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Karyomorphology of Five *Allium* species from Nagaland, North-Eastern Region of India

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Abstract

The *Allium* species {*A. chinense* (2n=4x=32), *A. tuberosum* (2n=4x=32), *A. hookeri* (2n=22), *A. ascalonicum* (2n=2x=16) and *A. sativum* (2n=2x=16)} with basic chromosome number (x= 8) were compared karyomorphologically using different quantitative and asymmetry parameters. The total sum of long arm (Σq) was observed high (61.70 µm) in *A. hookeri* followed by *A. ascalonicum* (58.87µm), *A. chinense* (58.44µm), *A. tuberosum* (57.87µm) and *A. sativum* (56.78µm), and an exact reverse trend was observed for total sum of short arm (Σp). The maximum mean value of arm ratio was observed in *A. hookeri* (1.75±0.144), covariance total chromosome length (A_2=CV_{CL}) in *A. tuberosum* (31.66), mean centromere asymmetry (M_{CA}) in *A. ascalonicum* (1.10), and covariance centromere index (CV_{CI}) in *A. hookeri* (21.10). The value of relative chromatin (VRC or ACL) was observed similar in tetraploids and diploids. Pearson correlation ($p \leq 0.05$ and $p \leq 0.01$), PcoA and cluster analysis showed the strong interrelationship of studied parameters among the *Allium* species. The karyotic formula (KF) and chromosome categorization (on the basis of chromosome length) was drawn for the *Allium* species (*A. chinense, A. tuberosum, A. hookeri, A. ascalonicum* and *A. sativum*) as follows 26m+5sm+1st (B_7+C_{19}+D_6), 25m+5sm+2st (B_{13}+C_{19}), 12m+9sm+1st (C_{19}+D_3), 12m+3sm+1st (C_{13}+D_3) and 14m+2sm (A_1+B_9+C_6), respectively. Stebbin's classification showed 2A and 1B type of chromosomal asymmetry among *Allium* species.

Key words: Karyotypic Formula, Principle Coordinate Analysis, Cluster Analysis, Inter or Intra Chromosomal Analysis, Stebbins Classification, *A. chinense, A. tuberosum, A. hookeri, A. ascalonicum, A. sativum.*

1. Introduction

The importance of chromosome investigation for basic dissimilarity has been transformed in modern periods. The qualitative or quantitative explanation of chromosome structure has been merged with molecular techniques for a better understanding of the structure, number and behaviour of chromosomes in an organism (genus or species. The interdisciplinary research approach of chromosome has revealed the possible types of karyotypic variation (within and between), systematic relationships, phylogeny and evolution of the related taxa.

The chromosomal symmetry or asymmetry leads to the symmetric or asymmetric differences in the genomic content of an individual and vice-versa. Therefore, the chromosome morphology (or chromosome karyotypes/ idiograms) is an important tool to establish uniqueness among the plant or animal species. The unique quality of a plant or animal species may be improved for various needs through a hybridization program. There is a need to know the chromosome number and structure of every possible organism {especially crops and Rare, Endangered and Threatened (RET) species} for genetic improvement by development of hybridization program (conventional as well as molecular) where both chromosome number and structure can be manipulated.

Allium chinense and *A. bakeri* Regel are known as synonyms to each other and both belong to the Alliaceae family (Bah *et al*., 2012; Allardice, 1997). It has been reported that *A. chinense* supports sub-genus *cepa* in the section of saccuniferum (Dutta and Bandyopadhyaya, 2014). It has been reported that *A. chinense* is a tetraploid (2n=4x=32) plant but some other plants with deviation in chromosome numbers (2n=3x=24; 2n=24 and 2n=33) were also reported (Dubouzet *et al*., 1993; Gohil and Kaul, 1980). Mukherjee and Roy (2012) reported that *A. tuberosum* is a tetraploid (2n=4x=32) plant. *A. hookeri* (subgenus *Amerallium*) is an important member of family Alliaceae. *A. hookeri* recorded chromosome number 2n=22, which is the most common, except for a few (33 and 44 chromosome number) as reported from Yunnan (Sen, 1974; Jha and Jha, 1989; Yi-Xiang *et al*., 1990; Rui-Fu *et al*., 1996). Both *A. ascalonicum* and *A. sativum* were reported as diploid (2n=2x=16) species.

Although very few studies are found on the karyomorphology (not from the Nagaland) of the *A. hookeri* (Ved Brat, 1965; Sharma *et al*., 2011; Toijam *et al*., 2013), *A. tuberosum* (Mukherjee and Roy, 2012; Ramesh, 2015) and *A. sativum* (Konvicka and Levan, 1972), we did not come across reports on *A. chinense* and *A. ascalonicum* from Nagaland as well as adjoining North-Eastern region of India and at National level. The present paper aims to conduct a karyomorphological study of five

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Allium species (*A. chinense, A. tuberosum, A. hookeri, A. ascalonicum* and *A. sativum*), collected from the different parts of the Nagaland, India which may provide additional information to the published data on the *Allium* karyomorphology at world, national or regional levels.

2. Materials and Method

Bulbs of *Allium* species (*A. chinense, A. tuberosum, A. hookeri, A. ascalonicum* and *A. sativum*) were collected from the different parts of the Nagaland and maintained in the Department for root tips. The chromosomal analysis was done according to the conventional root tip squash method (Sharma and Sharma, 1980). The root tips were pre-treated with saturated PDB for 3 h then fixed in carnoy's 1 (3:1 ethanol: glacial acetic acid) solution for 24 h and stored in preservative (70% v/v ethanol) at 4^0C for further use.

2.1. Preparation of slides

Each root tip was washed with distilled water (5 min) and then treated with 1N HCl (15 min). The hydrolysed root tips washed repeatedly with distilled water and stained (5 min) with acetocarmin (2% w/v) and then squashed. Approximately, 10 slides were analysed for each species and the best three slides were observed for number, size and morphology of the chromosomes. The metaphase stages were photographed by Leica digital microscope. The SPSS ver. 16 and ImageJ was used to analyse and measure the long and short arms (µm) of chromosomes and idiograms were prepared.The chromosome classification was done according to the Levan *et al.* (1964).

2.2. Karyotype variation study

The following are the different parameters used to study the karyotypic variations: chromosome number (2n), total chromosome length (TCL), basic chromosome number (x), total haploid chromosome length (THL), mean centromere asymmetry (MCA), covariance of centromere index (CV_{CI}), covariance of total chromosome length (CV_{CL}), mean (q_{Mean}) and summation (Σq) of long arm (q), mean (p_{Mean}) and summation (Σp) of short arm (p), mean arm ratio (AR_{Mean}), mean (RCL_{Mean}) and summation (ΣRCL) of relative chromosome length (RCL), average chromosome length (ACL), mean ($p+q_{Mean}$), summation (Σp+q), difference summation (Σp-q), standard deviation ($p+q_{S.D.}$), variance (V_{p+q}) and covariance (CV_{p+q}) of total chromosome length (p+q), mean (CI_{Mean}) and standard deviation ($CI_{S.D.}$) of centromeric index (CI), karyotypic formula (KF), chromosome categorization and Stebbins classification.

The other indices were also used to analyse the karyotype asymmetry, such as A, A_1, A_2, AI, AsK%, SYi, Rec, TF%, Value of Relative Chromatin (VRC), Centromeric Gradient (CG), Dispersion Index (DI) and Disparity Index (Dis. I).

The detailed formulas for calculations of the different parameters are presented in the form of a table (Table 1)

Table 1. Detailed Formulae used for calculation of different parameters of *Allium* species

Formulae	References
$\mathbf{TF\% = \frac{Total\ sum\ of\ short\ arm\ lengths}{Total\ sum\ of\ chromosome\ lengths} \times 100}$	Huziwara, 1962
$\mathbf{AsK\% = \frac{Length\ of\ long\ arm\ in\ chromosome\ complements}{Total\ sum\ of\ chromosome\ length\ in\ a\ set} \times 100}$	Arano, 1963
$\mathbf{SYi = \frac{Mean\ length\ of\ short\ arms}{Mean\ length\ of\ long\ arms} \times 100}$	Greilhuber and Speta, 1976
$\mathbf{Rec = \frac{Total\ sum\ length\ of\ each\ chromosome/\ Longest\ chromosome}{Total\ number\ of\ chromosomes} \times 100}$	Greilhuber and Speta, 1976
$\mathbf{A1 = 1 - \frac{\Sigma Mean\ length\ of\ short\ arms/\Sigma Mean\ long\ arms\ of\ each\ chromosome\ pair}{Number\ of\ homologous\ chromosome\ pairs} \times 100}$	Romero-Zarco, 1986
$\mathbf{A2 = \frac{Standard\ deviation\ of\ chromosome\ length}{Mean\ chromosome\ length} \times 100}$	Romero-Zarco, 1986
$\mathbf{A = \frac{\Sigma\ Difference\ of\ long\ and\ short\ arms}{\Sigma Sum\ of\ long\ and\ short\ arms} / Number\ of\ homologous\ chromosome\ pairs}$	Watanabe *et al*., 1999
$\mathbf{AI = \frac{Covariance\ of\ chromosome\ length \times Covariance\ of\ centromeric\ index}{100}}$	Arano and Saito, 1980
$\mathbf{CVCL = A2 \times 100 = \frac{Standard\ deviation\ of\ chromosome\ length}{Mean\ chromosome\ length} \times 100}$	Arano and Saito, 1980
$\mathbf{CVCI = \frac{Standard\ deviation\ of\ centromeric\ index}{Mean\ centromeric\ index} \times 100}$	Arano and Saito, 1980
$\mathbf{CG = \frac{Median\ length\ of\ short\ arm}{Median\ length\ of\ chromosome} \times 100}$	Lavania and Srivastava, 1999
$\mathbf{CV = \frac{Standard\ deviation\ of\ chromosome\ length}{Mean\ chromosome\ length} \times 100}$	Lavania and Srivastava, 1999
$\mathbf{Dispersion\ Index\ (DI) = \frac{CG \times CV}{100}}$	Lavania and Srivastava, 1999
$\mathbf{MCA = A \times 100}$	Peruzzi and Eroglu, 2013; Peruzzi and Altinordu, 2014
$\mathbf{Disparity\ index\ (Dis.I) = \frac{Longest\ chromosome - Shortest\ chromosome}{Longest\ chromosome + Shortest\ chromosome} \times 100}$	Mohanty *et al*., 1991
$\mathbf{VRC = \Sigma Total\ Length\ of\ chromosomes/n}$	Dutta and Bandyopadhyaya, 2014

2.3. Chromosome categorization

Chromosomes were categorized on the basis of their length as follows: Type A=5.00µm and above, Type B=4.00µm-4.99µm, Type C=3.00µm-3.99µm, Type D=2.00µm-2.99µm, Type E=1.00µm-1.99µm, and Type F=0.99µm and below.

3. Results

The *Allium* species were collected locally from the different regions of the Nagaland (North Eastern region of India) and the chromosome number from mitotic metaphase images and karyomorphology (karyotype and idiogram) were studied (Figure 1).

The quantitative parameters, such as Chromosome Number, CN (2n=2x), mean length and summation (Σ) of short arm (p), mean length and summation (Σ) of long arm (q), mean Arm Ratio (AR), Average Chromosome Length (ACL), mean and summation (Σ) Relative Chromosome Length (RCL) of *Allium* species (*A. chinense*, *A. tuberosum*, *A. hookeri*, *A. ascalonicum* and *A. sativum*), were analysed and reported in Table 2. The quantitative parameters, such as mean, Standard Deviation (SD), Variance (V), Covariance (CV) and summation (Σ) of total chromosome length (p+q), summation (Σ) of difference between short and long arm (p-q), mean and Standard Deviation (SD) of Centromeric Index (CI), Karyotypic Formula (KF), THL and chromosome categorization, were recorded and presented in Table 3. The inter- and intra-chromosomal quantitative asymmetric indices were calculated and presented for all *Allium* species in Table 4. The Pearson correlation between the inter and intra chromosomal asymmetry indices was performed and the indices, such as A_2, AI, SYi, TF%, CG, Dispersion index and Disparity index showed negative correlation and the indices AsK%, Rec, VRC, CV_{CI} showed positive correlation but not significant for all the indices (Table 5). The Stebbins classification, based on the ratio of longest and shortest chromosome and the proportion of their arm ratio, was provided (Table 6), and, based on that, 2A type of karyotype asymmetry was observed in all the species except *A. ascalonicum* (Table 7).

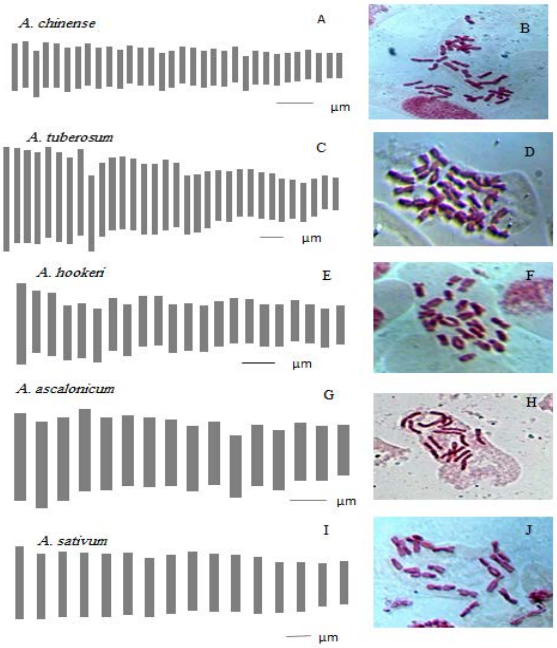

Figure 1. Idiograms (a,c,e,g,i) and Mitotic metaphase (b,d,f,h,j). A) *A. chinense*, B) *A. tuberosum*, C) *A. hookeri*, D) *A. ascalonicum*, E) *A. sativum*

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Table 2. Quantitative karyomorpho-parameters of *Allium* species.

Allium species	CN	BCN (x)	$p_{(mean±S.E.)}$	$q_{(mean±S.E.)}$	Σp	Σq	AR $_{(Mean±S.E.)}$	$RCL_{(mean±S.E.)}$	ΣRCL	ACL
chinense	32	8	1.29±0.053	1.82±0.059	41.58	58.44	1.47±0.082	0.72±0.031	23.14	3.12
tuberosum	32	8	1.31±0.093	1.80±0.097	42.11	57.87	1.50±0.105	0.72±0.036	22.50	3.12
hookeri	22	11	1.74±0.119	2.80±0.129	38.33	61.70	1.75±0.144	0.63±0.044	14.01	4.54
ascalonicum	16	8	2.57±0.150	3.67±0.194	41.12	58.87	1.522±0.150	0.68±0.065	10.89	6.24
sativum	16	8	2.70±0.127	3.54±0.114	43.23	56.78	1.33±0.052	0.75±0.028	12.10	6.25

Table 3. Quantitative karyomorpho-parameters of *Allium* species (Continued).

Allium species	$p+q_{(mean±S.E.)}$	p+q (S.D.)	Σ(p+q)	THL	Σ(p-q)	$V_{(p+q)}$	$CV_{(p+q)}$	CI $_{(Mean±S.E.)}$	CI(S.D.)	KF	Chromosomes category
chinense	3.12±0.087	0.495	99.97	49.985	16.72	0.246	15.87	41.53±1.119	6.334	26m+5sm+1st	$B_7+C_{19}+D_6$
tuberosum	3.12±0.174	0.988	100.02	50.01	15.60	0.976	31.61	41.48±1.324	7.491	25m+5sm+2st	$B_{13}+C_{19}$
hookeri	4.54±0.189	0.891	100	50.00	22.47	0.794	19.62	38.16±1.717	8.053	12m+9sm+1st	$C_{19}+D_3$
ascalonicum	6.25±0.263	1.054	99.99	49.995	17.75	1.112	16.87	41.17±1.807	7.230	12m+3sm+1st	$C_{13}+D_3$
sativum	6.25±0.219	0.877	100	50.00	13.07	0.770	14.03	43.03±0.922	3.689	14m+2sm	$A_1+B_9+C_6$

Table 4. Quantitative inter and intra karyomorphological indices of *Allium* species.

Allium species	Inter and Intrachromosomal quantitative asymmetric indices													
	A	A_1	$A_2=CV_{CL}$	AI	AsK% $_{(Mean±S.E.)}$	SYi	Rec	TF%	VRC	CV_{CI}	CG	DisI	DispI	M_{CA}
chinense	0.005	95.60	15.86	2.418	58.50±1.125	70.87	77.90	41.51	3.12	15.25	41.44	6.57	30.40	0.50
tuberosum	0.004	95.71	31.66	5.714	58.59±1.359	72.77	62.13	42.10	3.12	18.05	41.25	13.05	51.73	0.40
hookeri	0.010	92.70	19.62	4.139	61.85±1.710	62.14	61.59	38.33	4.54	21.10	28.92	5.67	35.41	1.00
ascalonicum	0.011	91.10	16.86	2.960	58.80±1.808	70.02	78.21	41.12	6.24	17.56	43.31	7.30	26.82	1.10
sativum	0.008	91.81	14.03	1.202	56.96±0.926	76.27	79.82	43.23	6.25	08.57	43.75	6.13	25.78	0.80

Table 5. Pearson correlation among the different quantitative chromosomal asymmetry indices.

	A	A_1	A_2=CV_{CL}	AI	AsK%	SYi	Rec	TF%	VRC	CV_{CI}	CG	Dispersion Index	Disparity Index	M_{CA}
A	1	-0.922*	-0.527	-0.304	0.373	-0.449	0.173	-0.468	0.800	0.159	-0.295	-0.628	-0.610	1.000**
A_1		1	0.575	0.485	-0.022	0.086	-0.369	0.111	-0.969**	0.192	0.004	0.565	0.677	-0.922*
A_2			1	0.931*	0.177	-0.034	-0.778	-0.028	-0.591	0.489	-0.118	0.922*	0.983**	-0.527
AI				1	0.504	-0.385	-0.887*	-0.378	-0.589	0.772	-0.392	0.751	0.918*	-0.304
AsK%					1	-0.985**	-0.696	-0.987**	-0.224	0.863	-0.940*	-0.191	0.222	0.373
SYi						1	0.566	0.999**	0.164	-0.835	0.905*	0.315	-0.076	-0.449
Rec							1	0.569	0.513	-0.727	0.711	-0.475	-0.819	0.173
TF%								1	0.139	-0.826	0.912*	0.323	-0.069	-0.468
VRC									1	-0.407	0.218	-0.496	-0.700	0.800
CV_{CI}										1	-0.672	0.219	0.483	0.159
CG											1	0.274	-0.204	-0.295
Dispersion Index												1	0.873	-0.628
Disparity Index													1	-0.610
M_{CA}														1

Table 6. 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 chromosome and shortest chromosome <2:1			
		1.00 (1)	0.99-0.51 (2)	0.50-0.01 (3)	0.00 (4)
<2:1	(A)	1A	2A	3A	4A
2:1-4:1	(B)	1B	2B	3B	4B
>4:1	(C)	1C	2C	3C	4C

Table 7. Karyotype asymmetry in *Allium* species based on Stebbins classification.

Allium species	Ratio longest/shortest chromosome	Proportion of arm ratio of longest chromosome and shortest chromosome	Stebbins karyotype asymmetry
chinense	1.72	0.74	2A
tuberosum	1.57	0.87	2A
hookeri	1.40	0.88	2A
ascalonicum	1.39	1.94	1B
sativum	1.39	0.80	2A

Recently, statistically correct six parameters (2n, x, THL, M_{CA}, CV_{CL} and CV_{CI}) have been suggested to analyse principle coordinates (PcoA) and chromosome asymmetry. In the present study, we used seven parameters including Total Chromosome Length (TCL) in the earlier parameters to analyse PcoA and phylogram (UPGMA) (Figures 2-3). The inter- (CV_{CL}) and intra- (M_{CA}) chromosomal asymmetry were performed and reported (Figure 4).

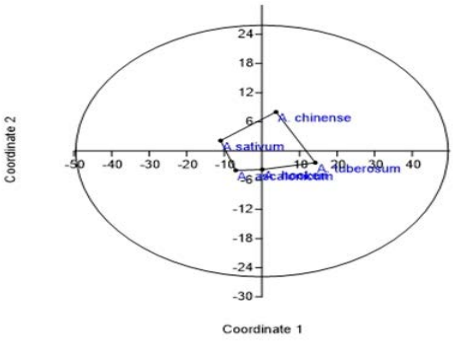

Figure 2. Principle coordinates analysis (PcoA) using six parameters among *Allium* species.

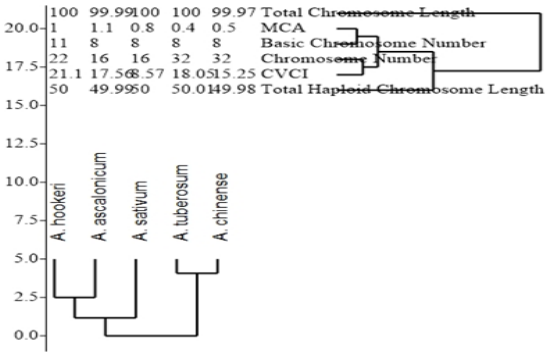

Figure 3. Two Way Euclidean Paired Group Cluster Analysis using six parameters among Allium species.

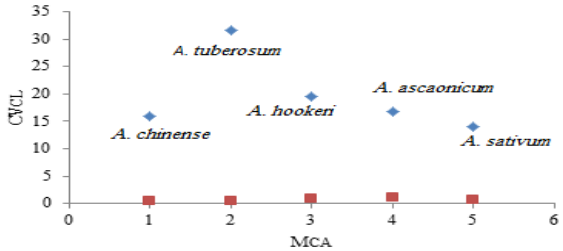

Figure 4. Quantitative inter and intra chromosomal asymmetry among the *Allium* species.

4. Discussion

The chromosome count of *Allium* species was similar as reported by the other studies (Dutta and Bandyopadhyaya, 2014; Mukherjee and Roy, 2012; Sharma *et al*., 2011). Some other studies suggested different chromosome count for presented species (Dubouzet *et al*., 1993; Gohil and Kaul, 1980; Sen, 1974; Jha and Jha, 1989; Yi-Xiang *et al*., 1990; Rui-Fu *et al*., 1996; Sharma and Gohil, 2013; Gohil and Koul, 1973; Talukdar and Sen, 2000). Therefore, doubt continues to remain for the chromosome count in the species analyzed as well as many other *Allium* species (Figure 1).

The mean short arm (p_{mean}) and long arm (q_{mean}) observed maximum in *A. ascalonicum* and *A. sativum*, on the other hand, total sum of long arm (Σq) and Arm Ratio (AR) was maximum in *A. hookeri* which suggests that chromosomes of *A. hookeri* are longer than others. The Average Chromosome Length (ACL) and Value of Relative Chromatin (VRC) are exactly similar and increases from polyploidy to *A. hookeri* to diploids. It may suggest the origin and speciation of the species from diploids to *A. hookeri* to ploids which was supported by the phylogram of the species (Table 2).

The total mean chromosome length ($p+q_{mean\pm S.E.}$) of *Allium* species (*A. chinense* and *A. tuberosum*) and (*A. ascalonicum* and *A. sativum*) was recorded similar (3.12±0.087 and ±0.174) and (6.25±0.263 and ±0.219), respectively. The earlier species were tetraploid and, later, diploid. The mean chromosome length was high for *A. ascalonicum* (2n=16) and *A. sativum* (2n=16) than *A. hookeri* (2n=22), A. *chinense* (2n=32) and *A. tuberosum* (2n=32). It suggests that the diploid species (*A. ascalonicum* and *A. sativum*) have more compact and larger chromatin and may be involved in the formation of the tetraploids (*A. chinense* and *A. tuberosum*). The diploid and polyploidy *Allium* species might have taken the same evolutionary process during the evolution in time and space. The maximum variance (V_{p+q}) and covariance (CV_{p+q}) in chromatin length were observed in *A. ascalonicum* and *A. hookeri*, respectively. *A. ascalonicum* showed more variations between the chromosomes while *A. hookeri* varied within the chromosomes. The mean centromeric index (CI) was recorded 43.03±0.922 (*A. sativum*), 41.53±1.119 (*A. chinense*), 41.48±1.324 (*A. tuberosum*), 41.17±1.807 (*A. ascalonicum*) and 38.16±1.717 (*A. hookeri*), respectively. The high centromeric index suggests that most of the chromosomes are in median region as the chromosomal arms are not exactly equal to make strict metacentric chromosomes (M). The position of centromere is variable in chromosomal arm which depends on the centromeric index of chromosome and suggest the symmetry or asymmetry among the chromosomes. The *Allium* species were recorded with sub-telocentric chromosomal region (st) (centromere near to the terminal region of the chromosome arm) except *A. sativum*. The Karyotypic Formula (KF) and chromosome categorization (on the basis of chromosome length) were drawn for the *Allium* species (*A. chinense, A. tuberosum, A. hookeri, A. ascalonicum* and *A. sativum*) as follows 26m+5sm+1st ($B_7+C_{19}+D_6$), 25m+5sm+2st ($B_{13}+C_{19}$), 12m+9sm+1st ($C_{19}+D_3$), 12m+3sm+1st ($C_{13}+D_3$) and 14m+2sm ($A_1+B_9+C_6$), respectively. The method of measurement of chromosome arms may affect the karyotype asymmetry or symmetry. The chromosomal categorization suggested that *A. sativum* (2n=2x=16) shared its maximum genome with the tetraploids, *A. chinense* (2n=4x=32) and *A. tuberosum* (2n=4x=32) while *A. ascalonicum* and *A. hookeri* shared their maximum genome with *A. chinense* (2n=4x=32) (Table 3).

The inter- or intra-chromosomal asymmetry may be measured from the shifting of centromeric position from median to subterminal or it may be the difference in relative size between the individual chromosome. Stebbins (1971) classified the chromosomal asymmetry on the basis of variation in chromosome length and centromeric position. The higher value of the indices suggested the more asymmetric chromosome complement, while the lower value indicates towards less asymmetric or more symmetric chromosome complement.

The intra-chromosomal asymmetry (A_1) was recorded maximum in *A. tuberosum* and *A. chinense* followed by *A. hookeri*, *A. sativum* and *A. ascalonicum*. The approximate similar chromosomal asymmetry between (*A. tuberosum* and *A. chinense*) and (*A. sativum* and *A. ascalonicum*) indicates the similar genome size or chromosome numbers. The inter-chromosomal asymmetry (A_2) was recorded with maximum chromosome variation in *A. tuberosum* followed by *A. hookeri*, *A. ascalonicum*, *A. chinense* and *A. sativum*. The covariance of the total chromosome length which is a variation within the chromosome of a complement recorded maximum for the *A. tuberosum* with maximum variable chromosomes than others. The measurement of the chromosomal variation with other species (A_2) did not follow the pattern of chromosomal variation within the same species (A_1). The asymmetry index (AI) of chromosomes of a species exactly followed the chromosomal variation with other species (A_2). It also suggests that the total asymmetry of chromosomes of a species is the measure of the covariance of the total chromosome length of a species. Also, the asymmetry indices (SYi, Rec and TF%) provides an average degree of symmetry over whole karyotype of a species. The Value of Relative Chromatin (VRC) ranged from 3.12-6.25µm in all the species, which is very little as compared to the earlier reports in *A. chinense* (27.38 and 26.89) and *A. tuberosum* (26.31 and 26.03) (Dutta and Bandyopadhyaya, 2014) (Table 4).

In the present study, the index A_1 and M_{CA} showed highly negative correlation (-0.922*) and perfect positive correlation (1.000**) with the index A at $p\leq0.05$ and $p\leq0.01$, respectively. The intra-chromosomal asymmetry index may not be dependent on the centromeric asymmetric position variation; the inter-chromosomal asymmetry index (A), however, may be fully or partially dependent on the centromeric position variation in a chromosome. The index A_1 showed highly negative correlation with VRC (-0.969**) and M_{CA} (-0.922*) at $p\leq0.01$ and $p\leq0.05$, respectively. It suggests that A_1 does not dependent on the VRC and M_{CA} for the chromosomal asymmetry. The index A_2 showed highly positive correlation with AI (0.931*), dispersion index (0.922*) and disparity index (0.983**) at $p\leq0.05$ and $p\leq0.01$, respectively. AI showed highly negative (-0.887*) and positive (0.918*) correlation with Rec and disparity index

at $p \leq 0.05$, respectively. AsK% showed highly negative correlation with SYi (-0.985**), TF% (-0.987**) and CG (-0.940*) at $p \leq 0.01$ and $p \leq 0.05$, respectively. SYi showed highly positive correlation with TF% (0.999**) and CG (0.905*) at $p \leq 0.01$ and $p \leq 0.05$, respectively. TF% showed highly positive correlation with CG (0.912*) at $p \leq 0.05$. It was reported that the indices TF% and AsK% perfectly positive or negative correlated with the index SYi. The present results suggested that indices TF% and AsK% highly positive (0.999**) and negative (-0.985**) correlated with SYi which agrees with other authors (Paszko, 2006; Peruzzi *et al*., 2009) (Table 5).

The Stebbins classification, based on the ratio of longest and shortest chromosome and the proportion of their arm ratio, was provided (Table 6) and, based on that, ratio (1.72, 1.52, 1.40, 1.39 and 1.39) and proportion of their arm ratio (0.74, 0.87, 0.88, 1.94 and 0.80), among the Allium species (*A. chinense*, *A. tuberosum*, *A. hookeri*, *A. ascalonicum* and *A. sativum*), were recorded respectively, and 2A type of karyotype asymmetry was observed in all the species except *A. ascalonicum* (Table 7). The Stebbins chromosomal asymmetry (2A) for *A. chinense* in present study supported the earlier reports of *A. chinense* collected from the other parts of North Eastern region (Shillong, Meghalaya) as well as the rest of India (Dutta and Bandyopadhyaya, 2014). The resemblance of karyotype asymmetry may be due to similar type of geographical and climatic conditions in Meghalaya and Nagaland as both are hilly states and near to each other. The earlier reports on *A. tuberosum* (collected from Kolkata, India) and *A. hookeri* (Darjeeling, West Bengal and NBPGR, Uttarakhand) suggested 2B and 3B type of Stebbins karyotype asymmetry, but in present result it showed 2A type of karyotype asymmetry in both the cases (Dutta and Bandyopadhyaya, 2014; Sharma *et al*., 2011). The difference in the karyotype asymmetry may be because of the distance factor in collection site, climate conditions and growth factor of States Kolkata, West Bengal and Uttarakhand which are very far from each other. *A. ascalonicum* and *A.sativum* showed 1B and 2A type of karyotype asymmetry, respectively.

The karyotypic formula, Stebbins classification and value of relative chromatin may be different in the species because of the different methods and application used to measure long and short arm of the chromosomes.

Recently, it has been reported that the karyological characters should be described by quantitative parameters which are statistically correct and without redundancy (Peruzzi *et al*., 2009). The quantitative parameters, such as chromosome number (2n), basic chromosome number (x), Total Haploid Length (THL) of the chromosome (rough estimation of genome size), mean centromeric asymmetry (M_{CA}), covariance of total chromosome length (CV_{CL}) and covariance of centromeric index (CV_{CI}), were suggested for karyomorphological calculation and its study. The suggested parameters were statistically correct and measures three different features of a karyotype without redundancy. The quantitative parameters measure the intra-chromosomal variation (M_{CA}), heterogeneity in the centromere position (CV_{CI}) and inter-chromosomal variation or asymmetry (CV_{CL}).

In present study, we used seven parameters including Total Chromosome Length (TCL) in the earlier parameters to analyse PcoA, phylogram (UPGMA), and inter- and intra-chromosomal asymmetry. The seven parameters as suggested (including TCL) were used to locate the ordinates on the x and y axis of principle coordinates (PcoA) of the five *Allium* species. All the *Allium* species were well distributed in all the quadrates of x and y axis. The distribution indicated that the taken species are not redundant and belong to different species; they also differ karyomorphologically (Figure 2). The same parameters were also used to draw the phylogram (UPGMA) and *A. ascalonicum* and *A. sativum* grouped or placed together in the phylogram. It seems that other species evolved, diverged and speculated from them in time and space (Fig. 3). The covariance of the chromosome length (CV_{CL}) was compared with mean centromeric asymmetry (M_{CA}) and suggested a variation in the species from each other. Intra-chromosomal variation was observed but the centromere variation seems to be near to the axis (Figure 4).

5. Conclusion

The other *Allium* species, such as *A. wallichii* Kunth. (2n=2x=16), *A. roylei* Stearn (2n=2x=16), *A. ampeloprasum* L. (2n=2x=16), *A. schoenoprasum* L. (2n=2x=16), *A. cepa* var. *cepa* Helm. (2n=2x=16), *A. cepa* var. *aggregatum* G. Don (2n=2x=16), *A. fistulosum* L. (2n=2x=16), *A. prattii* Wight (2n=2x=16), *A. stracheyi* Baker (2n=2x=14), *A. macranthum* Baker (2n=4x=28), *A. cepa* var. *viviparum* (Metzger) Alefeld (2n=3x=24; $8^{II}+8^{I}$), *A. porrum* L. (2n=4x=32) and *A. griffithianum* Boiss. Syn. *A. rubellum* M. Bieb. (2n=4x=32), has been observed around the North-Eastern region as well as Eastern Himalaya of the Indian sub-continent; therefore, it may be suggested that *Allium* species may be collected, maintained and preserved in these regions to be scientifically identified at molecular level to reduce the chance of misidentification and redundancy of the species.

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