

Genetic diversity, combining ability, heterosis and antioxidant enzymes of some rice genotypes under normal and water deficit.

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Abstract

Five rice lines and three testers were crossed in line x tester mating design and the resultant fifteen hybrids along with their eight parents were evaluated under normal and drought conditions (irrigation every 12 days). This investigation was undertaken at the experimental farm of Rice Research and Training Center (RRTC), Sakha Agricultural Research Station, Kafr El-Sheikh, Egypt during 2019 and 2020 growing season. Genetic diversity, combining ability, heterosis, and antioxidant enzymes were studied. Four primers were used to study genetic diversity. The results revealed a total number of 7 alleles were ranged from one to three alleles per locus, the average of major allele frequency, gene diversity, and (PIC) were 0.607, 0.45, 0.46, respectively. The GD dendrogram revealed the close similarity among the genotypes; IET 1444, GZ1368 and G177 clustered together in the main cluster. On another hand, NP856-9 diverged in sub cluster and the other genotypes G178, G179, A22, and G182 were clustered together. The drought had an intensive inhibition on studied traits, plant height, chlorophyll content, grain yield plant-1 and 1000 grain weight. Otherwise, all genotypes were more earliness under drought than under normal. Highly significant differences were detected among genotypes and their partitions for all studied traits. Both additive and non-additive are important in the inheritance of studied traits. The parents GZ 1368 under normal irrigation and combined data and IET 1444 under stress condition seemed to be the best combiner for grain yield plant-1. The cross NP856-9 X GZ 182 revealed the highest significant and positive SCA effects for chlorophyll content and grain yield/plant. The most desirable mid-parent and better-parent heterosis for grain yield plant-1 were detected for the crosses IET1444 x G182 and NP856-9 x G182, respectively in the combined data. Results indicated that the activity of antioxidant enzymes: CAT, APX, SOD, and MDA enhanced under drought conditions. Similarly, proline accumulation increased due to water stress.

Key words: Rice, Drought, Genetic diversity, Combining ability, Heterosis, Antioxidant enzymes.

1. Introduction

Water scarcity is one of the greatest challenges in the whole world. Drought is the several widespread and damaging of all environmental stresses. Egypt was suffering from severe water scarcity in recent years, which has been exacerbated by the new conflict over water with the Nile River countries. Rice harvested area in Egypt decreased from 745092 ha. in 2008 to 361075 ha. in 2018 [1] as a result of its high water needs relative to other crops. Therefore, many efforts are being made to develop new rice genotypes tolerant to drought stress since severe drought can cause up to 40% loss in rice yield [2]. A significant reduction in all physiological traits under drought stress relative to normal conditions[3]. Drought has a strong effect on yield and physiological traits. Rice responses to drought are assumed to be complex that concern various physiological, biochemical and molecular changes[4]. Grain yield/plant has reduced under water stress during vegetative, panicle initiation, flowering by 28%, 34%, and 40%, respectively[5]. Drought affected almost every growth stage causing a decrease to yield and yield components [6]. Drought led to reducing in plant height, due to reduce the rate of growth of stems [7].

Genetic diversity is the basis for the survival of plants in nature and crop improvement. Genetic

diversity helps breeders to develop varieties for specific traits like quality improvement and tolerance to biotic and abiotic stresses. Diversity is also important for the adaptability of crop plants to varied environments with special reference to changing climatic conditions. SSR markers are able to identify the allelic diversity and genetic variation among the studied rice genotypes [8]. (SSR) markers are believed to be the most suitable among the several classes of available DNA markers. Due to their ease of application, high reproducibility, codominant inheritance, rapid analysis, low cost, easy scoring patterns, greater allelic diversity and extensive genome coverage [9],[10],[11].

Combining ability is a powerful instrument in determining the best combiners that may be utilized in the hybrid program or accumulate fixable genes and obtain desirable segregates. Combining ability enables the breeder to define the pattern of gene effects in the expression of quantitative traits by determining potentially superior parents and hybrids [12]. The GCA is a function of additive genetic effects while SCA measures non-additive gene effects including dominance and epistasis [13]. The Line X Tester analysis enables estimate different types of gene actions, also provides information about the general combining ability (GCA) of parents and specific combining ability (SCA) effects of crosses [14].

One of the effective drought defense lines in plants is antioxidant enzymes: superoxide dismutase, SOD; catalase, CAT; ascorbate peroxidase, APX. Much of the damage to plants caused by abiotic stresses are associated with oxidative damage at cellular level [15]. Antioxidant enzymes activity improve drought tolerance in rice plant by preventing oxidative injury. (ROS)'s damage: cellular oxidative damage, lipid peroxidation, disruption in cellular homeostasis, protein denaturation, and DNA mutations. The enhancement of antioxidant activity is an indicator of decreasing the oxidative damage caused by drought stress [16]. The ROS can damage chlorophyll pigments, proteins, DNA, and lipid membranes leading to cell death [17]. Enhancement in antioxidant enzymes: SOD, CAT, and APX activities are the main reason for the adaptation process under drought stress conditions.

This work aimed to estimate genetic diversity among the parental genotypes using SSR marker. Study heterosis, general and specific combining ability for some yield traits and vegetative traits. Determine the behavior of antioxidant enzymes and yield in some rice crosses under water deficit relative to normal conditions.

2. Materials and methods

2.1. Plant materials:

A set of eight parents comprising of Five lines viz., NP856-9, A22, IET 1444, GZ 178, GZ 1368 and three testers viz., GZ 182, GZ 177, GZ 179 of *Oryza sativa* were selected for this study. Seeds of the parental lines were obtained from the genetic stock of the Rice Research and Training Center (RRTC), Egypt. The names, pedigree, type and origin of included lines are shown in Table 1.

Table (1) Names, pedigree, type and origin of included lines.

NO	Entry name	Pedigree	Type	Origin
1	NP856-9	Unknown	Indica	IRRI
2	A22	IR47664	Indica	Sri-lank
3	IET 1444	TN1/CO29	Indica	India
4	GZ 178	Giza175/ Milyang 49	Indica / Japonica	Egypt
5	Gz.1368-5-4	IR1615-31/BG94-2	Indica	China
6	GZ 182	Giza181/IR39422-161-1-3/ Giza181	Indica	Egypt
7	GZ 177	[Giza 171] Ymji No.1 // PiNo.4	Japonica	Egypt
8	GZ 179	(GZ 6296-12/GZ1368-5- S-4)	Indica/ Japonica	Egypt

2.2. Field Experiment:

In 2019 growing season, the eight parent's grains were sown. After thirty days old seedlings each parent was individually transplanted in the permanent field in two rows, 5 meters long and 20 x 20 cm apart between plants and rows. At flowering stage during this season, the five lines were crossed with the three testers to produce 15 F1 crosses using bulk emasculation method [18] by using hot water (42-44 °C for 10 min).

In 2020 summer season, the parents and their F1 crosses, were sown 30th April, then seedlings were transplanted into two adjacent experimental fields. The first one was normally irrigated every 6 days (continuous flooding) and the second one (drought stress) was irrigated every 12 days. After 30 days from the sowing, seedlings of each genotype were individually transplanted into their permanent field (s) in a randomized complete block design (RCBD) with three replications. Each genotype (parents and F1 crosses) was planted in three rows per replicate. Each row was 5.0 m long with the spacing of 20 × 20 cm among rows and hills. Water stress was applied after 10 days from transplanting. The remain recommended agricultural rice practices were applied at the proper time. Data were recorded for number of days to 50% heading, plant height, chlorophyll content, and grain yield/ plant.

2.3. Drought measurements:

For determination of enzymatic antioxidants, leaf samples were extracted in 50 mM phosphate buffer (pH 7.8). The extract was centrifuged at 15,000 rpm @ 4°C and the supernatant was used for further assay of MDA according [19], catalase (CAT) [20], superoxide dismutase (SOD) [21], and ascorbate peroxidase (APX) [22]. Proline content was measured according to [23], Relative water content (RWC) was estimated according to [24].

Total chlorophyll (Tchl) was estimated according to [25] concentrations as mg/g fresh weight of leaves were extracted. Leaves samples (0.5 g) were homogenized with acetone (90%v/v), filtered and make up to a final volume of 20 mL. Chlorophyll concentrations were calculated spectrophotometer from the absorbance of extract at 661.6, 644.8 and 470 nm.

2.4. Genetic diversity

2.4.1. DNA extraction

Genomic DNA was extracted from eight rice genotypes lines after twenty days from planting using modified CTAB method [26]. DNA integrity was checked using 1% agarose gel electrophoresis and the image was captured using gel documentation system (Gel Doc. BioRad). Concentration and purity of purified DNA were

measured by BioTek Epoch2 Microplate reader (Thermo Scientific, USA). For all samples, DNA purity was $>1.8 \pm 0.20$ under absorbance ratio A260/A280.

2.4.2. PCR Amplification and SSR analysis:

Eight genotypes were subjected to molecular diversity analysis using four SSR primers. (Table 2). All four SSR markers were found to be polymorphic and they used for the SSR analysis. PCR reaction was performed following the conditions of [27]. The PCR mixture (25 μ l) contained 0.2 μ M of each primer with concentration of 10 pM, 200 μ M of dNTPs mix, 2.5 μ L of 10 \times PCR reaction buffer, 1.5 μ M MgCl₂, 2 units of Promega Taq DNA polymerase, 2 μ L of template DNA and the final volume was adjusted with sterilized double distilled water. PCR thermocycler (AriaMx) was used to amplify the reactions consisting of 95 °C for 3 min followed by 35 cycles at 95 °C for 50 s, annealing temperature was calculated for each primer and lasted for 1 min with an extension of 72 °C for 1 min followed by final extension temperature at 72 °C for 5 min. Amplified PCR products were stored at -20 °C for further purification and downstream application, then 3 μ l of PCR amplicons was loaded on 2 % agarose gel electrophoresis stained with Ethidium bromide using GeneRuler™ 1 kb DNA ladder, then visualized using gel documentation system (Gel Doc. BioRad).

2.5. Data Analysis

The analyses of variance for all studied traits under normal irrigation and drought stress condition as well as combined data over both experiments were performed according [28]. General and specific combining analyses were

estimated for days to 50% heading, plant height, chlorophyll content as well as grain yield plant⁻¹ according to line x tester model [29]. Heterosis percentage relative to mid- parent and better parent for grain yield plant-1 was estimated according to [30],[31].

3. Results and discussion

3.1. Genetic Diversity

In this study four SSR primer pairs were investigated, four pairs revealed polymorphic pattern among the eight genotypes (Table 3 and Figure 1). A total number of 7 alleles were ranged from one to three alleles per locus with an average of 1.75 alleles per locus. The average of major allele frequency was 0.607 with a stretched range from 0.44 at locus RM225 to 0.72 at locus RM269. The gene diversity values ranged from 0.4 at loci RM115 and RM269 to 0.49 at loci RM225 and RM217 with an average of 0.45. While, the average of polymorphic information content (PIC) was 0.46 with values varied from 0.378 at locus RM115 and RM269 to 0.603 at RM225 locus. Similar results [10], [11],[8]. The GD dendrogram revealed the close similarity among the genotypes; IET 1444, GZ1368, GZ177 that clustered together in a main cluster. On the other hand, the genotype NP856-9 diverged in sub-cluster and the other lines GZ178, GZ179, A22, and GZ182 were clustered together (Figure 2). Genetic distance values ranged from 0.310 to 0.880 and averaging 0.595 (Table 4). The lowest genetic distance was detected between GZ182 and A22 as indica type, whereas the highest genetic distance was found between the parent A22 and GZ 177 as japonica type. These similarities among the parental lines GZ177 and GZ1368 may be attributed to studied primers which may be related to grain yield per plant under this study.

Table (2) SSR Heading Date Primers for rice genotypes

No.	Marker	Forward	Reverse	AT
1	RM225	TGCCCATATGGTCTGGATG	GAAAGTGGATCAGGAAGGC	51
2	RM217	ATCGCAGCAATGCCTCGT	GGGTGTGAACAAAGACAC	50
3	RM115	TTGCCGCGAGTGGCCGTTACCAC	AGGAGGCGGCGGAAATGGAAGG	63
4	RM269	GAAAGCGATCGAACCAGC	GCAAATGCGCCTCGTGTC	52

Table (3) Number of alleles, major allele frequency, gene diversity and polymorphic information content (PIC) of the sixteen SSR markers used in this study.

Marker	Ch.	Size Range (bp)	Repeat Type	No. of Alleles	Major Allele Frequency	Gene Diversity	PIC
RM225	6	200-204	(CT)18	2	0.44	0.49	0.603
RM217	6	122	(CT)20	1	0.55	0.49	0.491
RM115	6	190	(GA)7	1	0.72	0.40	0.378
RM269	10	120-160	(GA)17	3	0.72	0.40	0.378
Average				1.75	.607	0.45	0.46

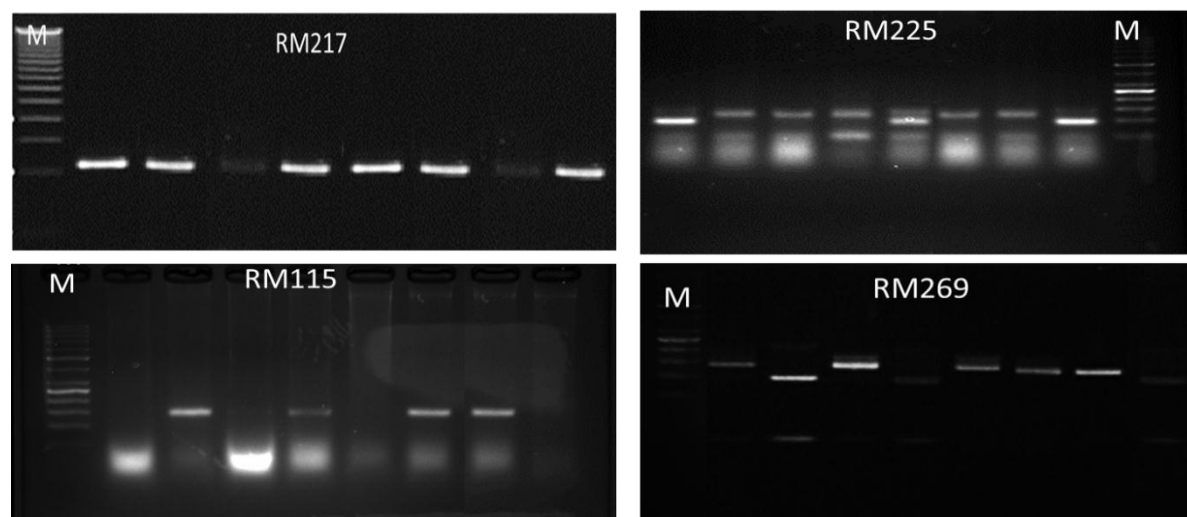


Fig. (1) PCR amplicons of some SSR markers with the eight genotypes. M denotes to 100 bp DNA ladder.

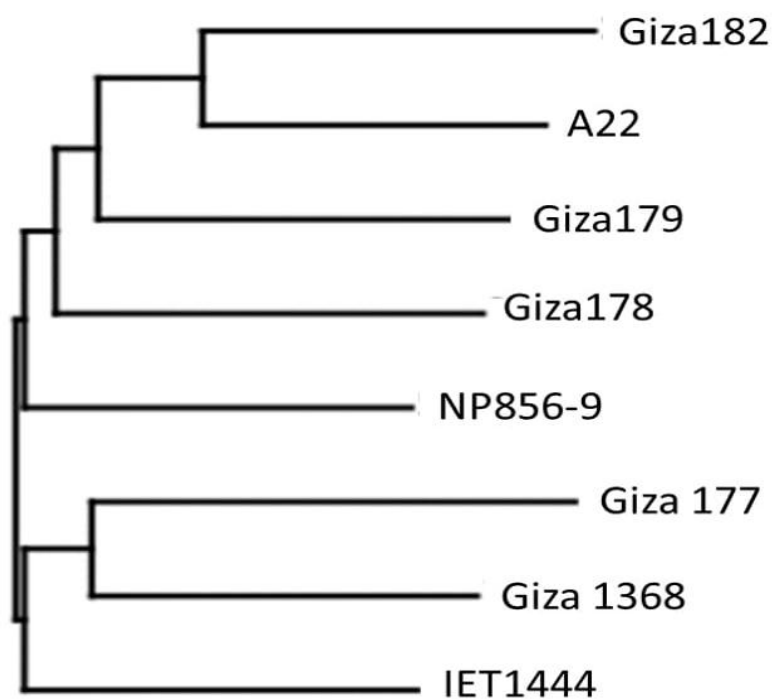


Fig. (2) Dendrogram of the eight rice lines constructed from SSR data using PAST).

Table (4) Genetic distance (GD) matrix among the eight rice genotypes based on SSR analysis

Parent	Giza182	A22	Giza 178	IET1444	NP856-9	Giza 177	Giza 1368	Giza 179
Giza182	0.000							
A22	0.310	0.000						
Giza 178	0.560	0.500	0.000					
IET1444	0.500	0.440	0.440	0.000				
NP856-9	0.630	0.440	0.440	0.380	0.000			
Giza 177	0.690	0.880	0.630	0.560	0.440	0.000		
Giza 1368	0.500	0.690	0.440	0.380	0.500	0.440	0.000	
Giza 179	0.630	0.310	0.440	0.500	0.380	0.690	0.630	0.000

3.2. Analysis of variance and mean performance

The analysis of variance for days to 50% heading, plant height, total chlorophyll content, grain yield⁻¹ and weight of 1000 grains under normal irrigation, drought stress and the combined data are presented in Table (5). Highly significant mean squares due to environments were detected for all studied traits with mean values of normal irrigation higher than those of drought stress condition (Table 6). Such results are expected since drought stress caused a severe reduction in the growth of the plants. Water stress negatively affects the physiological characteristics of rice in innumerable ways, such as decreases in transpiration rate, net photosynthetic rate, water use efficiency, stomatal conductance, internal CO₂ concentration, photosystem II (PSII) activity, relative water content and membrane stability index [32],[33], [34] ,[35]. These results are in line with those obtained by [36], [6], who found that drought affected almost every growth stage causing a decrease to yield and yield components: 1000-grain weight, tiller hill⁻¹, filled grains hill⁻¹, number of spikelets panicle⁻¹, and grain yield. Highly significant differences were detected among genotypes, parents, crosses, parents vs. crosses, lines, testers and line x tester for all studied traits under normal, drought and combined data except line x tester for plant height under normal conditions. Which reveals a wide diversity between the genetic materials involved in this study.

Genotypes mean performance for all studied traits under normal, drought and combined data are presented in (Table 6). Data revealed that the GZ 177 (T2) and GZ 182 (T1) as the parental genotypes as well as the cross combinations A22 x GZ 177 and A22 x GZ 182 had the earliest heading under normal, drought and across them. Regarding plant height, the parents GZ 179 (T3) and GZ 1368 (L5) recorded the lowest value under normal, while GZ 179 (T3) and GZ 177 (T2) had the lowest value under drought and combined data. However, the lowest mean value for plant height was exhibited by the cross GZ 1368 x GZ 182 under normal, drought and combined data.

For chlorophyll content, the parents A22 (L2) and IET 1444 (L3) recorded the highest mean value under normal irrigation and combined data. Meanwhile, IET 1444 (L3) and GZ 1368 (L5) gave higher mean values under drought conditions. Among the F₁ hybrids, (GZ 1368 x GZ 182 and GZ 1368 x GZ 179) under normal, (IET 1444 x GZ 182 and NP856-9 X GZ 182) under drought and (IET 1444 x GZ 179 and NP856-9 X GZ 182) under combined data.

Concerning grain yield plant⁻¹, the parent GZ 179 (T3) gave the highest mean value under normal, drought and combined data, followed by NP856-9 (L1) under normal and GZ 178 (L4) under drought and combined data. However, the

most desirable mean values for grain yield plant⁻¹ were detected for the cross GZ1368 x GZ179 under normal irrigation (59.070 g), drought stress (38.02 g) and combined data (48.86 g) (Table 6). The crosses GZ178 x GZ179 (under normal irrigation) and IET1444 x GZ 179 (under drought stress and combined data) ranked the second best for this trait with significant difference from the best parent (GZ179).

For 1000 grain weight, the highest mean value was detected for the parental genotypes GZ 179 (T3) and A22 (L2) under normal and combined data, while GZ 179 (T3) and GZ 178 (L4) under drought as well as the hybrids (GZ 1368 x GZ 179 and NP856-9 X GZ 179) under normal and (GZ 1368 x GZ 179 and IET 1444 x GZ 179) under drought and combined data. Such variability among rice genotypes for the studied traits were previously reported by [37] ,[38] ,[39].

In conclusion, the two studied crosses IET1444 x GZ 179 and GZ1368 x GZ179 are of prime importance and could be used in future rice breeding programs.

3.3. Combining ability analysis

Analysis of variance for general (GCA) and specific (SCA) combining abilities for days to 50% flowering, plant height, chlorophyll content, 1000 grain weight and grain yield plant⁻¹ under normal irrigation, drought and combined analyses are presented in Table (5). The mean squares due to GCA were higher than those of SCA for days to 50% under normal condition and combined data as well as plant height under normal irrigation; indicating the predominance of additive and additive x additive gene action in controlling these traits. On the other hand, mean squares due to SCA were much higher than those of GCA for days to 50% heading under drought condition, plant height under drought and combined data; chlorophyll content under both environments as well as grain yield plant⁻¹ and 1000-grain weight under normal irrigation and combined data, revealing the importance of non-additive gene action in governing these traits. The importance of additive genetic variance in controlling these traits were previously reported [40],[41]. However, Non-additive gene action was predominant in controlling rice yield and most of its attributes [42], [43]. Meanwhile, the interaction between SCA and environment was higher than of GCA x environment for all studied traits revealing that non additive gene action was more influenced by drought more than additive genetic variance.

3.3.1. General combining ability effects

Data of (GCA) for all studied traits under normal irrigation, drought stress and combined data are shown in Table 6. Positive GCA effects are desirable for chlorophyll content, grain yield per

plant and weight of 1000 grains. Unlike days to 50% heading and plant height which negative GCA are preferable.

Results exhibited that the lines A22 (L2), NP856-9 (L1) and GZ 177 (T2) had desirable significant and negative GCA effects for days to heading. However, the most desirable GCA effects for this trait were exhibited by the parent A22 recording -4.07**, -4.07** and -4.0** in under normal, drought and combined data, respectively. Such results indicated that these parents could be regarded as good combiners for earliness. Besides, NP856-9 (L1), GZ 1368 (L5) and GZ 182 (T1) revealed highly significant and negative GCA effects for plant height under normal, drought and combined data, exhibiting that these parents could be considered as good combiners for developing short stature genotypes. On the contrary, the highest desirable significant and positive GCA effects were obtained by the parents IET 1444 (L3) for chlorophyll content; and GZ 179 for 1000 grain weight under all environments. For grain yield plant⁻¹ the parents GZ 1368 under normal irrigation and combined data and IET 1444 under stress conditions seemed to be the best combiners since they expressed the highest positive and significant GCA effects for this trait. Regarding studied testers, GZ 182 seemed to be the best general combiner for plant height, chlorophyll content, grain yield plant⁻¹. The tester GZ 177 expressed the highest negative and significant GCA values for earliness, while GZ 179 was the best for 1000 grain weight under normal irrigation, drought stress and combined analyses (Table 7). Similar results were obtained by [44], [45],[46].

3.3.2. Specific combining ability effect (SCA)

Estimates of SCA effects of the 15 F1 hybrid combinations for all the studied characters under normal irrigation, drought stress and combined analyses are shown in Table (8). The results illustrated that three, two and three crosses had negative and significant SCA effects for days to 50% heading under normal, stress condition as well as combined data, respectively. However, the most desirable SCA effects were detected for the crosses G178 x G179 under normal irrigation and GZ1368x G182 under stress condition and combined data. For plant height, there was no desirable SCA effects under normal irrigation and combined analyses. However, the cross NP856-9 x G177 expressed the only significant desirable SCA for plant height under combined data. The crosses GZ 178 x GZ 182 and GZ 1368 x GZ 179 had the highest desirable effects and considered good specific combiners for short plant stature only under drought conditions. The cross NP856-9 X GZ 182 revealed the highest significant and positive SCA effects for chlorophyll content and grain yield/plant under normal, drought and

combined data and for 1000-grain weight under drought stress and combined data. Moreover, the cross G178 x GZ 177 showed the most desirable SCA for 1000-grain weight under normal irrigation.

3.4. Heterosis

Heterosis relative to mid-parent and better-parent for all the studied characters under normal irrigation, drought stress and combined data is presented in Tables (9 and 10). For days to 50% heading, negative and significant heterotic values relative to mid-parent under normal, stress condition and combined data, respectively. The respective crosses for better-parent were thirteen, thirteen and fourteen. However, the cross A22 x Giza 177 recoded the highest negative and significant heterotic effects relative to mid-parent and better-parent under drought and combined data.

Regarding plant height, none of the studied crosses expressed desirable negative heterosis relative to mid-parent under normal condition and combined data as well as relative to better parent in normal irrigation. However, four crosses expressed negative and significant mid-parent heterosis under drought condition as well as eight and one crosses for better-parent heterosis under drought stress and combined analysis, respectively. Meantime, the hybrid GZ 1368 x GZ 182 exhibited the most desirable mid-parent and better parent heterosis under drought condition being -5.29** and -9.94**, respectively. In contrast, positive and significant heterotic effects were detected by NP856-9 X GZ 182 for chlorophyll content relative to mid-parent and better-parent under normal, drought and combined data.

Concerning chlorophyll content, the cross NP856-9 x G182 expressed the most desirable mid-parent heterosis under normal irrigation, drought stress and combined analyses recording 9.12, 11.26 and 10.13%, respectively. This particular cross exhibited the most desirable better heterosis under drought stress (3.92**) and combined data (4.24**).

For grain yield, eleven, fourteen and thirteen crosses expressed positive and significant mid-parent heterosis under normal, stress condition and combined data, respectively. The respective better-parent heterosis values were detected for eleven, fourteen and twelve crosses. However, the best mid-parent heterosis values were detected for the crosses GZ1368 x GZ179 (normal), NP856-9 x GZ182 (drought) and IET1444 x GZ182 (combined data). While the most desirable better-parent heterosis were obtained for the crosses GZ1368 x GZ179 (normal irrigation) and NP856-9 x GZ182 (under drought and combined data). The highest positive and significant heterotic effects relative to mid-parent and better-parent were recorded by GZ 1368 x GZ 179 under normal conditions. While the

cross NP856-9 X GZ 182 gave the highest desirable heterosis relative to mid-parent under drought and better-parent under drought and combined data.

Concerning 1000-grain weight, the cross GZ 1368 x GZ 179 exhibited the highest positive and significant heterotic effects relative to mid-parent under normal condition and combined data.

However, the cross NP856-9 X GZ 182 showed the highest positive and significant heterotic effects relative to better-parents under normal conditions, while the cross GZ 1368 x GZ 182 had the highest positive and significant heterotic values under combined data. Similar results were obtained by [48],[43],[49].

3.5. Antioxidant enzymes

Results of proline content, relative water content, SOD, CAT, APX, and MDA for all studied genotypes under normal irrigation, drought stress condition and combined analyses are presented in Figures (3-8). Results indicated that the activity of antioxidant enzymes: CAT, APX, SOD, and MDA enhanced under drought conditions. Furthermore, proline accumulation increased due to water stress. Unlike RWC decreased under drought conditions.

The parent IET 1444 and A22 exhibited the highest mean value for CAT, APX, and RWC under drought and combined data. (GZ 1368 and GZ 178), (A22 and GZ 178) and (IET 1444 and GZ 178) gave the highest mean value under normal conditions for CAT, APX, and RWC, respectively (Fig. 6,7,4). IET 1444 and GZ 1368 gave the highest mean value for proline accumulation under drought and combined data (Fig. 3). While under normal conditions the parent IET 1444 and GZ 179 had the highest mean value. Regarding SOD, the highest mean value was recorded in the parent IET 1444 and GZ 1368 under drought conditions, (IET 1444 and GZ 179) under combined data, and (GZ 179 and IET 1444) under normal (Fig. 5). MDA lowest mean value was recorded in IET 1444 and A22 under drought and combined data, but under normal conditions it behaved differently (Fig. 8).

The crosses (IET 1444 X GZ 179) recorded the highest mean value for RWC, proline accumulation, and CAT under drought and combined data. Also, it ranked first for SOD, and APX under drought conditions. Followed by: IET 1444 X GZ 182 regarding proline accumulation,

SOD, and APX, GZ 178 X GZ 179 in terms of RWC, GZ 1368 X GZ 179 FOR CAT under drought conditions. IET 1444 X GZ 182 recorded the highest mean value for SOD and NP856-9 X GZ 182 for APX under combined data.

Concerning MDA, the crosses IET 1444 x GZ 179, IET 1444 X GZ 182 recorded the lowest mean value under drought and combined data.

Proline content, CAT, SOD, MDA, and APX increased in all involved genotypes under drought conditions compared to normal conditions (Fig.3.4.5.6.7.8). Malondialdehyde MDA, proline content, catalase (CAT), and ascorbate peroxidase (APX) were increased under drought conditions [50]. Results indicated a significant increase in CAT and SOD activity in all the genotypes under drought conditions compared with the control [51]. The most drought-tolerant genotypes had the highest mean values of proline content, CAT, SOD, and APX. While the drought-sensitive genotypes recorded the lowest values of proline content, CAT, SOD, and APX. In contrast, MDA gave the highest values in drought-sensitive genotypes. Similarly, treatments that suffer more had a higher MDA content [52]. While the drought-sensitive genotypes recorded the lowest values of proline content, CAT, SOD, and APX. Similarly, the activity of (CAT) and (APX) was higher in drought-tolerant species than drought-sensitive ones [53].

Relative water content declined in all studied genotypes under drought conditions relative to normal conditions. Similarly, relative water content (%) decreased under drought conditions compared to control [54].

Water stress leads to cell membrane damage as a result, MDA content increased under drought conditions. Drought increased reactive oxygen species (ROS) causing oxidative damage. Antioxidant enzymes improved drought tolerance in rice plants by preventing oxidative injury. Either proline or antioxidant enzymes are reactive oxygen species (ROS) scavengers. Therefore, the drought-tolerant genotypes show higher antioxidant enzymes activity and proline content, while drought-tolerant genotypes show low MDA content.

Based on the activity of the antioxidant enzymes, Genotypes IET 1444, A22, and (IET 1444 X GZ 179) were considered tolerant genotypes for drought stress.

Table (5) Mean squares for days to 50% heading, plant height, total chlorophyll content, grain yield per plant and weight of 1000 grains under normal irrigation (N) and drought stress (D) as well as the combined over them (C).

* and ** significant at 0.05 and levels of probability, respectively.

S.O.V	DF	Days to 50% heading (Days)			Plant height (cm)			Total Chl (mg/g FW)			Grain Yield per plant (g)			Weight of 1000 grains (g)				
		s	c	Normal	Drough	Combine	Normal	Drough	Combined	Norma	Drough	Combine	Normal	Drough	Combined	Norma	Drough	Combine
Env. (E)	1							23091.17*						12659.53*				130.6**
				376.7**			*							*				10**
Rep/ E	2 4	0.06	0.06	0.06	0.55	1.87	1.21	0.1**	0.01	0.06**	0.09	0.74	0.42	0.1	0.06	0.08		
Genotypes	2 2				168.22*						123.27*	81.61**	185.65**	4.46**	5.87**	8.15**		
	2 2	68.21**	54.83**	121.18**	*	66.42**	165.28**	0.42**	0.43**	0.78**	*							
Parents (P)	7 7				159.63*	109.92*					48.03**	29.22**	38.8**	5.23**	1.97**	5.07**		
		156.38**	111.8**	264.18**	*	*	165.33**	0.12**	0.35**	0.41**								
Crosses (C)	1 1				130.82*						113.49*	62.23**	164.79**	1.67**	6.08**	5.41**		
	4 4	28.9**	29.44**	57.58**	*	44.44**	131.38**	0.6**	0.48**	0.99**	*							
P vs C	1 1										786.74*	719.63*	1505.62**	38.15**	30.25**	68.17**		
		1.43**	11.53**	10.55**	752**	69.52**	639.4**	0.08**	0.23**	0.3**	*	*						
Lines	4 4				376.31*						136.19*	77.82**	202.35**	1.13**	2.51**	1.86**		
		89.7**	90.37**	178.84**	*	36.08**	296.76**	1.06**	0.64**	1.6**	*							
Testers	2 2				118.58*	149.06*					317.26*	136.56*	431.74**	5.15**	22.44**	20.75**		
		12.42**	11.47**	23.88**	*	*	263.63**	0.5**	0.99**	1.4**	*	*						
Line x Tester	8 8	2.62**	3.47**	5.38**	11.13**	22.47**	15.63**	0.39**	0.27**	0.59**	51.2**	35.86**	79.28**	1.07**	3.77**	3.35**		
Genotype x E	2												19.23**			2.18**		
	2			1.86**			69.36**			0.07**								
Crosses x E	1												10.93**			2.34**		
	4			0.75**			43.87**			0.08**								
Lines x E	4			1.22**			115.63**			0.1**			11.66**			1.79**		
Tester x E	2			0.01			4			0.09**			22.08**			6.84**		
Line x Tester x E	8												7.78**			1.49**		
				0.71**			17.96**			0.07**								
Parents x E	7			4**			104.22**			0.06**			38.45**			2.13**		
P vs C x E	1			2.42**			182.11**			0.02			0.75			0.23		
Error	4 8										2.4	2.17	2.29	0.14	0.13	0.13		
	4 8	0.18	0.32	0.25	3.59	2.66	3.13	0.01	0.02	0.01								
δ2 gca		0.929	0.918	0.92	4.232	0.777	2.05	0.007	0.007	0.007	2.202	0.932	1.5	0.021	0.082	0.066		
δ2 sca		0.812	1.050	0.855	2.512	6.601	2.0844	0.128	0.083	0.096	16.268	11.228	12.832	0.310	1.213	0.536		
δ2 gca x E				-0.007			3.488			0.002			0.757			0.235		
δ2 sca x E				0.153			4.944			0.020			1.832			0.452		

Table (6) Mean performance of the genotypes for days to 50% heading, plant height, total chlorophyll content, grain yield per plant and weight of 1000 grains under normal irrigation (N) and drought stress (D) as well as the combined over them (C).

Genotypes	Days to 50% heading (Days)			Plant height (cm)			Total Chl (mg/g FW)			Grain Yield per plant (g)			Weight of 1000 grains (g)		
	Normal	Drought	Combined	Normal	Drought	Combined	Normal	Drought	Combined	Normal	Drought	Combined	Normal	Drought	Combined
NP856-9	107.00	102.00	104.50	103.40	85.10	94.25	4.84	4.25	4.55	47.80	22.30	35.05	25.03	24.60	24.82
A22	105.00	102.33	103.67	99.00	87.00	93.00	5.45	5.06	5.25	45.40	25.43	35.42	26.03	23.50	24.77
IET 1444	112.33	106.33	109.33	119.00	80.20	99.60	5.45	5.16	5.30	36.70	26.51	31.61	23.40	23.11	23.26
GZ 178	104.00	102.33	103.17	103.40	82.00	92.70	5.43	4.80	5.11	46.70	28.10	37.40	23.60	22.06	22.83
GZ 1368	111.67	106.00	108.83	98.50	83.50	91.00	5.38	5.08	5.23	40.28	27.17	33.72	24.00	23.12	23.56
GZ 182	95.33	92.67	94.00	99.60	75.30	87.45	5.28	4.90	5.09	45.10	23.40	34.25	25.50	22.60	24.05
GZ 177	94.67	92.00	93.33	100.00	72.00	86.00	5.20	4.30	4.80	42.80	19.77	31.28	26.00	22.90	24.45
GZ 179	95.67	92.67	94.17	95.00	71.00	83.00	5.20	4.70	5.00	48.33	28.80	38.57	27.10	24.10	25.60
NP856-9 X G182	102.00	99.00	100.50	104.30	79.00	91.70	5.52	5.09	5.31	56.60	37.06	46.83	27.07	25.95	26.51
NP856-9 X G177	100.00	97.30	98.70	105.00	82.00	93.50	4.11	3.57	3.84	39.00	22.90	30.90	25.80	21.15	23.48
NP856-9 X G179	102.00	99.00	100.50	103.30	78.10	90.70	4.60	4.22	4.41	43.90	24.90	34.40	27.60	25.20	26.40
A22 X G182	100.00	96.70	98.30	107.30	82.10	94.70	4.86	4.67	4.76	49.40	30.60	40.00	26.00	23.15	24.58
A22 X G177	98.00	95.00	96.50	113.00	87.20	100.10	5.28	4.36	4.82	43.90	28.50	36.20	27.00	23.15	25.08
A22 X G179	100.30	97.30	98.80	112.27	81.30	96.78	4.72	4.57	4.64	50.10	31.00	40.60	27.00	25.90	26.45
IET1444 X G182	107.30	103.70	105.50	115.00	83.00	99.00	5.53	5.07	5.30	53.90	36.40	45.10	26.50	25.09	25.79
IET1444 X G177	104.30	100.30	102.30	124.60	85.60	105.10	5.40	4.74	5.07	49.40	30.30	39.90	26.40	24.17	25.28
IET1444 X G179	104.70	100.30	102.50	119.77	82.00	100.88	5.50	5.20	5.40	56.80	38.50	47.60	27.40	26.17	26.78
G178 X G182	105.00	102.70	103.80	104.00	77.00	90.50	5.50	4.80	5.10	55.00	32.50	43.80	25.00	25.70	25.35
G178 X G177	104.00	100.00	102.00	108.70	84.20	96.50	5.30	4.55	4.93	44.90	29.40	37.20	26.40	24.10	25.25
G178 X G179	103.00	100.70	101.80	104.00	84.60	94.30	5.52	4.77	5.15	58.90	33.40	46.10	26.70	24.50	25.60
GZ1368 X G182	107.33	104.00	105.67	102.30	75.20	88.75	5.67	4.80	5.23	54.60	34.60	44.60	26.80	25.30	26.05
GZ1368 X G177	106.33	104.67	105.50	109.50	87.00	98.25	5.17	4.66	4.92	52.40	31.60	42.00	26.10	23.77	24.93
GZ1368 X G179	108.33	105.33	106.83	104.50	76.00	90.25	5.54	4.82	5.18	59.70	38.02	48.86	27.90	26.30	27.10
LSD 5%	0.70	0.94	0.65	3.12	2.69	2.21	0.13	0.25	0.14	2.55	2.43	1.75	0.61	0.78	0.61
LSD 1%	0.93	1.25	0.86	4.17	3.59	2.95	0.18	0.33	0.19	3.41	3.24	2.34	0.81	1.04	0.81

Table (7) Estimates of general combining ability effects for days to 50% heading, plant height, total chlorophyll content, grain yield per plant and weight of 1000 grains under normal irrigation (N) and drought stress (D) as well as the combined over them (C).

Genotype	Days to 50% heading (Days)			Plant height (cm)			Total Chl (mg/g FW)			Grain Yield per plant (g)			Weight of 1000 grains (g)		
	Normal	Drought	Combined	Normal	Drought	Combined	Normal	Drought	Combined	Normal	Drought	Combined	Normal	Drought	Combined
Lines:															
NP856-9	-2.18**	-1.96**	-2.07**	-4.97**	-1.92**	-3.44**	-0.47**	-0.36**	-0.42**	-4.73**	-3.7**	-4.21**	0.18	-0.54**	-0.18
A22	-4.07**	-4.07**	-4.07**	1.69*	1.91**	1.8**	-0.26**	-0.13*	-0.19**	-3.45**	-1.93**	-2.69**	0.02	-0.57**	-0.27*
IET 1444	1.93**	1.04**	1.49**	10.62**	1.91**	6.27**	0.27**	0.35**	0.31**	2.13**	3.07**	2.6**	0.12	0.5**	0.31*
GZ 178	0.49**	0.71**	0.6**	-3.6**	0.31	-1.64**	0.22**	0.04	0.13**	1.7**	-0.22	0.74*	-0.61**	0.13	-0.24
GZ 1368	3.82**	4.27**	4.04**	-3.74**	-2.22**	-2.98**	0.24**	0.1*	0.17**	4.34**	2.78**	3.56**	0.29*	0.48**	0.39**
LSD 5%	0.28	0.38	0.26	1.27	1.10	0.90	0.05	0.10	0.06	1.04	0.99	0.71	0.25	0.32	0.25
LSD 1%	0.38	0.51	0.35	1.70	1.46	1.21	0.07	0.14	0.08	1.39	1.32	0.95	0.33	0.43	0.33
Testers															
GZ 182	0.82**	0.8**	0.81**	-2.59**	-2.36**	-2.47**	0.2**	0.22**	0.21**	2.67**	2.26**	2.46**	-0.37**	0.4**	0.01
GZ 177	-0.98**	-0.93**	-0.96**	2.99**	3.58**	3.29**	-0.16**	-0.28**	-0.22**	-5.31**	-3.43**	-4.37**	-0.3**	-1.37**	-0.84**
GZ 179	0.16	0.13	0.14	-0.4	-1.22**	-0.81*	-0.03	0.06	0.02	2.64**	1.17**	1.91**	0.68**	0.97**	0.82**
LSD 5%	0.22	0.30	0.20	0.99	0.85	0.70	0.04	0.08	0.05	0.81	0.77	0.55	0.19	0.25	0.19
LSD 1%	0.29	0.40	0.27	1.32	1.13	0.93	0.06	0.11	0.06	1.08	1.02	0.74	0.26	0.33	0.26

* and ** significant at 0.05 and levels of probability, respectively.

Table (8) Estimates of specific combining ability effects for days to 50% heading, plant height, total chlorophyll content, grain yield per plant and weight of 1000 grains under normal irrigation (N) and drought stress (D) as well as the combined over them (C).

Genotype	Days to 50% heading (Days)			Plant height (cm)			Total Chl (mg/g FW)			Grain Yield per plant (g)			Weight of 1000 grains (g)		
	Norm	Droug	Combin	Norm	Droug	Combin	Norm	Droug	Combin	Norm	Droug	Combin	Norm	Droug	Combin
NP856-9X	-0.16	-0.24	-0.20	2.69*	1.66	2.17**	0.59*	0.58**	0.58**	7.43*	6.54**	6.99**	0.62*	1.45**	1.03**
NP856-9X	-0.36	-0.18	-0.27	-2.19	-1.28	-1.74*	-	-	-0.46**	-2.19*	-1.95*	-2.07**	-	-	-1.15**
NP856-9X	0.51*	0.42	0.47*	-0.50	-0.38	-0.44	-0.11*	-0.13	-0.12*	-	-	-4.92**	0.10	0.13	0.11
A22 X G182	-0.27	-0.47	-0.37	-0.97	0.93	-0.02	-	-0.08	-0.19**	-1.10	-1.69	-1.39*	-0.30	-	-0.80**
A22 X G177	-0.47	-0.40	-0.43	-0.85	0.09	-0.38	0.49*	0.11	0.30**	1.42	1.89*	1.66**	0.64*	0.45	0.55*
A22 X G179	0.73*	0.87*	0.80**	1.81	-1.01	0.40	-	-0.03	-0.11*	-0.32	-0.20	-0.26	-0.34	0.86**	0.26
IET1444 X	1.07*	1.42**	1.24**	-2.20	1.83	-0.19	-	-0.16	-0.15**	-2.11*	-0.94	-1.52*	0.10	-0.45	-0.17
IET1444 X	-0.13	-0.18	-0.16	1.82	-1.51	0.15	0.07	0.01	0.04	1.35	-1.31	0.02	-0.06	0.40	0.17
IET1444 X	-	-	-1.09**	0.38	-0.31	0.03	0.08	0.15	0.11*	0.76	2.25*	1.50*	-0.04	0.05	0.01
G178 X G182	0.18	0.76*	0.47*	1.02	-	-0.78	-	-0.15	-0.16**	-0.60	-1.51	-1.05	-	0.53	-0.06
G178 X G177	0.98*	-0.18	0.40	0.14	-1.31	-0.59	0.04	0.14	0.09	-	1.08	-0.82	0.67*	0.71*	0.69**
G178 X G179	-	-0.58	-0.87**	-1.16	3.89**	1.36	0.12*	0.01	0.07	3.32*	0.43	1.88**	-0.01	-	-0.62**
GZ1368 X	-	-	-1.14**	-0.54	-1.84	-1.19	0.01	-0.18*	-0.08	-	-	-3.02**	0.24	-0.22	0.01

GZ1368 X	-0.02	0.93**	0.46	1.08	4.02**	2.55**	-0.13*	0.18*	0.03	2.14*	0.29	1.21	-0.53*	0.02	-0.26
GZ1368 X	0.84*	0.53	0.69**	-0.53	-2.18*	-1.36	0.11*	0.00	0.05	1.49	2.11*	1.80**	0.29	0.20	0.25
LSD Sij 0.05	0.493	0.663	0.458	2.205	1.899	1.563	0.09	0.18	0.10	1.803	1.715	1.236	0.43	0.55	0.43
LSD Sij 0.01	0.658	0.886	0.611	2.946	2.537	2.088	0.13	0.24	0.13	2.409	2.291	1.651	0.57	0.74	0.58
LSD sij-skl	0.697	0.938	0.647	3.118	2.685	2.211	0.13	0.25	0.14	2.55	2.43	1.75	0.61	0.78	0.61
LSD sij-skl	0.93	1.25	0.86	4.17	3.59	2.93	0.17	0.33	0.19	3.41	3.25	2.32	0.81	1.04	0.81

* and ** significant at 0.05 and levels of probability, respectively.

Table (9) Heterosis relative to mid parent for days to 50% heading, plant height, total chlorophyll content, grain yield per plant and weight of 1000 grains under normal irrigation (N) and drought stress (D) as well as the combined over them (C).

Genotype	Days to 50% heading (Days)			Plant height (cm)			Total Chl (mg/g FW)			Grain Yield per plant (g)			Weight of 1000 grains (g)		
	Norm	Droug	Combine	Norm	Droug	Combine	Norma	Droug	Combine	Norma	Droug	Combine	Norm	Droug	Combine
NP856-9 X	0.82**	1.71**	1.26**	2.76*	-1.50	0.88	9.12**	11.26*	10.13**	21.85*	62.19*	35.15**	7.12**	9.96**	8.49**
NP856-9 X	-	0.34	-0.3	3.24*	4.39**	3.74**	-	-	-17.63**	-	8.83**	-6.70**	1.11**	-	-4.70**
NP856-9 X	0.66*	1.71**	1.17**	4.13**	0.06	2.34*	-8.80**	-5.45**	-7.23**	-8.67**	-2.74*	-6.61**	5.88**	3.49**	4.73**
A22 X G182	-0.17	-0.85*	-0.5	8.06**	1.17	4.96**	-9.43**	-6.26**	-7.90**	9.06**	25.32*	14.76**	0.91**	0.45	0.69**
A22 X G177	-	-2.23**	-2.03**	13.57*	9.69**	11.84**	-1.15**	-7.08**	-3.92**	-0.48	26.11*	8.53**	3.78**	-0.22	1.90**
A22 X G179	0.00	-0.17	-0.1	15.74*	2.91*	9.98**	-	-6.26**	-9.10**	6.90**	14.32*	9.62**	1.63**	8.82**	5.03**
IET1444 X	3.37**	4.19**	3.77**	5.22**	6.75**	5.85**	3.06**	0.90**	2.01**	31.83*	45.66*	37.07**	8.38**	9.76**	9.05**
IET1444 X	0.81*	1.18**	0.99**	13.79*	12.48*	13.25**	1.06**	0.05	0.59**	24.28*	30.95*	26.73**	6.88**	5.05**	6.00**
IET1444 X	0.64*	0.84*	0.74**	11.93*	8.47**	10.50**	3.42**	6.18**	4.74**	33.52*	39.05*	35.70**	8.51**	10.85*	9.64**
G178 X G182	5.35**	5.30**	5.33**	2.46	-2.10	0.47	2.06**	-1.86**	0.19**	19.83*	26.21*	22.12**	1.83**	15.08*	8.14**
G178 X G177	4.70**	2.92**	3.82**	6.88**	9.35**	7.95**	-0.44**	-0.17	-0.31**	0.34	22.84*	8.18**	6.45**	7.20**	6.81**
G178 X G179	3.17**	3.25**	3.21**	4.84**	10.59*	7.34**	3.52**	0.64**	2.17**	23.96*	17.22*	21.44**	5.33**	6.14**	5.72**
GZ1368 X	3.70**	4.70**	4.19**	3.28*	-5.29**	-0.53	6.34**	-3.74**	1.47**	27.90*	36.85*	31.23**	8.28**	10.67*	9.43**
GZ1368 X	3.07**	5.72**	4.37**	10.33*	11.90*	11.02**	-2.49**	-0.75**	-1.67**	26.14*	34.66*	29.22**	4.40**	3.29**	3.87**
GZ1368 X	4.50**	6.04**	5.25**	8.01**	-1.62	3.74**	4.29**	-1.14**	1.69**	34.75*	35.88*	35.19**	9.20**	11.39*	10.25**

* and ** significant at 0.05 and levels of probability, respectively.

Table (10) Heterosis relative to better parent for days to 50% heading, plant height, total chlorophyll content, grain yield per plant and weight of 1000 grains under normal irrigation (N) and drought stress (D) as well as the combined over them (C).

Genotype	Days to 50% heading			Plant height (cm)			Total Chl (mg/g FW)			Grain Yield per plant (g)			Weight of 1000 grains (g)		
	Norm	Droug	Combin	Norm	Droug	Combin	Norm	Droug	Combin	Norm	Droug	Combin	Norm	Droug	Combin
NP856-9 X	-	-	-3.83**	0.87	-	-2.76*	4.53**	3.92**	4.24**	18.41*	58.38*	33.61**	6.14*	5.49**	6.82**
NP856-9 X	-	-	-5.58**	1.55	-	-0.80	-	-	-19.58**	-	2.65*	-11.71**	-0.77*	-	-5.41**
NP856-9 X	-	-	-3.83**	-0.10	-	-3.77**	-	-	-11.12**	-	-	-10.86**	1.85*	2.44**	3.12**
A22 X G182	-	-	-5.14**	7.73*	-	1.83	-	-	-9.34**	8.70**	20.31*	12.87**	-0.13	-	-0.77**
A22 X G177	-	-	-6.91**	13.00	0.23	7.63**	-	-	-8.34**	-3.33*	12.06*	2.20*	3.71*	-	1.24**
A22 X G179	-	-	-4.66**	13.40	-	4.07**	-	-	-11.63**	3.66**	7.64**	5.15**	-0.37	7.47**	3.32**
IET1444 X	-	-	-3.51**	-3.36*	3.49*	-0.60	1.45**	-	-0.04	19.56*	37.12*	31.78**	3.92*	8.55**	7.25**
IET1444 X	-	-	-6.40**	4.71*	6.73**	5.52**	-	-	-4.46**	15.42*	14.30*	26.09**	1.54*	4.57**	3.41**
IET1444 X	-	-	-6.25**	0.64	2.24	1.29	1.47**	1.26**	1.37**	17.46*	33.52*	23.45**	1.11*	8.58**	4.62**
G178 X G182	0.96*	0.33	0.65*	0.58	-	-2.37*	0.72**	-	-0.04	17.77*	15.66*	16.98**	-	13.72*	5.41**
G178 X G177	0.00	-	-1.13**	5.13*	2.68	4.05**	-	-	-3.65**	-	4.63**	-0.67	1.54*	5.24**	3.27**
G178 X G179	-	-	-1.29**	0.58	3.17*	1.73	1.82**	-	0.65**	21.87*	15.80*	19.60**	-	1.66**	0.00
GZ1368 X	-	-	-2.91**	2.71	-	-2.47*	5.43**	-	0.15*	21.06*	27.36*	30.22**	5.10*	9.43**	8.32**
GZ1368 X	-	-	-3.06**	9.50*	4.19**	7.97**	-	-	-5.95**	22.43*	16.32*	24.54**	0.38	2.80**	1.98**
GZ1368 X	-	-0.63	-1.84**	6.09*	-	-0.82	3.04**	-	-0.89**	23.53*	32.03*	26.70**	2.95*	9.13**	5.86**

* and ** significant at 0.05 and levels of probability, respectively.

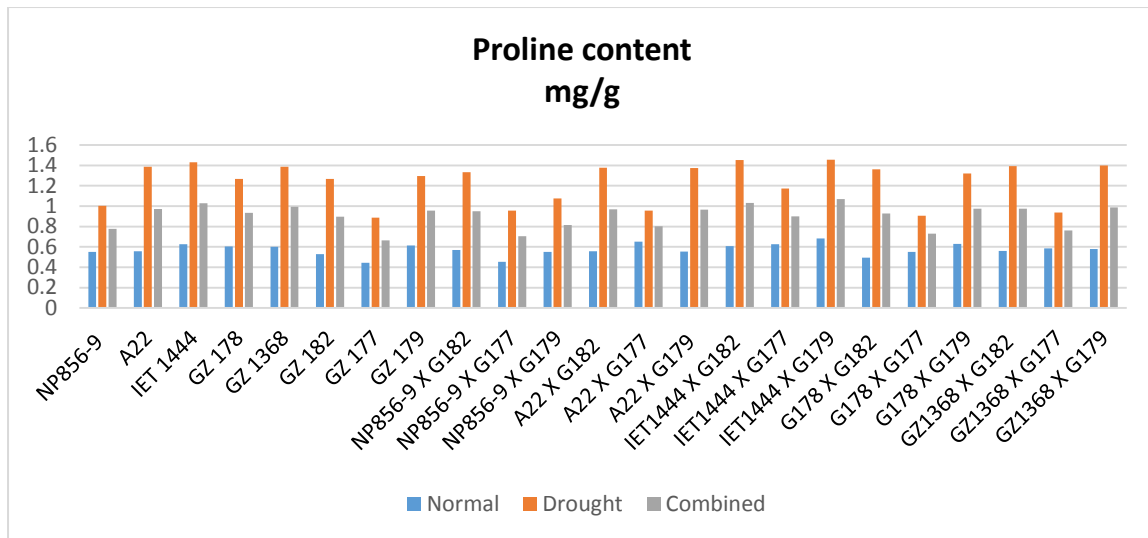


Fig. (3) Proline content of rice leaves in normal and drought conditions and combined date.

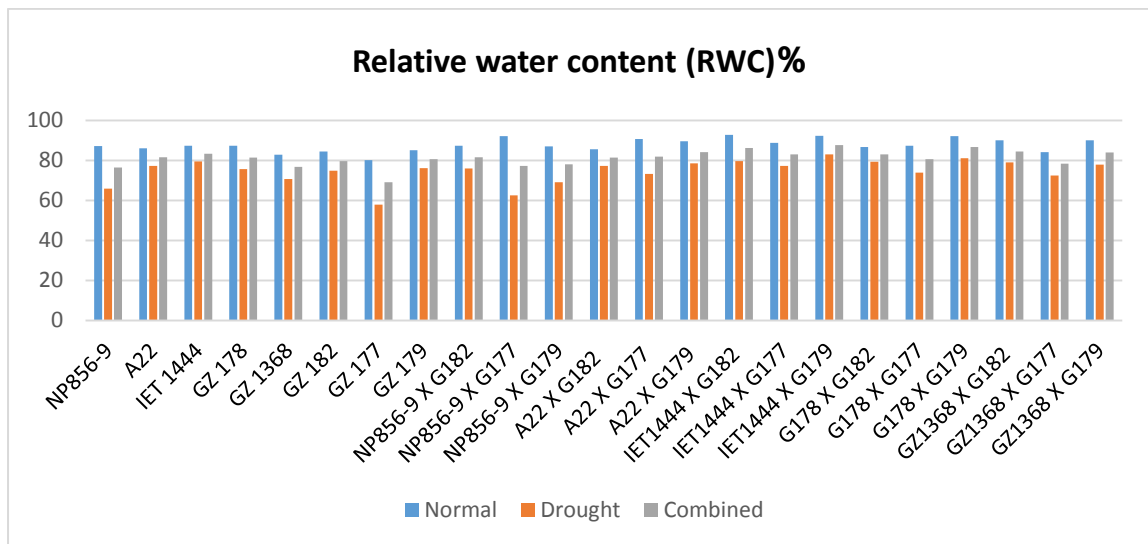


Fig. (4) Relative water content (RWC) under normal and drought conditions and combined date.

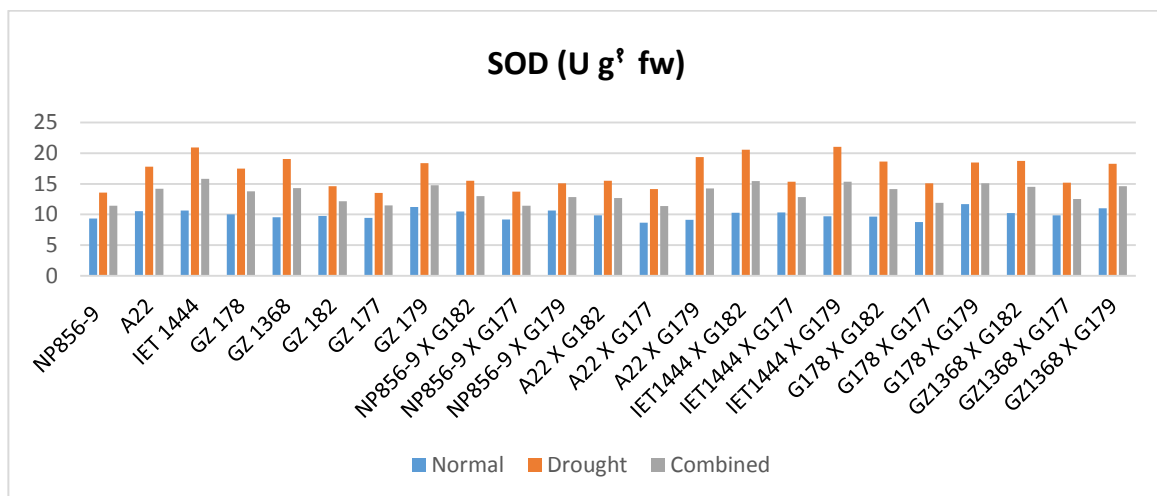


Fig. (5) Superoxide dismutase (SOD) activity of rice leaves in normal and drought conditions and combined date.

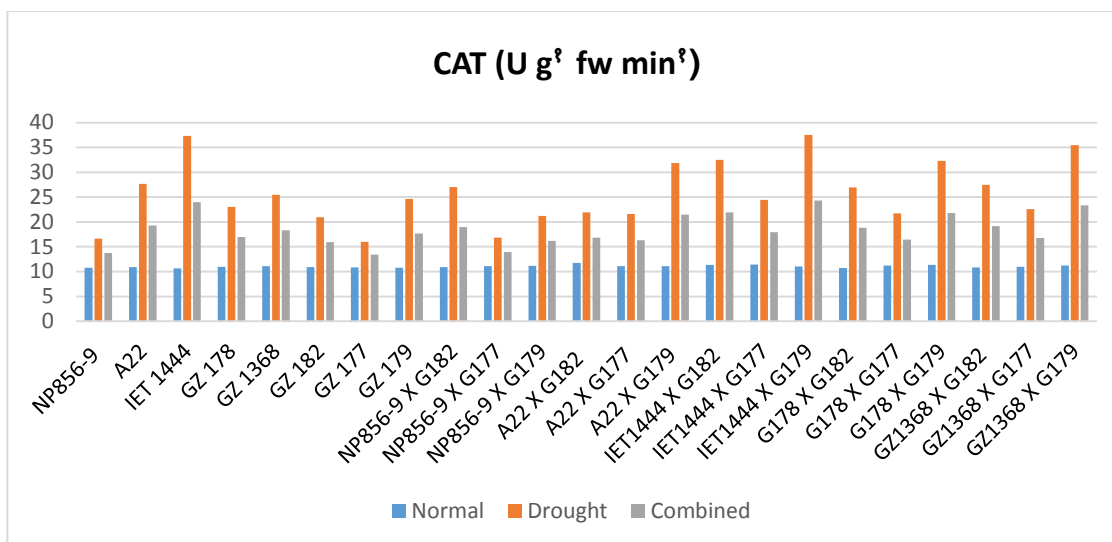


Fig. (6) catalase (CAT) activity of rice leaves in normal and drought conditions and combined date.

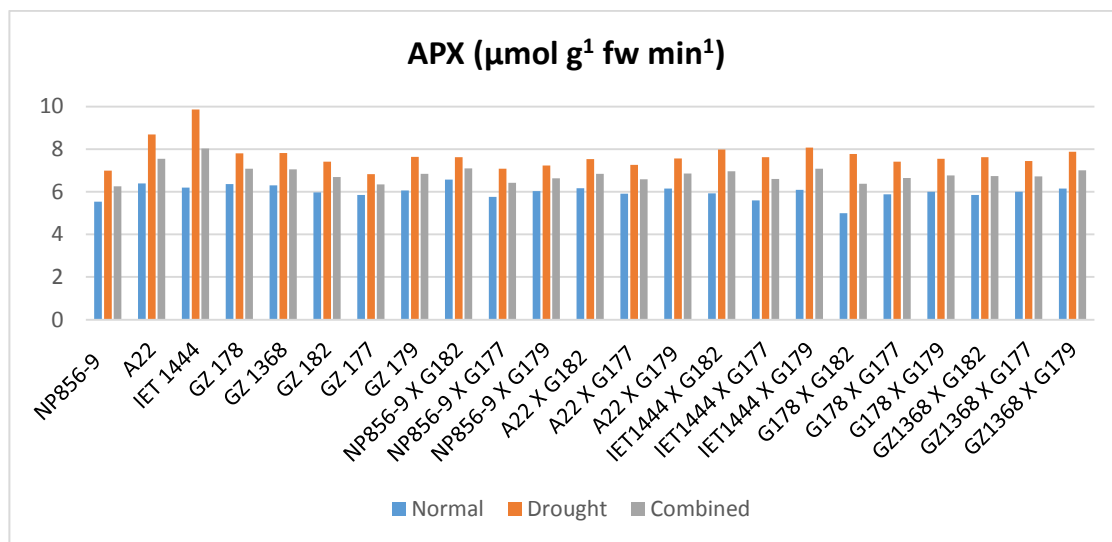


Fig. (7) Ascorbate peroxidase (APX) activity of rice leaves in normal and drought conditions and combined.

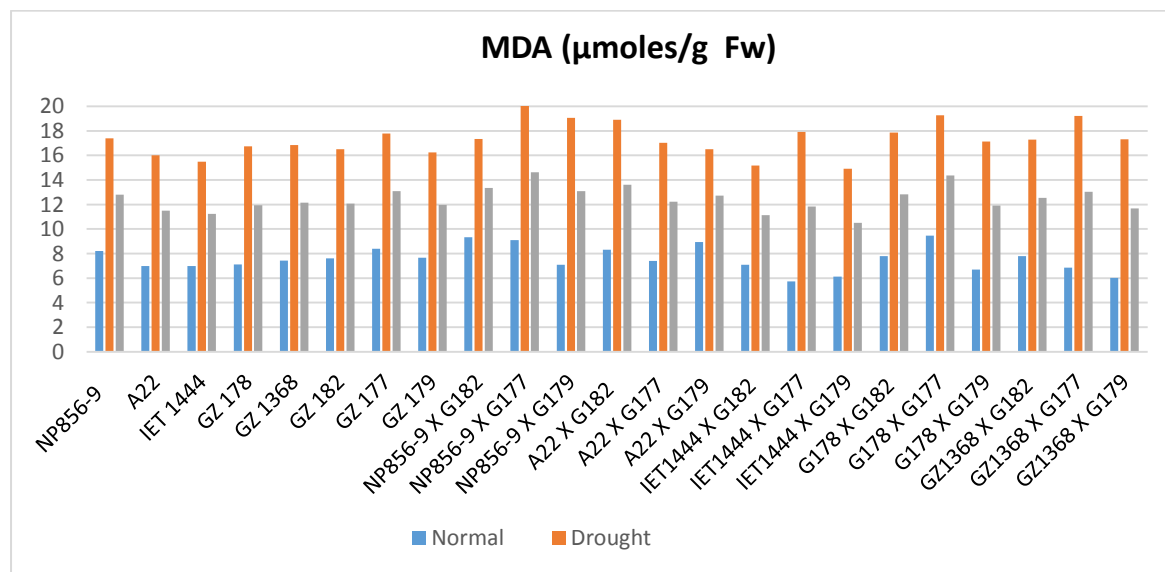


Fig. (8) Malondialdehyde (MDA) content of rice leaves in normal and drought conditions and combined date.

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