Karyotype Analysis in Some *Salvia* species (Lamiaceae) of Iran

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Summary Karyotype analysis was performed in 10 *Salvia* species (Lamiaceae) of Iran, *S. spinosa*, *S. reuterana*, *S. sclarea*, *S. ceratophylla*, *S. xanthocheiala*, *S. limbata*, *S. hypoleuca*, *S. staminea*, *S. nemorosa* and *S. verticillata* showing $2n=14$, 20, 22 and 32 chromosome numbers indicating the role played by polyploidy and aneuploidy in *Salvia* species diversification. Some of the species differed significantly in the size of chromosomes but occupied 1A and 1B classes of Stebbins system indicating them to have primitive karyotype.

Key words Karyotype, *Salvia*, Stebbins system, Species diversification.

The genus *Salvia* L. (Lamiaceae) contains about 900 herbaceous, suffrutescent or shrubby perennials, rarely biennial or annual, often strongly aromatic species mainly growing in temperate and subtropical areas with Western Asia and Mediterranean regions as the main center of their distribution (Standley and Williams 1973, Özdemir and Senel 1999, Wu and Li 1982). These species are of horticultural, commercial and medicinal values (Bhattacharya 1978).

Rechinger *et al.* (1982) reported 70 *Salvia* species in Flora Iranica, showing great morphological diversity possibly due to inter-specific hybridization. He believes that although some of the species are very distinct, others show close affinity with other species and some species are in a state of evolutionary flux.

Cytological studies carried out in the genus *Salvia* revealed the occurrence of diverse chromosome numbers indicating that the genus seems to be polybasic, with different groups of species in different parts of the world having polyploid origins. According to Afzal-Rafii (1976), *Salvia* species of the Mediterranean region have $x=7$, while Patudin *et al.* (1975) believe that *Salvia* species of Europe and Russia have $x=11$ and finally Epling *et al.* (1962) consider the species growing in California to have $x=16$. Moreover, studies made by Palomino *et al.* (1986), show that *Salvia* subgenus *Calosphaea* is characterized by $x=11$ and lower numbers.

In general, extensive cytological studies exist in the genus *Salvia* from different parts of the world (for example, Patudin *et al.* 1975, Lee 1967, Afzal-Rafii 1976, Mizianti *et al.* 1981, Haque 1981, Borgen 1980, Diaz-Lifante *et al.* 1992, Palomino *et al.* 1986, Özdemir and Senel 1999, Yildiz and Güccel 2006, Bhattacharya 1978, Foley *et al.* 2008). However, cytological reports from *Salvia* species of Iran are very limited (Ghaffari and Chariat-Panahi 1985, Estilai and Hashemi 1990, Sheidai *et al.* 2010). The present study considers the karyotype analysis of 10 *Salvia* species growing wild in Iran for the first time.

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Table 1.

Species	Locality	Voucher	2n Level	Ploidy (μ m)	TL (μ m)	L (μ m)	S	L/S (μ m)	X	ST	A1	A2	TF	KF
<i>S. nemorosa</i>	Gachsar	8500981	14	2x	15.40	2.65	1.69	1.57	2.20	1A	0.25	0.11	47.00	7m
<i>S. nemorosa</i>	Khojir	8500984	14	2x	15.67	2.82	1.66	1.69	2.24	1A	0.26	0.00	42.74	7m
<i>S. reuterana</i>	Khojir	8501000	20	2x	18.89	2.47	1.30	1.89	1.89	1A	0.26	0.17	42.62	10m
<i>S. spinosa</i>	Khojir	8501004	20	2x	16.55	2.32	1.08	2.15	1.65	1B	0.27	0.18	42.64	10m
<i>S. hypoleuca</i>	Khojir	8500986	22	2x	21.90	2.69	1.26	2.13	1.99	1B	0.32	0.17	40.38	10m+1 sm
<i>S. limbata</i>	Khojir	8500992	22	2x	21.51	2.58	1.32	2.00	1.98	1B	0.30	0.19	41.00	11m
<i>S. staminea</i>	Abali	8500999	22	2x	27.78	3.48	1.66	2.10	2.53	1B	0.26	0.18	42.45	11m
<i>S. ceratophylla</i>	Sorkhehesar	8500990	22	2x	22.73	2.69	1.46	1.85	2.07	1A	0.27	0.15	45.86	10m+1 sm
<i>S. sclarea</i>	Polur	8500998	22	2x	20.30	2.45	1.31	1.87	1.85	1A	0.27	0.14	42.04	10m+1 sm
<i>S. xanthocheila</i>	Polur	8501006	22	2x	21.27	2.70	1.47	1.84	1.93	1A	0.28	0.15	41.54	11m
<i>S. verticellata</i>	Gschsar	8500997	32	4x	37.74	3.16	1.70	1.86	2.36	1A	0.26	0.14	42.17	16m

Abbreviations: TL=Total chromosome length, L=Size of the longest chromosome, S=Size of the shortest chromosome, L/S=Ratio of the longest chromosome/shortest chromosome, X=The mean chromosome length, ST=Stebbins, class, A1 and A2=Romero-Zarco indices, TF=Total form percentage and KF=Karyotype formulae.

Materials and methods

Plant material

Plant materials collection was done in the Central Alborze region during 2009 and 2010. Cytological studies were performed in 11 populations of 10 *Salvia* species growing wild in this region, namely *S. spinosa* L., *S. reuterana* Boiss., *S. sclarea* (Mönch) Benth., *S. ceratophylla* L., *S. xanthocheila* Boiss. ex. Benth., *S. limbata* C. A. Mey., *S. hypoleuca* Benth., *S. staminea* Monthbr. & Auch. ex. Benth., *S. nemorosa* L., and *S. verticillata* L. The voucher specimens are deposited in Herbarium of Shahid Beheshti University (HSBU), details of which have been given in Table 1.

Cytological studies

For the karyotype study, freshly grown root tips were collected from germinated seed of at least ten randomly selected plants in each species, pretreated with 0.002 mol 8-hydroxyquinolin (2–2.5 h) and fixed in ethanol–acetic acid (3 : 1) for 24 h. The fixed tips were then washed thoroughly in distilled water, macerated in 60°C 1 N HCl for about 5 min., and squash in 2% aqueous aceo-orcein stain solution. The somatic chromosome number and karyotype details were studied in at least 5 well-prepared metaphase plates. The chromosomes were photographed by digital camera and measured by Image Tools3 software (Sheidai and Jalilian 2008).

Karyotype description

The chromosomes were described according to Levan *et al.* (1964), karyotype symmetry was determined according to Stebbins (1971), while other karyotype parameters like haploid total chromosome length (total sum of the size of the chromosomes by using only 1 chromosome from each pair), mean chromosome length (total haploid chromosome length/number of chromosome pairs), total form percentage (TF%=Sum of short arms of the chromosomes/total chromosome length) as well as the A1 and A2 indices of Romero-Zarco (1986) were determined (Sheidai and Jalilian 2008).

Statistical analyses

In order to reveal significant difference among the species and populations studied, the analysis of variance (ANOVA) (Sheidai and Jalilian 2008) followed by the least significant difference test (LSD) were performed on the size of chromosomes, size of the long arms and size of the short

Fig. 1. Representative somatic metaphase cells in *Salvia* species studied. A–E=*S. ceratophylla*, *S. hypoleuca*, *S. limbata*, *S. verticellata* and *S. nemorosa*, respectively. Scale bar=10 μ m.

arms as well as arms ratio among the species and populations studied (Sheidai and Jalilian 2008).

Results and discussion

Details of karyotypes are presented in Table 1, Fig. 1. The species studied had $2n=14$, 20, 22 and 32 chromosome numbers. *S. nemorosa* showed $2n=14$ in both populations of Gashsar and Kojir, supporting the earlier reports (Mizianti *et al.* 1981, Ghaffari and Chariat-Panahi 1985), *S. reuteriana* and *S. spinosa* showed $2n=20$, supporting the earlier reports (Afzal-Rafii 1981, Ghaffari and Chariat-Panahi 1985, Sheidai *et al.* 2010). *S. hypoleuca*, *S. ceratophylla*, *S. limbata* and *S. sclarea*, *S. staminea* and *S. xanthocheila* showed $2n=22$ chromosome number, supporting previous reports (Afzal-Rafii 1980, 1981, Özdemir and Senel 1999), *S. verticillata* showed $2n=4x=32$ supporting the reports of Afzal-rafii (1980) and Lövkvist and Hultgård (1999).

There are several reports on the occurrence of multiple basic chromosome numbers in a single *Salvia* species, including $2n=12$, 26 and 28 for *S. aegyptiaca* L., (Haque 1981, Borgen 1980, Díaz-Lifante *et al.* 1992), $2n=14$ and 16 for *S. chanroenica* ssp. *glomerifolia* Chung (Lee 1967), and for *S. nemorosa* L. (Mizianti *et al.* 1981, Ghaffari and Chariat-Panahi 1985). The reason for such multiple basic numbers is the occurrence of polyploidy and aneuploidy in *Salvia* species possibly for adaptation to the various environmental conditions the species are facing. In fact, such cytological variations may be one of the causes of extensive morphological diversity reported in *Salvia* species (Rechinger *et al.* 1982).

Controversy exists about the primitive *versus* advanced nature of chromosome base numbers in the genus *Salvia*. According to Bahattacharya (1978), base numbers 7 and 8 are the primitive base numbers from which secondary base numbers became established and diversified in different directions, forming the base number 11 which is frequent in many species.

The chromosomes were mostly metacentric with a only 1 pair of sub-metacentric chromosome occurring in *S. hypoleuca*, *S. ceratophylla* and *S. sclarea* (Table 1).

The size of the longest chromosome pair varied from 2.32 μ m in *S. spinosa* to 3.48 μ m in *S. staminea*, while the size of shortest chromosome pair ranged from 1.80 μ m in *S. spinosa* to 1.70 μ m in *S. verticellata*. Similarly the mean chromosome length varied from 1.65 μ m in *S. spinosa* to 2.53 μ m in *S. staminea*.

t-Test analysis showed no significant difference in size of the chromosomes and chromosome arms between 2 populations of *S. nemorosa* with $2n=14$ and also between *S. reuterana* and *S. spinosa* having $2n=20$. However the ANOVA test performed among the species having $2n=22$ showed significant difference in total chromosome length, size of the longest chromosome pair and size of the shortest chromosome pair. Therefore, cytological changes in the species having $2n=22$ consist of significant quantitative changes in the size of chromosomes (DNA).

The Stebbins classes occupied by *Salvia* species are 1A and 1B which are considered as primitive classes and TF value above 40 indicates a symmetrical karyotype.

Controversy also exists about the primitive *versus* advance nature of the genus *Salvia* itself, for example Fujita (1970), considered *Salvia* as the most primitive genus in the family Labiateae with the highest primary basic number 11 although *Ajuga* is often considered as the most primitive genus. However, according to Bahattacharya (1978), due to cytological numerical instability, the presence of highly irregular cytological behavior and presence of advanced karyotype in the *Salvia* species, they are recent and advanced members of a complex group. However, the present study shows that *Salvia* species studied have primitive (symmetrical) karyotypes as they occupy 1A and 1B classes of the Stebbins system and also show a very low degree of size difference among the chromosomes.

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