Male meiosis and meiotic abnormalities in two species of *Pouzolzia* Gaud. from western Himalayas, India

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Received December 21, 2013; accepted January 23, 2014

ABSTRACT. The present investigation involves the meiotic studies on two species of *Pouzolzia* (family Urticaceae) from western Himalayas, India. Both the species uniformly revealed chromosome count of $2n=26$ which is first ever chromosome report for *Pouzolzia pentandra* (Roxb.) Benn. on worldwide basis and first chromosome report for *Pouzolzia hirta* (Blume) Hassk. from western Himalayas. This is also the first report of cytotoxicity in *P. hirta*. In addition to bridge formation and extra chromatin material, chromosome stickiness was the most frequent meiotic chromosomal anomaly found in both the species. In spite of abnormalities there was no adverse effect on pollen fertility. Morphological analysis of *P. pentandra* revealed the existence of two morphotypes.

KEYWORDS: Chromosome stickiness, Cytotoxicity, Morphotypes, *Pouzolzia hirta*, *Pouzolzia pentandra*, Western Himalayas

Pouzolzia Gaud., family Urticaceae, is distributed in Asia, Australia and South Africa. Worldwide, the genus is represented by 36 species and 17 infraspecific taxa (Wilmot-Dear and Friis 2004). Of the total 13 species of the genus available in India two are discussed here.

Pouzolzia hirta (Blume) Hassk. [= *Gonostegia hirta* (Blume) Miq.] is an annual decumbent or semi-erect pubescent herb with cylindrical stem. The leaves are opposite, sessile or shortly stalked, narrowly ovate, entire with acute apex. Flowers are pale green in colour and are present in small rounded dense clusters. It is distributed worldwide in India, China, Indonesia, Malaysia and Australia. In India, *P. hirta* (Blume) Hassk. is found in tropical and sub-tropical Himalayas from Himachal Pradesh to north eastern states upto 1600m altitude. It is a medicinally important herb and the root paste is used to treat the bone fracture and bandaged around the affected area (Saha *et al.* 2011). Tender leaves and stems are used as vegetable to improve the appetite and lactation in women (Srivastava 2009; Shankar *et al.* 2012). The plant is used as fodder too. This species has been studied from eastern Himalaya and chromosome counts of $2n=16$, $2n=26$ and $2n=32$ are known (Sharma and Sarkar 1967-1968; Sharma 1970; Sharma and Mehra 1977; Guha 1977; Chatterjee *et al.* 1989).

Pouzolzia pentandra (Roxb.) Benn. [= *Gonostegia pentandra* (Roxb.) Miq.] is an annual, erect, nearly glabrous herb with robust, angular stem (Fig. 1). Leaves are opposite below, the upper alternate, crowded, gradually decreasing in size upwards, sessile or shortly stalked, oblong-lanceolate, entire with acute apex. Flowers are loosely clustered in upper leaf axils. It is distributed upto 1,000 m in India, China, Java, Pakistan, Afghanistan, Bangladesh, Indonesia, Myanmar, Papua New Guinea, Philippines, Thailand and Vietnam. In India the species is found in subtropical and tropical Himalayas from Kangra eastward to Assam Khasia Hills and Bengal to Kanara in

south. It is a great medicinal plant and is used as one of the ingredient in anti-cancer remedies in Thai traditional medicines. The plant exhibits the growth-inhibiting effects against bacterium named *Helicobacter pylori* known to cause various gastric ailments (Chaichanawongsaroj *et al.* 2012).

To the best of our knowledge chromosome studies are lacking in both the above species of medicinal importance from western Himalayas. The present study aims to fill the gap in knowledge of the basic information about chromosome number, meiotic behaviour and pollen fertility in various wild populations of the species of *Pouzolzia* from western Himalayas.

MATERIAL AND METHODS

Materials for meiotic studies and morphometric analysis

Fig. 1. Inflorescences of two morphotypes of *Pouzolzia pentandra* (Roxb.) Benn. as the yellowish-green colored stem (right) and the red colored stem (left)

Table 1. Comparative data on meiotic course, pollen fertility and pollen size of various accessions of *Pouzolzia hirta* (Blume) Hassk. and *Pouzolzia pentandra* (Roxb.) Benn. from western Himalayas, India

	<i>Pouzolzia hirta</i> (Blume) Hassk.				<i>Pouzolzia pentandra</i> (Roxb.) Benn.		
					Morphotype Green	Morphotype Red	
Population	Palampur, 1,100m	Beerta, 730m	Bhagsunag, 1,900m	Sali, 1,500m	Rehlu,900m	Dheera, 500m	Rehlu,900m
Accession number (PUN)	58579	58580	58582	58581	58583	58585	58584
Chromosome number (n)	13	13	13	13	13	13	13
Ploidy level	Diploid	Diploid	Diploid	Diploid	Diploid	Diploid	Diploid
PMCs with meiotic abnormalities (%)							
Bridges at A-I	1.18	-	-	-	-	-	-
Extra chromatin material	6.51	-	-	-	-	0.61	0.57
Chromatin stickiness	81.66	94.03	97.36	96.76	87.03	81.69	44.83
Cytomixis							
% PMCs involved	11.24	-	-	-	1.71	4.38	12.81
No. of PMCs involved/ event	2-3	-	-	-	2	2-3	2-3
Meiotic stages	Diakinesis, M-I	-	-	-	M-I	Diakinesis	Diakinesis, T-I,M-II,T-II
Pollen							
Pollen Fertility (%)	92.67	100	99.46	99.73	99.77	98.13	99.64
Pollen size (µm)	10.56X10.19	10.76X9.92	10.32X9.60	9.84X9.07	13.19X12.89	13.40X12.47	12.60X12.01

PMC= pollen mother cell; M-I= metaphase-I; A-I= anaphase-I; M-II= metaphase-II; T-I=telophase-I; T-II=telophase-II

in *Pouzolzia pentandra* (Roxb.) Benn. were collected from different locations in western Himalayas of India (Table 1; Fig. 1). Identification of the specimens was done at Botanical Survey of India, Dehra Dun, India. Voucher specimens were deposited in the Herbarium, Department of Botany, Punjabi University, Patiala (PUN).

The 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 then preserved in 70% alcohol at 4°C until use. For meiotic studies, anthers were squashed in 2% acetocarmine (BDH product). A number of slides were carefully examined for chromosome counts and meiotic abnormalities at different stages in each population. Pollen fertility was examined using glycerol-acetocarmine mixture (1:1) (Marks 1954). Well filled pollen grains with stained nuclei were taken as apparently fertile while shriveled and unstained pollen were counted as apparently sterile. Photomicrographs of chromosome counts were made from freshly prepared slides using Nikon 80i Eclips Microscope. In order to have proper insight into morphological variation, different morphometric characters were studied in the plants of different populations of *P. pentandra* (Roxb.) Benn. (Table 2).

RESULTS AND DISCUSSION

Morphological Analysis Morphological analysis was carried out on different populations of *P. pentandra* revealed the existence of two morphotypes- the green plants and the red plants (Table 2). Green plants, most common type, collected from Rehlu (900m) and Dheera (500m) showed the green stem, green midrib and pale green flowers while red plants collected from Rehlu (900m) exhibited the reddish stem, reddish mid-rib and reddish flowers. Different parameters have been studied

Table 2. Morphological comparison of two morphotypes of *Pouzolzia pentandra* (Roxb.) Benn. from different localities of western Himalayas, India

Locality	Green Morphotype	Red Morphotype
	Rehlu,900m and Dheera,500m	Rehlu,900m
Plant		
Habit	Erect herb	Erect herb
Height(cm)	60.8-62.1	61.9
Habitat	Near water channels in rice fields and waste land	Near water channels in rice fields
Stem		
Colour	Green	Reddish
Internode Length(cm)	4.2-5.5	4.8-5.6
Leaf		
Lamina (cm)	5.2X1.3	5.6X1.2
Petiole Length(cm)	0.2	0.2
Shape	Oblong-lanceolate	Oblong-lanceolate
Leaf margins	Entire	Entire
Mid-rib colour	Green	Reddish
Inflorescence		
Size of internodes in Inflorescence (cm)	0.1-1.2	0.1-0.8
Size of bracts(cm)	0.5-2.7	0.9-2.0
Number of flowers per cluster	5-7	6-7
Flower colour	Pale green	Reddish
Pedicel colour	Pale green	Reddish

for comparing characteristics of two morphotypes. Significant morphological differences were observed for characteristics such as stem colour, flower colour and leaf midrib colour (Table 2). The other characteristics were similar in both the morphotypes.

Chromosome number The meiotic analysis was carried out on four populations of *P. hirta* (Blume) Hassk. and three populations of *P. pentandra* (Roxb.) Benn. from western Himalayas, India. The meiotic analysis of three

Fig. 2. A-M. Meiotic chromosome number and meiotic abnormalities in *Pouzolzia hirta* (A-G) and *Pouzolzia pentandra* (H-M). A. A PMC at diakinesis showing 13_{II}, B. PMCs with chromatin stickiness at metaphase-I, C. A PMC with extra chromatin material at metaphase-I (arrow), D. A PMC showing chromatin bridge at anaphase-I (arrow), E. Chromatin transfer among two PMCs at metaphase-I, F. A PMC showing complete transfer of chromatin material to two adjacent PMCs (arrows), G. An apparently large fertile and small sterile pollen grain, H. A PMC showing 13_{II} at diakinesis, I. PMCs with stickiness of chromosomes at metaphase-II, J. Transfer of chromatin material among two PMCs at metaphase-II, K. Chromatin transfer between two PMCs at telophase-II, L. extra material in PMC at metaphase-II (arrow), M. An apparently large fertile and small sterile pollen grain. Scale bar = 10µm.

populations of *P. pentandra* (Roxb.) Benn. uniformly revealed the chromosome count of $2n=26$, confirmed by the presence of 13 bivalents at diakinesis (Fig. 2H). The chromosome count of $2n=26$ in *P. pentandra* is first ever chromosome report for the species on worldwide basis. Individuals of all the four populations of *P. hirta* uniformly showed chromosome count of $2n=26$, verified by the presence of 13 bivalents at diakinesis (Fig. 2A). The cytological analysis of *P. hirta* has been carried out for the first time from western Himalayas, India and the present count of $n=13$ in *P. hirta* is in conformity with the earlier chromosome record from eastern Himalayas (Sharma 1970; Guha 1977; Sharma and Mehra 1977). However, varied chromosome count of $n=8$ (Sharma 1970) and $n=16$ (Sharma and Sarkar 1967-1968; Sharma 1970; Chatterjee *et al.* 1989) was also reported earlier in the species.

Perusal of cytological literature revealed the chromosome counts in seven species of the genus *Pouzolzia*. The data show existence of chromosome number $2n=16$, 20, 22, 24, 26, 32, 48 and 52 of which $2n=26$ is the most common (23.08% species). Both the presently investigated species have $2n=26$ and are diploid based upon base number $x=13$ as suggested by Sharma and Mehra (1979).

Meiotic Behaviour Meiotic behaviour was analysed in plant belonging to four populations of *P. hirta* and three populations of *P. pentandra* (Table 1). A wide range of meiotic stages were observed in anthers within same flower. In most of the PMCs observed in both the species, clumpiness or stickiness of chromosomes was of common occurrence (Fig. 2B, 2I). Various accessions of three populations of *P. hirta* (Beerta, 730m; Bhagsunag, 1,900m and Sali, 1,500m) showed normal meiotic behavior. However in the accessions analysed from Palampur (1,100m) population meiotic abnormalities like bridges at anaphase-I (Fig. 2D) and extra chromatin material (Fig. 2C) was observed. A good number of PMCs at diakinesis and metaphase-I (11.24%) were involved in phenomenon of cytomixis. Cytoplasmic channels were observed linking 2-3 PMCs at a time (Fig. 2E, 2F). Microsporogenesis was normal leading to 92.67% pollen fertility (Table 1)

In *P. pentandra* apart from stickiness of chromosomes extra chromatin material was observed in few PMCs of various accessions (Fig. 2L). The accession of *P. pentandra* green morphotype showed few PMCs (below 5%) involved in cytomixis whereas the red morphotype had the involvement of 12.81% of PMCs in cytomixis (Table 1). PMCs involved in cytomixis were observed to be at diakinesis, metaphase-I, telophase-I, metaphase-II and telophase-II stages (Fig. 2J, 2K). However, no adverse effect on pollen viability was seen (Table 1).

The most frequent cytological abnormality, chromosome stickiness may be due to genetic and environmental factors as well as genomic-environmental interaction (Nirmala and Rao 1996; Baptista- Giacomelli *et al.* 2000).

In the present course of study, another chromosomal aberration was bridge formation at anaphase-I. According to Subramanian and Thilagavathy (1988), bridge formation is due to inversion heterozygosity and the frequency of bridge formation depends on chiasma frequency, number of inversions and size of inversions. The formation of bridge has also been attributed to failure of chiasmata in a bivalent to terminalize and chromosome get stretched between the poles (Sayler and Smith 1966), interlocking of bivalent chromosomes (Battacharjee 1953), or paracentric inversion (Sinha and Godward 1972). Cytomixis has been reported during microsporogenesis in several families of flowering plants (Heslop-Harrison 1966; Saggoo and Bir 1983; Ghanima and Talat 2003; Lattoo *et al.* 2006; Singhal *et al.* 2008; Saggoo and Srivastava 2009; Saggoo and Lovleen 2011). Several reasons have been cited as cause of cytomixis like natural cytological phenomenon, certain environmental or physiological stress, in addition to the controlling genetic mechanisms (Omara 1976; Mantu and Sharma 1983; Falistocco *et al.* 1995; Bllucci *et al.* 2003; Lattoo *et al.* 2006). In spite of abnormalities there is no negative effect on the pollen viability.

Western Himalayas is very important source of medicinal plants and many are still to be explored for cytomorphological diversity. The present report is useful entry in chromosomal data base of wild medicinal plants of the region.

ACKNOWLEDGMENTS. The authors are grateful to the University Grants Commission, New Delhi for providing facilities under the DRS SAP III and ASIST programme. Financial assistance to one of us (Lovleen) by Department of Science and Technology, Government of India, New Delhi, under WOS-A is gratefully acknowledged. Thanks are also due to the Head, Department of Botany, Punjabi University, Patiala for necessary laboratory facilities.

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