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Cytogenetics and reproductive output of two species of *Artemisia* L. inhabiting NW Himalayas

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ABSTRACT:

Present study is based upon two species of *Artemisia* i.e. *Artemisia nilagirica* (C.B.Clarke) Pamp. and *Artemisia scoparia* Walst & Kit. which form populations including a wide range of altitude (332-1705 masl) in regions which fall in lower Himalayan ranges of Jammu i.e. Jammu, Rajouri, Kud, Poonch and Bhaderwah. The two species differ with respect to their sex expression as well as meiotic system. Plants of *A. nilagirca* are gynomonoecious while that of *A. scoparia* are functionally monoecious. While *A. nilagirica* is highly complex cytologically and capable of generating variations both through its meiotic system as well as breeding system; all the populations *of A. scoparia* (Jammu, Rajouri and Kud) are cytologically stable diploids with 2n=16. Among five populations of *A. nilagirica* (Jammu, Rajouri, Kud, Poonch and Bhaderwah) investigated, four cytotypes could be identified (2n=18, 32, 34 and 54). Although the chromosomal changes have an effect upon the seed set in this species in the form of reduced percentage of healthy seeds; little amount of variability that survives and adapts is maintained and ramified asexually through rootstock. The presence of flexible meiotic system has helped *A. nilagirica* to expand its range and spread in diverse habitats.

Keywords: Artemisia, gynomonoecious, cytology, flexible, chromosome

I.INTRODUCTION:

Artemisia L. (known as Wormwood) is a huge genus of family Asteraceae and largest in the tribe Anthemideae comprising of more than 500 taxa at specific or sub-specific level (Mc Arthur, 1979; Mabberley, 1990; Ling 1991a, b, 1995a, b; Bremer and Humphries, 1993).

Taxonomically, it is considered as a complex genus since on one hand several species are reported to include different morphological forms, on other extreme, there is a close resemblance between different species posing difficulties in proper identification. Many taxonomic rearrangements have thus been carried out for this genus which has led to several infrageneric classifications. Genus *Artemisia* is distributed worldwide mainly across the temperate zones of Northern Hemisphere with some species reaching the Arctic, and a few species found even in the Southern Hemisphere (Bremer, 1994; Ling, 1994). The origin of *Artemisia* based on fossil data, is in semi-arid steppes of temperate Asia at mid - coenozoic i.e. about 20 million years ago (Wang, 2004). Genus is known to possess the ability to inhabit a wide range of ecosystems and environmental gradients as can be guessed from its distribution pattern and occurrence ranging from semi-deserts and deserts (*A. santolina* forming populations in Uzbekistan and Iranian deserts) to humid regions (*A. molinieri*, which is an endemic species of

SE France and *A. cana* sub-species *bolanderi*, endemic to Oregon area of United States, later is the only taxon in the genus to live in temporarily inundated soils); from near the sea level (*A. caerulescens* in European marine salt marshes to the hilly mountains at 4,000 masl (the Iranian, *A. melanolepis* and *A. pattersonii* and *A. scopulorum* inhabiting North America), some species are ruderal too (*A. vulgaris*) (Valles and Mc Arthur, 2001); water swamps also inhabit a few species of *Artemisia* (Kitamura, 2002). Most species of *Artemisia* grow sparsely and form small populations; however several other taxa form large populations and characterize landscapes. Species belonging to sub-genera *Seriphidium* (Mediterranean region, Central Asia) and *Tridentate* (North America) dominate the steppe and semi desert shrub communities.

Genus *Artemisia* has a long history of use in herbal medicine especially in matters connected to digestive system, menstrual complaints and the treatment of worms. Different parts of plants of various species are used as a multitude of medicinal agents (Miller, 2000). Two principal basic numbers x=8 and x=9, are reported for *Artemisia*; although some other numbers i.e. 7, 10, 13 and 17 are also on record for some species (Weins and Richter, 1966; Valles *et al.*, 2005; Chehregani and Hajisadeghian, 2009; Matoba and Uchiyama, 2009 and Park, 2009; Sharma *et al*., 2015). Of these two most common basic numbers, x=9 is much frequent. It is present in all the sub genera. Base number x=8, the less frequent is present in subgenera *Artemisia*, *Absinthium* and *Dracunculus* only.

II.MATERIAL

Present work is based on sprawling populations of four species of *Artemisia* namely, *Artemisia nilagirica* (C. B. Clarke) Pamp. and *Artemisia scoparia* Waldst & Kit. (F: Asteraceae). Different populations of these species were tagged and scanned at regions located at variable altitudes in Jammu region of Jammu and Kashmir .

Table 1: Location of different populations

A. nilagirica	**Altitude (masl)**	***A. scoparia***	**Altitude (masl)**
Jammu	332	Jammu	332
Rajouri	915	Rajouri	915
Poonch	1120	Kud	1705
Bhaderwah	1680		
Kud	1705		

METHODS

1. **Morphology:** Plants and flowers from these populations were studied for vegetative and floral morphology. Data was collected on various aspects like height of the plant, number of offshoots arising

from each root stock, shape, arrangement and size of leaves, length of inflorescence, number of disc and ray florets per inflorescence in the field itself. Floral structure with emphasis on reproductive structure was studied in the laboratory under a stereo - microscope (NIKON Type - 115).

2. **Fruit and Seed Set:** Fruit and seed set was observed in the plants growing open in the fields as well as in bagged inflorescences. Number of flowers per capitulum and later number of fruits per capitulum were counted for these plants. Percentage fruit and seed set both on open pollination and bagging was calculated as:-

$$\text{Percentage fruit set per inflorescence} = \frac{\text{Fruit count per inflorescence}}{\text{Flower count of the same inflorescence}} \text{x100}$$

For *A. scoparia*, the same was calculated as:

$$\text{Percentage fruit set per inflorescence} = \frac{\text{Fruit count per inflorescence}}{\text{Number of ray florets per capitulum}} \text{x100}$$

3. **Cytology:** For karyology, seeds of each species were germinated on moist filter paper lining petriplates. Seedlings with 4-5 mm 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 root tip squashes, the seedlings were hydrolyzed in a mixture of 1% acetoorcein and 1N HCl (9:1) and placed in an oven maintained at 60° Cfor 13 minutes. Finally, the root tips from these seedlings were squashed in 1% propiocarmine. Battaglia's (1955) scheme was used for the classification of somatic chromosomes. Karyotypes were classified following Stebbin's (1971) chart of karyotype asymmetry.

Pollen mother cell meiosis was studied from young immature flower buds fixed during morning hours (8-9 am) 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 permanent by removing the paraffin ring and inverting the slide in a petridish containing 1:1 mixture of n- butyl alcohol and acetic acid. Both the slide 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.

III.RESULTS AND DISCUSSION:

Plants of *Artemisia* are shrubs with perennial rootstock through which the species perennates in winter and sprouts in the month of March- April at different sites (18 - 30^{o}C) to produce aerial offshoots. The plants of *A. nilagirica* have height which ranges from 1.2-3.2m whereas the height of plants of *A. scoparia* ranges from 1.2-2.4m. The inflorescence is heterogamous capitulum ranging in size between 4 - 5.1 mm x 2.0 – 4. The plants of *A. nilagirica* exhibit gynomonoecy. Each capitulum consists of central hermaphrodite disc florets and peripheral female ray florets. Each disc floret is bisexual, regular, actinomorphic, pentamerous and epigynous. It bears five petals united to form a short cylindrical tube that merges into a campanulate limb. Corolla tube is yellowish or reddish brown in colour. Surrounded by corolla tube, are present five syngenesious stamens and a pistil. Dithecous, basifixed and introse anthers are fused to form a hollow cylinder or tube which surrounds the style. Long bristles are present at tips of anthers. In the centre of floret, is present a pistil consisting of bicarpellary, syncarpous, inferior and unilocular, one-ovuled ovary with basal placentation, an elongated style and bifid stigma. Ray florets are pistillate in the species. The floral structure of capitula of A. scoparia matches with that of A. nilagirica with the only diference that the pistil of disc florets of A. scoparia bears a rudimentary ovary without ovule. All these chracterstics provide these plants the sx-expression of functional monoecy.

More than one cytotypes were observed in *A. nilagirica*. These included diploid, 2n=18 (Bhaderwah population), hexapoloid 2n=6x=54 (Rajouri, Poonch and Kud populations) and new numbers i.e. 32 and 34 (Jammu population).

Out of 31 individuals tagged in Jammu population, 25 (80.65%) showed the presence of 34 chromosomes per pmc, while 6 (19.35%) showed 32 chromosomes. These were thus with n=17 and 16 respectively. The chromosomes paired during pmc meiosis to form bivalents during diakinesis and metaphase-I in a major chunk of cells but were also involved in different chromosomal associations leading to formation of multivalents in a significant percentage (i.e. 10.8.-23.3%) of pmcs. The mean chromosome pairing configuration calculated for plants with 2n=34 is 0.16I+16.13II+0.02III+0.18IV+0.06VI and that of 2n=32 is 15.33II+0.33IV+0.1VI. During Anaphase-I, most of the pollen mother cells showed regular segregation of chromosomes (17:17) in plants with 2n=34; in some (32.6%) the presence of laggards and chromosomal bridges were noticed. In plants with 2n=32, only a small proportion of cells (32.14%) showed normal segregation of chromosomes at Anaphase-I, whereas in 67.85% cells anomalies like laggards and bridges were observed.

Hexaploid cytotype (2n=54) was observed for three populations of *A. nilagirica* i.e. Rajouri, Poonch and Kud. The chromosomes in these plants paired normally to form 27IIs during pollen mother cell meiosis in 69.8-84.9 % cells; In rest of the cells, multivalents were also observed. These included III, IV, VI and VIII. Relative proportion of cells with multivalents varied in different populations. It was highest in Rajouri population (30.14%). At Anaphase-I, segregation of chromosomes was regular in most of the cells with a small percentage of cells (36.88%) showing anomalies that included bridges, laggards and chromosome fusions.

Hermaphrodite disc florets of each species produce copious amount of pollen. Pollen grains produced are smooth –walled, sub-spheroidal and triporate. Pollen ovule ratio per disc floret ranges from 3737.4 to 4143 in *A. nilagirica*. Pollen ovule ratio per capitulum averages 348.8 to 420.5 in *A. scoparia*.

Fruit in each species is a cylindrical achene enclosing the seed without pappus. It is ridged, brown, dry and indehiscent. On open pollination, percentage seed set is found to be minimum in plants of Jammu population of *A. nilagirica* (15.45%) and maximum in Bhaderwah population of *A. nilagirica* (61.43%). Plants of *A. scoparia* show high seed set in all the populations scanned which ranges from 61.43% to 65.77%. On bagging, the percentage seed set is reduced in the two species of *Artemisia* and all the seeds formed are shrivelled .

The most common base numbers reported for genus *Artemsia* are n=8 and 9 (Weins and Richter, 1966; Valles *et al*., 2005 and Chehregani and Hajisadeghian, 2009). Of these, x=9 is considered primitive and x=8 as advanced. Going by these base numbers, Bhaderwah population of *A. nilagirica* is diploid with base number as n=9; while 2n=54 cytotype (Rajouri, Kud and Poonch) is hexaploid (2n=6x=54). *A. scoparia* has 2n=16 which confers x=8 as base number to this species.

Other chromosome numbers i.e. 2n=32, 34 seem to have originated secondarily. 2n=34 which predominates in plants of Jammu population does not fit with x=8 or 9 as base numbers and seems to be derived. The same number has also been reported for few other species of *Artemisia* namely *A. princeps*, *A. dubia* and *A. momiyamae* (Matoba *et al*., 2007 and Zhen *et al*., 2010). Zhen *et al*., 2010 have proposed 2n=34 as a new number stablized enough to discard the case of aneuploidy and to consider-dysploid polyploid origin of a new secondary base number i.e. x=17. The chromosome numbers of 34 and 32 as observed presently in *A. nilagirica* may be reached through 2n=2x=18 through 2n=4x=36 or from 2n=2x=16 through 2n=4x=32. Another way of existence of intra-population chromosomal variability may be chromosome fusion, so that 2n=32 is derived. Going by all these possibilities, Jammu population of *A. nilagirica* can be considered as aneuploid.

Species of genus *Artemisia* L,. as studied presently are highly flexible, capable of generating variations through both meiotic and breeding system. The spread of these variations does get retarded by low seed set. Little amount of variability that survives and adapts is maintained and ramified asexually by rootstock. All this contributes to the widespread distribution and dispersal of genus *Artemisia*.

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