Detection of structural heterozygosity and a varied chromosome number in *Euphorbia pilosa* L. from Kashmir, India

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Received April 22, 2011; accepted August 2, 2011

ABSTRACT. Two populations of *Euphorbia pilosa* L. (the Euphorbiaceae) have been studied meiotically from Kashmir, India. Both the populations uniformly exist at a haploid chromosome count of $n=10$ which was confirmed at various stages of meiosis. The present chromosome count makes an addition to the intraspecific aneuploid cytotypes for the species. Harwan population (1,900 m) is meiotically normal with good pollen fertility, where as Dachigam (2,200 m) shows structural hybridity with only 45.61% pollen fertility. This is the first report of reciprocal translocation in the species.

KEYWORDS: Chromosome number, *Euphorbia pilosa* L., Structural heterozygosity

The state of Jammu and Kashmir, phytogeographically, the most complex and diverse, has a fairly rich diversity of plant life. The area is a store house of numerous medicinal and aromatic plants which grow wild in temperate and alpine habitats. One can find vegetation up to 5,000 m in the Kashmir area. As part of our programme on exploration of biological diversity in the high altitude plant species, presently, *Euphorbia pilosa* L. (family, Euphorbiaceae) has been worked out cytologically. It is an erect, 50-90cm tall perennial herb with alternate leaves and stems showing branches in the upper region. Its habitat ranges from western Himalaya from Garhwal west wards to Kashmir (Khare 2007). Medicinally the plant is very important as its roots and latex are used as purgative and in the treatment of pistular scores (Chauhan 1999, Bhattacharjee 2004, Pulliah 2006, Khare 2007, Pande *et al* 2007). It has been used to cure wounds and cuts (Chopda *et al* 2009, Pande *et al* 2007). It is known for its skin irritant effects (Kingham *et al* 1975) Due to its limited distribution, it is thought that it should be conserved in India (Bakshi *et al* 2001).

MATERIALS AND METHODS

Materials for meiotic studies were collected from two populations of *Euphorbia pilosa* L. growing at Harwan and Dachigam area of Srinagar (Kashmir, India) in the month of August 2008. Voucher specimens of the cytologically worked specimens (PUN 55060, 55386) are deposited in the Herbarium, Department of Botany, Punjabi University, Patiala (PUN). The young floral buds were fixed in Carnoy's fixative (6 ethanol: 3 chloroform: 1 acetic acid v/v) for 24 h and preserved in 70% alcohol at 4°C until use. For meiotic studies, anthers were squashed in 2% acetocarmine. A number of slides were carefully examined for chromosome counts and meiotic abnormalities at different stages in each population. Pollen fertility was examined using 1:1 glycerol-acetocarmine mixture where well filled pollen grains with stained nuclei were

taken as apparently fertile while shrivelled and unstained pollen were counted as apparently sterile (Marks 1954). Photomicrographs of meiotic stages were made from temporary slides using Nikon 80i Eclips Microscope.

RESULTS

The cytological analysis was made on two populations of *E. pilosa* which were collected from Harwan and Dachigam areas of Kashmir (India), ranging in altitude from 1,900-2,200m. The plants of both the populations have a haploid chromosome number of $n=10$. This was confirmed from the presence of 10 bivalents at metaphase-I and diakinesis (Figs 1A and B) and 10:10 chromosome distribution at anaphase I (Fig. 1C). The plants studied from Harwan (1,900m) were normal in its meiotic course. The PMCs of this accession showed regular bivalent formation and segregation of chromosomes at anaphase I and II. The pollen fertility was quite high (94.05%), while the second population growing in Dachigam area (2,200m) showed various meiotic abnormalities. As many as 23.87% PMCs showed the formation of chain among four chromosomes at diakinesis and M-I (Fig. 1D-F). Un-oriented bivalents were observed at metaphase-II in some PMCs (Fig. 1G). Late disjunction of 1-2 bivalents was also seen (Fig. 1H). At anaphase I and anaphase-II stages of meiosis laggards were also noticed (Fig. 1I-K). The consequences of meiotic abnormalities are quite clear from palynological data. The plants of Harwan population (with normal meiosis) have about 95% fertile pollen. The plants with structural hybridity showed only 45.61% pollen fertility and pollen size heterogeneity. Besides the normal sized (70%; $36.75 \times 34.44 \mu\text{m}$), fertile small sized (12.06%; $29.15 \times 25.86 \mu\text{m}$) and large sized (17.73%; $45.57 \times 40.31 \mu\text{m}$) pollens were also observed (Fig. 1L).

DISCUSSION

Euphorbia is reported to show great variability in chromosome numbers and chromosome morphology (Perry

Fig 1. Meiotic chromosomes of *Euphorbia pilosa* L. ($2n=20$). A. PMC showing 10_{II} at metaphase I. B. PMC showing 10_{II} at diakinesis. C. PMC at anaphase I showing 10:10 chromosome distribution. D-F. PMCs showing chain formation. G. PMCs showing un-oriented bivalent at metaphase II. H. PMC showing late disjunction of 1-2 bivalents. I-K. PMCs showing laggards at anaphase I and anaphase II. L. Heterogenous sized fertile along with sterile pollen grains. Scale=10 μ m.

1943). The chromosome numbers reported in the genus are $2n=12, 14, 16, 18, 20, 24, 26, 28, 30, 36, 40$ and 56 (Federov 1969). The genus is polybasic with $x=7, 10$ as primary base numbers and $x=6, 8$ and 9 as secondary base numbers (Hans 1973).

The present chromosome count of $n=10$ for *E. pilosa* makes an addition to the intraspecific aneuploid cytotype for the species. This count is in variance with the previous reports of $2n=18$ (Stepanov 1994, Perry 1943) from outside India and $2n=32$ (Choda and Mehra 1971, Mehra and Chada 1978) from India. Presently, out of the two populations, plants of one population showed structural heterozygosity (first report of the phenomenon in this species) while the other species had perfectly normal meiosis. Structural chromosomal changes and polyploidy are now considered as important mechanisms for the evolution of plants (Sybenga 1992). Laggards were also observed at different stages which may be due to abnormal spindle formation (Tarar and Dyansagar 1980) or delayed terminalization (Kumar and Tripathi 2007). Any one of or all cytogenetic phenomenon, such as irregular segregation, non-disjunction, unequal translocation, centric fusion, fission and hybridization may be involved in the origin of dysploid chromosome numbers (Stebbins 1971). Pollen grains of heterogenous sizes were formed which may be the consequence of unreduced nature or presence of laggards which later on form micronuclei and reduced pollen fertility (45.61%). Carr and Carr (1983, 2000) and Levin (2002) have also reported that chromosomal rearrangements and other abnormalities are responsible for reduced pollen fertility in plants. Although the cytological status of these heterogenous sized pollen grains could not be ascertained but the role of small and large sized pollen grains in the production of aneuploid and polyploid plants could not be ruled out, because these pollen grains are apparently fertile.

ACKNOWLEDGEMENTS. The authors are grateful to the University Grants Commission, New Delhi for providing financial assistance under the DRS SAP I and II and ASIST programmes. Thanks are also due to the Director Botanical survey of India (Dehradun) for identification of the species and Head, Department of Botany, Punjabi University, Patiala for necessary laboratory facilities.

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