Meiotic study in a completely male sterile wild plant of *Deyeuxia scabrescens* from Kullu District, Himachal Pradesh (Northwest Himalayas), India

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**ABSTRACT:** The accession showed the tetraploid chromosome number of 2n=28 and irregular meiotic course due to the presence of multivalents, univalents, scattered and unequal distribution of chromosomes, chromatin bridges and laggards. Besides, a few PMCs showed cytomixis and cell fusion resulting into syncytes. Meiotic course in syncytes was also irregular. Consequent to these aberrations, abnormal sporads and sterile pollen grains were resulted. Total pollen sterility in the accession seems to be under the control of genetic factors as reported by different workers. Complete pollen sterility leads to failure in seed set resulting into restricted/poor distribution of species.

**KEYWORDS:** *Deyeuxia scabrescens*, Multivalents, Cytomixis, Syncytes, Complete male sterility.

*Deyeuxia scabrescens* (Griseb.) Munro ex Duthie, the Poaceae, also treated in *Calamagrostis* as, *C. scabrescens* Griseb. grows as a perennial grass on open dry slopes between the altitudes of 2400–4600 m in Bhutan, India, Myanmar, Nepal and Pakistan. It is characterized by its loosely contracted, narrowly lanceolate panicles with yellowish green or purple spikelets which appeared from July to September. The present study is based on that sterile individual collected from the locality around the Malana Village in Kullu District, H.P., India. The species in the area showed very restricted distribution. The analyzed accession showed total male sterility and no fruit set. Aims of the study were to (i) record the exact gametic chromosome number and ploidy level, (ii) analyze the meiotic course in meiocytes including microsporogenesis and (iii) ascertain the apparent causes of pollen sterility during pollen development and pollen maturation.

**MATERIALS AND METHODS**

The materials for male meiotic studies were sampled from a wild accession collected around Malana Village (32° 4’ 0” N 77° 16’ 0” E, 2652 m) in the District of Kullu, Himachal Pradesh. The specimens for the cytological analyses were deposited in the Herbarium, Department of Botany, Punjabi University, Patiala (PUN 60300).

For meiotic chromosome counts, young and unopened spikes were fixed in a freshly prepared Carnoy’s fixative (glacial acetic acid: chloroform: ethanol 1: 3: 6) for 24 h and subsequently stored in 70 % ethanol in a refrigerator until meiotic analysis. Meiocytes preparations were made by squashing the developing anthers in 1% acetocarmine. A total of 60–100 meiocytes were examined for determining the chromosome number, while 50–80 slides were prepared from different anthers/ florets/ spikelets for analysis of meiotic abnormalities at various stages of meiosis. For pollen grain studies, anthers from mature florets were squashed in glycerol–acetocarmine mixture (1:1) and 1% aniline blue dye. Slides were kept overnight after heating at 60°C and observed after 24 h for pollen fertility. All the pollen grains were noticed to be shrivelled with unstained or poorly stained cytoplasm and were considered as sterile/unviable. Photomicrographs of chromosome counts, sporads, pollen grains and anthers were taken from the temporary mounts using Nikon Eclipse 80i microscope.

**RESULTS AND DISCUSSION**

*Gametic chromosome number and ploidy level*

Chromosome numbers of the species studied have been confirmed through male meiosis and meiocytes with countable chromosomes were observed at different stages of meiosis. Majority of the PMCs showed a tetraploid chromosome count of n=14 (based on x=7) as confirmed from the presence of 14 bivalents at metaphase I (Fig. 1) and 14:14 chromosomes distribution (Fig. 2) at anaphase I.

Analysis of 544 meiocytes revealed that the accession also showed the phenomenon of cell fusion at different stages of meiosis resulting into large sized syncyte PMCs (Fig. 3, 21.14%). Number of PMCs involved in fusion varies from 2-4. Further analysis of data on 115 syncytes revealed (Table 1) that the maximum frequency of syncytes were resulted involving two PMCs (13.97%). In some cases PMCs showed fusion at different stages of meiosis and the resultant syncyte PMCs depicted two separate groups of chromatin materials (Fig. 4).
Figs. 1-23. Meiosis in *Deyeuxia scabrescens*. 1. A PMC at metaphase I with 14 II. 2. A PMC showing 14:14 distribution of chromosomes at anaphase I. 3. A syncyte PMC showing 28 bivalents at metaphase I. 4. Two PMCs showing fusion at different phases of meiosis. 5. A large sized syncyte PMC showing four groups of chromatin materials. 6. A PMC at metaphase I showing 1IV (arrowed) + 12 II. 7. A PMC at metaphase I with 2IV (arrowed) + 10 II. 8. A PMC at metaphase I showing 2I (arrowed) + 13II. 9-10. PMCs showing unequal distribution of chromosomes at anaphase I. 11. A PMC showing laggards (encircled). 12. Transfer of chromatin between 3 PMCs (arrowed). 13. A PMC at metaphase I showing chromatin stickiness and out of plate bivalent. 14. A syncyte PMC showing univalents (arrowed) and a multivalent (arrowhead). 15. A syncyte PMC showing scattered distribution of chromatin material. 16. A PMC showing three groups of chromatin materials. 17. A PMC showing irregular sized nuclei/ chromatin masses during T-II. 18-19. Syncyte PMCs showing pinching off a part of meiocytes (arrowed). 20. Different sized units in a sporad. 21. A tetrad with included micronuclei (arrowed). 22-23. Sterile (unstained) pollen grains. Scale bar = 10 µm.
Syncyte meiocytes could be identified in a pool of meiocytes on the basis of their larger size compared to typical PMCs. In a few cases, syncytes were noticed to be exceptionally large sized (Fig. 5). Furthermore, the syncytes were also observed to be of irregular size depending upon the fusion process and the number of meiocytes involved in the fusion.

Meiosis
Meiotic course has been analyzed on the basis of observations made separately on typical meiocytes and syncytes.

Typical (4x) meiocytes Observation on typical meiocytes at different stages of meiosis revealed aberrant course of meiosis which included multivalents and univalents during metaphase I and anomalies in chromosome segregation during anaphases/telophases.

Multivalents and univalents Besides the presence of 14 bivalents, some meiocytes also showed the presence of 1-3 quadrivalents (Figs. 6 and 7) and up to two univalents (Fig. 8). Analysis of 104 such typical meiocytes revealed that 56 PMCs (53.85%) showed perfect 14 bivalents formation, while 48 (46.15%) depicted the presence of some multivalents and univalents (Table 2).

Scattered and unequal distribution of chromosomes and laggards Consequent to the presence of univalents, multivalents and abnormal spindle activity, the meiocytes showed scattered and unequal distribution of chromosomes during anaphase I (Figs. 9 and 10).

Of the 97 PMCs analyzed, 51 PMCs (52.58%) showed unequal distribution of chromosomes during anaphase I with 12:16, 13:15, 13:1:14, 13:2:13, 13:3:12 and 11:6:11 (Table 3). Besides, 54 PMCs also showed the presence of laggards (Fig. 11, 45.76%) and chromatin bridge formation (0.84%).

Cytomixis The phenomenon of cytomixis involving chromatin transfer among adjacent meiocytes during the early stages of prophase-I was also noticed in 2.56% cases (Fig. 12). PMCs involved in cytomixis exhibit chromatin stickiness (Fig. 13, 11.90% PMCs) and out of plate bivalents/chromosomes (26.92% PMCs).

Meiosis in syncytes Like typical PMCs, the syncyte PMCs also pass through different stages of meiosis and depicted the presence of multivalents and univalents, whereas rest of the meiocytes showed...
perfect 28 bivalents. Another interesting observation is that syncyte meiocytes showed highly abnormal spindle activity as reflected in the form of scattered distribution of chromosomes (Fig. 15), tripolar spindle (Fig. 16) and large number of nuclei/chromatin masses during T-II (Fig. 17). Furthermore, syncytes also showed pinching off a part of PMCs during meiosis (Figs. 18 and 19).

Microsporogenesis Consequent to aberrant meiotic behavior in typical meiocytes and syncytes, abnormal sporads was resulted (Figs. 20, 21). It has also been observed that microspore units in such anomalous sporads were of different sizes as, large (20.87×20.87 µm; 18.67%), medium (17.29×15.90 µm; 68%) and small sized (10.95×10.51µm; 13.33%). It has also been noticed that microspore units came out of meiocytes before their maturation.

Pollen grains The studied accession showed cent percent unstained/ sterile and shriveled pollen grains (Figs. 22 and 23).

Seed setting The analyzed accession was noticed to be completely sterile and do not shows any seed set.

Presently detected tetraploid chromosome count of n=14 in the species substantiates the earlier reports of 2n=28 by Parkash (1979) and Mehra (1982) from North Eastern Himalayas. Mehra and Sood (1975) have reported the presence of up to 3B-chromosomes in the plants analyzed from the Sikkim State in Eastern Himalayas. Presence of 1-3 multivalents in 4x meiocytes and syncytes indicates toward the partial homology of chromosomes of two different sets and segmented alloplloid nature of the taxon. Besides, the analyzed accession also showed the phenomenon of cytomixis and cell fusion.

Consequently syncyte meiocytes were resulted. This is the first report of cell fusion in the species. Ever since the first report of syncytes due to cell fusion by Gates and Rees (1921) in Lactuca sativa, the phenomenon has been reported in a number of flowering plants (Levan 1941; Price 1956; Katayama 1964; Mehra and Kalia 1973; Rao and Koduru 1978; Sarbhowy 1980; Padmaja 1988; Caetano-Pereira et al. 1999; Mendes-Bonato et al. 2001, 2003; Risso-Pascotto et al. 2006; Boldrini et al. 2006; Singhal and Kumar 2008, 2010; Kim et al. 2009; Kumar et al. 2010, 2011, 2012; Singhal et al. 2011; Kumar and Singhal 2012; Malik et al. 2014). The syncyte meiocytes could be recognized by their larger and irregular size compared to the typical meiocytes. Meiotic course in normal (4x) and

| Table 4. Meiotic abnormalities in PMCs of D. scabrescens. |
|-----------------------------------------------|
| Meiotic abnormalities | Percentage of meiocytes with abnormalities |
|------------------------|-------------------------------------------|
|                        | Typical PMCs | Syncyte PMCs |
| Cytomixis              | 11/429 (2.56%) | - |
| Laggards               | 54/118 (45.76%) | - |
| Chromatin bridges      | 1/118 (0.84%) | - |
| Chromosome stickiness  | 25/210 (11.90%) | 20/115 (17.39%) |
| Interbivalent/chromosomal connections | 45/210 (21.45%) | 30/115 (26.09%) |
| Out of plate bivalents/chromosomes | 28/104 (26.92%) | 17/66 (25.76%) |

| Table 5. Analysis of sporads in D. scabrescens. |
|-----------------------------------------------|
| No. of sporads observed | Normal | Abnormal |
|-------------------------|--------|----------|
|                         | Tetrads | Tetrads with micronuclei | Triads with micronuclei |
| 448                     | 200/448 (44.64%) | 232/448 (51.79%) | 16/448 (3.57%) |
Syncytes showed various meiotic abnormalities such as multivalents and univalents, unorganized chromosomes, scattered chromosome distribution, laggards and chromatin bridges. Consequently, the anomalous sporads produced due to meiotic disturbances yielded variable sized and genomically imbalanced microspores. Such microspores yielded sterile/unstained pollen grains.

Male sterility is the failure or inability of plants to produce functional anthers, pollen or male gametes. Male sterility may have multiple causes, it can results from anther suppression, abortion, failure of anther dehiscence, aberrant male meiosis during pollen formation, premature dissolution of callose in meiocytes or in plants infected with virus or fungal diseases (Kaul 1988). Gottschalk (1976) opined that male sterility in higher plants is expressed in two ways, (i) functional male sterility which included mutants in which either stamens or archesporial tissue are not differentiated or where male gametes are formed but fertilization is prevented either due to anther non-dehiscence or spatial separation of anthers and stigma. Kaul (1988) also included genes that influence the course of microsporogenesis under functional sterility. (ii) Structural sterility in which sterility is caused by abnormalities in flowers or in reproductive organs.

Presence of sterile/non-viable pollen grains in *D. scabrescens* seem to have been resulted due to aberrant meiotic divisions in pollen mother cells caused due to cytomixis and cell fusion as advocated by many workers in other plants (Li *et al.* 2005; Singhal *et al.* 2011; 2016; Lattoo *et al.* 2006; Liu *et al.* 2012). However, the total pollen sterility in the presently analyzed accession could be attributed to failure of starch biosynthesis in mature pollen grains which seems to be under the control of some genetic factors as reported earlier in male sterile clones of *some grasses* (Datta *et al.* 2001, 2002; Jain *et al.* 2007; Kong *et al.* 2007) and therefore there is a great need to studied this on molecular basis which is not possible in the present study. Kaul (1988) classified it as under sporogenous male sterility on phenotypic basis. The total pollen sterility resulted due to cytomixis and cell fusion induced meiotic irregularities coupled with structural and sporogenous male sterility seems to be the possible reason for the completely sterile nature of taxon with no seed set and restricted/poor distribution in the area.

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