

## GENETIC SYSTEMS IN *ARTEMISIA* L. I: *ARTEMISIA TOURNEFORTIANA*, A SPECIES WITH HIGH SEXUAL REPRODUCTIVE EFFICIENCY

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**Abstract:** Present communication encompasses detailed studies on genetic system of *Artemisia tournefortiana* Reichb., (F. Asteraceae) sprawling at Rumtse, Khardong and Kharu areas of Ladakh region of Jammu & Kashmir, India. Species has high sexual reproductive efficiency and exhibits a stable genetic system with diploid chromosome constitution and high pollen stainability resulting in good seed set averaging  $60.61 \pm 1.55$  on open pollination. Plants of the species studied by us are based on  $x=9$  and invariably exhibit  $2n=18$  as their chromosome number. Somatic analysis reveals presence of 16M and 2 SM chromosomes.

**Keywords:** *Artemisia*, Chromosome, Genetic system, Reproductive efficiency, Somatic analysis

### INTRODUCTION

Genus *Artemisia* L. is the major representative of tribe Anthemideae of family Asteraceae, the largest family of Angiosperms (Ling 1991 a and b, Bremer and Humphries, 1993). Species of the genus occupy a variety of habitats and are medicinally important (Valecha *et al.* 1994, Chanphen *et al.* 1998). Genus is famous for the drug Artemisinin isolated from *A. annua* which is highly effective in its action on malarial parasite. Different species are known to have a hepatoprotective activity and have been traditionally used against liver damage (Zargari, 1990, Janbaz & Gilani, 1995, Singh *et al.* 2009, Ene *et al.* 2009). Genus is reported to have originated in Central Asia from where it is speculated to have migrated to India. In India, a total of 45 species are on record Karthikeyan *et al.* (2009).

A major chunk of these species, 20 in number inhabit the Himalayan belt mainly the state of Jammu and Kashmir (Sharma & Kachoo, 1981, Kaul & Bakshi, 1984). Many of these species are reported to form distinct populations at varied altitudinal ranges; few are however, restricted in their distribution. These include *A. maritima* L., *A. glauca* Pall. ex Willd., *A. vestita* Wall., *A. sieversiana* Ehrh. ex Willd., *A. parviflora* Buch.-Ham. ex Roxb., *A. gmelini* L. and *A. tournefortiana* Reichb.

*Artemisia tournefortiana* Reichb., has been reported to occur in Afghanistan, Armenia and Iran (Podlech & Dieterla, 1969, Podlech & Bader, 1974, Gabrielian & Xirau, 1996, Valles, 1987a). Its previous reports of occurrence in India include Western Himalayan regions including area of Jammu & Kashmir state, India (Kaul & Bakshi, 1984). This study is based on populations of *A. tournefortiana* of Ladakh region of Jammu and Kashmir, India at an altitudinal range of 3558–3805 masl. It attempts to probe the genetic system of this species and compare it to the widely distributed ones.

### MATERIAL AND METHOD

Present work is based on natural populations of *Artemisia tournefortiana* Reichb. of family Asteraceae. Different populations of this species were tagged and scanned at Rumtse, Khardong and Kharu areas of Ladakh region of Jammu & Kashmir, India at an altitudinal range of 3358 to 3805masl.

**1. Morphology:-** Plants and flowers were studied for vegetative and floral morphology. Data was collected on various aspects like height of the plant, shape, size of leaves, length of inflorescence, number of disc and ray florets per inflorescence in the field itself. Floral structure with emphasis on reproductive parts was studied in the laboratory under a stereo-microscope.

**2. Cytology:-** For karyology, seeds of *Artemisia tournefortiana* were germinated on moist filter paper lined petriplates. Seedlings with 4-5mm long root tips were washed with water before pretreatment with saturated aqueous solution of p-dichlorobenzene for 3-4 hours at 4°C. The pretreated seedlings were washed and fixed in Carnoy's fixative (three parts of ethyl alcohol and one part of acetic acid). After fixation for 24 hours, these were washed in water and preserved in 70% alcohol. For preparation of metaphase spreads, root tips from these seedlings were hydrolysed in a mixture of 1% Aceto-orcein and 1N HCl (9:1) and placed in an oven maintained at 60°C for 13 minutes. Finally, the root tips were squashed in 1% propiocarmine. Battaglia's (1955) scheme was used for the classification of somatic chromosomes.

Pollen mother cell meiosis was studied from young immature flower buds fixed during morning hours (8-9a m) in a mixture of three parts of ethyl alcohol and one part of acetic acid. After fixation for 24 hours, the buds were washed in water and preserved in 70% ethyl alcohol at 4-6°C. Finally, the anthers were squashed in 1% propiocarmine. Chromosome preparations both (mitotic and meiotic) were made

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permanent by removing the paraffin ring and inverting the slides in a petridish containing 1:1 mixture of n- butyl alcohol and acetic acid. Both the slides and coverslip were then transferred to a petridish containing n- butyl alcohol. The slides were removed after 2-3 minutes and coverglass was restored using euparol.

All photomicrography of chromosomal preparations was done using unit-Nikon ECLIPSE E 400 attached to a digital color camera SAMSUNG SDS-312. The field photography was done with the camera SONY DSC-H10. The bar on each photomicrograph represent 10 $\mu$ m.

**3. Pollen stainability and viability:-** Pollen stainability and viability were determined by :-

**(a) Stainability test in 1% acetocarmine:-** Mature but undehisced anthers were squashed in 1% acetocarmine. All deeply stained pollen grains were considered to be fertile.

**(b) Fluorochromatic Reactions (F.C.R) test: -** Pollen grains from ready to dehisce anthers were squashed in a drop of saturated solution of fluorescein diacetate in acetone, and observed under fluorescence microscope. Pollen grains that fluoresce in UV-light were considered as viable and those that were deformed and did not fluoresce were considered as non-viable.

Percent viability of pollen grains was calculated as:-

%age viability =

No. of viable pollen grains  $\times$  100

No. of viable pollen grains +

No. of non- viable pollen grains

**4. Fruit and Seed Set:-** Fruit and seed set were observed in the plants growing open in the fields as well as in bagged inflorescences. Number of flowers /capitulum, number of fruits/ capitulum were counted for these plants.

Percent fruit and seed set both on open pollination and bagging was calculated as:-

%age fruit set/inflorescence

= Fruit count/inflorescence  $\times$  100

Flower count of the same inflorescence

**5. Sexual Reproductive Output:-** Number of seed producing florets per inflorescence were counted and SRE was calculated using the formula:

Sexual Reproductive Output = Number of seed producing florets in a capitulum  $\times$  percentage of healthy seed set  $\times$  number of capitula/plant

100

## RESULT AND DISCUSSION

Plants of *A. tournefortiana* are small sized annual shrubs which sprout from seeds in the month of March – April as snow melts in the area of study and temperature averages 15-20 °C. Aerial offshoots are produced, that reach an average height of 51.9cm

after a brisk vegetative phase of 4-5 months (Fig. 1a). The plants are highly branched with the branches bearing leaves which are broadly ovate with pinnatisect segments. Flowering initiates in mid-July and seed set occurs in 1st week of October. The temperature during the flowering months fluctuates between 25-35°C. The relative humidity averages 46-54% in the area during this period. The plants bear flowers in axillary spikes terminating into heads at terminal one-third to one –fourth of the shoot. The average size of individual capitulum is 3.06cm  $\times$  4cm (3 $\times$ 4-3 $\times$ 4.5) cm. Number of inflorescences per branch is high and averages 97.65. Number of branches per plant averages 7.8 $\pm$ 0.87.

Each capitulum is heterogamous consisting of central hermaphrodite disc florets and peripheral ray florets (Fig. 1b). The plants are thus gynomonoecious. Number of disc and ray florets per inflorescence averages 102 and 16 respectively. Each disc floret consists of five syngenesious stamens and a pistil with discoid stigma, elongated style and an ovary (Fig. 1c). The ray florets are pistillate (Fig. 1d).

Meiotic studies revealed the diploid chromosome number as 2n=18 in this species. A total of 191 cells were scanned for pmc meiosis, out of these, 36 cells (18.84%) at diplotene (Fig. 2a), 43 cells (22.51%) at diakinesis (Fig. 2b and c) and 69 cells (36.12%) at metaphase –I showed the presence of 9 perfect bivalents (Fig. 2d-f). At metaphase, both ring and rod shaped bivalents were observed. Chiasmata frequency per cell at diplotene averaged 15.86. Recombination index thus averages 24.68. Segregation of chromosomes was regular in 35 cells (18.32%) observed at Anaphase –I (Fig. 2g) while laggards were observed in 8 cells. Average pollen production per floret averages 6494.84 per floret and thus an average of 6,62,473.68 pollen grains are produced per inflorescence. Pollen stainability and viability averaged 94.29% and 89.64% respectively. Fruit is a cypsela as in other composit. It is dry, indehiscent, one seeded fruit developed from a bicarpellary, syncarpous, inferior, unilocular ovary. Seeds are ridged brown, 1-2 mm long. Percentage of healthy seeds formed in open and bagged inflorescences averages 60.61 $\pm$ 1.55 (45.9–75.5) and 1.02 $\pm$ 0.71 (0–6.7) respectively. Healthy seeds show good viability which could be observed from the germination tests carried out in the lab, where these showed 74.58% germination at a temperature of 25°C.

Somatic complement of the species as analysed from the root tip cells revealed the diploid chromosome number as 2n=18 with 9 pairs of chromosomes (Fig. 2h and i). The karyotype formula for the species was thus 16M+2SM. The presence of a large number of metacentric chromosomes as compared to sub metacentric chromosomes states its symmetrical

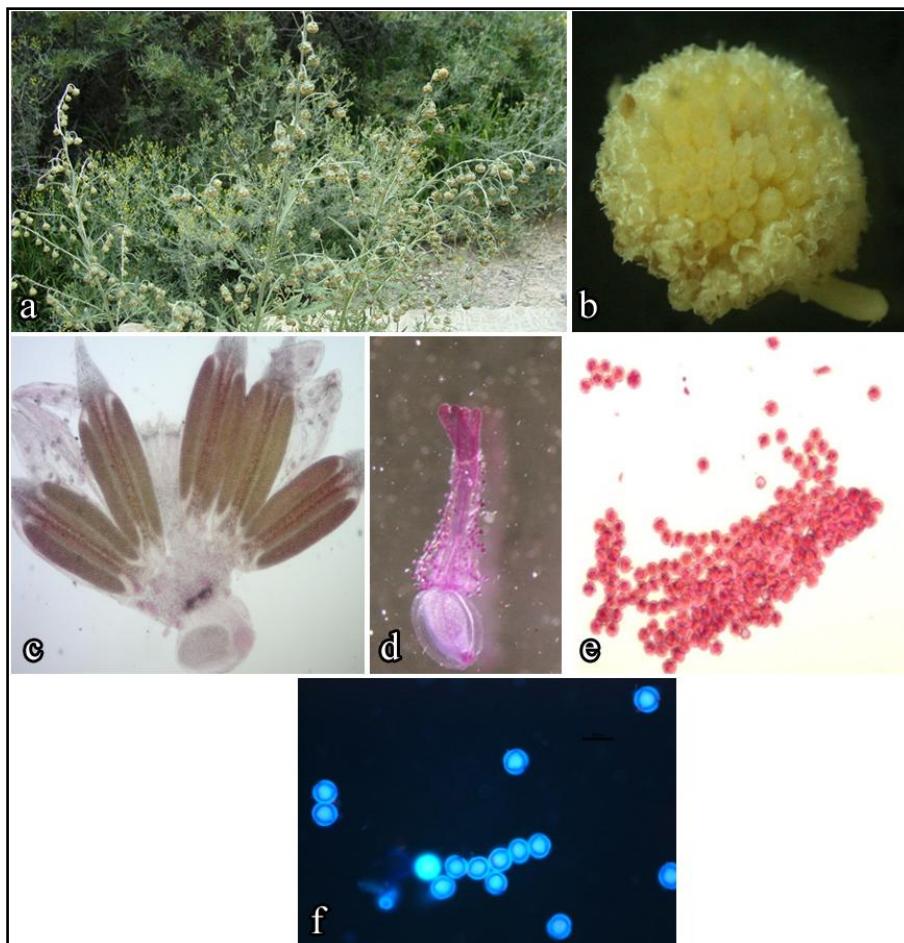
nature. The present chromosome report reveals *Artemisia tournefortiana* to be diploid species based upon  $x=9$ , the most common base number in the genus *Artemisia* (Valles & Garnatje, 2005, Chehregani & Mehanfar, 2008, Chehregani & Hajisadeghian, 2009). Unlike most of the *Artemisia* species scanned till date (Jabeen *et al.*, 2012, Mir *et al.*, 2013, Sharma *et al.*, 2015), *A. tournefortiana* is cytologically highly stable.

## CONCLUSION

**Table 1.** Morphometric details of *Artemisia tournefortiana*

S.No.	Character	Average
1	Height of plant (cm)	51.9* $\pm$ 3.7
2	Size of capitulum (mm)	3.7 $\pm$ 0.1
3	No. of disc florets per capitulum(mm)	99.6 $\pm$ 7.5
4	No. of ray florets per capitulum(mm)	15.6 $\pm$ 1.2
5	Size of disc floret(mm)	2.4 $\pm$ 0.05
6	Size of ray floret(mm)	2.2 $\pm$ 0.3
7	Size of anther(mm)	1.1 $\pm$ 0.01
8	Size of pistil of disc floret(mm)	2.2 $\pm$ 0.3
9	Size of pistil of ray floret(mm)	2.4 $\pm$ 0.05
<b>*Average</b>		

## LEGENDS

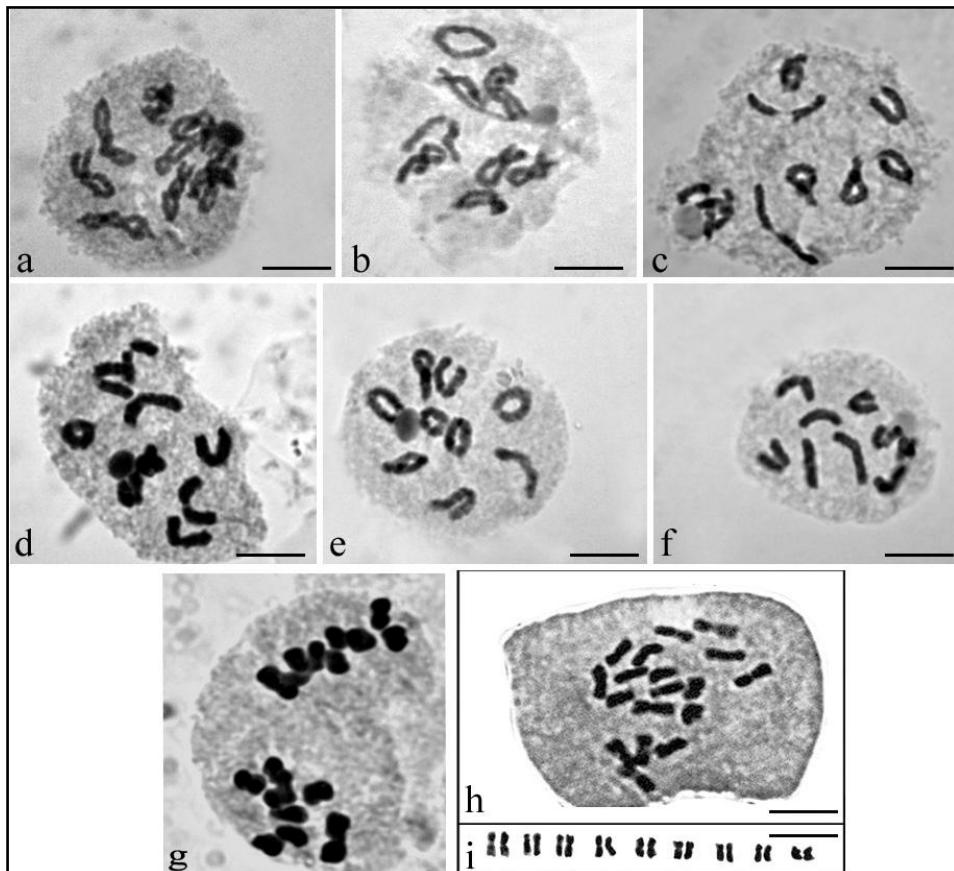


**Fig.1.** *Artemisia tournefortiana* Reichb.

The annual species has acclimatized itself well in the harsh weather of Leh by being highly efficient sexually in view of

- (a) its stable cytological nature unlike most of *Artemisia* species scanned till date.
- (b) high investment in sexual reproduction leading to production of an average of 46,164.81 healthy seeds/plant. This is huge when compared to cytologically unstable species like *A. nilagirica*, where this value comes out to be 22,215.6. (per observations; unpublished data).

- (a) Plant growing in its natural habitat
- (b) A capitulum
- (c) An opened hermaphrodite disc floret
- (d) A pistillate ray floret
- (e) Pollen grains stained in 1% Acetocarmine
- (f) Pollen grains treated with FDA



**Fig. 2.** Pmc's of *Artemisia tournefortiana* Reichb.

- (a) Diplotene
- (b) (b-c) Diakinesis
- (c) (d-f) Metaphase -I
- (d) Anaphase-I
- (e) Metaphase spread in root tip cells
- (f) Karyodiagram

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