Research Article

Exploring the Genetic Background and Performance Stability of Some Egyptian Rice Genotypes to Water Stress

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Abstract:

Global climate change effects on plant development, root system, growth and grain yield traits were investigated. Nine genotypes were evaluated for genetic diversity using selected SSR markers, along with various physiological, morphological, root, and yield-related traits under both normal and drought conditions to assess performance stability. Results showed highly significant variations for all traits due to genotype × environment interaction. The most desirable genotypes for the root system were Sakha105, Sakha104, IRAT170, and Moroberekan. The highest mean value for sterility percentage was found with Sakha109, while the lowest mean value was displayed with Sakha107 under drought stress. For grain yield per plant, Sakha Super300 recorded the highest mean values, whereas Giza177 had the lowest under both conditions. Six SSR primers detected 84 fragments ranging from 107 to 3846 bp, 46 of which were polymorphic (representing 54.76% polymorphism). The number of polymorphic fragments for each primer varied from 4 for primer RM451 to 11 for RM474. The dendrogram based on the GCARD similarity index and UPGMA method separated the nine genotypes into four main clusters. Genotypes Giza179, and Sakha104 were moderately drought-tolerant; the second group involved genotypes, Sakha107 and IRAT170, which were drought-tolerant; the third cluster included genotypes Sakha super300 was moderate tolerance and Moroberekan was tolerant to drought; and the fourth group included genotype Giza177 which was sensitive to drought tolerance. SSR marker analysis indicated the potential for some sensitive genotypes, such as Sakha109 and Sakha105, to possess genetic backgrounds that may contribute to moderate or high drought tolerance.

1. Introduction

Rice (*Oryza Sativa* L.) is an important cereal crop in the world, both in terms of production and consumption. It is the staple food for almost half of the world's population. Almost 90% of rice is produced in Asia (USDA, 2018). Therefore, saving irrigation water without much compromising with grain yield in rice cultivation is an important global agenda (Mohankumar et al., 2011). Rice is grown continuously flooded from transplanting until 7-10 days before harvest (IRRI 2014). Water requirement in rice varies depending on the rice growing ecosystem. Rice is grown in both lowland and upland ecosystems. The water requirement of lowland rice is quite high; 1 kg of rice requires about 1432 liters of water. Worldwide, more than 90% of rice is lowland rice (Halwart and Gupta, 2004). Rising temperature leads to an increase in water requirement for rice cultivation by about 2-5% in 2046. Climate change is also expected to decrease the precipitation, which may affect the rainfed rice growing ecosystem in the world (Wang et al., 2012). Wilhite and Glantz (1985) classified drought into four major categories: meteorological, hydrological, agricultural, and socio-economic drought. Agricultural drought is a major concern in crop growth and production. Water requirement level differs depending on the growth stage and the type of plant. Vegetative drought occurs at the seedling stage and is less harmful to the plants because they have an optimum time to recover. Intermittent drought affects the plant's vegetative development.

Terminal drought occurs during the flowering stage, resulting in a drastic reduction in grain yield.

Rice genotypes diversity for drought tolerance is imperative to find out the genotypes that can grow under water-scarce conditions to expand rice growing areas in water-limited lands. It can be helpful to meet the challenge of the increasing global food demand (Anis et al., 2019). The assessment of genetic diversity becomes important in establishing relationships among different cultivars. The first step towards determining the magnitude of these risks is to evaluate the genetic diversity in improved rice genotypes, as the success of a crop improvement program depends on the importance of genetic variability and how the desirable characters are heritable (Ravi et al., 2003). This identification of genotypes and their interrelationships is important. The development of new biotechnological techniques provides increased support to evaluate genetic variation a both phenotypic and genotypic levels. The results derived from analyses of genetic diversity at the DNA level could be used for designing effective breeding programs aiming to broaden the genetic basis of commercially grown varieties. The use of molecular markers to select accessions possessing genes and genomic regions that control target traits can fast-track the progress in breeding drought-tolerant rice. This is because molecular markers are consistently transmitted from generation to generation and are not subject to environmental influences (Afiukwa et al., 2016). SSR markers play a major role as molecular markers for

genome analysis and plant breeding. The microsatellites existing in the complete genome sequences would have a direct role in the genome organization, recombination, gene regulation, quantitative genetic variation, and the evolution of genes.

The SSR markers were identified as the system of choice for genetic analysis in rice because of their efficiency, abundance in rice genome, high level of polymorphism, and high but simple reproducible assays that are reliable (Singh et al., 2010). Studies using molecular markers have reported success in the identification of QTLs underlying various drought tolerance traits in rice chromosomes. Increased yield output and stable food supply can be achieved through the development of a drought-tolerant rice genotype. Quantitative trait loci (QTLs) are regions of DNA that encode genes for quantitative traits, the effects of which can be measured quantitatively. Plant height, grain yield, and susceptibility to abiotic and biotic stress are all examples of quantitative traits that are controlled by one or more genes and affected by environmental variation. Molecular markers are used to define and map QTLs. Recent advances in genomic technology and statistical analysis methodologies have made this mapping approach particularly cost-effective for plant research projects (Zhu et al., 2018; Dwiningsih et al., 2021). SNPs, or single-nucleotide polymorphisms, are a common type of DNA marker used to locate QTLs for desirable traits in plants. These SNPs are the most prevalent type of variations in rice genomes and are crucial for high-resolution genotyping and generating the most accurate maps possible (McCouch et al., 2010). Several QTLs associated with drought-tolerant traits have been identified in Oryza sativa (Mardani et al., 2013). The objectives of the present investigation were to evaluate the performance stability of the tested rice genotypes under drought stress conditions and to identify SSR markers potentially associated with drought tolerance traits in rice.

2. Materials and Methods

2.1. Plant materials

Pedigree, origin, type, and drought reaction of the studied genotypes are listed in Table 1. The varieties Sakha 107, IRAT 170 and Moroberekan were used as drought-tolerant, and the varieties Giza 179, Sakha 104 and Sakha Super 300 were used as moderately drought-tolerant, while the genotypes Giza177, Sakha105 and Sakha109 were used as drought-sensitive.

2.2. Experimental design

After thirty days from sowing, seedlings of each genotype were individually transplanted in the permanent field in seven rows. Each row was five meters long and contained 25 plants, adopting a spacing of 20 × 20 cm between plants. These materials were replicated three times in a randomized complete block design (RCBD) with the same set of genotypes in two experiments (under normal and drought conditions). Drought stress was imposed by using flush irrigation (flush irrigation is one of the surface irrigations without standing water after irrigation) every 12 days to reach the soil moisture content to the field capacity, after two weeks of transplanting to harvesting, and recommended cultural practices were followed. On the other hand, the normal irrigation was every four days with continuous flooding and without any severing from water shortage through the growth period from transplanting until harvesting time.

2.3. Studied traits

Data of the agronomic characters were recorded for five randomly selected plants in each replication for all the tested cultivars to estimate root length(cm), number of roots plant^{-1}, root thickness (mm), root volume (cm^3), root: shoot ratio, root xylem vessels number and root xylem vessel area(μm2) for rice root system at maximum tillering stage while, days to heading(days), plant height(cm), leaf rolling, number of panicles plant^{-1}, sterility percentage (%), 100-grain weight (g) and grain yield plant^{-1} (g) were taken at flowering stage and harvest time.

Table 1. Pedigree, origin, type and drought reaction of the studied varieties.

Number	Genotype	Pedigree	Origin	Type	Drought reaction
1	Giza 177	(Giza 171 / Yomji Number 1 / Pi Number 4)	Egypt	*Japonica*	Sensitive
2	Sakha 105	(GZ 5581-46-3 / GZ 4316-7-1-1)	Egypt	*Japonica*	Sensitive
3	Sakha 109	Sakha101/Sakha105	Egypt	*Japonica*	Sensitive
4	Giza 179	(GZ 1368-S-5-4 / GZ 6296-12-1-2)	Egypt	*Indica / Japonica*	Moderate
5	Sakha 104	(GZ 4096-8-1 / GZ 4100-9-1)	Egypt	*Japonica*	Moderate
6	Sakha Super 300	Introduction	Egypt	*Japonica*	Moderate
7	Sakha 107	(Giza 177 x BL1)	Egypt	*Japonica*	Tolerant
8	IRAT 170	(IRAT 13/ Palawan)	Ivory Coast	*Indica*	Tolerant
9	Moroberekan	(IR 8-24-6- (M307 H5)	Republic of Guinean	*Tropical Japonica*	Tolerant

2.4. Statistical analysis

At first, the data were analyzed using the ordinary analysis of variance to test the significance of differences among the nine genotypes. Statistical analysis was performed for normal irrigation and drought environments and the pooled data over the two environments. The combined analysis was calculated over 2022 and 2023 rice growing seasons for the two environments (non-stress and drought). Before the computations of the combined analysis, it is necessary to determine whether the error variance of the test was homogeneous. The test described by Bartlett (1937) is the best and preferred. All statistical analyses were performed using the analysis of variance technique by means of "Genes" computer software package. Means were compared using Duncan's multiple range test (Duncan, 1955).

2.5. Molecular analysis

2.5.1. DNA Extraction

One-month-old plant shoots were employed for genomic DNA isolation, Plant's EZ-10 Spin Column Genomic DNA Minipreps Kit was used. Extracted DNA quantity and quality were evaluated on agarose gel (0.8%).

2.5.2. Simple Sequence Repeats (SSR) Polymorphism Analysis

A set of six SSR primers related to drought tolerance were shown in Table 2 was used in PCR amplification reactions following instructions supporting OnePCR™. Amplification was programmed for a 5 min initial denaturation step at 94 C° followed by 35 cycles of denaturing at 94 C° for one minute,

annealing at 51-58 C° (according to each primer) for one minute, extension step at 72 C° for one minute, and a final extension step at 72 C° for 5 min by using programmable thermal cycler (MyGene®-MG96G). The amplification products were electrophoresed against a DNA ladder (100 bp) to estimate the molecular sizes of the amplified fragments as shown in figure 1. Using the PAST application, version 1.90, divided bands were assessed and scored depending on the absence and presence of bands (0 and 1, respectively). Using Past software (version 2.17) created by Hammer and Harper (2001), the genetic information was found using the unweighted pair group approach using arithmetic averages (UPGMA), as well as similarity indices (based on Jaccard formula) between the tested varieties (Sneath and Sokal, 1973).

Table 2. The SSR primers were used for detecting polymorphism among nine varieties.

Number	SSR Marker	Annealing temperature	Number of nucleotides	Forward sequencing	Annealing temperature	Number of nucleotides	Reverse sequencing
1	RM585	57	20	CAGTCTTGCTCCGTTTGTTG	55	22	CTGTGACTGACTTGGTCATAGG
2	RM7075	57	20	TATGGACTGGAGCAAACCTC	55	19	GGCACAGCACCAATGTCTC
3	RM201	56	22	CTCGTTTATTACCTACAGTACC	55	22	CTACCTCCTTTCTAGACCGATA
4	RM451	57	19	GATCCCCTCCGTCAAACAC	57	20	CCCTTCTCCTTTCCTCAACC
5	RM316	51	20	CTAGTTGGGCATACGATGGC	57	21	ACGCTTATATGTTACGTCAAC
6	RM474	55	20	AAGATGTACGGGTGGCATTC	55	20	TATGAGCTGGTGAGCAATGG

3. Results and Discussion

3.1. Analysis of variance

Analysis of variance in Tables 3 and 4 showed the mean squares due to years were not significant for major root traits, plant growth and grain yield traits, except for number of roots plant[-1], days to heading, leaf rolling, and number of panicles plant[-1] were significant, which would indicate no differences among two years were studied. Abdalla (2019) observed that the ordinary analysis of variance showed no significant differences among years for root traits in both treatments (normal and drought), except for number of roots plant[-1]. Mean squares due to environments were highly significant for each of the root-related traits, growth and grain yield traits, indicating that environments showed highly significant differences. The variation due to interaction between genotypes and year was not significant for all root traits as well as growth and grain yield traits, except traits of root length, root xylem vessel area, and number of panicles plant[-1]. Some genotypes surpassed

the others. The mean squares of genotypes were significantly higher than the interaction genotype × year mean squares and identified the most superior genotypes. Mean squares due to genotype × environment interactions were highly significant for all root traits as well as growth and grain yield traits, which indicated that the tested genotypes varied depending on the environment and ranked differently from the normal to drought conditions. Mean squares due to year × environment interactions were insignificant for all the studied traits, except root xylem vessel number. Mean squares of genotype × environment × year were not significant for all the root traits as well as growth and grain yield traits, except root volume, root xylem vessel area, plant height, and number of panicles plant[-1] traits, indicating that each genotype's performance in one environment will be changed from one year to another. The significant differences among rice genotypes in this investigation revealed genetic variability in the studied materials, providing an excellent opportunity for improvement.

Table 3. Mean squares of combined for root traits.

Source of variance	Df	Root length(cm)	Number of roots plant[-1]	Root thickness (mm)	Root volume(cm3)	Root: shoot ratio	Root xylem vessels number	Root xylem vessel area(μm2)
(B/E)/Y	8	0.83	3.25	0.001	1.27	0.0002	0.15	0.03
Genotype (G)	8	75.50**	13948.00**	1.17**	447.37**	0.07**	30.26**	2.11**
Years (Y)	1	0.31ns	40.95**	0.001ns	5.51ns	0.002ns	0.01ns	0.03ns
Environment (E)	1	1405.3**	328098.5**	0.8**	16467.4**	0.58**	17.60**	512.36**
G x Y	8	3.16**	4.32ns	0.001 ns	1.67ns	0.0001ns	0.21ns	0.96**
G x E	8	18.35**	17101.24**	0.01**	240.81**	0.01**	0.97**	1.42**
Y x E	1	0.12ns	2.52ns	0.002ns	3.48ns	0.0002ns	1.56*	0.05ns
G x Y x E	8	0.53ns	2.93ns	0.001ns	5.13*	0.0003ns	0.33ns	0.81**
Error	64	1.11	2.8	0.002	2.09	0.0001	0.18	0.09

*, ** and ns are significant, highly significant and not significant, respectively.

Table 4. Mean squares for growth and grain yield traits.

Source of variance	df	Days to heading(days)	Plant height(cm)	Leaf rolling	Number of panicles plant[-1]	Sterility percentage (%)	100-grain weight (g)	Grain yield plant[-1] (g)
(B/E)/Y	8	3.62	2.65	0.25	0.93	1.51	0.03	2.45
Genotype (G)	8	867.80**	2522.12**	7.23**	120.61**	48.54**	1.69**	151.43**
Years (Y)	1	25.52*	2.55ns	2.68*	17.12**	0.69ns	0.01ns	0.72ns
Environment (E)	1	2111.17**	10772.0**	91.12**	2161.87**	11663.0**	3.96**	6486.91**
G x Y	8	3.93ns	2.05ns	0.25ns	3.16*	1.47ns	0.02ns	2.13ns
G x E	8	19.60**	347.78**	3.52**	48.14**	43.32**	0.11**	87.03**
Y x E	1	7.00ns	10.21ns	0.38ns	3.13ns	0.93ns	0.01ns	0.04ns
G x Y x E	8	2.22ns	5.47*	0.58ns	4.36**	0.85ns	0.02ns	0.73ns
Error	64	1.94	2.58	0.3	1.38	1.07	0.04	2.02

*, ** and ns are significant, highly significant and not significant, respectively.

3.2. Mean performance

The mean values of root characters of the studied genotypes are presented in Tables 5. It is widely recognized that the root system plays an important role under water deficit conditions, and the nature and extent of root development are major factors governing plant response to moisture conditions. For root length, the genotypes IRAT 170 and Moroberekan gave the highest mean values (26.23 and 28.56 cm, respectively), while the genotypes Giza 177 and Sakha 105 gave the lowest mean values (19.94 and 22.10 cm, respectively). The genotype Sakha 104 recorded the highest number of roots plant[-1] (327.32 roots) compared with the other varieties. In contrast, IRAT 170 and Giza 177 recorded the lowest mean values (224.05 and 237.41 root, respectively). Concerning root thickness, the varieties IRAT 170 (1.39 mm) and Moroberekan (1.38 mm) recorded the most desirable mean values with significant differences compared with the other varieties. While the lowest averages were recorded by the varieties Giza 179 (0.60 mm) and Giza 177 (0.65 mm). These results were in agreement with those found by Ghazy (2017), Gaballah et al. (2021c) and Ghazy et al. (2023). With respect to root volume, the results revealed that the highest mean values obtained with

significant differences were for Moroberekan (73.88 cm^3) and Sakha Super 300 (72.56 cm^3) compared with the other varieties, while low averages were recorded by Giza 177 (55.43 cm^3) and Sakha 109 (60.56 cm^3). Concerning the trait of root: shoot ratio, the highest mean value of 0.84 was obtained by Sakha 105 followed by Giza 179 and Sakha Super 300 (0.83 for each), while the lowest mean values were obtained by Giza 177 (0.64) and IRAT 170 (0.70). These results agreed with those found by Verma et al. (2019) and Gaballah et al. (2021a). For root xylem vessel number, the most desirable mean values are for Moroberekan and IRAT 170 (8.52 and 6.73, respectively) compared with the other varieties. While the lowest mean value was recorded for Sakha 104 (3.82). Regarding root xylem vessel area, the most desirable mean values with significant differences were obtained by IRAT 170 and Moroberekan (3.20 and 2.97 μm^2, respectively). While the lowest values were recorded by Giza 177 and Sakha 105 (1.96 and 2.06 μm^2, respectevily). The number and size of the xylem vessels are closely related to the capacity of the plant to transport water and nutrients from the soil. Ghazy et al. (2023) observed that drought-tolerant varieties had larger size and a higher number of root xylem vessels than susceptible ones.

Table 5. Root mean performance under normal and drought stress environments.

Genotype	Root length (cm)	Root number plant[-1]	Root thickness (mm)	Root volume (cm^3)	Root: shoot ratio	Root xylem vessels number	Root xylem vessel area (μm^2)
Giza 177	19.94	237.41	0.65	55.43	0.64	4.07	1.96
Sakha 105	22.1	294.77	0.76	67.31	0.84	4.08	2.06
Sakha 109	23.43	244.98	0.67	60.56	0.71	4.55	2.22
Giza 179	23.59	278.38	0.6	62	0.83	4.24	2.11
Sakha 104	23.32	327.32	0.7	60.63	0.82	3.82	2.24
Sakha Super 300	25.43	292.78	0.81	72.56	0.83	4.47	2.42
Sakha 107	25.38	288.81	0.67	68.21	0.82	4.19	2.38
IRAT 170	26.23	224.05	1.39	62.25	0.7	6.73	3.2
Moroberekan	28.56	244.33	1.38	73.88	0.73	8.52	2.97
LSD 0.05	1.72	2.73	0.07	2.36	0.02	0.69	0.49
LSD 0.01	2.28	3.63	0.1	3.13	0.02	0.92	0.65

For days to heading in Table 6, the latest plants were obtained by Sakha Super 300 and Moroberekan (mean values of 110.75 and 108.04 days, respectively), while the earliness genotypes were Sakha 105 and Giza 179 (mean values of 89.83 and 89.67 days, respectively). These findings are in close agreement with those reported by Abade et al. (2016) and Ghazy (2017). A significant difference in plant height between the rice genotypes were found, suggesting that the growth rates were different in these genotypes. The most desirable mean values towards dwarfism were obtained by the genotypes Sakha 109, Giza 179 and Sakha 107 (81.73, 86.08 and 88,61, respectively), which

have tolerance via the plant height reduction after stress conditions. These results are in agreement with the results obtained by Abdalla (2019) and Gaballah et al. (2021a). Leaf rolling is considered the first symptom of the stress conditions reaction. The greatest recorded scores with significant differences were for Sakha 105 and Giza 177 (4.53 and 4.38, respectively) over all environmental conditions. On the other hand, IRAT170 and Moroberekan recorded the lowest scores of 2.98 and 2.19, respectively. These results are in agreement with the results obtained by Fen et al. (2015), Abdalla (2019), Ghazy et al. (2021) and Ichsan et al. (2021). For number of panicles plant[-1], the highest values were

obtained by Sakha Super 300 and Sakha 107 (21.59 and 22.60 panicles plant[-1], respectively), whereas the lowest values were shown with Moroberekan and Giza 177 (13.98 and 14.68 panicles plant[-1], respectively). Concerning sterility percentage, the lowest values were observed with Giza 179 and Moroberekan (16.53 and 15.44 %, respectively), while the highest values were obtained by Sakha 109 and Sakha Super 300 (21.28 and 20.63%, respectively). For 100-grain weight, the heaviest values were obtained by Moroberekan (3.49 g), therefore, the lightest values were shown with Giza 179 (2.43 g). Regarding grain yield plant[-1], the highest values were observed for Giza 179 (39.79 g), followed by Sakha Super 300 (39.39 g). While the lowest values were found for Giza 177 and Sakha 109 (29.50 and 32.30 g plant[-1], respectively).

Table 6. Growth and grain yield mean performance under normal and drought stress environments.

Genotype	Days to heading (days)	Plant height (cm)	Leaf rolling	Number of panicles plant[-1]	Sterility percentage (%)	100-Grain weight (g)	Grain yield plant[-1] (g)
Giza 177	90.75	96.67	4.38	14.68	17.19	2.73	29.5
Sakha 105	89.83	100.27	4.53	18.88	20.11	2.56	33.88
Sakha 109	89.92	81.73	4.23	18.71	21.28	2.54	32.3
Giza 179	89.67	86.08	3.19	20.09	16.53	2.43	39.79
Sakha 104	103.25	96.44	4.23	20.58	17.25	2.55	36.5
Sakha Super 300	110.75	99.62	3.62	21.59	20.63	2.6	39.39
Sakha 107	91.08	88.61	3.43	22.6	17.26	2.55	38.54
IRAT 170	96.83	119.79	2.98	15.04	17.83	3.28	33.56
Moroberekan	108.04	124.74	2.19	13.98	15.44	3.49	33.16
LSD 0.05	2.27	2.62	0.89	1.92	1.69	0.33	2.32
LSD 0.01	3.02	3.48	1.19	2.55	2.24	0.43	3.08

3.3. Genotypes and environment interaction

The data in Table 7 illustrates the mean performance of traits for the interaction between genotypes and environments. For root length trait, the highest mean values were found for Moroberekan (32.12 and 25 cm under normal and drought conditions, respectively), while the lowest mean values were displayed with Giza 177 (24.5 and 15.38 cm at both environments, respectively). These results were in agreement with those reported by Ghazy et al. (2023), Dubey et al. (2024) and Sun et al. (2024). With respect to root number plant[-1], the data illustrated that the highest mean values were found with sakha105 (396.17 for normal condition) and sakha104 (281.95 for drought condition), while the lowest mean values were displayed with IRAT 170 and Giza 177 (236.57 and 108.73 under normal and drought conditions, respectively). The similar findings were reported by Ghazy (2017) and Gaballah et al. (2021b). The highest mean values for root thickness were found for IRAT170 (1.55 and 1.24 mm) and Moroberekan (1.50 and 1.26 mm) under normal and drought conditions, respectively. While the lowest mean values were displayed with Giza 179 (0.68 and 0.52 mm at both environments, respectively). These results agreed with those of Abdella (2019) and Ghazy et al. (2023). The highest mean values for root volume were found with Moroberekan (87.07 and 60.70 cm[3] under normal and drought conditions, respectively), while the lowest mean values were displayed with Giza 179 under normal conditions (67.82 cm[3]) and Giza 177 under drought conditions (34.93 cm[3]). High mean values for root: shoot ratio were found with Sakha Super 300 (0.93 and 0.74 under normal and drought conditions, respectively), while low mean values were displayed with Giza 177 (0.79 and 0.50 under both environments, respectively). The highest mean values for root xylem vessels number under normal and drought environments were found with Moroberekan (8.82 and 8.22, respectively). In contrast, the lowest mean values were displayed with Sakha 104 (4.15 and 3.48, respectively). The highest mean values for root xylem vessel area at both environments were found for IRAT170 (0.41 and 5.98 mm, respectively), while the lowest mean values were displayed with Giza 177 (0.15 and 3.78 mm, respectively). It could be concluded that the most desirable genotypes for the root system were Sakha 105, Sakha 104, IRAT 170 and Moroberekan. The objective is to identify genotypes with good root systems, i.e., root length, root volume, root thickness, and higher root: shoot ratios. In addition, rice genotypes categorized as drought-tolerant should have higher values for each of root length, root volume, root: shoot ratios, and root xylem vessel area.

Table 7. Mean performance of genotype x environment interactions for root traits.

Genotype	Root length (cm)		Root number plant[-1]		Root thickness (mm)		Root volume (cm[3])		Root:shoot ratio		Root xylem vessels number		Root xylem vessel area(μm2)	
	N	D	N	D	N	D	N	D	N	D	N	D	N	D
Giza 177	24.5	15.38	366.08	108.73	0.71	0.58	75.92	34.93	0.79	0.5	4.42	3.72	0.15	3.78
Sakha 105	26.03	18.17	396.17	193.37	0.81	0.71	83.55	51.07	0.91	0.77	4.52	3.63	0.16	3.96
Sakha 109	26.23	20.62	299.25	190.72	0.75	0.59	71.47	49.65	0.76	0.66	4.85	4.25	0.18	4.26
Giza 179	26.03	21.15	312.92	243.85	0.68	0.52	67.82	56.18	0.91	0.76	4.28	4.2	0.22	4
Sakha 104	29.45	17.18	372.68	281.95	0.77	0.63	73.93	47.32	0.88	0.77	4.15	3.48	0.16	4.32
Sakha Super 300	29.27	21.6	354.77	230.78	0.89	0.73	86.7	58.42	0.93	0.74	4.7	4.23	0.2	4.63
Sakha 107	27.37	23.4	323.2	254.42	0.75	0.59	76.42	60	0.88	0.75	4.77	3.62	0.19	4.58
IRAT 170	29.45	23.02	236.57	211.53	1.55	1.24	71.08	53.42	0.77	0.64	7.78	5.67	0.41	5.98
Moroberekan	32.12	25	267.25	221.42	1.5	1.26	87.07	60.7	0.77	0.69	8.82	8.22	0.28	5.66
LSD 0.05	1.72		2.73		0.07		2.36		0.02		0.69		0.49	
LSD 0.01	2.28		3.63		0.1		3.13		0.02		0.92		0.65	

N and D is normal irrigation and drought, respectively

Concerning results in Table 8 demonstrate the mean performance of growth and grain yield traits for the interaction between genotypes and environments, the highest mean values for days to heading were found for Sakha Super 300 under both environments (117.00 and 104.50 days, respectively), while Sakha105 gave the lowest mean value under drought stress (83.67 days). These results were in agreement with those found by Abdalla (2019), Gaballah et al. (2021a), Ghazy et al. (2021), and. Alafari et al. (2023). Regarding mean values for plant height, results revealed that Moroberaken gave the highest values of 132.30 and 117.20 cm for normal and drought conditions, respectively, while Sakha 109 showed the lowest mean value under drought conditions (67.33 cm). These results were in agreement with those found by Gaballah (2016), Ghazy (2017) and Abdalla (2019). With respect to leaf rolling, high mean values were found with Sakha 105 (3.25 and 5.82, respectively) in both environments, while the lowest mean values were observed for Moroberaken in both environments (1.67 and 2.72, respectively). Concerning mean performance of number of panicles plant^{-1} for the interaction between genotype and environment, the highest mean values were

observed for Sakha Super 300 under normal condition (29.33) and for Sakha 107 under drought stress (19.00), while the lowest mean value under drought was obtained by Giza 177 (10.28). The results were in agreement with those reported by Nofal and Gaballah (2024). Under a drought environment, the highest value for sterility percentage was observed for Sakha 109 (34.58%), while the lowest value was observed for Moroberekan (24.20%). The results were in agreement with those reported by Ghazy (2017), Abdella (2019) and Ghazy et al. (2021). The highest mean values for 100-grain weight were observed for Moroberekan (3.90 and 3.08 g), while the lowest mean values were observed for Giza 179 (2.59 and 2.26g under normal and drought environments, respectively). The results were in agreement with those reported by Ghazy et al. (2021), Alafari et al. (2023) and Gaballah et al. (2023). High mean values for grain yield plant^{-1} were observed for Sakha Super 300 (48.53 and 30.25 g), and low values were observed for Giza 177 (38.68 and 20.32 g) in both environments, respectively. The results were in agreement with those reported by Abdella (2019), Romos et al. (2019), Ghazy et al. (2021) and Anand et al. (2023).

Table 8. Mean performance of genotype x environment interactions for growth and grain yield traits.

Genotype	Days to heading (days)		Plant height (cm)		Leaf rolling		Number of panicles plant^{-1}		Sterility percentage (%)		100-grain weight (g)		Grain yield plant^{-1} (g)	
	N	D	N	D	N	D	N	D	N	D	N	D	N	D
Giza 177	95	86.5	105.9	87.48	2.82	5.95	19.07	10.28	8.23	26.15	2.85	2.62	38.68	20.32
Sakha 105	96	83.67	107.4	93.18	3.25	5.82	23.43	14.32	9.08	31.13	2.65	2.47	44.1	23.67
Sakha 109	95.5	84.33	96.12	67.33	2.53	5.92	24.6	12.82	7.99	34.58	2.73	2.35	42.87	21.73
Giza 179	93.5	85.83	94	78.15	2.52	3.87	24.32	15.87	6.2	26.87	2.59	2.26	48.25	31.32
Sakha 104	106.3	100.2	101.7	91.23	2.92	5.55	26.93	14.23	5.66	28.83	2.7	2.39	45.77	27.23
Sakha Super 300	117	104.5	121.7	77.58	3.2	4.03	29.33	13.85	7.83	33.42	2.78	2.42	48.53	30.25
Sakha 107	94	88.17	99.97	77.25	3.13	3.72	26.2	19	9.08	25.43	2.68	2.41	43.85	33.23
IRAT 170	100.3	93.33	125	114.6	2.48	3.48	17.15	12.93	9.23	26.43	3.56	3	37.23	29.88
Moroberekan	112.3	103.8	132.3	117.2	1.67	2.72	15.38	12.58	6.68	24.2	3.9	3.08	37.08	29.23
LSD 0.05	2.27		2.62		0.89		1.92		1.69		0.33		2.32	
LSD 0.01	3.02		3.48		1.19		2.55		2.24		0.43		3.08	

N and D is normal irrigation and drought, respectively

3.4. Molecular markers associated with drought

For investigating the genetic diversity, several molecular markers are available. SSR markers play a major role as molecular markers for genome analysis and plant breeding. An attempt was conducted to evaluate the molecular genetic markers for the nine rice genotypes on the DNA nucleotide sequences to determine any fragment(s) or band(s) that could be associated with drought. Six SSR primers were used in this investigation related to drought tolerance in rice crop.
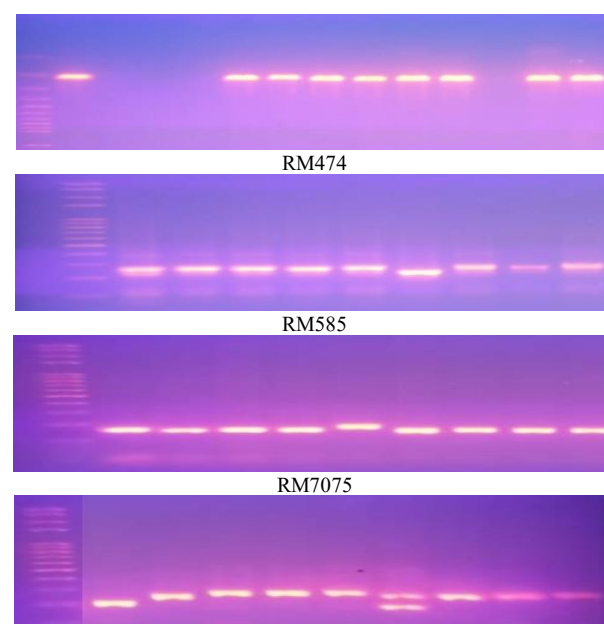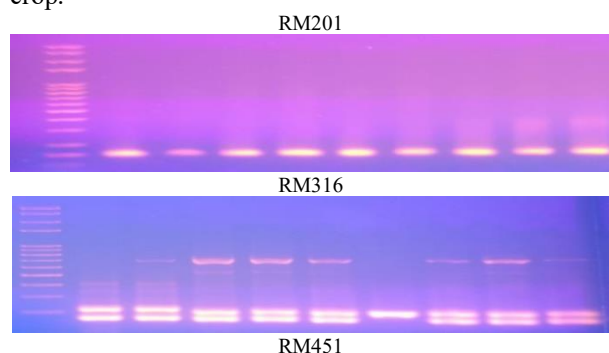

RM201

RM316

RM451

RM474

RM585

RM7075

Figure 1. The SSR primers and amplification products were electrophoresed against a DNA ladder (100 bp).

As shown in Table 9, the data represent the polymorphic amplified DNA bands that were obtained as a result of using the SSR primers. The six SSR primers detected a total of 84 fragments, ranging from 107 to 3846 bp, 46 of them were polymorphic (showing 54.76% polymorphism). The percentage of polymorphic bands ranged from 34.48 % for primer RM316 to 88.89 % for primers RM201. The number of polymorphic fragments for each primer varied from 4 for primers RM451 to 11 for RM474. However, the primer RM474 has the highest polymorphic bands, which distinguishes the used rice varieties into three classes: tolerance, moderate, and sensitive to water shortage.

Table 9. Total and polymorphic amplified fragments, and polymorphism percentage for the used SSR primers.

Primer	Molecular weight	Amplified fragments		Polymorphism (%)
		Total	Polymorphic	
RM201	142-147	9	8	88.89
RM316	123-755	29	10	34.48
RM451	2839-3846	9	4	44.44
RM474	107-254	18	11	61.11
RM585	138-161	9	7	77.78
RM7075	315-446	10	6	60.00
Total		**84**	**46**	**54.76**

3.5. Genetic similarity and phylogenetic tree

SSR markers are highly polymorphic and could be used for rice cultivar identification. These results are consistent with the findings of Sabouri et al. (2018), Al-Azzawi et al. (2020), Gaballah et al. (2021c), and Sanghamitra et al. (2022). They concluded that SSR markers could be used as highly informative markers for genome mapping and gene tagging because the evolutionary rate of change within microsatellites is considerably higher than many other types of DNA markers. In this study, the genetic similarity indices (Table 10) and phylogenetic tree (Fig. 1) were established by a simple matching coefficient based on the scorable banding pattern of the nine rice genotypes generated using six SSR primers. Concerning similarity indices, the genetic similarity ranged from 0.00 to 0.38%. The highest percentage of genetic similarity (0.38%) was obtained between Sakha 109 and Giza 179, followed by 0.20% between each of (Sakha 105 and Sakha 107), (Sakha 105 and IRAT170), (Giza 179 and Sakha 104), and (Sakha 107 and IRAT170). Then genetic similarity percentage of 0.19% was found between Sakha 105 and Moroberekan. The genetic similarity percentage of 0.00% was detected between (Giza 177 and each of Sakha 109, Giza 179, Sakha 104, Sakha Super 300, and Sakha 107), (Sakha 105 and Sakha 104), (Sakha 109 and each of Sakha 107, IRAT170, and Moroberekan), (Giza 179 and each of Sakha 107, IRAT170, and Moroberekan), (Sakha 104 and each of Sakha Super 300, Sakha 107, and Moroberekan), and (IRAT170 and Moroberekan).

Table 10. Similarity indices among the nine rice genotypes based on SSR data.

	Giza 177	Sakha 105	Sakha 109	Giza 179	Sakha 104	Sakha Super 300	Sakha 107	IRAT170	Moroberekan
Giza 177	1.00	0.06	0.00	0.00	0.00	0.00	0.00	0.06	0.12
Sakha 105		1.00	0.06	0.06	0.00	0.14	0.20	0.20	0.19
Sakha 109			1.00	0.38	0.13	0.07	0.00	0.00	0.00
Giza 179				1.00	0.20	0.07	0.00	0.00	0.00
Sakha 104					1.00	0.00	0.00	0.06	0.00
Sakha Super 300						1.00	0.07	0.07	0.13
Sakha 107							1.00	0.20	0.12
IRAT 170								1.00	0.00
Moroberekan									1.00

Concerning phylogenetic tree using Jaccard (Fig. 2), the dendrogram based on Jaccard similarity index and UPGMA method separated the nine genotypes into four main clusters: The first main cluster included genotypes 3 was sensitive while 4 and 5 were moderate to drought. The second group involved genotypes 2 was sensitive while 7 and 8 were tolerant to drought. The third group included genotypes 6 was moderate and 9 was tolerant to drought. The fourth group included genotype 1, which was sensitive to drought. Many authors have successfully used the SSR technique as a tool for the detection of similarities and dissimilarities of rice genotypes. In addition, the dendrogram based on UPGMA cluster analysis deduced that the SSR markers succeeded in distinguishing and discriminating all rice varieties concerning their genetic background and geographical origin (Verma et al.,2019). The main purpose of this study was to identify SSR markers associated with drought in rice. Six SSR primers were used to distinguish nine rice genotypes that differed in drought tolerance traits. The productivity of SSR markers may be due to the possibility of amplifying the different-sized fragments from different regions of the genome, or may be dependent on the genotypes. Similar findings were recorded by Alafari et al. (2024), who indicated that the SSR markers RM22, RM525, RM324 and RM3805 were able to discriminate the tolerant parents from the sensitive ones. Our SSR results showed probabilities for some sensitive genotypes (Sakha109 and Sakha105) to have genetic backgrounds to are moderate or tolerant to drought. It may need further investigation to detect the ability of these genotypes to show their drought tolerance performance.
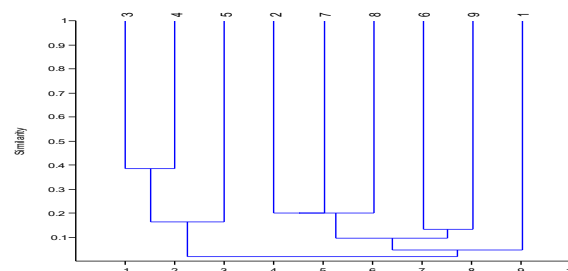

Figure 2. Jaccard phylogenetic tree for nine rice genotypes based on SSR makers. Whereas, 1is Giza177, 2 is Sakha 105, 3 is Sakha 109, 4 is Giza 179, 5 is Sakha 104, 6 is Sakha Super 300, 7 is Sakha 107, 8 is IRAT170 and 9 is Moroberekan.

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