THE EFFECTS OF THIDIAZURON ON CALLUS DEVELOPMENT AND ORGANOGENESIS FROM MATURE EMBRYOS OF SELECTED TURKISH BREAD AND DURUM WHEAT VARIETIES

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ABSTRACT

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The effects of cytokinin-like Thidiazuron growth regulator on the regeneration responses of callus cultures of Turkish bread *Triticum aestivum* L. cv. (Başak 95, Gerek 79, and Bezostaja 1) and durum *Triticum durum* Desf. cv. (Kunduru, Çakmak 79, and Kırmızı 5132) wheat varieties have been investigated in this study.

High callus induction frequencies are found to be independent of bread and durum wheat varieties (› 96%) whereas the callus weight is found to be variety-dependent. For bread wheat, Başak 95 and for the durum wheat Kunduru is found to be the best performers.

TDZ treatments are found to be negatively affecting the regeneration capacity of all the tested bread wheat varieties whereas for the durum wheat variety of Kunduru positive effect is observed.

Since the culture efficiency is a derivation from the regeneration capacity, this parameter yielded very similar results as in the case of regeneration capacity for both bread and durum wheat varieties.

In bread wheat varieties, the TDZ treatments increased the number of regenerated plants more than 2-fold when compared with the control and likewise very similar results were obtained from durum wheat varieties.

Unfortunately, following their transfer to soil, plants that were treated with various concentrations of TDZ displayed reduced vigor probably due to underdeveloped roots. In addition, majority of these plants did not sufficiently develop above the ground parts when compared with the control plants.

The simplicity and rapid development of shoots using mature embryos could potentially be used for regenerating superior plants following gene transfer studies in the future.

Key words: Thidiazuron, wheat, callus induction, *in vitro* regeneration, mature embryo.

ÖZ

THIDIAZURON'UN SEÇİLMİŞ TÜRK EKMEKLİK VE MAKARNALIK BUĞDAY ÇEŞİTLERİNİN OLGUN EMBRİYOLARINDAN ELDE EDİLMİŞ KALLUS VE ORGAN GELİŞİMİNE ETKİSİ

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Bu çalışmada, sitokinin-grubuna ait Thidiazuron büyüme düzenleyicisinin Türk ekmeklik *Triticum aestivum* L. cv. (Başak 95, Gerek 79, ve Bezostaja 1) ve makarnalık *Triticum durum* Desf. cv. (Kunduru, Çakmak 79, ve Kırmızı 5132) buğday çeşitlerinin kallus rejenerasyonuna etkisi incelenmiştir.

Türk ekmeklik ve makarnalık buğday çeşitlerinin olgunlaşmış embriyo eksplantlarından elde edilen yüksek kallus oluşum değerlerine (› % 96) çeşit etkisinin olmadığı gözlenirken kallus kütlesinin çeşitlere bağlı olduğu gözlenmiştir. Ekmeklik buğday'da Başak 95 makarnalık buğday'da Kunduru çeşitleri en iyi performansı sergilemişlerdir.

Tüm ekmeklik buğday çeşitlerinde TDZ uygulamasının rejenerasyon kapasitesi üzerinde azaltıcı bir etkisi gözlenirken, Kunduru makarnalık buğday çeşidinde etki artırıcı olarak gözlemlenmiştir.

Kültür verimi parametre değerinin kallus rejenerasyon kapasite değerlerinden elde edilmesi nedeniyle, bu parametrenin sonuçları hem ekmeklik hem makarnalık buğday çeşitlerinin rejenerasyon kapasite sonuçları ile paralellik göstermiştir.

Ekmeklik buğday çeşitlerinde, TDZ uygulamalarının rejenere olmuş bitki sayılarını, kontrol dozuna kıyasla 2 kat'dan fazla artırdığı gözlemlenmiştir ve benzer sonuçlar makarnalık buğday çeşitleri için de elde edilmiştir.

Değişik TDZ yoğunlukları uygulanmış bitkiler toprağa aktarıldıktan sonra, muhtemelen iyi gelişmemiş kök yapıları nedeniyle, düşük büyüme-gücü özellikleri sergilemişlerdir. Ayrıca, bu bitkilerin çoğunun kontrol bitkilerine kıyasla toprak üstü bölgelerinin de gerektiği kadar gelişmediği saptanmıştır.

Olgunlaşmış embriyolardan bu şekilde kolay ve hızlı sürgün sağlanması, gelecekte gen transferi sonrası elde edilebilecek olan üstün buğday çeşitlerinin rejenerasyonu için potansiyel olarak kullanılabilecektir.

Anahtar kelimeler: Thidiazuron, buğday, kallus oluşumu, *in vitro* rejenerasyon, olgunlaşmış embriyo.

“ I wish to dedicate this effort to
the immortal spirit of my father
and to my mother in appreciation for all her support and
encouragement ”

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ABBREVIATIONS

ABA	Abscisic Acid
BAP	Benzylaminopurine
2,4-D	2,4-Dichlorophenoxyacetic acid
IAA	Indole acetic acid
MS	Murashige and Skoog
NAA	α –Naphtylamide acetic acid
TDZ	Thidiazuron

CHAPTER 1

INTRODUCTION

1.1 The Wheat Plant

Wheat species is grown across a wide range of environments around the world. In fact, it has the broadest adaptation of all cereal crop species. More land is devoted worldwide to the production of wheat than to any other commercial crop. Wheat is the number one food grain consumed directly by humans, and its production leads all crops, including rice, maize and potatoe.

Wheat is an annual plant made up of roots, a stem, leaves, and the head, which is also called a spike. It has a strong root system, with a number of fibrous and long roots growing to a depth of more than 1 m in favorable conditions. The stem is cylindrical, erect, solid, and hallow. It is subdivided into internodes by knots, each one being the origin of a leaf. Wheat leaves are made up of a sheath and the leaf blade, which lies out flat so it can gather light.

Flowers are hermaphroditic, clustered in sessile that are layered upon each other to form a spike. The male part of the flower consists of three stamens, with bilocular anthers, attached with filaments close to the base.

Mature pollen is fusiform, with only one germinative pore, normally provided with three nuclei. The female part consists of a syncarpous ovary, unilocular, with three lobes, one ovule, and at the top two feathery stigmas (Fabriani and Lintas, 1988).

Fertilization begins about two days after the spike emerges from the sheath of the flag leaf. As the flowers open up, the anthers shed pollen that sticks to the stamen and germinates to grow into the ovary and fertilize the egg cell. Wheat is self-pollinating, which means that it is fertilized by its own pollen. The grain starts to grow after fertilization. The fruit is a large caryopsis, with a large flint endosperm and a flattened embryo located at the apex of the seed and close to the base of the flower.

1.1.1 Wheat Classification

Wheat is a member of the angiosperm class, the monocot sub-class, and the grass family, Gramineae (Poaceae). This plant is also a member of tribe *Hordeae* and the genus *Triticum*. More than 30 subspecies of wheat (*Triticum*) are known. Some are cultivated, and some still grow wild. All wheats, wild and cultivated, belong to the genus *Triticum* (Cook et al., 1993).

During this century many attempts have been made to classify the tribe *Triticeae* into subtribes and genera. Wheat is segregated into various classes according to the number and make up of chromosomes and the structure of the head (spike or ear) of the plant and its agronomic and

end-use attributes. These classifications are generally based on number of chromosomes, quality, color, growth habit and end use attributes (Oleson, 1996).

The *Triticeae* tribe contains 25 genera. All wheats belong to the genus *Triticum* that includes the wild, primitive, and modern cultivated types of wheat (Table 1.1) (Feldman, 1976).

Table 1.1 Names of wild, primitive cultivated and modern cultivated wheat (Feldman, 1976)

Wild wheats	**Primitive cultivated wheat**	**Modern cultivated wheat**
T. monococcum var. *beoticum* diploid (AA) *T. tauschii* diploid (DD)	*T. monococcum* var. *monococcum*, einkorn (hulled) diploid (AA)	–
T. turgidum var. *dicoccoides* tetraploid (AABB) *T. timopheevii* tetraploid (AADD)	*T. turgidum* var. *dicoccum,* emmer (hulled) *T. turgidum* var. *durum* (hull-less) tetraploid (AABB)	*T. durum*, durum (hull-less) tetraploid (AABB)
T. aestivum hexaploid (AABBDD)	*T. aestivum* var. *spelta* (hulled) *T. aestivum* var. *compactum* (hull-less) *T. aestivum* var. *aestivum* (hull-less) hexaploid (AABBDD)	*T. aestivum* var. *spelta*, spelt (hulled) *T. aestivum* var. *compactum*, club wheat (hull-less) *T. aestivum* var. *aestivum,* common wheat (hull-less) hexaploid (AABBDD)

Cytological and cytogenetic work showed that wheats fall into three basic natural groups, each one characterized by having 14 chromosomes or a multiple of 14 chromosomes in each somatic cell. The wheats (genus *Triticum*) form series with diploid (2n=2x=14), tetraploid (2n=4x=28) and hexaploid (2n=6x=42) forms (Table 1.2).

Table 1.2 Three basic groups of wheat species.

		Genome formula
Diploids	*T. urartu* Tum.	AA
	T. boeoticum	AA
	T. monococcum L.	AA
Tetraploids	*T. dicoccum*	AABB
	T. turgidum	AABB
	T. polonicum	AABB
	T. durum.	AABB
	T. turanicum	AABB
Hexaploids	*T. spelta*	AABBDD
	T. vavilovi	AABBDD
	T. macha	AABBDD
	T. compactum	AABBDD
	T. aestivum	AABBDD

Based on its suitability for baking bread, wheat is normally divided into two quality classes; hard and soft. Hard wheat has a physically hard kernel that yields flour with high gluten and consequently high protein content. This is suitable for producing a western style loaf of bread and some types of noodles. On the other hand, soft wheat is characterized by a lower protein level and is most suitable for producing cakes and biscuits, which do not require strong flour. There are also semi-hard wheats having some combination of the above quality characteristics and utilized in unleavened breads such as chapattis as well as Asian steamed bread and certain noodles (Oleson, 1996).

Color (red or white) refers to the color of the aleuronic or outer layer of the wheat kernel. Depending on the end product and the milling

extraction rate, different color wheats may be desirable in different markets.

Wheat may exhibit either a winter or spring growth habit. Winter wheats are planted in the autumn and produce grain the following spring or summer. They require a vernalization period of temperatures near or slightly below freezing as well as minimum accumulation of growing degree-days and length of daylight to convert from vegetative to reproductive growth (Oleson, 1996). Then, winter cereals can be sown and brought to flower in one season. Spring wheats are planted in the spring and produce grain the following summer. Unlike winter wheat, they do not require a vernalization period to convert from vegetative to reproductive growth (Cook *et al*., 1993).

1.1.2 Domestication of Wheat

Wheat emerged at early periods of civilization and dispersed worldwide. Its use as a food goes back to the Stone Age era. Wheat was domesticated around 10,000-15,000 B.C. in the Near East area known as the Fertile Crescent, a mountainous-hilly region (Figure 1.1). Then, wheat cultivation spread towards north to Europe, east to Asia and south to North Africa (Zohary and Hopf, 1994).

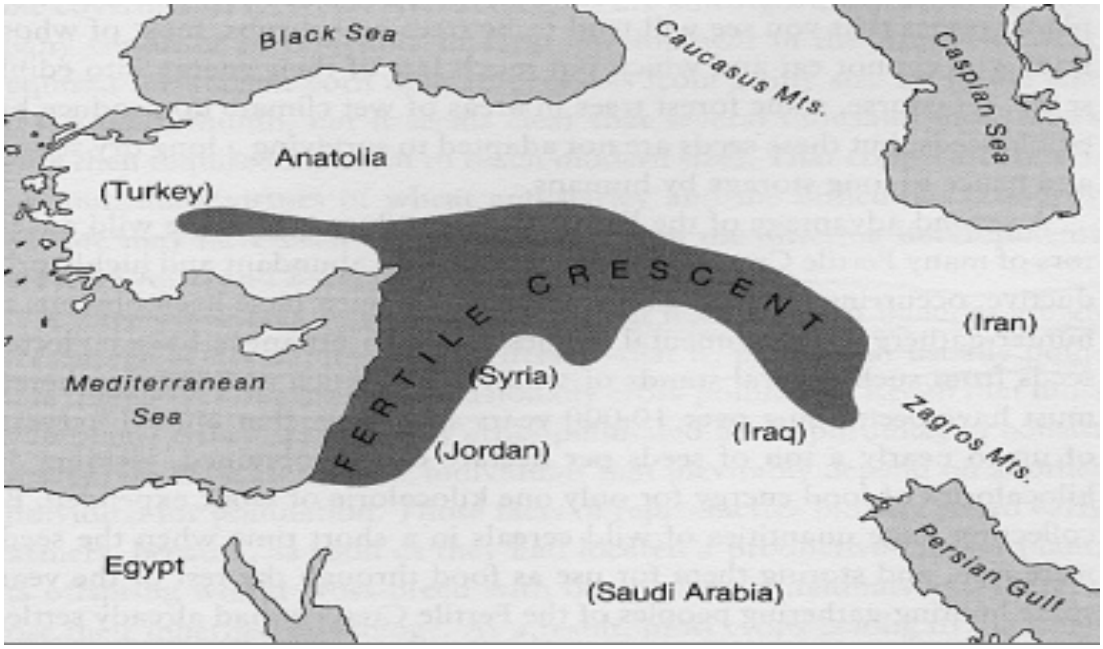


Figure 1.1 Fertile Crescent of Near East. This arc is the cross-section of species distribution of wild einkorn and emmer wheats.

The early dispersal of wheat species leading to the current cultivated varieties emerged from an expansion called the Neolithic dispersal (roughly 6000-3000 B.C.). This evolutionary expansion resulted in a broad ecological differentiation as wheat adapted to different environmental and cultural conditions. Wheat in archaeological sites suggests that it spread into southern Europe between 5000-6000 B.C., Egypt by 4500 B.C., the Netherlands by 4000 B.C., England and Scandinavia by 3000 B.C., and China by at least the second millennium. After 3000 B.C., wheat populations continued to evolve and spread, in concert with changing human settlements and cultivation practices; however, the crop probably remained confined to the Afro-Eurasian landmass until about 1500 A.D.

The foundation of crops first domesticated during the Neolithic age more than 10.000 years ago included primitive forms of wheat. The two basic steps for domestication of these wild grasses were 1) the selection of plants showing a lower degree of spike brittleness and 2) the selection of plants showing a larger size of kernel, thus providing a higher quantity of starch and proteins (Fabriani and Lintas, 1988).

The genus *Triticum* are divided into three groups; diploid, tetraploid, and hexaploid, according to their chromosome number. The diploid einkorn wheat *T. monococcum* with the AA genome, has no economic value, and is grown only occasionally as animal feed. The tetraploid emmer wheat *T. turgidum* var. durum with the AABB genome grows best in warmer climates and is prized for making pasta. The hexaploid common or bread wheat is grown in cool climates with moderate rainfall such as North America, Europe China, India and Australia. The modern hexaploid bread wheat (*Triticum aestivum* L.) evolved and became abundant about 8000 years ago.

Modern bread wheat is a true breeding hybrid with its ancestry linked to three wild grass species still growing in the Middle East (Patnaik and Khurana, 2001). The common wheat has the genome constitution of AABBDD, formed through hybridization of *T. urartu* (AA) with unknown diploid B genome (possibly *Aegilops speltoides*), and subsequent hybridization with a diploid D genome, *T. tauschii* (Figure 1.2). These grasses were adapted to the steppes or semiarid areas, characterized by winter rains and dry summers, developing with

available fall-winter moisture and reaching maturity in late spring or summer (Fabriani and Lintas, 1988).

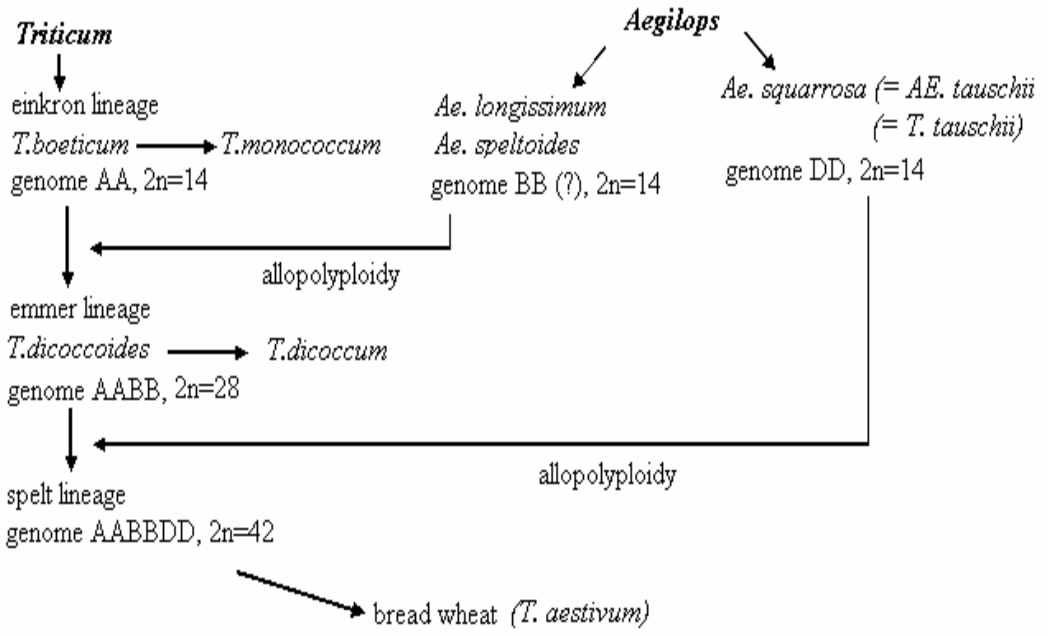


Figure 1.2 Evolution of modern hexaploid wheat (*T. aestivum*)

With the evolution of wheat, civilizations thrived and spread from the Middle East to new continents, resulting in massive wheat production feeding the growing world population.

1.1.3 Growing Conditions of Wheat Species

Wheat is a cool-season crop, but it flourishes in many different agroclimatic zones. Production is concentrated between latitudes 30 and 60^0 N and 27 and 40^0 S (Nuttonson, 1955; Percival, 1921). However, wheat can be and is grown beyond these limits. For example, in the Northern Hemisphere, wheat can be grown from within the Arctic Circle

to the equator, if, in the latter case, it is cultivated at locations of sufficiently high elevation.

The minimum temperature for growth is about 3 to 40 ^{0}C, the optimum temperature is about 25 ^{0}C, and the maximum is about 30 to 32 ^{0}C (Briggle, 1980). Wheat grows best on well-drained soils from sea level to about 3000 m above sea level. In some tropical countries, wheat is being grown from 2000 to 3200 m., and it has been reported at 4270 to 4570 m. in Tibet (Percival, 1921). The species can be grown in most locations where annual precipitation ranges from 250 to 1750 mm; about three-fourths of the land area used for wheat production receives an average of between 375 to 875 mm rainfalls annually (Leonard and Martin, 1963). While wheat can be grown on residual moisture alone in some areas (such as in northern Thailand), the seasonal distribution of precipitation is a critical factor in most production environments.

Wheat is harvested somewhere in the world during every month of the year (Briggle, 1980; Percival, 1921). Most of the global harvest, however, occurs between April and September in the temperate zone of the Northern Hemisphere; considerably less wheat is grown in the Southern Hemisphere where harvest occurs from October to January.

1.1.4 Wheat production in the World

Cereal grains have for centuries provided a main source of food for the human beings. Almost half of the total food energy of the people of the civilized world is derived from cereals.

Wheat is the leading cereal grain produced in the world, followed closely by rice and corn. Wheat and wheat products contribute to the world's food supply by providing energy, protein (which contains 17 amino acids, 9 vitamins and 15 minerals) and fats. These products require flour of select characteristics which are achieved through a proper balance of grain hardness and protein content (Percival, 1921). It is estimated that nearly two-thirds of the wheat produced in the world is used for food; the remaining one-third is used for feed, seed and non-food applications.

Wheat has the widest adaptation of all cereal crops and is grown in some 100 countries around the world. It is grown as far north as Finland and as far south as Argentina (Cook et al., 1993). This species is grown to some extent on every continent except Antarctica.

In many countries, wheat is the major component of the diet. It is non-perishable, easy to store and transport, has a good nutritional profile and allows the manufacture of a wide variety of interesting, enjoyable and satisfying products.

In general, the world's largest wheat producers were China, European Union, the United States, India, Turkey, France, Canada, Australia, Pakistan, Argentina and Poland (Oleson, 1996) (Table 1.3).

Table 1.3 World Wheat Production by top producing countries*.

WHEAT Production	1999/2000	2000/2001	2001/2002	2002/2003	2003/2004
European Union	96,392	104,732	90,864	103,894	90,500
China	113,880	99,640	93,873	90,290	86,000
India	70,780	76,369	69,680	71,810	65,100
United States	62,569	60,758	53,262	44,062	63,590
Russian Federation	31,000	34,450	46,900	50,550	34,000
Australia	24,757	22,108	24,299	10,058	25,000
Canada	26,941	26,519	20,568	16,198	23,500
Pakistan	17,854	21,079	19,023	18,226	18,200
Turkey	16,500	18,000	15,500	16,800	16,800
Argentina	16,400	16,230	15,500	12,300	13,500
Iran	8,500	8,000	9,500	12,400	12,400
Kazakhstan	11,200	9,100	12,700	12,600	12,000
Poland	9,051	8,503	9,283	9,304	7,858
Egypt	6,350	6,350	6,130	6,300	6,500
Brazil	2,403	1,660	3,250	2,935	5,500
Morocco	2,154	1,381	3,316	3,357	5,200
Uzbekistan	3,700	3,600	3,400	5,000	4,900
Others	64,917	63,067	83,966	80,427	57,514
WORLD Total	**585,348**	**581,546**	**581,014**	**566,511**	**548,062**

***USDA :World Crop Production Million Metric Tons (MMT); October 2003.**

According to the USDA/FAS (United States Department of Agriculture/ Foreign Agricultural Service) 2003 Reports, world wheat production was 548,062 million metric tons. Global production of wheat is now

approaching 600 million tons and developing countries accounted for 45 percent of this production.

Wheat is Asia's second most important stable and has been growing much faster than rice. Durum wheat account for 5 percent of developing countries' wheat production and 80 percent of it is grown in the Middle East-North Africa region.

The area of adaptation of durum wheat could nearly overlap the area of bread wheat. Among the reasons for its lower distribution are: 1) its lower fitness for bread making 2) its lower resistance to cold and long winters 3) the lower yield given by the available varieties, which is due also to the lower amount of breeding effort have been made for this crop. On the other side, durum wheats are better adapted to drought and more productive in marginal areas (Fabriani and Lintas, 1988).

1.1.5 Wheat production in Turkey

Turkey's varied ecology allows farmers to grow many crops. The greater part of the farm population traditionally have been dedicated to producing cereal crops, which supply 70 percent of Turkey's food consumption in terms of calories. Cereals are the most important crops grown in Turkey and wheat is the major cereal. Around 75% of the total wheat areas are located in the Central Plateau and in the transitional regions connecting the Plateau to the coasts. 53 % of the Turkish daily calorie expenditure comes from wheat and other wheat products and Turkey is one of the countries whose wheat consumption is the highest

per person (Kün, 1988). Other grain crops include rye, millet, corn, and rice. Grains are produced in most parts of the country (http://countrystudies.us/turkey/61.htm).

In Turkey wheat and its products have an important place. According to official data wheat had the largest sown and harvested area in field crops. Also wheat has an important place in the value of marketable field crops after industrial crops by 35%.

Wheat has long been the basic food in the Turkish diet, generally eaten in the form of bread –of which Turkish per capita consumption ranks among the highest in the world. Farmers consume about half of the crop; the other half moves through commercial channels. The Soil Products Office (TMO) buys up to one-fifth of the crop at support prices, which largely determine the prices for the open market, and handles most imports and exports of grain (http://countrystudies.us/turkey/61.htm).

Production increases in the late 1970s turned Turkey into a wheat exporter. After 1980 the country imported small amounts of high-quality wheat to improve baked products. Steady increases continued in the 1980s, with wheat production averaging 15 million tons. Even in the drought-stricken 1989 harvest, wheat production totaled 16.2 million tons. By the early 1990s, wheat production was averaging 20 million tons per year (http://countrystudies.us/turkey/61.htm).

According to the USDA/FAS 2003 Reports, 16,800 MMT was accounted by Turkey leading as the 9th country following European Union, China,

India, USA, Russian Federation, Australia, Canada and Pakistan (USDA October World Crop Production).

Turkey cultivates 20% spring type of durum wheat in the coastal regions, 55% winter type of durum wheat in the Central Anatolia, 25% facultative or spring type of durum wheat in the southeastern region.

1.2 Wheat Breeding and *in vitro* approaches

Modern scientific plant breeding can trace its development to cereal hybridization or planned cross-breeding which began in England in the 1790s and continued there in the mid-19th century. The last decades of the 19th century were marked by greater interest in both cross-breeding and better methods of selection in Europe, North America and Australia. Wheat improvement began to take the form of crossing locally adapted material with wheat from other areas in an effort to improve production characteristics or quality (Lupton, 1987). The rediscovery of Mendel's laws of heredity at the turn of the 20th century led to renewed interest in using genetics to improve crops. One such tool for improving crops is to transfer agronomically important genes into cells or tissues. However, in order to initiate such molecular-genetic breeding programs, which would lead to the production of transgenic plants, the most important prerequisite is to develop tissue/cell culture systems for the crop of interest.

1.2.1 Wheat Tissue Culture Studies

The ultimate aim in the development of in *vitro* plant cell/tissue culture systems is to be able to regenerate whole plants from utilized explant materials. For the regeneration of whole plants 3 factors play a crucial role and therefore must be considered carefully: 1) effects of genotype 2) effects of explant material and 3) effects of culture conditions (including medium and environment) (Maes *et al*., 1996).

1.2.1.1 Effects of Genotype

The genotype is an important factor in order to regenerate a plant *in vitro* culture. Because some genotypes or cultivars within species have greater *in vitro* regeneration capacity compared to other species or cultivars within a species (Sears and Deckard, 1982). Opposed to dicotyledonous plants, monocotyledon plants are regarded as difficult *in vitro* material. In other words, tissue culture of dicots is simple as compared to monocots (Reinert & Bajaj, 1976). A few selected publications within the last decade concerning the genotype-effects on regeneration in wheat tissue culture studies are given below:

Fadel and Wenzel (1990) investigated the effects of media-genotype on the rate of green plantlet formation from anther cultures of five different winter and spring wheat cultivars. An increased number of embryos and calli were produced on Ficoll-containing liquid potato-2 medium, whereas the addition of 0.2 M/l maltose increased the rate of

regeneration. They also reported the strong genotype effect on the formation of plantlets.

Lu *et al.* (1991) have studied twenty-two cultivars and lines of winter and spring wheat (*Triticum aestivum* L.) for their anther culture response. They have reported that the anther culture response was strongly genotype dependent. . Plants grown in the field gave higher callus induction frequency than those grown in the greenhouse and the controlled environment chamber. Donor plants grown in a season of low drought stress as compared to a season of severe drought stress resulted in a higher frequency of callus induction. Wheat lines that were more responsive to anther culture were identified.

Kintzios *et al.* (1996) have studied the effect of genotype and different growth regulator treatments on callus induction, proliferation and plant regeneration from mature wheat embryos. Callus cultures were initiated from mature excised embryos received from seeds of the *Triticum aestivum* L. cvs. 'Aeges' and 'Generoso' and the *Triticum durum* L. cvs. 'Mexicali' and 'Capeiti'. They have reported these four cultivars differed remarkably in their responses to embryo culture with various growth regulators, with the *T. durum* cultivar 'Capeiti' being the least responsive. They also observed no callus growth when no growth regulator was applied and maximum callus growth was obtained on a medium supplemented with 2,4-D and kinetin. For both *T. aestivum* and *T. durum* cultivars, better plant regeneration rates were obtained by inducing callus formation, shoot induction and root induction on a single medium, rather than submitting explants to four separate culture phases.

Dornelles *et al.* (1997) evaluated the plant regeneration capability of five genotypes of hexaploid wheat and their Fl hybrids in tissue culture after three different periods of callus maintenance. The results showed that this trait is mainly determined by additive gene effects, with high heterotic potential. They also reported that the plant regeneration capability is relatively easy to select for after a short period of callus maintenance. However, longer periods of callus maintenance led to a considerable reduction in plant regeneration capability in wheat.

J'Aiti *et al.* (1999) have studied the response of anthers to in vitro culture and the effect of coculture of ovaries on anther culturability in responsive and recalcitrant cultivars of durum wheat (*Triticum turgidum* ssp. durum) from Morocco and ICARDA. A large genotypic-dependence of anther culture has been found in 18 cultivars. However, they also observed no significant effect of anther-ovary coculture on green plant regeneration.

El-Sherbeny *et al.* (2001) evaluated 13 Egyptian bread wheat (*Triticum aestivum* L.) cultivars and Bobwhite for their potential use in tissue culture studies using different concentrations of 2,4-dicholorophenoxyacetic acid (2,4-D). Their results showed that genotypes, 2,4-D concentrations and their interactions significantly affected the percentage of embryogenic calli induced, regenerable calli and regenerated wheat plants.

Mzouri *et al.* (2001) also reported significant genotype effect on the somatic embryogenesis from immature embryos of wheat cultivars

(*Triticum aestivum* L.). They have examined the regeneration ability (including embryogenic potential) of twenty wheat cultivars in order to select high responding genotypes. The results, they found, showed a large genotypic effect in the production of embryogenic callus, the rate of regeneration and the mean number of regenerated plants/callus (MNRP as well).

Ahmad *et al.* (2002) reported a less genotype-dependent in vitro regeneration system capable of producing multiple shoot clumps and whole plants in four different wheat genotypes. They observed four genotypes responded positively to shoot multiplication depending upon media composition. The percentage of relative shoot apical meristem multiplication was 80-90%, and the average number of shoot meristems per multiplied shoot was 40-50 in all genotypes. They concluded that this in vitro system might prove useful for the production of transgenic plants of wheat in a relatively genotype-independent manner.

1.2.1.2 Effects of Explant Source

Besides genotype, explant source is another critical factor for successful regeneration system. For all species, generally younger or vigorously growing tissues from healthy plants should be used as explant source. The use of young tissues is especially important for cereals.

Explant sources, such as immature embryos (Ahloowalia, 1982; Sears and Deckard, 1982; Felfoldi and Purnhauser, 1992), immature leaves (Ahuja *et al*., 1982; Zamora and Scott, 1983), immature inflorescences

(Ozias-Akin and Vasil, 1982; Maddock *et al*., 1983; Redway *et al*., 1990; Sharma *et al*., 1995), mature embryos (Ozias-Akin and Vasil, 1983; Heyser *et al*., 1985; Kato *et al*., 1991), mesocotyls (Yurkova *et al*., 1982), seeds (Gosch-Wackerle *et al*., 1979), and apical meristems (McHugen, 1983) have been used for callus culture in wheat. *In vitro* culture of immature embryos is the method of choice for producing abundant callus and sufficient shoot formation in cereals (Ahloowalia, 1982, Ozias-Akins and Vasil, 1983; Maddock, 1985; Galiba and Yamada, 1988; Bohorova *et al*., 1995; Bregitzer *et al*., 1995; Rakoczy-Trojanowska and Malepszy, 1995; Ainsley and Aryan, 1998). Selections of some of the studies are mentioned below with details.

In order to reveal the pattern of growth and subsequent differentiation, Ozias-Akin and Vasil (1982) studied the early stages of callus formation from the scutellum of the immature embryo and young inflorescence of *Triticum aestivum* L. They demonstrated the immature embryo explants as the most efficient tissue to regenerate plants *in vitro*.

Then, Maddock *et al*. (1983), were used immature embryos and inflorescences of wheat as explant source. They developed a reproducible and efficient method of shoot and plantlet formation from these cultured explants. They also found obvious morphogenic differences among the 25 different cultivars of spring and winter wheat.

Using a wide concentration range of culture medium, Redway *et al*. (1990) investigated and assessed the capacity of immature embryos, inflorescences and anthers from embyogenic callus. They examined eight

commercial wheat cultivars in order to determine the relationship between cultivar and callus-plant formation.

Özgen *et al*. (1996) studied mature and immature embryos of seven genotypes of winter durum wheat. He found that mature embryos had low frequency of callus formation but a high regeneration capacity as compared to immature embryos.

Delporte *et al*. (2001) developed a wheat regeneration system using mature embryos. It is also demonstrated that calli derived from small mature embryo fragments have the good ability to undergo somatic embryogenesis and subsequent regeneration into plantlets.

Zale *et al*. (2004) examined tissue culture response of forty-seven different genotypes of wheat such as; callus induction and plant regeneration. Significant differences were detected in plant regeneration, culture efficiency, and regeneration capacity when mature embryos of 47 wheat cultivars were compared. They also reported: "Although immature embryos were superior explants in terms of plant regeneration, however, sufficient numbers of plants can be regenerated from mature embryos". This may cause to save on growth facility resources and time required for the collection of immature embryos.

Explants derived from mature wheat endosperm-supported embryos (Özgen *et al*., 1998) and thin mature embryo fragments (Delporte *et al*., 2001) were used for callus induction and plant regeneration. Use of mature embryos saves time and space, and reduces greenhouse costs

associated with growing plants to post-anthesis for the collection of immature embryos. Winter wheat research requires the additional input of controlled low temperature chambers to satisfy the vernalization requirements for floral induction prior to embryo collection.

1.2.1.3 Effects of culture conditions

The culture conditions are also highly influential in developing a regeneration system *in vitro* culture. Optimization of culture conditions for one species or genotype may not be optimal or even applicable for another. Growth regulator concentrations in culture medium are critical for the control of growth and morphogenesis. Generally, high concentration of auxins and low cytokinins in the medium promote abundant cell proliferation with the formation of callus.

Gamborg and Eleveigh (1968) succeeded in producing suspension cultures of wheat using a defined medium consisting of mineral salts containing sucrose, B vitamin and 2,4-D.

Shoot regeneration of wheat species is better on hormone-free medium or that containing 2, 4-D at low concentration than on a medium supplemented with IAA and BAP (Bennici *et al*., 1958; Chawala and Wenzel, 1987). Regeneration occurs either by somatic embryogenesis or adventitious bud and shoot development with subsequent rooting (Bhaskaran & Smith, 1990), while sometimes it may occur through direct organogenesis (Li *et al*., 1992). Low light intensities during callus induction and plantlet regeneration increased regeneration frequencies,

but decreased the proportion of green plantlets produced (Ekiz and Konzak, 1993).

Kumlehn *et al*. (1997) worked out and developed an efficient protocol for plant regeneration from wheat zygotes by means of ovule culture. Opposed to liquid or agarose-solidified medium, they observed that the isolated ovules cultured on phytagel-solidified medium gave an approximately six fold increase in zygotic embryogenesis, germination and plant regeneration.

Gonzales *et al.* (2001) investigated twelve durum wheat cultivars for their response to *in vitro* tissue culture. Zygotic immature embryos were used to induce callus formation using four different Murashige and Skoog-based media. They observed and found the strong influence of both genotype and medium on callus induction and plant regeneration from immature embryos of durum wheat cultivars.

Ahmad *et al*. (2002) reported *in vitro* regeneration system which is capable of producing multiple shoot clumps and whole plants in four different genotypes. They found that all four genotypes responded positively to shoot multiplication depending upon media composition.

Malik *et al*. (2003) tested mature healthy seeds of two genotypes of wheat on the gel-solidified LS media supplemented with different concentrations of 2,4-D.It was found that the callus induction frequency was significantly different in both genotypes. Higher levels of 2,4-D inhibited callus proliferation while lower concentrations allowed

morphogenesis to occur. It was found that 3.5 mg/L 2,4-D was the most appropriate concentration in LS media for adequate callus induction and proliferation, and 2 mg/L for subsequent subcultures from mature wheat seeds.

Within the last 20 years numerous synthetic compounds were tested for their suitability in plant tissue/cell culture systems. Although majority of these compounds were not initially intended for such purposes, a few of them later created an excitement by displaying phytohormone-like activities.

One such compound is Thidiazuron (TDZ), a substituted phenylurea compound.

1.3 Thidiazuron: Synthetic Growth Regulator

Although TDZ was developed and initially utilized to induce abscission of green-turged leaves of cotton to facilitate picking of bolls (Arndt *et al*., 1976), the morphoregulatory potential of the chemical was only realized and reaped once it was utilized in plant tissue cultures. Thidiazuron has emerged as a highly efficacious bioregulant in tissue cultures of a diverse array of species ranging from herbaceous (e.g. geranium, grasses) to tree crops (e.g. walnut).

1.3.1 Thidiazuron: the chemical and physical properties

Thidiazuron (N-phenyl-N'-(1,2,3-thiadiazol-ylurea) is a light yellow-crystalline chemical that is soluble in water, but highly soluble in ethanol, and at varying levels in other organic solvents such as acetone, benzene, DMSO etc. (Table 1.4). Ethanol is the preferred solvent in using TDZ for *in vitro* studies.

Table 1.4 Physical and Chemical properties of Thidiazuron

Character	Description
Color	Light yellow crystals
Odor	Faint odor
Trade name	DROPP
Chemical name	N-phenyl-N'-(1,2,3-thiadiazol-5'yl)urea
Molecular formula	$C_9H_8N_4OS$
Molecular weight	220.2
Melting point	213 ^{0}C
Storage	Dry conditions
Solubility (g/100ml)	Water: 0.002 Benzene: 0.0035 DMSO:>50.O

After long term storage of stock solutions, the stability of TDZ and the physiological responses are conserved. The reason is the formation of short-length polymers of TDZ molecules during the storage of stock solutions (Murch and Saxena, 1997). These findings indicate that the efficacy of TDZ may be due to storage of TDZ molecules as short-length

polymers in the culture period. This phenomenon may also explain the adverse effects seen in tissues incubated on media containing TDZ for long periods of time.

Thidiazuron has a very high intrinsic active and it has been used in *in vitro* studies for desirable effects at concentrations as low as 10 pM (Preece *et al*., 1991). Low levels of TDZ in the media and exposure of plant tissue to TDZ only for a short period are sufficient to stimulate regeneration, whereas higher levels of auxins and cytokonins are required for induction of a similar response (Hutchinson and Saxena, 1996; Visser *et al*., 1992). TDZ is biologically active at much lower concentrations and its effectiveness is considerable narrower, than other compounds used for stimulating regeneration *in vitro*. This characteristic of TDZ may be a result of the inherent stability of the molecule in tissue cultures or the direct physiological consequences of TDZ in a tissue. High intrinsic activity coupled with stability against heat and enzyme (Mok and Mok, 1985) renders TDZ a choice chemical for establishing regenerable tissue culture systems.

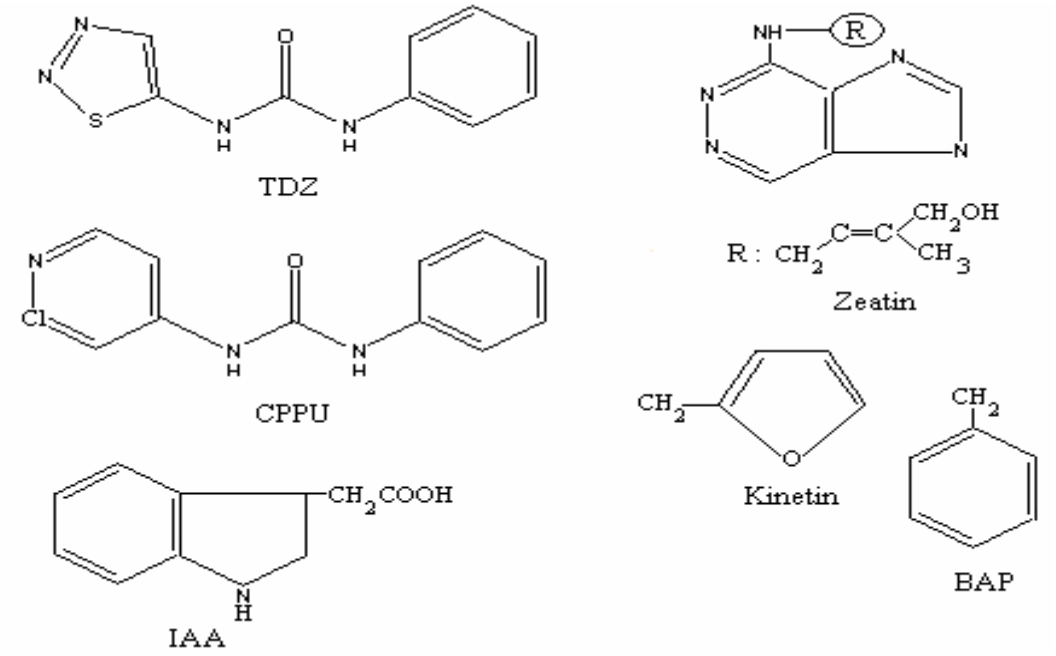


Figure 1.3 Chemical structures of phenylureas (TDZ, CPPU), auxin (IAA), and adenine type cytokinins (Zeatin, Kinetin and BAP)

TDZ is structurally quite different from both auxin and adenine-type cytokinins (Figure 1.3). However, TDZ induces morphogenic responses associated with cytokinins and auxins (e.g. induction of adventitious shoots and somatic embryos, respectively). This property of TDZ has been beneficial for studies on regulation of morphogenesis in plants.

There are two functional groups in the TDZ molecule, viz phenyl and thidiazol groups and replacement of any one of these groups with other ring structure results in the reduction in activity (Mok et al., 1982). The symmetrical N,N'-dithiazolurea is the compound with the last least cytokinin-like activity, an indication of the complementary roles of the two ring structures (phenyl and thidiazol) in TDZ-induced responses

(Mok et al., 1982). However, the method of testing the chemical activity of TDZ is an important consideration in the interpretation of the results. For example, maximum cell division activity was noticed with meta-substitution on the phenylurea-ring, whilst a para-substitution seemed to be most effective in senescence retardation (Bruce *et al*., 1965).

1.3.2 Physiological role of TDZ in plants

Thidiazuron was first synthesized and marketed as an efficient cotton defoliant under the trade name Dropp (Arndt *et al*., 1976). Further studies revealed that the compound could do much more than just inducing defoliation in cotton.

Thidiazuron brings about a high degree of efficacy and influences virtually all plant physiological processes (from germination to senescence) (Table 1.6). Examples of the diversity of physiological effects mediated by TDZ include: enhanced seed germination in *Striga asiatica* (Babiker *et al*., 1992), lettuce (Baskakov *et al*., 1981) and neem (Murthy and Saxena, 1997); substitution of chilling requirement for seed germination in *Pyrus serotina* (Lin *et al*., 1994); accelerated bud break in apple (Wang *et al*., 1986); stimulation of sprouting in potato (Ji and Wang, 1988); pumpkin cotyledon growth (Burkhanova *et al*., 1984); formation of branched trichomes and stomata on floral organs (Veneglat and Sawhney, 1994); and increased cluster and berry weight in grapes (Reynolds *et al*., 1992).

Table 1.5 Representative examples of physiological effects mediated by Thidiazuron in different plant species.

Stage of Development	Plant species	Physiological Response	Reference
Seeds	Apple	Post-bloom application to induce thinnig nearly eliminated seed development	Elfving and Cline, 1993
	Striga asiatica	Increased germination acompannied with increased ethylene biosynthesis	Gabbar *et al*., 1993
	Pyrus serotina	Reduced the length of the chilling period required to break seed dormancy and induced polypeptide profiles similar to those observed due to stratification treatments	Lin *et al*., 1994
Early Seedling Growth	Pumpkin	Activation of cotyledon growth, shortening and thickening of the hypocotyls	Burkhanova *et al*., 1984
	Peanut	Greening and thickening of cotyledons accompanied with stunting of shoot and roots	Murthy *et al*., 1995
	Oats, wheats, maize, radish	Inhibited shoot and root growth	Delvin *et al*., 1989
	Tomato	Stimulation of bud growth, shortening and thickening of the epicotyls and intensification of anthocyanin pigmentation. TDZ also reduced the growth of the epicotyls and root, and reduced root and shoot dry matter contents.	Aung and Steffens, 1987
Bud Break and Shoot Growth	Apple	Induction of bud break and development	Wang *et al*., 1986
	Oats, wheat	Shoot elongation decreased at lower concentrations and shoot fresh weight increased at higher levels	Devlin *et al*., 1989
	Sour orange	Reduced shoot growth	Pountney and Swietlik, 1988
	Apple	Increased shoot hardiness and increased wood sorbitol contents	Coleman *et al*., 1992
	Tomato	Stem diameter increased, epicotyls length decreased, terminal shoot elongation inhibited bud break	Devlin *et al*., 1989
Root Growth	Maize, radish oat, wheat	Inhibition of root growth (length, fresh weight, dry weight)	
	Geranium	Development of regenerative root out-growths	Sanago *et al*., 1995

1.3.3 TDZ and *in vitro* culture

All normal living cells theoretically possess the ability to regenerate into an entire organism via de novo formation of organs or somatic embryos (totipotency). Under the regulation of plant growth substances, the ratio of plant bioregulators is a critical factor for the regeneration of cells to form organized structures.

Thidiazuron has been used alone or in combination with other growth regulating substances to develop a wide variety of *in vitro* regeneration systems.

1.3.3.1 Cell division and callus proliferation

Recent studies showed that TDZ can stimulate cells to become activated and proliferate in the culture media. Using TDZ alone or in combination with other growth regulators, the callus response has been obtained from cells derived from different tissues; such as cells from stem (Hruskoci *et al*., 1993; Tegeder *et al*., 1995), roots of intact seedlings (Sankhla *et al*., 1994), leaf (Declerck and Korban, 1996; Jomori *et al*., 1995; Perri *et al*., 1994), leaf patiole (Arnold *et al*., 1995; Huang *et al*., 1994) and embryos including cotyledons (Bohmer *et al*., 1995; Lin *et al*., 1994; Neuman *et al*., 1993; Stimart and Mather, 1996) radicle, (Obeidy and Smith, 1993),endosperm (Iasi *et al*., 1994), pedicel (Okuse 1994; Mohamed *et al*., 1993), ovary (Zhou *et al*., 1994), and hypocotyls (Bretagne-Sagnard *et al*., 1996; Kolganova *et al*., 1992; Tibok *et al*., 1995).

The calli derived from these tissues were either used for regeneration of plantlets including transgenics (Bondt *et al*., 1996; Rosu *et al*., 1995) or for establishing suspension cultures for secondary metabolite production (Meyer *et al*., 1995; Smith, 1995).

These studies also indicate that TDZ is highly active in stimulating callus growth compared to other cytokinins like zeatin. For example, in *Phaseolus lunatus* cv. P.I.260415, the difference in the growth of callus cultures induced with TDZ and zeatin was as much as 30 times. The high intrinsic activity of TDZ compared to zeatin is attributed to differential uptake of these two chemicals by the tissues (Capella *et al*., 1983).

Since it may influence the efficiency of regeneration, compactness of the callus is a significant feature of *in vitro* culture studies. Compact callus mark the beginning of an organized growth.

Murthy and Saxena (1997) have shown that lower concentration of TDZ generally tended to promote the formation of compact-green nodular callus. Smith (1995) also demonstrated that incorporation of TDZ in the media at low concentrations helped begonia culture (which had a strong tendency to revert to organogenesis) to be in the callus phase.

1.3.3.2 Protoplast culture

Recent studies indicated that different combinations of Thidiazuron with other growth regulators have an effective role in protoplast cultures.

Thidiazuron (in association with NAA, NOA or 2,4-D) at different concentrations (0.001 to 20 µM) has been used for protoplast culture at various stages: during initial phases of cell wall formation around protoplasts, initiation of cell division (Chupeau *et al*., 1993; Reustle *et al*., 1995) and in later stages of the regeneration from protoplast derived callus (Lenzner *et al*., 1995).

It is also found that Thidiazuron was more effective than other cytokinin bioregulators. TDZ sustains division of leaf protoplasts of apple at better rates than either BAP or zeatin (Wallian and Johansson, 1989). At the same time, TDZ was observed more effective than kinetin and zeatin for growth of willow (*Salix viminalis* L.) protoplast cultures (Vahala and Eriksson, 1991). Chupeau *et al*. (1993) also found that TDZ was less toxic and more effective at lower doses than adenine type cytokinins for the growth and development of poplar protoplasts.

1.3.3.3 Somatic embryogenesis

TDZ alone has been found to substitute for both auxin and cytokinin requirement and stimulate somatic embryogenesis in many species (Gill *et al*., 1993; Saxena *et al*., 1992; Visser *et al*., 1992). Addition of TDZ in culture media stimulated *in vitro* somatic embryogenesis of tobacco (Gill and Saxena, 1993), peanut (Murthy *et al*., 1995; Saxena *et al*., 1992), geranium (Visser *et al*., 1992), and chickpea (Murthy *et al*., 1996) at a much higher rate than previously obtained with other phytohormones. In some cases, the production of shoots and somatic embryos has also been recorded (Bates *et al*., 1992).

1.3.3.4 Shoot regeneration

By using TDZ (alone or with other growth regulators) in the culture media, many crop species have been regenerated ranging from tropical fruit trees to root and tuber crops. Huetteman and Preece (1993) and Lu (1993) reported the large list of these species in the citation reviews.

Thidiazuron has been shown to promote shoot regeneration greater than the other cytokinins at much lower concentration (Fellman *et al*., 1987; Fiola *et al*., 1990; Kerns and Meyer, 1986; Malik and Saxena, 1992). The most effective use of TDZ has been seen in the woody plant species (Baker and Bhatia, 1993; Briggs *et al*., 1988; Preece *et al*., 1991).

Huetteman and Preece (1993) and Lu (1993) reported that TDZ can reduce the dominance of the apical meristem with the result that adventitious and/or axillary buds arise directly from cultured shoot tips in woody species.

TDZ has more effective role than other purine-type cytokinin bioregulators. On the other hand, insufficient rooting of shoots and poor elongation of regenerants are reported in many species where TDZ is used to promote shoot proliferation (Huetteman and Prece, 1993; Lu, 1993).

1.3.3.5 Cereal Cell/Tissue Culture

In cereals, TDZ containing media have been used to promote shoot induction from wheat (Shan *et al*., 2000), barley (Shan *et al*., 2000) and rice (Tian, 1994) calluses.

Gupta and Conger (1998) used a combination of TDZ and 2,4-D in the medium to induce multiple shoots directly from intact seedlings of switch grass, *Panicum virgatum*. Nodal explants of *Bambusa edulis* have also been induced to produce multiple shoots in response to TDZ (Lin and Chang, 1998). Similarly for sugarcane, calluses induced from immature inflorescences produced shoots on a TDZ-containing medium (Gallo-Meagher *et al*., 2000).

Seedhabadee *et al*. (2003) tested the plant regeneration frequencies for immature scutella, leaf-bases / apical meristems and mature embryos of four barley genotypes *in vitro*. It is demonstrated that mature embryos of recalcitrant barley genotypes produce three- to eight-fold more shoots in response TDZ than calluses derived from immature scutella. They also reported: “The use of mature embryos for barley regeneration eliminates the need for immature explant material, and consequently, growth of donor plants. Thus, the simplicity and rapid production of shoots from the mature embryo culture could favor its use over the alternative explant sources”. At last, researchers anticipated anticipate that the mature embryo system for barley could gain widespread use once genetic transformation has been demonstrated.

Xueyan *et al*. (2000) investigated the effects of TDZ on *in vitro* regeneration of barley (*Hordeum vulgare* L.) and wheat (*Triticum aestivum* L.) and found that it promotes shoot regeneration from callus in these two important cereal species. Plant regeneration from calluses derived from immature embryo culture of barley and wheat was observed in regeneration media with a wide range of TDZ concentration (0.045-45μM). They also demonstrated that roots developed normally when the regenerated wheat and barley shoots from TDZ-containing media were transferred to the rooting medium.

Beckett and Van Staden (1992) investigated the effect of Thidiazuron on the yield of salinity stressed wheat. They found that the application of Thidiazuron during early growth reduced the yield of unstressed plants, had little effect on moderately stressed plants and at high concentrations significantly increased the yield of highly stressed plants. Although Thidiazuron reduced spikelet and grain number in highly stressed plants, in plants treated with high concentrations of Thidiazuron an increase in grain weight more than compensated for the lower grain number.

1.3.4 How is TDZ involved in plant morphogenesis?

A number of researches were done about Thidiazuron bioregulator and how TDZ involved in plant growth and development. Some of the studies showed that TDZ might have three major mechanisms of action.

The first mechanism is the relation role of TDZ chemical with the metabolism of endogenous growth hormones. The association between

TDZ induced responses and endogenous plant growth regulators, specifically, cytokinins, auxins, ethylene and ABA have been documented with the help of selective inhibitors of these compounds (Capella *et al*., 1983; Hutchinson and Saxena, 1996; Ji and Wang, 1988; Mok *et al*., 1987; Murthy *et al*., 1995; Thomas and Katterman, 1986).

The second mechanism is the enzyme activity changes seen in tissues and organs treated with TDZ. Wang *et al*. (1991) observed vital changes in the activity of the enzymes such as accumulation of phenols, catalase and peroxidase, when he was studying the effect of TDZ on budbreak in apples. In other studies on enzyme activity, TDZ has been shown to induce *de novo* synthesis of nitrate reductase in leaf discs of been seedlings (Kulaeva *et al*., 1982). Chernyad'ev *et al*. (1987) and Chernyad'ev and Kozlovskikh (1990) observed increases in ATP, ribulose biphosphate carboxylase / oxidase (RubisCO), and pentose phosphate pathway enzymes in response to TDZ application.

The third mechanism is the physiological effects of TDZ on the induction of a stress response. Seeds of legumes (Malik and Saxena, 1992) and pumpkin (Burkhanova *et al*., 1984) that were germinated in the presence of TDZ had unique-characteristic morphology. The seedlings showed stunted growth, darkly colored leaves and swollen green cotyledons. TDZ also had deleterious effects on the root system including shortened main root with no or fewer secondary roots.

1.3.5 Advantages and Disadvantages of TDZ

Thidiazuron is resistant to degradation by cytokinin oxidase (Mok *et al*., 1987) so it is quite stable in tissue culture. In micropropagation studies, TDZ is more biologically active than BAP or zeatin. Besides effective in woody species, TDZ is required low concentrations *in vitro* studies.

Some disadvantages have been also reported about the use of TDZ. These are: a) hyperhydricity (Debergh *et al*., 1992) of the regenerated shoots (Briggs *et al*., 1988); b) abnormal leaf morphology (Van Nieuwkerk *et al*., 1986); c) short and compact shoots (Fasolo *et al*., 1989; Meyer and Van Staden, 1988).

1.4 Objectives of this study

The most of studies dealing with the effects of Thidiazuron in plant species *in vitro* culture have been on dicotyledonous plant species. Little information regarding the effects of TDZ on *in vitro* culture of cereals and other monocots is available.

Aim of this study is to investigate the effects of Thidiazuron (N-phenyl-N'-1,2,3-thiadiazol-5ylurea) on the callus development and organogenesis, using mature embryos of Turkish bread and durum wheat varieties.

The mature embryos of 3 bread wheat varieties (Gerek 79, Bezostaja 1 and Başak 95) and 3 durum wheat varieties (Kunduru, Çakmak 79 and

Kırmızı 5132) were utilized for callus induction via tissue culturing methods. In this research, plant regeneration from calluses derived from mature embryo culture of Turkish wheats was investigated in regeneration media with a wide range of TDZ concentrations (0 mg l^{-1} - 1 mg l^{-1}).

At last, some parameters in this study such as, callus induction (%), weight of callus (g), regeneration capacity of callus (%), culture efficiency (%), the number of plants regenerated, the number of plants transferred to soil per the number of embryo cultured were analyzed.

CHAPTER 2

MATERIALS AND METHODS

2.1 Materials

2.1.1 Plant Material

In this study; bread wheats, *Triticum aestivum* L. cv. Gerek 79, Bezostaja 1, Başak 95 and durum wheats *Triticum durum* Desf. cv. Kunduru, Çakmak 79, Kırmızı 5132 varieties were used as sources of mature embryos.

The seeds were kindly provided by Prof. Dr. Murat Özgen, University of Ankara, Faculty of Agriculture, Department of Field Crops, Ankara.

2.1.2 Chemicals

All the chemicals were molecular biology grade and tissue culture tested. They were purchased from Sigma-Aldrich Chemical Company, Gilingham, Dorset, UK and Merck, 64271 Darmstadt, Germany. Murashige and Skoog (MS) basal medium (Sigma cat. no. M5519), growth regulator 2,4-D (Sigma cat. no. D4517), Thidiazuron (P 6186

51707-55-2), sucrose (Sigma cat. no. S5390) and agargel as solidifying agent (Sigma cat. no. A3301) were used in media preparations. Sodium hydroxide (Merck Art. 6462) and hydrochloric acid (Merck Art. 314) were also used in stock solutions and buffers. %97 ethanol (Botafarma Laboratory Kimyasal ve Maddeler Tıbbi Ürünler A.Ş., Anıttepe, Ankara) was used for sterilization.

2.1.3 Glassware

Standard 10 cm diameter glass petri plates (Labor İldam) and baby jars (Sigma cat. no. V8630) with autoclavable caps (Sigma cat. no. B8648) were used throughout the study. Forceps, scalpels, razor blades (Hecos, no. 10, no. 21) were obtained from local commercial sources.

2.1.4 Tissue Culture Instruments and Photography

Holten Laminar flowhood, (HV 2460 Holten, Heto/Holten A/S, Copenhagen) was used to support asepticity in transfer of culture materials. Gerhardt Shaker (Type: THO 5, Bonn, Germany) and Incubator (Memmert type, PRO Scientific Inc., Oxford, CT 06478) were used for incubation of tissues. Autoclave (Tuttnauer 2540 M, Alfa Medical, NY 11550, USA) was also used in this study.

All photography were performed with Nikon 851S with macro 28-85 mm objective using Fuji 100 ASA 36 color print films (Fuji Photo Films Company LTD, Tokyo, Japan).

2.2 Methods

2.2.1 Surface Sterilization of Wheat mature embryos

Mature wheat seeds were surface-sterilized with 70% (vol/vol) ethanol for five min, washed three times with sterile distilled water, treated with commercial bleach (which involves 53% sodium hyperchloride) containing one or two droplets of Tween 20 for twenty five minutes and rinsed seven times with sterile distilled water (Özgen *et al*., 1998).

2.2.2 Preparation of Tissue Culture Media

Basic steps involved in the preparation of tissue culture media were as follows: (1) 90% of required volume of distilled water was measured; (2) powdered media and heat-stable tissue culture materials such as sucrose, agar, auxin and thidiazuron were added while continuously stirring the distilled water; (3) pH of the culture media was adjusted to desired level (pH=5.8) with 1 N NaOH and 0.25 N HCl; (4) the solution was completed to it's final volume with distilled water. Types of media used in the experiments and their contents are listed in Appendix A-1, A-2, A-3. All media were adjusted to pH 5.8 before autoclaving for 20 min at 121 ^{0}C and 1.1 kg/cm^{2} pressure.

2.2.3 Sterilization of Media and Glassware

All tissue culture media were sterilized by autoclaving at 121 ^{0}C and 1.1 kg/cm^{2}, for 20 min. All petri plates, forceps, baby jars, scalpels were heat

sterilized at 180 ^{0}C for 2 hours in a dry oven. All solid media were aseptically dispensed in to the sterile petri plates and baby jars inside the laminar flowhood.

2.2.4 Stock Solutions of Plant Hormones and Growth Regulators

2,4-D stock solution was prepared by dissolving the 2,4-D powder in 50% ethanol (stock 2 mg/l). Plant growth regulators phytohormones were used as 1 mg/ml stock solutions. Appropriate amounts (usually 25 mg) of Thidiazuron was dissolved with 2-3 drops of 1 N NaOH and brought to the final volume (usually 25 ml) (stock 1 mg/ml).

2.2.5 Transfer Conditions

All the transfers of the explants and culture material were performed in laminar flowhood. Panels of the cabinet and the working surface were first cleaned up with 70% ethanol before each use.

2.2.6 Culture Conditions

After sterilization, the seeds were incubated at 33 ^{0}C for two hours in sterilized distilled water for imbibition. Then, mature embryos were aseptically removed from imbibed seeds with scalpel and a razor blade. Removed embryos were placed scutellum upwards and arranged in a circle of 2.5 cm-diameter at the center of the petri plates containing callus induction medium (Appendix A-1). They were incubated at 25±1 ^{0}C for 15 days in darkness.

Then, the callus tissues were transferred to TDZ-containing MS medium (Appendix A-2) for shoot regeneration for 5 weeks at 25±1 ^{0}C in a 16 h light (2000 lux) 8 h dark photoperiod. After regeneration, shoot regenerated plants were transferred into hormone-free rooting medium (Appendix A-3) in baby jars for 5 weeks at 25±1 ^{0}C in a 16 h light 8 h dark photoperiod.

At last, they were transferred to pots containing garden soil. In order to attain high humidity (acclimatization) the pots were covered with plastic film and kept at 25±1 ^{0}C in a 16 h light 8 h dark photoperiod.

2.2.7 Data Collection and Statistical Analysis

The first set of data is the callus induction frequency of the wheat varieties and it is calculated by the formula given below:
Callus Induction Frequency = (# of embryos with callus formation/15 embryos) X 100

The second set of data consisted of the weight of formed callus structures and it is simply collected by weighing the individual calli.

The third set of data consisted of regeneration capacity and calculated by using the following formula:

Regeneration Capacity = (# of regenerants / # of embryo with callus) X 100

The fourth set of data is the Culture Efficiency which was calculated by using the following formula:

Culture Efficiency = (# of regenerants / 15 embryos) X 100

The final set of data is consisted of the Number of Plants Regenerated and Transferred to Soil and it is simply recorded by counting the number of plants obtained.

All sets of data were analyzed by using MINITAB Release 13 Statistical Software Package to test the roles of variety and TDZ on the tissue culture response parameters.

CHAPTER 3

RESULTS

In this study, the aim was to understand the effects of cytokinin-like Thidiazuron hormone on the regeneration responses of callus cultures of Turkish bread (Başak 95, Gerek 79, and Bezostaja 1) and durum (Kunduru, Çakmak 79, and Kırmızı 5132) wheat varieties. Furthermore, some of the tissue culture responses of Turkish bread and durum wheat varieties such as; a) callus induction (%), b) weight of callus (g), c) regeneration capacity of callus (%), d) culture efficiency (%), e) the number of plant regeneration and f) the plants transferred to soil/15 embryo cultured were calculated and analyzed statistically (MINITAB Release 13 Statistical Software Package).

3.1 Turkish Bread and Durum Wheat Varieties

3.1.1 Callus Induction

The growth of cereal tissue cultures which are capable of plant regeneration has been accomplished on relatively few culture media formulations. Murashige and Skoog (MS) media has been particularly effective for culture initiation, maintenance, and plant regeneration in many cereals

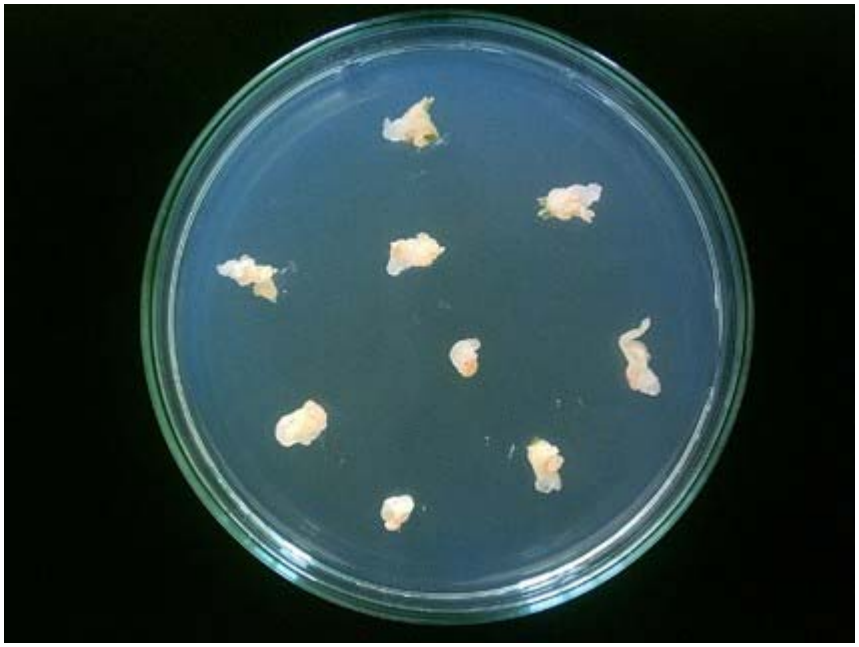
(Cheng and Smith, 1975; Rangan, 1974; Green and Philips, 1975; Gamborg *et al*., 1977; Sears and Deckard, 1982; Ahloowalia, 1982).

Auxin has been the principal hormone requirement for the initiation and maintenance of tissue cultures of cereals capable of plant regeneration. In many instances, 2,4-D at 1-15 mg per liter has been sufficient to obtain the desired cultures (Cummings *et al*., 1976; Rangan, 1974; Green and Philips, 1975; Sears and Deckard, 1982).

In vitro culture of immature embryos (Ahloowalia, 1982; Ozias-Akins and Vasil, 1983; Ainsley and Aryan, 1998) and mature embryos (Özgen *et al*., 1998; Delporte *et al*., 2001) of wheat species are the methods of choice for producing abundant callus and sufficient shoot formation in cereals. Explants derived from mature wheat endosperm supported embryos (Özgen *et al*., 1998) and thin mature embryo fragments (Delporte *et al*., 2001) were used for callus induction and plant regeneration.

In this study, callus formation from mature embryos (c.a. 15 embryos) derived from wheat varieties started after 2-3 days of culture. Maximum callus induction occurred 15 days after inoculation on mature embryo callus induction media (Figure 3.1).

As can be seen clearly from Table 3.1 and Table 3.2 callus induction responses were not greatly influenced by the varieties for mature embryo explants of both Turkish bread and durum wheat varieties. For all bread and durum varieties, high callus induction frequency events were observed.



A

B

Figure 3.1 Representative photographs of Çakmak 79 (Panel A) and Kunduru (Panel B) callus induction events on 15^{th} days of inoculation.

Table 3.1 Callus induction frequency of Turkish bread wheat varieties.

Bread wheat var.	Başak 95	Gerek 79	Bezostaja 1
Callus Induction (%)	100	80	100
	100	100	100
	100	86.67	100
	100	100	100
	100	100	100
	100	100	100
	93.33	100	100
	100	100	100
	100	100	100
	100	100	100
	100	100	100
	100	100	100
	100	100	100
	93.33	100	100
	100	100	100
Mean	**99.11± 0.606**	**97.77 ± 1.55**	**100 ± 0.00**

Table 3.2 Callus induction frequency of Turkish durum wheat varieties.

Durum Wheat var.	Kunduru	Çakmak 79	Kırmızı 5132
Callus Induction (%)	100	100	100
	80	100	100
	100	100	100
	100	100	100
	100	100	100
	80	100	100
	100	100	100
	100	100	100
	100	93.33	100
	100	100	93.33
	100	100	93.33
	100	100	100
	100	100	100
	93.33	100	100
	93.33	100	100
Mean	**96.44 ± 1.82**	**99.56± 0.445**	**99.11± 0.606**

While the bread wheat varieties of Başak 95 and Gerek 79 yielded less than 100 % callus induction frequencies, Bezostaja 1 variety had an excellent callus induction frequency (100 %). However, as it can be seen from the statistical analysis performed for this group of data (Appendix B-1), the differences between the callus induction frequencies for the bread wheat varieties are insignificant.

In Turkish durum wheat varieties; although Kunduru had 96.44% callus induction frequency, Çakmak 79 and Kırmızı 5132 had 99.56% and 99.11% callus induction frequencies, respectively. However, as in the case of bread wheat varieties, when a similar statistical test was applied to this group of data, an insignificant difference between the durum wheat varieties and callus induction frequencies became apparent (Appendix B-2).

3.1.2 Weight of Callus

As can be seen in Table 3.3, Başak 95 had the highest average weight of callus (1.05g), which was followed by Bezostaja 1 (0.95g) and Gerek 79 (0.78g).

Table 3.3 Callus weight of Turkish bread wheat varieties.

Bread wheat var.	Başak 95	Bezostaja 1	Gerek 79
Weight of Callus (g)	1.16	0.91	0.58
	1.22	1.00	0.61
	1.03	0.8	0.69
	1.01	1.02	0.98
	1.12	0.91	0.98
	0.97	1.04	0.61
	1.31	0.91	0.86
	1.21	0.84	0.97
	1.11	0.93	0.71
	0.98	0.82	0.97
	1.04	1.08	0.78
	1.00	1.05	0.92
	0.89	1.06	0.66
	0.82	0.99	0.61
	0.94	0.93	0.88
Mean	**1.05 ± 0.0344**	**0.95 ± 0.0232**	**0.78 ± 0.0403**

As can be inferred from Appendix B-3, the statistical analysis revealed a non-significant difference between Başak 95 and Bezostaja 1 for the callus weight parameter. However a statistically significant difference was observed between Gerek 79 and the aforementioned varieties.

For the durum wheat varieties, while Kunduru had the highest average weight of callus (0.94g), Kırmızı 5132 (0,89 g) and Çakmak 79 (0.80g) followed Kunduru (Table 3.4). Statistical analysis performed for this set of data revealed significant differences between varieties (Appendix B-4). While the differences between Kunduru and Kırmızı 5132 as well as Çakmak 79 and Kırmızı 5132 are insignificant, the difference between Çakmak 79 and Kunduru is found to be statistically significant.

Table 3.4 Weight of callus of Turkish durum wheat varieties.

Durum Wheat var.	Kunduru	Çakmak 79	Kırmızı 5132
Weight of Callus (g)	1.03	0.62	0.99
	0.85	0.79	1.1
	0.88	0.86	0.93
	1.12	0.86	0.821
	1.08	0.95	0.75
	0.63	0.85	0.89
	0.92	0.88	0.79
	1.27	0.85	0.98
	0.82	0.84	0.91
	1.09	0.85	0.7
	0.95	0.96	0.81
	0.91	0.85	0.858
	0.77	0.50	1.11
	0.81	0.77	0.90
	1.1	0.57	0.92
Mean	**0.94 ± 0.0428**	**0.80 ± 0.0345**	**0.89 ± 0.0301**

3.1.3 Regeneration Capacity and Culture Efficiency

Callus that are obtained from all bread and durum wheat varieties were plated, in triplicates, in regeneration medium supplemented with four different concentrations of Thidiazuron (0.25, 0.5, 0.75, 1.0 mg/l) and without Thidiazuron (control). Calli with green spots rapidly developed shoots and leaves (Figure 3.2 and 3.3).

Contrary to the expectations, the first striking result that can be seen in Table 3.5, is the general negative effect of TDZ on the regeneration capacity of all the tested bread wheat varieties. When the overall means of the treatments are compared with the control means, a reduction is evident.

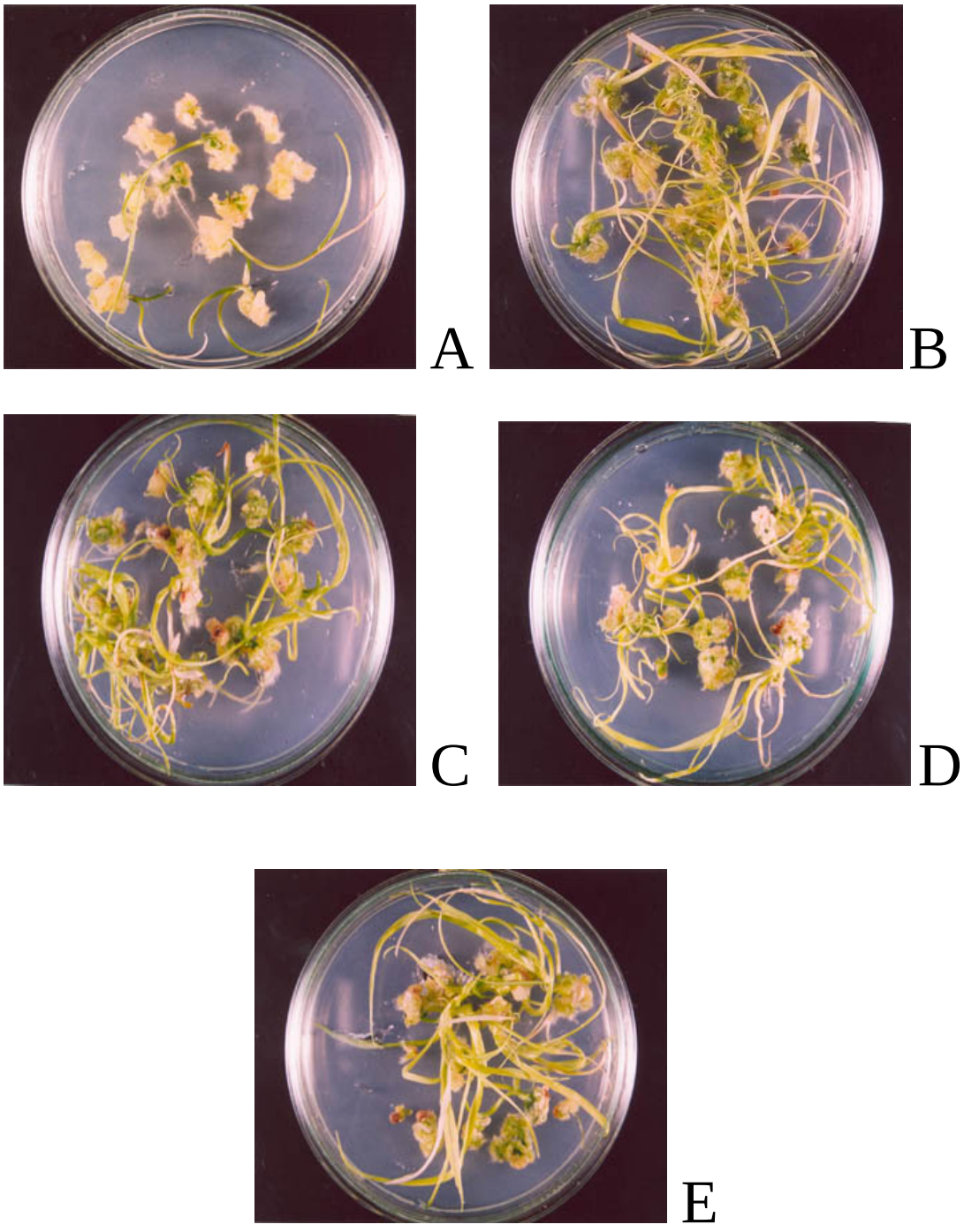


Figure 3.2 Representative photographs of Kunduru callus regeneration events on 5th weeks of inoculation. Panel A: Control, B: 0.25 mg/l TDZ, C: 0.50 mg/l TDZ, D: 0.75 mg/l TDZ and E: 1.0 mg/l TDZ

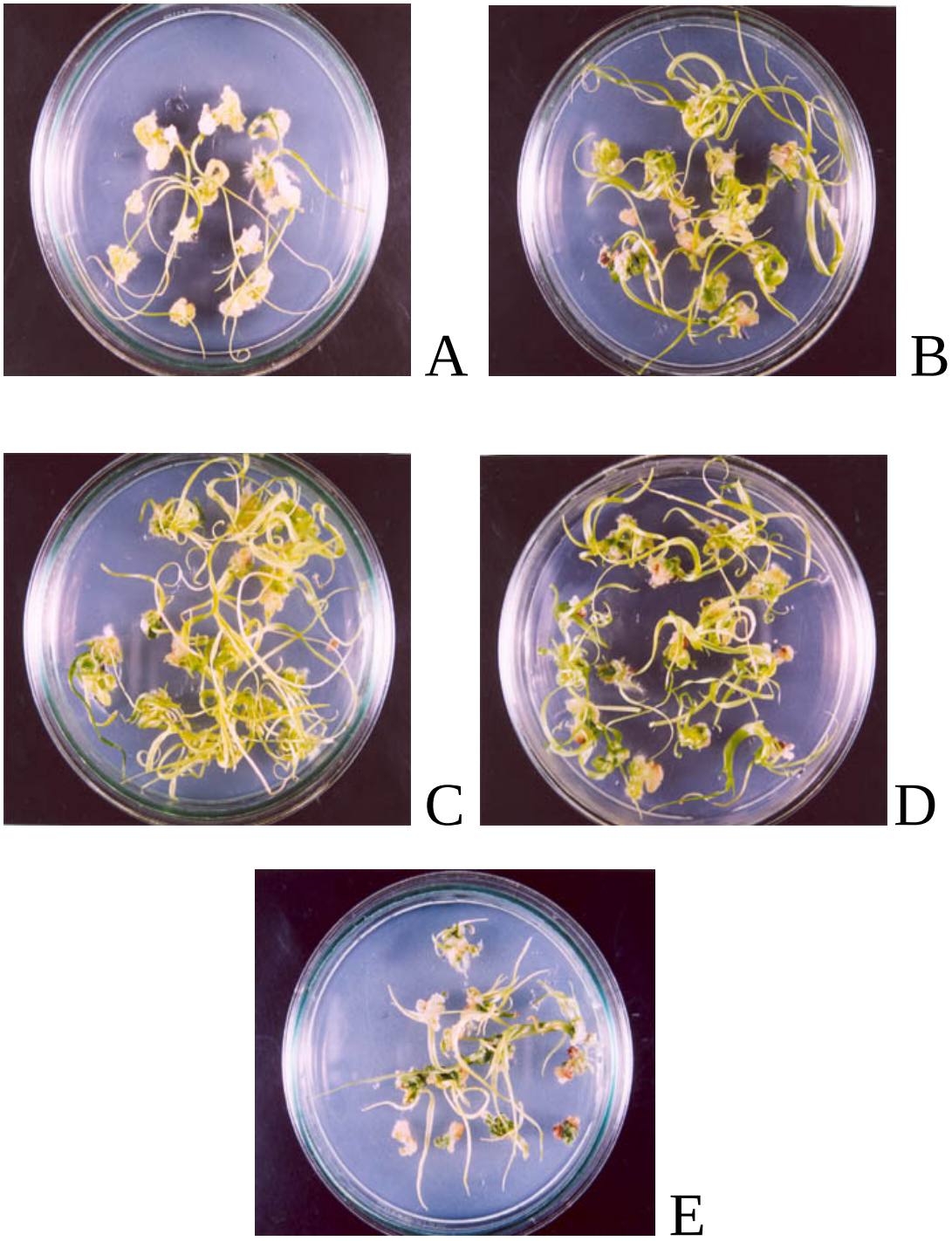


Figure 3.3 Representative photographs of Çakmak 79 callus regeneration events on 5th weeks of inoculation. Panel A: Control, B: 0.25 mg/l TDZ, C: 0.50 mg/l TDZ, D: 0.75 mg/l TDZ and E: 1.0 mg/l TDZ.

Table 3.5 Regeneration capacity of callus and culture efficiency of Turkish bread wheat varieties.

Bread wheat var.	**Başak 95**	**Gerek 79**	**Bezostaja 1**		**Başak 95**	**Gerek 79**	**Bezostaja 1**	
TDZ (mg/l)	Regeneration capacity of callus (%)	Regeneration capacity of callus (%)	Regeneration capacity of callus (%)	***Overall Means***	Culture efficiency (%)	Culture efficiency (%)	Culture efficiency (%)	***Overall Means***
0.0 (Control)	100	100	100		100	80	100	
	93.33	100	80		93.33	100	80	
	93.33	100	100		93.33	86.66	100	
Mean	**95.55 ± 2.22**	**100 ± 0.00**	**93.33 ± 6.67**	***96.29 ± 1.96***	**95.55 ± 2.22**	**88.88 ± 5.88**	**93.33 ± 6.67**	***92.58 ± 1.96***
0.25	100	93.33	100		100	93.33	100	
	100	73.33	100		100	73.33	100	
	86.66	73.33	80		86.66	73.33	80	
Mean	**95.55 ± 4.45**	**79.99 ± 6.67**	**93.33 ± 6.67**	***89.62 ± 4.85***	**95.55 ± 4.45**	**79.99 ± 6.67**	**93.33 ± 6.67**	***89.62 ± 4.85***
0.50	71.42	100	100		66.66	100	100	
	93.33	100	93.33		93.33	100	93.33	
	93.33	100	100		93.33	100	100	
Mean	**86.02 ± 7.30**	**100 ± 0.00**	**97.77 ± 2.22**	***94.59 ± 4.33***	**84.44 ± 8.89**	**100 ± 0.00**	**97.77 ± 2.22**	***94.07 ± 4.85***
0.75	86.66	93.33	73.33		86.66	93.33	73.33	
	93.33	73.33	73.33		93.33	73.33	73.33	
	86.66	73.33	86.66		86.66	73.33	86.66	
Mean	**88.88 ± 2.22**	**79.99 ± 6.67**	**77.77 ± 4.44**	***82.21 ± 3.39***	**88.88 ± 2.22**	**79.99 ± 6.67**	**77.77 ± 4.44**	***82.21 ± 3.39***
1.0	86.66	60	86.66		86.66	60	86.66	
	92.85	40	93.33		86.66	40	93.33	
	80	40	80		80	40	80	
Mean	**86.50 ± 3.71**	**46.66 ± 6.67**	**86.66 ± 3.85**	***73.27 ± 13.3***	**84.44 ± 2.22**	**46.6 6± 6.67**	**86.66 ± 3.85**	***72.58 ± 12.9***
Overall Means	**90.50 ± 2.12**	**81.32 ± 9.75**	**89.77 ± 3.49**		**89.77 ± 2.49**	**79.10 ± 8.91**	**89.77 ± 3.49**	

The statistical analysis also revealed a significant difference between control and 1.0 and 0.75 mg/l treatments whereas 0.25 and 0.50 mg/l concentrations displayed a non-significant difference when compared with the controls (Appendix B-5).

Comparison of the overall means of the varieties revealed the fact that the poorest performance was obtained from Gerek 79 (81.3 %) which was followed by Bezostaja 1 (89.8 %), and Başak 95 (90.5 %). Statistical analysis also reflected the same pattern by a non-significant difference between Başak 95 and Bezostaja 1 but a significant difference between Gerek 79 and the remaining two varieties (Appendix B-5).

Due to the derivation from the regeneration capacity of callus data, the culture efficiency parameter yielded very similar results as in the case of regeneration capacity parameter (Table 3.5). However a slight difference is observed in the case of the TDZ treatments (Appendix B-6). While the difference between the control and 1.0 mg/l concentration is significant, the difference between all the rest of the concentrations and the control is found to be non-significant. The relationship between the varieties and their culture efficiency became more distinctive when compared with the regeneration capacity parameter. Nevertheless the same pattern is also observed and Başak 95 and Bezostaja 1 responded similarly whereas Gerek 79 displayed significantly reduced culture efficiency (Appendix B-6).

For the durum wheat varieties TDZ was found to be affecting the regeneration capacity in a positive way for Kunduru variety (Table 3.6). While the control yielded 91.10 % regeneration, overall mean of the TDZ treatments yielded 93.48 %. Statistical results also confirmed the significant difference between Kunduru and the remaining two varieties whereas the difference between Çakmak 79 and Kırmızı 5132 was found to be non-significant (Appendix B-7).

The statistical analysis on the TDZ treatments resulted with the non-significant differences between control and the TDZ treatments except 1.0 mg/l (Appendix B-7). At this concentration TDZ caused adverse effects on the regeneration capacity of the callus (61.4 %).

In durum wheat varieties, the culture efficiency response values followed a parallel path as of the regeneration capacity of callus (Table 3.6).

Likewise the statistical analysis confirmed the previous findings on the regeneration capacity parameter (Appendix B-8).

Table 3.6 Regeneration capacity of callus and culture efficiency of Turkish durum varieties.

Durum Wheat var.	**Kunduru**	**Çakmak 79**	**Kırmızı 5132**		**Kunduru**	**Çakmak 79**	**Kırmızı 5132**	
TDZ (mg/l)	Regeneration capacity of callus (%)	Regeneration capacity of callus (%)	Regeneration capacity of callus (%)	***Overall Means***	Culture efficiency (%)	Culture efficiency (%)	Culture efficiency (%)	***Overall Means***
0.0 (Control)	86.66	80	93.33		93.33	80	93.33	
	100	100	86.66		80	100	86.66	
	86.66	80	86.66		86.66	80	86.66	
Mean	**91.10 ± 4.45**	**86.66 ± 6.67**	**88.88 ± 2.22**	***88.88 ± 1.28***	**86.66 ± 3.85**	**86.66 ± 6.67**	**88.88 ± 2.22**	***87.4 ± 0.74***
0.25	86.66	93.33	93.3.3		86.66	93.33	93.33	
	93.33	100	100		86.66	100	100	
	83.33	86.66	86.66		66.66	86.66	86.66	
Mean	**87.77 ± 2.94**	**93.33 ± 3.85**	**93.33 ± 3.85**	***91.47 ± 1.8***	**79.99 ± 6.67**	**93.33 ± 3.85**	**93.33 ± 3.85**	***88.88 ± 4.44***
0.50	100	100	86.66		100	100	86.66	
	100	93.33	86.66		100	93.33	86.66	
	100	80	93.3.3		100	80	93.3.3	
Mean	**100 ± 0.00**	**91.11 ± 5.88**	**88.88 ± 2.22**	***93.33 ± 3.39***	**100 ± 0.00**	**91.11 ± 5.88**	**88.88 ± 2.22**	***93.33 ± 3.39***
0.75	93.33	100	100		93.33	100	93.33	
	100	100	100		100	100	93.33	
	86.66	100	93.3.3		86.66	100	93.33	
Mean	**93.33 ± 3.85**	**100 ± 0.00**	**97.77 ± 2.22**	***97.03 ± 1.96***	**93.33 ± 3.85**	**100 ± 0.00**	**93.33 ± 0.00**	***95.55 ± 2.22***
1.0	100	33.3	53.3		100	33.3	53.3	
	85.71	40	60		80	40	60	
	100	33.3	46.6		93.33	33.3	46.6	
Mean	**95.23 ± 4.76**	**35.53 ± 2.23**	**53.3 ± 3.87**	***61.35 ± 17.6***	**91.11 ± 5.88**	**35.53 ± 2.23**	**53.3 ± 3.87**	***59.98±16.38***
Overall Means	**93.48 ± 2.05**	**81.32 ± 11.6**	**84.43 ± 7.96**		**90.21 ± 3.34**	**81.32 ± 11.6**	**83.54 ± 7.63**	

3.1.4 Number of plants regenerated and transferred to soil

The shoot regenerated plants were transferred into hormone-free rooting medium for root initiation. After 30 days, the plants that developed roots (Figure 3.4 and 3.5) were transferred to pots containing garden soil. However, majority of the plants that were treated with TDZ developed weak roots when compared with the control plants (Figure 3.6 and 3.7).

In bread wheat varieties, the TDZ treatments (especially at 0.75 mg/l concentration for Gerek 79) more than 2-fold increase in numbers of regenerated plants were observed when compared with the control (Table 3.7). While the control and 1.0 mg/l TDZ treatments yielded statistically non-significant differences (Appendix B-9), the rest of the concentrations within themselves yielded non-significant differences but significant differences when compared with the control. When the effects of TDZ on the varieties were analyzed, it was found out to be non-significant.

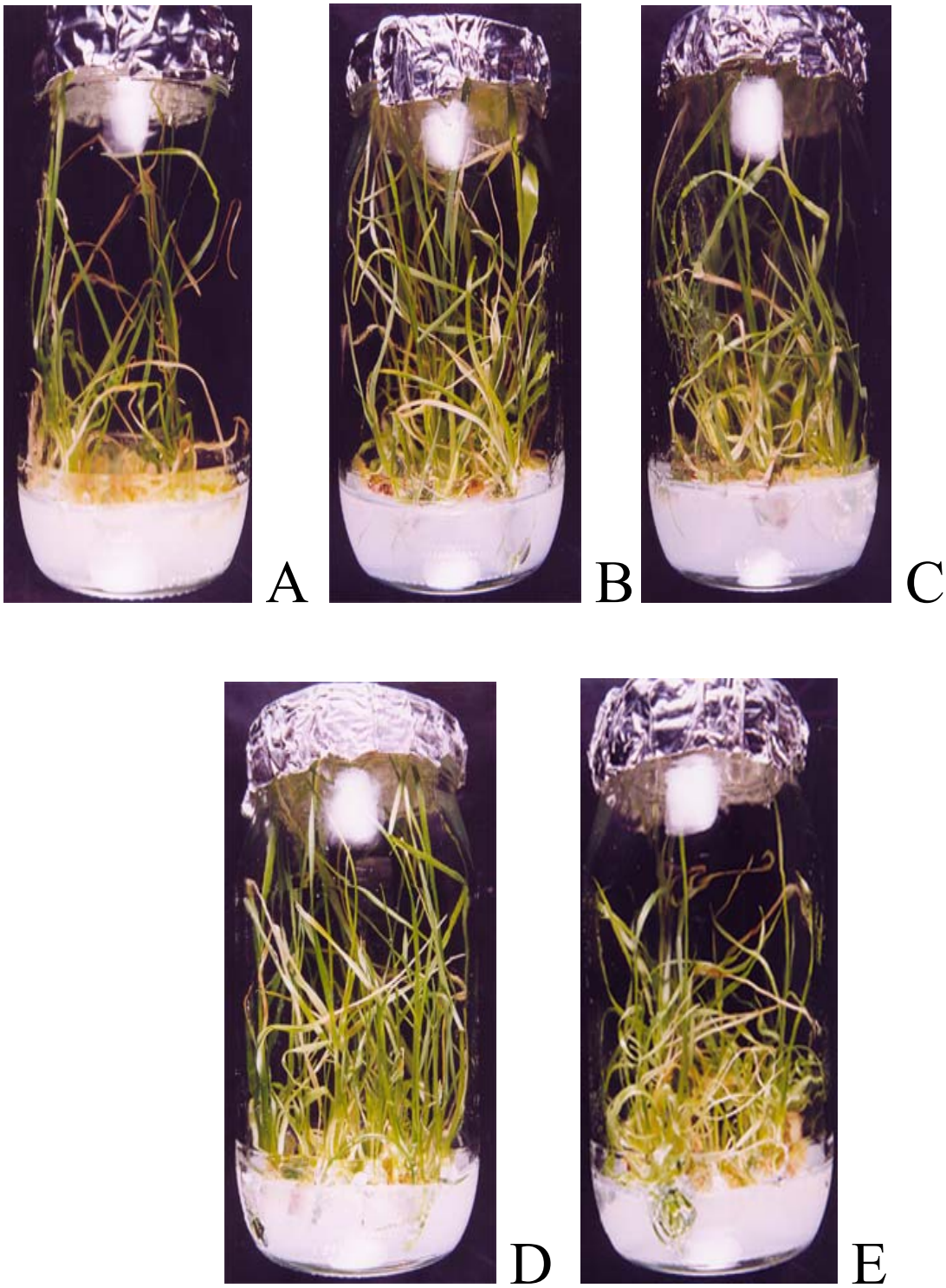


Figure 3.4 Representative photographs of Başak 95 rooting of the regenerants on 5th weeks of inoculation. Panel A: Control, B: 0.25 mg/l TDZ, C: 0.50 mg/l TDZ, D: 0.75 mg/l TDZ and E: 1.0 mg/l TDZ.

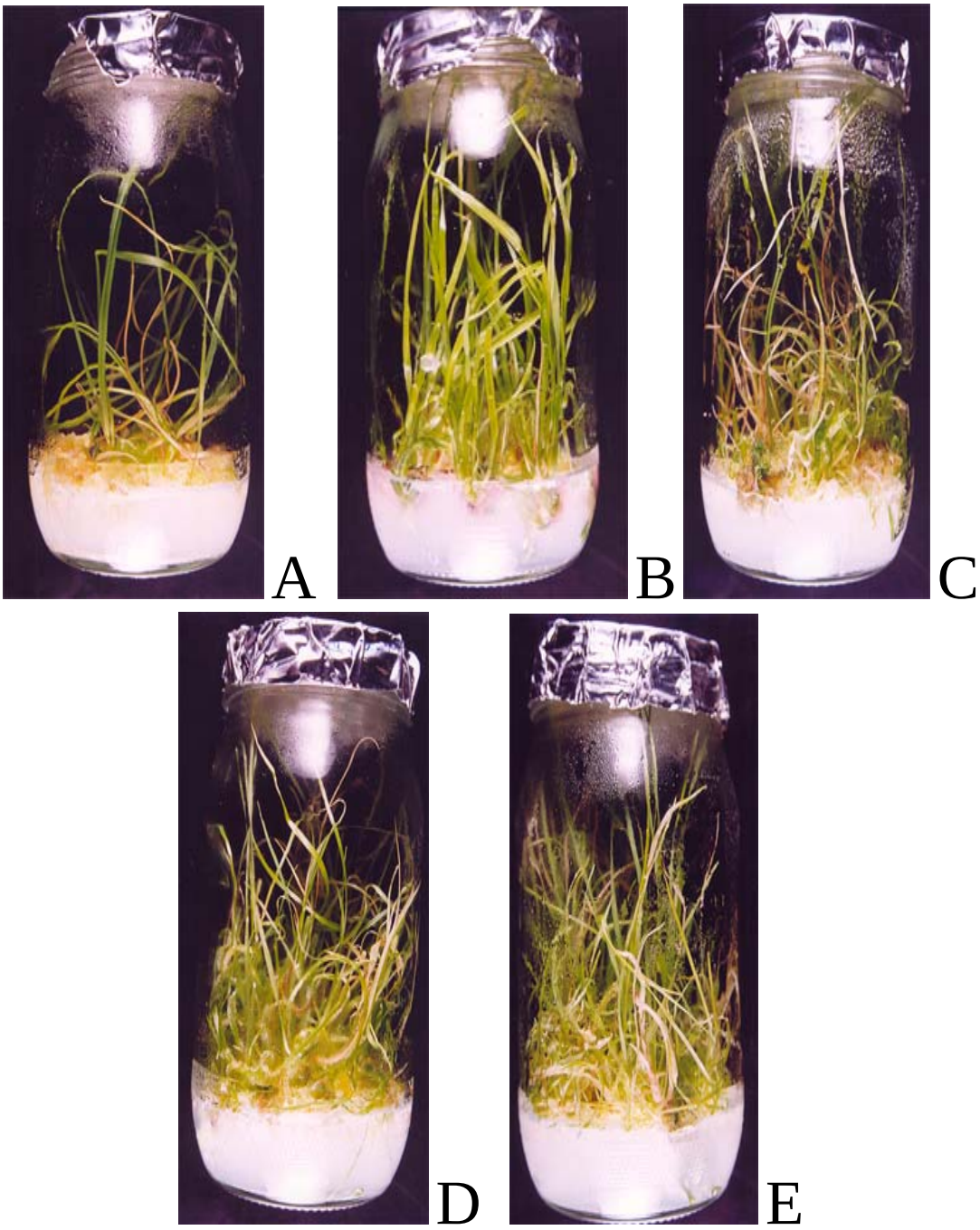


Figure 3.5 Representative photographs of Kunduru rooting of regenerants on 5th weeks of inoculation. Panel A: Control, B: 0.25 mg/l TDZ, C: 0.50 mg/l TDZ, D: 0.75 mg/l TDZ and E: 1.0 mg/l TDZ.

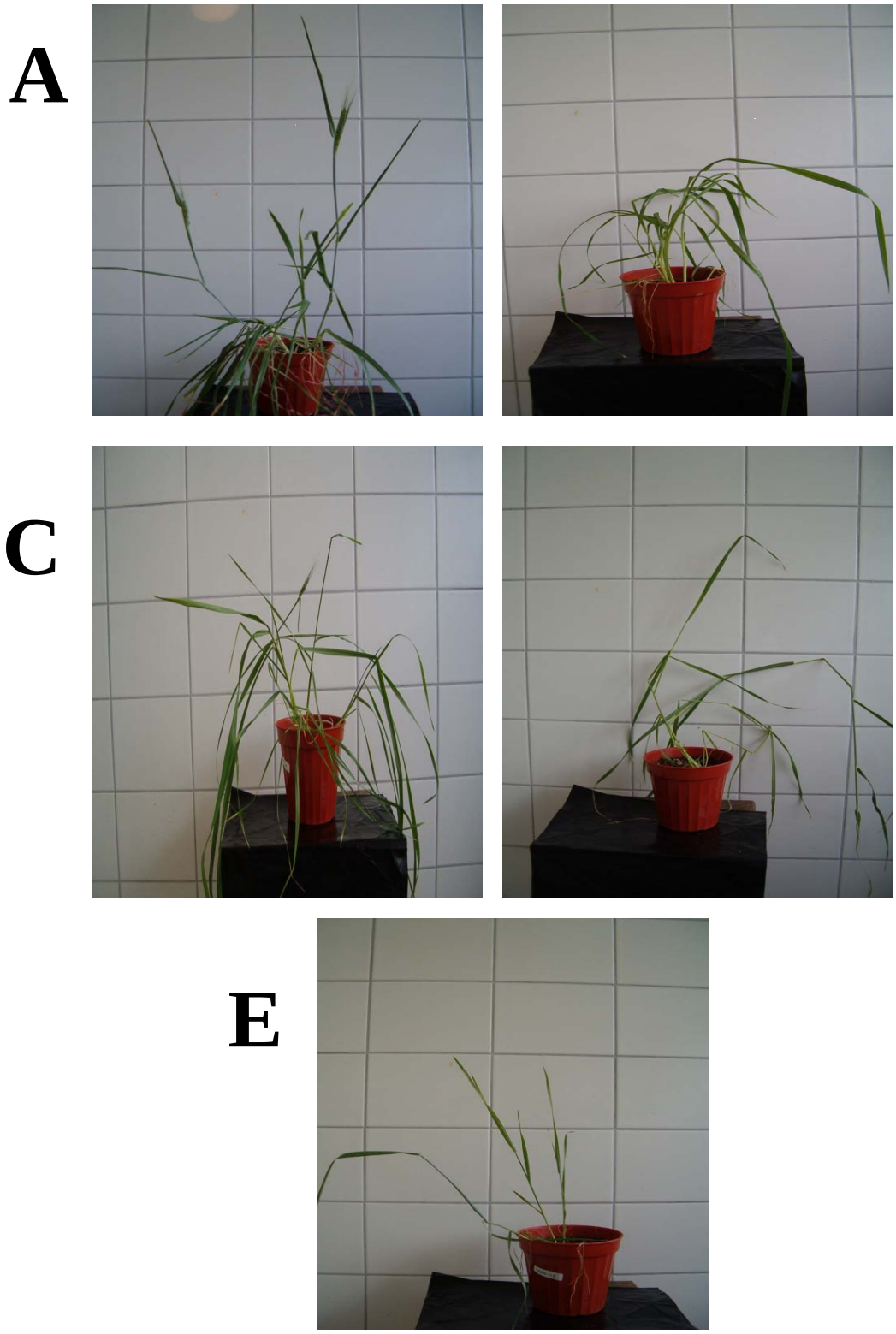


Figure 3.6 Representative photographs of Başak 95 regenerants that were transferred to soil after rooting. Panel A: Control, B: 0.25 mg/l TDZ, C: 0.50 mg/l TDZ, D: 0.75 mg/l TDZ and E: 1.0 mg/l TDZ

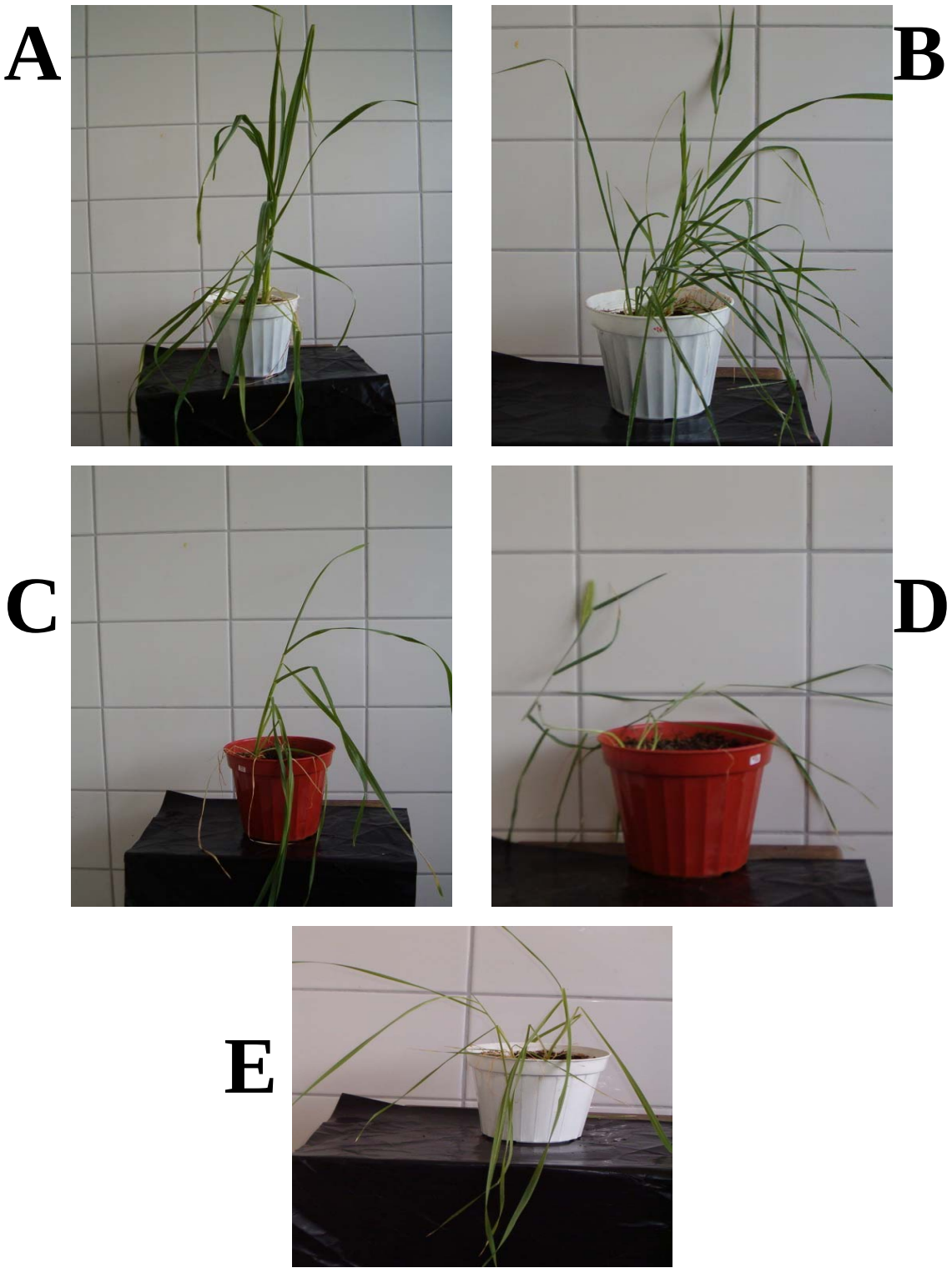


Figure 3.7 Representative photographs of Bezostaja 1 regenerants that were transferred to soil after rooting. Panel A: Control, B: 0.25 mg/l TDZ, C: 0.50 mg/l TDZ, D: 0.75 mg/l TDZ and E: 1.0 mg/l TDZ

In durum wheat varieties, very similar results were obtained (Table 3.8) to that of bread wheat varieties. TDZ treatments (especially at 0.5 and 0.75 mg/l concentrations for Kunduru and Çakmak 79) yielded nearly 2-fold increase in numbers of regenerated plants when compared with the control.

Statistical analysis performed on this set of data (Appendix B-10) revealed that while the control and 1.0 mg/l were statistically non-significant, the remaining concentrations within themselves although being non-significant, are significantly different than that of control and 1.0 mg/l TDZ treatments.

As in the case for bread wheat data, when the effects of TDZ on the durum wheat varieties were analyzed, it was found out to be non-significant (Appendix B-10).

Following their transfer to soil, plants that were treated with various concentrations of TDZ displayed reduced vigor and majority of these plants did not sufficiently develop above the ground parts when compared with the control plants.

Table 3.8 The number of plants regenerated and transferred to soil/15 embryo cultured of Turkish durum varieties.

Durum Wheat var.	**Kunduru**	**Çakmak 79**	**Kırmızı 5132**		**Kunduru**	**Çakmak 79**	**Kırmızı 5132**	
TDZ (mg/l)	No of plants regenerated	No of plants regenerated	No of plants regenerated	***Overall Means***	No of plants transferred to soil/15 embryo cultured	No of plants transferred to soil/15 embryo cultured	No of plants transferred to soil/15 embryo cultured	***Overall Means***
0.0 (Control)	5	4	6		0.333	0.266	0.400	
	6	8	7		0.400	0.533	0.466	
	6	6	4		0.400	0.400	0.266	
Mean	**5.66 ± 0.33**	**6.00 ± 1.15**	**5.66 ± 0.88**	***5.77 ± 0.11***	**0.377 ± 0.02**	**0.400 ± 0.07**	**0.377 ± 0.05**	**0.384 ± 0.007**
0.25	6	14	11		0.400	0.933	0.733	
	6	10	11		0.400	0.666	0.733	
	7	10	10		0.466	0.666	0.666	
Mean	**6.33 ± 0.33**	**11.33 ± 1.33**	**10.66 ± 0.33**	***9.44 ± 1.56***	**0.422 ± 0.02**	**0.755 ± 0.08**	**0.710 ± 0.02**	**0.629 ± 0.10**
0.50	13	13	9		0.866	0.866	0.600	
	11	12	9		0.733	0.800	0.600	
	11	12	11		0.733	0.800	0.733	
Mean	**11.66 ± 0.66**	**12.33 ± 0.33**	**9.66 ± 0.66**	***11.21 ± 0.80***	**0.777 ± 0.04**	**0.822 ± 0.02**	**0.644 ± 0.04**	**0.747 ± 0.05**
0.75	9	11	9		0.600	0.733	0.600	
	12	13	7		0.800	0.866	0.466	
	13	12	6		0.866	0.800	0.400	
Mean	**11.33 ± 1.20**	**12.00 ± 0.57**	**7.33 ± 0.88**	***10.22 ± 1.45***	**0.755 ± 0.08**	**0.800 ± 0.03**	**0.488 ± 0.05**	**0.681 ± 0.09**
1.0	9	3	5		0.600	0.200	0.333	
	7	4	7		0.466	0.266	0.466	
	9	3	7		0.600	0.200	0.466	
Mean	**8.33 ± 0.66**	**3.33 ± 0.33**	**6.33 ± 0.66**	***5.99 ± 1.45***	**0.555 ± 0.04**	**0.222.± 0.02**	**0.421 ± 0.04**	**0.399 ± 0.09**
Overall Means	***8.66 ± 1.24***	***8.99 ± 1.83***	***7.92 ± 0.96***		***0.577 ± 0.08***	***0.599 ± 0.12***	***0.528 ± 0.06***	

CHAPTER 4

DISCUSSION

A number of complex factors determine the in vitro growth of plants. There are three major factors influencing *in vitro* plant regeneration: *genotype*, *explant source* and *culture conditions* (including culture medium and environment) (Maes et al., 1996).

4.1.1 Genotype

The genotype is the most crucial factor to be concentrated on since certain species or cultivars within the species have greater regeneration capacity compared to others. That is the reason that first which genotype will be used has to be determined. In our studies, we have decided to study three Turkish bread (Başak 95, Gerek 79, and Bezostaja 1) and three durum (Kunduru, Çakmak 79, and Kırmızı 5132) wheat varieties.

4.1.2 Explant type

Explant source is another critical factor that effects the growth of plants in vitro culture. For all species, generally younger or vigorously growing tissues from healthy plants should be used as explant source. Different explant sources can be used for the induction of regenerable wheat cultures: shoot tips, inflorescences, anthers, and isolated microspores, immature and mature embryos.

The regeneration system of our in vitro study based upon initiation of embryogenic calli from mature embryos. Unlike immature embryo and other explant sources, the physiological state of the mature embryo shows minimal variability. In addition, donor plants do not have to be grown in a growth chamber or green house that requires additional resources. The complete regulation of the culture conditions of donor plants is sometimes difficult to achieve. The seeds can be harvested from the field and stored for future use; thus material is available in great quantity and all year round as well as they are easy to work with.

4.1.3 Culture Conditions

Third factor, for in vitro studies is physical parameters including culture medium, environment, and temperature and light intensity. The culture conditions (both chemical and physical) are highly influential in vitro studies and culture conditions optimized for one species or genotype may not be optimal or even applicable for another. In our study, especially during regeneration stage, we have included various concentrations of TDZ into the tissue culture media and analyzed the effects of TDZ on the regeneration response of bread and durum wheat varieties.

4.2 Studied Factors

In our study, we have tested the effect of TDZ on the tissue culture responses of mature embryo-derived Turkish bread and durum wheat varieties. The tissue culture responses such as, callus induction (%) and weight (g), the regeneration capacity (%) and culture efficiency (%), the

number of plant regenerated and transferred to soil were discussed in below.

4.2.1 Callus induction and Callus Weight

In callus induction of both Turkish bread and durum wheat varieties, we have kept two major factors, explant source and MS culture medium, constant. For the explant, mature embryo-derived wheat seeds and for the media, MS medium supplemented with 2,4 dichlorophenoxyacetic acid (2,4-D) (2 mg l^{-1}) is used for callus induction.

The callus induction frequency of both Turkish bread and durum wheat varieties were observed to be independent of varietal influence and extremely high callus induction frequencies were obtained in both species (*c.a.* › 96%). The statistical analysis performed on this set of data also confirmed the non-significant differences observed between tested varieties.

However, a definitive varietal influence was observed on callus weights. Statistical analysis (Appendix B-3 and B-4) performed on these sets of data clearly indicated that the varieties Gerek 79 (bread wheat) and Çakmak 79 (durum wheat) performed poorly whereas the best performers were found to be Başak 95 (bread) and Kunduru (durum).

The observed varietal effect on callus weight is not surprising since it is well known from the results obtained in our laboratory previously (Birsin & Özgen, 2004) and elsewhere (Arzani & Mirodjagh 1999) that genotype plays an important role on wheat development.

4.2.2 Regeneration Capacity and Culture Efficiency

Although Thidiazuron has been shown an effective regulator on *in vitro* morphogenesis of many dicotyledonous plant species, information regarding the effect of TDZ on *in vitro* regeneration of monocot species is limited (Shan *et al*., 2000).

In this study, we investigated the effects of Thidiazuron, a cytokinin-like growth regulator on *in vitro* regeneration of bread and durum wheat varieties.

When the results presented in Chapter 3 are considered, a general negative effect of TDZ on the regeneration capacity of bread wheat varieties was apparent. The results of the statistical analysis (Appendix B-5) also confirmed this negative effect. However, although being negative, low concentrations of TDZ (0,25 and 0,50 mg/l) yielded comparable (non-significant) results with the controls (TDZ-free) whereas high concentrations (0,75 and 1.0 mg/l) caused significant reductions in regeneration capacity of bread wheat varieties. Varietal influence is also observed on this parameter and Gerek 79 performed poorly when compared to very similar Başak 95 and Bezostaja-1 performances (Appendix B-5).

For the durum wheat varieties TDZ was found to be affecting the regeneration capacity in a positive way for Kunduru variety (Table 3.6). While the control yielded 91.10 % regeneration, overall mean of the TDZ treatments yielded 93.48 %. Statistical results also confirmed the significant difference between Kunduru and the remaining two varieties

whereas the difference between Çakmak 79 and Kırmızı 5132 was found to be non-significant (Appendix B-7).

The statistical analysis on the TDZ treatments resulted with the non-significant differences between control and the TDZ treatments except 1.0 mg/l (Appendix B-7). At this concentration TDZ caused adverse effects on the regeneration capacity of the callus (61.4 %).

This adverse effect might be due to the "accumulation-suppression" effect of the growth regulator. According to Mok *et al.* (1987), TDZ is resistant to degradation by cytokinin oxidase and therefore can not be metabolized as easily as other natural cytokinins. This inability for metabolization might cause TDZ to accumulate and start to exaggerate its own function probably by generating excessive amount of signals for cytokinesis, thus suppressing the regeneration capacity by disturbing the auxin/cytokinin ratio required for regeneration (Moore, 1989).

Due to its derivation from regeneration capacity, the culture efficiency parameter yielded very similar results for both bread and durum wheat varieties.

4.2.3 Number of plants regenerated and transferred to soil

The shoot adventitious regenerated plants were transferred into hormone-free rooting medium for root initiation. After 30 days, the plants that developed roots were transferred to pots containing garden soil. However, majority of the plants that were treated with TDZ developed weak roots when compared with the control plants.

Regardless of their root strength, plants that were regenerated and transferred to soil were counted and the data were analyzed statistically (Appendices B-9 and B-10). The results revealed a strong influence of TDZ on the number of plants regenerated for both bread and durum wheat varieties. Although, there is a non-significant varietal influence on the number of regenerated plants, concentration of TDZ applications are found to be affecting the number of plants regenerated and transferred to soil significantly. While extremes (hormone free and 1,0 mg/l) caused low level recovery of plants that were regenerated and transferred to soil, mid-ranges (0,25 0,50 and 0,75 mg/l) caused up to 2 fold increases which were also confirmed to be statistically significant for both bread and durum wheat varieties.

Following their transfer to soil, plants that were treated with various concentrations of TDZ displayed reduced vigor and majority of these plants did not sufficiently develop above the ground parts when compared with the control plants. As mentioned earlier our regenerated plants that were treated with TDZ did not developed well-established roots prior to their transfer to soil. The reason for this behavior is probably due to another side effect of TDZ on root morphogenesis (Meyer and van Staden, 1988).

For the roots to initiate and then to establish themselves a proper ratio of auxin and cytokinin is required. The auxin hormones required for this process are provided by the plant (endogenous supply) whereas not only the exogenous supply of TDZ but its persistence in the cell environment, due to the inability of metabolization by cytokinin oxidase, disturbs the balance of the required auxin/cytokinin ratio. This in turn, not only cause

the roots to develop poorly but also affects the above the ground parts of the plants due to insufficient root conductance of water and minerals.

As pointed by Lu (1993) for dicotyledonous plants, these unwanted effects of TDZ might be reduced if TDZ exposure is kept less than 8 weeks. Likewise, the same line of argument should be extended to monocotyledonous plant, in this case, especially for bread and durum varieties.

CHAPTER 5

CONCLUSION

1. In this study, we have used mature embryos of wheat varieties as explant material and successfully demonstrated the recovery of callus followed by plant regeneration in response to TDZ treatments. The use of mature embryos for wheat regeneration eliminates the need for immature explant material, and growth of donor plants. Thus, the simplicity and rapid production of shoots from the mature embryo culture could favor its use over the alternative explant sources.

2. While the callus induction frequency was found to be independent of Turkish bread and durum wheat species and varieties within species, the callus weight is found to be dependant on varieties and the best responded varieties are Başak 95 (bread) and Kunduru (durum).

3. While TDZ is found to be slightly reducing the regeneration capacity of bread wheat varieties, a positive effect is observed for durum wheat varieties especially for Kunduru.

4. Within the tested range of TDZ concentrations, 0.75 and 1,0 mg/l were found to have detrimental effects on regenerations events for bread wheat varieties whereas for the durum wheat varieties 1,0 mg/l concentration created a similar result.

5. Although for both wheat species, TDZ is found to be promoting the number of plants regenerated and transferred to soil (up to 2-fold), due to its possible side effects on the root morphogenesis these plants did not sufficiently developed as compared with the controls.

Future Prospects

This study centered on the possibility of using TDZ on wheat tissue culture systems and particular emphasis is given on the regeneration of bread and durum wheat plants from callus cultures. Therefore for future studies, the following suggestions would be useful:

- TDZ treatments should not exceed 1,0 mg/l and if possible should be kept below 0,75 mg/l.

- For regeneration studies TDZ exposure should not exceed 8 weeks.

- For increasing the rooting of TDZ-treated regenerants, low concentrations of natural auxins like IBA should be tested in order to counter the accumulated-effects of TDZ.

REFERENCES

Abdrabou, R.T. and Moustafa, R.A.K. (1993). Effect of 2,4-D concentrations and two levels of sucrose on callus induction and plantlet formation in two wheat cultivars. Annals of Agricultural Science Cairo. No.1(special issue), 41-46. Cairo, Egypt.

Ahloowalia, B.S., (1982). Plant regeneration from callus culture of wheat. Crop Sci., 22: 405-410.

Ahmad, A., Zhong H., Wang W. and Sticklen, M.B. (2002). Shoot apical meristem. *In vitro* regeneration and morphogenesis in wheat (*Triticum aestivum*). *In vitro* Cell Development. Biol. Plant, 38: 163-167.

Ahuja, P.S., Pental, D. and Cocking, E.C., (1982). Plant regeneration from leaf base callus and cell suspensions of *Triticum aestivum*. Z. Pflanzenzücht. 89: 139-144.

Ainsley, P.J. and Aryan, A.P. (1998). Efficient plant regeneration system for immature embryos of *Triticale* (*X Triticosecale* Wittmack). Plant Growth Regul. 24: 23-30.

Arndt, F., Rusxh, R. and Stilfried, H.V. (1976). SN 49537, A new cotton defoliant. Plant Physiol. 57:99.

Arnold, D.L., Flegmann, A. and Clarkson, J.M. (1995). Somaclonal variation in watercress for resistance to crook root disease. Plant Cell Rep., 14: 241-244.

Arzani A. and Mirodjagh, Sayed-Shahram. 1999. Response of durum wheat cultivars to immature embryo culture, callus induction and in vitro salt stres. Plant Cell, Tissue and Organ Culture 58 (1): 67-72.

Aung, L.H. and Steffens, G.L. (1987). Comparative growth responses of *Lycopersicon* seedlings to thidiazuron and 6-benzylaminopurine. Proceedings of the Plant Growth Regulator Society of America., 340, Lincoln, Nebraska, USA.

Babiker, A.G.T., Parker, C. and Suttle, J.C. (1992). Induction of *Striga* seed germination by thidiazuron. Weed Res., 32:243-248.

Baker, B.S. and Bhatia, S.K. (1993). Factors effecting adventitious shoot regeneration from leaf explants of quince (*Cydonia oblonga*). Plant Cell Tissue Org. Cult., 35: 273-277.

Baskakov, Y.A., Shapovalov, A.A., Zhirmunskaya, N.M. and Ovsyannikov, T.V. (1981). Interrelationship of growth-regulating activity and phytotoxicity of synthetic cytokinins. Dokl. Akad. Nauk SSSR., 267 : 1514-1517.

Bates, S., Preece, J.E., Navarrete, N.E., Sambeek, J.W. van Gaffney, G.R. and Van Sambeek, J.W. (1992). Thidiazuron stimulates shoot organogenesis and somatic embryogenesis in white ash (*Fraximus Americana* L.). Plant Cell Tissue Org. Cult., 31: 21-29.

Beckett, R.P and Van Staden, J. (1992). The effect of Thidiazuron on yield of salinity stressed wheat. Annals of Botany 70: 47-51.

Bennici, A., Caffaro, L., Dameri, R.M., Gastaldo, P. and Profuma, P. (1958). Callus formation and plantlet regeneration from immature *Triticum durum* Desf. embryos. Euphytica, Piazza delle Cascine 28, 50144 Florence, Italy, 39(3): 255-263.

Bhaskaran, S. & Smith, R.H. (1990). Regeneration in cereal tissue culture: a review. Crop Sci., 30: 1328-1336.

Birsin, M. and Özgen, M.2004. A comparison of callus induction and plant regeneration from different embryo explants of Triticale (x Triticosecale Wittmack). Cellular & Molecular Biology Letters Vol. 9, pp 353-361.

Bohmer, P., Meyer, B. and Jacobsen, H.J. (1995). Thidiazuron-induced high frequency of shoot induction and plant regeneration in protoplast derived pea callus. Plant Cell. Rep., 15: 26-29.

Bohorova, N.E., Ginkel, M., Rajaram, S., Hoisington, D.A. (1995). Tissue culture response of CIMMYT elite bread wheat cultivars and evaluation of regenerated plants. Cereal Res. Commun., 23: 243-249.

Bondt, A., De Eggermount, K., Penninckx, I., Gonderis, I., Broekaert, W.F. and De Bondt, A. (1996). Agrobacterium-mediated transformation of apple (*Malus* x *domestica* Borkh.): An assessment of factors affecting regeneration of transgenic plants. Plant Cell Rep., 15: 549-554.

Bregitzer, P., Campbell, R.D. and Wu, Y. (1995). Plant regeneration from barley callus: Effects of 2,4-dichlorophenoxyacetic acid and phenylacetic acid. Plant Cell Tiss. Org. Cult., 43: 229-235.

Bretagne-Sagnard, B. and Chupeau, Y. (1996). Selection of transgenic flax plants is facilitated by spectinomycin. Transgenic Res., 5: 131-137.

Briggle, L.W. (1980). Origin and botany of wheat. In E. Hafliger (ed.) Wheat. Documenta Ciba-Giegy, Basel, Switzerland. 6-13.

Briggs, B.A., McCuloch, S.M. and Edick, L.A. (1988). Micropropagation of azaleas using thidiazuron. Acta Hort. 226, vol. I: 205-208.

Bruce, M.I., Zwar, J.A. and Kefford, N.P. (1965). Chemical structure and plant kinin activity-the activity of urea and thiourea derivatives. Life Sci., 4: 461-466.

Burkhanova, E.A., Fedina, A.B., Baskakov, Yu, A. and Kulaeva, O.N. (1984). Comparative study of the action of 6-benzylaminopurine, thidiazuron, and cartolin on growth of intact pumpkin seedlings. Sov. Plant Physiol., 31: 8-12.

Capella, S.C., Mok, D.W.S., Kirchner, S.C. and Mok, M.C. (1983). Effects of thidiazuron on cytokinin autonomy and the metabolism of N6-(DELTA2-isopentenyl) (8-14C) adenosine in callus tissues of *Phaseolus lunatus* L. Plant Physiol., 73: 796-802.

Chawala, H.S. & Wenzel, G. (1987). Regeneration potential of callus from wheat and barley. Archiv fur Zuchtungsforschung. Bockhorn, 8059 Grunbach, German Federal Republic, 17(6): 337-343.

Chernyad'ev, I.I. and Kozlovskikh, A.L. (1990). Effects of 6-benzylaminopurine, thidiazuron, and Cartolin on activity of phosphosynthetic enzymes and ATP content in leaves of perennial grasses. Sov. Plant Physiol., 37: 251-256.

Chernyad'ev, I.I., Kozlovskikh, A.L.., Obraztsov, A.S. and Daman, N.G. (1987). Cytokinins as regulators of phosphosynthesis, respiration and productivity of some perennial grasses. Prikladnaya Biokhimiya I. Mikrobiologiya., 23: 647-656.

Chupeau, M.C., Lemoine, M. and Chupeau, Y. (1993). Requirement of thidiazuron for healthy protoplast development to efficient tree regeneration of a hybrid poplar (*Populus tremula* x *P. alba*). J. Plant Physiol., 141: 601-609.

Coleman, W.K., Estrabrooks, E.N., O'Hara, M., Embleton, J. and King, R.R. (1992). Seasonal changes in cold hardiness, sucrose and sorbitol in apple trees treated with plant growth regulators. J. Hort. Sci., 67: 429-435.

Cook, RJ., Johnson, V.A. and Allen R.E. (1993). Traditional crop breeding practices: an historical review to serve as a baseline for assessing the role of modern biotechnology. Organization for economic co-operation and development, OECD Paris, p.27-36.

Debergh, P., Aitken-Christie, J., Cohen, D., (1992). Reconsideration of the term 'vitrification' as used in micropropagation. Plant Cell Tissue Organ Cult., 30: 135-140.

Declerck, V. and Korban, S.S. (1996). Influence of growth regulators and carbon sources on callus induction, growth and morphogenesis from leaf tissues of peach (*Prumus persica* L. Batsch). J. Hort.Sci., 71: 49-55.

Delporte, F., Mostade, O., and Jacquemin, (2001). Plant regeneration through callus initiation from thin mature embryo fragments of wheat. Plant Cell, Tissue and Organ Culture 67: 73-80.

Devlin, R.M., Zbiec, I.I. and Nowicka, S.E. (1989). The effect of thidiazuron on some plant growth systems. Proc. Plant Growth Reg. Soc., 16: 99-103.

Dornelles, A.L.C., deCarvalho, F.I.F., Federizzi, L.C., Lange, C.E., Handel, C.L., Bered, F., (1997). Genetics of regeneration of wheat (*Triticum aestivum* L.) plants. Brazilian Journal of Genetics 20 (2): 293-297.

Ekiz, H. and Konzak, C.F. (1993). Effects of different light applications on anther culture response of spring bread wheat (*Triticum aestivum* L.). Doğa, Türk Tarım ve Ormancılık Dergisi, 17(2): 511-520.

Elfving, D.C. and Cline, R.A. (1993). Cytokinin and ethephon affect crop load, shoot growth, and nutrient concentration of “Empire” apple trees. Hort Sci., 28: 1011-1014.

El-Sherbeny, G.A.R., Sato, S., Al-Otayk, S.M., Clemente, T., Baenziger, P.S., (2001). Effect of genotype and 2,4-D concentration on callus induction from immature embryos of modern Egyptian wheat cultivars. Cereal Research Communications 29 (3-4): 305-311.

Fabriani, G. and Lintas, C. (1988). Durum wheat: Chemistry and technology. American Association of Cereal Chemists, Inc., Minnesota, USA.

Fadel, F. and Wenzel, G., (1990). Medium-genotype-interaction on androgenetic haploid production in wheat. Plant Breeding 105 (4): 278-282.

Fasolo, F., Zimmerman, R.H., Fordham, I., (1989). Adventitious shoot formation on excised leaves of *in vitro* grown shoots of apple cultivar. Plant Cell Tissue Organ Cult., 16: 75-87.

Feldman, M. (1976). "Wheats" in N.W. Simmonds, ed., Evolution of Crop Plants. Longman Press, London and New York, p.120.

Felfoldi, K. and Purnhauser, L. (1992). Induction of regenerating callus cultures from immature embryos of 44 wheat and 3 triticale cultivars. Cereal Res. Comm., 20: 273-277.

Fellman, C.D., Read, P.E. and Hosier, M.A. (1987). Effects of thidiazuron and CPPU on meristem formation and shoot proliferation. Hort. Sci., 22: 1197-1200.

Fiola, J.A., Hassan, M.A., Swartz, H.J., Bors, R.H. and McNicols, R. (1990). Effect of thidiazuron, light fluence rates and kanamycin on *in vitro* shoot organogenesis from excised *Rubus* cotyledons and leaves. Plant Cell Tissue Org. Cult., 20: 223-228.

Gabbar, A., Babiker, T., Butler, L.G., Ejeta, G. and Woodson, W.R. (1993). Enhancement of ethylene biosynthesis and germination by cytokinins and

1-aminocyclopropane-1-carboxylic acid in *Striga asiatica* seeds. Physiol. Plant., 89: 21-26.

Galiba, G. and Yamada, Y. (1988). A novel method for increasing the frequency of somatic embryogenesis in wheat tissue-culture by NaCl and KCl supplementation. Plant Cell Reports, 7(1): 55-58.

Gallo-Meagher, M., English, R.G., Abouzid, A. (2000). Thidiazuron stimulates shoot regeneration of sugarcane embryogenic callus. *In vitro* Cell Dev-Pl., 36(1): 37-40.

Gamborg, O.L. and Eveleigh, DE (1968). Culture methods and detection of glucanases in suspension cultures of wheat and barley. Can. J. Biochem., 46: 417-421.

Gill, R. and Saxena, P.K. (1993). Somatic embryogenesis in *Nicotiana tabacum* L. induction by thidiazuron of direct embryo differentiation from cultured leaf discs. Plant Cell Rep., 12: 154-159.

Gill, R., Senaratna, T., Saxena, P.K. (1994). High-frequency direct somatic embryogenesis in thin layer cultures of hybrid seed geranium. J. Plant Physiol., 143: 726-729.

Gonzales, J.M., Friero, E. and Jouve, N. (2001). Influence of genotype and culture medium on callus formation and plant regeneration from immature embryos of *Triticum turgidum* Desf. Cultivars. Plant Breed., 120: 513-517.

Gosch-Wackerle, G., Avivi, L., Galun, E. (1979). Induction, culture and differentiation of callus from immature rachis, seeds and embryos of *Triticum*. Z. Pflanzenphysiol 91: 267-278.

Gupta, S.D., Conger, B.V. (1998). *In vitro* differentiation of multiple shoot clumps from intact seedlings of switchgrass. *In Vitro* Cell Dev-Pl., 34(3): 196-202.

Heyser, J.W., Nabors, M.W., Mackinnon, C., (1985). Long-term, high-frequency plant-regeneration and the induction of somatic embryogenesis in callus-cultures of wheat (*Triticum aestivum* L.). Z Pflanzenzucht 94(3): 218-233.

Hruskoci, J.D., Read, P.E. and Clayton-Greene, K.A. (1993). *In vitro* shoot regeneration from internode segments and internode-derived callus of blueberry (*Vaccinim* spp.). Acta Hort., 346: 127-132.

26.07.2004 http://countrystudies.us/turkey/61.htm

26.07.2004 http://www.usfca.edu/~trembath/www-class/Protect/PIX/FertileCrescent.jpg

Huang, X.L., Li, X.J., Fu, J.R. and Lao, C.L. (1994). Effect of thidiazuron on ethylene production and somatic embryogenesis in callus of alfalfa (*Medicago sative* L.). Acta Phytophysiol. Sinca., 20: 367-372.

Huetteman, C.A. and Preece, J.E. (1993). Thidiazuron: a potent cytokinin for woody plant tissue culture. Plant Cell Tissue Org. Cult., 33: 105-119.

Hutchinson, M.J. and Saxena, P.K. (1996). Acetylsalicylic acid enhances and synchronizes thidiazuron-induced somatic embryogenesis in geranium (*Pelargonium x horturum* Bailey) tissue cultures. Plant Cell Rep., 15: 512-515.

Hutchinson, M.J. and Saxena, P.K. (1996). Role of purine metabolism in thidiazuron-induced somatic embryogenesis of geranium (*Pelargonium x horturum*) hypocotyl cultures. Physiol. Plant., 98: 517-522.

Iasi, L., Infante, R., Rosati, P. and Ochatt, S.J. (1994). Protoplast isolation and culture of blackberry and raspberry cultivars (*Rubus* spp.). J. Gen. Br., 48: 149-154.

Kumlehn, J., Schieder, O., Lorz, H. (1997). *In vitro* development of wheat (*Triticum aestivum* L.) from zygote to plant via ovule culture. Plant Cell Rep., 16(10): 663-667.

J'Aiti, F., Benlhabib, O., Sharma, H.C., El Jaafari, S., El Hadrami, I. (1999). Genotypic variation in anther culture and effect of ovary coculture in durum wheat. Plant Cell, Tissue and Organ Culture 59: 71-76.

Ji, Z.L. and Wang, S.Y. (1988). Reduction of abscisic acid content and induction of sprouting in potato, *Solanum tuberosum* L., by thidiazuron. J. Plant Grow. Regul., 7: 37-44.

Jomori, H., Takahata, Y., Kaizuma, N. and Nishio, T. (1995). Plant regeneration from leaf derived calli of gentians and their protoplast culture. Acta Hort., 392: 81-86.

Kato, K., Chowdhury, SH., Harada, S., (1991). Effect of culture condition on plant capacity of mature embryo derived callus wheat (*Triticum aestivum* L.). Wheat Inf. Serv. 72: 95-97.

Kerns, H.R. and Meyer, M.M. (1986). Tissue culture propagation of *Acer x freemanii* using thidiazuron to stimulate shoot tip proliferation. HortSci., 21: 1209-1210.

Kintzios, S.E., Triantafyllou, M., Drossopoulos, J., (1996). Effect of genotype and different growth regulator treatments on callus induction, proliferation and plant regeneration from mature wheat embryos. Cereal Research Communications 24 (2): 147-153.

Kolganova, T.V., Srivastiva, D.K., Mett, V.L. and Piruzyan, E.S., (1992). Callusogenesis and regeneration of cotton (*Gossypium hirsutum* L. cv. 108-F) Sov. Plant Physiol., 39 : 232-236.

Kulaeva, O.N., Yu, A., Baskakov, A., Borisova, N.N., Kuznetsov, V.V., Tsibulya, L.V., and Shapovalo, A.A. Investigations of cytokinin properties of the defoliant DROP and herbicide DPX-4189. Fizol Rast., 29: 266-273.

Kumlehn, J., Brettschneider, R., Lörz, H., and Kranz, E. (1997). Zygote implantation to cultured ovules leads to direct embryogenesis and plant regeneration of wheat. Plant Journal 12: 1473–1479.

Kün, E. (1988). "Serin İklim Tahılları" Ankara Üniversitesi Ziraat Fakültesi Yayınları, 1032. Ankara pp: 132.

Lenzner, S., Zoglauer, K. and Schieder, O. (1995). Plant regeneration from protoplasts of sugar beet (*Beta vulgaris*). Physiol. Plant., 94: 342-350.

Leonard, W.H., and Martin, J.H. (1963). Cereal crops. TheMacmillan Co., New York.

Li, L.H., Dong, Y.S., Zhou, R.H., Li, X.Q., Yang, X.M. and Xu, S.Y., (1992). Plant regeneration and variation of a wheat X *Agropyron* Gaertn intergeneric hybrid. Acta Genetica Sinica., Beijing 100081, China, 19 (3): 250-258.

Lin, C., Chang, W.C. (1998). Micropropagation of *Bumbusa edulis* through nodal explants of field-grown clums and flowering of regenerated plantlets. Plant Cell Reports, 17: 617-620.

Lin, C.H., Lee, L.Y. and Tseng, M.J. (1994). The effect of stratification and thidiazuron treatment on germination and proteinsynthesis of *Pyrus serotina* Rehd cv. Niauli. Ann. Bot., 73: 515-523.

Lu, CS., Sharma, H.C., Ohm, H.W. (1991). Wheat anther culture - effect of genotype and environmental-conditions. Plant Cell Tissue and Organ Culture. 24 (3): 233-236.

Lu, C.Y. (1993). The use of thidiazuron in tissue culture. *In Vitro* Cell. Dev. Biol., 29: 92-96.

Lupton, F.G.H. (ed.) (1987). Wheat Breeding: It's Scientific Basis. London Chapman and Hall

Maddock, SE., (1985). Cell culture, somatic embryogenesis, and regeneration in wheat, barley, oats, rye, and triticales. In: Bright SWJ, Jones MGK (eds) Cereal tissue and cell culture. Nijhoff/Junk, Dordrecht, pp 131-174.

Maddock, S.E., Lancaster, V.A., Risiott, R. and Franklin, J. (1983). Plant regeneration from cultured immature embryos and inflorescences of 25 cultivars of wheat (*Triticum aestivum* L.) J. Exp. Bot., 34: 915-926.

Maes, O.C., Chibbar, R.N., Caswell, K., (1996). Somatic embryogenesis from isolated scutella of wheat: Effects of physical, physiological and genetic factors. Plant Sci., 121(1): 75-84.

Malik, K.A. and Saxena, P.K. (1992). *In vitro* regeneration of plants. A novel approach. Naturwissenshaften, 79: 136-137.

Malik, K.A. and Saxena, P.K. (1992). Thidiazuron induces high-frequency shoot regeneration in intact seedlings of pea (*Pisum sativum*), chickpea (*Cicer arietinum*) and lentil (*Lens culinaris*). Aust. J. Plant. Physiol., 19: 731-740.

McHugen, a., (1983). Rapid regeneration of wheat *in vitro*. Ann. Bot., 51: 851-853.

Meyer, H.J., Staden, J. and Van Staden, J. (1995). The *in vitro* production of an anthocyanin from callus cultures of *Oxalis linearis*. Plant Cell Tissue Org. Cult., 40: 55-58.

Meyer, H.J., and van Staden, J. 1988. In vitro multiplication of Ixia flexuosa. Hortscience 23: 1070-1071.

Mohamed, M.F., Coyne, D.P. and Read, P.E. (1993). Shoot organogenesis in callus induced from pedicel explants of common bean (*Phaseolus vulgaris* L.). J. Am. Soc. Hort. Sci., 118:158-162.

Mok, M.C., Mok, D.W.S., Turner, J.E., Mujer, C.V. (1987). Biological and biochemical effects of cytokinin-active phenylurea derivatives in tissue culture systems. Hort. Sci., 22: 1194-1197.

Mok, M.C., and Mok, D.W.S. (1985). The metabolism of (14C)-thidiazuron in callus cultures of *Phaseiolus lunatus*. Physiol Plant., 65: 427-432.

Mok, M.C., Mok, D.W.S., Armstrong, D.J., Shudo, K., Isogai, Y. and Okamoto, T. (1982). Cytokinin activity of N-phenyl-N' (-1,2,3-thidiazol-5-yl) urea (thidiazuron). Phytochem., 21: 1509-1511.

Moore, T.C. 1989. Biochemistry & Physiology of Plant Hormones Springer-Verlag.

Murch, S.J. and Saxena, P.K. (1997). Modulation of mineral and fatty acid profiles during thidiazuron mediated somatic embryogenesis in peanuts (*Arachis hypogaea* L.). J. Plant Physiol., 151(3): 358-361.

Murthy, B.N.S. and Saxena, P.K. (1997). Somatic embryogenesis and plant regeneration of Neem (*Azadirachta indica* A.Juss). Plant Cell Rep., 17(6-7): 469-475.

Murthy, B.N.S., Jerrin, V., Singh, R., Fletcher, R.A. and Saxena, P.K. (1996). *In vitro* regeneration of chickpea (*Cicer arietinum* L.) stimulation of direct organogenesis and somatic embryogenesis by thidiazuron. Plant Growth Reg., 19: 233-240.

Murthy, B.N.S., Murch, S.J. and Saxena, P.K. (1995). Thidiazuron-induced somatic embryogenesis in intact seedlings of peanut (*Arachis hypogaea*):

endogenous growth regulator levels and significance of cotylendones. Physiol. Plant., 94: 268-276.

Mzouri, K., Amssa, M., Bouiamrine, E.H. (2001). Somatic embryogenesis from immature embryos of wheat cultivars (*Triticum aestivum* L.): genotype effect. Acta Botanica Gallica 148 (3): 215-225.

Neuman, M.C., Preece, J.E., Van Sambeek, J.W. and Graffney, G.R. (1993). Somatic embryogenesis and callus production from cotyledon explants of eastern black walnut (*Juglans nigra* L.). Plant Cell, Tissue and Org. Cult., 32: 9-18.

Nuttonson, M.Y. (1955). Wheat-climatic relationships and the use of phenology in ascertaining the thermal and photo-thermal requirements of wheat. American Institute of Crop Ecology, Washington, DC.

Obeidy, A.A. and Smith, M.A.L. (1993). Organogenesis from mature pecan cotyledons and embryogenic axes. HortSci., 28: 213-215.

Okuse, I. (1994). Influences of the sexuality in flower stalk explants on the callus formation and growth in spinach (*Spinacia oleracea* L.). Bull. Facult. Agric. Hirosaki Univ., 57: 55-62.

Oleson, F. (1996) Wheat situation and Outlook, Bi-weekly Bulletin, Market Analyses Division.

Özgen, M., Türet, M., Altınok, S. and Sancak, C. (1998). Efficient callus induction and plant regeneration from mature embryo culture of winter wheat (*Triticum aestivum* L.) genotypes. Plant Cell Rep. 18: 331-335.

Özgen, M., Türet, M., Özcan, M. and Sancak, C. (1996). Callus induction and plant regeneration from immature and mature embryos of winter durum wheat genotypes. Plant Breeding, 115 (6): 455-458.

Ozias-Akin, P. and Vasil I.K. (1983). Improved efficiency and normalization of somatic embryogenesis in *Triticum aestivum* (Wheat). Protoplasma, 117: 40-44.

Ozias-Akins, P. and Vasil, I.K. (1982). Plant regeneration from cultured immature embryos and inflorescences of *Triticum aestivum* L. (wheat): Evidence for somatic embryogenesis. Protoplasma, 110: 95-105.

Patnaik, D. and Khurana, P. (2001). Wheat biotechnology: A minireview. EJB Electronic Journal of Biotechnology (online) vol:4 No.2. ISSN: 0717 3458.

Percival, John. (1921). The wheat plant, a monograph. E.P. Dutton and Co., New York.

Perri, E., Parlati, M.V., Rugini, E. and Lavee, S. (1994). Isolation and culture of olive (*Olea europaea* L.) cultuvar protoplasts. Acta Hort., 356: 51-53.

Pountney, C. and Swietlik, D. (1988). The effect of Dropp (thidiazuron), an urea derivative with cytokinin activity, on sour orange seedlings and field-grown grapefruit trees. J. Rio-Grande Valley Hort Soc., 41: 33-39.

Preece, J.E., Huetteman, C.A., Ashby, W.C. and Roth, P.L. (1991). Micro and cutting propagation of silver maple. I. Results with adult and juvenile propagules. J. Am. Soc. Hort. Sci., 116 : 142-148.

Rakoczy-Trojanowska, M., Malepszy, S., (1995). Genetic factors influencing theregeneration ability of rye (*Secale cereale* L.). Euphytica 83: 233-239.

Redway, FA., Vasil, V., Lu, D. and Vasil. I.K. (1990) Identification of callus types for long-term maintenance and regeneration from commercial cultivars of wheat (*Triticum aestivum* L.). Theor. Appl. Genet. 79: 609-617.

Reinert, J. and Bajaj, Y.P.S (1976). Applied and Fundamental aspects of plant cell, tissue and organ culture, pp: 144-147.

Reustle, G., Harst, M. and Alleweldt, G. (1995). Plant regeneration of grapevine (*Vitis* sp.) protoplasts isolated from embryogenic tissue. Plant Cell Rep., 15: 238-241.

Reynolds, A.G., Wardle, D.A., Zurowski, C. and Looney, N.E. (1992). Phenylureas CPPU and thidiazuron affect yield components, fruit composition, and storage potential of four seedless grape selections. - J. Am. Soc. Hort. Sci., 117: 85-89.

Rosu, A., Skirvin, R.M., Bein, A., Norton, M.A., Kushad, M. and Otterbacher, A.G. (1995). The development of putative adventitious shoots from a chimerical thornless rose (*Rosa multifora* Thunb. Ex J. Murr.) *in vitro*. J. Hort. Sci., 70 : 901-907.

Sanago, M.H.M., Murch, S.J., Slimmon, T.Y., KrishnRaj, S. and Saxena, P.K. (1995). Morphoregulatory role of thidiazuron : morphogenesis of root outgrowths in thidiazuron-treated geranium (*Pelargonium x hortorum* Bailey). Plant Cell Rep., 15: 205-211.

Sankhla, D., Davis, T.D. and Sankhla, N. (1994). Thidiazuron-induced *in vitro* shoot formation from roots of intact seedlings of *Albizzia julibrissin*. Plant. Growth. Reg., 14: 267-272.

Saxena, P.K. and Malik, K.A. and Gill, R. (1992). Induction by thidiazuron of somatic embryogenesis in intact seedlings of peanut. Planta, 187: 421-424.

Sears, R.G. and Deckard, E.L. (1982). Tissue culture variability in wheat: Callus induction and plant regeneration. Crop Sci., 22: 546-550.

Seedhabadee, G., Monica, B., Bryan, L., Brian, G., Scoles, J. & Chibbar, N. (2003). Production of multiple shoots from thidiazuron-treated mature embryos and leaf-base / apical meristems of barley (*Hordeum vulgare*). Plant Cell Tissue and Organ Culture 73: 57-64.

Sharma, V.K., Rao, A., Varshney, A., Kothari, S.L. (1995). Comparison of developmental stages of inflorescence for high frequency plant regeneration in *Triticum aestivum* L. and *Triticum durum* L. Desf. Plant Cell Rep 15: 227-231.

SIS DATA, Turkey Field Crops 1997

Smith, M.A.L. (1995). Begonias as a natural source of food coloring. Begonian., 62: 7 - 10.

Stimart, D.P. and Mather, J.C. (1996). Regenerating adventitious shoots from *in vitro* culture of *Liatris spicata* (L.) Wild. Cotyledons. HortSci., 31: 154-155.

Tegeder, M., Gebhardt, D., Schieder, O. and Pickardt, T. (1995). Thidiazuron-induced plant regeneration from protoplasts of *Vicia faba* cv. Mythos. Plant Cell Rep., 15: 164-169.

Thomas, J.C. and Katterman, F.R. (1986). Cytokinin activity induced by thidiazuron. Plant Physiol., 81: 681-683.

Tian, W. (1994). Improvement of plant regeneration frequency *in vitro* in Indica rice. Acta Genet. Sin., 21: 215-221.

Tibok, A., Blackhall, N.W., Power, J.B. and Davey, M.R. (1995). Optimized plant regeneration from callus derived from seedling hypocotyls of *Eucalyptus urophylla*. Plant Sci., 110: 139-145.

USDA October World Crop Production

Vahala, T. and Eriksson, T. (1991). Callus production from willow (*Salix viminalis* L.) protoplasts. Plant Cell Tissue Org. Cult., 27: 243-248.

Van Nieuwkerk, JP., Zimmerman, R.H. and Fordham, I. (1986). Thidiazuron stimulation of apple shoot proliferation in vitor. Hort Sci., 21: 516-518.

Veneglat, S.P. and Sawhney, V.K. (1994). Ectorpic formation of thichomes and stomata in floral organs of *Arabidopsis thaliana* induced by thidiazuron. Can. J. Bot., 72: 671-677.

Visser, C., Quershi, J.A., Gill, R. and Saxena, P.K. (1992). Morphoregulatory role of thidiazuron: substitution of auxin and cytokinin requirement for the induction of somatic embryogenesis in geranium hypocotyls cultures. Plant Physiol., 99: 1704-1707.

Wallin, A. and Johansson, L. (1989). Plant regeneration from leaf mesophyll protoplasts of *in vitro* cultured shoots of a columnar apple. J.Plant Physiol., 135: 565-570.

Wang, S.Y., Jiao, H.J. and Faust, M. (1991). Changes in activities of catalase, peroxidase and polyphenoş oxidase in apple buds during bud break induced by thidiazuron. J. Plant Growth Regul., 10: 33-39.

Wang, S.Y., Steffens, G.L. and Faust, M. (1986). Breaking bud dormancy in apple with a plant bioregulators, thidiazuron. Phytochemistry, 25: 311 - 317.

Xueyan, S., Li, D., and Qu, R., (2000). Thidiazuron promotes *in vitro* regeneration of wheat and barley. *In Vitro* Cell Dev. Biol-Plant 36: 207-210.

Yurkova, G.N., Levenko, B.A., Novozhilov, O.V. (1982) Plant regeneration in wheat tissue culture. Biochem.Physiol.,77: 337-344.

Zale, J.M., Borchardt-Wier, H., Kidwell, KK., Steber, C.M. (2004). Callus induction and plant regeneration from embryos of a diverse set of wheat genotypes. Plant Cell, Tissue and Organ Culture 76: 277-281.

Zamora, A.B., Scott, K.J., (1983). Callus formation and plant regeneration from wheat leaves. Plant Sci. Lett., 29: 183-189.

Zhou, J.Y., Ma, H., Guo, F.X. and Luo, X.T. (1994). Effect of thidiazuron on somatic embryogenesis of *Cayratia japonica*. Plant Cell Tissue Org. Cult., 36: 73-79.

Zohary, D. and Hopf, M. (1994). Domestication of plants in the Old World (2nd edition), Clarendon, Oxford, ISBN 0-19-854896-6, 279 pp.

APPENDIX A

MEDIUM COMPOSITIONS

A-1 Callus induction medium

(per liter)

MS (Murashige and Skoog basal medium)……4.43 g

Sucrose……..20 g

Agar…….....7 g

2,4-D…….2 mg

The pH was adjusted to 5.8 before autoclaving for 20 minutes at 121 ^{0}C and 1.1 kg/cm^{2} pressure.

A-2 Regeneration medium

(per liter)

MS (Murashige and Skoog basal medium)…….…...4.43 g

Sucrose…….....20 g

Agar…….7 g

Thidiazuron

0.00 µl (free-hormone)

250 µl from 1 mg/ml stock solution

500 µl from 1 mg/ml stock solution

750 µl from 1 mg/ml stock solution

1000 µl from 1 mg/ml stock solution

The pH was adjusted to 5.8 before autoclaving for 20 minutes at 121 ^{0}C and 1.1 kg/cm^{2} pressure.

A-3 Rooting medium

(per liter)

MS (Murashige and Skoog basal medium)……4.43 g

Sucrose……..20 g

Agar…….7 g

The pH was adjusted to 5.8 before autoclaving for 20 minutes at 121 ^{0}C and 1.1 kg/cm^{2} pressure.

APPENDIX B

Appendix B-1 Callus induction (%) versus Varieties

One-way ANOVA:

Analysis of Variance for Callus induction (%)

```
Source     DF        SS        MS        F        P
Varieties   2      37.5      18.8     1.36    0.269
Error      42     580.7      13.8
Total      44     618.3
                                  Individual 95% CIs For Mean
                                  Based on Pooled StDev
Level          N      Mean     StDev  -+---------+---------+---------+-----
Başak 95      15     99.11      2.35         (---------*--------)
Gerek 79      15     97.78      6.00  (---------*---------)
Bezostaja 1   15    100.00      0.00             (---------*---------)
                                     -+---------+---------+---------+-----
Pooled StDev =        3.72          96.0      98.0     100.0     102.0
```

Appendix B-2 Callus induction (%) versus Varieties

One-way ANOVA:

Analysis of Variance for Callus induction (%)

```
Analysis of Variance for Callus induction
Source     DF        SS        MS        F        P
Varieties   2      84.9      42.5     2.18    0.126
Error      42     817.9      19.5
Total      44     902.9
                                  Individual 95% CIs For Mean
                                  Based on Pooled StDev
Level          N      Mean     StDev  ----+---------+---------+---------+--
Kunduru       15     96.44      7.07   (--------*--------)
Çakmak 79     15     99.56      1.72              (--------*--------)
Kırmızı 5132  15     99.11      2.35            (--------*---------)
                                    ----+---------+---------+---------+--
  Pooled StDev =      4.41            95.0      97.5     100.0     102.5
```

Appendix B-3 Weight of callus (g) versus Varieties

One-way ANOVA:

Analysis of Variance for Weight of callus

```
Source     DF        SS        MS        F        P
Varieties   2    0.5436    0.2718    16.22    0.000
Error      42    0.7035    0.0168
Total      44    1.2471
                                  Individual 95% CIs For Mean
                                  Based on Pooled StDev
Level          N      Mean     StDev  -+---------+---------+---------+-----
Başak 95      15    1.0540    0.1334                          (-----*----)
Gerek 79      15    0.7873    0.1562   (-----*----)
Bezostaja 1   15    0.9527    0.0899                 (----*-----)
                                     -+---------+---------+---------+-----
Pooled StDev =      0.1294        0.72      0.84      0.96      1.08
```

Appendix B-4 Weight of callus (g) versus Varieties

One-way ANOVA:

Analysis of Variance for Weight of callus

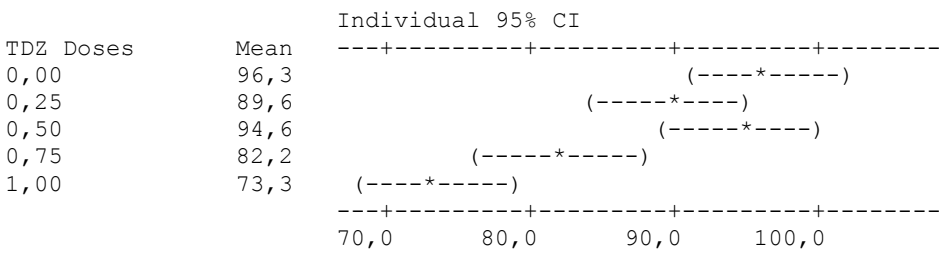
```
Source     DF        SS        MS        F        P
Varieties   2    0.1708    0.0854     4.34    0.019
Error      42    0.8265    0.0197
Total      44    0.9973
                                  Individual 95% CIs For Mean
                                  Based on Pooled StDev
Level          N      Mean     StDev  ----------+---------+---------+-
Kunduru       15    0.9487    0.1659                     (---------*-------)
Çakmak 79     15    0.8000    0.1335   (--------*--------)
Kırmızı 5132  15    0.8967    0.1170               (--------*--------)
                                     ----------+---------+---------+------
Pooled StDev =    0.1403                   0.800     0.880     0.960
```

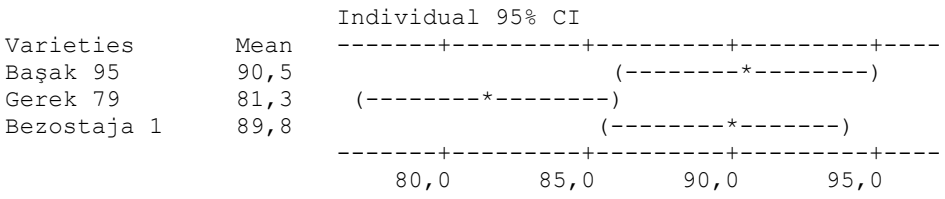
Appendix B-5 Regeneration capacity (%) versus TDZ Doses (mg/L), Varieties

Two-way ANOVA:

Analysis of Variance for Regeneration capacity (%)

Source	DF	SS	MS	F	P
TDZ Doses	4	3258,5	814,6	11,36	0,000
Varieties	2	779,8	389,9	5,44	0,010
Interaction	8	3446,5	430,8	6,01	0,000
Error	30	2150,9	71,7		
Total	44	9635,6			



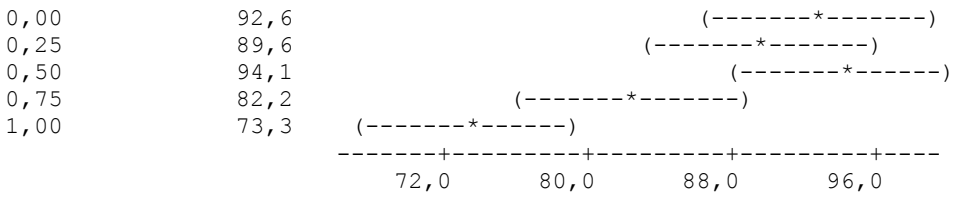


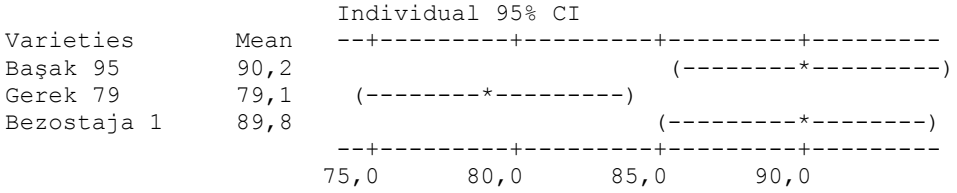
Appendix B-6 Culture efficiency (%) versus TDZ Doses (mg/L), Varieties

Two-way ANOVA:

Analysis of Variance for Culture efficiency (%)

Source	DF	SS	MS	F	P
TDZ Doses	4	2675,6	668,9	7,99	0,000
Varieties	2	1183,3	591,6	7,06	0,003
Interaction	8	3129,7	391,2	4,67	0,001
Error	30	2512,5	83,7		
Total	44	9501,0			



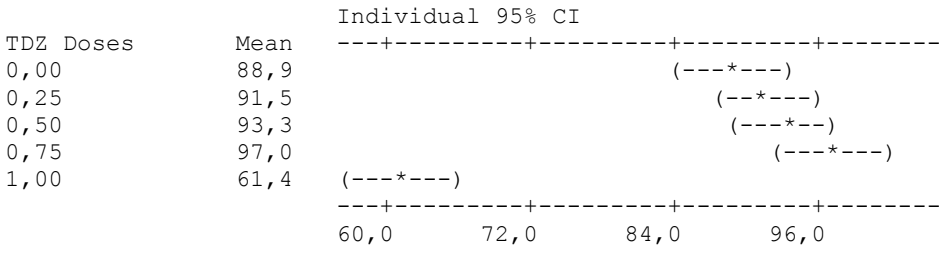


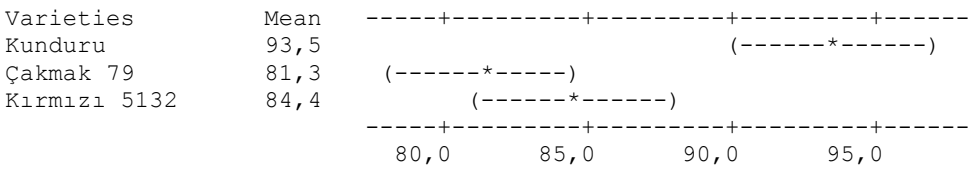
Appendix B-7 Regeneration capacity (%) versus TDZ Doses (mg/L), Varieties

Two-way ANOVA:

Analysis of Variance for Regeneration capacity (%)

Source	DF	SS	MS	F	P
TDZ Doses	4	7382,6	1845,6	44,07	0,000
Varieties	2	1197,7	598,8	14,30	0,000
Interaction	8	4809,2	601,2	14,36	0,000
Error	30	1256,3	41,9		
Total	44	14645,8			



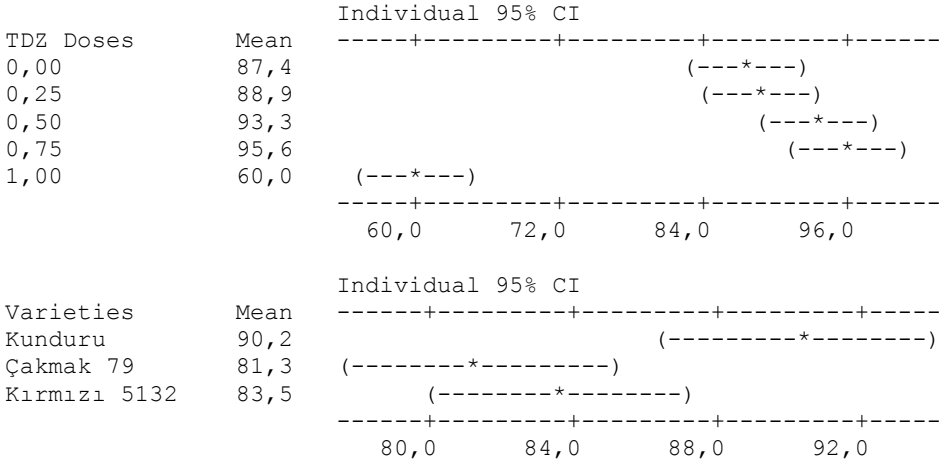


Appendix B-8 Culture efficiency (%) versus TDZ Doses (mg/L), Varieties

Two-way ANOVA:

Analysis of Variance for Culture efficiency (%)

Source	DF	SS	MS	F	P
TDZ Doses	4	7448,3	1862,1	37,67	0,000
Varieties	2	642,6	321,3	6,50	0,005
Interaction	8	4853,6	606,7	12,27	0,000
Error	30	1482,9	49,4		
Total	44	14427,4			

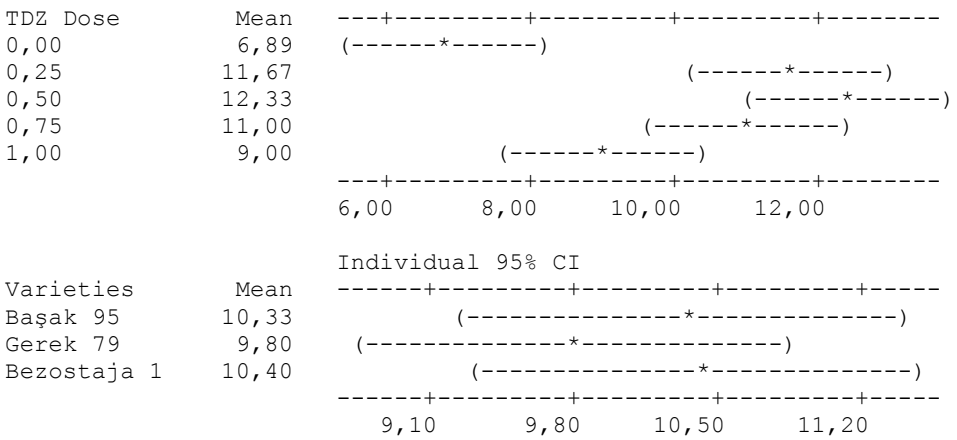


Appendix B-9 No of plants regenerated versus TDZ Doses (mg/L), Varieties

Two-way ANOVA:

Analysis of Variance for No of plant regenerated

Source	DF	SS	MS	F	P
TDZ Doses	4	177,69	44,42	10,52	0,000
Varieties	2	3,24	1,62	0,38	0,684
Interaction	8	82,98	10,37	2,46	0,035
Error	30	126,67	4,22		
Total	44	390,58			



Appendix B-10 No of plants regenerated versus TDZ Doses (mg/L), Varieties

Two-way ANOVA:

Analysis of Variance for No of plant regenerated

Source	DF	SS	MS	F	P
TDZ Doses	4	224,31	56,08	31,94	0,000
Varieties	2	8,93	4,47	2,54	0,095
Interaction	8	123,29	15,41	8,78	0,000
Error	30	52,67	1,76		
Total	44	409,20