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PHYSIOLOGICAL ANALYSIS OF RICE GENOTYPES FOR THE SELECTION OF WATER STRESS TOLERANCE

Abdul Jabbar*[1], Aisha Shereen[2], Zahoor Ahmed Soomro[1] and Shah Nawaz Mari[1]

[1]Department of Plant breeding and genetics, Faculty of Crop Production, Sindh Agriculture University, Tandojam, Sindh, Pakistan
[2]Plant Physiology Division, Nuclear Institute of Agriculture, Tandojam, Pakistan
**Corresponding author's email: ajchandiio@gmail.com*

ABSTRACT

Rice is an essential cereal food and cash crop in Pakistan. Drought is main growth limiting factor in rice productivity, about 50%. The present study was conducted to explore rice genotypes within physiological perspective for the identification of potential donors to be used in cross breeding program for improving water stress tolerance in rice. For this purpose, eight rice genotypes; were obtained from Nuclear Institute of Agriculture (NIA), Tandojam, studied for their physiological responses at anthesis stage with two treatments (water stress and control) and three replications. All physiological responses of collected rice genotypes were affected variably under stress condition. The relative water contents (RWC), chlorophyll, and leaf area (LA), were reduced significantly. On the contrary electrolyte leakage (EL), proline, and potassium (K) increased among the rice genotypes. Genotypic comparison has clearly shown that IR-83140-B-28-B, IR-04L-191 and GML-510 exhibited better tolerance potential against WS in term of (LA), chlorophyll and RWC. Differential osmo-regulatory responses were observed among the genotypes. The trait of K and proline showed highly significant positive correlation with RWC. Therefore, these characteristics may be used with selection tool for the determining drought tolerant.

Key words: *Oryza sativa*, proline, chlorophyll, water stress, correlation

INTRODUCTION

Rice (*Oryza sativa* L.) is the 2nd major staple and a fundamental food for more than half of the world's population, grown in both temperate and tropical regions (Shivani *et al*., 2017 and Buffon *et al*., 2018). It is an important food as well as cereal cash crop in Pakistan; it contributes 3.5% of value added in Agriculture and 0.7%in GDP. Rice production comprises of Basmati (fine) and coarse types. The crop is cultivated on 3.34 mha and produced 8.4 mt with a yield of 2.5 t/ha (Pakistan Economic Survey, 2020-21). Its harvest yield is significantly influenced worldwide by various environmental constraints among which abiotic stresses including salinity, drought (water stress), temperature, mineral toxicity and nutrient deficiency are the key growth limiting factors negatively affect rice productivity more than 50% (Alejandro *et al*., 2021). Among these stresses water shortage / drought represents primary cause of losses in rice crop productivity up to 70% due to its high-water requirements (Lum *et al*., 2014). It is investigated that approximately, 70 m ha of rice growing areas suffered from water scarcity. This crop needs more water (3000L/kg rice production) to grow consumes approximately 24-30% of total fresh water available. In future impact of the water shortage on rice production will further aggravate due to population growth and climate changes. Thus, there is dire need to identify/ develop rice genotypes with water stress tolerant character appropriate to flourish rice with sustainable grain productivity under water deficit conditions (Ahmad *et al*., 2014).

Drought a stress is multidimensional stress affect growth at all stages and ultimately affects its yield. Rice plant responses to osmotic stress due to shortage of water are variable depends on intensity of shortage, stage of growth, environmental factors, and their genetic potential. Physiologically crop plant under water stress manifest different responses related to osmotic adjustment for regulating their normal metabolic functions may play adaptive role for tolerance in plant. Studies have indicated that these metabolic functions are affected through hampered nutrient uptake and assimilation rates through different enzymes results in many physiological disorders (Ying *et al*., 2020). Sustainability in rice production under water stress environment requires improvement with genetic aspects for improving crop potential. For this all-inclusive understanding of physiological traits of adaptive nature which are responsible for water deficit is prerequisite for developing rice genotypes possessing specific stress tolerant genes/traits under stressed environments (Shereen *et al*., 2019).

Conventional breeding strategies may play significant role for production of high yielding stress tolerant varieties combined physiological and genetic approaches. Specific adaptive traits may be contributed effectively through exploitation of genetic techniques heterosis as well as the specific combining ability of crosses. Diallel analysis is an effective tool for measuring the combining ability (GCA and SCA) of crosses and efficient utilization of heterosis. Muthuramu *et al*. (2011) reported that hybrid rice varieties have been released with 15 to 20% greater yield potential than the commercial growing rice varieties. Manickavelu *et al*. (2006) have reported that days to 75% heading, relative water content, plant height, and other leaf traits were regulated by different additive genes, whereas recovery rate, days to flowering, productive tillers plant^{-1}, 100 grain weight, root and shoot length, root dry weight, root shoot ratio and grain productivity may be enhanced by heterosis breeding.

The aim of present research is to explore rice genotypes within physiological perspective for the identification of potential donors to be used in cross breeding program for improving water stress tolerance in rice.

MATERIALS AND METHODS

The study was conducted in the Plant Physiology Division of Nuclear Institute of Agriculture (NIA), Tandojam as collaboration research between Sindh Agriculture University and NIA, Tandojam during 2018-19. Eight rice genotypes; HHZ5-SAL10-DT2-DT1, IR 83140-B-28-B, GML-498, GML-500, GML-510, GML-511 and GML-514 along with IR-04L-191 (International drought tolerant check) were collected from Nuclear Institute of Agriculture (NIA), Tandojam.

The research experiment was conducted in completely randomized block design (RCBD) with 3 replicates in hydroponically controlled water stress condition. The rice seeds were planted in sand Nursery at the age of 21 days was transplanted in cemented sand filled beds (bed size: 1.22 m width and 8.84 m long) irrigated with nutrients solution (Hoagland-1956). Two weeks after transplantation irrigation was stopped for the period of 15 days and then re-irrigated with known quantity of water at field capacity till complete flowering stage following alternate wetting and drying technique (AWD). Second leaf (flag leaf) from top were harvested and investigated for subsequent physiological parameters i.e. cell membrane stability, chlorophyll, potassium, leaf area, proline, relative water contents and total soluble sugar after exposure of water stress for the time period of 15 days.

Cell membrane stability

Cell membrane stability was measured in term of electrolyte leakage (EL) as prescribed by Wu *et al.* (2017). 100 mg fresh rice leaves cutting (0.1g pieces) were kept in ten milliliter de-ionized water and these were incubated in plastic water bath at 32^{o}C. After 120 minutes electrical conductivity (EC) of medium was noted (EC-1). The same samples were sterilized at 121^{o}C for twenty minutes for releasing electrolytes. The samples kept at room temperature for cooling and electrical conductivity (EC-2) was recorded. The rate of electrolyte leakage was calculated by using following formula:

EL = EC (1) / EC (2) x100

Chlorophyll content (mg g^{-1} fresh weight)

Chopped shoot fresh weight (100mg) was taken in 10 mL acetone (80%) and kept overnight. The extracts of leaves were then centrifuge at (4000xg) for 5 min. Chlorophyll contents were read at spectrophotometer (Hitachi double beam 150, Japan), (Lichtenthaler, 1987) by taking absorbance at 663.2 and 646.8 nm.

Shoot Potassium concentration (K %)

Shoots samples (0.1 g) of rice plant were extracted in 10 mL of (100 mM) acetic acid (CH_3COOH) in water bath at 90^{o}C for 1h as by Yeo and Flowers (1993). The extracted material was filtered, and ions were read at flame photometer (Jenway, Model PFP7) followed by calculation against known standard curve.

Leaf area (cm^{2})

Leaf area (next to flag leaf of main tiller) of selected plants was read on the leaf area meter (AM-300).

Proline contents (μg/g fresh weight)

Freshly chopped rice shoot samples (0.5g) were mixed in 10 milliliter sulphosalicylic acid (3%) and then filtered. Two mL of the filtrated sample was reacted with a rate of 2ml acid ninhydrin and 2 mL of glacial acetic acid (GAA) at 100^{o}C for one hr. The ice bath was used to stop reaction. 4 mL of toluene was added and mixed to same reacted filtrate and mixed for a while up to 20-25 seconds on vortex mixer equipment. The Toluene layer was taken away and read at 520 nano meters on spectrophotometer with double beams (Hitachi 150, Japan). Afterward Proline concentrations were calculated on fresh weight basis using formula (Bates *et al.,* 1973).

(μ moles proline /g FW = [(μg proline / mL x 4 mL toluene)/115.5 μ g / μ moles]/ [0.5 g/5].

Relative water contents (%)

The relative water contents were analyzed prescribed method of Bonnet *et al*. (2000). Expended 10 leave samples of were collected from each replication. The segments of leaf (10cm) were weighed (FW) immediately, kept in distilled water for 10 h at room temperature, after that the turgid weight (TW). Sample was then kept for oven drying in paper bag for 72 h at a temperature of 80^0C and the dry weight (DW) of the leaf samples was measured. The RWC (%) was calculated with following formula:

$$RWC= [(FW-DW) / (TW-DW)] X100$$

Total soluble sugars (%)

Fresh leaves were chopped, and 1.0 g sample was taken and shacked in ten milliliters ethanol 80% (v/v) for overnight. The 100 µL of solution was then mixed and heated with 3ml anthrone (150 mg in 100 mL of 72% H_2SO_4) at 97^oC for ten minutes then cooled in ice (Riazi *et al.*, 1985). The absorbances were read at wavelength of 625 nm in double beam spectrophotometer (Model: Hitachi 150-20, Japan).

Statistical analysis

The Collected data was analyzed through using two-way ANOVA for treatments, genotypes, treatments, and their interactions. Later, Tukey HSD test was to compare applied treatments means (at 5%). Correlation co-efficient (Pearson's) studies were done for all physiological traits statistix 8.1 [analytical software Inc., Tallahassee, FL, USA] software. Based on parameters studied better performing rice genotypes were selected for crossing purpose.

RESULTS AND DISCUSSION

The mean squares of 08 genotypes for electrolyte leakage, chlorophyll, potassium, leaf area, proline, relative water content (%) and total sugar soluble (Table-3) showed that the genotypes are highly significant at 5% level of probability for all the traits studied.

Leaf area (cm^2)

In non-stress condition (Table1), the range of leaf area was 24.5 to 40.7, while in stress condition it ranged from 21.5 to 37.2. On an average, 9.8% decrease was caused due to water stress. Among the tested genotypes, in control condition genotype IR-83140-B-28-B (40.7) showed greater leaf area, followed by GML-511 (37.1) whereas less leaf area was observed in genotype HHZ-5-SAL-10-DT1-DT1 (24.5). Under stress condition the leaf area was reduced with varying extents, the greater leaf area was observed in the genotype IR 83140-B-28-B (37.2). The genotype IR-04L-191 showed comparatively less reduction (5.2%) in leaf area under water stress condition. In genotype IR-83140-B-28-B, leaf area and chlorophyll both traits were observed comparatively good under control as well as under stress. The leaf area is the main source of preparing food for the plants is considered an imperative variable for eco-physiological studies related to interception of sunlight, photosynthetic productivity, evapotranspiration, fertilizers, and irrigation responses and plant growth (Pandey and Singh, 2011). Several leaf traits i.e., higher flag leaf area, leaf area index, leaf relative water content and leaf pigment content have been used for the screening of drought tolerant variety (Farooq *et al.*, 2009; Mishra and Panda, 2017; Hussain *et al*., 2018). Kumar *et al*. (2021) reported that drought tolerant varieties show longer flag leaf with higher stomates density as compared to drought susceptible genotypes. Due to limited water under drought stress reduced leaf growth is reported (Zhu *et al*., 2020). From xylem towards other cell flow of water disrupted, which leads to water deficiency and causes lower turgor pressure, responds in form of deprived cell growth/development and changes in anatomy of leaf may be the main cause of reduced leaf area in crops (Hussain *et al*., 2018; Upadhyaya and Panda, 2019).

Chlorophyll content (mg g^{-1} fresh weight)

Chlorophyll contents generally decreased among rice genotypes under water stress condition with varying intensities. The range of chlorophyll under non-stress condition (Table 1) was 1.9 to 2.3 and the range in stress condition was 1.6 to 1.9. Comparison among the genotype has shown that the maximum chlorophyll in control condition was observed GML-498, GML-500 and GML-510 (2.3) followed by IR-83140-B-28-B, IR-04L-191 and GML-511 (2.1) whereas the minimum chlorophyll in control condition was observed in GML-514 (1.9). The maximum chlorophyll in water stress condition was observed in genotype IR-04L-191 (1.99) followed by IR 83140-B-28-B (1.96). The genotype IR-04L-191 was significantly different from other genotypes as least relative reduction in chlorophyll contents (5.2 %) was observed under water stress. Sheeren *et al*. (2019) reported that during stress chlorophyll content is reduced in rice leaves with increasing concentrations of PEG-6000 and the stress tolerant genotypes had different osmo-regulatory response with the solute production. Chlorophyll pigments plays vital role in photosynthetic process quenching of for light and generation of reducing powers thus reduced Carbon dioxide utilization efficiency of mesophyll cells (Debabrata *et al*., 2021). Nutrient deficiency under water stress may be another factor for abnormal functioning of the photosynthetic apparatus. Sami *et al*. (2020) explored that grain yield traits and water use efficiency can be improved by utilization of potassium under both water stress as well as control conditions but result was more protruding under water stress conditions than control conditions.

Electrolyte leakage (EL)

In non-stress condition, the range of electrolyte leakage was 22.5-36.8 while in water-stress condition it ranged from 32.6 to 55.8 (Table1). On an average, water stress caused 53.5 % increases in electrolyte leakage (EL). Among the genotypes, under control condition the maximum electrolyte leakage (EL) was observed in GML-514 (36.8), followed by IR-04L-191 (32.1) whereas minimum electrolyte leakage (EL) observed in genotypes GML-511 (22.5). The maximum electrolyte leakage (EL) in stress condition was observed in the genotype GML-514 (55.8), followed by GML-500 (47.6) whereas minimum electrolyte leakage (EL) was observed by GML-511(32.6). Among the genotypes IR-04L-191 exhibited reasonably less electrolyte leakage (EL) with least relative increase (23.7%). The electrolyte leakage is the main stress response that occurs due to many stress factors, indicate stress-induced injury of plant tissues, and is also considered as a measure of cell membrane stability of plants. Under water stressed conditions, lipids structure of membrane becomes unorganized and causes structural changes in bilayer lipid membrane due to which membrane become more permeable to solutes. The electrolyte leakage increases due to increase in ammonium accumulation and simultaneously decreases the protein degradation. Drought and heat stress also triggered oxidative damage by increased generation of hydrogen peroxide, which results high electrolyte leakage. Shahadat *et al*. (2022) compared rice genotypes in stress conditions and reported, decrease in photosynthetic pigments, water status, and plant biomass, whereas significant increase and mortality rate also seen, hydrogen peroxide content, electrolyte leakage, lipoxygenase activity, level of malondialdehyde and methylglyoxal, indicating increased lipid peroxidation. In the present study variable decrease of dissimilar rice genotypes were also reflected in their physiological responses. Traits of cell membrane stability/membrane stability index have been used to know its correlation with yield of rice under drought stress (Debabrata *et al*., 2021).

Shoot Potassium concentrations (%)

The data of potassium concentration in shoot (Table 1) has shown a very high reduction in genotypes under stress condition. Almost all genotypes have shown more than 93 % average reduction in comparison to control (Table 1). The tested genotype HHZ-5-SAL-10-DT1-DT1 was distinctly different from the other genotypes, as this genotype maintain higher potassium concentration under water stress with comparatively less relative reduction (41.2). Potassium is an important element play a vital role in osmo-regulation under stress and improves plant vigor and make tolerant to disease and pests. Qiwen *et al*. (2021) reported that potassium reduced the deleterious effect of drought stress on plant but increasing the drought, it decreases the potassium and simultaneously plant become weaker and less tolerant to abiotic and biotic stresses. Potassium causes the effect on the photosynthesis process by synthesis of ATP, stimulation of the enzymes involved in photosynthesis, stomatal movement, and electric charges required for photophosphorylation in chloroplasts (Marschner, 1995). Function of photosynthetic apparatus disrupted by nutrient deficiency (Smethurst *et al*., 2005).

Proline contents (μg/g fresh weight)

In non-stress condition, the range of proline was 0.94 to 1.14, while in stress condition proline content increased in all rice genotypes in range of 4.13 to 9.71. On an average, water stress caused increase of 535.78 % (Table 2). Among the genotypes, in control condition maximum proline shown by genotype GML-498 (1.14), followed by GML-510 and GML-514 (1.13) whereas minimum proline was observed in genotype IR-04L-191 (0.94). For the stress condition the maximum proline was observed in the genotype GML-500 (9.71). Plants are capable and having quality to synthesize various solutes under the water-deficient conditions, to adjust smooth water flow from dried soil hence, helping rice plants for the efficient osmotic modification and maintenance of development and growth under water stress conditions. Higher accretion of proline is usually related with drought tolerance, and it benefits for preservation of shoot turgor and stomatal conductance (Debabrata *et al*., 2021; Ying *et al*., 2020). Thus, proline content may be considered as a biochemical marker under drought screening of plants (Debabrata *et al*., 2021). It has also been observed in the present study that those genotypes showing higher relative water content also produced comparatively higher osmolytes i.e., proline and potassium, thus maintained osmoregulation under water stress. Correlation studies (Table 4) also revealed significant positive correlation of relative water content with proline (0.8323) and potassium (0.8648).

Table 1. Mean performance of rice genotypes for Electrolyte leakage (%), chlorophyll, potassium, and leaf area.

Genotypes	**Electrolyte leakage (%)**			**Chlorophyll (mg g^{-1} fresh weight)**			**Potassium (%)**			**Leaf Area (cm^2)**		
	Control	**Water Stress**	**Inc%**	**Control**	**Water Stress**	**RD %**	**Control**	**Water Stress**	**RD%**	**Control**	**Water Stress**	**RD %**
HHZ-5-SAL-10-DT1-DT1	24.9	41.5	66.7	2.0	1.6	20.0	1.7	1.0	41.2	24.5	21.5	12.2
IR 83140-B-28-B	28.1	43.8	55.9	2.1	1.96	6.7	1.9	0.3	84.2	40.7	37.2	8.6
IR-04L-191	32.1	39.7	23.7	2.1	1.99	5.2	2.0	0.4	80.0	33.1	30.8	6.9
GML-498	26.9	36.7	36.4	2.3	1.7	26.1	1.9	0.3	84.2	35.4	30.5	13.8
GML-500	30.3	47.6	57.1	2.3	1.8	21.7	2.1	0.2	90.5	30.9	28.5	7.8
GML-510	23.7	45.5	92.0	2.3	1.8	21.7	1.9	0.2	89.5	30.9	27.9	9.7
GML-511	22.5	32.6	44.9	2.1	1.7	19.0	1.7	0.4	76.5	37.1	34	8.4
GML-514	36.8	55.8	51.6	1.9	1.7	10.5	1.4	0.2	85.7	25.36	22.6	10.9

HSD value for Genotype at Alpha 5% = 5.3023
HSD value for Treatment at Alpha 5% = 2.6512
HSD value for Genotype x Treatment at Alpha 5% = 7.5978

Table 2. Mean performance of rice genotypes for proline, relative water content (RWC) and total soluble sugar (TSS).

Genotypes	**Proline (µg/g f w)**			**RWC (%)**			**TSS**		
	Control	**Water Stress**	**Inc%**	**Control**	**Water Stress**	**RD%**	**Control**	**Water Stress**	**Inc%**
HHZ-5-SAL-10-DT1-DT1	1.05	4.13	293.3	97.5	75.3	22.8	0.42	0.89	111.9
IR 83140-B-28-B	0.98	7.98	712	94.4	83.1	12.0	0.36	0.72	100.0
IR-04L-191	0.94	6.97	645	94.1	80.6	14.3	0.32	0.79	146.9
GML-498	1.14	5.22	357	97	76.2	21.4	0.41	0.78	90.2
GML-500	1.12	9.71	767	93	75.1	19.2	0.4	0.81	102.5
GML-510	1.13	6.24	451	92	84.6	8.0	0.44	0.83	88.6
GML-511	1.0	8.12	714	94	82.5	12.2	0.32	0.74	131.3
GML-514	1.13	5.03	347	92.4	79.9	13.5	0.34	0.71	108.8

HSD value for Genotype at Alpha 5% = 7.6749
HSD value for Treatment at Alpha 5% = 3.8375
HSD value for Genotype x Treatment at Alpha 5% = 10.8540

Table 3. Mean squares from analysis of variances for Electrolyte leakage, Chlorophyll, Potassium, Leaf Area, Proline, Relative Water Content and Total Soluble Sugar.

SOV	**Replication**	**Genotype**	**Treatment**	**Genotype x Treatment**	**Error**
DF	2	7	1	7	16
Electrolyte Leakage	3.332 NS	336.336**	195.133**	3.252NS	2.294NS
Chlorophyll	0.00301 NS	0.07554 **	1.30680**	0.04744 NS	0.01613*
Potassium	0.0708 NS	0.1236 NS	15.8700 **	0.1504 NS	0.0662 NS
Leaf Area	0.844 NS	179.433 **	119.417 **	0.963 NS	5.921 NS
Proline	0.827 NS	5.154 **	378.226 **	5.375 NS	0.855 **
Relative Water Content	8.29 NS	22.58 NS	2927.50 **	47.41 NS	28.33 NS
Total Soluble Sugar	0.00219 *	0.01470 **	1.98453 **	0.00309 NS	0.00055 **

** = Significant at 1% probability level. NS = non-significant, * = Significant @ 5% probability

Table 4. Correlation coefficients (r) for cell membrane stability, chlorophyll, potassium, leaf area, proline, relative water content and total soluble sugar rice genotypes.

Character	**Electrolyte leakage**	**Chlorophyll**	**Potassium**	**Leaf Area**	**Proline**	**Relative water content**
Chlorophyll	-0.2559 NS	-	-	-	-	-
Potassium	-0.3317 NS	0.7508 **	-	-	-	-
Leaf Area	0.0659 NS	0.5260 *	0.279 NS	-	-	-
Proline	0.2213 NS	-0.5948 *	-0.9222 **	-0.0999 NS	-	-
Relative water content	-0.2603 NS	0.7971 **	0.8648 **	0.3792 NS	0.8323**	-
Total sugar	0.2008 NS	-0.7629 **	-0.8881 **	-0.3820 NS	0.8670 **	-0.9272 **

**= Highly Significant at P<0.01 and P<0.05; NS= non-significant, * = Significant @ 5% probability

Relative water contents (%)

In non-stress, the range of relative water content (Table 2) was 92.0 to 97.5, while in stress condition it ranged from 75.1 to 84.6. On an average, water stress caused 15.4% decreases. Among the genotypes, the maximum reduction in relative water content % (22.8%) produced by HHZ-5-SAL-10-DT1-DT1 followed by the genotype GML-498 (21.4%) whereas minimum reduction in relative water content% (8.0%) was observed by GML-510. Water stress is of the several factors that negatively affects the RWC, turgor pressure and rate of useful transpiration in many crop plants (Debabrata *et al*., 2021). Relative water contents (RWC) are well-thought-out as an effective trait to quantify water position in plants. This trait helps in enzyme and membrane integrity under water deficit conditions (Shereen *et al*., 2019).

Total soluble sugars

For the trait of total soluble sugar, the range of total soluble sugar in control condition was 0.32 to 0.44, while in stress condition it ranged from 0.71 to 0.83 as shown in Table 2. Normally, stress caused an increase of 146.9%. Among the genotypes, the maximum increase in total soluble sugar (146.9%) produced by (IR-04L-191) followed by the genotype GML-511(131.3%) whereas minimum increase in total soluble sugar (88.60%) was observed by GML-510 (Table 2). Accumulation of soluble sugars during drought acts as osmo protectants under negative conditions and protects plants to a confident extent (Debabrata *et al.*, 2021). Carbohydrates/soluble sugar are the structural unit, which provides energy to build plant biomass. Water-soluble carbohydrates under abiotic stress of basically three types of, viz. disaccharides, oligosaccharides play a vital role for stress tolerance. Soluble sugars are important for matching physiological processes like photosynthetic reactions and mitochondrial respiration. Various roles of sugars in plants as they used numerous sugar-based strategies for acclimatization with environmental stress. The abundance of mannitol, sorbitol and trehalose plays important roles in proper growth and metabolic function of the plant (Philippe *et al*., 2022).

Conclusion

Genotypic variability for water stress tolerance was observed under various physiological responses under stress. Based on results of this study the physiological traits i.e. chlorophyll contents, shoot potassium concentration, leaf area, proline contents and relative water contents were observed beneficial/useful traits for coping genotypes under water stress conditions. Genotypic comparison has shown that IR-83140-B-28-B, IR-04L-191 and GML-510 exhibited better tolerance potential and may be used as genetic resource for traits of leaf area, chlorophyll and RWC. These traits may be beneficially combined through crossing for developing water stress tolerant genotypes.

REFERENCES

Ahmad, M., G. Zaffar, S.M. Razvi, Z.A. Dar, S.D. Mir, S. A. Bukhari and M. Habib (2014). Resilience of cereal crops to abiotic stress: A review. *Afr. J. Biotechnol*., 13(29): 2908-2921.

Alejandro H.S., F.E. Beirute, A.A. Esquivel, M.V. Melara, J. Boch and A.G. Arias (2021). Rice breeding in the new era: Comparison of useful agronomic traits. *Current Plant Biology*, 100211.

Bates, L. S., R.P. Waldern and L.D. Teare (1973). Rapid determination of free proline for water stress studies. *Plant Soil,* 39: 205-207.

Bonnet, M., O. Camares and P. Veisseire (2000). Effect of zinc and influence of *Acremonium lolii* on growth parameters, chlorophyll a fluorescence and antioxidant enzyme activities of ryegrass (*Lolium perenne* L. CV Apollo). *J. Exp. Bot*., 51: 945-953.

Buffon, G., E.A.R. Blasi, A.G.S. Rativa, T.I. Lamb, R. Gastmann and J.M. Adamski (2018). Unraveling rice tolerance mechanisms against *schizotetranychus oryzae* mite infestation. *Bior. Xiv* [Preprint] doi: 10.1101/281733.

Debabrata P., S. S. Mishra and P.K. Behera (2021). Drought tolerance in rice: Focus on recent mechanisms and approaches. *Rice Science*. 28 (2): 119-132.

Farooq, M., N. Kobayashi, A. Wahid, O. Ito and S.M.A. Basra (2009). Strategies for producing more rice with less water. *Adv. Agron.*, 101: 351–388.

Hussain, H.A., S. Hussain, A. Khaliq, U. Ashraf, S.A. Anjum, S.N. Men and. L.C. Wang (2018). Chilling and drought stresses in crop plants: Implications, cross talk, and potential management opportunities. *Front Plant Sci*, 9: 393.

Hoagland, M.B., E.B. Keller and P.C. Zamecnik (1956). Enzymatic carboxyl activation of amino acids. *J. Biol. Chem.* 218, 345–358.

Jefferson M. F., A. Promkhambut and P. Yokying (2020). Impact of COVID-19 on Rice Farmers in Southeast Asia. East-West Wire.

Kumar, S., S. Tripathi, S. P. Singh, A. Prasad, F. Akter, Md. Abu Syed, J. Badri, S. P. Das, R. Bhattarai, M. A. Natividad, M. Quintana, C. Venkateshwarlu, A. Raman, S. Yadav, S.K. Singh, P. S. A. Anandan, R. B. Yadaw, N. P. Mandal, S. B. Verulkar, A. Kumar and A. Henry (2021). Rice breeding for yield under drought has selected for longer flag leaves and lower stomatal density. *J. Experimental Botany*, 72 (13): 4981–4992.

Lichtenthaler, H.K (1987). Chlorophylls and carotenoids: Pigments of photosynthetic bio-membranes. *Methods Enzymol*., 148: 350-382.

Lum, M.S., M.M. Hanafi, Y.M. Rafii and A.S.N. Akmar. (2014). Effect of drought stress on growth, proline, and antioxidant enzyme activities of upland rice. *J. Anim. Plant Sci*., 24(5):1487-1493.

Manickavelu, A., N. Nadarajan, S.K. Ganesh, R.P. Gnanamalar and R. Babu. (2006). Drought tolerance in rice: morphological and molecular genetic consideration. *Plant Growth Regul*., 50: 121–138.

Marschner, H. (1995). Mineral Nutrition of Higher Plants, second ed. Academic, *Great Britain.*

Mishra, S.S., and D. Panda (2017). Leaf traits and antioxidant defense for drought tolerance during early growth stage in some popular traditional rice landraces from Koraput, India. *Rice Sci.*, 24(4): 207–217.

Muthuramu, S., S. Jebaraj and M. Gnanasekaran (2011). AMMI biplot analysis for drought tolerance in rice (*Oryza sativa* L.). *Res. J. Agri. Sci*., 2(1): 98-100.

Pandey S. K and H. Singh (2011). A simple cost-Effective method for leaf area estimation. Hindawi Publishing Corporation. *Journal of Botany*, 2011, Article ID 658240. https://doi.org/10.1155/2011/658240.

Pakistan Economic Survey (2020-21). Ministry of Finance, Economic Advisor's Wing, Islamabad.

Philippe J., M. F-Luboińska, M. Labudda and I. Morkunas (2022). The Role of Sugars in Plant Responses to Stress and Their Regulatory Function during Development. *Int. J. Mol. Sci*., 23 (9): 5161.

Qiwen, X., H. Fu, B. Zhu, H. A. Hussain, K. Zhang, X. Tian, M. Duan, X. Xie and L. Wang (2021). Potassium Improves Drought Stress Tolerance in Plants by Affecting Root Morphology, Root Exudates and Microbial Diversity. *Metabolites*. 11, 131.

Riazi, A., K. Matruda and A. Arslan (1985). Water stress induced changes in concentration of proline and other solutes in growing regions. *J. Exp. Bot.*, 36 (172): 1716-1725.

Sami U-A., M. Ijaz, A Nawaz, A. Sattar, A. Sher, M. Naeem, U. Shahzad, U. Farooq, F. Nawaz and K. Mahmood (2020). Potassium Application Improves Grain Yield and Alleviates Drought Susceptibility in Diverse Maize Hybrids. *Plants*, 9 (1): 75.

Shahadat H., M. F. Karim, M. Fujita, MHM. B. Bhuyan, K. Nahar, A. A. C. Masud, J. Mahmud and M. Hasanuzzaman (2022). Comparative Physiology of Indica and Japonica Rice under Salinity and Drought Stress: An Intrinsic Study on Osmotic Adjustment, Oxidative Stress, Antioxidant Defense and Methylglyoxal Detoxification. *10.3390/stresses2020012*.

Shereen, A., M. A. Khanzada, M. A. W. Baloch, ASMA, M. U. Shirazi, M. A. Khan and M. Arif (2019). Effects of PEG induced Water stress on growth and physiological responses of rice genotypes at seedling stage. *Pak. J. Bot.*, 51(6): 2013-2021.

Shivani, D.K., H. ag. edi, H. Raja, Y. Kunvar, Garima, Gaurav K. , S.O.N. Sanghmitra, V. Nitin and N.A. Khan (2017). Physiology, morphology, and molecular mechanisms for drought tolerance in rice. *Int. J. Micro. Biol. Appl. Sci.*, 6(7): 4160-4173.

Smethurst, C.F., T. Garnett and S. Shabala (2005) Nutritional and chlorophyll fluorescence responses of lucerne (*Medicago sativa*) to waterlogging and subsequent recovery. *Plant Soil*, 270 (1): 31–45.

Upadhyaya, H. and S.K. Panda (2019). Drought stress responses and its management in rice. *In*: Hasanuzzaman M, Fujita M, Nahar K, Bis was J K. *Advances in Rice Research for Abiotic Stress Tolerance*. UK: *Elsevier*: 177–200

Yeo, A.R. and T.J. Flowers (1993). Varietal differences in the toxicity of sodium ions in rice leaves. *Physiol. Plant*., 59: 189-195.

Ying, M., M.C. Dias and H. Freitas (2020). Drought and Salinity Stress Responses and Microbe-Induced Tolerance in Plants. *Front. Plant Sci*., (11) 59191.

Wu, W., Q. Zhang, E.H. Ervin, Z. Yang and X. Zhang (2017). Physiological mechanism of enhancing salt stress tolerance of perennial ryegrass by 24-epibrassinolide. *Front Plant Sci*.; 8: 1017. Doi: 10.3389/fpls.2017.01017

Zhu, R., F.Y. Wu, S. Zhou, T. Hu, J. Huang and Y. Gao (2020). Cumulative effects of drought-flood abrupt alternation on the photosynthetic characteristics of rice. *Environ. Exp. Bot.*, 169: 103901.

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