Genetic Diversity Analysis of Tomato (Solanum Lycopersicon L.) Hybrids under Drought Tolerant Stress using SSR Markers

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Abstract

Abiotic stressors such as drought are particularly detrimental to tomato vegetative growth and yield. Breeding and selection for drought-tolerant genotypes is a significant strategy for addressing this challenge. In this study, we used ten SSR primers for drought tolerance on six parents of tomato genotypes were the chosen parental genotypes included one wild type, i.e., *Solanum pimpenillifolium* (LA:411) and five cultivated genotypes *Solanum lycopersicum*, i. e., Edkawi, Super Marmande, Super Strain B, Castle Rock and Peto 86 and their fifteen crosses. Five primers were succeful and observed positive and negative markers for drought tolerance. Genetic diversity was estimated to be between 0.485 and 0.947 using SSR data, while there was very high genetic similarity (0.999) between ($F_1 8$ and $F_1 7$) (super Marmande x LA:411 and super Marmande x Edkawi) respectively. LA:411 and Edkawi could be source of drought tolerance.

Keywords: Abiotic Stress; Drought Dolerant; *Solanum lycopersicum*; *Solanum pimpenillifolium*; SSR Markers.

1. Introduction

One of the most economically important in *Solanaceae* family is tomato (*Solanum lycopersicum* L.,* formerly *Lycopersicon esculentum* L.). It is worldwide agricultural and economic importance as a vegetable crop, and in Egypt it is one of the most important vegetable crops. Tomato is a superior model system for genetic studies in plants [7].

Such conditions are generated when living organisms, mostly viruses, bacteria, fungi, nematodes, insects, arachnids, and weeds, affect the plants' regular metabolism.

Abiotic stresses, such as low or high temperatures, insufficient or excessive water, salt, heavy metals, and UV radiation, are detrimental to plant growth and development, resulting in significant crop yield losses globally [1]. Such impacts are a risky sign for food safety and impact the geographical distribution of flowers in nature and climate alternate, i.e., is a supply of widespread abiotic stress [8,36].

Several coding pathways are variably triggered according to the strain type [6]. Several common defense mechanisms are controlled by kinase enzymes and phytohormones [19]. Ion channels, for instance, are inspired by reactive oxygen species (ROS) technology and (jasmonic acid, abscisic acid, ethylene, and salicylic acid [17]. These substances accumulate and alter genetic and metabolic machinery. Protection responses minimize organic loss as a result of pressure; these responses constitute the concept of plant tolerance [24].

In addition, drought stress (normal shortage of water) will reason the decreased survival, development, and boom of vegetation. Drought is normally associated with a lack of availability of water in the soil but can also be exacerbated through excessive evapotranspiration [13].

Single nucleotide polymorphisms (SNPs), sequence-characterized amplified regions (SCAR), and simple sequence repeats (SSRs) are PCR-based molecular markers, and gene sequence records of the sample is needed to apply these molecular markers [30].

Simple sequence repeats (SSRs), also known as microsatellites, are rapidly getting popular as a source of unique genetic markers due to their high reproducibility, multi-allelic nature, co-dominant inheritance, abundance, and broad genome coverage [14].

SSR markers have been successfully used to study genetic diversity associated with the spread of diverse plant species [18,12,9,26,27,28].

DNA molecular markers provide information on the allelic status of each offspring in the population (heterozygosis, maternal homozygosis, paternal homozygosis [20]. Such genetic component may be analyzed and documented. The creation of molecular maps and genetic structure, as well as the composition of markers in a pattern that depicts genetic diversity and linkage assemblies based on recombination identified in hybrid plant genotypes [30,22].

Additionally, hybrids discovered between common tomato and wild species were employed to harmonize horticultural characteristics and for gene mapping. A few SSR markers have been shown to be Solanaceae-specific, including tomato [4,2,35,3,16].

The main objective of this study is to use the SSR markers to evaluate six tomato genotypes and their hybrids under drought stress in order to determine drought tolerance genes in these genotypes that will aid farmers in reducing irrigation time and conserving water .

2. Materials and Methods

1. Plant Material and Growth Conditions:

This study was conducted during the period from 2018 to 2020 in the greenhouse at Kaha Vegetable Research Farm, Qalubia Governorate, Egypt.

Six tomato genotypes were chosen and crossed following half diallel approach to produce 15 F_1 hybrids. The chosen parental genotypes included one wild type, i.e., (P1) *Solanum pimpenillifolium* (LA:411) and five cultivated genotypes *Solanum lycopersicum*, *i.e*, Edkawi (P_2), Super Marmande (P_3), Super Strain B(P_4), Castle Rock (P_5) and Peto 86 (P_6).

2. Screening tomato varieties under drought stress:

Irrigation treatments were initiated seven days after transplanting in the second season. Two irrigation treatments were used: drought stress (DS) and irrigated control (I), where DS = 50% and I = 100% [7].

DNA extraction and Simple Sequence Repeat (SSR):

The DNAeasy Plant Mini Kit (Qiagen, Germany, GmbH, Cat. no. 69104) was used to extract the gDNAs from (200 mg) tomato leaves according to the manufacturer's instructions. A Nanodrop 1000 spectrophotometer was used to determine the quantity of DNA. Ten SSR markers were chosen due to their earlier use and extensive distribution in tomato genome (Table 1).

3. PCR amplification:

The SSR primers were purchased from Sigma Company (Sigma-Aldrich Corporate). A total volume of 20 μL was used for amplification reactions, containing 11.6 μL ddH2O, 1μL gDNA (20 ng), 1μL forward and reverse primer (10 μM), 2 μL 10x Buffer, 1.2 μL $MgCl_2$ (25 mM), 2 μL dNTPs (2.5 mM), and 0.2 μL Taq DNA polymerase (5 U/μL). The amplification reactions were carried out in a Thermal Cycler (Applied Biosystems) with the following program as described by [32] (94°C for 3 min; then 35 cycles of 94°C for 45 s, 54°C for 45 s, and 72°C for 1 min; finally, 72°C for 5 min); the PCR products had been confirmed through 2% agarose gel electrophoresis and visualized by ethidium bromide staining. To estimate band sizes, a 100 bp DNA ladder was loaded in the first well of agarose gel. Electrophoresis unit was run at a 100 V for approximately 45 min. The SSR gels image were photographed under UV transilluminator and the bands were scored using gel documentation system manufactured by Alpha Ease FC (Alphimager Tm 2200), U.S.A. Only reproducible and distinct bands were scored as 1/0 (presence /absence) for data analysis.

Data analysis:

The pairwise difference matrix was calculated according to [5]. SSR analysis data were uploaded as binary matrices into a computer file. Coefficients of similarity were determined using the Dice matrix [21]. The genetic similarity among genotypes as revealed by phylogenetic tree were done using ETE toolkit Tree Viewer program.

3. Results and Discussion

SSR primers analysis was used to obtain molecular genetic markers for drought tolerance from the 21-genotype using ten primers (Fig.1). Five out of ten primers were successfully used in the amplification of five SSR markers to determine the genetic distances across tomato genotypes (Five *Solanum Lycopersicon*, one *Solanum pimpinellifolium* and their fifteen F_1 crosses) and to identify drought-tolerant genes. All five primers (100%) successfully amplified the expected PCR fragments.

The parents (P1 and P2) gave two bands with same molecular weight with all primers. So, these bands can be used as positive molecular markers for drought stress in tomato cultivars (Fig. 1). Unique markers are defined as bands that specifically identify genotypes from the others by their presence or absence. The bands that are present in one genotype but not found in the others are defined positive unique markers (PUM), opposite to the negative unique markers (NUM). Unique DNA markers were obtained by SSR and were used on the six tomato genotypes and their fifteen crosses of drought tolerance. These markers could be used in breeding programs aiming to genetic improvement of drought tolerance in tomato. These data are good evidence of the SSR markers as highly variable markers that detect the co-dominant single locus.

The twenty-one genotypes were examined using ten SSR primers uniformly spread across different tomato chromosomes. The overwhelming majority of indicators amplified a single allele. SSR-A and SSR-D markers had the lowest main allele frequency (Table 2). The polymorphic information content (PIC) values computed varied between 0.266 (SSR1-B) to 0.953 (SSR-D), with an average of 0.553 per marker. The results indicated that five of the ten SSR primers tested generated amplicons in each of the 21 genotypes and 10 markers detected polymorphism. The number of alleles per locus ranged from four to six, with an average of 4.8 alleles per locus.

Additionally, molecular markers are independent of their environment and have a greater polymorphism density. The enormous degree of morphological variation, however, is not necessarily represented at the molecular level as reported by [34,11,10].

Nei's genetic similarity value between six tomato genotypes and their hybrids (Table 3). The genetic similarity coefficients varied, the minimum GS (Genetic similarity) value was 0.485 derived between P1 and P2 with P6 and it was (0.556) between $F_1$11 and $F_1$4, while the maximum GS value was 0.947 derived between $F_1$10 and F_1 9. The GS values between (P1 and P2) and (P4 and P5) were (0.875) and (0.895), respectively. On the other hand, there was identical similarity between F_1 8 and F_1 7 the genetic similarity was 0.999 (Table 3).

The hybrids F_1 8 and F_1 7 were developed by crossing *Solanum lycopersicon* v. Edkawi and *Solanum lycopersicon* v. Super Marmande x *Solanum*

pimpinillfolium LA:411 and were considered drought tolerant genotypes. Fig. (2) illustrates a dendrogram clustering of six tomato genotypes and their hybrids. There are two major clusters; the first contains (CLU I) (1, LA:411) and (2, Edkawi) while the second cluster (CLU II) has two subclusters, the first subcluster (Sub I) contains (4, Super strain B, 5, castle rock, 6, Peto 86) and (3, Super Marmande), however, the second sub cluster (Sub II) splits into two sub clusters; the first sub cluster (Sub III) consists of two groups; GI (18, Castle rock x LA:411; 19, Castle rock x Edkawi) and GII (20, Castle rock x Super Marmande; 21, Castle rock x Super strain B), while the second sub cluster (Sub IV) consists of two groups (GIII) (15, Super strain B x Super Marmande; 16, Super strain B x Edkawi) and (G IV) (17, Super strain B x LA:411). The second sub cluster (Sub V) likewise has two groups: (GV) contains (9, Peto 86 x Super Marmande; 10, Peto 86 x Super strain B) and (8, Peto 86 x Edkawi), while the (GVI) contains (7, Peto 86 x LA:411), (11, Peto 86 x Castle rock), (12, Edkawi x LA:411) & (13, Super Marmande x LA:411; 14, Super Marmande x Edkawi).

Table (1) List of SSR primers.

No.	Markers name	Primers Sequence (5'----3')	Tm	Ref.
1	rbah21g15 (SSR-A)	F: CCACATACATACATACATA R: TCCAGTCTTCGCATCATCTG	55±5	[15]
2	bah55b22 (SSR-B)	F: CCGAAAAGTTCGATCCTTCA R: GCTCTCGGACTTGGAGGTAG	62±5	[15]
3	bah17g14 (SSR-C)	F: GATGCTCGTCTCTGTGGTGA R: GCAGAAGAATGCATCAACGA	58±5	[15]
4	ABC04320 (SSR-D)	F: CTCCCTCCTCGAGGTAGTCC R: ATGCAGTTGTGCTTCACGTC	62±5	[25]
5	Bmag382 (SSR-E)	F: TGAAACCCATAGAGAGTGAGA R: TCAAAAGTTTCGTTCCAAATA	56±5	[31]
6	rbaak21g03 (SSR-1)	F: TGTGCAGTTCAAGGATGCTC R: GTACCCCATCCTCTTGCTCA	61±5	[15]
7	bah49c21 (SSR-2)	F: GGACGGCTTCAGCTATGGTA R: CCCCTCCTCTCATCCTTCTC	61±5	[15]
8	ABC16030 (SSR-3)	F: TTGCAATCCACAAGGTTGAA R: AACCGGTCAGCACACCTTAG	59±5	[23]
9	Bmag579 (SSR-4)	F: CCTAGATAAGCAACATAGCCA R: CAAAGACCCTAACTCATGTTC	57±5	[31]
10	GMS149 (SSR-5)	F: ACCCTAACTCATGTTCT R: AAGGAACATAGCCAACTC	50±5	[29]

Table (2) Molecular diversity of 21 tomato genotypes as measured by the number of alleles, allele frequency, polymorphism %, and polymorphic information content.

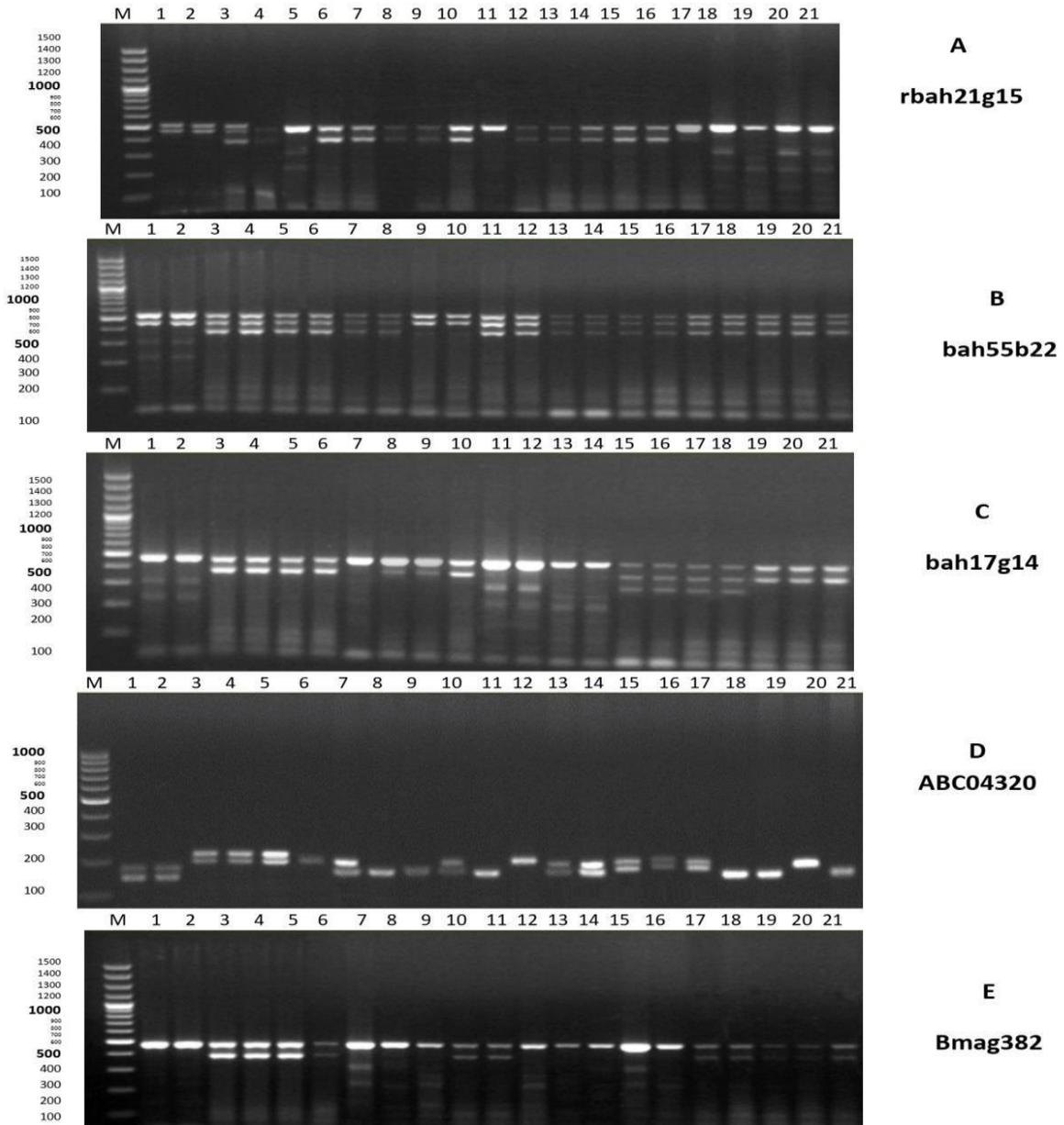| Marker identifier	Allele No.	Major Allele Frequency	Amplicon Size (bp)	Poly morphism %	Polymorphic information content value (PIC)
(SSR-A)	6.0	0.386	170-431	100	0.614
(SSR-B)	5.0	0.733	469-866	100	0.266
(SSR-C)	5.0	0.627	469-866	100	0.373
(SSR-D)	4.0	0.047	462-513	100	0.953
(SSR-E)	4.0	0.429	109-465	100	0.559
Mean	4.8	0.444	-	-	0.553

Fig. 1. Amplification Profiles of the six tomato genotypes and their crosses as revealed by SSRs. A, B,C,D and E show the allelic segregation of the SSR markers rbah21g15, bah55b22 bah17g14, ABC04320 and Bmag382, respectively, in the analyzed tomato genotypes and their crosses. Lanes 1 to 21 represent LA411(P1), Edkawi(P2),Super Marmande(P3), Super strain B(P4), Castel rock(P5), Peto 86(P6), (P1xP6), (P2xP6), (P3xP6),(P4xP6),(P5xP6),(P1xP2),(P1xP3),(P2xP3),(P4xP3),(P4xP2),(P4xP1),(P5xP1),(P5xP2),(P5xP3),(P5xP4),respectively; M, DNA marker was size (100-bp).
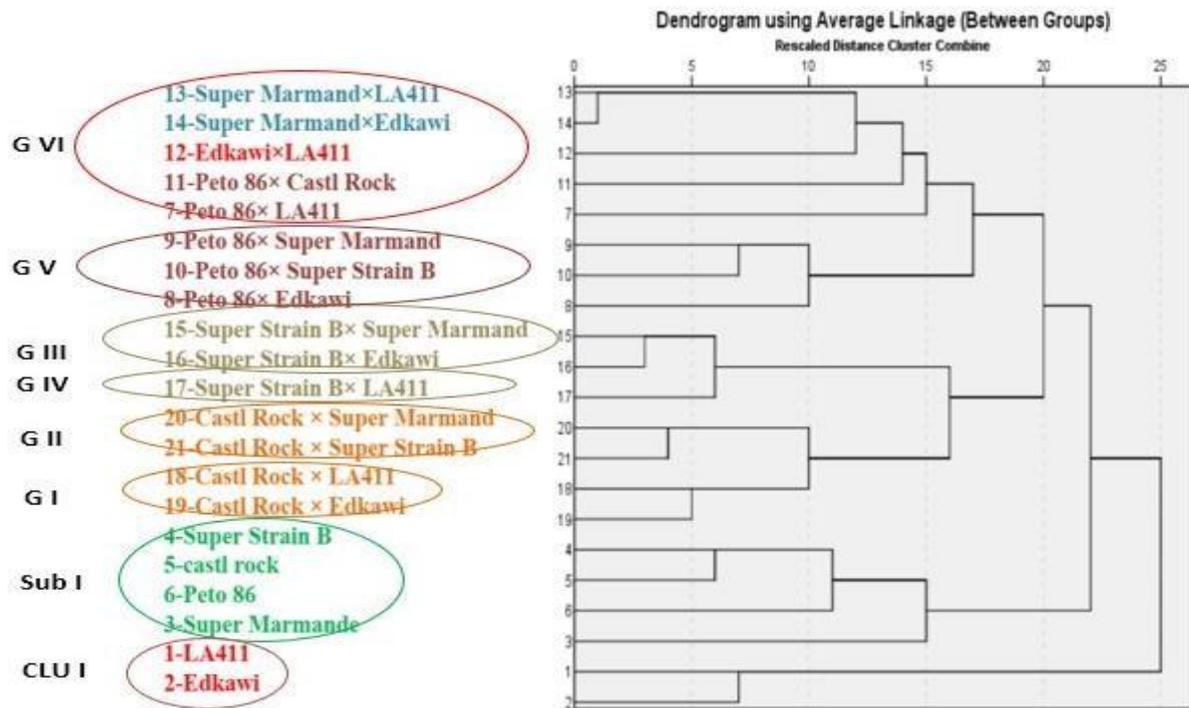
Fig. (2): dendrogram cluster analysis between Six tomato genotypes and their 15 hybrids using SSR data .

Table (3) Similarity value (Pairwise comparison) of tomato genotypes (5 *Solanum Lycopersicon*, 1 *Solanum pimpinellifolium* and their 15 F$_1$ crosses) based on SSR data.

Varieties	P1	P2	P3	P4	P5	P6	F1 1	F1 2	F1 3	F1 4	F1 5	F1 6	F1 7	F1 8	F1 9	F1 10	F1 11	F1 12	F1 13	F1 14
P2	0.875																			
P3	0.556	0.556																		
P4	0.529	0.529	0.789																	
P5	0.500	0.500	0.700	0.895																
P6	0.485	0.485	0.703	0.857	0.757															
F1 1	0.563	0.563	0.667	0.706	0.611	0.727														
F1 2	0.621	0.621	0.667	0.710	0.667	0.733	0.828													
F1 3	0.581	0.581	0.629	0.606	0.571	0.625	0.710	0.857												
F1 4	0.545	0.545	0.595	0.571	0.541	0.588	0.667	0.800	0.875											
F1 5	0.581	0.581	0.514	0.606	0.571	0.563	0.710	0.786	0.667	0.750										
F1 6	0.581	0.581	0.571	0.606	0.571	0.625	0.710	0.786	0.667	0.625	0.800									
F1 7	0.581	0.581	0.629	0.667	0.571	0.688	0.774	0.786	0.667	0.625	0.733	0.800								
F1 8	0.581	0.581	0.629	0.667	0.571	0.688	0.774	0.786	0.667	0.625	0.733	0.800	0.999							
F1 9	0.500	0.500	0.600	0.632	0.550	0.649	0.667	0.727	0.629	0.595	0.629	0.743	0.686	0.686						
F1 10	0.529	0.529	0.632	0.667	0.579	0.686	0.706	0.774	0.667	0.629	0.667	0.727	0.727	0.727	0.947					
F1 11	0.514	0.514	0.564	0.649	0.564	0.611	0.629	0.688	0.588	0.556	0.647	0.647	0.647	0.647	0.872	0.919				
F1 12	0.529	0.529	0.526	0.611	0.579	0.571	0.588	0.710	0.606	0.571	0.667	0.667	0.606	0.606	0.737	0.778	0.865			
F1 13	0.545	0.545	0.541	0.629	0.595	0.588	0.606	0.733	0.625	0.588	0.625	0.625	0.625	0.625	0.703	0.743	0.833	0.914		
F1 14	0.563	0.563	0.556	0.647	0.611	0.606	0.625	0.759	0.645	0.606	0.645	0.645	0.645	0.645	0.611	0.647	0.743	0.824	0.848	
F1 15	0.563	0.563	0.556	0.647	0.611	0.606	0.625	0.759	0.645	0.606	0.645	0.645	0.645	0.645	0.611	0.647	0.743	0.824	01.848	0.938

4.   Conclusion

On the basis of the aforementioned molecular marker-based variety analysis, we may conclude that this study verifies the effectiveness of the SSR markers used in assessing and differentiating tomato genotypes. Such study must be beneficial for identifying replica accessions and establishing the established order of core choice hybrids, as well as for ensuring the genotype's choice is conserved sustainably. Accurate molecular representation of conserved genotypes will enable more efficient genotype management and usage within breeding operations. While the cultivated tomato (*Solanum Lycopersicon*) exhibits considerable variation, it is frequently monomorphic at the molecular level.

The polymorphic molecular markers found in nature have difficulty identifying this restricted variety. However, multiple polymorphic microsatellite markers produced from the database have been successfully utilized to genotype a variety of tomato accessions and cultivars.

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