Phenomenon of Cytomixis and Intraspecific Polyploidy (2x, 4x) in *Spergularia diandra* (Guss.) Heldr. & Sart. in the Cold Desert Regions of Kinnaur District (Himachal Pradesh)

Dalvir Kaur and Vijay Kumar Singhal*

Department of Botany, Punjabi University, Patiala-147002, Punjab, India

Received September 15, 2011; accepted December 15, 2011

Summary *Spergularia diandra* (Guss.) Heldr. & Sart. (Family: Caryophyllaceae), worked out presently from the valleys of Ganjul and Hangrang in the Kinnaur district of Himachal Pradesh, exhibits intraspecific diploid ($2x=n=9$) and tetraploid ($4x=n=18$) cytotypes. The species has been worked out chromosomally for the first time from India and adds a new $4x$ cytotype to the already existing $2x$ cytotype known from outside of India. The individuals of $2x$ cytotype are restricted to the Ganjul Valley while those of $4x$ cytotype are present in Hangrang valley. The individuals of the $2x$ and $4x$ cytotypes could be distinguished on the basis of habit, plant size, number of branches, bushy nature, and fleshiness and size of leaves. The diploid plants showed spindle abnormalities during meiosis resulting into heterogeneous sized fertile/stained pollen grains and some pollen sterility (10.00–12.00%). On the other hand, some plants of $4x$ cytotype showed the phenomenon of cytomixis involving chromatin transfer among proximate meiocytes, and chromatin stickiness resulting into 5.00–8.00% unstained/sterile pollen grains. The cytomixis in the $4x$ cytotype is the first ever record for the species and seems to be a natural phenomenon under direct genetic control as the plants with and without cytomixis grow under the same environmental conditions.

Key words Ganjul and Hangrang valleys, Intraspecific cytotypes, Diploid, Tetraploid, Cytomixis, Heterogeneous sized fertile pollen grains.

Spergularia diandra (Guss.) Heldr. & Sart. (Family: Caryophyllaceae), also described under the name *Arenaria diandra* Guss., is referred to as the Lesser sand spurrey, Small sand spurrey or Alkali sand spurrey. The species, which is commonly found in damp clay or sandy and saline or calcareous soils along sandy beaches, river shores and roadsides, is distributed in Mediterranean Europe, North Africa, South Western and East Asia. It grows as an annual herb with ascending, slender, glandular pubescent stems which are erect to diffusely spreading, much-branched proximally and distally (Fig. 1). Leaves are linear, somewhat fleshy, apex obtuse and stipules inconspicuous, silvery to dull tan, triangular or rarely lanceolate. Flowers with white and oblong-elliptic petals are present in 4–8 compound cymes. Capsule ovoid and greenish tan become purple-black at maturity.

The chromosomal information gathered from various Indexes to Plant Chromosome numbers (Darlington and Wylie 1955, Fedorov 1969, Moore 1967–1971, 1968, 1969, 1970, 1972, 1973, 1974, 1977, Goldblatt 1981, 1984, 1985, 1988, Goldblatt and Johnson 1990, 1991, 1994, 1996, 1998, 2000, 2003, 2006, Kumar and Subramanian 1986, Khantoon and Ali 1993) revealed that the species in India has not been studied chromosomally and so far there are only diploid reports of chromosome counts ($n=9$) from North Africa (Humphries *et al.* 1978, Galland 1988), Canary

* Corresponding author, e-mail: vksinghal53@gmail.com

DOI: 10.1508/cytologia.77.163

Figs. 1–13. 1. *Spergularia diandra* growing on sandy slopes. 2. A PMC of 2x cytotype with $n=9$ at M-I. 3. A PMC with 9:9 chromosomes distribution at A-I. 4. PMCs of diploid cytotype showing lag-gards (arrowed) and a chromatin bridge (arrowhead) at A-I. 5. A sporad of 2x cytotype with a micronucleus (arrowed). 6. Heterogeneous sized apparently fertile/stained and sterile pollen grains of 2x cytotype. 7. A PMC of 4x cytotype showing 18 bivalents at M-I. 8. A PMC of 4x cytotype with 18 countable chromosomes at 1 pole at A-I. 9. 2 PMCs of 4x cytotype involved in cytomixis at M-I (arrowed). 10. 2 PMCs involved in chromatin transfer at T-II (arrowed). 11. 2 PMCs involved in chromatin transfer through long chromatin strands at T-II (arrowed). 12. A group of PMCs showing chromatin stickiness at M-I. 13. A fertile and a sterile pollen grain of 4x cytotype. (Scale bar= $10\mu\text{m}$).

Islands (Spain) of Europe (Dalgaard 1987) and Mediterranean regions (Runemark 1996). While analyzing the cytomorphological diversity in the flowering plants from the cold desert regions of the Kinnaur district in Himachal Pradesh, we have been able to detect the existence of intraspecific dip-

loid ($n=9$) and tetraploid ($n=18$) cytotypes in *S. diandra*. Some of the diploid (2x) and tetraploid (4x) plants showed the phenomena of cytomixis, associated meiotic abnormalities and pollen malformation.

In this paper the aim was to study in detail the course of meiosis including microsporogenesis and pollen fertility in both the cytotypes. The aim here was also to study the distribution pattern and to differentiate the 2 cytotypes on morphological characters.

Materials and methods

Materials for male meiotic studies were collected during the months of September in 2008 and 2009 from 10 wild plants growing in Ganjul Valley (Pooh, 2840m, 31°45'47"N and 78°35'20"E) and Hangrang Valley (Chango, 3050m, 31°58'34"N and 78°35'33"E) of the Kinnaur district of Himachal Pradesh (India). Voucher specimens of the cytologically worked out individuals were deposited in the Herbarium, Department of Botany, Punjabi University Patiala (PUN* 53741, 53742). For male meiotic studies, young and unopened floral buds were fixed in freshly prepared Carnoy's fixative (6 ethanol:3 chloroform:1 glacial acetic acid, v/v/v) for 24h and preserved in 70% alcohol at 4°C. Anthers were squashed in 1% acetocarmine and preparations were studied for meiotic chromosome counts and detailed meiotic behavior in pollen mother cells (PMCs) at different stages. Pollen viability was estimated through stainability tests for which anthers from mature flowers were squashed in glycerol-acetocarmine (1:1) mixture and 1% aniline blue dye. Well-filled pollen grains with fully stained cytoplasm were scored as fertile while those that were shriveled up and had partially or unstained cytoplasm were counted as sterile. Pollen grain size was measured using an oculomicrometer. Images of chromosome counts, meiotic abnormalities, sporads and pollen grains were photomicrographed from the freshly prepared slides with a Nikon 80i eclipse microscope and a Leica Qwin Digital Imaging System.

Results

While surveying dicotyledonous plants for cytological study from the Ganjul Valley and Hangrang Valley in the Kinnaur district of Himachal Pradesh (India), we have detected the existence of 2x and 4x cytotypes in *S. diandra*. Out of the total of 10 individuals scored presently, 2 plants collected from Ganjul Valley showed a meiotic chromosome count of $n=9$ while the rest of the plants studied from the Hangrang Valley showed a tetraploid chromosome number of $n=18$.

The 2 cytotypes could be differentiated in the field on the basis of habit, plant height, number of branches, and fleshiness and size of leaf (Table 1). Tetraploid plants which are confined to Hangrang Valley are relatively more common than the diploids growing in Ganjul Valley. Further, the 2 cytotypes could also be differentiated on the basis of their bushy nature as 4x plants were observed to have more branching and are bushy compared to the 2x plants. The leaves of both the cytotypes are linear but differentiated in being more fleshy in 2x plants compared to 4x plants. Pollen grains in tetraploid plants are of uniform size whereas in the diploids, pollen grains are of heterogeneous sizes.

The diploid (n=9)

The individuals collected for chromosomal studies from Ganjul Valley growing on sandy slopes and among stones along roadsides existed at diploid level with a meiotic chromosome count of $n=9$ as confirmed by the presence of 9 bivalents at M-I (Fig. 2) and 9:9 chromosomes distribu-

* Herbarium Code of Department of Botany, Punjabi University, Patiala as per "Index Herbariorum" by Holmgren and Holmgren (1998).

Table 1. Comparison of morphological characters in the 2x and 4x cytotypes of *S. diandra*

Characters	Cytotype	
	The diploid (n=9)	The tetraploid (n=18)
Habitat	Sandy slopes, among stones along roadsides	Sandy slopes, along roadsides
Habit	Less bushy	More bushy
Plant size (cm)	28.82–34.23 (31.58±2.79)	42.80–56.00 (51.26±4.32)
No. of branches	3–5 (4±1)	7–9 (8±1)
Leaves		
(i) Shape	Linear	Linear
(ii) Size (mm)–Length	10.0–15.8 (10.80±1.1)	15.0–22.0 (17.2±1.65)
Breadth	6.0–7.0 (6.2±0.46)	4.0–6.0 (5.0±0.92)
(iii) Fleshiness	More	Less
Flowers	White	White
Pollen grains		
(i) Size (μm); Frequency	Heterogeneous sized Large: 33.93–35.82 (34.81±0.95)×33.93–35.82 (34.81±0.95); 6.90% Medium: 28.27–30.16 (29.12±0.96)×28.27–30.16 (29.12±0.96); 82.76% Small: (20.73–22.62 (21.67±0.68)×20.73–22.62 (21.67±0.68); 10.34%	Uniform sized 28.27–29.40 (28.77±0.57)×28.27–29.40 (28.77±0.57)
(ii) Fertility (% age)	88.00–90.00 (89.00±1)	92.00–100.00 (95.12±3.22)

Values in parentheses represent the mean±standard deviation

tion at A-I (Fig. 3). However, a few PMCs showed the presence of laggards (1.23–2.65%) and chromatin bridges (0.55–1.22%) during A-I (Fig. 4). The lagging chromosomes in such PMCs failed to get included at poles and organized into micronuclei during sporad formation (Fig. 5). Consequently, the individuals showed some pollen malformation in the form of pollen sterility (10.00–12.00%) and apparently fertile pollen grains of 3 different sizes (Large: 33.93–35.82 μm×33.93–35.82 μm, 6.90%; Typical: 28.27–30.16 μm×28.27–30.16 μm, 82.76%; Small: 20.73–22.62 μm×20.73–22.62 μm, 10.34%) (Fig. 6).

The tetraploid (n=18)

The male meiotic course studied on the basis of 8 individuals collected from the Hangrang Valley showed a tetraploid chromosome count of $n=18$ as confirmed from the presence of 18 bivalents at M-I (Fig. 7) and 18:18 chromosomes distribution at A-I (Fig. 8). Out of 8 tetraploid individuals, 2 showed perfectly regular meiotic course, normal sporad formation and 100.00% fertile pollen grains. On the other hand, the remaining 6 individuals showing normal 18 bivalents depicting the phenomenon of chromatin transfer among neighbouring meiocytes (Figs. 9–11) and chromatin stickiness (Fig. 12). The chromatin transfer occurred through long narrow cytomictic channels by forming 1–2 strands. Consequent to meiotic irregularities in meiocytes, these individuals showed 5.00–8.00% unstained/sterile pollen grains (Fig. 13).

The data on chromosome number, meiotic course, microsporogenesis and pollen grain size and pollen fertility in both the cytotypes are given in Table 2.

Discussion

S. diandra has been counted chromosomally for the first time from India. Further, the present study also records the existence of intraspecific 2x and 4x cytotypes in the Kinnaur district of Himachal Pradesh in India (Asia). Outside of India, only diploid cytotype with $n=9$ is known from Morocco and Algeria in North Africa (Humphries *et al.* 1978, Galland 1988), the Canary Islands

Table 2. Meiotic course, microsporogenesis, pollen grain size, frequency and pollen fertility in different individuals of the 2x and 4x cytotypes of *S. diandra*

Cytotype	Cytomixis		Meiotic course		Microsporo- genesis	Pollen grains		Fertility % age
	% age of PMCs involved	Meiotic stages	% age of PMCs with chromatin stickiness	%age of PMCs with laggard at A-I	% age of PMCs with chromatin bridges at A-I	Size (μm)	Frequency % age	
2x ₁	—	—	—	1.23	0.55	Large: 33.93–35.82 (34.81±0.95) × 33.93–35.82 (34.81±0.95) Typical: 28.27–30.16 (29.12±0.96) × 28.27–30.16 (29.12±0.96) Small: 20.73–22.62 (21.67±0.68) × 20.73–22.62 (21.67±0.68)	6.90	88.00
2x ₂	—	—	—	2.65	1.22	Large: 33.93 × 33.93 Typical: 28.27–30.16 (29.12±0.96) × 28.27–30.16 (29.12±0.96) Small: 20.73–22.62 (21.67±0.68) × 20.73–22.62 (21.67±0.68)	10.34 3.32 85.45	90.00
4x ₁	3.47	M-I	2.50	—	—	Typical: 28.27 × 28.27	11.23	95.00
4x ₂	5.88	M-I, T-II	10.31	—	—	Typical: 28.27–29.40 (28.77±0.57) × 28.27–29.40 (28.77±0.57)	100.00	92.00
4x ₃	—	—	—	—	—	Typical: 28.27 × 28.27	100.00	100.00
4x ₄	—	—	—	—	—	Typical: 28.27 × 28.27	100.00	100.00
4x ₅	3.66	T-II	19.49	—	—	Typical: 28.27 × 28.27	100.00	92.00
4x ₆	1.52	T-II	6.38	—	—	Typical: 28.27 × 28.27	100.00	94.00
4x ₇	1.88	T-II	—	—	—	Typical: 28.27 × 28.27	100.00	95.00
4x ₈	2.12	M-I, T-II	—	—	—	Typical: 28.27 × 28.27	100.00	93.00

M-I=Metaphase-I, A-I= Anaphases-I, T-I=Telophases-I, values in parentheses represent the mean±standard deviation.

Map 1. Distribution pattern of the 2x and 4x cytotypes of *Spergularia diandra* in India and other parts of the world

(Spain) of Europe (Dalgaard 1987) and Mediterranean regions (Runemark 1996) (Map 1).

The intraspecific 2x and 4x cytotypes recorded presently in *S. diandra* from cold desert regions of the Indian Himalayas could be differentiated in the field on the basis of some morphological characters (habit, plant size, number of branches and fleshiness and size of leaf). The tetraploid plants were observed to be much taller and possessed large fleshy leaves in a smaller quantity. It is thus apparent that morphological characters in both the cytotypes of the species are directly correlated with the increase in ploidy level which has also been reported in *Andropogon gerardii* Vitman (Keeler and Davis 1999), *Centaurea jacea* L. (Hardy *et al.* 2000), *Parasenecio auriculata* (DC.) J.R. Grant (Nakagawa 2006), *Dactylis* L. (Amirouche and Misset 2007), *Centaurea phrygia* L. (Koutecký 2007), *Rorippa amphibia* L. (Luttikhuizen *et al.* 2007), *Centaurea stoebe* L. (Španiel *et al.* 2008, Mráz *et al.* 2011) and *Ranunculus hirtellus* (Kumar and Singhal 2011). Pollen grains are of uniform size in the tetraploid cytotype whereas those of 2x cytotype are of 3 different sizes, large, medium (typical) and small sized. The heterogeneous sizes of pollen grains in the 2x cytotype could be the consequence of spindle irregularities. Further, the typical sized pollen grains in 2x and 4x cytotypes were observed to be of the same size indicating that the increase in the ploidy level has not affected the pollen grain size in the 4x cytotype. Kumar and Singhal (2011) have also reported in *Ranunculus hirtellus* that increase in ploidy level does not affect the size of pollen grains.

Both the cytotypes are found to grow under the same climatic conditions but the individuals of 4x cytotype are restricted to the Hangrang Valley at the altitude of 3050m whereas the 2x cytotype is restricted to the Ganjul Valley at 2840 m.

In spite of normal bivalent formation and their regular segregation during anaphases/telophases, a few PMCs in the individuals of the 2x cytotype showed some spindle abnormalities re-

sulting in the presence of laggards and chromatin bridges. The lagging chromosomes failed to get included at poles and organized into the formation of micronuclei or micropollen as reported by Bhat *et al.* (2006) and Singhal and Kumar (2008a). Out of 8 tetraploid individuals, 6 plants showed the phenomenon of cytomixis involving chromatin transfer among proximate PMCs leading into some sterile pollen grains (5.00–8.00%). The phenomenon of cytomixis, coined by Gates (1911), is reported here for the first time in the species. Cytomixis only occurs in the tetraploid and not in the diploid individuals in *S. diandra*, confirming the views of earlier workers that the phenomenon of chromatin transfer is more prevalent in polyploids than their diploid counterparts (Kamra 1960, Semyarkhina and Kuptsou 1974, Basavaiah and Murthy 1987, Sheidai and Attai 2005, Singhal *et al.* 2007). Chromatin transfer occurred through long sized cytoplasmic channels forming 1–2 chromatin strands involving 2 PMCs in 1.52–5.88% cases from late stages of meiosis-I and continued up to T-II. The same results are found in some cases where the transfer of chromatin or chromosomes may take place until the later stages of meiosis and the size of cytomictic channels may increase to form conspicuous transfer of chromatin material among inter-PMCs (Falistocco *et al.* 1995, Haroun 1995, Singhal and Kumar 2008a, b, 2010, Shabrangi *et al.* 2010, Mursalimov and Deineko 2011). The inter-PMCs transfer of chromatin material results in chromosome stickiness and consequently pollen sterility. Similar findings on the impact of chromatin transfer in causing chromatin stickiness and consequently pollen sterility have been recorded earlier in *Vicia faba* (Haroun *et al.* 2004), *Caltha palustris* (Kumar and Singhal 2008), *Meconopsis aculeata* (Singhal and Kumar 2008a), *Hippophae rhamnoides* (Singhal *et al.* 2008), *Anemone rivularis* (Singhal *et al.* 2009a) and *Clematis orientalis* (Kumar *et al.* 2010).

The phenomenon of cytomixis has been reported in a large number of angiospermic plants, and many researchers consider cytomixis to be of considerable evolutionary significance (Falistocco *et al.* 1995, Morikawa and Leggett 1996, Malallah and Attia 2003, Singhal and Kumar 2010). Some of the possible causes and explanations put forth by earlier researchers include the effect of fixation (Haroun 1995), pathological changes (Morisset 1978), physiological control (Bahl and Tyagi 1988), chemicals and herbicides (Haroun 1995), environmental stress and pollution (Haroun *et al.* 2004), temperature (Kumar and Tripathi 2008), stress factors and genetic control (Malallah and Attia 2003), pressure difference (Morisset 1978) and clumped chromatin bridges during premeiotic anaphase (Mendes and Rijo 1951). In the present case, cytomixis seems to be a natural phenomenon under direct genetic control as the plants with and without cytomixis grow under the same environmental conditions as reported by several researchers (Singhal and Gill 1985, Bellucci *et al.* 2003, Haroun *et al.* 2004, Lattoo *et al.* 2006, Singhal *et al.* 2007, 2008, 2009a, b, 2010, 2011, Kumar and Singhal, 2008, Singhal and Kumar, 2008a, b, 2010, Kumar *et al.* 2008a, b, 2010, 2011, 2012, Himshikha *et al.* 2010).

Acknowledgements

The authors are grateful to the University Grant Commission, New Delhi for providing financial assistance under DRS-SAP II and ASIST programme. Dalvir Kaur is also grateful to UGC for providing fellowship under Rajiv Gandhi National Fellowship Scheme. Thanks are also due to the Head, Department of Botany for providing necessary laboratory and library facilities.

References

- Amirouche, N. and Misset, M. T. 2007. Morphological variation and distribution of cytotypes in the diploid-tetraploid complex of the genus *Dactylis* L. (Poaceae) from Algeria. *Plant Syst. Evol.* **264**: 157–174.
- Bahl, J. R. and Tyagi, B. R. 1988. Cytomixis in pollen mother cells of *Papaver dubium* L. *Cytologia* **53**: 771–775.
- Basavaiah, D. and Murthy, T. C. S. 1987. Cytomixis in pollen mother cells of *Urochloa panicoides* P. Beauv. (Poaceae). *Cytologia* **52**: 69–74.

- Bellucci, M., Roscini, C. and Mariani, A. 2003. Cytomixis in pollen mother cells of *Medicago sativa* L. J. Hered. **94**: 512–516.
- Bhat, T. A., Parveen, S. and Khan, A. H. 2006. MMS induced cytomixis in pollen mother cells of Broad Bean (*Vicia faba* L.). Turk. J. Botany **30**: 273–279.
- Dalgaard, V. 1987. Chromosome Number Reports 96. Taxon **36**: 660.
- Darlington, C. D. and Wylie, A. P. 1955. Chromosome Atlas of Flowering Plants. George Allen and Unwin Ltd., London. pp. 275.
- Falisticco, E., Tosti, T. and Falcinelli, M. 1995. Cytomixis in pollen mother cells of diploid *Dactylis*, one of the origins of $2n$ gametes. J. Hered. **86**: 448–453.
- Fedorov, An. A. 1969. Chromosome Numbers of Flowering Plants. Academy of Sciences of the USSR. Komarov Botanical Institute, Leningrad (Reprint 1974).
- Galland, N. 1988. Recherche sur l'origine de la flore orophile du Maroc étude caryologique et cytogéographique. Travaux de l'Institut Scientifique, Université Mohammed V. Série Botanique **35**: 1–168.
- Gates, R. R. 1911. Pollen formation in *Oenothera gigas*. Ann. Bot. **25**: 90–940.
- Goldblatt, P. (ed.) 1981, 1984, 1985, 1988. Index to Plant Chromosome Numbers 1975–1978, 1979–1981, 1982–1983, 1984–1985. Monogr. Syst. Bot. Mo. Bot. Gard. **5**, **8**, **13**, **23**.
- and Johnson, D. E. (eds.) 1990, 1991, 1994, 1996, 1998, 2000, 2003, 2006. Index to Plant Chromosome Numbers 1986–1987, 1988–1989, 1990–1991, 1992–1993, 1994–1995, 1996–1997, 1998–2000, 2001–2003. Monogr. Syst. Bot. Mo. Bot. Gard. **30**, **40**, **51**, **58**, **69**, **81**, **94**, **106**.
- Hardy, O. J., Vanderhoeven, S., De Loose, M. and Meerts, P. 2000. Ecological, morphological and allozymic differentiation between diploid and tetraploid knapweeds (*Centaurea jacea*) from a contact zone in the Belgian Ardenes. New Phytol. **146**: 281–290.
- Haroun, S. A. 1995. Cytomixis in pollen mother cells of *Polygonum tomentosum* Schrank. Cytologia **60**: 257–260.
- , AL Shehri, A. M. and AL Wadie, H. M. 2004. Cytomixis in the microsporogenesis of *Vicia faba* L. (Fabaceae). Cytologia **69**: 7–11.
- Himshikha, Kumar, P., Gupta, R. C., Kumari, S. and Singhal, V. K. 2010. Impact of chromatin transfer and spindle abnormalities on pollen fertility and pollen size in *Plantago lanceolata* L. Cytologia **75**: 421–426.
- Holmgren, P. K. and Holmgren, N. H. 1998. Index Herbariorum: A Global Directory of Public Herbaria and Associated Staff. New York Botanical Garden's Virtual Herbarium. http://sweetgum.nybg.org/ih/herbarium_list.php.
- Humphries, C. J., Murray, B. G., Bocquet, G. and Vasudevan, K. N. 1978. Chromosome numbers of phanerogams from Morocco and Algeria. Nord. J. Bot. **131**: 391–404.
- Kamra, O. P. 1960. Chromatin extrusion and cytomixis in pollen mother cells of *Hordeum*. Hereditas **46**: 592–600.
- Keeler, K. H. and Davis, G. A. 1999. Comparison of common cytotypes of *Andropogon gerardii* (Andropogoneae, Poaceae). Am. J. Bot. **86**: 974–979.
- Khatoun, S. and Ali, S. I. 1993. Chromosome Atlas of the Angiosperms of Pakistan. Department of Botany, University of Karachi, Karachi.
- Koutecký, P. 2007. Morphological and ploidy level variation of *Centaurea phrygia* agg. (Asteraceae) in the Czech Republic, Slovakia and Ukraine. Folia Geobot. **42**: 77–102.
- Kumar, P. and Singhal, V. K. 2008. Cytology of *Caltha palustris* L. (Ranunculaceae) from cold regions of Western Himalayas. Cytologia **73**: 137–143.
- and — 2011. Male meiosis, morphometric analysis and distribution pattern of $2x$ and $4x$ cytotypes of *Ranunculus hirtellus* Royle, 1834 (Ranunculaceae) from the cold regions of northwest Himalayas (India). Comp. Cytogenet. **5**: 143–161.
- , — and Kaur, D. 2008a. Meiotic studies in species from the cold deserts of Lahaul-Spiti and adjoining areas (Northwest Himalayas). Cytologia **73**: 463–470.
- , — and Kaur, J. 2008b. Cytomixis induced meiotic abnormalities in pollen mother cells of *Clematis flammula* L. (Ranunculaceae). Cytologia **73**: 381–385.
- , —, — and Kaur, S. 2010. Cytomixis and associated meiotic abnormalities affecting pollen fertility in *Clematis orientalis*. Biol. Plant. **54**: 181–184.
- , —, Rana, P. K., Kaur, S. and Kaur, D. 2011. Cytology of *Ranunculus laetus* Wall. ex Royle from cold desert regions and adjoining hills of North-west Himalayas. Caryologia **64**: 25–32.
- , — and Kaur, D. 2012. Impaired male meiosis due to irregular synapsis coupled with cytomixis in a new diploid cytotype of *Dianthus angulatus* Royle (Caryophyllaceae) from Indian cold deserts. Folia Geobot. **47**: 59–68.
- Kumar, G. and Tripathi, R. 2008. Induced cytotoxic variations through abiotic stresses in grasspea (*Lathyrus sativus* L.). Indian J. Genet. Plant Breed. **68**: 58–64.
- Kumar, V. and Subramanian, B. 1986. Chromosome Atlas of Flowering Plants of the Indian Subcontinent. Vol. **I**. Dicotyledons. BSI, Calcutta. pp. 464.
- Lattoo, S. K., Khan, S., Bamotra, S. and Dhar, A. K. 2006. Cytomixis impairs meiosis and influences reproductive success

- in *Chlorophytum comosum* (Thunb.) Jacq.—an additional strategy and possible implications. *J. Biosci.* **31**: 629–637.
- Luttikhuizen, P. C., Stift, M., Kuperus, P. and Van Tienderen, P. H. 2007. Genetic diversity in diploid vs. tetraploid *Rorippa amphibian* (Brassicaceae). *Mol. Ecol.* **16**: 3544–3553.
- Malallah, G. A. and Attia, T. A. 2003. Cytomixis and its possible evolutionary role in a Kuwait population of *Diplotaxis harra* (Boraginaceae). *Bot. J. Linn. Soc.* **143**: 169–175.
- Mendes, E. J. and Rijo, L. 1951. A new interpretation for “Cytomixis”. *Port. Acta Biol. Ser. A* **3**: 211–288.
- Moore, R. J. (ed.). 1970, 1971, 1972, 1973, 1974, 1977. Index to Plant Chromosome Numbers 1967–1971, 1968, 1969, 1970, 1972, 1973–1974. *Regnum Veg.* **68**, **77**, **84**, **90**, **91**, **96**.
- Morikawa, T. and Leggett, M. 1996. Cytological and morphological variations in wild populations of *Avena canariensis* from the Canary Islands. *Genes Genet. Syst.* **71**: 15–21.
- Morisset, P. 1978. Cytomixis in pollen mother cells of *Ononis* (Leguminosae). *Can. J. Genet. Cytol.* **20**: 383–388.
- Mráz, P., Bouchier, R. S., Treierz, U. A., Schaffner, U. and Müller-Schärer, H. 2011. Polyploidy in phenotypic space and invasion context: a morphometric study of *Centaurea stoebe* S.L. *Int. J. Plant Sci.* **172**: 386–402.
- Mursalimov, S. R. and Deineko, E. V. 2011. An ultrastructural study of cytomixis in tobacco pollen mother cells. *Protoplasma* [Internet] 2011 Feb 1 [cited 2011 Sep 15]. Available from: <http://www.ncbi.nlm.nih.gov/books/NBK7281/#A55869>.
- Nakagawa, M. 2006. Ploidy, geographical distribution and morphological differentiation of *Parasenecio auriculata* (Senecioneae, Asteraceae) in Japan. *J. Plant Res.* **119**: 51–61.
- Runemark, H. 1996. Mediterranean chromosome number reports 6 (590–678). *Flora Mediterranea* **6**: 223–243.
- Semyarkhina, S. Y. A. and Kuptsou, M. S. 1974. Cytomixis in various forms of sugarbeet; *Vests I. ANBSSE Ser Biyal* **4**: 43–47.
- Shabrangi, A., Sheidai, M., Majd, A., Nabiuni, M. and Dorrani, D. 2010. Cytogenetic abnormalities caused by extremely low frequency electromagnetic fields in canola. *Science Asia*. **36**: 292–296.
- Sheidai, M. and Attai, S. 2005. Meiotic studies of some *Stipa* (Poaceae) species and populations in Iran. *Cytologia* **70**: 23–31.
- Singhal, V. K. and Gill, B. 1985. Cytomixis in some woody species. *Biologica* **1**: 168–175.
- , Gill, B. S. and Dhaliwal, R. S. 2007. Status of chromosomal diversity in the hardwood tree species of Punjab state. *J. Cytol. Genet.* **8**: 67–83.
- , Kaur, D. and Kumar, P. 2008. Effect of cytomixis on the pollen size in ‘Seabuckthorn’ (*Hippophae rhamnoides* L., Elaeagnaceae). *Cytologia* **73**: 167–172.
- , Kaur, S. and Kumar, P. 2010. Aberrant male meiosis, pollen sterility and variable sized pollen grains in *Clematis montana* Buch.-Ham. ex DC. from Dalhousie hills, Himachal Pradesh. *Cytologia* **75**: 31–36.
- , —, Kaur, D. and Kumar, P. 2009b. New detection of haploid chromosomes, pollen size and sterility in *Lychnis indica* Benth. var. *fimbriata* Wall. *Chromosome Bot.* **4**: 53–56.
- and Kumar, P. 2008a. Impact of cytomixis on meiosis, pollen viability and pollen size in wild populations of Himalayan poppy (*Meconopsis aculeata* Royle). *J. Biosci.* **33**: 371–380.
- and — 2008b. Cytomixis during microsporogenesis in the diploid and tetraploid cytotypes of *Withania somnifera* (L.) Dunal, 1852 (Solanaceae). *Comp. Cytogenet.* **2**: 85–92.
- and — 2010. Variable sized pollen grains due to impaired male meiosis in the cold desert plants of North West Himalayas (India). In: Benjamin, J. K. (Ed.), *Pollen: structure, types and effects*. Nova Science Publishers, New York, pp. 101–126.
- , —, Kaur, D. and Rana, P. K. 2009a. Chromatin transfer during male meiosis resulted into heterogeneous sized pollen grains in *Anemone rivularis* Buch.-Ham. ex DC. from Indian cold deserts. *Cytologia* **74**: 229–234.
- , Rana, P. K., Kumar, P. and Kaur, D. 2011. Persistent occurrence of meiotic abnormalities in a new hexaploid cytotype of *Thalictrum foetidum* L. from Indian cold deserts. *Biologia* **66**: 458–464.
- Španiel, S., Marhold, K., Hodgálová, I. and Lihová, J. 2008. Diploid and tetraploid cytotypes of *Centaurea stoebe* (Asteraceae) in central Europe: morphological differentiation and cytotype distribution patterns. *Folia Geobot.* **43**: 131–158.