*Pak. J. Bot.*, 49(SI): 1-6, 2017.

# WATER DEFICIT INDUCED PHYSIOLOGICAL AND YIELD RESPONSES IN *ORYZA SATIVA* L.

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## Abstract

Drought is a serious constraint for rice growth, affecting about 50% of world rice productivity. The yield losses are more severe when drought strikes at flowering stage. Identification of tolerant genotype is urgently needed for the improvement of crop productivity. Under such conditions it requires understanding of physiological strategies adopted by plant to offset the stress. Experiments were conducted using five rice genotypes to study some physiological parameters [i.e. relative water contents (RWC), osmotic potential (OP), proline, total soluble sugars, leaf area, chlorophyll] contributing to yield responses under water stress (aerobic) conditions. Drought tolerant check (IR-04L191) has shown least reduction in grain weight. Among the tested genotypes, comparatively high grain weight was produced by IR83140-B-28-B followed by HHZ-5-Sal 10- $DT_1$ - $DT_1$ under water stress conditions. In addition the genotype HHZ-5-Sal 10- DT1- DT1 and IR 04L 191 also exhibited higher increases in proline and RWC compared to other genotypes. Chlorophyll concentrations and Leaf area were least affected in three tolerant genotypes (IR 83140-B-28-B, IR-72 and HHZ-5-Sal 10- DT1- DT1). Correlation studies under water stress conditions have shown strong negative correlation between RWC and OP. whereas, the parameters of proline ($r_=$ 0. 515), potassium ($r_=$ 0.802) and TSS ($r_=$ 0.617) were positively related with grain weight. Therefore physiological traits such as RWC, proline, potassium, leaf area and chlorophyll may be incorporated along with yield traits in rice screening programmes for water stress tolerance.

**Key words:** Drought, Water deficit, *Oryza sativa*, Relative water content, Osmotic potential

## Introduction

Scarcity of water is an important worldwide constraint for rice crop productivity. This is becoming severe threat especially in the countries with arid and semiarid climates (Ashraf, 2010). It is reported that 40-43% world land masses are drought affected and 12 Mha are lost each year (Anon., 2012). Statistical estimates have shown that drought affected areas have become doubled worldwide during last three decades (Mostajeran & Rahimi- Eichi. 2009).

Rice is a high water requiring (3000 to 5000 L of water / kg) major food crop for three billion people (Cho & Oki, 2012). It is very sensitive to water stress. Global estimates reveal that more than 70 million hectares of land growing under rice cultivation are drought affected (Ahmad *et al*., 2014). Increasing population pressure and global climate change (Kashmir *et al.,* 2016) have further worsened the scenario of water availability for rice crop productivity (Ray *et al*., 2015). It is expected that by the year 2025, 15 Mha. of rice will be affected from physical scarcity of water (Bouman *et al*., 2007). Thus, this is an alarming situation for global food security. The harmful effects of water stress on rice crop varies with time, duration, intensity of drought and growth stages may cause yield losses of 12- 46 %. These losses may be severe (> 70 %) when water stress occurs particularly at booting / flowering stage (Oak *et al*., 2006; Zubaer *et al*., 2007; Ji *et al*., 2012). Therefore, development of drought tolerant rice genotypes is considered a major breeding objective for the improvement of rice yield under water scarce environments (Lafitte *et al.,* 2006).

Drought tolerance is a complex trait involve combination of several mechanism at morphological, physiological and genetic level (Hao & Lin, 2010; Krasensky & Jonak, 2012; Mahmod *et al*., 2014; Zain *et al*., 2014; Pandey & Shukla, 2015). Studies for various drought causing physiological changes in plants have been reported so far including affects on plant-water relations, mineral nutrition, photosynthetic rate, transpiration rate, stomatal conductance, pigment degradation, alteration in assimilate partitioning among the organs, spikelets fertility and grain filling processes which cumulatively affects crop productivity (Zubaer *et al*., 2007; Akram *et al*., 2013; Mahmod *et al*., 2014; Storme & Geelen, 2014; Alter *et al*., 2015; Saleem *et al*., 2016). These studies have shown that more yield losses occurs when stress appears particularly at flowering / reproductive stage and rice genotypes exhibit variability in adaptation at physiological level. Thus, there is a need to study variation in physiological responses of contrasting rice genotypes at flowering stage. Therefore, this study was conducted to evaluate physiological responses at flowering stage and their correlation with grain yield at maturity using five rice genotypes imposing water stress treatment (Irrigation aerobically throughout the growth period; at an interval of fifteen days).

## Materials and Methods

Five rice genotypes [Three tolerant (HHZ 5-SAL10-DT1-DT1, IR 83140-B-28-B and IR-72) and one sensitive (HHZ 5-SAL9-Y3-Y1) rice genotypes of IRRI origin along with drought tolerant check (IR 04L191)] was obtained from NIBGE Faisalabad. The genotypes (selected on grain weight basis from previously conducted mass scale screening programme for water stress) were studied for physiological and yield responses under control and water stress conditions (aerobic) at Nuclear Institute of Agriculture (NIA), Tandojam, Pakistan. The experiment was conducted using RCBD with five replicates in sand filled cemented beds (size: 9 m x1.2 m), under controlled hydroponic system (equipped with recycling of water) in net house. Basic dose of Nitrogen, Phosphorous & Potassium was applied @ 120:60: 60 Kg $ha^{-1}$ (derived from Urea, Di-ammonium phosphate & Potassium Nitrate respectively) in the upper 15 cm layer. Six weeks old rice seedlings (previously grown in sweet soil) were transplanted in sand filled irrigated beds. Six rows of one meter of each genotype were planted in sand filled beds at a distance of 25 cm between rows and hills. Initially the flood irrigation was given to plants (Hoagland & Arnon 1950). After two weeks, irrigation water of one bed (served as water stress treatment) was drawn back (aerobic conditions) to water reservoir. Whereas; the plants in other bed were kept in flooded conditions (served as control). Water stress treatment beds were irrigated aerobically at an interval of fifteen days by following alternate wetting and drying method (Coombs *et al*., 1987) from vegetative stage till maturity. The moisture contents of water stress treatment bed were monitored and calculated thrice a week by following Coombs *et al*., 1987 using the weight fraction of sand from root zone (15 cm depth).

At flowering stage, when soil water contents were at 7 % (after withholding irrigation for15 days) second leaf from top were analyzed for following physiological and biochemical parameters i.e. SPAD chlorophyll, Leaf area ($cm^2$), Relative water content (Bonnet *et al*., 2000), Osmotic potential (Slavik, 1974), proline (Bates *et al.,* 1973), potassium (Yoe and Flowers, 1983), total soluble sugars (Riazi *et al*., 1985). Observations on different growth and yield parameters (Plant height, numbers of tillers, straw weight, panicle numbers, grain yield and 100-grain weight) were recorded on per plant basis at maturity.

**Statistical analysis**

Analysis of variance (ANOVA) was done for genotypes, treatments and their interactions. Tukey's HSD test was applied for comparison between treatment means at $\alpha$ 0.05 using Statistix 8.1 software.

**Results and Discussion**

**Physiological Responses:** The present study has shown variable responses of different rice genotypes in their physiological traits under water stress conditions (Fig.1). Leaf relative water contents (RWC) reduced variably among the genotypes (Fig.1-B). Highest RWC were found in HHZ 5-SAL10-DT1-DT1and IR 83140-B-28-B and least in HHZ 5-SAL9-Y3-Y1 and IR-72. There was also reduction in osmotic potential (OP) of leaves in all rice genotypes under water stress conditions (Fig. 1-C). Least reduction in OP was exhibited by drought tolerant check (IR 04L191) followed by IR83140-B-28-B and HHZ 5-SAL10-DT1-DT1. Comparatively more reduction was observed in genotypes IR-72 and HHZ 5-SAL9-Y3-Y1.The parameters of OP and RWC were significantly negatively correlated ($R^2$ = 0.743) with each other (Fig.1-A). Lowering of OP is generally considered as a useful adaptive trait under water stress (Ji, *et al*., 2012; Maisura *et al*., 2014). Hypothetically this trait could be beneficial for maintaining cell turgor and osmotic adjustment under water stress. In contrast to this hypothesis the results of this study revealed that least increase in OP in tolerant genotypes (IR 04L191, IR83140-B-28-B and HHZ 5-SAL10-DT1-DT1). Ji *et al*. (2012) have also reported comparatively more increase in OP in drought sensitive rice genotype (Zhenshan 97B) than tolerant (IRAT 109).The beneficial role of osmotic adjustment in plants for drawing more water and improving yield under water stress conditions was also not supported by Serraj and Sinclair, (2002). Therefore reduction in OP may be related to osmotic adjustment in sensitive genotypes from survival point of view under water stress environments.

Plants grown under decreased water potential (water deficit conditions) reduce their osmotic potential in relative terms for turgor maintenance through accumulating organic solutes (Cha-um *et al*., 2010; Wang *et al*., 2010; Akram *et al*., 2013; Kumar *et al*., 2014; Alter *et al*., 2015). Variable responses of rice genotypes in their shoot proline, potassium and total soluble sugars concentrations were observed (Fig 1-D, 1-E & 1-F). The sensitive genotype (HHZ 5-SAL9-Y3-Y1) accumulated less proline in comparison to tolerant ones. The highest proline accumulation was observed in HHZ 5-SAL10-DT1-DT1 followed by IR 04L191 (D-T check) and IR 83140-B-28-B. In the present study the role of proline seems to be osmo- regulatory contributed for higher drought tolerance as these genotypes were also found comparatively better in their relative water contents (Fig 1-B). The findings are in agreement with Mostajeran and Rahimi- Eichi, 2009; Chutia and Borha, 2012; Cha-um *et al*., 2013; Maisura *et al*., 2014; Fen *et al*., 2015. Reports related to proline accumulation in other crop species including *Cajanus cajan* L. (Kumar *et al*., 2011), *Portulaca oleracea* L. (Rahdari *et al*., 2012), *Solanum lycopersicum* (Ali & Rab, 2017) and wheat genotypes (Bayoumi *et al*., 2008) have also indicated positive correlation with survival and growth. Thus, the proline and relative water contents may be regarded as screening criteria for drought tolerance in crop plants.

Genotypic variability for shoot potassium (K) concentrations was observed in the present study (Fig 1-E). Under water stress conditions the genotype HHZ 5-SAL10-DT1-DT1 accumulated comparatively more K in shoot followed by IR 83140-B-28-B. The least K concentration was observed in genotype HHZ 5-SAL9-Y3-Y1. Potassium is most preferred ion in plants plays a vital role in different physiological functions i.e. osmotic adjustment, stomatal regulation, enzymes activity, cell homeostasis. Zain *et al*. (2014) have reported that accumulation of K in plants coping with water stress may be more important than organic solutes due to its low energy cost in term of ATP utilization which is 10 times less than organic solutes (Wyn Jones, 1981).

Under water stress conditions total soluble sugars (TSS) were reduced with varying intensities in all genotypes except IR-72. Highest values of TSS were observed in HHZ 5-SAL10-DT1-DT1 and drought tolerant check (Fig 1-F). Factor responsible for decreased photosynthesis (lower water potential, stomatal conductance, leaf area, and chlorophyll and carbon dioxide assimilation rates impaired regeneration of RUBP and RUBISCO) may lead to decrease synthesis of TSS. In contrast to this general increase in total soluble sugars is reported under stress conditions (Mostajeran & Rahimi- Eichi, 2009; Chutia & Borha, 2012; Lum *et al*., 2014; Maisura *et al*., 2014). Sugar metabolism is a very dynamic process varies with genotype and stress factors (Rosa *et al*., 2009). Soluble sugars are an important metabolic resource act as an osmolyte for turgor maintenance play role in source to sink partitioning differentially affected during stress (Rosa *et al*., 2009). Invertase, a key enzyme involved in hydrolysis of sucrose has been regarded as a regulator in partitioning of assimilates is modulated by stress stimuli (Rolland *et al*., 2002).

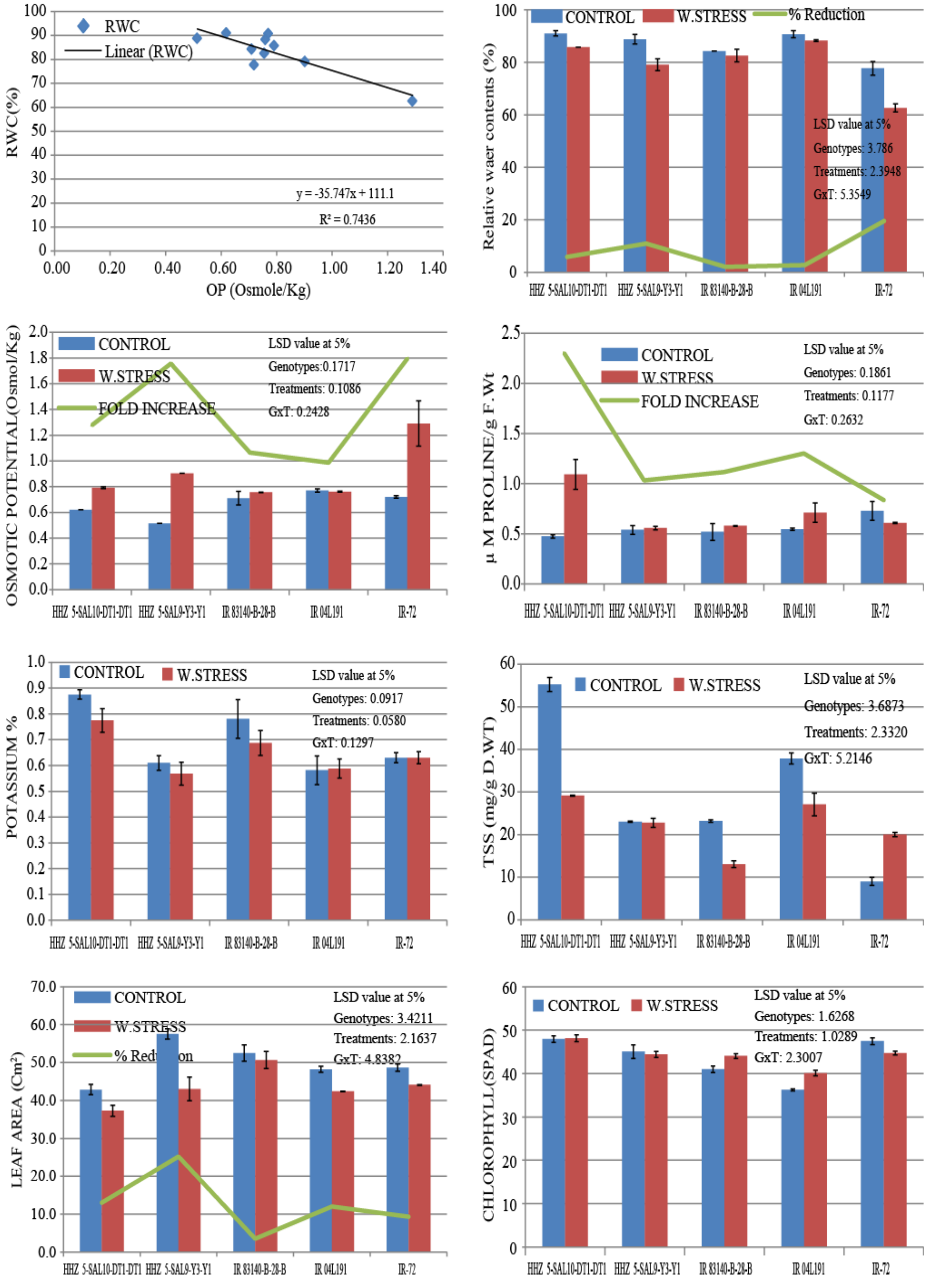


Fig. 1. Physiological responses of rice genotypes under water stress conditions. The ANOVA results with LSD values are given for genotypes, treatments and genotype – treatment interaction (GxT) at $P < 0.05$.

Reduced soil moisture affected leaf area and chlorophyll contents of these rice genotypes variably (Fig 1-G &1-H). Maximum leaf area was recorded by IR 83140-B-28-B with least reduction (3.5%) under stress while more reduction (25%) was observed in HHZ 5-SAL9-Y3-Y1 (sensitive). Drought stress reduces turgor pressure in the cell and therefore restricted cell elongation and expansion which ultimately caused reduction in crop growth. The similar findings were observed by Zubaer *et al.,* 2007 and Zain *et al.,* 2014. Increased chlorophyll contents were observed under water stress in three tolerant genotypes while, chlorophyll contents were decreased in genotypes HHZ 5-SAL9-Y3-Y1 and IR-72. Decrease in chlorophyll, and chlorophyll stability index in rice genotypes growing under water stress was observed by Deivanai *et al.* (2010).While, Mahmod *et al*., 2014; Fen *et al*., 2015 have reported increased chlorophyll contents in tolerant rice genotypes under water stress. Increase chlorophyll contents may be helpful for plants coping with stress through more photosynthesis and assimilate production. So, high chlorophyll stability under stress correlates with drought tolerance. (Anjum *et al*., 2011; Cha-um *et al*., 2013).

**Yield Traits:** The data (per plant) of these genotypes have shown that water stress had significant negative effects on yield related traits and these traits were variably affected in rice genotypes (Table 1). Tiller and panicle numbers (per plant) were least affected in genotypes HHZ 5-SAL10-DT1-DT1 and IR 83140-B-28-B. Water stress in all genotypes showed a significant effect on straw weight. The genotype HHZ 5-SAL10-DT1-DT1 and IR 83140-B-28-B produced comparatively higher straw weight while the least straw weight was produced by IR-72 and HHZ 5-SAL9-Y3-Y1. The grain yield of all genotypes decreased with greater intensities under water stress. The genotypes HHZ 5-SAL10-DT1-DT1 and IR 83140-B-28-B were comparatively good in grain yield. Minimum yield was produced by HHZ 5-SAL9-Y3-Y1 (sensitive). Grain yield is an important trait generally considered as selection criteria for drought tolerance. According to Chutia & Bohra (2012) grain yield is dependent on genetic makeup and environment, the interaction of these two expressed in the form of agronomic traits. Reduced turgor pressure in the cell is a primary response of water stress may have impact on all physiological activities which ultimately caused reduction in crop growth and productivity (Ashfaq *et al.,* 2012; Afridi *et al.,* 2014; Fen *et al.,* 2015; Pandey and Shukla, 2015). The differential reduction in yield traits among rice genotypes (Table 1) could be due to comparatively greater effects on their relative water contents, chlorophyll, leaf area and less translocation of assimilates towards grain (Zubair *et al*., 2007; Anjum *et al*., 2011, Ji *et al*., 2012).

Correlation between physiological and yield traits have shown that potassium ($r_= 0.802$) and TSS ($r_= 0.617$) in shoot were positively related with grain weight. This suggests the important role of potassium, soluble sugars and proline as osmoticum to maintain cell turgor under water stress conditions. The correlation between RWC and osmotic potential were highly significant (Table 2). Positive correlation between physiological traits and yield was also reported by Biswal and Kohli, (2013) & Zain *et al.* (2014) and they suggested that these traits are directly related with rice yield.

**Table 1. Effects of water stress on growth and yield parameters of rice (*Oryza sativa* L.).**

| Genotypes | Plant Height (cm) | | Tiller/Plant (No.) | | Panicles/Plant (No) | | Straw weight (g/P) | | Grain weight (g/P) | | 100 Grain Weight(g) | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Cont | W Stress | Cont | W Stress | Cont | W Stress | Cont | W Stress | Cont | W Stress | Cont | W Stress |
| HHZ 5-SAL10-DT1-DT1 | 107 | 104 | 24 | 13 | 23 | 13 | 50 | 34 | 43.3 | 18.6 | 2.7 | 2.3 |
| HHZ 5-SAL9-Y3-Y1 | 125 | 97 | 10 | 8 | 8 | 6 | 30 | 21 | 30.3 | 9.0 | 2.6 | 2.1 |
| IR 83140-B-28-B | 116 | 104 | 15 | 12 | 13 | 12 | 32 | 25 | 27.9 | 18.8 | 2.7 | 2.3 |
| IR 04L191 (D-T check) | 130 | 125 | 10 | 10 | 9 | 9 | 27 | 17 | 20.6 | 16.1 | 2.2 | 2.1 |
| IR-72 | 114 | 100 | 15 | 11 | 13 | 10 | 22 | 18 | 21.7 | 15.9 | 2.3 | 1.9 |
| LSD Values for | | | | | | | | | | | | |
| Treatments | 3.156 | | 1.429 | | 1.392 | | 4.625 | | 2.736 | | 0.102 | |
| Genotypes | 4.99 | | 2.259 | | 1.089 | | 7.313 | | 4.327 | | 0.161 | |
| G x T at α 5% | 7.057 | | 3.195 | | 3.112 | | 10.342 | | 6.119 | | 0.228 | |

**Table 2. Relationship between physiological, biochemical and yield parameters in rice genotypes grown under water stress conditions (Correlations Pearson).**

| Parameters | RWC | Spad chlorophyll | Leaf area | Osmotic potential | Potassium | Proline | TSS |
|---|---|---|---|---|---|---|---|
| Spad chlorophyll | -0.235 | | | | | | |
| Leaf area | 0.126 | -0.251 | | | | | |
| Osmotic potential | -0.862*** | -0.085 | -0.379 | | | | |
| Potassium | 0.192 | 0.450 | -0.215 | -0.150 | | | |
| Proline | -0.045 | 0.291 | -0.461 | 0.059 | 0.142 | | |
| TSS | 0.552 | -0.060 | -0.338 | -0.244 | 0.515* | -0.299 | |
| Grain weight | 0.421 | 0.349 | 0.123 | -0.477 | 0.802** | -0.207 | 0.617* |

*** = Significant @0.1% prob., * * = Significant @ 1% prob. * = Significant @0.5% prob.

## Conclusions

On the basis of these studies it was concluded that genotype HHZ 5-SAL10-DT1-DT1 and IR 83140-B-28-B were comparatively more tolerant to water stress. This was probably due to their better capability of water uptake and there by maintaining chlorophyll, leaf area and other metabolite synthesis, which consequently affected on yield. Therefore physiological traits such as leaf area, RWC, proline, potassium and chlorophyll, may be incorporated along with yield traits in rice screening programmes for water stress tolerance.

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(Received for publication 15 January 2016)