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Cytological Studies in *Asteracantha longifolia* (L.) Nees-A Medicinal Herb

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Abstract: The present study was accentuated upon karyotype analysis and meiotic chromosome behavior in *Asteracantha longifolia* (L.) Nees. The chromosome number of *Astercantha longifolia* (L.) Nees-a medicinal herb-found to be 2n = 2x = 32 through mitotic and meiotic analysis. Karyotype analysis revealed eight ($6A_m$ + $4B_{sm}$+$4C_{st}$+$4D_m$+$4E_{sm}$+$4F_{st}$+$2G_t$+$4H_{sm}$) morphologically distinct chromosome types. Karyotype analysis showed prevalence of chromosomes with submedian and median primary constrictions. The chromosome length varied from 1.43 to 3.32 μm. The karyotype was symmetric in nature with TF% = 32.8. Meiotic analysis showed the gametic number n = 16 consistently with an average of 15.65±0.65 II and 0.7±0.98 I. The meiocytes revealed predominance of ring bivalents (10.05±0.76) over rod bivalents (5.6±0.68), which had mostly terminalised chiasmata with terminalization coefficient of 0.89. Anaphase-I segregation showed normal 16:16 separation to two poles, although univalents in the form of laggards were often observed. Pollen stainability was 80.3±8.63% indicating the partial heterozygosity in constituent genome of *A. longifolia*.

Key words: *Asteracantha longifolia*, karyotype, mitosis, meiosis, chromosome association

INTRODUCTION

Asteracantha longifolia (L.) Nees [Syn. *Hygrophila auriculata* (Schum) Heine], belongs to the family Acanthaceae, is an important medicinal herb described in ayurvedic literature as Ikshura, Ikshugandha and Kokilakshya etc. It is a marshy, hispid herb, growing in the banks of fresh or stagnant water ditches and swampy grounds, mixed with grasses and sedges. The plant parts including leaves, inflorescences, seeds, roots and ashes have been extensively used in traditional system of medicine for various ailments including diuretic, jaundice, diopsy, rheumatism, hepatic obstructions and dissolution of gallstones, kidney stones, liver dysfunction and diseases of urino-genital tracts (Singh *et al*., 2003; Mishra, 1985; Shailajan *et al*., 2005). The plant extract are possessed with antitumor (Ahmed *et al*., 2001; Majumdar *et al*., 1997) hypoglycaemic (Fernando *et al*., 1991), antibacterial (Vlietinck *et al*., 1995) hepatoprotective (Singh and Handa, 1995; Hewawasam *et al*., 2003; Shanmugasundaram and Venkataramana, 2006) and anti-nociceptive properties (Shanmugasundaram and Venkataramana, 2005). The seeds were act as effective inducer of sexual behavior (Chauhan *et al*., 2009). The data on chromosome number and karyological analysis are prerequisites to overall understanding of a genome and its genetic amelioration through several approaches of crop breeding. There is no worth while information available on cytological studies including meiosis and mitosis. Therefore, an attempt has been made to study this herb from cyto-genetics point of view and this research communication reported on the karyotype analysis and meiotic chromosome behavior which were previously undescribed in *A. longifolia*.

MATERIALS AND METHODS

Plant materials: Thirty ecotypes of *A. longifolia* plants were collected during Winter, 2007 from different parts of Orissa and Andhra Pradesh. All the ecotypes were maintained in the experimental garden of MITS, Rayagada, India. An accession LLR-11 of *A. longifolia* having violet flower colour was used in the present study for mitotic and meiotic analysis.

Mitotic analysis: Well developed plants were kept under water to raise roots. After couple of days roots were developed from nodal region. Well developed roots (1-3 cm) were cut at 7.00-8.00 am and pre treated with saturated solution of Para dichlorobenzene (PDB) for three hours at room temperature. Then the root tips were fixed in 1:3 aceto-alcohols and kept for 24 h at room temperature (25±2°C). After that the root tips were

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transferred to 70% ethanol and stored at 4°C. The root tips were hydrolysed in preheated 1N HCl at 60°C for 10 min. The hydrolysed root tips were squashed with the help of 1.5% aceto-orcein for mitotic analysis. Suitable metaphase plates prepared for the species were observed under microscope (Unilab, India) and were photographed using Nikon Coolpix-4500 camera. The chromosome images were analysed manually through image analyzing system as suggested by Fukui (1986) and an idiogram was prepared. The chromosomes were classified as median (m), submedian (sm), subterminal (st) and terminal (t) according to the position of the centromere (Hirahara and Tatuno, 1967) and based on chromosome length (long: 2.5 to 3.32 μm, medium: 1.75 to <2.5 μm and Small: <1.75 μm). The chromosomes were morphologically graded into following types- type A: Long median, type B: Long submedian, type C: Long subterminal, type D: Medium median, type E: Medium submedian, type F: Medium subterminal, type G: Medium terminal and type H: Short submedian. Total chromatin length, TF% (Proportion of short arms in total chromatin length) and S % (relative length of shortest chromosome compared to longest) were also estimated.

Meiotic analysis: Flower buds of appropriate size were fixed in 1:3 aceto-alcohols during 7.00-8.00 a.m. and kept for 24 h at room temperature (25±2°C). After fixation the buds were transferred to 70% ethanol and stored at 4°C. Anthers from the bud of appropriate size were squashed in a drop of 1. 5% Acetocarmine for meiotic studies. On an average 100 Pollen Mother Cells (PMCs) were analysed at diplotene/diakinesis/metaphase-I to estimate the range of chromosome associations and recombinational frequencies through chiasma. At anaphase-I, on an average 20 cells were analysed to study the distribution pattern of chromosomes/ chromatids. Pollen stainability was assessed by staining the pollen grains in 1:1 Glycerin: Acetocarmine mixture and an average five slides were scored for this purpose.

RESULTS

Mitotic analysis: Mitotic analysis of several slides at metaphase showed that the chromosome number of *A. longifolia* is 2n = 2x = 32 (Fig. 1a). The chromosomes were well condensed and the position of nucleolus is peripheral in most cases. Karyomorphological studies revealed eight (2n = 32: $6A_m+4B_{sm}+4C_{st}+4D_m+4E_{sm}+4F_{st}+2G_t+4H_{sm}$) distinct chromosome types (Table 1, Fig. 1b). This karyotype showed predominance of chromosomes with median (F% = 40.05 to 46.25%) and submedian (F% = 29.71 to 39.98%) primary constrictions though

Table 1: Karyotype details of *A. longifolia* as revealed by metaphasic plates

Chromosome type	No. of chromosomes	Length (μm)	Centromeric index (F%)	Nature of centromere
A (long)	06	2.54-3.15	40.05-44.44	Median
B (long)	04	2.71-3.32	35.23-38.74	Sub-median
C (long)	04	2.62-2.8	18.93-25.00	Sub-terminal
D (medium)	04	2.27-2.5	42.00-46.25	Median
E (medium)	04	1.76-2.27	29.71-38.33	Sub-median
F (medium)	04	2.01-2.45	21.63-22.91	Sub-terminal
G (medium)	02	1.92	0.0	Terminal
H (short)	04	1.43-1.74	35.13-39.98	Sub-median

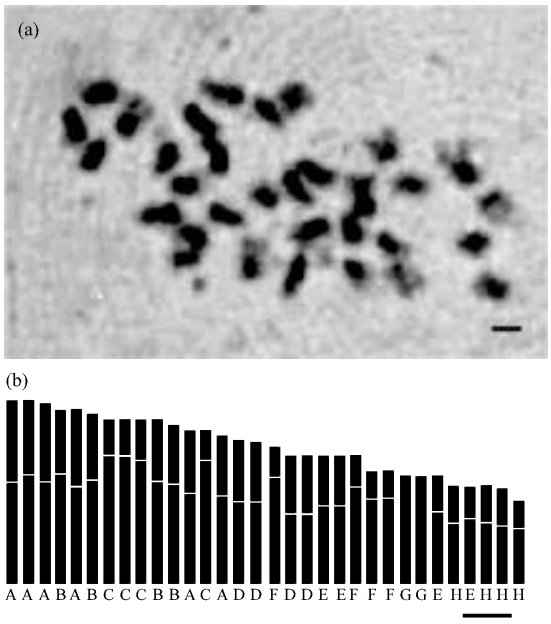

Fig. 1: Mitosis in *A. longifolia*. (a) 2n = 2x = 32 chromosomes at metaphase (Bar = 2.0 μm) and (b) Idiogram showing chromosome type and centromeric position (Bar = 1.0 μm)

there were few sub terminal (F% = 18.93 to 25.00%) and terminal (F% = 0) primary constrictions. Chromosome length varied from 1.43 to 3.32 μm. Two chromosomes in type-G were telocentric in nature having primary constriction at its proximal end. Total chromatin length was 76.77 μm and S% noted in the species was 48.89%. The karyotype was symmetric in nature with TF% = 32.8%.

Meiotic analysis: In *A. longifolia* most of the PMCs analyzed had shown the gametic chromosome complement n = 16 (Fig. 2a, b) and showed mixture of bivalents and univalents as chromosome associations. Predominant chromosomal association was 16II in 65% Diakinesis/Metaphase-I cells, while rest had 15II + 2I with an average of 15.65±0.65 II and 0.7±0. 98 I (Table 2). Diplotene chromosomal configuration showed predominance of ring bivalents (10. 05±0.76) over rod

Table 2: Meiotic analysis in pollen mother cells of *A. longifolia* at diplotene, diakinesis, metaphase-I and anaphase-I

Chromosome associations				Chiasmata properties					Anaphase-I distribution			
Bivalents (II)		Univalents (I)										
Mean±SD	Range	Mean±SD	Range	Mean±SD	Range	Termin-alised	Untermin-alized	Terminalization coefficient	16II:16II	15II:2I:15II	14II:4I:14II	Age of pollen fertility (%)
15.65±0.49	15-16	0.7±0.98	0-2	21.25±8.01	6-32	19.0±7.86	2.25±2.12	0. 89	83.33%	12.5%	4.1%	80.3±8.63

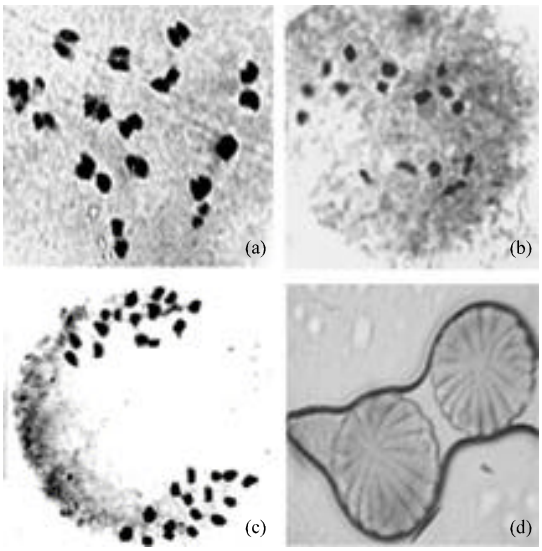

Fig. 2: Meiotic behaviour in *A. longifolia*. (a-b). Late and early diakinesis showing 16 II, (c) Anaphase-I showing 16:16 segregation to two poles and (d) Stained pollen grains showing radial sculpturing from central circular position

bivalents (5.6±0.68) with mean chiasma of 21.25±8.01 (Range: 6-32) per cell. On average 19 chiasmata were terminalized with terminalization coefficient 0.89 (Table 2). Majority of PMCs (83.33%) showed equal distribution (16:16) of chromosomes during anaphase-I (Fig. 2c), while rest showed unequal separation of chromosomes having complement 15II:2I:15II (12. 5%) and 14II:4I:14II (4. 1%). Average pollen fertility studied in this species was 80.3±8. 63%. Sculpturing of pollengrains was appeared as thickening bands radiating from a central circular position (Fig. 2d).

DISCUSSION

Asteracantha longifolia root tip preparations constantly showed the same somatic number of 2n = 32. Although, different types of chromosomes were observed with structural differences in length and centromere index, symmetric karyotype was the characteristic feature of *A. longifolia*. This somatic chromosome count was also reflected as chromosome associations in PMCs analysed at metaphase-I, where either 16 bivalents or a combination of bivalents and univalents amounting into 32 chromosomes were observed. Multivalent associations were never encountered. The presence of univalents in some of the PMCs might be due to failure of synapsis. Such behavior of chromosomes can be correlated with the phenomenon of desynapsis as reported in number of tropical plants (Gottschalk and Kaul, 1980; Dave, 1998; Rao and Kumar, 2003; Rawat *et al.* , 2006). The desynaptic behavior among the few bivalents can trace to influence of several environmental factor such as temperature, humidity and nutrition as in *Tribulus rajasthanensis* (Rawat *et al.*, 2006).

The chiasmata were distally localized in most PMCs and this may be due to participation of short conserved segment of chromosomes during the process of recombination. Presence of localized chiasma (distal/proximal) has been extensively observed in several plant species (Singh, 1993; Gupta, 1995; Kumar and Rao, 2002, 2003; Rawat *et al*., 2006). This has been attributed to chromosome pairing and/or interference pattern coupled by availability of short segments for genetic crossing over in *A. longifolia*. Majority of PMCs showed 16:16 distribution of chromosomes and few PMCs exhibited laggards during anaphasic separation. The presence of high number of univalents and their subsequent failure in orientation and disjunction in anaphase are considered as main source of laggard formation (John, 1990; Singh, 1993; Gupta, 1995; Kumar and Rao, 2002, 2003; Rawat *et al.*, 2006).

Observations on chromosome morphology and size made in this study are an insight into what will be expected at meiosis. Distribution of several morphological categories namely median, submedian, subteleocentric and telocentric occurring in different size regimes as was observed, is likely to result in many structural changes and rearrangements at meiosis, which could lead to speciation as in *Crotolaria* and *Abrus* sp. (Akpabio and Olorode, 1988; Agbagwa *et al.*, 2005).

Above cytological studies including mitotic and meiotic analysis deciphered the somatic chromosome compliment of *A. longifolia* to be 2n = 2x = 32. The karyotype was symmetric in nature, which predominated with median and submedian chromosomes. The PMCs revealed predominance of ring bivalents over rod bivalents at diakinesis and metaphase, which had mostly terminalised chiasmata. Appearance of few univalent in

the form of laggards and staining behavior of pollens indicated the partial heterozygosity in constituent genome of *A. longifolia*.

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