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A Case of Cytomixis, Cell fusion, Syncyte and Dimorphic pollen grains in *Angelica glauca* from the cold deserts of North-West Himalayas

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ABSTRACT: *Angelica glauca* Edgew. (Apiaceae) has been cytologically studied from the cold deserts of Ladakh division of North-West Himalayas in India. Besides re-reporting the diploid chromosome count of 2*n*=22 from India, the analyzed accession depicted the phenomenon of cytomixis, cell fusion leading to syncyte PMCs and dimorphic pollen grains. Majority of the pollen mother cells (PMCs) exhibited 11 bivalents at Metaphase-I, 11:11 chromosomes at Metaphase-II and equal sized pollen grains. However, a few PMCs showed the phenomenon of cytomixis involving chromatin transfer and fusion resulting into syncytes. These syncytes could be detected in preparations due to their larger size compared to typical (2*x*) PMCs and showed 22 bivalents. The products of typical and syncyte meiocytes yielded dimorphic pollen grains. Such pollen grains with different genetic constitution could play an important role in the origin of intraspecific euploids.

KEY WORDS: *Angelica glauca*, Apiaceae, Cytomixis, Cell fusion, Dimorphic pollen grains, Ladakh, Syncytes

INTRODUCTION

The genus *Angelica* L. (Family: Apiaceae) which is distributed in the higher hills of Asia from Afghanistan, China, Nepal and West Pakistan is represented by three species in India (Aghinotri *et al.,* 2004). Among the Indian species, *A. glauca* Edgew. is widely distributed in the Western Himalayas of Jammu and Kashmir, Himachal Pradesh and Uttar Pradesh to Eastern Himalayas in Sikkim. The plant is a tall (20-60 cm) perennial herb, with 1-3 pinnate leaves, bearing white flowers during the months of June to July. The species grows as a glabrous, rhizomatous herb on moist shady sub-alpine slopes between 2600-4000m. It is an active herb bearing long-conic thick roots which are used as spices, condiments and drugs. Roots are considered as cardiac stimulant, carminative, expectorant and diaphoretic. Powdered roots are administered with warm water for curing stomach ailments among children. Besides, the root powder also checks vomiting. As a part of the project to explore the cytomorphological diversity in the flowering plants of cold deserts of Ladakh division of Jammu & Kashmir, meiotic analysis and pollen grain studies have been made in this medicinal herb. Analyzed individuals which exist at diploid level with a chromosome count of 2*n*=22 showed the phenomenon of cytomixis and fusion among meiocytes resulting into syncytes. Aims of the present investigations were to (i) analyze the meiotic course in normal '2*n*' and syncyte '4*n*' meiocytes and (ii) estimate pollen fertility and analyze pollen grain variation involving size and morphology.

MATERIALS AND METHODS

Materials for male meiotic studies and pollen grains were collected from the wild plants during June, 2015 growing in the cold desert region of Ladakh division of North West Himalayas (Drass, 3130m; 34°94'N 75°45'E). The umbels of appropriate sizes were fixed in a freshly prepared Carnoy's fixative (mixture of alcohol, chloroform, and acetic acid in a volume of 6:3:1) for 24 hrs and preserved in 70% ethanol in a refrigerator. The voucher specimen of the cytolologically worked out accession was deposited in the Herbarium, Department of Botany, Punjabi University, Patiala (PUN 60220). Pollen mother cells for cytological studies were prepared by squashing the developing anthers in 1% acetocarmine. A total of 1042 PMCs at different stages of meiosis-I and II were analyzed to work out the frequency of cell fusion and cytomixis. For the determination of pollen fertility slides from mature anthers taken from different florets/ umbels were prepared in glycero-acetocarmine mixture (1:1). Estimation of pollen fertility was carried out by considering the well stained pollen grains as fertile, and unstained and shrunken as sterile. Terminology about pollen morphology and symmetry is as per standard books of (Erdtman, 1952; Nair, 1965). Size of pollen grains and PMCs was measured by micrometry. The photomicrographs of PMCs, sporads and pollen grains were taken from the freshly prepared slides by using Leica Qwin Imaging System.

Table 1. Frequency of meiocytes affected by cell fusion, cytomixis, meiotic aberrations and pollen grains in ***Angelica glauca***.

I. Cytomixis			
Phase	No. of meiocytes analyzed	Cell fusion	Meiocytes affected due to cytomixis
Leptotene and Zygotene	239	6 (2.51%)	23 (9.62%)
Diplotene	190	5 (2.63%)	-
Diakinesis	180	4 (2.22%)	5 (2.77%)
Metaphase-I	200	4 (2.01%)	16 (8.01%)
Anaphase-I	121	3 (2.74%)	2 (1.65%)
Anaphase-II	112	2 (1.70%)	3 (2.67%)
Total	1042	24 (2.30%)	49 (4.70%)
II. Meiotic Aberrations			
Type	PMCs analyzed	Aberrant PMCs	% of PMCs
a. Cytomixis	1042	49	4.70
b. Hypoploid PMCs	760	18	2.36
c. Hyperploid PMCs	1042	24	2.30
d. Chromatin bridges	840	22	2.61
e. Laggards at anaphase	320	15	4.68
f. Triads	90	04	4.44
III Pollen grains			
Type	Size		Frequency (Fr)
Small sized (*n*)	(24.49-26.80 µm×18.10-19.80 µm)		(97.96%)
Large sized (2*n*)	(35.10-36.70 µm×27.00-28.80 µm)		(2.04%)

RESULTS

Majority of the PMCs in the accession showed 11 bivalents at diakinesis (Fig. 1A), Metaphase-I (Fig. 1B) and 11:11 chromosomes at M-II (Fig. 1C). However, in a few cases two adjacent PMCs showed fusion at different stages of meiosis-I (Figs. 1D, E, F) and resulted into syncytes (Figs. 1G, H). Although the frequency of syncyte Meiocytes is rather low (24 out of 1042, 2.30%), these can be detected in the preparations due to their larger size (99.09-118.00 µm×48.05-60.03 µm) and presence of 22 bivalents (Fig. 1I) compared to the typical PMCs which are small-sized (68.80-80.00 µm×32.02-45.50 µm) and possessed 11 bivalents.

Besides fusion, 4.70% of the PMCs (Table 1) showed the phenomenon of cytomixis involving chromatin transfer through cytomictic channels (CCs) at different meiotic stages (Figs. 1J, K) resulting into hypo- (*n*=6; Figs. 2A, B) and hyperploid (*n*=12; Fig. 2C) PMCs. The PMCs involved in chromatin transfer also showed the presence of certain spindle abnormalities which included multiple chromatin bridges (Fig. 2D), laggards (Fig. 2E) and formation of triads (Fig. 2F). Pollen grain analysis revealed that the accession generates two types of pollen grains (Table 2). The larger (2*n*) pollen grains measure (35.10-36.70 µm×27.00-28.80 µm) while the small sized (*n*) measure (24.49-26.80 µm×18.10-19.80 µm). Shape-wise also, '*n*' and '2*n*' pollen grains were noticed to be different. The typical and small sized pollen grains were perprolate and laterally symmetrical, while the larger ones were prolate, bilaterally symmetrical, and triquette with three radiating arms (Fig. 2G). Out of a total of 882 pollen grains analyzed, the larger pollen grains were recorded to be in rather low frequency (2.04%) compared to the typical '*n*' pollen grains (97.96%).

DISCUSSION

The presently recorded diploid chromosome count of 2*n*=22 in *Angelica glauca* (based on *x*=11) confirms the earlier reports by cytologists from Lahaul-Spiti and Pangi valley (Kumar & Singhal, 2011; Kumar, 2015). However, the presently analyzed wild accession showed the phenomenon of cytomixis and cell fusion resulting into hypo-, hyperploid and syncyte meiocytes. Consequent to the processes of cell fusion cytomixis and Syncytes, meiocytes with spindle irregularities were resulted. The products of such meiocytes yielded small sized and large sized fertile/stained and sterile/unstained pollen grains.

Ever since the first report of syncyte formation through cell fusion in *Lactuca sativa* (Gates & Rees, 1921), number of cases regarding syncyte meiocytes have been reported in flowering plants (Levan, 1941; Price, 1956; Katayama, 1964; Rao & Koduru, 1978; Sarbhoy, 1980; Patra *et al.*, 1986; Caetano-Pereira *et al.,* 1999; Singhal & Kumar, 2008; Kim *et al.*, 2009; Singhal *et al.,* 2011, 2016; Kumar & Singhal, 2012; Malik *et al.*, 2014; Kaur *et al.*, 2017). The generation of syncyte PMCs through the fusion of adjacent meiocytes in *Angelica glauca* (2*n*=22) is the first report for the species. The syncyte PMCs were much larger and possessd double chromosome number. Such polyploid/syncyte meiocytes showed normal 22 bivalents and regular segregation. Presence of cytomixis and cell fusion resulting into syncyte meiocytes in the analyzed accession seems to have caused some pollen sterility (5.60%) and variability in pollen size and morphology (dimorphic pollen grains). Such an effect of cytomixis and cell fusion generated syncytes on pollen grains has also been reported in *Lindelofia longiflora* (Singhal *et al.,* 2011), *Mertensia echioides* (Malik *et al.,* 2014),

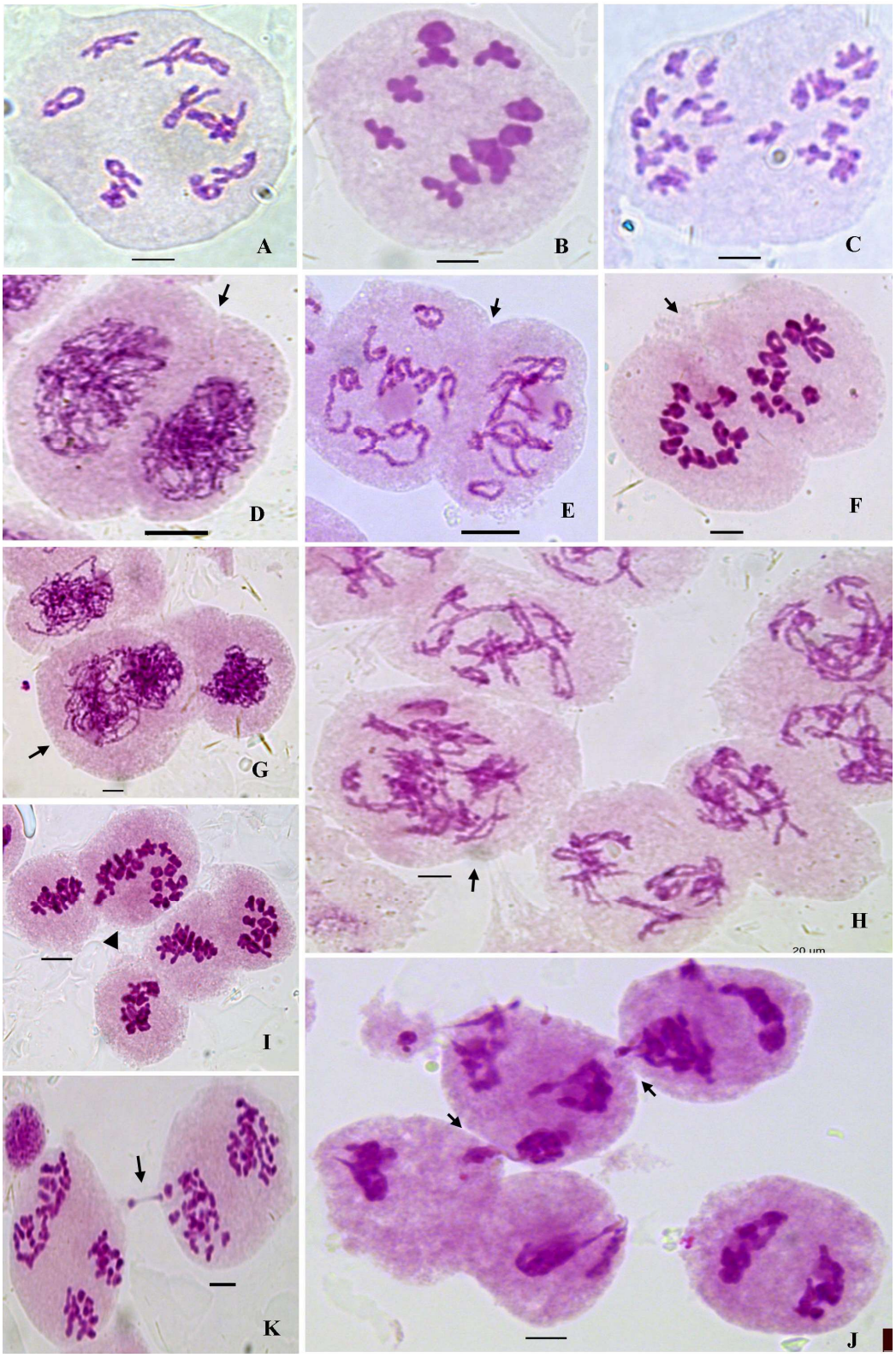

Fig. 1 Meiotic behaviour in ***Angelica glauca***. **A.** A PMC with 11 bivalents at diakinesis. **B.** A PMC at M-I showing 11 bivalents. **C.** A PMC with 11:11 chromosomes at M-II. **D-F.** Two PMCs showing fusion at different stages of meiosis. **G.** A large syncyte PMC with double chromatin material (arrowed) along with '2*n*' normal PMCs. **H.** A syncyte PMC (arrowed) in a pool of normal '2*n*' PMCs. **I.** A large sized syncyte PMC with 22_{II} at M-I. **J.** PMCs involved in cytomixis showed extra chromatin masses (arrowed). **K.** Two PMCs involved in chromatin transfer through a narrow channel at A-II (arrowed). Scale bar=10 µm.

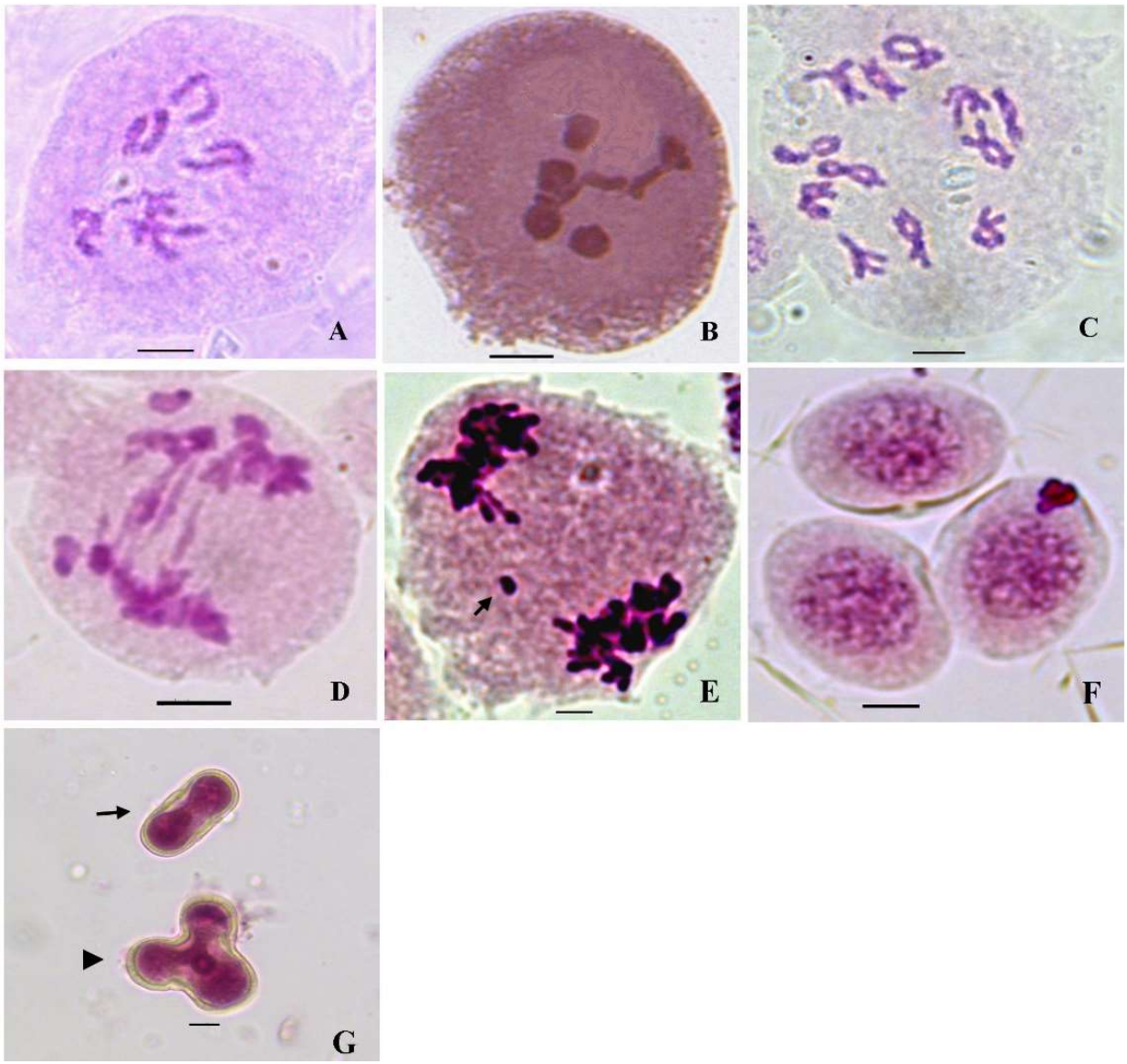

Fig. 2. Meiotic behaviour in ***Angelica glauca*. A, B**. A hypoploid PMC showing 6 bivalents. **C.** A hyperploid PMC showing 12 bivalents. **D.** A PMC showing multiple chromatin bridges at A-I. **E.** A PMC showing a laggard at A-I (arrowed). **F.** A triad. **G.** Normal '*n'* pollen grain (arrowed) and a '2*n'* pollen grain (arrowhead). Scale bar=10 µm.

Heracleum pinnatum (Singhal *et al.,* 2016) and *Achillea millefolium* (Kaur *et al.,* 2017). Pollen grain analysis in the accession showed the presence of two types of fertile pollens differing in size and morphology. Although the exact cytological status of larger sized pollen grains could not be ascertained herein, but their higher cytological status is clearly depicted from their size and different shape compared to typical *'n'* pollen grains, as increasing DNA content has been known to influence the pollen diameter (Jansen & Den Nijs, 1993; De Storme & Geelan, 2013). As bigger sized '2*n*' pollen grains are well stained and appeared to be fertile, it seems possible that these might play a role in the origin of intraspecific polyploids as suggested earlier in *Erianthus arundinaceus* (Retz) Jeswiet, *Saccharum rubustum* E. W. Brands & Jeswiet ex Grassl, and *Saccharum sinense* Roxb. (Price, 1956) and *Chrysanthmum* (Kim *et al.,* 2009). The origin of such gametes, also reffered as diplogametes (De Storme & Geelen, 2013), through syncyte PMCs seems to be one of the possible factors for the origin of intraspecific polyploids as advocated in *Chrysanthemum* (Kim *et al.,* 2009), interspecific hybrids of *Brassica* (Mason *et al.,* 2011), *Lindelofia longiflora* (Singhal *et al.,* 2011) and *Mertensia echioides* (Malik *et al.,* 2014).

Divergent views regarding the possible causes responsible for the origin of syncytes have been put forward by different workers which included the effects

of chemicals, X-rays, temperature, moisture stress, culture conditions or genetic factors (Stern, 1946; Merwine & Bennet, 1996; Singhal & Kumar, 2008; Kumar & Srivastava, 2009; Mason *et al.,* 2011; Pecrix *et al.,* 2011; Kumar & Singhal, 2012; Kumar *et al.,* 2012; Malik *et al.,* 2014). In the presently analyzed accession of *A. glauca*, the origin of syncytes due to whole chromatin transfer and/or direct fusion could be attributed to low temperature stress conditions prevailing in the cold deserts of Ladakh region of North-West Himalayas where temperature dips to below freezing point during May-June when plants enter the flowering stage. Such low temperature conditions might have induced the formation of syncytes through fusion of meiocytes and whole chromatin transfer during premeiotic genome duplication as has also been suggested by some workers (Jansen & Den Nijs 1993; Kim *et al.,* 2009; Kumar & Singhal, 2012; Rana *et al.,* 2013; Malik *et al.,* 2014, Singhal *et al.,* 2016).

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