Chromosome Number, Meiosis and Pollen Fertility in *Vicia rigidula* Royle and *V. tenera* Grah. From Cold Desert Regions of India

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Summary We have recorded the meiotic chromosome counts in 2 species, *Vicia rigidula* ($n=12$) and *V. tenera* ($n=7$) from the cold desert region of Kinnaur district of Himachal Pradesh (India). *V. rigidula* which exists at tetraploid level ($x=6$) and depicts the phenomenon of cytomixis involving chromatin transfer in 6.52% of the observed pollen mother cells (PMCs) during different meiotic stages. 3.43–12.20% of PMCs also show non-synchronous disjunction of a few bivalents, laggards and chromatin bridges during anaphases/telophases, and micronuclei and included micronuclei in tetrads. Products of such aberrant PMCs result in the formation of sterile/unstained pollen grains and fertile/stained pollen grains of variable sizes. The meiotic course in *V. tenera*, which is diploid ($x=7$), depicts normal meiotic behaviour in the majority of the PMCs. Only a few PMCs (0.20–1.20%) depict some meiotic disturbances during anaphases resulting in the formation of 6.00–7.20% sterile/unstained pollen grains.

Key words Chromosome number, Cytomixis, Meiotic irregularities, *Vicia rigidula*, *V. tenera*.

The genus *Vicia* L., Tribe: Vicieae, Family: Fabaceae with 190 species (ILDIS 1999) is distributed in Europe, Asia and North America, extending to the temperate regions of South America and Tropical Africa (Maxted 1993). In India species of the genus *Vicia* are distributed in the temperate zones of Western Himalaya and the Northern plains. Many species of the genus are cultivated for food and fodder (Cremonini *et al.* 1998). Maxted *et al.* (1991) have divided the genus *Vicia* into 2 subgenera: *Vicilla* and *Vicia* with 17 and 9 sections, respectively. Most of the species of the genus are annuals, but a few of them are perennials. *V. rigidula* Royle is a perennial subglabrous climber with 2–3 inch long leaves ending in intricate tendrils and with golden yellow flowers appearing during June to September. The species is found in the temperate regions of Western Himalaya between altitudes of 1500–3000 m. *V. tenera* Grah. grows as a perennial subglabrous climber with pinnate leaves and pinkish-purple flowers which appear during May–July. The species is distributed at elevations of 1800–2600 m in moist shady places.

Perusal of chromosomal data on the basis of information gathered from Index to Plant Chromosome Numbers (1976–2006) and other authors (Srivastava 1963, Raina and Bisht 1988, Kumari and Bir 1990, Nikiforova 1990, Maxted *et al.* 1991, Venora *et al.* 1991, Akpinar and Bilaloglu 1997, Bisht *et al.* 1998, Kamel 1999, Bagci and Sahin 2000, Kotseruba *et al.* 2000, Rahiminejad *et al.* 2000, Tabur *et al.* 2000, 2002) reveals that a large number of species of the genus *Vicia* are yet to be known chromosomally, especially those from the high altitudinal regions and cold deserts of Indian Himalayas. We have worked out the chromosome counts in *V. rigidula* and *V. tenera* collected in Kinnaur district of Himachal Pradesh. Simultaneously, we have also recorded our observations on male meiotic course, pollen sterility and pollen grain size in these species.

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Materials and methods

For cytological studies, wild plants of *V. rigidula* Royle and *V. tenera* Grah. were collected from the cold regions of the Kinnaur district of Himachal Pradesh during the months of June–July 2008. Voucher specimens of the worked-out plants were deposited in the Herbarium of the Department of Botany, Punjabi University, Patiala (PUN). Floral buds of variable sizes ranging from 1–18 mm×0.5–5 mm were fixed in Carnoy's fixative for 24 h and preserved in 70% alcohol at 4°C. For male meiotic studies, the anthers from flowers of size ranging from 2–5 mm×0.5–2 mm were squashed in 1% acetocarmine. A number of slides were carefully examined for chromosome counts and meiotic analysis in each species. For male meiotic analysis, 590–650 PMCs (*V. rigidula*) and 415–458 PMCs (*V. tenera*) at different stages of meiosis were observed. Pollen grain fertility was estimated through stainability for which the mature anthers from different flowers of sizes ranging from 13–18 mm×4–5 mm were squashed in 1% aniline blue in lactophenol solution and glycerol–acetocarmine (1 : 1) mixture. A total of 835–899 pollen grains (*V. rigidula*) and 590–732 pollen grains (*V. tenera*) were observed for the estimation of pollen fertility. Well filled and stained pollen grains were scored as apparently fertile and poorly stained/unstained pollen grains were counted as sterile. Pollen grain size was measured using oculomicrometre. Photomicrographs of PMCs, tetrads and pollen grains were made from the freshly prepared slides using the Nikon 80i eclipse microscope and Leica Qwin Digital Imaging System.

Results

V. rigidula Royle

The male meiotic studies were carried out in 2 plants (PUN 50919, 51044) collected from the roadside in the Kalpa region, growing at 31°31'N, 78°15'E latitude and at 2760 m altitude in Kinnaur district of Himachal Pradesh. Both the individuals scored through male meiosis uniformly reveal the same haploid chromosome count of $n=12$ (Fig. 1) indicating that the species was tetraploid based on $x=6$. The chromosomes, which are large-sized, could not be counted clearly during the late prophase I and metaphase I (Fig. 2) due to stickiness in chromatin material and interbivalent connections (Figs. 3, 4) as observed in majority of the PMCs. The meiotic chromosomes number in the species is confirmed in only a few PMCs at anaphase I/metaphase II with 12 : 12 chromosomes at each pole (Fig. 5). The meiotic course in majority of the observed

Figs. 1–20. Meiotic chromosomes, meiotic abnormalities and pollen grains in *Vicia rigidula* (1–14) and *V. tenera* (15–20). 1. A PMC showing 12 bivalents at metaphase I. (PUN 50919), 2. A PMC showing uncountable bivalents at metaphase I. (PUN 50919), 3. A PMC showing interbivalent connections (arrowed) at late prophase I. (PUN 51044), 4. A PMC showing interbivalent connections (arrowed) at metaphase I. (PUN 50919), 5. A PMC showing 12 : 12 chromosomes distribution at metaphase II. (PUN 51044), 6. PMCs showing chromatin transfer (arrowed) during early prophase I and 1 of the PMC showing extra chromatin material (arrow head). (PUN 50919), 7. Two PMCs showing chromatin transfer (arrowed) at telophase I. (PUN 50919), 8. A PMC with laggards (arrowed) at anaphase I. (PUN 50919), 9. A PMC with laggards (arrowed) at telophase I. (PUN 51044), 10. A PMC with lagging chromatin material (arrowed) at telophase II. (PUN 50919), 11. A PMC with chromatin bridges (arrowed) at anaphase I. (PUN 50919), 12. A tetrad with 1 micronucleus (arrowed). (PUN 51044), 13. A tetrad with micronuclei (arrowed) and included micronuclei (arrow head). (PUN 50919), 14. Apparently fertile heterogeneously sized pollen grains. (PUN 50919), 15. A PMC showing 7 bivalents at metaphase I. (PUN 50920), 16. A PMC showing 7 : 7 chromosomes distribution at anaphase I. (PUN 51952), 17. A PMC with non-synchronous disjunction of 2 bivalents (arrowed) at anaphase I. (PUN 51950), 18. A PMC with 1 laggard (arrowed) at anaphase I. (PUN 51951), 19. A PMC with chromatin bridges (arrowed) at anaphase I. (PUN 51950), 20. Fertile and sterile pollen grains. (PUN 51950). Scale bar=10 μ m.

PMCs (81.28–96.57%) is regular resulting into normal tetrad formation. However, in 1 individual, 6.52% of the observed PMCs depict abnormal meiotic course due to the phenomenon of cytomixis involving chromatin transfer among proximate meiocytes during the different stages of meiosis (Figs. 6, 7, Table 1). The chromatin migration among the adjacent meiocytes occurs through cytoplasmic channels. As a consequence of chromatin transfer, several PMCs with aneuploid chromosome numbers and extra chromatin material are observed (Fig. 6). Besides, 3.43–12.20% PMCs are also observed to show non-synchronous disjunction of some bivalents, the presence of 3–4 laggards (Figs. 8–10) and chromatin bridges (Figs. 11) at anaphases/telophases, micronuclei (Fig. 12) and included micronuclei in tetrads (Fig. 13). These meiotic abnormalities consequently result into some pollen sterility (19.00–19.65%) and 2 different sized fertile pollen grains as normal ($37.70\text{ }\mu\text{m}\times 22.62\text{ }\mu\text{m}$; 96.15%) and small-sized ($26.39\text{ }\mu\text{m}\times 18.85\text{ }\mu\text{m}$; 3.85%) (Fig. 14).

Table 1. Cytomixis, Meiotic course, Microsporogenesis and Pollen grains in different individuals of *Vicia* species

Species	PUN No.	Cytomixis		Meiotic course				Microsporogenesis		Pollen grains		
		% age of PMCs involved	Meiotic stages	PMCs with non-synchronous bivalents (%)	PMCs with laggards at A/T (%)	PMCs with bridges at A/T (%)	Tetrads with micro-nuclei (%)	Tetrads with included micro-nuclei (%)	Fertility (%)	Size (μm)	RF (%)	
<i>Vicia rigidula</i> (n=12)	50919	6.52	EPI, TI	0.80	6.20	1.20	2.60	1.40	80.35	37.70×22.62	96.15	
	51044	—	—	—	1.80	0.60	1.03	—	81.00	26.39×18.85	3.85	
<i>V. tenera</i> (n=7)	50920	—	—	—	—	—	—	—	94.40	33.93×26.39	100	
	51950	—	—	1.20	—	0.30	—	—	93.00	33.93×26.39	100	
	51951	—	—	—	0.20	0.30	—	—	94.00	33.93×26.39	100	
	51952	—	—	—	—	—	—	—	92.80	33.93×26.39	100	

EPI=Early Prophase I; TI=Telophase I; A=Anaphases I/II; T=Telophases I/II; RF=Relative frequency of normal and small-sized pollen grains [Observed number of (normal or small) pollen grains/total number of fertile pollen grains].

V. tenera Grah.

The chromosome number is observed on male meiosis from 4 plants (PUN 50920, 51950, 51951, 51952) collected from moist places along cultivated fields in the Sangla region (31°25'N, 78°15'E latitude and 2680 m altitude) of the Kinnaur district (Table 1). All the plants share the same chromosome number of $n=7$ as confirmed from the uniform presence of 7 relatively small-sized bivalents at metaphase I (Fig. 15) and 7:7 chromosomes distribution at each pole during anaphase I (Fig. 16). In the majority of the observed PMCs, meiotic course up to the tetrad formation is recorded to be normal. However, a few PMCs (0.20–1.20%) are observed to show the presence of non-synchronous disjunction of 1–2 bivalents (Fig. 17), 1–2 laggards (Fig. 18) and chromatin bridges during anaphase I (Fig. 19). Consequent to these meiotic abnormalities, some sterile/unstained pollen grains (6.00–7.20%) are resulted in the species (Fig. 20). The fertile/stained pollen grains, however, were noticed to be of uniform size (33.93 μm×26.39 μm).

Discussion

Chromosome information gathered on the genus *Vicia* reveals that 193 species, inclusive of 69 species from India, are known chromosomally (Darlington and Wylie 1955, Love and Love 1966, 1974, 1975, Fedorov 1969, Moore 1973, 1974, 1977, Goldblatt 1984, 1985, 1988, Kumar and Subramanian 1986, Goldblatt and Johnson 1990, 1991, 1994, 1996, 1998, 2000, 2003, Index to Plant Chromosome Numbers 2006). To this list, we here add 2 more species, *V. rigidula* ($n=12$) and *V. tenera* ($n=7$). An analysis of chromosome records in the genus reveals that majority of the species are diploid at basic numbers of $x=5, 6, 7$ while 25 species exist at tetraploid or hexaploid level. 48 species are counted to depict the existence of chromosomal variation due to intraspecific polyploidy and aneuploidy. Among the different basic numbers, $x=7$ with 122 species is the most common followed by $x=6$ (79 species). Only 29 species depict $x=5$

as the basic number. Species depicting dibasic ($x=5$, 6; 8 species, $x=6$, 7; 18 species and $x=5$, 7; 1 species) and 3 basic numbers ($x=5$, 6, 7; 5 species) also exist. Based on $x=6$, *V. rigidula* with $2n=24$ exists at tetraploid level while *V. tenera* with $2n=14$ exists at diploid level ($x=7$). The meiotic course in *V. rigidula* is characterized by the presence of the phenomenon of cytomicis involving chromatin transfer and associated meiotic abnormalities, resulting into some pollen sterility and heterogenous-sized, apparently fertile/stained pollen grains. Similar observations regarding the effect of chromatin transfer and associated meiotic abnormalities on reduced pollen fertility and formation of variable sized pollen grains have been reported in *Vicia faba* (Haroun *et al.* 2004), *Caltha palustris* (Kumar and Singhal 2008), *Clematis flammula* (Kumar *et al.* 2008), *Meconopsis aculeata* (Singhal and Kumar 2008a), *Withania somnifera* (Singhal and Kumar 2008b), *Hippophae rhamnoides* (Singhal *et al.* 2008), *Lychnis indica* (Singhal *et al.* 2009a), *Anemone rivularis* (Singhal *et al.* 2009b), *Carthamus tinctorius* (Sheidai *et al.* 2009a) and species of *Alopecurus* and *Catbrosa* (Sheidai *et al.* 2009b). Male meiosis in *V. tenera*, which is a diploid, depicts almost normal meiotic course and high pollen fertility and uniform sized fertile pollen grains. The presence of some meiotic disturbances during anaphase resulting in some pollen sterility in *V. tenera* might be the consequence of abnormal spindle activity during chromosome distribution.

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