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KARYOTYPIC ANALYSIS ON THREE EDIBLE *ALLIUM* SPECIES FROM BANGLADESH

MEGHLA SAHA PINKY, SUSMITA SAHA AND KAZI NAHIDA BEGUM*

Department of Botany, Faculty of Life and Earth Sciences, Jagannath University, Dhaka-1100, Bangladesh

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Abstract

Three edible species of *Allium*, namely, *A. sativum*, *A. cepa* and *A. tuberosum* were cytogenetically investigated to highlight the chromosomal variations among them. These three species of *Allium* revealed two different numbers of chromosomes with divergent karyotype formula *i.e.* 2n = 16 = 12m + 4sm in *A. sativum*, 2n = 16 = 14m + 2sm in *A. cepa* and 2n = 32 = 28m + 2sm+ 2st in *A. tuberosum*. A pair of satellite chromosome was found only in *A. tuberosum*. According to Stebbins's classification 1B (*A. sativum*) and 2A (*A. cepa* and *A. tuberosum*) type of chromosomal asymmetry were observed. The present cytogenetical analysis revealed that *A. cepa* was primitive in nature compared to other two species. *A. sativum* was found to be contemporary to *A. cepa* and *A. tuberosum* was more advanced type.

Introduction

Allium L. is the largest monocotyledonous genus, belonging to Amaryllidaceae with more than 800 wild and cultivated species (Fritsch *et al.* 2010). Generally, this genus is widespread in temperate and Alpine territories of Northern Hemisphere but the diversified center is found in Eastern or Central Asia, Southwest Asia and North America (Nguyen *et al.* 2008).

Allium L. comprises a divergent range of economically important plants *viz.* the common onion (*A. cepa* L.), garlic (*A. sativum*), the bunching onion (*A. fistulosum*), the chives (*A. tuberosum*), leek (*A. porrum*) and ornamental species such as *A. sphaerocephalon* L. or *A. moly* L. (Fritsch *et al.* 2010). In Bangladesh, *A. cepa* and *A. sativum* are well known for condiment crops to increase the flavour of prepared food whereas *A. tuberosum* is used as a substitute of onion with medicinal and horticultural advantages (Mahbub *et al*. 2014). Hence, the demands of these edible species are increasing day by day as a vital part of the country diet. Even after ongoing analysis of molecular genetics and breeding programs in *Allium* species, the classical cytogenetical analysis are essential to estimate the numerical and structural features of chromosome set considering karyotype construction to fulfill the increasing needs (Saha *et al.* 2020).

A number of chromosomal and molecular analysis have been accomplished worldwide on *Allium* (Okumus and Hassan 2000, Mukherjee and Roy 2012, Manzum *et al.* 2014, Mahbub *et al.* 2014, Ramesh 2015, Pinky *et al*. 2016, Awe and Akpan 2017), whether chromosomal information as well as cytological analysis of *Allium* in Bangladesh still have some lacking. Therefore, in the present investigation, three *Allium* species, *viz. A. sativum*, *A. cepa* and *A. tuberosum* from Bangladesh were cytogenetically investigated to identify their complete set of chromosome in view of karyotype and comparing them from previous chromosomal reports.

Materials and Methods

Three *Allium* species *viz. A. cepa* L., *A. sativum* L. and *A. tuberosum* Rottl. ex Spreng. were collected from the Bangladesh Agricultural Research Institute (BARI) and further maintained in the Botanical garden of Jagannath University, Bangladesh.

* Author for correspondence: <kazinahida@bot.jnu.ac.bd>.

For the present investigation, collected fresh root tips (RTs) of ten individuals were pretreated with 2 mM Para dichloro benzene (PDB) for 3 hrs at room temperature (28 - 30°C) followed by fixing in Carnoy's fluid (1 glacial acetic acid: 3 ethanol) at 4°C for 24 hrs. Then, the pre-treated RTs were heated uniformly with a mixture of 1% aceto-orcein and 1 N HCl (3:1) for hydrolysis. Next the slides were prepared by squashing in 1% aceto-orcein to observe under the Optika electric microscope and at least five somatic metaphases for each species were photographed with the magnification of 40X by the Euromax camera (CMEX 10, DC 10000C).

To determine centromeric positon, the nomenclature suggested by Levan *et al.* (1964) was followed. Based on decreasing order of chromosome size a haploid idiogram was prepared. Different karyomorphological parameters including symmetry and asymmetry indices were evaluated as the total form per cent (TF%) following Huziwara (1962). The karyotype asymmetry index (AsK%) following Arano (1963), the index of karyotype symmetry and chromosomal size resemblance (Syi% and Rec%) as suggested by Greilhuber and Speta (1976), the intra and inter chromosomal asymmetry index (A_1 and A_2) after Zarco (1986), the asymmetry index (AI) ensuing Paszko (2006) and the degree of karyotype was estimated by the categories of Stebbins (1971).

Results and Discussion

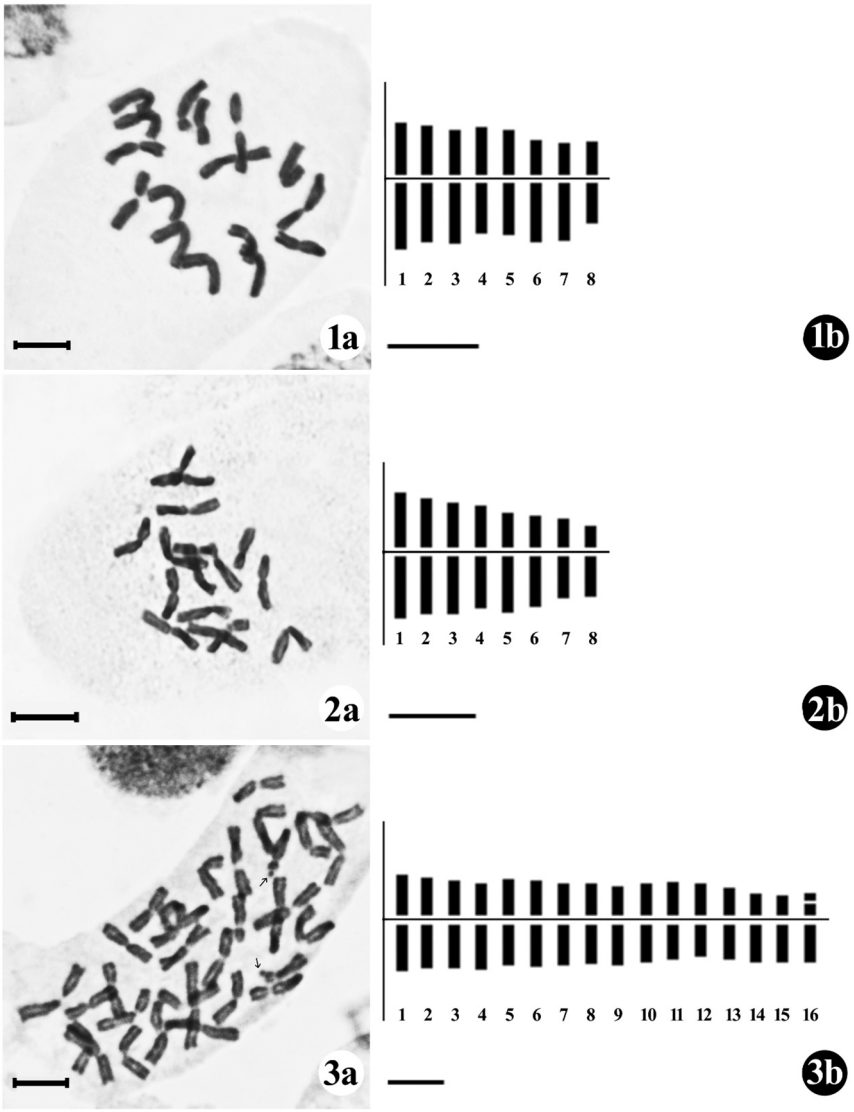
Allium L. is one of the versatile genera which display an impressive range of chromosome numbers. In the present investigation, the karyological data of *A. cepa*, *A. sativum* and *A. tuberosum* are presented in Table 1.

Table 1. Comparative karyomophological features of three edible *Allium* species.

Features	*A. cepa*	*A. sativum*	*A. tuberosum*
Chromosome number	2n=16	2n=16	2n=32
Satellite	-	-	2
CF	14m+2sm	12m+4sm	28m+2sm+2st
TCL (µm)	173.55±6.64	167.10±5.79	397.63±4.64
RCL (µm)	7.55±0.28-13.32±0.40	6.71±0.55-13.10±0.16	8.41±0.95-15.45±0.74
ACL (µm)	10.84	10.44	12.43
AsK %	55.91	57.65	56.81
TF %	44.09	42.35	43.19
Syi %	78.85	73.46	76.02
Rec %	80.49	77.36	81.44
A_1	0.21	0.28	0.23
A_2	0.14	0.20	0.14
AI	0.13	0.17	0.14
Stebbins's classification	2A	1B	2A

CF= Centrometic Formula, TCL= Total chromosome length, ACL= Average chromosome length, RCL= Range of chromosomal length, AsK%= Karyotype asymmetry index, TF%= Total form value, Syi%= Karyotype symmetry index, Rec%= The index of chromosomal size resemblance, A_1= Intra chromosomal asymmetry index, A_2= Inter chromosomal asymmetry index, AI= The asymmetry index

A. cepa was found with 16 diploid chromosome complement where the total chromosome length was 173.55 ± 6.64 µm ranging from 7.55 ± 0.28 µm to 13.32 ± 0.40 µm and the average chromosome length was 10.84 µm. The karyotype formula of *A. cepa* was found to be comprised of 2 sub-metacentric and 14 metacentric chromosomes. The estimated value of some karyological parameters *i.e.* AsK, TF, Syi and Rec was 55.91, 44.09, 78.85 and 80.49% along with A_1 (0.21), A_2 (0.14) and AI (0.13). Based on Stebbins's classification (1971), *A. cepa* was placed into 2A (Figs.1a-b, Table 1).



Figs. 1-3. Orcein-stained mitotic metaphase chromosomes and haploid idiograms of three edible *Allium* species. (1a) metaphase and (1b) idiogram of *A. cepa*; (2a) metaphase and (2b) idiogram of *A. sativum*; (3a) metaphase and (3b) idiogram of *A. tuberosum*; arrows indicate the presence of satellite. Bars=10 µm.

In *A. sativum*, 2n= 16 were observed with 2 pairs of sub-metacentric chromosome and remaining pairs were metacentric in nature. The total length of chromosome compliments was 167.10± 5.79 µm and the value of average chromosomal length was 10.44 µm. The longest chromosome (13.10 ± 0.16 µm) was nearly double to the shortest chromosome (6.71 ± 0.55 µm). The calculated values of AsK, TF, Syi and Rec% were 57.65, 42.35, 73.45 and 77.36%, respectively. The values of A_1, A_2 and AI were 0.28, 0.20 and 0.17, respectively. This species was categorized into 1B as per Stebbins's classification (Figs. 2a-b, Table 1).

The somatic chromosome number of *A. tuberosum* was found to be 32 with 28 metacentric , 2 sub-metacentric and 2 acrocentric chromosomes. The individual chromosome length was found to range from 8.41 ± 0.95 µm to 15.45 ± 0.74 µm. In this case the longest chromosome was nearly twice to the shortest chromosome and 12.43 µm was the value of average chromosome length. In consequence, AsK%, TF%, Syi% and Rec% were accounted for 56.81%, 43.19%, 76.02% and 81.44%, respectively. The estimated values of A_1, A_2 and AI were 0.23, 0.14 and 0.14, respectively. In this species, satellite chromosomes were located at the short arms of both the chromosomes in pair 16 and categorized as 2A according to Stebbins's classification (1971) (Figs. 3a-b, Table 1).

Earlier, several authors reported 2n = 16 as well-accepted chromosome number of *A. cepa* (Okumus and Hassan 2000, Mukherjee and Roy 2012, Mahbub *et al*. 2014, Ramesh 2015, Pinky *et al*. 2016, Awe and Akpan 2017). The present finding regarding chromosome number in *A. cepa* was found to be supported by the earlier investigations.

Somatic chromosome number 2n = 16 for *A. sativum* in the present investigation was found to be supported by different previous reports (Yuzbasioglu and Unal 2004, Manzum *et al.* 2014, Ramesh 2015, Awe and Akpan 2017).

A divergent chromosome number was observed earlier in *A. tuberosum i.e.* 2n = 24, 30, 32, 48 (Ruifu *et al.* 1985, Do *et al.* 2000, Sharma and Gohil 2013a, b, Dutta and Bandyopadhyay 2014a, Mahbub *et al*. 2014, Ramesh 2015). However, the present report (2n = 32) is in agreement with the findings of Do *et al.* (2000), Mukherjee and Roy (2012), Mahbub *et al*. (2014), Dutta and Bandyopadhyay (2014a) and Ramesh (2015).

Some previous reports suggested *A. tuberosum* as an auto-tetraploid which showed 32 chromosomes in somatic cells (Talukdar and Sen 2000, Mukherjee and Roy 2012, Sharma and Gohil 2013a). Though the present report resembles to the chromosome number but it did not show such morphological variation. Thus *A. tuberosum* can be clearly considered this species as an auto-tetraploid. In the present investigation, it is not possible to place four chromosomes in a homologous pair due to the presence of two sub-metacentric and two acrocentric chromosomes though they are more or less similar in size and also the investigated *A. tuberosum* displayed two satellites instead of four. Therefore, the current analysis indicated that the analyzed *A. tuberosum* might not be an auto-tetraploid but influenced the possibilities to accept this species as a diploid, which correlates with the work of Mahbub *et al*. (2014).

According to Dutta and Bandyopadhyay (2014b), the genus *Allium* L. showed a divergent range of basic chromosome number (x) ranging from 8 to 11 as the appearance of polyploidy. In the present investigation, *A. cepa* (2n = 2x = 16) and *A. sativum* (2n = 2x = 16) both represent the basic number x=8 as per previous reports (Mahbub *et al*. 2014, Ramesh 2015, Pinky *et al*. 2016, Awe and Akpan 2017). The recent investigated *A. tuberosum* showed diploid nature rather than an auto-tetraploid which is related with previous records (Talukdar and Sen 2000, Mukherjee and Roy 2012, Sharma and Gohil 2013a).

Based on karyotypic formula among the investigated three *Allium* species, *A. cepa* (14m + 2sm) and *A. sativum* (12m + 4sm) showed gradual decrease in chromosome size for the

appearance of metacentric chromosomes along with sub-metacentric chromosomes. *A. tuberosum* revealed the dominance of metacentric chromosomes along with submetacentric and subterminal chromosomes (28m + 2sm + 2st). Pinky *et al.* (2016) worked on different BARI varieties of *A. cepa* and the chromosome number was 2n = 2x = 16. Assuming CF most of the *A. cepa* varieties showed appearance of four to six number of sub-metacentric chromosomes and in some cases a pair of acrocentric chromosomes also . But in the present findings, the CF of *A. cepa* did not relate with the previous analysis due to absence of acrocentric chromosomes. According to Manzum *et al.* (2014) on different specimens of *A. sativum*, three out of four specimens displayed 2n = 2x = 16 chromosome number with four to eight sub-metacentric chromosomes. Among the previously analyzed four specimens of *A. sativum*, local mono-cloved garlic showed the 12m + 4sm CF which correlates with current analysis. Previously, Mahbub *et al.* (2014) reported 2n = 32 chromosome in *A. tuberosum* with CF (16m + 12sm + 2st) from Sylhet region of Bangladesh. Compared to the present findings the earlier findings show a slight variation and that might be due to divergent environmental factors.

A. tuberosum had slight higher average chromosome length (12.43 µm) whereas the remaining two species showed similar average chromosome length *i.e. A. sativum* (10.44 µm) and *A. cepa* (10.84 µm). Karyotype asymmetry can be considered as an important tool for speciation in which symmetrical karyotypes are regarded as ancestral state in evolution (Stebbins 1971). The diversification among AsK, TF, Syi, Rec%, A_1, A_2 and AI were found due to their mostly positive or negative correlation among each another. In the current analysis, AsK% showed an absolute negative correlation with two asymmetry indices which are TF% and Syi%. Recently, AI can be regarded also as an important tool to find out karyotype asymmetry in which higher AI value indicates more chromosomal heterogeneity (Paszko 2006). According to Stebbins (1971), karyotype asymmetry can be counted as a tool for speciation in which symmetrical karyotypes are regarded as ancestral state in evolution. Hence, *A. cepa* is the most primitive in character due to more symmetrical karyotypic formula with lower AI (0.13) and dominance of metacentric chromosome.

In consequence, the comparative karyomorphological analysis is not only helpful to authentic identification but also provide sufficient information to know the intra-generic relationships among analyzed *Allium* species of this divergent genus. However additional genomic investigation is required to reveal the perfect ploidy level along with the taxonomic status of *A. tuberosum* Rottl. ex Spreng.

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