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Assessment of genetic relationships, and catalase gene expression in response to drought stress in various cultivars of eggplant (*Solanum melongena* L.)

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Abstract

Objective

Eggplant (*Solanum melongena* L.) is an economically important vegetable crop and its quantity and quality are influenced by water deficit. The study of genetic relationships and the genes involved in drought stress tolerance is essential in plant breeding programs and will help in the adoption of management strategies in dry areas. In this study, genetic variation and population structure of eggplant cultivars were identified using ISSR marker. Additionally, the expression of the catalase gene in response to drought stress was evaluated in selected three cultivars.

Materials and methods

DNA was extracted from leaf samples of 23 eggplants using the modified CTAB method and a polymerase chain reaction was done by 10 ISSR primers. Three cultivars Greta, Sally, and Melusina were selected from each class identified through cluster analysis to assess gene expression under drought stress. Drought treatments were imposed in the first stage of plant growth by stopping irrigation for 17 and 23 days in moderate and long-term stress, respectively. Gene-specific primer to assess gene expression was catalase. RT-qPCR was done in technical triplicates using a Rotor-Gene Q. The reference gene for normalization of gene expression was actin.

Results

The mean polymorphism percentage in this study was 73%. Primer (AG)8YC exhibited the highest polymorphism percentage and marker index, along with high PIC and Shannon index values. According to the analysis of molecular variance, the genetic variance between the three

subpopulations was highly significant. Population structure analysis and cluster analysis based on phenotypic and molecular data divided cultivars into three main clusters. Catalase gene expression has been significantly increased under long-term drought stress in compared to the control in three cultivars of eggplant. The Melusina cultivar, with a value of 3.56 showed the highest gene expression in long-term drought stress.

Conclusions

The ISSR marker serves as a suitable tool for evaluating the differentiation within eggplant populations. This marker in combination with other codominance primers can be used for future studies in eggplant. Additionally, the catalase gene plays a significant role in breeding programs of eggplant in arid regions and the Melusina cultivar can be introduced as a drought-adapted cultivar, if the results are confirmed by subsequent studies.

Keywords: ISSR marker, genetic diversity, the gene overexpression, water stress.

Paper Type: Research Paper.

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Introduction

Eggplant (*Solanum melongena* L.) is a diploid species (2n=2x=24). It is an economically important nutritious vegetable and most important Solanum plant native to the Old World (Mwinuka et al., 2021). Eggplant originated from Africa and was likely domesticated in Asia (Syfert et al., 2016). This vegetable includes species used for both food and medicinal purposes, serving as a vital source of vitamins, antioxidants, minerals, carbohydrates, and proteins (Guillermo et al., 2014; Samtiya et al., 2021). The most effective antioxidants in eggplant fruit are phenolic compounds which are useful for cardiovascular and metabolic ailments (Syfert et al.,

2016). Genetic diversity is used to adapt the population to changing environments and plays a crucial role in crop improvement strategies through the introduction of new improved crops and the selection of desirable traits (Begna 2021). Genetic diversity within a population reduces the vulnerability of plants to various types of stress (Markert et al., 2010). Conservation of genetic sources and breeding programs in plants must access the genetic structure and variation among different species (Ouborg 2010). Population structure is used for minimizing both types of errors (types I and II) in association analysis and association mapping and is essential to prevent false-positive associations (Pritchard et al., 2000). Also, population structure analysis has been employed for the quantitative assessment of genetic parameters in crops (Hayatgheibi et al., 2024). Hayatgheibi et al., (2024) stated that models incorporating population structure significantly decrease additive genetic variance, resulting in a significant decline of narrow-sense heritability of traits in Norway spruce. In another study, population structure was used to recognize genetic populations, admixed individuals, and assign individuals to populations (Pritchard et al., 2000). Molecular markers are essential tools for obtaining precise estimates of genetic diversity and population structure among cultivars and accessions, which can be applied in eggplant improvement projects (Hussain & Nisar 2020). Among molecular markers, ISSR (inter simple sequence repeat) has been widely and effectively used to access the genetic variation of plant and animal germplasm, screen mutant plants, and identify closely related individuals (Mohammadabadi et al., 2021; Ghasemi et al., 2010; Ebrahimi 2022). The benefits of this marker are reliability, speed, simplicity, cost-effectiveness, and determining genetic diversity between closely related individuals (Kumar et al., 2016). ISSR markers have been successfully utilized for genetic diversity and identifying related species in eggplant (Isshiki et al., 2008; Ali et al., 2011).

Drought stress is one of the major abiotic stresses and the most important yield limiting factor in arid regions. Adequate water availability during the initial stage of plant growth is very important, as drought stress reduces growth and yield. Drought triggers a wide variety of crop responses involving cellular, biochemical, morphological, and molecular mechanisms (Yang et al., 2021). Plants experiencing water deficit increase the expression of various genes to produce the necessary proteins. Catalase (CAT) plays a crucial role in drought tolerance and plant adaptation by scavenging stress-induced excess H2O2 (Zhang et al., 2021). The CAT gene family has been identified in plants, including seven members in tobacco from the Solanaceae family (Liu et al., 2023), seven in cotton (Wang et al., 2019), four in cucumber (Hu et al., 2016) and one in Scots pine (Vuosku et al., 2015). The study of the genes involved in stress tolerance will help the breeder in the choice of drought-tolerant plants, determining desirable genotypes, and the

adoption of management strategies in dry areas. Although catalase gene expression in response to water deficit stress has been assessed in different plants, expression of this gene under drought conditions has not been studied in eggplant. Eggplant has a wide variation in morphological characteristics and drought tolerance. Given the challenges caused by climate change, it is essential to explore the germplasm to obtain tolerant genotypes to biotic and abiotic stresses. The aims of this research were: 1) to determine the genetic structure of cultivars adapted to the region and their genetic diversity 2) to assess catalase gene expression in response to moderate and long-term drought stress and identify tolerant cultivars.

Materials and methods

In this study, 23 eggplant hybrid cultivars were used, which occupied the most cultivated areas in Jiroft (28° 40’ 7.79” N 57° 44’ 7.79” E), a city located in the southeast region of Iran with hot weather conditions. Plantlets per cultivar were grown under greenhouse conditions at the research greenhouse of Shahid Bahonar University of Kerman in 2024. The name and origin of the cultivars are shown in Table 1.

Table 1. Name of eggplant cultivars used in this study

Number	Cultivar name	Country of origin	Number	Cultivar name	Country of origin
1	712BS	Taiwan	13	Shadow	Netherland
2	Anamur	Netherland	14	Armaghan 513	India
3	SV 1574EV	United States	15	Galine	France
4	GBPUAT-605-Brinjal	India	16	Tiger	Spain
5	Elettra	Italia	17	Sally	Netherland
6	Kodak	Taiwan	18	Finix	Spain
7	Denise	United States	19	Bartok	Netherland
8	Elegance	France	20	Greta	Netherland
9	Rebellion	Spain	21	Melusina	Netherland
10	Violetta Di Firenze	Italia	22	Checkmate	Japan
11	Bagira	Turkey	23	Kemer Patlican	Turkey
12	Arlene	United States			

Genomic DNA extraction and ISSR analysis: DNA was extracted from the young leaf sample of twenty tree eggplant cultivars by the modified CTAB method (2% CTAB and 1.4 M NaCl) (Saghai-Maroof et al., 1984) in the laboratory of Research and Technology Institute of Plant Production of Shahid Bahonar University of Kerman. The quantity and quality of DNA was investigated by nanodrop at the ratios of the absorbance values of 260 nm vs 280 nm (A260/A280) and the 260 nm vs 230 nm (A260/A230), and by agarose gel electrophoresis (1%). Polymerase chain reaction was done using 10 ISSR primers (with the highest polymorphic percentage)

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screened from 20 primers. PCR was conducted in a BioMetra thermocycler (Biometra, Göttingen, Germany) after an initial denaturation step at 94°C for 4 min. The amplification was followed by 34 cycles of denaturation at 94°C for 40 s. annealing step at primer-dependent different temperatures (50-61°C) for 50 s, an extension step at 72°C for 45 s, and a final extension step at 72°C for 7 min. The PCR amplicons were separated by gel electrophoresis on agarose gel (2.5% (w/v) and TBE buffer (1X). The gel straining was done in ethidium bromide solution and imaged on the Molecular Imager® Gel DocTM XR system (Bio-Rad) (Figure 1). The DNA bands were scored as present (1) and absent (0) of each locus.

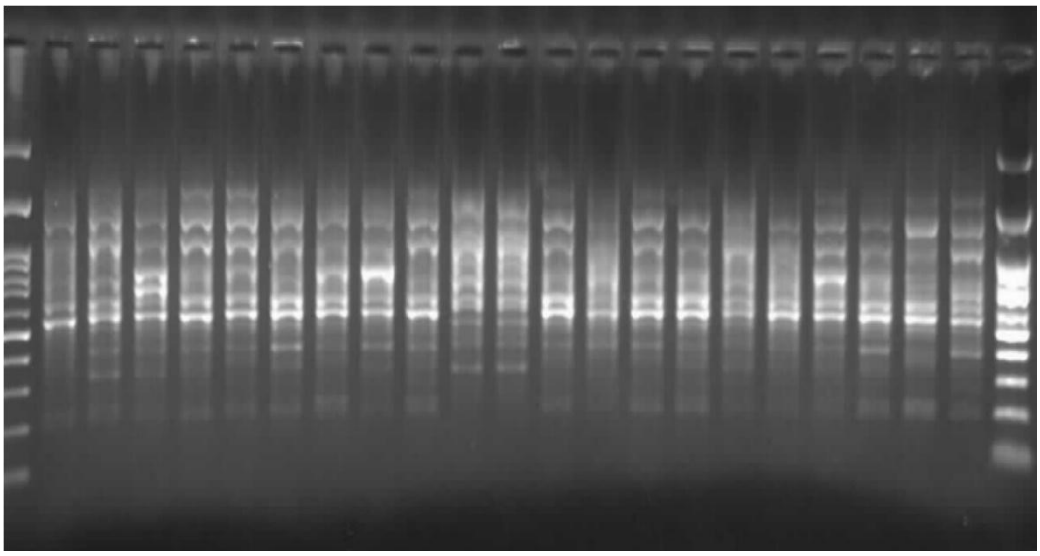

Figure 1. Electrophoresis of PCR products from eggplant cultivars for ISSR marker on agarose gel

RNA extraction and CAT gene expression analysis: Three cultivars Greta, Sally, and Melusina were selected from each class resulting from cluster analysis and population structure to assess gene expression under drought stress. Drought stress treatments were imposed at the first stage of plants by stopping irrigation for 17 and 23 days in moderate and long-term drought conditions, respectively. In the normal humidity conditions, plots were irrigated when 50% of the total available water was depleted from the root zone. Each normal and stress treatment had three plot replicates for each cultivar. The leaves were frozen in liquid nitrogen, and stored at −80 °C to extract RNA. Total RNA was extracted using the Total RNA Isolation Kit (DENA Zist Asia) following the manufacturer's instructions in the laboratory of Research and Technology Institute of Plant Production of Shahid Bahonar University of Kerman. A DNase digestion was done with an RNase-free DNase Set (SINACLON). The quality and

quantity of RNA were determined using a NanoDrop ONE^{C} Spectrophotometer (Thermo Scientific) and 1.5% agarose gel electrophoresis. Synthesis of cDNA was performed using the Easy^{TM} cDNA Synthesis Kit (Parstous) with 2 µg of DNA-free RNA and Oligo $(dT)_{16}$ primers. Gene-specific primer in this study was catalase (GenScript) (Table 2). RT-qPCR was performed in technical triplicates using a Rotor-Gene Q (QIAGEN) with Rotor-Gene Q series Software (Figure 2). The reactions were done as: 12 min at 95 °C, followed by 40 cycles for 50 s at 95 °C, 50 s at 60 °C, 50 s at 72 °C, and a final melting curve analysis protocol including heating to 95 °C for 15 s, 60 °C for 1 min and heating to 95 °C. A final volume of reactions were 20 µL, including 4 µL of 5x Hot FIREPOL Eva Green qPCR Super Mix (Solis Biodyne), 2 µL of primers (10 µM each), 2 µL of cDNA, and 12 µL of water. The reference gene used for normalization of gene expression was actin (Table 2).

Table 2 The sequence of primers related to Catalase and Actin genes for qPCR

Gene	Sequence
Catalase	Fq- GCGACCAAGGATCTTTACGA
	Rq- CAACACCAATCGACCAACTG
Actin	Fq- ATGCCTATGTTGGTGACGAG
	Rq- CTCTGGAGCCACACGAAGT

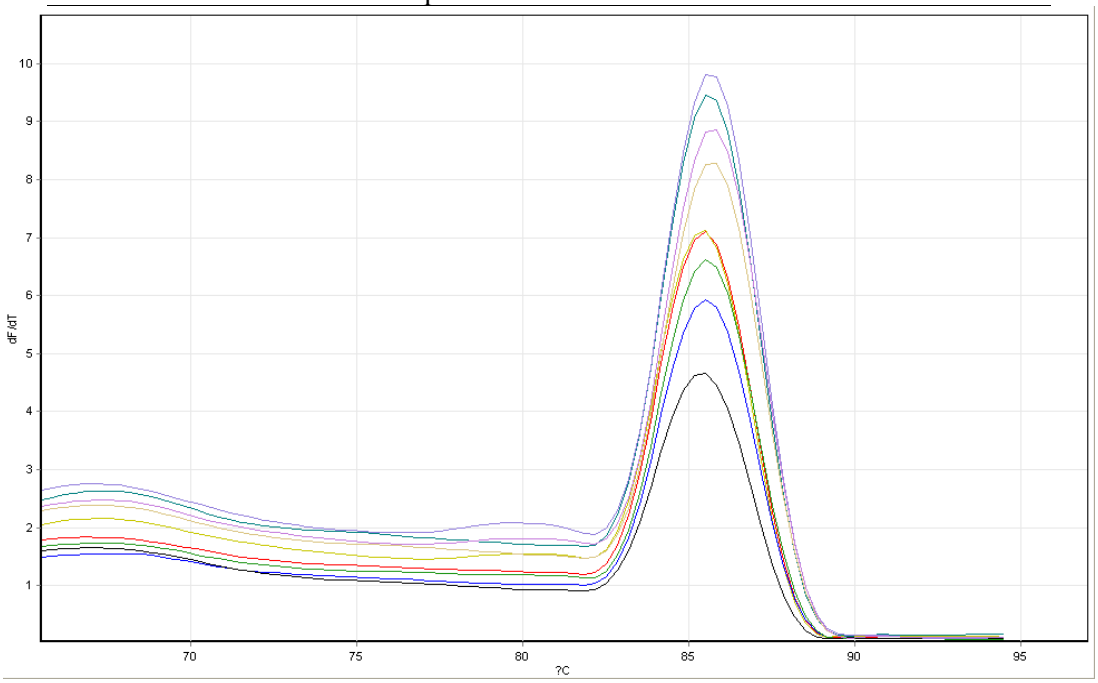

Figure 2. Melting curve of CAT gene production using real- time PCR for Melusina eggplant cultivar

Phenotyping: Five plants from each cultivar were used to determine phenotypic traits. Traits including fruit phenolic compounds, fruit antioxidant compounds, the hardness of the fruit, fruit

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shape, fruit color, fruit weight, plant height, leaf length, leaf width, and petiole length were measured. The hardness of fruit was measured with a Universal machine (STM-250). The total phenolic contents were determined according to the Folin-Ciocalteu method and measurements were performed at 750 nm with a spectrophotometer (UNICO 2802, China) (Taga et al., 1984). Antioxidant activity was determined by DPPH radical scavenging. 0.5 g of sample was mixed with 10 ml ethanol 96% and was centrifuged for 10 minutes at 5000g. 600 µL of filtered sample (diluted 1:5) mixed with 600 ml of DPPH solution (0.2 mmol) and was incubated in a dark cabinet for 15 min. Then, the absorbance was determined by a UV-vis spectrophotometer at 520 nm. Antioxidant activity was computed as (Valko et al., 2007):

Total antioxidant activity (%) = [(control − sample)/control] × 100

Statical analysis- Analysis of molecular data: Descriptive statistics including PIC (polymorphic information content) (Weising et al., 2005), MI (marker index) (Powell et al., 1996), and H (Shannon's index) (Shannon 1948) were calculated for each primer by GenAlEx version 6.5b3 software (Peakall & Smouse 2012). Indices were computed as:

$$PIC = 1 - p^2 - q^2$$

$$MI = PIC \times \text{number of polymorphic loci}$$

$$H = -1 \times (p \times \ln(p) + q \times \ln(q))$$

Where p is the frequency of visual alleles and q is the frequency of null alleles.

Analysis of genetic diversity: The cophenetic correlation coefficient was estimated for the different clustering methods. The method with the highest cophenetic coefficient was selected. Cluster analysis for molecular data was performed based on the complete linkage method and Euclidean distance matrix. Also, cluster analysis for phenotypic data was assessed based on Ward's method and Euclidean distance matrix by the XLSTAT software. Population structure analysis was performed based on molecular data using STRUCTURE software (version 2.3.4). The analysis was conducted by 100,000 Markov chain Monte Carlo (MCMC) iterations with a burn-in of 10,000 iterations. Genetic clusters (K) were from K= 2 to 10 with five replicates for each K. The optimal levels of K were defined by calculating ΔK based on the method described by Evanno et al., (Evanno et al., 2005) according to the rate of change in the log probability of the data between K values (Earl & VonHoldt 2012) as:

$$\Delta K = m\,|L''(K)|/s[L(K)]$$

AMOVA (analysis of molecular variance) was done between and within subpopulations using GenAlEx 6.5b3. PhiPT statistic (Cockerhamc 1973) to assess genetic diversity between subpopulations obtained as:

$$PhiPT = {}^{AP}/_{WP + AP}$$

Where AP and WP are the variances between and within subpopulations, respectively.

Analysis of gene expression: Relative expression analyses were done by comparative quantification of the amplified products based on the $2^{-\Delta\Delta CT}$ method (Schmittgen & Livak 2008) by REST 2009 2.0 software.

Results and discussion

Allele diversity: In total, 93 bands were generated from 10 ISSR primers in 23 eggplant cultivars, of which 70 bands were polymorphic (Table 3). Minor alleles with a frequency less than 0.05 were deleted before conducting analysis, because these alleles are generally biased for LD assessment and problematic (Mohlke et al., 2001). The highest polymorphic bands percentage was produced by the primer (AG)8YC (91.66%) (Table 3). The mean polymorphism percentage was 73.45. The PIC index (polymorphic information content) varied from 0.30 (primer (TC)8C) to 0.47 (primer (GACA)4) (Table 3). The highest and lowest marker index values were related to the primer (AG)8YC (5.06) and primer HVH(TCC)5 (1.64), respectively (Table 3). Shannon index values ranged from a minimum of 0.46 (primer (TC)8C) to a maximum of 0.66 (primer (GACA)4)) (Table 3). In total, primer (AG)8YC had the highest polymorphism percentage and marker index as well as high PIC and Shannon index (Table 3).

Table 3. Diversity statistic for 10 ISSR primers in 23 eggplant cultivars

Primer	Number of Polymorphic bands	Polymorphic percentage	Shannon's information index	Polymorphic information content (PIC)	Marker index (MI)
(TC)8C	9	81.82	0.46	0.30	2.70
(AC)8C	7	70	0.61	0.43	3.01
(AC)8G	8	88.88	0.58	0.40	3.20
(GA)8YG	6	66.66	0.59	0.41	2.46
(GA)8C	9	81.82	0.59	0.40	3.60
(AG)8YC	11	91.66	0.65	0.46	5.06
(GACA)4	4	66.66	0.66	0.47	1.88
HVH(TCC)5	4	57.14	0.59	0.41	1.64
(GAC)5	4	57.14	0.63	0.44	1.76
(GA)8RC	8	72.73	0.64	0.45	3.60

The PIC index can be utilized to assess the level of allele diversity. In the case of bi-allelic markers (such as ISSR), the PIC values are from 0.25 to 0.5 (Martinez-Arias et al., 2001). In this study, the PIC value with an average of 0.42 was a good indicator for studying genetic variation. The PIC value of primers (0.30-0.47) indicated that ISSR primers used in this study had high

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efficiency and could be useful tools in detecting eggplant genotypes. The high percentage polymorphic of primers showed ISSR markers are effective for distinction of the eggplant genotypes studied. These results are consistent with previous studies about eggplant (Isshiki et al., 2008; Weihai et al., 2008). Isshiki et al., (2008) show the percentage of ISSR polymorphisms from Japanese eggplants and 12 related Solanum species was 99.1%. Mahmoud and El-Mansy (2012) reported that the effectiveness of this dominant microsatellite-based marker is due to its ability to access variation in the numerous microsatellite regions dispersed across the genomes. Primer (AG)8YC with the highest polymorphism percentage and marker index as well as high PIC and Shannon index is the most suitable primer in discriminating cultivars of eggplant in this study. Wang and Jost (2013) reported that the Shannon–Wiener index (H) is a reliable measure among the parameters for assessing genetic diversity.

Population structure: The optimal levels of K were defined by calculating ΔK according to the rate of change in the log probability of the data between K values (Table 4) and to determine optimum K, ΔK was plotted against the number of subpopulations (K) based on Evanno's method (Evanno et al., 2005). The maximum of ΔK was at K=3 and the studied population was divided into three subpopulations with different genetic structures (Figure 3). There were 7, 11, and 5 cultivars in each of the subpopulations (Figure 4).

The results of AMOVA indicated the PhiPT statistic was highly significant and 92% and 8% of total variance existed within and among subpopulations, respectively (Table 5). The pairwise PhiPT was significant between all pairs of subpopulations. Thus, the ISSR marker can be useful for discriminating genotypes and population structure of eggplant. The third subpopulation had the highest heterozygosity, polymorphism loci percentage, Shannon index, and number of effective alleles (Table 6). The results determined that genetic differentiation within subpopulations was higher than between subpopulations. The high variation within populations could be due to genetic differences between individuals.

Cluster analysis: According to the results of the cluster analysis based on Ward's method and Euclidean distance for phenotypic data, the 23 eggplant cultivars were classified into 3 main clusters (Figure 5). The first cluster was the largest group with 47.82% cultivars. The second and third groups each included 26.09% of cultivars. The third group included cultivars Denise, Bartok, Kodak, finix, SV 1574EV, and Greta which had the highest fruit weight and hardness of fruit with high fruit antioxidant compounds. Cultivars of the second group included cultivars Checkmate, GBPUAT-605-Brinjal, Violetta Di Firenze, Arlene, Elettra, and Melusina had the lowest fruit weight. Cluster analysis for phenotypic data was able to distinguish cultivars with high fruit weight and high fruit antioxidant compounds from other cultivars. Also, cultivars with

the lowest fruit weight were located in separate groups. The trait of fruit weight had the greatest contribution to the differentiation of genotypes.

Table 4. The results of Evanno's method in optimal K selection

K	Reps	Mean LnP(K)	Stdev LnP(K)	Ln'(K)	|Ln''(K)|	Delta K
2	5	-1617.800000	0.494975	—	—	—
3	5	-1498.020000	0.708520	119.780000	20.180000	28.481923
4	5	-1398.420000	7.616561	99.600000	2.660000	0.349239
5	5	-9937.360000	7.002000	405.640000	131.660000	18.803200
5	5	-1301.480000	3.652670	96.940000	8.480000	2.321589
6	5	-1213.020000	6.113264	88.460000	16.600000	2.715407
7	5	-1141.160000	18.402391	71.860000	17.920000	0.973786
8	5	-1051.380000	16.031438	89.780000	27.860000	1.737835
9	5	-989.460000	23.037101	61.920000	178.060000	7.729271
10	5	-1105.600000	241.357536	-116.140000	—	—

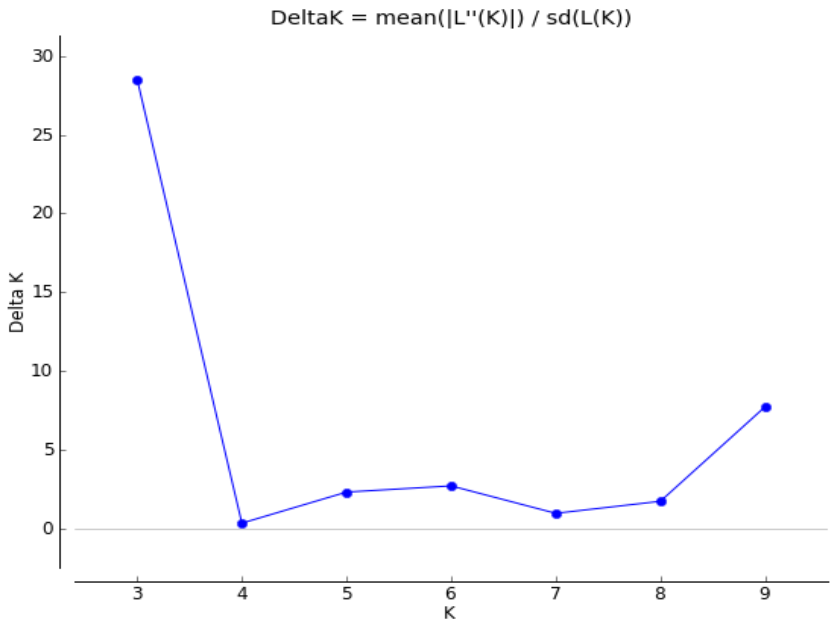

Figure 3. The result of Evanno's method in optimal K selection

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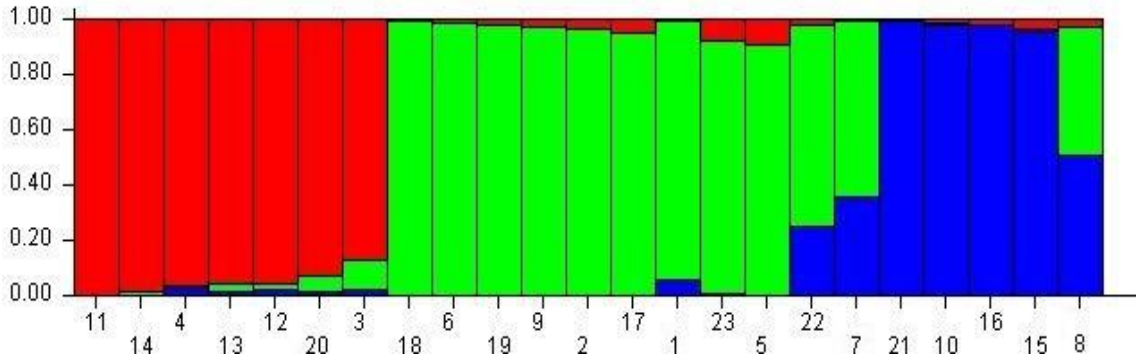

Figure 4. Bar plot of the structural analysis of data obtained from 10 ISSR primers in different cultivars of eggplant by STRUCTURE software. The numbers on the horizontal and vertical axes are related to the cultivars and the membership coefficient of each individual, respectively. Cultivars with similar colors belong to the same group

Table 5. Analysis of molecular variance (AMOVA) of data obtained from ISSR markers for three subpopulations derived from STRUCTURE software in 23 eggplant cultivars **P < 0.01

Source of variation	Degree of freedom	Mean sum of square	Standard deviation	Variation percentage	PhiPT
Among population	2	20.72	1.10	8%	0.08
Within population	20	12.337	12.73	92%	
Total	22		1383	100%	

Table 6. Genetic variation parameters within subpopulations of eggplant using ISSR markers

Subpopulation (Number of genotypes)	Polymorphic loci percentage	Number of different alleles	Number of effective alleles	Shannon's information index	Heterozygosity
Pop1 (10)	71.43	1.61± 0.08	1.53± 0.05	0.42± 0.04	0.29± 0.03
Pop2 (6)	80.00	1.74± 0.07	1.54± 0.05	0.45± 0.03	0.30± 0.02
Pop3 (7)	95.71	1.96± 0.02	1.73± 0.04	0.58± 0.02	0.40± 0.02

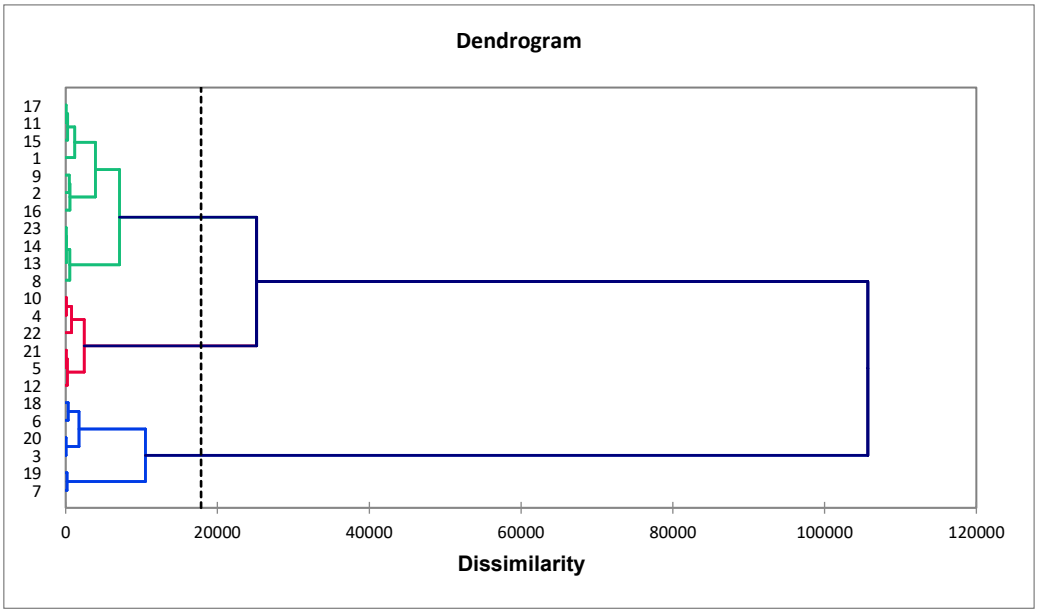

Figure 5. Cluster analysis for phenotypic traits in different cultivars of eggplant based on Ward's method

The cluster analysis based on complete linkage and Euclidean distance for molecular data divided cultivars into three main clusters (Figure 6). 60.87, 17.39, and 21.74 percent of cultivars were placed in the first, second, and third groups, respectively. Cultivars SV 1574EV and Violetta Di Firenze had the furthest Euclidean distance from cultivars Galine and Rebellion based on both the phenotypic and molecular clustering.

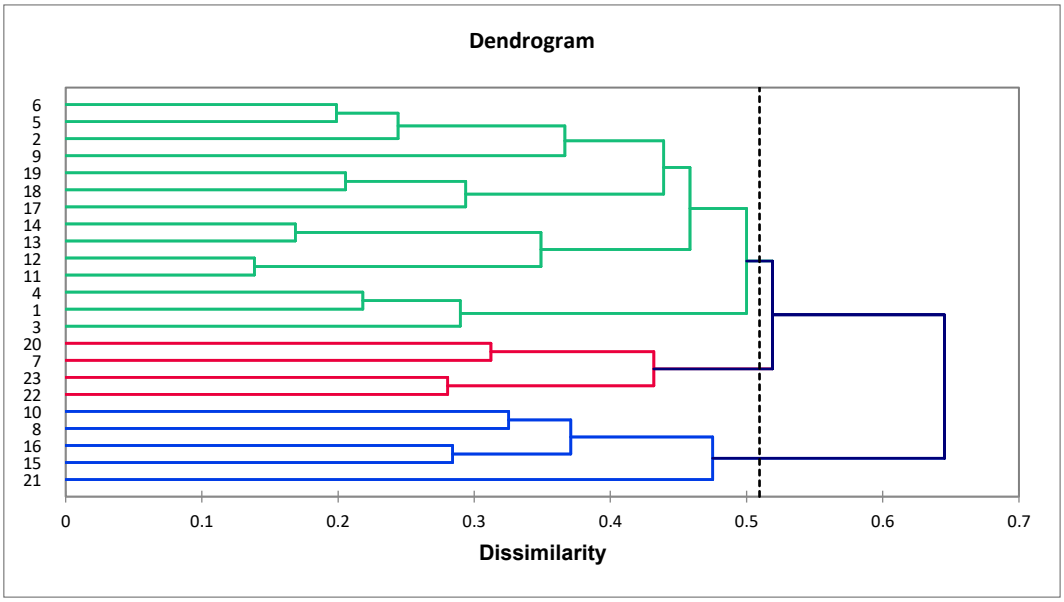

Figure 6. Cluster analysis in different cultivars of eggplant using ISSR marker based on complete linkage clustering method

Gene expression: According to the results of phenotypic, molecular clustering, and population structure, the cultivars Greta, Sally, and Melusina clustered in separate groups. Also, response of these cultivars to drought stress was different (data not shown). Therefore, these cultivars were selected to investigate the expression of the catalase gene in response to drought stress. Based on the obtained results, catalase gene expression has been significantly increased with increasing drought stress levels in all cultivars of eggplant (Table 7). Melusina cultivar with 3.56 (Triple the control) showed the highest gene expression in long-term drought stress conditions (Table 7). Catalase gene overexpression under drought stress has been reported in different plants (Hu et al., 2016; Liu et al., 2023; Wang et al., 2019).

Table 7. CAT gene expression values in eggplant cultivars under moderate and long-term drought stress conditions

Cultivars	Moderate-term drought stress	Long-term drought stress
Greta	1.33 *	1.62**
Sally	1.91*	2.68**
Melusina	1.58*	3.568**

*, **: Probability level of significantly in 0.05 and 0.01 respectively

Catalase gene plays a vital role in the ability of plants to respond to oxidative stress generated under drought by scavenging stress-induced H2O2 (Luna et al., 2004; Zhang et al., 2021). It seems that more resistance will be observed in the cultivars by increasing the expression of the CAT gene under long-term drought stress conditions. The Melusina cultivar showed higher catalase activity than the other two cultivars under long-term drought stress. Therefore, it seems that the Melusina cultivar is more tolerant under long-term drought stress drought stress than the Greta and Sally cultivars. The ratio of changes in catalase gene expression was different in the studied cultivars under different drought stress conditions. Guan and Scandalios (2000) stated that the expression patterns of CAT gene in barley under drought conditions depended on the genotype and plant development stage. Previous studies have also revealed that drought stress increased CAT expression as a rapid plant response in different plant species (Dudziak et al., 2019; Harb et al., 2015).

Conclusions: Our findings demonstrate that ISSR markers can effectively reveal polymorphism (73.45%) among eggplant cultivars, with primer (AG)8YC showing particularly high discriminatory power (PIC=0.47). However, the dominant nature of ISSRs prevents heterozygosity analysis and future studies should validate these results using co-dominant markers like SSRs or SNPs. Regarding drought response, all cultivars showed increased catalase (CAT) gene expression under stress (up to 3.56-fold in Melusina), consistent with its known ROS-

scavenging role. While Melusina's strong CAT response suggests drought adaptation potential, we recommend: Multi-location field trials under Iranian southeast conditions, comprehensive phenotyping (RWC, photosynthesis, yield metrics), and comparative analysis with known drought-tolerant commercial varieties. These results provide preliminary evidence for molecular breeding applications, but further validation is required before recommending the Melusina cultivar for drought-prone regions.

Author contributions

Fatemeh Ebrahimi: Investigation, Project administration, Data curation, analysis of the data, Writing -original draft, Writing - review & editing.

Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Ethical considerations

The author avoided data fabrication, falsification, plagiarism, and misconduct.

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Conflict of interest

There is no conflict of interest.

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ارزیابی روابط ژنتیکی و بیان ژن کاتالاز در پاسخ به تنش خشکی در ارقام مختلف بادمجان (*Solanum melongena* L.)

فاطمه ابراهیمی

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چکیده

هدف: بادمجان (*Solanum melongena* L.) یکی از سبزیجات اقتصادی مهم است و کمیت و کیفیت آن تحت تاثیر کمبود آب است. مطالعه رابطه ژنتیکی و ژن‌های دخیل در تحمل به استرس خشکی در برنامه‌های اصلاح نباتات ضروری است و به اتخاذ استراتژی‌های مدیریتی در مناطق خشک کمک می‌کند. در این مطالعه، تنوع ژنتیکی و ساختار جمعیتی ارقام بادمجان با استفاده از نشانگر ISSR تعیین گردید. علاوه بر این، بیان ژن کاتالاز در پاسخ به تنش خشکی در سه رقم انتخابی مورد ارزیابی قرار گرفت.

مواد و روش‌ها: DNA از نمونه‌های برگ ۲۳ رقم بادمجان به روش CTAB استخراج و واکنش زنجیره ای پلیمراز با استفاده از ۱۰ آغازگر ISSR انجام شد. سه رقم گرتا، سالی و ملوسینا از هر گروه ناشی از تجزیه خوشه‌ای جهت ارزیابی بیان ژن در شرایط تنش خشکی انتخاب شدند. تیمارهای تنش خشکی در مرحله اول رشد گیاه با قطع آبیاری به مدت ۱۷ و ۲۳ روز در تنش خشکی متوسط و طولانی مدت اعمال شد. آغازگر اختصاصی برای ارزیابی بیان ژن کاتالاز بود. ارزیابی RT-qPCR در سه تکرار تکنیکی با استفاده از Rotor-Gene Q انجام شد. ژن مرجع برای نرمال‌سازی بیان ژن در این مطالعه اکتین بود.

نتایج: میانگین درصد پلی‌مورفیسم در این مطالعه ۷۳٪ بود. پرایمر (AG)8YC بالاترین درصد پلی‌مورفیسم و شاخص نشانگر همراه با PIC و شاخص شانون بالا را نشان داد. بر اساس آنالیز واریانس مولکولی، واریانس ژنتیکی بین سه زیرجمعیت بسیار معنی‌دار بود. تجزیه ساختار جمعیت و تجزیه خوشه‌ای بر اساس داده‌های فنوتیپی و مولکولی ارقام را به سه گروه اصلی تقسیم کرد. بیان ژن کاتالاز تحت تنش خشکی بلندمدت در مقایسه با شاهد به طور معنی‌داری در سه رقم بادمجان افزایش یافته است. رقم ملوسینا با مقدار ۳/۵۶ بیشترین بیان ژن را در تنش خشکی بلندمدت نشان داد.

نتیجه‌گیری: نشانگر ISSR به‌عنوان یک ابزار مناسب برای ارزیابی تمایز داخل جمعیت‌های بادمجان بکار می‌رود و این نشانگر در ترکیب با سایر نشانگرهای همبارز می‌تواند برای مطالعات بعدی در بادمجان استفاده شود. علاوه بر این، ژن کاتالاز نقش مهمی را در برنامه‌های اصلاحی بادمجان در مناطق خشک ایفاء می‌کند و در صورت تایید نتایج در مطالعات بعدی، می‌توان رقم ملوسینا را به عنوان یک رقم سازگار به خشکی معرفی کرد.

کلمات کلیدی: نشانگر ISSR، تنوع ژنتیکی، بیان بیش از حد ژن کاتالاز، تنش آبی

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