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Genetic Prediction of Heterosis for Agronomic and Drought Tolerance Characters of Some Rice Genotypes by Molecular Markers and Multivariate Analysis Under Different Water Treatments

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Abstract

This investigation focused on assessing heterosis, genetic variability, and the key physiological and biochemical indicators associated with drought tolerance in rice at water-limited environments. Seven rice accessions were utilized in a half-diallel pattern, resulting in the development of twenty-one F_1 hybrid combinations. Molecular divergence among the parental lines was analyzed through eleven SCoT polymorphism primers, generating 149 DNA fragments, out of which 71 were polymorphic, representing (47.6%) polymorphism. PIC value was 0.40, with SCoT-02, SCoT-08, and SCoT-09 showing the highest polymorphism. Genetic distances ranged from 0.81 to 0.90, with SK-101 and GZ-179 being the most divergent. Cluster separated the rice accessions into two main groups. Several hybrids, notably GZ-179×IET-1444, IET-1444×HR-5824, and SK-106×SK-107, displayed significant heterosis for yield-related traits across environments. Under normal circumstances, there was a strong correlation between marker-based genetic distance (GD) and heterosis, especially for sterility. Traits such as thousand-grain weight, weight panicle, sterility, and yield/plant^{-1} exhibited strong positive correlations with better-parent heterosis under water regime, indicating that SCoT-based genetic distance can predict hybrid performance under stress. Multivariate analyses (Bi-plot PCA and heatmap) revealed that grain yield was positively associated with photosynthetic rate, membrane stability, water-use efficiency, and proline content, while negatively correlated with oxidative stress indicators. PCA effectively discriminated drought-tolerant hybrids, especially GZ-179×IET-1444, IET-1444×HR-5824, and GZ-179×HR-5824, which combined superior photosynthetic capacity, membrane integrity, and biochemical resilience along highly production and related features.

Key words: Rice; SCoT; Heterosis; GD; PCA; Photosynthetic; Antioxidant.

Introduction

An important cereal crop that provides food for almost half of the world's population is rice (*Oryza sativa* L.). It is the second most important grain crop over Egypt, after crop wheat. Due to improved agronomic techniques and genetic advancement, the amount of paddy rice produced increased from 4.38 to 6.20 million tons during the 2024 season, while the cultivated area increased to 1.77 million fed., a 40.9% increase over the previous year. **(FAOSTAT, 2023).** However, the Grand Ethiopian Renaissance Dam and climate change have made rice agriculture even more difficult due to diminishing water supplies. Water deficit stress significantly lowers photosynthetic efficiency, nutrient uptake, and grain yield since rice is naturally drought-sensitive. In recent decades, this has resulted in national yield losses of up to 25%. Thus, creating genotypes that are drought-tolerant and high-yielding is a top breeding priority.

To increase drought resilience, traditional and molecular breeding techniques are used. In order to choose better parental combinations, the half-diallel mating design is still a useful tool for assessing heterosis and combining ability. The improved performance of F_1 hybrids in comparison to their parents is shown in hybrid vigor, which is strongly associated with genetic divergence. It has been shown that Start Codon Targeted (SCoT) markers are very effective at predicting hybrid performance and evaluating genetic diversity. Combining multivariate technologies like PCA and heatmap clustering with morpho-physiological, biochemical, and molecular investigations speeds up rice development initiatives and makes it easier to identify genotypes that are resistant to drought. As a result, there were four primary goals for this study: (1) to identify superior parents and hybrid combinations that combine high yield potential with strong drought tolerance for use in future breeding programs; (2) to examine the connections between hybrid performance under both

irrigation regimes, mid and better-parent heterosis for important agronomic variables, and SCoT-based genetic diverse; (3) to assess heterosis in 21 F_1 rice hybrids derived from seven diverse parental genotypes under well-watered, drought-stressed, and combined environments; and (4) to clarify the interrelationships among yield, phy-biochemical traits influencing to moisture resilience in rice.

Materials and Methods

Field trials were implemented at the Rice Research and Training Center (RRTC), Sakha, Kafr El-Sheikh, Egypt, during the 2023 and 2024 growing seasons, utilizing seven rice genotypes as presented in Table (1).

Field experiment:

In the 2023 season, the parental lines were intercrossed in all possible combinations based on a 7 × 7 half-diallel design without reciprocals as outlined by **(Griffing, 1956),** generating twenty-one F_1 hybrids. Emasculation was achieved through the hot-water technique proposed by **Jodon (1938)** and refined by **Butany (1961),** involving immersion of panicles in water at 43–45 °C for 12 min.

During the summer season of 2024, a total of 28 rice genotypes; comprising seven parents and their twenty-one F_1 hybrids were sown on April 30. Seedlings aged thirty days were transplanted into two neighboring experimental fields: one maintained under normal irrigation (flooding every four days) and the other subjected to water-deficit conditions (watering every twelve days). The trial followed a randomized complete block design (RCBD) comprising three replicates. Each accession was occupied three rows per replicate, with rows measuring 5 meters in length and spaced at twenty × twenty cm between both rows and hills. Drought stress was induced through intermittent surface irrigation, where water was applied every twelve days without maintaining standing water after each irrigation event. This treatment began two weeks following transplanting and continued until crop maturity. The amount of irrigation water used throughout the growing period was recorded using water meters. Routine cultural and protection measures were adopted in accordance with of the Rice Research and Training Center **(RRTC, 2022).** Data were recorded for a set of morpho-physiological and yield-related parameters, including short stature (cm), flag leaf area (cm²), number of panicles per plant, panicle length (cm), panicle weight (g), sterility (%), 1000-grain weight (g), and grain yield per plant (g). Photosynthetic traits were evaluated through several indicators; Chlorophyll concentration in the flag leaf was quantified following the protocol of **Arnon (1949),** while PSII photochemical efficiency (Fv/Fm) was estimated as described by **Maxwell and Johnson (2000).** Leaf gas-exchange characteristics were determined according to the procedures outlined by **Agehara and Leskovar (2012)** and **Bunce (1993).** Relative water content (RWC) was computed using the equation by **Bhushan *et al.*, (2007),** and water-use efficiency (WUE) followed the formula of **Dos Santos *et al.*, (2017).** Membrane stability index (MSI) was determined according to **Sairam (1994).**

For biochemical determination, fresh flag leaves were sampled at the flowering stage, homogenized in 50 mM phosphate buffer (pH 7.8), and centrifuged at 15,000 rpm for 15 minutes at 4°C. The resulting supernatant was stored at −80°C until analysis. Lipid peroxidation (malondialdehyde, MDA) was measured following **Yagi (1998),** while free proline content was estimated as per **Bates *et al.*, (1973).** Total soluble phenolics were quantified using the Folin–Denis method **(Shahidi and Naczk, 1995).** (ROS); including (O_2^-) radical superoxide, (OH^-) radicals hydroxyl, and hydrogen peroxide (H_2O_2) were quantified according to **Wang and Luo (1990), Alexieva *et al.*, (2001), and Loreto and Velikova (2001).** Antioxidant enzyme activities were assessed following standard procedures: peroxidase (POX) by **Putter (1974),** superoxide dismutase (SOD) by **Giannopolitis and Ries (1977),** catalase (CAT) by **Beers and Sizer (1952),** and ascorbate peroxidase (APX) by **Nakano and Asada (1987).** Observations were taken from ten randomly selected plants per genotype per replicate following the standard guidelines of IRRI (2008).

DNA Extraction and Quantification:

Leaf tissues from all rice genotypes grown under both irrigation conditions were sampled 21 days after transplanting, quickly frozen in liquid nitrogen, and preserved at −80 °C for later biochemical and molecular analyses. Genomic DNA was isolated from youth leaves using a modified cetyltrimethylammonium bromide (CTAB) protocol described by **Doyle and Doyle (1990),** followed by purification with the DNeasy Mini Kit (Qiagen, Germany). The quantity and quality of DNA were verified through agarose gel electrophoresis and NanoDrop spectrophotometric readings. DNA purity was determined using the A260/A280 absorbance ratio, and the concentration (µg/µl) was calculated according to the equation.

Concentration = (OD260 × 50 × dilution factor) / 1000.

PCR Amplification and SCoT Marker Analysis:

To assess the molecular diversity among the seven rice genotypes, a total of eleven ATP-SCoT primers were used **(Table 2).** According to **Ibrahim *et al.*, (2019),** the PCR amplification was carried out in a 25 µl reaction volume with 12.5 µl of 2× Master Mix (Sigma), 2.5 µl of primer (10 pmol), 3 µl of genomic DNA template (10 ng), and 6 µl of nuclease-free water. Using a Perkin-Elmer/GeneAmp® PCR System 9700, thermal cycling was carried out according to the following program: pre-denaturation

for 5 min at 94 °C, denaturation at 94 °C for 45 s, annealing at 50 °C for 50 s, extension at 72 °C for 1 min, and final extension at 72 °C for 7 min. A BIO-RAD Gel Documentation System was used to view the PCR products after they were separated on 1.5% agarose gels with 0.5 µg ml^{-1} of ethidium bromide in 1× TBE buffer and electrophoresed at 95 V. The primer amplification protocols described by **Sharma *et al.*, (2015)** were followed.

Data Scoring and Cluster Analysis:

For analysis, only unique and repeatable SCoT amplification bands were taken into account, and each was given a binary score of present (1) or absent (0). The Dice similarity coefficient was used to calculate the genetic similarity between genotypes based on the resultant binary data matrix. The unweighted pair group technique with arithmetic mean (UPGMA) was then used to group the clusters. The PAST program version 1.91 was used to create a dendrogram that showed the genetic links between the rice genotypes based on Euclidean similarity **(Hammer *et al.*, 2001).**

Estimation of Heterosis:

Using the methods described by **Mather (1949)** and **Mather and Jinks (1982),** the percentage deviation of the F_1 mean from the mid-parent (MP) and better-parent (BP) means was used to calculate the heterosis for each hybrid and feature. The least significant difference (LSD) test, as outlined by **Wynne *et al.*, (1970),** was used to assess the statistical significance of heterotic effects.

Correlation and Multivariate Analyses:

In accordance with **Singh and Chaudhary (1979),** Pearson correlation coefficients were computed to assess the relationships among genetic distance (GD), mid-parent heterosis (MPH%), and better-parent heterosis (BPH%) for a variety of morpho-physiological and yield-related traits. A two-tailed t-test, as outlined by **Snedecor and Cochran (1989),** was used to assess the significance of the associations. IBM SPSS Statistics version 30.0 (Armonk, NY, USA, 2024) was used for all correlation and least significant difference (L.S.D.) analyses. Correlations between the attributes under both irrigation settings, heatmaps matrix were created. Following the methodology of **Gomez and Gomez (1984),** principal component analysis (PCA-biplot) was used to ascertain the contribution of each characteristic to the total phenotypic variation and to examine the relationships between genotypes and traits. R software version 4.4.0 was used for all multivariate statistical studies.

Results and Discussion

Eleven SCoT primers were evaluated for their efficiency in generating start codon–based banding patterns and characterizing the genetic diversity among seven Egyptian and exotic rice varieties. All primers produced distinct and reproducible amplification profiles and were therefore used for polymorphism analysis based on clear and well-defined banding patterns. The amplification results obtained from the tested primers are summarized in Table (3), and representative gel images are presented in Tables and Figures (1).

Across the seven rice genotypes, amplification produced seventy-one allelic fragments, with sizes varying between 150 and 1500 base pairs. Primer SCoT-01 produced the highest number of alleles (7), while the lowest number (1) was obtained with SCoT-05 and SCoT-06. In total, 149 DNA fragments were amplified across all primers, with an average of approximately 13.5 fragments per primer. The number of amplified fragments per primer varied between 11 (for SCoT-05, SCoT-06, and SCoT-09) and 17 (for SCoT-01). Of these, 71 fragments were polymorphic, corresponding to an average of 6.5 polymorphic fragments per primer. The most polymorphic bands (8) were created by primers SCoT-02, SCoT-08, and SCoT-09, whereas the fewest (4) were produced by primer SCoT-13. The percentage of polymorphism found in the tested primers ranged from 29% (SCoT-01 and SCoT-13) to 73% (SCoT-09), which is in good agreement with **Abdelghany *et al.*, (2022)** findings. The band occurrence frequency values were 0.68 to 0.85, with SCoT-11 having the greatest frequency (0.85) and SCoT-05 and SCoT-06 having the lowest frequencies. The average frequency for all primers was 0.75. **Othman *et al.*, (2022)** also observed comparable patterns in SCoT marker efficiency within rice germplasm.

For each of the eleven SCoT primers, the polymorphism information content (PIC) values were calculated to assess how well they differentiated the genotypes of rice under study. The informativeness of each molecular marker in exposing allelic variation and its capacity to distinguish across genotypes with similar ancestry is represented by this parameter. The polymorphism information content (PIC) values in the current study ranged from 0.23 to 0.65, with an average of 0.40 for each primer. While SCoT-02, SCoT-05, and SCoT-06 also demonstrated relatively high PIC values (>0.50), indicating their significant efficiency in detecting allelic polymorphism, the primer SCoT-04 yielded the highest PIC (0.65) and amplified four different alleles. On the other hand, SCoT-11 had the lowest PIC (0.23) **(Table 3).** For evaluating genetic variation among rice genotypes, primers with PIC values between 0.25 and 0.50 are deemed informative, according to **Nawade and Lee (2024).** SCoT markers with higher PIC values demonstrate greater efficiency in differentiating varieties due to their reproducibility and strong ability to detect polymorphisms **(Eissa *et al.*, 2023).** Overall, the mentioned values of P.I.C. here in this paper, suggesting that the selected molecular markers

(SCoT) are reliable tools for detecting genetic variation and assessing genetic relationships among rice genotypes. These findings provide valuable insights for breeders in identifying genetically diverse parental lines for rice improvement programs.

Identification of Unique SCoT Markers:

As presented in Table (4), the SCoT marker analysis effectively differentiated six of the seven rice cultivars by identifying distinct positive and negative markers. In total, 24 unique loci were detected among the evaluated genotypes, comprising 14 specific positive and 10 negative markers.

Distinctive SCoT marker profiles were identified for each rice genotype. For Sk-101, two exclusive positive bands were detected using primers SCoT-08 (790 bp) and SCoT-11 (950 bp), while two negative bands were missing with SCoT-09 (230 bp) and SCoT-11 (250 bp). Sakha-106 exhibited three characteristic positive fragments amplified by SCoT-01 (1500 bp), SCoT-04 (290 bp), and SCoT-06 (530 bp), together with four absent bands corresponding to SCoT-03 (630 bp), SCoT-04 (420 bp), SCoT-09 (1200 bp), and SCoT-14 (800 bp). The GZ-179 cultivar was defined by one unique positive marker amplified with SCoT-02 (220 bp) and two negative markers absent with SCoT-08 (1000 bp) and SCoT-09 (890 bp). In IET-1444, three positive loci were amplified by SCoT-03 (380 bp), SCoT-05 (520 bp), and SCoT-11 (220 bp), while one negative locus was missing at SCoT-11 (410 bp). For HR-5824, two positive amplicons were generated using SCoT-04 (260 bp) and SCoT-13 (1300 bp), along with a single negative band absent with SCoT-03 (520 bp). Finally, the cultivar PL-77-8-04 displayed three genotype-specific positive fragments produced by primers SCoT-02 (150 bp), SCoT-04 (740 bp), and SCoT-13 (170 bp). Collectively, the obtained results highlight the high discriminatory power of SCoT markers for detecting DNA polymorphisms specific to each genotype, thereby providing a reliable molecular basis for cultivar differentiation and genetic fingerprinting in rice.

The genetic similarity (GS) matrix was then computed using the produced SCoT profiles, as shown in **Table (5).** Among the seven rice entries; GS coefficients ranged from 0.81 (between Sakha-106 and IET-1444, and between Sakha-106 and HR-5824) to 0.90 (between Sakha-101 and Giza-179), with an average genetic similarity of 0.86. These results indicate that all parental genotypes differ genetically at the DNA level, confirming sufficient diversity for hybridization in breeding programs. The observed genetic variability provides useful guidance for rice breeders in selecting genetically divergent parents to enhance selection efficiency, particularly under water-deficit conditions. Such diversity may be associated with differences in yield performance among the studied genotypes. These results are in a line with those previously documented by **Patidar *et al.*, (2022), Abd EL-Aty *et al.*, (2023); Eissa *et al.*, (2023), Mollier *et al.*, (2023), Kalita *et al.*, (2024), Nivedha *et al.*, (2024), Youssef *et al.*, (2024), and Safitri *et al.*, (2025).**

The UPGMA technique was used to create a cluster dendrogram based on the GD coefficients that were produced from the SCoT (Fig. 2). The rice cultivars were divided into two major clusters by the resulting dendrogram. PL-77-8-04 and Sakha-106 made up the first cluster, and IET-1444, Sakha-107, and HR-5824 made up the second cluster, which was further broken into two sub-clusters: Giza-179 and Sakha-101 were in the second sub-cluster.

Heterotic Effects for Morpho-Physiological and Yield Traits:

Mid-parent (MP) and better-parent (BP) values for morpho-physiological, yield, and related variables were used to assess heterotic impacts in both well-watered and water-deficient environments (Tables 6 and 7). For every feature under study, with the exception of plant height, a positive heterosis value was considered desirable.

Under normal and drought conditions, respectively, eleven and six hybrids exhibited desired substantial negative MP heterosis for short stature. The crosses Sakha 101 × HR 5824 (when well-watered) and Sakha 101 × Giza 179 (when drought stressed) showed the most desired decreases in plant height. In terms of BP heterosis, Sakha 101 × Giza 179 showed the highest negative BP heterosis (-3.13%) during drought, while PL-77-8-4 × HR 5824 showed the largest reduction (-6.35%) after regular irrigation.

For flag leaf area, significant positive MP heterosis was observed in 18 and 20 hybrids under normal and drought circumstances, respectively. The hybrid Giza 179 × IET 1444 recorded the most ideal BP heterosis across settings and expressed the highest MP heterosis in both environments (32.84% and 34.90%).

Nineteen and eighteen hybrids showed substantial positive MP heterosis in terms of the number of panicles per plant under normal conditions and drought conditions, respectively. While Sakha 101 × Sakha 106 performed better in well-watered settings (16.92%), the cross IET 1444 × HR 5824 displayed the highest MP and BP heterosis (30.96%) under drought.

For spikelet sterility (%), fifteen and fourteen hybrids exhibited desirable negative MP heterosis, while nine hybrids showed significant negative BP heterosis under both environments. The crosses IET 1444 × HR 5824 and Giza 179 × HR 5824 showed the greatest reductions under normal (-58.11% and -56.49%) and drought conditions (-51.03% and -54.44%), respectively. Moreover, Giza 179 × IET 1444 recorded the lowest BP heterosis (-37.25%) under normal conditions, while Sakha 101 × Sakha

106 exhibited the most desirable reduction (-49.66%) under drought.

For panicle length (cm), nineteen and eighteen hybrids exhibited profitable positive MP heterotic effect under normal and drought conditions, respectively. The crosses Giza 179 × HR 5824 and Giza 179 × IET 1444 recorded the highest MP and BP heterosis under both environments (22.19–23.54% and 13.55–15.18%, respectively).

Under both water regime conditions, respectively, eleven and nine hybrids exhibited significant MP heterosis for panicle weight (g). Under both irrigation regimes, the hybrid Giza 179 × IET 1444 exhibited the highest levels of MP heterosis (40.07% and 37.82%) and BP heterosis (33.56% and 37.11%).

For 1000-grain weight, 8 and 5 hybrids exhibited significant positive mid-parent (MP) heterosis under normal and drought stress conditions, respectively. Under normal irrigation, the cross **Giza 179 × IET 1444** recorded the highest MP heterosis (14.49%), whereas **Giza 179 × HR 5824** displayed the greatest heterotic response (13.97%) under drought stress. Notably, both crosses also expressed the highest better-parent (BP)

In terms of yield/plant^{-1}, 8 hybrids demonstrated significant and positive mid-parent (MP) heterosis across both environmental conditions. Under normal irrigation, the hybrid GZ 179 × IET 1444 exhibited the highest MP heterosis (33.17%), whereas IET 1444 × HR 5824 showed the greatest value (30.94%) under drought stress. Regarding better-parent (BP) heterosis, GZ 179 × IET 1444 achieved the top value (30.34%) under well-watered conditions, while IET 1444 × HR 5824 outperformed all other hybrids under drought (24.43%). These results balanced with previous findings reported by **Gaballah *et al.*, (2025), and Ojah *et al.*, (2025).**

Hybrid Vigour Prediction Based on SCoT Genetic Distance (Genetic Similarity):

This study examined the relationship between the expression of hybrid vigor inside rice accessions cultivated under both drought and well-watered circumstances and molecular genetic distance (GD), as assessed by SCoT marker. For important agronomic variables, mid-parent (MPH%) and better-parent heterosis (BPH%) were associated with genetic variation among parental lines **(Table 8).** With the exception of spikelet sterility, which had a substantial positive connection with both MPH% (r = 0.31) and BPH% (r = 0.33), the majority of characteristics displayed weak or non-significant correlations under normal irrigation. Stronger relationships were found under drought stress for panicle weight (BPH% r = 0.23), 1000-grain weight (BPH% r = 0.30), spikelet sterility (MPH% r = 0.41; BPH% r = 0.36), and grain yield per plant (BPH% r = 0.23). These findings indicate that greater genetic divergence among parents enhances heterosis, particularly for yield-related traits under stress conditions. Thus, SCoT-based GD can serve as a moderate predictor of hybrid performance and sterility percentage under drought environments. The results confirm that the predictive value of GD for heterosis is both trait- and environment-dependent, being more relevant under water deficit than under optimal conditions. Consequently, breeding programs targeting drought tolerance should prioritize genetically diverse parents to improve hybrid performance and yield stability. These outcomes corroborate earlier evidence studies (e.g., **Salem *et al.*, (2022); Manna *et al.*, 2022; Chen *et al.*, 2023; Zhu *et al.*, 2023 and Fan *et al.*, 2024)** that also reported stronger GD–heterosis correlations under stress environments.

Pearson's Correlation Coefficients among Different Studied Traits:

Major changes in the links between the physiological, biochemical, and rice genotypes' yield-related factors were examined in this study using Pearson's correlation analysis, as shown by the correlation heatmap plot analysis under normal watering and moisture stress situations.

Under optimal irrigation (Fig 3A), photosynthetic traits (Pn, Gs, Tr, CE, Fv/Fm) and chlorophyll contents (Chlorophyll a, Chl. b, total Chl.) revealed significant positive associations with GYP, suggesting that effective photosynthesis and chlorophyll accumulation boost output. Similarly, traits related to water status (RWC, WUE, MSI) were positively associated with photosynthetic efficiency and yield, emphasizing the importance of maintaining water balance and membrane integrity under both conditions. Conversely, oxidative stress markers (H_2O_2, O_2^-, OH^-, MDA) exhibited negative correlations with yield traits, confirming their detrimental impact on growth and productivity.

Under drought stress (Fig 3B), the correlation network became more complex. While photosynthetic and water relation traits maintained positive associations with yield, their strength declined. The detrimental function of (R.O.S. molecules) under water deprivation was highlighted by the strengthening negative correlations between oxidative stress indicators and yield-related characteristics. Enzymatic (SOD, CAT, APX, POX) and non-enzymatic (SPC, LFP) antioxidants involved in oxidative stress defense displayed stronger positive correlations with yield and physiological efficiency, indicating their protective role in mitigating ROS damage. Overall, these findings demonstrate a dual regulatory mechanism: photosynthetic efficiency dominates under normal conditions, while antioxidant defense and water conservation (RWC, WUE, MSI) govern yield stability under drought. Therefore, breeding for drought-tolerant rice should focus on integrating traits linked to photosynthetic capacity, WUE, and antioxidant activity to ensure high productivity and

stress resilience. Such results align with **El-Agoury *et al.*, (2023).**

Biplot representation and PCA:

In order to determine important markers of drought tolerance and assess the contribution of physiological and biochemical variables, as well as grain yield per plant, to the overall diversity across rice genotypes, principal component analysis (PCA) was carried out under both normal and drought conditions. Based on eigenvalues greater than one, PC1 and PC2 were retained, together explaining most of the total variance.

Under normal conditions, PC1 accounted for the majority of variability and was positively associated with photosynthetic rate (Pn), grain yield per plant (GYP), water-use efficiency (WUE), membrane stability index (MSI), and soluble protein content (SPC). Traits such as MSI, GYP, and Pn showed the highest positive loadings, indicating their strong contribution to productivity and photosynthetic efficiency under favorable environments. In contrast, antioxidant enzymes, chlorophyll pigments, and oxidative stress markers loaded negatively, suggesting limited relevance under non-stress conditions.

Under drought stress, PC1 was mainly driven by positive loadings from Pn, WUE, MSI, GYP, O_2^-, MDA, and OH^- radicals, highlighting the importance of maintaining membrane integrity, photosynthetic performance, and yield stability under water deficit. Negative loadings of antioxidant enzymes and chlorophyll pigments indicated that drought-tolerant genotypes relied more on physiological efficiency than on biochemical defense. PC1 effectively differentiated tolerant genotypes (high Pn, WUE, MSI, GYP) from sensitive ones, highlighting these characteristics as important markers of drought resistance.

In PCA biplots, acute angles (<90°) between variable vectors indicate positive associations, whereas obtuse angles (>90°) denote negative ones. Most studied traits showed acute angles, suggesting positive correlations, though their strength varied among traits. yield/plant^{-1}(GYP) showed a substantial positive connection beside Leaf free proline (LFP), soluble protein content (SPC), antioxidant enzymes (APX, CAT, SOD, POX), chlorophyll pigments (Chl a, Chl b, total Chl), water-use efficiency (WUE), membrane stability index (MSI), and photosynthetic rate (Pn) under both normal and drought conditions. Conversely, GYP correlated negatively in regarding (MDA, H_2O_2, O_2^-, and OH^-) oxidative stress indicators

Under normal conditions, PC1 (34.8%) and PC2 (12.1%) together explained 46.9% of total variance, with Pn, GYP, WUE, MSI, and SPC contributing most to yield potential. In contrast, oxidative stress traits were negatively oriented, indicating their detrimental effects on productivity. Hybrids such as P3×P4, P2×P5, and P4×P7 clustered positively with yield-related traits, while parental lines P6 and P7 associated with oxidative stress markers, reflecting lower yield potential.

Under drought stress, PC1 (44.6%) and PC2 (9.7%) together explained 54.3% of the variance. Traits including Pn, WUE, GYP, MSI, CE, SPC, and LFP were dominant positive contributors, whereas oxidative stress markers loaded negatively. Antioxidant enzymes clustered closely, indicating coordinated ROS detoxification. Overall, PCA effectively distinguished drought-tolerant genotypes with higher physiological efficiency and yield stability from sensitive ones characterized by oxidative stress and reduced productivity. Drought-tolerant hybrids such as P3×P4, P4×P6, P3×P6, and P2×P5 were positioned on the positive side of both PC1 and PC2 (first and fourth quadrants), closely aligned with photosynthetic efficiency, water-use efficiency (WUE), membrane stability index (MSI), and yield traits. This clustering reflects their superior physiological stability under water regime stress. In contrast, sensitive genotypes (P6 and P7) were located in the negative PC1 region (third quadrant), closely associated with oxidative stress markers, confirming their drought susceptibility.

Hybrids such as P1×P5 and P2×P6 showed moderate association with chlorophyll efficiency and MSI but weaker links to yield. Acute angles among GYP, Pn, WUE, MSI, and SPC confirmed strong positive correlations, identifying these traits as key determinants of drought tolerance. Relative water content (RWC) also clustered with yield-related traits, reinforcing its role in adaptation. Conversely, oxidative stress markers (O_2^-, H_2O_2, OH^-, MDA) showed negative orientation to yield traits, indicating their adverse effect on productivity. Antioxidant enzymes (APX, CAT, SOD, POX) clustered together but opposed yield traits, implying that high enzyme activity signals stress response rather than tolerance efficiency. Overall, PCA revealed that drought tolerance depends on sustaining photosynthesis, membrane integrity, and WUE while minimizing oxidative damage. These findings align with earlier reports highlighting these mechanisms as central to drought adaptation. Identified tolerant hybrids represent promising candidates for breeding drought-resilient rice cultivars. These outcomes support previous findings documented by **Kaysar *et al.*, (2022); Al-Khayri and El-Malky (2023); Hallajian *et al*., (2024) and Naik *et al*., (2025)**.

Table (1): Information on the identity, ancestry, provenance, and type of included genotypes.

NO.	Genotypes	Pedigree	Origin	Type
1	Sakha 101 **(P1)**	(Gz 176 / Milyang 79)	Egypt	Japonica
2	Sakha 106 **(P2)**	(Gz 177 / Hexi 30)	Egypt	Japonica
3	Giza 179 **(P3)**	(Giza 1368-S-5-4 /Giza 6296-12-1-2)	Egypt	Indica / Japonica
4	IET 1444 **(P4)**	(TN1/CO29)	India	Indica
5	Sakha 107 **(P5)**	(GZ 177/BL1)	Egypt	Japonica
6	HR 5824 **(P6)**	(Akiyudaka x Suweon 310)	IRRI	Indica
7	PL-77-8-4 **(P7)**	Unknown	IRRI	Japonica

Table (2): Details of SCoT markers employed in this paper including sequences

S. No.	Primers Name	Sequence (5-3)	GC content	T_m
1.	**SCoT-01**	5'-ACGACATGGCGACCACGC-3'	50.0	53.6
2.	**SCoT-02**	5'-ACCATGGCTACCACCGGC-3'	55.6	52.6
3.	**SCoT-03**	5'-ACGACATGGCGACCCACA-3'	55.6	54.4
4.	**SCoT-04**	5'-ACCATGGCTACCACCGCA-3'	67.0	52.9
5.	**SCoT-05**	5'-CAATGGCTACCACTAGCG-3'	50.1	60.7
6.	**SCoT-06**	5'-CAATGGCTACCACTACAG-3'	55.7	59.0
7.	**SCoT-08**	5'-ACAATGGCTACCACTGCC-3'	50.0	60.7
8.	**SCoT-09**	5'-ACAATGGCTACCACCAGC-3'	50.0	58.3
9.	**SCoT-11**	5'- ACAATGGCTACCACTACC -3'	67.0	57.0
10.	**SCoT-13**	5'- ACCATGGCTACCACGGCA -3'	61.0	55.0
11.	**SCoT-14**	5'- ACCATGGCTACCAGCGCG -3'	56.0	61.5

Table 3. SCoT primers details, size range (bp), total and polymorphic bands, polymorphism (P%), frequency (F), P.I.C., and resolving power (R) for seven rice genotypes.

No.	Identification	length (bp)	T.N.B	N.M.P	N.P.B	P%	F.	P.I.C.	R
1	**SCoT-01**	170-1500	**17**	**12**	5	**29**	0.81	0.26	2.7
2	**SCoT-02**	150-1400	16	8	**8**	50	0.70	0.53	4.3
3	**SCoT-03**	260-860	12	6	6	50	0.77	0.39	3.2
4	**SCoT-04**	170-880	15	8	7	47	0.69	**0.65**	**4.7**
5	**SCoT-05**	190-600	**11**	4	7	64	**0.68**	0.48	4.4
6	**SCoT-06**	160-840	**11**	5	6	55	**0.68**	0.51	4.5
7	**SCoT-08**	150-1300	13	5	**8**	62	0.74	0.31	3.7
8	**SCoT-09**	230-1200	**11**	**3**	**8**	**73**	0.78	0.28	3.1
9	**SCoT-11**	180-1100	14	9	5	36	**0.85**	**0.23**	**2.1**
10	**SCoT-13**	170-1300	14	10	**4**	**29**	0.82	0.38	2.6
11	**SCoT-14**	170-850	15	8	7	47	0.77	0.39	3.2
Total		-	149	78	71	-	-	-	-
Mean		-	**13.5**	**7.0**	**6.5**	**49.3**	**0.75**	**0.40**	**3.5**

Table 4. Marker size (bp), total markers, and distinct + and - SCoT markers that distinguish.

Rice cultivars	Positive unique markers		Positive unique markers		Total
	Primer (band size bp)	Total	Primer (band size bp)	Total	
Sakha-101	SCoT-08 (790bp) SCoT-11 (950bp)	2	SCoT-09 (230bp) SCoT-11 (250bp)	2	4
Sakha-106	SCoT-01 (1500bp) SCoT-04 (290bp) SCoT-06 (530bp)	3	SCoT-03 (630bp) SCoT-04 (420bp) SCoT-09 (1200bp) SCoT-14 (800bp)	4	7
Giza-179	SCoT-02 (220bp)	1	SCoT-08 (1000bp) SCoT-09 (890bp)	2	3
IET-1444	SCoT-03 (380bp) SCoT-05 (520bp) SCoT-11 (220bp)	3	SCoT-11 (410bp)	1	4
HR-5824	SCoT-04 (260bp) SCoT-13 (1300bp)	2	SCoT-03 (520bp)	1	3
PL-77-8-4	SCoT-02 (150bp) SCoT-04 (740bp) SCoT-13 (170bp)	3			3
Total		14		10	24

Table 5. Genetic similarity coefficients based on SCoT marker analysis among parents.

Cultivars	Sakha-101	Sakha-106	Giza-179	IET-1444	Sakha-107	HR-5824	PL-77-8-04
Sakha-101	1.00						
Sakha-106	0.82	1.00					
Giza-179	0.90	0.85	1.00				
IET-1444	0.84	0.81	0.83	1.00			
Sakha-107	0.88	0.82	0.88	0.88	1.00		
HR-5824	0.86	0.81	0.84	0.83	0.89	1.00	
PL-77-8-04	0.89	0.89	0.87	0.85	0.86	0.85	1.00

Table 8. Correlation coefficients between (MPH%), (BPH%) heterosis, and parental genetic distance (GD) for all parameters under both circumstance (N and S).

Traits	Irrig.	MPH %		BPH %	
		r	P-value	r	P-value
Plant Height (cm)	N	-0.03	0.09 ns	−0.016	0.09 ns
	S	-0.21	0.25 ns	0.33	0.12 ns
Leaf Area index (cm^2)	N	0.24	0.18 ns	0.18	0.11 ns
	S	0.35	0.11 ns	0.38	0.13 ns
No. of Panicles/$plant^{-1}$	N	-0.03	0.12 ns	−0.15	0.15 ns
	S	0.20	0.28 ns	0.27	0.18 ns
Spikelet's Sterility (%)	N	0.31	0.04 *	0.33	0.02 **
	S	0.41	0.02 **	0.362	0.03 **
Panicle Length (cm)	N	0.15	0.18 ns	0.26	0.15 ns
	S	0.30	0.14 ns	0.40	0.09 ns
Panicle Weight (g)	N	0.21	0.20 ns	−0.14	0.12 ns
	S	0.34	0.10 ns	0.23	0.04 *
1000 Grain Weight (g)	N	0.13	0.22 ns	−0.06	0.08 ns
	S	0.25	0.18 ns	0.30	0.04 *
Grain Yield/$plant^{-1}$	N	0.16	0.08 ns	0.21	0.07 ns
	S	0.31	0.12 ns	0.23	0.02 **

Asterisks (*) and **, respectively, denote significance at $P \leq 0.05$ and $P < 0.01$., ns refere to not significant.

Table 6. Heterosis% relative to mid parent (MPH) and better parent (BPH) is calculated for 21 F1 hybrids using half-diallel analysis of yield and agronomic parameters under water stress (S) and normal irrigation (N).

Trait / Cross Combination	Plant height (cm)				Flag leaf area (cm^2)				Number of panicles $plant^{-1}$				Spikelet's Sterility (%)			
	MP		BP		MP		BP		MP		BP		MP		BP	
	N	S	N	S	N	S	N	S	N	S	N	S	N	S	N	S
P1xP2	0.83	-1.96	7.96**	1.72	4.86**	9.95**	-1.62	2.94	19.69**	22.42**	16.92**	21.2**	-48.07**	-52.26**	-33.8**	-49.66**
P1xP3	-3.01**	-5.44**	-2.82**	-3.13**	-2.99 *	2.38	-11.02**	-7.48	15.86**	15.42**	9.04*	4.38	7.38*	26.28*	51.86**	44.74**
P1xP4	-0.44	5.94**	11.08**	12.59**	22.24**	17.25**	12.17**	5.88	12.5**	14.35**	-2.27	-6.28*	-3.24	19.76*	57.47**	48.31**
P1xP5	0.02	4.75**	2.71**	8.64**	-12.25**	-14.49**	-13.58**	-17.06**	15.54**	8.6*	-4.07	-9.51*	-49.28**	-33.32**	-28.9**	-14.92**
P1xP6	2.25**	2.68*	7.95**	7.03*	14.28**	12.98**	2.04	-2.29	7.61*	5.84*	-10.4**	-15.8**	-44.01**	-41.5**	-17.69*	-5.29*
P1xP7	-6.67**	-0.93*	-3.22**	1.68	3.93 *	3.97 *	-4.53*	-7.84	10.99*	20.87**	-13.1**	-8.2**	-21.13*	-9.45*	63.22**	60.18**
P2xP3	2.52**	2.00	9.54**	8.51**	-10.95**	-0.79	-13.09**	-4.49	7.61*	6.58*	-0.91	-2.74	26.41*	21.37*	44.03*	32.51*
P2xP4	-1.59*	0.88	16.3**	11.19*	11.56**	20.04**	8.94**	15.47**	4.0	-2.31	-11.7**	-19.1**	13.9*	13.1*	54.06**	33.9**
P2xP5	-1.19**	-3.98**	8.03**	3.37	19.61**	25.9**	13.86**	14.58**	16.77**	12.35**	-5.43**	-5.46**	-25.55**	-27.64**	-19.44**	-13.17**
P2xP6	-1.98**	-2.72**	9.98	5.23	15.03**	9.80**	-1.41**	-11.62	6.96*	17.69**	-13.13	-5.46*	-20.88**	-40.04**	-8.99*	-7.49*
P2xP7	1.88*	1.47	12.41**	8.15**	-0.37	0.93	-11.97	-16.59	18.1**	13.18**	-9.96	-13.11	-16.35*	-15.4*	46.35**	43.21**
P3xP4	-1.12*	-0.95*	10.09**	2.85*	32.84**	34.9**	32.77**	34.79**	23.41**	26.58**	14.47**	15.85**	-46.22**	-32.09**	-37.25**	-26.81**
P3xP5	-3.82**	4.68**	-1.43**	6.01**	-5.91**	-4.99 *	-12.48**	-16.44**	10.95**	1.77	-1.36	-5.46	21.04 *	10.56 *	50.61**	46.86**
P3xP6	2.5**	6.52**	8.0**	8.42**	-0.27	10.96**	-16.69	-14.09	4.05	6.75	-7.25	-4.92	27.64 *	-11.12 *	67.54**	48.55**
P3xP7	-6.44**	-3.23**	-3.18**	-3.03**	24.59**	32.64**	7.38**	5.56**	19.9**	16.94**	0.91	0.1	-56.49**	-54.44**	-16.81**	-17.02**
P4xP5	1.32	1.44	9.86**	3.97**	0.37	9.82**	-6.59**	-3.48	13.57**	11.33**	8.46*	9.55*	-6.9*	-6.42*	38.94**	36.38**
P4xP6	-4.76**	11.18**	5.87	14.52**	-0.33	7.21*	-16.69	-17.06	11.05*	10.51*	6.35	3.82	-15.84*	-20.45*	31.35**	43.21**
P4xP7	1.33	2.47	10.85**	6.19**	27.63**	28.64**	10.07**	2.29**	25.88**	36.89**	13.22**	22.93**	-58.11**	-51.03**	-9.52**	-4.49**
P5xP6	6.28**	9.97**	9.2**	10.53**	7.84**	2.99	-2.38	-7.84*	14.78**	15.32**	14.44**	10.19**	-16.39 *	-33.87 *	-10.68*	-11.18*
P5xP7	-0.14	7.57**	0.82	8.72**	-0.19	-6.47*	-7.0**	-14.29**	18.28**	12.9**	10.4	3.19	-12.27*	3.94	46.29*	55.19*
P6xP7	-7.96**	2.02	-6.35**	3.64**	4.58*	6.08*	1.36	3.33	14.81**	5.49*	7.51	0.69	-16.39*	12.79*	27.68	22.82

Asterisks (*) and **, respectively, denote significance at $P \leq 0.05$ and $P < 0.01$.

Table 7. Estimates of the better-parent (BPH) and mid-parent (MPH) heterosis for yield attributes under drought (S) and normal (N) circumstances for all hybrids.

Trait / Cross Combinations	Panicle length (cm)				Panicle weight (g)				Weight of 1000 grains (g)				Grain yield / plant^{-1}			
	MP		BP		MP		BP		MP		BP		MP		BP	
	N	S	N	S	N	S	N	S	N	S	N	S	N	S	N	S
P1xP2	14.82**	3.86**	13.49**	2.51	15.61**	16.71**	15.13**	6.96**	5.48**	-1.70	5.32**	-5.46	10.32**	10.27**	3.84	7.07
P1xP3	5.56**	2.38*	2.78	2.22	-14.05**	-19.08**	-18.37**	-19.21**	-0.05	-9.18**	-0.54	-14.55**	-16.69**	-11.13**	-18.3**	-17.6**
P1xP4	3.93*	-6.67*	0.54	-8.47*	-14.64**	-19.81**	-22.90**	-20.10**	2.76**	1.26	-4.74	-10.5	-21.14**	-4.87*	-24.28**	-15.84
P1xP5	16.54**	2.95**	10.81**	-1.12	15.54**	4.09*	5.67	-0.21	-0.9	-6.26**	-1.52	-9.93*	9.49**	17.26**	5.19	3.05**
P1xP6	21.35**	14.93**	8.10**	3.95**	11.94**	12.82**	-3.25	-0.21	1.64	-3.68**	-1.99	-9.93	0.16	0.55	-4.68	-15.93
P1xP7	13.74**	15.75**	1.97**	4.0**	-5.17*	-18.43**	-16.86**	-22.68*	-0.97	-9.61**	-7.75**	-17.53*	-15.89**	-3.8	-21.65**	-19.27
P2xP3	3.68*	-3.15*	1.22	-4.27	-14.9**	-15.78**	-19.5**	-22.71**	-1.90	-6.83**	-2.23	-8.77*	-16.97**	-6.94*	-20.37**	-16.03**
P2xP4	1.85	6.49*	-1.21	3.11	-8.91*	-13.77**	-18.07**	-21.24**	3.75**	-2.58**	-3.67	-10.16**	-11.45**	-8.28*	-16.96**	-21.13**
P2xP5	3.46	-1.06	-1.36	-6.21	14.52**	13.95**	4.31	-0.21	1.35	-6.10**	0.87	-6.17	9.25**	22.3**	2.52	4.45**
P2xP6	21.87**	12.62**	9.77**	0.45	15.50**	28.87**	-0.6	3.30	2.01	-0.99	-1.48	-3.62	6.31*	25.08**	-1.24	1.47
P2xP7	6.85**	4.90*	-3.93	-7.06	15.32**	-2.98*	0.68	-16.08	2.12	-2.43	-4.71	-7.25	-9.16**	2.56	-17.52*	-16.48
P3xP4	13.3**	17.62**	13.55**	15.18**	40.07**	37.82**	33.56**	37.11**	14.49**	12.65**	6.63**	5.95**	33.17**	23.72**	30.34**	20.22**
P3xP5	5.68**	8.44**	3.24	4.0*	-10.22*	-2.21*	-13.38*	-6.39	3.02**	4.02**	2.18	1.93	-10.65**	-10.88**	-12.5**	-15.17**
P3xP6	12.33**	14.7**	3.0	3.58*	-12.56**	-0.53	-20.03*	-12.16	-3.46**	-2.66*	-7.09	-7.13	-14.5**	-6.85 *	-17.07**	-15.33**
P3xP7	22.19**	23.54**	11.81**	10.82**	26.38**	16.56**	17.16*	10.31*	12.74**	13.97**	4.83**	6.14**	15.66**	21.78**	9.75*	11.07*
P4xP5	7.03**	5.03*	5.26	2.91	-12.10**	-18.48**	-13.17*	-21.55*	1.68	0.6	-6.01	-7.17	-9.35**	-1.94	-9.41*	-2.56
P4xP6	17.05**	9.07**	8.10**	0.76	-8.58*	-11.09**	-14.63*	-21.03**	5.95**	2.68**	-5.17	-7.9	-26.33**	-20.67**	-27.1**	-24.88**
P4xP7	18.07**	17.62**	9.72**	7.95**	31.33**	37.05**	24.39**	30.41**	10.8**	10.08**	-4.45**	-3.77	31.41**	30.94**	27.16**	24.43**
P5xP6	9.14**	6.51*	2.37	0.3	16.89**	32.97**	10.57*	22.3**	-0.94	-4.52**	-3.86	-6.98	8.41**	26.2**	7.35	18.46**
P5xP7	8.18**	5.87*	2.11	-0.96	-5.93*	-4.57*	-9.76	-5.69	1.87	-3.53**	-4.46	-8.22*	-10.33**	-2.06	-13.17**	-6.37
P6xP7	8.67**	10.00**	7.94**	9.22**	3.10*	-0.31	1.59	-7.31	-9.46**	-16.59**	-12.6**	-18.6**	-10.26**	3.78	-12.26**	3.39

Asterisks (*) and **, respectively, denote significance at $P \leq 0.05$ and $P < 0.01$.

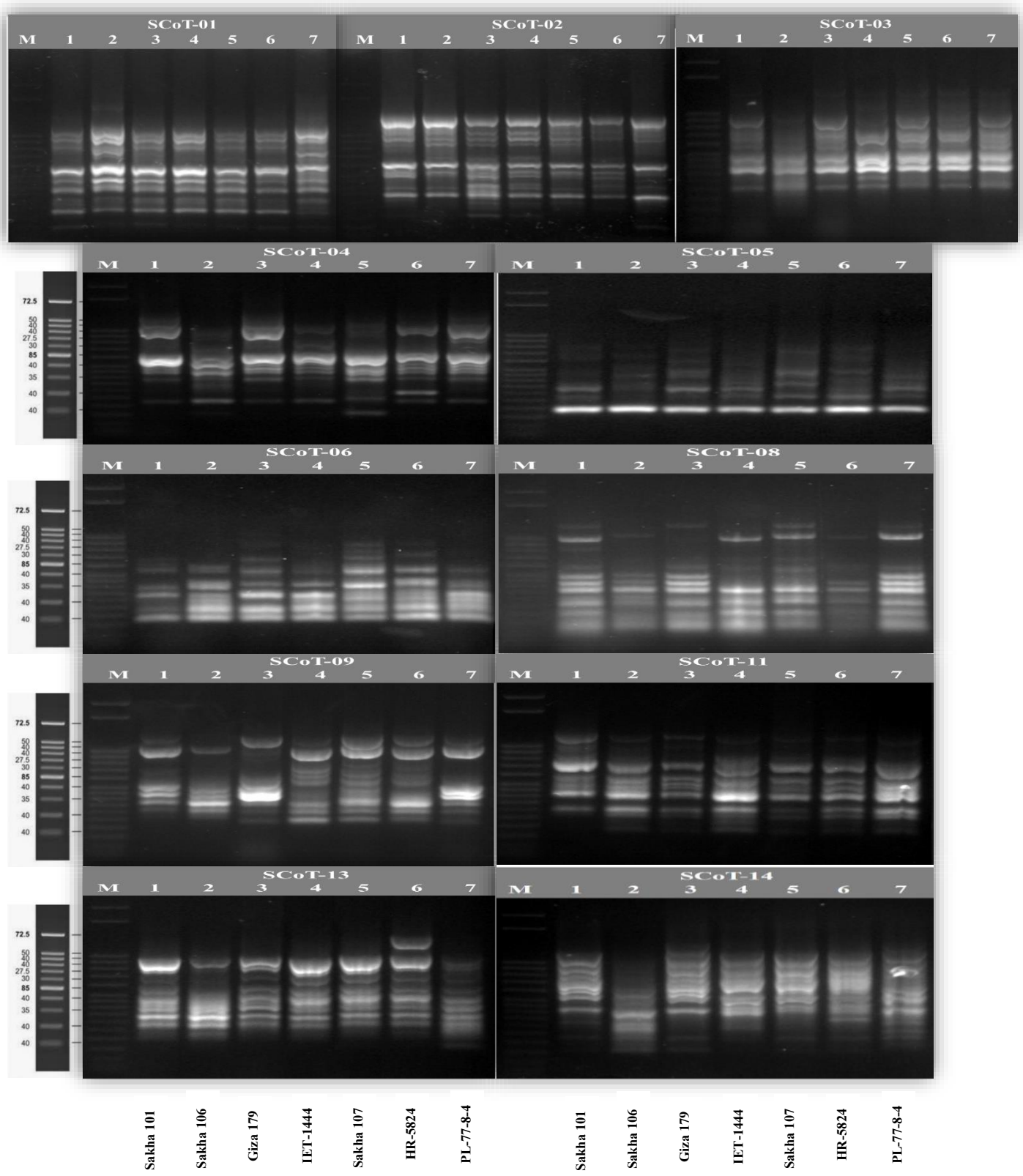


Figure 1. Banding patterns of seven rice (*Oryza sativa* L.) cultivars amplified with the SCoT primers profile of 11 SCoT markers under control and drought conditions after 60 days from sowing.

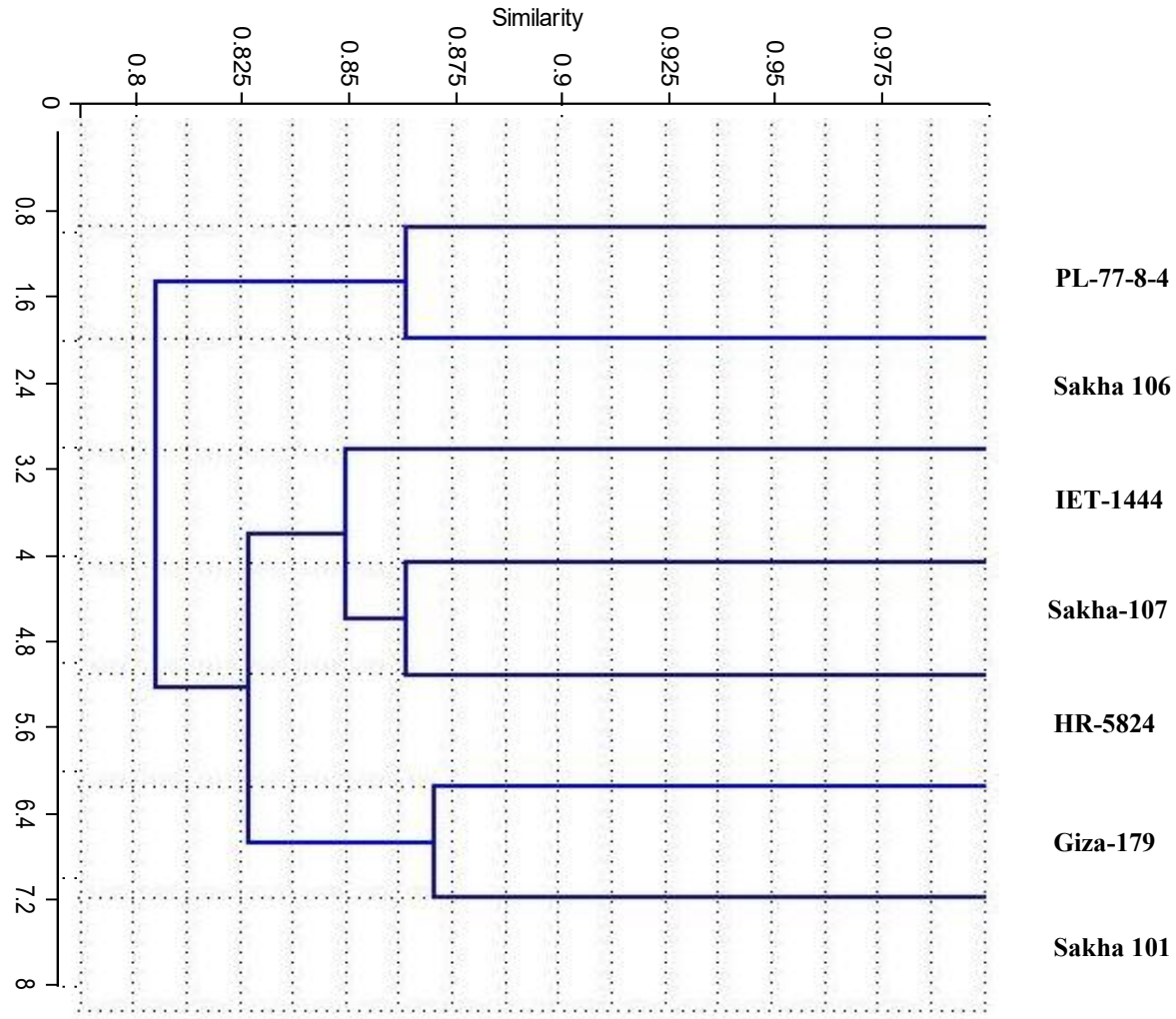


Fig. 2. UPGMA tree declaring the genetic variabilities among 7 rice (*Oryza sativa* L.) accessions based on SCoT markers.

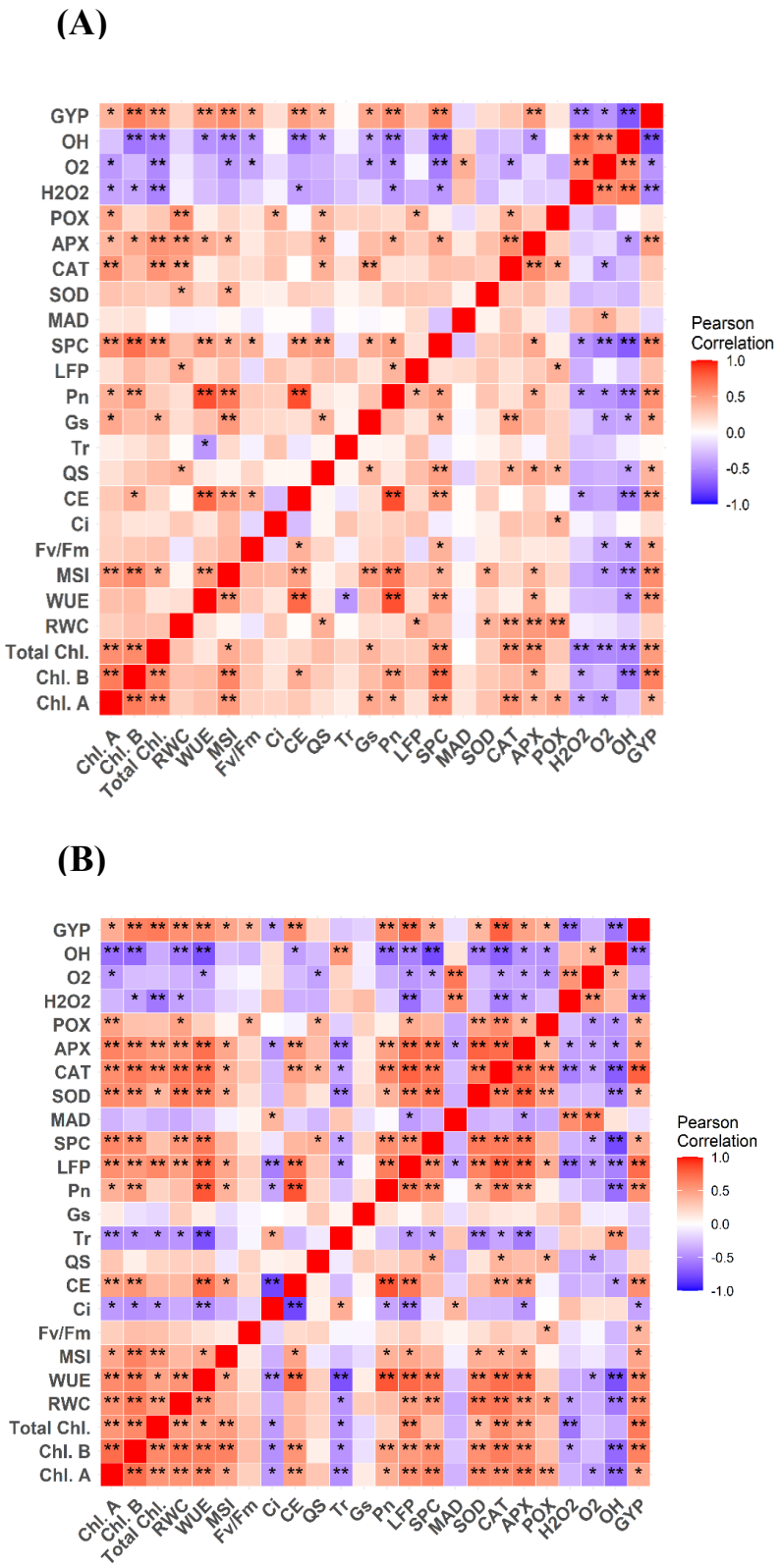


Fig. 3: Heatmap showing correlations between physiological, biochemical, and grain yield traits of rice genotypes under (A) normal and (B) drought conditions. Abbreviations: Chl. A – Chlorophyll a; Chl. B – Chlorophyll b; Total Chl. – Total chlorophyll; Fv/Fm – PSII maximum quantum efficiency; Ci – Intercellular CO_2; CE – Carboxylation efficiency; Tr – Transpiration rate; Gs – Stomatal conductance; Pn – Net photosynthesis; RWC – Relative water content; WUE – Water use efficiency; MSI – Membrane stability index; MDA – Malondialdehyde; LFP – Leaf free proline; SPC – Soluble phenols; O_2^- – Superoxide; OH^- – Hydroxyl radical; H_2O_2 – Hydrogen peroxide; POX – Peroxidase; SOD – Superoxide dismutase; CAT – Catalase; APX – Ascorbate peroxidase; GYP – Grain yield per plant.

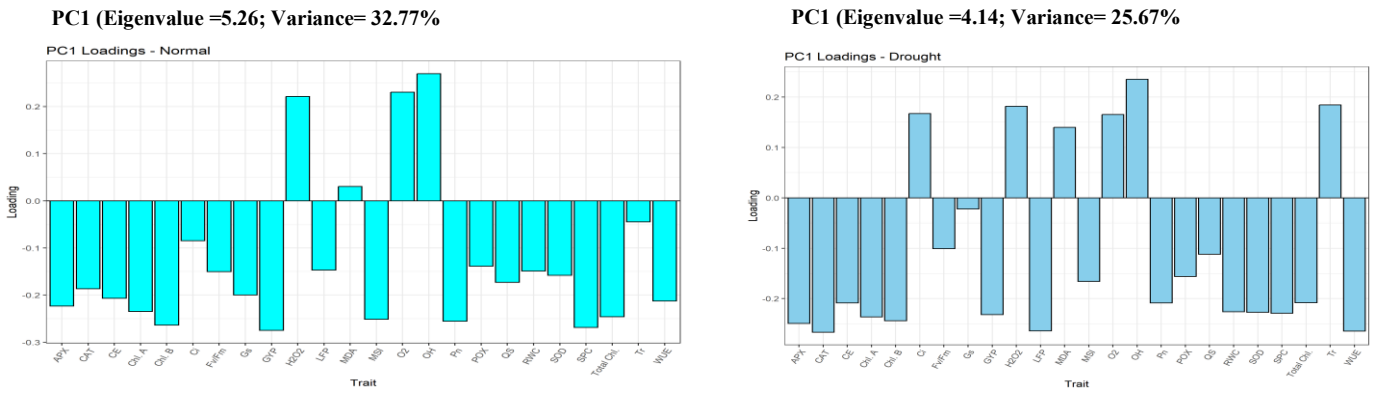
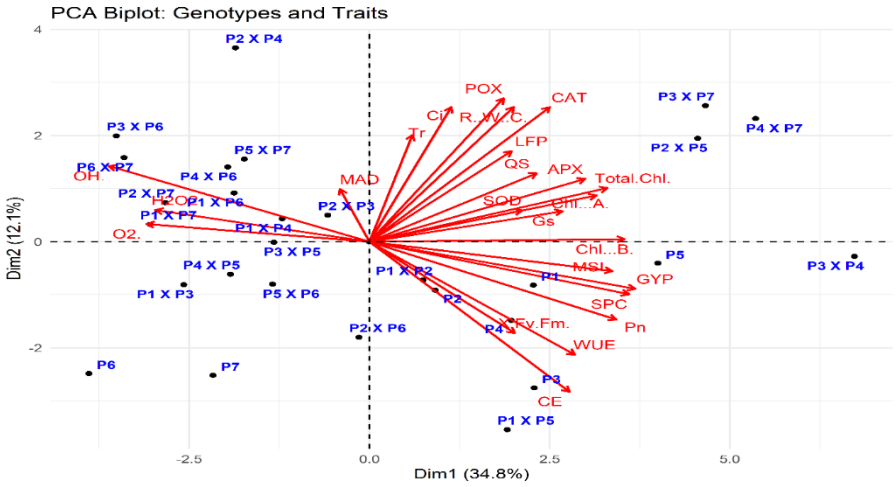


Fig. 4. The factor loadings, eigenvalue, and variance percent for studied traits among parents and crosses under (**A)** normal and (**B)** water deficit stress conditions.

(A) Variance of PC1= 34.8%, PC2= 12.1%



(B) Variance of PC1= 44.6%, PC2= 9.7%

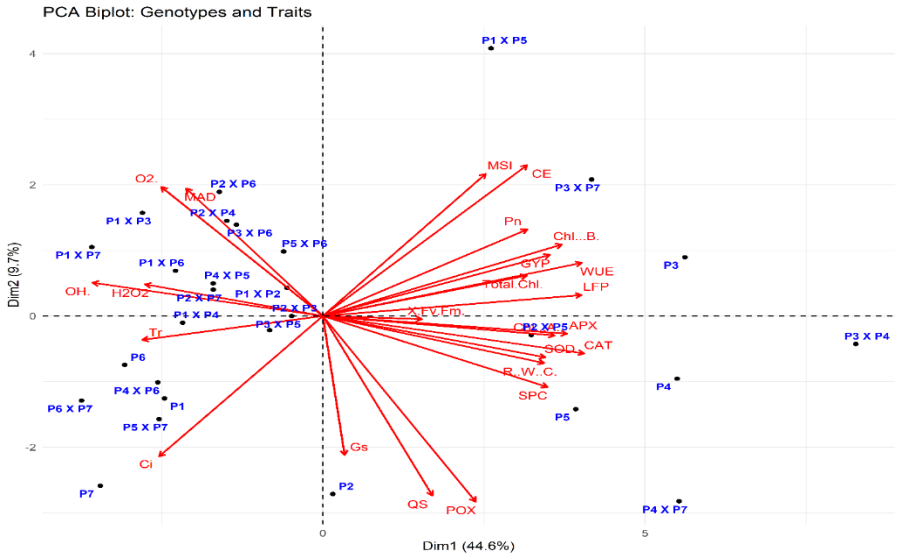


Fig. (5): PCA biplot diagram shows the parental and hybrids genotypes and all studied traits under optimum and water stress conditions.

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