CYTOTAXONOMIC STUDIES ON THE GENUS *SALVIA* (LABIATAE) IN TURKEY

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ABSTRACT

CYTOTAXONOMIC STUDIES ON THE GENUS *SALVIA* (LABIATAE) IN TURKEY

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The genus *Salvia* L. is significantly important with regard to both its worldwide distribution and usage areas including food, medical and perfumary industries. In this current study, it is targeted to address the chromosome numbers and karyomorphology of the ten species and one variety of the genus *Salvia*. All of the eleven taxa examined in this study are economically significant and nine of these are endemic to Turkey. To define the chromosome numbers and karyomorphology of these eleven taxa somatic chromosomes of the each were examined. Mitotic metaphase chromosomes were obtained from root meristems of germinating seeds, which were pre-treated in α-bromonaphtalene at 4ºC for 16 h, then fixed in Carnoy solution (3 parts of ethanol: 1 parts of glacial acetic acid) at 4ºC for 24h and stored in 70 % ethanol. Fixed root tips were stained in 2 % aceto-orcein and squashed in a drop of 45 % acetic acid. Long arm, short arm, total length of the each chromosome were measured; relative length, arm ratio, centromeric index of the each chromosome were calculated. Karyogram and haploid idiograms were drawn by computer-aided analysis programme (Bs200pro). A cluster analysis of the karyotype data was carried out to examine karyotype similarity among taxa.

Somatic chromosome numbers have been counted as 2n=2x=14 for the endemic taxa *S. divaricata* Montbret & Aucher, *S. euphratica* Montbret & Aucher ex Bentham (var. *leiocalycina* (Rech. fil.) Hedge) and *S. recognita* Fisch. & Mey.; 2n=2x=14-1B for *Salvia rosifolia* Sm.; 2n=20 for *S. longipedicellata* Hedge, *S. vermifolia* Hedge & Hub.-Mor. and *S. yosgadensis* Freyn & Bornm.; 2n=2x=22 for *S. aethiopis* L., *S. cilicica* Boiss. & Kotschy, *S. hypargeia* Fisch. & Mey. and 2n=2x=32 for *S. napifolia* Jacq. respectively. In general, the chromosomes are short with median and submedian centromeres.

The current study is essential for being the first report about chromosome numbers and karyomorphology of the six endemic taxa, namely *S. divaricata*, *S. euphratica* var. *leiocalycina*, *S. longipedicellata*, *S. rosifolia, S. vermifolia* and *S. yosgadensis.* Moreover, in spite of the chromosome numbers of *S. aethiopis*, *S. cilicica*, *S. hypargeia* and *S. recognita* are known, this research is the first study for their karyomorphologies.

Keywords: Chromosome, Endemic, Karyotype, Labiatae, *Salvia*, Cytotaxonomy.

ÖZ

TÜRKİYE *SALVIA*LARI (LABIATAE) ÜZERİNDE SİTOTAKSONOMİK ÇALIŞMALAR

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Salvia L. cinsi dünya üzerindeki geniş dağılımından ve yiyecek, medikal ve parfümeri endüstrisindeki yaygın kullanımından dolayı oldukça önemlidir. Bu çalışmada, *Salvia* L. cinsine ait Türkiye ' de yayılış gösteren on tür ve bir varyetenin kromozom sayıları ve karyomorfolojilerinin verilmesi hedeflenmiştir. Bu çalışmada incelenmiş olan 11 taksonun tamamı ekonomik açıdan önemlidir. Ayrıca bu taksonlardan 9 tanesi Türkiye için endemiktir. Bu taksonların kromozom numaralarını ve karyomorfolojilerini belirlemek için, her taksonun somatik kromozomları incelenmiştir. Çimlenen kök meristemleri α-bromonaftalinle 4ºC'de 16 saat ön işleme tabi tutulduktan sonra, Carnoy çözeltisinde 4ºC'de 24 saat fikse edilmiş ve % 70'lik alkolde depolanmıştır. Fikse edilen kök uçları % 2 ' lik aseto-orsein boyasıyla boyanmış ve 1 damla %45'lik asetik asit içinde ezilmiştir. Her kromozomun uzun kolu, kısa kolu, toplam uzunluğu ölçülmüş; nispi uzunluk, kol oranı ve sentromer indeksi hesaplanmıştır. Karyogramlar ve haploid idiogramlar bilgisayar destekli analiz programı (Bs200pro) ile çizilmiştir. Taksonlar arasında karyotip benzerliğini incelemek için kümeleme analizi yapılmıştır.

Endemik *S. divaricata* Montbret & Aucher, *S. euphratica* Montbret & Aucher ex Bentham (var. *leiocalycina* (Rech.fil.) Hedge) ve *S. recognita* Fisch. & Mey.' nın somatik kromozom sayıları 2n=2x=14 olarak sayılırken, *S. rosifolia* Sm.'nın 2n=2x=14-1B; *S. longipedicellata* Hedge, *S. vermifolia* Hedge & Hub.-Mor. ve *S. yosgadensis* Freyn & Bornm.'de 2n=2x=20; *S. aethiopis* L., *S. cilicica* Boiss. & Kotschy, *S. hypargeia* Fisch. & Mey.'*da* 2n=2x=22; *S. napifolia Jacq.*'da 2n=2x=32 olarak sayılmıştır.

Bu çalışma, *S. divaricata*, *S. euphratica* var. *leiocalycina*, *S. longipedicellata*, *S. rosifolia, S. vermifolia* ve *S. yosgadensis* taksonlarının kromozom sayıları ve kromozom morfolojileri için ilk kayıt olma özelliğini taşıdığı için önemlidir. Ayrıca *S. aethiopis*, *S. cilicica*, *S. hypargeia* ve *S. recognita*' nın kromozom sayıları bilinmesine rağmen, kromozom morfolojileri ile ilgili ilk çalışma bu olmuştur.

Anahtar Kelimeler: Kromozom, Karyotip, Labiatae, *Salvia*, Endemik, Sitotaksonomi.

To my beloved husband and son

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LIST OF ABBREVIATIONS

A1	Intra-chromosomal asymmetry index
A2	Inter-chromosomal asymmetry index
C	Total length of the chromosome
CI	Centromeric Index
CR	Critically Endangered
EN	Endangered species
GA	Gibberellic acid
L	Long Arm
LC	Least Concern
m	Metacentric
r	Arm ratio
R	Relative length
S	Short arm
sm	Submetacentric
ST	Stebbins Classes
TCL	Total Length of Haploid Complement
UPGMA	Unweighted Paired Group with Aritmetic Avarage
x	Mean chromatin length

CHAPTER I

INTRODUCTION

1.1. Labiatae

The Labiatae (Lamiaceae) known as Mint Family, is a family of flowering plants that comprises over 236 genera and 7173 species worldwide (Kubitzki, 2004). The family is known as the third largest family of the flowering plants. Though occurring almost all over the world, with the exception of the coldest polar regions, the Labiatae is particularly well represented in tropical and temperate areas especially those with a seasonal climate, such as the Mediterranean region and in tropical upland savannas. While some species are characteristics of semi-arid conditions, many others are adapted to wet habitats, in seasonally flooded areas or along river banks in forest (Cantino *et al.*, 1992).

The family is a large and natural family of mostly herbs and undershrubs including many useful plants such as sage (*Salvia*) and mint (*Mentha*) (Heywood, 1978). The Labiatae are important and many are of great economic importance (Judd *et al.*, 1999). The family is used for its fine ornamental or edible herbs like basil, lavender, mint, oregano, rosemary, sage and thyme and the species of this family is a source of essential oils for the flavouring and perfume industry (Wagstaff *et al*., 1998). They are widely used in traditional systems of medicine and horticulture. Moreover, a large number of Labiates are cultivated either as ornamentals or as kitchen herbs (Heywood, 1978). Numerous genera provide ornamentals, including *Ajuga* (bugleweed), *Callicarpa* (beautyberry), *Clerodendrum*, *Plectranthus* (coleus), *Holmskioldia* (Chinese-hat plant), *Leonotis* (lion's ear), *Monarda* (bee balm), *Pycnanthemum* (mountain-mint), *Salvia*, *Scutellaria* (skullcap), and *Vitex* (monk's pepper) (Judd *et al*., 1999). The family includes many species that are economically important either for their essential oils or for use as spices, including *Mentha* (peppermint, spearmint), *Lavandula* (lavender), *Marrubium* (horehound), *Nepeta* (catnip), *Ocimum* (basil), *Origanum* (oregano), *Rosmarinus* (rosemary), *Salvia* (sage), *Satureja* (savory) and *Thymus* (thyme) (Judd *et al*., 1999).

Labiates do not occur only in a few regions of the world: the members of the family grow in almost all types of habitat and at all altitudes, from the Arctic to the Himalayas, Southeast Asia to Hawaii and Australasia, throughout Africa and in the New World from North to South: a few genera such as *Salvia*, *Scutellaria* and *Stachys* are almost cosmopolitan (Heywood, 1978). The Mediterrean basin is one of the regions of the greatest concentration of species, *Micromeria, Phlomis, Rosmarinus, Sideritis and Thymus* are some of the genera which are characteristic components of the maquis and the garrigue in this region. In general Labiataes are open ground plants; only a few genera are found in tropical rain forests (Heywood, 1978).

The family contains many culinary or flavouring herbs, native to Turkey and Mediterrean area, which are cultivated throughout the world. (Davis,1982). Turkey is regarded as an important gene center for the family Labiatae (Baser, 1993). With regard to recent literatures (Erik and Tarıkahya, 2004), Labiatae is consists of 45 genera and 574 species, 256 of which are endemic in Turkey with 44.5 % of endemism ratio.

1.2. The Genus *Salvia*

Kingdom: Plantae

Subkingdom: Tracheobionta

Superdivision: Spermatophyta

Division: Angiospermae (Magnoliophyta)

Class: Dicotyledoneae (Magnoliopsida)

Order: Lamiales

Family: Lamiaceae

Genus: *Salvia*

The genus *Salvia* L. is the largest and the most important genus of Lamiaceae with nearly 1000 species spread through the World (Walker *et al*., 2004). *Salvia*, which grows in temperate regions (Heywood, 1978), shows cosmopolitan distribution with main regions of diversity in SW Asia, Central and South America (Kubitzki, 2004); Central Asia/Mediterrean and Eastern Asia (Walker *et al*., 2004).

According to the Flora of Turkey, Anatolia is major centre of diversity for the genus *Salvia* in Asia (Hedge, 1982), and the genus was represented by 86 species (Hedge, 1982). Beside these species there are six more new species namely *S. nydeggerii* Hub.-Mor. (Davis *et al*., 1988), *S. aytachii* Vural & Adıgüzel (Vural and Adıgüzel, 1996), *S. hedgeana* Dönmez (Dönmez, 2001), *S. anatolica* Hamzaoğlu & Duran (Hamzaoğlu and Duran, 2005), *S. marashica* A. İlçim, F. Celep & Doğan, (İlçim *et al*., 2009) and *S. ekimiana* Celep & Doğan (Celep and Doğan, 2009) were described and three new records namely *S. viscosa* Jacq. (Celep *et al*., 2009), *S. macrosiphon* Boiss. (Kahraman *et al*., 2009) and *S. aristata* Aucher ex Benth. (Behçet and Avlamaz, 2009) have been added to the Flora of Turkey. As a consequence of these studies, 97 *Salvia* species have been recorded from Turkey, 55 of which are endemic. Its endemism ratio is 56.7 %.

Infrageneric delimitation of the genus has been carried out by Doğan *et al*. (2007). According to this study, the member of the genus found in Turkey were grouped under seven sections called *Salvia* Hedge, *Hymenosphace* Benth, *Drymosphace* Benth, *Aethiopis* Benth, *Plethiosphace* Benth, *Horminum* Dumort and *Hemisphace* Benth.

Salvia has been shown to be the most potent natural antioxidant of the common species (Wang *et al*.,1998) as well as monoterpens with antiseptic characteristics (Nakipoğlu, 1993a). Many *Salvia* species are used as herbal tea and for food flavouring, as well as in cosmetics, perfumery and the pharmaceutical industry (Demirci *et al*., 2003). Species of the genus *Salvia* have also been used as folk medicines throughout the world. The members of the genus are also widely important because of their antibacterial, antioxidant (Dobrynin *et al*., 1976) antidiabetic and antitumor characteristics (Hanson and Hocking, 1957). Because of their medicinal purposes, they are widely known among people (Özcan *et al*., 2003). Ethanol extracts of *Salvia* crypthanta has antimicrobial activity aganist gram positive bacteria (Yiğit *et al*., 2002).

S. officinalis (sage) is the most popular species of the genus *Salvia*, and it is well-known for its medicinal purposes. Sage has various medicinal uses such as spasmodical, antiphlogistic, stomachic antiseptic, astringent, antiasthematic (Newall *et al*., 1996; Tepe et.al., 2005.) The leaves of S. officinalis are well known for their anti-oxidative properties used in the food processing industry as well as the area of human health (Baricevic *et al*., 2001). Due to the anti-viral activity of its water and alcohol extracts, sage is also used for the treatment of acute and chronic bronchitis, this treatment was officially approved for clinical use in Bulgaria (Manolova *et al*., 1995). Moreover in Turkey, *S. fruticosa* Mill. (Anadolu Adaçayı) is one of the most important species of the genus (Bayram, 2001). *S. fruticosa* and *S. triloba* L. are known as synonyms (Schönfeld, 1994). *S. fruticosa* is used in the treatment of constipation and used as antiseptic. In Turkey the leaves of this species are used in many treatments instead of *S. officinalis* (Baytop, 1984).

Some *Salvia* species were reported to be used for memory enhancing purposes in European folk medicine (Orhan *et al*., 2007). Essential oil of *S. sclarea* have been used as antidepressant, antiseptic, antispasmodic, carminative, and aphrodisiac (Dzamic *et al*., 2008) Ethanol extracts of *S. crypthanta* has antimicrobial activity aganist gram positive bacteria. (Yiğit *et al*., 2002) In the Eastern countries, mucilage of *Salvia* seeds have been used in the treatment of eye diseases (Baytop, 1999). Moreover, the leafs and seeds of *S. verbenaca* are effective in constipation, aganist sweat, sedative, eye diseases, dyspeptic complaint (Saraç and Uğur, 2007)

In addition to their medicinal importance, *Salvia* species are also used as ornamental plants in parks and gardens (Nakipoğlu, 1993). *Salvia* species are also used in processed food of all types, as well as in alcoholic and soft drinks (Dzamic *et al*., 2008).

1.3. Cytotaxonomy

Taxonomy is referred as synthetic science, drawing upon data from such diverse disciplines as morphology, anatomy, palynology, embryology, physiology, ecology, cytology, genetics, cytogenetics and chemistry etc. (Stuessy, 1989; Sharma, 1993).

Cytology, which can be defined as study of the cell, in a broad sense deals with the all aspects of the cells, but in taxonomic work, cytology focuses on chromosomes and their various attributes (Stuessy, 1989). When the characters of cytology are used for explanation of taxonomic problems, it is referred as cytotaxonomy (Sharma, 1993). Cytotaxonomy can also be defined as the branch of taxonomy that uses cytologic structures, esp. the chromosomes, as an aid in classifying organisms (Judd *et al*., 1999). Moreover, Stebbins (1971) indicated that only details of chromosomes have been used in resolving many taxonomic problems. The chromosomes play a special role as a source of comparative data in taxonomy, because these structures contain the genetic material which is responsible for maintaining reproductive barriers and the integrity of species and other taxa (Stuessy, 1989).

Chromosome number, size and morphology, gene and noncoding sequence content, behavior in meiosis, and total DNA content are some of the chromosomal data that have been used taxonomically (Stuessy, 1989). Since chromosomes are visible only with a microscope during cell division, chromosomal data are obtained most commonly during two types of cell division, mitosis and meiosis. While the shoot, root, and lateral meristems can be used to observe mitotic division, the sporogenous tissue is used for observing meiotic division. However, Stuessy (1989) indicated that most mitotic chromosomal observations come from root tips, and almost all meiotic observations are made of microsporogenesis in young anthers.

Due to the ease of observation, chromosome number has been used most often in taxonomic work. Most workers have used *2n* to refer to counts made in mitosis, and *n* to refer to counts from. In angiosperms haploid chromosome number have varied from n=2 *Haplopappus gracilis* of *Compositae* and n= 320 *Sedum suaveolens* of *Crassulaceae* (Jackson, 1957; Stace, 2000). The highest chromosome number recorded for vascular plants was found as 2n=1260 in a pteridophyte, *Ophioglossum reticulatum* (Sharma, 1993). If chromosome numbers are similar this may indicate close relationship; different chromosome numbers often create some reproductive isolation through reduced fertility of hybrids (Judd *et al*., 1999). Variation in chromosome numbers has been especially characteristic of the herbaceous dicots, whereas woody dicots show much less diversity (Stuessy, 1989). The chromosome number in woody dicots remains constant in all species e.g. all species of *Pinus* and *Quercus* possess n=12. While, such numbers called as constant chromosome numbers, the species bearing constant chromosome numbers are called *homoploids*. However, these numbers are of limited importance, but prove useful in knowing a particular genus. Moreover, polyploid series are present in several genera of vascular plants. *Polyploids* are the plants which possess higher chromosome number because of multiplication of genomes or chromosome sets, e.g. different species of *Aster* have n=9 n=18 n=27 etc. Such a series of polyploidy, in which the chromosome numbers of a taxon are in the proportion of its extract multiples, is called *euploidy*. On the other hand, if the chromosome numbers of a group bear no simple numerical relationships with each other, the series is called *aneuploidy*, e.g. different species of *Brassica* bear n=6,7,8,9 or 10 (Sharma, 1993).

The chromosome size has been very useful in understanding relationships in several taxa. In most plants, the length of chromosomes varies from 0.5 μm to 30 μm (Sharma, 1993).

Chromosome morphology is usually studied at the metaphase of mitosis. The principle landmarks which may be seen at this stage called centromere, to which the splindle fibres are attached (Stebbins, 1971). In general, chromosomes are classified as median, submedian, subterminal or terminal in terms of their length and position of the centromere whose location marks the position of primary constriction. Additional constrictions are called secondary constrictions. Occasionally, a secondary constriction may be present near terminal end of a chromosome, seperating its small segment called satellite. The chromosomes may be symmetrical or asymmetrical. Symmetrical ones possess two equal arms and a median centromere. Asymmetrical ones possess unequal arms and subterminal centromeres.

According to Stebbins (1971), (1) differences in absolute size of the chromosomes; (2) differences in the position of the centromere; (3) differences in relative chromosome size; (4) differences in basic number; (5) differences in the number and position of satellites are some important characters that have taxonomic significance.

The standard chromosomes which are forming chromosome complement of a cell called A chromosomes. Beside normal constant complement of A chromosomes, many plant species contain variable number of extra chromosomes. Then these are called accessory chromosome or supernumerary chromosome or extrachromosomes or B- chromosome (Shukla and Chandel, 1974). B chromosomes which do not usually pair with normal chromosomes during meiosis and are often invisible in meiosis, differ from the A chromosomes in their variable number, smaller size and greater degree of heterochromatinisation (Sen and Kar, 2005). Although these chromosomes are usually acro- or telocentric in nature, submetacentrics and metacentrics have also been recorded in the literature before. They may vary in number within the tissues of the same individual, between different individuals of the same population and even between populations of the same species from different regions (Sen and Kar, 2005). For example, in certain circumstances, an increase in B chromosomes has been observed in plants growing under different environmental conditions e.g. clay soil and dry climates (Sen and Kar, 2005).

The term karyotype is used for a group of characteristics that allow identification of a particular chromosomal set i.e.; the number of chromosomes, relative length of chromosome arms, position of centromere, presence of secondary condition and the size of satellite (Sen and Kar, 2005). Stebbins (1971) defined karyotype as morphological aspect of the chromosome complement as seen at mitotic metaphase and he indicated that usually somatic karyotypes are studied and compared, most often from root tip mitoses. When karyotype represented diagrammatically showing all the morphological features of the chromosome is called idiogram (Sen and Kar, 2005).

1.4. Studies on the chromosomes of the *Salvia*

Chromosome counts are available for few of a thousand known *Salvia* species (Scheel, 1931; Yakovleva, 1933; Epling, 1938; Stewart, 1939; Epling *et al*., 1962; Gill, 1971; Afzal-Rafii, 1972; Löve and Kjellqvist, 1974; Vij and Kashyap, 1976; Bhattacharya, 1978; Haque and Ghoshal, 1980; Löve, 1980; Haque, 1981; Markova and Ivanova, 1982; Alberto *et al*., 2003).

Scheel (1931) examined chromosome numbers of different *Labiates*: *Salvia*, *Rosmarinus*, *Scutellaria*, *Lavandula* and *Colcus*. The chromosome numbers of 25 taxa belonging to *Salvia* genus were found, these were; *S. officinalis* L. n=8, *S. ringens* Sibth. & Sm. 2n=12; *S. recognita* Fisch et Mey 2n=14, *S. glutinosa* L. 2n=16, *S. hians* Royle 2n=32; *S. przewalskii* Maxim 2n=16; S. viridis L. var. *horminum* f. *violac* n=8, S. viridis L. var. *horminum* f. *rubra* n=8; *S. sclarea* L. 2n=22; *S. argentea* L. 2n=22; *S. pratensis* L. supsp. vulgaris. Briqu. var vulgaris. R.f. 2n=32; *S. pratensis* L. supsp. vulgaris. Briqu. f.a. n=9; *S. pratensis heamatodes* 2n=18; *S. pratensis haemotodes* L. *var*. *x* 2n=18; *S. pratensis subsp. virgata* Ait. n=16; *S. baumgartenii* Heuff 2n=16; *S. cleistogama* De Barry et Paul n=32; *S. silvestris* L. 2n=16; *S. nutans* n=9; *S. jurisicii* Kosanin n=11; *S. pseudosilvestris* Stapf n=7; *S. tiliaefolia* Vahl. n=11; *S. lanceolata* Willd. 2n=20; *S. hirsuta* Jacqu. 2n=22; *S. azurea* Lamk. 2n=20; *S. hispanica* L. n=6, *S. splendes* Sellow 2n=44; *S. pseudococcinea* Jacqu. n=11; *S. patens* Cav. n=9; *S. carduaca* Benth. 2n=16; *S. verticillata* L. n=8;

S. regeliana Trautv. n=8. According to this study most frequently found basic numbers were 8 and 11.

Stewart (1939) investigated chromosomes of 18 *Salvia* taxa which have taxonomic difficulty. In this study, pollen mother cells at diakinesis stage and metaphase plates in tranverse sections at root tips were used for counting chromosomes. As a result of this study, chromosome numbers of 18 species belonging to section *Audibertia* were recorded and basic chromosome numbers were found as n=8, 11, 12, 13, 16. Stewart (1939) also indicated that whole *Salvia* genus and the Section *Audibertia* have more than one basic chromosome number.

Hruby (1945) found the somatic chromosome numbers of *S. nemecii* Hruby which is an interspecific hybrid between *S. nutans* L. and *S. jurisicii* Kos and its parent species as 2n=22.

Likewise Stewart (1939) Epling *et al*. (1962), studied chromosomes of 19 species belonging to section *Audibertia*. His work has shown similarity with the findings of previous studies, but in addition more than one chromosome numbers were observed for many species. Meiosis of natural hybrids of *S. mellifera* ve *S. apiana* was included. While haploid chromosome number in *S. columbariae* belonging to subsection *Pycnosphace* was found as n=13, all species belonging to subsection *Echinosphace* were found as n=16. However, in subsections of *Greeneostachys, Parishiella* and *Jepsonia* haploid chromosome numbers of all species were characterized with n=15. The findings of this research were not compatible with the early findings about section *Audibertia* covered by Yakovleva (1933), Stewart (1939), Carlson (1936).

Löve and Kjellqvist (1974) examined spanish plants which include *Salvia* species. With this study chromosome numbers of four *Salvia* species were recorded as; *S. lavandulifolia* Vahl. 2n=14; *S. sclarea* L. 2n=22; *S. aethiopis* L. 2n=22; *S. verbenaca* L. 2n=54.

Haque and Ghoshal (1980) indicated that until this time works on chromosomes of the genus *Salvia* in relation to karyotypes and chromosome morphology was very little and all studies were limited to chromosome counts only. This might due to very small chromosome size. Thus, Haque and Ghoshal carried out a detail karyological study which contained chromosome size and centromeric position of seventeen taxa of the genus: *S. coccinea*, S. *splendens*, *S. pratensis*, *S. hispanica*, *S. aegyptica*, *S. tiliifolia*, *S. reflexa*, *S. horminum*, *S. leucantha*, *S. nemorosa S. verbenaca S. aethiopis S. officinalis.* Chromosome number of studied species varied between 2n=12 (*S. hispanica*) and 2n=54 (*S. verbenaca*). In that study also B chromosome was observed in the karyotyope of *S. horminum*.

In 1981 Haque achieved a new study including comparative account of the chromosome numbers of the seventeen species with several varieties and three cultivated species of *Salvia*. The paper reported chromosome numbers of *S. hispanica* 2n=12; *S. officinalis* 2n=14 (Hruby, 1935; Chauhan and Abel, 1968; Gill, 1971); *S. nemorosa* 2n=14 (Hruby ,1935; Chauhan and Abel, 1968; Gill, 1971; Markova and Ivanova, 1972) *S. glutinosa* 2n=16 (Hruby, 1935; Linnert, 1955a; Majovsky, 1970; Skalinska *et al*., 1971); *S. pratensis* 2n=16 (n=9,16 (Schee,l 1931), 2n=16 (Afzal-Rafii, 1971), 2n=18 (Scheel, 1931; Hruby, 1934; Benoist, 1937; Gadella *et al*., 1970; Majovsky *et al*., 1970; Chuksanova and Kaplanbekova, 1971) 2n=20 (Sugiura 1936); *S. horminum* 2n=16+1B (Afzal-Rafii, 1972; 2n=16+2B); *S. farinaceae* 2n=18 (2n=20 Sugiura, 1936; Gill, 1971); *S. reflexa* 2n=20; *S. aethiopis* 2n=22; *S. coccinea* 2n=22; *S. splendens* 2n=44, 22; *S. grahamii* 2n=22; *S. leucantha* 2n=22; *S. aegyptica* 2n=26; *S. taraxacifolia* 2n=26; *S. verbenaca* 2n=54. Likewise other studies performed on chromosome numbers of *Salvia* genus, this paper reported more than one basic chromosome number.

Markova and Ivanova (1982) studied chromosomes of *Salvia* species and diploid chromosome numbers were found as *S. grandiflora* Etlinger, 2n=16; *S. scabiosifolia* Lam. 2n=14; *S. ringens* 2n=12; *S. ringens var. macedonica* 2n=14; *S. glutinosa* L. 2n=16; *S. forskahlei* 2n=16; *S. pratensis* 2n=18, 2n=32; *S. virgata* 2n=16; *S. nemorosa* 2n=14; *S. amplexicaulis* 2n=20; *S. verbenaca* 2n=56, 54.

Dalgaard (1986) counted chromosomes of 65 flowering plants from Macaronesia. Chromosome numbers of two *Salvia* species were reported in this study as *S. aegyptiac* Decne 2n=26 and *S. canariensis* L. 2n=22. In this study, Dalgaard indicated that *Salvia* has the extensive series of aneuploid chromosome numbers.

Estilai *et al.* (1990) studied chromosome number and meiotic behaviour of cultivated *S. hispanica*. This species has the lowest chromosome number in the genus with 2n=12. Chromosomes of the species were small, ranging from 2 μm to 3.5 μm long. The karyotype formula of the species was found as 1m+4sm+1t.

Boşcaiu *et al*. (1998) reported chromosome numbers of twenty taxa belonging to Labiatae family which existed in Spain. Chromosome number of *S. pratensis* from Spain was first reported as 2n=18 and *S. blancoana* subsp. *mariolensis* was reported as 2n=14 (Boşcaiu *et al*., 1998). According to this study the size of chromosomes of *S. pratensis* varied from 1 μm to 3 μm and *S*. *blancoana* subsp. *mariolensis* varied from 2 μm to 3.5 μm. According to this study, there are systematic gropus belonging to Labiatae family with great karyological stability, while other groups show high variability, due to the process of dysploidy, aneuploidy or polyploidy.

Turki *et al*. (2000) reported chromosome numbers of thirty-one taxa belonging to fourteen families of angiosperms, some of them were belonging to *Salvia* genus. These are; *S. deserti* L. 2n=48; *S. aegyptiaca* 2n=28, *S. spinosa* L. 2n=20.

Alberto *et al*. (2003) studied meiotic and mitotic chromosomes of thirteen species of *Salvia*. Chromosome numbers of studied species were reported as *S. cardiophylla* Benth. 2n=44; *S. coccinea* Juss. 2n=22; *S. farinacea* Benth. 2n=10; *S. gilliesii* Benth. 2n=22; *S. guaranitica* A. St.- Hil. 2n=88; S. *involucrata* Cav. 2n=22; *S. microphylla* Kunth 2n=22; *S. pallida* Benth. 2n=88; *S. procurrens* Benth. 2n=52; *S. rypara* Briq. 2n=88; *S. splendens* Roem. & Schult. 2n=44; *S. stachydifolia* Benth. 2n=66; *S. uliginosa* Benth 2n=52. According to this study the most frequently found basic number was x=11, but two species had x=13 and one species had x=10.

Foley *et al*. (2008) found the mitotic chromosome number of *Salvia tingitana* as 2n=42 which is unusual but not the first in the genus. Foley *et al.* (2008) also indicated that most frequently counted chromosome number in the genus is 2n = 16 or 22 chromosomes (44 and 43 species, respectively), and the next most common counts are 2*n* = 14 and 20 chromosomes (24 and 20 species).

In Turkey, there are few studies related to chromosomes and karyology of the *Salvia* genus. In 1988, Çobanoğlu found the mitotic chromosome number of S. palaestine as 2n=20 and chromosome size of the species varied from 1 μm to 1.6 μm long. No B chromosome and satellite were observed on this study.

Nakipoğlu (1993b) reported chromosome numbers and karyomorphologies of five *Salvia* species. In this study chromosome number of *S. viridis* L. was found as 2n=16 and chromosome length of this species range between 1.00 μm to 1.60 μm, as for *S. virgata* Jacq. chromosome number was found as 2n=16 and chromosome length varied from 1.48 μm to 2.57 μm, and chromosome number of *S. glutinosa* L. was reported as 2n=16+2B and chromosome length varied from 1.00 μm to 2.80 μm. According to this study chromosome number of *S. argentea* L. was counted as 2n=22 and *S. verbenaca* 2n=42, 46, 48 but because of very small chromosome size of chromosome morphologies of these species did not reported. Nakipoğlu (1993c), in her another study investigated chromosome numbers and karyotype morphologies of *S.fruticosa* Mill., *S. tomentosa* Mill., *S. officinalis* L., *S. smyrnaea* Boiss.. Chromosome numbers of the species were reported as 2n=14 for *S. fruticosa* and *S. smyrnaea*, 2n=14-1B for *S. fruticosa*, 2n=14-2B for *S. fruticosa* and *S. officinalis*; 2n=16, 2n=16-1B and 2n=16-2B for *S. tomentosa.* This study also indicated that the number of B chromosome could be changed in the same species from different populations and it is also indicated soil type and different ecologic features affected the number of B chromosome.

Özdemir and Şenel (1999) performed a detailed karyotype analysis in *S. sclarea*. It has 22 chromosomes which consists of submedian and median chromosomes and the size of chromosomes varied from 0.2 μm to 1.6 μm.

In the study performed by Kandemir (2003) chromosome number and chromosome morphology of *S. hypargeia* were represented. As regard to this study somatic chromosome number of this species is 2n=22 and centromeric position of chromosomes were recorded as submetacentric and metacentric. Chromosome size of the species varied from 0.30 μm to 1.60 μm long.

S. wiedemannii and *S. tchihatcheffii* were studied by Özkan (2006) the somatic chromosome number of the former is 2n=14 and the latter is 2n=18. Özkan and Soy (2007) found chromosome number of *S. blepharoclaena* Hedge and Hub.-Mor.as 2n=14; karyotype consisted of submedian and subtelocentric chromosomes. Chromosome size of the species varied from 0.5 μm -1.4μm. Özkan and Şenel (2007) analyzed the chromosome number and morphology of four species belonging to the *Salvia* L. genus. These species are *S. aethiopis* L., *S. ceratophylla* L., *S. verticillata* L. subsp.

v*erticillata*, and *S. verticillata* L. subsp. *amasiaca.* The chromosome number of the studied species were reported as *S. aethiopis* 2n = 22, *S. ceratophylla* 2n = 18, *S. verticillata* subsp. *verticillata* 2n = 32, and S*. verticillata* subsp. *amasiaca* 2n = 16.

1.5. Aim of the Study

A few cytological studies have been published on the genus in Turkey and these indicated that the chromosome numbers and chromosome morphology were unknown for the most of the *Salvia* species.

The purpose of this study is to characterize the karyotype of the eleven *Salvia* L. taxa namely *S. aethiopis*, *S. cilicica*, *S. divaricata, S. euphratica* var. *leiocalycina, S. hypargeia, S.longipedicellata, S. napifolia, S. rosifolia, S. recognita, S. vermifolia, S. yosgadensis* occuring in Turkey and associate karyotype features with taxonomic and evolutionary issues.

For this purpose; mitotic metaphase chromosomes were obtained from germinated root tips, and detailed karyological analysis were carried out by computer-aimed image analysis system for each species. Karyological data of this eleven taxa suggest a valuable information to fulfill the deficiency in cytological point of view. Moreover, these data will be useful in solving taxonomic problems, especially in morphologically close species.

CHAPTER 2

MATERIALS AND METHODS

2.1. Materials

2.1.1. Plant Materials

The specimens and their seeds were collected from their natural habitats in 2006, 2007 and 2008. The specimens have been deposited in the plant science laboratory of Biology Department, METU, as well as at the Herbarium of the Faculty of Education, Hacettepe University, Ankara, Turkey. Seed materials were put into envelopes and the informations such as the location, specimen number and date of collection were written on the envelopes. The photographs of studied taxa are given in Appendix A. Locality, position, altitude, endemism and threat category, flowering time and specimen numbers are given in Table 2.1.

Table 2.1. Locality, position, altitude, endemism and threat category, flowering time, collectors and specimen numbers of the taxa

Taxon	Locality	Position	Altitude	Endemism & Threat category	Flowering Time	Collectors & Specimen numbers
S. aethiopis	Van	380 33 21 N 430 26 19E	1896 m	Non Endemic	May to August	AKahraman 1330
S. cilicica	Adana	37 23 520 N 34 51 033 E	858 m	Endemic EN	July to September	FCelep 1199
S. divaricata	Sivas	39 42 554 N 37 45 137 E	1420 m	Endemic LC	June to July	GAkaydın 10994
S. euphratica var. *leiocalycina*	Malatya	38 23 00 N 37 55 21 E	1348 m	Endemic LC	April to May	AKahraman 1362
S. hypargeia	Mersin	37 09 522 N 34 41 075 E	1347 m	Endemic LC	June to July	FCelep 1760
S. longipedicellata	Sivas	39 52 280 N 37 56 995 E	1650 m	Endemic LC	July to August	SBagherpour 519
S. napifolia	Mersin	36 57 949 N 34 29 682 E	870 m	Non Endemic	April to July	FCelep 1113
S. recognita	Ankara	40 08 724 N 33 21 351 E	1150 m	Endemic LC	May to August	FCelep 949
S. rosifolia	Artvin	41 06 571 N 42 08 513 E	1500 m	Endemic LC	May to August	GAkaydın 11070
S. vermifolia	Sivas	39 23 142 N 36 55 898 E	1495 m	Endemic CR	June to July	SBagherpour 521
S. yosgadensis	Nevşehir	38 44 155 N 34 40 299 E	950 m	Endemic LC	May to June	SBagherpour 441

Abbrevations: EN: Endangered, LC: Lower concern, CR: Critically Endangered

2.1.2. CHEMICALS

The chemicals with their suppliers are listed in Appendix B.

2.1.2.1. Preparation of solutions

Aceto-Orcein

55 ml distilled water and 45 ml acetic acid were mixed, and slowly brought them to a boil then we added 2 gr orcein. It was mixed by magnetic mixer for about 30 minutes, was filtered by whatman filter paper (Dyer, 1979).

Lacto-Propionic Orcein

2 gr. natural orcein were dissolved in a mixture of 50 ml lactic acid and 50 ml. propionic acid at room temperature and filtered by whatman filter paper (Dyer, 1963).

Carnoy Solution

Three parts of absolute ethanol and one part of Glacial Acetic acid were mixed (Elçi, 1982).

α- bromonaphtalene Solution

Aqueous stock solution of 1 ml bromonaphtalene dissolved in 100 ml absolute ethanol for ½ hour (Dyer, 1979).

1 N HCl

8,3 ml. of HCl was diluted in 100 ml distilled water and kept in room temperature.

Giberellic Acid (GA3)

50 mg Gibberellic acid was diluted in 1 lt. 95 % ethanol (Nord and Gunter, 1971).

2.2. METHODS

2.2.1. Seed Germination

To show chromosomes, tissues or organisms must be alive and healthy and contain actively dividing cells at the time of fixation (Dyer, 1979). The most frequently used material for demonstrating dividing chromosomes is the permanently embriyonic tissue of growing root tips (Stebbins, 1971).

Only a few number of *Salvia* seeds germinated initially (without pre-treatment) under normal conditions, while a great number of them required some pre-treatment methods for germination. Germination time ranged from 7 days to 40 days. Most of *Salvia* seeds have deep dormancy and thick testa and they exhibit very few or no germination rate in normal conditions. Therefore, some different seed pre-treatment procedures, used to break seed dormancy and to enable the seeds to germinate, were applied. Soaking, mechanic scarification, surface sterilization, cold stratification and treatment with Gibberellins are some effective methods to remove dormancy and fastened the germination (Piotto *et al*., 2003).

2.2.1.1. Soaking

The seeds were soaked in distilled water for 7–30 days. Soaking is beneficial in two ways; it can soften a hard seed coat and also remove any chemical inhibitors in the seed which may prevent germination (Sen and Kar, 2005). The seeds were soaked in distilled water for 7–30 days. It was observed that in some species soaking in water was sufficient. This method was shortened the time of germination in some species.

2.2.1.2. Cold Stratification

Short period of cold stratification at +4° or +5°C is another method for breaking dormancy (Piotto *et al*., 2003). The seeds were kept in water at +4°C few days to several weeks according to the species (Keeley; 1986), it is observed that this method hastened mucilage production.

2.2.1.3. Alcohol Sterilization

Seeds of some *Salvia* species became mouldy after they were sown in filter paper layered petri dishes. In order to prevent molding, seeds were rinsed with 10 % alcohol widely, then rinsed with distilled water 3 times, when molding was observed again, the concentration of alcohol was increased to 20 %. Unfortunately, this method prohibited germination in some species.

2.2.1.4. Sulphuric Acid Sterilization (H_2SO4)

Some species were mildewed after they were planted. Petri dishes were sterilized in an autoclave at 121ºC. Viable seeds were scarified with concentrated (1%) sulphuric acid (H_2SO4) for 5 minutes and were washed with distilled water for three times to remove acid residue. Seeds were sown in sterilized petri dishes containing double layer of filter paper. This method prevented seed moulding but delayed the germination. As a conclusion it has a negative effect on germination.

2.2.1.5. Mechanic Scarification

Mechanic scarification is effective method in order to germinate the seed which has hard seed coat (testa). Soften, scarify or remove the covering of the seed increase the germination (Hartmann *et al*., 1997). With this aim thick testas were rubbed with sandpaper and hilums were stracthed by lancet, this method facilitated absorption of water and fastened mucilage production.

2.2.1.6. Treatment with Gibberellic Acid

Seed dormancy of *Salvia* that is overcome in nature by either dry after-ripening or chilling may be circumvented through a 1-hour soak in gibberellic acid (GA) C100 to 500 ppm (Nord and Gunter, 1974) and 400 ppm (Emery, 1988). Seeds were soaked few hours in gibberellic acid (GA_3) solution (50 mg/ lt) (Nord and Gunter, 1974), before sowning and they regularly were watered by GA solution after sowning. Treatment with GA affected germination significantly. It is observed that in most of the species GA treatment facilitate the germination and accelerate growing of root tips.

In some cases two or more pre-treatment procedures were applied together. Germination was checked everyday and seeds were watered with 50mg/lt GA solution.

Seeds can be germinated in moist blotting paper in Petri dishes. Healthy growing roots are brittle, translucent and white with opaque cream to white, gently tapered tips (Dyer, 1979).

2.2.2. Pre –Treatment

Pre-treatment is carried out for :

- Causing mitotic block and metaphase arrest through destruction of splinde fibre.
- Bringing about scattering of chromosomes and clarification of constriction regions through differential hydration in chromosome segments.
- Clearing the cytoplasm by removing heavy contents to bring about the transparency of cytoplasmic background.
- Achieving rapid penetration of the fixative through removal of undesirable deposits from the surface of the tissue.
- Seperation of the middle lamella causing softening of the tissue (Sen and Kar, 2005).
- Preventing anaphase.

A number of chemicals are used for the purpose of pre-treatment, the most common being colchicine. These chemicals are not universally applicable to all plant materials. Generally, a particular group of plants gives better results in a particular chemical. These chemicals are; aesculine, α-bromonaphtalene, colchicine, coumarin, paradichlorobenzene, 8-hydroxyquinoline (Sen and Kar, 2005). According to the literature colchicine is usually more succesful, but it is also unfortunately by far the most expensive and as a possible carcinogen must be handled with care. In many cases saturated solution of paradichlorobenzene and α-bromonaphtalene are acceptable substitutes, but due to the former is particularly good for leaf material, the latter was chosen in this study as pre-treatment agent (Dyer, 1979).

Actively growing root tips in 1-1.5 cm length were excised at about 09.30 am in some species and 04.00 pm in others. The time of pre-treatment is extremely important, thus the most dividing cells can obtain in the late morning. Then root tips were pretreated with 0.1 % solution of α-bromonaphtalene for 16 hours at 4ºC. Then root tips widely rinsed with dH_2O.

2.2.3. Fixation

Fixation is the process by which tissues and their components are fixed selectively at a particular stage. The purpose of fixation is to kill the tissue (lethality) without causing any distortion of the components to be studied. Fixation in chromosome study brings about blocking of cell divisions and enable the preservation of the structural integrity of the chromosomes.
A truly effective fixative should fulfill the following conditions:

- Rapid penetration to cause immediate killing of the tissue.
- Coagulation of the protein component and consequent precipitation causing a marked change in the refractive index of the chromosomes.
- Checking denaturation of protein consequent to the death of cell. Due to lethality the medium becomes acidic which causes enzymes to act in reverse direction, with breakdown of complex protein molecule into simpler amino acids.
- Checking bacterial action with on set of lethality, thus preventing tissue decomposition.
- Precipitating the chromatin matter to render the chromosome visible.
- Increasing the basophilia of the chromosomes, helping in the adherence of the stain.

Carnoy solution (1 glacial acetic acid: 3 ethanol) was used as fixative in our study, thus it is a common fixative for root tips (Dyer, 1979). Pre-treated root tips were fixed in Carnoy solution for 24 hours at 4ºC.

2.2.4. Storage

After fixation root tips were rinsed with dH_2O and were tranferred to 70 % alcohol for long term storage in the freezer.

2.2.5. Hydrolysis

HCl hydrolysis greatly softens the cell wall allowing a better spread of cells and chromosomes; as a result the cytoplasm becomes extremely transparent (Fox, 1969). Root tips were hydrolyzed in 1 N HCl for 13- 18 min. at room temperature. The time of hydrolysis varies among species. After hydrolysis, root tips were widely washed with distilled water giving 3 changes to wash out excess HCl, because in the presence of HCl residue, chromosome staining can be failed (Wittmann, 1965).

2.2.6. Aceto-Orcein Staining

Fixed root tips were taken in a watch glass and stained in 2 % aceto-orcein for 3-4 hours in darkness (Galbany-Cassals and Romo, 2008). Well stained root caps (apical meristem) were cut off with a razor blade and taken in a drop of 45% acetic acid on a slide, dissected with a pin. A cover- slip is placed over the root tip and squashed by applying uniform pressure with the thumb through a piece of blotting paper. For further staining a drop of lacto-propionic orcein was added (Dyer, 1963).

2.2.7. Preparation of Permanent Slides / Mounting

Good slides were immediately frozen in liquid nitrogen; coverslip was then removed with a razor blade and were left air dried for two days. Permanent preparations were made by mounting in Entellam, a cover glass was added.

2.2.8. Karyotype Analysis

Well spread slides with minimum five metaphases were observed to determine chromosome number and investigated using 10 X ocular by 100 X oil immersion objective of Euromex FE 2025 microscope and photographed by using a ''Euromex CMEX DC.1300'' camera. Chromosome measurements based on at least three metaphase plates for each species were analysed by Bs200pro Image Analysing System, which helped in drawing accurate idiograms and karyograms (Martin *et al*., 2008). Short arms (S), long arms(L), total length(C), arm ratio (r), centromeric index (CI) , relative length (R), total chromosome length of haploid complement (TCL) were measured for each chromosome. The ratio between short arm and long arm was estimated by the formula:

$$\text{Arm ratio} = \frac{long\ \ arm}{short\ \ arm}$$

Chromosome morphology was determined using the centromeric index for every sample using the following equation :

$$\text{Centromeric Index} = \frac{short\ \ arm}{total\ \ length}x100$$

Chromosome type was determined based on centromeric position terminology described by Levan *et al.* (1964). Accordingly, if the value of centromeric index is 50-37.5 chromosomes were classified as metacentric

(m) ; if 37.5-25 as sub-metacentric (sm); if 25-12.5 as sub-telocentric (st) (Betiana and Dematteis, 2009).

Relative Length was evaluated by the formula below:

$$\text{Relative length}=\frac{total\ \ length\ of\ \ the\ chromosome}{total\ \ haploid\ \ length}\ x100$$

Karyotype formula was based on the measurements of chromosomes. Chromosomes were arranged in the karyotype according to decreasing total length. Karyograms and idiograms were drawn based on mean centromeric index and arranged in order of decreasing size.

Karyotype asymmetry was evaluated by Stebbins Classes (1971) and Romero Zarco asymmetry indices (1986). Stebbins classes were evaluated by recognizing three degrees of difference (A-C) between largest and smallest chromosome of the complement and four degrees (1-4) with respect to the proportion of chromosomes which are metacentric with an arm ratio of less than 2:1(Pazsko, 2006).

The intrachromosomal asymmetry index (A1) and the interchromosomal asymmetry (A2) were calculated according to the formulas proposed by Romero Zarco (1986):

$$A_1 = 1 - \frac{\sum_{i=1}^{n} \frac{b_i}{B_i}}{n}$$

bi and Bi are the mean length of short and long arms of each pair of homologous, *n* is the number of homologous.

$$A_2 = \frac{s}{\bar{x}}$$

s is the standard deviation and *x* is the mean chromosome length.

2.2.9. Cluster analysis

A cluster analysis of the karyotype data was carried out to examine karyotype similarity among species. The MVSP (MultiVariate Statistical Package) programme was used to construct phenogram. In order to group the species studied based on their karyotypic similarity, UPGMA (unweighted paired group with aritmetic avarage) clustering methods were performed. Numerical characterization of karyotypes was performed by calculating following parameters: total chromosome length of haploid complement (TCL); mean chromatin length (X); the ratio between longest to the shortest chromosome pair (L/S); intrachromosomal asymmetry index (A1); interchromosomal aymmetry index (A2); diploid chromosome numbers (2n) of species (Seijo and Fernandez, 2003; Sheidai and Bagheri-Shabestarei, 2007).

CHAPTER 3

RESULTS

3.1. Karyological features of *S. aethiopis* L.

Chromosome number: 2n=2x=22

Karyotype formula: 8m+3sm

Karyotype of this species consists of eight metacentric and three submetacentric chromosomes (Figure 3.1.1., Table 3.1.). The length of chromosomes varies from 2.19 µm to 0.95 µm (Table 3.1.). No satellite was observed on this species. The karyotypic formula is represented as 8m+3sm (Table 3.1.). The ratio of the longest to the shortest chromosome is 2.3:1 and the karyotype symmetry is type 2B. Total haploid length of the species is 17.12 µm.

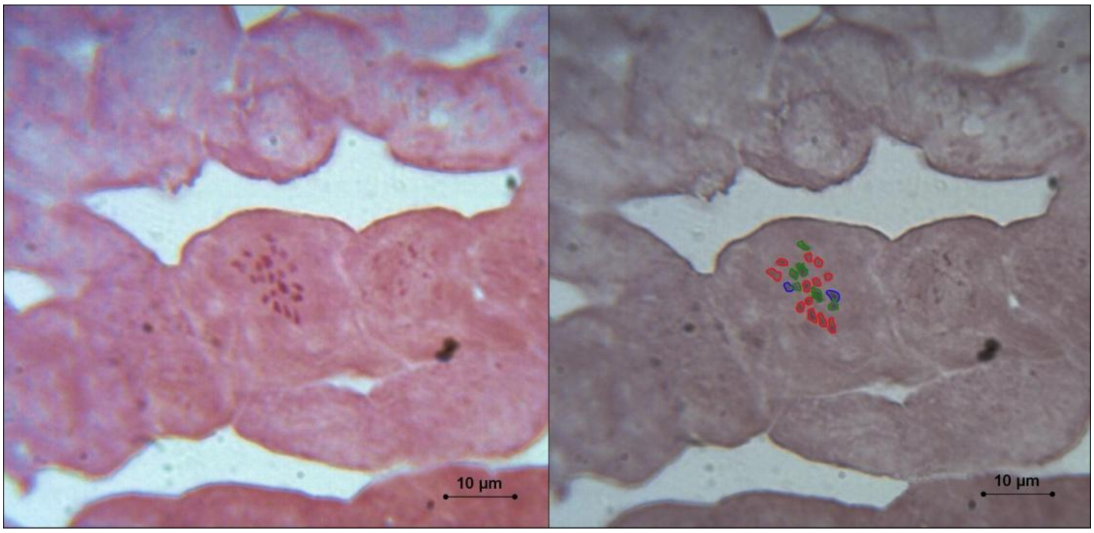

Figure 3.1.1. Mitotic metaphase chromosomes of *S. aethiopis*

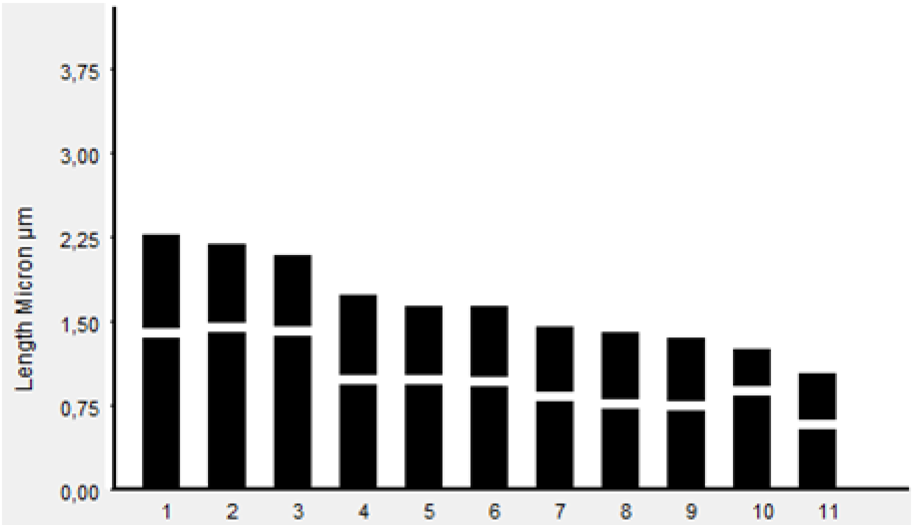

Figure 3.1.2. Haploid Idiogram of *S. aethiopis*

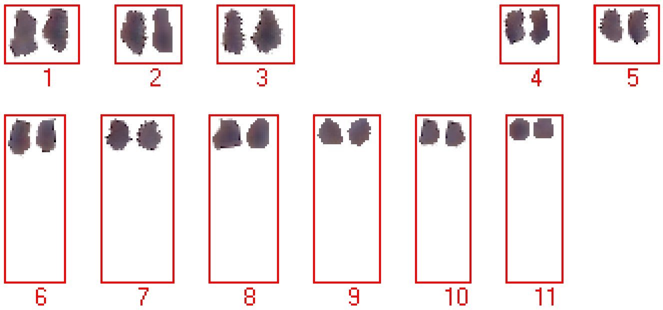

Figure 3.1.3. Karyotype of *S. aethiopis*

Chromosome I: It is a metacentric chromosome and longest chromosome of the species. Total length of the chromosome is 2.19 μm, long arm of the chromosome is 1.35 μm, short arm of the chromosome is 0.84 μm. Its arm ratio is 1.61, centromeric index is 38.3 and relative length is 12.79.

Chromosome II: It is a submetacentric chromosome. Total length of the chromosome is 2.10 μm, long arm of the chromosome is 1.40 μm, short arm of the chromosome is 0.70 μm. Its arm ratio is 2.00, centromeric index is 33.3 and relative length is 12.27.

Chromosome III: It is a submetacentric chromosome. Total length of the chromosome is 2.00 μm, long arm of the chromosome is 1.37 μm, short arm of the chromosome is 0.63 μm. Its arm ratio is 2.19, centromeric index is 31.5 and relative length is 11.65.

Chromosome IV: It is a metacentric chromosome. Total length of the chromosome is 1.65 μm, long arm of the chromosome is 0.93 μm, short arm of the chromosome is 0.72 μm. Its arm ratio is 1.28, centromeric index is 43.6 and relative length is 9.61.

Chromosome V: It is a metacentric chromosome. Total length of the chromosome is 1.56 μm, long arm of the chromosome is 0.94 μm, short arm of the chromosome is 0.62 μm. Its arm ratio is 1.52, centromeric index is 39.7 and relative length is 9.11.

Chromosome VI: It is a metacentric chromosome. Total length of the chromosome is 1.54 μm, long arm of the chromosome is 0.91 μm, short arm

of the chromosome is 0.63 μm. Its arm ratio is 1.44, centromeric index is 40.9 and relative length is 9.00.

Chromosome VII: It is a metacentric chromosome. Total length of the chromosome is 1.38 μm, long arm of the chromosome is 0.79 μm, short arm of the chromosome is 0.59 μm. Its arm ratio is 1.33, centromeric index is 42.7 and relative length is 8.03.

Chromosome VIII: It is a metacentric chromosome. Total length of the chromosome is 1.32 μm, long arm of the chromosome is 0.72, short arm of the chromosome is 0.60. Its arm ratio is 1.20, centromeric index is 45.45 and relative length is 7.71.

Chromosome IX: It is a metacentric chromosome. Total length of the chromosome is 1.27 μm, long arm of the chromosome is 0.70 μm, short arm of the chromosome is 0.57 μm. Its arm ratio is 1.23, centromeric index is 44.8 and relative length is 7.42.

Chromosome X: It is a submetacentric chromosome. Total length of the chromosome is 1.17 μm, long arm of the chromosome is 0.84 μm, short arm of the chromosome is 0.33 μm. Its arm ratio is 2.56, centromeric index is 28.2 and relative length is 6.86.

Chromosome XI: It is a metacentric chromosome and it is shortest chromosome. Total length of the chromosome is 0.95 μm, long arm of the chromosome is 0.54 μm, short arm of the chromosome is 0.41 μm. Its arm ratio is 1.32, centromeric index is 43.1 and relative length is 5.55.

The chromosome type, chromosome length, arm ratio, relative length and centromeric index for *S. aethiopis* were given in detail in Table 3.1.

Table 3.1. Karyomorphological parameters of *S. aethiopis*

Pair no.	Long arm (L)	Short Arm (S)	Total length (C)	Relative Length	Arm Ratio (r=L/S)	Centromeric Index CI=100.S/C	Chromosome Type
1	1.35	0.84	2.19	12.79	1.61	38.3	m
2	1.40	0.70	2.10	12.27	2.00	33.3	sm
3	1.37	0.63	2.00	11.65	2.19	31.5	sm
4	0.93	0.72	1.65	9.61	1.28	43.6	m
5	0.94	0.62	1.56	9.11	1.52	39.7	m
6	0.91	0.63	1.54	9.00	1.44	40.9	m
7	0.79	0.59	1.38	8.03	1.33	42.7	m
8	0.72	0.60	1.32	7.71	1.20	45.4	m
9	0.70	0.57	1.27	7.42	1.23	44.8	m
10	0.84	0.33	1.17	6.86	2.56	28.2	sm
11	0.54	0.41	0.95	5.55	1.32	43.1	m

3.2. Karyological Features of *S. cilicica* Boiss. & Kotschy

Chromosome number: 2n=2x=22

Karyotype formula: 9m+2sm

This species is endemic to the Mediterrean region of Turkey. It consists of nine metacentric and two submetacentric chromosomes (Figure 3.2.1., Table 3.2.). The length of chromosomes varies from 2.14 µm to 1.05 µm (Table 3.2.). No satellite was observed on this species. The karyotypic formula is represented as 9m+2sm (Table 3.2.). The ratio of the longest to the shortest chromosome is 2.0:1 and the karyotype symmetry is type 2B. Total haploid length of the species is 16.21 µm.

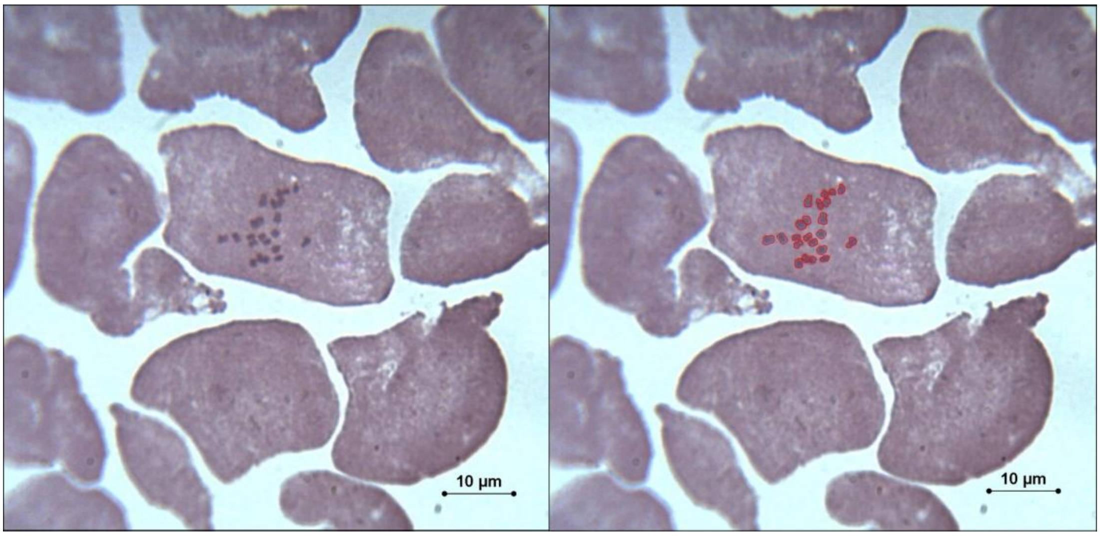

Figure 3.2.1. Mitotic metaphase chromosomes of *S. cilicica*

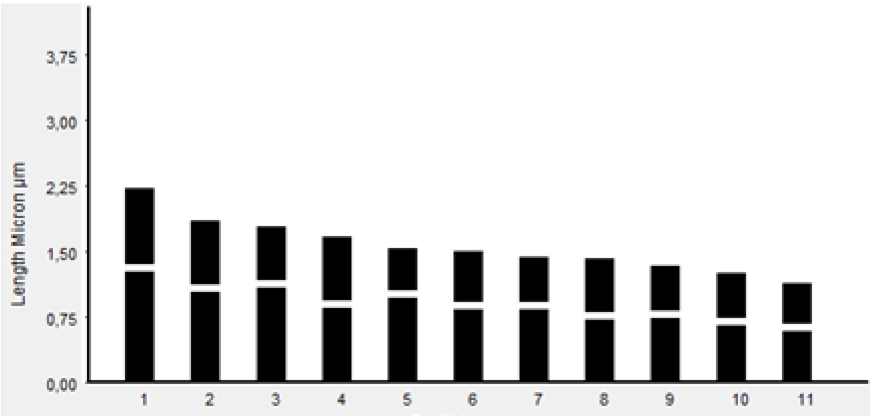

Figure 3.2.2. Haploid Idiogram of *S. cilicica*

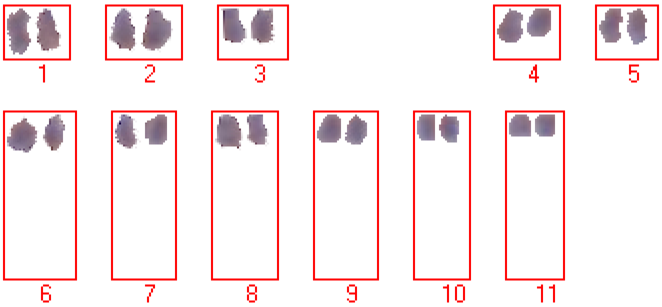

Figure 3.2.3. Karyotype of *S. cilicica*

Chromosome I: It is a metacentric chromosome and longest chromosome of the species. Total length of the chromosome is 2.14 μm, long arm of the chromosome is 1.27 μm, short arm of the chromosome is 0.87 μm. Its arm ratio is 1.46, centromeric index is 40.6 and relative length is 13.22.

Chromosome II: It is a metacentric chromosome. Total length of the chromosome is 1.77 μm, long arm of the chromosome is 1.03 μm, short arm of the chromosome is 0.74 μm. Its arm ratio is 1.38 centromeric index is 41.5 and relative length is 10.95.

Chromosome III: It is a submetacentric chromosome. Total length of the chromosome is 1.70 μm, long arm of the chromosome is 1.08 μm, short arm of the chromosome is 0.62 μm. Its arm ratio is 1.75, centromeric index is 36.4 and relative length is 10.52.

Chromosome IV: It is a metacentric chromosome. Total length of the chromosome is 1.58 μm, long arm of the chromosome is 0.85 μm, short arm of the chromosome is 0.73 μm. Its arm ratio is 1.16, centromeric index is 46.2 and relative length is 9.71.

Chromosome V: It is a submetacentric chromosome. Total length of the chromosome is 1.45 μm, long arm of the chromosome is 0.97 μm, short arm of the chromosome is 0.48 μm. Its arm ratio is 2.03, centromeric index is 33.1 and relative length is 8.97.

Chromosome VI: It is a metacentric chromosome. Total length of the chromosome is 1.41 μm, long arm of the chromosome is 0.83 μm, short arm of the chromosome is 0.58 μm. Its arm ratio is 1.42, centromeric index is 41.1 and relative length is 8.67.

Chromosome VII: It is a metacentric chromosome. Total length of the chromosome is 1.35 μm, long arm of the chromosome is 0.84 μm, short arm of the chromosome is 0.51 μm. Its arm ratio is 1.66, centromeric index is 37.7 and relative length is 8.36.

Chromosome VIII: It is a metacentric chromosome. Total length of the chromosome is 1.34 μm, long arm of the chromosome is 0.72 μm, short arm of the chromosome is 0.62 μm. Its arm ratio is 1.16, centromeric index is 46.2 and relative length is 8.26.

Chromosome IX: It is a metacentric chromosome. Total length of the chromosome is 1.25 µm, long arm of the chromosome is 0.74 µm, short arm of the chromosome is 0.51 µm. Its arm ratio is 1.45, centromeric index is 40.8 and relative length is 7.71.

Chromosome X: It is a metacentric chromosome. Total length of the chromosome is 1.16 µm, long arm of the chromosome is 0.65 µm, short arm of the chromosome is 0.51 µm. Its arm ratio is 1.27, centromeric index is 43.9 and relative length is 7.15.

Chromosome XI: It is a metacentric chromosome and it is shortest chromosome. Total length of the chromosome is 1.05 µm, long arm of the chromosome is 0.59 µm, short arm of the chromosome is 0.46 µm. Its arm ratio is 1.28, centromeric index is 43.8 and relative length is 6.48.

The chromosome type, chromosome length, arm ratio, relative length and centromeric index for *S. cilicica* were given in detail in Table 3.2.

Table 3.2. Karyomorphological parameters of *S. cilicica*

Pair no.	**Long arm (L)**	**Short Arm (S)**	**Total length (C)**	**Relative Length**	**Arm Ratio (r=L/S)**	**Centromeric Index CI=100.S/C**	**Chromosome Type**
1	1.27	0.87	2.14	13.22	1.46	40.6	m
2	1.03	0.74	1.77	10.95	1.38	41.5	m
3	1.08	0.62	1.70	10.52	1.75	36.4	sm
4	0.85	0.73	1.58	9.71	1.16	46.2	m
5	0.97	0.48	1.45	8.97	2.03	33.1	sm
6	0.83	0.58	1.41	8.67	1.42	41.1	m
7	0.84	0.51	1.35	8.36	1.66	37.7	m
8	0.72	0.62	1.34	8.26	1.16	46.2	m
9	0.74	0.51	1.25	7.71	1.45	40.8	m
10	0.65	0.51	1.16	7.15	1.27	43.9	m
11	0.59	0.46	1.05	6.48	1.28	43.8	m

3.3. Karyological features of *S. divaricata* Montbret & Aucher

Chromosome number: 2n=2x=14

Karyotype formula: 5m+2sm

This species is endemic to Irano-Turanian region of Turkey. It consists of five metacentric and two submetacentric chromosomes (Figure 3.3.1., Table 3.3.). The length of chromosomes varies from 2.23 µm to 1.27 µm (Table 3.3.). No satellite was observed on this species. The karyotypic formula is represented as 5m+2sm (Table 3.3.). The ratio of the longest to the shortest chromosome is 1.7:1 and the karyotype symmetry is type 2A. Total haploid length of the species is 12.28 µm. This is the first report on the chromosome number and morphology of this species. The basic chromosome number of this species is x=7.

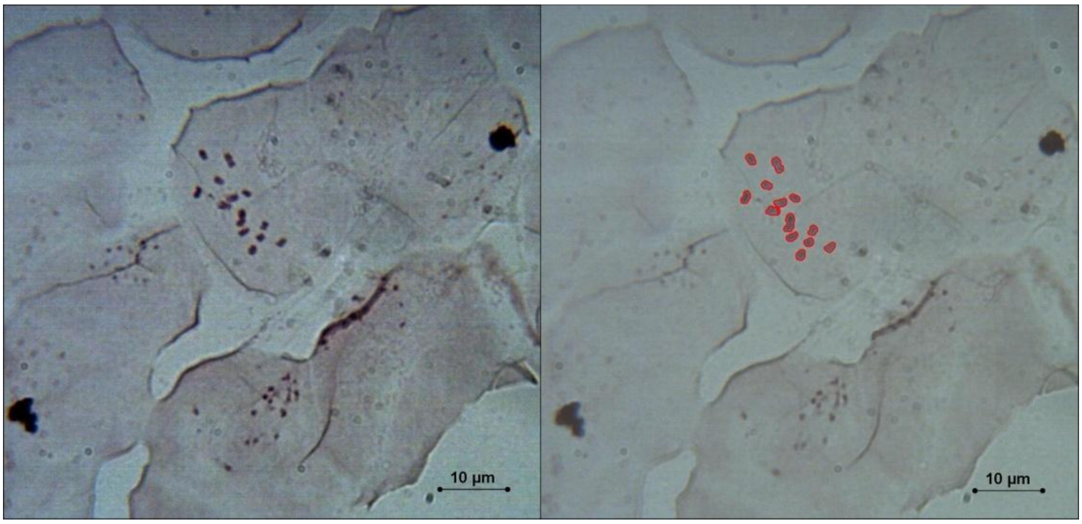

Figure 3.3.1. Mitotic metaphase chromosomes of *S. divaricata*

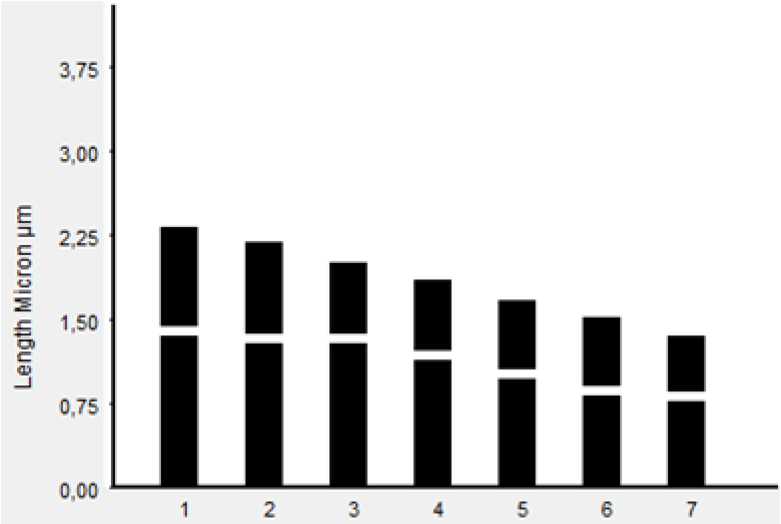

Figure 3.3.2. Haploid Idiogram of *S. divaricata*

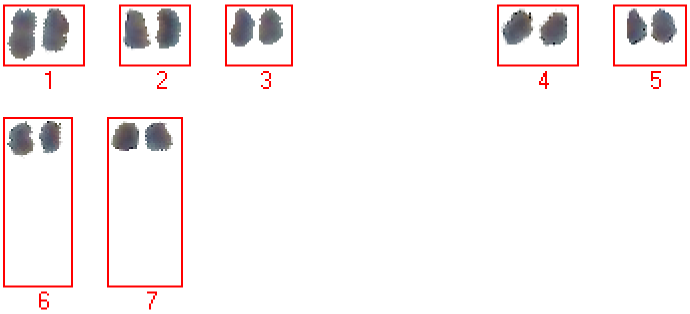

Figure 3.3.3. Karyotype of *S. divaricata*

Chromosome I: It is a metacentric chromosome and longest chromosome of the species. Total length of the chromosome is 2.23 µm, long arm of the chromosome is 1.35 µm, short arm of the chromosome is 0.88 µm. Its arm ratio is 1.54, centromeric index is 39.46 and relative length is 18.14.

Chromosome II: It is a metacentric chromosome. Total length of the chromosome is 2.10 µm, long arm of the chromosome is 1.28 µm, short arm of the chromosome is 0.82 µm. Its arm ratio is 1.57 centromeric index is 39.04 and relative length is 17.06.

Chromosome III: It is a submetacentric chromosome. Total length of the chromosome is 1.91 µm, long arm of the chromosome is 1.28 µm, short arm

of the chromosome is 0.63 μm. Its arm ratio is 2.04, centromeric index is 32.90 and relative length is 15.60.

Chromosome IV: It is a submetacentric chromosome. Total length of the chromosome is 1.76 μm, long arm of the chromosome is 1.13 μm, short arm of the chromosome is 0.63 μm. Its arm ratio is 1.79, centromeric index is 35.79 and relative length is 14.29.

Chromosome V: It is a metacentric chromosome. Total length of the chromosome is 1.58 μm, long arm of the chromosome is 0.96 μm, short arm of the chromosome is 0.62 μm. Its arm ratio is 1.55, centromeric index is 39.24 and relative length is 12.87.

Chromosome VI: It is a metacentric chromosome. Total length of the chromosome is 1.44 μm, long arm of the chromosome is 0.82 μm, short arm of the chromosome is 0.62 μm. Its arm ratio is 1.31, centromeric index is 43.05 and relative length is 11.69.

Chromosome VII: It is a metacentric chromosome. Total length of the chromosome is 1.27 μm, long arm of the chromosome is 0.77 μm, short arm of the chromosome is 0.50 μm. Its arm ratio is 1.54, centromeric index is 39.34 and relative length is 10.34.

The chromosome type, chromosome length, arm ratio, relative length and centromeric index for *S. divaricata* were given in detail in Table 3.3.

Table 3.3. Karyomorphological parameters of *S. divaricata*

Pair no.	Long Arm (L)	Short Arm (S)	Total length (C)	Relative Length	Arm Ratio (r=L/S)	Centromeric Index CI=100.S/C	Chromosome Type
1	1.35	0.88	2.23	18.14	1.54	39.46	m
2	1.28	0.82	2.10	17.06	1.57	39.04	m
3	1.28	0.63	1.91	15.60	2.04	32.9	sm
4	1.13	0.63	1.76	14.29	1.79	35.79	sm
5	0.96	0.62	1.58	12.87	1.55	39.24	m
6	0.82	0.62	1.44	11.69	1.31	43.05	m
7	0.77	0.50	1.27	10.34	1.54	39.37	m

3.4. Karyological features of *S. euphratica* Montbret & Aucher var. *leiocalycina* (Rech. fil.) Hedge

Chromosome number: 2n=2x=14

Karyotype formula: 4m+3sm

This species is endemic to the Irano-Turanian region of Turkey. It consists of four metacentric and three submetacentric chromosomes (Figure 3.4.1, Table 3.4.). The length of chromosomes varies from 3.69 to 2.07 (Table 3.4.). No satellite was observed on this species. The karyotypic formula is represented as 4m+3sm (Table 3.4.). The ratio of the longest to the shortest chromosome is 1.7:1 and the karyotype symmetry is 2A. Total haploid length of the species is 19.60 µm. This is the first report on the chromosome number and morphology of this taxon.

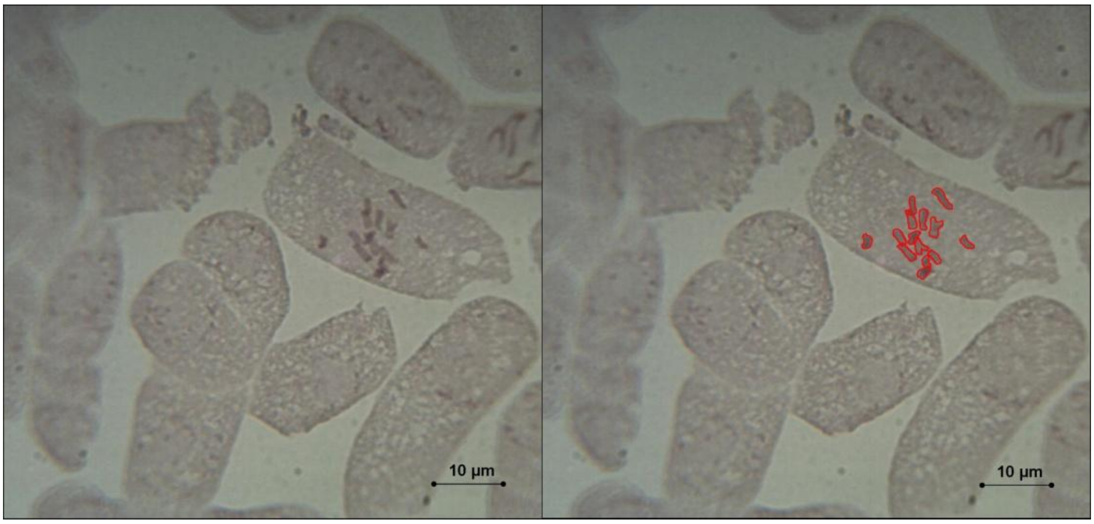

Figure 3.4.1. Mitotic metaphase chromosomes of *S. euphratica* var. *leiocalycina*

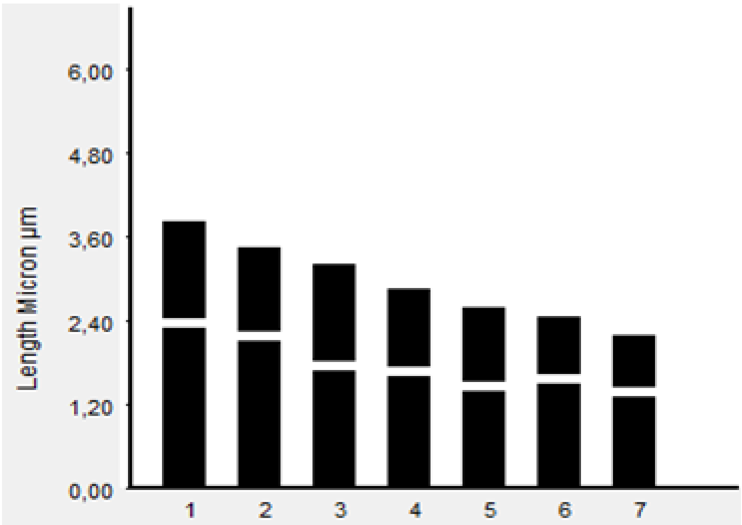

Figure 3.4.2. Haploid Idiogram of *S. euphratica* var. *leiocalycina*

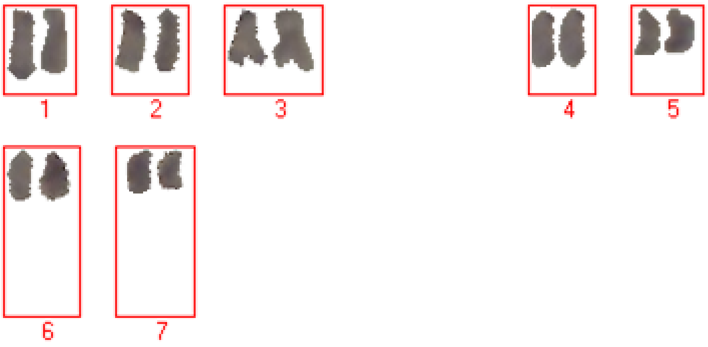

Figure 3.4.3. Karyotype of *S. euphratica* var. *leiocalycina*

Chromosome I: It is a metacentric chromosome and longest chromosome of the species. Total length of the chromosome is 3.69 µm, long arm of the chromosome is 2.29 µm, short arm of the chromosome is 1.39 µm. Its arm ratio is 1.65, centromeric index is 37.66 and relative length is 18.80.

Chromosome II: It is a submetacentric chromosome. Total length of the chromosome is 3.32 µm, long arm of the chromosome is 2.11 µm, short arm of the chromosome is 1.21 µm. Its arm ratio is 1.75 centromeric index is 36.44 and relative length is 16.96.

Chromosome III: It is a metacentric chromosome. Total length of the chromosome is 3.05 µm, long arm of the chromosome is 1.67 µm, short arm of the chromosome is 1.39 µm. Its arm ratio is 1.21, centromeric index is 45.5 and relative length is 15.59.

Chromosome IV: It is a metacentric chromosome. Total length of the chromosome is 2.72 µm, long arm of the chromosome is 1.59 µm, short arm of the chromosome is 1.13 µm. Its arm ratio is 1.41, centromeric index is 41.5 and relative length is 13.88.

Chromosome V: It is a metacentric chromosome. Total length of the chromosome is 2.44 µm, long arm of the chromosome is 1.38 µm, short arm of the chromosome is 1.06 µm. Its arm ratio is 1.30, centromeric index is 43.4 and relative length is 12.44.

Chromosome VI: It is a submetacentric chromosome. Total length of the chromosome is 2.31 µm, long arm of the chromosome is 1.49 µm, short arm

of the chromosome is 0.82 μm. Its arm ratio is 1.81, centromeric index is 35.4 and relative length is 11.78.

Chromosome VII: It is a metacentric chromosome. Total length of the chromosome is 2.07 μm, long arm of the chromosome is 1.31 μm, short arm of the chromosome is 0.76 μm. Its arm ratio is 1.74, centromeric index is 36.7 and relative length is 10.56.

The chromosome type, chromosome length, arm ratio, relative length and centromeric index for *S. euphratica* var. *leiocalycina* were given in detail in Table 3.4.

Table 3.4. Karyomorphological parameters of *S. euphratica var. leiocalycina*

Pair no.	Long arm (L)	Short Arm (S)	Total length (C)	Relative Length	Arm Ratio (r=L/S)	Centromeric Index CI=100.S/C	Chromosome Type
1	2.29	1.39	3.69	18.80	1.65	37.6	m
2	2.11	1.21	3.32	16.96	1.75	36.4	sm
3	1.67	1.39	3.05	15.59	1.21	45.5	m
4	1.59	1.13	2.72	13.88	1.41	41.5	m
5	1.38	1.06	2.44	12.42	1.30	43.4	m
6	1.49	0.82	2.31	11.78	1.81	35.4	sm
7	1.31	0.76	2.07	10.56	1.74	36.7	sm

3.5. Karyological Features of *S. hypargeia* Fisch. & Mey.

Chromosome number: 2n=2x=22

Karyotype formula: 9m+2sm

This species is endemic to the Irano-Turanian region of Turkey. It consists of nine metacentric and two submetacentric chromosomes (Figure 3.5.1., Table 3.5.). The length of chromosomes varies from 2.12 µm to 0.83 µm (Table 3.5.). No satellite was observed on this species. The karyotypic formula is represented as 9m+2sm (Table 3.5.). The ratio of the longest to the shortest chromosome is 2.5:1 and karyotype symmetry is type 2B. Total haploid length of the species is 14.21 µm.

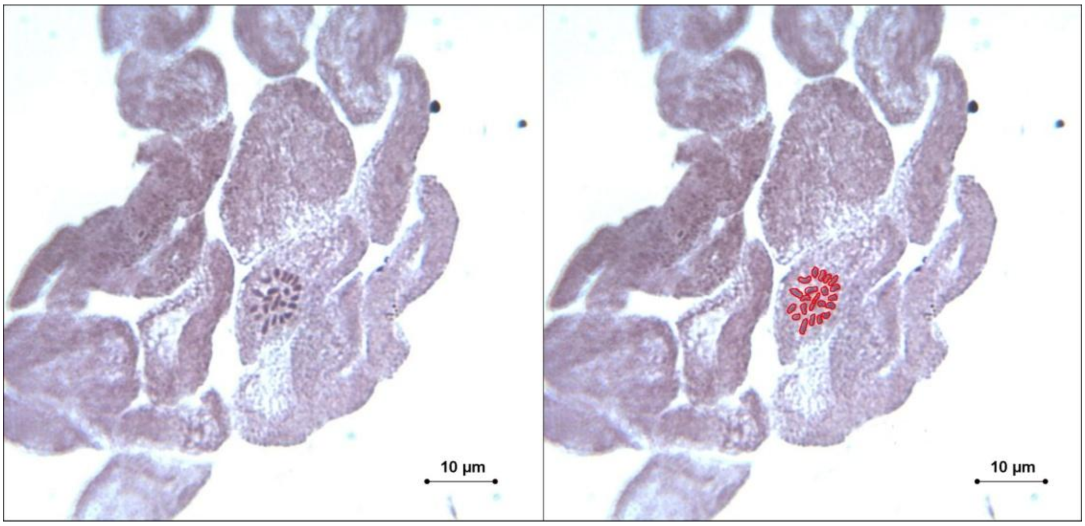

Figure 3.5.1. Mitotic metaphase chromosomes of *S. hypargeia*

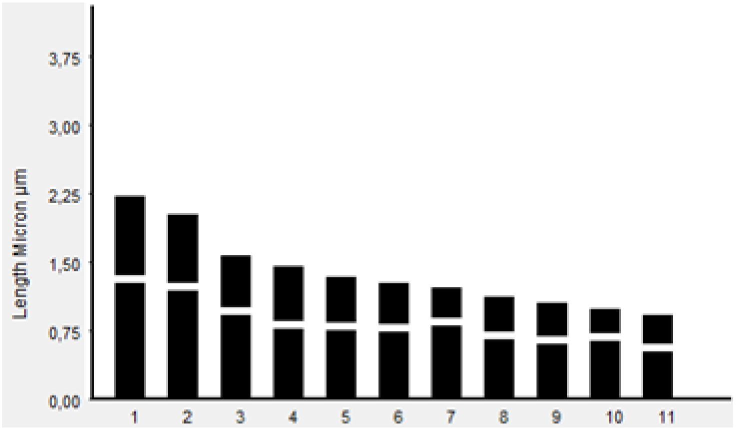

Figure 3.5.2. Haploid Idiogram of *S. hypargeia*

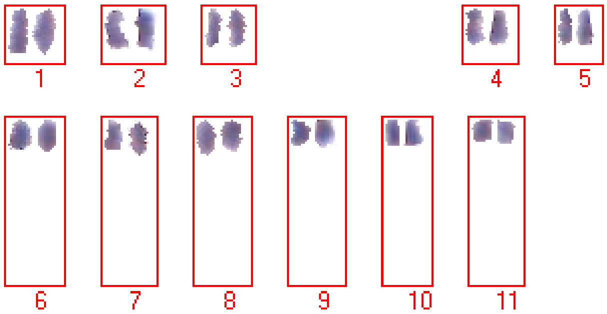

Figure 3.5.3. Karyotype of *S. hypargeia*

Chromosome I: It is a metacentric chromosome and longest chromosome of the species. Total length of the chromosome is 2.12 µm, long arm of the chromosome is 1.26 µm, short arm of the chromosome is 0.86 µm. Its arm ratio is 1.46, centromeric index is 40.5 and relative length is 14.88.

Chromosome II: It is a metacentric chromosome. Total length of the chromosome is 1.94 µm, long arm of the chromosome is 1.19 µm, short arm of the chromosome is 0.75 µm. Its arm ratio is 1.59 centromeric index is 38.6 and relative length is 13.65.

Chromosome III: It is a metacentric chromosome. Total length of the chromosome is 1.48 µm, long arm of the chromosome is 0.91 µm, short arm of the chromosome is 0.57 µm. Its arm ratio is 1.60, centromeric index is 38.5 and relative length is 10.41.

Chromosome IV: It is a metacentric chromosome. Total length of the chromosome is 1.36 μm, long arm of the chromosome is 0.76 μm, short arm of the chromosome is 0.60 μm. Its arm ratio is 1.27, centromeric index is 44.1 and relative length is 9.60.

Chromosome V: It is a metacentric chromosome. Total length of the chromosome is 1.25 μm, long arm of the chromosome is 0.75 μm, short arm of the chromosome is 0.50 μm. Its arm ratio is 1.50, centromeric index is 40.0 and relative length is 8.79.

Chromosome VI: It is a metacentric chromosome. Total length of the chromosome is 1.20 μm, long arm of the chromosome is 0.74 μm, short arm of the chromosome is 0.45 μm. Its arm ratio is 1.64, centromeric index is 37.5 and relative length is 8.44.

Chromosome VII: It is a submetacentric chromosome. Total length of the chromosome is 1.13 μm, long arm of the chromosome is 0.80 μm, short arm of the chromosome is 0.33 μm. Its arm ratio is 2.40, centromeric index is 28.9 and relative length is 8.02.

Chromosome VIII: It is a metacentric chromosome.Total length of the chromosome is 1.04 μm, long arm of the chromosome is 0.65 μm, short arm of the chromosome is 0.39 μm. Its arm ratio is 1.69, centromeric index is 37.5 and relative length is 7.28.

Chromosome IX: It is a metacentric chromosome. Total length of the chromosome is 0.97 μm, long arm of the chromosome is 0.60 μm, short arm of the chromosome is 0.36 μm. Its arm ratio is 1.64, centromeric index is 37.1 and relative length is 6.79.

Chromosome X: It is a submetacentric chromosome. Total length of the chromosome is 0.90 μm, long arm of the chromosome is 0,63 μm, short arm of the chromosome is 0.27 μm. Its arm ratio is 2.31, centromeric index is 30.0 and relative length is 6.30.

Chromosome XI: It is a metacentric chromosome and it is shortest chromosome. Total length of the chromosome is 0.83 μm, long arm of the chromosome is 0.51 μm, short arm of the chromosome is 0.32 μm. Its arm ratio is 1.59, centromeric index is 38.5 and relative length is 5.84.

The chromosome type, chromosome length, arm ratio, relative length and centromeric index for *S. hypargeia* were given in detail in Table 3.5.

Table 3.5. Karyomorphological parameters of *S. hypargeia*

Pair no.	Long arm (L)	Short Arm (S)	Total length (C)	Relative Length	Arm Ratio (r=L/S)	Centromeric Index CI=100.S/C	Chromosome Type
1	1.26	0.86	2.12	14.88	1.46	40.5	m
2	1.19	0.75	1.94	13.65	1.59	38.6	m
3	0.91	0.57	1.48	10.41	1.60	38.5	m
4	0.76	0.60	1.36	9.60	1.27	44.1	m
5	0.75	0.50	1.25	8.79	1.50	40.0	m
6	0.74	0.45	1.20	8.44	1.64	37.5	m
7	0.80	0.33	1.14	8.02	2.40	28.9	sm
8	0.65	0.39	1.04	7.28	1.69	37.5	m
9	0.60	0.36	0.97	6.79	1.64	37.1	m
10	0.63	0.27	0.90	6.30	2.31	30.0	sm
11	0.51	0.32	0.83	5.84	1.59	38.5	m

3.6. Karyological Features of *S. longipedicellata* Hedge

Chromosome number: 2n=2x=20

Karyotype formula: 6m+4sm

This species is endemic to the Irano-Turanian region of Turkey. It consists of six metacentric and four submetacentric chromosomes (Figure 3.6.1., Table 3.6.). The length of chromosomes varies from 2.18 μm to 0.68 μm (Table 3.6.). No satellite was observed on this species. The karyotypic formula is represented as 6m+4sm (Table 3.6.). The ratio of the longest to the shortest chromosome is 3.2:1 and the karyotype symmetry is type 2B. Total haploid length of the species is 14.48 μm. This is the first report on the chromosome number and morphology of this species.

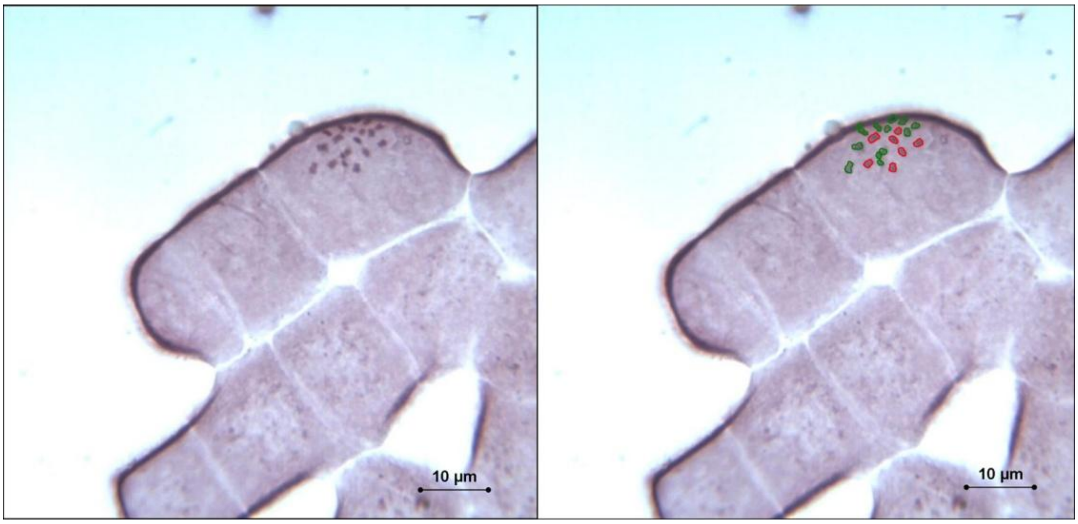

Figure 3.6.1. Mitotic metaphase chromosomes of *S. longipedicellata*

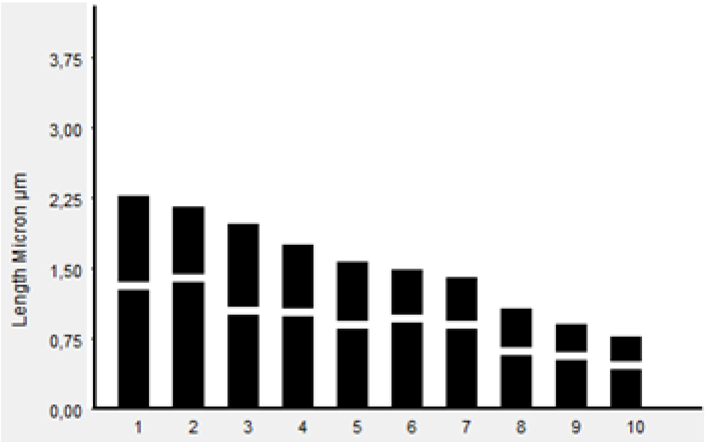

Figure 3.6.2. Haploid Idiogram of *S. longipedicellata*

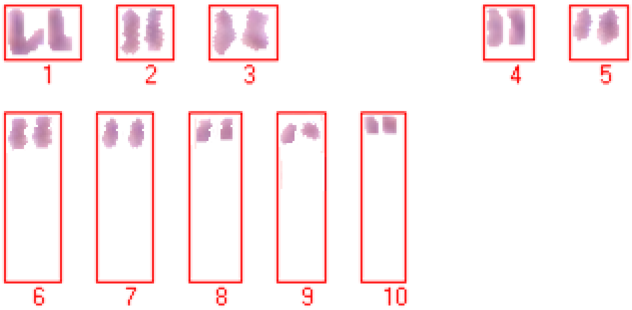

Figure 3.6.3. Karyotype of *S. longipedicellata*

Chromosome I: It is a metacentric chromosome and longest chromosome of the species. Total length of the chromosome is 2.18 µm, long arm of the chromosome is 1.26 µm, short arm of the chromosome is 0.92 µm. Its arm ratio is 1.37, centromeric index is 42.20 and relative length is 15.08.

Chromosome II: It is a submetacentric chromosome. Total length of the chromosome is 2.07 µm, long arm of the chromosome is 1.35 µm, short arm of the chromosome is 0.72 µm. Its arm ratio is 1.88, centromeric index is 34.78 and relative length is 14.29.

Chromosome III: It is a metacentric chromosome. Total length of the chromosome is 1.88 µm, long arm of the chromosome is 1.00 µm, short arm of the chromosome is 0.88 µm. Its arm ratio is 1.14, centromeric index is 46.8 and relative length is 12.98.

Chromosome IV: It is a metacentric chromosome.Total length of the chromosome is 1.67 µm, long arm of the chromosome is 0.99 µm, short arm of the chromosome is 0.68 µm. Its arm ratio is 1.46, centromeric index is 40.7 and relative length is 11.53.

Chromosome V: It is a metacentric chromosome.Total length of the chromosome is 1.48 µm, long arm of the chromosome is 0.85 µm, short arm of the chromosome is 0.63 µm. Its arm ratio is 1.36, centromeric index is 42.56 and relative length is 10.25.

Chromosome VI: It is a submetacentric chromosome. Total length of the chromosome is 1.40 µm, long arm of the chromosome is 0.92 µm, short arm of the chromosome is 0.48 µm. Its arm ratio is 1.92, centromeric index is 34.28 and relative length is 9.67.

Chromosome VII: It is a submetacentric chromosome. Total length of the chromosome is 1.32 µm, long arm of the chromosome is 0.85 µm, short arm of the chromosome is 0.47 µm. Its arm ratio is 1.82, centromeric index is 35.6 and relative length is 9.15.

Chromosome VIII: It is a metacentric chromosome. Total length of the chromosome is 0.98 µm, long arm of the chromosome is 0.57 µm, short arm of the chromosome is 0.41 µm. Its arm ratio is 1.39, centromeric index is 41.8 and relative length is 6.77.

Chromosome IX: It is a submetacentric chromosome.Total length of the chromosome is 0.82 µm, long arm of the chromosome is 0.52 µm, short arm of the chromosome is 0.30 µm. Its arm ratio is 1.76, centromeric index is 39.7 and relative length is 5.63.

Chromosome X: It is a metacentric chromosome.Total length of the chromosome is 0.68 µm, long arm of the chromosome is 0,41 µm, short arm of the chromosome is 0.27 µm. Its arm ratio is 1.55, centromeric index is 39.7 and relative length is 4.66.

The chromosome type, chromosome length, arm ratio, relative length and centromeric index for *S. longipedicellata* were given in detail in Table 3.6.

Table 3.6. Karyomorphological parameters of *S. longipedicellata*

Pair no.	Long arm (L)	Short Arm (S)	Total length (C)	Relative Length	Arm Ratio (r=L/S)	Centromeric Index CI=100.S/C	Chromosome Type
1	1.26	0.92	2.18	15.08	1.37	42.20	m
2	1.35	0.72	2.07	14.29	1.88	34.78	sm
3	1.00	0.88	1.88	12.98	1.14	46.80	m
4	0.99	0.68	1.67	11.53	1.46	40.70	m
5	0.85	0.63	1.48	10.25	1.36	42.56	m
6	0.92	0.48	1.40	9.67	1.92	34.28	sm
7	0.85	0.47	1.32	9.15	1.82	35.60	sm
8	0.57	0.41	0.98	6.77	1.39	41.80	m
9	0.52	0.30	0.82	5.63	1.76	36.50	sm
10	0.41	0.27	0.68	4.66	1.55	39.7	m

3.7. Karyological Features *S. napifolia* Jacq.

Chromosome number: 2n=2x=32

Karyotype formula: 11m+5sm

This species is East mediterrean element. It consists of eleven metacentric and five submetacentric chromosomes (Figure 3.7.1., Table 3.7.). The length of chromosomes varies from 2.23 μm to 1.26 μm (Table 3.7.). No satellite was observed on this species. The karyotypic formula is represented as 11m+5sm (Table 3.7.). The ratio of the longest to the shortest chromosome is 1.7:1 and the karyotype symmetry is type 2A. Total haploid length of the species is 28.19 μm.

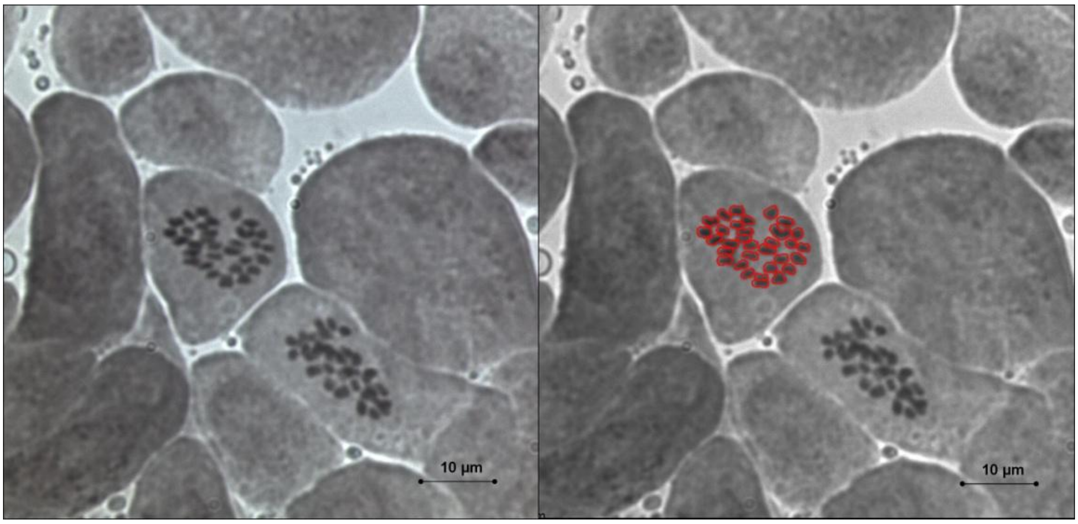

Figure 3.7.1. Mitotic metaphase chromosomes of *S. napifolia*

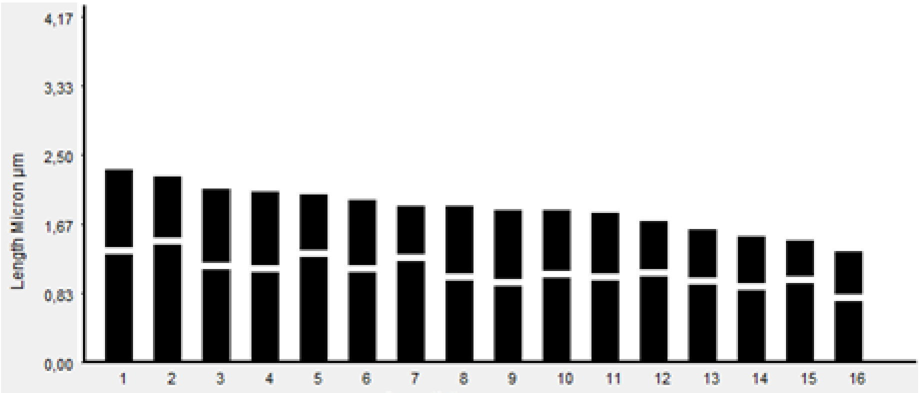

Figure 3.7.2. Haploid Idiogram of *S. napifolia*

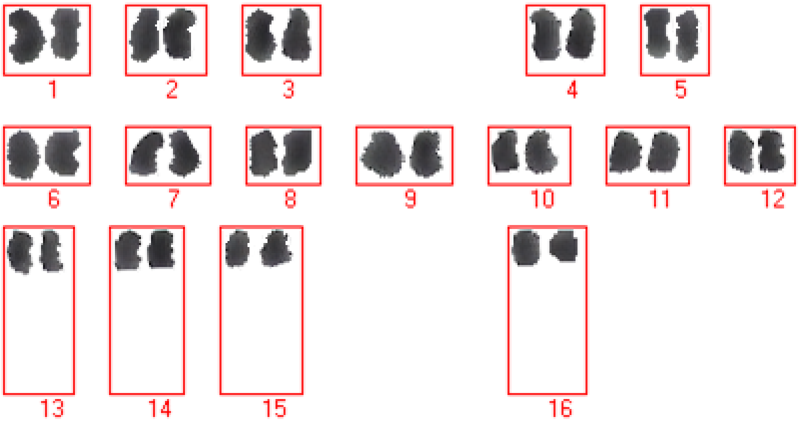

Figure 3.7.3. Karyotype of *S. napifolia*

Chromosome I: It is a metacentric chromosome and longest chromosome of the species. Total length of the chromosome is 2.23 µm, long arm of the chromosome is 1.30 µm, short arm of the chromosome is 0.93 µm. Its arm ratio is 1.40, centromeric index is 41.70 and relative length is 7.93.

Chromosome II: It is a submetacentric chromosome. Total length of the chromosome is 2.15 µm, long arm of the chromosome is 1.41 µm, short arm of the chromosome is 0.74 µm. Its arm ratio is 1.91, centromeric index is 34.41 and relative length is 7.63.

Chromosome III: It is a metacentric chromosome. Total length of the chromosome is 2.00 µm, long arm of the chromosome is 1.12 µm, short arm

of the chromosome is 0.88 µm. Its arm ratio is 1.27, centromeric index is 44.0 and relative length is 7.09.

Chromosome IV: It is a metacentric chromosome. Total length of the chromosome is 1.96 µm, long arm of the chromosome is 1.12 µm, short arm of the chromosome is 0.88 µm. Its arm ratio is 1.22, centromeric index is 44.89 and relative length is 6.94.

Chromosome V: It is a submetacentric chromosome. Total length of the chromosome is 1.93 µm, long arm of the chromosome is 1.27 µm, short arm of the chromosome is 0.66 µm. Its arm ratio is 1.92, centromeric index is 34.19 and relative length is 6.85.

Chromosome VI: It is a metacentric chromosome. Total length of the chromosome is 1.88 µm, long arm of the chromosome is 1.09 µm, short arm of the chromosome is 0.78 µm. Its arm ratio is 1.39, centromeric index is 41.48 and relative length is 6.67.

Chromosome VII: It is a submetacentric chromosome.Total length of the chromosome is 1.81 µm, long arm of the chromosome is 1.22 µm, short arm of the chromosome is 0.59 µm. Its arm ratio is 2.05, centromeric index is 32.59 and relative length is 6.44.

Chromosome VIII: It is a metacentric chromosome.Total length of the chromosome is 1.80 µm, long arm of the chromosome is 0.99 µm, short arm of the chromosome is 0.81 µm. Its arm ratio is 1.21, centromeric index is 45.0 and relative length is 6.39.

Chromosome IX: It is a metacentric chromosome.Total length of the chromosome is 1.75 µm, long arm of the chromosome is 0.92 µm, short arm of the chromosome is 0.83 µm. Its arm ratio is 1.10, centromeric index is 47.44 and relative length is 6.21.

Chromosome X: It is a metacentric chromosome.Total length of the chromosome is 1.75 µm, long arm of the chromosome is 1.01 µm, short arm of the chromosome is 0.74 µm. Its arm ratio is 1.36, centromeric index is 42.28 and relative length is 6.21.

Chromosome XI: It is a metacentric chromosome.Total length of the chromosome is 1.72 µm, long arm of the chromosome is 0.98 µm, short arm

of the chromosome is 0.74 μm. Its arm ratio is 1.32, centromeric index is 43.02 and relative length is 6.08.

Chromosome XII: It is a submetacentric chromosome.Total length of the chromosome is 1.63 μm, long arm of the chromosome is 1.04 μm, short arm of the chromosome is 0.59 μm. Its arm ratio is 1.75, centromeric index is 36.19 and relative length is 5.76.

Chromosome XIII: It is a metacentric chromosome.Total length of the chromosome is 1.52 μm, long arm of the chromosome is 0.93 μm, short arm of the chromosome is 0.59 μm. Its arm ratio is 1.58, centromeric index is 38.80 and relative length is 5.39.

Chromosome XIV: It is a metacentric chromosome.Total length of the chromosome is 1.43 μm, long arm of the chromosome is 0.86 μm, short arm of the chromosome is 0.56 μm. Its arm ratio is 1.53, centromeric index is 39.16 and relative length is 5.07.

Chromosome XV: It is a submetacentric chromosome.Total length of the chromosome is 1.38 μm, long arm of the chromosome is 0.95 μm, short arm of the chromosome is 0.43 μm. Its arm ratio is 2.21, centromeric index is 31.15 and relative length is 4.90.

Chromosome XVI: It is a metacentric chromosome.Total length of the chromosome is 1.26 μm, long arm of the chromosome is 0.74 μm, short arm of the chromosome is 0.51 μm. Its arm ratio is 1.46, centromeric index is 40.47 and relative length is 4.45.

The chromosome type, chromosome length, arm ratio, relative length and centromeric index for *S. napifolia* were given in detail in Table 3.7.

Table 3.7. Karyomorphological parameters of *S. napifolia*

Pair no.	Long Arm (L)	Short Arm (S)	Total length (C)	Relative Length	Arm Ratio (r=L/S)	Centromeric Index CI=100.S/C	Chromosome Type
1	1.30	0.93	2.23	7.93	1.40	41.70	m
2	1.41	0.74	2.15	7.63	1.91	34.41	sm
3	1.12	0.88	2.00	7.09	1.27	44.0	m
4	1.08	0.88	1.96	6.94	1.22	44.89	m
5	1.27	0.66	1.93	6.85	1.92	34.19	sm
6	1.09	0.78	1.88	6.67	1.39	41.48	m
7	1.22	0.59	1.81	6.44	2.05	32.59	sm
8	0.99	0.81	1.80	6.39	1.21	45.0	m
9	0.92	0.83	1.75	6.21	1.10	47.44	m
10	1.01	0.74	1.75	6.21	1.36	42.28	m
11	0.98	0.74	1.72	6.08	1.32	43.02	m
12	1.04	0.59	1.63	5.76	1.75	36.19	sm
13	0.93	0.59	1.52	5.39	1.58	38.80	m
14	0.86	0.56	1.43	5.07	1.53	39.16	m
15	0.95	0.43	1.38	4.90	2.21	31.15	sm
16	0.74	0.51	1.26	4.45	1.46	40.47	m

3.8. Karyological Features of *S. recognita* Fisch. & Mey.

Chromosome number: 2n=2x=14

Karyotype formula: 7m+0sm

This species is endemic to the Irano-Turanian region of Turkey. It consists of seven metacentric chromosomes (Figure 3.8.1., Table 3.8.). The length of chromosomes varies from 2.85 µm to 1.78 µm (Table 3.8.). No satellite was observed on this species. The karyotypic formula is represented as 7m+0sm (Table 3.8.). The ratio of the longest to the shortest chromosome is 1.6:1 and the karyotype symmetry is type 1A. Total haploid length of the species is 16.19 µm.

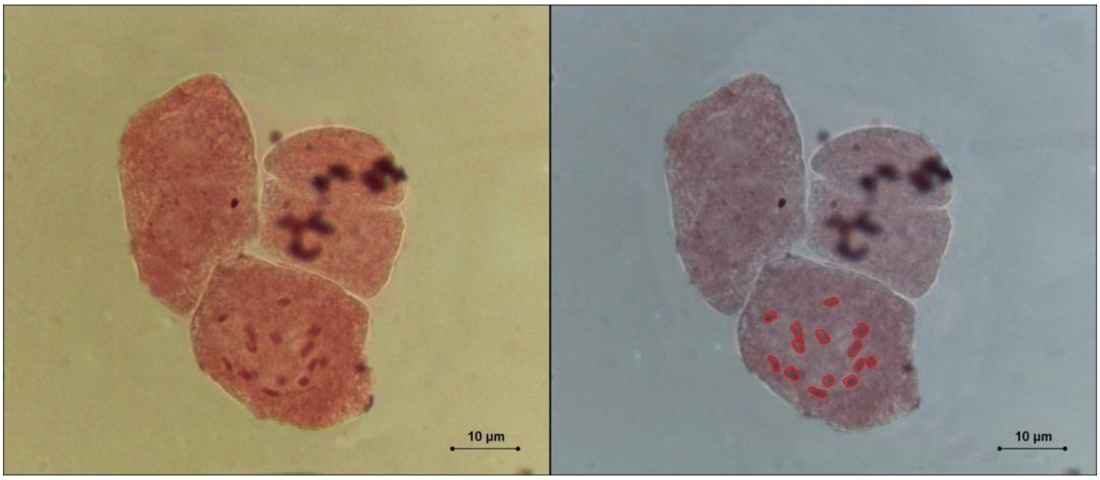

Figure 3.8.1. Mitotic metaphase chromosomes of *S. recognita*

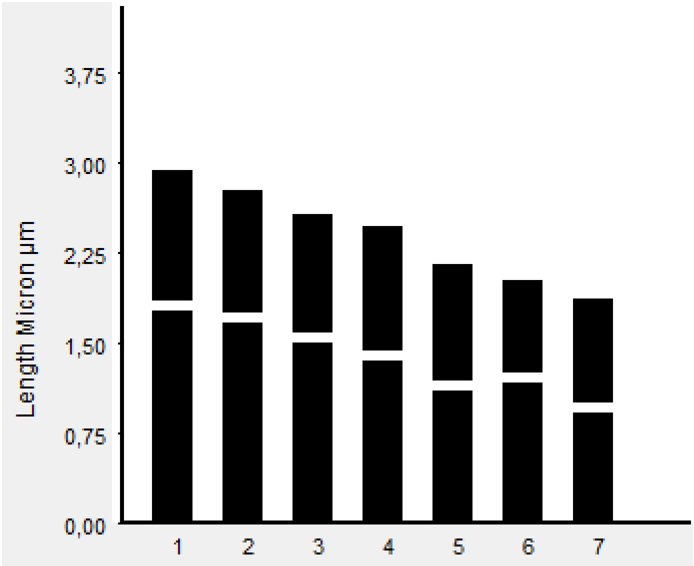

Figure 3.8.2. Haploid Idiogram of *S. recognita*

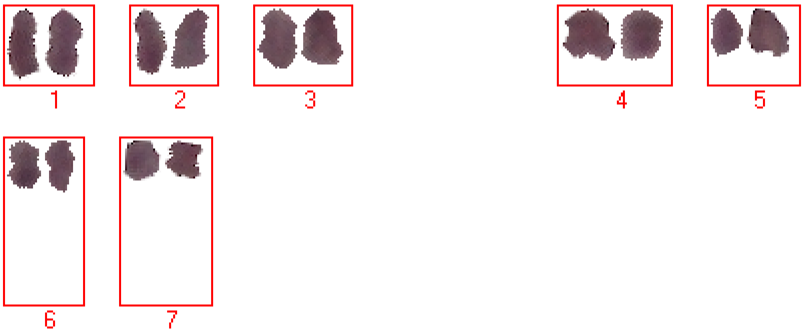

Figure 3.8.3. Karyotype of *S. recognita*

Chromosome I: It is a metacentric chromosome and longest chromosome of the species. Total length of the chromosome is 2.85 µm, long arm of the chromosome is 1.77 µm, short arm of the chromosome is 1.08 µm. Its arm ratio is 1.63, centromeric index is 37.89 and relative length is 17.63.

Chromosome II: It is a metacentric chromosome. Total length of the chromosome is 2.69 µm, long arm of the chromosome is 1.67 µm, short arm of the chromosome is 1.02 µm. Its arm ratio is 1.65 centromeric index is 37.91 and relative length is 16.58.

Chromosome III: It is a metacentric chromosome. Total length of the chromosome is 2.49 µm, long arm of the chromosome is 1.50 µm, short arm

of the chromosome is 0.99 μm. Its arm ratio is 1.51, centromeric index is 39.60 and relative length is 15.44.

Chromosome IV: It is a metacentric chromosome. Total length of the chromosome is 2.39 μm, long arm of the chromosome is 1.35 μm, short arm of the chromosome is 1.04 μm. Its arm ratio is 1.30, centromeric index is 43.51 and relative length is 14.73.

Chromosome V: It is a metacentric chromosome. Total length of the chromosome is 2.06 μm, long arm of the chromosome is 1.10 μm, short arm of the chromosome is 0.96 μm. Its arm ratio is 1.15, centromeric index is 46.60 and relative length is 12.75.

Chromosome VI: It is a metacentric chromosome. Total length of the chromosome is 1.92 μm, long arm of the chromosome is 1.16 μm, short arm of the chromosome is 0.76 μm. Its arm ratio is 1.53, centromeric index is 39.58 and relative length is 11.86.

Chromosome VII: It is a metacentric chromosome. Total length of the chromosome is 1.78 μm, long arm of the chromosome is 0.92 μm, short arm of the chromosome is 0.86 μm. Its arm ratio is 1.06, centromeric index is 48.31 and relative length is 11.02.

The chromosome type, chromosome length, arm ratio, relative length and centromeric index for *S. recognita* were given in detail in Table 3.8.

Table 3.8. Karyomorphological parameters of *S. recognita*

Pair no.	Long arm	Short arm	Total length	Relative Length	Arm Ratio (L/S)	Centromeric Index CI=100.S/C	Chromosome Type
1	1.77	1.08	2.85	17.63	1.63	37.89	m
2	1.67	1.02	2.69	16.58	1.65	37.91	m
3	1.50	0.99	2.49	15.44	1.51	39.60	m
4	1.35	1.04	2.39	14.73	1.30	43.51	m
5	1.10	0.96	2.06	12.75	1.15	46.60	m
6	1.16	0.76	1.92	11.86	1.53	39.58	m
7	0.92	0.86	1.78	11.02	1.06	48.31	m

3.9. Karyological Features of *S. rosifolia* Sm.

Chromosome number: 2n=2x=14

Karyotype formula: 2m+5sm

This species is endemic to the Irano-Turanian region of Turkey. It consists of two metacentric chromosomes and five submetacentric chromosomes (Figure 3.9.1., Table 3.9.). The length of chromosomes varies from 2.14 μm to 1.30 μm (Table 3.9.). No satellite was observed on this species. B chromosome was observed in this species. The karyotypic formula is represented as 5m+2sm (Table 3.9.). The ratio of the longest to the shortest chromosome is 1.6:1 and the karyotype symmetry is type 3A. Total haploid length of the species is 11.79 μm. The length of B chromosome was found as 1.02 μm.

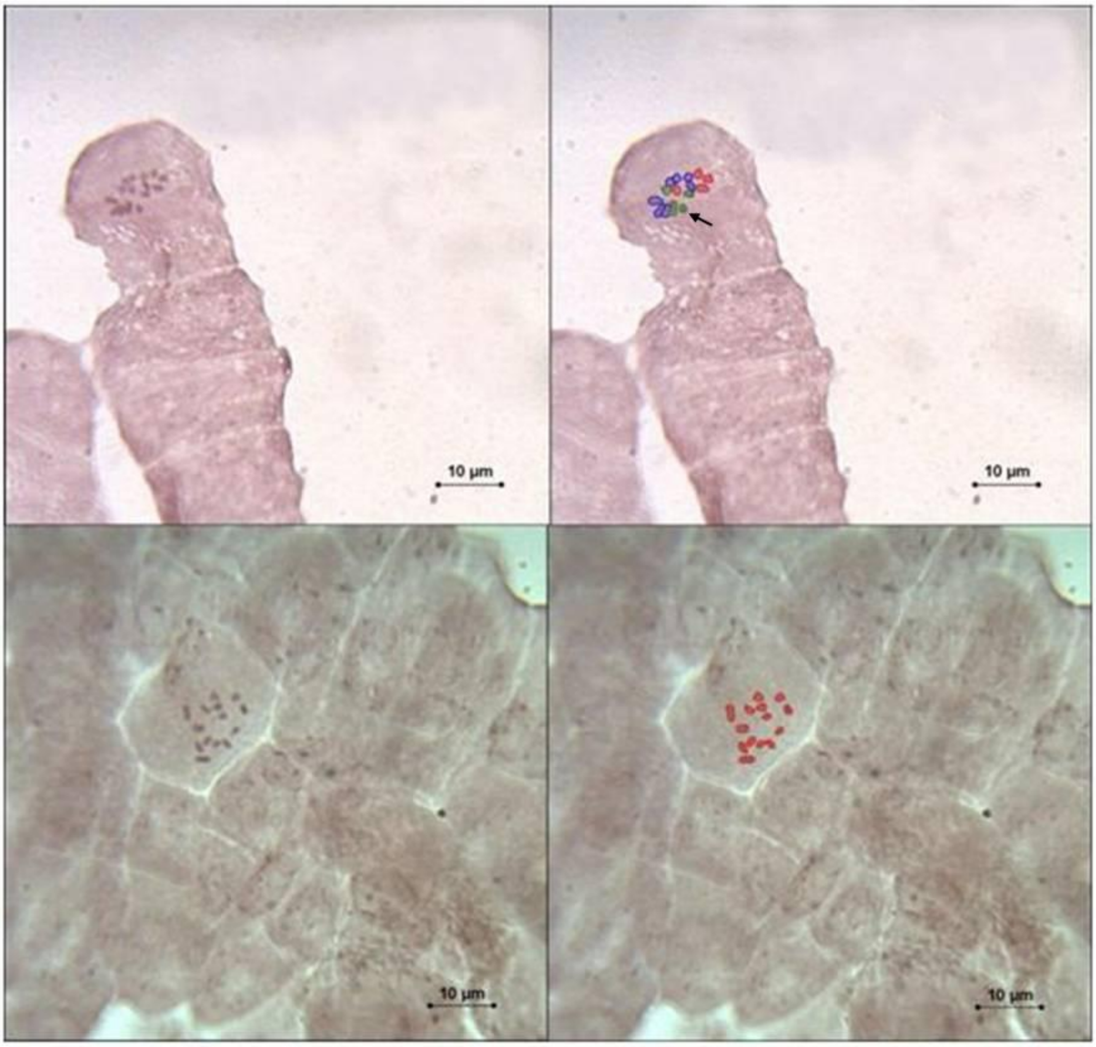

Figure 3.9.1. Mitotic metaphase chromosomes of *S. rosifolia.* Arrow indicates B chromosome.

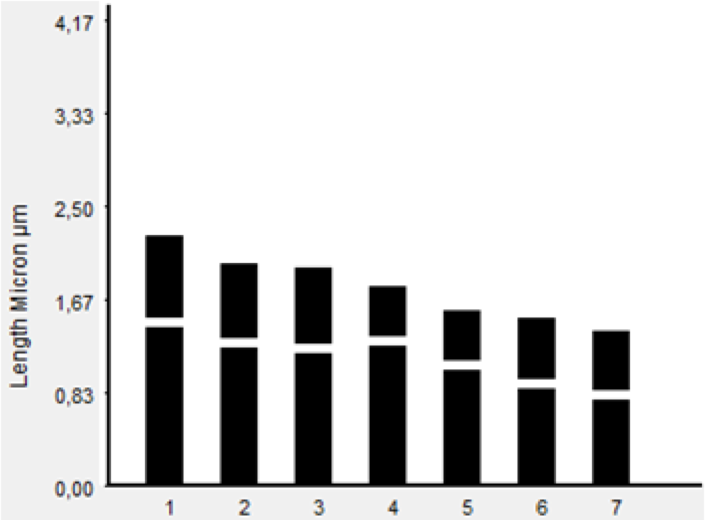

Figure 3.9.2. Haploid Idiogram of *S. rosifolia*

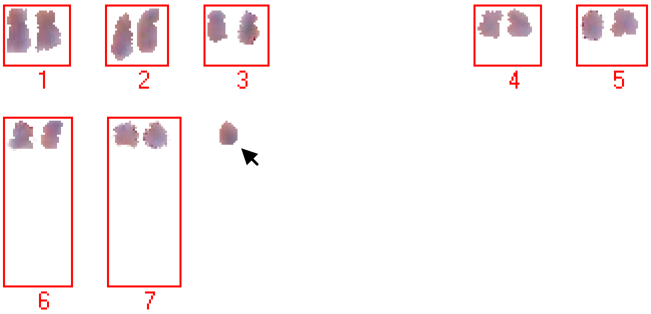

Figure 3.9.3. Karyotype of *S. rosifolia*

Chromosome I: It is a submetacentric chromosome and longest chromosome of the species. Total length of the chromosome is 2.14 µm, long arm of the chromosome is 1.41 µm, short arm of the chromosome is 0.74 µm. Its arm ratio is 1.90, centromeric index is 34.57 and relative length is 18.18.

Chromosome II: It is a submetacentric chromosome. Total length of the chromosome is 1.89 µm, long arm of the chromosome is 1.23 µm, short arm of the chromosome is 0.66 µm. Its arm ratio is 1.87 centromeric index is 34.92 and relative length is 16.07.

Chromosome III: It is a submetacentric chromosome. Total length of the chromosome is 1.86 µm, long arm of the chromosome is 1.18 µm, short arm

of the chromosome is 0.68 μm. Its arm ratio is 1.74, centromeric index is 36.55 and relative length is 15.77.

Chromosome IV: It is a submetacentric chromosome. Total length of the chromosome is 1.70 μm, long arm of the chromosome is 1.25 μm, short arm of the chromosome is 0.45 μm. Its arm ratio is 2.77, centromeric index is 26.47 and relative length is 14.37.

Chromosome V: It is a submetacentric chromosome. Total length of the chromosome is 1.49 μm, long arm of the chromosome is 1.04 μm, short arm of the chromosome is 0.45 μm. Its arm ratio is 2.31, centromeric index is 30.20 and relative length is 12.63.

Chromosome VI: It is a metacentric chromosome. Total length of the chromosome is 1.42 μm, long arm of the chromosome is 0.86 μm, short arm of the chromosome is 0.55 μm. Its arm ratio is 1.57, centromeric index is 38.73 and relative length is 12.00.

Chromosome VII: It is a metacentric chromosome. Total length of the chromosome is 1.30 μm, long arm of the chromosome is 0.77 μm, short arm of the chromosome is 0.53 μm. Its arm ratio is 1.44, centromeric index is 40.76 and relative length is 10.98.

The chromosome type, chromosome length, arm ratio, relative length and centromeric index for *S. rosifolia* were given in detail in Table 3.10.

Table 3.9. Karyomorphological parameters of *S. rosifolia*

Pair no.	Long arm (L)	Short arm (S)	Total length	Relative Length (%)	Arm Ratio (r=L/S)	Centromeric Index CI=100.S/C	Chromosome Type
1	1.41	0.74	2.14	18.18	1.90	34.57	sm
2	1.23	0.66	1.89	16.07	1.87	34.92	sm
3	1.18	0.68	1.86	15.77	1.74	36.55	sm
4	1.25	0.45	1.70	14.37	2.77	26.47	sm
5	1.04	0.45	1.49	12.63	2.31	30.20	sm
6	0.86	0.55	1.42	12.00	1.57	38.73	m
7	0.77	0.53	1.30	10.98	1.44	40.76	m

3.10. Karyological Features of *S. vermifolia* Hedge & Hub.-Mor.

Chromosome number: 2n=2x=20

Karyotype formula: 7m+3sm

This species is endemic to the Irano-Turanian region of Turkey. It consists of seven metacentric and three submetacentric chromosomes (Figure 3.10.1., Table 3.10.). The length of chromosomes varies from 1.90 μm to 0.85 μm (Table 3.10.). No satellite was observed on this species. The karyotypic formula is represented as 7m+3sm (Table 3.10.). The ratio of the longest to the shortest chromosome is 2.2:1 and the karyotype symmetry is type 2B. Total haploid length of the species is 14.70 μm. This is the first report on the chromosome number and morphology of this species.

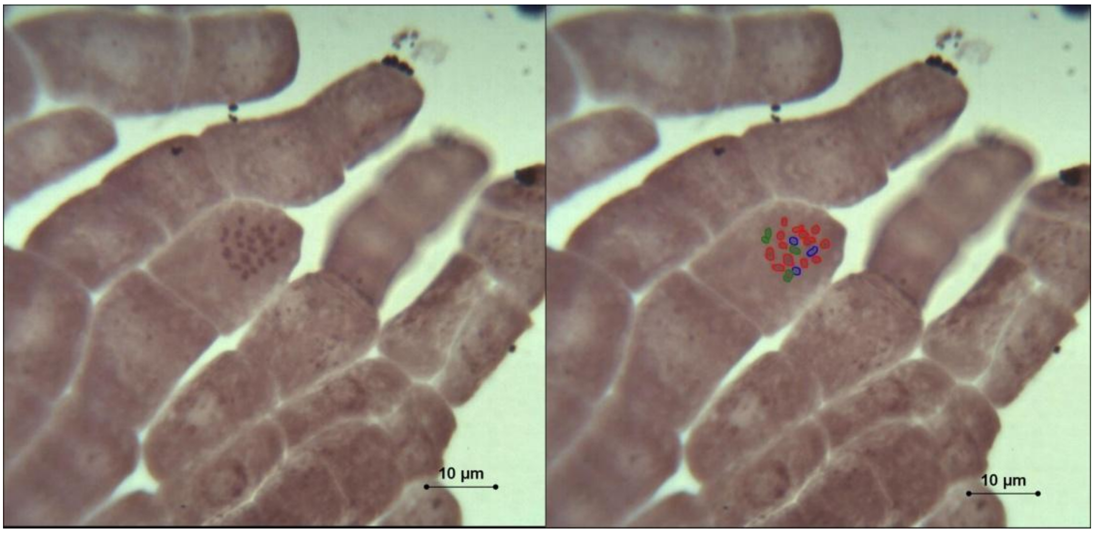

Figure 3.10.1. Mitotic metaphase chromosomes of *S. vermifolia*

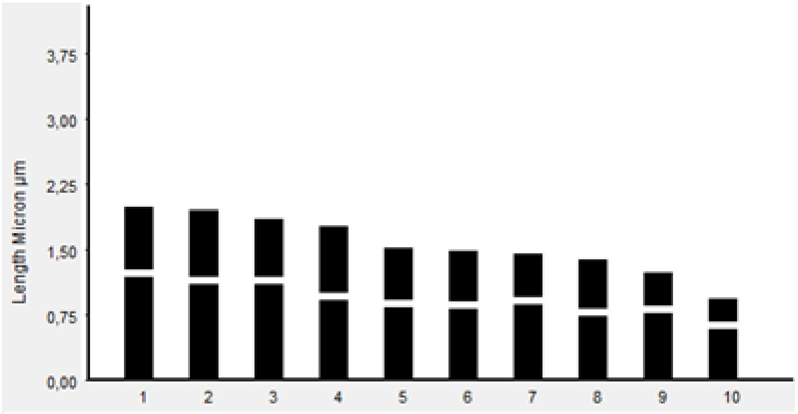

Figure 3.10. 2. Haploid Idiogram of *S. vermifolia*

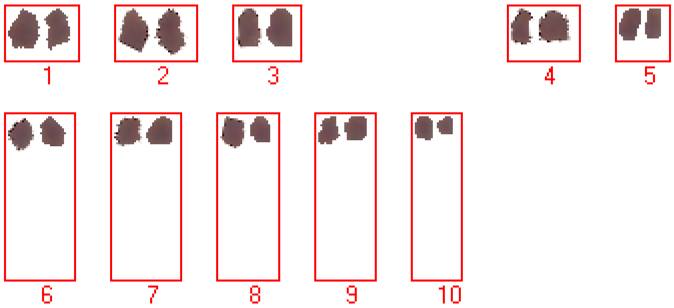

Figure 3.10.3. Karyotype of *S. vermifolia*

Chromosome I: It is a metacentric chromosome and longest chromosome of the species. Total length of the chromosome is 1.90 µm, long arm of the chromosome is 1.19 µm, short arm of the chromosome is 0.71 µm. Its arm ratio is 1.67, centromeric index is 37.36 and relative length is 12.89.

Chromosome II: It is a metacentric chromosome. Total length of the chromosome is 1.86 µm, long arm of the chromosome is 1.10 µm, short arm of the chromosome is 0.76 µm. Its arm ratio is 1.45, centromeric index is 40.86 and relative length is 12.65.

Chromosome III: It is a metacentric chromosome.Total length of the chromosome is 1.77 µm, long arm of the chromosome is 1.10 µm, short arm of the chromosome is 0.67 µm. Its arm ratio is 1.64, centromeric index is 37.85 and relative length is 12.04.

Chromosome IV: It is a metacentric chromosome.Total length of the chromosome is 1.67 µm, long arm of the chromosome is 0.91 µm, short arm of the chromosome is 0.76 µm. Its arm ratio is 1.19, centromeric index is 45.50 and relative length is 11.33.

Chromosome V: It is a metacentric chromosome.Total length of the chromosome is 1.44 µm, long arm of the chromosome is 0.84 µm, short arm of the chromosome is 0.60 µm. Its arm ratio is 1.40, centromeric index is 41.66 and relative length is 9.80.

Chromosome VI: It is a metacentric chromosome.Total length of the chromosome is 1.40 µm, long arm of the chromosome is 0.81 µm, short arm of the chromosome is 0.58 µm. Its arm ratio is 1.41, centromeric index is 41.42 and relative length is 9.49.

Chromosome VII: It is a submetacentric chromosome.Total length of the chromosome is 1.36 µm, long arm of the chromosome is 0.86 µm, short arm of the chromosome is 0.50 µm. Its arm ratio is 1.72, centromeric index is 36.76 and relative length is 9.25.

Chromosome VIII: It is a metacentric chromosome.Total length of the chromosome is 1.31 µm, long arm of the chromosome is 0.74 µm, short arm of the chromosome is 0.57 µm. Its arm ratio is 1.30, centromeric index is 43.51 and relative length is 8.91.

Chromosome IX: It is a submetacentric chromosome.Total length of the chromosome is 1.15 µm, long arm of the chromosome is 0.77 µm, short arm of the chromosome is 0.38 µm. Its arm ratio is 2.03, centromeric index is 33.04 and relative length is 7.82.

Chromosome X: It is a submetacentric chromosome.Total length of the chromosome is 0.85 µm, long arm of the chromosome is 0.59 µm, short arm of the chromosome is 0.27 µm. Its arm ratio is 2.23, centromeric index is 31.76 and relative length is 5.82.

The chromosome type, chromosome length, arm ratio, relative length and centromeric index for *S. vermifolia* were given in detail in Table 3.10.

Table 3.10. Karyomorphological parameters of *S. vermifolia*

Pair no.	Long arm (L)	Short Arm (S)	Total length (C)	Relative Length	Arm Ratio (r=L/S)	Centromeric Index CI=100.S/C	Chromosome Type
1	1.19	0.71	1.90	12.89	1.67	37.36	m
2	1.10	0.76	1.86	12.65	1.45	40.86	m
3	1.10	0.67	1.77	12.04	1.64	37.85	m
4	0.91	0.76	1.67	11.33	1.19	45.50	m
5	0.84	0.60	1.44	9.80	1.40	41.66	m
6	0.81	0.58	1.40	9.49	1.41	41.42	m
7	0.86	0.50	1.36	9.25	1.72	36.76	sm
8	0.74	0.57	1.31	8.91	1.30	43.51	m
9	0.77	0.38	1.15	7.82	2.03	33.04	sm
10	0.59	0.27	0.86	5.82	2.23	31.76	sm

3.11. Karyological Features of *S. yosgadensis* Freyn & Bornm.

Chromosome number: 2n=2x=20

Karyotype formula: 9m+1sm

This species is endemic to the Irano-Turanian region of Turkey. It consists of nine metacentric and one submetacentric chromosomes (Figure 3.11.1., Table 3.11.). The length of chromosomes varies from 3.35 μm to 1.38 μm (Table 3.11.). No satellite was observed on this species. The karyotypic formula is represented as 9m+1sm (Table 3.11.). The ratio of the longest to the shortest chromosome is 2.4:1 and the karyotype symmetry is type 2B. Total haploid length of the species is 20.33 μm. This is the first report on the chromosome number and morphology of this species.

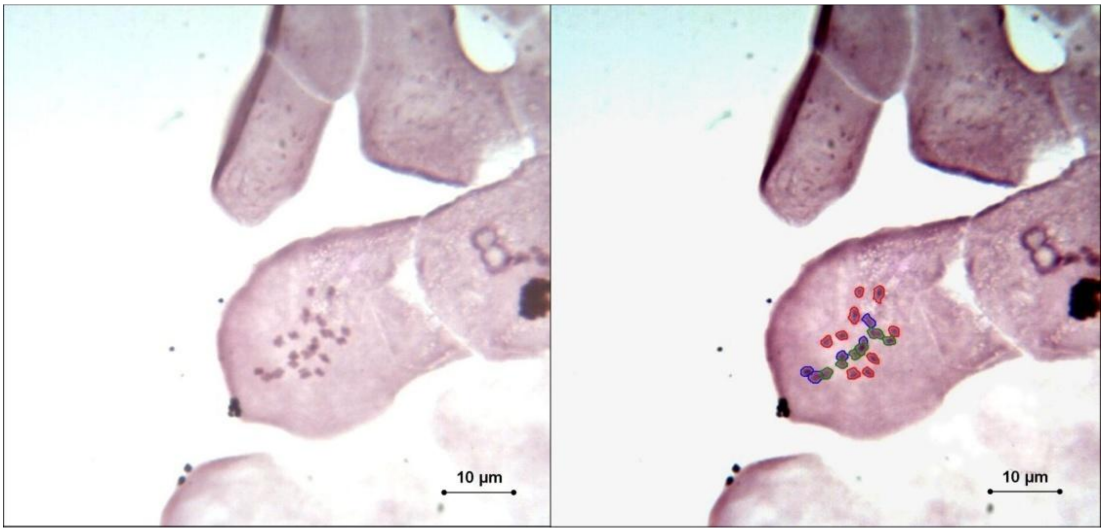

Figure 3.11.1. Mitotic metaphase chromosomes of *S. yosgadensis*

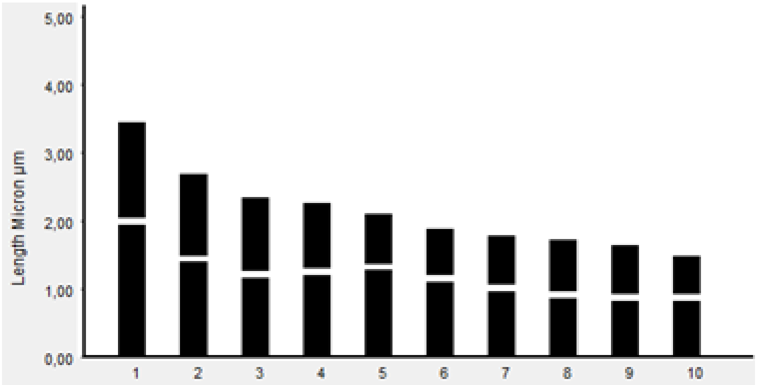

Figure 3.11.2. Haploid Idiogram of *S. yosgadensis*

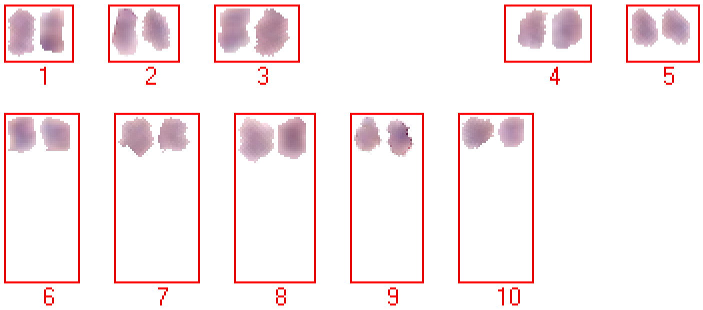

Figure 3.11.3. Karyotype of *S. yosgadensis*

Chromosome I: It is a metacentric chromosome and longest chromosome of the species. Total length of the chromosome is 3.35 µm, long arm of the chromosome is 1.94 µm, short arm of the chromosome is 1.41 µm. Its arm ratio is 1.37, centromeric index is 42.08 and relative length is 16.48.

Chromosome II: It is a metacentric chromosome.Total length of the chromosome is 2.58 µm, long arm of the chromosome is 1.39 µm, short arm of the chromosome is 1.19 µm. Its arm ratio is 1.17, centromeric index is 46.12 and relative length is 12.69.

Chromosome III: It is a metacentric chromosome.Total length of the chromosome is 2.23 µm, long arm of the chromosome is 1.16 µm, short arm of the chromosome is 1.07 µm. Its arm ratio is 1.08, centromeric index is 47.98 and relative length is 10.97.

Chromosome IV: It is a metacentric chromosome.Total length of the chromosome is 2.16 µm, long arm of the chromosome is 1.20 µm, short arm

of the chromosome is 0.96 µm. Its arm ratio is 1.25, centromeric index is 44.44 and relative length is 10.62.

Chromosome V: It is a submetacentric chromosome.Total length of the chromosome is 2.01 µm, long arm of the chromosome is 1.27 µm, short arm of the chromosome is 0.74 µm. Its arm ratio is 1.72, centromeric index is 36.80 and relative length is 9.89.

Chromosome VI: It is a metacentric chromosome.Total length of the chromosome is 1.79 µm, long arm of the chromosome is 1.10 µm, short arm of the chromosome is 0.69 µm. Its arm ratio is 1.59, centromeric index is 38.50 and relative length is 8.80.

Chromosome VII: It is a metacentric chromosome.Total length of the chromosome is 1.68 µm, long arm of the chromosome is 0.96 µm, short arm of the chromosome is 0.72 µm. Its arm ratio is 1.33, centromeric index is 42.85 and relative length is 8.24.

Chromosome VIII: It is a metacentric chromosome.Total length of the chromosome is 1.63 µm, long arm of the chromosome is 0.86 µm, short arm of the chromosome is 0.76 µm. Its arm ratio is 1.14, centromeric index is 46.60 and relative length is 7.99.

Chromosome IX: It is a metacentric chromosome.Total length of the chromosome is 1.54 µm, long arm of the chromosome is 0.82 µm, short arm of the chromosome is 0.72 µm. Its arm ratio is 1.13, centromeric index is 46.75 and relative length is 7.55.

Chromosome X: It is a metacentric chromosome.Total length of the chromosome is 1.38 µm, long arm of the chromosome is 0.82 µm, short arm of the chromosome is 0.56 µm. Its arm ratio is 1.46, centromeric index is 40.57 and relative length is 6.76.

The chromosome type, chromosome length, arm ratio, relative length and centromeric index for *S. yosgadensis* were given in detail in Table 3.11.

Table 3.11. Karyomorphological parameters of *S. yosgadensis.*

Pair no.	Long arm (L)	Short Arm (S)	Total length (C)	Relative Length (%)	Arm Ratio (r=L/S)	Centromeric Index CI=100.S/C	Chromosome Type
1	1.94	1.41	3.35	16.48	1.37	42.08	m
2	1.39	1.19	2.58	12.69	1.17	46.12	m
3	1.16	1.07	2.23	10.97	1.08	47.98	m
4	1.20	0.96	2.16	10.62	1.25	44.44	m
5	1.27	0.74	2.01	9.89	1.72	36.80	sm
6	1.10	0.69	1.79	8.80	1.59	38.50	m
7	0.96	0.72	1.68	8.24	1.33	42.85	m
8	0.86	0.76	1.63	7.99	1.14	46.60	m
9	0.82	0.72	1.54	7.55	1.13	46.75	m
10	0.82	0.56	1.38	6.76	1.46	40.57	m

The summary of all these results are integrated in Table 3.12. As seen in this table, the range of chromosome numbers of studied taxa is between 14 and 32. Also all of these taxa have diploid chromosomes with metacentric and submetacentric chromosome pairs. The karyotypes fall in the Stebbins 1A, 2A 3A or 2B category of asymmetry. Haploid length of the species ranges between 11.79 µm (*S. rosifolia*) - 28.19 (*S. napifolia*) µm.

Table 3.12. Somatic chromosome number, ploidy level, karyotype formula, ranges of chromosome length, haploid chromosome length for *Salvia* species.

Taxon	2n	Ploidy level	Karyotype formula	Chromosome length range (µm)	A1	A2	ST	Haploid chromosome length (µm)
S. aethiopis	22	2x	8m+3sm	2.19-0.95	0.34	0.25	2B	17.12
S. cilicica	22	2x	9m+2sm	2.14-1.05	0.29	0.21	2B	16.12
S. divaricata	14	2x	5m+2sm	2.23-1.27	0.37	0.20	2A	12.28
S. euphratica var. *leiocalycina*	14	2x	4m+3sm	3.69-2.07	0.34	0.20	2A	19.60
S. hypargeia	22	2x	9m+2sm	2.12-0.83	0.39	0.32	2B	14.21
S.longipedicellata	20	2x	6m+4sm	2.18-0.68	0.34	0.35	2B	14.48
S. napifolia	32	2x	11m+5sm	2.23-1.26	0.33	0.15	2A	28.19
S. recognita	14	2x	7m	2.85-1.78	0.27	0.17	1A	16.19
S. rosifolia	14	2x	2m+5sm	2.14-1.30	0.46	0.18	3A	11.79
S. vermifolia	20	2x	7m+3sm	1.90-0.85	0.35	0.23	2B	14.70
S.yosgadensis	20	2x	9m+1sm	3.35-1.38	0.23	0.29	2B	20.33

Abbrevations: A1=Intra-chromosomal asymmetry index, A2= Inter-chromosomal asymmetry index, ST= Stebbins Classes

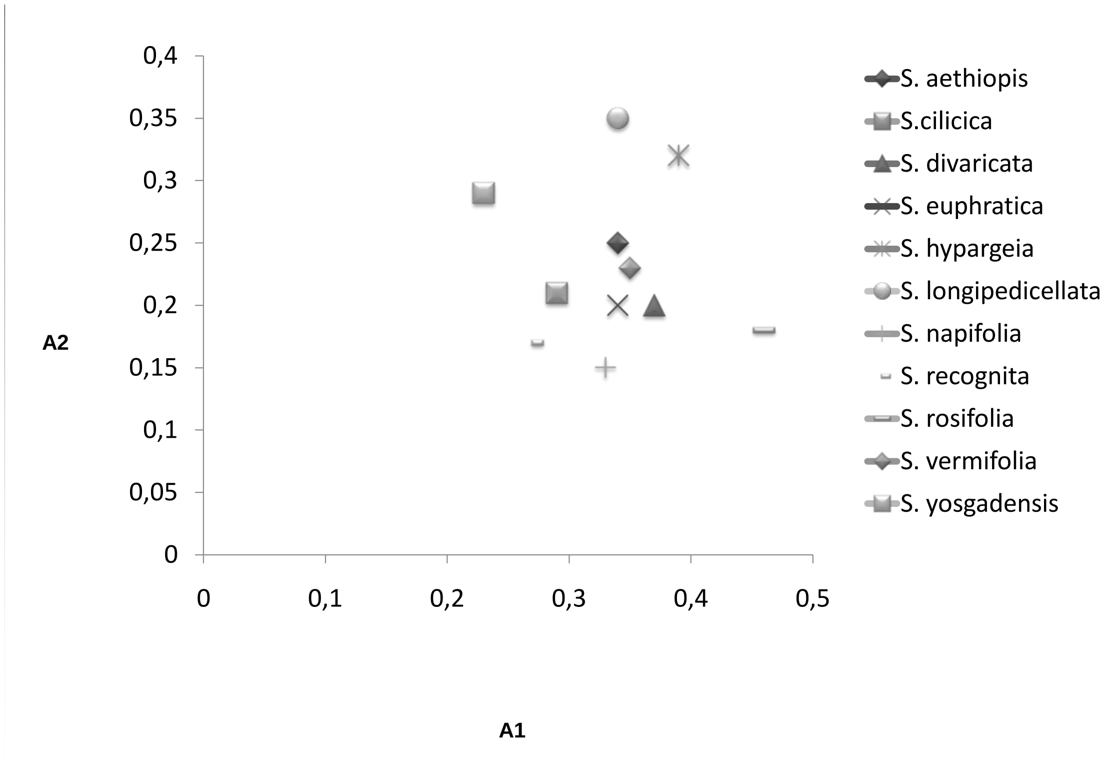

Figure 3.12. Scatter diagram of the Romero Zarco asymmetry indices.

Species were grouped by constructing UPGMA phenogram on the basis of karyotype similarities (Table 3.13, Figure 3.12). We used 6 variables x 11 cases. Variables used in cluster analysis were given in Table 3.13.

Table 3.13. Relative cytological characters used in cluster analysis.

Species	A1	A2	TCL	X	L/S	2n
S. aethiopis	0.34	0.25	17.12	1.55	2.3	22
S. cilicica	0.29	0.21	16.21	1.47	2.03	22
S. divaricata	0.37	0.20	12.28	1.75	1.7	14
S. euphratica* var. *leiocalycina	0.34	0.20	19.60	2.8	1.7	14
S. hypargeia	0.39	0.32	14.21	1.29	2.5	22
S. longipedicellata	0.34	0.35	14.48	1.44	3.2	20
S. napifolia	0.33	0.15	28.19	1.76	1.7	32
S. recognita	0.27	0.17	16.19	2.31	1.6	14
S. rosifolia	0.46	0.18	11.79	1.68	1.6	14
S. vermifolia	0.35	0.23	14.70	1.47	2.2	20
S. yosgadensis	0.23	0.29	20.33	2.03	2.4	20

Abbrevations: A1=Intra-chromosomal asymmetry index, A2= Inter-chromosomal asymmetry index, TCL: Total length of haploid complement, x= Mean chromatin length, L/S=longest/shortest chromosome ratio, 2n= diploid chromosome number.

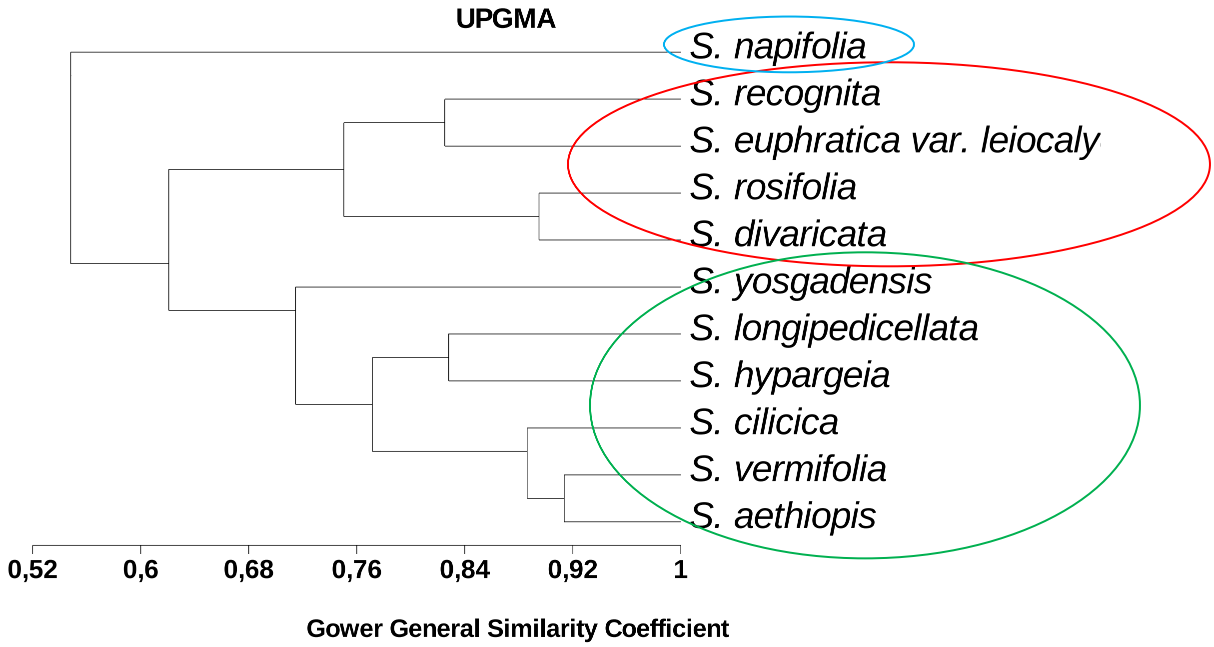

Figure 3.13. Dendogram showing the phenetic relationships among the studied species of *Salvia*, constructed using the matrix of karyotype similarities with UPGMA.

Table 3.14. Data obtained from cluster analysis

Node	Group 1	Group 2	Similarity level	Objects in group
1	*S. aethiopis*	*S. vermifolia*	0,914	2
2	*S. divaricata*	*S. rosifolia*	0,895	2
3	*Node 1*	*S. cilicica*	0,886	3
4	*S. hypargeia*	*S. longipedicellata*	0,828	2
5	*S. euphratica*	*S. recognita*	0,825	2
6	Node 3	Node 4	0,771	5
7	Node 2	Node 5	0,750	4
8	Node 6	*S. yosgadensis*	0,715	6
9	Node 8	Node 7	0,621	10
10	Node 9	*S. napifolia*	0,548	11

Clustering was carried out using Unweighted Pair Group method (UPGMA). As a result of our numerical analysis, the UPGMA clustering analysis has separated specimens under two major groups.

CHAPTER 4

DISCUSSION

Up to now, chromosome studies have been used for many different purposes such as for taxonomic purposes. These kind of studies including the investigation of chromosomal behaviours of both meiosis and mitosis can be used to acquire taxonomic information like relationships among populations and evolution of populations (Stebbins, 1971; Heywood, 1972). In the present study, only mitotic behaviours of chromosomes such as chromosome number and morphology were studied. According to Moore (1968), the characters related with A chromosomes can be succesfully applied to taxonomic studies.

In the current study, economically important ten species and one variety of the genus *Salvia* nine of which are endemic to Turkey were karyologically analyzed in detail. Six new chromosome counts and five previously published counts (Scheel, 1931; Yakovleva, 1933; Hruby, 1934; Afzal-Rafii, 1980; Kandemir, 2003) in the genus *Salvia* were reported.

In order to gain information about the chromosome numbers and morphologies, the seeds of *Salvia* species were germinated and then mitotic metaphase chromosomes were obtained. Since some difficulties in the germination of *Salvia* seeds existed, the most time consuming part of this study was germination. Particularly, germination was not able to be performed at the beginning due to the thickness of seed coat and dormancy which was reported to be one of the well-known germination problems in

Salvia seeds (Nord and Gunter, 1974). In order to get rid of these kinds of problems different methods such as soaking, mechanic scarification, surface sterilization, gibberellin treatment and cold scarification were performed. Some of the pre-treatment methods could not be effective and not give efficient results to be evaluated. For example, it was observed that sterilization had a negative effect on germination. Moreover, soaking the seeds in water fastened mucilage production and shortened time of germination. Nevertheless, all these pre-treatment methods could not be effective and didn't give positive results. It is observed that while surface, other methods has positive effects. Particularly, soaking the seeds in water fastened mucilage production and shortened time of germination. Consequently, it was clearly observed that the treatment with Gibberellic acid, which was carried out on Lamiaceae family and *Salvia* genus before (Nord *et al.,* 1971; Nord and Gunter, 1974; Emery, 1988; Yıldız and Gücel, 2006), and cold scarification were found to be more effective among the pretreatment methods for the breakage of dormancy in our study (Keeley, 1986).

There are some parameters which have important effects on the pre-treatment procedure. One of them is the chemical that is used for pretreatment of the root tips (Dyer, 1979). There are a variety of pre-treatment chemicals, which have been used in different studies, such as α-bromonaphtalene (Çobanoğlu, 1988; Özkan, 2006; Özkan and Soy, 2007), 8-hydroxyquinoline (Nakipoğlu, 1993a; 1993b) and colchicine (Yıldız and Gücel, 2006). According to the literature colchicine is usually more succesful, but the most expensive and as a possible carcinogen (Dyer, 1979). In our study we preffered to use α-bromonaphtalene since it is easy to use. Beside pre-treatment chemicals the time of excised the root tips has also immense impact on the procedure. After performing the procedure in different time of the day the best time for pre-treatment is found as 8.00 to 9.00 am. On the other hand, hydrolysis temperature is another parameter affecting the process. Hydrolysis at 60 ºC which was widely used in previous studies

(Turki *et al*., 2000; Özkan, 2006; Özkan and Soy, 2007) also tried, however; root tips of some species became soft in a very short time period. According to Fox (1969), the optimal time is not so readily observed using hot hydrolysis technique. On account of time of hydrolysis is changed for each species and room temperature was chosen. Hydrolysis time was changed for each species from 10 to 14 minutes.

As mentioned above, the aim of the study is to acquire taxonomic information about the *Salvia* species by investigating the chromosome number and morphology of these species. In order to find these five different characteristics of karyotypes are usually studied, compared and differentiated with each other as reported by Stebbins (1971). These characters are chromosome size, centromeric position, relative chromosome size, basic chromosome number, and the number and position of satellites. Thus, in this study we used these five characters in our detailed karyological analysis.

The cytological studies which were carried out by an image analysis system are not novel in plant taxonomy (Bauchan and Campbell, 1994; Kato & Fukui, 1998) but there are few examples for the Labiatae family. Karyotype analysis by an image analysis system was done by Turki *et al*. (2000). In that study, 31 taxon and 14 family belonging to Angiosperms which contained some taxa from Labiatae family were studied. In this paper, there were also reported chromosome numbers of 3 *Salvia* species named, *S. aegyptiaca* L., 2n=28; *S. desertii* Decne, 2n=48 and *S. spinosa* L., 2n=20. Likewise, in the current study, the use of the software program called Bs200pro overcomes visualization and measuring problems of the small chromosomes of *Salvia* species. The measurement of chromosomes with image analysis systems have many advantages when compared with the methods performed with calper rule such as; karyotypes can be prepared in a short time, karyograms and haploid idiograms can be done automatically and with minimum error.

In the present study, total length of the chromosomes, short arms, long arms, arm ratio, centromeric index, relative length of the each chromosome and haploid chromosome length of eleven taxa belonging to four sections of *Salvia* were represented. These are, *S. napifolia* belonging to section *Hemisphace* Benth*; S. aethiopis, S. cilicica, S. hypargeia, S. longipedicellata, S. vermifolia, S. yosgadensis* belonging to section *Aethiopis* Benth*; S. euphratica* var. *leiocalycina* belonging to section *Hymenosphace* Benth*; S. divaricata, S. recognita and S. rosifolia* belonging to section *Salvia* Hedge*.*

According to the literature showed that *Salvia* has more than one basic chromosome number of x=6, 7, 8, 9, 10, 11, 12, 13, 15, 16, 17, 19, 21, 22, 27, 32 (Stewart, 1939; Epling *et al*., 1962; Haque and Ghoshal, 1980; Haque, 1981; Nakipoğlu, 1992; Alberto *et al*., 2003). It is clear that the genus *Salvia* is highly polybasic. According to Haque (1981), x=6, 7 and 8 can be considered as primary and the higher numbers seem to be of secondary origin. In Gill's research (1971) 20 species of *Salvia* were studied and he revealed that the literature showed a frequency of the order of 21.7 % polyploidy in *Salvia*. Moreover, in most of the studies (Stewart, 1939; Haque and Ghoshal, 1981; Kandemir, 2003; Nakipoğlu, 1993b; Özkan and Soy, 2007) *Salvia* species had diploid chromosome in accordance with our results.

In section *Aethiopis* Benth, basic chromosome numbers are x=10, 11, rarely x=12 with one exception S. microstegia x=8. *S. aethiopis, S. cilicica, S. hypargeia, S. longipedicellata, S. vermifolia and S. yosgadensis* are the members of this section and basic chromosome numbers of former three is x=11, and the rest of the species is x=10 which show that our results supported previous studies.

S. divaricata, S. rosifolia and *S. recognita* are the members of the section *Salvia* Hedge which includes 30 species. Basic chromosome numbers in this section is x=7, 8. According to our results, basic chromosome number of *S. divaricata, S. rosifolia* and *S. recognita* is x=7*.* One or two B chromosome was observed in some species belonging to this section (Nakipoğlu, 1993). As a result of our study, we found one B chromosome in the karyotype of *S. rosifolia.* Presence of B chromosome is widely known in the genus *Salvia*, but these chromosomes are not providing diagnostic information. Basic chromosome number of *S. euphratica* var. *leiocalycina* which belongs to section *Hymenosphace* Benth *was found as x=7.* In literature, several basic chromosome numbers were reported for this section such as x= 7, 8, 9, 16. Basic chromosome number of *S. napifolia* which belongs to section *Hemisphace* Benth was found as x=16. Previous reports about the basic chromosome numbers of three species of this section were reported as x=8,16 (Özkan and Şenel, 2007).

As a result, our findings collaborate with the previous studies concerning the basic chromosome numbers as well as the chromosome numbers given for the *Salvia* species (Afzal-Rafii, 1972, 1980; Haque, 1981; Nakipoğlu, 1993; Özkan, 2006). In this study, it was found that the species from the same section has the same chromosome number.

As reported before by some researchers, (Scheel, 1931; Stewart, 1939; Epling *et al*., 1962; Afzal-Rafii, 1971; Bhattarcharya, 1978; Haque and Ghoshal, 1980; Nakipoğlu, 1993; Kandemir, 2003, Özkan and Soy, 2007) chromosome size of the genus *Salvia* is very small. Moreover, according to the some researchers, due to the very small chromosome size, there are very few detailed studies on the karyotypes of the *Salvia* genus (Haque and Ghoshal, 1980). In fact, Hruby (1934) emphasized that when a large number of chromosome is existed, even the centromeres are not apparent. With regard to chromosome size, our findings support previous studies.

In literature, chromosome number of ***S. aethiopis*** was presented by two close number as 2n= 22 (Yakovleva, 1933; Löve & Kjellqvist, 1974; Haque and Ghoshal, 1980) and 2n=24 (Hruby, 1934; Felfoldy, 1947; Afzal-Rafii, 1976). Our result is in accordance with the former. With regard to chromosome morphology, Haque and Ghoshal (1980) reported chromosome size of the species as 5.0 μm to 2.0 μm long. However, our results when we compare chromosome size, no correlation was observed as it can be seen in Table 3.1. Chromosome size of the species varied from 2.19 μm to 0.95 μm long. Similar to the previous studies, no satellite was observed in this species. Since Haque and Ghoshal (1980) did not explain karyotype of the species and no other study could be found in literature, we could not compare our results by the mean of karyotype of species. As it was indicated before, the species has nine metacentric and two submetacentric chromosome pairs.

In ***S. cilicica***, the diploid chromosome number 2n=22 confirms the earlier reports for this species (Afzal-Rafii, 1980). As seen in Table 3.1 the length of chromosomes vary from 2.14 μm to 1.05 μm long. Since, no previous study about chromosome morphology of the species were found, we could not compare our findings. This is the first study on both chromosome number and chromosome morphology of this species.

S. divaricata, an endemic species, showed 2n=14 chromosomes, whose length varied from 2.23 μm to 1.27 μm long. Any previous data concerning the chromosome number and chromosome morphology of this endemic species was not reported for this endemic species. This is the first study on both chromosome number and chromosome morphology of this species.

Chromosome number of ***S. euphratica* var. *leiocalycina*** was first found to be 2n=14. This taxon has relatively long chromosomes with 3.69 μm to 2.07 μm long. No satellite was observed on the karyotype of the species. No previous study was carried out regarding chromosome number and karyomorphology of this taxon. This is the first report about both chromosome number and chromosome morphology of this species.

Somatic chromosome number of ***S.hypargeia*** was reported as 2n=22 which agrees with the earlier observation of Kandemir (2003). Our results support this result. Regarding chromosome morphology, Kandemir (2003) reported chromosome size of the species varied between 1.60 μm to 0.30 μm long. However, due to our findings chromosome size of the species varied from 2.12 μm to 0.83 μm with nine metacentric and two submetacentric chromosome pairs. In both in our and Kandemir's studies existence of satellite was not observed on the karyotype of the species.

Chromosome number of the endemic ***S. longipedicellata*** was found as 2n=20 with 6 metacentric and 4 submetacentric chromosome pairs. There have been no previous studies including chromosome number or chromosome morphology of this species. Chromosome length of the species varied from 2.18 μm to 0.68 μm long.

Chromosome number of ***S.napifolia*** was recorded as 2n=32 on the basis of Iranian material by Afzal-Rafii (1980). We also found the same chromosome number for the spcimens from Turkey in this study. Chromosome length of the species varies from 2.23 μm to 1.26 μm long and it consists of eleven metacentric and five submetacentric chromosome pairs.

In literature, there are two chromosome numbers which were recorded for ***S. recognita***, these are 2n=14 (Scheel, 1931) and 2n=16 (Afzal-Rafii, 1980). Our result collaborates with the number 2n=14. The previous studies performed for this species limited only with chromosome number. Thus, we could just compare chromosome number of species wtih previous results. According to our findings, the karyotype of this species is highly symmetric with seven metacentric chromosome pairs and the chromosome size varied from 2.85 μm to 1.78 μm long.

The chromosome number of the endemic ***S. rosifolia*** was firstly recorded in this study as 2n=14 with two metacentric and five submetacentric chromosome pairs. The chromosomes of this endemic species varied from 2.14 µm to 1.30 µm long. We also found B chromosome in the karyotype of the species. The number of B chromosome differed in many species. Our B chromosome findings supported all the previous studies preformed on the cytotaxonomy of *Salvia* (Afzal-Rafii, 1980; Haque and Ghoshal, 1980; Nakipoğlu, 1992; Alberto, 2003). Existence of B chromosome can be correlated with soil structure, ecological features and cultural forms of species (Bosemark, 1956; Fröst, 1956).

This is also first report on chromosome number and chromosome morphology of endemic ***S. vermifolia***. Chromosome number of the species was found as 2n=20 and chromosome size varied from 1.90 µm to 0.85 µm long. The species consists of seven metacentric and three submetacentric chromosome pairs.

S. yosgadensis has twenty somatic chromosomes with nine metacentric and one submetacentric chromosome pairs. Chromosome size of this species varied from 3.35-1.38. No satellite was observed on the karyotype of the species. Chromosome number and chromosome morphology of this species were firstly reported in this research.

Table 4.1. Chromosome numbers of studied *Salvia* taxa

Taxon	Previous report (2n)	Present study (2n)	References
S. aethiopis	22,24	22	Yakovleva, 1933 Hruby, 1934
S. cilicica	22	22	Afzal-Rafii, 1980
S. divaricata	-	14	-
S. euphratica var. leiocalycina	-	14	-
S. hypargeia	22	22	Kandemir, 2003
S. longipedicellata	-	20	-
S. napifolia	32	32	Afzal-Rafii, 1980
S. recognita	14,16	14	Scheel, 1931 Afzal-Rafii, 1980
S. rosifolia	-	14	-
S. vermifolia	-	20	-
S. yosgadensis	-	20	-

All the species included in this study contained metacentric or submetacentric chromosome pairs. *S. recognita* has the highest number of metacentric chromosomes with seven metacentric chromosome pairs. However, the highest number of submedian chromosomes was observed in *S. rosifolia* with five submetacentric chromosome pairs. As a whole, karyotypes of the analyzed species had a predominance of metacentric chromosomes except for *S. rosifolia* which had a predominance of submetacentric chromosome (Table 3.12).

In this study, the total length of chromosomes of the investigated taxa varied between 3.69 µm to 0.68 µm. While *S. euphratica* var. *leiocalycina* had the longest chromosomes with 3.69 µm, *S. longipedicellata* had the shortest chromosome with 0.68 µm. In this study, no satellites were observed in the karyotypes of these eleven taxa unlike the previous studies.

S. divaricata showed the shortest (12.28 µm) and *S. napifolia* the longest (28.19 µm) total chromatin length of all the species. *S. euphratica* var. *leiocalycina* had the largest (2.8 µm) and *S. hypargeia* (1.29 µm) had the smallest mean chromatin length.

Stebbins (1971) and Romero-Zarco (1986) suggested two methods to measure and classify karyotype asymmetry which could be defined as comparative morphology of the karyotype. Stebbins's karyotype asymmetry can be found by recognizing three degrees of difference between the largest and smallest chromosome of the complement and four degrees with respect to the proportion of chromosomes which are acro- or telocentric. On the other hand, in Zarco's method karyotype asymmetry is measured by two asymmetry indices namely intrachromosomal asymmetry index (A1) and interchromosomal asymmetry index (A2) both of which do not depend on chromosome number or chromosome size. In our study, Stebbins's karyotype asymmetry and Zarco's A1 and A2 asymmetry indices were estimated for each taxa to find karyotype asymmetry.

The A1 equation which represents the intrachromosomal asymmetry was formulated to obtain lower values when chromosomes tend to be metacentric and this value does not depend on chromosome number and chromosome size (Zarco, 1986). Our results supported this statement in such a way that; *S. rosifolia* (2n=14) having two metacentric and five submetacentric chromosome pairs, had the highest A1 value with 0. 46 and the highest asymmetric karyotype, whereas *S. recognita* (2n=14) having seven metacentric chromosome pairs, had the lowest A1 value with 0.27.

In general, the karyotypes are found to be all reasonably symmetrical and fall in the Stebbins 1A and 2A category of symmetry, apart from *S. rosifolia* which falls in 3A category (Table 3.13). Within section *Aethiopis* Benth, *S. hypargeia* has the most asymmetrical karyotypes (avarage of A1 and A2= 0.35) whereas *S. yosgadensis* has the most symmetrical karyotypes (avarage of A1 and A2=0.26). Within *Salvia* Hedge While, *S. rosifolia* has the most asymmetrical karyotype (avarage of A1 and A2= 0.32), *S. recognita* has the most symmetrical karyotype (avarage of A1 and A2= 0.22). Both *S. napifolia* which belongs to section *Hemisphace* Benth and *S. euphratica* var. *leiocalycina* which belongs to section *Hymenosphace* Benth have symmetrical karyotype (respectively avarage of A1 and A2= 0.24 and 0.27). Stebbins (1971), indicated that the most symmetrical karyotype is the most primitive and the most aymmetrical karyotypes are derived (Stebbins, 1971). According to our results while *S. rosifolia* is the most derived species among all the studied taxa, *S. recognita* is the most primitive one.

In UPGMA analysis two major clusters were observed (Figure 3.12). *S. napifolia* stands alone in the first cluster, and all the other taxa have been composed of the second major cluster. The second major cluster have also been composed of two groups, the first grouped is composed of *S. recognita*, *S. euphratica* var. *leiocalycina*, *S. rosifolia* and *S. divaricata* and the second cluster is composed of *S. yosgadensis, S. longipedicellata, S. hypargeia, S. cilicica, S. vermifolia* and *S. aethiopis,* these two groups are seperated from each other with chromosome numbers and the ratio between longest to the shortest chromosome. Whereas the chromosome numbers of the group is 2n= 14 in all the taxa, chromosome numbers in the second group is 2n=20 and 22. Furthermore, the L/S ratio is below two in the first group, and above two in the second group. Eventhough *S. aethiopis* and *S. vermifolia* have different chromosome numbers, they are closely related in the A1 (intrachromosomal asymmetry index) and A2 (interchromosomal asymmetry index) characteristic. With 0.914 similarity level this two species come together. They are members of the same section. *S. cilicica* is close to both *S. vermifolia* and *S. aethiopis* with 0.886 similarity level (Table 3.14). *S. rosifolia* and *S. divaricata* which have the same chromosome numbers and they are the members of the section *Salvia* and close to each other in L/S (the ratio between longest to the shortest chromosome) characteristics. Their similarity level is 0.895 (Table 3.14). *S. longipedicellata* and *S. hypargeia* are belonging to section *Aethiopis* are close to each other with 0.828 smiliarity level. A1 and A2 values of these species are similar. Although *S. recognita* and *S. euphratica* var*. leiocalycina* take place in different sections (*S. recognita* is the member of section *Hedge*; *S. euphratica* is the member of section *Hymenosphace*), they are close with 0.825 similarity level (Table 3.14). Since our phenogram grouped the taxa according to sections and the taxa which have the same or close chromosome numbers were grouped together, it seems that the selected characters reflect the taxonomic relationships well.

CHAPTER 5

CONCLUSION

In this study the chromosome numbers and chromosome morphologies of eleven taxa (*S. aethiopis*, *S. cilicica*, *S. divaricata*, *S. euphratica* var. *leiocalycina*, *S. hypargeia*, *S. longipedicellata*, *S. napifolia*, *S. recognita, S. rosifolia, S. vermifolia*, *S. yosgadensis*) belonging to *Salvia* genus were examined. This study indicated that all of the studied taxa had symmetrical karyotypes with metacentric and submetacentric chromosomes with very small chromosome size.

Moreover, it is observed that the species from different sections have different chromosome numbers whereas the species from same sections have the same or close chromosome numbers. All of the studied taxa have diploid chromosome numbers with basic chromosome numbers of 7, 10, 11 and 16. Our results supported previous studies by the mean of basic chromosome numbers (Stewart, 1939; Haque, 1981). B chromosome was only found in *S. rosifolia*. The results were in a good correlation with the previous results afforded for the karyomorphology of the genus.

In this study, A1 and A2 indices and Stebbins karyotype symmetry classes were used in order to understand karyotype symmetries of the taxa and compare them in an evolutionary aspect. According to the UPGMA cluster analysis which was done to group species, the studied taxa were separated into two major groups.

Consequently, the results acquired from this study have allowed us to compare the karyomorphology of some Turkish *Salvia*, and indicated that chromosomal change have played in the evolutionary process. The karyomorphological data of these eleven *Salvia* taxa are extremely benefical for the following karyological studies as well as the taxonomical studies related with the genus. Moreover, this study is essential for preserving gene sources of endemic and rare species in breeding and solving taxonomical problems among the genus.

CHAPTER 6

RECOMMENDATIONS FOR FUTURE STUDY

This study is extremely important for reclassifying of especially endemic species whose chromosome numbers and karyological features are not known. However, it is known that the cytological knowledge of *Salvia* is still limited and additional chromosome counts are necessary. More karyotype studies should provide essential information in understanding the systematic and evolution of the genus. In addition, the taxa used in this study are belonging to one population, It is obvious that studying karyotypes of the same species from different populations will give us better results in terms of evolution. Furthermore, it may be more satisfactory to study different stages of meiosis in addition to mitotic metaphase chromosomes.

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APPENDIX A

PHOTOGRAPHS OF STUDIED TAXA

Figure A.1. *S. aethiopis*

Figure A.2. *S. cilicica*

Figure A.3. *S. divaricata*

Figure A.4. *S. euphratica* var. *leiocalycina*

Figure A.5. *S. hypargeia*

Figure A.6. *S. longipedicellata*

Figure A.7. *S.napifolia*

Figure A.8. *S. recognita*

Figure A.9. *S. rosifolia*

Figure A.10. *S. vermifolia*

Figure A.11. *S. yosgadensis*

APPENDIX B

CHEMICALS AND THEIR SUPPLIERS

Chemicals	**Chemical Suppliers**
1-Bromonaphtalene	Merck
Entellan	Merck
Gibberellic acid 3 (GA3)	Duchefa Biochemie
Orcein	Sigma
Acetic Acid	Merck
Propionic acid	Merck
Lactic acid	Merck
Sodium chloride	Applichem
Sulphuric Acid	Merck
HCl	Merck

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