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CYTOLOGICAL STUDIES IN *Astercantha longifolia* L. SYN. *Hygrophila spinosa* T. ANDERS.

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Key words : *Population, Variation, Cytology, Astercantha longifolia L.*

Cytological investigations were carried out in *Astercantha longifolia* L. in different populations from different localities in different seasons. Mitotic and meiotic behaviour of chromosomes were studied including chiasma frequency and structural heterzygosity. Variations were also visualized in the chromosome structure.

INTRODUCTION

Population is a group of individuals of the same species. One of the most dynamic aspects of taxonomy in the study of populations of living plants is to establish variation patterns. This type of study provides rich source of understanding of a given taxon, when these patterns are properly evaluated. Thus, variation patterns from genetic sources within and between populations set the stage for the deveopment of discontinuities upon which taxonomic system may be founded. There is a great range of variability existing in natural populations. There are various causes namely intraspecific and interspecific hybridization, introgression, apomixis, polyploidy and many other genetic factors which bring about variability in natural populations (Anderson and Abbey, 1934 and Grant, 1984).

Populations are the gene pool of genetic system in plants. The genetic system refers to all the intrinsic genetic processes that affect genetic recombination in a population or species. The major components of the genetic systems are the chromosomal organization, their behaviour in meiosis and the breeding system.

Astercantha longifolia L. occurs as a weed. From the cytological point of view, weeds are endowed with a variety of characteristics. Greater knowledge can be accumulated by deducting a relationship between the species, races and individuals of the species growing in different environments and by comparative study of meiosis and mitosis.

Keeping all the above mentioned points in view, the present investigation has been carried out.

MATERIALS AND METHODS

The material for the present investigation is *Astercantha longifolia* L. syn. *Hygrophila spinosa*. Three populations of the species have been studied cytologically. Details of collection and localities are listed below:

List of materials with locality and period of Collection:

Name of Species	Populations	Locality	Date of Collection
Astercantha longifolia , L. syn. *Hygrophila spinosa* T. Anders.	Al 0914	Bhusunda	15th Sept, 2014
	Al' 0914	Kendui	18th Sept, 2014
	Al'' 0914	Gaya College Campus	21th Sept, 2014

For the cytological studies of the above three populations mitotic studies were carried from root tips and meiotic studies from flower buds. Slides were prepared by squash method. Meiotic studies were carried out by anther squash preparation. 1:3 acetoalcohol was used as fixative and staining was done in acetocarmine.

OBSERVATIONS

Cytological studies of *Astercantha longifolia* L. were done from three different populations Al 0914, Al' 0914, Al'' 0914 and some important characters were noted.

Mitotic Analysis

Mitotic analysis was carried out from the root tips. Suitable metaphase plates of all the three different populations of *Astercantha longifolia*, L. were prepared and were observed under microscope. The chromosomes were classified as median (m) sub-median (sm) and subterminal, according to the posiion of centromere and, based upon their length chromosomes were graded into following types.

Type		Length
A	-	3.50-3.99µ
B	-	3.00-3.49µ
C	-	2.50-2.99µ
D	-	2.00-2.49µ
E	-	1.50-1.99µ
F	-	1.00-1.49µ
G	-	LESS THAN 1µ

Meiotic Analysis

For the meiotic analysis of the different populations of *A. longifolia* L. slides were prepared from the flower buds by squash method. Pollen mother cells were analysed at different stages to estimate the range of chromosomal association and recombination frequencies through chiasma. At anaphase-I pollen mother cells were analysed to study the pattern of chromosome. Pollen stainability was assessed by staining the pollen grains.

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Results:

Mitotic Analysis

During mitotic analysis of the different populations of *A. longifolia* L. somatic chromosome number was found to be 2n = 32 (Fig-1). The length of the chromosome varies from 1.00µ to 3.99µ. The karyotype showed predominance of median and sub-median chromosomes. Out of the sixteen pairs of chromosomes, seven pairs were of median type, six pairs were of sub-median type and the rest three were of subterminal type. The detailed chromosome type, their measurement and T.F.% have been given in Table-1.

Table-1

Sl. No. of Chromo-some pair	Chromosomal type	Position of centromere		Length of component protion in µ		Total length	T.F.%
		Primary	Secondary	Long arm	Short arm		
1	B	M	–––––	1.66 µ	1.64 µ	3.60 µ	
2	B	M	–––––	1.58 µ	1.52 µ	3.20 µ	
3	C	M	–––––	1.50 µ	1.44 µ	2.94 µ	
4	C	Sm	–––––	1.65 µ	1.22 µ	2.87 µ	
5	C	Sm		1.58 µ	1.13 µ	2.71 µ	
6	C	Sm		1.50 µ	1.10 µ	2.60 µ	
7	C	M		1.28 µ	1.22 µ	2.50 µ	
8	D	M		1.25 µ	1.15 µ	2.40 µ	41.80%
9	D	M		1.20 µ	1.12 µ	2.32 µ	
10	D	Sm		1.30 µ	0.80 µ	2.10 µ	
11	E	Sm		1.22 µ	0.76 µ	1.98 µ	
12	E	St		1.30 µ	0.49 µ	1.79 µ	
13	E	Sm		1.10 µ	0.68 µ	1.78 µ	
14	E	M		0.82 µ	0.78 µ	1.60 µ	
15	F	St		1.26 µ	0.20 µ	1.46 µ	
16	F	St		1.20 µ	0.21 µ	1.41 µ	

Meiotic Analysis

In all the three different populations, the gametic number n = 16 was found and the meiotic division was found to be non-synchronized.

The dividing pollen mother cells with sixteen regular bivalents were observed (Fig-2) in AI 0914. At metaphase-I sixteen bivalents were recorded (Fig-3). Deviations from the regular sixteen bivalents organization were observed at metaphase-I in the form of univalent and multivalent (Fig.5 & 6). The nature and frequency of chromosomal association have been depicted in Table-2. Chiasma frequency and analysis of ring and rod bivalents have been shown in Table-3. The common anomalies observed at this stage were clumping of chromosome, precocious separation of chromosome and interbivalent connection. Anaphase-I was found to show equal number of chromosomes at both poles (fig-4). However, in population AI'0194, collected from village Kendui of Gaya town showed abnormalities in the form of simple chromosomal bridge and laggards.

The analyses of pollen stainability have been summarized in Table-4.

Table-2

Nature and frequency of chromosomal association at Metaphase-I

Population	Frequency of pollen mother cell	Chromosomal association			
		Univalent	Bivalent	Trivalent	Quadrivalent
		I	II	III	IV
Al 0914	30	00	16	00	00
	08	00	14	00	01
	07	04	14	00	00
	05	00	10	00	03
Al' 0194	30	00	16	00	00
	06	08	12	00	00
	08	00	12	00	02
	06	03	13	01	00
Al'' 0194	26	00	16	00	00
	12	04	14	00	00
	06	01	14	01	00
	06	00	14	00	01

Table-3

Nature and frequency of Chiasma and Chromosomal pairing at metaphase-I in populations of Al0914, Al'0914, Al''0914 respectively.

Population	No. of PMC studied	No. of vivalent per PMC				Total	Chiasma per PMC		Terminalis-ed Chiasma		½ chiasma per chromosome
		Ring		Rod			Range	Mean	Range	Mean	
		Range	Mean	Range	Mean						
Al 0914	50	08	10	04	06	16	24	26	22	24	0.81
		01		01			01		01		
		12		08			28		26		
Al' 0914	50	10	11	04	05	16	24	26	22	24	0.81
		01		01			01		01		
		12		06			28		26		
Al'' 0914	50	06	09	04	07	16	22	24	20	22	0.75
		01		01			01		01		
		12		10			26		24		

Table-4

Pollen analysis in the populations of Al 0914, Al' 0914, Al'' 0914

Population	No. of pollen studied	No. of normal pollen	No. of sterile pollen	% of sterile pollen
Al 0914	1104	888	216	19.5
Al' 0914	1000	810	190	17.27
Al'' 0914	1050	910	140	13.33

DISCUSSION

In all the studied populations of *A. longifolia* L. the chromosome number n = 16 was reported (Darlington & Wylie 1955). Different types of chromosomes were observed with structural differences in length and centromere index. The somatic chromosomes count was also reflected as chromosome association in PMCs analysed at metaphase where either sixteen bivalents on a combination of bivalent, univalent, multivalent amounting 32 chromosomes were observed. Meiotic behavior was found to be almost normal. However, some abnormalities like clumping of chromosome, formation of uni and multivalent at metaphase-I and at anaphase-I, clumping of chromosome and simple chromosomal bridges were scored. Half chiasma per chromosome was reported as 0.81µ in AI 0914 and AI' 0914 while it was 0.75 in AI" 0914. Pollen sterility was found to vary from 13.33 to 19.50 per cent (Table-4).

When we look upon the meiotic behaviour of the mentioned populations, we do not find many differences; however, the univalents and multivalents were found in considerable number of pollen mother cells of different populations. Presence of multivalent in the population is indicative of real homology among several pairs of chromosomes of different individuals within the population which have common parents. At the same time occurrence of univalent is the indication of the non-homology between certain chromosomes in the complement. It is possible that gene mutations are responsible for failure of pairing between pairable chromosome. Sometimes one or more bivalents fail to orient on the equatorial plate at metaphase I and remain away from the spindle. All these situations indicate that even within a cell different chromosomes may often have different meiotic rhythms showing lack of co-ordination among themselves. (Bandopadhyay *et al*., 1992).

The difference in chiasma frequency and half chiasma per chromosome provide clues to the nature of structural heterozygosity. It is quite reasonable to believe that the same variety studied meiotically from two localities favours its own form of individuals of a population growing in that particular area and adapted to that locality. Even within one locality, two populations growing in two different seasons have often been found to differ morphologically and in many other ways as revealed from their meiotic patterns (Biswas, 1975). But if the taxa are growing under identical conditions and studied at the same time, meiotic and chiasma differences among them should be taken to indicate intrinsic difference in the chromosome structures of a taxon concerned (Sharan, 2015).

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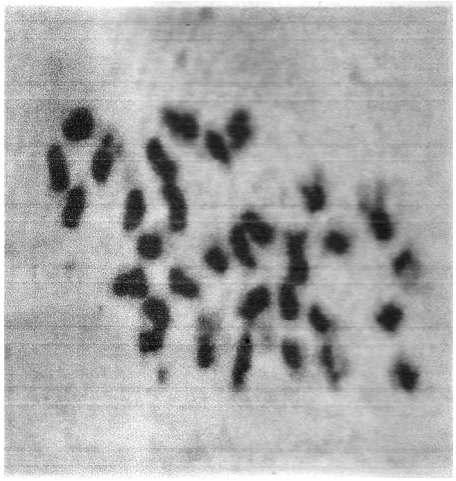

Fig. 1 Mitotic metaphase showing 32 chrmosomes

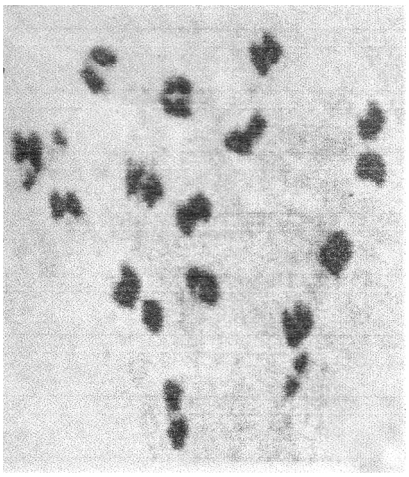

Fig. 2 PMC at late diakinesis showing 16 bivalents

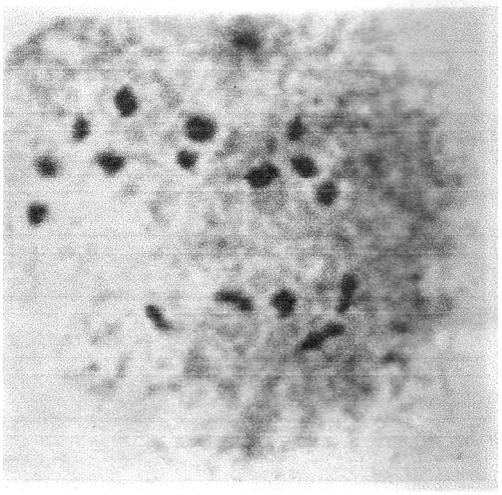

Fig. 3 PMC at metaphase I showing 16 bivalents

Fig. 4 PMC at anaphase showing equal no. of chromosomes

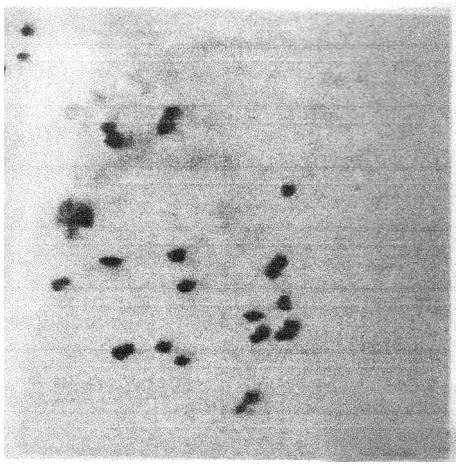

Fig. 5 PMC at metaphase showing scattered mutivalents and univalents

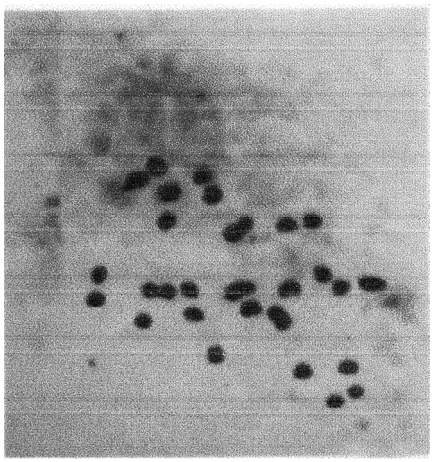

Fig. 6 PMC at metaphase showing bivalents and univalents