AGRONOMIC PERFORMANCE AND BIOCHEMICAL GENETIC MARKERS FOR DROUGHT TOLERANCE IN RICE (*Oryza sativa* L.)

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Rice (*Oryza sativa* L.) is considered one of the most important food crops not only in Egypt but also world wide. The demands of rice are in continuous increasing. The majority of rice ecotypes are semi-aquatic plants adapted to saturated soil conditions where it is difficult for crop species to survive (Champoux *et al.,* 1995). Agricultural expansion in Egypt depends largely on water which is considered one of the main factors limiting agricultural development, so, one way to save water is increasing water intervals without any sharp effect on the yield. High degree of drought tolerance allows the plant to maintain its growth and development under water stress. The ability of the plant to produce new tillers and resume growth and development after irrigation is an important factor in drought tolerance (Chang *et al.*, 1974). Identifying varieties with high yield potential and drought tolerance is one of the principal objectives of rice breeders.

Several researchers have conducted studies on the effect of water deficit on rice. The effect of water stress on grain yield depends on the duration and timing of water deficit (Lenka and Garnayak, 1991; Castillo *et al.*, 1992; Tsuda *et al.*, 1994). Grain yield and yield components were significantly decreased with increasing irrigation intervals (Nour *et al.*, 1994; Sorour *et al.*, 1998; Adhikary *et al.*, 1999; Sehly *et al.*, 2001; Gaballah, 2009). Drought stress resulted in high spikelet sterility (Nour and Mahrous, 1994; Chauhan *et al.*, 1999). Panicle length was decreased sharply when rice plants were subjected to irrigation intervals every 6 days (El-Wehishy and Ghanem, 1996) or longer than 6 days (Abou El-Hassan, 1997).

The conventional methods of plant selection for drought tolerance are not easy because of the large effects of the environment and low narrow sense heritability. Selection for drought tolerance genotypes of rice based on phenotypic performance alone is less reliable and will delay progress in breeding. Recent advent of molecular and biochemical markers, are used to find out drought tolerant rice genotypes.

Molecular marker assisted identification with high power of genetic resolutions has emerged as a robust technique for cultivar fingerprinting, identity profiling, estimating and comparing genetic

similarity, and variety protection. Several types of molecular marker i.e., allozymes (Devanand *et al.,* 1999), RAPD (Wang and Lu, 2006; Ichii *et al.,* 2003) and SSR (Nandakumar *et al.,* 2004) have been used in this term. **.** The application of molecular markers in rice improvement has been reviewed recently (Mackill, 2007). Another indication for a response of the plant against abiotic stress is an increased level of free amino acids. Some of the amino acids are by themselves compatible solutes like proline, others are precursors of compatible solutes, like glycin or alanin (Hanson *et al.,* 1994). But also other amino acids turned out to be enhanced (Rizhsky *et al.,* 2004) which might be necessary for *de novo* synthesis of induced proteins.

This study aimed to achieve reliable information about the relationships between morphological performance as some growth, yield as well as yield components and drought stress tolerance of 25 rice genotypes and to capture effective molecular and biochemical markers associated with drought tolerance to use in marker-aided selection breeding programs.

MATERIAL AND METHODS

Plant materials

The experimental materials involved in this study were, two newly released hybrid rice varieties, hybrid rice No.1 and hybrid rice No.2 (were kindly provided by Rice Research and Training Center, Sakha, Kafr El-Sheikh), a promising hybrid rice combination G46A x Giza 178 and twenty two rice lines, varieties and cultivars involving Giza 171, Suweon 287R, Milang, Giza 159, IR66R, Pusa 150-9-3-1R, IR4467-3-2-2R, Sakha 104, Giza 177, Giza 181,Giza 182, Giza 178, Giza 176, Sakha 102, Sakha 103, Riho, Giza 175, Sakha 101, Giza 172, Gz1368-5-4 and G46B (were kindly provided by agronomy department faculty of agriculture, Kafrelsheikh university). The seeds of G 46A x Giza 178 rice hybrid were produced in the summer season of 2007 through crossing the promising cytoplasmic male sterile line (G46A) with the indica rice cultivar (Giza 178).

Response of rice genotypes to water deficit

Three irrigation intervals; every 6 or 9 or 12 days were subjected. A randomized complete block design with three replications was used for each irrigation interval. Thirty days old seedlings of each genotype were individually transplanted in seven rows, 5.8 m long and 20 cm apart. To avoid the effect of lateral movement of flooding water, each treatment was isolated by ditches.

The stress was applied after two weeks from transplanting till harvest. The recommendation cultural practices for rice production were followed during the growing seasons. At harvest time, ten guarded hills were randomly taken from each plot of the three replications to determine plant height (cm), panicle length, number of grains/panicle, 1000-grain weight (g). Grain yield was determined

based on the five internal rows from each plot. Drought susceptibility index (DSI) for each genotype was calculated according to the formula given by Ali *et al.* (1990): DSI = NS-S/NS, Where:

NS: is yield under normal conditions.

S : is yield under drought conditions.

Thus, NS and S representative in this study grain yield under irrigation every 6 and 12 days, respectively.

The data were subjected to statistical analysis of variance according to Snedecor and Cochran (1967). Error variances from separate analysis of the data were tested for homogenity (Bartlett, 1937). As the error variances were homogenous, combined analysis was conducted for the data of the two seasons according to Cochran and Cox (1957). Environments means were compared by Duncan's multiple range test (Duncan, 1955).

Biochemical and molecular studies

Three of the most tolerant genotypes (Agami, Giza 159 and Gz 1368-5-4) as well as two of the most rice susceptible genotypes (Sakha 101 and Sakha 102) were chosen for biochemical analysis and genetic fingerprints to detect associated markers for drought tolerance in rice.

Proline determination

Before proline extraction seedlings were grown for 30 days under normal irrigation and drought stress conditions**.**

The detection of the free L-proline content was carried out according to Bates *et al.* (1973) with some modifications as follows. A sample of 150 mg fresh leaves material was grinded under liquid nitrogen in a precooled mortar with a pestle. The homogenate was resuspended in 10 ml of 3 % salicylic acid in 50 ml flasks and shaken for 20 min. The suspension was filtered through filter paper to remove cell debris. Then 300 µl of acidic ninhydrin were added to the same volume of supernatant, followed by addition of 300 µl of glacial acetic acid. The mixture was boiled for 60 minutes. For extraction of L-proline, 600 µl of toluene were added to the mixture and the mixture shaken vigorously for 30 sec. The toluene phase was collected and its absorption was measured spectrophotometrically at 520 nm. Free proline was quantified by a standard curve obtained with pure standard solutions of L-proline as reference substance in the same assay.

Plant DNA extraction and RAPD-PCR condition

Isolation of DNA was carried out according the CTAB method of Doyle and Doyle (1990).In this study,three DNA markers (Ladders) were used, M) DNA Molecular weight Marker XIV (Roche), M1)) DNA Molecular weight Marker IV (Roche) and M2) 100bp DNA Marker (Gene ON).

100-150 mg leaves from 14 days old rice seedlings were harvested, placed immediately in liquid nitrogen and grinded to powder under liquid nitrogen using mortar and pestle. The ground material was transferred into 2 ml Eppendrof

tubes. 800 µ1 of pre-heated (65°C) CTAP extraction buffer (Tris-HCl pH 8.0 (base) 100 mM, CTAP 3% (W/V), NaCl 1.4 M, EDTA, 20 mM, ß-Mercaptoethanol, 0.2% (v/v) was added, followed by vigorous vortexing under a fume hood. The tubes were incubated for 30 minutes at 60ºC. After incubation 800 µ1 CI-mix (23 parts chloroform + 1 part isoamylalcohol) were added and tubes were gently mixed by inverting the tube for 4-5 times to avoid shearing of genomic DNA. The mixture was centrifuged at room temperature for 10 minutes at 10000 g. The aqueous phase (app. 800 µ1) was transferred into a fresh 1.5 ml Eppendrof tube.

The centrifugation step was repeated to get a clear sample. 550 µ1 of pre-cooled (-20°C) isopropanol was added and gently mixed to allow precipitation of DNA. The tubes were centrifuged for 10 minutes at 14000 rpm to precipitate the genomic DNA. The supernatant was discarded and the DNA pellet was washed with 200 µ1 washing buffer (76% absolute ethanol, 10 mM Na-acetate, 7.5 M NH4-acetate, 0.5 M EDTA, pH 8) until the pellet floats. Washing buffer was carefully removed and the pellet was re-suspended in 200 µ1 TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8) supplemented with RNase A (10 µg/ml), (Fermentas, St. Leon-Rot, Germany) incubated for 30 minutes at 37°C, and then 100 µ1 7.5 M NH4- acetate and 750 µ1 absolute ethanol was added and gently mixed. The mixture was centrifuged at maximum speed for 10 minutes at room temperature. The supernatant was discarded completely and the pellet was dried for 40-50 minutes at 37°C. After drying, the pellet was re-suspended in 100-200 µ1TE buffer and stored at 4ºC over night.

PCR reactions were conducted using 13 mer primers (Metabion, Germany) with the following sequences:

Primers	Sequences
P1	5′- CCGACTCTGGCGA-3′
P2	5′- GTAAGCCGAGACA-3′
P4	5′- ACCTGCCAACATA-3′
P5	5′- GTAGGTCGCAGGT-3′
P6	5′- TCGTGGCACATAC-3′
P7	5′- TGTACGGCACACG-3′
P8	5′- ACGGAGGCAGAGA-3′
P9	5′- GTCTTCCGTCGTC-3′
P10	5′- GTGTGCCTGGTGC-3′
P11	5′- AGCCCAAAGGATC-3′

Amplification was carried out in 25 µl reaction volume containing the following reagents: 1.0 µl of dNTPs (10 mM), 1.0 µl of $MgCl_2$ (25 mM), 5ul of 10x buffer, 1.0 µl of primer (10 pmol), 1.0 µl of DNA (25 ng/µl), 0.3 µl of taq polymerase (5 u/ul) and 15.7 dd H_2O. Amplification was carried out in MJ Mini Bio RAD, thermal cycler as follows: one cycle at 94°C for 5 min followed by 40 cycles at 95°C for 1 min; 35 for 1 min and 72°C for 2 min. The reaction was finally incubated at 72°C for 7 min.

The RAPD products were electrophoresed in 1% agarose gel in TAE buffer at 75 V. The gel was stained with ethedium bromide and then distained with tap water and photographed by gel documentation system (UVITEC, UK).

Isozyme Patterns

Native polyacrylamide gel electrophoresis was used to study isozyme variation between the selected tolerant and sensitive cultivars after thirty days of water deficit. Peroxidase Isozyme Patterns were extracted by homogenizing 200 mg fresh leaf samples in 1 ml of 0.125 M Tris-borate buffer pH 8.9.Then the samples were centrifuged for 10 min. at 10000 rpm. The isozymes were separated in 7.5% polyacrylamide gel electrophoresis according to Stegmann *et al.* (1985). A volume of 60 µl of the extract was applied to each gel well. Staining of the gel was performed as described by Larsen and Benson (1970). The staining solution was composed of 50 ml of 1M Na-acetate pH 4.7, 50 ml of Methanol, 50 ml of tetramethylbenzidine (TMBZ) and 2 ml of 30% H_2O_2.

RESULTS AND DISCUSSION

Response of rice genotypes to water deficit

a) Irrigation intervals

Results in Table (1) show that irrigation intervals had highly significant effects on all the studied characters. Prolonging the irrigation intervals from 6 to 12 days resulted in delaying heading date by about two days as average over 25 genotypes and two seasons. However, this reduction was insignificant when the irrigation intervals were prolonged from 6 to 9 days. Furthermore, increasing irrigation intervals from 6 to 9 or from 9 to 12 days resulted in a steady reduction on all the other traits.

a, b and c refers to Duncan's multiple range test. Means followed by a common letter are not significantly differed at 0.05 level of probability.

Such reduction was about 10.46% for plant height, 16.88% for panicle length, 18.01% for number of grains/panicle, 10.49% for fertility percentage, 20.21% for number of panicles/plant, 7.42% for 1000 grain weight, while such reduction was about 33% for grain yield. The reduction percentage in 1000-grain weight as a result of prolonging irrigation intervals was lower compared with the other characters indicating that 1000-grain weight was more genetically controlled. The reduction in growth characters, grain yield and yield components by increasing water stress may be due to decreasing the activity of meristimic tissue, reduction in net photosynthate availability by reducing leaf area and increasing stomatal resistance as well as decreasing in enzymes and photochemical activities (Sinha *et al.*, 1982). These results are in harmony with those reported by El-Wehishy and Abdel-Hafez (1997) who found that plant height, panicle length, number of panicles/plant, number of spikelets/panicle, 1000 grain weight and grain yield were significantly decreased by delaying flooding water up to fourteen days. Similar decreases in plant characters were also obtained by Adhikary *et al.* (1999).

b) Varietals differences and interactions

Data presented in Table (1) revealed that Giza 177 and Sakha 103 cultivars were the earliest cultivars as they headed after 89 days from sowing. The aforementioned rice cultivars were bred for early maturity. On the other hand, Giza 171 cultivar revealed significantly the highest number of days to 50% heading (119 days). Number of days to 50% heading was significantly affected by the interaction between genotypes and water intervals (Table 2). Under watering every 6 or 9 days, Sakha 103 cultivar was the earliest genotype as it headed after 87 days from sowing, while such estimates were maximized (124.3) in case of Giza 171 cultivar associated with the third water intervals (irrigation every 12 days).

Milang rice variety recorded significantly shortest plant stature compared with the other genotypes followed by Giza 182 while, Giza 159 and Giza 172 recorded the tallest plants (119 and 113 cm, respectively). The differences among rice genotypes for plant height may be due to genetic variability. Plant height was significantly affected by the interaction between genotypes and irrigation intervals. Under the most water stress environment, Giza 182 recorded the shortest plants (74.3 cm), while, the tallest ones were recorded by Agami under the shortest water intervals every 6 days (Table 2).

Highly significant differences among rice genotypes were detected for panicle length and number of grains/panicle (Table 1). IR4467-3-2-2R and G46 A x Giza 178 hybrids revealed significantly longer panicles compared with the other genotypes, 25.9 and 24 cm, respectively (Table 2). Also, the aforementioned rice hybrids showed the greatest number of grains/panicle (152.6) followed by hybrid rice variety No.2 (Table 3). These results were in general agreement with those of El-Keredy *et al.* (2003). IR4467-3-2-2R hybrid recorded the longest panicles (30.1 cm) under irrigation every 6 days while, such estimates were minimized (15 cm) in case of Riho variety. Whereas, G46A x Giza 178 hybrid exhibited the maximum number of grains/panicle under the first and the second irrigation intervals (167 and 156, respectively) followed by G46B and hybrid rice No. 2 under irrigation every 6 days. However, Giza 181 cultivar gave the minimum number of grains/panicle when it irrigated every 12 days.

Eight among 25 rice genotypes detected estimates of fertility percentage more than 90% as average over the three irrigation intervals (Table 1). The most favorable percentage (94%) was recorded for Giza 177 cultivar followed by Sakha 103 (93.2%), while, the lowest fertility percentage (79.3%) was recorded for Sakha 104. Furthermore, the three rice hybrids involved in this study exhibited fertility percentage less than 90%. These results were in general agreement with those reported by Chauhan *et al.* (1999) and El-Degwy (2006). G46A x Giza 178 hybrid recorded the lowest fertility percentage (71%) under 12 days intervals while the highest percentage (97%) was

recorded for Sakha 101 cultivar under the shortest irrigation intervals every 6 days (Table 3).

Large variations among rice genotypes were detected for number of panicles/plant.Gz1368-5-4 recorded significantly the greatest number of panicles/plant (22.2) as average over three irrigation intervals followed by Giza 178 and hybrid rice No.1 (21.5 and 21, respectively). Such character was significantly affected by the interaction between genotypes and water intervals. The greatest number of panicles/plant (24.7) was recorded for Gz1368-5-4 under watering every 6 days and Giza 178 under the intermediate irrigation interval, while, such estimates were minimized (10.5) in case of Giza 176 under the highest water stress (Table 3). These results were in accordance with those of El-Wehishy and Abdel-Hafez (1997) and Chauhan *et al.* (1999). Also, Sahu and Rad (1974) reported that moisture stress during reproductive phase brought death of tillers.

Rice genotypes were significantly differed in their 1000-grain weight (Table 1). Agami rice variety recorded the heaviest 1000-grain weight (29 g) over the three water treatments followed by Suweon 287R (27.6 g). This character was significantly affected by the interaction between rice genotypes and irrigation intervals. There was a tendency of decreasing 1000-grain weight with increasing irrigation intervals in most genotypes. While, IR4467-3-2-2R recorded its heaviest 1000-grain weight (27 g) under the intermediate irrigation interval (Table 4). The heaviest 1000-grain weight (29.8) was recorded by Agami rice variety under the first irrigation interval. Giza 175 cultivar recorded the lowest value under irrigation every 12 days. The reduction in 1000-grain as a result of prolonging irrigation intervals was in agreement with the finding of Tsuda *et al.* (1993), El-Wehishy and Abdel-Hafez (1997) and Gaballah (2009).

Large variations among rice genotypes were detected for grain yield. Generally, prolonging water intervals from 6 to 9 or 12 days significantly reduced grain yield in most genotypes. As average over the three irrigation intervals, hybrid rice No 2, G46A x Giza 178, Giza 178 and Gz.1368-5-4 gave higher grain yield compared with the other genotypes but, the differences among them were not significant. The interaction between genotypes and irrigation intervals was significant for grain yield. Hybrid rice No.2 gave the highest grain yield (4.42 ton/fed) followed by G46A x Giza 178 (4.2 ton/fed.) under irrigation every 6 days (Table 4). While, such estimates were minimized in case of Sakha 103 (1.6 ton/fed) under the highest drought stress. Similar decrease in grain yield due to drought stress was obtained by Sorour *et al.* (1998) who found that water deficit during reproductive phase reduced grain yield to 20-70% of the irrigated control.

Results in drought susceptibility index (DSI) between 6 and 12 days intervals are presented in Table (1). The results showed that Sakha 103, Sakha 101 and

Sakha 102 cultivars exhibited high DSI values of 52, 47 and 45%, respectively, indicating that these cultivars were more affected by water deficit, while, Agami and 1368-5-4 revealed the lowest DSI values; 23 and 27%, respectively reflects their drought tolerance ability. These results were in agreement with those obtained by Gaballah (2009).

RAPD-PCR Molecular markers

RAPD polymorphism for the most tolerant genotypes (Agami, Giza 159 and Gz1368-5-4) and the most susceptible ones (Sakha 101 and 102) to drought are shown in Figure (1). In this study we focused only on those markers which found in the tolerance cultivars and disappeared in susceptible ones. All primers successfully amplified DNA fragments from rice DNA samples. However, four primers; P1, P4, P6, and P10 were the only ones that detect molecular markers related to drought tolerance. Six primers, P2, P5, P7, P8, P9 and P11 did not detect unique DNA markers related to drought tolerant in the five tested rice cultivars. Primer No.1 produce one unique band (150bp) found only in the tolerant cultivars (Agami, Giza 159 and Gz1368-5.4), although primers No.4 and 8 gave the lowest number of the amplified DNA fragments (2), primer No.4 gave a unique DNA marker (900bp) in the three tolerant cultivars. Furthermore, Primer No.6 and 10 gave unique DNA markers with molecular weight of 453bp and 450bp, respectively. These markers showed clearly the ability to be presented in the three tolerant cultivars, but lost this ability in the tow susceptible genotypes. Figure 1a, 1c. 1e and 1i) .The results of this study were similar to those of Abdel-Tawab *et al.* (1997, 1998a, b) for salt and drought tolerance in maize and salt tolerance in sorghum, respectively. Moreover Recent developments in molecular marker technologies, such as SSR, RFLP, PCR, RAPD, AFLP provide opportunities for analyzing both simply inherited and quantitative traits, and located and manipulate individual genetic factors associated with traits of interest, McCouch *et al.* (1988), McCouch *et al.* (2002), Nguyen thi Lang *et al.* (2008), Bhowmik *et al.* (2009), Ciucă *et al.* (2009) and Ciucă and Petcu (2009).

Peroxidase markers

The electophorotic pattern of peroxidase enzyme extracted from rice leaves of the most tolerant and susceptible cultivars for drought revealed marked polymorphism among the studied cultivars. The tolerant cultivars were discriminated from the susceptible ones by the presence of three bands numbered Px3, Px4 and Px5 .These bands were completely absent in the susceptible genotypes (Fig. 2).These results confirmed that peroxidase Isozyme can be used as a biochemical marker for discrimination of the tolerant and susceptible rice genotypes. These results agreed with the results of other authors such as Abdelsalam *et al*. (1998) who used four isozymes to discriminate between some Egyptian barley cultivars. Comparable results were obtained by Smith. (1989), who used isozyme electrophoresis for characterization and assessment of genetic diversity among maize (*Zea mays* L.). Draz *et al.* (1993) reported that some new peroxidase bands were associated with spe-

cific agronomic characters, also the salt tolerant cultivars exhibited new isozyme bands.

Accumulation of proline

As well known reaction of plants to compensate for drought stress is the intracellular accumulation of free proline. Comparative measurements of proline accumulation in the different rice genotypes were carried out in this study. Although lowest in controls, tolerant cultivars Agami, Giza 159 and Gz 1368-5-4 accumulated significantly higher proline levels compared with its controls, whereas the accumulation of proline was not significant after drought stress of the susceptible genotypes (Fig. 3). Proline is thought to play an important role as an osmoregulatory solute in plants subjected to drought and salt stress (Delauney and Verma, 1993) and in stabilizing cellular structures as well as scavenging free radicals (Hare and Cress 1997; Tripathi and Gaur 2004). The significantly higher proline accumulation of drought tolerant genotypes than in susceptible ones suggests that tolerant genotypes possess a higher potential to tolerate drought stress.

SUMMARY

Twenty five rice genotypes having a wide range of genetic variability were used to study the effect of water stress on grain yield and yield attributes characters and to capture effective molecular and biochemical markers associated with drought tolerance to use in marker –aided selection breeding programs. Three irrigation intervals, i.e. every 6, 9 or 12 days were applied. A randomized complete block design with three replications was used for each irrigation interval. Significant differences were detected among rice genotypes in grain yield and yield attributes characters. Hybrid rice variety, No.2 and the promising hybrid rice combination, G46A x Giza 178 recorded the highest grain yield as average over the three irrigation treatments and two seasons. While, Agami rice variety recorded the heaviest 1000-grain weight and the minimum value of fertility percentage. Grain yield was decreased as the irrigation intervals were increased from 6 to 9 or 12 days due to decrease in number of panicles/plant, panicle length, number of grains/panicle and 1000-grain weight. Prolonging the irrigation intervals from 6 to 12 days caused significant reduction in most studied characters compared to irrigation every 6 or 9 days. However, number of day to 50% heading was significantly increased with increasing irrigation intervals from 6 to 12 days. Under the shortest irrigation intervals every 6 days, hybrid rice varieties No.1 and G46A x Giza 178 surpassed significantly most of the other genotypes while, under upland conditions (watering every 12 days), Agami, Giza 178, hybrid rice variety No.2 and Gz.1368-5-4 recorded higher grain yield compared with the other genotypes but, the differences among them were insignificant. Drought susceptibility index (DSI) estimates showed that Sakha 103, Sakha 101 and Sakha 102 recorded high DSI values of 0.52, 0.47 and 0.45, respectively, indicating that such cultivars were more affected by water deficit. While, Agami and Gz.1368 recorded the lowest

DSI values; 0.23 and 0.27, respectively, reflects their drought tolerance ability. The results of genetic fingerprints using molecular (RAPD-PCR) and biochemical (peroxidase isozyme and proline accumulation) markers were successfully able to discriminate the tolerant cultivars from the sensitive ones. Three peroxidase isozymes as well as five RAPD markers were found to be positive markers with tolerance to drought. The accumulation of proline was higher in the tolerant genotypes under drought stress and suggests the role of proline accumulation in drought tolerance mechanism.

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Table (1): Grain yield, its contributing variable and drought susceptibility index as influenced by irrigation intervals and genotypes over the two seasons of 2008 and 2009.

Treatment	Number of days to 50% heading (day)	Plant height (cm)	Panicle length (cm)	Number of grains/ panicle	Fertility %	Number of panicles/plant	1000-grain weight (g)	Grain yield (ton/fed)	Drought susceptibility index
Water intervals									
6	102.6bc	99.3a	23.7a	136.8a	90.6a	18.8a	25.6a	3.60a	-
9	103.4b	94.9b	21.9b	125.5b	86.5b	17.7b	24.8b	2.92b	0.19
12	104.8a	88.9c	19.7c	112.2c	81.1c	15.0c	23.7c	2.33c	0.35
F. test	**	**	**	**	**	**	**	**	
Genotypes									
Giza 171	119.2	115.0	21.2	122.9	90.7	13.4	23.4	2.64	0.38
Suweon 287R	108.1	85.1	21.6	119.4	90.7	15.8	27.6	2.97	0.41
Milang	114.1	78.4	17.0	121.3	80.3	18.5	24.2	2.52	0.32
Agami	112.0	118.6	21.5	115.8	75.3	15.0	29.0	3.28	0.23
Giza 159	112.2	119.3	23.5	124.1	91.4	15.6	27.1	3.20	0.27
IR66R	109.9	96.2	22.5	108.6	90.8	19.5	23.7	2.66	0.35
Pusa 150-9-3-IR	104.7	95.2	25.3	126.2	83.0	17.7	21.8	3.08	0.32
IR4467-3-2-2R	108.8	81.4	25.9	126.1	86.1	19.3	26.5	3.10	0.28
Sakha 104	99.4	90.9	21.5	130.0	79.3	17.6	26.9	3.11	0.34
Giza 177	88.7	93.4	21.1	128.7	94.4	17.2	27.2	2.85	0.39
Giza 181	100.2	90.7	22.6	116.8	88.2	19.7	23.8	2.71	0.39
Giza 182	104.8	80.7	21.6	123.1	85.1	17.5	23.0	3.24	0.30
Giza 178	103.8	87.2	22.8	128.0	84.8	21.5	21.4	3.34	0.29
Giza 176	105.2	96.6	20.3	121.3	81.1	14.6	23.2	3.05	0.40
Sakha 102	96.9	95.0	20.9	124.6	88.4	15.4	27.2	2.57	0.45
Sakha 103	88.9	81.2	20.8	114.8	93.2	13.9	26.0	2.44	0.52
Riho	93.4	103.2	16.8	111.3	91.2	17.8	24.2	3.06	0.35
Giza 175	100.3	86.0	18.6	115.4	81.2	20.2	20.4	2.99	0.34
Sakha 101	104.6	86.6	23.2	132.1	92.3	14.1	26.4	2.70	0.47
Hybrid rice No. 1	100.8	93.7	22.8	130.7	82.8	21.0	25.3	3.28	0.41
Hybrid rice No.2	107.0	92.3	23.4	140.7	81.9	17.6	24.8	3.35	0.30
Giza 172	117.7	113.3	20.1	119.4	89.9	14.6	23.2	2.25	0.39
Giza 1368-5-4	92.2	100.3	22.0	126.1	86.0	22.2	22.2	3.28	0.27
G 46B	94.5	83.3	22.3	140.6	87.3	15.2	25.1	2.84	0.38
G 46A x Giza 178	103.9	94.9	24.0	152.6	79.7	16.6	24.5	3.37	0.33
L.S.D									
5%	1.30	1.74	0.74	1.58	0.81	0.65	0.25	0.08	
1%	1.83	2.45	1.04	2.22	1.14	0.91	0.35	0.10	

Table (2): Number of days to 50% heading, plant height and panicle length as influenced by the interaction between rice genotypes and irrigation intervals (Combined data, 2008 and 2009).

Genotypes	Irrigation interval (day)								
	6	9	12	6	9	12	6	9	12
	Number of days to 50% heading			Plant height			Panicle length		
Giza 171	115.0	118.3	124.3	116.0	115.0	114.0	22.3	22.2	19.2
Suweon 287R	106.0	108.0	110.3	91.7	83.7	80.0	23.3	21.1	20.4
Milang	114.3	113.0	115.0	79.0	79.7	76.7	18.6	17.3	15.2
Agami	110.7	111.7	113.7	124.7	120.7	110.3	23.0	22.2	19.3
Giza 159	111.0	113.0	112.7	120.7	119.0	118.3	25.2	23.2	22.0
IR66R	108.0	111.7	110.0	104.3	96.0	88.3	26.0	22.3	19.1
Pusa 150-9-3-IR	103.0	105.0	106.0	96.0	99.3	90.3	27.1	24.3	24.6
IR4467-3-2-2R	107.7	108.0	110.7	85.0	82.0	77.3	30.1	29.1	18.5
Sakha 104	101.3	99.0	98.0	93.0	96.3	83.3	23.0	21.2	20.3
Giza 177	87.7	88.0	90.3	99.7	90.3	90.3	22.3	21.3	19.5
Giza 181	98.6	99.7	102.0	95.7	93.3	83.0	24.4	23.1	20.4
Giza 182	105.3	106.0	103.0	84.7	83.0	74.3	25.1	21.5	18.4
Giza 178	105.3	104.0	102.0	92.3	88.0	81.3	26.2	22.5	19.8
Giza 176	105.0	105.3	105.0	96.7	98.0	95.0	22.9	21.6	16.5
Sakha 102	95.0	96.0	99.7	101.0	98.0	86.0	26.1	21.5	15.1
Sakha 103	87.7	87.0	91.3	82.7	83.7	77.3	22.2	21.1	19.1
Riho	94.3	94.0	92.0	114.3	99.0	96.3	18.1	17.3	15.0
Giza 175	98.0	99.7	103.3	91.3	89.7	77.0	20.4	19.1	16.4
Sakha 101	104.0	104.3	105.3	93.0	84.7	82.0	22.4	23.2	23.9
Hybrid rice No. 1	100.0	100.3	102.0	94.0	95.0	92.0	24.1	23.1	21.1
Hybrid rice No.2	104.0	107.0	110.0	96.7	94.3	86.0	25.6	21.4	23.1
Giza 172	120.0	118.0	115.0	125.0	112.0	103.0	21.9	20.2	18.1
Giza 1368-5-4	91.7	90.0	95.0	109.3	99.7	92.0	22.6	22.2	21.2
G 46B	92.5	95.7	96.0	87.0	85.7	77.3	26.1	24.4	22.3
G 46A x Giza 178	101.0	102.7	108.0	108.0	87.7	89.0	25.5	23.4	23.1
L.S.D.									
5%	2.23			2.98			1.26		
1%	3.18			4.24			1.80		

Table (3): Number of grains/panicle, fertility percentage and number of panicles/plant as influenced by the interaction between rice genotypes and irrigation intervals (Combined data, 2008 and 2009)

Genotypes	irrigation intervals (day)								
	6	9	12	6	9	12	6	9	12
	Number of grains/panicle			Fertility percentage			Number of panicles/plant		
Giza 171	132.3	122.3	114.0	96.0	94.0	82.0	13.7	14.5	12.1
Suweon 287R	138.0	119.3	101.0	85.3	80.7	75.0	17.4	16.3	13.6
Milang	129.0	122.0	113.0	77.7	76.0	72.3	19.5	17.6	18.5
Agami	132.3	112.0	103.0	95.0	92.0	87.0	16.9	15.6	12.7
Giza 159	134.3	116.0	122.0	96.0	91.0	85.4	17.6	15.5	13.7
IR66R	124.0	104.7	97.0	88.0	82.0	79.1	23.7	16.4	18.5
Pusa 150-9-3-IR	142.7	120.0	116.0	90.0	88.0	80.3	19.0	19.2	15.1
IR4467-3-2-2R	139.7	121.0	117.7	83.1	79.0	76.2	21.5	20.0	16.3
Sakha 104	135.3	129.0	125.7	93.0	85.0	82.3	18.6	17.6	16.6
Giza 177	140.3	132.0	113.7	96.3	95.0	92.0	19.2	15.6	16.8
Giza 181	130.0	125.3	95.0	92.3	87.0	85.3	20.8	22.0	16.4
Giza 182	137.0	120.0	112.3	94.0	86.3	75.0	19.3	18.9	14.3
Giza 178	142.0	137.0	105.0	90.0	84.0	80.3	20.7	24.7	19.0
Giza 176	128.0	128.0	108.0	86.0	81.3	76.5	16.1	17.3	10.5
Sakha 102	138.0	130.0	105.7	92.0	87.0	86.3	16.1	17.6	12.4
Sakha 103	130.0	108.0	106.3	94.7	94.0	91.0	14.2	14.7	12.7
Riho	117.0	110.0	107.3	95.0	92.3	86.3	21.3	17.2	15.0
Giza 175	130.0	110.0	106.3	86.0	83.0	74.7	20.4	23.4	16.9
Sakha 101	145.0	139.0	112.3	97.9	92.3	87.7	16.2	15.1	11.0
Hybrid rice No.1	137.3	144.0	110.7	88.0	84.0	76.3	21.4	22.3	19.3
Hybrid rice No. 2	150.0	142.0	130.0	85.0	82.3	78.3	18.9	17.6	16.2
Giza 172	136.0	121.3	101.0	94.2	90.3	85.3	18.2	13.0	12.7
Giza 1368-5-4	132.3	130.0	116.0	92.0	87.0	79.0	24.7	23.6	18.4
G 46B	152.0	139.0	130.7	92.0	88.1	82.0	17.6	16.4	11.7
G 46A x Giza 178	167.0	156.0	134.7	87.0	81.0	71.0	17.4	13.6	15.8
L.S.D									
5%		2.70			1.38			1.11	
1%		3.85			1.97			1.58	

Table (4): 1000-grain weight and grain yield as influenced by the interaction between rice genotypes and irrigation intervals (Combined data, 2008 and 2009).

Genotypes	Irrigation intervals					
	6	9	12	6	9	12
	1000-grain weight (g)			Grain yield (ton/fed.)		
Giza 171	24.4	23.8	22.0	3.23	2.69	2.01
Suweon 287R	27.7	27.7	27.5	3.72	3.0	2.18
Milang	25.5	24.2	23.0	2.8	2.85	1.90
Agami	29.8	29.3	28.0	3.69	3.31	2.83
Giza 159	28.5	27.0	25.7	3.65	3.3	2.65
IR66R	24.3	23.6	23.2	3.0	3.02	1.95
Pusa 150-9-3-IR	22.4	21.5	21.5	3.72	2.99	2.52
IR4467-3-2-2R	26.4	27.0	26.0	3.66	3.0	2.63
Sakha 104	27.9	27.0	25.8	3.62	3.3	2.4
Giza 177	28.0	27.2	26.4	3.5	2.91	2.15
Giza 181	24.0	23.8	23.5	3.65	2.25	2.22
Giza 182	24.5	22.9	21.7	3.84	3.2	2.69
Giza 178	21.2	22.0	21.1	3.86	3.4	2.75
Giza 176	23.6	23.8	22.1	3.85	3.01	2.3
Sakha 102	28.4	27.0	26.2	3.24	2.65	1.8
Sakha 103	27.5	26.0	25.4	3.3	2.43	1.6
Riho	25.0	24.2	23.5	3.54	3.32	2.31
Giza 175	21.0	21.3	19.0	3.55	3.07	2.35
Sakha 101	28.3	26.4	24.5	3.63	2.56	1.92
Hybrid rice No. 1	26.5	25.5	24.0	4.42	2.80	2.61
Hybrid rice No. 2	25.8	25.0	23.6	3.93	3.31	2.75
Giza 172	24.8	23.0	21.7	2.95	2.0	1.8
Giza 1368-5-4	22.9	22.3	21.3	3.77	3.32	2.75
G 46B	26.3	24.8	24.2	3.72	2.49	2.31
G 46A x Giza 178	25.0	24.7	23.8	4.2	3.1	2.82
L.S.D						
5%		0.43			0.11	
1%		0.61			0.16	

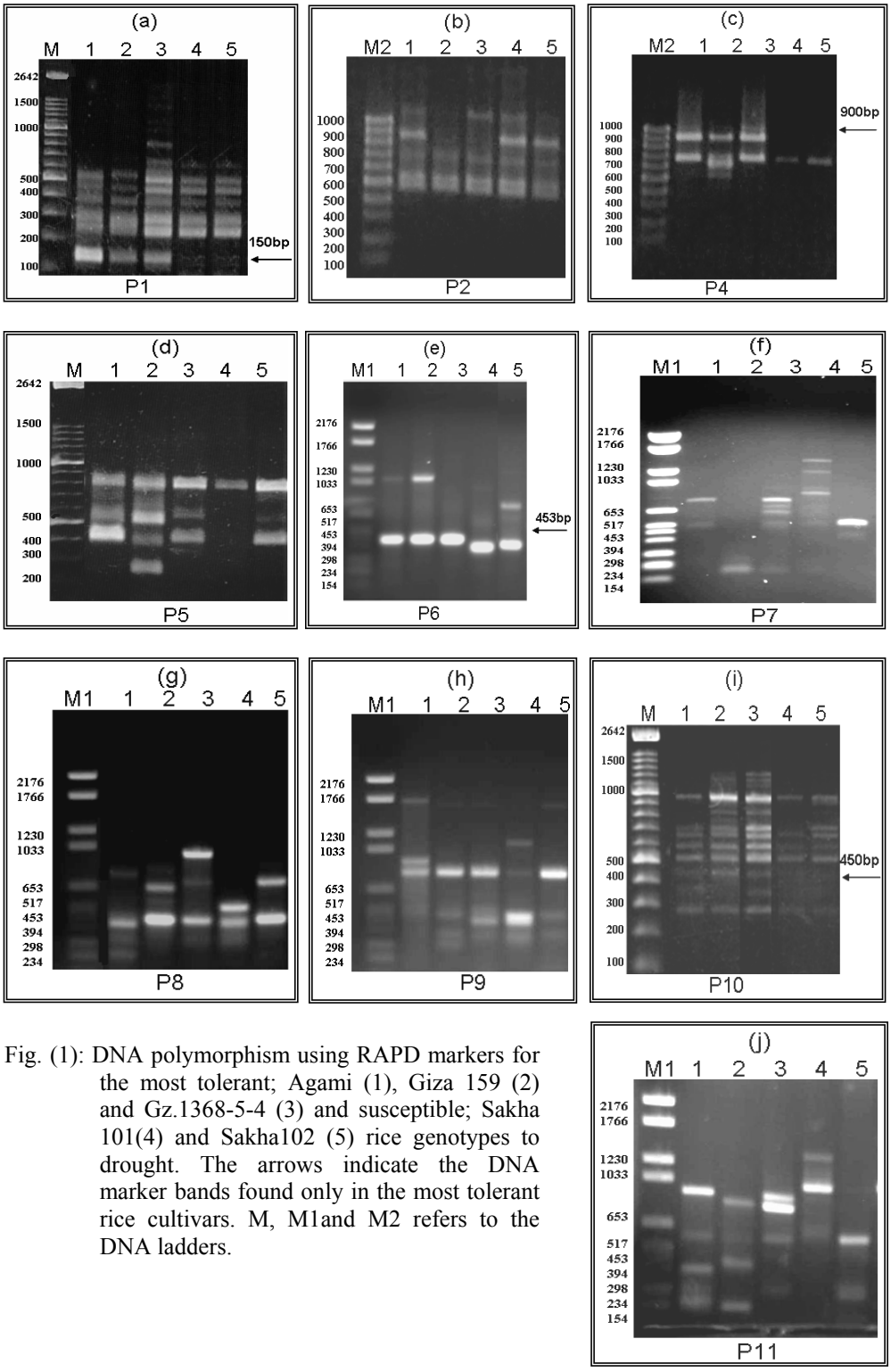

Fig. (1): DNA polymorphism using RAPD markers for the most tolerant; Agami (1), Giza 159 (2) and Gz.1368-5-4 (3) and susceptible; Sakha 101(4) and Sakha102 (5) rice genotypes to drought. The arrows indicate the DNA marker bands found only in the most tolerant rice cultivars. M, M1and M2 refers to the DNA ladders.

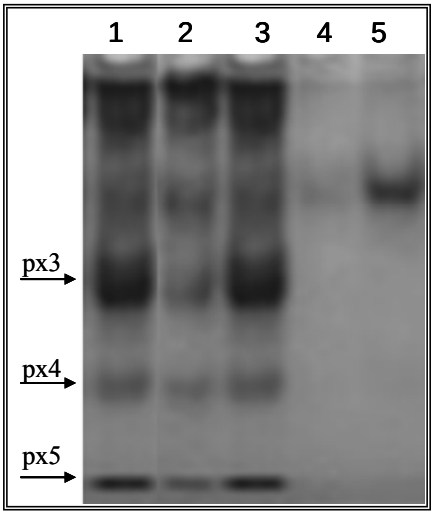

Fig. (2): Photograph of peroxidase isozyme banding patterns of the five rice genotypes. Lanes 1-3: Agami, Giza 159 and Gz 1368-5-4, respectively. Lanes 4-5: Sakha 101 and Sakha 102, respectively.

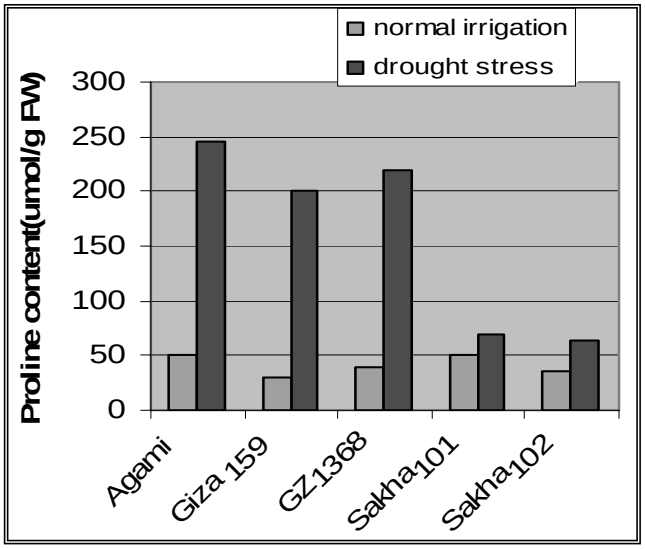

Fig. (3): Proline content in the five different rice genotypes.