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Agro-Morphological and Molecular Characterization of Local Tomato Cultivars Grown in Pakhal Region of Pakistan Using RAPD Markers

[1]Samia Nawaz, [1]Zubeda Chaudhry, [2]Ayesha Bibi, [3]Sohail Ahmad Jan, [2]Khatiba Bibi and [1]Asma

[1]Department of Botany, Hazara University Dhodial Mansehra, Pakistan
[2]Department of Genetics, Hazara University Dhodial Mansehra, Pakistan
[3]Department of Biotechnology, Quaid-i-Azam University, Islamabad, Pakistan

Abstract: Tomato is a very important dietary vegetable crop. Like any other crop species, improvement in tomato for quality and quantity of fruit has always been on the top of scientific breeding programs. Pakhal is an important region in Pakistan for tomato production but problem lies with the proper available cultivars for the area as farmers use different sources to get seed which result in variable per acre production. The studies were carried out to select the best genotype for this area. For this purpose different varieties from the local farmer's field were collected. Genomic DNA was extracted from 3 weeks old plants and PCR study was performed for Randomly Amplified Polymorphic DNA(RAPD) analysis from 13 genotypes (from Pakhal region) were used for morphological and DNA based fingerprinting. The fruit shape showed largest variation with three types (round, ovate and pear-shaped) among all tested genotypes. A total of 10 different RAPD primers were used for screening of diverse tomato genotypes. Six out of eleven morphological traits showed maximum diversity. A total of thirty-five reproducible and scorable amplification products were generated out of which 23 (65.7%) fragments were polymorphic with 10 RAPD primers. With an average of 5 bands per primer the highest level of polymorphism was detected with primer OPA-18 (83%) whereas primer OPA-05 detected the least polymorphism (25%). It was found that F1 Hybrid and Rio USA accessions were much related to each other. The present study is useful for the selection of improved tomato genotypes for maximum yield production.

Key words: Genetic Variation · Randomly Amplified Polymorphic DNA · Tomato

INTRODUCTION

Tomato (*Solanumlycopersicum* L.), originated from South America is nowadays one of the significant vegetable crops of special economic importance and widely grown plant in Solanaceaefamily. Botanically, it is a fruit and horticulturally it is a vegetable [1]. Tomato plays an important role in human nutrition by providing essential amino acids, vitamins and minerals. Its vitamin C content is particularly high. It also contains carotenes andlycopene, a very potent antioxidant that may be an important contributor to prevention of cell cancers [2]. Other than fresh fruit consumption, processed forms as canned tomatoes, ketchup and variety of sauces, juice, soups, paste and powder are equally popular. Pakistan produces about 0.3 million tons of tomato annually. Khyber Pakhtunkhwa (KP) produces 30% of tomato production. Parachanar, Pakhal and Battal valley are major tomato growing areas of KP. Tomato crop has a tremendous export potential due to its demand in the international market [3]. Bulging population demands increase in the production of tomatoes, which can be met by increasing per unit production. Tomato cultivars are much sensitive to hot climate, is one of the limitations in optimum production of summer tomato crop in plains of Naz *et al.* [4]. Like any other crop species, improvement in tomato for quality and quantity of fruit has always been on the top of scientific breeding programs. The processes of domestication and artificial selection contributed to the depletion of genetic diversity in tomato. A chance of

Corresponding Author: Ayesha Bibi, Department of Genetics, Hazara University Dhodial Mansehra, Pakistan
Sohail Ahmad Jan, Department of Biotechnology, Quaid-i-Azam University, Islamabad, Pakistan
E-mail: sjan.parc@gmail.com, sohailahmadjan3@gmail.com.

success of any breeding programs depends on availability of information regarding existing genetic variability. Without genetic variation, breeding efforts remain ineffective and crops may lack important traits such disease and insect resistances [5]. Morphological trait measurements can provide a simple technique of quantifying genetic variation while simultaneously assessing genotype performance under relevant growing environments [6, 7]. Molecular markers can provide an effective tool for efficient selection of desired agronomic traits because they are based on the plant genotypes and thus, are independent of environmental variation. Pakhal is the best region for production but problem lies with the proper available cultivars for the area as farmers use different sources to get seed which result in variable per acre production. The present study was carried out to develop a morpho-molecular based screening system for identification of best tomato genotype for Pakhal regions of Khyber Pakhtunkhwa, Pakistan.

MATERIALS AND METHODS

Mature Seeds of 13 important commonly grown tomato genotypes were collected from Pakhal area from local farmers. General information about the plant material (Tomato genotypes) used is given in Table 1. The research work was done at Department of Botany during 2013. Thirteen local varieties were used for morphological study. The experiment was conducted in Randomized Complete Block, (RCB) Design with three replications. Forty days, old seedlings were transferred from pots to field. The distance between rows and plants were kept 20 cm. Standard agronomic practices and plant protection measures were adopted. The Data was collected for 11 quantitative traits and 6 qualitative traits. The data collected on agro-botanical traits were subjected to statistical analysis using Statistic v. 8.1 [8].

DNA Isolation: The total genomic DNA from fresh tomato leaves was isolated by using protocol of Doyle and Doyle [9] with minor modifications. The plants were raised in Botany Department, Hazara University, Khyber Paktunkhwa, Pakistan. Approximately 0.5 g of fresh leaves was collected and placed in an eppendorf tube. The tubes were immediately placed in liquid Nitrogen. The leaf samples were crushed to fine powder. To each tube 500µL DNA-extraction buffer was added and mixed well and 500µL of the stock solution of Phenol, Chlorofom, Isoamyalcohol in the ratio of 25: 24: 1 is added. The tubes were individually vortexes until homogenization and

Table 1: General information about the tomato genotypes

S.No	Accessions Name	Accession Symbol	Source
1	Rio USA	RU	Local farmers
2	Rio Italy	RI	Local farmers
3	Bari-5	B5	Local farmers
4	Pusa-Ruby	PR	Local farmers
5	Paut-Behar	PB	Local farmers
6	Rio NARC	RN	Local farmers
7	Rio Grand USA4	RGU	Local farmers
8	F1-Hybrid	FH	Local farmers
9	Rio-Grain Italy	RGI	Local farmers
10	Rattan Long Tomato	R	Local cultivars
11	Behar	B	Local cultivars
12	T- 245	T245	Local cultivars
13	Mision 102	M102	Local cultivars

incubated at 65°C for 30 min.. The tubes were then centrifuged at 12000 rpm for 10 minutes. Aqueous phase of the mixture was transferred to fresh tubes, The DNA was extracted with ice chilled Isopropanol and then washed with 70% Ethanol. After drying DNA pellet was dissolved in 50µL of TE buffers. DNA was quantified by spectrophotometer at A260/A280 wavelengths. Stock DNA samples were stored at –20°C and diluted to 20 ng /µL for Polymerase Chain Reaction (PCR) analysis.

RAPD Analysis: RAPD (Randomly Amplified Polymorphic DNA) primers obtained from Gene Link, Inc, USA; were used to amplify genomic DNA isolated from tomato genotypes. Details of the used primers are presented in Table 2. Amplification of DNA was performed in 10 mMTris-HCl pH 9.0, 1.5mM MgCl2, 10 mMdNTPs, 5 mM of Taq DNA polymerase and 20 ng of DNA per 20 ul reaction were used Polymerase Chain Reaction (PCR) analysis. After initial denaturation for 4 min at 94°C, 40 cycles at each cycle comprised 1min denaturation at 94°C, 60 sec annealing at 55°C and 2 min extension at 72°C with a final extension for 7 min at 72°C. Amplified products were mixed 6 X loading dye and were electrophoreses on a 2% agarose gel using 1 x Tris Borate buffer at pH 8.0. Gel was then run at a constant voltage of 100 volts for one hour and was documented using Gel Documentation System.

Statistical Analysis: Each DNA fragment amplified by a given primer was treated as a unit character and the RAPD fragments were scored as present (1) or absent (0) for each of the primer-genotype combinations. Only major bands were scored and faint bands were not considered. The molecular size of the amplification products was calculated from a known size of DNA fragments of a 1Kb molecular weight marker. The presence and absence of the

Table 2: Information about the RAPD primers

S.No	Primer name	Primer sequence	Amplified fragments	Polymorphic fragments	Percent polymorphism	Fragment size(bp)
1	OPA-02	TGCCGAGCTG	5	4	80	300-1600
2	OPA-05	AGGGGTCTTG	4	1	25	500-1800
3	OPA-15	TTCCGAACCC	2	1	50	900-1100
4	OPA-18	AGGTGACCGT	6	5	83	270-2300
5	OPB-10	CTGCTGGGAC	8	3	37.5	250-1200
6	OPB-17	AGGGAACGAG	5	3	60	250-1000
7	OPB-18	CCACAGCAGT	5	2	40	300-800
8	OPC-02	GTGAGGCGTC	7	4	57.1	400-2500
9	OPC-08	TGGACCGGTG	6	3	50	400-2200
10	OPC-09	CTCACCGTCC	3	2	67	400-1500

bands was scored in a binary data matrix. Dendrogram was constructed by using a distance matrix using Nie and Lie's Coefficientswhich is defined as $Sij = 2Nij/(Ni + Nj)$, where $Nij$is the number of bands present in the cultivars i and j and Ni and Njrepresenting the number of bands present in cultivar i and j, respectively [10]. All computations were carried out using the computer program NTSYS, PC version 2.1 (Applied Biostatistics Inc., USA).

RESULTS

Six out of 11 traits had morphological variation in the total 13 tomato varieties. Numbers of observed types for each trait ranged from 2 to 3. Four traits (36.3 %) had more diversity than 2 types, of which fruit shape had the largest variation with three types (Round, ovate and pear-shaped) (Fig. 2). No noticeable differences for 5 traits including leaf shape, stem and leaf hairiness, inflorescence type, corolla color, plant posture and fruit shoulder were observed. This study revealed that maximum height of plant was 3.9 ft in F1 Hybrid while minimum plant height was 2.3 ft in T-245. Minimum days to maturity were 97 days for T-245 and maximum days to maturity was observed for F1 Hybrid *i.e.* 123 days. The maximum leaf length (9.7 cm) was noted in F1-Hybrid and minimum length (7.9 cm) was found in T-245. In fruit morphology highest number of fruit per plant (58) was recorded for F1-Hybrid while lowest number of fruits per plant (31) was recorded for Mission-102.

Genetic Polymorphism among Tomato Accessions: A total 30 RAPD primers were selected for the genetic analysis of all tomato accessions. Only 10 polymorphic RAPD primers were accessed for the genetic distance calculation to find out the phylogenetic relationship among 13 tomato accessions under study (Fig. 1). A total of thirty-five reproducible and scorable amplification products were generated out of which

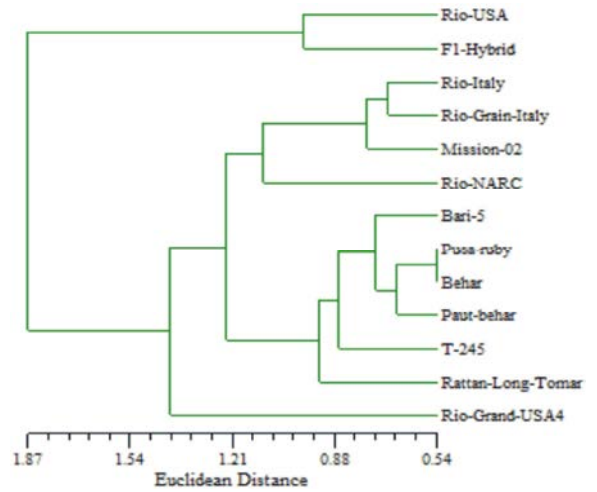

Fig. 1: Dendrogram for the 13 tomato cultivars constructed from RAPD data analysis using UPGMA similarity matrices computed according to Dice coefficients

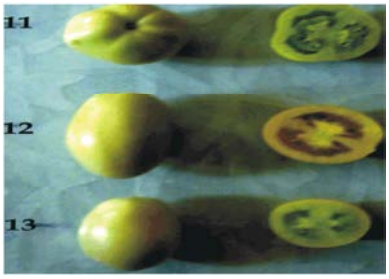

Fig. 2: Shape of three improve fruit genotypes of tomato

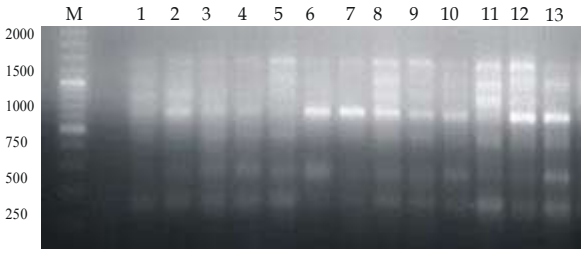

Fig. 3: RAPD banding pattern of 13 tomato genotypes generated by random primers PC-02

Table 3: Mean ± standard deviation of group members

Groups	Days to Maturity (days)	Leaf Length (cm)	Leaf Width (mm)	Number of fruits/Plant
Group I	117.5±5.5	9.6±0.1	3.8±0.4	68±1
Group II	104.75±8.4	9.025±0.3	3.525±0.24	41.75±4.2
Group III	99±2.3	8.4±0.39	4.13±0.21	52±2.83

Table 4: Composition of different cluster on the basis of RAPD

S.No	Cluster	Number of Varieties	Cluster Members
1	Cluster I	2	Rio-USA, F1 Hybrid
2	Cluster II	4	Rio-Italy, Rio-Grain-Italy, Mission-102, Rio-NARC
3	Cluster III	6	Bari-5, Pussa-Ruby, Behar, Paut-Behar, T-245, Rattan- Long-Tomato

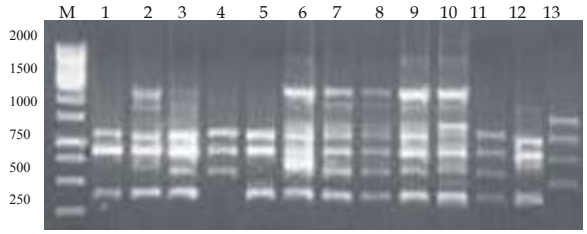

Fig. 4: RAPD banding pattern of thirteen tomato genotypes generated by random primers OPA-02

23 (65.7%) fragments were polymorphic. The number of amplified products generated by each primer varied from 2 (OPA-15) to 8 (OPB-10). With an average of 5 bands per primer the highest level of polymorphism was detected with primer OPA-18 (83%) whereas primer OPA-05 detected the least polymorphism (25%) (Table 2). Average polymorphism across 13 selected tomato accessions was found to be 54%. The size of the amplified fragments ranged from 250 (OPB 10) to 2500 bp (OPC-02) (Fig. 3, 4). Pair-wise estimates of similarity for 13 tomato genotypes ranged from 0.01 to 1.87. Lowest similarity coefficient was calculated for these groups 0.01 (Bari-5 and Rio – Italy), 0.02 (Rattan-Long Tomato and Mission-102), 0.03 (F1 Hybrid and Paut-Behar), 0.03 (Behar and Rio-Grand-USA4), 0.08 (Rio Grain Italy and Paut Behar), 0.12 (Rio-Italy and Rio-NARC), 0.12 (Rio USA and Bari) and 0.16 (T-245 and Rio Italy). Mean± Standard Deviation was calculated for three groups (Table 3, 4). Group IV is not included as it has only one member. Members of Group I had longest days to maturity, longest leaf length, longest plant height, highest number of flowers and highest number of fruits per plant, while group II members had medium leaf length, leaf width and lowest number of fruits per plant. Group III members had shortest days to maturity and medium number of fruit per plan (Table 3).

DISCUSSION

The present study was conducted to evaluate different tomato varieties from Pakhal region through morphological and molecular markers and the construction of phylogenetic tree for these cultivars on the bases of RAPD analysis. Morphological characterization included the plant height, leaf length, leaf width, number offlowers and number of fruits per plant, fruit shape and size were studied. Among PCR based assays, various primer systems viz; Allele Specific Amplification (ASA), Cleavage Amplification Polymorphic Sequences (CAPS), Sequence Tag Site (STS) etc. have been used. Introduction of Randomly Amplified Polymorphic DNA (RAPD) has an additional advantage that it does not require any sequence information. Recently morphological and molecular based diversity via RAPDs markers have been tested for the estimation of genetic diversity in many plant species including tomato. The primers that were used in this study have been useful for assessing different plant genotypes [11-14]. RAPD molecular technique was used to molecularly characterize by using 30 decamer primers, out of which 10 primers showed polymorphism and generated a total 51 loci in all varieties. Maximum number of loci 14 was obtained in the genome of Rattan Long tomato and minimum number of loci 7 in tomato genotype Behar. Similarly, Ezekiel *et al*. [15] used 10 RAPD primers for characterization of Nigerian tomato cultivars and suggested that RAPD markers are efficient in characterization of tomato genotypes. The possibility and application of the RAPD technique in varietal identification of tomato have been well explored [15-17]. The maximum genetic polymorphism was recorded in both local and exotic cultivars.Bernardett *et al.* [18] also studied the genetic variation among 35 different tomato genotypes and about 257 (78.6%) reproducibly

polymorphic bands were obtained with 20 sets of primers. Elham *et al.* [19] also reported the phylogenetic relationship in many important tomato varieties by using RAPD markers.

CONCLUSION

Morphological and molecular study is important for identification and selection of improve tomato genotypes. We have screened 13 important commonly grown tomato genotypes of Pakhal region of Pakistan. Maximum morphological and molecular variation was observed in all tested genotypes. The high performance genotypes will be useful for local farmer of that area.

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