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GENETIC DIVERSITY ANALYSIS BASED ON MOLECULAR MARKER AND QUANTITATIVE TRAITS OF THE RESPONSE OF DIFFERENT TOMATO (*LYCOPERSICON ESCULENTUM* MILL.) CULTIVARS TO DROUGHT STRESS

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Abstract: The drought tolerance of tomato (*Lycopersicon esculentum* Mill.) is a trait needing urgent improvement due to recent climate changes and limited water availability. We therefore conducted a greenhouse screening experiment to identify tomato cultivars with improved drought tolerance. Several sensitivity and tolerance indices were computed based on morphological markers. With the aim of establishing a correlation to these markers, a total of 16 inter-simple sequence repeat (ISSR) primers were used, the genetic diversity among cultivars was elucidated and clustering the cultivars into groups based on their molecular profiles was performed. The obtained results indicated that selection indices, such as geometric mean productivity (GMP), mean productivity (MP), tolerance index (TOL),and stress tolerance index (STI), represented suitable indices for screening the drought tolerance of tomato cultivars. An interesting correlation of the ISSR analyses to these morphological findings was established according to 83 detectable fragments derived from 10 primers. The highest value of the effective multiplex ratio (EMR) and marker index (MI) was detected for primer INC7 followed by INC1. Based on Jaccard's similarity coefficients, the genetic distance of the genotypes varied from 0.702 to 0.942 with a mean value of 0.882. The results showed a clear-cut separation of the 15 tomato cultivars due to their genetic variability, making them a valuable genetic source for their incorporation into potential breeding programs. Molecular data were in good agreement with the results as regards selection indices, and both will be useful tools for improvement of the tomato germplasm.

Key words: Tomato (*Lycopersicon esculentum* Mill.); drought stress; drought tolerant/sensitivity indices; genetic diversity; ISSR markers; polymorphic information; cluster analysis

Abbreviations: GMP – geometric mean productivity; EMR – effective multiplex ratio; MI – marker index; MP – mean productivity; NPL – number of polymorphic loci; NTL – number of total loci; ISSR – Inter-Simple Sequence Repeat; SSI – stress sensitivity index; STI – stress tolerance index; PIC – polymorphism information content; RP – resolving power; TOL – tolerance index; YI – yield index; UPGMA – unweighted pair group method with arithmetic average

INTRODUCTION

Most commercial cultivars of *L. esculentum* are sensitive to abiotic stress, particularly to drought stress, during all stages of plant development [1,2]. In Arab Gulf countries, tomato cultivars grow under specific and often extreme abiotic stress, such as salinity, drought and heat stress. These stress factors affect the plants during their life cycle from germination, growth until harvest and during transport to distant markets. Under such stress, the plants are exposed to many changes in their metabolism and gene expression, which leads to a decrease in growth and increase in damage to the fruits. In order to deduce an effective breeding strategy to expand tomato cultivation to a

wider range of environments, detailed knowledge of the nature and magnitude of the genetic variability present in germplasm and the degree of transmission of the economic traits is a prerequisite of selecting suitable and promising parents [3]. Thus, the selection of suitable germplasm and crossing high-yield cultivars with robust, drought-tolerant cultivars, controlling the changes in chemical and biochemical metabolisms, and determining the genetic similarity via molecular markers in tomato are essential to future breeding programs. Foold [4] suggests that the most reliable criteria for breeding tomatoes for drought tolerance are agronomic characteristics (yield), and absolute and relative plant growth under stress and non-stress environments.

Owing to recent developments, plant breeders can now complement phenotypic traits [5]. Various studies reported the genetic diversity among different accessions, including varieties and populations, which were selected based on morphological and agronomic traits [6-8] or physiological behavior [9]. However, the applied model systems of identification were often restricted by a number of limitations, including low polymorphism, low heritability and late expression. Moreover, variations in environmental factors and variable stages of plant development hampered the elucidation of real genetic variations, due to interactions of environment-dependent genetic control of polygenic morphological and agronomic traits [10,11].

Because of these disadvantages, the use of biomolecular methods has been proposed for breeding programs, where marker-assisted selection (MAS) aims at the replacement or complementation of the conventional phenotypic selection [12-14]. Among the most promising and widely used markers, inter-simple sequence repeats (ISSR) markers have been successfully used to map plant genomes, identify stress tolerant cultivars, assess genetic diversity, and study interspecific and intraspecific relationships in different crops, such as potato plant breeding [15]. For the determination of ISSRs, repeat-anchored primers were used to amplify DNA sequences between two inverted SSRs [16]. The presented study was conducted to compare the usefulness of morpho-agronomic and ISSR markers in order to decipher the extent of genetic variation, genetic relationships and diversity among 15 tomato cultivars. Furthermore, correlations between distance estimates based on morpho-agronomic traits and DNA molecular marker should be investigated.

MATERIAL AND METHODS

Fifteen tomato (*Lycopersicon esculentum* Mill.) cultivars were provided and identified by the Leibniz Institute of Plant Genetics and Crop Plant Research (IPK), Gatersleben, Germany. Their wide diversity of geographical origins is shown in Table 1. A greenhouse experiment was conducted from September 2014 to March 2015 at the Biological Science Department, Faculty of Science, King Abdulaziz University, Jeddah, KSA, in cooperation with the Institute of Food Science and Biotechnology, Plant Foodstuff Technology and Analysis, University of Hohenheim, Stuttgart, Germany.

Tomato seeds were grown in germinating trays for 7 days. Then plantlets were transplanted into pots (30 cm diameter, volume of 1.1 L) containing a mixture of peat moss and quartz sand at a ratio of 1:3. Plants (27) were grown in a split-plot design and a combination of the treatments was laid out in a randomized complete block design (RCBD) with three replicates, setting up the pots in rows. Three levels of drought treatments were applied to the main plots, and tomato cultivars were assigned to the subplots. Each treatment was represented by three pots each with three plants, giving a total of 27 plants per treatment. Plants supplied with 600 mL of water three times a week were considered as control treatment (T_0), while two levels of reduced irrigation of 200 and 400 mL (twice a week) mimicked mild drought stress (T_1 and T_2, respectively). The plants were developed at 22/16°C (day/night) and under a relative humidity of 60% for the entire growth period. They were fertilized twice; the first dose was at the end of October and the second in mid-December, using liquid fertilizer (A 15-10-5 fertilizer contains 15% nitrogen, 10% phosphorus and 5% potassium). Four months after from transplanting, 16 morphological and yield characters were measured.

Table 1. Accession number, commercial name, serial code, botanical name, and origin of 15 tomato genotypes used for drought tolerance evaluation.

IPK Accession no.*	Commercial name	Ser. code	Botanical name #	Origin
LYC3912	Dedication	**C1**	*Lycopersicon esculentum* Mill.	**Russia**
LYC4112	Anna Aasa	**C2**	*Lycopersicon esculentum* Mill. convar. *infiniens* Lehm. var. *flammatum*	**Russia**
LYC2019	Gelbfruechtig	**C3**	*Lycopersicon esculentum* Mill. convar. *infiniens* Lehm. var. *cordiforme*	**Germany**
LYC192	Australische Frühe	**C4**	*Lycopersicon esculentum* Mill. convar. *infiniens* var. *commune* L.H.Bailey	**Australia**
LYC3152	Australische Rosen	**C5**	*Lycopersicon esculentum* Mill.	**Australia**
LYC2431	Vencal	**C6**	*Lycopersicon esculentum* Mill. convar. *fruticosum* Lehm. var. *speciosum* Lehm	**Netherlands**
LYC2432	Zevat	**C7**	*Lycopersicon esculentum* Mill. convar. *fruticosum* Lehm. var. *speciosum* Lehm	**Netherlands**
LYC4242	Petomech	**C8**	*Lycopersicon esculentum* Mill. convar. *fruticosum* Lehm. var. *speciosum* Lehm	**Italy**
LYC4079	Sankt Ignatius	**C9**	*Lycopersicon esculentum* Mill. convar. *infiniens* Lehm. var. *commune*	**Italy**
LYC1346	Sintesti	**C10**	*Lycopersicon esculentum* Mill. convar. esculentum var. *esculentum*	**Romania**
LYC359	Tiganesti	**C11**	*Lycopersicon esculentum* Mill. convar. *infiniens* Lehm. var. *flammatum* Lehm	**Romania**
LYC2937	Florida MH-1	**C12**	*Lycopersicon esculentum* Mill. convar. *fruticosum* Lehm. var. *finiens* Lehm	**USA**
LYC2493	Sandpoint	**C13**	*Lycopersicon esculentum* Mill. convar. *fruticosum* Lehm. var. *pygmaeum* Lehm.	**USA**
LYC2987	California	**C14**	*Lycopersicon esculentum* Mill.	**USA**
LYC4113	California Red Cherry	**C15**	*Lycopersicon esculentum* Mill. convar. *parvibaccatum* Lehm. var. *cerasiforme* (Dunal) Alef.	**USA**

*Accession code of the Leibniz Institute of Plant Genetics and Crop Plant Research (IPK).
#The botanical name *Lycopersicon esculentum* used in the database of IPK is used here.

Table 2. Drought tolerance/sensitivity indices and their equations.

Drought Tolerance/sensitivity indices	Equation	References
Stress Sensitivity Index (SSI)	$SSI= [(1- (Y_{si}/Y_{pi})/ SI]$	Fischer and Maurer (17)
Stress Tolerance Index (STI)	$STI= [Y_{pi} \times Y_{si}] / (Y_p)^2$	Fernandez (18)
Tolerance Index (TOL)	$TOL = Y_{pi} - Y_{si}$	Hossain et al. (19)
Geometric Mean Productivity (GMP)	$GMP = (Y_{pi} \times Y_{si})^{0.5}$	Fernandez (18)
Mean Productivity (MP)	$MP = (Y_{pi} + Y_{si}) / 2$	Hossain et al. (19)
Yield Index (YI)	$YI = Y_{si} / Y_s$	Gavuzzi et al. (20)
Yield Stability Index (YSI)	$YSI = Y_{si} / Y_{pi}$	Bouslama and Schapaugh (21)

Y_{pi} and Y_{si} are the shoot fresh weight of a genotype at normal and stressed treatments, respectively. SI is the stress intensity as calculated by $SI = 1-(Y_s/Y_p)$; Y_s and Y_p are the mean shoot fresh weights of all genotypes under stress and normal conditions, respectively.

Drought tolerance/sensitivity indices

Drought tolerance and stress sensitivity indices were calculated for each genotype based on shoot fresh weight across two irrigation levels (T1 and T2), as described previously [17-21] (Table 2).

Molecular markers

Extraction and purification of genomic DNA

DNA was extracted from 0.2 g of randomly picked fresh young leaf tissue of plants, using the Qiagen DNeasy kit (Qiagen, Santa Clara, CA, USA).

Inter-simple sequence repeat analysis

PCR was performed in 25 µL reaction volume containing the 2X ready mix (Emerald Amp Max PCR master mix) by Takara Clontech (Madison, CA, USA), 25 pM oligonucleotide primer and 50 ng genomic DNA. A set of 16 ISSR primers synthesized by Bioron (Ludwigshafen, Germany) were used in this study, although we only show results of 10 primers (Table 3). DNA amplification was performed applying 35 cycles using Cetus 480 DNT Thermal Cycler (Perkin Elmer Ltd, Norwalk, CA, USA) as follows: initial denaturation step at 95°C for 5 min; 35 cycles of denaturation

Table 3. Code name of primers, repeat motif and sequence of the primers used in ISSR detection.

Marker	Repeat Motif	Sequence of primers	Marker	Repeat Motif	Sequence of primers
INC1	$(AG)_8YC$	**5'-AGAGAGAGAGAGAGAGYC-3'**	**INC9**	$(GATA)_4GC$	**5'-GATAGATAGATAGATAGC-3'**
INC2	$(AG)_8YG$	**5'-AGAGAGAGAGAGAGAGYG-3'**	**INC10**	$(GACA)_4AT$	**5'-GACAGACAGACAGACAAT-3'**
INC3	$(AC)_8YT$	**5'-ACACACACACACACACYT-3'**	**INC11**	$(AC)_8YA$	**5'-ACACACACACACACACYA-3'**
INC4	$(AC)_8YG$	**5'-ACACACACACACACACYG-3'**	**INC12**	$(AC)_8YC$	**5'-ACACACACACACACACYC-3'**
INC5	$(GT)_8YG$	**5'-GTGTGTGTGTGTGTGTYG-3'**	**INC13**	$(AG)_8YT$	**5'-AGAGAGAGAGAGAGAGYT-3'**
INC6	$CGC(GATA)_4$	**5'-CGCGATAGATAGATAGATA-3'**	**INC14**	$(CTC)_4TT$	**5'-CTCCTCCTCCTCCTCTT-3'**
INC7	$GAC(GATA)_4$	**5'-GACGATAGATAGATAGATA-3'**	**INC15**	$(CT)_8RG$	**5'-CTCTCTCTCTCTCTCTRG-3'**
INC8	$(AGAC)_4GC$	**5'-AGACAGACAGACAGACGC-3'**	**INC16**	$(TC)_8A$	**5'-TCTCTCTCTCTCTCTCA-3'**

Y (C,T) and R (A,G)

at 94°C for 1 min; annealing temperature (Ta) for 1 min; an extension step at 72°C for 1 min; a final extension step at 72°C for 10 min. Amplification products were separated by horizontal gel electrophoresis using 1.5% (w/v) agarose gel on 0.5×TBE buffers (50 mM Tris, 50 mM boric acid, 2.5 mM EDTA, pH 8.3) under a constant voltage of 80 V for 2 h, stained with 1 μg mL^{-1} ethidium bromide. A 1 kb DNA Ladder (250 to 1000 bp), supplied by Thermo Fisher Scientific (Watham, MA, USA), was used as a DNA marker and applied in the first column of the gel. The samples were arranged on the gel from left to right in numeric order. Bands were visualized in a UV transilluminator (Sigma-Aldrich, St. Louis, MO, USA) at 300 nm and photographed using gel documentation equipment (Bio Rad, Hercules, CA, USA). Amplified products were scored as 1 or 0 depending on their presence or absence, respectively.

Molecular genetic analysis

The ABI Gene Scan software (Applied Biosystems, Riyad, KSA) assigned non-integer base-pair size values to detected fragments. The number of total loci (NTL) and number of polymorphism loci (NPL) were calculated for each primer. Polymorphic ratio (P%) was calculated based on the ratio of NPL/NTL. The polymorphism information content (PIC) of a marker was calculated according to a simplified version of Anderson et al. [22].

$$PIC_i = 1 - \sum_{j=1}^{n} P_{ij}^2$$

The resolving power (RP) for individual marker systems was calculated according to Prevost and Wilkinson [23] where RP = ΣIb. The effective multiplex ratio (EMR) is the product of the fraction of polymorphic bands and the number of polymorphic bands [24]. Marker index (MI) was determined according to Powell et al. [25] as the product of PIC and EMR. The presence or absence of alleles for each ISSR was recorded for all cultivars and converted into a genetic matrix. Employing the computer package NTSYSpc [26], Jaccard's similarity coefficients were calculated and used to identify genetic relationships among the genotypes based on the unweighted pair group method of arithmetic averages (UPGMA) and sequential agglomerative hierarchical nested (SAHN) clustering for molecular markers using the Nei and Li [27] method.

Statistical analysis

Analyses of variance (ANOVA) and comparison of means for morphological variables were performed using MStat-C version 2.10 (Software, MSU, USA) to investigate the effects of genotypes and irrigation levels. Ward's minimum variance clustering method was performed to classify the genotypes into discrete clusters based on selection indices values calculated as means of two irrigation levels according to Ward [28] and Romersburg [29].

Table 4. Analysis of variance (ANOVA) for effect of cultivars and drought levels on different root, shoot, leaves, and fruits parameters of tomato (*Lycopersicon esculentum*.).

SOV	df	Root length (RL)	Shoot length (SL)	Root fresh weight (RFW)	Shoot fresh weight (SFW)	Root dry weight (RDW)	Shoot dry weight (SDR)	Shoot / Root Length (S/R L)	Root/Shoot dry weight (R/SDW)
Genotypes	14	166.2*	956.2*	282.5*	24325*	243.08*	818.1*	0.974*	0.262*
Treatments	2	591.2*	3315.1*	7807.9*	138274*	406.8*	4953.5*	0.4075*	0.0115ns
G. X T.	28	108.8*	114.73*	931.4*	2560*	61.28*	128.4*	0.1346*	0.055*
Error	88	41.62	26.47	60.99	302.7	10.91	18.54	0.078	0.020
LSD (0.05)									
Genotypes		6.08	4.85	7.36	16.41	3.114	4.06	0.264	0.1354
Treatments		2.72	2.16	3.29	7.35	1.39	1.81	0.118	0.060
G. x T.		10.53	8.40	12.75	28.4	5.39	7.03	0.457	0.234

Table 4 pt. 2

SOV	df	No. of Leaves (NL)	Leaf fresh weight (LFW)	Leaf dry weight (LDW)	No. of branches (NB)	No. of inflorescences (NI)	No. of fruits (NF)	Fruit fresh weight (FFW)	Fruit yield
Genotypes	14	1122.3*	44.48*	897.31*	23.37*	74.024*	9.502*	3277.1*	706115*
Treatments	2	2734.8*	281767*	7470.5*	108.1*	109.55*	36.94*	1324.4*	429607*
G. X T.	28	193.6*	9590.9*	134.86*	6.51*	9.01*	1.623*	382.36*	79186.2*
Error	88	36.96	1420.8	22.38	1.63	1.50	0.930	23.56	6533.4
LSD (0.05)									
Genotypes		5.73	35.53	4.46	1.20	1.155	0.909	4.57	76.20
Treatments		2.56	15.89	1.99	0.539	0.516	0.406	2.04	34.08
G. x T.		9.92	61.55	7.72	2.09	2.002	1.575	7.92	131.9

SOV: Source of variance, MS: Mean Square, df: degrees of freedom, and * significant at 0.05 probability level.

RESULTS AND DISCUSSION

Drought tolerance and sensitivity indices

ANOVA revealed highly significant differences among tomato cultivars for all traits investigated under the same drought conditions (Table 4), suggesting a high degree of phenotypic diversity among the cultivars. Results show a decline in various plants' attributes as response to stress, in particular, when applying high stress treatment (T2), which is commonly observed and is due to the tolerance level in plants. This effect was differently pronounced among the cultivars (Fig. 1A and B). These results were in agreement with George et al. [30]. Improvement of these traits with a small value of variation might be limited if not impossible by simple selection of genotypes from the germplasm used in this study according to Ajmal et al. [31].

To identify tomato cultivars with a superior tolerance to drought stress, different sensitivity and tolerance indices were determined based on shoot fresh weight. As regards stress sensitivity indices (SSI), five cultivars C8, C9, C10, C11 and C12 gave a high degree for drought sensitivity (Table 5), while cultivars C3, C13 and C14 recorded a lower degree. Clarke et al. [32] and Amini et al. [33] concluded that the identification of drought-tolerant cultivars on the sole basis of the SSI index might also include those that have low total yields. The cultivars C9, C5, C15 and C11 ranked among those with the highest STI and GMP, indicating their drought tolerance (Table 5). While cultivars C6, C2, C7, C1 and C13 displayed the lowest values of STI and GMP and, thus, were classified as poorly drought tolerant, all other cultivars were characterized as semi-tolerant to drought stress. Accordingly, similar rankings for the tomato cultivars were observed when considering mean productivity (MP) and tolerance index (TOL) indices as well as STI and GMP, which suggested that these indices might be equally suitable for the screening of drought-tolerant genotypes. Simi-

Table 5. Selection indices of 15 tomato cultivars calculated for shoot fresh weight as means of two irrigation treatments.

Cultivars	C1	C2	C3	C4	C5	C6	C7	C8	C9	C10	C11	C12	C13	C14	C15
Ypi	152.5	165	179	221	251	123.5	117.5	217	394	252.5	271	200	87	204	255.5
Ysi	90	119.5	143.5	134.25	169.5	74.25	86	122.25	224.25	96.75	155	116	74	156.25	165
SSI	1.1	0.619	0.53	0.92	0.87	0.96	1.102	1.19	1.14	1.81	1.14	1.17	0.293	0.58	0.984
STI	0.325	0.464	0.605	0.699	1.01	0.216	0.238	0.625	2.08	0.575	0.989	0.544	0.151	0.75	0.993
TOL	62	45.5	35.5	86.7	81.5	49.25	31.5	94.75	169.7	155.7	116	84	13	47.7	90.5
MP	121.5	142.3	161.2	177.6	210.3	98.9	101.6	169.6	309.1	174.6	213	158	80.5	180.2	210.25
GMP	116.32	139	160.11	168.08	205.6	93.94	100.35	161.87	294.6	155.8	203.2	151.8	79.9	177.9	204.9
YSI	0.593	0.724	0.802	0.607	0.675	0.601	0.732	0.565	0.569	0.384	0.572	0.58	0.85	0.666	0.646
YI	0.695	0.916	1.15	0.995	1.336	0.556	0.685	0.951	1.726	0.76	1.194	0.913	0.343	1.232	1.307

GMP, geometric mean productivity; SSI, stress sensitivity index; STI, stress tolerance index; TOL, tolerance index; YI, yield index; Mean Productivity (MP); Yield Stability Index (YSI); Y_{pi} and Y_{si} are the shoot fresh weight of a genotype after normal and stressed regeneration

Table 6. Comparison profile of tomato cultivars classified by Ward's minimum variance clustering method based on selection indices values.

Cluster groups	selection indices								
	Y_pi	Ysi	SSI	STI	TOL	MP	GMP	YSI	YI
Cluster I (9)	227.88	139.84	1.1	0.75	88.1	183.9	176.6	0.61	1.09
Cluster II (5)	129.1	88.75	0.82	0.28	40.25	108.9	105.9	0.7	0.64
Cluster III (1)	394	224.25	1.15	2.08	169.7	309.1	294.6	0.57	1.73

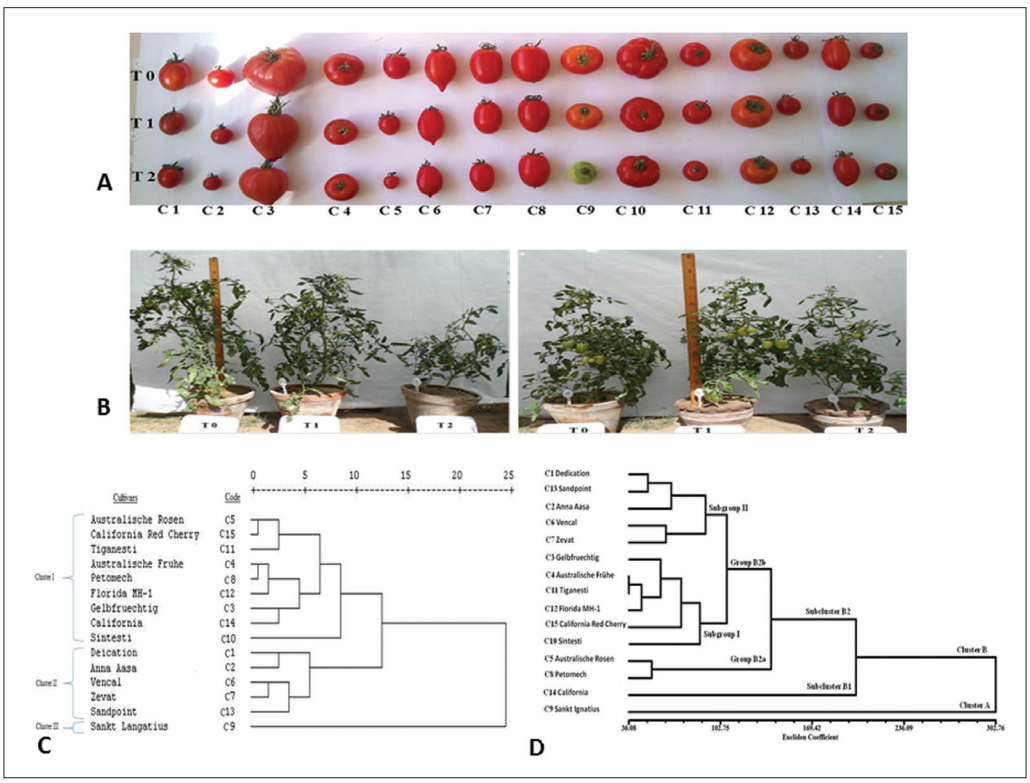

Fig 1. The effect of different levels of drought on A) fruit development and B) plant growth of tomato cultivars (C1 and C8). T0=600 mL, T1=400 mL, T2=200 mL. C1-C15; cultivar code according to Table 1. C) Dendrogram using Ward's method (28) for clustering tomato cultivars according to their drought tolerance indices. D) Dendrogram derived from UPGMA cluster analysis of 15 tomato cultivars based on Nei and Li (27) similarity coefficient method using 10 ISSR markers.

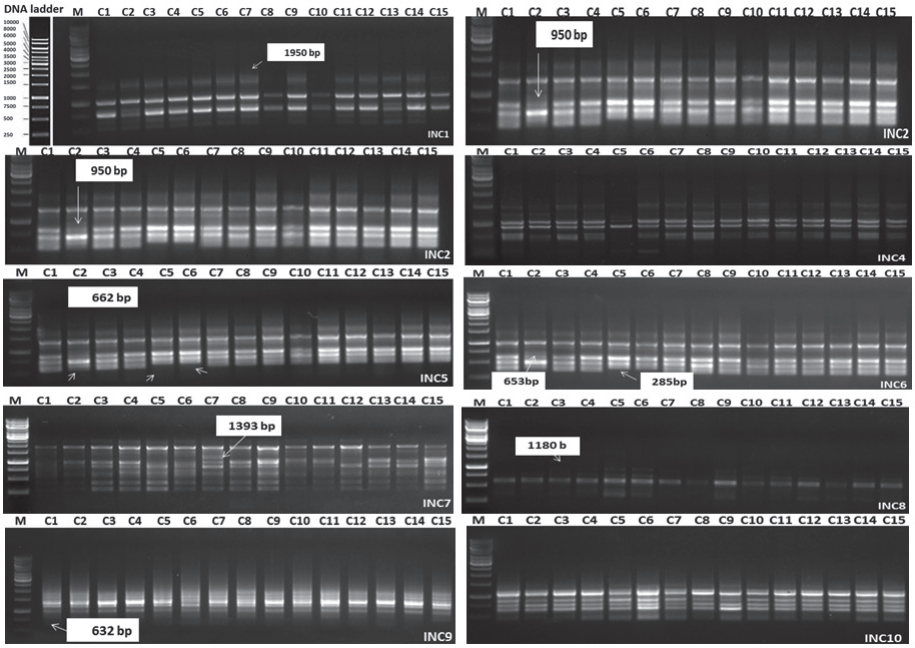

Fig. 2. PCR amplification profile generated from genomic DNA of 15 tomato cultivars with ISSR using 10 primers (INC1-INC10). (M: marker, C1-C15: tomato cultivars).

lar results have been reported by Mevlut and Sait [34], Sharafi et al. [35], Manal et al. [36], Farshadfar et al. [37] and Bradar-Jakanovic et al. [38] for Turkish oat, barley, sorghum, and wheat and tomato, respectively.

Grouping the tomato cultivars using selection indices based on the mean of two irrigation treatments revealed the existence of three groups, namely, drought-tolerant, semi-tolerant and sensitive cultivars (Fig. 1C). The characterization of each cluster group, i.e. the clustered means of selection indices, is summarized in Table 6 and illustrated in Fig. 2. In this analysis, the drought-tolerant group (cluster III) contained only one cultivar (C9, Sankt Langatius), being the most drought-tolerant cultivar according to its high STI, GMP and MP values. The semi-tolerant group (cluster I) contained nine cultivars, while the third group (cluster II) comprised the five cultivars with the lowest drought tolerance according to their sensitivity and yield indices. Thus, these cultivars were sensitive to drought and only suitable for cultivation under irrigated conditions. In conclusion, the best cultivar for drought tolerance was C9 and it could be involved in a breeding program and for growing under shortage of water.

Genetic diversity analysis

Inter-simple sequence repeat analysis

Selection of ISSR primers was based on the number of amplicons recovered through PCR, and reproducibility of the patterns. The size of the detected alleles ranged from 256 bp to 2300 bp (Table 7). These wide average size-ranges were probably fue to the adequate number of evaluated cultivars, and might be due to the particular set of loci tested [39]**.** The level of polymorphism among the cultivars was evaluated by calculating allele numbers. The marker attributes for the ISSR primers were summarized as PIC, RP, EMR and MI values for each of the 10 primers evaluated (Table 7). The number of total amplified loci (NTL) was 83, of which 35 loci were polymorphic with an average polymorphic ratio (P %) of 42.16 %. This low ratio of polymorphic loci is probably due to an inherently narrow genetic base.

Table 7. Attributes of markers produced by 10 ISSR primers.

No.	ISSR Primer	Allele size (bp)		NTL	NML	NUL	NPL	P (%)	PIC	EMR	MI	RP
		Min	Max									
1	INC1	490	1950	8	3	0	5	62.5	0.352	5.00	1.76	10
2	INC2	550	2300	9	5	1	4	44.4	0.347	3.996	1.386	8
3	INC3	675	1926	9	7	0	2	22.2	0.365	1.998	0.729	4
4	INC4	524	1562	9	4	0	5	55.5	0.281	4.995	1.403	10
5	INC5	662	1794	6	4	0	2	33.3	0.320	1.998	0.639	4
6	INC6	285	1626	9	7	0	2	22.2	0.658	1.998	1.314	4
7	INC7	256	2633	11	4	0	7	63.6	0.439	6.996	3.07	14
8	INC8	295	1180	7	2	1	5	71.4	0.449	4.998	2.244	10
9	INC9	632	1847	8	6	0	2	25.0	0.356	2.00	0.712	4
10	INC10	464	1453	7	6	0	1	14.3	0.391	1.001	0.391	2
Total				83	48	2	35	414.4	3.958	34.98	13.648	70
Average				8.3	4.8	0.2	3.5	41.44	0.3958	3.498	1.3648	0.7

NTL, number of total loci; NML, number of monomorphic loci; NUL, number of unique loci; NPL, number of polymorphic loci; PIC, polymorphic information content; RP, resolving power; P (%), polymorphic ratio; EMR, effective multiplex ratio; MI, marker index.

The number of total loci (NTL) ranged from 6 for primer (INC5) to 11 (INC7) with an average of 8.3 loci per marker. The number of polymorphic loci (NPL) ranged from 1 (INC10) to 7 (INC7) with an average of 3.5 loci. Three unique loci specific to the cultivars C2 and C3 were detected by the primers INC2, INC6 and INC8; these may be converted into specific-specific probes for quick identification of these genotypes or interspecific hybrids of interest during the early stages of tomato selection programs. This was in concordance with previous studies [40,41].

In addition, the PIC values, reflecting allele frequency and information content among the cultivars, were estimated. The INC6 primer was the most informative, showing the highest PIC value (0.658), whereas INC4 gave the lowest PIC (0.281). The overall average PIC was 0.3958. This moderate PIC value for the ISSR primers used could be attributed to the narrow genetic base of the tomato cultivars and/or highly informative ISSR markers used in this study [41]. EMR is the product of the fraction of polymorphic bands and the number of polymorphic bands. Consequently, primers with higher polymorphism had higher EMR values. The value of EMR varied from 1.001 (INC10) to 6.996 (INC7) with an overall mean of 3.498. MI is the product of PIC and EMR, and ranged from 0.391 to 3.07. The highest MI value (3.07) was observed for INC10, while the lowest MI (0.391) was that of INC10. In addition, INC7 showed the highest RP (14), while INC10 exhibited the lowest value (2) with an average RP of 0.7 (Table 7). Also, three of the ISSR primers (INC1, INC4 and INC8) possessed high RP values (10); these were the most informative primers for distinguishing the tomato cultivars. Prevost and Wilkinosin [23] stated that the RP index provides a moderately accurate estimate of the number of genotypes distinguishable by a primer. However, RP does not provide accurate information on the ability of a primer to reflect genetic or taxonomic relationships among a set of cultivars. Furthermore, Razmjoo et al. [41] recommended the parameters MI and RP to be used for selecting informative primers. Previously, GD (genetic diversity), PIC, EMR and MI to identify the most suitable primer for ISSR-marker-based classification of germplasms, observing a highly significant positive correlation between them [42].

Detection of DNA polymorphism

Among the detected polymorphism bands, a total of 9 bands were found to be useful as positive or negative markers of drought stress (Fig 2). These 9 bands were generated by all primers except the primers INC4 and INC10. INC7 and INC8 yielded cultivar-specific amplification fragments at 1393bp (C7) and 1180 bp

(C3 only). Moreover, INC1 and INC2 produced one amplified DNA fragment of 1950 bp (C6 and C7 only) and 950 bp (C2 only), which might be specific for these drought-sensitive cultivars.

Regarding the ISSR profiles generated by primers INC3 and INC5, bands with molecular weights 1113 bp and 662 bp were absent only in the drought-sensitive cultivars C5 and C6 (1113 bp) as well as C2, C5 and C6 (662 bp), respectively. Using primer INC6, an amplified fragment of 653 bp was produced only in the drought-sensitive cultivar C2, while an INC6-specific band at 285 bp was absent in the drought-sensitive cultivars C2, C5 and C6. Polymorphic bands generated by INC9 ranged between 632 bp to1847 bp. The smallest band (632 bp) was recorded solely in the cultivars C1, C2, C5, C6, C7 and C13 with a size of around 632 bp, which therefore may be considered a negative marker of drought tolerance. While primers INC2, INC3, INC5, INC8 and INC9 also contributed to the generatation of negative markers by specfic bands (Fig. 2), positive markers of drought tolerance were generated by the primers INC4, INC7 and INC8. These positive markers generated specific and exclusive bands in the drought-tolerant cultivar C9, and, eventually, also in moderately drought-tolerant cultivars, such as C3, C4, C8, C10, C11, C12, C14 and C15. According to the field trials and morphological parameters, these cultivars showed an acceptable drought tolerance. The correlation to our ISSR results may be useful to accelerate genetic advancement in tomato by using these cultivars as parent lines for future breeding programs. The proposed genetic markers may be more effective and less costly than evaluations based on phenotypic traits. Our results were in agreement with previous studies [14, 16, 43] that demonstrated the effectiveness of ISSR-PCR to enhance the identification of drought-tolerant genotypes in different crops. The reliabilty of ISSR data may be improved by using more primers and cultivars. As described below, ISSR analyses may also be used in detecting possible genetic relationships among cultivars with unknown ancestry [44].

Based on simple matching coefficients among the genetic attributes of the 15 tomato cultivars, a cluster analysis was carried out and a dendrogram generated. The coefficients of genetic similarity obtained in the present study were characterized by a narrow range (0.702 to 0.942), i.e., genetic diversity among the 15 cultivars was comparably low (Table 8). Cultivars C7 and C12 revealed the maximum similarity of 0.942, followed by C5 and C10 (0.930), while cultivars C5 and C10 exhibited the lowest genetic similarity of 0.702, followed by C5 and C13 (0.706) and C2 and C10 (0.736), indicating that these cultivars were not closely related to each other, which was reflected by their highly distinct response to drought stress. Therefore, these cultivars may be considered as diverse genotypes for breeding programs, and especially for improving resistance to abiotic stress.

Clustering of the varieties based on similarity of ISSR markers

Fifteen tomato cultivars were grouped into two major clusters (Fig. 1D). The first cluster (A) included only C9, whereas, other cultivars were predominantly grouped in the second cluster (B). The cultivars C8 and C5 were included in group B2a, while the remaining cultivars were included in group B2b. The first subgroup (I) included most of the moderately drought-tolerant cultivars C3, C4, C10, C11, C12 and C15, while all the previously identified drought-sensitive cultivars, C1, C2, C6, C7 and C13, were grouped together in the second subgroup (II), once more revealing the good agreement of our genetic ISSR data with the field evaluation data. The grouping of the moderately drought-tolerant cultivars C3, C4, C10, C11, C12 and C15 in the same subgroup (I) confirmed their greater genetic similarity. Taking into account that cultivars aggregated together in the same cluster, this indicated a possible common origin of these genotypes [45]. Due to their higher genetic similarity, only low positive heterotic effects may be expected when generating hybrids from these cultivars and, thus, they may be less useful in transgressive breeding than those with less genetic similarity (C5 and C10, C5 and C13). Since C9 was found to be the most promising drought-tolerant cultivar, the genetically most dissimilar genotype (C2, similarity index 0.786)

Table 8. Similarity matrix for 15 tomato cultivars based on 10 ISSR markers.

	C1	C2	C3	C4	C5	C6	C7	C8	C9	C10	C11	C12	C13	C14	C15
C1	1.000														
C2	0.840	1.000													
C3	0.810	0.776	1.000												
C4	0.845	0.760	**0.930**	1.000											
C5	0.780	0.746	0.769	0.776	1.000										
C6	0.786	0.753	0.820	0.828	0.916	1.000									
C7	0.871	0.808	0.805	0.837	0.800	0.828	1.000								
C8	0.823	0.736	0.808	0.816	0.753	0.736	0.890	1.000							
C9	0.797	0.786	0.855	0.840	0.826	0.831	0.842	0.794	1.000						
C10	0.820	**0.732**	0.805	0.867	**0.702**	0.733	0.814	0.904	0.767	1.000					
C11	0.855	0.816	0.837	0.847	0.783	0.789	0.873	0.826	0.901	0.823	1.000				
C12	0.869	0.805	0.826	0.835	0.797	0.826	**0.942**	0.840	0.888	0.811	0.898	1.000			
C13	0.850	0.838	0.859	0.842	**0.706**	0.714	0.842	0.848	0.845	0.818	0.880	0.867	1.000		
C14	0.884	0.819	0.840	0.849	0.786	0.792	0.875	0.828	0.826	0.800	0.859	0.873	0.882	1.000	
C15	0.855	0.791	0.813	0.847	0.760	0.789	0.873	0.852	0.800	0.878	0.830	0.845	0.826	0.8857	1.000

may represent a promising mating partner for future breeding to increase drought tolerance. Although less relevant for increasing drought tolerance, further cultivars of other clusters or subgroup may be combined with the cultivars in subgroup (I) to allow a general improvement of tomato germplasm diversity.

CONCLUSIONS

A total of 15 different tomato cultivars was grown under two different levels of drought stress, and the obtained morphological data and selection indices were compared with ISSR analyses. By these means, one drought-tolerant cultivar and several moderately drought-tolerant cultivars were identified. Specific ISSR markers were proposed to facilitate future screening for drought-tolerant cultivars among a larger germplasm database. The high consistency of morphological and genetic markers should encourage other researchers to seek for cultivars of other crops possessing a tolerance against drought or other types of stress, such as salinity or light stress.

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