

**COMPARATIVE KARYOTYPE AND RAPD ANALYSIS FOR
CHARACTERIZING THREE VARIETIES OF
LYCOPERSICON ESCULENTUM MILL.**

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Abstract

Three tomato varieties (*Lycopersicon esculentum* Mill.) viz. BARI tomato-11, BARI tomato-2 and BARI tomato-3 released from Bangladesh Agriculture Research Institute (BARI) were characterized by karyotype analysis and RAPD markers. BARI tomato-11 and BARI tomato-2 were found to possess $2n = 24$ metacentric chromosomes. On the other hand, $2n = 25$ metacentric chromosomes were observed in BARI tomato-3 revealing a trisomic variety. The karyotype of the three varieties was very symmetric and each of the varieties showed specific and unique RAPD fingerprinting. Therefore, based on karyotype and RAPD analysis the three tomato varieties could be characterized.

Introduction

Tomato (*Lycopersicon esculentum* Mill.) is one of the most important vegetables, native of Central and South America. It is extensively cultivated throughout the world. It is eaten as raw or cooked in salads and used sauces, soups and meat or fish dishes as flavouring agents (Ahmed *et al.* 2009).

Bangladesh Agriculture Research Institute (BARI) has made several breeding programmes to create variability for transferring desirable characters such as fruit size, colour, disease resistance, etc. for the development of suitable tomato variety. As a result, 15 varieties and 8 hybrid varieties of tomato were released by BARI (Md. Shahidul Islam, BARI, Personal communication 2012). These varieties are characterized solely by morphological features. No genomic information about these varieties is available. Genetic information has been considered as an important factor and pre-requisite for plant improvement programme (Chaudhuri *et al.* 1976). Therefore, detailed genetic information of the available varieties of tomato is necessary for proper identification and characterization.

It is well established that cultivated tomato is a diploid with $2n = 2x = 24$ chromosomes (Rick 1960, Haskell 1964, Bose and Banerjee 1968). In a few varieties/races, $2n = 25$ chromosomes (trisomic) were also reported (Chaney and Reeves 1973, Gill 1978). Generally, trisomic produces half of the sterile gametes ($n + 1$) due to irregular distribution of chromosomes at anaphase. This type of gametes generally does not participate in fertilization. Therefore, a screening is necessary prior to release the variety to the farmers. Moreover, the varieties released from BARI should be characterized genomically to prevent the trade piracy.

Karyotype of a variety is a stable character and often play role to distinguish different taxa. However, the problem arises when different taxa possess same chromosome number and centromeric position. In such a situation, other karyotype features like total length of $2n$ chromosome complements and range of individual chromosome length might help in this regard.

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In addition molecular characterization is more reliable and authentic. In recent years, Random Amplified Polymorphic DNA (RAPD) analysis through the Polymerase Chain Reaction (PCR) has been widely used in molecular characterization and trace the phylogeny of diverse plant and animal species. This technique has contributed widely in the studies of genetic diversity and phylogenetic analysis (Savolainen and Chase 2003, Nybom 2004). These markers are used as versatile tools to provide the correct estimate of genetic diversity. The main advantages of RAPD analysis over other molecular methods are low sample DNA requirements, the high frequency of detectable polymorphic bands (Williams *et al.* 1990) and independent from the effects of environmental factors (Powell *et al.* 1995, Garcia *et al.* 1998, Kuras *et al.* 2004).

In this study, three tomato varieties released from BARI were considered to - (a) confirm diploid chromosome number of the varieties, (b) elucidate aneuploidy or other chromosomal aberration and (c) characterize the varieties based on karyotypic analysis and RAPD fingerprinting.

Materials and Methods

Three varieties of tomato namely, (i) BARI tomato-11 (Jhumka), (ii) BARI tomato-02 (Ratan) and (iii) BARI tomato-03 were used in this study (Figs a - c). These varieties were collected from BARI and maintained in the Botanical Garden, Department of Botany, University of Dhaka, Bangladesh.

Healthy roots of the grown plants from the field were collected 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 hydrolysed in a mixture of 1 N HCl and 45% acetic acid (2 : 1) at 60°C for 8 s. The root tips were stained and squashed in 1% aceto-orcein.

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 (Analytikjena, 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 MgCl₂) 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 s, extension at 72°C for 3 min and final extension at 72°C for 5 min. Six primers of Operon Technologies, USA *viz.* Batch-7736-030 (GAA ACG GGT G), Batch-7736-031 (GTT GCG ATC C), Batch-7736-032 (TGC CGA GCT G), Batch-7736-033 (GGG TAA CGC C), Batch-7736-034 (TCA CGT CCA C) and Batch- 7736-036 (CCC GCC TTC C) 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 volts and 100 mA for 1.5 h. 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 "Statistica". Linkage distances were computed from frequencies of polymorphic markers to estimate genetic relationship between the studied three tomato varieties using UPGMA (Sneath and Sokal 1973).

Results and Discussion

BARI tomato-11 and BARI tomato-2 were found to possess $2n = 24$ metacentric chromosomes (Figs 1d - e, Table 1). Same $2n$ chromosome number was reported earlier in tomato (Rick 1960, Haskell 1964, Bose and Banerjee 1968). On the other hand, $2n = 25$ metacentric chromosomes were found in BARI tomato-3 (Fig. 1f, Table 1). The extra chromosome was the smallest and a regular number of pair XII (Table 1). It suggested that this variety is a primary trisomic. Trisomic in tomato was reported earlier (Chaney and Reeves 1973, Gill 1978). However, trisomic tomato (BARI tomato-3) is a new report for BARI tomato variety. The trisomic variety was also karyotypically different from the rest two varieties in respect of - (i) the range of individual chromosome length (0.41 - 0.75 μm) was the lowest among three varieties (Table 1) and (ii) total length of $2n$ chromosome complements (14.11 μm , Table 1). Therefore, karyotypically BARI tomato-3 is different from the rest two.

Table 1. Karyotypic characterization of three varieties of *Lycopersicon esculentum*.

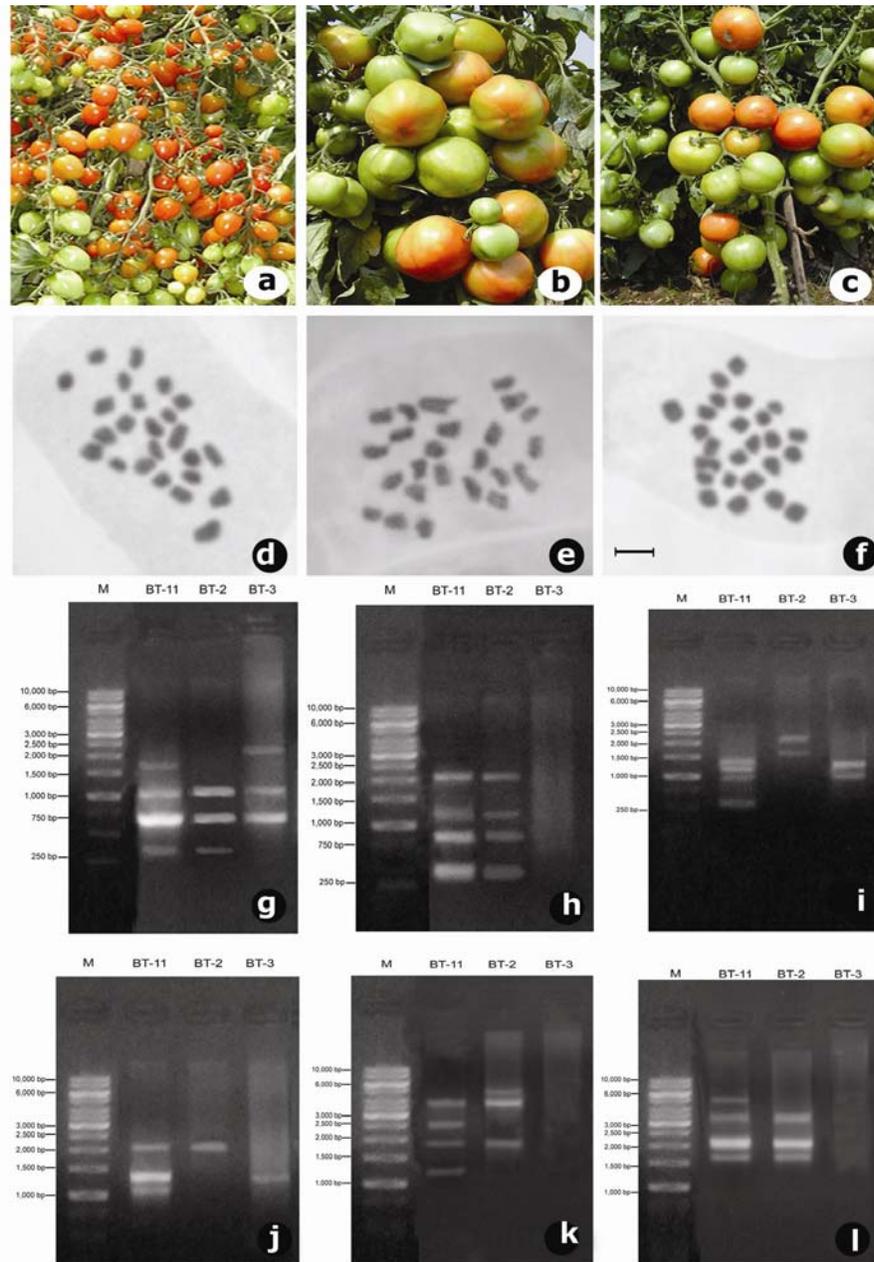
Varieties	2n	Range of chromosomal length (μm)	Total length of 2n chromosome complement (μm)	Karyotype formulae
BARI tomato-11	24	0.58 - 1.05	17.10	24 m
BARI tomato-02	24	0.63 - 1.17	19.99	24 m
BARI tomato-03	25	0.41 - 0.75	14.11	25 m

m = Metacentric chromosome.

The three varieties had all metacentric chromosomes (Figs 1d - f, Table 1). The difference between smallest and biggest chromosomes was almost 0.5 μm only (Table 1). As a result, no gradual decrease in chromosomal length was observed. These are the characteristic features of strict symmetric karyotype. Some other members of the genus *Solanum* e.g., *S. vilossum*, *S. americanum* and *S. melongena* have moderately symmetric karyotype a few sub-metacentric chromosomes are present in their karyotypes (Sultana and Alam 2007, Fawzia and Alam 2011). Symmetric karyotype is primitive in nature (Stebbins 1971). Therefore, tomatoes are primitive than these species of *Solanum*.

RAPD analysis revealed no band in BARI tomato-3 in primer batch-7736-031, 7736-034 and 7736-036 (Figs 1h, k, l). This result suggested the absence of those sequences in BARI tomato-3. In primer combination 7736-030, two fragments of 1000 and 700 bp were common in all the three varieties (Fig. 1g). This revealed that the three varieties are sharing some common fragments of genome.

In spite of a few common bands, each variety showed specific characteristic RAPD fingerprinting. These bands were unique and not present in other varieties. For example, a fragment of 1700 and 2300 bp was found in primer 7736-030 in BARI tomato-11 and BARI tomato-3, respectively (Fig. 1g). In primer 7736-032, fragment size of 1200 and 300 bp were found in only BARI tomato-11. Two bands of 2400 and 1700 bp were found in only BARI tomato-2 in the same primer (7736-032, Fig. 1i). BARI tomato-11 showed a band of 1000 bp in primer 7736-033 which was absent in other two varieties (Fig. 1j). Two fragments of 2500, 1200 bp and one of 5000 bp were found in BARI tomato-11 and BARI tomato-2, respectively with primer 7736-034 (Fig. 1k). BARI tomato-11 showed a band of 5000 bp in primer 7736-036 which was absent in other two varieties (Fig. 1l).



Figs 1a - l. Morphology and comparative cytogenetical and molecular analysis of three varieties of *Lycopersicon esculentum*. a, BARI tomato-11 (Jhumka); b, BARI tomato-02 (Ratan); c, BARI tomato-03, d, e and f, Orcein-stained mitotic metaphase chromosomes of BARI tomato-11 (Jhumka), BARI tomato-02 (Ratan), BARI tomato-03, respectively (Bar = 1 μ m); g, RAPD analysis with Primer Batch-7736-030; h, RAPD analysis with Primer Batch-7736-031; i, RAPD analysis with Primer Batch-7736-032; j, RAPD analysis with Batch-7736-033; k, RAPD analysis with Primer Batch-7736-034 and l, RAPD analysis with Primer Batch- 7736-036.

The dendrogram tree showed that on the basis of the cluster analysis, BARI tomato-11 and BARI tomato-2 were placed in a cluster at linkage distance 13. BARI tomato-3 was placed in another cluster far away from the first cluster at linkage distance 17.5 (Fig. 2). Therefore, the three varieties could easily be identified with the help of cluster analysis.

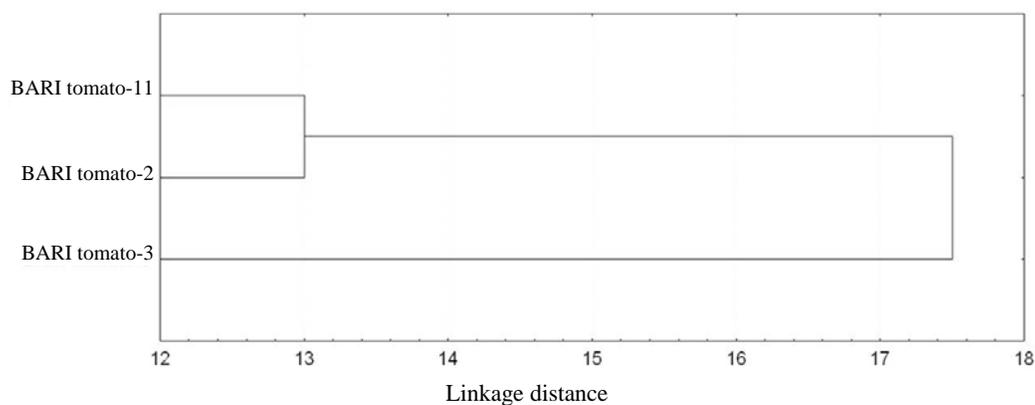


Fig. 2. Cluster analysis by UPGMA of three varieties in *Lycopersicon esculentum* Mill. based on different RAPD markers.

The above results showed that BARI tomato-3 is quite different from the rest two varieties of tomato in respect to - (i) $2n = 25$ chromosome instead of $2n = 24$, revealing a trisomic nature (Fig. 1c). (ii) lowest total length of $2n$ chromosome complement ($14.25 \mu\text{m}$) and range of individual chromosome length ($0.75\text{-}0.41\mu\text{m}$) (Table 1), (iii) no RAPD fragment was found in primer batch 7736-031, 7736-034 and 7736-036 (Figs 1h, k, l), (iv) showed characteristic specific bands in primer 7736-030 and 7736-034 (Figs 1g, k) and (v) the tree dendrogram showed that this variety was placed in different cluster far away from other two varieties at linkage distance 17.5 (Fig. 2).

With the help of above parameters, BARI tomato-3 could be easily characterized. This variety is a primary trisomic. Trisomic produces unbalanced gametes due to irregular distribution of chromosomes at anaphase and generally resulting in 50% sterile gametes. This lowers the yield of BARI tomato-3. Therefore, BARI authority must characterize tomato varieties before releasing those to the farmers.

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