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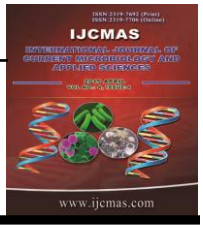

Original Research Article

DNA Polymorphism of Three Tomato (*Solanum lycopersicum*) Landraces from Sudan Using RAPD Markers

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Keywords

Tomato, *Solanum lycopersicum*, DNA polymorphism, RAPD markers

A B S T R A C T

This work was carried out in the Agricultural Genetic Engineering Research Institute (AGERI) in Cairo, Egypt to study genetic relationship between three landraces of tomato *(Solanum lycopersicum*) from Sudan in relation to a heat sensitive commercial genotype, RAPD markers using 16 primers were used to detect DNA polymorphism. The 16 primers gave polymorphism percentage for each single primer range between 0 – 83% with a total polymorphism percentage reaching 39%. Primers OPB-0 2, OPA- 10 and OPB- 20 gave the highest polymorphism percentage in a range of 71 - 83% while OPC-07 did not give any polymorphic fragments. The dendogram showed that Toktuk and Abu Zarif belong to the same group with a genomic DNA similarity reaching 91%. While similarity between this group and Strain B was 88%. HSD 0977 (The cherry tomato) belongs to separate group related to the main group that correlates the three other genotypes with a percentage of 85%.

Introduction

Abiotic stresses are often interrelated, either individually or in combination, they cause morphological, physiological, biochemical, and molecular changes that adversely affect plant growth and productivity and ultimately yield. Heat, drought, cold, and salinity are the major abiotic stresses that induce severe cellular damage in plant species including crop plants. Extreme temperature variations during hot summers can damage the intermolecular interactions needed for proper growth, thus impairing plant development and fruit set. The increasing threat of climate change is already having a substantial impact on agricultural production worldwide as heat waves cause significantly yield losses with great risks for future global food security (Christensen and Christensen, 2007).

At higher temperature and inadequate moisture, the productivity of tomato is far below the potential yields. This necessitates the identification of abiotic stress tolerant

genotypes (Bray, 1997; Ribaut *et al.*, 2002). Major lacunae for inadequate progress in improving tolerance have been the lack of suitable techniques to screen a large number of germplasm accessions and segregating populations for thermotolerance (Ganeshkumar *et al.*, 1999; Heslop-Harrison, 2002).

Tremendous variations in heat tolerance exist within and between species, providing opportunities to improve crop heat stress tolerance through genetic means (Ehlers and Hall, 1998).

Genetic variation in the ability of tomatoes to set fruit under high temperature conditions, has made selection for heat tolerance possible. For example, in several genotypes differing in their capacity for thermotolerance, as well as in sugarcane, an increased chlorophyll a:b ratio is observed in the tolerant genotypes under high temperatures, indicating that these changes are related to thermo tolerance (Camejo *et al.*, 2006; Wahid, 2007).

Heat stress due to increased temperature is an agricultural problem in many areas in the world including Sudan. Due to the expected climate changes of a few degrees especially temperature, cold weather crops are expected to decline by about 15% in the next fifty years (Lane and Jarvis, 2007).

Tomato landraces which grow and produce successfully under the high summer temperatures in Sudan usually have a low productivity. However, they may furnish a source of thermotolerance that could be utilized to develop that trait in more productive and less thermotolerant cultivars. There is also a strong need to use molecular genetic basis to identify some heat tolerant marker genes found in the local landraces so as to utilize them in the molecular breeding programs to produce tomato cultivars with improved heat tolerance in the future.

The analysis of genetic diversity and relatedness between or within different species, populations and individuals is a prerequisite towards effective utilization and protection of plant genetic resources.

RAPD is a powerful tool for identification and monitoring pedigree breeding record of inbred parents or varieties and determining genetic relationships among genotypes (Alam *et al.*, 2012). It is an updated plant varietal identification method independent of restriction sites employing in the detection of polymorphisms by using the PCR technology (Welsh and McCleland, 1990).

This study aimed to detect DNA polymorphism among three tomato landraces from Sudan in relation to a heat sensitive commercial genotype using RAPD markers.

Materials and Methods

This work was carried out in the experimental green house of the Agricultural Genetic Engineering Research Institute (AGERI), Giza, Egypt.

Plant materials used in this study were three landraces of tomato *(Solanum Lycopersicum*) grown by traditional farmers in different sites of Sudan in addition to Strain B, a heat sensitive commercial cultivar grown in Sudan during winter. HSD 977 is a type of cherry tomato from the Blue Nile State, Toktuk a type of local tomato from North Darfur characterized by its large fruits and Abu Zarif, a landrace with large fruits grown in Wad Ramly, North to Khartoum.

Tomato seeds of the four genotypes were sown in fiber trays divided to 4x4 cm squares containing a mixture of soil constitute of peat moss and vermiculite. Trays were kept inside the green house at 30^{o}C and 80% relative humidity and irrigation was carried out at interval of two days.

Two weeks after germination, single seedlings were transferred to 15cm diameter pots containing the same soil mixture. The fungicide topsin (1g/l) was added mixed with irrigation water. Seedlings were fertilized with N.P.K. (1g/l) and irrigation was carried out at interval of two days. After seedlings transplanting temperature was raised to 37/23^{o}C day and night respectively. Two weeks after transplanting, temperature was raised gradually to 45/29 ^{o}C (Average day and night temperatures during summer in Sudan). Irrigation continued at interval of two days until reaching maturity. Leaves were collected in liquid nitrogen to carry on DNA extraction.

DNA extraction

DNA was extracted according to Lodhi *et al.,* (1994). The method used was CTAB-based (Cetyle-Trimethyl Ammonium Bromide). DNA concentration was determined by diluting the DNA 1:5 with sterilized distilled water and loaded in 0.7% agarose gel (Sambrook *et al.,* 1989) and run against DNA size marker.

RAPD technique was carried out using 16 primers (Operon Technologies, Ca, USA). The sequences of primers were given in table 1.

PCR reaction conditions for tomato genomic DNA were similar to those described by Williams *et al.* (1990). The PCR products were separated in 10% agarose gel after staining with ethidium bromide. The DNA marker was loaded in the first and last well. After electrophoresis completed the RAPD fragments were visualized with UV transilluminator, photographed and scored from the photos. DNA polymorphism was calculated and the dendogram was constructed.

Results and Discussion

DNA bands were scored from the photographs as bands present in all lanes (monomorphic bands) or bands absent from one or more lanes (polymorphic bands).

Polymorphism percentage for each primer was calculated for the 16 primers. Number of amplification bands per primer varied between 6 and 14 for the 16 tested primers. The total amplified fragments were 155 bands, 61 of them were polymorphic. The 16 primers gave polymorphism percentage for each single primer range between 0 – 83% with a total polymorphism percentage reaching 39%. Primers OPB-0 2, OPA- 10 and OPB- 20 gave the highest polymorphism percentage in a range of 71 - 83% while OPC-07 did not give any polymorphic fragments.

Similarity matrix was generated by group average clustering analysis (UPGMA) and it was performed with the TREBCON (Version 1.1) software package (Van der Peet and De Wachter, 1993).

Genetic relationship between the four genotypes was summarized in a dendogram. The dendogram showed that Toktuk and Abu Zarif belong to the same group with a genomic DNA similarity reaching 91%. While similarity between this group and Strain B was 88%. HSD 0977 (The cherry tomato) belongs to separate group related to

the main group that correlates the three other genotypes with a percentage of 85%.

One approach to facilitate selection and breeding for complex traits, such as heat tolerance is the identification of genetic markers Linked to heat tolerance. DNA markers are abundant and operate independently from environmental conditions. The application of polymerase chain reaction (PCR) based markers such as RAPD is a powerful measure for the detection of polymorphism in tomato (Foolad and Lin, 2001) RAPD markers were used to identify polymorphism between the four genotypes under study as it was used earlier with tomato genomic DNA by Klein-Lankbrust *et al.* (1992); Foolad *et al.* (1993) and Lin *et al.* (2006).

In this study the sixteen primers used produced 61 polymorphic fragments compared to Bagheri *et al.* (1995) results with *Pisum sativum* who obtained 180 polymorphic fragments using 34 primers. There was a wide range of variations between polymorphism percentages per primer for the sixteen primers. This result gave data that make selection for the suitable primer for the genomic DNA of tomato possible. OPB- 02 gave the highest polymorphism % which is coinciding by the result obtained by Tabassum *et al.* (2013).

Similarity matrix was used to determine genetic relationship between the four genotypes. Close relationship between Toktuk and Abu-Zarif were found, which reached 91% and that expressed by the occurrence of the two genotypes in one major group. Previous similarities in their behavior against heat stress make the two landraces more genetically related.

Table.1 The sequences of primers for RAPD technique

No	Code	5′ to 3′ sequence
1.	OPA-04	AATCGGGCTG
2.	OPA-10	GTGATCGCAG
3.	OPA-05	AGGGGTCTTG
4.	OPB-02	TGATCCCTGG
5.	OPB-15	GGAGGGTGTT
6.	OPB-16	TTTGCCCGGA
7.	OPB-18	CCACGCAGT
8.	OPB-20	GGACCCTTAC
9.	OPB-17	AGGGAACGAG
10.	OPC-07	GTCCCGACGA
11.	OPC-09	CTCACCGTCC
12.	OPC-17	TTCCCCCCAG
13.	OPC-19	GTTGCCAGCC
14.	OPC-15	GAGGGATCAG
15.	OPC-20	ACTTCGCCAC
16.	OPG-19	GTCAGGGCAA

Table.2 Primers polymorphism percentage

Primer	Amplified fragments	Polymorphic fragments	Polymorphism %
OPA-0 4	14	2	14
OPA-10	11	8	73
OPA-05	9	2	22
OPB-02	12	10	83
OPB-15	11	3	27
OPB-16	6	2	33
OPB-17	12	3	25
OPB-18	8	4	50
OPB -20	7	5	71
OPC-07	6	1	17
OPC -09	9	2	22
OPC-15	11	3	27
OPC-17	5	0	0
OPC-19	9	5	55
OPC-20	13	5	38
OPG-19	12	6	50
TOTAL	155	61	39

Fig.1 RAPD profiles of genomic DNA of the four genotypes of tomato (*Solanum lycopersicum*) amplified with primer OPG-19

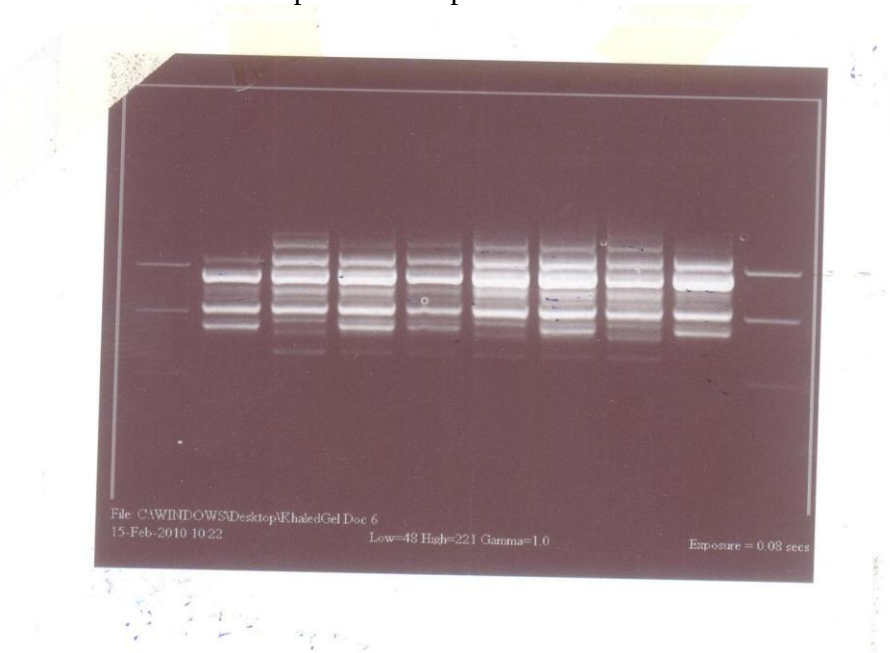


Fig.2 RAPD profiles of genomic DNA of the four genotypes of tomato (*Solanum lycopersicum*) amplified with primer OPA-10

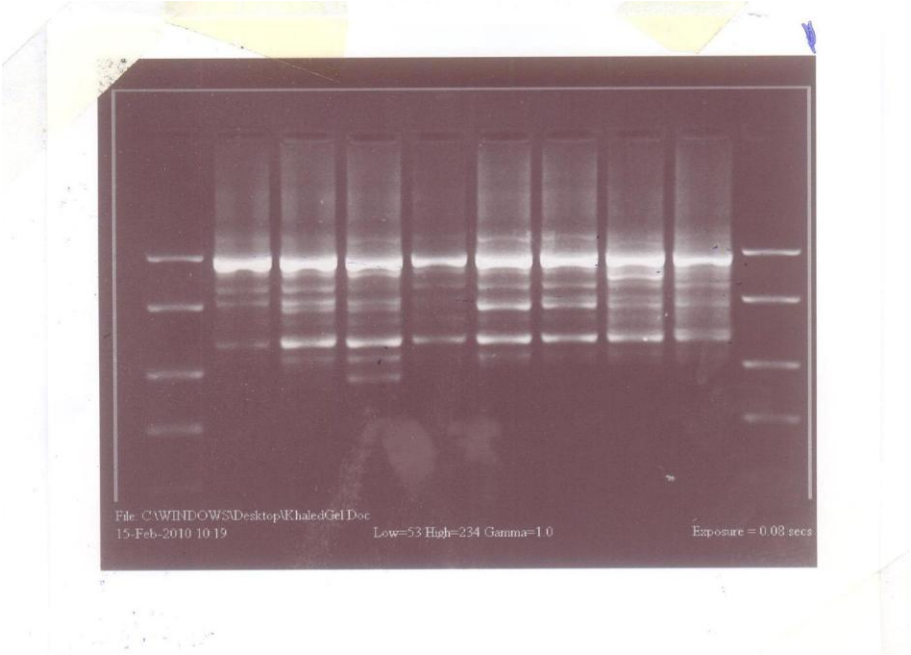
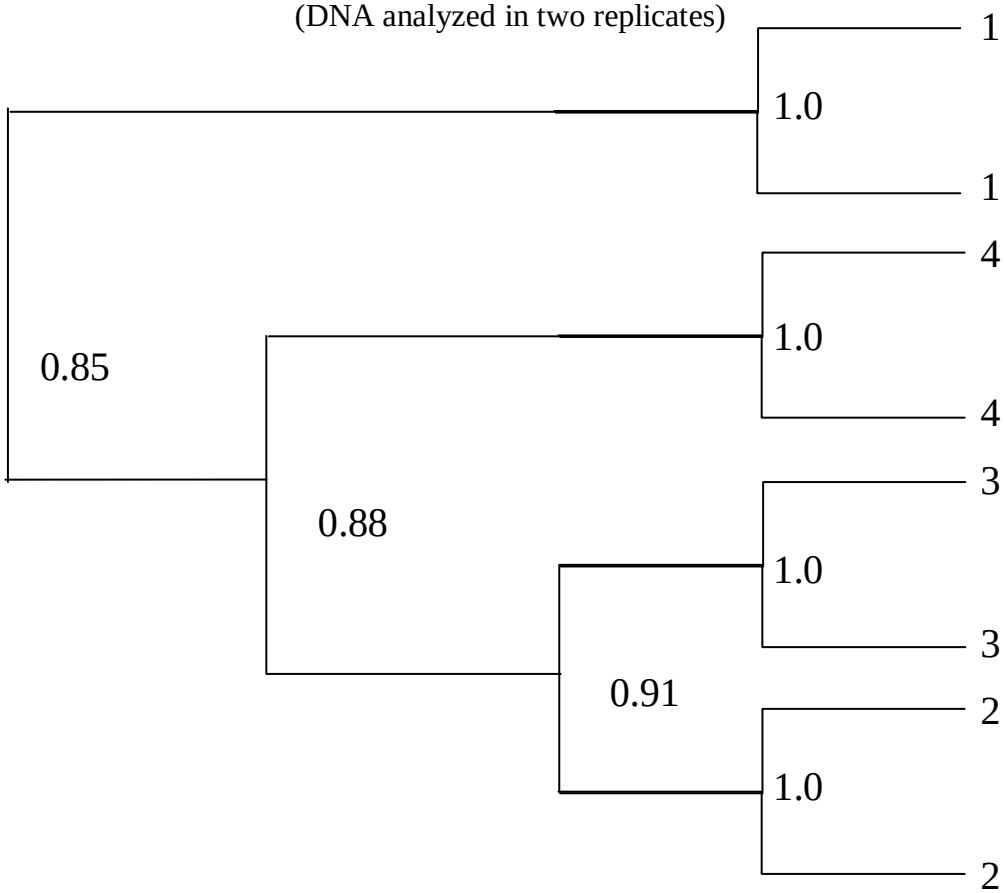


Fig.3 Dendogram showing similarity percentage between the four genotypes: HSD 977(1), Toktuk (2), Abu Zarif (3) and Strain B (4)
(DNA analyzed in two replicates)



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