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RESEARCH ARTICLE

KARYOMORPHOLOGICAL AND STOMATAL STUDIES OF*ALLIUM HOOKERI*THWAITES

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Abstract

*Alliumhookeri*Thwaites belongs toAmaryllidaceae family is one of the most important medicinal plants, enormously used in cancer or inflammation because it contains large amounts of sulfonylmethane.It is also used to treat coughs, colds, fatigue and to recover immunity. Karyotypic study showed that somatic chromosome number is 2n = 22. Only nearly sub median and nearly median chromosomes are found in the complement. Karyotype formula = 1 nsm(-)+ 10 nm=2n=22. The total length of long arms is 60.36 µm, ranging from 3.16µm to 8.23µm. Total length of short arms is 31.57µm, ranging from 1.75µm to 4.11µm. The total length of the chromosome is 92.17µm, ranging from 4.78µm to 12.35µm. Relative chromosome length ranges from38.70µm to 100 µm. Arm ratio ranges from1.70 to2.38.Tf %= 41.63.Centromeric index ranges from 0.40 to 0.28 and The total chromatin index is 100, ranging from 5.18 to 13.40. Anomocytic type of stomata is observedStomatal index and stomatal size were calculated. The maximum length (53.6±2.06µm) was observed in basal portion of the adaxial surface and the minimum length (45.2 ± 1.40µm) was observed in the middle portion of abaxial surface. The maximum width size (24.8±1.11µm) was observed in the basal portion of the adaxial surface and the minimum width was (18.8 ± 1.14µm) at the middle portion of the abaxial surface. Stomatal index 12.63±0.5) maximum and minimum (6.13±0.40) was observed.

Introduction:-

The study ofchromosomes are considered as an important tool for determining the evolutionary trend phylogeny and systematic position of the related species (Sharma and Sharma, 1959; Stebbins, 1971; Watanabe et al ., 1995; Das et al., 1999; Vanzel et al., 2000; Shan et al., 2003; Sanjaykumar and Tsipila Thonger.,2018). Chromosomes are considered as the most important component of the genetic system of the eukaryotes. For better understanding of any species chromosome number, structure and behavior is determined qualitatively and quantitatively (Naruhashi and Iwatsubo,1991). The chromosomal study are quite helpful in genetic improvement of the crops whether the crops are rare, endangered or threatened species. The research on chromosome provides the basic foundation of the entire discipline of the molecular genetics of the present day world..*Allium hookeri* belongs to family amaryllidaceae (Revised by APG system in 2016 i.e.4th classification till now). Its common name is hooker's chive and is also known as winter leek. Locally it is called as 'Van lahsun'. The plant is native to India, China,Myanmar, Srilanka and

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Bhutan (Sharma et al.,2011) and is enormously used in cancer or inflammation because it contains large amounts of sulfonyl methane(Bae and Bae , 2012).It is also used to treat coughs, colds, fatigue and to recover immunity. This plant has gained significant attention because it produces high amount of phenolic antioxidants, phytosterols, fibres, ascorbic acid, flavonoids and allicin. The chromosome number recorded were 2n= 22 which is regarded as the most common to the best of our knowledge, there is no report on the karyology of this species in Jharkhand. Therefore the aim of present investigation is to provide information on the mitotic chromosome and stomata to understand the detailed cytotaxonomic position of*Allium hookeri*Thwaites.

Material and Method:-

The plant of *Allium hookeri*was collected from Lalkhatanga , Ranchi, Jharkhand which is 20 km away from the main city. Following studies were done on this plant.

Karyotype study

For karyotypic study the root apices of 1-2 cm were cut and pretreated with saturated PDB for 4 hours and fixed in Carnoy's fluid 1 (3:1 ethanol: glacial acetic acid) for 24 hours and preserved in 70% ethanol at 4^0c for future use. The preparation of slide was carried out by hydrolyzing the preserved root tips in 1N Hcl for 10-12 mins and stained in 2% acetocarmine solution (Sharma and Sharma, 1980) and slides wereprepared using squash technique.For analyzing chromosomes a well scattered metaphase stage were selected and observed under compound microscope at a magnification of 10X and 45X and photomicrography was done using DSLR camera.

Karyotype preparation

Following parameters were used for the analysis of karyotype i.e., length of long arm (LA), Short arm(SA) and the Total chromosome length(TL),Total length of long arms (TLLA), Total length of short arms(TLSA) and Total length of the whole chromosomes (TLWC), Relative chromosome length (RCL), Arm Ratio (AR) , Centromeric Index (CI), Chromosome type (CT), Karyotype formula (KF) etc.The chromosomes were classified on various categories following the Kutarekar and Wanjari(1983)classification.Based on the centromeric position the chromosomes are classified into metacentric (M), Submetacentric (Sm) and Subtelocentric (St) having an arm ratio (Long/short) above 0.76, 0.75 to 0.51 and less than 0.50 respectively.On the basis of length the chromosomes were grouped into following categories:-Type A = 11.00µm & above. Type B = 9µm -10.99µ,Type C = 7.00µm -8.99µm, Type D = 5.00µm -6.99µm, Type E = 3.00µm – 4.99µm.The other indices were also used to analyse the karyotype asymmetry such as:-

Detailed Formulae used for calculation of different parameters of *Allium hookeri*Thwaites

Formulae	References
$TF\% = \frac{\text{Totalsumofshortarmlengths}}{\text{Totalsumofchromosomelengths}} \times 100$	Huziwara, 1962
$Ask\% = \frac{\text{Lengthoflongarminchromosomecomplements}}{\text{Totalsumofchromosomelengthinaset}} \times 100$	Arano, 1963 Greilhuber and Septa, 1976
$SYi = \frac{\text{Meanlengthofshortarms}}{\text{Meanlengthoflongarms}} \times 100$	Greilhuber and Septa, 1976
$Rec = \frac{\text{Totalsumlengthofeachchromosome} \quad / \ \text{Longestchromosome}}{\text{Totalnumberofchromosomes}} \times 100$	
$A = \frac{\frac{\Sigma \text{Difference of long and short arms}}{\Sigma \text{Sum of long and short arms}}}{\text{Number of homologus chromosome pairs}} \times 100$	Watanabe et al., 1999
$CG = \frac{\text{Medianlengthofshortarm}}{\text{Medianlengthofchromosome}} \times 100$	Lavania and Srivastava, 1999
$CV = \frac{\text{Standarddeviationofchromosomelength}}{\text{Meanchromosomelength}} \times 100$	Lavania and Srivastava, 1999 Lavania and Srivastava, 1999 Peruzzi and Eroglu, 2013;

Dispersion Index(DI) = $\frac{CG \times CV}{100}$ MCA = A × 100	Peruzzi and Altinordu, 2014
Disparity Index = $\frac{\text{Longestchromosome} - \text{shortestchromosome}}{\text{Longestchromosome} + \text{shortestchromosome}} \times 100$ VRC = Σ Total length of chromosome/n	Mohanty et al, 1991 Dutta and Bandyopadhyaya, 2014

Stebbins classification based on ratio of longest and shortest chromosome and arm ratio of longest and shortest chromosome.

Ratio Longest/shortest chromosome	Proportion of arm ratio of longest and shortest chromosome <2:1			
	1.00	0.99-0.51	0.50-0.01	0.00
<2:1 (A)	1A	2A	3A	4A
2:1-4:1 (B)	1B	2B	3B	4B
>4:1(C)	1C	2C	3C	4C

Stomatal Study

For stomatal studies young and healthy leaves were selected. The leaves were divided into apex, middle and basal portion of both the abaxial and adaxial surface of the leaves. Mechanical peeling was done with the help of sharp razor. Peeled materials were first stained in 1% aqueous safranin solution (Nalawade&Gurav, 2017) and mounted in 5% glycerine (Awasthi et al, 1984). Stomatal index, length and width of the stomata were calculated by ocular micrometer. Calculation of Stomatal index(Salisbury, 1927) was done using following formula:

$$S.I = \frac{S}{E+S} \times 100$$

Where,

S.I = Stomatal index

S = Number of Stomata per unit area

E = Number of epidermal cells in the same unit

Result:-

Karyotype

In this species, the chromosome number was observed to be 2n = 22 chromosomes in which 10 nearly median and 1 nearly sub median chromosomes were observed. The total length of long arms is 60.36 µm, ranging from 3.16µm to 8.23µm. Total length of short arms is 31.57µm, ranging from 1.75µm to 4.11µm. The total length of the chromosome is 92.17µm, ranging from 4.78µm to 12.35µm. Relative chromosome length ranges from38.70µm to 100 µm. Arm ratio ranges from1.70 to2.38.Tf %= 41.63.Centromeric index ranges from 0.40 to 0.28. and The total chromatin index is 100, ranging from 5.18 to 13.40. On the basis of the length, the chromosome were classified into Type A-E (Table: 1), The different types of chromosome categorised on the basis of the length are represented as 1A+3B + 3C + 3D+ 1E = 2n = 22.The karyotypic formula represented as 1nsm(-) + 10 nm =2n =22.On the basis of Stebbins (1971) karyotype asymmetry it was 1B (Table : 2,fig: 2) in this species. The inter and intra chromosomal quantitative asymmetry indices were observed. The value of these parameters were represented as A= 0.32, Ask% = 65.48, Syi=52.38, Rec=67.81, VRC= 8.37, TF%= 41.63, MCA= 2.9, CG= 30.62, CV= 28.67, DI= 25.93, Dispersion Index= 8.78 (Table: 2)

Stomata

The stomatalstudies are depicted in table (3) and in fig (3). Stomatal index and stomatal size of *Alliumhookeri*Thwaites were calculated. The measurement of abaxial and adaxial surface of leaves at the apex, middle and base were observed. The maximum length (53.6±2.06µm) was observed in basal portion of the adaxial surface and the minimum length (45.2 ± 1.40µm) was observed in the middle portion ofabaxial surface. The maximum width size (24.8±1.11µm) was observed in the basal portion of the adaxial surface and the minimum

width was (18.8 ± 1.14µm) at the middle portion of the abaxial surface. Stomatal index 12.63±0.59 which was maximum in the adaxial surface and minimum in the apex portion of theabaxial surface (6.13±0.40).

Table 1:- Karyomorphological data of *Allium hookeri*Thwaites.

Chrom No.	Arm LengthLong(µm)	Arm Length Short(µm)	ChromosomeLength(µm)	Arm Ratio L/S	R.L.	F%	T.C.I	Centromeric index(µm)	Centromeric position	Chromosome type	Nomemclature of chromosome
1	8.23±0.37	4.11±0.26	12.35±0.60	2.03±0.08	100	33.34	13.40	0.33	Nsm(-)	A	Nearly sub median
2	7.20±0.42	3.95±0.13	11.51±0.48	1.89±0.06	97.4	34.37	12.50	0.34	Sm	B	Sub median
3	7.18±0.35	3.59±0.12	10.76±0.40	1.91±0.07	90.9	33.39	11.68	0.33	Sm	B	Sub median
4	6.69±1.05	3.36±0.09	10.04±0.41	2.08±0.11	87.6	33.46	10.90	0.36	Sm	B	Sub median
5	6.35±0.90	2.47±0.11	8.81±0.29	1.90±0.27	80.1	28.04	9.57	0.28	Sm	C	Sub median
6	5.73±0.33	2.76±0.70	8.49±0.42	2.38±0.19	73.8	32.54	9.20	0.32	Sm	C	Sub median
7	4.80±0.33	2.50±0.14	7.30±0.29	2.01±0.21	70.4	34.33	7.92	0.34	Sm	C	Sub median
8	4.25±0.27	2.60±0.20	6.87±0.21	1.76±0.18	66.1	34.97	7.45	0.37	Sm	D	Sub median
9	3.56±0.24	2.30±0.23	5.86±0.28	1.70±0.19	63.3	39.25	6.35	0.39	Sm	D	Sub median
10	3.21±0.23	2.18±0.19	5.40±0.35	1.71±0.20	63.0	40.41	5.85	0.40	Sm	D	Sub median
11	3.16±0.21	1.75±0.15	4.78±0.38	1.81±0.15	58.9	36.62	5.18	0.36	Sm	E	Submedian
Total	60.36	31.57	92.17	21.18			100				

Table 2:-Quantitative inter and intra karyomorphological indices of*Allium hookeri*Thwaites.

A	Ask%	Syi	Rec	T.F%	VRC	CV	CG	DI	Dis I	MCA	Stebbins karyotype asymmetry
0.029	65.48	52.38	67.81	41.63	8.37	28.67	30.62	25.93	8.78	2.9	1B

Asymmetry indices = (Syi, Rec and TF%)
TF% = Total Form%
VRC = Value of Relative Chromatin
CV = Centromeric Variation
CG = Centromeric Gradient
DI = Dispersion Index
Dis.I = Disparity Index
MCA = Mean Centromeric asymmetry

Table 3:-Stomatal index (%), Length and Width of stomata (in µm) in Abaxial and Adaxial leaf surfaces of *Alliumhookeri*Thwaites.

ra ce of L aa	Apex portion of Leaf	Middle portion of Leaf	Base portion of leaf

	S. I.	Length (µm)	Width (µm)	S. I.	Length (µm)	Width (µm)	S. I.	Length (µm)	Width (µm)
Abaxial surface	11.49 ± 0.68	45.6 ± 1.72	19.6 ± 0.89	11.05± 0.76	45.2 ± 1.40	18.8 ± 1.14	6.13± 0.40	48.4 ± 1.44	20.0 ± 0.98
Adaxial surface	9.34±0. 48	47.6±2.0 1	20.8±0. 95	12.63±0 .59	47.6±2.3 1	22±1.30	9.16±0. 65	53.6±2.0 6	24.8±1. 11

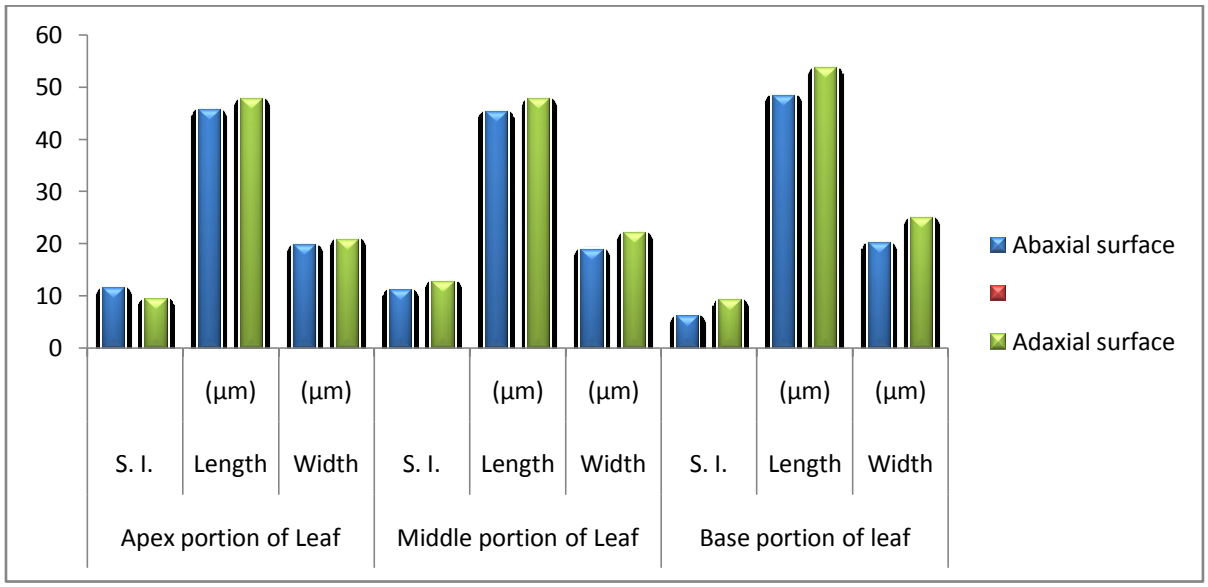
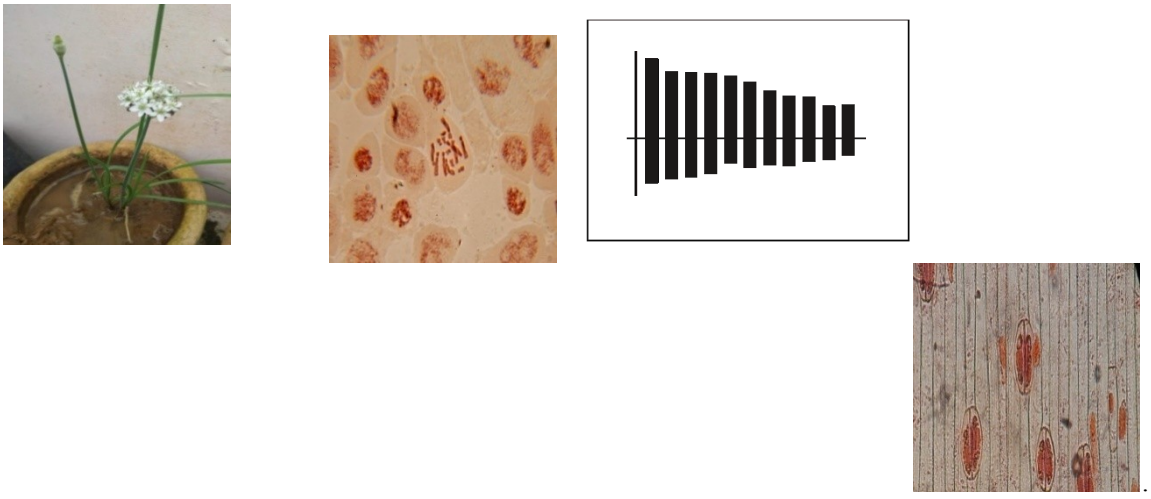


Fig: 1:- Column graph showing Stomatal index, Length and Width of *Allium hookeri* Thwaites.



a b c d

Fig 2:-a-photograph of plant of Allium hookeri, **b-** photo micrograph of mitotic metaphase ,**c-**idiogram**d**photo micrograph of stomata

Discussion:-

Karyotype

The chromosome number of*Allium hookeri* was 2n = 22 which matches with the earlier reports of (Bhaben- Tanti-2013; Sen, 1974; Jha and Jha,1989; Yi-Xiang et mal., 1990; Rui-Fu et al., 1996). On the basis of the centromericposition,the chromosome may be classified into metacentric,Sub-metacentric, sub-telocentric and telocentric (Levan et al.,1964; Tanti et al., 2009: Tanti et al., 2012).In the present investigation karyotypic formula deduced as 1 nsm(-)+ 10 nm=2n=22But earlier report of Sen (1974) showed 3m+9sm+10st=2n=22.(Sharma et al.,2010) showed 2m+13sm+7st=2n=22.The study of number of chromosomes and karyomorphological characters are helpful in the identification of any species or variety and are also helpful in establishing the relationships among related species (Lavania and Srivastava, 1999, Liu et al 2009, Frame.,2001; Sharma and Sen,2002; Salimuddin and Ramesh, 2005; Yiang et al ,2006).In the present finding the Stebbins chromosomal asymmetry was of 1B type. Earlier workers have reported 2B and 3B type of Stebbins karyotype asymmetry (Dutta and Bandyopadhyaya, 2014; Sharma et al., 2011). The species consists of 1nearly sub median and 10 nearly median chromosomes. so it is considered as asymmetrical (Stebbins,1971).

Stomata

Anomocytic type of stomata was reported in the above species (Stebbins &Khush, 1961). Stomatal characters are used as an important tool in distinguishing medicinal plant (Johr A, 2013). Stomatal function is important in controlling physiological process such as photosynthesis and transpiration.

Conclusion:-

Karyotypic and stomatal study of present investigation will be helpful in understanding the number and morphology of chromosomes which proved quite helpful incyto-taxonomy and are beneficial for further research in cytogenetics.

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