Morphological and Ecological Adaptations, and Cytological Studies in *Astragalus rhizanthus* Royle ex Benth. (Papilionaceae), an Endemic to Himalayas

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Summary In the present investigation, we examined the morphological and ecological adaptations, and male meiosis and pollen fertility in an endemic species of genus *Astragalus*, *A. rhizanthus* Royle ex Benth. from cold deserts of Lahaul–Spiti district of Himachal Pradesh, India. The three populations from Lahaul Valley and one from Spiti Valley depicted the same meiotic chromosome number of $n=8$ in all the accessions. In three populations (Lahaul, Sissu, 3170 m, Chandartal Lake, 4300 m; Spiti, Lossar, 3900 m), meiosis was normal resulting into cent percent pollen fertility. However, in the accession from Lahaul Valley (Koksar, 3140 m) majority of the pollen mother cells (PMCs) showed normal chromosome behaviour during meiosis-I and II. While, 12.09% PMCs showed the occurrence of chromatin material transfer involving 2–5 meiocytes during prophase-I, metaphases-I/II, anaphases-I/II and telophases-I/II. Consequent to this, hypo- and hyperploid PMCs resulted. In certain cases, PMCs fused together to form syncytes which had two complements in the same cell. Pollen sterility was observed to be 9%. The paper also discusses morphological and ecological adaptations that the species has adopted to cope with the harsh and hostile environmental conditions prevailing in the cold deserts of Lahaul–Spiti.

Key words Chromosome, Cytomixis, Cold deserts, Hypo- and hyperploid PMCs, Himalayas, Lahaul–Spiti.

Astragalus L. (Family: Papilionaceae), with ca. 2500–3000 species in the world, is considered the largest and most complex genus of angiosperms (Podlech 1986, Zarre and Podlech 1997, Chaudhary and Srivastava 2007) distributed in arid and temperate regions of the Northern Hemisphere and South America (Podlech 1986). In India, it has about 80–90 species, chiefly distributed in the temperate to alpine regions of the Himalayas with the main center of diversity in the cold deserts of Lahaul–Spiti (Himachal Pradesh) and Leh and Ladakh (Jammu and Kashmir) (Chaudhary and Srivastava 2007, Chaudhary *et al.* 2007). In the current study, we examined *A. rhizanthus* Royle ex Benth. for its chromosome counts, male meiosis, and pollen fertility. *A. rhizanthus* complex includes four closely related species, *A. rhizanthus*, *A. candolleanus*, *A. malacophyllus* and *A. pindreensis* which are distributed in the Himalayas from Afghanistan to Nepal and in India from Jammu and Kashmir to Uttarakhand (Podlech 1988). *A. rhizanthus* ssp. *rhizanthus* var. *rhizanthus* exhibits an enormous amount of morphological variation among different Indian populations (Chaudhary *et al.* 2008). Anand *et al.* (2010) used molecular tools to address the relationships among the aforementioned allied species of the *A. rhizanthus* Benth. complex from different parts

of the Indian Himalayas and found the highest degree of genetic similarity was observed within *A. rhizanthus* followed by *A. malacophyllus*, *A. candolleanus* and *A. pindreensis*. Indian women chew the roots of the taxon to stimulate lactation (Anand *et al.* 2010). Roots have also been used as a heat stimulant and for skin diseases in Kashmir and Ladakh regions (Kaul 1997). In the Pangi Valley of the Chamba district and Lahaul–Spiti, the species is used as fodder (Sood *et al.* 2001, Kumar and Singhal 2013, Rana *et al.* 2014a). Chaudhary *et al.* (2008) emphasized the significance of chromosome numbers in interpretation of phylogenetic relationships in *Astragalus* and also pointed towards strong correlations between its geographic distribution and chromosome numbers. However, this morphologically variable species is poorly known for its chromosome number and meiotic behaviour from cold deserts of Lahaul–Spiti. As the species is widely distributed in cold regions of India and no cytological data is available for the species from the study area, an attempt has been made to study the species on a population basis for cytomorphological variations if any.

Materials and methods

For the chromosome counts and meiotic studies, material of *A. rhizanthus* Royle ex Benth. was collected from the drier habitats of Lahaul Valley (Sissu, 3170 m,

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Fig. 1. A wild individual of *Astragalus rhizanthus* in its natural dry habitat showing long thick root (arrowed)

PUN51273; Chandartal Lake, 4300m, PUN51275; Kok-sar, 3140m, PUN51203) and Spiti Valley (Lossar, 3900m PUN51167) in Lahaul–Spiti district, Himachal Pradesh, India in June–July 2008. The young developing floral buds from healthy plants were fixed in freshly prepared Carnoy's fixative (6 absolute alcohol:3 chloroform:1 glacial acetic acid, v:v:v) for 24h and subsequently stored in 70% ethanol in a refrigerator until analysis. Developing anthers from floral buds were squashed in 1% acetocarmine and preparations were studied for chromosome counts, and detailed meiotic behaviour in pollen mother cells (PMCs) at prophase-I, metaphase-I (M-I), anaphases-I/II (A-I/II), telophases-I/II (T-I/II) and sporad stage. A total of 50–100 PMCs were examined for determining the chromosome counts while 30–60 slides were prepared from different anthers/flowers for analysis of chromosomal associations. Pollen fertility was estimated through stainability tests for which anthers of mature flowers were squashed in glycerol–acetocarmine mixture (1:1). A total of 200–500 pollen grains were analyzed in each case for pollen fertility. Well-filled pollen grains with uniformly stained cytoplasm were scored as fertile/viable while shrivelled ones with unstained or poorly stained cytoplasm were counted as sterile/unviable. Photomicrographs from the temporary mounts were taken using a Nikon Eclipse 80i microscope (Melville, U.S.A.).

Results and discussion

Morphological and ecological observations

The individuals of *A. rhizanthus* were growing in different localities and displayed observable differences

owing to microclimatic conditions prevailing in the areas from which they were harvested. The habitats of the species are very hostile due to freezing temperature, dry climate, hard and rocky soils and the low ability of the soil to retain the moisture and high velocity of wind. The species has developed morphological and physiological adaptations such as hairs on leaves and young parts to counter the high wind velocity and high incidence of UV-rays as well as a deep root system (Figs. 1, 2), which provides ability to retain some moisture. The plants collected from the Sissu population were observed to be large with longer leaves and the individuals from the Chandartal Lake were quite dwarfed with reduced stems and small leaves. Other ecological and morphological adaptations include their ability to grow in very hard, dry and stony soils, the underground parts become perennial, woody, and deep rooted to acquire moisture and nutrition. These rootstocks are generally longer than upper part of the plants (Fig. 1) and may reach more than 0.30m long. The longer rootstock provides these plants the ability to penetrate rock crevices and hard substratum and thus, the plants withstand the high wind velocity in higher altitudes. The plants are generally a stemless perennial herb and commonly grow on dry, stony or sandy riversides, amongst boulders, mountain slopes, and near agriculture fields and subalpine and alpine meadows up to altitudes of 3000–5500 m.

Chromosome count and male meiosis

Presently, cytological studies have been performed in four accessions collected from different localities in Lahaul Valley and Spiti Valley. All the accessions uniformly exist at diploid level ($n=8$) as confirmed by

Fig. 2. A plant of *Astragalus rhizanthus* showing hairs on leaves and young parts (arrowed)

the presence of eight small sized bivalents at diakinesis (Fig. 3a) and M-I (Fig. 3b), 8:8 chromosomes distribution at A-I (Fig. 3c) and M-II (Fig. 3d). Meiotic course was recorded to be normal in the three accessions (Sissu, 3170m; Lossar, 3900m; Chandartal Lake, 4300m) resulting into cent per cent pollen fertility. However, the accession scored from Koksar (3140m) depicted inter-PMC transfer of chromatin material at various stages of meiosis from early prophase-I to T-II (Fig. 3e–i). Transfer of chromatin material involving 2–5 PMCs occurred in 12.09% cases. Chromatin transfer occurred through narrow and broad cytotoxic channels forming a single chromatin strand. The resultant PMCs after chromatin transfer at T-II showed one, three and five haploid nuclei (Fig. 3i). Cytotoxic channels without any transfer of chromatin material were also noticed in 7.19% cases. Another interesting observation of the present study is the transfer of nucleolus along with the chromatin material between meiocytes (Fig. 3f). Such transfer of nucleolus along with chromatin material during cytotoxic had also been recorded in some other plants as well (Bhandari *et al.* 1969, Bhat *et al.* 2006, Kumar 2010, Kumar *et al.* 2014). Extra chromatin masses other than the normal complement were also noticed in some PMCs (Fig. 3j). In rare occasions, PMCs were directly fused to form syncytes (Fig. 3k). Transfer of chromatin material resulted in the formation of hypo- and hyperploid PMCs (Fig. 3i, j). Consequent to these meiotic irregularities, some pollen sterility (9%) resulted in the accession (Fig. 3l). The present meiotic chromosome count of $n=8$ in the species is in conformity with the earlier diploid records of $2n=16$ from Meenamarg (3325m) in Ladakh, Jammu and Kashmir (Ashraf and Gohil 1986, 1989, Gohil and

Ashraf 1989). These researchers have also reported the lagging of one chromosome at A-I and have attributed it to the failure of spindle apparatus. The occurrence of cytotoxic and consequent pollen sterility is reported here for the first time in the species.

Cytotoxic, which refers to inter-PMC transfer of chromatin material, has been observed to produce hypo-, hyperploid and enucleated PMCs and in extreme case, syncytes besides many other meiotic irregularities. Cytotoxic induced meiotic irregularities and formation of hypo- and hyperploid PMCs has also been reported in several angiosperms (Omara 1976, Singhal and Gill 1985, Falistocco *et al.* 1995, Malallah and Attia 2003, Ghaffari 2006, Kumar and Singhal 2008, 2011, 2012, Kumar *et al.* 2008, 2010, 2011, 2012, 2014, 2015, Singhal and Kumar 2008a, b, 2010, Singhal *et al.* 2009, 2010, 2011a, b, 2014, Himshikha *et al.* 2010, Kumar 2010, Kaur and Singhal 2012, 2014, Kaur *et al.* 2013, Rana *et al.* 2013, 2014b, Mursalimov and Deineko 2011, 2012, 2015, Mursalimov *et al.* 2013a, b). The phenomenon of cytotoxic does not seem to have affected the pollen fertility to a greater extent in current case. Similar results regarding the high pollen fertility in plants depicting cytotoxic were reported in *Dactylis* (Falistocco *et al.* 1995). As individuals with and without cytotoxic are found to grow in the same environmental conditions, cytotoxic in *A. rhizanthus* seems to be a natural process of intercellular interaction under direct genetic control (Omara 1976, De and Sharma 1983, Singhal and Gill 1985, Lattoo *et al.* 2006; Singhal and Kumar 2008a, Kumar 2010, Kumar *et al.* 2010, Kravets 2012, Kumar and Singhal 2012, Mandal *et al.* 2013).

Fig. 3. (a–l) PMCs (pollen mother cells) showing meiotic chromosome numbers and meiotic abnormalities, and pollen grains. a) Diakinesis, $n=8$. b) Metaphase-I, $n=8$. c) Anaphase-I, 8:8 chromosomes distributions. d) Metaphase-II, 8:8 chromosomes distributions. e) Chromatin transfer (arrows) at early prophase-I. f) Chromatin as well as nucleolus (arrow) transfer at early prophase-I. g) Chromosome transfer (arrow) can be seen without forming agglutinated chromatin mass during migration through the cytostictic channels at metaphase-I. h) Chromatin transfer at late T-II. i) Hypo- (arrowhead) and hyperploid (arrow) PMCs. j) Hyperploid PMC with extra chromatin material (encircled) at M-I. k) Syncyte (arrow) with double chromosome complement and a hypoploid PMC (arrowhead). l) Apparently fertile (darkly stained) and sterile (shriveled and lightly stained) pollen grains. Scale bar=10 μ m.

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