Cytological Investigations in Some Important Tree Species of Rajasthan IV. Male Meiosis Studies in the Genus *Salvadora* L.

Arun Kumar¹, S. Rama Rao^{1,*} and T. S. Rathore²

¹ Cytogenetics Laboratory, Department of Botany, J.N. Vyas University, Jodhpur-342005, India

² Plant Biotechnology Division, Institute of Wood Science and Technology, Bangalore-560001, India

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Summary Male meiosis in 12 accessions belonging to 2 species of the genus *Salvadora* viz. *S. persica* and *S. oleoides*, an important medicinal and oil-yielding tree has been studied in detail. The somatic chromosome number in both the species is confirmed as $2n=24$. Two basic numbers $n=12$ and 13 are contemplated for the genus. Analysis of chromosome associations revealed regular occurrence of univalents and bivalents. Among bivalents ring type were more in number as compared to rod bivalents, which normally had terminalized chiasma. A single nucleolus which always showed affinity towards 1 to 3 bivalents, was the common observation in PMCs. Structural rather than numerical changes (polyploidy) seems to have played a significant role in speciation and evolution of the genus *Salvadora*.

Study of meiotic chromosomes that represents the fundamental life process has always been a favorite subject for cytogeneticists since a long time. Various interesting events that occur during the process of meiosis, whether the chromosome movement during bouquet stage (Zickler and Kleckner 1998) or the intrinsic pairing mechanism of chromosomes involving homolog juxtaposition followed by recombination at DNA level (Chua and Roeder 1998, Moore 1998, Schnable *et al.* 1998, Sybenga 1996, 1999, Zickler and Kleckner 1999) have always attracted cytogeneticists to study the meiotic process in diverse organisms ranging from algae to wheat plant. Further male meiotic studies in pollen mother cells (PMCs) have long been recognized as an important tool to understand the genome structure and organization of various plant genera and their constituent species. Although karyological studies are critical in ascertaining the chromosome number of various taxa, they may be of little help in understanding the genome especially if the chromosome size is small and if there is an overall stability in the chromosome morphology and symmetry, both between and within the species of the genus, as is a case in many tropical tree genera (Gill and Singhal 1998a,b). In such a situation meiosis could be a reliable parameter for understanding the cytogenetic mechanisms controlling the speciation in the genus.

The arid and semi-arid areas in Thar desert regions of Rajasthan are bestowed with some important tree genera like *Prosopis*, *Anogeissus*, *Maytenus*, *Acacia* etc. Despite their multifacet utility and advantage they did not enthuse much interest among cytologists to work out meiotic details in many of these genera. The genus *Salvadora* belonging to family Salvadoraceae is an important medicinal and oil yielding tree, which inhabits saline and rocky soils (Bhandari 1990). Two species viz. *S. persica* and *S. oleoides* grow abundantly and constitute important components of the stressed ecosystem of western Rajasthan. Both the species constitute important source of leaf fodder for camel and produce non-edible oil which is extensively used in several industries that produce soaps, cosmetics, paint, vanish and lubricants (Singh *et al.* 1996). The oil also contains lauric acid which forms the basic raw material for the industrial production of lauryl alcohol (Chatterjee

* Corresponding author, e-mail: srrao58@satyam.net.in

and Pakrashi 1995), besides its medicinal use in curing diseases like piles, rheumatism and skin diseases. The fruits of *S. oleoides* are sweet in taste and are a delicacy with local populace. The red/yellow wood is of light weight and is commonly used in the manufacture of furniture items, boats, doors, etc. Despite the greater importance of *Salvadora*, attempts for its genetic improvement and sustainable utilization are by and large lacking which resulted in gradual decline of the trees in the natural populations. Cytogenetical and molecular mechanism controlling the organization and evolution of genome in the genus *Salvadora* remained ambiguous till date. Koul and Singh Chauhan (1962) have reported $n=12$ in *S. persica* by analyzing PMCs, where as deviant number of $n=13$ is reported by Sidhu (1961) and Sarkar *et al.* (1977). In *S. oleoides* a single report on chromosome count suggest that the $2n=24$ (Baquar and Hussain 1967). Besides these reports, Mohnot (1993) while analyzing *in vitro* raised plants of both *S. persica* and *S. oleoides*, observed $2n=24$ in majority of the cells, while aneusomaty was recorded only in few of them (3–5%). In view of this scant information available an attempt has been made to carry out detailed male meiotic studies in various accessions belonging to both the important species viz. *S. persica* and *S. oleoides*.

Material and methods

Extensive surveys were conducted to locate populations of both the species of *Salvadora*. The representative plants from each population have been marked and appropriately labeled. Herbarium specimens for all the mandate trees were prepared and voucher specimens were deposited with departmental herbarium and Botanical Survey of India, Jodhpur for obtaining accession numbers (Table 1). Flower buds of appropriate size were collected from mature trees and fixed in freshly prepared Carnoy's fluid, supplemented with a drop of ferric chloride solution for a minimum of 24 h at room temperature and subsequently stored in 70% alcohol at 10°C. For meiotic analysis anthers were squashed in 1% acetocarmine. On average 25 cells were analyzed at diplotene/diakinesis and metaphase I for recording chromosome associations and recombinational frequencies through chiasma analysis. 15–20 cells were also analysed at AI/AII for distributional pattern of chromosomes. For percentage pollen stainability, the pollen grains were stained in 1:1 (glycerin:acetocarmine) mixture and on average 10 slides were scored for stainable pollen. Photomicrographs from temporary preparations were taken using Agfa-Copex Pan photonegative film (ASA~20).

Observations

Associations

S. persica BSJO 19573 Nineteen (76%) out of 25 PMCs analyzed have shown the occurrence of 12 bivalents, while the remaining PMCs had a mixture of bivalents and univalents (Figs. 1–4). On average 11.68 and 0.08 bivalents were resolved into ring and rod type respectively. Mean number of total bivalents and total univalents were observed to be 11.76 and 0.48 respectively (Table 1). A single, spherical, well stained nucleolus was consistently observed in majority of the PMCs analyzed at diplotene/diakinesis. More often, than not, this nucleolus was found in close association with 1 or 2 bivalents (Figs. 1, 2). The average number of chromosome associations of this taxon was $11.76 \text{ II} + 0.48 \text{ I}$.

S. persica JNVU/RI 2002 Twenty one (84%) out of 25 PMCs analysed had 12 bivalents (Figs. 7–10). On average ring and rod bivalents were observed to be 11.80 and 0.80 respectively (Table 1). Mean number of total bivalents and univalents were observed to be 11.88 and 0.24 respectively. One or 2 bivalents were observed in close association with nucleolus (Fig. 7). The average number of chromosome associations for this accession was $11.88 \text{ II} + 0.24 \text{ I}$.

S. persica JNVU/RI 2003 Fifteen (60%) out of 25 PMCs analyzed were found with 12 bivalents and the remaining PMCs were encountered with a mixture of both univalents and bivalents

Table 1. Mean number, range of associations at diplotene/diakinesis/metaphase-I in *Salvadora* species ($n=12$)

Species	Accession number	Cells with 12 II				Cells with 13II				Chromosome associations																		
		No. of cells analyzed	No.	%	No.	%	No.	%	No.	%	Quadrivalents			Bivalents			Ring			Rod			Univalents					
											No.	Mean	Range	No.	Mean	Range	No.	Mean	Range	No.	Mean	Range	No.	Mean	Range	No.	Mean	Range
								Total bivalents																				
No.	Mean	Range	No.	Mean	Range	No.	Mean	Range	No.	Mean	Range	No.	Mean	Range	No.	Mean	Range	No.	Mean	Range	No.	Mean	Range					
<i>S. persica</i>	BSJO 19573	25	19	76.00	—	—	—	—	—	—	294	11.76±0.43	11-12	292	11.68±0.47	11-12	2	0.08±0.27	0-1	12	0.48±0.85	0-1						
<i>S. persica</i>	JNVU/RI 2002	25	21	84.00	—	—	—	—	—	—	297	11.88±0.32	10-12	295	11.80±0.48	10-12	2	0.80±0.27	0-1	6	0.24±0.64	0-2						
<i>S. persica</i>	JNVU/RI 2003	25	15	60.00	—	—	—	—	—	—	291	11.64±0.68	8-12	284	11.36±0.97	8-12	7	0.28±0.44	0-1	20	0.80±1.38	0-6						
<i>S. persica</i>	JNVU/RI 2004	55	22	40.00	20	36.36	—	—	—	—	671	12.21±0.71	10-13	665	12.09±0.83	10-13	6	0.10±0.36	0-2	20	0.36±0.77	0-2						
<i>S. persica</i>	JNVU/RI 2005	45	19	42.22	4	8.88	—	—	—	—	529	11.75±0.82	9-13	511	11.35±0.96	9-13	18	0.40±0.61	0-4	36	0.80±1.14	0-4						
<i>S. persica</i>	JNVU/RI 2006	40	18	45.00	8	20.00	—	—	—	—	468	11.70±0.90	10-13	466	11.65±0.93	10-13	2	0.05±0.31	0-2	46	1.15±1.54	0-6						
<i>S. persica</i>	JNVU/RI 2007	35	8	22.85	2	5.71	—	—	—	—	391	11.17±1.20	8-13	378	10.80±1.21	8-13	13	0.37±0.63	0-2	76	2.17±2.21	0-8						
<i>S. persica</i>	JNVU/RI 2008	30	15	50.00	2	6.66	1	0.03±0.17	0-1	344	11.46±0.95	9-12	338	11.26±0.99	9-12	6	0.20±0.47	0-2	32	1.03±1.83	0-6							
<i>S. oleoides</i>	BSJO 19572	25	20	80.00	—	—	—	—	—	—	295	11.80±0.40	10-12	294	11.76±0.51	10-12	1	0.04±0.19	0-1	4	0.16±0.54	0-2						
<i>S. oleoides</i>	JNVU/RI 2009	30	13	43.33	—	—	—	—	—	—	352	11.73±0.51	10-12	340	11.33±0.64	10-12	12	0.40±0.60	0-2	14	0.46±0.99	0-4						
<i>S. oleoides</i>	JNVU/RI 2010	25	17	68.00	—	—	—	—	—	—	292	10.81±3.11	9-12	286	11.44±0.89	9-12	6	0.24±0.51	0-2	14	0.56±1.20	0-4						
<i>S. oleoides</i>	JNVU/RI 2011	25	10	40.00	—	—	—	—	—	—	289	11.56±0.57	10-12	282	11.28±0.66	10-12	7	0.28±0.44	0-1	22	0.88±0.14	0-4

Figs. 1–31. Male meiosis in *S. persica*. 1–6) *S. persica* BSJO 19573. 1–2) Diplotene 12II, 3–4) Metaphase I 12II, 5) Anaphase I, 6) Anaphase II. 7–12) *S. persica* JNVU/RI 2002. 7) Diplotene 12II, 8–10) Metaphase I 12II, 11) Anaphase I, Note laggard bivalents, 12) Anaphase II. 13–17) *S. persica* JNVU/RI 2003. 13–14) Diplotene 12II, 15) Metaphase I 12II, 16) Anaphase I, 17) Anaphase II. 18–25) *S. persica* JNVU/RI 2004. 18–19) Diplotene 12II, 20) Diplotene 13II, 21–23) Metaphase I 13II, 24) Anaphase I, 25) Anaphase II, 26–31) *S. persica* JNVU/RI 2005. 26) Diplotene 12 II, 27) Diakinesis 12II, 28) Metaphase I 12II, 29) Diakinesis 13II, 30) Anaphase I, 31) Anaphase II.

(Figs. 13–15). On average the bivalents resolved into 11.36 ring and 0.28 rod types. Mean number of total bivalents was observed to be 11.64 and univalents were 0.80 (Table 1). 1–3 bivalents were always found in close association with the nucleolus (Figs. 13, 14). The average number of chromosome associations for this accession was $11.64 \text{ II} + 0.80 \text{ I}$.

S. persica JNVU/RI 2004 Twenty two (40%) PMCs were observed with 12 bivalents (Figs. 18–19) and the remaining with a mixture of univalents and bivalents among a total 55 cells analysed. On the average ring bivalents were observed to be 12.09 and rod bivalents were 0.10. Mean number of total bivalents was observed to be 12.21 and that of univalents to be 0.36 (Table 1). Twenty (36.36%) out of 55 PMCs have shown the occurrence of 13 bivalents (Figs. 21–23). 1–3 bivalents were found to be in close associations with the nucleolus (Figs. 18–20). The average number of chromosome association of this taxon was $12.21 \text{ II} + 0.36 \text{ I}$.

S. persica JNVU/RI 2005 Nineteen PMCs (42.22%) were observed with 12 bivalents while remaining were observed with a mixture of univalents and bivalents, in a total of 45 PMCs observed (Figs. 26–28). On average ring bivalents were found to be 11.35 and rod bivalents as of 0.40. Mean number of total bivalents was 11.75 and that of univalents was 0.80 (Table 1). Four (8.88%) out of 45 PMCs analyzed have shown the occurrence of 13 bivalents (Fig. 29). 1–2 bivalents (Figs. 26, 27, 29) were observed to be in close association with the nucleolus. The average number of chromosome associations for this accession was $11.75 \text{ II} + 0.80 \text{ I}$.

S. persica JNVU/RI 2006 Eighteen (45%) out of 40 PMCs analyzed were with 12 bivalents at diplotene/diakinesis (Fig. 32). On average, the total bivalents were resolved into 11.65 ring and 0.05 rod type. Mean number of total bivalents was observed to be 11.70 and of total univalents as 1.15 (Table 1). Eight (20%) out of 45 PMCs analyzed had shown 13 bivalents (Fig. 33). 1–3 bivalents were observed to be in close association with nucleolus. The average number of chromosome association for this taxon was $11.70 \text{ II} + 1.15 \text{ I}$.

S. persica JNVU/RI 2007 Eight (22.85%) have shown the occurrence of 12 bivalents (Figs. 36–38). 10.80 were resolved into ring and 0.37 bivalents as rod type. Mean number of total bivalents was observed to be 11.17 and that of univalents was 2.17 (Table 1). Two (5.71%) (Fig. 39) out of 35 PMCs analyzed have been observed with 13 bivalents. 1–3 bivalents were observed to be in close association with the nucleolus (Figs. 36, 37). The average number of chromosome associations for this association was $11.17 \text{ II} + 2.17 \text{ I}$.

S. persica JNVU/RI 2008 Fifteen PMCs out of 30 analyzed have shown the occurrence of 12 bivalents and remaining had a mixture of both univalents and bivalents (Figs. 42–44). On average bivalents resolved into 11.26 and 0.20 ring and rod type respectively. One of the PMCs (3.33%) has been observed with a quadrivalent (Fig. 42). Mean number of total bivalents and univalents were observed to be 11.46 and 1.03 respectively (Table 1). Two (6.66%) (Fig. 44) out of 30 PMCs analyzed have shown the occurrence of 13 bivalents at diplotene/diakinesis. 1–2 bivalents were observed to be associated with the nucleolus. The average number of chromosome associations of this taxon was $11.46 \text{ II} + 0.03 \text{ I}$.

S. oleoides BSJO 19572 Twenty (80%) out of 25 PMCs analyzed have shown the occurrence of 12 bivalents (Figs. 46, 47). On average bivalents were resolved into 11.76 and 0.04 ring and rod type respectively. Mean number of total bivalents were found to be 11.80 and that of univalents 0.16 (Table 1). Contrary to the observations made in other taxa belonging *S. oleoides* three (12%) PMCs with 11 bivalents (Figs. 48, 49) were observed in this accession. 1–3 bivalents (Fig. 46) were observed to be in close association with nucleolus. The average number of chromosome associations of this taxon was $11.80 \text{ II} + 0.16 \text{ I}$.

S. oleoides JNVU/RI 2009 Thirteen (43.33%) out 30 PMCs analyzed have shown the occurrence of 12 bivalents while in the remaining a mixture of both univalents and bivalents were observed (Figs. 52–54). On average the bivalents were resolved into 11.33 ring and 0.40 rod types respectively. Mean number of total bivalents and univalents were observed to be 11.73 and 0.46 re-

spectively (Table 1). 1–2 bivalents were observed to be in close association with nucleolus (Fig. 52). The average number of chromosome associations in this taxon was $11.73 \text{ II} + 0.46 \text{ I}$.

S. oleoides JNVU/RI 2010 Seventeen (68.00%) and of 25 PMCs analyzed have shown the occurrence of 12 bivalents, in the remaining cells a mixture of both univalents and bivalents were observed (Figs. 57, 58). On average bivalents were resolved in to 11.44 ring and 0.24 rod respectively. Mean number of total bivalents and univalents were found to be 10.81 and 0.56 (Table 1). The average number of chromosome associations of this taxon was $10.81 \text{ II} + 0.56 \text{ I}$.

S. oleoides JNVU/RI 2011 In ten (40%) out of 25 PMCs analyzed, 12 bivalents were observed, while in the remaining cells a mixture of both univalents and bivalents were observed (Figs. 61–63). On average bivalents were resolved into 11.28 ring and 0.28 rod respectively. Mean number of bivalents was found to be 11.56 and that of univalents were 0.88 (Table 1). 1–2 bivalents were observed in close association with nucleolus (Fig. 61). The average number of chromosome association of this taxon was $11.56 \text{ II} + 0.88 \text{ I}$.

Chiasma frequency

S. persica (BSJO 19573; JNVU/RI 2002 to 2008) Average number of chiasmata per cell ranged from 22–24, 20–24, 17–24, 22–26, 17–27, 22–28, 16–28 and 18–26, mean number being 23.44, 23.68, 23.00, 24.30, 23.11, 23.35, 21.35 and 22.83 in *S. persica* BSJO 19573, *S. persica* JNVU/RI 2002, *S. persica* JNVU/RI 2003, *S. persica* JNVU/RI 2004, *S. persica* JNVU/RI 2005, *S. persica* JNVU/RI 2006, *S. persica* JNVU/RI 2007 and *S. persica* JNVU/RI 2008, respectively, out of which on average 15.32, 22.80, 21.60, 23.12, 21.31, 22.62, 19.74 and 22.4 were terminalized giving terminalization coefficient 0.65, 0.96, 0.93, 0.95, 0.92, 0.96, 0.90 and 0.98 respectively (Table 2).

S. oleoides (BSJO 19572; JNVU/RI 2009 to 2011) Average number of chiasmata per cell ranged from 21–24, 20–24, 19–24 and 20–24 and mean number being 23.56, 23.06, 23.12 and 22.80 in *S. oleoides* BSJO 19572, *S. oleoides* JNVU/RI 2009, *S. oleoides* JNVU/RI 2010 and *S. oleoides* JNVU/RI 2011 respectively, out of which on average 21.40, 19.56, 21.48 and 19.64 were terminalized giving terminalization coefficient 0.90, 0.84, 0.92 and 0.86 (Table 2) respectively.

Anaphase I, II

S. persica (BSJO 19572; JNVU/RI 2002 to 2008) Barring 3, all the accessions have shown the equal distribution of chromosomes at anaphase I (Table 3, Figs. 5, 16, 24, 30, 34, 40, 45). Three taxa viz. *S. persica* JNVU/RI 2001, JNVU/RI 2004 and JNVU/RI 2007 had unequal distribution of chromosomes at anaphase I in as many as 20, 17 and 14 percent cells respectively showing the presence of lagging bivalents or univalents (Table 3, Fig. 11). Few cells analyzed at anaphase II were observed to be devoid of any abnormalities there by indicating normal distribution of chromatids (Figs. 6, 12, 17, 25, 31, 35, 41).

S. oleoides (BSJO 19572; JNVU/RI 2009 to 2011) All the 4 accessions in this species (*S. oleoides* 19572, JNVU/RI 2009, JNVU/RI 2010 and JNVU/RI 1011) exhibited normal distribution of chromosomes at At anaphase I in all the cells analyzed (Table 3, Figs. 50, 55, 59, 64). Similarly few cells analyzed at anaphase II observed, were devoid of any abnormalities there by indicating normal distribution of chromatids (Figs. 51, 56, 60, 65).

Pollen stainability

Various taxa of *S. persica* had shown percentage pollen stainability ranging between 92.63–96.34 while in *S. oleoides* it ranged between 74.50 to 80.19 in different accessions (Table 3).

Table 2. Mean number, range of chiasmata, terminalization coefficient and pollen stainability in *Salvadora species* (n=12)

Species	Accession number	No. of cells analyzed	Chiasmata				Terminalization coefficient	Percentage pollen stainability
			Mean	Range	Terminalized	Unterminalized		
<i>S. persica</i>	BSJO 19573	25	23.44 ± 0.85	22–24	15.32 ± 4.07	8.52 ± 4.24	0.65	92.63
<i>S. persica</i>	JNVU/RI 2002	25	23.68 ± 0.78	20–24	22.80 ± 1.67	0.88 ± 1.39	0.96	93.21
<i>S. persica</i>	JNVU/RI 2003	25	23.00 ± 1.62	17–24	21.60 ± 3.26	1.40 ± 2.38	0.93	93.75
<i>S. persica</i>	JNVU/RI 2004	55	24.30 ± 1.47	22–26	23.12 ± 2.12	1.10 ± 1.69	0.95	94.52
<i>S. persica</i>	JNVU/RI 2005	45	23.11 ± 1.63	17–27	21.31 ± 2.44	1.80 ± 1.72	0.92	94.00
<i>S. persica</i>	JNVU/RI 2006	40	23.35 ± 1.81	22–28	22.62 ± 2.06	0.72 ± 1.11	0.96	96.34
<i>S. persica</i>	JNVU/RI 2007	35	21.35 ± 2.33	16–28	19.74 ± 3.20	2.17 ± 1.88	0.90	93.00
<i>S. persica</i>	JNVU/RI 2008	30	22.83 ± 1.86	18–26	22.40 ± 2.37	0.43 ± 0.88	0.98	92.40
<i>S. oleoides</i>	BSJO 19572	25	23.56 ± 0.89	21–24	21.40 ± 2.17	2.16 ± 2.05	0.90	76.64
<i>S. oleoides</i>	JNVU/RI 2009	30	23.06 ± 0.99	20–24	19.56 ± 2.70	3.50 ± 2.56	0.84	74.50
<i>S. oleoides</i>	JNVU/RI 2010	25	23.12 ± 1.45	19–24	21.48 ± 2.36	1.64 ± 1.49	0.92	80.13
<i>S. oleoides</i>	JNVU/RI 2011	25	22.80 ± 1.15	20–24	19.64 ± 3.49	3.16 ± 2.82	0.86	78.56

Table 3. Anaphase I distribution in *Salvadora* species (U=univalents, B=bivalents)

Species	Accession number	2n	No. of cells analyzed	Chromosome distribution	No. of cells	Percentage
<i>S. persica</i>	BSJO 19573	24	15	12:12	15	100.00
<i>S. persica</i>	JNVU/RI 2002	24	20	12:12	16	80.00
				11:1B:11	2	10.00
				11:2U:11	2	10.00
<i>S. persica</i>	JNVU/RI 2003	24	15	12:12	15	100.00
<i>S. persica</i>	JNVU/RI 2004	24	30	12:12	25	83.33
				11:1B:11	3	10.00
				10:2U:12	2	6.67
<i>S. persica</i>	JNVU/RI 2005	24	15	12:12	15	100.00
<i>S. persica</i>	JNVU/RI 2006	24	12	12:12	12	100.00
<i>S. persica</i>	JNVU/RI 2007	24	15	12:12	13	86.67
				12:1U:11	2	13.33
<i>S. persica</i>	JNVU/RI 2008	24	12	12:12	12	100.00
<i>S. oleoides</i>	BSJO 19572	24	15	12:12	15	100.00
<i>S. oleoides</i>	JNVU/RI 2009	24	20	12:12	20	100.00
<i>S. oleoides</i>	JNVU/RI 2010	24	15	12:12	15	100.00
<i>S. oleoides</i>	JNVU/RI 2011	24	15	12:12	15	100.00

Discussion

12 taxa belonging to 2 species of *Salvadora* viz. *S. persica* and *S. oleoides* have been studied presently. All the taxa were characteristic in having majority of the PMCs with 12 bivalents. However in 5 accessions belonging to *S. persica*, a few cells with deviant number of bivalents ($n=13$) were encountered whose proportion never exceeded 36% of the total PMCs analyzed. Similarly in *S. oleoides* BSJO 19572, 12% PMCs had shown the presence of 11 bivalents. By combining present data with earlier published information (Baquar and Hussain 1967, Koul and Singh Chauhan 1962, Mohnot 1993, Sarkar *et al.* 1977, Sidhu 1961) it is clear that the genus *Salvadora*, seemingly consists of 3 basic numbers of $n=11$, 12, and 13. In order to ascertain the true basic number it is essential to obtain information on gametic and zygotic chromosome numbers of as many species as possible. The karyological data (Kumar and Rao 2001) confirms the somatic chromosome number of $2n=24$ in both the species. The deviant cells having 11 and 13 bivalents observed in present studies can be at best be considered as chimera in nature, a feature common in many of woody tree species. Interestingly the present author did encounter few PMCs with 13 bivalents only in taxa belonging to *S. persica* as earlier reported by Sidhu (1961), and Sarkar *et al.* (1977). However, not a single taxon had shown an exclusive gametic number of $n=13$ in presently investigated accessions. In *S. oleoides* few stray PMCs with 11 bivalents were encountered but no previous reports are available indicating this number. Therefore, 2 basic numbers for the genus *Salvadora* i.e. *S. persica* ($n=12$, 13) and one for *S. oleoides* ($n=12$) are being suggested.

Various accessions belonging to the genus *Salvadora* were characteristic in having regular univalents with a mean value ranging from 0.24–2.17 in *S. persica* and 0.16–0.88 in *S. oleoides*. Here also structural heterozygosity in the bivalents rather than precocious separation of rod bivalents has been suggested as a reason for regular occurrence of univalents. A salient feature observed in the present investigations is that of 1–3 bivalents consistently being associated with nucleolus in almost all the PMCs analyzed at diplotene/diakinesis in *S. persica* and *S. oleoides*, with lesser degree of variation between the species. This is indicative of the nucleolar nature of bivalents (Koul and Singh Chauhan 1962). The karyological study of *S. persica* does indicate the presence of nucleolar chromosomes in I pair of the karyotype with secondary constriction being clearly visible in both the chromosomes. However, karyological observations of *S. oleoides* did not confirm the presence of

nucleolar pair. Technical difficulties in obtaining clear preparations might be the reason for non-observance of nucleolar bivalents. Such problems were highlighted in many of the tree species (Gill and Singhal 1998a, b).

The chiasma frequency which ranged from 21.35 (*S. persica* JNVU/RI 2007) to 24.30 (*S. persica* JNVU/RI 2004) in *S. persica*, from 22.80 (*S. oleoides* JNVU/RI 2001) to 23.56 (*S. oleoides* BSJO 19572) in *S. oleoides*. In general a maximum of 2 chiasmata, which are mostly terminalized in bivalents were recorded and apparently the chiasma is localized at distal region. The smaller size of the bivalents with availability of short segments for recombinations may be the reason (Singh 1993, Sybenga 1972) for the varying degree of chiasma frequency.

The anaphase distribution is quite regular and normal with equal number of chromosomes reaching opposite poles in 9 out of 12 taxa belonging to *S. persica* and *S. oleoides*. The remaining 3 taxa *S. persica* JNVU/RI 2002, *S. persica* JNVU/RI 2004 and *S. persica* JNVU/RI 2007 had about less than 20% cells with either lagging bivalents or univalents resulting in slightly reduced percentage pollen stainability. Due to very few number of cells showing unequal distribution that too only in 3 out of 12 taxa studied, suggest that the univalents encountered regularly at diakinesis/M I might, be the reason. However, they do not directly affect the fertility (Bedi *et al.* 1985, Chatha and Bir 1987, Gill *et al.* 1981) of the concerned taxon.

Unlike many other tropical tree genera viz. *Terminalia*, *Quisqualis*, *Acacia* (Kumar and Subramaniam 1987, Ohri 1996), *Salvadora* has not adopted polyploidy as mode of evolution since not a single report is available about the incidence of polyploidy in this genus, for that matter in any of the 2 genera (*Azima*, *Dobera*) of the family Salvadoraceae.

Unfortunately, no details on either quantification or molecular composition of 2C/4C DNA are available for the genus *Salvadora*. Since the chromosome size is very small with a symmetrical nature of morphology which exhibit more or less localized chiasma at diplotene/diakinesis, more information on genome organization and species differentiation can be deduced by studying qualitative and quantitative aspects of DNA *vis-a-vis* chromosome studies (Ohri and Khoshoo 1987, Raina *et al.*, 1986). Such studies would further confirm the genetic uniqueness that helps the soft wood tree species of *Salvadora* to strive and sustain the harsh and high saline habitat of Rajasthan where they form the dominant vegetation.

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