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RESEARCH ARTICLE

CYTOMORPHOLOGICAL STUDIES, DISTRIBUTION PATTERN AND ETHNOBOTANY OF GENUS *ARGEMONE* L. FROM NORTH WEST INDIA.

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Abstract

During the present course, detailed male meiosis, morphological observations and distribution pattern have been studied of two different species of Argemone (A. mexicana and A. ochroleuca). Six populations of Argemone mexicana have been collected from different localities of Rajasthan and Himachal Pradesh of which two populations from Talmata and Boh (H.P) depicts the diploid cytotype (2n=2x=14), one population from Nakki lake, Mt. Abu (Rajasthan) shows the tetraploid cytotype (2n=4x=28) whereas three populations from different localities of Rajasthan shows the octaploid cytotype (2n=8x=56) of the species. Octaploid cytotype of the species adds the new chromosomal report on worldwide basis. Detailed meiotic analysis of diploid cytotype shows the presence of meiotic irregularities like chromosomal stickiness, chromatin bridges, abnormal microsporogenesis and heterogenous sized pollen grains while one octaploid cytotype from Chappar shows secondary associations. Meiotic analysis of A. ochroleuca collected from different localities of Rajasthan shows the chromosomal count of 2n=56 which is in conformation with the previous reports from India and outside India.

Introduction

The genus Argemone belonging to family Papaveraceae consists of 32 species at world level (McDonald 1991) and 3 species from India (Sharma & Balakrishnan 1993). The genus shows high medicinal value, having high alkaloid contents, and has been studied in detail by several workers (Benn & Mitchell 1972, Stermitz et al. 1974, Raynie et al. 1991).

Argemone mexicana commonly known as 'Mexican Prickly Poppy' and 'Satyanashi/ Kandayi' by the local people of Rajasthan is an erect, spiny herb, leaves pinnatifid, spiny with amplexicaul bases, flowers showy yellow and fruits capsules. While A. ochroleuca commonly known as Pale Mexican Poppy is strinkingly similar to A. mexicana but is distinguished by ash-colored leaves and whitish or creamy colored flowers. As in literature, it is well known that both qualitative and quantitative differences exists in the active principle of many medicinal plants (Bahaguna et al. 2000; Berkov 2001).

Accumulated cytological data of the genus indicate that, out of 24 taxonomically known species, 20 species/32 cytotypes are known by now. The genus is strictly monobasic (x=7) with chromosome numbers varying from 2n=14 to 112. All the species of the genus show polyploidy with intraspecific euploid variations reported in 8 species as well as intraspecific aneuploid variations exhibited in 2 species. From India, all the taxonomically known species are cytologically worked out with chromosome numbers as 2n=14, 28, 56, 112. Keeping the medicinal value and existence of polyploid cytotypes in view, the present cytomorphological study was undertaken to explore the genetic diversity of genus Argemone from different parts of North-west India (various localities of Kangra and

Rajasthan). So from the medicinal point of view, the genetic diversity can be evaluated for screening better cytotypes for future exploitation.

MATERIALS AND METHODS

Cytological study:

For meiotic studies, flower buds were collected in the field from plants growing under natural conditions from different localities of selected areas of the North west India. These flower buds were collected from 10-15 randomly selected plants of each species/population and fixed in Carnoy's fixative (6:3:1 ethanol/chloroform/acetic acid v/v/v) for 24 hrs. Flower buds were washed and preserved in 70% ethanol at 4°C until used. Smears of appropriate-sized flower buds were made, using standard acetocarmine technique. About 20-50 fresh slides in each case were prepared from different anthers/flowers for different individuals of a particular population and then were analysed in each case. To confirm the chromosome number in case of normal meiosis, around 50 pollen mother cells (PMCs) were observed at different stages of meiosis, preferably at diakinesis/ metaphase-I/anaphase-I, II. In case of abnormal meiosis, however, more than 500 PMCs were considered to ascertain the type and frequency of various abnormalities per plant. Pollen fertility was estimated by mounting mature pollen grains in glycerol-acetocarmine (1:1) mixture. Nearly 500-700 pollen grains were analysed in each case for evaluating pollen fertility and pollen size. Well-filled pollen grains with stained nuclei were taken as apparently fertile, while shrivelled and unstained pollen grains were counted as sterile. Photomicrographs of pollen mother cells and pollen grains were made from freshly prepared slides using Nikon 80i eclipse Digital Imaging System. Voucher specimens are deposited in the Herbarium, Department of Botany, Punjabi University, Patiala (PUN).

Ethnobotanical study: Ethnobotanical information was collected from the local people and from different research papers. In order to document the utilization of Argemone species, a total of 10 field surveys were carried out from July 2011 to July 2013 in the area. The surveys were spread across seasons so as to get maximum information and also to cross check the information provided by the local informants during the earlier visits. Surveys were conducted amongst the Masas, Gaddi, Brahmin, Rajput, Gujjar and Lohar communities residing in different localities of North India. During the initial surveys, friendly relations were developed with the village people. Information on people having specialized knowledge on the uses of plants for curative purposes was gathered in their local language. Twenty such knowledgeable people (15 males and 5 females) who are locally called vaids (local physicians) were identified and interviewed in detail during subsequent surveys. Structured questionnaires, interviews and participatory observation were used to illicit information from the resource persons using standard methods (Martin 1995; Reyes-Garcia et al. 2007). The information on scientific name, local name, plant part exactly used to cure and method of dosage of these plants has been provided in results and discussion.

RESULTS AND DISCUSSION

CYTOLOGY:

Argemone mexicana L.: Six populations of A. mexicana collected from different localities of North-West India show the presence of three cytotypes as diploid (2n=2x=14), tetraloid (2n=2x=28) and octaploid (2n=2x=56) based on the base number x=7.

The population collected from Talmata and Boh, H.P. depicts the diploid cytotype (2n=14) with the presence of 7 bivalents at M-I which adds a new cytotype report for the species by Kumar et al. 2013. Detailed meiotic analysis of the population shows the irregular meiotic behaviour with the presence of chromosomal stickiness (5.38%) at M-I, chromatin transfer between different PMCs and chromatin bridges (9.73%/4.02%) at Anaphases and Telophases. These abnormalities lead to abnormal microsporogenesis, low pollen fertility (67.67%) and heterogenous sized pollen grains.

Tetraploid cytotype (2n=28) has been collected from Nakki lake, Mount Abu (Rajasthan) exhibiting 14:14 distribution of chromosomes at A-I with further abnormal meiotic course. Chromosome count of 2n=28 is in accordance with the previous report from India (Sidhu 1979, Sidhu & Bir 1983, Trivedi & Trivedi 1992) and outside India (Safonova 1991). The species also contains 2n=112 (Diers 1961) from outside India.

Out of six accessions of A. mexicana, three accessions collected from different localities of Rajasthan depicts the chromosomal count of 2n=56 with the presence of 28 bivalents at M-I and 28:28 distribution of chromosomes at A-I which adds a new octaploid cytotype for the species. Further the detailed meiotic course of the population collected from Chappar is marked by anomalous meiotic behaviour with presence of secondary associations at M-I, interbivalent connections, early and late disjunction of bivalents and laggards at T-I but the microsporogenesis and pollen fertility shows the normal behaviour.

Argemone ochroleuca Sweet: Both the populations of A. ochroleuca collected from different localities of Rajasthan have the same chromosome number 2n=56 which is in accordance with the previous reports from India (Sidhu 1979, Bir & Sidhu 1980) and outside India (Ownbey 1958). The species is also known to have other report of 2n=28 by Koul & Wakhlu (1976) and Kumar et al. (2013). The meiotic course represents the normal behaviour with high pollen fertility.

Table 1. Data showing taxon with accession number, meiotic chromosome number, ploidy level and localities of different populations of Argemone mexicana and Argemone ochroleuca

Taxon/Accession Number (PUN)	Meiotic chromosome number (2n)	Ploidy level (x)/Meiotic behaviour	Locality with latitude and longitude, district, altitude
Argemone mexicana L.			
52743	14	2x/A	Talmata,31°52'N 76°12'E, Kangra (H.P.), 1500 m
56376	14	2x/N	Boh, 32°19'N 75°50'E, Kangra (H.P.), 1900 m
59106	28	4x/A	Nakki lake, 24°59' N 72°70' E, Mount Abu (Raj.), 1220 m
59103	56	8x/A	Chappar, 27°79'N 74°43'E, Churu (Raj.), 302 m
59104	56	8x/N	Sri Ganganagar, 29°91'N 73°88'E, Sri Ganganagar (Raj.), 164 m
59105	56	8x/N	Gyan Sarovar, 24°60'N72°73'E, Mount Abu (Raj.), 1220 m
Argemone ochroleuca Sweet			
59107	56	8x/N	Gyan Sarovar, 24°60'N72°73'E, Mount Abu (Raj.), 1220 m
59108	56	8x/N	Rai Singhnagar, 29°53'N, 73°44'E, (Raj.), 166 m

Table 2. Data on cytomixis, abnormal meiotic behaviour and pollen fertility in diploid cytotype of A. mexicana from Boh (H.P.)

Taxa/Voucher no.	Cytomixis at meiosis I /meiosis II		Meiotic course showing PMCs with				Pollen fertility (%)
	% of PMCs involved	No. of PMCs involved	Chromatin stickiness (%)	Unoriented bivalents (%)	Bridges at meiosis-I/meiosis-II (%)	Laggards at meiosis-I/meiosis-II (%)	
Argemone mexicana (2x)/52743	5.45 (6/110) / 3.88 (4/103)	2-4	5.38 (7/130)	4.20 (5/119)	4.20 (5/119)/ 5.79 (6/105)/ 4.08 (4/98)	5.17 (6/116)/ 4.25 (4/94)	67.67

Table 3. Data on abnormal meiotic course and pollen fertility in octaploid cytotype of A. mexicana from Chappar (Rajasthan).

Taxon with ploidy level /Voucher no.	Meiotic course showing PMCs with				Pollen fertility (%)
	Interbivalent connections (%)	Early and late disjunction of bivalents (%)	Secondary associations (%)	Laggards at meiosis-I/meiosis-II (%)	
Argemone mexicana (8x)/ 59103	6.8 (4/58)	3.44 (6/74)	12.5 (6/48)	4.76 (3/63)	79.85

Meiotic Abnormalities: The presence of meiotic abnormalities like secondary associations, cytomixis, chromatin stickiness, laggards, bridges, multipolarity, etc. in the presently studied species indicates the existence of genetic diversities.

The general phenomenon of secondary association was first observed in Oryza sativa by Kuwada (1910) followed by Ishikawa (1911) in Dahlia variabilis and Marchal (1912) in Amblystegium. The theory of secondary association of chromosomes at meiosis implies that the paired bivalents are originally related to each other. It is insisted by several authors (especially Lawrence 1931) that in the absence of multivalents, the secondary association provides the only available criterion of chromosome homology. Stebbins (1950) recognizes that secondary associations serve as an indication of the polyploid origin of a species or genus, but he cautioned against elaborate phylogenetic conclusions based on such evidence. One more reason put forth by Jelenkovic et al. (1980) states that secondary associations may be due to the presence of heterochromatin region in the genome which facilitates the association between non homologous parts. According to Kumar and Chaudhary (2014), the estimation of the strength of forces involved in the secondary association makes a foundation for assessing the impact of environmental factors on chromosomal association. The environmental factors modify the chiasma frequency by either altering chiasma formation or chromosome pairing. Since secondary pairing between bivalents is independent of chiasma formation, it provides accurate details about the effect of environmental factors on chromosome pairing.

Cytomixis for the first time was discovered by Kornicke (1901) in Crocus sativus. In the presently studied species, cytomixis results in the formation of hyperploid and hypoploid PMCs. These hypoploid and hyperploid PMCs formation is attributed to cytomixis (Falistocco et al. 1995, Fadaei et al. 2010). The existence of lagging chromosomes has been found to be highly genotype dependent (Pagliarini 2000). The occurrence of laggards and bridges at anaphase and telophase stages, as observed in present investigation could be due to delayed terminalization, stickiness of chromosomes ends or because of abnormal chromosomal movements (Sax, 1940). Chromosome stickiness is caused due to genetic and environmental factors and several agents have been reported to cause chromosome stickiness (Pagliarini, 2000). Gaulden (1987) postulated that stickiness may result from the defective functioning of one or two types of specific non histone proteins involved in chromosome organization which are needed for chromosome separation and segregation.

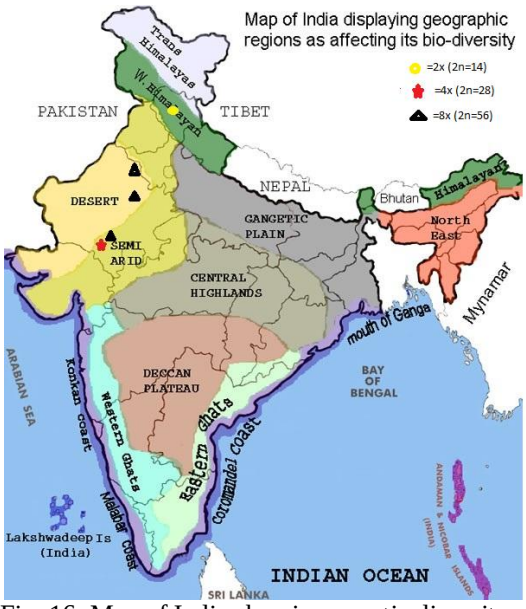

Fig. 16: Map of India showing genetic diversity of the Argemone mexicana in North-West India.

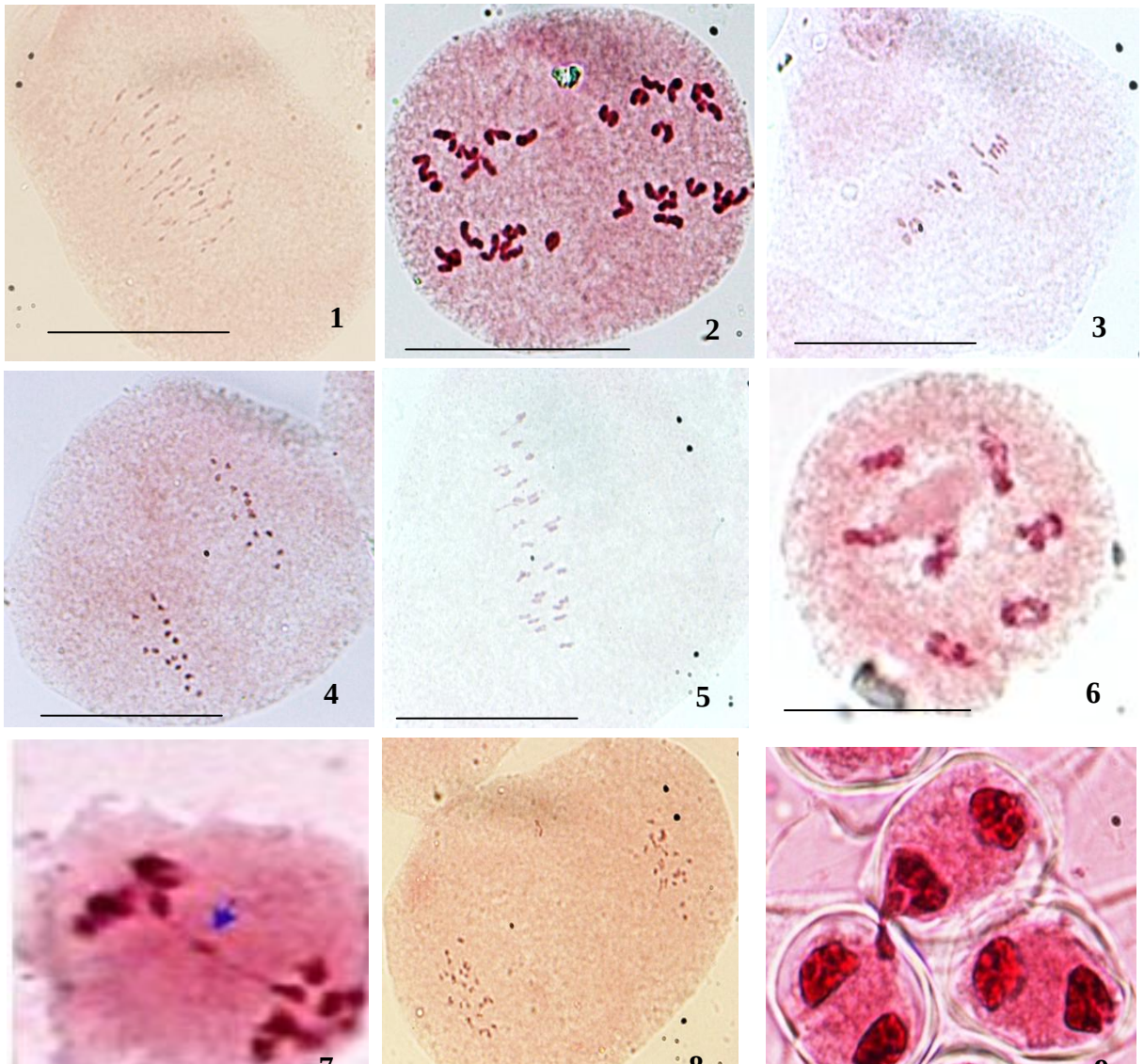

Fig. 1-15: **1. Argemone ochroleuca**: PMC at M-I showing 28 bivalents **2-15 Argemone mexicana 2.** PMC at A-II showing 7 chromosomes at each pole **3.** PMC at M-I showing 14 bivalents (Tetraploid cytotype) **4**. PMC at A-I showing 7:7 distribuion of chromosomes **5.** PMC at M-I showing 28 bivalents (octaploid cytotype) **6**. PMC at Diakinesis showing 7 bivalents (Diploid cytotype) **7.** PMC showing chromatin bridge at A-I **8.** PMC showing laggard at A-I . **9.** Cytomixis between two PMCs **10**. Chromatin bridge at A-I **11-12**. PMCs showing secondary associations between different bivalents at M-I **13**. Tetrad with micronucleus **14**. Polyad **15**. Heterogenous sized pollen grains.

DISTRIBUTION

The genus is generally distributed in North America from United States to Central Mexico and the West Indies, nine species in South America, one in Hawaii, and the others scattered along the north west coasts of the America. In the North West India, the distribution pattern of euploid cytotypes shows definite relation to altitudinal variations (Table 1). The diploid cytotype is not found in Rajasthan and is only restricted to Himachal Pradesh. Tetraploids are the most common and are widely distributed in Rajasthan and Himachal Pradesh. The octaploid cytotype is restricted only to Rajasthan (164-1220m) in Churu, Sri Ganganagar and Sirohi districts of Rajasthan. Thus, it is clear that North West India harbours maximum genetic diversity for the species (Fig. 16).

ETHNOBOTANY:

Argemone mexicana:

Local name: Kandayi (in Kangra) and Satyanashi (in Rajasthan).

Parts used: Latex, seeds, seed oil, Root.

Disease/ailment: Different plant parts are used for different diseases as gum troubles (seeds); conjunctivitis, dropsy, skin diseases (latex), scorpion sting (root).

Dry powder of seeds applied on gums once a day reduces the gum troubles.

Yellow sap of the plant is used to cure the eye irritation, on ulcer for quick healing. The seed-oil is also used to cure scabies.

The plant also shows poisonous effect as if it is taken orally in large dose acts as an irritant and causes vomiting. There occurs intense body pain and oedematous area in legs and feet become inflammed (Singh & Pandey, 1998).

The plants when eaten by animals causes diarrhoea and sleepiness (Katewa et al. 2006).

CONCLUSION:

There exists a variation in chromosome number in the form of occurrence of euploid cytotypes at 2x, 4x and 8x levels showing difference in meiotic behaviour accompanied by different distributional pattern at intraspecific level in the Argemone mexicana but A. ochroleuca shows the stable chromosome number at octaploid level in different

distributional areas. Thus, there is a further need for the extensive cytological exploration of A. mexicana at population basis to score different cytotypes/morphotypes/ecotypes, so as to mark the best chemotype for future medicinal use.

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