



First report of structural heterozygosity in *Artemisia parviflora* (Asteraceae) from Parvati Valley in Kullu District (Himachal Pradesh, India)

Ragbir Chand GUPTA, Himshikha, Pawan Kumar RANA, Puneet KUMAR and Vijay Kumar SINGHAL*

Department of Botany, Punjabi University, Patiala-147002, Punjab (India)

ABSTRACT: The existence of structural heterozygosity for reciprocal translocations in the wild diploid ($n=9$) accession of *Artemisia parviflora* collected from the Parvati Valley in Kullu district, Himachal Pradesh, India is reported for the first time. The other two Indian accessions studied from Pangi Valley in Chamba district and Pulga, 2300m in Parvati Valley depict normal meiosis with the presence of nine bivalents, regular segregation of chromosomes during anaphases and almost a hundred percent pollen fertility. We studied in detail the male meiosis in pollen mother cells (PMC) and pollen fertility in the accessions showing structural heterozygosity. The PMCs exhibited abnormal meiosis due to the presence of multivalents in 62.92% of the analyzed 213 PMCs. A total of 12.31% of the chromosomes are observed to be involved in multivalents and 2.35% remained as univalents. In spite of the presence of multivalents and early/late disjunction of few bivalents, the chromosomes in PMCs are regularly segregated to poles at A-I/A-II resulting into normal tetrad formation. The structural rearrangements of chromosomes in *A. parviflora* are responsible for causing some pollen malformation (30%) as has been the case in other species. Another effect of structural heterozygosity is the increase in chiasma frequency per PMC from 10.75-12.40.

KEY WORDS: *Artemisia parviflora*, chiasma frequency, multivalents, reciprocal translocations, structural heterozygosity.

Received 16 December 2009

Revision Accepted 25 May 2010

UDK 582.998.16:576.316

INTRODUCTION

The genus *Artemisia* L. belonging to the tribe Anthemideae of the family Asteraceae is represented by ca. 400 species with 32 species distributed in different parts of India, especially Himalayas (HAJRA *et al.* 1995). Some species are reported to be confined to the high altitudinal regions of cold deserts of India. Perusal of chromosomal literature reveals that among Indian species, *A. capillaris* Thunb. (2x, 3x, 4x), *A. dracuncululus* L. (2x, 4x, 6x, 10x), *A. gmelinii* Web. ex Stechm. (4x, 6x), *A. maritima* L. (2x, 4x, 6x and B-chromosomes), *A. scoparia* Waldst. et Kit. (2x, 4x, 6x) and *A. vulgaris* L. (2x, 4x, 5x, 6x and B-chromosomes) exhibited considerable morphogenetic variation and intraspecific polyploidy. *A. parviflora* Buch.-Ham. ex

Roxb. is a widely distributed species mainly in hilly parts between altitude of 900-3500m. Its occurrence in lowland areas is attributed to its seeds being washed down by the streams. It is a more or less aromatic erect tall perennial herb with variable sessile linear cuneate leaves and greenish white flower heads in racemes. The species is reported to have essential oils (RANA *et al.* 2003) and coumarins (JAIN *et al.* 1974) in the aerial parts. The species is diverse morphologically besides depicting diploid, $2n=18$ (MEHRA & REMANANDAN 1974; KAUL & BAKSHI 1984; GUPTA & GILL 1989; MATHEW & MATHEW 1987) and tetraploid, $2n=36$ cytotypes (MEHRA & REMANANDAN 1969, 1974; XIONG *et al.* 1995). Presently the species has been studied cytologically from two geographical different valleys, Parvati Valley in Kullu and Pangi Valley in Chamba district

*correspondence: vksinghal53@gmail.com

of Himachal Pradesh, India. All the three wild accessions scored presently existed at diploid level ($n=9$). But one of the accession studied from Parvati Valley exhibited structural heterozygosity due to reciprocal translocation as confirmed from the presence of multivalents and reduced pollen fertility. Detailed male meiosis, microsporogenesis and pollen studies have been investigated in the accession which showed structural heterozygosity.

MATERIALS AND METHODS

Materials for male meiotic studies were collected from the wild accessions growing in Parvati Valley (Barsani 2200m; 51723 PUN, 51724 PUN and Pulga 2250m; 51725 PUN) in Kullu district and Pangli Valley (Sahali 2300m; 51734 PUN) in Chamba district of Himachal Pradesh during July-August of 2008-2009. The voucher specimens of the cytologically studied plants were deposited in the Herbarium, Department of Botany, Punjabi University, Patiala (PUN). For meiotic chromosome counts, young floral heads with developing flowers were fixed in Carnoy's fixative (6 Alcohol: 3 Chloroform: 1 Acetic acid v: v: v) for 24 hours. The materials were subsequently transferred to 70% alcohol and stored in refrigerator. Slides were prepared through standard squash technique in 1% aceto-carmine. A number of freshly prepared slides with PMCs undergoing meiosis were examined to determine the exact chromosome number and meiotic abnormalities at different stages. Chromosomal associations were analyzed at late prophase-I and metaphase-I and their separation was observed at A-I/T-I as well as A-II/T-II. Further observations on meiotic course resulting into tetrad formation were also recorded. Pollen fertility was estimated using glycerol/aceto-carmine (1:1) mixture. Well filled

pollen grains with stained nuclei were taken as apparently fertile while shriveled and unstained pollen were counted as sterile. Some 400-500 pollen grains were screened for pollen fertility in each accession. Photomicrographs of chromosome counts were made from the freshly prepared slides using Nikon 80i eclipse microscope.

RESULTS AND DISCUSSION

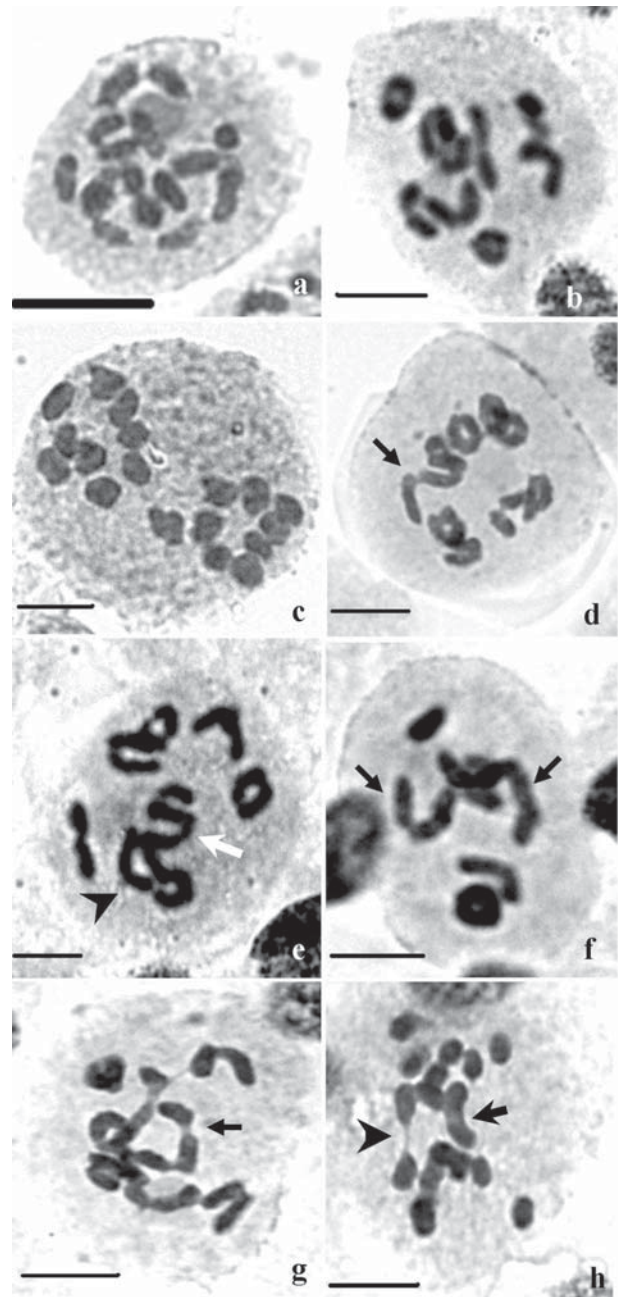
A. parviflora which had been worked out quite extensively by different workers from India and other parts of the world exhibited the presence of diploid ($2x$) and tetraploid ($4x$) cytotypes. The diploid cytotype ($2n=2x=18$) seems to be more common in Indians taxa and has already been reported from the hills in South India (MEHRA & REMANANDAN 1974; MATHEW & MATHEW 1988), Kashmir Himalayas (KAUL & BAKSHI 1984) and Pachmarhi hills, Madhya Pradesh (GUPTA & GILL 1989). On the other hand, the tetraploid cytotype ($2n=4x=36$) is recorded by MEHRA & REMANANDAN (1969, 1974) from Kodiakanal (Tamil Nadu), India and by XIONG *et al.* (1995) from China. Present cytological investigations in the two wild accessions from the Parvati Valley in Kullu and one accession from the Pangli Valley in Chamba district of Himachal Pradesh revealed that these accessions uniformly existed at diploid level with $n=9$. This can be confirmed from the presence of 9 large sized bivalents at late prophase-I and metaphase-I (Figs. 1a & 1b) and 9:9 chromosomes distribution at anaphase-I (Fig. 1c). Out of these, 2-3 bivalents are observed to be of ring type with two chiasmata and 6-7 are rod type with only one chiasma. The chiasma frequency per PMCs in the two accessions (with normal meiosis) on the basis of analysis of 179 and 195 pollen mother cells (PMCs) works to 10.75 and 10.78, respectively. The frequency of

Table 1. Data on chromosomal associations at diakinesis/metaphase-1 in wild accession of *Artemisia parviflora*. (PMCs=pollen mother cells; C=chain; R=ring)

Serial Number	Number of PMCs analysed	Chromosomal associations at diakinesis/metaphase-1					
		VI	V	IV	III	II	I
1.	8	--	--	--	1	7	1
2.	79	--	--	--	--	9	--
3.	81	--	--	1(C)	--	7	--
4.	25	--	--	--	--	8	2
5.	2	1 (C)	--	--	--	6	--
6.	2	--	--	1(R)+1(C)	--	5	--
7.	7	1(C)	--	--	--	5	2
8.	9	--	--	--	2	5	2
Total PMCs	213	9	--	85	26	1636	90
% age of chromosomes involved		1.41	--	8.87	2.03	85.34	2.35

chiasma per chromosome in these accessions is calculated to be 0.597 and 0.599. In spite of the early/late disjunction of 1-2 bivalents, anaphases/telophases are perfectly regular resulting into normal micro-sporogenesis and almost a hundred percent pollen fertility (99%). However, the accession studied from the Parvati Valley (Barsani 2200 m) showed abnormal male meiosis characterized by the presence of multivalents (trivalents, quadrivalents and hexavalents) and univalents at diakinesis/metaphase-I in 62.92 % of the observed PMCs (Table 1). Analysis of 213 PMCs revealed that 12.31% of the chromosomes are involved in the multivalent formation (ring or chain type) and 2.35 % of the chromosome remained as univalents. The maximum frequency of PMCs (8.87%) is recorded to be with one quadrivalent (Chain type) and 7 bivalents (Fig. 1d). PMCs with two quadrivalents (ring and chain type) are also existent (Figs. 1e & 1f). The hexavalents involving six chromosomes are always of chain type and are observed in only 9 PMCs (Fig. 1g). Trivalents (1-2) are observed in 17 PMCs and are always associated with 1-2 univalents. Due to early disjunction of one bivalent, 2 univalents are also recorded in 25 PMCs. The chiasma frequency in this accession with structural heterozygosity per PMC ranges between 12-14. Similarly, the chiasma frequency per chromosome works to be little higher (0.688) than the normal plant. In spite of the presence of multivalent and early disjunction and late disjunction of 1-2 bivalents (Fig. 1h), the chromosomes in majority of the PMCs in this accession are regularly segregated to opposite poles at A-I/A-II (Fig. 1c) resulting into normal tetrad formation. However, these structural rearrangements of chromosomes in this accession resulted into some pollen sterility (30%). CARR & CARR (1983, 2000) and LEVIN (2002) have also reported that chromosomal rearrangements are responsible for reduced pollen fertility in plants. This is the first record of structural heterozygosity in *A. parviflora*, the species which has been reported earlier by different workers to show normal meiosis. Such an existence of natural heterozygosity due to reciprocal translocations has also been reported in species of *Chrysanthemum* viz. *C. boreale*, *C. carinatum*, *C. coronarium*, *C. sagetum*, *C. indicum*, *C. zawadskii* (JAIN & GUPTA 1960; MEHRA & REMANANDAN 1974; GILL & GUPTA 1981; KIM *et al.* 2008), *Calycadenia ciliosa* and *C. pauciflora* (CARR & CARR 1983, 2000).

Acknowledgements – The authors are grateful to the University Grants Commission, New Delhi for providing financial assistance under the DRS SAP I and II and ASIST programme and CSIR for providing Senior Research Fellowship to Mr. Puneet Kumar. Thanks are also due to the Head, Department of Botany for necessary laboratory and library facilities.



Figures (1a-h). Meiosis in *Artemisia parviflora*:-

- a & b) PMCs at late prophase-I and metaphase-I showing 9 large sized bivalents.
- c) A PMC showing regular 9:9 chromosomes distribution at anaphase-I.
- d) A PMC showing one chain type quadrivalent (arrowed) and 7 bivalents.
- e) A PMC with one ring type (arrowhead) and one chain type quadrivalent (arrowed) at metaphase-I stage.
- f) A PMC at metaphase-I with two chain type quadrivalent (arrowed).
- g) A PMC with chain type hexavalent (arrowed) at metaphase-I.
- h) A PMC showing early disjunction (arrow head) and late disjunction (arrowed) of bivalents.

REFERENCES

- CARR GD & CARR RL. 2000. A new chromosome race of *Calycadenia pauciflora* (Asteraceae: Heliantheae-Madiinae) from Butte County, California. *Amer. J. Bot.* **87**: 1459–1465.
- CARR RL & CARR GD. 1983. Chromosome races and structural heterozygosity in *Calycadenia ciliosa* Greene (Asteraceae). *Amer. J. Bot.* **70**: 744–755.
- GILL BS & GUPTA RC. 1981. Structural hybridity in *Chrysanthemum coronarium* L. *Perspect. Cytol. Genet.* **3**: 523–530.
- GUPTA RC & GILL BS. 1989. Cytopalynology of north and central Indian Compositae. *J. Cytol. Genet.* **24**: 96–105.
- HAJRA PK, RAO RR, SINGH DK & UNIYAL BP. (eds.) 1995. Flora of India. Asteraceae (Anthemideae-Heliantheae), Vol. 12, BSI, Calcutta.
- JAIN HK & GUPTA SB. 1960. Genetic nature of self-incompatibility in annual *Chrysanthemum*. *Experientia* **8**: 364–365.
- JAIN MP, THAKUR RS & RAO PR. 1974. Coumarins of *Artemisia parviflora* Roxb. *Curr. Sci.* **40**: 640.
- KAUL MK & BAKSHI SK. 1984. Studies on the genus *Artemisia* L. in north-west Himalaya with particular reference to Kashmir. *Folia Geobot. Phytotax.* **19**: 299–316.
- KIM JS, OGINUMA K & TOBE H. 2008. Analysis of meiotic chromosome behaviour in diploid individuals of *Chrysanthemum zawadskii* and related species (Asteraceae): evidence for chromosome rearrangements. *Cytologia* **73**: 425–435.
- LEVIN DA 2002. The Role of Chromosomal Changes in Plant Evolution. Oxford Univ. Press, Oxford.
- MATHEW A & MATHEW PM. 1987. Cytological studies on the South Indian Compositae. In: NAIR PKK (ed.). Glimpses in Plant Research, vol. XI. Cytological Research Monographs. Today and Tomorrows Printers and Publishers, New Delhi, pp. 1–177.
- MEHRA PN & REMANANDAN P. 1969. In IOPB chromosome number reports. XXII. *Taxon* **18**: 433–442.
- MEHRA PN & REMANANDAN P. 1974. Cytological investigations on Indian Compositae. II. Tribes Asterae, Heliantheae, Helineae and Anthemideae. *Caryologia* **27**: 255–284.
- RANA VS, JUYAL JP, AMPARO BLAZQUEZ M & BODAKHE SH. 2003. Essential oil composition of *Artemisia parviflora* aerial parts. *Flav. Frag. J.* **18**: 342–344.
- XIONG X, LING Y-R & JIANG L. 1995. Studies on the chromosome numbers and karyotypes of six species of *Artemisia* (Compositae). *J. Trop. Subtrop. Bot.* **3**: 23–29.

REZIME

Prvi nalaz heterozigotnosti kod *Artemisia parviflora* (Asteraceae) iz doline Parvati (Kullu, Himašal Pradeš, Indija).

Raghibir Chand GUPTA, Himshikha Pawan Kumar RANA, Puneet KUMAR and Vijay Kumar SINGHAL

Postojanje strukturne heterozigotnosti za recipročnu translokaciju kod divljeg diploida ($n=9$) *Artemisia parviflora* iz doline Parvati u oblasti Kulu, Himašal Pradeš u Indiju zabeleženo je po prvi put. Druge dve probe iz doline Pangi u oblasti Čambi i Pulge (2300m) u dolini Parvati imale su normalnu mejozu sa prisustvom devet bivalenata, segregacijom hromozoma za vreme anafaze i gotovo 100 procentnu fertilitnost polena. Detaljno su studirane mejoze polenske majke ćelije (PMC) i polenska fertilitnost kod uzoraka koje su pokazale strukturnu heterozigotnost. PMC su pokazale abnormalnu mejozu na osnovu prisustva multivalenata kod 62.92% analiziranih PMC. Kod ukupno 12.31% posmatranih hromozoma uočeni su multivalenti a kod 2.35% univalenti. Uprkos prisustva multivalenata i rane-kasne disjunkcije kod nekoliko bivalenata, hromozomi kod PMC normalno imaju normalnu segregaciju ka polovima na A-I/A-II što je rezultovalo obrazovanjem normalnih tetraida. Strukturni rearanžman hromozoma kod *A. parviflora* je uzrok polenske malformacije (30%) što je slučaj i kod drugih vrsta. Drugi uočeni efekat strukturne heterozigotnosti je povećani broj hijazmi po PMC za 10.75–12.40

Ključne reči: *Artemisia parviflora*, frekvencija hijazma, multivalenti, recipročna translokacija, strukturna heterozigotnost.