation and positive values show up-regulation. Fold changes less than 2 are indicated with - (HS: heat shock).

Probe Set ID	AT locus – Putative Annotation	Fold Change			
		S2 C	M48 C	S2 AS	M48 AS
RIBOSOMAL PROTEINS					
les.1323.1.s1_at	AT5G65220– ribosomal protein L29 family protein	-	-	2.04	-
les.1816.1.s1_at	AT1G68590– plastid-specific 30S ribosomal protein 3, putative / PSRP-3, putative	-	-	3.30	-
les.2974.1.a1_at	AT1G04270– RPS15 (cytosolic ribosomal protein S15); structural constituent of ribosome	-	-	2.01	-
les.3007.3.a1_at	AT1G78630– EMB1473 (embryo defective 1473); structural constituent of ribosome	-	-	2.17	-
les.3154.1.a1_at	AT1G35680– 50S ribosomal protein L21, chloroplast / CL21 (RPL21)	-	-	2.41	-
les.3154.2.s1_at	AT1G35680– 50S ribosomal protein L21, chloroplast / CL21 (RPL21)	-	-	2.58	-
les.392.1.s1_at	AT5G24490– 30S ribosomal protein, putative	-	-	3.10	-
les.4298.1.s1_s_at	ATCG00380– RPS4, Chloroplast encoded ribosomal protein S4	3.78	4.81	3.26	2.95
les.4379.1.s1_at	AT3G27830– RPL12, RPL12-A (ribosomal protein L12-A); structural constituent of ribosome	-	-	2.16	-
les.4379.2.s1_a_at	AT3G27850– RPL12-C (ribosomal protein L12-C); structural constituent of ribosome	-	-	2.51	-
les.4399.1.a1_at	ATCG01310– RPL2.2 chloroplast ribosomal protein L2	-	-	2.48	2.46
les.4410.1.a1_at	AT1G64510– ribosomal protein S6 family protein	-	-	3.03	-
lesaffx.19618.1.s1_at	AT3G18760– ribosomal protein S6 family protein	-	-	2.60	-
lesaffx.33796.2.s1_at	ATCG00900– RPS7.1, chloroplast ribosomal protein S7	5.68	4.77	24.78	12.62
lesaffx.55735.1.s1_at	AT3G15190– chloroplast 30S ribosomal protein S20, putative	-	-	2.47	-
lesaffx.67632.1.a1_at	AT5G13720– structural constituent of ribosome	2.49	2.25	2.62	-
lesaffx.71228.1.s1_at	AT5G54600– 50S ribosomal protein L24, chloroplast (CL24)	-	-	2.46	-
lesaffx.71577.1.s1_a_at	AT4G02380.1 late embryogenesis abundant 3 family protein / LEA3 family protein, similar to several small proteins	-2.96	-	-	-
les.241.1.a1_at	AT2G33800– ribosomal protein S5 family protein	-	-	2.06	-
les.2522.1.s1_at	AT1G07320– RPL4 (ribosomal protein L4); poly(U) binding / structural constituent of ribosome	-	-	2.79	-
les.2860.1.s1_at	AT1G05190– EMB2394 (embryo defective 2394); structural constituent of ribosome	-	-	3.25	-
les.3007.2.s1_at	AT1G78630– EMB1473 (embryo defective 1473); structural constituent of ribosome	-	-	2.17	-
les.3007.3.a1_at	AT1G78630– EMB1473 (embryo defective 1473); structural constituent of ribosome	-	-	2.17	-
les.3149.2.s1_at	AT3G09630– 60S ribosomal protein L4/L1 (RPL4A)	-	-	-3.25	-
les.3149.3.s1_at	AT3G09630– 60S ribosomal protein L4/L1 (RPL4A)	-	-2.11	-	-
les.3546.1.s1_at	AT5G04800– 40S ribosomal protein S17 (RPS17D)	-	-	2.01	-
les.4437.2.s1_at	AT4G01310– ribosomal protein L5 family protein	-	-	2.97	-
lesaffx.1195.2.s1_at	AT4G27940– mitochondrial substrate carrier family protein	-	-	-7.68	-3.54

Table H.3 (continued)

Probe Set ID	AT locus – Putative Annotation	Fold Change			
		S2 C	M48 C	S2 AS	M48 AS
lesaffx.26489.1.s1_at	AT3G54210– ribosomal protein L17 family protein	-	-	2.16	-
lesaffx.48130.1.s1_at	AT1G75350– EMB2184 (embryo defective 2184); structural constituent of ribosome	-	-	3.07	-
lesaffx.5957.1.s1_at	AT3G25920– RPL15 (ribosomal protein L15)	-	-	2.17	-
lesaffx.67298.1.s1_at	AT1G48350– ribosomal protein L18 family protein	-	-	4.93	-
PROTEIN DEGRADATION					
affx-le_ubiquitin_5_at	AT5G03240– UBQ3 (polyubiquitin 3); protein binding	-	-	-3.65	-2.91
les.1114.1.a1_at	AT2G22010– zinc finger (C3HC4-type RING finger) family protein	-	-	2.11	-
les.1132.1.a1_at	AT1G17870.1– S2P-like putative metalloprotease	-	2.15	3.76	-
les.15.1.s1_at	AT5G67360.1 – cucumisin-like serine protease (ARA12), Asp48	-	-	2.39	-
les.1616.2.s1_at	AT2G17190– ubiquitin family protein	-	-	-2.78	-
les.1675.1.s1_at	AT4G23630.1– reticulon family protein (RTNLB1)	7.09	-2.14	-	-
les.2068.1.a1_at	AT3G45010– SCPL48 (serine carboxypeptidase-like 48); serine carboxypeptidase	-	-	3.02	-
les.2173.1.a1_at	AT5G21930– HMA8/PAA2 (P-TYPE ATPase of arabidopsis 2)	2.34	-	-	-
les.2294.2.a1_a_at	AT1G79110– protein binding / zinc ion binding	2.04	-	-	-
les.2294.2.a1_at	AT1G79110– protein binding / zinc ion binding	-	2.58	-	-
les.232.1.s1_at	AT3G14067– subtilase family protein	-	2.16	3.72	-
les.2574.2.s1_at	AT5G09900.2 – 26S proteasome regulatory subunit, putative (RPN5), p55 protein-like	-	-	-3.95	-2.27
les.2574.3.s1_at	AT5G09900.2 – 26S proteasome regulatory subunit, putative (RPN5), p55 protein-like |	-	-	-6.42	-3.18
les.2689.2.s1_at	AT4G29490– X-Pro dipeptidase	-	-	-2.86	-2.74
les.2689.3.s1_at	AT4G29490– X-Pro dipeptidase	-	-	-	-2.24
les.2722.3.a1_at	AT1G17110– UBP15 (ubiquitin-specific protease 15); ubiquitin-specific protease	-	-	2.70	-
les.2845.2.s1_at	AT5G61900– CPN1, BON, BON1 (BONZAI1); calcium-dependent phospholipid binding	-	-	-3.68	-2.26
les.2960.2.s1_a_at	AT3G10410– SCPL49 (serine carboxypeptidase-like 49); serine carboxypeptidase	-	-	-2.77	-2.49
les.2960.2.s1_at	AT3G10410– SCPL49 (serine carboxypeptidase-like 49); serine carboxypeptidase	-	-	-5.17	-3.81
les.2960.3.s1_a_at	AT3G10410– SCPL49 (serine carboxypeptidase-like 49); serine carboxypeptidase	-	-	-13.99	-9.25
les.2960.3.s1_at	AT3G10410– SCPL49 (serine carboxypeptidase-like 49); serine carboxypeptidase	-	-	-2.68	-2.59
les.3164.1.a1_at	AT5G63160– BT1 (BTB and TAZ domain protein 1); protein binding / transcription regulator	-	2.31	-	-
les.3164.2.s1_at	AT5G63160– BT1 (BTB and TAZ domain protein 1); protein binding / transcription regulator	-	-	-2.69	-
les.3228.1.s1_at	AT1G47128– RD21A, RD21 (RD 21); cysteine-type peptidase	-	-	-2.97	-
les.3228.2.s1_at	AT1G47128– RD21A, RD21 (RD 21); cysteine-type peptidase	-	-	-2.14	-
les.3260.2.s1_at	AT3G46460– UBC13 (ubiquitin-conjugating enzyme 13); ubiquitin-protein ligase	2.11	3.52	-	-
les.3293.2.s1_at	AT5G50920– HSP93-V, DCA1, CLPC (HS protein 93-V); ATP binding / ATPase	-	-	-4.64	-5.22

Table H.3 (continued)

Probe Set ID	AT locus – Putative Annotation	Fold Change			
		S2 C	M48 C	S2 AS	M48 AS
les.3293.3.s1_at	AT5G50920– HSP93-V, CLPC, DCA1, CLPC (HS protein 93-V); ATP binding / ATPase	-	-	-	-2.07
les.3308.2.s1_at	AT5G62350– invertase/pectin methylesterase inhibitor family protein / DC 1.2 homolog	-2.13	-	-	-
les.3308.3.s1_at	AT4G05320– UBQ10 (polyubiquitin 10)	-	-	-5.56	-5.38
les.3343.2.s1_at	AT1G15670– kelch repeat-containing F-box family protein	-	2.01	-2.04	-
les.3369.2.s1_at	AT2G30950– FTSH2, VAR2 (variegated 2); ATP-dependent peptidase/ ATPase/ metallopeptidase/ zinc ion binding	-	-	-5.83	-6.39
les.3515.1.s1_at	AT4G12910– SCPL20 (serine carboxypeptidase-like 20); serine carboxypeptidase	2.75	-	-	-
les.3621.1.s1_at	– wound-induced proteinase inhibitor 1 precursor	10.29	-	-	-
les.3824.1.a1_at	AT1G69330– zinc finger (C3HC4-type RING finger) family protein	-	2.01	-	-
les.3940.2.a1_at	– wound-induced proteinase inhibitor 1 precursor	5.46	-	-	-
les.4022.1.s1_at	– proteinase inhibitor type-2 cevi57 precursor	-	-	-2.13	-
les.411.1.s1_at	AT2G25490– FBL6, EBF1 (EIN3-binding F box protein 1); ubiquitin-protein ligase	-	-	-2.35	-
les.411.2.a1_at	AT2G25490– FBL6, EBF1 (EIN3-binding F box protein 1); ubiquitin-protein ligase	-	2.07	-	-
les.4287.2.s1_at	AT1G20200.1 – 26S proteasome regulatory subunit S3, putative (RPN3)	-	-	-7.28	-4.04
les.4563.1.s1_at	AT1G35340– ATP-dependent protease La (LON) domain-containing protein	-	-	3.71	-
les.4712.1.s1_at	AT1G49850– zinc finger (C3HC4-type RING finger) family protein	-	-	2.57	-
les.4792.1.s1_at	AT1G51710– UBP6 (ubiquitin-specific protease 6)	-	-2.25	-4.05	-2.37
les.4820.1.s1_x_at	AT3G12490– cysteine protease inhibitor, putative / cystatin, putativ	6.83	-	-	-
les.494.2.s1_at	AT1G45000– 26S proteasome regulatory complex subunit p42D, putative	-	-	-7.88	-2.62
les.494.3.s1_at	AT1G45000– 26S proteasome regulatory complex subunit p42D, putative	-	-2.32	-5.27	-2.26
les.5217.1.s1_at	AT1G49050– aspartyl protease family protein	-	-	2.10	-
les.5240.1.s1_at	AT5G67090– subtilase family protein	-	-	3.95	-
les.5264.1.s1_at	AT5G67360– ARA12; subtilase	-	-	4.93	2.68
les.5363.1.s1_at	AT1G11910– aspartyl protease family protein	-	-	-2.01	-
les.5654.1.s1_at	AT3G16720– ATL2; protein binding / zinc ion binding	-2.38	-	-	-
les.795.2.s1_at	AT1G29150– RPN6, ATS9 (19S proteosome subunit 9)	-	-	-5.44	-3.36
lesaffx.12254.2.s1_at	AT2G03890– phosphatidylinositol 3- and 4-kinase family protein	-	-	2.46	-
lesaffx.1574.1.s1_at	AT3G61710– autophagy protein Apg6 family	-	-	-3.10	-2.66
lesaffx.1574.2.s1_at	AT3G61710– autophagy protein Apg6 family	-	-	-2.12	-
lesaffx.21877.1.s1_at	AT4G05050.1 – polyubiquitin (UBQ11)	-	-	2.39	-
lesaffx.22812.2.s1_at	AT3G14250– protein binding / zinc ion binding	-2.50	-	-	-
lesaffx.24556.1.s1_at	AT1G18660– zinc finger (C3HC4-type RING finger) family protein	-	-	-4.09	-3.17
lesaffx.30683.1.s1_at	AT3G14250– protein binding / zinc ion binding	-2.67	-	-	-

Table H.3 (continued)

Probe Set ID	AT locus – Putative Annotation	Fold Change			
		S2 C	M48 C	S2 AS	M48 AS
lesaffx.31022.1.a1_at	AT4G16563– aspartyl protease family protein	-	-	2.48	-
lesaffx.31192.1.s1_at	AT5G47050– ATP binding / protein binding / shikimate kinase/ zinc ion binding	-	-	2.51	-
lesaffx.3308.1.s1_at	AT4G19006– 26S proteasome regulatory subunit, putative (RPN9)	-	-	-3.12	-
lesaffx.3308.2.s1_at	AT4G19006– 26S proteasome regulatory subunit, putative (RPN9)	-	-	-6.83	-2.22
lesaffx.33164.1.a1_at	AT5G57860– ubiquitin family protein	-	-	2.11	-
lesaffx.33164.1.s1_at	AT5G57860– ubiquitin family protein	-	-	2.03	-
lesaffx.40157.1.s1_at	AT1G14570– UBX domain-containing protein	-	-	-2.02	-
lesaffx.40158.1.s1_at	AT2G18280– AtTLP2 (tubby like protein 2); phosphoric diester hydrolase/ TF	-2.66	-	-4.66	-2.33
lesaffx.46734.1.a1_at	AT3G01400– armadillo/beta-catenin repeat family protein	-	-	-3.79	-
lesaffx.48114.1.a1_at	AT1G32740– protein binding / zinc ion binding	-	-	3.33	2.59
lesaffx.48314.2.s1_at	AT3G26730– zinc finger (C3HC4-type RING finger) family protein	-	-	-2.91	-2.32
lesaffx.49541.1.s1_at	AT3G09770– zinc finger (C3HC4-type RING finger) family protein	-	-	-4.45	-3.15
lesaffx.49548.1.s1_at	AT1G16470– PAB1 (proteasome subunit PAB1); peptidase	-	-	-	-
lesaffx.51724.1.s1_at	AT4G39370– UBP27 (ubiquitin-specific protease 27); ubiquitin-specific protease	-	-	-	-
lesaffx.51779.1.s1_at	AT1G47710– (ATSERPIN1); cysteine protease inhibitor/ serine-type endopeptidase inhibitor	-3.01	-2.39	-5.84	-2.23
lesaffx.51888.1.s1_at	AT3G56740– ubiquitin-associated/TS-N domain-containing protein	-	-	-2.69	-
lesaffx.53232.1.s1_at	AT3G47990– zinc finger (C3HC4-type RING finger) family protein	-	-2.01	-	-
lesaffx.56846.1.s1_at	AT5G22000– RHF2A, CIC7E11; protein binding / zinc ion binding	-	-	-2.11	-
lesaffx.57874.1.s1_at	AT1G71980– protease-associated zinc finger (C3HC4-type RING finger) family protein	-	-	-	-2.04
lesaffx.58896.1.s1_at	AT2G45500– similar to ATPase	-	-	-2.04	-2.28
lesaffx.59101.1.s1_at	AT2G19560– proteasome protein-related	-	-	-4.65	-4.12
lesaffx.61619.1.s1_at	AT5G42270– FTSH5, VAR1 (VARIEGATED 1); ATP-dependent peptidase/ ATPase/ metallopeptidase	-	-	-5.01	-3.35
lesaffx.62975.1.s1_at	AT5G27420– zinc finger (C3HC4-type RING finger) family protein	-	-	-3.70	-3.36
lesaffx.63367.1.s1_at	AT2G03120– signal peptide peptidase family protein	-	-2.94	-3.05	-
lesaffx.63935.1.s1_at	AT1G24140– matrixin family protein	-3.10	-4.28	-	-2.01
lesaffx.63987.1.s1_at	AT1G06110– SKIP16 (SKP1/ASK-interacting protein 16); protein binding	-	-	-2.58	-2.10
lesaffx.64823.1.s1_at	AT4G02075– PIT1 (PITCHOUN 1); protein binding / zinc ion binding	-	2.65	-	-
lesaffx.66215.1.s1_at	AT1G44130– nucellin protein. putative	-2.18	-2.03	-2.63	-
lesaffx.66215.2.s1_at	AT1G44130– nucellin protein. putative	-3.07	-	-5.93	-3.70
lesaffx.66459.1.s1_at	AT4G33565– zinc finger (C3HC4-type RING finger) family protein	-2.18	-	-2.33	-
lesaffx.67780.1.s1_at	AT5G60160– aspartyl aminopeptidase, putative	-	-	-3.79	-2.76
lesaffx.67923.1.s1_at	AT1G77480– nucellin protein, putative	-	-	-2.14	-2.02

Table H.3 (continued)

Probe Set ID	AT locus – Putative Annotation	Fold Change			
		S2 C	M48 C	S2 AS	M48 AS
lesaffx.68388.1.s1_at	AT3G07990– SCPL27 (serine carboxypeptidase-like 27); serine carboxypeptidase	-	-	-2.80	-2.73
lesaffx.68556.1.s1_at	AT4G38630– MCB1, MBP1, RPN10 (regulatory particle non-ATPase 10)	-	-	-11.75	-3.76
lesaffx.68623.1.s1_at	AT1G27340– F-box family protein	-	-	-3.44	-2.87
lesaffx.68854.1.s1_at	AT2G22120– protein binding / zinc ion binding	-	-	-2.09	-
lesaffx.70855.1.s1_at	AT5G59550– zinc finger (C3HC4-type RING finger) family protein	-	-	-	-2.49
lesaffx.71026.1.s1_at	AT3G47550– zinc finger (C3HC4-type RING finger) family protein	-	-	-2.02	-
lesaffx.71026.2.s1_at	AT3G47550– zinc finger (C3HC4-type RING finger) family protein	-	-	-2.21	-
lesaffx.71088.1.s1_at	AT4G37880– protein binding / zinc ion binding	-	-	-3.72	-2.73
lesaffx.8080.1.s1_at	AT1G22040– kelch repeat-containing F-box family protein	-	-	-3.81	-3.15
POST-TRANSLATIONAL MODIFICATIONS					
les.1297.1.s1_at	AT3G21630– CERK1 (chitin elicitor receptor kinase 1); kinase/ receptor signaling protein/ transmembrane receptor PK	-3.19	-2.74	-4.06	-2.90
les.1806.1.s1_at	AT3G09830– PK, putative	-2.84	-2.42	-	-
les.1892.1.a1_at	AT5G21170– 5'-AMP-activated PK beta-2 subunit. putative	-	2.45	-	-
les.2027.3.s1_at	AT2G23770– PK family protein / peptidoglycan-binding LysM domain-containing protein	-2.70	-	-5.64	-7.01
les.2459.1.s1_at	AT5G14640– PK family protein	-	-	-6.27	-4.08
les.2459.2.s1_at	AT5G14640– PK family protein	-	-	-4.11	-2.69
les.3124.1.s1_at	AT1G17550– HAB2 (Homology to ABI2); protein serine/threonine phosphatase	-	-	-11.77	-6.12
les.3124.2.s1_at	AT1G72770– HAB1 (homology to ABI1)	-	-	-	-
les.3124.3.s1_at	AT1G72770– HAB1 (homology to ABI1)	-	-	-8.67	-3.07
les.322.1.s1_at	AT4G27800– protein phosphatase 2C PPH1 / PP2C	-	-	2.05	-
les.3248.2.s1_at	AT4G18710– DWF12, UCU1, BIN2 (brassinosteroid-insensitive 2); kinase	-	-	-6.10	-4.40
les.3248.3.s1_at	AT4G18710– DWF12, UCU1, BIN2 (brassinosteroid-insensitive 2); kinase	-	-	-2.44	-2.08
les.3377.2.s1_at	AT5G26751– ATSK11 (SHAGGY-related kinase 11); PK	-	-	-15.00	-5.53
les.3403.1.s1_at	AT5G27930– protein phosphatase 2C, putative / PP2C, putative	-	-	-3.13	-
les.3502.1.s1_at	AT2G05940– PK,putative	-	-	2.54	-
les.3727.1.s1_at	AT4G16360– 5'-AMP-activated PK beta-2 subunit, putative	-	-	2.39	-
les.3869.1.s1_at	AT1G07160– protein phosphatase 2C, putative / PP2C, putative	-2.30	-	-	-
les.390.1.s1_at	AT5G14640– PK family protein	-	-	-7.36	-4.32
les.390.2.s1_at	AT5G14640– PK family protein	-	-	-4.03	-2.45
les.4684.1.s1_at	AT2G26980– SnRK3.17, CIPK3 (CBL-interacting PK 3); kinase	-3.36	-	-	-
les.5339.1.s1_at	AT3G27580– ATPK7 (serine/threonine-PK 7); kinase	-	-	-2.29	-
les.5382.1.s1_at	AT3G13690– PK family protein	-	-	2.87	-

Table H.3 (continued)

Probe Set ID	AT locus – Putative Annotation	Fold Change			
		S2 C	M48 C	S2 AS	M48 AS
les.5647.1.s1_at	AT3G08720– S6K2, PK2, PK19 (PK 19); kinase	-	-	-3.22	-
les.5833.1.s1_at	AT1G53570– MAPKKK3, MAP3KA (Mitogen-activated PK kinase kinase 3); kinase	-2.51	-	-	-
les.5954.1.s1_at	AT1G73500– MKK9 (MAP kinase kinase 9); kinase	-	-	-2.11	-2.26
lesaffx.10444.1.s1_at	AT3G11410– AHG3/PP2CA (protein phosphatase 2CA); protein binding / protein serine/threonine phosphatase	-	-	-4.27	-
lesaffx.11410.1.s1_at	AT3G17510– SnRK3.16, CIPK1 (CBL-interacting protein KINASE 1); kinase	-	-	-7.88	-4.18
lesaffx.12647.1.s1_at	AT5G55560– PK family protein	-2.17	-	-3.00	-2.29
lesaffx.13824.1.s1_at	AT3G22750– PK, putative	-	-	-3.44	-
lesaffx.16082.1.s1_at	AT3G08760– SIK; kinase	-	-	-2.31	-2.41
lesaffx.26003.1.a1_at	AT3G08720– S6K2, PK2, PK19 (PK 19); kinase	-	-	-3.72	-
lesaffx.26661.1.a1_at	AT5G47070– PK, putative	-	-	2.10	-
lesaffx.26661.1.s1_at	AT5G47070– PK, putative	-2.01	-	-	-
lesaffx.33.1.s1_at	AT3G62260– protein phosphatase 2C, putative / PP2C, putative	-	-	-4.56	-2.29
lesaffx.344.12.s1_at	AT1G34750– protein phosphatase 2C, putative / PP2C, putative	-	-2.24	-3.59	-2.73
lesaffx.38907.2.s1_at	AT3G51550– FER (FERONIA); kinase/ PK	-2.12	-2.12	-2.36	-
lesaffx.40681.1.s1_at	AT4G31770– calcineurin-like phosphoesterase family protein	-	-	-2.03	-
lesaffx.40940.1.s1_at	AT4G38470– PK family protein	-	-	-	-2.19
lesaffx.40975.1.s1_at	AT4G14340– CKL11, CKI1 (casein kinase I); casein kinase I/ kinase	-	-	-2.77	-2.35
lesaffx.41333.2.s1_at	AT2G29380– protein phosphatase 2C, putative / PP2C, putative	-	-	-2.69	-
lesaffx.43162.2.s1_at	AT5G59160– PPO, TOPP2 (Type one serine/threonine protein phosphatase 2); protein serine/threonine phosphatase	-	-	-2.81	-
lesaffx.46935.1.s1_at	AT2G33700– protein phosphatase 2C, putative	-	-	-4.07	-2.21
lesaffx.47699.1.s1_at	AT2G17220– PK, putative	-	-	-3.13	-
lesaffx.48598.3.s1_at	AT3G58500– EP7, PP2A-3 (protein phosphatase 2A-3); protein serine/threonine phosphatase	-	-	-3.70	-2.15
lesaffx.48925.1.s1_at	AT2G17265– HSK (homoserine kinase); homoserine kinase	-	-	-2.86	-
lesaffx.50307.1.s1_at	AT1G72710– CKL2; casein kinase I/ kinase	-	-	-2.96	-2.29
lesaffx.58478.1.s1_at	AT3G02750– protein phosphatase 2C family protein / PP2C family protein	-	-	-2.02	-
lesaffx.5860.1.a1_at	AT4G28400– protein phosphatase 2C, putative / PP2C, putative	-2.29	-	-	-
lesaffx.61790.1.s1_at	AT5G28850– calcium-binding EF hand family protein	-	-	-2.07	-
lesaffx.63721.1.s1_at	AT5G42440– PK family protein	-	-	-4.03	-2.93
lesaffx.64437.1.s1_at	AT4G15900– PRL1 (pleiotropic regulatory locus 1); nucleotide binding	-	-	-2.32	-
lesaffx.64445.1.s1_at	AT1G78230– protein binding	-2.79	-	-3.25	-2.38
lesaffx.64831.1.s1_at	AT3G26020– serine/threonine protein phosphatase 2A (PP2A) regulatory subunit B', putative	-	-	-2.60	-
lesaffx.64984.1.s1_at	AT2G23070– casein kinase II alpha chain, putative	-2.15	-	-6.13	-2.23

Table H.3 (continued)

Probe Set ID	AT locus – Putative Annotation	Fold Change			
		S2 C	M48 C	S2 AS	M48 AS
lesaffx.64984.2.s1_at	AT2G23070– casein kinase II alpha chain, putative	-	-	-2.60	-
lesaffx.65398.3.s1_at	AT3G25800– PR65, PDF1 (65 KDA regulatory subunit of protein phosphatase 2A); protein phosphatase type 2A regulator	-	-	-11.82	-5.26
lesaffx.65524.1.s1_at	AT1G16220– protein phosphatase 2C family protein / PP2C family protein	-	-	-7.29	-6.49
lesaffx.65678.1.s1_at	AT3G14350– SRF7 (strubbelig-receptor family 7); ATP binding / protein serine/threonine kinase	-	-	-2.13	-
lesaffx.65817.2.s1_at	AT1G09020– ATSNF4, SNF4 (Sucrose NonFermenting 4)	-	-	-5.21	-3.46
lesaffx.66270.1.s1_at	AT5G01820– SnRK3.15, CIPK14, ATSR1 (serine/threonine PK 1); kinase	-	-	-4.23	-
lesaffx.67629.1.s1_at	AT5G58380– SIP1, SNRK3.8, PKS2, CIPK10 (CBL-interacting PK 10); kinase	-	-	-2.11	-
lesaffx.68000.1.s1_at	AT3G05580– serine/threonine protein phosphatase, putative	-3.12	-2.20	-10.09	-3.24
lesaffx.68419.1.s1_at	AT2G43850– ankyrin PK, putative (APK1)	-3.36	-3.06	-2.83	-3.53
lesaffx.68979.1.s1_at	AT3G51630– ZIK1, WNK5 (Arabidopsis WNK kinase 5)	-	-	-2.69	-2.84
lesaffx.70383.1.s1_at	AT1G16220– protein phosphatase 2C family protein / PP2C family protein	-	-	-5.19	-4.06
lesaffx.70568.1.s1_at	AT3G21070– ATNADK-1, NADK1 (NAD kinase 1); NAD+ kinase/ NADH kinase/ CAM binding	-	-	-2.51	-2.57
lesaffx.70796.1.s1_at	AT5G03470– ATB' ALPHA (PP2A, B' subunit, alpha isoform); protein phosphatase type 2A regulator	-2.52	-	-4.50	-3.57
lesaffx.7177.1.s1_at	AT1G14000– PK family protein / ankyrin repeat family protein	-	-	-8.16	-4.14
PROTEIN TARGETING					
les.2862.2.s1_at	AT1G11890– ATSEC22, SEC22 (secretion 22); transporter	-	-2.06	-	-
les.2874.2.s1_at	AT4G30600– signal recognition particle receptor alpha subunit family protein	-	-	-2.59	-
les.2874.3.s1_at	AT4G30600– signal recognition particle receptor alpha subunit family protein	-	-	-5.26	-3.39
les.393.2.s1_at	AT4G32940– GAMMAVPE, GAMMA-VPE (Vacuolar processing enzyme gamma); cysteine-type endopeptidase	-	-	-6.55	-3.45
les.4504.2.s1_at	AT2G28800– ALB3 (ALBINO 3)	-	-	-2.00	-2.34
les.4519.1.a1_at	AT2G34250– protein transport protein sec61, putative	-	-	-	-
les.4519.2.s1_at	AT2G34250– protein transport protein sec61, putative	-	-2.00	-3.27	-2.11
les.4519.3.s1_at	AT2G34250– protein transport protein sec61, putative	-	-	-11.55	-8.34
les.4744.1.s1_at	AT3G25150– nuclear transport factor 2 (NTF2) family protein / RNA recognition motif (RRM)-containing protein	-	-	-2.15	-
lesaffx.22051.2.s1_at	AT1G51980– mitochondrial processing peptidase alpha subunit, putative	-	-	-9.48	-7.04
lesaffx.35587.1.s1_at	AT4G32940– GAMMA-VPE (Vacuolar processing enzyme gamma); cysteine-type endopeptidase	-2.32	-	-7.73	-3.65
lesaffx.35741.1.s1_at	AT1G09270– importin alpha-1 subunit, putative (IMPA4)	-	-2.35	-4.54	-3.26
lesaffx.35741.2.s1_at	AT1G09270– importin alpha-1 subunit, putative (IMPA4)	-	-	-2.15	-
lesaffx.44463.1.s1_at	AT3G59340– unknown protein	-	-2.08	-	-

Table H.3 (continued)

Probe Set ID	AT locus – Putative Annotation	Fold Change			
		S2 C	M48 C	S2 AS	M48 AS
lesaffx.51291.1.s1_at	AT4G24880– unnamed protein product	-	-	-4.39	-3.32
lesaffx.64328.1.s1_at	AT1G60780– clathrin adaptor complexes medium subunit family protein	-	-	-2.46	-
lesaffx.64328.2.s1_at	AT1G60780– clathrin adaptor complexes medium subunit family protein	-	-	-3.01	-2.53
lesaffx.66676.1.s1_at	AT5G14030– translocon-associated protein beta (TRAPB) family protein	2.92	-	-	-

Table H.4 Significantly (P<0.05) regulated transcripts involved in transport. Probe set ID, *Arabidopsis thaliana* (AT) locus name and putative annotation of each transcript is listed. Values represent fold changes of transcripts in S2 C and M48 C compared to WT C and in S2 AS and M48 AS compared to WT AS. Negative values show down-regulation and positive values show up-regulation. Fold changes less than 2 are indicated with -.

Probe Set ID	AT locus – Putative Annotation	Fold Change			
		S2 C	M48 C	S2 AS	M48 AS
TRANSPORT					
lesaffx.2135.1.s1_at	AT5G42740– glucose-6-phosphate isomerase, cytosolic (PGIC)	-	-	-3.03	-2.97
lesaffx.2135.4.s1_at	AT5G42740– glucose-6-phosphate isomerase, cytosolic (PGIC)	-	-	-2.31	-2.00
lesaffx.34390.1.s1_at	AT3G11320– organic anion transmembrane transporter	-	-2.06	-7.65	-4.77
lesaffx.46624.1.s1_at	AT5G17630– glucose-6-phosphate/phosphate translocator, putative	3.23	-	-	-
lesaffx.52329.1.s1_at	AT5G17520– MEX1, RCP1 (root cap 1)	-	-2.09	-	-2.29
les.4912.1.s1_at	AT3G05290– mitochondrial substrate carrier family protein	-	-	-2.23	-
lesaffx.10723.1.s1_at	AT5G42130– mitochondrial substrate carrier family protein	-	-	-3.94	-3.04
lesaffx.59407.1.s1_at	AT3G53940– mitochondrial substrate carrier family protein	-	-	-3.01	-2.18
lesaffx.68112.1.s1_at	AT5G48970– mitochondrial substrate carrier family protein	-	-	-3.25	-2.08
lesaffx.68360.1.s1_at	AT5G15640– mitochondrial substrate carrier family protein	-	-	-2.68	-
lesaffx.68360.2.s1_at	AT5G15640– mitochondrial substrate carrier family protein	-	-	-4.51	-2.02
les.58.1.s1_at	AT2G39890– ATPROT1, ProT1 (proline transporter 1)	2.44	-	-	-
les.631.3.s1_at	AT3G30390– amino acid transporter family protein	-	-	-2.87	-2.04
lesaffx.35418.1.s1_at	AT2G01170– amino acid permease family protein	-	-	-8.32	-5.85
lesaffx.35418.2.s1_at	AT2G01170– amino acid permease family protein	-	-	-3.22	-2.44
lesaffx.39098.1.s1_at	AT1G47670– amino acid transporter family protein	-	-	-	-2.14
lesaffx.40648.1.s1_at	AT2G39130– amino acid transporter family protein	-	-	-2.71	-2.08
les.1784.1.s1_at	AT1G54730– sugar transporter, putative	2.52	-	-	-
les.3756.1.s1_a_at	AT2G43330– ATINT1 (inositol transporter 1); carbohydrate transmembrane transporter/ sugar:hydrogen ion symporter	3.93	-	-	-
lesaffx.10299.1.a1_at	AT3G18830– ATPLT5 (polyol transporter 5); D-ribose transmembrane transporter	-	-	2.16	-

Table H.4 (continued)

Probe Set ID	AT locus – Putative Annotation	Fold Change			
		S2 C	M48 C	S2 AS	M48 AS
lesaffx.10299.1.s1_at	AT3G18830– ATPLT5 (polyol transporter 5); D-ribose transmembrane transporter	-2.10	-2.62	-	-2.21
lesaffx.48830.1.s1_at	AT3G59360– ATUTR6/UTR6 (UDP-galactose transporter 6); nucleotide-sugar transmembrane transporter	-	-	-4.67	-2.58
lesaffx.49378.1.s1_at	AT5G13750– ZIFL1 (zinc induced facilitator-like 1); tetracycline:hydrogen antiporter/ transporter	-	-	-13.17	-5.74
lesaffx.53008.1.s1_at	AT5G65000– nucleotide-sugar transporter family protein	-	-	-2.08	-
lesaffx.55549.1.s1_at	AT5G13750– ZIFL1 (zinc induced facilitator-like 1); tetracycline:hydrogen antiporter/ transporter	-	-	-4.18	-2.54
lesaffx.61214.1.s1_at	AT3G01280– porin, putative	-	-2.74	-2.99	-
les.270.1.s1_at	AT5G65380– ripening-responsive protein, putative	-	2.14	-	-
les.3530.1.s1_at	AT5G20960– AO1, ATAO, ATAO1, AAO1 (aldehyde oxidase 1)	3.52	-	-	-
les.4264.1.a1_s_at	AT3G22200– HER1, GABA-T, POP2 (pollen-pistil incompatibility 2); 4-aminobutyrate transaminase	-	-	-4.42	-3.05
les.4789.1.s1_at	AT1G53580– ETHE1/GLX2-3/GLY3 (glyoxalase 2-3); hydroxyacylglutathione hydrolase	-	-	2.10	-
les.5209.1.s1_at	AT2G26510– PDE135 (pigment defective embryo 135); transmembrane transporter	-2.72	-	-	-
les.5512.1.s1_at	AT2G34460– flavin reductase-related	-	-	2.75	-
lesaffx.1490.1.s1_at	AT4G21800– QQT2 (QUATRE-QUART2); ATP binding	-	-	-4.31	-2.62
lesaffx.15299.1.s1_at	AT1G80380– phosphoribulokinase/ uridine kinase-related	-	-	-	-
lesaffx.16086.1.s1_at	AT1G78820– curculin-like (mannose-binding) lectin family protein / PAN domain-containing protein	-	-	-4.45	-4.88
lesaffx.16442.1.a1_at	AT3G21690– MATE efflux family protein	-	-	-2.14	-
lesaffx.31434.1.s1_at	AT3G10530– transducin family protein / WD-40 repeat family protein	-	-	-2.15	-2.33
lesaffx.32974.1.s1_at	AT4G27585– band 7 family protein	-	-	-3.26	-
lesaffx.37702.2.s1_at	AT5G18660– PCB2, DVR (pale-green and chlorophyll B reduced 2); 3,8-divinyl protochlorophyllide a 8-vinyl reductase	2.04	-	2.80	-
lesaffx.49378.1.s1_at	AT5G13750– ZIFL1 (zinc induced facilitator-like 1); tetracycline:hydrogen antiporter/ transporter	-	-	-13.17	-5.74
lesaffx.52437.1.s1_at	AT5G65980– auxin efflux carrier family protein	-	-	-10.33	-2.74
lesaffx.55549.1.s1_at	AT5G13750– ZIFL1 (zinc induced facilitator-like 1); tetracycline:hydrogen antiporter/ transporter	-	-	-4.18	-2.54
lesaffx.60146.1.s1_at	AT1G34470– permease-related	-	-	-2.88	-
lesaffx.62361.1.s1_at	AT5G19300– unnamed protein	-	-	-4.15	-2.41
lesaffx.65940.1.s1_at	AT2G44920– thylakoid lumenal 15 kDa protein, chloroplast	2.24	-	-	-
lesaffx.66107.1.s1_at	AT2G21120– unknown protein	-	-	-2.72	-2.60
lesaffx.69151.1.s1_at	AT3G03620– MATE efflux family protein	-2.10	-2.29	-4.40	-2.82
lesaffx.70494.1.s1_at	AT4G38890– dihydrouridine synthase family protein	-	-	-	-
lesaffx.70660.1.s1_at	AT3G46450– SEC14 cytosolic factor family protein / phosphoglyceride transfer family protein	-2.01	-	-4.98	-3.85

Table H.5 Significantly (P<0.05) regulated transcripts involved in signalling. Probe set ID, *Arabidopsis thaliana* (AT) locus name and putative annotation of each transcript is listed. Values represent fold changes of transcripts in S2 C and M48 C compared to WT C and in S2 AS and M48 AS compared to WT AS. Negative values show down-regulation and positive values show up-regulation. Fold changes less than 2 are indicated with -.

Probe Set ID	AT locus – Putative Annotation	Fold Change			
		S2 C	M48 C	S2 AS	M48 AS
HORMONE SIGNALING / AUXINS					
les.2668.2.a1_at	– auxin and ethylene responsive GH3-like protein	-	2.20	-2.53	-
les.3216.1.s1_at	AT1G51760 – JR3, IAR3 (IAA-alanine resistant 3); metallopeptidase	-	-	-7.91	-4.29
les.3216.2.s1_at	– JR3, IAR3 (IAA-alanine resistant 3); metallopeptidase	-	-	-2.81	-2.28
les.3216.3.s1_at	AT1G51760 – JR3, IAR3 (IAA-alanine resistant 3); metallopeptidase	-	-	-2.54	-2.15
les.3757.1.s1_at	– Stem-specific protein TSJT1, putative	-4.12	3.26	2.16	-
les.5177.1.s1_at	– Probable indole-3-acetic acid-amido synthetase GH3.1	2.09	-	-3.69	-2.99
les.5312.1.s1_at	AT2G46370 – JAR, FIN219, JAR1 (jasmonate resistant 1)	-	-	-2.03	-
lesaffx.44071.1.s1_at	AT2G44500 – similar to unknown protein	-	-	-6.04	-3.33
lesaffx.44071.2.s1_at	AT2G44500 – similar to unknown protein	-2.24	-	-2.89	-
lesaffx.58573.1.s1_a_at	AT1G52630 – similar to unknown protein	-	-	-2.32	-2.12
lesaffx.71035.1.s1_at	AT4G38840 – auxin-responsive protein, putative	-	2.79	-	-
HORMONE SIGNALING / JASMONATE					
les.13.1.s1_at	AT5G42650 – CYP74A, AOS (allene oxide synthase); hydro-lyase/ oxygen binding	-	-	-3.55	-5.07
les.3478.1.s1_at	AT4G15440 – CYP74B2, HPL1 (hydroperoxide lyase 1); heme binding / iron ion binding / monooxygenase	4.38	-	-	-
les.3632.1.s1_at	AT1G17420 – LOX3 (Lipoxygenase 3); iron ion binding / lipoxygenase/ metal ion binding / oxidoreductase	-	-2.83	-	-
les.3762.1.s1_at	AT2G06050 – OPR3 (OPDA-reductase 3)	-	-	-2.15	-
les.3980.1.s1_at	AT3G45140 – LOX2 (lipoxygenase 2)	10.95	-2.88	7.13	-
les.3986.1.s1_at	AT5G42650 – CYP74A, AOS (allene oxide synthase); hydro-lyase/ oxygen binding	2.33	-	-	-
HORMONE SIGNALING / GIBERELLIC ACID					
les.417.1.s1_at	AT5G59845 – gibberellin-regulated family protein	2.60	-	10.20	2.84
les.4311.1.s1_at	AT1G14920 – RGA2, GAI (GA insensitive); TF	-	2.20	-	-
les.4766.1.s1_at	AT2G18420 – Gibberellin-regulated GASA/GAST/Snakin family protein	-	-	7.77	2.11
les.64.1.s1_at	AT4G25420 – GA20OX1, AT2301, GA5 (GA requiring 5); gibberellin 20-oxidase/ gibberellin 3-beta-dioxygenase	4.15	2.81	4.84	-
les.827.1.s1_at	AT3G02885 – GASA5 (GAST1 protein homolog 5)	-2.10	3.02	-	-
SIGNALING / CALCIUM REGULATION					
les.1360.1.s1_at	AT1G18890 – CPK10, ATCDPK1; CAM-dependent PK/ kinase/ PK	-2.35	-2.43	-3.59	-2.70
les.1672.1.s1_at	AT3G10190 – CAM, putative	-3.04	-	-	-
les.1989.1.a1_at	AT2G38800 – CAM-binding protein-related	2.13	-	-	-
les.3334.2.s1_at	AT5G66210 – CPK28 (calcium-dependent PK 28)	-	-	-3.15	-4.01

Table H.5 (continued)

Probe Set ID	AT locus – Putative Annotation	Fold Change			
		S2 C	M48 C	S2 AS	M48 AS
les.4149.2.s1_a_at	AT4G38810 – calcium-binding EF hand family protein	-	-	-2.42	-
les.5056.1.s1_a_at	AT5G42380 – CML37/CML39; calcium ion binding	-2.75	-	-	-
les.5318.1.s1_at	AT3G52870 – CAM-binding family protein	-	-	2.89	-
les.5939.1.s1_at	AT4G27280 – calcium-binding EF hand family protein	-	-2.15	-	-
les.934.1.s1_at	AT1G18210 – calcium-binding protein, putative	-2.18	-	-	-
lesaffx.16164.1.s1_at	AT3G29000 – calcium-binding EF hand family protein	-3.16	-	-	-
lesaffx.3635.2.s1_at	AT2G26190 – CAM-binding family protein	-	-	2.39	-
lesaffx.47093.1.s1_at	AT3G43810 – CAM7; calcium ion binding	-	-	2.39	-
lesaffx.47666.1.s1_at	AT4G34150 – C2 domain-containing protein	-	-	-3.94	-3.16
lesaffx.57054.2.s1_at	AT5G54130 – calcium-binding EF hand family protein	-2.50	-	-6.89	-3.98
lesaffx.57454.1.s1_at	AT1G18530 – CAM, putative	-3.03	-	-	-
lesaffx.60825.1.s1_at	AT5G28830 – calcium-binding EF hand family protein	-3.74	-2.16	-2.31	-
lesaffx.63947.2.s1_at	AT3G57530 – CDPK32, CPK32 (calcium-dependent PK 32); CAM-dependent PK/ kinase	-	-	-2.49	-2.71
lesaffx.64561.1.s1_at	AT5G62390 – BAG7 (BCL-2-associated athanogene 7); CAM binding	-2.11	-	-	-
lesaffx.66814.1.s1_at	AT1G73805 – CAM binding	-2.21	-3.13	-	-
SIGNALING / G-PROTEINS					
les.1324.1.s1_at	AT1G56050 – GTP-binding protein-related	2.14	-	2.02	-
les.1434.1.s1_at	AT5G52580 – RAB GTPase activator	-	-	-2.02	-
les.223.1.s1_at	AT3G46060 – AtRABE1c, AtRab8A, ARA3	-	-	-	-
les.2350.1.s1_at	AT4G35750 – Rho-GTPase-activating protein-related	-	-	3.37	-
les.2350.2.s1_at	AT4G35750 – Rho-GTPase-activating protein-related	-	-	2.09	-
les.3564.1.a1_at	AT5G08650 – GTP-binding protein LepA, putative	-	-	-	-2.04
les.3670.1.s1_at	AT2G26300 – GPA1 (G protein alpha subunit 1); signal transducer	2.10	-	-	-
les.4749.1.s1_at	AT2G46710 – rac GTPase activating protein, putative	-	-	5.15	2.40
les.4857.2.s1_at	AT5G45130 – Rab5A, RABF2a, RHA1	5.00	-	-	-
les.4861.1.s1_at	AT1G07410 – Rab GTPase homolog A2b; GTP binding	-	2.14	-	-
les.5316.1.s1_at	AT5G66470 – GTP binding / RNA binding	2.22	-	2.10	-
les.5401.1.s1_at	AT4G36390 – radical SAM domain-containing protein / TRAM domain-containing protein	-	-	2.45	-
les.5877.1.s1_at	AT5G47520 – RABA5a, Rab GTPase homolog A5a; GTP binding	-	2.03	-	-
lesaffx.37222.1.s1_at	AT5G61530 – small G protein family protein / RhoGAP family protein	-	-	-8.59	-3.43
lesaffx.39914.1.s1_at	AT5G54310 – AGD5 (ARF-GAP domain 5); DNA binding	-	-	-2.90	-2.63
lesaffx.51015.1.s1_at	AT4G21520 – transducin family protein / WD-40 repeat family protein	-	-	-6.21	-2.98
lesaffx.66384.1.s1_at	AT5G02040 – prenylated rab acceptor (PRA1) family protein	-	-	-3.28	-
lesaffx.67491.1.s1_at	AT4G28950 – RAC7, ROP9 (RHO-related protein from plants 9); GTP binding	-	-2.56	-	-

Table H.5 (continued)

Probe Set ID	AT locus – Putative Annotation	Fold Change			
		S2 C	M48 C	S2 AS	M48 AS
lesaffx.68259.1.s1_at	AT4G34460 – ELK4, AGB1 (GTP binding protein beta 1)	-	-	-9.02	-4.08
lesaffx.68259.2.s1_at	AT4G34460 – ELK4, AGB1 (GTP binding protein beta 1)	-	-	-2.74	-
lesaffx.68801.1.s1_at	AT5G52580 – RAB GTPase activator	-	-	-2.13	-2.01
SIGNALING / RECEPTOR KINASES					
les.1297.1.s1_at	AT3G21630 – CERK1 (chitin elicitor receptor kinase 1); kinase/ receptor signaling protein/ transmembrane receptor PK	-3.19	-2.74	-4.06	-2.90
les.1334.1.a1_at	AT5G38280 – PR5K (PR5-like receptor kinase); kinase/ transmembrane receptor protein serine/threonine kinase	-	-	2.10	-
les.5333.1.s1_at	AT1G28340 – LRR family protein	-	-	2.15	-
lesaffx.16086.1.s1_at	AT1G78820 – curculin-like (mannose-binding) lectin family protein / PAN domain-containing protein	-	-	-4.45	-4.88
lesaffx.40862.1.s1_at	AT4G00300 – fringe-related protein	-	-2.48	-10.92	-6.11
lesaffx.59625.1.s1_at	AT5G48540 – 33 kDa secretory protein-related	-2.04	-	2.18	2.18
lesaffx.65273.1.s1_at	AT1G16670 – PK family protein	-	-	-3.04	-2.62
lesaffx.65678.1.s1_at	AT3G14350 – SRF7 (strubbelig-receptor family 7); ATP binding / protein serine/threonine kinase	-	-	-2.13	-
SIGNALING / CYTOPLASMIC KINASES					
les.1297.1.s1_at	AT3G21630 – CERK1 (chitin elicitor receptor kinase 1); kinase/ receptor signaling protein/ transmembrane receptor PK	-3.19	-2.74	-4.06	-2.90
les.1806.1.s1_at	AT3G09830 – PK, putative	-2.84	-2.42	-	-
les.2027.3.s1_at	AT2G23770 – PK family protein / peptidoglycan-binding LysM domain-containing protein	-2.70	-	-5.64	-7.01
les.3502.1.s1_at	AT2G05940 – PK, putative	-	-	2.54	-
les.5382.1.s1_at	AT3G13690 – PK family protein	-	-	2.87	-
lesaffx.16082.1.s1_at	AT3G08760 – ATSIK; kinase	-	-	-2.31	-2.41
lesaffx.26661.1.a1_at	AT5G47070 – PK, putative	-	-	2.10	-
lesaffx.26661.1.s1_at	AT5G47070 – PK, putative	-2.01	-	-	-
lesaffx.38907.2.s1_at	AT3G51550 – FER (FERONIA); kinase/ PK	-2.12	-2.12	-2.36	-
lesaffx.47699.1.s1_at	AT2G17220 – PK, putative	-	-	-3.13	-
lesaffx.63721.1.s1_at	AT5G42440 – PK family protein	-	-	-4.03	-2.93
lesaffx.65678.1.s1_at	AT3G14350 – SRF7 (strubbelig-receptor family 7); ATP binding / protein serine/threonine kinase	-	-	-2.13	-

Table H.6 Significantly (P<0.05) regulated transcripts involved in enzymatic processes. Probe set ID, *Arabidopsis thaliana* (AT) locus name and putative annotation of each transcript is listed. Values represent fold changes of transcripts in S2 C and M48 C compared to WT C and in S2 AS and M48 AS compared to WT AS. Negative values show down-regulation and positive values show up-regulation. Fold changes less than 2 are indicated with -.

Probe Set ID	AT locus – Putative Annotation	Fold Change			
		S2 C	M48 C	S2 AS	M48 AS
ENZYME FAMILIES/ PHOSPHATASES					
les.3743.1.s1_at	AT4G25150– acid phosphatase, putative	-	-	-	2.18
lesaffx.24391.1.s1_at	AT1G72880– acid phosphatase survival protein SurE, putative	-	-	-4.34	-3.18
lesaffx.42534.1.s1_at	AT2G01180 – PAP1, LPP1, (phosphatidic acid phosphatase 1); phosphatidate phosphatase	-2.34	-	-	-
lesaffx.67373.2.s1_at	AT1G09870– histidine acid phosphatase family protein	-	-	-7.47	-3.55
ENZYME FAMILIES/ PEROXIDASES					
les.2092.1.s1_at	AT4G21960 – PRXR1 (peroxidase 42); peroxidase	-	-	3.85	-
les.2832.1.s1_at	AT5G64120.1 – peroxidase, putative	-2.87	-	2.64	-
les.4492.2.s1_at	AT4G21960 – PRXR1, (peroxidase 42); peroxidase	-	-	2.61	-
les.4492.3.s1_at	AT4G21960.1 – peroxidase 42 (PER42) (P42) (PRXR1)	3.11	-	3.09	-
les.4999.1.s1_at	AT2G37130– peroxidase 21 (PER21) (P21) (PRXR5)	-	-	-4.27	-
lesaffx.32359.1.s1_at	AT4G37530– peroxidase, putative	-2.89	-	-3.29	-
lesaffx.53517.1.s1_at	AT1G71695– peroxidase 12 (PER12) (P12) (PRXR6)	-	-	-2.65	-
lesaffx.57363.1.s1_at	AT1G14550– anionic peroxidase, putative	-	-	2.96	-
ENZYME FAMILIES/ CYP					
les.1859.2.s1_at	AT3G14690– CYP72A15 (cytochrome P450, family 72, subfamily A, polypeptide 15); oxygen binding	-	-	-2.46	-2.19
les.1859.3.s1_at	AT3G14690– CYP72A15 (cytochrome P450, family 72, subfamily A, polypeptide 15); oxygen binding	-	-	-4.54	-4.11
les.2988.1.s1_at	AT2G30490– C4H/CYP73A5 (cinnamate 4-hydroxylase); trans-cinnamate 4-monooxygenase	2.56	2.88	-	-
les.3094.2.s1_at	AT1G11680– EMB1738, CYP51A2, CYP51,CYP51G1 (cytochrome P450 51); oxygen binding	-	-	-6.00	-3.29
les.438.1.s1_at	AT1G69780– ATHB13; DNA binding / TF	-	2.28	-	-
les.4443.1.a1_s_at	AT5G38970– CYP85A1, BR6OX, BR6OX1 (brassinosteroid-6-oxidase); oxygen binding	-	2.81	-	-
les.4980.1.s1_at	AT4G36220– CYP84A1, FAH1 (ferulate-5-hydroxylase 1); ferulate 5-hydroxylase	2.85	-	-	-
les.5057.1.s1_at	AT3G61880–CYP78A9 (cytochrome P450 78A9); oxygen binding	-	2.91	-2.49	-
lesaffx.22297.1.s1_at	AT2G34500– CYP710A1 (cytochrome P450, family 710, subfamily A, polypeptide 1); C-22 sterol desaturase/ oxygen binding	-	-	-5.14	-3.33
lesaffx.24132.1.a1_at	AT3G14640– CYP72A10 (cytochrome P450, family 72, subfamily A, polypeptide 10); oxygen binding	-	-	-2.56	-2.48
lesaffx.31317.11.s1_at	AT1G64940– CYP89A6 (cytochrome P450, family 87, subfamily A, polypeptide 6); oxygen binding	-	-	-4.06	-3.26
lesaffx.3253.2.s1_at	AT4G19230– CYP707A1 (cytochrome P450, family 707, subfamily A, polypeptide 1); oxygen binding	-	-	2.74	-
lesaffx.51311.1.s1_at	AT4G37320– CYP81D5 (cytochrome P450, family 81, subfamily D, polypeptide 5); oxygen binding	-	-	-2.01	-
lesaffx.8720.2.s1_at	AT3G52970– CYP76G1 (cytochrome P450, family 76, subfamily G, polypeptide 1); oxygen binding	-	-	-4.44	-2.10
lesaffx.9038.1.s1_at	AT2G32440– CYP88A4, KAO2 (ent-kaurenoic acid hydroxylase 2); oxygen binding	4.52	-	-	-

Table H.6 (continued)

Probe Set ID	AT locus – Putative Annotation	Fold Change			
		S2 C	M48 C	S2 AS	M48 AS
lesaffx.9038.3.s1_at	AT4G37400– CYP81F3 (cytochrome P450, family 81, subfamily F, polypeptide 3); oxygen binding	-2.52	-2.98	-	-
ENZYME FAMILIES/ REDOX					
les.1925.1.a1_at	AT1G19570– DHAR1 (dehydroascorbate reductase)	3.26	-	-	-
les.1925.2.s1_at	AT1G19570– DHAR1 (dehydroascorbate reductase)	2.39	-	-	-
les.296.1.s1_at	AT5G16710– DHAR3 (dehydroascorbate reductase 1); glutathione dehydrogenase (ascorbate)	-	-	2.31	-
les.3451.1.s1_at	ATGLDH | GLDH (L-galactono-1,4-lactone dehydorogenase); FAD binding / catalytic/ oxidoreductase	-	-	2.09	-
les.3759.1.s1_at	AT5G53560– B5-A (Cytochrome b5 A)	-4.09	-	-	-
les.5581.1.s1_at	AT4G09010– APX4 (ascorbate peroxidase 4); peroxidase	2.39	-	5.85	-
lesaffx.32653.1.s1_at	AT3G54660– GR2 | GR (glutathione reductase); glutathione-disulfide reductase	-2.95	-	-	-
lesaffx.54488.1.s1_at	AT1G63940– monodehydroascorbate reductase, putative	-	-	-3.33	-2.30

Table H.7 Significantly (P<0.05) regulated transcripts involved in metabolic reactions. Probe set ID, *Arabidopsis thaliana* (AT) locus name and putative annotation of each transcript is listed. Values represent fold changes of transcripts in S2 C and M48 C compared to WT C and in S2 AS and M48 AS compared to WT AS. Negative values show down-regulation and positive values show up-regulation. Fold changes less than 2 are indicated with -.

Probe Set ID	AT locus – Putative Annotation	Fold Change			
		S2 C	M48 C	S2 AS	M48 AS
SECONDARY METABOLISM/ PHENYLPROPANOIDS					
les.1097.1.a1_at	AT3G21240– 4CL2 (4-coumarate:CoA ligase 2)	3.49	2.26	2.76	2.71
les.220.1.s1_at	AT5G01210– transferase family protein	-2.53	-	-5.60	-2.43
les.281.1.s1_at	AT1G51680– 4CL1 (4-coumarate:CoA Ligase 1)	2.36	-	-	-
les.281.3.s1_at	AT1G51680– 4CL1 (4-coumarate:CoA Ligase 1)	-	-	-7.23	-7.37
les.3741.1.s1_at	AT4G37980– ELI3-1 (elicitor-activated gene 3); binding / catalytic/ oxidoreductase/ zinc ion binding	-	-	-4.59	-
les.4271.1.s1_at	AT2G37040– PAL1 (PHE ammonia lyase 1); phenylalanine ammonia-lyase	-	-	2.71	-
les.5068.1.s1_at	AT5G23230– NIC2 (nicotinamidase 2); catalytic/ nicotinamidase	-	-	2.39	-
lesaffx.47885.1.s1_at	AT1G20510– OPCL1 (OPC-8:0 CoA ligase1); 4-coumarate-CoA ligase	-	-	-2.59	-4.21
SECONDARY METABOLISM/ ISOPRENOIDS					
les.3123.1.s1_at	AT1G74470– geranylgeranyl reductase	-	-	3.02	-
les.3544.1.s1_at	AT5G52570– BETA-OHASE 2 (beta-carotene hydroxylase 2)	-	-	-4.04	-
les.4438.1.a1_s_at	AT5G17230– PSY (phytoene synthase)	2.32	-	-	-
les.4930.1.a1_at	AT5G17230– PSY (phytoene synthase)	2.08	-	2.28	-
lesaffx.21854.1.s1_at	AT2G26800– 3-hydroxy-3-methylglutarate-CoA lyase, putative / HMG-CoA lyase, putative	-	-	-3.07	-2.21
lesaffx.44987.1.s1_at	AT1G78510– SPS1 (solanesyl diphosphate synthase 1)	-2.42	-	-4.29	-2.72

Table H.7 (continued)

Probe Set ID	AT locus – Putative Annotation	Fold Change			
		S2 C	M48 C	S2 AS	M48 AS
CELL WALL/ PRECURSOR SYNTHESIS					
les.1852.3.s1_at	AT3G29360– UDP-glucose 6-dehydrogenase, putative	-	-	-16.58	-9.30
les.2813.1.s1_at	AT1G08200– AXS2 (UDP-D-APIOSE/UDP-D-xylose synthase 2)	-	-2.03	-9.54	-3.42
les.4583.1.s1_at	AT4G30440– GAE1 (UDP-D-glucuronate 4-epimerase 1); UDP-glucuronate 4-epimerase/ catalytic	-3.37	-	-	-
les.963.2.s1_at	AT1G30620.1– UDP-D-xylose 4-epimerase, putative (MUR4)	-2.27	-3.39	-4.37	-2.81
lesaffx.14736.1.s1_at	AT3G46440 – UXS5 (UDP-Xyl synthase 5); catalytic	-2.06	-3.20	-	-
lesaffx.38740.1.s1_at	AT4G10960– UGE5 (UDP-D-glucose/UDP-D-galactose 4-epimerase 5); UDP-glucose 4-epimerase/ protein dimerization	-2.29	-2.18	-7.39	-3.46
lesaffx.62950.1.s1_at	AT4G23920– UGE2 (UDP-D-glucose/UDP-D-galactose 4-epimerase 2); UDP-glucose 4-epimerase/ protein dimerization	-	-	-4.84	-3.40
lesaffx.9043.1.s1_at	AT3G62830– AUD1, ATUXS2, UXS2 (UDP-glucuronic acid decarboxylase 2)	-2.80	-3.04	-5.95	-2.31
les.4368.1.s1_s_at	AT1G62440– LRX2 (LRR/extensin 2); protein binding / structural constituent of cell wall	-	-	8.56	5.09
lesaffx.43685.2.s1_s_at	AT1G62440– LRX2 (LRR/extensin 2); protein binding / structural constituent of cell wall	-	-	9.28	4.92
CELL WALL/ PROTEINS					
les.4368.1.s1_s_at	AT1G62440– LRX2 (LRR/extensin 2); protein binding / structural constituent of cell wall	-	-	8.56	5.09
lesaffx.43685.2.s1_s_at	AT1G62440– LRX2 (LRR/extensin 2); protein binding / structural constituent of cell wall	-	-	9.28	4.92
les.2946.2.s1_at	– extensin-like protein	3.24	-	-	-
les.3320.2.s1_at	AT3G22440– hydroxyproline-rich glycoprotein family protein	-	-	-19.51	-11.31
les.3320.3.s1_at	AT3G22440– hydroxyproline-rich glycoprotein family protein	-	-	-3.45	-2.27
CELL WALL/ MODIFICATION					
les.210.1.s1_at	AT3G23730– xyloglucan:xyloglucosyl transferase, putative / xyloglucan endotransglycosylase, putative / endo-xyloglucan transferase, putative	-4.13	-	2.39	-
les.276.1.s1_at	AT4G14130– XTR7 (xyloglucan endotransglycosylase 7); hydrolase, acting on glycosyl bonds	-	-	2.15	-
les.3537.1.s1_at	AT2G01850– XTH27, EXGT-A3 (endo-xyloglucan transferase A3); hydrolase, acting on glycosyl bonds / xyloglucan:xyloglucosyl transferase	-4.45	-	2.06	-
les.3590.1.s1_at	AT5G13870– EXGT-A4 (endoxyloglucan transferase A4); hydrolase. acting on glycosyl bonds	-5.53	-	5.55	-
les.369.1.s1_at	AT1G26770– EXP10, HEXP ALPHA 1.1, EXPA10 (expansin A10)	2.62	-	2.72	-
les.4530.1.s1_at	AT3G23730– xyloglucan:xyloglucosyl transferase, putative / xyloglucan endotransglycosylase, putative / endo-xyloglucan transferase. putative	-	-	5.83	3.05
les.4769.1.s1_at	AT3G45970– EXPL1, HEXP BETA 2.1, ATEXLA1 (expansin-like A1)	-5.57	-	-	-
les.4968.1.s1_s_at	AT2G01850– XTH27, EXGT-A3 (endo-xyloglucan transferase A3); hydrolase, acting on glycosyl bonds / xyloglucan:xyloglucosyl transferase	-3.75	-	-	-

Table H.7 (continued)

Probe Set ID	AT locus – Putative Annotation	Fold Change			
		S2 C	M48 C	S2 AS	M48 AS
CELL WALL/ DEGRADATION					
les.1847.1.a1_at	AT3G47000– glycosyl hydrolase family 3 protein	-	-	2.26	-
les.2187.1.a1_at	AT5G66460– (1-4)-beta-mannan endohydrolase. putative	-	2.15	-	-
les.2298.2.a1_a_at	AT3G16850– glycoside hydrolase family 28 protein / polygalacturonase (pectinase) family protein	-	2.16	-	-
les.3991.1.s1_at	AT5G64570– BXL4, XYL4 (beta-xylosidase 4); hydrolase, hydrolyzing O-glycosyl compounds	-	-	6.92	-
les.5081.1.s1_at	AT3G47000– glycosyl hydrolase family 3 protein	-	-	2.57	-
les.5495.1.s1_at	AT3G55140– pectate lyase family protein	-	-	2.05	-
les.5579.1.s1_at	AT4G13710– pectate lyase family protein	2.57	-2.47	-	-
CARBOHYDRATE METABOLISM/ STARCH SYNTHESIS					
les.1310.1.s1_at	AT1G32900– starch synthase, putative	-	-	5.48	-
les.3350.1.s1_at	AT3G01180– ATSS2 (starch synthase 2); transferase, transferring glycosyl groups	-	-	2.15	-
les.4764.1.s1_at	AT2G36390– BE3, SBE2.1 (starch branching enzyme 2.1); 1,4-alpha-glucan branching enzyme	-	-	2.18	-
les.4890.1.s1_at	AT5G24300– ATSS1/SSI (starch synthase I); transferase, transferring glycosyl groups	-	-	2.38	-
les.78.1.s1_at	AT5G19220– APL1, ADG2 (ADPG pyrophosphorylase 2); glucose-1-phosphate adenylyltransferase	-	-	3.16	-
lesaffx.71563.1.a1_at	AT5G03650– SBE2.2 (starch branching enzyme 2.2); 1,4-alpha-glucan branching enzyme	-	-	2.57	-
CARBOHYDRATE METABOLISM/ STARCH DEGRADATION					
les.1401.2.s1_at	AT5G18670– BAM9, BMY3 (beta-amylase 9); beta-amylase	-2.76	-	-8.36	-3.49
les.1401.3.s1_at	AT5G18670– BAM9, BMY3 (BETA-AMYLASE 9); beta-amylase	-	-	-11.88	-7.70
les.2844.1.s1_at	AT3G23920– BAM1/BMY7/TR-BAMY (beta-amylase 1); beta-amylase	-2.16	-	-	-
lesaffx.34425.1.s1_at	AT4G25000– ATAMY1, AMY1/ATAMY1 (alpha-amylase-like); alpha-amylase	-	-	-3.09	-
lesaffx.52329.1.s1_at	AT5G17520– MEX1, RCP1 (root cap 1)	-	-2.09	-	-2.29
lesaffx.9.1.s1_at	AT1G76130– AMY2/ATAMY2 (alpha-amylase-like 2); alpha-amylase	-	-2.14	-	-
CARBOHYDRATE METABOLISM/ SUCROSE SYNTHESIS					
les.1617.1.s1_s_at	AT1G43670– fructose-1,6-bisphosphatase / D-fructose-1,6-bisphosphate 1-phosphohydrolase/ FBPase/ putative	-	-2.29	3.73	-
les.1617.2.s1_s_at	AT1G43670– fructose-1,6-bisphosphatase / D-fructose-1,6-bisphosphate 1-phosphohydrolase/ FBPase/ putative	-	-	6.27	-
les.1617.3.a1_s_at	AT1G43670– fructose-1,6-bisphosphatase / D-fructose-1,6-bisphosphate 1-phosphohydrolase/ FBPase/ putative	-	-	10.54	-
les.4946.1.s1_at	AT1G43670– fructose-1,6-bisphosphatase / D-fructose-1,6-bisphosphate 1-phosphohydrolase/ FBPase/ putative	-	-	10.13	-
CARBOHYDRATE METABOLISM/ SUCROSE DEGRADATION					
les.3460.1.s1_at	AT3G52600– ATCWINV2 (cell wall invertase 2); hydrolase, hydrolyzing O-glycosyl compounds	-2.43	-	-2.37	-2.45
lesaffx.53904.1.s1_at	AT5G40510– unknown protein	-	-2.08	-4.58	-
LIPIDS/ FATTY ACID SYHTHESIS					
les.2747.2.s1_at	AT2G33150– KAT2/PED1 (peroxisome defective 1); acetyl-CoA C-acyltransferase	-	-	-2.18	-

Table H.7 (continued)

Probe Set ID	AT locus – Putative Annotation	Fold Change			
		S2 C	M48 C	S2 AS	M48 AS
les.3383.1.s1_at	AT3G48990– AMP-dependent synthetase and ligase family protein	-5.51	-	-2.10	-
les.4040.1.s1_at	AT2G26640– beta-ketoacyl-CoA synthase, putative	-	-	-2.80	-
les.5427.1.s1_at	AT3G16170– acyl-activating enzyme 13 (AAE13)	-	-	2.07	-
les.5952.1.s1_at	AT4G25050– ACP4 (acyl carrier protein 4)	-	-	4.10	2.06
lesaffx.10235.1.s1_at	AT1G65880– BZO1; benzoate-CoA ligase	2.05	-	2.32	2.83
lesaffx.13683.2.s1_at	AT3G16910– AAE7/ACN1 (acyl-activating enzyme 7); AMP binding / acetate-CoA ligase	-	-	-2.15	-
lesaffx.36985.1.s1_at	AT5G47720– acetyl-CoA C-acyltransferase, putative / 3-ketoacyl-CoA thiolase, putative	-	-	-4.24	-2.83
lesaffx.55337.2.s1_at	AT1G68530– CER6, G2, POP1, CUT1 (cuticular 1); catalytic	-	-	-5.69	-3.71
lesaffx.55353.1.s1_at	AT1G08510– FATB (fatty acyl-acp thioesterases B); acyl carrier/ acyl-ACP thioesterase	-	-	-2.27	-2.05
lesaffx.55921.1.s1_at	AT1G74960– KAS2, FAB1 (fatty acid biosynthesis 1); fatty-acid synthase	-	-	-3.29	-3.03
GLYCOLYSIS					
les.19.1.s1_at	AT1G53310– ATPPC1 (phosphoenolpyruvate carboxylase 1); phosphoenolpyruvate carboxylase	-	-	2.30	-
les.2677.1.s1_at	AT3G08590– 2,3-biphosphoglycerate-independent phosphoglycerate mutase, putative / phosphoglyceromutase, putative	-	-	-11.63	-11.48
les.2888.1.s1_at	AT3G26650– GAPA-1,GAPA (glyceraldehyde 3-phosphate dehydrogenase A subunit); glyceraldehyde-3-phosphate dehydrogenase	-	-	2.71	-
les.2900.1.s1_at	AT4G04040– MEE51 (maternal effect embryo arrest 51); diphosphate-fructose-6-phosphate 1-phosphotransferase	-	-	-	-
les.2909.1.s1_at	AT1G53310– ATPPC1 (phosphoenolpyruvate carboxylase 1); phosphoenolpyruvate carboxylase	-3.47	-	-4.67	-2.17
les.2909.2.s1_at	AT1G53310– ATPPC1 (phosphoenolpyruvate carboxylase 1); phosphoenolpyruvate carboxylase	-4.31	-2.31	-12.50	-3.85
les.2933.1.s1_at	AT1G42970– GAPB (glyceraldehyde-3-phosphate dehydrogenase B subunit); glyceraldehyde-3-phosphate dehydrogenase	-	-	2.30	-
les.3129.2.s1_at	AT5G08570– pyruvate kinase, putative	-	-	-13.19	-7.80
les.3242.1.a1_at	AT1G42970– GAPB (glyceraldehyde-3-phosphate dehydrogenase B subunit); glyceraldehyde-3-phosphate dehydrogenase	-	-	5.58	-
les.3242.3.s1_at	AT1G42970– GAPB (glyceraldehyde-3-phosphate dehydrogenase B subunit); glyceraldehyde-3-phosphate dehydrogenase	-	-	3.06	-
les.3932.1.s1_at	AT2G36530– LOS2 (Low expression of osmotically responsive genes 1); phosphopyruvate hydratase	-	-	-2.42	-2.00
les.5326.1.s1_at	AT3G22960– PKP-ALPHA/PKP1 (plastidial pyruvate kinase 1); pyruvate kinase	2.14	-	-	-
lesaffx.59008.1.s1_at	AT2G34590– transketolase family protein	-	-	-2.78	-
lesaffx.66367.1.s1_at	AT5G56350– pyruvate kinase, putative	-	-	-2.15	-
LIGHT REACTIONS					
les.1603.1.a1_at	AT1G61520– LHCA3 (Photosystem I LHC gene 3)	2.19	2.27	3.40	-
les.1923.1.s1_at	AT1G77090– thylakoid lumenal 29.8 kDa protein	-	-	2.18	-
les.2478.1.s1_at	AT1G29930– AB140, CAB140, LHCB1.3, CAB1 (chlorophyll A/B binding protein 1); chlorophyll binding	-	-	2.02	-

Table H.7 (continued)

Probe Set ID	AT locus – Putative Annotation	Fold Change			
		S2 C	M48 C	S2 AS	M48 AS
les.2286.1.s1_at	AT1G45474– LHCA5 (Photosystem I LHC gene 5)	2.08	-	2.93	-
les.2620.1.s1_at	AT2G30570– PSBW (photosystem II REACTION CENTER W)	-	-	3.69	-
les.2620.2.s1_at	AT2G30570– PSBW (photosystem ii reaction center W)	-	-	2.56	-
les.3016.1.s1_at	AT5G54270– LHCB3 (LHC chlorophyll binding protein 3)	2.76	2.41	2.19	-
les.3031.2.s1_at	AT4G38510– (vacuolar ATP synthase subunit B2); hydrogen ion transporting ATP synthase, rotational mechanism	-	-	-5.83	-4.85
les.3087.1.a1_at	AT2G26500– cytochrome b6f complex subunit (petM), putative	-	-	2.35	-
les.3087.2.s1_at	AT2G26500– cytochrome b6f complex subunit (petM), putative	-	-	2.73	-
les.3170.1.a1_at	AT3G16140– PSAH-1 (photosystem I subunit H-1)	-	-	2.19	-
les.3170.2.s1_a_at	AT3G16140– PSAH-1 (photosystem I subunit H-1)	-	-	2.16	-
les.3217.2.s1_at	AT5G13200– GRAM domain-containing protein / ABA-responsive protein-related	-	-	2.40	-
les.3297.1.s1_at	AT3G47470– CAB4, LHCA4 (Photosystem I LHC gene 4); chlorophyll binding	-	-	4.30	-
les.3775.1.s1_at	AT3G54890– LHCA1	-	-	2.24	-
les.4301.1.s1_at	AT1G20340– DRT112 (DNA-damage-repair/toleration protein 112); copper ion binding / electron carrier	-	-	2.65	-
les.4330.1.s1_s_at	AT1G06760– histone H1, putative |	-	-	2.41	-
les.4345.2.a1_a_at	AT1G29930– AB140, CAB140, LHCB1.3, CAB1 (chlorophyll A/B binding protein 1); chlorophyll binding	2.19	-	3.35	-
les.4345.2.a1_x_at	AT1G29930– AB140, CAB140, LHCB1.3, CAB1 (chlorophyll A/B binding protein 1); chlorophyll binding	-	-	3.49	-
les.4345.3.s1_x_at	AT2G34420– LHCB1.5, LHB1B2 (Photosystem II LHC gene 1.5); chlorophyll binding	-	-	3.53	-
les.4345.4.a1_at	AT1G29930– AB140, CAB140, LHCB1.3, CAB1 (chlorophyll A/B binding protein 1); chlorophyll binding	2.48	-	2.88	-
les.4345.4.a1_x_at	AT1G29930– AB140, CAB140, LHCB1.3, CAB1 (chlorophyll A/B binding protein 1); chlorophyll binding	2.62	-	2.74	-
les.4359.1.s1_at	AT1G29920– AB165, LHCB1.1, CAB2 (Chlorophyll a/b-binding protein 2); chlorophyll binding	2.01	-	2.32	-
les.4428.1.s1_a_at	AT1G20340– DRT112 (DNA-damage-repair/toleration protein 112); copper ion binding / electron carrier	-	-2.18	2.00	-
les.4492.2.s1_at	AT1G15820– CP24, LHCB6 (LHC PSII); chlorophyll binding	-	-	2.61	-
les.4492.3.s1_at	AT1G15820– CP24, LHCB6 (LHC PSII); chlorophyll binding	3.11	-	3.09	-
les.4508.1.s1_s_at	AT1G08380– PSAO (photosystem I subunit O)	-	-	2.84	-
les.4508.2.s1_s_at	AT1G08380– PSAO (photosystem I subunit O)	-	-	2.17	-
les.4615.1.s1_at	AT2G31040– ATP synthase protein I -related	-	-	2.26	-
les.5157.1.s1_at	AT1G08380– PSAO (photosystem I subunit O)	-	-	2.55	-
les.5738.1.s1_at	AT3G55330– PPL1 (PSBP-like protein 1); calcium ion binding	-	-	2.61	-
les.5852.2.s1_at	AT5G08690– ATP synthase beta chain 2, mitochondrial	-	-3.16	-4.44	-3.12
les.5852.3.s1_at	AT5G08690– ATP synthase beta chain 2, mitochondrial	-	-2.03	-	-
lesaffx.11323.1.a1_at	ATCG00730– PETD, A chloroplast gene encoding subunit IV of the cytochrome b6/f complex	-	-	3.27	2.42

Table H.7 (continued)

Probe Set ID	AT locus – Putative Annotation	Fold Change			
		S2 C	M48 C	S2 AS	M48 AS
les.986.1.s1_at	ATCG00130– ATPase F subunit	2.07	2.40	2.73	-
lesaffx.11323.1.s1_at	ATCG00730– PETD, A chloroplast gene encoding subunit IV of the cytochrome b6/f complex	4.37	2.92	4.31	-
lesaffx.29730.2.s1_at	ATMG01190– ATP1, ATPase subunit 1	2.37	-	14.77	10.85
lesaffx.35136.1.s1_at	ATCG01070– NDHE, NADH dehydrogenase ND4L	3.56	3.69	23.32	24.49
lesaffx.44224.1.a1_at	ATCG01100– NDHA, NADH dehydrogenase ND1	11.21	3.45	6.14	4.64
lesaffx.44224.1.s1_at	ATCG01100– NDHA, NADH dehydrogenase ND1	4.14	3.48	2.09	-
lesaffx.44474.1.a1_at	ATCG01050– NDHD, Represents a plastid-encoded subunit of a NAD(P)H dehydrogenase complex	7.70	6.01	6.56	5.20
lesaffx.44474.1.s1_at	ATCG01050– NDHD, Represents a plastid-encoded subunit of a NAD(P)H dehydrogenase complex	5.68	5.25	3.27	-
lesaffx.48402.1.s1_at	AT4G15510– photosystem II reaction center PsbP family protein	-	-	2.75	-
lesaffx.51226.1.a1_at	ATCG00540– PETA, cytochrome f apoprotein	3.96	3.16	22.04	16.74
lesaffx.66410.1.s1_at	ATCG00280– PSBC, CP43 subunit of the photosystem II reaction center	6.29	3.76	15.90	6.94
lesaffx.70106.1.s1_at	AT1G76450– oxygen-evolving complex-related	2.01	-	2.65	-
lesaffx.70106.2.s1_at	AT1G76450– oxygen-evolving complex-related	2.36	-	4.03	-
lesaffx.70216.1.s1_at	AT5G58260– NDH-N :plastoquinone dehydrogenase complex (Ndh complex)	-	-	2.22	-
lesaffx.70450.1.s1_at	ATCG01080– NDHG, NADH dehydrogenase ND6	4.60	3.20	2.11	2.14
lesaffx.70834.1.s1_at	ATCG00150– ATPI, subunit of ATPase complex CF0	5.29	4.13	15.50	6.21
les.2959.1.s1_at	AT3G60750– transketolase, putative	-	-	2.02	-
les.3217.2.s1_at	AT5G13200– GRAM domain-containing protein / ABA-responsive protein-related	-	-	2.40	-
les.376.1.s1_at	AT5G38420– ribulose bisphosphate carboxylase small chain 2B / RuBisCO small subunit 2B (RBCS-2B) (ATS2B)	-	-	7.00	2.74
les.424.1.s1_at	AT1G32060– PRK (PHOSPHORIBULOKINASE); ATP binding / phosphoribulokinase/ protein binding	-	-	2.70	-
les.4275.1.s1_at	AT4G38970– fructose-bisphosphate aldolase, putative	-	-	2.97	-
les.4616.1.s1_at	AT3G54050– fructose-1,6-bisphosphatase, putative / D-fructose-1,6-bisphosphate 1-phosphohydrolase, putative / FBPase, putative	-	-	3.16	-
lesaffx.16966.1.a1_at	AT1G14030– ribulose-1,5 bisphosphate carboxylase oxygenase large subunit N-methyltransferase, putative	-	-	2.08	-
lesaffx.31052.1.a1_at	AT5G13200– GRAM domain-containing protein / ABA-responsive protein-related	2.05	-	2.68	-
lesaffx.31052.1.s1_at	AT5G13200– GRAM domain-containing protein / ABA-responsive protein-related	2.01	-	-	-
lesaffx.344.2.s1_at	AT1G67090– RBCS1A; ribulose-bisphosphate carboxylase	-	-	2.16	-
lesaffx.70764.1.s1_at	ATCG00490– RBCL, large subunit of RUBISCO	4.89	6.03	3.18	2.05

Table H.8 Significantly (P<0.05) regulated transcripts involved in development. Probe set ID, *Arabidopsis thaliana* (AT) locus name and putative annotation of each transcript is listed. Values represent fold changes of transcripts in S2 C and M48 C compared to WT C and in S2 AS and M48 AS compared to WT AS. Negative values show down-regulation and positive values show up-regulation. Fold changes less than 2 are indicated with -.

Probe Set ID	AT locus – Putative Annotation	Fold Change			
		S2 C	M48 C	S2 AS	M48 AS
DEVELOPMENT					
les.162.1.s1_at	AT3G58040.1 – seven in absentia (SINA) family protein	-	-	-5.74	-2.97
les.162.2.s1_at	AT3G58040– seven in absentia (SINA) family protein	-	-	-3.18	-
les.162.3.s1_at	AT3G58040– seven in absentia (SINA) family protein	-	-	-4.70	-3.55
les.222.1.s1_at	AT1G48410– AGO1 (ARGONAUTE 1)	-2.42	-3.57	-3.24	-2.14
les.23.1.s1_at	AT4G27410– ANAC072, RD26 (RD 26)	-2.28	-	-2.12	-
les.2569.1.s1_at	AT1G69490– ANAC029, ATNAP, NAP (NAC-like, activated by AP3/PI); TF	-5.01	-	-6.52	-2.34
les.3070.2.a1_at	AT4G25150– acid phosphatase, putative	11.40	-2.50	-	-
les.3095.1.s1_at	AT1G01720– ANAC002, ATAF1 (NAC domain containing protein 2); TF	-	-	-3.28	-
les.3348.1.s1_at	AT5G62200– embryo-specific protein-related	-2.74	-	-	-
les.3743.1.s1_at	AT4G25150– acid phosphatase, putative	-	-	-	2.18
les.3759.1.s1_at	AT5G53560– ATB5-A (Cytochrome b5 A)	-4.09	-	-	-
les.3766.1.s1_at	AT1G01470– LSR3, LEA14 (late embryogenesis abundant 14)	-5.12	-	-	-
les.4683.1.s1_at	AT2G33430– plastid developmental protein DAG, putative	-	-	2.94	-
les.476.2.s1_at	AT5G14520– pescadillo-related	-	-	-2.07	-
les.4975.1.s1_at	AT4G29270– acid phosphatase class B family protein	6.40	-2.46	-	-
les.5128.1.s1_at	AT2G17840– ERD7 (early-RD 7)	-2.98	-	-	-
les.5699.1.s1_at	AT2G17040– ANAC036 (Arabidopsis NAC domain containing protein 36); TF	-	-	2.75	-
les.5791.1.s1_at	AT5G48150– PAT1 (phytochrome a signal transduction 1); TF	-	-	2.06	-
lesaffx.13113.1.s1_at	AT1G09380– integral membrane family protein / nodulin MtN21-related	2.07	-	-	-
lesaffx.2597.1.s1_at	AT4G35770– ATSEN1, DIN1, SEN1 (dark inducible 1)	-	-	3.15	-
lesaffx.31434.1.s1_at	AT3G10530– transducin family protein / WD-40 repeat family protein	-	-	-2.15	-2.33
lesaffx.3377.1.s1_at	AT1G10510– EMB2004 (embryo defective 2004); protein binding	-	-	2.27	-
lesaffx.43329.1.s1_at	AT5G61430– ANAC100/NAC5 ; TF	-	-	-3.76	-2.23
lesaffx.4509.1.s1_at	AT2G19520– ACG1, MSI4, NFC4, NFC04, FVE	-	-	-6.42	-4.01
lesaffx.4763.1.s1_at	AT2G41380– embryo-abundant protein-related	-2.19	-	-	-
lesaffx.51271.1.s1_at	AT1G10200– WLIM1; TF	-	-	2.84	-
lesaffx.52241.1.a1_at	AT4G27990– YGGT family protein	2.53	-	-	-
lesaffx.52241.1.s1_at	AT4G27990– YGGT family protein	2.16	-	2.67	-
lesaffx.56390.2.s1_at	AT2G47070– SPL1 (squamosa promoter binding protein-like 1); DNA binding / TF	-	-	-2.78	-
lesaffx.58104.1.a1_at	AT5G44120– ATCRA1, CRU1, CRA1 (cruciferina); nutrient reservoir	-	-	2.79	-
lesaffx.6024.1.a1_at	AT2G44670– senescence-associated protein-related	2.18	-	-	-
lesaffx.60947.1.s1_at	AT4G36920– FLO2, FL1, AP2 (APETALA 2); TF	-	-	-2.58	-

Table H.8 (continued)

Probe Set ID	AT locus – Putative Annotation	Fold Change			
		S2 C	M48 C	S2 AS	M48 AS
lesaffx.60966.2.s1_at	AT5G48150– PAT1 (phytochrome a signal transduction 1); TF	-	-2.19	-	-
lesaffx.64505.2.s1_at	AT1G56010– ANAC021, ANAC022, NAC1; TF	-	2.51	-8.12	-
lesaffx.65682.1.a1_at	AT5G50790– nodulin MtN3 family protein	2.37	2.50	2.25	-
lesaffx.66436.1.s1_at	AT1G70670– caleosin-related family protein	-	2.17	-3.58	-
lesaffx.69815.1.s1_at	AT3G14770– nodulin MtN3 family protein	-	-	-6.96	-2.31
lesaffx.70080.1.s1_at	AT1G11430– plastid developmental protein DAG, putative	-	-	2.14	-
lesaffx.70563.1.s1_at	AT5G48150– PAT1 (phytochrome a signal transduction 1); TF	-	-2.27	-3.24	-3.39
lesaffx.71577.1.s1_a_at	AT4G02380.1– LEA3 family protein	-2.96	-	-	-
lesaffx.71623.1.s1_at	AT4G27410– ANAC072, RD26, (RD 26)	-	-	-2.95	-
CELL ORGANISATION					
affx-les-actin-m_at	AT5G09810– ACT2/7, ACT7 (actin 7)	-	-	-3.12	-
les.2756.2.s1_at	AT5G38530– tryptophan synthase-related	-2.79	-	-	-
les.2944.1.a1_at	AT5G23860– TUB8 (tubulin beta-8)	-	-	-	2.77
les.4703.1.s1_at	AT5G12250– TUB6 (BETA-6 TUBULIN)	-	-	2.30	-
lesaffx.15353.1.s1_at	AT3G61060– ATPP2-A13	-2.68	-	-	-
lesaffx.1574.1.s1_at	AT3G61710– autophagy protein Apg6 family	-	-	-3.10	-2.66
lesaffx.1574.2.s1_at	AT3G61710– autophagy protein Apg6 family	-	-	-2.12	-
lesaffx.37916.1.s1_at	AT1G09155– ATPP2-B15 (Phloem protein 2-B15); carbohydrate binding	-	-	-2.07	-
lesaffx.44837.1.s1_at	AT5G57740– XBAT32 (XB3 ortholog 2 in *Arabidopsis thaliana* 32); protein binding / zinc ion binding	-	-	-2.10	-
lesaffx.54238.1.s1_at	AT4G08580– microfibrillar-associated protein-related	-	-	-3.06	-2.13
lesaffx.57312.1.s1_at	AT3G04710– ankyrin repeat family protein	-	-	-3.39	-2.06
lesaffx.6012.1.s1_at	AT4G15930– dynein light chain, putative	2.06	3.07	-	-
lesaffx.65646.1.s1_at	AT1G18450– ARP4 (actin-related protein 4); structural constituent of cytoskeleton	-	-	-2.89	-
lesaffx.65646.2.s1_at	AT1G18450– ARP4 (actin-related protein 4); structural constituent of cytoskeleton	-	-	-3.77	-2.65
lesaffx.70593.1.s1_at	AT5G61230– ankyrin repeat family protein	-	-	-2.03	-

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PUBLICATIONS

Ufuk Çelikkol Akçay, Gülsüm Kalemtas, Meral Yücel, Hüseyin Avni Öktem, 2010, Comparison of different molecular methods in screening genetically modified lentil, Journal of Engineering Science and Design, 1(2): 73-78

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PROJECTS

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