Genetic Diversity in Different Populations of *Artemisia absinthium* Linn. from Kashmir Himalaya

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Received April 11, 2010; accepted June 29, 2010

Summary The present study revealed the varied frequencies of natural chromosomal abnormalities in 4 populations of *Artemisia absinthium* Linn. collected from different altitudes of Kashmir Himalaya. All the populations are diploid showing $n=9$ with abnormal meiotic behaviour like quadrivalent formation at diakinesis, cytomixis at early prophase-I and laggards at telophases, resulting into reduced pollen fertility. Besides the heterogeneous sized pollen grains, occasionally inter pollen channels were also observed.

Key words *Artemisia absinthium*, Genetic diversity, Kashmir Himalaya, Cytomixis.

Artemisia absinthium in India is mostly distributed in the Himalayan belt. In Kashmir it grows at an altitude from about 1800 m to 3000 m (Kaul 1986). There are 20 species of the genus *Artemisia* occurring in this region (Kaul and Bakshi 1984, Dar *et al.* 2002).

A. absinthium Linn, commonly called worm wood, is herbaceous, perennial and a medicinally important plant. In Kashmir it is called “Tethwen” by the local people has immense medicinal properties like anti-helminth, vermifuge, as an emenagogue, anti-inflammatory, increases uterine circulation and treats gastritis (Lustin 1979). The active principles present in this species are essential oils (thujon and thujol), sesquiterpenes, absinthin, terpenoids and flavinoids that are known to increase the secretion of gastric acid pepsin and pancreatic juice (Khare 2004). Its extracts have also been proved to have free radical scavenging activity (Canadanovic-Burnet *et al.* 2005) and an influence on osmotic stability of human erythrocytes (de Frietas *et al.* 2008).

Keeping in view the various important medicinal properties of this species, the present study is undertaken on population basis in order to mark out the genetic diversity among different populations collected from different altitudinal sites.

Materials and methods

Meiotic studies were carried out in young floral buds fixed in Carnoy's fixative using standard acetocarmine smearing technique. Pollen fertility was estimated by their stainability in 1% glycerol-acetocarmine. Well-filled and well-stained pollen grains were regarded as fertile while the shrunken and unstained pollen grains were considered as sterile.

Results and discussion

Presently meiotic studies have been made on 4 different populations covering different altitudinal ranges of Kashmir Himalaya (Table 1). All 4 populations are found to be diploid with $n=9$ (Figs. 1–3) and show various meiotic abnormalities. The meiotic abnormalities observed include multivalent formation at diakinesis, cytomixis at early prophase-I and laggard formation at

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Table 1. Population number with site of collection, altitude in meters (m), chromosome no. (n) and ploidy level of 4 populations of *A. absinthium*

Population number	Collection site	Altitude	Haploid chromosome No.	Ploidy level
P-1	Dalgate, Hillock (Srinagar)	1750 m	9	2x
P-2	Tral, Karmullah (Pulwama)	1850 m	9	2x
P-3	Kellar (Shopian)	2000 m	9	2x
P-4	Aharbal (Kulgam)	2600 m	9	2x

Table 2. Data on meiotic course and pollen fertility in 4 populations of *A. absinthium*

Population number	% of PMCs with quadrivalent	Cytomixis		% of PMCs with laggards at T-I and T-II	% of pollens showing channels	Pollen fertility % age
		% of PMCs involved	No. of PMCs connected			
P-1	0.00 (0/247)	00.00 (0/220)	0	0.00 (0/240)	0.00 (0/226)	91.80
P-2	0.00 (0/251)	02.94 (6/204)	2	0.00 (0/245)	0.00 (0/232)	87.00
P-3	0.80 (2/248)	10.55 (19/180)	2–3	3.44 (8/232)	0.00 (0/220)	75.86
P-4	1.95 (4/205)	10.60 (21/198)	2–3	5.80 (13/224)	1.92 (4/208)	73.21

Figures in parentheses denote observed number of abnormal PMCs/pollens in numerator and total number of PMCs/pollens in denominator.

telophase-I and telophase-II, resulting in reduced pollen fertility. Interestingly, there are interpopulation differences in the meiotic abnormalities (Table 2).

At diakinesis, one quadrivalent (Fig. 2) in few PMCs was observed in 2 populations *viz.* P-3 and P-4 (Table 1) whereas in the other 2 populations no PMC with quadrivalents was observed. However, the frequency of PMCs with quadrivalents in P-3 is less (0.80%) than that in P-4 (1.95%). In both these populations the quadrivalent observed is of open chain form that may have arisen due to shorter size of interchanged segments. The presence of such a low frequency of quadrivalents might be indicative of the cryptic type of structural heterozygosity. According to Levin (2002), reduced pollen fertility is also as a result of structural heterozygosity. Though the species has been already worked out by researchers including Khoshoo and Sobti (1958), Koul (1964, 1965), Bhat *et al.* (1974), and Kaul and Bakshi (1984), no one has yet reported the multivalent formation in this species.

Presently, the cytomixis is reported in the 3 populations *viz.* P-2, P-3 and P-4, but is found to be absent in P-1. As such there is no earlier report of cytomixis in *A. absinthium*. Further the frequency and the number of PMCs involved in cytomixis also varies in different populations (Table 2). It is seen that generally the cytomixis involves a varied number of PMCs (2–3) and are mostly observed at early prophase-I (Figs. 4–5). However no PMC with hyperploidy or hypoploidy has been observed. The phenomenon of cytomixis is referred to be due to various factors, such as the physiological (Bahl and Tyagi 1988), temperature (Narain 1976), stress factors coupled with genetic control (Ghanima and Talaat 2003), and direct genetic control (Bellucci *et al.* 2003, Haroun *et al.* 2004). Some of the recent reports of cytomixis prevalent in plants from India include that of Lattoo *et al.* (2006), Singhal *et al.* (2007) and Singhal and Kumar (2008). According to some of the workers like Levan (1941), Zheng *et al.* (1987), Ghanima and Talaat (2003) and Kim *et al.* (2009), cytomixis plays a major role in chromosomal diversity and speciation of taxa. As per Bhat *et al.* (2006), the extra chromatin mass received by one PMC from other during cytomixis can also form micronuclei or micro pollen in the later stages.

Figs. 1–10. 1. PMC at diakinesis with 9 bivalents. 2. PMC at diakinesis with 1 quadrivalent (arrow). 3. PMC at Anaphase-II. 4. Cytomixis involving 2 PMCs. 5. Cytomixis involving 3 PMCs. 6. PMC with a laggard at Telophase-I (arrow). 7. PMC with a laggard at early Telophase-II (arrow). 8. Tetrad with 2 micronuclei (arrows). 9. Two pollen grains connected through a channel. 10. Heterogeneous sized pollen grains (bar=10µm).

Consequent to the meiotic abnormality laggards have also been observed. Though in low frequencies, laggards were observed during T-I (Fig. 6) and early T-II (Fig. 7) only in the populations from P-3 (3.44% PMCs) and P-4 (5.80% PMCs) whereas in the other 2 populations no PMC has been observed with laggards. These laggards might have later on formed the micronuclei (Fig. 8).

The post-meiotic consequences, such as reduced pollen fertility, the production of heterogeneous sized pollen grains (Fig. 10) and inter-pollen channels, are the result of the abnormalities discussed above. Hence, population P-1 shows the highest frequency of fertile pollen

grains (91.81%) and population P-4 shows the lowest pollen fertility (73.21%) among the 4 populations studied. Further the inter-pollen channel-like structures are observed only in the P-4 population, with a low frequency (1.92% PMCs), and not more than 2 pollens are seen involved per group (Fig. 9).

From the above discussion it appears that there is some correlation in the frequency of meiotic abnormalities with the altitudinal distribution of the species and these might have been induced due to harsh environmental conditions.

Acknowledgements

Authors acknowledge University Grants Commission, New Delhi for providing financial assistance under SAP, ASIST (U.G.C) and FIST (DST).

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