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RAPD Based Fingerprinting of Tomato Genotypes for Identification of Mutant and Wild Cherry Specific Markers*

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Abstract: Ten tomato (*Lycopersicon esculentum*) genotypes showing distinct variation in morphological and anatomical features were screened for random amplified polymorphic DNA (RAPD). Germplasm under study comprised 3 mutant derivatives, 3 hybrids, their parents and one wild cherry tomato genotype. Twelve random decamer operon primers of OPAB series could generate total 690 bands of which 33.3% were polymorphic. Mutant specific polymorphic markers were detected. Polymorphy could clearly identify mutant derivatives, cultivated genotypes and wild cherry tomato genotype. The UPGMA based dendrogram divided genotypes into two main clusters. Interestingly, wild cherry tomato was present in cluster of mutant derivatives, affirming its potential for utilization as unique source for tomato breeding. Low level of genetic diversity was noted in cultivated tomato genotypes (Mean = 0.138) indicating the existence of limited or narrow genetic base.

Key words: Tomato, DNA fingerprinting, genetic diversity, mutant derivatives, RAPD markers

Introduction

Tomato (*Lycopersicon esculentum* Mill.) is one of the most important vegetable crops grown in World. The origin and early events in the domestication of tomato crop are obscure. The most likely ancestor of the cultivated tomato is wild cherry tomato known as *L. esculentum* var. *cerasiforme*. This is found growing in tropical and sub-tropical America (Primary center of origin). Wild cherry tomato is also found frequently in the fields in India (Secondary center of origin).

There is drastic reduction in Tomato gene pool and sources of variability in tomato due to continuous inbreeding and selection of similar type of cultivars. Wild relatives like *Lycopersicon hirsutum* and *Lycopersicon pimpinellifolium* are good sources of variability but with certain drawbacks of genetic drift. *Lycopersicon esculentum* var. *cerasiforme* (Cherry tomato) is promising source for variability spread widely in primary and secondary center of origin. Sub species Cerasiforme originated in Andean region spread to Mexico and then disseminated to the old world (Rick and Holle, 1990). Cerasiforme germplasm has been found to be more or less variable than *L. esculentum* for morphological and molecular markers (Miller and Tanksley, 1990; Williams and St. Clair, 1993) depending on germplasm studies. Villand *et al.* (1998) reported eight RAPD markers with polymorphism between *L. esculentum* and *L. esculentum* var. *cerasiforme*.

We have reported anatomical and morphological basis for genetic variability in tomato (Deshpande and Kulkarni, 2005). Shashidhar *et al.* (2000) identified RAPD markers for maximum root length in rice. These markers were co-dominant in nature. Marker aided selection strategies are discussed with reference to breeding for drought tolerance in Rice and Pearl Millet (Hash *et al.*,

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2000a, b). The accomplished and proposed strategies in Tomato (Rajput *et al*., 2006), Chili (Adetula, 2006), Maize (Veldboom and Lee, 1996; Ribaut and Betran, 1999), Pearl Millet (Yadav *et al*., 1999, Hash and Barmel-cox, 2000) and Sorghum (Tuinstra *et al*., 1998; Subudhi and Nguyen, 2000; Tao *et al*., 2000) were discussed.

Molecular genetic markers are powerful tools to analyze genetic relationship and genetic diversity. A number of marker systems such as RAPD, AFLP, SSR, ISSR, CAPS and STMS can be used in this endeavor. RFLP, can be used but they are expensive and time consuming. RAPD showed high level of resolution equivalent to RFLPs for determining genetic relationships. The dominant marker nature, technical simplicity and speed of RAPD methodology is a principle advantage (Gepts, 1993). Estimates of genetic similarity based on RAPDs, have been obtained for rice (Cho *et al*., 1999), wheat (Liu *et al*., 1999), Maize (Hahn *et al*., 1995), Barley (Strelachenko *et al*., 1999) and some other crops.

DNA markers have been used to quantify the genetic diversity and determine phenotypic relationship in several plant species (Lee, 1998). The low variability of tomato genome is because of natural isolation of populations due to self-pollination. The low level of heterozygosity and genetic polymorphism in *Lycopersicon esculentum* is already well known (Smulders *et al*., 1997). Molecular identification of tomato cultivars is difficult as tomato genome is highly conserved (Grandillo and Tanksley, 1996).

Dongre and Kharbikar (2004) reported 0.13 to 0.35% genetic divergences in 25 accessions of cotton (*Gossypium hirsutum*) genotypes with RAPD markers. Pandey *et al*. (2004) reported mutant specific markers in cowpea [*Vigna unguiculata* (L.) walp] screened for RAPD variation. Eight mutants and the parent could be identified using mutant specific markers.

Kochieva *et al*. (2002) used RAPD for estimating genetic polymorphism in and phylogenetic relationship among species of genus Lycopersicon. RAPD genotype analysis of 53 species and cultivars of genus Lycopersicon (Tourn). Mill revealed their high genetic polymorphism. Intraspecific polymorphism was maximum (79%) in *Lycopersicon peruvianum* and minimum (9%) in *Lycopersicon parviflorum*. In general, genome divergence among cross-pollinating tomato species was substantially high than in self pollinating species.

However, very scanty information is available regarding mutant specific and *Lycopersicon cerasiforme* (Cherry tomato) specific markers. Considering very narrow genetic base in cultivated tomato genotypes it is essential to study variability available to achieve breeding goal to develop elite genotypes. RAPD markers being dominant type of markers were used for studying molecular polymorphism as method of choice. In present investigation level of genetic polymorphism at DNA level was surveyed between cultivated tomato genotypes, mutant derivatives and wild cherry tomato from secondary center of origin.

Materials and Methods

Plant Material

Seeds of genotype TG-80 were soaked in distilled water for 24 h and then soaked in 0.15% (w/v) Colchicin for 48 h for development of Colchiploids. One mutant plant with desirable traits was selected. M_2 progeny was grown and 4 plants resembling original mutants were selected for further study. Wild cherry tomato genotype was collected from village Dhanegaon Dist. Nanded (M.S. India) i.e., secondary center of origin. For present investigation ten genotypes including 3 mutant derivatives, 3 hybrids, 2 cultivated genotypes and one wild genotype (*Lycopersicon esculentum* var. *cerasiforme*) i.e., cherry tomato were subjected for genetic diversity analysis using RAPD. Study aimed to reconfirm morphological and anatomical polymorphism amongst genotypes at molecular level. The genotypes included in this investigation, along with key morphological and anatomical characteristics are shown in Table 1.

Table 1: Key morphological and anatomical features of germplasm

Genotype	Source of/pedigree	Stomata per microscopic field (Lower side of leaf)	Palisade mesophyll height (μm)	Root length (cm)	Root: Shoot ratio (Fresh weight g)	No. of hair root/5 cm
MTG 1-1	Mutant derivative (M_2)	102	248	67	0.08	49
MTG 1-2	Mutant derivative (M_2)	116	236	39	0.06	43
MTG 1-3	Mutant derivative (M_2)	132	213	43	0.05	39
Hy –2	TG -42 × Mutant 1	140	168	47	0.07	33
Hy –3	Mutant 1 × TG -42	122	239	71.3	0.05	39
TG –42	Pure line	216	162	32	0.04	31
Hy –4	TG-80 × TG-64	176	147	37	0.04	27
TG –80	Pure line	205	167	28	0.04	32
TG –64	Pure line	222	126	35	0.04	29
Wild cherry	*L. esculentum, var. cerasiforme*	165	179	63	0.05	34

RAPD Analysis

DNA was isolated from lyophilized leaf material following the modified CTAB method (Doyle and Doyle, 1990). The PCR reaction was performed in a final volume of 25 μL containing 1×Taq polymerase buffer (Gibco. BRL), 0.5 units of Taq polymerase (Gibco, BRL). 200 μM each of dNTPs (Promega), 15 ng random primer (Operon technologies), 2.5 mM $MgCl_2$ and 30 ng of total genomic DNA. The PCR was performed using the following cycling parameters: Initial denaturation at 95°C for 5 min followed by the amplification programme of 40 cycles of denaturation -94°C for 1 min, primer annealing at 37°C for 2 min and extension at 72°C for 2.5 min. The 40th cycle was followed by an extended final extension step at 72°C for 6 min and held at 4°C until electrophoresis. The PCR products were resolved on 1.5% agarose gels and visualized under UV light after ethidium bromide staining (Sambrook *et al*., 1989).

Data Analysis

Bands were scored as 1 for their presence or 0 for their absence across the cultivars for generating a binary matrix. Genetic similarity (GS) was computed based on Jaccard's coefficient of similarity (Jaccard, 1908). The data was subsequently used to construct a dendrogram using the unweighted pair group method of arithmetical averages (UPGMA) algorithm. All the computations were carried out using the NTSYS-pc software (ver. 2.0, Rohlf, 1998).

Results

Mutant derivatives, Hybrids developed by using mutant as parents i.e., (MTG 1×TG-42, TG-42×MTG-1) cultivated genotypes TG-80, 64 and one wild cherry tomato genotype (*Lycopersicon esculentum* var. *cerasiforme*) were used for RAPD assay using random primers to study the genetic diversity (Fig. 1).

Initially 68 random primers from different operon series were screened. On the basis of robustness, polymorphy and reproducibility 12-decamer primers were selected. These OPAB series primers with 10 genotypes generated total 690 bands. The number of bands per primer, varied from 3 to 8 with a mean of 3.5. The number of polymorphic bands per primer ranged between zero and 3.7 with a mean of 0.6. The frequency of polymorphic markers is 33.3% which is lower than earlier reports 37.2% Williams *et al*. (1993) and 63.8% Archak *et al*. (2002), 44.4% Rus Kortekas (1994). The band profile obtained with 12 primers are summarized in Table 2.

Pair wise similarity between the cultivars calculated on the basis of Jaccard's coefficient ranged between 0.400 to 0.884 with a mean of 0.796. Mean marker diversity among the cultivars was 0.204. Overall high level of pair wise similarity and low level of polymorphic value (1-F) indicates low

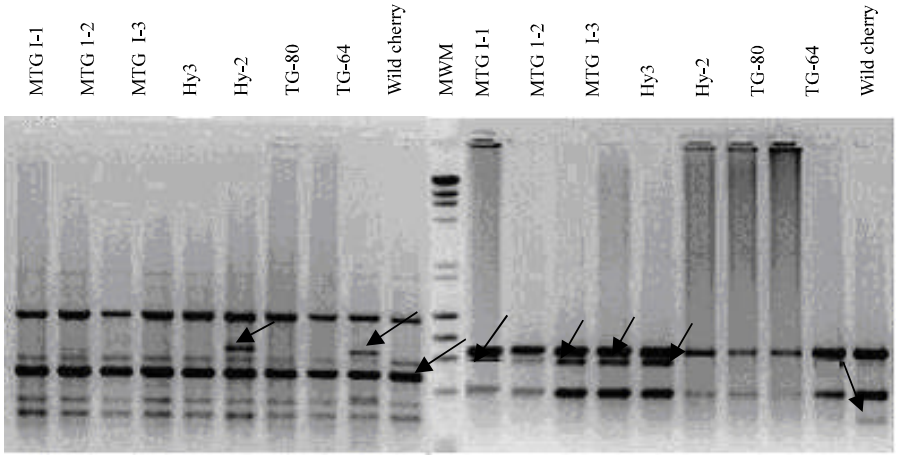

Fig. 1: RAPD profile of the ten Lycopersicon cultivars obtained with random primers OPAB-4 and OPAB-13. Unique or cultivar specific bands are indicated with arrow. Lane marked as MWM displays molecular weight marker

Table 2: Analysis of polymorphism obtained with random primers among various cultivars of *Lycopersicon* spp.

Primer	Sequence 5' to 3'	Total bands	Polymorphic bands	Percent polymorphism
OPAB -3	TGGCGCACAC	17	11	64.7
OPAB –4	GGCACGCGTT	6	6	-
OPAB –5	CCCGAAGCGA	11	3	27.27
OPAB –7	GTAAACCGCC	11	7	63.63
OPAB –8	GTTACGGACC	3	-	-
OPAB –13	CCTACCGTGG	5	3	60.00
OPAB –15	CCTCCTTCTC	7	1	14.14
OPAB –16	CCCGGATGGT	8	3	37.50
OPAB –17	TCGCAACCAG	6	2	30.00
OPAB –18	CTGGCGTGTC	9	2	22.22
OPAB -19	ACACCGATGG	8	-	-
OPAB -20	CTTCTCGGAC	6	2	33.33
	Total bands	97	40	
	No. of bands per primer	8.0	3.5	

level of genetic diversity in tomato (Table 3). Similar results have been obtained in tomato accessions from other regions of world, including both primary and secondary centers of diversity (Villand *et al*., 1998). Archak *et al.* (2002) also confirms low level of genetic diversity in tomato genotypes. Less intercultivar polymorphism is due to self-pollination, breeding for similar fruit type and growth habit.

Table 4 provides results of group wise analysis of diversity in the material under study. Mean marker diversity was highest for wild cherry tomato and least in cultivated genotypes. Mean marker diversity when compared amongst group exhibited significant differences. Within group mean Jaccard's Pair wise similarity was highest in cultivated genotypes followed in that order by mutant derivatives and wild tomato.

Primer OPAB-5 generated a unique 1353 bp band observed only in mutant derivatives (MTG-1, MTG-2 and MTG-3) and Hybrid-3 (MTG 1×TG-42) along with wild cherry tomato genotype. This wild genotype is ancestor of modern day cultivated tomato and known as potential source for fruit quality traits like high vit. C and organoleptic flavor.

Primer OPAB-7 produced a 603 bp band in wild cherry tomato absent in all other genotypes. This can be used as species-specific marker. Primer OPAB-8 and OPAB-17 produced bands monomorphic

Table 3: Polymorphic Values (1 -F) among pairs of Lycopersicon genotypes

Genotype	MTG 1-1	MTG 1-2	MTG 1-3	Hy-3	Hy-2	TG-42	Hy -4	TG-80	TG-64	Wild cherry
MTG 1-1	1.000									
MTG 1-2	0.200	1.000								
MTG 1-3	0.333	0.166	1.000							
Hy-3	0.333	0.116	0.126	1.000						
Hy-2	0.144	0.333	0.456	0.456	1.000					
TG-42	0.252	0.400	0.500	0.500	0.144	1.000				
Hy-4	0.252	0.400	0.500	0.500	0.144	0.112	1.000			
TG-80	0.144	0.333	0.456	0.456	0.116	0.144	0.144	1.000		
TG-64	0.144	0.333	0.456	0.456	0.144	0.144	0.144	0.144	1.000	
Wild cherry	0.400	0.333	0.286	0.286	0.556	0.600	0.600	0.556	0.600	1.000

Table 4: Comparative mean marker diversity between different groups of cultivars

Genotypes	Average mean marker diversity
Between mutant and wild tomato	0.373
Between cultivated genotypes	0.138
Between cultivated and wild tomato	0.582
Between mutant and cultivated	0.371
Between mutant derivatives	0.204

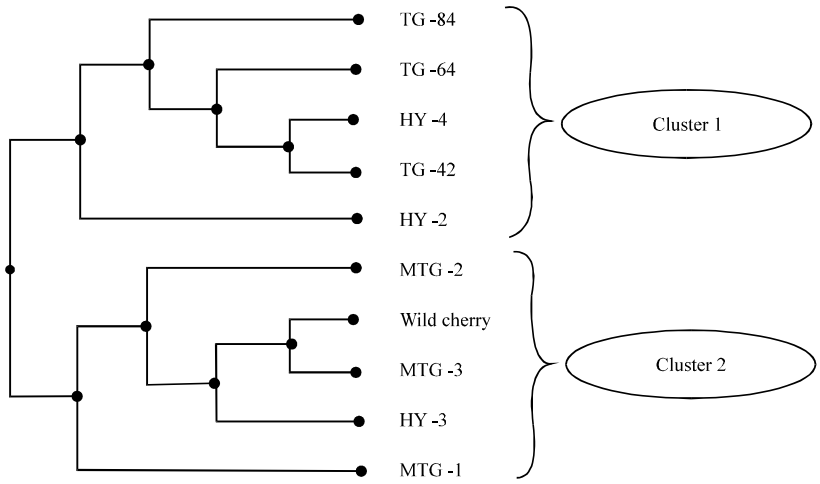

Fig. 2: Phenetic dendrogram generated by software phylodraw showing relationship among different Lycopersicon genotypes

in all cultivars under study. This indicates conserved genomic regions in Lycopersicon genus. Primer OPAB-18 produced 593 bp band only present in cultivated genotypes TG-80, TG-64, TG-42 that was absent in mutant derivatives, its hybrids and wild cherry tomato.

Primer OPAB-20 produced a 603 bp band present in Hybrid-3 only and absent all other genotypes. Hybrid-3 exhibited 7 bands while other genotypes exhibited only 3-4 bands. Development of new bands may be due to addition of new priming sites in genotype. Two bands (1822 and 872 bp) are tomato genome specific bands present in all genotypes. Primer OPAB-3 amplified 1822 in TG-42, TG-80, TG-64 and Hybrid-4 that was absent in mutant derivatives and wild cherry tomato. Primer OPAB-13 produced 1078 bp mutant specific and 603 bp wild cherry tomato specific band. Such bands can be taken as mutant specific markers that can identify mutants. These specific markers can be utilized for maintaince of genetic purity of these genotypes along with trait of interest. Primer OPAB-15 produced only 4 bands in all 10 genotypes exhibiting very low level of amplification.

Cluster Analysis

Mean marker diversity data was submitted in Phylodraw software for cluster analysis. Dendrogram clearly divided genotypes under study into two separate clusters (Fig. 2). Cluster II consisted genotypes including mutant derivatives (MTG-1-1, MTG-1-2, MTG 1-3) and its hybrid (Hybrid-3) along with wild cherry tomato. Cluster I consisted cultivated genotypes TG-80, TG-64, TG-42, Hy-2 and Hy-4. *Lycopersicon esculentum* var. *cerasiforme* which is wild relative of modern day cultivated tomato formed separate sub cluster. Mutant derivatives in the same cluster are also observed to be similar at morphological levels. As all mutants derivatives were selected on basis of resemblance with original mutant plant having characters like thick leaves, long calyx and deep root system.

Discussion

Due to high sensitivity and ability to distinguish even closely related genotypes DNA fingerprinting appears to be especially valuable for analysis of vegetable crops with narrow genetic base like tomato. This fingerprinting effort could result in identification of mutant specific and wild cherry specific bands. The fingerprinting of wild cherry race (*Lycopersicon esculentum* var. *cerasiforme*) found in Southern and central part of India is unique source for drought tolerance and organoleptic flavor of fruit is worth mentioning.

The polymorphic nature of certain bands clearly points out possible molecular markers specific to mutants and wild cherry genotypes in tomato. These results supports and are in accordance with morphological and anatomical characterization. Genetic diversity at molecular level can be considered as one of the indication of presence of genes, which can be used for germplasm improvement.

Primer OPAB-5 exhibited creating priming site during meiosis leading to generation of new amplification product in mutant derivatives. This band was absent in modern day cultivated genotypes (TG-64, TG-80, TG-42), which shows low level of variability. This unique marker would have initiated due to recombination, point mutation or random segregation of chromatins at meiosis. Present results are in accordance with Huchett and Botha (1995) and Darnell *et al*. (1990). This may be the main reason for absence of wild cherry specific and mutant specific bands in cultivated genotypes.

El Sayed *et al*. (2002) used RAPD markers for genetic variability studies in tomato cultivars strain A and Ace VF. RAPD markers are questioned for their reproducibility but our results were reproducible indicating their usefulness in genetic diversity studies.

Substantial amount of genetic distance during biotic stress resistance studies have been reported. TH-802 is a nematode resistant cultivar, its greater distance from normal cultivated genotypes like Selection-12 was reported. Its greater distance could be due to introgression of genetic material from wild species *Lycopersicon peruvianum*, a source of nematode resistance in tomato (Foolad *et al*., 1993).

Archak *et al*. (2002) reported 0.165 average value for mean marker diversity studying 27 tomato cultivars dividing them into 4 different groups. Our study reports even lower value (0.138), which are comparable to 0.137 reported by Villand *et al*. (1998). Present investigation reconfirms very narrow genetic base amongst cultivated tomato genotypes. Substantial amount of genetic variation obtained when compared to wild cherry tomato and mutant derivatives (0.582 and 0.371, respectively) is a new source of genetic diversity for germplasm advancement. Williams and St. Clair. (1993) found similarity of large number of RAPD loci between *L. esculentum* and *L. esculentum* var. *cerasiforme*. Present investigation shows promising polymorphism between cultivated and wild cherry tomato genotype may be because this is new and unique collection from secondary center of origin.

Present investigation reports new source of genetic diversity i.e., unique tomato mutants and *Lycopersicon esculentum* var. *cerasiforme* found in India. This investigation also reconfirms the narrowing down genetic base in tomato germplasm resources. Collection and molecular characterization

of cherry tomato genotypes from secondary source of origin will add up to unique genetic variability in tomato genetic stocks. *Inter-se* crosses followed by molecular characterization will be helpful in future to maintain this new genetic variability for further utilization. The source is of prime importance for fruit quality improvement considering very narrow genetic base in tomato gene pool. Obviously, breeding for elite genotypes would best be achieved with utilization of diverse material like this along with proper management of available genetic resources.

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