Impact of Chromatin Transfer and Spindle Abnormalities on Pollen Fertility and Pollen Size in Plantago lanceolata L.

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Summary The phenomenon of cytomixis involving chromatin transfer and spindle abnormalities were recorded for the first time in the diploid \((n=6)\) accession of Plantago lanceolata scored from Parvati Valley, Kullu District, Himachal Pradesh (India). Cytomixis with chromatin transfer involved 2–3 PMCs (pollen mother cells) at different stages of meiosis from early prophase-I to T-II, but the maximum frequency of PMCs involved in cytomixis was recorded during the early prophase stages of meiosis-I (11.03%). The transfer of chromatin material among meiocytes was observed to be both partial and complete resulting into PMCs with hypo- and hyperploid chromosome numbers \((2n=14, 15)\). During cytomixis, nucleolus also transmigrated to neighbouring PMCs and the PMCs with supernumerary nucleoli also resulted. Other meiotic abnormalities associated with cytomixis included chromatin stickiness, out of plate bivalents, laggards, bridges, unoriented chromosomes during A-I/T-I and A-II/T-II, and micronuclei. The effect of chromatin transfer, spindle abnormalities and associated meiotic anomalies on meiotic behaviour, pollen fertility and pollen size has been discussed. The cytomixis in \(P.\) lanceolata seems to be a natural phenomenon under genetic control and certainly have played a role in the origin of aneuploids and polyploids.

Key words Cytomixis, Meiotic abnormalities, Parvati Valley, Hypo- and hyperploid chromosome numbers.

Plantago lanceolata L. (Family: Plantaginaceae), a common weed of cultivable lands, is known by several different local names at different places including, English plantain, Ribble grass, Black plantain, Snake plantain, Ribwort plantain, and Lambs’s tongue. The species, which is highly polymorphic, is widely distributed in Europe from South Iceland and east to Spain, and Northern and Central Asia. Besides, it had also been introduced into several countries and is considered as an invasive weed in North America. In India, the species commonly occurs from Kashmir to Shimla between 1500–3000 m along roadsides, meadows, ditches and wastelands. Its leaves and seeds have been used extensively as antibacterial, antidotic, astringent, antihaemorrhagic, demulcent, expectorant, laxative, ophthalmic, poultice and diuretic treatments. Internally, these have been used in the treatment of a wide range of complaints including diarrhoea, gastritis, enteritis, peptic ulcers, irritable bowel syndrome, haemorrhage, haemorrhoids, cystitis, goitre, liver disorders, worms and hay fevers (Brown 1995, Chevalier 1996). Externally, plants have been used in treating skin inflammations, malignant ulcers, cuts, dog bites, eczema, herpes, and stings (Grieve 1984). Because of its immense medicinal importance and wide distribution, the species has been studied chromosomally by several researchers from different regions of the world depicting an array of chromosome numbers \(2n=12, 13, 14, 24, 96\) (Darlington and Wylie 1955, Löve and Löve 1956, 1975, Fedorov 1969, Moore 1973, 1974, 1977, Goldblatt 1981, 1984, 1985, 1988, Kumar and Subramanian 1986, Goldblatt and Johnson 1990, 1991, 1994, 1996, 1998, 2000, 2003, 2006,

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Khatoon and Ali 1993). Besides, Soyano (1959) reported the presence of B-chromosomes in some accessions. Extensive studies on chromosomal investigations were also conducted by researchers from the Kashmir Himalayas in India (Koul and Gohil 1973, Vasudevan 1976, Sharma et al. 1986, Sharma et al. 1992, Sareen et al. 1990, 1994, Sen and Sharma 1990, Munshi et al. 1994, 1995, Sharma and Koul 1995, Pramanik and Raychandhari 1997). Munshi et al. (1994, 1995) had reported aneuploid (2n=14) and primary trisomic (2n=13) plants in the Kashmir Himalayas. Keeping in view the chromosomal variability and medicinal value of species, we explored chromosomally plants from the Parvati Valley and the Kullu Valley in the Kullu District of Himachal Pradesh, India. We recorded for the first time the phenomenon of cytomixis involving chromatin transfer and spindle abnormalities in the accession scored from Parvati Valley. In this paper we have also discussed the effect of chromatin transfer and spindle abnormalities on meiotic behaviour, pollen fertility and pollen size.

Materials and methods

Materials for male meiotic studies were collected from wild plants growing along roadsides from the Parvati Valley (Jari 1600 m, 52986, 52987 PUN*) and Kullu Valley (Manali 2000 m, 52984, 52985 PUN) in the Kullu District of Himachal Pradesh, India during the months of March–April, 2010. Voucher specimens of the meiotically worked-out individuals were deposited in the Herbarium, Department of Botany, Punjabi University, Patiala (PUN). Spikes of appropriate sizes were fixed in freshly prepared Carnoy’s fixative (6 ethanol : 3 chloroform : 1 acetic acid v/v/v) for 24 h and subsequently transferred to 70% alcohol and preserved at 4°C in refrigerator. For pollen mother cells (PMCs) preparation, the developing anthers were squashed in 1% acetocarmine. A number of freshly prepared slides were examined for meiotic analysis and chromosome counts. Pollen fertility was estimated through stainability tests for which mature anthers were squashed in 1% aniline blue dye. Well-filled pollen grains with stained nuclei were scored as fertile while those with shrivelled and unstained cytoplasm were counted as sterile. Pollen grain size was measured with the aid of occulomicrometre. A total of 1105 meiocytes and 413 pollen grains were analyzed in the accessions. Photomicrographs of chromosome counts, meiotic irregularities, sporads and pollen grains were made from the temporary mounts using Nikon 80i eclipse microscope.

Results and discussion

Meiocytes in both the accessions of Plantago lanceolata uniformly revealed the presence of 6 equal-sized bivalents at diakinesis and M-I (Fig.1a) and 6:6 chromosomes distribution at A-I (Fig. 1b). The majority of the PMCs in these accessions which existed at diploid level (based on x=6) showed regular meiotic course resulting into normal sporad formation. However, 52 of the 502 observed PMCs in the plants scored from the Parvati Valley exhibited the phenomenon of cytomixis involving transfer of chromatin material among proximate PMCs. Cytomixis with chromatin transfer generally involved 2–3 PMCs at different stages of meiosis from early prophase-I to T-II. However, the frequency of PMCs involved in chromatin transfer was noticed to be at a maximum during the earlier prophase stages of meiosis-I (11.03%) compared to late stages of meiosis-I and meiosis-II (Table 1). Maheshwari (1950), Sarvella (1958), Kundu and Sharma (1988), and Sen and Bhattacharya (1988) had also suggested the early stages of meiosis-I to be more favourable for cytomixis. The transfer of chromatin from donor PMCs to recipient PMCs occurred through 1–2

* PUN is the Herbarium Code of Department of Botany, Punjabi University, Patiala as per “Index Herbariorum” by Holmgren and Holmgren (1998).
narrow and broad cytomictic channels. During cytomixis, chromatin transfer occurred to only one neighbouring meiocyte, but in some cases it had been noticed that the donor PMCs were simultaneously involved in transfer of chromatin material to 2 adjacent PMCs (Fig. 1c). In some cases, bivalents as such migrated through cytomictic channels (Fig. 1d). The transfer of chromatin material among meiocytes was observed to be both partial and complete. Depending upon the nature of chromatin material transfer, the resultant PMCs either become hypo-, hyperploid or enucleated. Figure 1e shows a PMC with hyperploid chromosome constitution depicting 7 bivalents. Figure 1f depicts a recipient hyperploid PMC with 7 chromosomes at one pole and the donor hypoploid PMC with 5 chromosomes during A-I. Formation of hypo-, hyperploid and enucleated PMCs as a result of chromatin transfer had also been reported in *Astragalus*.

Figs. 1 (a–m). Meiosis in *Plantago lanceolata*. a) A PMC showing 6 bivalents at M-I. b) A PMC showing 6:6 chromosomes distribution at poles during A-I. c) Simultaneous transfer of chromatin from a single PMC to 2 adjacent PMCs (arrows). d) Transfer of individual bivalent through cytoplasmic channel (arrowed). e) A hyperploid PMC with 7 bivalents at diakinesis. f) Hyper- (arrowed) and hypoploid (arrowhead) PMCs showing 7 and 5 chromosomes at one pole during A-I, respectively. g) A PMC at diakinesis with supernumerary nucleoli. h) A PMC with out of plate one bivalent at M-I (arrowed). i) A PMC with laggards at A-I (arrowed). j) A PMC showing chromatin bridge at T-II (arrowed). k) A PMC showing spindle abnormality in the form of unoriented chromosomes at late A-II (arrows). l) A PMC with micronuclei at T-II (arrows). m) Heterogeneous sized apparently fertile and unstained/sterile pollen grains. Scale bar = 10 μm.
subuliformis (Ashraf and Gohil 1994), Dactylis (Falistocco et al. 1995), Caltha palustris (Kumar and Singhal 2008), Meconopsis aculeata (Singhal and Kumar 2008a), Withania somnifera (Singhal and Kumar 2008b), Lychnis indica (Singhal et al. 2009a), Anemone rivularis (Singhal et al. 2009b), Clematis montana (Singhal et al. 2010) and C. orientalis (Kumar et al. 2010). During cytomixis, nucleolus also transmigrated and the resultant PMCs showed supernumerary nucleoli (Fig. 1g). Basavaiah and Murthy (1987) and Bhatt et al. (2006) had also observed the migration of nucleolus along with chromatin clumps to neighbouring PMCs. Besides depicting the phenomenon of cytomixis, the PMCs in the accession also showed spindle irregularities which could be noticed in the form of out of plate bivalents (Fig. 1h), laggards (Fig. 1i), unoriented chromosomes during A-I/T-I and A-II/T-II (Fig. 1k) and micronuclei (Fig. 1l, Table 1). Chromatin stickiness was the other meiotic irregularity frequently observed in PMCs at M-I. Consequent to these meiotic irregularities, the products of PMCs resulted into variable sized fertile (18.20 μm×21.84 μm, 12.35%, 25.48–29.12 μm×21.84 μm, 83.30%) and unstained/sterile pollen grains (14.56 μm×14.56 μm, 4.65%) (Fig. 1m). Similar observations related to heterogeneity in the size of pollen grains and some pollen sterility as a consequence of chromatin transfer had been reported earlier in several other plants (Haroun 1996, Malallah and Attia 2003, Haroun et al. 2004, Ghaffari 2006, Singhal and Kumar 2008a, b, Singhal et al. 2009b, 2010, Kumar et al. 2010).

The phenomenon of cytomixis involving transfer of chromatin material among meiocytes had been reported in large number of flowering plants. There are conflicting opinions and explanations regarding the causes and evolutionary significance of cytomixis. Some of the possible causes suggested earlier include the effect of fixation materials for cytological studies (Haroun 1995), pressure difference (Morisset 1978), pathological changes (Bobak and Herich 1978), physiological control (Bahl and Tyagi 1988), chemicals and herbicides (Ajay and Sarbhoy 1987), pollution (Haroun et al. 2004), abiotic stress factors and genetic control (Malallah and Attia, 2003, Kumar and Tripathi, 2008). The phenomenon of cytomixis in the accession of P. lanceolata from Parvati Valley appeared to be a natural phenomenon under the genetic control as mentioned by several other workers (Singhal and Gill 1985, Bedi 1990, Haroun et al. 2004, Kumar and Singhal 2008, Singhal and Kumar 2008a, b). Although the cytological status of variable sized pollen grains could not be ascertained, but the role of small and large sized pollen grains in the origin of aneuploid and polyploid cytotypes could not be ruled out because the pollen grains are apparently fertile. The large sized pollen are almost double the sized of the normal sized pollen grains and appeared to be of ‘2n’ chromosome constitution as suggested by Stanley and Linsken (1974). Such pollen grains might had participated in the origin of intraspecific polyploid cytotypes as had already been reported in Chrysanthemum by Kim et al. (2009) and Alopecurus and Catbrosa (Sheidai et al. 2009).

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