Cytomixis and Associated Abnormalities during Male Meiosis in a New Tetraploid Cytotype of *Vicia pallida* Turcz. (Fabaceae)

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Summary  Cytological work on *Vicia pallida* Turcz. presents male meiotic studies in wild accesses gathered from the Kinnaur district of Himachal Pradesh. All 3 accesses uniformly shared the same meiotic chromosome number of \( n = 12 \) in which 5 bivalents are relatively small sized as compared with the remaining 7 bivalents. This is the first report of tetraploid cytotype in the species against the earlier record of diploid cytotype \( (n=7) \) from the other region of Himalaya. Out of the 3 accesses scored presently, 1 accession showed a regular 12 bivalent formation, equal chromosome segregation during anaphases/telophases, normal tetrad formation and 99% pollen fertility with uniform sized pollen grains. The remaining 2 accesses depicted pollen mother cells (PMCs) with irregular meiotic behaviour which included the phenomenon of chromatin transfer among proximate meiocytes at different stages of meiosis, interbivalent connections and chromatin stickiness, out of plate bivalents at metaphase-I, lagging of chromosomes/laggards at anaphases, abnormal sporads (tetrads with micronuclei and polyads) and consequently some pollen sterility and pollen grains of variable sizes. The phenomenon of cytomixis which induces various meiotic abnormalities in the PMCs and consequently pollen sterility and pollen grains of heterogeneous sizes in *V. pallida* seems to be under direct genetic control.

Key words  Abnormal meiosis, Cytomixis, Heterogeneous sized pollen grains, Tetraploid cytotype, *Vicia pallida*.

*Vicia pallida* Turcz. (=*V. sylvatica* Benth.), family Fabaceae, is widely distributed in the temperate regions of central Asia and central Siberia between altitudes of 1200 to 2700 m. In India, the species is distributed in Jammu & Kashmir and Himachal Pradesh where it grows in moist waste places and along water courses, occasionally found in forests and orchards. The species grows as a tall straggling glabrous climber with well-branched rigid stems, compound pinnate leaves with 5–8 pairs of leaflets ending in 1–1.2 cm long, branched tendrils and lilac flowers in axillary racemes on drooping peduncles. It is an important wild forage plant (Arora and Chandel 1972). Tender fruits of the species are boiled and pickled (Manandhar 2002). The only chromosome report so far known for the species from the Kumaon hills in western Himalayas is by Mehra and Dhawan (1971) who recorded the existence of a diploid chromosome count of \( n = 7 \) (based on \( x = 7 \)). The present studies which pertain to an altogether different phyto-geographical region of the Kinnaur district in Himachal Pradesh (India) revealed the existence of a tetraploid cytotype with \( n = 12 \). The present communication covers detailed studies on male meiosis, microsporogenesis and pollen fertility in this newly reported tetraploid cytotype in the species from 3 different localities of the Kinnaur district in Himachal Pradesh.

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Material and methods

The work on *V. pallida* presents male meiosis studies in wild accessions gathered from 3 different localities of the Kinnaur district of Himachal Pradesh (Table 1). Voucher specimens of the worked-out accessions were deposited in the Herbarium, Department of Botany, Punjabi University, Patiala (PUN). For the study of male meiosis, flower buds of variable sizes measuring 1 to 13 mm were collected from the wild plants and fixed in freshly prepared Carnoy's fixative (6 ethanol:3 chloroform:1 acetic acid, v/v) for 24 h. Subsequently, the materials were transferred to 70% ethanol and stored in a refrigerator at 4°C. Slides of PMCs were prepared by squashing the developing anthers in 1% aceticarmine. A number of freshly prepared slides were carefully examined for chromosome counts and detailed meiotic course including microsporogenesis. Pollen fertility was estimated through stainability tests for which anthers from mature flowering buds were squashed in glycerol–aceticarmine (1:1) mixture and 1% aniline blue dye. Well-filled pollen grains with stained nuclei and cytoplasm were scored as fertile while those with shrivelled and unstained/poorly stained cytoplasm were counted as sterile. Pollen grain size was measured using an ocularmicrometer. Best preparations of chromosome counts, meiotic abnormalities, sporads and pollen grains were photomicrographed with Nikon 80i eclipse microscope.

Results

All 3 accessions uniformly shared the same meiotic chromosome number, \( n=12 \), as confirmed from the existence of 12 bivalents unequivocally present in all the PMCs at diakinesis/metaphase-I (Fig. 1). In all the accessions, 5 bivalents are relatively small sized as compared with the remaining 7 bivalents (Fig. 2). Based on \( x=6 \) (one of the basic numbers for the genus *Vicia*), the presently studied individuals existed at tetraploid level. One accession (PUN 53707) showed regular 12 bivalents for-
Cytomixis and Abnormalities during Male Meiosis in Tetraploid Cytotype of *Vicia pallida*

It has been observed that the fertile pollen grains are of uniform size measuring 37.70 μm × 22.62 μm. On the other hand, the accessions (PUN 53706) and (PUN 53708) collected from Bhawanagar, 1900 m, and Palingi, 1900 m, respectively depicted PMCs with irregular meiotic behaviour which included the phenomenon of chromatin transfer among proximate meiocytes at different stages of meiosis, interbivalent connections and chromatin stickiness, out of plate bivalents at metaphase-I, lagging of chromosomes/laggards at anaphases, abnormal sporads and consequently some pollen sterility and pollen grains of variable sizes.

**Cytomixis**

Although the phenomenon of cytomixis in both the accessions is existent, the frequency of meiocytes involved in actual chromatin transfer is observed to be rather low (1.52–2.20%). The phenomenon of cytomixis involving chromatin transfer involving 2–3 meiocytes is noticed to be relatively high during meiosis II compared to meiosis I. The partial chromatin transfer among proximate meiocytes occurred through narrow cytomictic channels (Fig. 3). In some cases at anaphase-II whole of the chromatin mass from one pole of a meiocyte was observed to be transferred to the adjacent meiocyte resulting into a PMC with 5 units (Fig. 4). Consequent to chromatin transfer, hypoploid PMCs are resulted. A hypoploid PMC with 6 bivalents has been observed in accession (PUN 53706) (Fig. 5). The PMCs with extra chromatin masses (Fig. 6) and supernumerary nucleoli (2 nucleoli) (Fig. 7) were also resulted as a consequence of cytomixis.

**Interbivalent connections**

Associated with the phenomenon of cytomixis, PMCs in both the accessions showed interbivalent connections involving varying number of bivalents at diakinesis and metaphase-I (Figs. 8, 9). The frequency of PMCs depicting such interbivalent connections was noticed to be relatively high (11.50%) in accession PUN 53708 compared to only (4.80%) in accession PUN 53706.

**Chromosome stickiness**

Chromosome stickiness was the other meiotic anomaly encountered in the PMCs of these accessions that occurred from the early stages of prophase-I to anaphase-II. However, the frequency of PMCs showing chromatin stickiness was observed to be significantly higher during metaphase-I (Fig. 10). Chromosome stickiness either involved a few bivalents or the whole complement at metaphase-I. The percentage of PMCs exhibiting chromosome stickiness was recorded to be higher in the accession PUN 53708 (38.80%) compared to the accession PUN 53706 (21.20%).

**Out of plate bivalents and laggards**

Consequent to cytomixis, PMCs also depicted abnormalities in spindle behaviour due to which some bivalents failed to arrange properly during metaphase-II and remained out of plate (Fig. 10). Such bivalents also showed irregular behaviour during disjunction and left as laggards during anaphases/telophases (Fig. 11). Besides, PMCs with extra chromatin masses also showed irregularities during anaphases/telophases and remained as laggards. These lagging chromosomes during further meiotic stages constituted into micronuclei.

**Sporads**

Consequent to all these irregularities at different meiotic stages, 5.40–5.52% of the observed PMCs showed irregular microsporogenesis resulting into abnormal sporad formation in the form of tetrads with micronuclei (Figs. 12, 13) and polyads (Fig. 14).
Reduced pollen viability and heterogeneous sized pollen grains

As a result of cytomixis and associated meiotic irregularities in the individuals in both the accessions depicted reduced pollen viability (73.00–78.55%) and heterogeneous sized pollen grains.
(Fig. 15). The pollen grains in these accessions are categorized into small (33.93 μm×22.62 μm), normal (37.70 μm×22.62 μm) and large sized (41.47–45.24 μm×22.62–22.62 μm). On the other hand, the pollen grains in the accession (PUN 53707) collected from Chaura, 1890 m, with normal meiotic course showed uniform sized pollen grains (37.70 μm×22.62 μm) and are almost fertile.

Discussion

The present studies from the Kinnaur district of Himachal Pradesh (India) added a new tetraploid cytotype with a chromosome count of n=12 for *V. pallida* against the earlier diploid chromosome report of n=7 recorded by Mehra and Dhawan (1971) from the other region of Himalayas in India. Out of the 3 accessions scored meiotically, 1 accession collected from Chaura, 1890 m (PUN 53707), depicted perfectly normal meiosis and nearly 99% pollen fertility and uniform sized pollen grains. The accessions studied from Bhawanagar, 1900 m (PUN 53708) and Palingi, 1900 m (PUN 53706), showed the phenomenon of cytomixis involving chromatin transfer and associated meiotic irregularities. This is the first record of cytomixis in the species. In these accessions, the cytomixis affected the meiotic course considerably resulting into pollen malformation and pollen grains of heterogeneous sizes. The frequency of PMCs involved in chromatin transfer in these individuals was noticed to be high during meiosis II. On the other hand, the majority of researchers have earlier reported that the phenomenon of cytomixis seems to be more frequent in the PMCs during earlier stages of meiosis I (Yen et al. 1993, Consolaro and Pagliarni 1995, de Souza and Pagliarni 1997, Bellucci et al. 2003, Kumar et al. 2010). The transfer of chromatin material in *V. pallida* is mostly partial resulting in the formation of hypo- and hyperploids PMCs with variable chromosome numbers. The formation of such PMCs with aneuploid chromosome numbers as a consequence of chromatin transfer has already been reported by other workers in *Pisum sativum* (Gottschalk 1970), *Astragalus subuliformis* (Ashruf and Gohil 1994), *Brassica napus* var. *oleifera* and *B. campestris* var. *oleifera* (de Souza and Pagliarni 1997), *Caltha palustris* (Kumar and Singhal 2008), *Meconopsis aculeata* (Singhal and Kumar 2008), *Hippophae rhamnoides* (Singhal et al. 2008), *Lychnis indica* var. *fimbriata* (Singhal et al., 2009a), *Anemone rivularis* (Singhal et al. 2009b), *Plantago lanceolata* (Himshikha et al. 2010), *Clematis montana* (Singhal et al. 2010). Although the fate of extra chromatin masses consequent to cytomixis is not known, but they failed to get included at poles and organized into micronuclei or micropollen as suggested by Bhat et al. (2006), and Singhal and Kumar (2008).

The inter-PMCs transfer of chromatin material in the individuals resulted in PMCs with various meiotic irregularities which included unorganized/pycnotic chromatin masses, interbivalent connections, chromosome stickiness, out of plate bivalents and laggards. The role of cytomixis in inducing such meiotic abnormalities in the meiocytes in *V. pallida* is quite clear as the accession without cytomixis showed perfectly normal meiosis. Further, the frequency of PMCs with such meiotic irregularities is also noticed to be significantly higher in the accession showing high percentage of PMCs involved in cytomixis (Table 1). Similar findings on the impact of chromatin transfer and causing meiotic irregularities and consequently pollen malformation have been recorded earlier in *Vicia faba* (Haroun et al. 2004), *Caltha palustris* (Kumar and Singhal 2008), *Meconopsis aculeata* (Singhal and Kumar 2008), *Hippophae rhamnoides* (Singhal et al. 2008), *Lychnis indica* var. *fimbriata* (Singhal et al. 2009a), *Anemone rivularis* (Singhal et al. 2009b), *Carthamus tinctorius* (Sheidai et al. 2009a), *Alopecurus* and *Catbrosa* (Sheidai et al. 2009b), *Clematis orientalis* (Kumar et al. 2010) and *Vicia rigidula* (Kaur and Singhal 2010).

Although the phenomenon of chromatin transfer among meiocytes has been reported in a large number of cases, there are varied opinions and explanations regarding the causes and significances of cytomixis. Possible causes suggested earlier included the effect of fixation (Gottschalk 1970), temperature (Narain 1976), level of ploidy (Verma et al. 1984), physiological (Bahl and Tyagi...
1988), stress factors coupled with genetic control (Ghanima and Talaat 2003) and direct genetic control (Bellucci et al. 2003, Haroun et al. 2004). In *V. pallida* also, the cytomixis seems to be a natural phenomenon under direct genetic control as has been proposed by other workers (Singhal and Gill 1985, Bedi 1990, Bellucci et al. 2003, Haroun et al. 2004, Lattoo et al. 2006, Singhal et al. 2007, Singhal and Kumar 2008).

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Cytomixis and Abnormalities during Male Meiosis in Tetraploid Cytotype of *Vicia pallida*

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