Endangered Medicinal Plant *Polygonatum cirrhifolium* (Wall.) Royle Undergoing Erratic Male Meiosis in Kinnaur Population

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Summary *Polygonatum cirrhifolium* commonly known as Sobnyam in Kinnaur is rich source of starch, pectin, protein and fiber content. In the present study, two accessions of *P. cirrhifolium* belonging to family Asparagaceae were studied for male meiosis from the cold desert region of Kinnaur. Both the accessions showed the presence of 16 bivalents (based on $x=8$) at diakinesis and metaphase, which is in conformity with the previous reports. Earlier, floristic diversity of medicinal and aromatic plants was studied by various workers, but the cytological study along with the meiotic abnormalities in the species has been carried out for the first time from the study area. Therefore, there is need to generate awareness among the local people regarding the conservation and utilization of medicinal plants.

Key words Endangered, Wild edible plant, Meiotic irregularities, Ethno medicinal value, Kinnaur, Himachal Pradesh.

Present study was conducted in district Kinnaur, the high altitude area that comes under Trans Himalayan arid zone rich in endemic plants. District Kinnaur in Himachal Pradesh lies between 31°05'50"–32°05'15"N latitude to 77°45'–79°00'35"E longitude and cover an area of about 6401 km². *Polygonatum* a genus of about 57 species has its centre of diversity in Eastern Himalayas and Western China (Poonam *et al.* 2011). Around the world found in Northern Asia, China, Nepal, Bhutan, Japan, temperate parts of Eurasia and North America at an elevation of 2000–4000 m. In India the species is occasionally found in the forest and grassy slopes of temperate Himalayas in between 1500–3000 m in Himachal Pradesh, Uttarakhand, Manipur and Sikkim (Balkrishna *et al.* 2012).

P. cirrhifolium is an erect tall perennial herb growing 60–120 cm in height. Leaves are narrow lance like, in whorls of three to six, linear 7–12 cm long, 3–4 mm wide. Stem is erect, weak and glabrous often climbing by means of tendril like tips of leaves. The flowers are tube shaped borne as drooping clusters from leaf axis with white coloured triangular petals. The fruits are orange red coloured berries when ripe (Fig. 1). The plant grows at an altitude range of 2000–3100 m in Kinnaur. *Polygonatum* species have traditional use, both as medicine and food, in China and other countries, like the Northern and Eastern United states, South-Central Canada, India and Korea (Youn *et al.* 2007, Misra *et al.*

2008).

Cytologically this species revealed intraspecific variability as $2n=26, 28, 32, 38, 56$. Kinnaur exhibit unique, isolated, extreme climatic locations rich in endemic forms. The non-availability of cytological information about taxa from the region promoted us to study this endangered plant.

Earlier the medicinal importance of the plant was studied from Kinnaur (Negi and Chauhan 2009), but the cytological investigation have remained unexplored, so the present study focused on the various irregularities during meiosis in the studied species.

Materials and methods

Plant material was collected from the wild plants growing on open moist slopes around the apple orchards in district Kinnaur, Himachal Pradesh in June–July 2015 and was identified with the help of regional floras and Botanical Survey of India (BSI) Herbarium, Dehradun. The voucher specimens were deposited in the Herbarium, Department of Botany, Punjabi University, Patiala (PUN). For Ethnobotanical uses local amchi and elderly people nearby habitations were consulted. For meiotic studies, the young buds of suitable sizes were fixed in Carnoy's fixative for 24 h and then stored in 70% alcohol at 4°C until use. Anthers were squashed in 2% acetocarmine and a number of freshly prepared slides were examined. Pollen fertility was estimated through stainability test with glycerol–acetocarmine mixture (1:1). Well-filled pollen grains with uniformly stained cyto-

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Fig. 1. *Polygonatum cirrhifolium* growing in wild.

plasm were considered fertile while unstained or partially stained was counted as sterile. Photomicrographs were made from the temporary mounts using Nikon 80i Eclipse Microscope.

Results and discussion

Medicinal uses

Traditionally, *P. cirrhifolium* has various medicinal uses. The plant is considered to be wild tasty leafy vegetable and are eaten roasted, young shoots are also added to salads (Wujisguleng *et al.* 2012). Dried rhizomes and bulbs also show higher protein content, macro and micronutrients such as copper, iron, potassium, calcium and energy value including some fats and fiber content (Sharma *et al.* 2014).

In Lahaul valley, the plant is useful in the treatment of pain in kidneys and waist region, fluid retention in joints and it restores body strength (Sharma *et al.* 2011). In Almora district of Uttarakhand, the tubers mixed with water is used for removing weakness, leaves eaten as vegetable, root infusion with milk used as an aphrodisiac and blood purifier, paste made out of its roots is used in healing cuts and wounds, skin irritations and inflammations, when consumed as a tea, it is used to cure

wide range of symptoms associated with menopause, indigestion, diabetes, broken bones, insomnia, kidney pains and infertility (Kumari *et al.* 2012). In Garhwal region, *P. cirrhifolium* is used as a tonic and whole plant is useful in curing skin diseases, wounds, ulcers, fever, cough, bronchitis and general debility. In Parvati valley of Himachal Pradesh, the plant is used in rheumatism, cough and debility from old age (Sharma *et al.* 2004). In China, the plant has reported to show hypoglycemic, hypertensive, antibacterial and antifungal effects (Singh 2006). Locals in Kinnaur, uses leaves as vegetable, tonic, roots for washing purposes (Negi and Chauhan 2009). Local amchi suggest the powdered roots mixed with milk as tonic, to cure headache, skin irritation, healing burns.

Meiotic study

In the present study, the species was collected from Nichar (2150 m) and Kande (2200 m) villages in Kinnaur valley. Cytological analysis of both the accessions revealed chromosome count of $n=16$ at diakinesis and metaphase-I (Figs. 2A–2B). This confirms the earlier chromosome count reported from Lahaul area of Himachal Pradesh (Lattoo *et al.* 2005). The present count is tetraploid. Chromosome number already reported in the species are $2n=26$, 28 from North Sikkim, $2n=38$ from Shimla and $2n=56$ from China Hill (Kumar 1959a, 1959b, 1960). The detailed meiotic course shows the abnormal behavior in both the populations (Figs. 2C–2N). The population collected from Nichar (PUN-59125) showed the presence of late disjunction at M-I (4.68%), out of plate bivalents at M-I (7.46%), chromosome stickiness (13.20%), laggards at A-I (12.30%), and bridges at A-I (8.33%) and abnormal microsporogenesis in the form of monad (19.79%) and dyad (11.11%). Late disjunction, which is the failure of chromosomes to separate, is generally due to interlocking of chiasmata. The population collected from Kande (PUN-59126) showed the presence of out of plate bivalents at M-I (6.97%), chromosome stickiness (9.43%), laggards at A-I (7.69%), and bridges at T-I (7.14%). The chromosomal stickiness observed in the species involving intense clustering of chromosomes, may be due to presence of mutant genes (Kaul and Murthy 1985) or abiotic factors such as temperatures (Caetano-Pereira *et al.* 1995). In both the populations bridges and laggards were observed at A-I and T-I, causes of which may be due to interlocking of bivalents (Bhattacharjee 1953), or paracentric inversion (Sinha and Godward 1972). Further, microsporogenesis was greatly affected with the appearance of monad (17.64%), dyad (10.34%), and polyad (7.84%). Due to these meiotic irregularities, the pollen fertility was also reduced and it varied from 69.44 to 72.60% (Fig. 2O).

Although the plant is listed as endangered but there is no proper management plan for conservation due to lack of information. The plant is facing multiple threats.

Fig. 2. *Polygonatum cirrhifolium*. A–B) A PMC showing 16 (eight large sized and eight small sized) bivalents at diakinesis and M–I. C) A PMC with 16:16 distribution of chromosomes at A–I. D) Out of plate bivalents at M–I. E) Late disjunction of bivalents at A–I. F) A PMC showing chromosome stickiness at M–I. G–H) A PMC with laggards at A–I and T–I. I–J) A PMC with Bridges at A–I and T–I. K) A PMC with monad. L) A PMC with dyad. M–N) A PMC with polyad. O) Sterile and fertile pollen grains. M–I=metaphase I; A–I=anaphase I. Scale bar=10 μ m.

The reproductive potential of the wild plants is far from satisfactory. The local inhabitants are overexploiting this plant for medicinal as well as food purpose. The uprooting of plants for rhizome also contributes towards its least availability. The present study emphasize that this medicinal plant is suffering from negligence of people both common man and researchers. Conservation strategies are urgently needed. Such medicinally important plants should be conserved with both *in situ* and *ex situ* methods of conservation. The study is useful in under-

standing the meiotic behavior in the species, which is the first attempt to work out cytomorphology of the plant from the cold desert region which remained unexplored cytologically. Further, the geographical distribution, cytomorphological diversity and ethnobotanical aspects in the studied medicinal plant, mark out the better chemotypes for further conservation.

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