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# Evaluation of genetic variation among three species of *Allium* on the basis of karyomorphology and SDS-PAGE profiling

Subhas Chandra Roy* and Tshering O Bhutia
Plant Genetics & Tissue Culture Laboratory Department of Botany, University of North Bengal, PO- NBU, Siliguri-734013 WB, India

**Abstract**
Determining the base number, ploidy level and type of ploidy are important aspects in the cytogenetic study of a species. Genetic variation among the three species of the genus *Allium* (*A. cepa*, *A. sativum* and *A. hookeri*) has been carried out on the bases of chromosomal karyotype and protein banding patterns on SDS-PAGE. Protein profiling was prepared from the observed bands on SDS-PAGE after electrophoresis and staining with coomassie brilliant blue. Shoot protein was extracted in phosphate buffer (pH 7.5) and run on gel for 3 hours at 20 mA constant current. Band numbers were varies from 31 to 39 in different position on the gel according to their *Rf* value. Bands were scored as binary data and computed in MVSP software for dendrogram analysis to examine their genetic relationship using Jaccard's coefficient. Three species were grouped into three distinct clusters in the dendrogram. Species *cepa* and *sativum* was grouped at the similarity coefficient value of 0.53 and species *hookeri* was placed in separate group at coefficient level of 0.40. Genetic variation was also examined on the basis of PCA analysis. The number of chromosome were found to be present 16 in *Allium cepa*,16 in *Allium sativum*, and 22 in *Allium hookeri*. The shortest chromosome in *Allium cepa* was 8.75(µm) and the longest one was 23.12(µm) with the ratio of shortest/longest chromosome of 0.37, the mean chromosome length of 15.23(µm) and a mean centromeric index of 0.16%. The shortest chromosome in *Allium sativum* was 10.62(µm) and the longest one was 30.0 (µm) with the ratio of shortest/longest chromosome of 0.35, the mean chromosome length of 17.41(µm) and a mean centromeric index of 0.60%. The shortest chromosome in *Allium hookeri* was 5.62 (µm) with the ratio of shortest/longest chromosome of 0.21, the mean chromosome length of 16.58(µm) and the mean centromeric index of 0.46%. *Allium hookeri* is considered to be much more advanced than the rest of the species (*Allium sativum*,and *Allium cepa*) because karyotype of the *A. hookeri* was ranges from submediam to telocentric chromosome, which is evolutionarily advance characters.

**Keywords**: *Allium* sp, chromosome karyotype, ideogram, SDS-PAGE, Dendrogram, PCA analysis

The genus *Allium* is widely distributed over the region from the dry sub- tropic to the boreal region. Evolution of the genus has been accompanied by ecological diversification. The majority of the species grow in open, sunny, rather dry sites in arid and moderately humid climates. The onion is a variety of plants in the genus *Allium*, specifically *Allium cepa*. *Allium cepa* is also known as the "garden onion" or "bulb" onion. *Allium cepa* is known only in cultivation but related with other species occurs in Central Asia. The most closely *Allium* related species include *Allium vavilovii* (Popov and Vved) and *Allium asarense* (R.M.Fritsch and Matin) from Iran. The name *Allium sativum* is derived from the Celtic word 'all' meaning burning or stinging, and the Latin "*sativum*" meaning planted or cultivated. *Allium hookeri*, an important member of family Alliaceae subgenus Amerallium, is reported in the wild from Ceylon, Greece, Yunnan, Southern China, Bhutan, Sri Lanka and India (Hooker 1892). Cytological study has been carried out by some other workers using different Alliums as plant material (Sharma *et al.*, 2011 and Sen, 1974). In India, plants of this species are commonly found in forest margins and meadows of the Khasi hills in Northeastern Himalaya and upper gangetic plains (Pandey *et al.*, 2008). Because of characteristic flavor of genus Allium and some therapeutic properties, these plants are used by the locals, Khasi tribals in particular, for seasoning dishes, treating cough and cold, healing burn injuries and wounds (Kala, 2005). Since leaves and fleshy roots of this species are also consumed as vegetables, it has good marketability in areas of its common occurrence (Pandey *et al.*, 2008). Estimates of genetic relatedness are important in designing plant improvement programmes. Information on genetic diversity is also valued for the management of germplasm and for designing conservation strategies. Molecular markers are the best tools for determining genetic relationships between different species of the same genus.

The main objective of this present investigation was to study the genetic diversity among the three species of *Allium* on the basis of plant protein profiling through SDS-PAGE polymorphisms in combination with karyomorphology.

**Corresponding author:*
E-mail: subhascr@rediffmail.com

Table 1 :Karyomorphological data of *Allium cepa*

| Chromosome number | Long arm (µm) | Short arm(µm) | Chromosome length(µm) | F% | Classi-fication | Centromery index | Mean arm ratio |
|---|---|---|---|---|---|---|---|
| 1 | 11.25 | 6.87 | 18.12 | 37.91 | nsm | 0.61 | 1.63 |
| 2 | 11.25 | 6.87 | 18.12 | 37.91 | nsm | 0.61 | 1.63 |
| 3 | 12.5 | 7.50 | 20.0 | 37.5 | nsm | 0.60 | 1.66 |
| 4 | 12.5 | 7.50 | 20.0 | 37.5 | nsm | 0.60 | 1.66 |
| 5 | 5.0 | 3.75 | 8.75 | 42.85 | nm | 0.75 | 1.33 |
| 6 | 11.25 | 7.50 | 18.75 | 40.0 | nm | 0.66 | 1.50 |
| 7 | 8.125 | 2.5o | 1o.62 | 23.52 | nsm | 0.30 | 3.25 |
| 8 | 5.0 | 3.75 | 8.75 | 42.85 | nm | 0.75 | 1.33 |
| 9 | 5.0 | 3.75 | 8.75 | 42.85 | nm | 0.75 | 1.33 |
| 10 | 5.0 | 3.75 | 8.75 | 42.85 | nm | 0.75 | 1.33 |
| 11 | 11.25 | 7.50 | 18.75 | 40.o | nm | 0.66 | 1.50 |
| 12 | 10.0 | 8.75 | 18.75 | 46.66 | nm | 0.87 | 1.14 |
| 13 | 10.0 | 8.75 | 18.75 | 46.66 | nm | 0.87 | 1.14 |
| 14 | 8.75 | 2.50 | 11.25 | 22.22 | nsm | 0.28 | 3.5o |
| 15 | 16.25 | 6.25 | 22.5 | 27.77 | nsm | 0.38 | 2.6o |
| 16 | 16.25 | 6.87 | 23.12 | 29.71 | nsm | 0.42 | 2.36 |
| Total | 159.375 | 94.36 | 253.73 | | | 0.61 | 1.82 |
| | | Mean= | 15.85 | | | | |

Table 2:Karyomorphological data of *Allium sativum*

| Chromosome number | Long arm (µm) | Short arm(µm) | Chromosome length (µm) | F % | Classification | Centromery Index | Arm ratio |
|---|---|---|---|---|---|---|---|
| 1 | 13.75 | 6.87 | 20.62 | 33.31 | nsm | 0.49 | 2.0 |
| 2 | 9.37 | 5.62 | 14.99 | 37.49 | nsm | 0.59 | 1.66 |
| 3 | 9.37 | 5.62 | 14.99 | 37.49 | nsm | 0.59 | 1.66 |
| 4 | 13.75 | 12.5 | 26.25 | 47.61 | nm | 0.90 | 1.10 |
| 5 | 13.75 | 12.5 | 26.25 | 47.61 | nm | 0.90 | 1.10 |
| 6 | 17.5 | 12.5 | 30.0 | 41.66 | nm | 0.71 | 1.40 |
| 7 | 17.5 | 11.87 | 29.37 | 40.41 | nm | 0.67 | 1.47 |
| 8 | 13.75 | 6.87 | 20.62 | 33.31 | nsm | 0.49 | 2.0 |
| 9 | 7.5 | 3.12 | 10.62 | 29.37 | nsm | 0.41 | 2.41 |
| 10 | 8.12 | 5.0 | 13.12 | 38.10 | nsm | 0.61 | 1.62 |
| 11 | 8.12 | 3.75 | 11.87 | 31.59 | nsm | 0.46 | 2.16 |
| 12 | 9.37 | 3.75 | 13.12 | 28.58 | nsm | 0.40 | 2.49 |
| 13 | 8.12 | 4.37 | 12.49 | 34.98 | nsm | 0.53 | 1.85 |
| 14 | 7.5 | 3.12 | 10.62 | 29.37 | nsm | 0.41 | 2.40 |
| 15 | 6.87 | 5.0 | 11.87 | 42.12 | nm | 0.72 | 1.37 |
| 16 | 6.87 | 5.0 | 11.87 | 42.12 | nm | 0.72 | 1.37 |
| Total | 171.21 | 107.46 | 278.67 | | | o.60 | 1.75 |
| | | Mean= | 17.29 | | | | |

Table 3: Karyomorphological data of *Allium hookeri*

## Material and Methods

*Plant materials*

Two species of *Alllium* were collected (*Allium cepa*, and *Allium sativum*) from different places of the NBU campus and *Allium hookeri* from Medicinal plant gardens of the Department of Botany, NBU.

*Root tips for Karyomorphology*

Root tips of *Allium cepa*, *Allium hookeri* and *Allium sativum* were pre-treated in saturated aqueous solution of p-dichlorobenzene at 4°c for 5 minutes, and then transferring it at 12-14°c for about 3-4 hours, and followed by fixation in 3:1 (v/v) solution of absolute ethanol: glacial acetic acid for overnight at room temperature. After that root tips were kept in 45 % acetic acid for 10-15 minutes followed by staining with 2% acetic-orcein and HCl(1N) mixture at the ratio of 9:1 and kept for 30 minutes for staining after heated at 60-65° C for 30-40 seconds. The stained root tips were macerated in 45 % (v/v) acetic acid on a grease free new slide. The well spread metaphase plates of each taxon were analysed. The camera-lucida drawing was done for karyotype and ideogram analysis.

*Shoot protein for SDS-PAGE electrophoresis*

SDS-PAGE was performed according to Laemmli (1970). Shoot tissues were used for the protein profiling purposes. One gram shoot tissue was taken from three samples *Allium cepa*, *Allium hookeri* and *Allium sativum*. Shoot tissues were crushed separately with 3 ml phosphate buffer (7.5 pH) in ice cold condition. The

Table 3: Karyomorphological data of *Allium hookeri*

| Chromosome number | Long arm(µm) | Short arm (µ m) | Total length (µ m) | F% | Classification | Centromery ratio | Arm ratio |
|---|---|---|---|---|---|---|---|
| 1 | 18.12 | 3.75 | 21.87 | 17.14 | nst | 0.20 | 4.85 |
| 2 | 18.12 | 3.75 | 21.87 | 17.14 | nst | 0.20 | 4.85 |
| 3 | 18.75 | 7.5 | 26.25 | 28.57 | nsm | 0.4 | 2.5 |
| 4 | 8.12 | 7.5 | 15.62 | 48.33 | nm | 0.92 | 1.08 |
| 5 | 6.25 | 5.0 | 11.25 | 44.44 | nm | 0.8 | 1.25 |
| 6 | 7.5 | 4.37 | 11.87 | 36.81 | nsm | 0.58 | 1.71 |
| 7 | 8.12 | 7.5 | 15.62 | 48.01 | nm | 0.92 | 1.08 |
| 8 | 3.75 | 1.87 | 5.62 | 33.27 | nsm | 0.49 | 2.00 |
| 9 | 9.37 | 1.87 | 11.24 | 16.63 | nst | 0.19 | 5.01 |
| 10 | 8.75 | 1.87 | 10.62 | 17.60 | nst | 0.21 | 4.67 |
| 11 | 3.75 | 1.87 | 5.62 | 33.27 | nsm | 0.49 | 2.00 |
| 12 | 4.37 | 3.12 | 7.49 | 41.65 | nm | 0.71 | 1.4. |
| 13 | 18.75 | 1.8 | 20.55 | 8.75 | st | 0.09 | 10.41 |
| 14 | 18.75 | 7.5 | 26.25 | 28.57 | nsm | 0.4 | 2.5 |
| 15 | 7.5 | 4.37 | 11.87 | 36.81 | nsm | 0.58 | 1.71 |
| 16 | 8.75 | 1.87 | 10.62 | 17.60 | nst | 0.21 | 4.67 |
| 17 | 6.25 | 3.12 | 9.37 | 33.29 | nsm | 0.49 | 2.00 |
| 18 | 4.37 | 3.12 | 7.49 | 41.65 | nm | 0.71 | 1.40 |
| 19 | 6.25 | 5.0 | 11.25 | 44.44 | nm | 0.8 | 1.25 |
| 20 | 6.25 | 3.12 | 9.37 | 33.29 | nsm | 0.49 | 2.00 |
| 21 | 18.75 | 1.8 | 20.55 | 8.75 | st | 0.09 | 10.41 |
| 22 | 9.37 | 1,87 | 11.24 | 16.63 | nst | 0.19 | 5.01 |
| Total | 219.96 | 83.54 | 303.5 | | | 0.46 | 4.20 |
| | | Mean= 13.79 | | | | | |

Table 4: Comparison of chromosome properties of *Allium taxa*

| Taxa | 2n | S | L | S/L | Mean length | MCI | MAR | TF% | D.I | HCL |
|---|---|---|---|---|---|---|---|---|---|---|
| *A. cepa* | 16 | 8.75 | 23.12 | 0.37 | 15.23 | 0.61 | 1.82 | 37.18 | 45.08 | 127.49 |
| *A. sativum* | 16 | 10.62 | 30.0 | 0.35 | 17.41 | 0.60 | 1.75 | 38.56 | 47.71 | 140.59 |
| *A. hookeri* | 22 | 5.62 | 26.25 | 0.21 | 16.58 | 0.46 | 4.20 | 27.52 | 64.63 | 151.75 |

2n=Chromosome number; S=short chromosome length; L=long chromosome length; MCI=Mean Centromery Index; MAR=mean arm ratio; all measurement are in µm

crude extract was filtered with cotton cloth. The crushed materials were then centrifuged at 10000 rpm for 10 minutes. Supernatant was collected in 1.5ml eppendorf tube. 25 µl 1X SDS sample buffer was mixed with 25 µl protein sample in the ratio 1:1. Then sample were heated for 3 minutes in water bath. 15 µl each of the samples were loaded into the well of the gel by micropipette. Electrophoresis was performed at 60-70 volt, 20-30 mA current for a period of about 3 hours till the tracking dye reach the bottom of the gel. Gel was stained overnight with 0.2% Comassie Brillant Blue. The gel was kept in destainer solution until clear blue bands were appeared on the gel. The gel was viewed over the illuminator, photographed for band analysis purposes.

## Results and Discussion

*Shoot protein profiling for genetic diversity analysis*

*Data Analysis*

The presence (1) and absence (0) of each band was considered in each species of *Allium*. The binary data matrix was used to calculate Jaccard's similarity index for genetic diversity analysis. The genetic relationship among 3 species were analyzed, and viewed in Dendrogram using Multi Variate Stastical Package MVSP version 3.2 and Unweighted Pair Group Method of Arithematic Means (UPGMA). The numbers of band varied from 25-34 among the species, that of the *Allium sativum* was the maximum bands whereas the *Allium hookeri* was the minimum band numbers. Electrophoregram showing protein banding pattern of different species of *Allium* were given in Figure-1.

*Cluster Analysis*

According to the statistical analysis data of the present and absent of each band was used to construct Dendrogram based on Jaccard's similarity index. Dendrogram showed single cluster showing highest similarity between *A. cepa* and *A. sativa* at 0.53 coefficient value (Figure 2). The *Allium hookeri* was placed in the same cluster but at different claude at 0.40 coefficient value. Principal component analysis (PCA) also showing the same genetic diversity in compare to dendrogram in a graphical mode (Figure 3). The protein analysis through SDS-PAGE showed the presence of 34 bands out of which 7 bands were common (in case of *Allium cepa shoot, Allium sativum Shoot, Allium hookeri shoot*) while the other 27 were polymorphic.

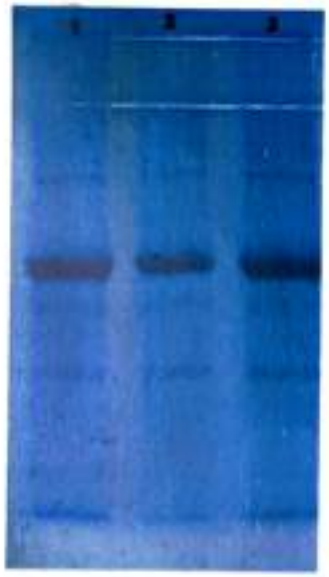

Figure 1: Electrophoregram showing protein banding pattern of different species of *Allium* genotypes (Lane 1:*A. cepa*, lane 2:*A. sativum* and lane 3 :*A. hookeri* )

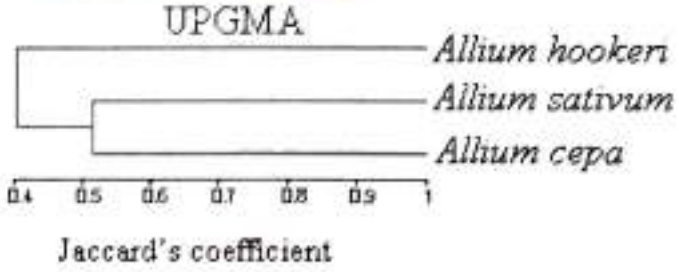


Figure 2. Dendrogram showing the genetic relationships among the 3 species of *Allium* on the bases of SDS-PAGE protein profiling using MVSP software in UPGMA algorithm

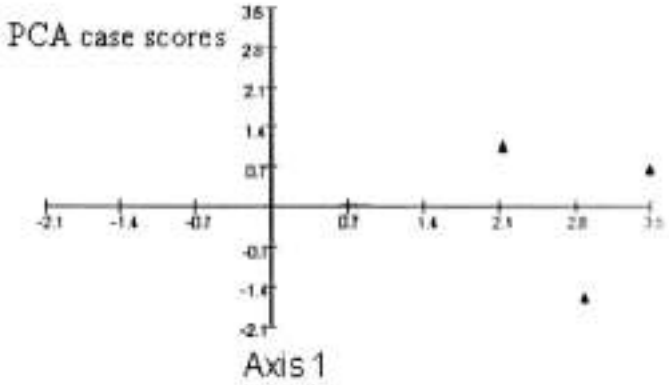


Figure 3. Principal component analysis (PCA) showing graphical representation of the genetic variation among the three species of *Allium* based on SDS-PAGE protein profiling

Dendrogram revealed that the *Allium hookeri* differ from *Allium cepa*, and *Allium sativum* at about coefficient value 0.40. Nearly 50% similarity was there between the *Allium cepa* and *Allium sativum*.

*Karyomorphological analysis*

The number of chromosome were found to be 16 in *Allium cepa*,16 in *Allium sativum*, and 22 in *Allium hookeri* (Figure 4 & 5). Chromosomes of all the investigated types were nearly submedian, nearly median, nearly sub-terminal, and terminal. Form percentage (F %) was recorded maximum (48.0) in *Allium hookeri*. Minimum form percentage (8.75) was recorded in *Allium hookeri*. The shortest chromosome in

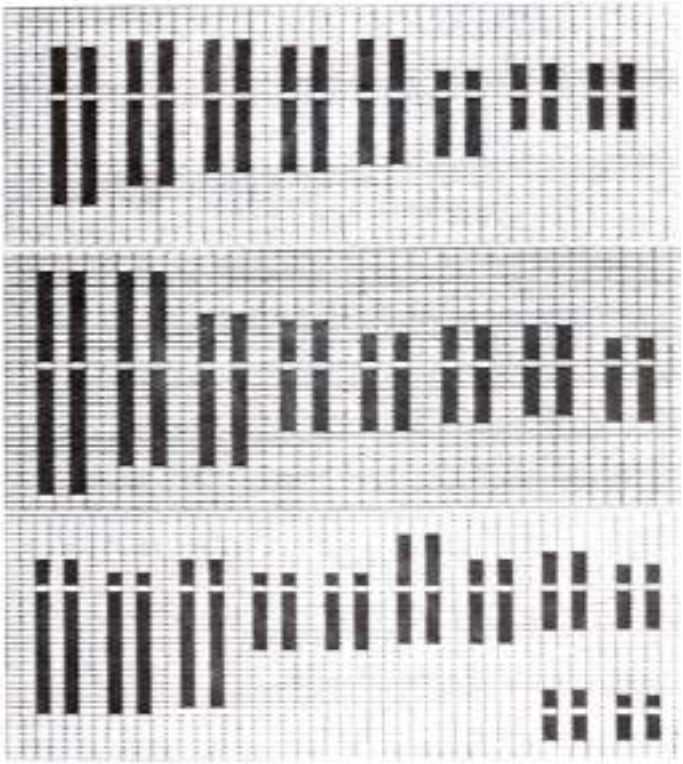

Figure 4: Ideogram of *Allium* taxa, top= *A. cepa*, middle= *A. sativum*, bottom= *A. hookeri*

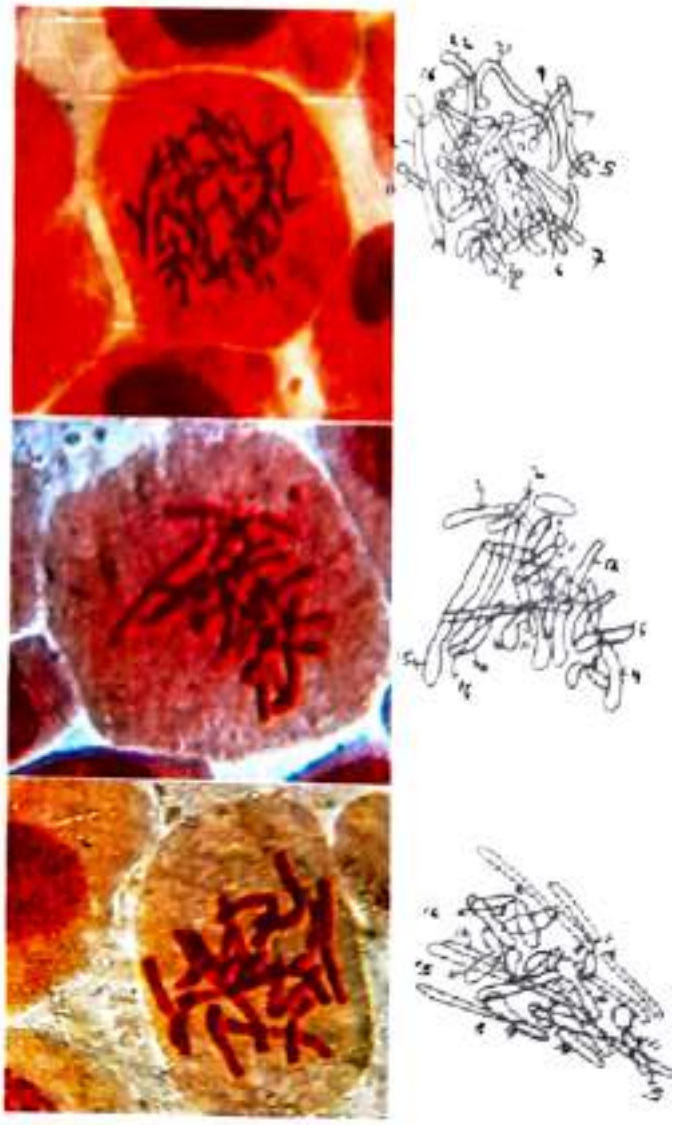

Figure 5: Showing the light micrograph of metaphase chromosome complement and free hand cameralucida drawing of three *Allium* sp. karyotype: top=*A. cepa*, middle= *A. sativum*; bottom= *A. hookeri*

*Allium cepa* was 8.75(μm) and the longest one was 23.12(μm) with the ratio of shortest/longest chromosome of 0.37, the mean chromosome length of 15.23(μm) and a mean centromeric index of 0.16%. The shortest chromosome in *Allium sativum* was 10.62 (μm) and the longest one was 30.0 (μm) with the ratio of shortest/longest chromosome of o.35, the mean chromosome length of 17.41(μm) and a mean centromeric index of 0.60%. The shortest chromosome in *Allium hookeri* was 5.62(μm) with the ratio of shortest/longest chromosome of 0.21, the mean chromosome length of 16.58(μm) and the mean centromeric index of 0.46% (Table 1, 2, 3 & 4).

Nomenclature for the centromeric positions of chromosome follows Levan *et al.* (1964) and the karyotype classification follows Stebbins (1971). The number of chromosome were found to be 2n = 16 in *Allium cepa*, 2n = 16 in *Allium sativum* and 2n= 22 in *Allium hookeri*. The total length of chromatin were found to be 253.73μm in *Allium cepa*, 278.67μm in case of *Allium sativum* and 303.5μm in case of *Allium hookeri*. Chromosome of all investigated types were nearly submedian, nearly median, nearly subterminal and terminal. Form percentage (F%) was recorded maximum (48) in *Allium hookeri*. Minimum form percentage (F%) was recorded in *Allium hookeri*. *Allium hookeri* has 2 sub-terminal chromosome and 6 nearly sub-terminal chromosomes and according to Stebbins species having greater number of submedian and telocentric chromosomes should be treated more evolved than those in which there are lesser number of sub metacentric and telocentric chromosome.

The above finding in the assessment of evolutionary tendencies suggested that *Allium hookeri* was much advanced than the rest of species, however *Allium sativum*, *Allium cepa* were much more primitive than *Allium hookeri* as their chromosome were nearly median and submedian type.

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