A study on genetic relationship between *Allium sativum* L. and *Scadoxus multiflorus* (Martyn) Raf. of Amaryllidaceae

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Introduction

Karyological studies of *Allium sativum* L. and *Scadoxus multiflorus* (Martyn) Raf. (=*Haemanthus multiflorus*) belonging to the family Amaryllidaceae had been carried out by different researchers from time to time (1-6). *A. sativum*, commonly known as garlic, is extensively used as spice in the Indian subcontinent (1). On the other hand, *S. multiflorus* is extensively cultivated as ornamental plant for its beautiful flower (6). Though Konvicka and Levan (1) observed significant differences in the karyotype of different clones of *A. sativum*, previous cytological studies revealed that the karyotype of *A. sativum* is symmetrical in nature (3, 5). Moreover, comparative analysis of karyotype of *Allium* spp. had also yielded the order of chromosomal evolution in related species (7). On the contrary, asymmetrical karyotype was found to be the inherent characteristics of *S. multiflorus* (4, 6). However, in order to evaluate the karyotype symmetry or asymmetry of a species one has to deal with both inter-chromosomal and intra-chromosomal asymmetries of the somatic...
chromosome complements of species studied and usually these are determined by quali-quantitative method (8) and/or quantitative methods based on statistical approach (9,10). Stebbins (8) quali-quantitative method of determining karyotype asymmetry is based on the ratio of largest and smallest chromosome in a karyotype (interchromosomal asymmetry) and proportion of chromosome arm ratio (L/S) <2:1 (intra-chromosomal asymmetry). Recently, Peruzzi and Eroglu (11) have developed another model in which coefficient of variation of chromosome length (CV\textsubscript{CL}) is coupled with a new parameter called mean centromeric asymmetry (M\textsubscript{CA}). Accordingly, the karyotype asymmetry relationship among different species/families/tribes was represented by drawing scatter plot putting M\textsubscript{CA} data in X axis and CV\textsubscript{CL} data in Y axis. An attempt has therefore been made to establish the genetic relationship between A. sativum and S. multiflorus of Amaryllidaceae using CV\textsubscript{CL} and M\textsubscript{CA} data of each species.

Materials and Methods

Bulbs of Allium sativum and Scadoxus multiflorus were collected from Madhupur (23°43’28.22”N, 91°13’33.70”E), Sepahijala, Tripura and grown in the experimental garden, Department of Botany, Tripura University. Somatic chromosome study of A. sativum and S. multiflorus was carried out with modified aceto-orcein staining technique (12). Young root tips of each species were pre-treated separately in a saturated solution of para dichlorobenzene (p-DB) at 12-15°C for 5 hrs. The root tips were then washed with distilled water and kept in acidulated alcohol mixture of 1NHCL and Ethanol (1:1) for 30 mins. Thereafter, root tips were kept in 45% acetic acid for 15 mins, stained with 2% aceto-orcein:1NHCL (9:1) mixture for 2 hrs. and finally squashed in 45% acetic acid. The well spread metaphase plate was captured using Carl Zeiss make AXIO Lab A1 microscope located in the department of Botany, Tripura University and Zen software was used for determining the length of short and long arm of each individual chromosome of the species studied. In quali-quantitative method (8) inter chromosomal and intra chromosomal indices of each species were calculated by the following formula:

\[
\text{Inter chromosomal asymmetry index} = \frac{\text{Length of the largest chromosome} \times \mu m}{\text{Length of the smallest chromosome} \times \mu m}
\]

\[
\text{Intra-chromosomal asymmetry index} = \text{Proportion of chromosomes with arm ratio (L/S) \ < \ 2:1}
\]

In quantitative method (11), inter chromosomal and intra chromosomal indices were calculated by deriving the coefficient of variation of chromosome length (CV\textsubscript{CL}) and the Mean Centromeric Asymmetry (M\textsubscript{CA}) of each species where,

\[
CV_{CL} = A_2 \times 100 \text{ and } A_2 = \frac{\text{Standard deviation of the chromosome length}}{\text{Mean of chromosome length}}
\]

\[
M_{CA} = A \times 100 \text{ and } A = \frac{\sum \text{Long arm (L)} - \text{Short arm (S)}}{\text{Long arm (L)} + \text{Short arm (S)}} \times \frac{1}{n}
\]

Finally, the karyotype asymmetry relationship between the species was represented by drawing scatter plot putting M\textsubscript{CA} data in X-axis and CV\textsubscript{CL} data in Y-axis (11).

Results and Discussion

The somatic chromosome number of Allium sativum was found to be 2n=16 (Fig. 1) which corroborates previous findings (1, 3). Numerical data of the karyotype of A. sativum revealed that its chromosomes are either metacentric or submetacentric in nature (Fig. 1) and their length ranged from 9.24 μm to 16.17 μm (Table 1). This also indicates the absence of acrocentric or telocentric chromosomes in its karyotype. Based on quali-quantitative estimation, the inter-chromosomal and intra-chromosomal indices of A. sativum were found to be 1.75 and 1.00.

Fig. 1. (a) Mitotic metaphase plate showing 2n = 16 chromosomes (b) karyogram of Allium sativum
respectively. Thus, the karyotype of *A. sativum* falls under Stebbins category 1A (Table 2). Our results are also in agreement with previous reports (1, 5). Simultaneously, we also estimated the defined inter-chromosomal and intra-chromosomal indices (CV$_{CL}$ and M$_{CA}$) through quantitative methods, which were not reported previously. The value of CV$_{CL}$ and M$_{CA}$ in *A. sativum* respectively.
was estimated for the first time as 19.87 and 12.20 respectively (Table 1). In the present study, the somatic chromosome count of *Scadoxus multiflorus* was found to be 2n=18 chromosomes (Fig.2). From the numerical data it was observed that the karyotype of *S. multiflorus* had four pairs of large chromosomes and five pairs of small chromosomes (Fig. 2) and this shows the karyotype is composed of two distinct classes with respect to the size of the chromosomes indicating the bimodal nature. The length of chromosomes of *S. multiflorus* ranged from 6.50 µm to 38.00 µm (Table 1) and the TF% (13) value (Table 1) justified the presence of acrocentric and telocentric chromosomes (4, 6). Through quasi-qualitative estimation, the inter- and intra-chromosomal asymmetry indices were found to be 5.84 and 0.22 (Table 1) respectively and so under Stebbins categorization the karyotype of *S. multiflorus* falls under 3C which was not reported earlier. In contrast, the quantitative estimation of inter and intra-chromosomal asymmetry indices revealed that the value of CVCL and MCA of *S. multiflorus* were 59.0 and 43.90, (Table 1) respectively. Thus, quasi-qualitative asymmetry data of *A. sativum* and *S. multiflorus* indicate only the varying degree of asymmetry of their karyotype but the genetic distance between the two species cannot be assessed from such relationship. On the contrary, quantitative data of inter- and intra- chromosomal asymmetry index when plotted in bi-dimensional scattered plot the genetic distance between *A. sativum* and *S. multiflorus* is clearly reflected in the graph (Fig. 3) indicating that inter- and intra-chromosomal indices were comparatively high in *S. multiflorus* than those of *A. sativum*. This also suggests that CVCL and MCA are the determinants of inter-chromosomal and intra-chromosomal asymmetry. Similar such findings were also reported by previous researchers (14, 15) where the karyotype asymmetry relationship among organisms has been explained by means of bi-dimensional scattered plot of MCA and CVCL data (11).

**Conclusion**

The karyotype asymmetry data estimated in *A. sativum* and *S. multiflorus* clearly establishes the genetic distance between these two species and this suggests that the coefficient of variation of chromosome length (CVCL) and the mean centromeric asymmetry (MCA) data could be easily used in bi-dimensional scatter plot in order to establish the genetic relationship among different species/families in future cyto-taxonomical work.

**Competing Interests**

The authors have no conflict of interests.

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## Authors’ contributions

HDP and KS designed the experiment and wrote the manuscript.

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