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COMPARATIVE KARYOMORPHOLOGICAL ANALYSIS OF FOUR BARI RELEASED GERMPLASMS OF GARLIC

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Several garlic varieties (*Allium sativum* L.) have been released by Bangladesh Agricultural Research Institute (BARI), namely BARI Rosun-1, BARI Rosun-2, BARI Rosun-3 and BARI Rosun-4, along with some lines which are experimentally cultivated under the breeding program, such as ASGAZ001, ASGAZ002, ASGAZ003, ASGAZ004, ASGAZ005, ASGAZ006, ASGAZ007, ASGAZ008 and ASGAZ009. The morphological features are used to characterize these varieties, and many lines that often create complications as phenotypic characteristics are not always authentic. Thus, classical cytogenetics will be helpful for numerical and structural features of chromosome set, including karyotype characteristics for authentic characterization and further molecular investigations and conservation (Saha *et al.* 2020). Many previous studies have been reported on somatic chromosome numbers (Manzum *et al.* 2014, Awe and Akpan 2017), but there is no previous report on cytogenetical analysis of BARI released garlic germplasms.

For the present analysis, the four germplasms of *Allium sativum* L. (BARI Rosun-1, LINE ASGAZ002, LINE ASGAZ003, and LINE ASGAZ005) were collected from the Regional Spices Research Centre (RSRC) of Bangladesh Agricultural Research Institute (BARI) and then maintained in the Botanical garden of Jagannath University. Young, healthy roots were cut 2 cm away from the root tips of *Allium sativum* by a fine forceps and then gently washed with water for proper cleaning. Afterwards, the cleaned root tips (RTs) were soaked on a filter paper to remove surface water and pretreated with PDB (Para-dichloro Benzene) for 5.30 hours at room temperature (28-30ºC). For fixation of RTs, 45% acetic acid was used at 4ºC for 15 min and preserved in 70% alcohol for further use. A mixture of 1N HCl and 45% acetic-acid (2:1) was used to hydrolyze the pretreated RTs for 5 min at 60ºC. Then the hydrolyzed RTs were taken on a clean slide and soaked by a filter paper. With a fine blade, the meristematic region of RTs about 0.5 cm was cut and a drop of 1% aceto-orcein was added to keep it in an acetic acid chamber to stain for 1 hour and then a clean cover glass was placed on the material. Gently, the

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materials were tapped by a tooth pick and then squashed by placing thumbs. After all, XSZ-107BN, namely binocular microscope was used with 100× magnification for slides observation and the microphotographs were captured by Euromex CMEX-10 digital USB camera (Holland).

Based on the staining nature of interphase nuclei and prophase chromosomes the germplasms were differentiated into 'Diffuse Type' and 'Continuous Type'. All germplasms had 2n = 2x = 16 chromosomes in which the highest TCL was found in line ASGAZ005 (208.16 ± 4.96 µm) and lowest in ASGAZ002 (145.57 ± 3.17 µm) (Table 1). Line ASGAZ002, and line ASGAZ005 were placed under 2A karyotype, but karyotype 1B and 2B were found in line ASGAZ003 and BARI Rosun-1. On the basis of symmetry-asymmetry indices, AI vs CV_{CI} graph was used to reveal the karyotypic nature of the germplasms.

Twenty to twenty-five interphases and prophases were used to examine their nature based on Tanaka's (1971) classification, whereas finely scattered three metaphases were used for karyomorphological analysis. At first, the karyotype and a haloid idiogram were established regarding the decreasing chromosomal length to classify the chromosomes following the nomenclature of Levan *et al.* (1964). Using Karyo Type Software, different parameters of karyomorphological characteristics were estimated (Altinordu *et al.* 2016). Nature of interphase nuclei and prophase chromosomes, somatic chromosome number, karyotype, idiogram have been presented in Figure 1 and karyomorphological features in Table 1.

On the basis of orcein-staining properties, the interphase nuclei of analyzed four *Allium sativum* L. germplasms *i.e.* BARI Rosun-1, LINE ASGAZ002, LINE ASGAZ003, LINE ASGAZ005 were found as the uniformly stained nucleus (Figs 1A-D), in which heterochromatin blocks were homogeneously distributed throughout the nucleus and categorized as 'Diffuse Type' by Tanaka (1971). In the interphase of analyzed germplasms, prominent nucleolus was found but varied in number (Figs 1A-D, arrow) but no prophase nuclei was found. A prominent nucleolus was observed both in LINE ASGAZ003 and LINE ASGAZ005 (Figs 1C-D, arrow), whereas two and three nucleoli were found in BARI Rosun-1 and LINE ASGAZ002, respectively (Figs 1A-B, arrow). After orcein-staining, the prophase chromosomes of analyzed four germplasms of *A. sativum* had to possess homogenous nature within the inter-length, which was counted as 'Continuous Type' according to the classification of Tanaka (1971) (Figs 1E-H). Tanaka's classification (1971) revealed that the specimens who possessed 'Diffuse type' in interphase nuclei were generally displayed 'Continuous Type' in prophase

chromosomes. Thus, the present finding correlates with Tanaka's classification, which also indicates constitutive heterochromatin in all the analyzed germplasms.

The four *Allium sativum* L. germplasms had 2n = 16 chromosomes in a somatic cell (Figs 1, I-P and Table 1) and basic number x = 8 (Figs 1 Q-T and Table 1). These findings are similar to previous reports (Manzum *et al.* 2014, Awe and Akpan 2017). However, several researchers reported 2n =11, 12, 18, 22 and 32 chromosomes in garlic. This difference might be due to the study materials belonging to different aneuploid series or different cytotypes of garlic.

Table 1. Comparative karyomorphological analysis of four *Allium sativum* L. germplasms.

Features	BARI Rosun-1	Line ASGAZ002	Line ASGAZ003	Line ASGAZ005
2n	2n = 2x = 16	2n = 2x = 16	2n = 2x = 16	2n = 2x = 16
CF	12m + 4sm	14m + 2sm	16m	14m + 2st
TCL (µm)	184.96 ± 2.56	145.57 ± 3.17	195.30 ± 4.12	208.16 ± 4.96
ACL (µm)	11.56	9.10	12.21	13.01
CV_{CI}	14.98	13.63	8.44	18.36
CV_{CL}	14.87	15.95	17.37	16.31
M_{CA}	15.33	12.97	6.74	12.22
AsK %	57.67	56.26	53.23	55.76
TF %	42.33	43.74	46.77	44.24
Syi %	73.41	77.74	87.88	79.34
Rec %	74.44	72.22	79.78	80.18
A_1	0.25	0.21	0.12	0.19
A_2	0.15	0.16	0.17	0.16
A	0.15	0.13	0.07	0.12
AI	2.23	2.17	1.47	2.99
DI	6.22	7.48	7.89	7.77
Category	2B	2A	1B	2A

2n = Somatic chromosome number, x = Basic chromosome number, CF = Centromeric formula, TCL = Total chromosome length (µm), ACL = Average chromosome length (µm), CV_{CI} = Coefficient of variation of centromeric index, CV_{CL} = Coefficient of variation of chromosome length, M_{CA} = Mean centromeric asymmetry, AsK % = Karyotype asymmetry index (%), TF % = Total form value (%), Syi % = Karyotype symmetry index (%), Rec % = The index of chromosomal size resemblance, A_1 =

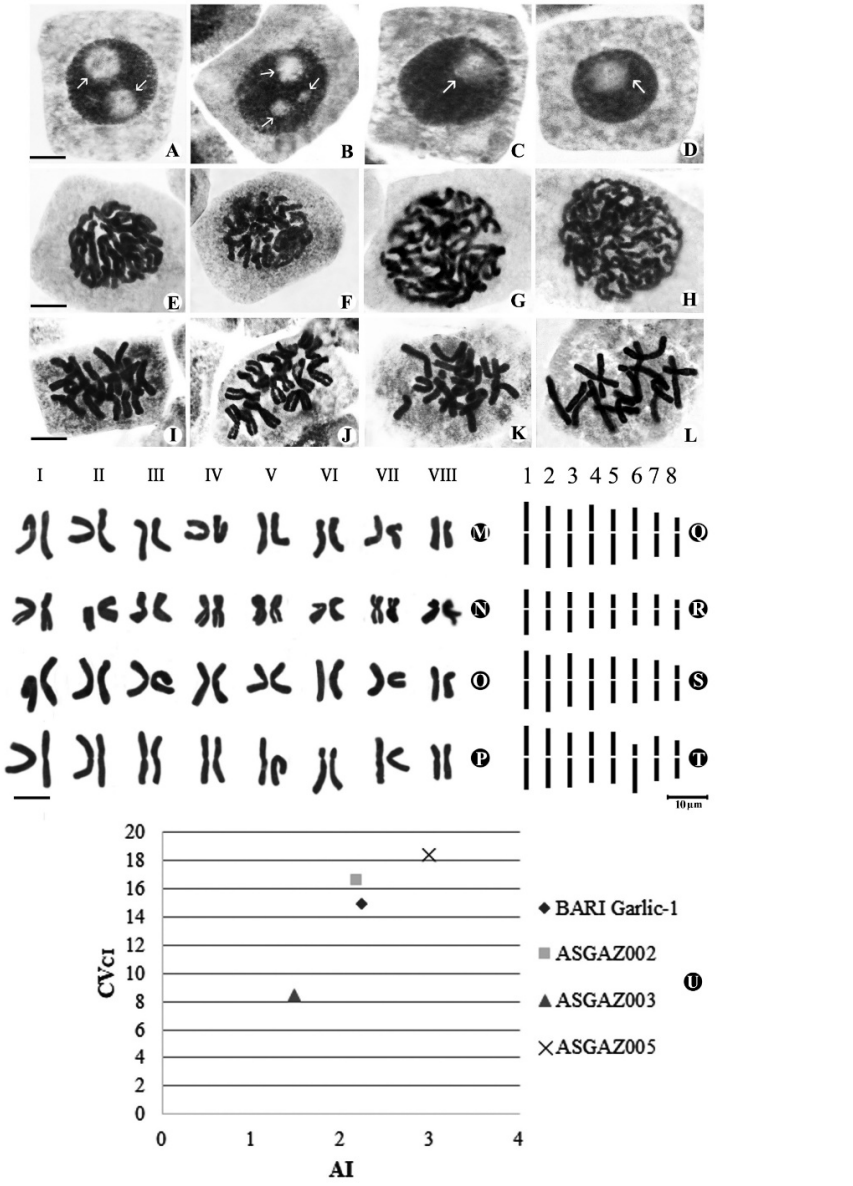

Fig. 1. Orcein-stained mitotic interphase nuclei, prophase chromosomes, metaphase chromosomes, karyotypes and idiograms of four germplasms of *Allium sativum* L. (A, E, I, M, Q) BARI Rosun-1, (B, F, J, N, R) line ASGAZ002, (C, G, K, O, S) line ASGAZ003, (D, H, L, P, T) line ASGAZ005, Arrows indicate the presence of nucleolus, except idiogram all Scale Bar = 5 µm and (U) AI vs CV_{CI} of four BARI germplasms of *Allium sativum* L.

Intrachromosomal asymmetry index, A_2 = Interchromosomal asymmetry index, A = Degree of asymmetry of karyotypes, AI = The asymmetry index, DI = The dispersion index, m = metacentric chromosome, sm = sub-metacentric chromosome, st = acrocentric chromosome.

The four germplasms of *Allium sativum* L. had dominance of metacentric chromosomes. The highest number of sub-metacentric chromosomes was found in BARI Rosun-1 with twelve metacentric chromosomes (12m + 4sm), whereas line ASGAZ003 possessed sixteen metacentric chromosomes (16m) (Figs. 1I, 1K, 1M, 1O and Table 1). A pair of the sub-metacentric chromosome was found in line ASGAZ002 along with fourteen metacentric chromosomes (14m + 2sm) (Figs. 1J, 1N, and Table 1). Only inline ASGAZ005, a pair of the acrocentric chromosome was found where remaining chromosomes were metacentric (14m + 2st) (Figs. 1L, 1P, and Table 1). Among these four garlic germplasms, the highest total chromosome length (TCL) was 208.16 ± 4.96 µm observed in line ASGAZ005 with an average chromosome length (ACL) of 13.01µm and line ASGAZ002 had been exhibited the lowest value of (ACL) which was 9.10 µm along to total chromosome length (TCL) of 145.57 ± 3.17 µm that was also lowest as value. The total length of diploid chromosome complement and average chromosome length were reported as 184.96 ± 2.56 µm and 11.56 µm in BARI Rosun-1, whereas 195.30±4.12 µm, and 12.21µm line ASGAZ003, consecutively (Table 1). No secondary constriction was observed in any of the analyzed garlic germplasms. Based on the classification of Stebbins (1971), line ASGAZ002 and line ASGAZ005 both were categorized as 2A, whereas BARI Rosun-1 and line ASGAZ003 were placed to 2B and 1B, respectively (Table 1).

Karyosystematic analysis is widely used in the study of divergence and genetic relationships among species. In the present analysis, values of CV_{CI} and CV_{CL} showed a wide variation, ranging from 8.44 to 18.36 for $CV_{CI,}$ whereas 14.87 to 17.37 for CV_{CL} (Table 1). Higher chromosomal homogeneity was found in line ASGAZ003 along with CV_{CI} and CV_{CL} values of 8.44 and 17.37, respectively (Table 1). In LINE ASGAZ005 slightly higher CV_{CI} (13.01) and CV_{CL} (18.36) were found, which is of comparatively lower chromosomal homogeneity (Table 1).

Now-a-days, CV_{CI} and AI are considered as two reliable indices that exhibit the inter-relationships and asymmetry of karyotype. As such, these two asymmetry indexes AI and CV_{CI} are put on the X- axis and vertical (Y) axis to draw a bi-directional scattered plot where line ASGAZ003 located at the bottom with lowest AI (1.47) and CV_{CI} (8.44) indicating primitive type in nature meanwhile with highest AI (2.99) and CV_{CI} (18.36)

LINE ASGAZ005 was found on the top with relatively advanced features (Fig. 1U). Each of the analyzed germplasms displayed an adequate variation ranging from 0.12 to 0.25 A_1 and 0.15 to 0.17 in the case of A_2 (Table 1). The total form value (TF%) showed a proportional relationship with Karyotype symmetry index (Syi%) and index of chromosomal size resemblance (Rec%) but formed an opposite connection with Karyotype asymmetry index (AsK%) whereas Dispersion index (DI) was negatively correlated to Mean centromeric asymmetry (M_{CA}) (Table 1).

The present involving karyomorphological analysis with asymmetrical and symmetrical indices of BARI released four *Allium sativum* L. germplasms indicate rearrangement of the chromosome as well as reveals different steps in evolution which may be useful in breeding studies. Thus, the outcome of this research may help in research with these *A. sativum* germplasms as an aid to identification based on for performing the present analysis.

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