Original Article

Bulletin of Pure and Applied Sciences.
Vol.36 B (Botany), Issue (No.1) 2017
Print version ISSN 0970 4612
Online version ISSN 2320 3196
DOI 10.5958/2320-3196.2017.00002.7

SEXUAL REPRODUCTIVE EFFICIENCY OF *ARTEMISIA SIEVERSIANA* ABOUNDING LADAKH (TRANS-HIMALAYAN) REGION IN INDIA

Rinchen Gurmet*, Gazala J Mir*, Uma Bharti*, and Namrata Sharma*

Author's Affiliation: *Department of Botany, University of Jammu, Jammu 180006, Jammu and Kashmir, India

Corresponding Author: Rinchen Gurmet
Department of Botany,
University of Jammu,
Jammu 180006, Jammu and Kashmir, India.
Email:
rinzmailbox@rediffmail.com
Received on 15.02.2017,
Accepted on 26.04.2017

Abstract
Artemisia sieversiana Ehrh. ex Willd. is an annual temperate species growing in the Himalayan belt including the state of Jammu and Kashmir, India. The wild populations of the species occurring at high altudunal ranges of Ladakh region of the state have been analysed for their cytological details and reproductive efficiency. Plant have a somatic chromosome count of 2n = 18 revealing it to be a diploid species based on x = 9, the most common base number in genus *Artemisia* L. Meiotic events are normal leading to high pollen viability. The species exhibits high sexual reproductive efficiency with number of florets per inflorescence averaging 121.9 which is much higher than the number reported for most of the *Artemisia* species including the one under discussion. The standard cytological composition leads to stable genetic system which impacts the reproductive efficiency of the species. This stable genetic system along with large pollen production and high seed set depicts the stable nature of the species.

Keywords: *Artemisia sieversiana*; stable genetic system; diploid species; Ladakh; reproductive efficiency.

INTRODUCTION

Artemisia L. is an eminent wind pollinated, cosmopolitan genus concentrated mainly in semi arid to arid climate areas of temperate regions of mid to high latitude of the northern hemisphere (Valles & McArthur 2001). With more than 500 species, it is among the largest and economically important genera of family Asteraceae (McArthur & Plumer 1978, Mabberley 1990 Ling 1995 a,b, Kadereit & Jeffrey 2007, Funk et al.2009,Jabeen et al. 2012, Mir et al. 2015,Sotoodeh 2015). Almost every species of the genus *Artemisia* finds use as a source of medicine, food, forage or other useful products in one way or other. Numerous karyological surveys are available for this genus, (Kawatani & Ohno 1964, Torrell et al. 1999,

Valles & McArthur 2001,Rabiei et al. 2003,Pellicer et al. 2008, Atri et al. 2009, Chehregani & Hajisadeghian 2009, Park et al. 2009,Abdolkarim et al. 2010, Chehreghani et al. 2010, Zhen et al. 2010, Bala et al. 2012, Jabeen et al. 2012,Gupta et al. 2014, Sharma et al. 2014, Mir et al. 2015) revealing it to be highly unstable cytologically with most of the species exhibiting polyploidy and even new base numbers.

In our attempt to analyse the cytological status of the taxa abounding different altitudinal ranges in our state, we made studies on taxa forming distinct populations in different regions. Interestingly, species occurring in Ladakh region (Kharu, Sakti and Hemis) turned out to be most stable cytologically, with all the populations displaying diploid chromosome numbers only. Herein we report the details of our studies on *A sieversiana* Ehrh. ex Willd. a dominant species of this area and accordingly propounds why this species with stable genetic system guides for a better sexual reproductive efficiency.

A. sieversiana, an annual, temperate species is reported abound to the Himalayan belt including the state of Jammu and Kashmir and Himachal Pradesh, where it is found growing on marshy, sandy soil at 2500–3500masl (Shah 2014). In other parts of the world it is distributed in Pakistan, West Tibet, China westward to S. Russia (Hazra et al. 1995). The species is esteemed as a tonic, deobstruent, febrifugal, antihelminthic and applied externally as a discutient and as an antiseptic (Bal 1932). The decoction of leaves and flowers of this species acts as wormicide and it is also a source of 'siersin' and 'sieversinin' having antimicrobial properties (Nazarenko and Leont'eva 1966). The chemical composition of *A. sieversiana* essential oil has also been studied (Suleimenov et al. 2009).

This research was initiated to determine the different aspects of reproduction mechanism and cytological constituents of *A. sieversiana* growing in the Ladakh (trans –Himalayan) region in India. The results obtained from the study will provide the general description of the species, its cytological complements of stable genetic class rendering for a sexual reproductive efficiency and in addition, throws a limelight of its position in the perpetual theory of migration of the genus from its place of origin.

MATERIAL AND METHODS

Study area and the Plant species

The present study is based on the plants growing in Leh district of Ladakh. The wild populations of the species were tagged at Sakti, Kharoo and Hemis regions in Leh at altitudinal ranges of 3258–3835m asl (Table 1). The region is characterized by harsh climatic conditions and very complex soil formation patterns. The plants inhabit rocky areas and dry slopes of these regions.

Plants of *A. sieversiana* were tagged at hill slopes and valleys of Kharoo (Fig. 2A), Sakti and Hemis in Ladakh region of Jammu & Kashmir state (Fig. 1A,B). The plants were identified using regional floras, papers on local floras and voucher specimens from herbarium of Botanical Survey of India: Dehradun, Indian Institute of Integrative Medicine: Jammu and Defence Institute of High Altitudinal Research: Leh.

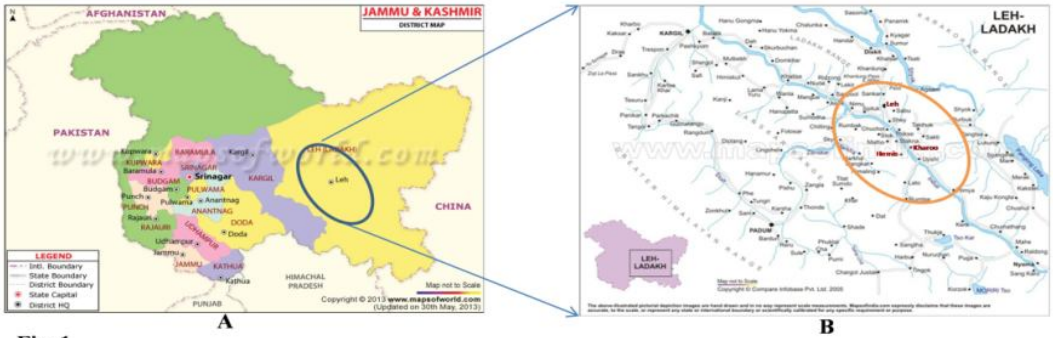

Fig. 1

Fig 1: Map of Jammu and Kashmir state in India (A), Study sites in the area of Ladakh region from where plants of *Artemisia sieversiana* have been assessed.

Table 1: Population of *Artemisia sieversiana* assessed at different sites of Ladakh region in Jammu and Kashmir state in India.

Site	Population	Latitude	Longitude	Elevation (masl)	No. of Plants Assessed
1	Kharoo	33°54.590´	077°41.729´	3258	60
2	Hemis	33°54.590´	077°41.729´	3835	50
3	Sakti	33°55.275´	077°44.041´	3780	50

Floral phenology and reproductive efficiency

Details on vegetative and morphological features like plant height, leaf number per plant, size of the leaf, number of inflorescence per plant, inflorescence size and number of flower per inflorescence were collected from the plants growing in the field. Floral structure with emphasis on reproductive apparatus, number of florets per capitulum was studied in the lab. All these measurements were carried out using a scale and/or stage micrometer. Observations on anthesis and anther dehiscence were made in the field at regular interval. Inflorescences and flowers were regularly monitored throughout the day to record the time taken by an individual flower, inflorescence and the full plant to bloom.

Pollen stainability and viability were determined by subjecting the discharged pollen to 1% acetocarmine stainability test (Sharma et al. 2014). Ovules were counted by carefully dissecting out the individual ovaries with the help of fine needles. Pollen–ovule ratio was calculated by dividing the pollen count with the number of ovules in the same capitulum. Stigma receptivity was checked from the stigma of different size fixed in a mixture of three parts of absolute alcohol and one part of acetic acid for 2–3 hours. These were later washed in distilled water, stained in Lewis stain and mounted in lactophenol (mixture consisting of lactic acid, distilled water, glycerine and phenol in 1:1:1:1 ratio) (Mir et al. 2015). Then the slides were scanned under the microscope to record the number of pollen grains attached to the stigmatic surface and the number of pollen grains germinating on it. Hanging slide method was employed for conformation the role of wind in pollination (Sharma et al. 2014). The plants growing open in the field were observed for fruit and seed set. Percentage fruit and seed set was calculated using equation:

(Total number of seed set per capitulum / Total number of florets per capitulaum) x 100

Cytology

Pmc meiosis was studied from young immature buds, fixed during morning hours in a mixture of 3 parts of ethyl alcohol and 1 part of acetic acid. After 24 hrs of fixation, the buds were washed in water and preserved in 70 % ethyl alcohol at 4–6 °C. Finally the panthers were squashed in 1% propiocarmine (Sharma et al. 2015). All the studies were made on the freshly prepared slides.

Karyotype analysis

Metaphase spreads were prepared from the young root tips obtained from the germinating seeds placed in a petriplate containing moist filter paper. 2–3 cm long root tips were initially washed with water and pretreated in a saturated solution of p–dichlorobenzene for three hours (Sharma et al. 1992). The pretreated root tips were again washed with water and fixed in the mixture of three parts of ethyl alcohol and one part of acetic acid for 24 hours and stored in 70 % ethanol at low temperature. The stored root tips were then washed and stained in a mixture of 1 % acetoorcein and 1N HCl (9:1) for 13 minutes in an oven maintained at 60 °C. The stained root tips were subsequently squashed in 1 % propiocarmine (Jamwal and Sharma 2015).

All photomicrography of chromosomal preparations was done using unit–Nikon Eclipse E 400 attached to a digital color camera Samsung SDS–31.

RESULTS

Floral biology and reproductive efficiency

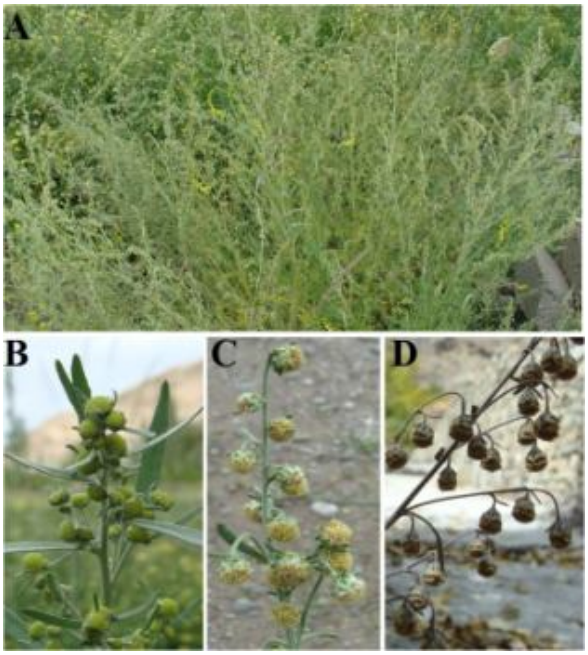

Fig. 2: Plants of *Artemisia sieversiana* in the fields in Kharoo region in Ladakh (A), Portion of twigs showing inflorescences at different stages of maturation (B-D).

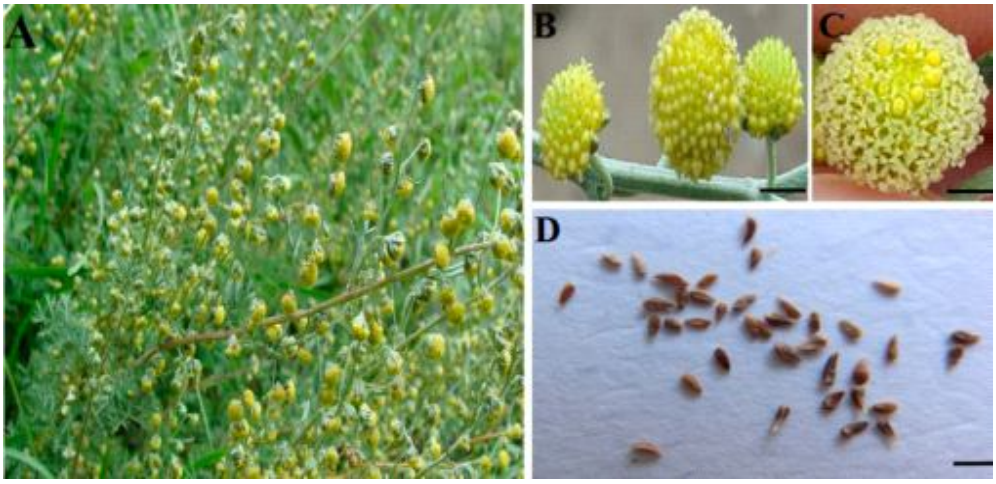

Fig. 3: *A. sieversiana*: Portion of plant with full bloom inflorescences (A), Floral head at different stages of maturation (B-C) and seeds (D). Scale bars: B, C = 1mm; D = 2mm.

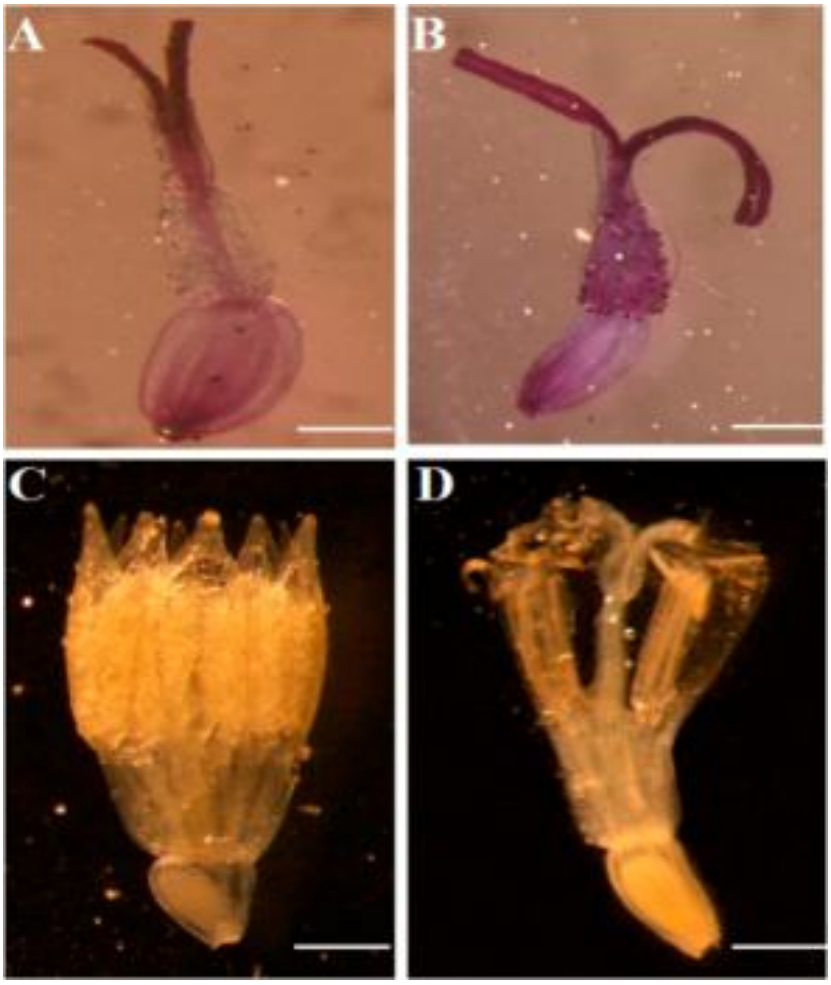

Fig. 4: Ray florets(A,B) and Disc florets (C,D) of *A. sieversiana*. Scale bars = 0.5mm

Plants of *Artemisia sieversiana* Ehrh. ex Willd. are strongly aromatic herbs. Stem is tall, ribbed and branched above. Leaves are petiolate, broadly ovate with pinnatisect segments and hairy on both the surfaces. Flowers are borne on hemispherical capitula arranged on a panicle with the lower portion of the stem turning woody with age (Fig. 2. B-D). The flower heads are pudunculateand are protected by involucralbracts, covering lower half of the heads, which are longand green in colour initially but turn brownish at maturity (Fig. 3.B,C). The average size of individual capitulum is 4.83 ± 0.02 × 4.5 ± 0.03 mms and its circumference averages 13.76 ± 0.11 mms. Each capitulum is heterogenous consisting of central hermaphrodite disc florets and peripheral female ray florets. Each disc floret bears five petals united to form a short cylindrical corolla tube that is usually pale yellow in colour. It encloses anthers that are syngenecious, dithecous, basifixed and introse. They are fused to form hollow cylinder which surrounds the pistil (Fig. 4. C, D). Ray florets bear filiform corollas that appear translucent enclosing a single pistil (Fig. 4. A, B). Pistil in both disc and ray florets is bicarpellary, syncarpous, inferior and unilocular with single ovule. Number of florets per capitulum is high averaging 121.9 with the disc florets outnumbering the ray florets (99.93: 21.97) in great numbers (Table 2).

Table 2: Morphometric parameters of capitula in three different populations of *Artemisia sieversiana*a bounding Ladakh region of Jammu and Kashmir, India

S. No.	Character	Population		
		Kharoo	**Hemis**	**Sakti**
1.	**No. of inflorescence per branchlet (n=30)**	9.9±0.45* (5–13)**	9.8±0.71 (6–17)	10.3±0.63 (6–16)
2.	**Size of inflorescence (mm) (n=30)**			
	Length	5.2±0.21 (3.5–6)	4.5±0.11 (3–5)	4.8±0.32 (3–5.5)
	Diameter	5.6±0.37 (3–6)	3.8±0.1 (3–5)	4.1±0.09 (3–5)
	Circumference	14.6±0.53 (12–16)	13.1±0.50 (10–17)	13.6±0.52 (9–19)
3.	**Total no. of florets per inflorescence (n=30)**	119.7±4.8 (98-146)	118.8±3.69 (96-133)	127.2±4.49 (107-143)
	Disc florets	97.9±4.49 (82-121)	96.6±2.85 (78-107)	105.3±4.41 (82-117)
	Ray florets	21.8±1.15 (17-29)	22.2±1.24 (16-27)	21.9±1.08 (15-28)

***Mean±standard error**
****Range**

Flowering starts in the month of July and remains till mid September (**Table 3**). Anthesis is marked by the opening of peripheral ray florets and the emergence of its style along with adpressed stigmatic lobes. After a gap of one or two days, the disc florets also initiate opening by the formation of small slit towards the apical portion of the corolla tube. Disc florets are protandrous with anther dehiscence preceding stigma receptivity by a minimum of three days. Pollination is mainly carried out by wind as no other source or regular insect visitor is found. Pollen production is high and the pollen ovule ratio was found to be 3164.8:1. Percentage viability of pollen grains by 1 % acetocarmine averages 86.08 ± 1.2 and by enzyme assay test using TTC (2,3,5 triphenyl tetrazolium chloride) averages 66.77 ± 0.11.

Table 3: Details of flowering period and anthesis of *Artemisia sieversiana* in different populations in Ladakh

S. No.	Character	Populations		
		Kharoo	**Hemis**	**Sakti**
1.	**Flowering period**	July to Mid - September	July to Mid-September	July to September
2.	**Onset of flowering**	July(3rd week)	July(3rd week)	July(4th week)
3.	**Days taken by an inflorescence to complete anthesis**	6-10	7-10	8-12
4.	**Days taken by a plant to complete anthesis**	25-30	25-30	25-35
5.	**Peak hours of anthesis**	1030-1300	1100-1400	1100-1400

Stigma receptivity in the species is retained for a long time. As the style grows out of the floral tube, the stigmatic lobes start opening and then divert to the opposite direction. These curvings expose the inner stigmatic portion (which is its receptive part) to the outside. These events take 2–3 days, thereafter the stigma both on ray and disc florets remains in the receptive condition for 8–10 days, then it dries up and shrivelles. Fruit set is complete in the month ofOctober. Fruit is a cypsela; a dry indehiscent, one- seeded fruit developed from a bicarpellary, syncarpous, inferior, unilocular ovary. Each fruit remains enclosed by persistent bracts. Data on fruit/ seed set in *Artemisia sieversiana* was collected from the entire three different populations Ladakh region. On open pollination both healthy and shriveled seeds are formed. Percentages of healthy seeds formed in the species are more or less similar in all these populations. The average fruit/seed set (Fig. 3. D) thus turns out to be 63.6 per capitulum with 43.3 percent of them being healthy. Bagging of single inflorescence per branch on different individual resulted in zero seed set; but when ten inflorescences were bagged together the seed set is very much reduced (25.7%) and all are shriveled, thus displaying inbreeding depression (Table 4).

Seeds take 5-6 days to germinate on moist filter paper in lab conditions. Germination of healthy seeds averaged 74% but shriveled seeds were unable to show any germination in lab. By the end of October and starting November, the seeds get dispersed and the above ground part of the plant dries up. During the winter months of December to March, when there is

heavy snowfall and temperature in the area drops to minimum of -12.4°C, the species survives and later propagates through seeds in the month of April when the snow melts.

Table 4: Reproductive output in open pollinated and bagged inflorescences in three different populations of *Artemisia sieversiana* growing in Ladakh.

S. No	Treatment	Percentage fruit/seed set in			Average
		Kharoo (3258masl)	**Hemis** (3835masl)	**Sakti** (3780masl)	
1	**Open pollination (n=30)**	67.7 (36.4 %healthy)	59.4 (52.5% healthy)	63.9 (41.1% healthy)	63.6% (43.3% healthy)
2	**Unassisted selfing**				
a.	**Single inflorescence bagged (n=25)**	Nil	nil	nil	Nil
b.	**10 inflorescences bagged together (n=25)**	32.5 (shriveled)	24.2 (shrivelled)	20.3 (shrivelled)	25.7% (all shrivelled)
3	**Percentage seed germination (n=25)**	78.8	65.4	77.9	74%

Cytology

During meiosis, pmcs of all the plants (n = 60) scanned from three diverse populations reveal the presence of 9 bivalents at diplotene and metaphase I (Fig. 5.A-C), thus, the diploid chromosome number of the species as 2n = 18. The chiasmata frequency for the pmcs observed at diplotene averages 14.3 per cell. At anaphase I, segregation of chromosome was found to be 9 chromosomes at each pole (Fig.5.D).

Bivalents of both ring and rod shaped were observed at diakinesis and metaphase I. Out of 100 cells scanned for meiosis, 21 at diplotene, 26 at diakinesis, 42 at metaphase I and 11 at anaphase I were observed. No chromosomal aberration during segregation was noticed in these plants.

Karyotype analysis

Somatic complement of the species was analysed from the root tip cells and the mean karyotype parameters were obtained. It reveals the diploid chromosome number as 2n = 18 forming 9 pairs of chromosomes (Fig. 5E, F).

The karyotype formula for the species is thus 2n = 2x = 18 (3M + 15SM). A pair of satellite chromosome was also observed. The individual chromosome length ranges from 2.37–4.21 µm and the total chromatin length is 61.05µm. The ratio of longest to shortest chromosome is 1.77µm.

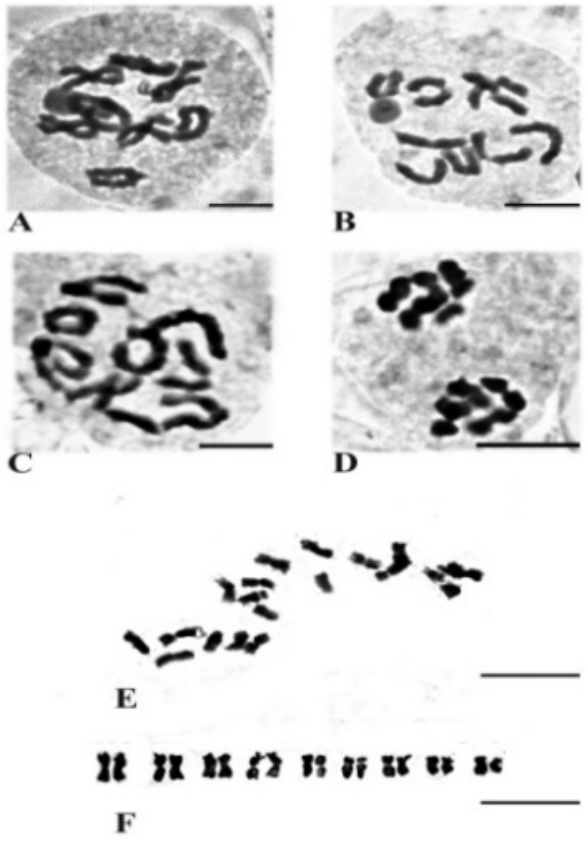

Fig. 5: *A. sieversiana*: Pollen mother cells at various stages of Meiosis I: Diplotene (A), Diakinesis (B), Metaphase (C) and Anaphase (D); Somatic complement (E) and its karyotype (F). Scale bars = 10µm.

DISCUSSION

Present study reveals *A.sieversiana* to be a stable diploid with high reproductive efficiency, a feature uncommon in genus *Artemisia*. Number of florets per capitulum is highest in this taxon of all the *Artemisia* species in which this trait has been studied. The species thus has potential of high sexual reproductive efficiency. Infact, plants of this species at Leh have much bigger capitula as compared to the ones reported from Kashmir valley of J&K, India (Kaul&Bakshi 1984: 50-93 florets). Other species of *Artemisia* possess comparatively lesser number of florets per inflorescence (Kaul&Bakshi 1984,Jabeen et al. 2012, Mir et al.2015, Sharma et al. 2015). A good number of viable pollen grains (86.08 %) and a well retaining time of stigma receptivity assure good fruit set on open pollination (63.6%) in *A. sieversiana*. Though moderate, this fruit set is much higher when compared to other *Artemisia* species (Penas et al. 2011, Sharma et al. 2014, Mir et al. 2015). This is important since all the florets in every capitula of *A. sieversiana* are capable of bearing seeds unlike species like *A. glauca* Pall ex Willd. and *A. scoparia* Waldst and Kit. (subgenus- Dracunculus) in which disc florets carry an aborted pistil (Mir et al. 2015; Sharma et al. 2015). Extremely low fruit set (14.60% - *A. glauca*) has been reported in these species.

Genus Artemisia is known to be unstable cytologically with species showing ploidy level even to the tune of 16x (Pellicer et al. 2007: *Artemisia medioxima,*). This flexible cytological status affects the reproductive efficiency, and may be the reason behind several species being poor seed setters. Present study reveals *A. sieversiana* to be a diploid species based on x = 9, the most common base number in genus *Artemisia* (Valles & Garnatje 2005, Chehregani & Mehanfar 2008, Chehregani & Hajisadeghian 2009). This report is in line with the previous reports of chromosome count in the same species from other parts of the world (Kawatani & Ohno 1964, Zhen et al. 2010).

Large number of florets, high pollen output with high pollen viability, stable genetic system and huge number of seed set on open pollination depicts the sound and stable nature of the species. The species is well adapted to the habitat, climatic conditions and flourishes well in the cold desert of Ladakh. Few other *Artemisia* species grow luxuriantly along with *A. sieversiana* in the area of study. Cytological studies of two species among them, namely *A. gmelinii* Weber ex Stechm. and *A. tournefortiana* Reichb. have been done by us (observation unpublished yet). Both of them also carry stable genetic system with 2n = 18. The significance of stable genetic system in *Artemisia* species in cool and high altitudinal areas of Ladakh and its relevance to its distribution and conservation are under investigation. An assumption can however be forwarded that *Artemisia* species acclimatized and restricted to Ladakh have stable genetic system while the ones in which further diversification and dispersal took place to other parts, cytological variability has been induced for acclimatization. Our work on *Artemisia* species inhabiting sub tropical and sub temperate regimes of J&K, India has depicted them to be highly variable cytologically (Jabeen et al. 2012, Sharma et al. 2014, Mir et al. 2015). This may be interpreted in light of dispersal of genus *Artemisia* as per Wang (2004). *Artemisia* species are speculated to have migrated out of northern Asia (the place of their origin) in three lines: (1) westward into Europe, western Asia, the Mediterranean and Africa, (2) eastward into Siberia, western North America and Eastern Europe and (3) further southward into Asia primarily during the Quaternary.

ACKNOWLEDGEMENTS

The authors are grateful to the Head, Department of Botany, University of Jammu, Jammu, for providing necessary laboratory facilities for carrying out the research work. Authors Rinchen Gurmet and Gazala J Mir are thankful to University Grant Commission for financial assistance as Basic Scientific Research Fellowship vide Order No. F.25-1/2013–14(BSR)/11–57/2007(BSR) dated 30/05/2014.

REFERENCES

1. Abdolkarim. C., M. Atri, S. Yousefi and F. Jalali. (2010). Polyploidy variation in some species of genus *Artemisia* (Asteraceae) in Iran. *Caryologia*63:168–175.
2. Atri, M., A. Chehregani, F.Jalali and Yousefi, S. (2009). New chromosome counts in some speciesof the genus *Artemisia* L. (Asteraceae) from Iran. *Cytologia*74: 443–448.
3. Bal, S.N. (1932). *Catalogue of medicinal plants exhibits*. The Industrial Section of the Indian Museum. Government of India Publication, Calcutta, pp. 1–20.
4. Bala, S., B. Kaushal. and Gupta, R.C. (2012). Effect of B-chromosome on meiotic behaviour and genetic recombination in *Artemisia nilagirica* (C. B. Clarke) Pamp. *Chromosome Botany* 7(1): 9–12.
5. Chehregani, A., M. Atri, S. Yousefi. and Jalali, F. (2010). Polyploidy variation in some species of thegenus *Artemisia* L. (Asteraceae) in Iran. *Caryologia*63: 168–175.
6. Chehregani, A. and Hajisadeghian, S. (2009). New chromosome counts in some species of Asteraceae from Iran. *Nordic Journal of Botany*27: 247–250.
7. Chehregani, A. and Mehanfar, N. (2008). New chromosome counts in the tribe Anthemideae (Astraceae) from Iran. *Cytologia*73: 1–8.

8. Funk, V.A., A. Susana, T.F. Steussy and Robinson, H.E. (2009). *Classification of Compositae,* in: Funk, V.A., A. Susanna, T.F. Stuessy, R.J. Bayer, (Eds.), *Systematics, evolution and biogeography of the Compositae*.: International Association for Plant Taxonomy, (IAPT). Vienna, Austria, pp. 171–189.
9. Gupta, R.C., H. Goyal and Singh, V. (2014). Cytology of the genus *Artemisia* (Anthemidae, Asteraceae) in the Western Himalayas. *Biologia*69: 1134–1141.
10. Hazra, P.K., R.R. Rao, D.K. Singh and Uniyal, B.P. (1995). *Flora of India Vol.12. Asteraceae (Anthemideae-Helmintheae)*. Botanical Survey of India, Calcutta.
11. Jabeen, N., U. Bharti and Sharma, N. (2012). Inter-population chromosome variation in *Artemisia nilagirica* L. *The Nucleus*55: 67–71.
12. Jamwal, M. and Sharma, N. (2015). Reproductive efficiency of two Luffa species- Factors affecting low reproductive rate in meiotically stable *Luffaacutangula* (L.) Roxb. *The Nucleus* 58(1): 59–65.
13. Kadereit, J.W. and Jeffrey, C. (2007). *Flowering plants. Eudicots. Asterales*,in: Kubitzki, K.(Ed.), The families and genera of vascular plants. Vol. VIII. Springer, pp. 131–139.
14. Kaul, M.K. and Bakshi, J.K. (1984). Studies on the genus *ArtemisiaL.* in North-West Himalayas with particular reference to Kashmir. *FoliaGeobotanica and Phytotaxonomica*19: 299–316.
15. Kawatani, T. and Ohno, T. (1964). Chromosome numbers in *Artemisia*. *Bulletin of the National Institute of Hygienic Sciences*82: 183–193.
16. Ling, Y.R. (1995).a*The New World Artemisia L.,* in: D.J.N. Hind, C. Jeffrey, G.V. Pope, (Eds.), Advances in Compositae systematics. Kew: Royal Botanic Gardens, pp. 255–281.
17. Ling, Y.R. (1995).b*The Old World Seriphidium (Besser) Fourr.,* in: D.J.N. Hind, C. Jeffrey, G.V. Pope, (Eds.), Advances in Compositae systematics. Kew: Royal Botanic Gardens, pp. 283–291.
18. Mabberley, D.J. (1990). *The plant-book.* Cambridge: Cambridge University Press, London, pp. 607.
19. McArthur, E.D. and Plummer. A.P. (1978). Biogeography and management of native western shrubs: a case study, section *Tridentatae* of *Artemisia*. *GreatBasin Naturalist Memoirs* 2: 229–243.
20. Mir, G.J., R. Gurmet and Sharma. N. (2015). Interpopulation variability in *Artemisia glauca* Pall. Ex Willd.- studies on three distinct cytotypes. *The Nucleus*58: 95–100.
21. Nazarenko, M.V. and Leont′evaL.I.. (1966). A new sesquiterpene γ-lactone, sieversinin. *Chemistry of Natural Compounds* 2: 327–330.
22. Park, M. S., J. Jang and Chung, G. Y. (2009). A taxonomic study of Korean *Artemisia* L. using somatic chromosome numbers. *Korean Journal of Plant Taxonomy39*: 247–253.
23. Pellicer J., S. Garcia, T. Garnatje, S. Dariimma, A.A. Korobkov and Vall`es, J. (2007). Chromosome numbers in some *Artemisia* (Asteracceae, Antehmideae) species and genome size variationin its subgenus *Dracunculus*: Karyological, systematicsand phylogenetic implications. *Chromosome Botany*2: 45–54.
24. Pellicer, J., S. Garcia, T. Garnatje, O. Hidalgo, S. Siljak-Yakovlev and Valles, J. (2008). Molecularcytogenetic characterization of some representatives of the subgenera *Artemisia* and *Absinthium* (genus *Artemisia,* Asteraceae).*Collectanea Botanica Barcelona*27: 19–27.
25. Peñas, J., J. Lorite, F.A. Sánchez and Taisma, M.A. (2011).Self-incompatibility, floral parameters, and pollen characterization in the narrow endemic and threatened species *Artemisia granatensis* (Asteraceae). *Anales del JardínBotánico de Madrid* 68(1): 97–105.
26. Rabiei, M., A. Jalili and Ghamari. Z.A. (2003). Studying on ploidy level of five *Artemisia* species from the north of Iran.*Plant Breeding and Genetic Resources*11: 429–441.
27. Shah, N.C. (2014). The Economic and Medicinal *Artemisia* species in India. *The Scitech, Journal of Science and Technology* 1: 29–38.
28. Sharma, I., U. Bharti, J. Parihar and Sharma, N. (2014). Sex-Expression and Reproductive Output in Three Species of *Artemisia* L. Abounding Jammu Province (J&K), India. *National Academy of Science Letters* 37(3):285–288.
29. Sharma, I., E. Sharma and Sharma, N. (2015). Resource allocation in relation to meiotic system in two species of Artemisia from North-West Himalayas. *Caryologia*68: 351–356.

30. Sharma, N., P. Koul, and Koul, A.K. (1992). Genetic systems of six species of *Plantago* (Plantiginaceae).*Plant Systematics and Evolution* 181(1):1–9.
31. Sotoodeh, A. (2015). *Biogeography and evolutionary history of two genera Verbascum and Artemisia in Iran, using molecular phylogeny.* PhdThesis, Université Paul Sabatier de Toulouse, Toulouse, 193 p.
32. Suleimenov, E.M., T. Ozek, F. Demirci, B. Demirci, K.H.C. Baser and Adekenov, S.M. (2009). Component composition of essential oils of *Artemisia lercheana* and *A. sieversiana* of the flora of Kazakhstan. Antimicrobial activity of *A. sieversiana* essential oil. *Chemistry of Natural Compounds* 45: 120–123.
33. Torrell, M., N. Garcia-Jacas, A. Susana and Valles, J. (1999). Infrageneric Phylogeny of the genus *Artemisia L. (Asteraceae, Anthemideae*) based onnucleotide sequences of nuclear ribosomal DNA internal transcribed spacers (ITS).*Taxon*48: 721–736.
34. Valles, J. and Garnatje, T. (2005). *Artemisia and its allies: genome organization and evolution and their biosystematics, taxonomical and phylogenetic implications in Artemisiinae and related subtribes (Asteraceae, Anthemideae)*. in: Sharma, A. (Ed.), Plant Genome: Biodiversity and Evolution. Phanerogams. Science Publishers. Enfield, New Hampshire, 1B, 255–285.
35. Valles, J. and McArthur, E.D. (2001). *Artemisia systematic and phylogeny: cytogenetic and molecular insights*, in: E.D McArthur, D.J. Fairbanks. (Eds.), Proceedings Shrub land ecosystem genetics and biodiversity. Provo, UT Ogden: US department of agriculture forest service, Rocky Mountain research station, pp. 67–74.
36. Wang, WM. (2004). On the origin and development of *Artemisia* (asteraceae) in the geological past. *Botanical Journal of the Linnean Society*145: 331–336.
37. Zhen, L., S. Chen, F. Chen, W. Fang, J. Li and Wang, H. (2010). Karyotype and meiotic analysis of five species in the genus *Artemisia*. *Caryologia*63: 382–290.