Bangladesh J. Bot. **46**(2): 683-689, 2017 (June)

MOLECULAR AND CYTOGENETICAL CHARACTERIZATION IN DIFFERENT VARIETIES OF *LYCOPERSICON ESCULENTUM* MILL.

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Keywords: Karyotype, Characterization, *Lycopersicon esculentum*

Abstract

Five tomato varieties (*Lycopersicon esculentum* Mill.) *viz.* Apurba, Shila, Lalima, BARI tomato-14 and BARI tomato-15 released from Bangladesh Agricultural Research Institute (BARI) were investigated in this study for cytogenetical characterization. The interphase nuclei and prophase chromosomes of these varieties showed different types of orcein-staining pattern. Although the tomato varieties were found to possess 2n = 24 chromosomes, differed in respect to other karyotypic features such as total length of 2n chromosome complements, range of relative length, centromeric index etc. All varieties were found to possess 2n = 24 metacentric chromosomes except BARI tomato-14 where two sub metacentric chromosomes were observed. Each variety showed different types of RAPD banding pattern. Moreover, these were characterized by specific unique RAPD fragments. The combined cytogenetical and RAPD analysis made BARI tomato-14 distinct from the rest. Therefore, each tomato variety could be characterized authentically by cytogenetical and molecular analysis.

Introduction

Tomato (*Lycopersicon esculentum* Mill.), of Solanaceae is considered to be an important vegetable crop and a model species for introduction of agronomical important genus into dicotyledonous crop plant (Wing *et al.* 1994). It originated in Western South America and used as a food originated in Mexico and spread throughout the world following the Spanish colonization of the Americans. It is regarded as the 2nd most important vegetable crop in the world after potato (Bhatia *et al*. 2004, Foolad 2004). Tomato plant is very versatile and fruits are grown either for fresh market or processing. The fruits are eaten fresh and have diversified use like - preparing salad, soup and processed into valuable products like ketchup, sauce, conserved puree, marmalade, chantey, jelly, jam, pickles, juice, paste, powder and many other products. Tomato is rich in vitamins A, vitamin C and fiber and is also cholesterol free (Block *et al*. 1992, Gerster 1997, Rao and Agarwal 2000) as well as provides antioxidant elements such as lycopene which prevents cancer (Bhutani and Kallo 1983). It is measured that, one hundred grams of tomato contain 93.1 g water, 0.7 g fiber, 3.6 g carbohydrate, 0.1 g fat, 23 kilo calorie energy, 0.07 mg vitamin A, 0.01 mg vitamin B, 31 mg vitamin C, 20 mg Ca, 1.8 mg Fe and 129 µg carotene.

The cultivated tomato is a short-lived dicotyledonous annual plant with about 1 - 3 m in height. The plant has weak and woody stem that usually scrambles over other plants. The leaves are 10-25 cm long, pinnate, 5 - 9 leaflets, each leaflet up to 8 cm long with a serrated margin. Both the stem and leaves are densely glandular-hairy. The flowers are 1 - 2 cm across, yellow, with two pointed lobes on the corolla, born in a cyme of 3 - 12 together. The fruit is edible, brightly colored (usually red, from the pigment lycopene) berry, 1 - 2 cm diameter in wild plants, commonly much larger in cultivated forms (Mandal *et. al*. 2011).

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For its enormous nutritive value, the demand of tomato in our country is increasing day by day. By producing more tomato we may earn a considerable amount of foreign exchange. However, due to limitation of lands it is not possible to increase tomato producing area. The most logical way to increase the total production at the national level from our limited land resources is to increase yield per unit area.

It is well established that cultivated tomato is a diploid germplasm with 2n = 2x = 24 chromosomes (Rick 1960, Haskell 1964, Bose and Banerjee 1968). It is well known that karyotype is a stable character and specific for each specimen. In addition, other karyomorphological parameters *viz*. staining property of interphase nuclei and prophase chromosomes should be considered to get more data about each germplasm. Tanaka (1971) classified the different types of interphase nuclei and prophase chromosomes on the basis of orcein staining property.

DNA fingerprinting by RAPD is one of the molecular methods for characterizing germplasm. The term DNA fingerprinting describes the combined use of several single locus detection systems. This method has been using as versatile tool for investigating various genomic aspects of organism. It includes characterization of genetic variability, genome fingerprinting, genome mapping, gene localization, analysis of genome evolution, population genetics, taxonomy, etc. The advantages of RAPD analysis over other methods are its low amount of sample DNA requirement and appearance of high frequency of polymorphic bands (Williams *et al.* 1990). In the present study, a combination of cytogenetical and molecular analysis was carried out to characterize five tomato varieties released from BARI.

Materials and Methods

Five tomato varieties, namely Apurba, Shila, Lalima, BARI tomato-14 and BARI tomato-15 were investigated which were released from BARI (Figs. 1 - 5). Seeds were initially collected from the gene bank of BARI and sown in the Botanic garden, Department of Botany, Jagannath University. Healthy roots were collected from the mature plants and pretreated with 0.002 M 8-hydroxyquinoline for 30 min at 18°C followed by 15 min fixation in 45% acetic acid at 4°C. These were then hydrolyzed in a mixture of 1 N HCl and 45% acetic acid (2 : 1) at 60°C for 8 sec. The root tips were stained and squashed in 1% aceto-orcein. These were observed under a binocular microscope named XSZ-107BN.

Tender leaves were harvested and total genomic DNA was extracted by using modified CTAB method (Doyle and Doyle 1987). DNA concentration was quantified through spectrophotometer (Analylikjena, Specord 50, Germany). The PCR reaction mixture for 25 µl contained template DNA (25 ng) 2 µl, de-ionized distilled water 18.8 µl, Taq buffer A 10× (Tris with 15 mM $MgCl_2$) 2.5 µl, primer (10 µM) 1.0 µl, dNTPs (2.5 mM) 0.5 µl and Taq DNA polymerase (5U/µl) 0.2 µl. PCR amplification was done in an oil free thermal cycler (Biometra UNOII, Germany) for 46 cycles after initial denature at 94°C for 5 min, denature at 94°C for 1 min, annealing at 36°C for 30 sec, extension at 72°C for 3 min and final extension at 72°C for 5 min. Three primers of Operon Technologies, USA *viz.* OPA-3 (5'-AGT CAG CCA C-3'), OPA-5 (5'-AGG GGT CTT G-3') and OPA-10 (5'-GTG ATG GCA G-3') series were used. The amplified products were separated electrophoretically on 1% agarose gel. The gel was prepared using 1.0 g agarose powder containing ethidium bromide (10 mg/ml) 8 µl and 100 ml 1× TAE buffer. Agarose gel electrophoresis was conducted in 1× TAE buffer at 50 v and 100 mA for 1 hr. DNA ladder (1 kb) was electrophoresed alongside the RAPD reactions as marker. DNA bands were observed on UV-transilluminator and photographed by a gel documentation system. The PCR products were analyzed after gel electrophoresis. The photographs were critically examined

on the basis of presence (1) or absence (0), size of bands and overall polymorphism of bands. These were carried out for further investigation. RAPD analysis was then combined to create a single data matrix. This was used for estimating linkage distance (D) and constructing a UPGMA (Unweighted Pair Group Method of Arithmetic Means) dendrogram among the varieties using computer program Popgene (Version 1.31) (Yeh *et al.* 1999). Linkage distances were computed from frequencies of polymorphic markers to estimate genetic relationship between the studied five tomato varieties using UPGMA.

Results and Discussion

Five varieties of tomato *viz.*, Aburba, Shila, Lalima, BARI tomato-14 and BARI tomato-15 were cytogenetically studied after orcein staining. The nature of staining property of interphase nuclei and prophase chromosomes were accomplished to get additional information. Besides, DNA fingerprinting by using RAPD was also carried out for molecular characterization.

In case of Shila, Lalima, BARI tomato-14 and BARI tomato-15, darkly stained small regions were found which sometimes form heteropycnotic body in the interphase nuclei (Figs. 7 - 10). These regions were heterochromatic in nature. According to Tanaka's (1971) classification, this type of staining is called 'Complex Chromocenter Type' interphase nuclei. The prophase chromosomes of Lalima, Shila, BARI tomato-14 and BARI tomato-15 stained in interstitial region (Figs. 12 - 15). Tanaka (1971) classified this nature of staining as 'Interstitial Type' prophase chromosomes. It means that the aggregated heterochromatins scattered in the prophase chromosomes. In this respect, the four varieties followed the general features.

On the other hand, the staining properties of interphase nuclei in Apurba did not match with any of the category proposed by Tanaka (1971) (Fig. 6). Moreover, a clear zone was present around the nucleus. The prophase chromosome of this specimen showed uniform staining revealed continuous type of staining (Fig. 11). The staining property of interphase nuclei and prophase chromosomes did not follow the general features. The reason for this disagreement is unknown. Probably it was due to presence of some facultative heterochromatin. Presence of facultative heterochromatin in the interphase nuclei was reported earlier. Therefore, on the basis of staining of interphase nuclei and prophase chromosome the variety Apurba could be differentiated from the rest four.

Table 1. Comparative karyotype analysis of five varieties of *Lycopersicon esculentum*.

Variety	2n	Range of chromosomal length (μm)	Total length of 2n chromosome complement (μm)	Centromeric formulae
Apurba	24	0.65-1.16	21.05	24m
Shila	24	0.51-1.53	24.26	24m
Lalima	24	0.54-0.99	18.63	24m
BARI tomato-14	24	0.82-1.29	24.45	22m+2sm
BARI tomato-15	24	0.48-0.92	16.15	24m

m = Metacentric chromosome, sm = Submetacentric chromosome.

All the five tomato varieties were found to possess 2n = 24 metacentric chromosomes (Figs. 16-20, 21-25). The centromeric formulae and the range of chromosomal length revealed strict symmetric karyotype of four varieties (Table 1). On the other hand, a pair of sub-metacentric

chromosomes was observed in BARI tomato-14 (Fig. 24, Table 1). The probable reason for origin of sub metacentric chromosome was due to deletion of chromosomal part which was reported earlier by several workers in different plant species (Fawzia and Alam 2011, Rahman *et al.* 2013). However, in tomato it might be a new report. The presence of sub metacentric chromosome in BARI tomato-14, made its karyotype comparatively heterogenous from the rest four. According to Stebbins (1971), heterogenous or asymmetric karyotypes are relatively advanced having more adaptive features. The BARI tomato-14 has some agronomically better characteristic such as (i) large fruit size, (ii) color is dark red and shiny (iii) more yield and (iv) grown all over Bangladesh round the year (while the rest four grow in restricted part and season). Therefore, the better agronomical characters of BARI tomato-14 correlates with its karyotype asymmetry.

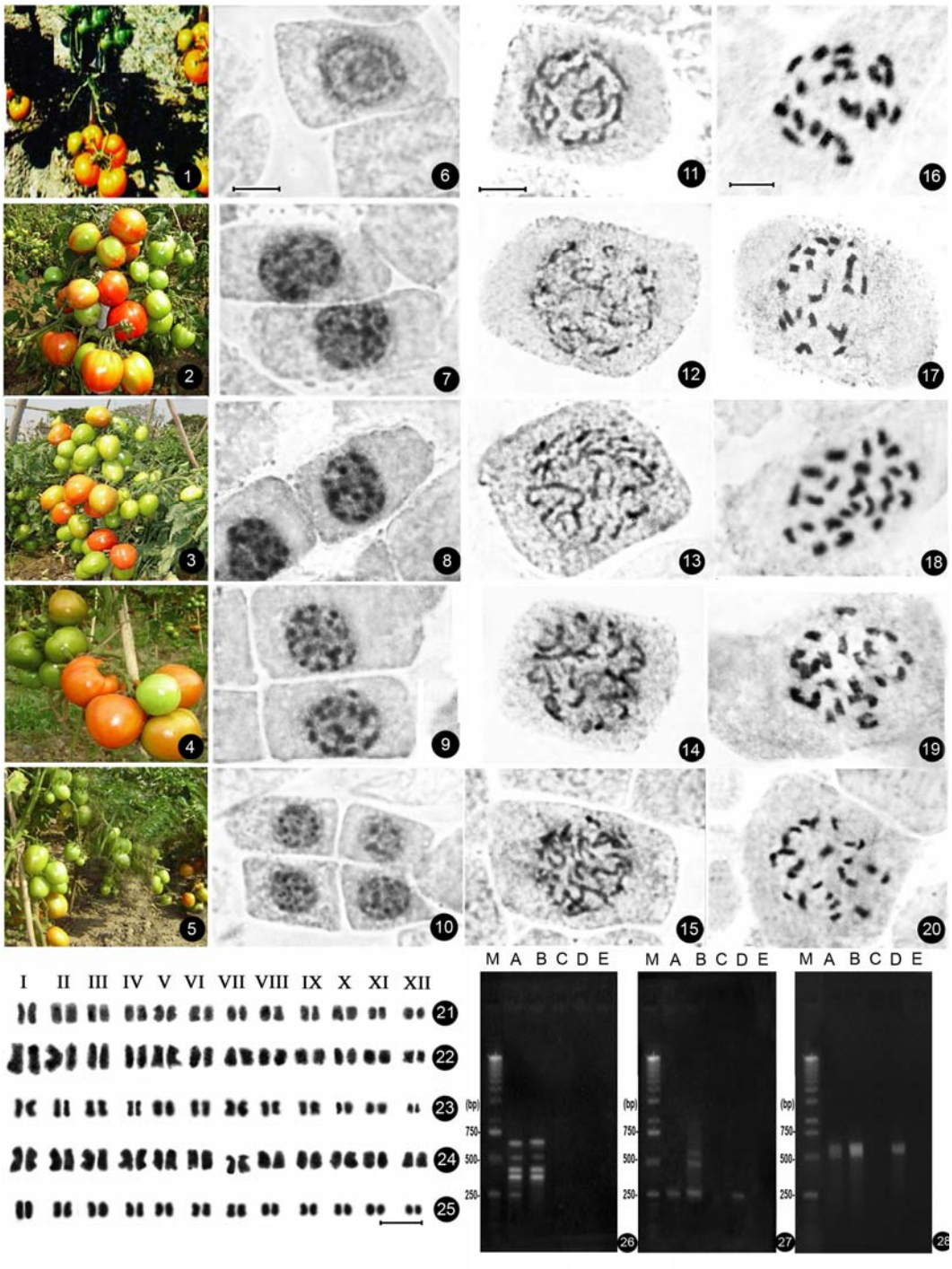
Table 2. Compilation of RAPD analysis of five BARI tomato varieties.

Primer codes	Total bands	Size ranges (bp)	No. of polymorphic bands	No. and size (bp) of variety specific unique bands	Polymorphisms (%)
OPA-3	5	250-680	5	250 bp in Apurba	100
OPA-5	3	250-550	3	550 bp and 500 bp in Shila	100
OPA-10	1	550	1	--	100

Three different primer combinations were used for RAPD fingerprinting analysis of five BARI tomato varieties. Each specimen showed characteristic RAPD banding pattern. In case of Primer OPA-3, four common bands (600, 480, 380 and 300 bp) were observed in variety Apurba and Shila (Fig. 26). Moreover, these two varieties had common bands in other primers with different base pair. This result indicated that the two varieties were sharing much DNA fragments amplified with related primers. After using Primer OPA-5, two unique bands were observed in Shila with different base pair (550 and 500 bp) (Fig. 27). The fragment size of 250 bp with OPA-3 was unique and thus could be used as marker for variety Apurba. Only one band of 550 bp with OPA-10 was found in Apurba, Shila and BARI tomato-14. No band was observed in Lalima and BARI tomato-15 (Fig. 28).

On the basis of RAPD banding pattern five tomato varieties were initially grouped into two different clusters, such as cluster-1 (C_1) and cluster-2 (C_2). C_1 comprises of Apurba and Shila whereas C_2 formed with rest three varieties. Again C_2 was divided into two sub-clusters (SC_1 and

Figs 1-28. Comparative fruit morphology, orcein staining and RAPD analysis of five varieties of *Lycopersicon esculentum* Mill. 1. Morphology of Apurba, 2. Morphology of Shila , 3. Morphology of Lalima, 4. Morphology of BARI tomato-14, 5. Morphology of BARI tomato-15, 6. Interphase nuclei of Apurba, 7. Interphase nuclei of Shila, 8. Interphase nuclei of Lalima, 9. Interphase nuclei of BARI tomato-14, 10. Interphase nuclei of BARI tomato-15, 11. Prophase chromosomes of Apurba, 12. Prophase chromosomes of Shila, 13. Prophase chromosomes of Lalima , 14. Prophase chromosomes of BARI tomato-14, 16. Prophase chromosomes of BARI tomato-15, 16. Mitotic metaphase chromosomes of Apurba, 17. Mitotic metaphase chromosomes of Shila, 18. Mitotic metaphase chromosomes of Lalima, 19. Mitotic metaphase chromosomes of BARI tomato-14, 20. Mitotic metaphase chromosomes of BARI tomato-15, 21. Karyotype prepared from orcein-stained mitotic metaphase chromosomes of Apurba, 22. Karyotype prepared from orcein-stained mitotic metaphase chromosomes of Shila, 23. Karyotype prepared from orcein-stained mitotic metaphase chromosomes of Lalima, 24. Karyotype prepared from orcein-stained mitotic metaphase chromosomes of BARI tomato-14, 25. Karyotype prepared from orcein-stained mitotic metaphase chromosomes of BARI tomato-15 (Bar = 2 µm), 26. RAPD analysis with Primer OPA-3, 27. RAPD analysis with Primer OPA-5, 28. RAPD analysis with Primer OPA-10. M-1Kb DNA. Ladder, A-Apurba, B-Shila, C-Lalima, D-BARI tomato-14, E-BARI tomato-15.



(Figures legend left page)

SC_2). Lalima and BARI tomato-15 belong to SC_1 whereas BARI tomato-14 forms SC_2 (Fig. 29). The cluster analysis on the basis of RAPD banding pattern indicated that Lalima and BARI tomato-15 again Apurba and Shila were closely related with each other. In contrast, BARI tomato-14 is distantly related than the other four varieties (Fig. 29). This variety was karyotypically and agronomically different from the other four varieties. Therefore, the cluster analysis is correlated with cytological and agronomical features of this variety.

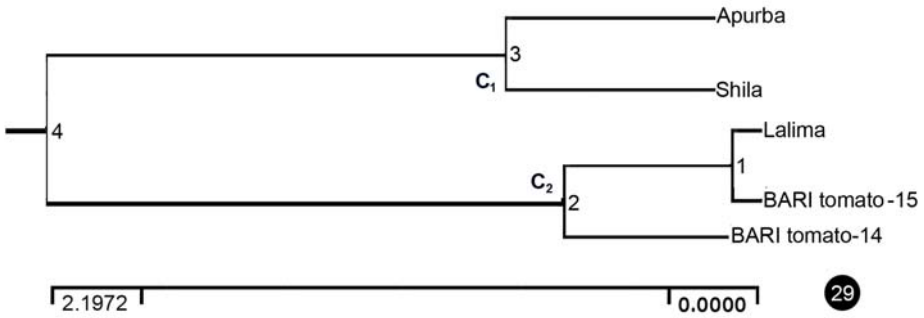


Fig. 29. Cluster analysis by UPGMA of five varieties in *Lycopersicon esculentum* Mill. based on different RAPD markers.

Therefore, the five BARI tomato varieties could be characterized on the basis of karyotype and molecular data. This information will be helpful for future breeding program and identify these varieties released from BARI.

Acknowledgements

The authors are indebted to the Ministry of Science and Technology, the People's Republic of Bangladesh for funding the present research project. They are thankful to the Director of the Chromosome Research Centre, Department of Botany, University of Dhaka for providing facilities to carry out the research work.

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(Manuscript received on 26 June, 2016; revised on 3 November, 2016)