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Assessment of Genetic Diversity of Diverse rice Genotypes Using Agro-Physiological and Molecular Characterization under Water Deficit Conditions

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DROUGHT is a significant abiotic stress that has a significant impact on rice growth, production, and quality. Furthermore, water scarcity is expected to become increasingly severe and frequent as a result of climate change, particularly in arid environments. Therefore, the objective of this study was to assess the impact of water deficit on morphological, physiological and agronomic of eight rice varieties with different genetic backgrounds. In addition to apply two PCR-based molecular marker systems ISSR and SCoT to assess the genetic diversity among the studied rice varieties. The results revealed that, water shortage stress significantly reduced relative water content, total chlorophyll content, grain yield, and yield characteristics. While, it significantly increased proline content and antioxidant enzyme activity (CAT, APX, and SOD) compared to normal irrigation treatment. The combined analysis of variance demonstrated that the mean squares for environments, varieties, and their interaction were highly significant for all investigated traits. The evaluated genotypes exhibited varied responses to drought-stress conditions. The Puebla and Hispagran varieties possessed the highest performance for most of the evaluated parameters and surpassed the other tested genotypes under water-deficit conditions. Therefore, it could be exploited in rice breeding programs for water-deficit tolerance. The ISSR primers produce 46 amplified bands with an average of 6.6 bands/primer and 49.64% polymorphism. The SCoT primers reveal 46 bands with a mean of 11.5 bands/primer and 57% polymorphism. Both marker systems were informative, and the average polymorphism information content (PIC) was 0.33 and 0.38 for ISSR and SCoT, respectively. The dendrogram generated by ISSR and SCoT markers combined data divided the varieties into two major clusters. Cluster I consisted of the genotype Sakha 106. Cluster II retained seven varieties, which were further divided into two sub-clusters; Sakha 101, Sakha 105, Sakha 106, Sakha 107 constituted the first subgroup, while Giza 177, Hispagran, and Puebla formed the second one. It could be concluded that, Puebla, Hispagran, and sakha 108, which recorded the highest desirable values for the majority of studied traits under water deficit stress, could be used as a doner in rice breeding programs to develop new promising lines under water shortage conditions.

Keywords: Rice, Water deficit, Physiological and Biochemical Responses, Molecular analysis, ISSR Marker, SCoT Marker, Cluster Analysis.

1. Introduction

Rice (*Oryza sativa* L) is the most consumed crop by more than 50% of the world's population (Rasheed *et al*., 2020), providing up to 80% of their daily caloric intake (sahebi *et. al.*, 2018). Moreover, rice cultivation is a basic activity and a major source of revenue in many regions of the world (Das *et al*., 2017). It is grown in a variety of agroecological situations, including rain-fed areas where drought stress is common owing to variable rainfall (Swamy and Kumar 2013). Rice production is predicted to grow by 100 tonnes by 2050 to feed the world's 9.1 billion people (Jaggard *et al.*, 2010; Rasheed *et al*., 2020). As a result, global rice consumption will need to rise from 763 million tonnes to 850 million tonnes. In a while, only a 1% yearly improvement in rice production has been seen over the last decade (Khush 2013; Gaballah *et al*., 2022).

Egypt is the Middle East's largest rice producer, with growing taking place in the Nile River's lower basin

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as a summer crop (Freeg e*t al.,* 2022). In Egypt, the rice sector has performed well in terms of output and yield during the previous three decades. The widespread adoption of early maturing and semi-dwarf Egyptian cultivars increased rice yield per unit to 9.52 t/ha in the 2000s, up from 5.7 t/ha in the 1980s (El-Mowafi *et al*., 2021; Abd El-Aty *et al.,* 2022). Rice is one of Egypt's key crops and a critical commodity in terms of food security.

Climate change, which affects the frequency and magnitude of hydrological oscillations, is a serious danger to agriculture, particularly in developing countries, and creates a variety of biotic and abiotic stresses for plants. (Turral *et al,* 2011). Abiotic variables have a considerable impact on rice growth and yield. Drought stress is one of the major threats to global rice production, causing significant yield losses. (Swamy and Kumar 2013; Sahebi *et al.,* 2018). The major cause of low rice output is a lack of fresh water for rice plants. Water scarcity is a prevalent problem for sustainable agriculture (Foley *et al.,* 2011), as generating 1 kg of rice requires around 3000 liters of water (Todaka *et al*., 2015). Rice is more sensitive to water scarcity than other crops since it is a semi-aquatic plant and is farmed in lowland paddies with standing water during all phases of growth (Singh *et al.,* 2012; Al Azzawi *et al*., 2020).

Depending on relevant factors such as genotype, stress severity, and developmental stage, Drought disrupts several molecular, biochemical, and physiological processes in plants, affecting their growth and development. Drought also has a detrimental impact on grain yield and quality. Plants have evolved many adaptation strategies to enable them to survive and cope with stress conditions (Batool *et al.,* 2020). When plants are subjected to abiotic challenges such as drought and salinity, various physiological processes occur swiftly to allow the plants to survive. As a result, physiological indicators linked to abiotic stress (such as proline content, superoxide dismutase (SOD) activity, and Catalase (CAT)) can be employed as a quick and reliable technique for assessing plant tolerance to drought stress (Tang *et al.,* 2017; James *et al.,*2018).
Drought stress tolerance is a highly complicated system controlled by several elements and regulated by several quantitative trait loci (QTLs) (Fleury *et al*., 2010). Plant responses to drought stress are likewise exceedingly complex, and the physiological and molecular bases of these responses must be explored to fully comprehend these responses (Sahebi *et al.,* 2018). Drought resistance mechanisms range among plant species, and different agricultural cultivars have shown genetic diversity in terms of drought tolerance (Oladosu *et al*., 2019). Genetic variations also have been detected in rice genes related to drought tolerance through screening of rice germplasm (Swamy and Kumar 2013). Plants have the ability to induce the production of proteins that protect them against abiotic stressors; various variables are involved in this transcriptional control (Friedel *et al*., 2012). A large number of genes are known to be involved in plant drought responses at the molecular level. (Fujita *et al*., 2005), Osmotic and other protector biosynthetic genes, such as late embryogenesis abundant (LEA) associated genes (Capell *et al.,* 2004), and oxidative stress-related genes (Gaber *et al.,* 2006). The study of allelic variation in key genes associated with drought tolerance is critical for developing an effective drought strategy; knowledge of these genotypic variations in expression levels of important candidate genes under drought stress, combined with related field performance data, may be very useful in drought stress tolerance breeding programs (Dey *et al.,* 2016).

As a result, greater climate-resilient varietal development is required to reduce the negative effects of climate change and preserve sustainable rice production and food security. These newly developed rice varieties can aid in the reduction of production losses caused by an unexpected increase in drought stress events due to climate change by exhibiting various mechanisms and specific physiologic responses to drought stress, such as reduced plant growth and productivity, decreased CO_2 assimilation, membrane injury and damage of the affected tissues, and, in some cases, inhibition of enzyme activity (Abid *et al.,* 2018). Due to its complicated processes and unpredictable nature, improving drought tolerance in rice through diverse tactics is a difficult challenge (Kumari *et al.,* 2022). To overcome these challenges and increase crop productivity in drought-prone environments, crop-level molecular breeding methods and functional genomics should be integrated. Understanding the physiological process is also required, as the mechanisms underlying dehydration tolerance in plants are still poorly understood (Sahebi *et al.,* 2018; Seleiman *et al.,* 2021).

Various DNA markers have been employed in breeding programs for different purposes, including the discovery of biotic- and abiotic-stress-related genes (e.g., disease resistance, salinity, drought, heat, and cold tolerance). Furthermore, DNA markers are often utilized in assessing genetic variation between types. There are a wide array of DNA markers with various utilization aims; however, their accuracy and economic strains, among other criteria, play an important part in DNA marker selection. Among the most commonly used DNA markers in genetic diversity assessments are inter-simple sequence repeats (ISSRs) and start codon targeted polymorphism (SCoT). The high polymorphisms of ISSRs and SCoT allow them to be used in phylogeny, genome mapping, evolutionary biology, genetic diversity, and gene tagging. ISSRs and SCoT have previously been shown to accurately estimate the extent of genetic diversity in rice (Moonsap *et al.* 2019; Al-daej *et al*., 2023). SCoT and ISSR markers are gaining popularity for its superiority over other dominant DNA marker system for higher polymorphism, better marker resolvability and reliable bands which can be used for effective population studies, genetic mapping in different plants and in marker assisted selection programs (Patidar *et al.* 2022). Keeping these points in mind, the present study was conducted to i) assess the performance of some rice varieties under drought stress conditions by detecting variations in physiological and biochemical responses between drought-sensitive and resistant rice varieties. ii) assess the molecular genetic diversity among tested genotypes using two DNA marker systems, ISSRs and and SCoT.

2. Material and Methods

2.1. Experimental location

The research was carried out during the two successive rice growing seasons of 2017 and 2018 at the experimental farm of the rice research and training center (RRTC), Agricultural Research Centre, Sakha, Kafr El-Sheikh governorate, Egypt, (31°5′ 54″N, 30°57′0″E).

According to a previously described method, soil chemical and physical properties, as well as soil texture of the 0–30-cm deep soil, were measured in the experimental field across two rice growth seasons according to Piper, 1950; Black *et al*., 1965 and presented in Table 1.

Climate data, including minimum and maximum air temperatures as well as relative humidity, were obtained from the Sakha Agro-meteorological station, the central laboratory for agricultural climate, during the two rice growing seasons of 2017 and 2018 in Sakha research station, kafr El-Sheikh governorate and shown in Table 2.

2.2. Rice germplasm and experiment design

The experimental material consisted of eight rice varieties (Puebla, Hispagran, Giza177, Sakha101, Sakha105, Sakha106, Sakha107, and Sakha108), classified into three groups based on their tolerance to drought stress. The names, origin, and pedigree of the varieties used in this study are presented in Table 3.

Grains from each of the eight varieties were sown in the nursery at May 1st over the two rice growing seasons. In two different irrigation experiments, thirty-day-old seedlings of eight rice varieties were individually transplanted in the field plots. the first experiment (normal condition) was watered every 4 days (14,400 m^3 ha^{-1}), and the plots in this experiment were kept moist with water from transplanting until 2 weeks before harvesting. However, the second experiment (water deficit) was irrigated every 10 days (9,120 m^3 ha^{-1}) with just flush irrigation without standing water. The seedlings of the two experiments were transplanted in a randomized complete block design with three replications. Each replicate contained ten rows of each genotype. The row was 5 m long with a single seedling per hill and a spacing of 20 × 20 cm between rows and hills.

According to the Ministry of Agriculture, a permanent field was created, and Ca-superphosphate (15.5% P_2O_5) was applied at a rate of 238 kg ha^{-1} during soil tillage. Two equal doses of potassium sulphate (48% K_2O) were administered at rates of 120 kg K_2O ha^{-1} at 15 and 35 days after transplanting. Nitrogen fertilizers in the form of urea (46% N) at a concentration of 165 units ha^{-1} of N (357 kg urea ha^{-1}) were also applied at 15, 35, and 55 days after transplanting in three equal dosages. All agricultural practices were carried out in compliance with the RRTC rice recommendations (RRTC, 2018). To collect data, ten plants were chosen at random from each replication, and the mean values were utilized for statistical analysis.

TABLE 1. Chemical mechanical and texture properties of soil of the experimental field used for the study in the two seasons of 2017 and 2018.

Characters	2017	2018
Particle size distribution		
Sand %	11.69	12.30
Clay %	55.57	54.15
Silt %	31.35	32.15
texture	clay	clay
Organic matter %	1.39	1.40
Chemical analyses		
pH (1:2.5)	8.20	8.35
Soil salinity (EC dS m^{-1})	0.60	0.68
Available N (ppm)	51.8	47.7
Available P (ppm)	15.09	14.0
Available K (	249.8	236.3
Anions (Cmolc/ kg soil)		
CO_3^{--}	--	--
HCO_3^-	5.40	5.65
Cl^-	8.64	9.54
SO_4^{--}	16.89	18.04
Cations (Cmolc/ kg soil**)**		
Ca	11.18	11.65
Mg	3.60	4.95
Na	1.55	1.90
K	14.34	15.87
Available Micronutrients (ppm)		
Fe	5.35	5.14
Mn	3.72	3.25
Zn	0.65	0.70

TABLE 2. The monthly minimum and maximum air temperature (°C) and relative humidity (%) at Sakha Agricultural research station during the 2017 and 2018 rice growing seasons.

Month	Day	2017				2018			
		Air Temp.		RH %		Air Temp.		RH %	
		Min	Max	7.30	13.30	Min	Max	7.30	13.30
May	1-10	20.98	28.89	76.0	46.9	25.4	30.7	77.3	47.7
	11-20	24.36	32.93	66.6	41.5	26.3	30.3	78.0	44.3
	21-31	22.93	29.72	71.5	48.9	25.8	30.9	77.9	44.7
June	1-10	26.7	33.4	67.3	43.5	27.7	32.6	78.7	46.7
	11-20	25.5	33.1	76.0	44.3	27.8	31.9	81.5	54.2
	21-30	26.8	34.2	83.9	52.1	28.8	32.9	80.6	53.2
July	1-10	26.5	33.9	82.5	57.7	29.4	34.7	84.6	57.2
	11-20	25.9	33.7	83.0	54.2	29.1	35.2	83.4	54.9
	21-31	26.0	32.9	81.7	58.6	28.4	34.4	85.6	59.1
August	1-10	26.2	34.3	84.8	57.6	28.8	34.2	86.8	57.2
	11-20	26.3	33.0	82.7	56.8	29.2	34.9	84.6	58.3
	21-31	25.5	36.2	85.0	54.4	27.4	35.6	86.2	50.5
September	1-10	24.3	33.27	85.4	53.7	25.9	33.1	86.0	49.8
	11-20	24.93	33.42	82.6	51.3	26.0	33.6	87.1	49.5
	21-30	23.63	31.09	82.2	50.1	26.7	31.5	85.0	51.7
October	1-10	20.3	31.5	85.6	50.0	24.5	30.0	83.3	53.1
	11-20	23.0	29.84	78.4	56.0	23.6	27.9	81.5	56.6
	21-31	21.90	28.04	83.2	59.8	23.9	28.1	78.5	54.6

Rain was zero (mm/day) during the 2017 and 2018 rice growing seasons.

TABLE 3. Origin and main characteristics of the rice varieties used in this study.

No	Genotype	Parentage	Origin	Variety group	Drought tolerant
١	Puebla	Unknown	California (USA)	Japonica	Tolerant
٢	hispagran	Unknown	California (USA)	Japonica	Tolerant
٣	**Giza177**	[Giza 171] Ymji No.1// PiNo.4	Egyptian	Japonica	Sensitive
٤	**Sakha101**	(Giza176/Milyang 79)	Egyptian	Japonica	moderate
٥	**Sakha105**	GZ5581-46-3/GZ4316-7-1-1	Egyptian	Japonica	Sensitive
٦	**Sakha106**	(Giza 177/ Hexi 30)	Egyptian	Japonica	moderate
٧	**Sakha107**	(Giza 177/BL1)	Egyptian	Japonica	Tolerant
٨	**Sakha108**	(Sakha101/HR5824-B-3-2-3/Sakha101)	Egyptian	Japonica	Tolerant

2.3. Studied traits

2.3.1. Physiological and Biochemical Traits

Data was collected on flag leaves from ten randomly selected plants of each genotype. From 8:00 to 10:00 a.m., leaves and sheaths were gathered individually, swiftly placed in pre-weighed zip-sealed containers, and instantly measured to ascertain Physiological and Biochemical features.

Chlorophyll Content

Chlorophyll a, Chlorophyll b, and total Chlorophyll were determined according to Fadeel (1962). About 1 g fresh weight of mixed leaves was homogenized and centrifuged in 5 mL of 85% cold acetone. The extract was diluted to the required amount before the optical densities at 663 and 647 nm were measured. The chlorophyll content of the samples was calculated as mg/g fresh weight using the following equations:

Chlorophyll a = 11.79 E_{663} − 2.29 E_{647},

Chlorophyll b = 20.05 E_{647} − 4.77 E_{663},

Relative Water Content (RWC)

Calculated based on Barrs and Weatherley (1962). Leaf discs were weighed as a fresh weight (FW) then floated in distilled water for 4 hours before being weighed again to get turgid weight (TW). To ascertain dry weight, the discs were oven-dried at 85^{o}C for a consistent weight (DW). The relative water content was calculated using the following equation:

$$RWC\ \% = \frac{FW\text{-}DW}{TW\text{-}DW} \times 100$$

Proline Content Antioxidant Enzyme Activities determination

Free proline in leaf tissues was evaluated taking after the convention of Bates *et al.,* (1973). Leaf samples (0.5 g) were homogenized in 5 mL of 3% sulphosalicylic acid using a mortar and pestle. In a tube, about 2 mL of extract was added, followed by 2 mL of ninhydrin reagent and 2 mL of glacial acetic acid. The reaction mixture was cooked in a 100^{o}C water bath for 60 minutes. After cooling, 6 mL of toluene was added and placed to a separating funnel. The chromophore containing toluene was separated after careful mixing, and absorbance at 520 nm was measured in a spectrophotometer against a toluene blank. The concentration of proline was measured with a calibration curve and represented as mg proline g/FW.

The activities of catalase (CAT), Ascorbate peroxidase (APX), and superoxide dismutase (SOD) were measured using the methods described by Aebi *et al*., (1974), Nakano and Asada, (1987) and Beauchamp and Fridovich (1971), respectively. Fresh leaf samples (0.5 g) were homogenized in 5 mL of cold K-phosphate buffer (50 mM) (pH 7.8). The homogenates were centrifuged at 10,000 g for 20 minutes at 4^{o}C. The antioxidant enzyme activity was measured in the supernatant (Units mg^{-1} protein).

2.3.2.Yield attributes

Observations were recorded on ten plants that were selected randomly from each replication. Individual plants were harvested and threshed separately to determine yield characters as recommended by (IRRI 2008) viz., Number of panicles plant^{-1}, panicle weight (g; **PW**), spikelet fertility **(%; SF**), 1000-grain weight (g; **GW**) and grain yield plant^{-1} (g; **GY**),

2.4. Molecular analyses

Genomic DNA extraction

Genomic DNA was extracted from the young leaves collected from 20-day-old seedlings from each genotype using DNeasy Plant Mini Kit (QIAGEN, Germany) according to the manufacturer's instructions. The DNA purity and concentration were measured using the NanoDrop spectrophotometer (ND-1000, USA).

ISSR and SCoT primers and PCR amplification

A set of eight ISSR and four SCoT primers (Integrated DNA Technologies, Inc, USA) were used

to estimate genetic diversity among the studied varieties (**Table 4**). The PCR reactions was carried out in 25 µl reaction volume containing 12.5 µl Master Mix (sigma), 2.5 µl ISSR/SCoT primers (10pcmol), 3 µl template DNA (10ng) and 7 µl dH_2O, according to (**Ibrahim *et al.*, 2019**). PCR amplification was performed using a Perkin-Elmer/GeneAmp® PCR System 9700 (PE Applied Biosystems). The amplification of ISSR markers was performed in 40 cycles as follows: an initial denaturation cycle at 94°C for 1 min, annealing at 50°C for 1 min, elongation at 72°C for 2 min, and a final extension for 5 min. On the other hand, SCoT amplification was performed in 35 cycles as follows: 5 min at 94°C denaturation, 7 min annealing at 50°C, and elongation in the final cycle at 72°C. The PCR amplification products of ISSR and SCoT markers were separated on 1.5% agarose gel. Gels were stained with 100 µM/L EtBr (100 µM/L, Sigma–Aldrich®) in 1X TBE. The PCR products were visualized and documented using a Bio-Rad ChemiDoc™ MP gel documentation and imaging system (Cat. no. 1708280).

Molecular data analyses

The amplified bands were scored for ISSR and SCoT markers based on the presence or absence of bands, generating a binary data matrix of (1) and (0) for each marker. Clear, unambiguous, and strong bands were selected for scoring. Standard diversity parameters, including polymorphism information content (PIC), resolving power (RP), and marker index (MI) were calculated for each marker system. The value of PIC was calculated as $PIC = 1 - \sum Pi^2$, where pi is the frequency of i^{th} allele (band present) and summation extends over n alleles (Botstein *et al*., 1980). Resolving power (Rp) of each primer was estimated with $Rp = \sum Ib$ where Ib (informative fragments) $= 1 - [2 \times (0.5 - pi2)]$, where pi2 is the proportion of accession containing bands (Prevost and Wilkinson 1999).

Genetic similarities were calculated based on Jaccard's similarity coefficient (Jaccard 1908). The dendrogram tree was constructed with the unweighted pair group method utilizing arithmetic averages (UPGMA) using PAST software version 1.91 (Hammer *et al*., 2001).

Table 4. List of ISSR and SCoT markers used in this study and their sequences.

Primer Name	Sequence
ISSR 3	5'-ACACACACACACACACYT-3'
ISSR 5	5'-GTGTGTGTGTGTGTGTYG-3'
ISSR 10	5'-GACAGACAGACAGACAAT-3'
ISSR 11	5'-ACACACACACACACACYA-3'
ISSR 13	5'-AGAGAGAGAGAGAGAGYT-3'
ISSR 18	5'-HVHCACACACACACACAT-3'
ISSR 19	5'-HVHTCCTCCTCCTCCTCC-3'
ISSR 20	5'-HVHTGTGTGTGTGTGTGT-3'
SCoT F2	5'-CAACAATGGCTACCACGC-3'
SCoT F6	5'-CAACAATGGCTACCACGG-3'
SCoT E9	5'-CAACAATGGCTACCACCT-3'
SCoT E11	5'-CAACAATGGCTACCACGA-3'

*Y= (C or T)
H= not G (A or C or T)
V = not T (A or C or G)

2.5. Statistical analyses

Analysis of variance

The ordinary analysis of variance was used to test the significance of the differences among the studied varieties then the combined analysis of variance across years and the two environments (normal irrigation and water shortage) was calculated. The test suggested by Bartlett (1937) was used to test the homogeneity.

All statistical analyses were performed using analysis of variance technique employing "MSTAT" computer software package. The treatment means were compared using Duncan's multiple range test Duncan (1955). Results

2.6. Analysis of variance

2.6.1.Physiological and biochemical traits

The analysis of variance (Table 5) revealed that the mean squares for years and irrigation treatments were highly significant for all the studied physiological and biochemical traits. In addition, highly significant interaction among the irrigation treatments and the years was found for chlorophyll a content, Total chlorophyll content and superoxide dismutase enzyme activity (SOD).

The mean squares of the varieties were observed to be highly significant for all physiological and biochemical traits under study. Highly significant interaction between the varieties and the

environments was obtained for all studied physiological and biochemical traits, furthermore, non-significant interaction between the varieties and the years was found in all the studied Physiological and biochemical traits except chlorophyll a content and total Chlorophyll content. Additionally, the interaction among the varieties × irrigation × years was non-significant for all physiological and biochemical characters under study.

2.6.2. Yield traits

Highly significant mean squares due to the years and irrigation treatments were detected for grain yield and its related trait. While, there was non-significant interaction between the Irrigation treatments and the years for all the studied yield characters. The varieties showed highly significant mean squares for all yield traits under study. Likewise, varieties × irrigation interaction mean squares were highly significant for all the studied yield traits. In contrast, the mean squares for varieties × years and varieties × Irrigation × years' interactions were not significant for all studied yield and its components characters.

Table 5. Mean squares from the analysis of variance for the studied Physiological and biochemical traits under well-watered and drought stress conditions.

Sources of variance	d.f	Chlorophyll a (mg g^{-1} FW)	Chlorophyll b (mg g^{-1} FW)	Total Chlorophyll (mg g^{-1} FW)	Relative water content (RWC)
Years (Y)	**1**	2.77**	2.16**	0.35**	77.76**
Error	**4**	2.97	2.03	11.09	78.71
Irrigation (I)	**1**	2.00**	11.95**	20.80**	4875.78**
I x Y	**1**	0.001**	0.001 ns	0.001**	0.001 ns
Error	**4**	0.0001	0.07	0.0001	0.06
Varieties (V)	**7**	0.19**	0.19**	2.49**	602.19**
V x I	**7**	0.02**	0.01**	0.31**	35.89**
V x Y	**7**	0.0001**	0.001 ns	0.0002**	0.001 ns
V x I x Y	**7**	0.0005 ns	0.0001 ns	0.0003 ns	0.0002 ns
Error	**56**	0.0001	0.00015	0.00013	1.135
Sources of variance	**d.f**	**Proline (mg g^{-1} FW)**	**CAT (Unit mg^{-1} Protein)**	**APX (Unit mg^{-1} Protein)**	**SOD (Unit mg^{-1} Protein)**
Years (Y)	**1**	5.08**	11.10**	8.07**	12.44**
Error	**4**	0.02	56.80	67.62	5.81
Irrigation (I)	**1**	3.50**	381.05**	254.80**	305.02**
I x Y	**1**	0.00011 ns	0.00012 ns	0.0001 ns	0.00014 **
Error	**4**	0.06	1.30	0.03	0.001
Varieties (V)	**7**	0.12**	81.56**	44.62**	42.63**
V x I	**7**	0.03**	5.84**	3.46**	0.93**
V x Y	**7**	0.00014 ns	0.00016 ns	0.0001 ns	0.00011 ns
V x I x Y	**7**	0.0001 ns	0.0001 ns	0.0001 ns	0.0001 ns
Error	**56**	0.00012	0.021	0.083	0.0001

* and **: Significant at 0.05 and 0.01 levels of probability, respectively. ns: Not significant.

Table 6. Mean squares from the analysis of variance for the studied yield traits under well-watered and drought stress conditions.

Sources of variance	d.f	Number of panicles/plant	Panicle weight (g)	Spikelet fertility (%)	1000-grain weight (g)	Grain yield/plant (g)
Years (Y)	**1**	23.01**	24.00**	61.44**	24.00**	212.7**
Error	**4**	1.51	0.02	0.56	0.24	18.6
Irrigation (I)	**1**	866.40**	8.03**	927.57**	65.51**	3188.2**
I x Y	**1**	0.01 ns	0.001 ns	0.0009 ns	0.00014 ns	0.001 ns
Error	**4**	0.57	0.00	0.18	0.26	3.6
Varieties (V)	**7**	49.79**	0.23**	58.28**	53.09**	116.1**
V x I	**7**	7.66**	0.24**	19.21**	2.95**	29.6**
V x Y	**7**	0.012 ns	0.0001 ns	0.0001 ns	0.0001 ns	0.066 ns
V x I x Y	**7**	0.012 ns	0.00013 ns	0.00014 ns	0.00011 ns	0.052 ns
Error	**56**	0.156	0.004	0.063	0.191	0.398

* and **: Significant at 0.05 and 0.01 levels of probability, respectively. ns: Not significant.

2.7. Mean performance

2.7.1.Physiological and biochemical traits

Drought stress significantly reduced chlorophyll a, chlorophyll b and Total Chlorophyll content by 9.30%, 24.63% and 11.52% respectively (**Figures 3A**). Puebla and Hispagran recorded the highest desirable mean values for chlorophyll a, chlorophyll b and total chlorophyll content under both conditions, with the mean values of 3.38 and 3.25 for chlorophyll a, 2.98 and 3.05 for chlorophyll b, and 8.52 and 8.68 for total chlorophyll content) under normal condition, in addition to (3.01 and 2.91 for chlorophyll a, 2.36 and 2.33 for chlorophyll b, and 7.77 and 8.07 for total chlorophyll content under water deficit condition, respectively (**Figure 1A-C)**. Similarly, Relative water content (RWC) was also depressingly impacted by drought stress; it decreased by 17.36% under water deficit (**Figure 3A**).

The highest mean values for RWC were recorded by Sakha107 and Hispagran under normal conditions (95.07 and 87.70 % respectively) and water shortage conditions (80.17 and 75.43 % respectively) followed by Puebla under normal conditions while Sakha105 and Puebla under stress condition (**Figure 1D)**.

Otherwise, water shortage caused a considerable increase in proline content and the activities of antioxidant enzymes: CAT, APX, and SOD by 25.66, 21.37, 19.81 and 20.59 %, respectively, compared to well-watered conditions (**Figure 3A**). The genotype Hispagran followed by Puebla and Sakha106 displayed the maximum values of proline content under normal conditions (1.60, 1.52 and 150 respectively), while Puebla followed by Sakha108 and Sakha107 gave the highest mean values under stressed conditions (2.06, 1.97 and 1.96) respectively. (**Figure 1E**).

The highest values of CAT were recorded by Hispagran and Sakha107 under normal conditions (23.52 and 23.29 respectively) and water deficit conditions (26.13 and 25.72 respectively) **(Figure 1F**). Regarding APX and SOD, the genotype Puebla exhibited the highest mean values under both conditions with the values 18.98 and 22.50 for APX as well as 20.23 and 24.66 for SOD under normal and water deficit conditions respectively (**Figure 1G and 1H**).

2.7.2. Yield traits

The number of panicles per plant was significantly reduced by 25.25 % due to the decrease in the amount of applied irrigation water (**Figure 3B**). Among the studied varieties, Sakha108, Hispagran, Giza177 and Sakha107 had the highest number of panicles per plant with values (27.53, 24.87, 24.53 and 24.53) under normal conditions, respectively. While, Sakha108, Puebla and Hispagran were the best varieties under water stress conditions for the number of panicles per plant (19.90, 19.83 and 19.53 respectively). Water shortage conditions, caused declined in panicle weight by 13.45 % ((**Figure 3B and 2 B)**.

The heaviest panicles were recorded by the varieties Hispagran, Sakha105 and Sakha108 under normal irrigation (4.53, 4.46 and 4.40 g respectively) and the varieties Sakha108, Sakha106, Giza177 and Hispagran under water deficit condition (3.83, 3.79, 3.75 and 3.74 g respectively). Likewise, the water drought treatment dropped spikelet fertility percentage by 6.58 % (**Figure 3B**). The rice variety Sakha108 followed by Puebla, Sakha107 and Hispagran gave the highest fertility percentage under the normal condition with mean values of 96.28, 95.53, 95.52 and 95.40 %, respectively. While, the highest percentage of spikelet fertility under water deficit conditions (91.71 and 91.39 %) were assigned for the varieties Sakha107 and Sakha108, respectively (**Figure 2C**).

Eventually, 1000-grain weight was significantly reduced by 5.47 % under water stress conditions (**Figures 3B**). The varieties Puebla, Hispagran and Sakha108 were the best varieties for 1000-grain weight under both conditions which recorded the heaviest 1000-grain weight with the mean values of 33.08, 32.44 and 31.63 g under normal condition and 32.81, 30.80 and 30.06 g under stress condition, respectively, as presented in (**Figure 2D**). Likewise, grain yield per plant was significantly affected by water deficit; it decreased by 24.46 % compared to well-watered conditions (**Figure 3B**). The highest mean values for single plant yield were detected by the varieties Sakha108 under both conditions (50.93 and 39.63 g respectively) followed by the varieties Hispagran under normal condition (50.98 g) and Sakha101 and Sakha107 under water scarcity condition (38.93 and 38.23 g respectively) (**Figure 2E**).

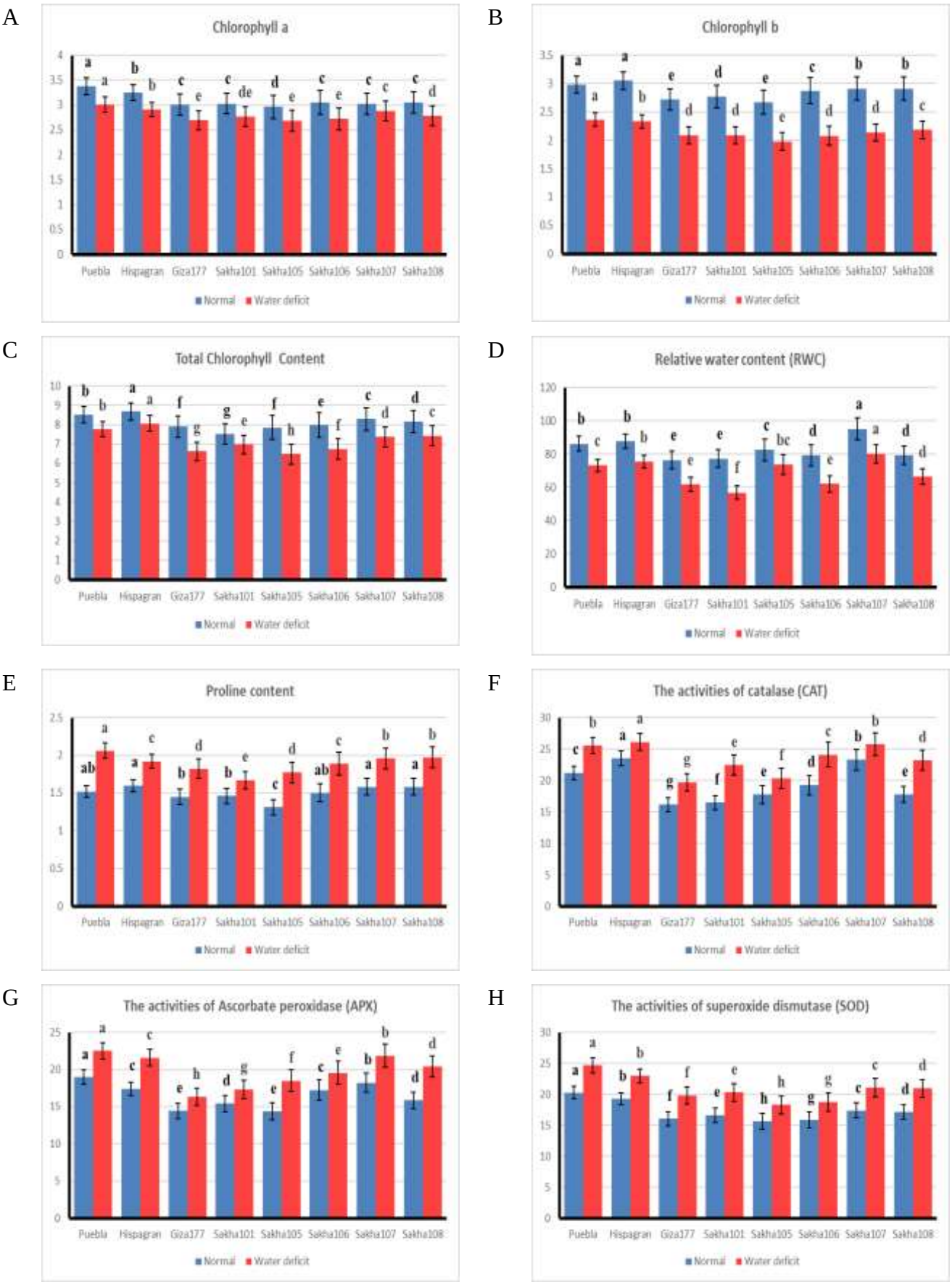

Fig. 1. The mean performances of the studied varieties for the studied Physiological and biochemical traits under normal irrigation and water shortage conditions.

Data are presented as mean ± SE (n = 3). Values with the same lowercase are not significantly different at $P \leq 0.05$ by the analysis of variance.

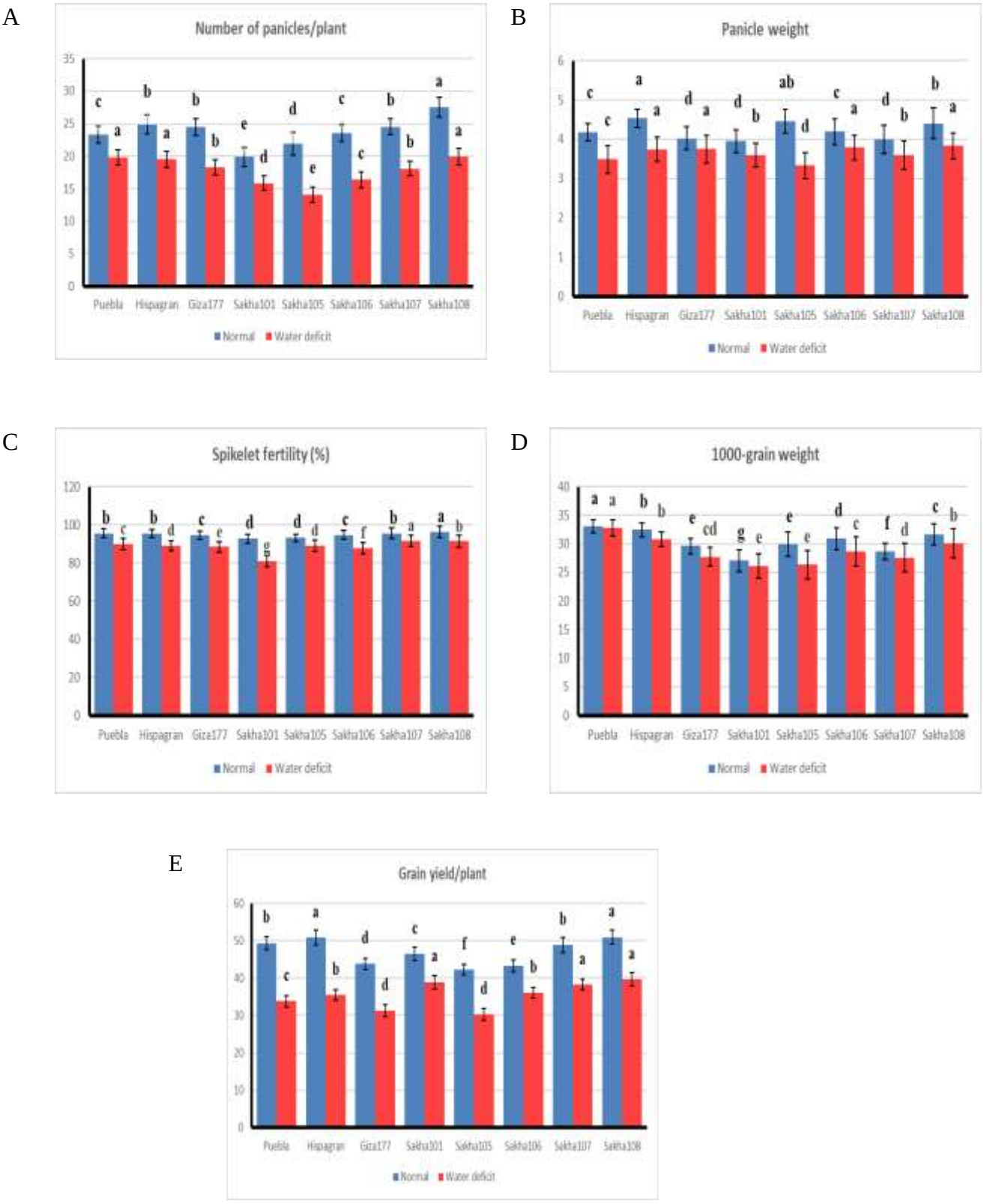

Fig. 2. The mean performances of the studied varieties for the studied yield traits under normal irrigation and water shortage conditions.

Data are presented as mean ± SE (n = 3). Values with the same lowercase are not significantly different at $P \leq 0.05$ by the analysis of variance.

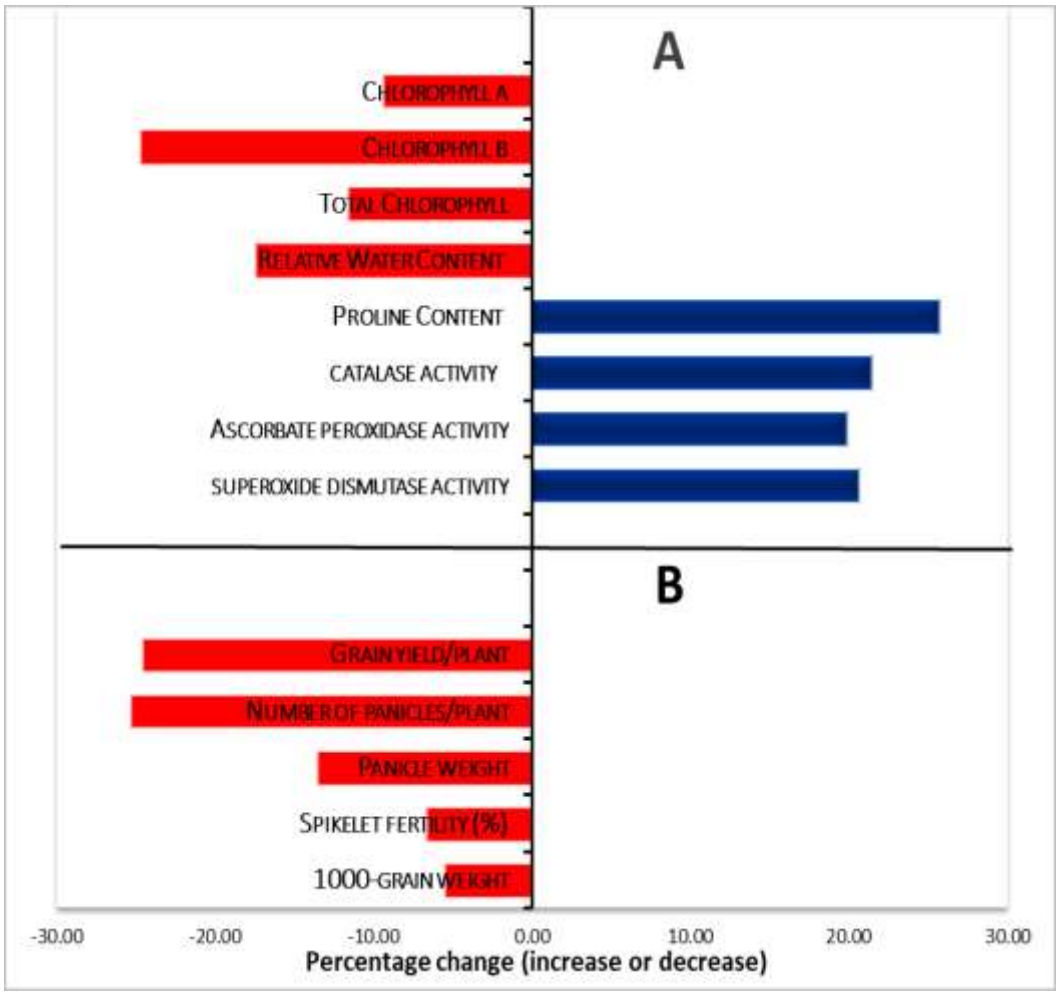

Fig. 3. Percentage change (increase or decrease) in physiological and biochemical traits (A) and yield traits (B) exposed to drought stress compared with well-watered rice plants.

3.3. Molecular analyses

3.3.1. Polymorphism detected by ISSR and SCoT Markers

The eight ISSR primers amplified a total of 101 bands (**Fig. 4A**), out of which 59 bands (57.03%) were polymorphic (**Table 7**). The total number of amplified fragments varied from 8 (ISSR-20) to 14 (ISSR-3), with an average 11.2 bands per primer. The number of polymorphic bands ranged from 2 (ISSR-20) to 10 (ISSR-3), with an average of 6.6 bands/primer. The polymorphism information content (PIC) varied from 0.28 (ISSR-19) to 0.47 (ISSR-18) with an average of 0.38. The lowest and highest values of marker index (MI) were observed in ISSR-20 (0.15) and ISSR-18 (2.73) primers, respectively. The resolving power (Rp) of the primers varied from 3.0 (ISSR-20) to 8.25 (ISSR-18), with an average of 6.08.

A total of 46 bands were generated from the four SCoT primers (**Fig. 4B**), of which 24 bands (49.64%) were polymorphic (**Table 7**). The total number of amplified amplicons varied from 10 (SCoT-E9) to 14 (SCoT-E11), with a mean of 11.5 bands/primer. The number of polymorphic bands ranged from 2 (SCoT-E9) to 11 (SCoT-E11), with an average of 6.0 bands/primer. The PIC values ranged from 0.2 (SCoT-E9) to 0.45 (SCoT-E11), with an average of 0.33. The Rp values varied between 2.25 (SCoT-F2) and 9.75 (SCoT-E11) with an average of 5.44.

3.3.2. Genetic Similarity and Cluster Analyses

Genetic similarity coefficient based on ISSR markers data varied from 0.62 to 0.84 with an average of 0.72 (**Table 8**). The highest genetic similarity was detected between the two cultivars Sakha101 and Sakha105, whereas the lowest genetic similarity was observed between Sakha108 and Hispagran. Based on SCoT data analysis, the genetic similarity ranged from 0.68 to 0.86 with an average of 0.74 (**Table 8**). The highest genetic similarity was recognized between varieties Sakha 105 and Giza 177, while the lowest genetic similarity was identified between varieties Sakha 108 and Puebla. The genetic

similarity coefficient based on the combined ISSR and SCoT markers data varied from 0.65 to 0.82 with an average of 0.72 (**Table 8**). The highest genetic similarity was related to varieties Sakha101 and Sakha105, while the lowest genetic similarity was observed between Sakha108 and Hispagran.

The dendrogram obtained with SSR markers separated the studied rice varieties into two main clusters (**Fig. 5A**). Cluster I consisted of the genotype Sakha 106. Cluster II included seven varieties, which were further divided into two sub-clusters. The first sub-cluster contained Sakha 101, Sakha 105, Sakha 106, Sakha 107. The second sub-cluster had the varieties Giza 177, Hispagran, and Puebla. The dendrogram obtained with SCoT markers (**Fig. 5B**), also divided the rice varieties into two main clusters. Cluster 1 contained Sakha 108. Cluster II included seven varieties, which were subdivided into three sub-clusters. The first sub-cluster contained Sakha 108, the second sub-cluster contained the varieties Giza 177, Sakha 105, Sakha 107, and Sakha 101. Meanwhile, Hispagran, and Puebla were distinguished as the third sub-cluster. The dendrogram generated by ISSR and SCoT markers combined data divided the varieties into two major clusters (**Fig. 5C**). Cluster I consisted of the genotype Sakha 106. Cluster II retained seven varieties, which were further divided into two sub-clusters; Sakha 101, Sakha 105, Sakha 106, Sakha 107 constituted the first subgroup, while Giza 177, Hispagran, and Puebla formed the second one.

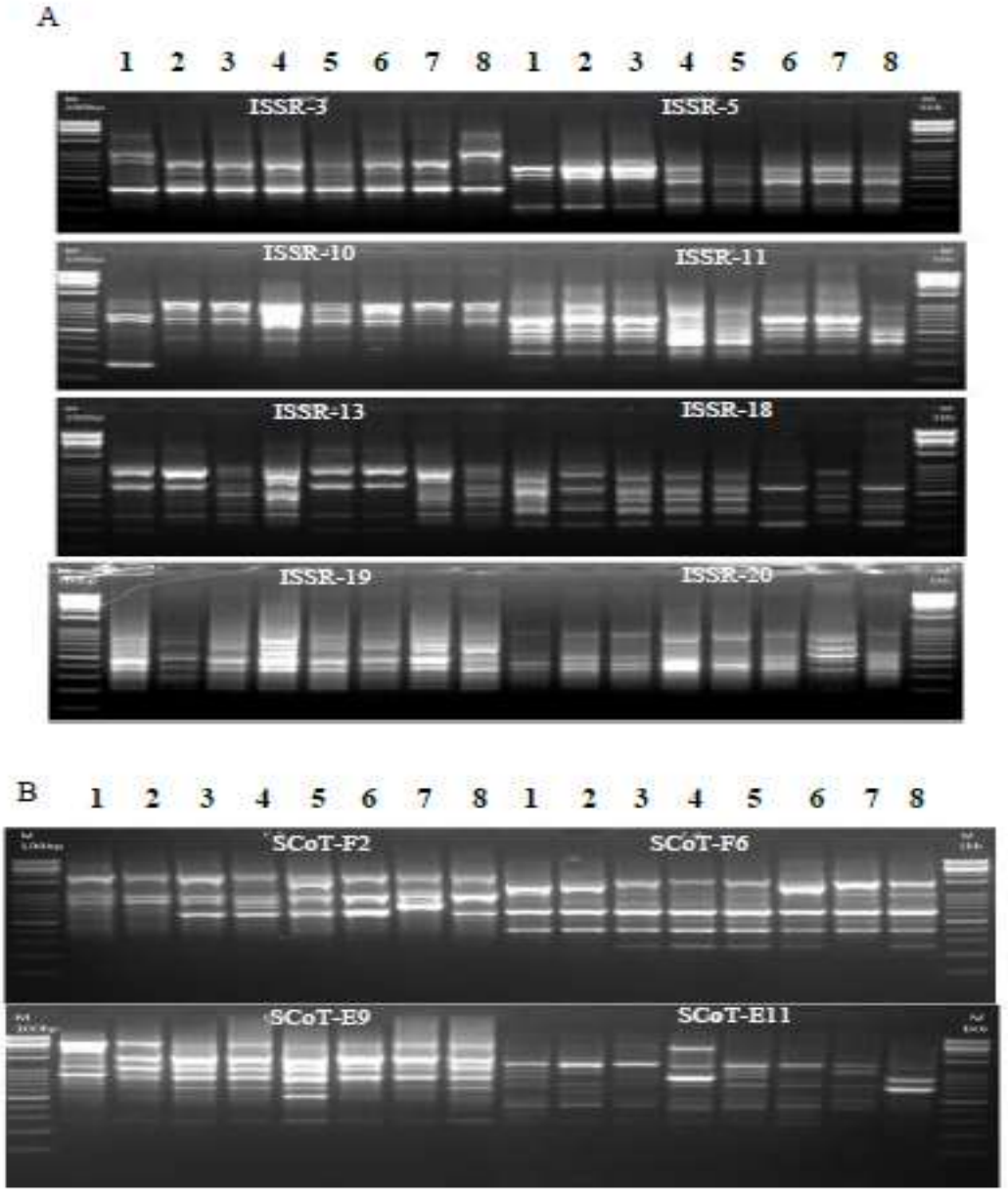

Fig. 4. Amplification pattern of ISSR (A) and the SCoT markers (B) with the eight rice varieties.

Table 7. Total number of bands (TB), number of polymorphic bands (PB), percentage of polymorphic bands (PB%), polymorphic information content (PIC), resolving power (Rp), marker index (MI), for ISSR and SCoT markers.

Marker	Primer	TB	PB	PB%	PIC	MI	RP
ISSR	ISSR-3	14.00	10.00	71.43	0.34	2.41	6.00
	ISSR-5	12.00	7.00	58.33	0.39	1.61	6.50
	ISSR-10	12.00	8.00	66.67	0.40	2.16	6.75
	ISSR-11	12.00	6.00	50.00	0.41	1.24	7.00
	ISSR-13	11.00	8.00	72.73	0.44	2.57	7.25
	ISSR-18	11.00	8.00	72.73	0.47	2.73	8.25
	ISSR-19	11.00	4.00	36.36	0.28	0.41	3.75
	ISSR-20	8.00	2.00	25.00	0.30	0.15	3.00
	Mean	11.22	6.56	57.03	0.39	1.65	6.08
SCoT	SCoT-E9	10.00	2.00	20.00	0.20	0.08	2.25
	SCoT-E11	14.00	11.00	78.57	0.45	3.92	9.75
	SCoT-F2	11.00	6.00	54.55	0.28	0.93	3.75
	SCoT-F6	11.00	5.00	45.45	0.3967	0.90	6.00
	Mean	11.50	6.00	49.64	0.33	1.46	5.44

Table 8. Similarity matrices among the eight varieties based on applied markers.

	Puebla	Hispagran	Giza177	Sakha101	Sakha105	Sakha106	Sakha107	Sakha108
ISSR markers								
Puebla	**1.00**							
Hispagran	0.71	**1.00**						
Giza177	0.79	0.82	**1.00**					
Sakha101	0.67	0.71	0.80	**1.00**				
Sakha105	0.66	0.63	0.74	0.84	**1.00**			
Sakha106	0.69	0.72	0.73	0.70	0.81	**1.00**		
Sakha107	0.66	0.69	0.74	0.76	0.71	0.77	**1.00**	
Sakha108	0.71	0.62	0.67	0.66	0.64	0.70	0.73	**1.00**
SCoT markers								
Puebla	**1.00**							
Hispagran	0.79	**1.00**						
Giza177	0.73	0.76	**1.00**					
Sakha101	0.71	0.78	0.76	**1.00**				
Sakha105	0.75	0.73	0.86	0.78	**1.00**			
Sakha106	0.72	0.74	0.69	0.71	0.71	**1.00**		
Sakha107	0.73	0.75	0.78	0.71	0.81	0.73	**1.00**	
Sakha108	0.68	0.70	0.73	0.71	0.79	0.68	0.68	**1.00**
Combined (ISSR and SCoT)								
Puebla	**1.00**							
Hispagran	0.74	**1.00**						
Giza177	0.77	0.80	**1.00**					
Sakha101	0.68	0.73	0.79	**1.00**				
Sakha105	0.69	0.66	0.77	0.82	**1.00**			
Sakha106	0.70	0.73	0.72	0.70	0.78	**1.00**		
Sakha107	0.68	0.71	0.75	0.74	0.74	0.76	**1.00**	
Sakha108	0.70	0.65	0.69	0.67	0.69	0.69	0.71	**1.00**

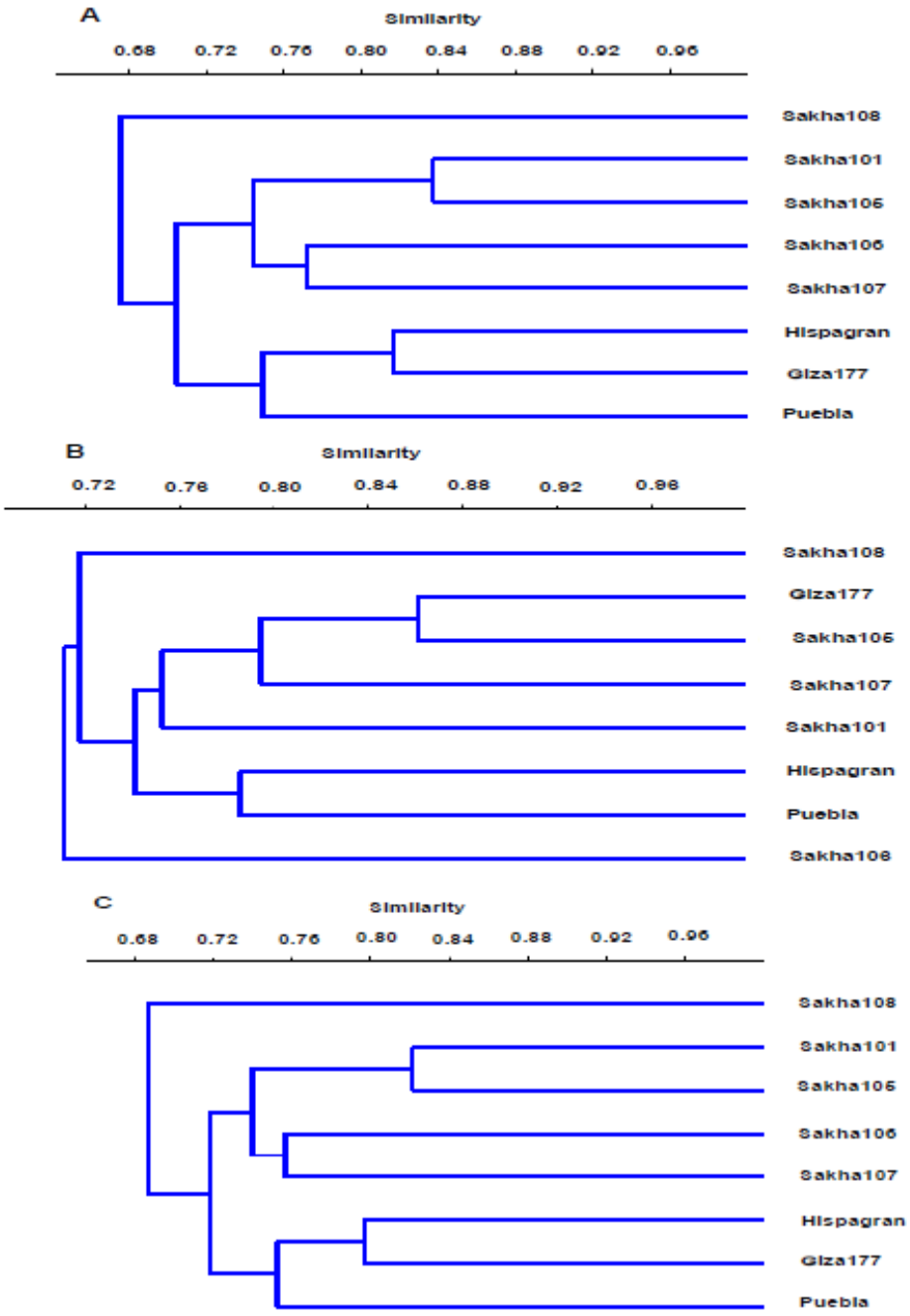

Fig. 5. UPGMA dendrogram the eight rice varieties based on ISSR (A), SCoT (B) and ISSR and SCoT (C) molecular markers.

4. Discussion

Rice (Oryza sativa L.) is one of the most fundamental food crops for the majority of world population. It is the chief food of supreme of the Egyptian's population (**Elsakhawy and Abd El-Rahem 2020**). rice is the second-most significant food crop in Egypt, Following wheat. In the 2016–2017 growing season, it was cultivated in an area of around 1.3 million feddan, primarily in the north Delta (**Abou Hussien *et al.* 2020**). Rice is a semi-aquatic plant that grows in highly humid environments. In other phrases, the behaviour of grown rice plants necessitates much water for irrigation in comparison to different crops (**Mandal and Ghosh 2021**; **Ghazi *et al.* 2023**). Despite, being the populations increase the most important determinant factor for the development of rice productivity to meet the increasing demand for rice a few years ago, nowadays, climate change and global warming may make the situation worse. Climate change and global warming, which affects the frequency and magnitude of hydrological

oscillations, is a danger to agriculture, particularly in developing countries, and creates a variety of biotic and abiotic stresses for plants. (**Turral *et al,* 2011; Pandey and Shukla, 2015; Al Azzawi *et al*., 2020**). Among the abiotic factors that have shaped and continue shaping plant evolution in general and rice crop in particular, Water scarcity is the most imperative and main limitation for rice growth and production in rainfed ecosystems (**Nelson *et al,* 2014; Pandey and Shukla, 2015**). Water deficit is considered a natural phenomenon and is commonly defined as a period without significant rainfall (shortage of water), resulting in extensive damage to crops, and an immense loss of yield more than 70% compared to the yield under normal conditions (**Wilhite and Pulwarty 2018**).When plants are exposed to abiotic stresses such as drought, several physiological, biochemical, and molecular reactions happen quickly to enable these plants to survive under these adverse conditions depending on relevant factors such as genotype, stress severity, and developmental stage. Consequently, physiological indices (such as proline content, activity of Catalase (CAT), Ascorbate peroxidase (APX) and super oxide dismutase (SOD)) related to abiotic stress can be used as a rapid and accurate method for evaluating plant tolerance to drought stress (**Tang *et a*l., 2017; James *et al*.,2018; Al Azzawi *et al*., 2020; Abd El-Aty *et al*., 2023**).To develop the drought tolerant varieties, it is necessary to know how plants handle with drought stress issues and develop varieties that could produce high yields at a range of soil moisture conditions that actually occur in farmers' rice fields (**Torres and Henry 2018).** In present study, we evaluated eight different rice varieties under water shortage condition for their resistance capacity through physiological, biochemical and yield approaches, in addition to determining the functional expression of some drought tolerance related genes in the most highly candidate tolerant varieties in order to identify the most desirable varieties for rice breeding programs for water shortage conditions.

According to the combined analysis of variance data, all the studied traits showed highly significant mean squares due to the environments indicating a huge difference in the performance of the studied rice varieties from well- watered conditions to water stress conditions. For varieties mean squares, all the studied traits showed the highly significant differences indicating large diversity among the used varieties to normal and water deficit conditions for all studied traits; this means that the germplasm utilized in the study possessed significant genetic diversity. The interaction between the varieties and the environments was highly significant for all studied traits; which means that the varieties under investigation behaved differently from one environment to another and can ranked differently in each environment, furthermore the non-significant interaction between the varieties and the years which was found in all the studied traits, except Chlorophyll a and Total Chlorophyll content, indicating that the tested materials performed independently from the seasonal changes hence we can recommend the superior lines. The interaction among the varieties × years × environments were non-significant for all studied characters which reflect a non-changing performance for the rice varieties in each environment in different years. As a result, the varieties studied can improve grain yield and other studied traits in water deficit-prone crops, similar results in other studies with different varieties reported by (**Sitaresmi *et al.,* 2020**; **Freeg e*t al.,* 2022; Padmashree *et al.,* 2023**).

The mean performance values of the variables studied changed depending on genotype and irrigation situation. For all evaluated attributes, the highest mean values are preferred. With the exception of proline content and antioxidant enzyme activity (CAT, APX and SOD), which dramatically increased, water deficit induced significant reductions in all characters under study when compared to normal irrigation treatment. A lack of water restricts water uptake from the root system to the leaves (**Arjenaki *et al.,* 2012)**. As a result, it reduces water-holding capacity and stomatal movement, limiting chlorophyll synthesis, CO_2 influx to leaves, and photosynthesis **(El-Hendawy *et al.,* 2020; Desoky *et al.,* 2021).** Drought stress depressed RWC values in all varieties, the varieties Sakha107, Hispagran and Puebla were the best able to hold a high level of water in its leaf tissues. Water scarcity stress causes stomatal closure, limiting CO_2 fixation and reducing $NADP^+$ regeneration through the Calvin Cycle (**Satoh and Murata, 1998**). By increasing electron leakage to molecular oxygen, these adverse circumstances increase the rate of reactive oxygen species (ROS) such as H_2O_2 (hydrogen peroxide), O_2^-(superoxide), O_2 (singlet oxygen), and OH^- (hydroxyl) radicals. ROS production in plants has been discovered to be enhanced by a range of environmental stresses (**Sgherri *et al,* 1996**). ROS causes lipid peroxidation, protein denaturation, DNA mutation, and other forms of cellular oxidative damage. (**Avramova *et al,* 2017**). Furthermore, an increase in ROS levels promotes chlorophyll breakdown, chloroplast chloroplast, and a reduction in photosystem II activity (**Osakabe *et al.,* 2014; Abd El-Aty *et al.,***

2023). Lower water content caused a significant drop in turgor pressure, forcing stomata to close and the size of stomatal holes to shrink, resulting in decreased gas exchange and photosynthetic suppression. Drought has a typical detrimental influence on photosynthetic pigment production; hence, several studies have demonstrated a dramatic drop in chlorophyll content in drought stressed plants when compared to well-watered circumstances (**Sikuku *et al.*, 2012; Maisura et al., 2014**). To deal with ROS-induced oxidative stress, plants have an effective antioxidant (enzymatic and non-enzymatic) defence mechanism (**Anjum *et al,* 2017**). A characteristic reaction to drought stress is the rapid accumulation of free proline. Many plants accumulate large levels of proline when subjected to drought stress in the soil, in some instances several times the total of all the other amino acids (**Lum *et al,* 2014**). It is widely recognized that proline content plays an important function as an anti-oxidative defence molecule in preventing cells from damage caused by water deficit stress (**Sahebi *et al.*, 2018**). A higher level of proline content was observed in in drought- resistant varieties compared to moderately drought tolerant and drought sensitive varieties (**Saddique *et al.*, 2020**). Many researches have shown that proline content can be utilized to screen and select drought tolerance varieties in rice (**Sahebi *et al,* 2018**). The regulations activities of CAT, APX and SOD enzymes provide a quick and effective reaction to control the excess of ROS generated by environmental stresses (**Sofo *et al,* 2004**). An excess of reducing power during the water stress state, with the consequent increases in H_2O_2 and other ROS concentrations, is likely to cause changes in the regulation of various antioxidant enzymes. The antioxidant enzymes have a variety of yet complimentary roles in the cell defence mechanism, including direct ROS scavenging (**Palatnik *et al,* 1999**). Yield traits are the end results of physiological processes that occur at various stages of development. Water stress severely impair the plant growth and development, usually plants reduce or stops its growth as a survival technique under water stress conditions (**Pandey and Shukla, 2015**). It is well documented that drought causes severe reduction in rice grain yield, numbers of panicles per plant, panicle weight and 1000-grain weight (**Ji *et al.*, 2012; Maisura *et al.*, 2014**). Drought at booting and flowering stages negatively affect flowering and grain filling hence a high level of spikelet sterility is commonly observed under dehydration stress conditions (**Kumar *et al.*, 2014; Pandey *et al.*, 2015**). As a result, for all studied traits, the varieties that gave the lowest reduction under water-deficit were more tolerant to water deficit than others. Thus, based on the per se performance the varieties, which gave the highest mean performance for all traits under water deficit condition, have been estimated as superior varieties and they might be useful for the incorporation of the respective traits in breeding programme for water shortage tolerance. Results are in good agreement with those reported by (**Herwibawa *et al.*, 2019**; **Al Azzawi *et al.*, 2020; Abd El-Aty *et al.*, 2023**).

The assessment of genetic diversity is crucial for the future rice breeding programs and the conservation of genetic resources. In the present study, two PCR-based molecular marker systems ISSR and SCoT were applied to assess the genetic diversity among the studied rice varieties. Both SCoT and ISSR are dominant markers. However, ISSR has randomly amplified markers, whereas SCoT is gene-targeted marker **(Luo *et al.*, 2011)**. ISSR and SCoT markers have shown usefulness in genetic diversity studies due to their high repeatability and efficiency in detecting polymorphism (**Etminan *et al.*, 2018; Moonsap *et al.*, 2019; Abouseada *et al.*, 2023; Al-daej *et al.*, 2023)**. The results showed that the both molecular markers exhibited comparable genetic diversity values but a higher level of polymorphism was represented by ISSR. This indicates the high efficiency of both markers in discriminating the tested varieties. The increased percentage of polymorphism noticed in ISSR markers could be explained by the greater number of ISSR primers utilized in this investigation. The efficiency of a molecular marker in genetic diversity analysis is also determined by the number of polymorphic bands per primer (**Jing *et al.*, 2019**). In this study, ISSR markers showed a higher number of polymorphic bands (6.6) than that of SCoT marker (6.0), indicating higher efficacy of the ISSR markers in the analysis of the studied varieties. In accordance to our results ISSR markers produced higher polymorphic bands per primer than SCoT markers in wheat and laurel varieties **(Jing *et al.*, 2019; Yilmaz and Ciftci 2021)**.

The polymorphism information content (PIC) demonstrates the informativeness and discriminating power of the marker among the tested varieties. PIC values for dominant marker such as ISSR and SCoT are at maximum of 0.5 (**Bolaric *et al.*, 2005**). The average PIC value of ISSR primers (0.39) was found to be slightly higher than those of SCoT primers (0.33). (**Botstein *et al.*, 1980**) stated that markers

with a PIC value ranging from 0.25 to 0.50 contain useful information for genetic diversity studies. The relatively high PIC values observed in this investigation demonstrated the informativeness and discriminative capacity of both markers system. The average PIC value obtained in this study was comparable to that found by (**Al-daej *et al.*, 2023)** using ISSR, and by (**Patidar *et al.* 2022)** using SCoT markers in rice germplasm.

The resolving power (Rp) is another factor used to measure the ability of primers to distinguish between varieties **(Prevost and Wilkinson 1999**. The average Rp of ISSR primers (6.08) was higher than SCoT primers (5.44). Moreover, the average of marker index (MI) values, which can be considered to be a common measure of efficiency in discovering polymorphism **(Powell *et al.*, 1996)**, was found to be higher in ISSR system. Considering all the results, based on the PIC, Rp and MI values, ISSR marker was more effective than SCoT marker in the assessment of genetic diversity among the evaluated rice varieties. Our findings are in agreement with (**Etminan *et al*. 2016 and Yilmaz and Ciftci 2021**) who demonstrated the usefulness of ISSR markers for estimation of genetic diversity and fingerprinting of varieties more than other DNA marker systems.

The average genetic similarity obtained by ISSR and SCoT, and their combined data was 0.72, 0.74 and 0.72, respectively. This indicates that the genetic diversity of the varieties is similar when SCoT, and ISSR markers are used. Moreover, it was evident from the results that the existence of substantial genetic diversity among the tested varieties, which could be considered for parental selection in the future rice breeding programs. Interestingly, the lowest genetic similarity was observed between the local cultivar Sakha 108 and the exotic varieties Puebla and Hispagran. On the other hand, the highest genetic similarity was observed between the two Egyptian rice cultivars Sakha101 and Sakha105. These results demonstrate that geographic distance is one of the primary causes of genetic differentiation among varieties by preventing gene transmission. The large genetic distance between local and exotic varieties detected in this study, suggesting the opportunity to use these varieties in rice breeding programs for developing high-yielding and heterotic hybrids.

The results of UPGMA cluster analysis based on two genetic marker systems grouped the eight varieties into two main clusters, which generally agreed with their origin. Both ISSR and SCoT markers showed kind of similarity in the topology of their respective dendrograms. However, some differences in the subclades and the position of some varieties were observed. This variation in the number of sub-clusters obtained from the both markers could be due to the ability of each marker to divergent target regions of the genome, which in turn resulted to existence of some varieties in separate groups instead of aligning with their respective larger groups. It is clear from the cluster analysis that the exotic varieties Hispagran and Puebla from California (USA) were always clustered in the same group. This result suggested that these rice cultivars are genetically related to each other. It is possible that they may have originated from the same ancestor. Moreover, the local rice cultivars Sakha 101, Sakha 105, and Sakha 107 were placed together in all dendrograms. This observation suggested that these rice cultivars are closely related. These results were in agreement of that **(Mansoory *et al*., 2022;** Yilmaz **and Ciftci 2021)** who found ISSR and SCoT markers grouped the varieties according to their geographic origin.

5. Conclusions

Water deficit stress significantly decreased relative water content, total chlorophyll content, grain yield, and yield attributes. In contrast, it significantly increased proline content and antioxidant enzyme activities (CAT, APX and SOD) compared with normal irrigation conditions. The combined analysis of variance revealed that the mean squares for environments, varieties and their interaction were highly significant for all studied traits, indicating that the germplasm utilized in the study possessed significant genetic diversity from one environment (normal irrigation) to another (water deficit) and can ranked differently in each environment. The varieties Puebla and Hispagran were identified as the best varieties for most physiological and biochemical traits under study in addition to yield traits under both irrigation conditions. which suggested that they could be considered for use in rice hybrid breeding programs for water scarcity tolerant. The cluster analysis revealed that the exotic varieties Hispagran and Puebla from California (USA) were consistently grouped together. This study indicated that these rice varieties are genetically linked. It is probable that they descended from the same progenitor.

Conflicts of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest

Author Contributions: Conceptualization, M.S.A.E.A., M.I.A.Y., and M.M.B.; methodology, M.S.A.E.A., M.I.A.Y. and M.M.B., formal analysis, M.M.B., M.S.A.E.A., and M.I.A.Y., investigation, M.M.B., and M.I.A.Y., data curation, M.M.B., and M.S.A.E.A. writing original draft preparation, M.M.B., writing review and editing M.M.B., All authors have read and agreed to the published version of the manuscript.

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