Male meiosis in reed grass *Calamagrostis emodensis* Griseb. from cold desert region of Western Himalayas

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**ABSTRACT.** The present investigation involves the meiotic studies on two populations of *Calamagrostis emodensis* Griseb. (Family: Poaceae) from District Kinnaur, Himachal Pradesh, India in the Western Himalayas. Both the populations reveals tetraploid chromosome count of 2n=28 which is the first ever report for the species. The objective of the present research were to study the detailed meiotic course, microsporogenesis, and the effect of meiotic abnormalities encountered during different stages of meiosis I and II on pollen grain size and pollen fertility.

**KEYWORDS:** *Calamagrostis emodensis*, male meiosis, microsporogenesis and pollen study, Western Himalayas.

*Calamagrostis* (Calamos means Reed; agrostis means grass in Greek-commonly known as “Reed grasses”), is a genus of native forage grasses in West, especially in the Montane and more Northerly areas. This genus comprises about 270 species in the world distributed in temperate regions of which 20 species are available in India. The genus is very widely distributed and many species are endemic to Himalayan region, including the Kashmir Himalayas, Himachal Pradesh, Garhwal and Sikkim Himalayas.

*Calamagrostis emodensis* (=*C. garhwalensis*) is tufted perennial herb up to 1.5m tall with creeping rhiome, with florets in a dense 30-35 cm long, purplish or green panicle. The plants of this species are found in Himalayas from Kashmir to Sikkim occasional on the slopes in Zoizila, Ladakh and Lahaul-Spiti. Outside India, the species is distributed in Western Asia, China, Pakistan and Bhutan, The species is also reported from Iran.

Survey of published records revealed that 54 species of the genus *Calamagrostis* has been reported cytologically. All the species are based on x=7. Chromosome counts of two species of the genus (*C. pseudophragmitis* and *C. epigjios*) are reported from India (Prakash 1979; Mehra 1982; Gohil and Koul 1986; Koul and Gohil 1991) and rest of the species were reported from outside India. Current species is not known cytologically, on the basis of World wide data.

Keeping in view the existence of cytormorphological diversity and the economic value of species the present study was carried out from different localities of Kinnaur Himachal Pradesh. The objective of the present research was to study the detailed meiotic course, microsporogenesis and the effect of meiotic abnormalities encountered during different stages of meiosis I and II on pollen grain size and pollen fertility.

**MATERIAL AND METHODS**

Materials for meiotic studies were taken from two Populations of *Calamagrostis emodensis* from the valley in Kalpa (2,758m) and Sangla (2,680m) in the Kinnaur district of Himachal Pradesh in May-July 2012. The specimens were identified and authenticated with the help of regional floras (*Rau*1975; *Chowdhery and Wadhwa* 1984; *Aswal and Mehrotra* 1994) and herbarium, Botanical Survey of India, Dehradun. Vouchers specimens of cytologically examined plants were deposited in the Herbarium, Department of Botany, Punjabi University, Patiala (PUN). Young floral buds of suitable sizes 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% aceticarmine. 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 (Marks 1954). Photomicrographs of chromosome counts were made from freshly prepared slides using a Nikon 80i Eclips Microscope.

**RESULTS AND DISCUSSION**

The present study pertains to a detailed male meiotic analysis of plants of *Calamagrostis emodensis* (Family: Poaceae) belonging to different populations collected from the cold deserts of Kinnaur. Meiotic analysis in the plants studied from the Kalpa Valley and Sangla Valley (Kinnaur district) showed the presence of 14 bivalents at metaphase I (Fig. 1B).This count is the first ever chromosome count for the species and both the populations exist uniformly at tetraploid level based on x=7. The data regarding the Locality, meiotic chromosome number, meiotic abnormalities, cytomixis, microsporogenesis, pollen fertility and pollen size are provided in Table 1.

Cytomixis was discovered for the first time in *Crocus sativus* L. by Kornicke (1901). Since then the phenomenon of cytomixis involving inter PMC transfer of chromatin material through cytoplasmic channels has been reported in wide range of flowering plants (*Ghanima and Talaat* 2003; *Singhal et al.* 2007; *Saggoo and Srivastava* 2009;
Fig. 1. A. A PMC showing 14 bivalents at Diakinesis (S), B. A PMC showing 14 bivalents at metaphase I (K), C. A PMC showing 14:14 distribution of chromosome at metaphase II (S), D. A PMC showing 14:14 distribution of chromosome at anaphase I (K), E. A PMC with late disjunction at anaphase I (K), F. A PMC showing 1-4 chiasma at metaphase I (K), G. A PMC showing 1-2 Chiasma at metaphase I (S), H. A PMC with chromosome stickiness at metaphase I (S), I. A PMC with unoriented bivalents at metaphase I (K), J. A PMC showing unidirectional migration of chromatin material from one PMC to another PMC at early prophase I (K), K. A PMC showing hypoploid and hyperploids chromatin transfer at Diakinesis (K), L. A single PMC donating its chromatin material simultaneously to more than two PMCs (K). (S=Sangla population; K= Kalpa population). Scale bar=10μm.
Saggoo and Lovleen 2011). Presently, cytomixis was observed in 38.98% PMCs in the accession from Kalpa valley and 37.03% PMCs in the accession from Sangla valley. Two types of connections between proximate PMCs were observed: cytoplasmic channels and direct fusion. Cytomixis through cytoplasmic channels was observed at various stages of cell division and PMCs were mostly connected with a single cytoplasmic channel (Figs. 1K, 1L, 2M, 2N, 2P). Multiple cytoplasmic channels were also found between PMCs (Fig. 1J). The direct fusion of PMCs was observed at various stages of cell division but in low frequency (Fig. 2O). In the plants from Kalpa Valley the migration of chromatin materials was usually unidirectional, i.e. from a donor to recipient cell (Fig. 1J). Two to four PMCs were simultaneously involved in cytomixis and the chromatin passed from the first meiocyte to the second, from second to the third, and so on in plants from Sangla population (Fig. 2N). Unidirectional migration of chromatin materials from one PMC to another in a series has been reported by Gottschalk (1970). Presently, a single PMC was found donating its chromatin materials simultaneously to more than two
PMCs through their independent cytoplasmic channels (Fig. 1L). The frequency of PMCs showing cytomixis was higher during the early stages of meiosis and gradually decreased towards the end of meiosis. This observation supports earlier views (Levan 1941; Maheshwari 1950) that early stages are more favourable for cytomixis, and is contrary to the belief that all the stages of meiosis are equally susceptible to cytomixis (Verma et al. 1984). In rare cases, all the chromatin material of the donor cell migrated to the recipient cell and the donor cell was almost empty (Fig. 2M).

The factors responsible for cytomixis are rather ambiguous. Some possible causes attributed to cytomixis are the effect of chemical fixative used for fixation of material (Gottschalk 1970), mechanical injury (Sarvella 1958), temperature (Basavaiah and Murthy 1987), polypliod level (Verma et al. 1984), cell response as a consequence of pesticides, antibiotic dosage or chemicals (Kumar and Sinha 1991), failure of cell wall formation during premeiotic mitosis (Kamra 1960), and genetically controlled behaviour (Mantu and Sharma 1983).

Detailed meiotic analysis revealed more than one third PMCs showing one or the other type of abnormality. The abnormal meiotic course was characterised by the presence of laggards and bridges at anaphases and telophases (Figs. 2Q,2R) in both the populations, causes of which may be due to interlocking of bivalents (Bhattcharjee 1953). The plant collected from Kalpa (2,758m) showed late disjunction of 1-4 bivalents anaphase I (Fig. 1E). Unoriented bivalents being unable to orient at the metaphase plate were observed during metaphase I (Fig. 1I). The laggards observed may result from delayed terminalisation (Kumar and Tripathi 2007) and abnormal spindle formation (Tarar and Dynsangar 1980).

Chromosome stickiness is a common phenomenon in both the plant populations (Fig. 1H). Chromosome stickiness first reported by Beadle (1932) in Maize is reported to be due to genetic and environmental factors (Pagliarini 2000). Chromosome stickiness is characterized by an intense clustering of chromosomes during any phase of cell cycle. Prophase I stage in the species under analysis was normal and chromosome stickiness was observed as from metaphase I (Fig. 1H). Genetic as well as environmental factors have been considered as the reasons for chromosome stickiness in different plant species (Nirmala and Rao 1996; Consolaro and Pagliarini 1996).

The microsporogenesis was also observed to be moderately abnormal due to the presence of dyads and triads (Figs. 2S, 2T). In both the populations pollen grains of two sizes were observed (Fig. 2U). The small sized pollens were more common. The big sized pollen about 25 μm size were available with relative frequency of 35.11% and 29.70% in population from Kalpa and Sangla, respectively.

Observations were also made to study the chiasma frequency in the plants belonging to both the populations. The accessions of Kalpa population showed the presence of 2-4 rod shaped and 10-12 ring shaped bivalents, while in the Sangla population the rod shaped bivalents ranged between 1-2 per PMC (Figs. 1F,1G). The average chiasma frequency per PMC was 1.88 and 1.78 in accession of Kalpa and Sangla populations, respectively. Chiasma corresponds to the point of physical exchange between homologous non-sister chromatids (Tease and Jones 1978). Genetic as well as environmental factors have generally been considered the reason in chromosome pairing.

Therefore it can be concluded that abnormalities affects mechanism of chromosome segregation in the cells, pollen performance, its fertility and ultimately decline in seed production.

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