Evaluation of some rice (*Oryza sativa* L.) genotypes under drought stress conditions by using morphological, physiological and molecular characteristics

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ABSTRACT

Drought is a major challenge, affecting rice at the morphological, physiological, biochemical and molecular levels. This study was conducted to compare eight cultivars of rice (*Oryza sativa* L.) for drought tolerance based on some morphological, physiological and molecular tests at the seedling stage. Drought stress was imposed by four levels of polyethylene glycol (PEG) 0, 5, 10 and 15% for 5 days. After 5 days, where seeds of all concentrations were watered daily with tap water for 21 days, plant samples were taken for measurements. Seeds from all cultivars treated with 15% PEG recorded complete germination (100%) for both G179 and Hageen Masry, while the other varieties recorded germination ranged between 56.7 and 83.30. At 10% PEG, a decrease in chlorophyll a, b, carotenoids, proline and shoot length was observed. This result indicates that Hageen Masry showed the best performance under drought stress due to its own nature of tolerance. For PCR analysis, a total of 47DNA fragments were detected; Among them, 33 are polymorphic, and 9 bands are monomorphic. To determine the level of polymorphism in the analyzed set of the eight rice genotypes, the percentage of polymorphic bands ranged from 20% to 100% with an average of 73.4%. The amplified DNA bands ranged in size from 100 to 1500 bp. The SCoT-2 primer was the most polymorphic, while the lowest number of amplified polymorphic fragments (1) was detected by primers SCoT-5 and SCoT-6. The polymorphic information content (PIC) value ranged from 0 to 0.374, with an average of 0.26.

Keywords: rice; combining ability; inbreeding depression; heterosis; type of gene action F_1 and F_2.

INTRODUCTION

Rice (*Oryza sativa* L.) is an important crop and staple food for more than half of the world's population, with a global production of more than 700 million tons annually. It belongs to the grass family. Rice is the second most popular cereal after wheat (Kavitha *et al*., 2002). It provides 20 percent of per capita energy and 13 percent of protein consumed globally (Juliano, 1994). Rice is an annual, semi-aquatic grass plant that thrives in a variety of soil and water conditions, including rain-fed and irrigated lowlands, upland, and flood-prone areas (Bouman, 2007).

Drought usually is the most important abiotic stress that affects crop production. Agricultural drought as defined by (Van Bavel and Verlinden, 1956) is a condition that exists when there is insufficient water supply to meet crop water requirements.

Rice normally requires 1,900 mm (millimeters) of water throughout the season which is much more than other crops. Cotton, for example, requires an application of 1,380 mm, while maize requires 1,000 mm (Abou El Hassan *et al.*, 2007). For research on drought and the improvement of modern crop varieties, plants that exhibit high drought tolerance are the most suitable targets and promising sources of drought-related genes (Qin *et al.*, 2016). Global environmental changes will intensify the need to develop crops that can withstand abiotic stresses, especially water shortages. Drought is the main challenge affecting rice at morphological, physiological, biochemical and molecular levels and thus affecting its (Pandey and Shukla, 2015).

Plants with high drought tolerance are the most suitable candidates and most potential sources of drought-related genes for research on drought and enhancement of modern crop varieties (Qin *et al.*, 2016). The need to produce crops that can withstand abiotic stresses, especially water shortages, will become more important as the world's environment evolves. Drought is a major concern because it affects rice at the morphological, physiological, biochemical and molecular levels, leading to production reduction (Pandey and Shukla, 2015). Development of cultivars tolerant to drought is an objective in many breeding programs in dry and semi-dry regions. Drought tolerance in rice is a complex trait and is determined by the traits of different

constituents. These traits are governed by many genes with massive environmental interaction, with low heritability, and thus difficult to investigate [Verulkar and Verma, 2014].

A method for differential gene expression in plants depends on the start codon targeted polymorphism (SCoT) DNA marker technique, called cDNA SCoT (Wu *et al.*, 2013). Recently, it has been applied to sugarcane (Wu *et al.*, 2013) and Stevia rebaudiana bertoni (Al-Taweel *et al.*, 2019). The SCoT marker has been successfully used in analyzing genetic diversity and fingerprinting a number of agricultural and horticultural crop varieties (Mulpuri *et al.*, 2013). The aim of this study is to evaluate some rice genotypes under drought using the cDNA SCoT marker and some morphological and physiological traits associated with these traits.

MATERIALS AND METHODS

Plant materials and drought experiments

Sakha 101 (SAK 101), Sakha 104 (SAK 104), Sakha105 (SAK105), Sakha106 (SAK106), Giza 177 (G177), Giza178 (G178), Giza 179 (G179), and Hagen masry (H.M) (*Oryza sativa* L.) were chosen. The Sakha Agricultural Research Center in Kafr El-Shekh provided these varieties.

Ten rice seeds from each cultivar were soaked in different concentrations of PEG 6000, with the concentrations being 0, 5, 10, and 15% for each cultivar, with three replications for each concentration. For each concentration, a total of thirty seeds were used. After five days of being soaked in worm and dark conditions, all cultivars were irrigated daily with pure tap water. Plant samples were taken after 21 days for morphological, physiological, and molecular testing. Molecular test samples were promptly frozen in liquid nitrogen and stored at -80°C.

Morphological traits

Length of shoot and root (cm), number of branches and roots, and number of seedlings. The length of the shoot (in cm) was measured from the surface of the substrate medium to the vegetative point. The length of the root was measured in centimeters from the end of the vegetative point to the end of the root.

Physiological traits

Pigment estimations (mg g-1 FW): Chlorophylls a, b and total and carotenoids were extracted in (80% v/v) aqueous acetone. Pigment measurements were quantified spectrophotometrically using a Perkin-Elmer Spectrophotometer (Model Lamda 1A). Absorbance of chlorophylls a and b and carotenoids extracts were determined at wave lengths of 663, 645 and 470 nm, respectively. Concentrations (mg g-1fw) of pigments were calculated by equations of Lichtenthaler and Wellburn (1983).

Chlorophyll a **(**CH. A.) = 12.21OD663-2.81OD646

Chlorophyll b **(**CH. B.) = 20.13 OD646-5.03 OD663

Carotenoids (**Cart.)** = (1000 OD470-3.27Chlorophyll a-104 Chlorophyll b)/ 229.

where OD is the optical density of sample solution.

Assay of proline

Fresh leaf samples (200 mg) were crushed with liquid nitrogen and extracted with a pestle in ice-cold mortar with10 ml of 3% sulfosalicylic acid. The homogenate was filtered with a filter paper; the filtrate was used for the analysis. Free proline was determined using acidic ninhydrine reagent (30ml of glacial acetic acid + 20 ml of H3PO4 (6ml) +1.25g of ninhydrine). One ml of plant extract was added to 1ml glacial acetic and 1ml of ninhydrine reagent then it was boiled for 1h. until the red color developed. Proline content was determined spectro-photometically at 520 nm, proline concentration expressed as mg/1mg F.W (Bates, 1973).

Proline standard curve:

Proline standard solution was prepared by dissolving 100 mg of L- proline in 100 ml of 3% aqueous sulfosalicylic acid. Aliquots of 10µl (20µg) to (100µg) of the proline solution were put into test tubes. Then, total volume up to one ml was reached by using 3% aqueous sulfosalicylic acid. Each tube was treated as previously described. The obtained optical densities were precedently diagrammed against proline concentrations.

Molecular parameters:

Samples of each cultivar from each treatment were collected after 21 days, immediately frozen using liquid nitrogen and stored at -80°C till farther biochemical and molecular analysis.

DNA extraction and purification

Total DNA was extracted from eight Rice cultivars by DNeasy Plant Kit (QIAGEN,

Germany). The extracted DNA concentration and quality were estimated by NanoDrop.

SCoT "Start Codon Target" analysis:

Ten primers SCoT were used in the detection of polymorphism Table (1). The PCR reaction was carried out in 25 µl reaction volume containing 12.5 µl Master Mix (sigma), 2.5 µl primer (10pcmol), 3 µl template DNA (10ng) and 7 µl dH2O, according to (Ibrahim *et al*., 2019a). PCR amplification was performed in a Perkin-Elmer/GeneAmp® PCR System 9700 (PE Applied Biosystems) programmed to fulfill 40 cycles after an initial denaturation cycle for 5 min at 94ºC. Each cycle consisted of a denaturation step at 94ºC for 45s, an annealing step at 50ºC for 50s, and an elongation step at 72ºC for 1min. The primer extension segment was extended to 7 min at 72ºC in the final cycle. The amplification products were resolved by electrophoresis in a 1.5% agarose gel containing ethidium bromide (0.5ug/ml) in 1X TBE buffer at 95 volts. PCR products were visualized on UV light and photographed using a Gel Documentation System (BIO-RAD 2000).

RNA extraction and cDNA synthesis

Total RNA was isolated from control and drought-stressed seedlings (1 h and 6 h) using TriPure Isolation Reagent (Roche Molecular Biochemicals, Mannheim, Germany) according to the manufacturer' s instructions. Total RNA of each sample was measured using Nano Drop 2000 Spectrophotometer (Thermo Scientific, Germany) to calculate the concentration; also each sample of total RNA was loaded into agarose gel to test the integrity of RNA. High quality of cDNA was prepared using Improm™ Reverse Transcription System (Promega, Madison, Wisconsin, USA) according to the manufacturer's instructions.

cDNA SCoT-PCR Amplification and Detection

Ten SCoT primers were selected for genetic diversity analysis on the basis of sharp and clear banding pattern (Table 1).

The PCR reaction was performed in a total volume of 25µl using the SCoT primers (Table 1) for the study of expression profiling. These primers were selected from the literature according to Collard and Mackill (2009). The cDNA concentration was about 40 ng for PCR amplification with SCoT primer (30 pmol). The PCR amplification was performed in a GeneAmp® PCR System 9700 (Applied Bio systems, Foster City, California, USA). The cycling profile was 94°C for 5 min, 40 cycles at 94°C for 50 sec., 50°C for 50 sec., 72°C for 1 min, and a cycle of 72°C for 7 min, then held at 4°C. The amplified products were resolved on 1.5% agarose gel.

Data analysis

For SCoT and cDNA SCoT analysis, only clear and unambiguous bands were visually scored as either present (1) or absent (0) for all samples and final data sets included both polymorphic and monomorphic bands. Then, a binary statistic matrix was constructed. Dice's similarity matrix coefficients were then calculated between genotypes using the unweighted pair group method with arithmetic averages (UPGMA). This matrix was used to construct a phylogenetic tree (dendrogram) that was performed according to Euclidean similarity index using the PAST software Version 1.91 (Hammer *et al.,* 2001).

RESULTS AND DISCUSSION

Analysis of variance

The analysis of variances for the studied traits under the four drought stress levels of PEG are presented in Table (2). The analysis of variance was significant (P≤0.05 to P≤ 0.01) or of highly significant differences among the genotypes, PEG concentrations and their interactions for all the studied traits, revealing an appropriate genetic diversity. In this regard, significant differences between rice genotypes under PEG concentrations were recorded for germination% (Ashaduzzaman *et al*., 2020 and Basal *et al*., 2020), shoot length (Mardita and Violita, 2018), root length (Leishman and Westoby 1994), leaves number (Karamanos, 1980 and Maqsood & Ali, 2007), root number (Hu *et al.,* 2012), chlorophyll a, chlorophyll b (Pandey and Shukla, 2015 and Idhan *et al.,* 2018), cart. and proline (Sobahan *et al.,* 2022).

Morphological Responses to Drought Stress

Results showed that drought stress levels of PEG mean square were significant for germination % revealing that performance of the tested genotypes differed under the four stress levels of PEG. **).** Results obtained in Table (3) indicated that the genotypes were differed significantly for germination %, where G179 and Hageen Masry gave the highest significant values for germination 96.7 and 100%, respectively compared with the other genotypes which ranged from 60% (Sakha 101) to 93.3% (Sakha 106). Also, the effect of PEG concentrations affected germination %. The highest germination % recorded at the control treatment or 5% PEG gave 83.3 and

88.8, respectively while the lowest germination % was recorded at PEG concentrations of 10 or 15%, it gave 80.8 and 81.3 germinations %, respectively .

Also, there was a significant mean square due to the interaction of genotype × PEG concentrations. The highest germinations % (100%) was recorded for Hageen Masry across the four different stress concentration of PEG or G 177 under 15%. On the other hand, the lowest one (43.3%) across all the genotypes were recorded for Sakha101 at the control). Similar results were also obtained by (Ashaduzzaman *et al.*, 2020).

Generally, germination % was decreased less than control in some genotypes under PEG 10% such as SAK104, SAK 105 and G179 and PEG 15% such as SAK 104, SAK 105, SAK 106 and G 177. But the other genotypes were increased such as SAK 101 10%, 15% and G 178 15%. Vibhuti *et al.,* (2015) confirmed that drought negatively affects the germination process by inhibiting water uptake and reducing the strength of seedling (Basal *et al*., 2020).

The obtained results in Tables (4, 5, 6 and 7) showed a variety in the morphological parameters (shoot and root length; branch and root numbers) as affected by PEG treatment in the examined rice genotypes. It was observed that variances due to genotypes tested were highly significant for shoot and root length. Also significant mean square due to the interaction of genotype × PEG concentrations used of PEG indicating that control treatment without PEG gave the highest value for shoot and root length whereas lowest values for their traits were recorded at PEG 15% Table (4).

Results obtained indicated that Sakha 106 gave the highest significant (9.6 cm) values for shoot length compared with the other genotypes, while the lowest values for shoot length were recorded for Sakha 101and Sakha 178 which recorded 3.4 and 3.6 cm, respectively. Also, the effect of PEG concentrations affected shoot length. The highest shoot length recorded at the control treatment or 5% PEG (9.40 and 8.20) while, the lowest number of leaves was recorded at PEG concentration 15% (1.40)

The values of shoot length ranged from 14.5cm for Sakha 106 control (without PEG) and1.6cm for Sakha 104 under 15% PEG. It was less than control in SAK104, 106 under all PEG treatments and G177 under PEG 10% (Ibrahim, *et al.,* 2019b; Violita and Azhari, 2021, Saha *et al.,* 2019). The highest value under 15% PEG was obtained for Hageen Masry but some disappeared under the high concentrations. PEG treatment prevents shoot elongation because drought stress will affect aspects of growth morphology (Mardita and Violita, 2018), anatomy (Zagoto and Violita 2019) and physiology. Root length was significantly decreased than control in almost genotypes. The highest significant values were obtained by Sakha 105 and Hageen Masry for root length which recorded 10.1 a 7.9 cm for Sakha 105 and Hageen Masry, respectively, whereas the lowest significant values exhibited for Sakha 101and Giza178 recorded 6.6 and 5.9 cm for root length, respectively. Also, the effect of PEG concentrations affected the root length. The highest root length was recorded for the control treatment with 5% PEG (10.80 and 10.60 cm), while the lowest root length was recorded for the 15% PEG concentration (2.90 cm).

Also, significant mean square due to the interaction of genotype × PEG concentrations .Root length was significantly decreased less than control under all drought stress levels except Sakha 105, Giza 177 and Giza178 under PEG 5% and Giza 179 under PEG 10%. They were14, 13.2, 12.4 and 13.8 cm respectively. (Violita and Azhari, 2021). As the previous traits the genotype Hageen Masry was the best under PEG 15% it became 10cm. This was in consistent with (Pandey and Shukla, 2015) and Khan *et al.,* (2001) who reported a reduction in root and shoot length in plants subjected to drought stress. A decrease in shoot length and root length, ranging from 14.5 cm to 9.9 and from 11.2 cm to 1.6 cm, was observed with an increase in drought stress (Saha *et al.,* 2019). The ability to extend the root is used to differentiate cultivars for drought tolerance and root length is an important trait in selecting a drought-tolerant cultivar (Leishman and Westoby 1994). In our study, the total root length of Sakha 105 and Hageen masry indicates higher tolerance to drought stress.

Results for leaves number showed that the highest number of leaves was obtained for Sakha 101 under PEG 5% whereas Sakha 104 15% was the lowest. Leaves number was significantly decreased less than control under PEG 15 % in Sakha104 and H.M, whereas the other genotypes disappeared. It was observed that variances due to genotypes tested were highly significant for root length. Also, significant mean square due to the interaction of genotype × PEG concentrations using PEG indicating that control (without PEG) gave the highest value for branch number, in contrast

PEG 15 % was the lowest as it became 0.21 Table (5).

Results obtained in Table (5) indicated that the genotypes differed significantly for leaf numbers. The highest values for this trait were obtained for Sakha 101, Sakha 106 an**d** Hageen Masry, it gave 1.8 leaf in all case, whereas the lowest leaf number were recorded for G178 an**d** G179 which gave 1.4 in all case. Also, the effect of PEG concentrations affected the number of leaves. The highest leaves number was recorded at the control or 5% PEG (1.07 and 1.08), while the lowest number of leaves was recorded at PEG concentration 15% (0 .21).

The average number of leaves per plant was found to decrease (2.3) as drought stress levels increased by up to 15% (Ibrahim *et al.*, 2019b). The reason for the decrease in leaf number with increasing drought may be that drought inhibits growth with changes in cell size and division leading to decreased leaf production and promoting senescence and abscission (Karamanos, 1980). This decrease in leaf number under drought stress (Maqsood and Ali, 2007) is likely to be one of the mechanisms of drought tolerance or water conservation strategy (Jones, 1992) under the limited available soil moisture. This is consistent with our findings in the genotype of Sakha 101, Sakha 106 Hageen Masry genotype and this is reflected in their physiological traits.

Also, results obtained in Table 5 showed that the genotypes differed significantly for roots number. The highest value for this trait were obtained from Sakha 104 and Hageen Masry they gave 5.15 and 6.65 root numbers .Whereas the lowest number of root were recorded for Sakha 101 and Sakha106 which gave2.85 and 2.42. Moreover, the effect of PEG concentrations affected the number of roots per plant. The highest number of roots obtained on the control treatment (5.73),while the lowest root number (0.92) was obtained at PEG concentration 15%.

Also, significant mean square due to the interaction of genotype × PEG concentrations. Results indicated that the control (without PEG) gave the highest value for root numbers. It was 8.3 but the lowest was zero for PEG at 15% Table (5). Root numbers was decreased less than control under PEG treatments and this disagrees with (Hu *et al.*, 2012) who found that salinity treatments cause increasing in root number.

Root-shoot length ratio

An increase in the root-shoot length ratio was noted when using PEG concentrations for root numbers. The highest ratio was observed at 5% PEG for Sakha 101, G177and G178 compared with the control. At 10% PEG concentration, the highest ratios obtained Sakha 105, Sakha 106 and G179. At the highest PEG concentration (15%),all the plants died except Sakha104 and Hageen Masry. They gave root – shoot ratio higher than the other two PEG treatments. Fig (2).

Root-shoot length ratio helps assess the overall health of plants and is used to assess the stress avoidance potential of plants (Bush, 1995). This is consistent with our results especially in G 178 at 5% and HM at 15% have the maximum root- shoot ratio. The decrease in shoot/root length in other genotypes may be due to some perturbations caused by osmotic stress conditions in cell division and elongation (Bayoumi *et al.*, 2008). This is consistent with the SAK 101 genotype under all PEG treatments compared to control as a response to drought (Alvareza *et al.*, 2009). Decrease or increase in this percentage agreed upon and reflected on other features Figure (2).

Physiological and Biochemical Parameters to Drought Stress

The results of physiological parameters i.e. chlorophyll a, b, a/b ratio and carotenoid showed decline in those parameters with increasing PEG concentration in almost cultivars. Chlorophyll a significantly decreased compared to the control under all PEG concentrations (Idhan *et al.*, 2018). Results obtained in Table (6) showed that the genotypes differed significantly for chlorophyll a content and the highest values were recorded for Sakha 105 (0.45) and Hageen Masry (0.47),whereas the lowest value was obtained for G178 (0.2). Also, the effect of PEG concentration affected the chlorophyll a content. PEG decreased gradually the chlorophyll a with increase in PEG concentrations. The lowest value of chlorophyll a was observed at 15% PEG (0.08) compared with the control (0.6).

Also, significant mean square due to the interaction of genotype × PEG concentrations used of PEG indicating that control (without PEG) gave the highest value that was 0.6 but the lowest was 0.1 for 15%PEG Table (6).

Results obtained in Table (6) showed that the genotypes differed significantly for chlorophyll b content. The highest value was

recorded for Hageen Masry (0.25), whilethe lowest value was obtained for Sakha 101 an G 178(0.12).

Also, the effect of PEG concentration affected the chlorophyll b content. Chlorophyll b decreased gradually with increasing PEG concentrations. The lowest value of chlorophyll b was observed at 15% PEG (0.08).

Also significant mean square due to the interaction of genotype × PEG concentrations used of PEG indicating that control (without PEG) gave the highest value that was 0.4(Sakha 5 and Sakha 6) but the lowest was 0.0 at 15%PEG Table (6).

Chlorophyll a /b ratio

Effect of PEG on Chlorophyll a/b ratio is presented in Table (7) Results obtained showed that the genotypes differed significantly in a/b ratio of chlorophyll. The highest ratios were recorded for Sakha 101 (1.90) and Hageen Masry (1.85),whereas, lowest ratio was obtained for G 178 (1.32). Moreover, the effect of PEG concentrations affected the chlorophyll a/b ratios, A/b ratios of chlorophyll decreased gradually with increasing PEG concentrations. The lowest value of a/b ratios of chlorophyll was observed at 15% PEG (0.35).

Chlorophyll a/b ratio and Carotenoid significantly decreased less than the control Table (7). Also, significant mean square due to the interaction of genotype × PEG concentrations used of PEG indicating that control (without PEG) gave the highest value, it was 2.3 but the lowest was 0.5 for 15%PEG Table (7). The highest value for carotenoid was obtained for Giza 179 but the lowest was Sakha 104 10%. The mean squares due to genotypes tested for carotenoid were highly significant for this trait under the four stress levels of PEG.

Also significant mean square due to the interaction of genotype × PEG concentrations used of PEG indicating that control (without PEG) gave the highest value, it was 0.2 but the lowest was 0.02 for 15%PEG Table (7).

Previous studies showed that water stress significantly reduced the ChL content and values of other physiological traits during different growth stages of rice (Pandey and Shukla, 2015). Among all the genotypes studied, SAK101 was expected to be the most sensitive to drought stress. Otherwise Hageen Masry was the least affected. Yellow and old leaves, which indicate chlorophyll, were lost, and the power of photosynthesis decreased (Majid, 2012). The genotypes with the highest chlorophyll and carotenoid content under drought stress were classified as tolerant, and those with the lowest chlorophyll content as susceptible genotypes (Arjenaki *et al.,* 2012, Sairam, 1994) Table (6).

Also, results obtained in Table (7) showed that the genotypes did not differ significantly for carotenoid, while the PEG concentrations were significant on the value of carotenoid. The highest value for carotenoid was recorded for the control and the values of carotenoid decreased with increasing PEG concentrations, while, the lowest value for carotenoid was recorded at the highest concentration of PEG(0.02).

Results obtained in Table (8) showed that the genotypes differed significantly for proline accumulation. The highest values were recorded for Sakha 104 and G179 (0.34μM/gfw), whereas the lowest values obtained for Sakha 101 (0.10μM/gfw). PEG concentrations were significant in proline accumulation. The highest values recorded at PEG concentration 10 %(0.26μM/gfw) compared with PEG 5% concentration (0.05μM/gfw). At the highest concentration, (15%) most of sample recorded zero. On the other hand, it was significantly increased compared to control G 179 under PEG 10% it recorded (0.80 μM/gfw) (Sobahan *et al.,* 2022). Also, significant mean square due to the interaction of genotype × PEG concentrations used of PEG indicating that control gave the highest value it was (0.26 μM/gawk) but the lowest was (0.05 μM/gfw) for 10%PEG Table (8).

Importantly, the accumulation of proline in plant cells plays a critical role in combating drought stress resistance to oxidative stress (Vendruscolo *et al.,* 2007) and may help maintain the osmotic capacity of the cytoplasm in cells which is critical for the survival of plants under stress. (Saha *et al*., 2016). It was observed that the amount of proline content increased with increasing level of drought stress(Zhan *et al.,* 2011) and the genotype with the highest proline content performed better and overcame stress conditions (Kadam *et al*., 2017 and Jaleel *et al*., 2007). Proline accumulation in plants may have a scavenger function and act as an osmolyte. Decreased proline oxidase may be the reason for the increased accumulation of proline (Sankar *et al.,* 2007). This study agrees with some genotype such as SAK104 and G179 under PEG 10% which mean that these genotypes are tolerant to drought, whereas some genotypes

were decreased under PEG treatment which means that these genotypes are so sick and sensitive to drought, this matter was reflected on other parameters Table (8).

The results generally showed that PEG 15% treatment lead to disappearance almost of genotypes and this agree with Engelbrecht *et al.,* (2005), they reported that seedling death during drought can occur as a direct result of water stress and can exacerbate the effects of factors other than drought such as pathogens, herbivores, competitors, or light. The Hageen Masry genotype performed better and showed maximum germination and percentage of seedlings still living in the higher PEG treatments.

Molecular characterization analysis

SCoT DNA polymorphisms

SCoT markers are very important in detecting polymorphisms in rice genotypes (Patidar *et al.,* 2022). Ten SCoT primers were chosen to develop DNA fingerprints for the eight rice genotypes. Amplification results from the SCoT primers used are presented in Table 8. The profile of the SCoT bands is shown in Figure 3. A total of 144 bands were detected by the ten primers, about 81 were polymorphic (56.6%). The number of bands per primer ranged from 9 to 22, with an average of 14.4 bands per primer. Primers SCoT-03 yielded the highest number of bands (22 bands), while primer SCoT-05 revealed the lowest number (9 bands).

The number of polymorphic bands ranged from 2 (SCoT-06) to 9 (SCoT-03, 9 and 11), with an average of 6. SCoT primers produced 21 unique bands with an average of 2.1 bands per primer. The largest number of unique bands (4) was achieved by the SCoT-10 primer. The percentage of polymorphisms ranged from 20% to 80% with the primers SCoT-06 and SCoT-04, respectively. Polymorphic information content (PIC) values ranged from 0.13 (SCoT-06) to 0.38 (SCoT-04), with a mean of 0.33. Moreover, the size of the amplified bands with different primers ranged from 140 to 1650 bp.

SCoT cDNA polymorphisms:

Seven SCoT primers with the same genotypes were used, and the sequences of SCoT primers 1, 2, 3, 4, 5, 6, and 11 are showed in Table 1. The profile of the SCoT banding are shown in Figure 5 and the amplification results in Table 10. A total generated 47 bands by 7 primers, 33 of which were polymorphic (56.6%).

The percentage of polymorphism detected among rice samples using the 7 SCoT primers and it ranged between 20% (SCoT-05) and 100%. The primers SCoT01, SCoT2 and SCoT4 detected the highest percentage of polymorphisms (100 percent) and this percentage corresponds with Abdelghany *et al.,* (2022).). Primer SCoT-02 showed the highest number of polymorphic bands (6). Earlier, Baghizadeh and Dehghan, (2018) also executed a study in the rice germplasm and observed a similar result. The polymorphism information content ranged 0 (SCoT-05) to 0.374 (SCoT-03) with an average of 0.26 tables (5). According to Botstein *et al.,* (1980), prefixes with a PIC value of 0.25 to 0.50 supply important information for genetic diversity research.

SCoT markers with higher PIC values possess higher power to distinguish varieties (Feng *et al.,* 2016) because of their higher reproducibility and major power for polymorphism detection (Hamidi *et al.,* 2014). The PIC of a stands for primer or primers likelihood for polymorphism detection. The combination of two randomly selected individuals is based on the frequency distribution of the allele number of detectable alleles. These results indicated reliable sources of diversity that would assist breeders in assessing genetic diversity and relationships between different genotypes.

Using SCoT markers to dissect genetic relationships is fundamental for crop improvement. In conclusion, a high level of genetic diversity and the relationship between eight genotypes of rice were dissected. The results indicated good sources as an alternative method for selecting more promising rice genotypes and reducing the cost and time needed to develop hybrids for a future plant breeding program.

CONCLUSION

Significant differences between rice genotypes under PEG concentrations were recorded for germination%, shoot length, root length, leaves number, root number, chlorophyll a, chlorophyll b**,** cart. and proline**.** Result indicated that Hageen Masry showed the best performance under drought stress due to its own nature of tolerance. For PCR analysis, a total of 47DNA fragments were detected; Among them, 33 are polymorphic, and 9 bands are monomorphic. To determine the level of polymorphism in the analyzed set of the genotypes, the percentage of polymorphic bands ranged from 20% to 100%.

The amplified DNA bands ranged in size from 100 to 1500 bp. The SCoT-2 primer was the most polymorphic, while the lowest number of amplified polymorphic fragments was detected by primers SCoT-5 and SCoT-6. The polymorphic information content (PIC) value ranged from 0 to 0.374, with an average of 0.26.

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Table 1: List of primers SCoT and their nucleotide Sequences used.

NO.	Primer cod	Primer sequence (5′-3′)
1	SCoT-01	ACGACATGGCGACCACGC
2	SCoT-02	ACGACATGGCGACCCACA
3	SCoT-03	CAATGGCTACCACTAGCG
4	SCoT-04	ACCATGGCTACCAGCGCG
5	SCoT-05	CCATGGCTACCACCGGCA
6	SCoT-06	CAACAATGGCTACCACGC
7	SCoT-07	ACAATGGCTACCACTGAC
8	SCoT-09	ACAATGGCTACCACTGCC
9	SCoT-10	ACAATGGCTACCACCAGC
10	SCoT-11	AACCATGGCTACCACCAC

Table 2: The analysis of variances for all the studied traits under the four drought stress levels of PEG

S.O.V	D .F	Germi-nation%	Shoot length(cm)	Root length(cm)	Leaves number (L.NO.)	Root number (R.NO.)	(CH. A.)	(CH. B.)	(CH. a/b)	Cart.	Proline
PEG	3	318.055	281.825**	324.871**	965.125**	79.222**	1.165**	0.238**	15.046**	0.089**	0.25**
Gen.	7	2680.35	47.463**	26.356*	415.446**	27.261**	0.084**	0.031**	0.713**	0.005**	0.11**
PEG x	21	168.849	7.173**	26.529**	35.196**	2.896	0.060**	0.018**	1.426**	0.005**	0.06**
Error	64	89.583	2.081	10.123	0.875	1.812	0.011	0.001	0.107	0.001	0.01

Table 3: The effect of PEG 6000 concentrations on rice genotypes germinations.

Germination %					
Concentration Genotype	Con	PEG 5%	PEG 10%	PEG 15%	Mean
Sakha 101	43.3	73.3	60	63.3	60
Sakha 104	93.3	90	83.3	83.3	87.5
Sakha 105	96.7	100	83.3	83.3	90.8
Sakha 106	96. 7	96. 7	100	80	93.3
Giza 177	63. 3	76. 7	66.7	56.7	65.8
Giza 178	73. 3	73. 3	66.7	83.3	74.2
Giza 179	100	100	86. 7	100	96.7
Hageen Masry	100	100	100	100	100
Mean	83.3	88.8	80.8	81.3	83.5
L.S.D. at 0.05	PEG 5.46		G7.72	PEG x G 15.43	
at 0.01	PEG 7.25		G10.26	PEG x G 20.52	

Table 4: The effect of PEG 6000 concentrations on rice genotypes shoot and root length.

	Shoot length (SH.L.)					Root length (R.L.)				Mean
Concentration Genotype	con	PEG 5%	PEG 10%	PEG 15%	Mean	Con	PEG 5%	PEG 10%	PEG 15%	
Sakha 101	4.3	5.7	3.8	0	3.4	11.3	9.5	5.6	0	6.6
Sakha 104	11.2	7.3	6	1.6	6.5	13.7	7.8	5.3	3.1	7.5
Sakha 105	10.8	9.5	5.1	0	6.8	13.5	14	8.1	0	10.1
Sakha 106	14.5	11.2	9.9	0	9.6	11.4	7.8	12	0	9.1
Giza 177	10.5	9	6.3	0	6.5	9.6	13.2	8.8	0	7.9
Giza 178	4.7	5.3	4.3	0	3.6	6.7	12.4	4.4	0	5. 9
Giza 179	7.5	9.3	7.3	0	6.04	9.3	10.7	13.8	0	8.5
Hageen Masry	8.9	8.4	6.8	5.03	7.3	10.7	9.3	9	10	9.7
Mean	9.04	8.2	6.2	1.4	6.2	10.8	10.6	8.4	2.9	8.2
L.S.D.at 0.05	PEG 0.83		G1.18	PEG x G 2.35		PEG 1.83		G 2.59	PEG x G5.19	
at 0.01	PEG 1.11		G1.56	PEG x G 3.13		PEG 2.43		G 3.45	PEG x G 6.89	

Table 5: The effect of PEG 6000 concentrations on rice genotype leaves and root numbers.

	Leaves number (L.NO.)					Root number (R. NO.)				Mean
Concentration Genotype	con	PEG 5%	PEG 10%	PEG 15%	Mean	con	PEG 5%	PEG 10%	PEG 15%	
Sakha 101	2.3	2.7	2	0	1.8	3.7	4.7	3	0	2.85
Sakha 104	2	2	2	0.7	1.7	7.3	4.3	6.3	2.7	5.15
Sakha 105	2	2	1.7	0	1.7	7.3	6	4.7	0	4.50
Sakha 106	2	2	2	0	1.8	7	3.3	5.7	0	4.00
Giza 177	2	2	1.7	0	1.4	3.3	2.7	3.7	0	2.42
Giza 178	2	2	2	0	1.5	3.7	4	4	0	2.92
Giza 179	2	2	2	0	1.5	5.3	4	2.7	0	2.55
Hageen Masry	2.3	2	2	1	1.8	8.3	8.3	5.3	4.7	6.65
Mean	2.07	2.08	1.92	0.21	2.1	5.73	4.66	4.42	0.92	4.10
L.S.D.at 0.05	PEG 0.14		G 0.20	PEG x G 0.41		PEG 0.78		G 1.09	PEG x G 2.19	
at 0.01	PEG 0.19		G 0.27	PEG x G 0.54		PEG 1.03		G1.46	PEG x G 2.92	

Table 6: The effect of PEG 6000 concentrations on rice genotypes chlorophyll a and chlorophyll b.

	Chlorophyll a (CH. A.)					Chlorophyll b (CH. B.)				Mean
Concentration Genotype	con	PEG 5%	PEG 10%	PEG 15%	Mean	con	PEG 5%	PEG 10%	PEG 15%	
Sakha 101	0.6	0.5	0.2	0	0.32	0.2	0.2	0.1	-	0.10
Sakha 104	0.5	0.32	0.3	0.2	0.33	0.2	0.2	0.3	0.1	0.20
Sakha 105	0.7	0.6	0.5	0	0.45	0.4	0.3	0.3	-	0.20
Sakha 106	0.7	0.5	0.5	0	0.42	0.4	0.2	0.4	-	0.20
Giza 177	0.6	0.5	0.4	0	0.37	0.3	0.2	0.2	-	0.20
Giza 178	0.2	0.2	0.4	0	0.20	0.1	0.1	0.2	-	0.12
Giza 179	0.9	0.3	0.3	0	0.57	0.3	0.3	0.2	-	0.20
Hageen Masry	0.6	0.4	0.4	0.5	0.47	0.2	0.3	0.2	0.3	0.25
Mean	0.60	0.41	0.37	0.08		0.3	0.2	0.2	0.04	
L.S.D .at 0.05	PEG 0.06		G 0.09	PEG x G 0.17		PEG 0.03		G 0.04	PEG x G 0.07	
at 0.01	PEG 0.08		G 0.11	PEG x G 0.23		PEG 0.03		G 0.05	PEG x G 0.09	

Table 7: The effect of PEG 6000 concentrations on rice genotypes chlorophyll a/b ratio and carotenoid.

	a/b ratio					cartnoid				Mean
Concentration Genotype	con	PEG 5%	PEG 10%	PEG 15%	Mean	con	PEG 5%	PEG 10%	PEG 15%	
Sakha 101	2.5	2.3	2.8	0	1.90	0.18	0.13	0.1	-	0.1
Sakha 104	2.8	1.4	1.2	1.2	1.65	0.2	0.04	0.03	0.1	0.1
Sakha 105	1.8	2.4	1.8	0	1.50	0.1	0.1	0.1	-	0.1
Sakha 106	1.8	2.2	1.5	0	1.38	0.2	0.1	0.1	-	0.1
Giza 177	2.1	2.5	2.7	0	1.82	0.2	0.2	0.2	-	0.1
Giza 178	1.9	1.8	1.6	0	1.32	0.1	0.1	0.1	-	0.1
Giza 179	2.8	1.2	2.0	0	1.50	0.3	0.1	0.1	-	0.1
Hageen Masry	2.6	1.6	1.6	1.6	1.85	0.2	0.1	0.1	0.1	
Mean	2.28	1.92	1.90	0.35	1.7	0.2	0.1	0.1	0.02	0.1
L.S.D.at 0.05	PEG 0.19		G 0.27	PEG x G 0.53		PEG 0.02		G 0.03	PEG x G 0.05	
at 0.01	PEG 0.25		G 0.35	PEG x G 0.71		PEG 0.03		G 0.04	PEG x G 0.07	

Table 8: The effect of PEG 6000 concentrations on proline accumulation for rice tested genotypes

	Proline				
Concentration Genotype	Con	PEG 5%	PEG 10%	PEG 15%	Mean
Sakha 101	0.14	0.20	0.06	-	0.10
Sakha 104	0.48	0.20	0.56	0.14	0.34
Sakha 105	0.27	0.13	0.20	-	0.15
Sakha 106	0.19	0.20	0.12	-	0.13
Giza 177	0.33	0.20	0.06	-	0.15
Giza 178	0.25	0.19	0.12	-	0.14
Giza 179	0.29	0.26	0.80	-	0.34
Hageen Masry	0.16	0.15	0.16	0.23	0.18
Mean	0.26	0.19	0.26	0.05	0.19
L.S.D. at 0.05	PEG 0.05		G 0.07	PEG x G 0.14	
L.S.D. at 0.01	PEG 0.06		G 0.09	PEG x G 0.54	

Table 9: The amplification results of SCoT-DNA primers

Primer name	AB	MB	PB	UB	P %	PIC	Product size(bp)
SCoT-01	16	7	7	1	56	0.33	150- 1200
SCoT-02	16	11	4	1	31	0.26	210- 1300
SCoT-03	22	10	9	3	55	0.34	150- 1500
SCoT-04	10	2	6	2	80	0.38	260- 1000
SCoT-05	9	3	3	3	67	0.37	190- 720
SCoT-06	10	8	2	-	20	0.13	160- 500
SCoT-07	17	8	7	2	53	0.35	240- 1600
SCoT-09	17	5	9	3	71	0.37	140- 1650
SCoT-10	13	6	3	4	54	0.36	210- 1300
SCoT-11	14	3	9	2	79	0.37	230- 1400
Total	144	63	60	21	-	-	-
Mean	14.4	6.3	6	2.1	56.6	0.33	-

Table 10. Similarity matrix among the eight rice genotypes according to Dice coefficient as revealed by SCoT DNA markers.

	Sak-101	Sak-104	Sak-105	Sak-106	G-177	G-178	G-179	H.-M
Sak-101	1.00							
Sak-104	0.76	1.00						
Sak-105	0.79	0.83	1.00					
Sak-106	0.83	0.84	0.83	1.00				
G-177	0.82	0.83	0.83	0.83	1.00			
G-178	0.81	0.79	0.83	0.81	0.84	1.00		
G-179	0.81	0.77	0.80	0.81	0.81	0.77	1.00	
H.-M	0.77	0.80	0.83	0.80	0.79	0.85	0.84	1.00

Table 11: Total number of bands (TB), polymorphic bands (PB), monomorphic bands (MB), percentage of polymorphism (%P), unique bands, fragment size range and polymorphic information content (PIC) as revealed by SCoT analysis.

Primer	TB	PB	MB	UB	% P	PIC
SCoT-01	8	8	0	4	100	0.368
SCoT-02	10	10	0	4	100	0.331
SCoT-03	7	4	1	2	85.7	0.374
SCoT-04	7	4	0	3	100	0.342
SCoT-05	5	1	4	0	20	0
SCoT-06	4	1	3	0	25	0.132
SCoT-11	6	5	1	0	83.3	0.242
Total	47	33	9	13		

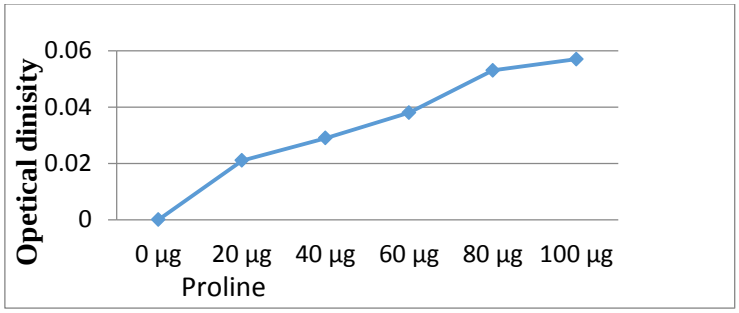

Figure 1: Proline standard curve.

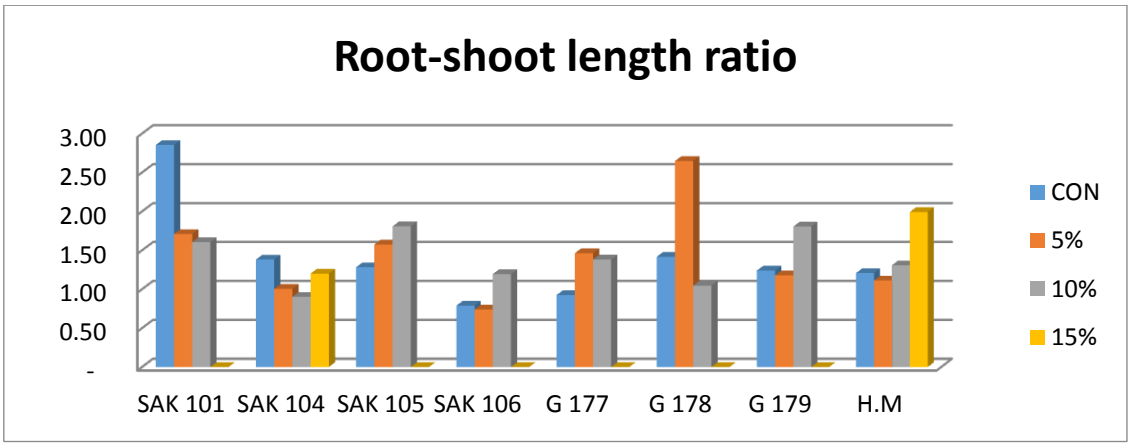

Figure 2: The effect of PEG 6000 concentrations on rice genotypes root/ shoot ratio.

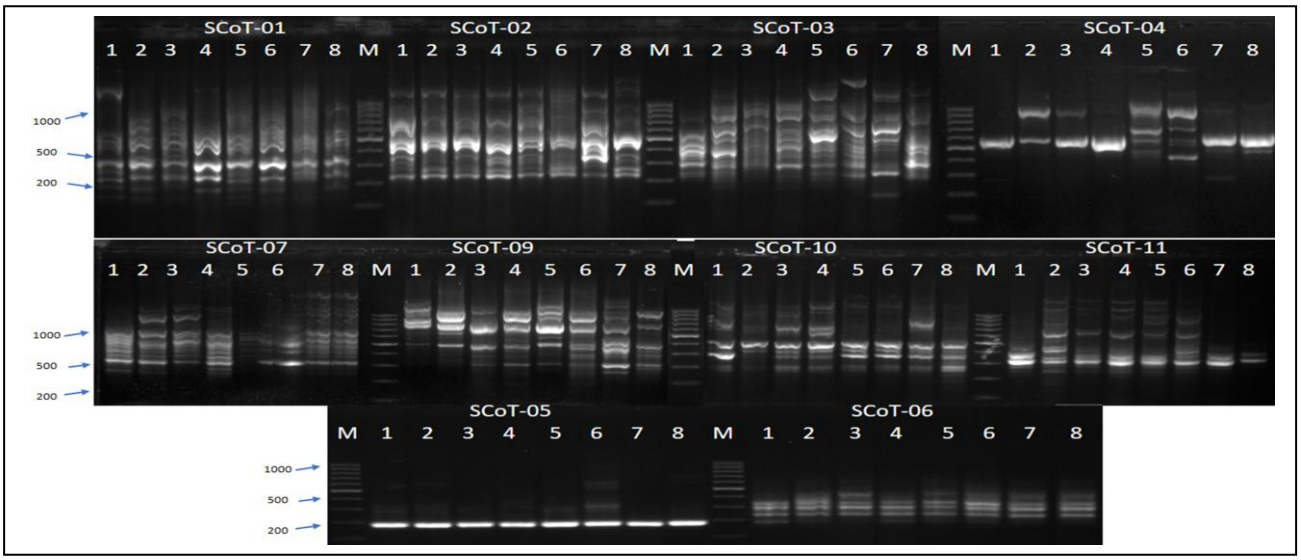

Figure 3: SCoTs profiles (1, 2, 3, 4, 5, 6, 7, 9, 10 and 11) for Rice (DNA bulked samples). M refers to DNA ladder

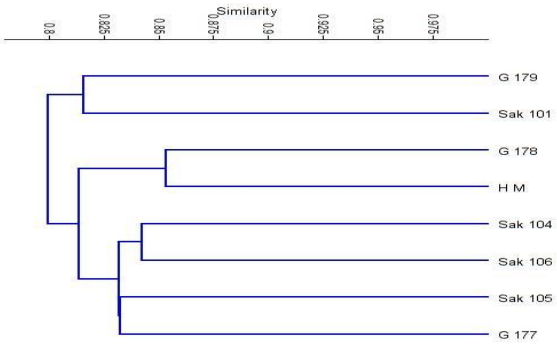

Figure 4: Dendrogram for the eight rice genotypes constructed from SCoT DNA data using UPGMA and similarity matrix computed according to Dice coefficient.

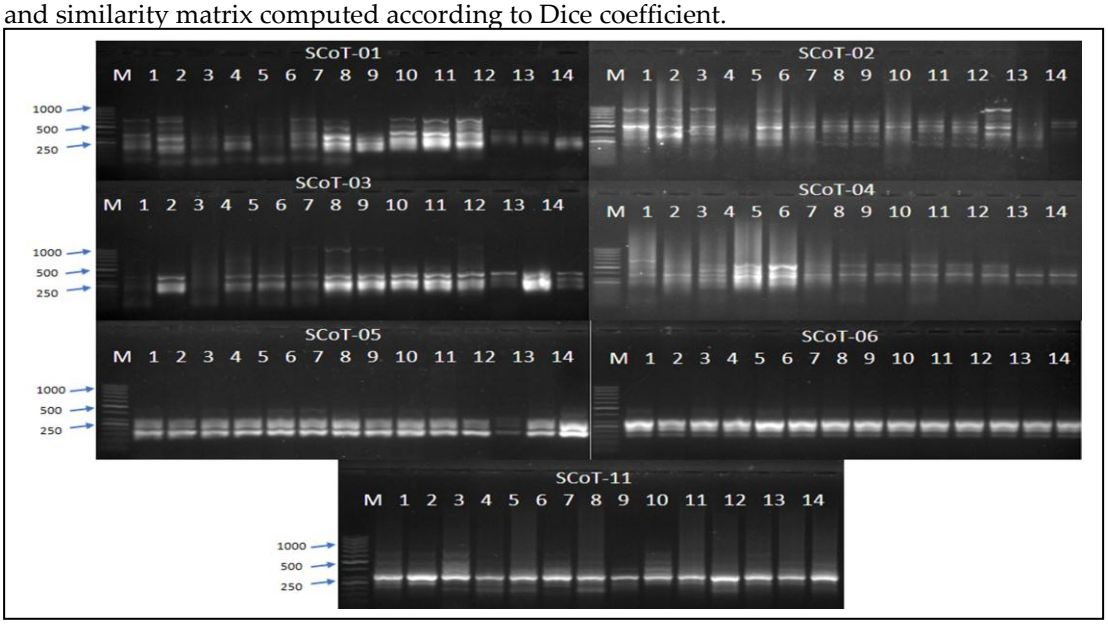

Figure 5: SCoTs profiles (1, 2, 3, 4, 5, 6 and 11) for Rice (C-DNA bulked samples). M refers to DNA ladder (50-bp).

تقييم بعض التراكيب الوراثية للأرز (*Oryza sativa* L.) تحت ظروف الجفاف باستخدام الخصائص المورفولوجية والفسيولوجية والجزيئية

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الملخص العربى

يعتبر الجفاف تحديًا كبيرًا للعديد من النباتات حيث يؤثر على المستوى المورفولوجي والفسيولوجي و الجزيئي لنبات الأرز، فى هذه الدراسة أجريت تجربة لمقارنة ثمانية أصناف من الأرز (*Oryza sativa* L.) على تحمل الجفاف وذلك بالاعتماد على بعض القياسات المورفولوجية والفسيولوجية والجزيئية في مرحلة البادرات، تم استخدام البولى ايثيلين جليكول لاستحثاث الحبوب لإجهاد الجفاف وكانت التركيزات المستخدمة من البولى ايثيلين جليكول هى (0 – 5- 10- 15 %) حيث ظلت الحبوب فى هذه التركيزات لمدة 5 ايام تحت الظروف المناسبه للانبات وبعد 5 أيام تم رى جميع الحبوب لجميع التركيزات يوميا بماء الصنبور لمدة 21 يوماً ،بعد ذلك تم أخذ العينات النباتية للقياسات المستخدمة، جميع الأصناف المعامله بالبولى ايثيلين جليكول بتركيز 15٪ قد سجلت إنباتا كاملا (100٪) لكل من G179 و Hageen Masry بينما الأصناف الأخرى قد سجلت إنباتا تراوح بين 56.7 و 83.30 .عند تركيز10٪ .لوحظ انخفاض في الكلوروفيل أ ، ب ، كاروتينويد ، برولين وطول النبات تشير هذه النتيجة إلى أن Hageen Masry أظهر أفضل أداء تحت إجهاد الجفاف لقدرته العالية على تحمل الجفاف، بالنسبة لتحليل PCR ، تم الكشف عن إجمالي شظايا ال DNA فكانت47 ؛ من بينها 33 متعدد الأشكال ، و 9 أحادية الشكل وتراوحت النسبة المئوية للشظايا متعددة الأشكال من 20٪ إلى 100٪ بمتوسط 73.4٪ .تراوحت حجم شظايا الحمض النووي المتضاعفة ما بين 100 و 1500 زوج من القواعد.كان البادئ SCoT-2 هو الأكبر من حيث الشظايا المعتددة الأشكال. بينما كانت البوادئ5 -SCoT و SCoT-6 هم الأقل (1). وتراوحت قيمة ال (PIC)من 0 إلى 0.374 بمتوسط 0.26.وهذه النتائج تؤكد أهمية استخدام تطبيقSCoT في تمييز التباين بين التراكيب الوراثية المختلفة من الأرز من حيث قدرتها على تحمل الإجهادات المائية للاستعانة بزراعتها تحت ظروف الجفاف المختلفة .

الكلمات الاسترشادية: