Effectual tolerant traits for grain yield in rice genotypes grown under drought

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Abstract

In agriculture, screening against drought stress is not only the matter of adaptation but also its productivity. The present work was conducted with a question to elucidate efficient drought sustaining characters that contribute positively to grain yield. Eight genotypes of same maturity group showing partial tolerance to drought were used as study materials in two different situations, one as control in the field condition and drought stress was imposed in the rain out shelter. Result showed variation amongst genotypes response for root morphological traits. Genotype BRR-0028 showed maximum R:S with 0.613, RDM with 0.317 and RMF with 0.273 as compared to other genotypes. Observation of increasing percent in photosynthetic rate under drought stress is remarkably good index for tolerance genotypes, whereby genotype BRR-0028 showed maximum increased in the photosynthetic rate with 13.03 % revealing more tolerant. Increased activities of antioxidant enzymes like peroxidise (POD), catalase (CAT) and superoxide dismutase (SOD) scavenging reactive oxygen species i.e. hydrogen peroxide and superoxide anion in the genotypes with higher CSI was observed. Amongst the genotypes studied, BRR-0026 (23.06) and BRR-0028 (22.09) showed higher in soluble protein content. Out of eight genotypes, Sabour Ardhjahl recorded the highest proline content with 69.9 µg g⁻¹ fresh wt. Correlation study revealed a positive association amongst physiological parameters. Photosynthetic rate was positively correlated with total chlorophyll content, transpiration rate and stomatal conductance. However, correlation of tolerant traits with yield showed inefficiency of many studied tolerant traits in regards to grain yield. Morphological traits R:S has direct association with grain yield in rice in both the control and drought stress at the level of p<0.05 and p<0.01. RWC was observed to be positively correlated with grain yield under drought stress but non-significant at control. Transpiration and proline were negatively correlated with yield and photosynthesis showed non-significant positive correlation. Therefore, result strongly suggest that amongst all the efficient traits, chlorophyll stability, R:S and RWC showed better and stable performance of grain yield and yield attribute morphological, physiological and biochemical traits in drought stress. Genotypes Sabour Ardhjahl, BRR-0028 and BRR-0026 are seemingly promising genotypes based on the overall performance that could be brought forth in rice improvement program for higher yield under drought situation.

Keywords: drought, relative water content, proline, yield, rice

Introduction

Rice is the most important staple food crop in Asia but also the highest water consuming crop. There are reports that more than 80% of rice production come from 79 million ha of irrigated lowland. Irrigated lowland rice in Asia usually has standing water for most of the growing season. Now, global water crisis has gradually threatened the rice production over the world scenario. In Asia, around 130 million ha of paddy are annually affected by drought, thus limiting rice production worldwide [1, 2]. Global climate change affects a variety of factors associated with drought and extreme drought land area is likely to increase from 1-30% by 2100 [3].

In India, rice is cultivated round the year in one or the other part of the country, in diverse ecologies spread over 44.6 M ha with a production of 132 MT of rice and average productivity of 2.96 t ha⁻¹. However, global rise in water scarcity highly effect crop production and they may no longer be stand for crop production in the future especially rice, highest water consuming crop. Therefore, to facilitate the selection or development of rice genotypes that not only adapt to the drought conditions but also obtain better yield, a thorough understanding of the various traits that govern the yield of rice under water stress condition is a prerequisite. Plants intervention to drought stress is vast and immensely depends upon intensity and duration of stress as well as plants developmental stages. Drought stress is one of the major abiotic stresses that threaten the crop productivity and thus world food security. Report says, drought severely impede crop production 50-60 percent due to significant restriction in plant growth and development. Yield loss, induction of oxidative stress with loss of membrane
integrity, semi-permeability, electrolyte leakage, loss in pigment content, decreased photosynthesis activity, alteration in osmotic adjustment water relation are commonly described responses of drought stress.

Plants undergo various morphological, physiological and biochemical changes upon drought stress, such changes become imperative to crop adaptation. Many reports revealed crop tolerant towards drought stress through various plant growth components. The Root:shoot (R:S) length ratio is adaptive mechanism in response to water deficit; it is considered an important indicator for the ability of a genotype to tolerate drought stress. Root:shoot ratio increased under water stress condition to facilitate water absorption.[4].

Relative water content (RWC) is a measure of the amount of water present in the leaf tissue. Higher RWC in leaf has been reported as selection criteria to breed plants tolerate to drought stress and high RWC under drought stress conditions are preferable to maintain water balance. RWC related to water uptake by the roots as well as water loss by transpiration. A decrease in the relative water content (RWC) in response to drought stress has been noted in wide variety of plants as reported by Nayyar and Gupta [5] that when leaves are subjected to drought, leaves exhibit large reductions in RWC and water potential.[6].

Chlorophyll being the most important photosynthetic pigment plays vital role in regulating crops yield. However, chlorophyll is quite delicate, not very stable and easily affected by abiotic stresses. Chlorophyll contents as chlorophyll 'a' and chlorophyll 'b' plays a vital role in photosynthetic process which ultimately increases crop growth and yield. These alterations in amount of chlorophylls depend on edaphic and climatic factors. In addition plant species and position of leave affect the amount of chlorophyll [7]. There have been many studies that reported on positive correlation between chlorophyll contents, photosynthetic rate and grain yield in different crops like wheat [8], cotton [9]. But working with maize found that chlorophyll had little direct effect on grain yield [10].

Drought, generally affect the whole photosynthetic activity by disrupting all major components of photosynthesis. Decreased in photosynthesis has been reported by many in varied crops. However, the ability of crop plants to acclimate to different environments is directly or indirectly associated with their ability to acclimate at the level of photosynthesis, which in turn affects biochemical and physiological processes and, consequently, the growth and yield of the whole plant [11].

Also, there are reports on increasing oxidative damage to plants. ROS e always been formed by the inevitable leakage of electrons onto molecular oxygen from the electron transport activities in chloroplast, mitochondria [12]. The production of ROS under normal growth conditions in cell is low however, an enhanced production under stressful conditions altering cellular homeostasis were reported. Plant cell contains a range of protective and repairing system to minimize the occurrence of oxidative damage. The antioxidant enzymes as superoxide dismutase (SOD), catalase (CAT), guaiacol peroxidise (POD) etc. react on active oxygen molecule and quench ROS [13, 14].

In response to different stresses plants accumulate large quantities of different types of compatible solutes. These solutes provide protection to plants from stress by contributing to cellular osmotic adjustment, ROS detoxification, protection of membrane integrity and enzymes/protein stabilization [15, 16, 17]. These include proline, sucrose, polyols, trehalose and quaternary ammonium compounds (QACs) such as glycine betaine, alinine betaine, proline betaine and pipecolate betaine.[ashraf harris 2004, Rhodes hanson 1993] (6-7) stressful environment results in an overproduction of proline in plants which in turn imparts stress tolerance by maintaining cell turgor or osmotic balance; stabilizing membranes thereby preventing electrolyte leakage; and bringing concentrations of reactive oxygen species (ROS) within normal ranges, thus preventing oxidative burst in plants [16].

Despite of all adaptive response crops sometime failed to give better yield performance. There is an increasing evidence for considerable interlinking about contrasting responses with various crops yield. The previous related study also revealed rice genotype BRR-0028 showed the highest CSI (Chlorophyll stability index) with higher yield irrespective of less chlorophyll content [20]. A negative correlation had been reported for antioxidant enzyme activities and yield of sugar beet [21].

In agricultural crops, it is not simply a matter of survival of plants but of an economic output in terms of yield and quality. Yield is a complex quantitative trait, considerably affected by environment. Therefore, selection of genotypes based on yield is not effective. Selection has to be made for the components of yield. Therefore the work was undertaken to examine drought tolerant characters that have direct impact on grain yield. In regard to this the study was shaped to evaluate the response of within and between rice genotypes variation in tolerance ability with its impact on grain yield to drought and correlation matrix were analyzed for studied traits to yield bridging gaps of understanding the question if all tolerant traits are specific for grain yield? This investigation will exaggerate screening for more desirable traits that associate positively to grain yield of rice genotypes for drought situation.

Materials and Methods

The present study was conducted using eight rice genotypes (IR-83381-B-B-18-3, BRR-0026, Sabour Ardhjal, IR-87759-5-2-1-3, BRR-0028, Sabour Srbhlt, MAS-946, R Bhagwati) in direct seeded condition. Randomized block design was followed with three replications, under two situations, one in the open field as control and second under the rain out shelter for drought stress induction. All the data were collected at 60 days of growth. Drought stress was imposed by withholding irrigation and bringing the soil moisture content (SMC) upto 25%. Overall yield data was expressed in terms of kg/h.

Root Morphology: Plants were carefully dug out from the root level and washed properly in running tap water to separate roots from soil and any debris. Root morphological traits were determined for root:shoot ratio (R:S), by dry weight, root dry mass (RDM) and expressed in g, root mass fraction (RMF) at 60 days of growth under control and drought stress.

Physiological traits

Photosynthesis rate (Phot.), transpiration rate (Trans.), stomatal conductance (St Cond.), Relative water content (RWC), total chlorophyll (Total Chl): Physiological parameters such as Phot. (μmol CO₂ m⁻² s⁻¹), Trans. (mmol H₂O m⁻² s⁻¹) and St Cond. (gs) (mmol m⁻² s⁻²) were determined using Infra red gas analyser (IRGA), LI-6400, Biosciences. RWC was measured using formula [(fresh weight-dry weight/turgid weight-dry weight) x 100]. The total
Chlorophyll content was determined in a fully expanded leaf from the main tiller and estimated as per the method of Arnon [22] with little modification and was calculated using formula, Total chl (μg g⁻¹ fr wt) = 17.3 A₆₆₅ + 7.18 A₆₆₃.

Biochemical assays
Three enzymes Peroxidase enzyme (POD) as peer the methods of Egley et al. [23], Catalase (CAT) activity by the method of Beers and Sizers [24], Superoxide dismutase (SOD) activity Mishra and Fridovich [25] method. Moreover expression level hydrogen peroxide (H₂O₂) by Jana and Chaudhuri [26] and superoxide anion (O²⁻) Chaitanya and Naithani [27] were determined accordingly.

Extraction and estimation of total soluble protein and proline: The leaf sample was homogenized in 5 ml of 0.2 M sodium phosphate buffer (pH 6.5) containing 0.5 M NaCl. After centrifugation at 10000x g, the soluble proteins were precipitated from the supernatant with 1 ml of 10 percent TCA and dissolved in 0.1 N NaOH. The absorbance of the color developed was recorded at 660 nm according to the method of Lowry et al. [28] using BSA (Sigma) as standard. Proline was extracted using 0.5g of leaf by homogenizing in 10 ml of 3 % aqueous sulphy-salicylic acid. In 2 ml of homogenate, add 2 ml of glacial acetic acid and 2 ml acid ninhydrin. After boiling in water bath for 1 hr, the reaction was terminated by placing the tube in ice bath. Add 4 ml toluene to the reaction mixture and stir well for 30 sec. With separation of toluene layer the red color intensity was measured at 520 nm as per the Bates Bates et al. [29].

Statistical analyses: Data were analyzed using Analysis of Variance (ANOVA) for the variables measured to test for significant differences. The correlation analysis was performed with simple coefficient matrix at P ≤ 0.05 and P ≤ 0.01.

Results and Discussion
Root morphological traits of eight genotypes studied under drought stress
The wide ranges of corresponding responses of genotypes towards drought stress were noticed. Drought stress generally affected the whole root system including root length, R:S ratio and dry mass [30]. Result strongly suggest variation amongst genotypes in the all the root morphological traits studied (Table 1) and showed significantly different from each other. Genotype BRR-0028 showed maximum R:S with 0.613, RDM with 0.317 and RMF with 0.273 as compared to other genotypes. As far as RDM is concerned genotype BRR-0028 was followed by Sabour Ardhjal and then IR 83381-B-B-18-3 with 0.285 and 0.276 respectively. This increased in root traits under drought stress may be generally an indication of roots less sensitivity than shoots to growth inhibition by low water potentials. Franco and co-workers [31] also reported about less affected of root growth by drought stress and further suggested a possibility of decreasing in R:S ratio results either from an increased in root growth or from a relatively larger decreased in shoot growth than root growth. The result is in support to the report on two rice cultivars, Zhenshan 97 (drought susceptible) and IRAT109 (drought resistant) that significantly increased root:shoot under drought in comparison with that under well-watered condition [32]. The proportion of dry matter and soluble sugar of roots markedly increased under drought stress. Interestingly, genotype BRR-0028 and Sabour Ardhjal showed a peculiar responds on root morphological trait, showing hardly any change under drought condition, which could probably being more tolerant to drought stress and further investigation of its characters could be of great contribution in screening purpose for breeding program.

Changes in physiological traits under drought stress
Drought stress is one of the main culprits to reduce photosynthesis thus by decreasing both leaf area and photosynthetic rate per unit leaf area. The present study also showed significant variation among different genotypes for physiological traits (Table 1). The percent of increased and decreased in the photosynthetic rate has been calculated for the eight genotypes. Amongst them, genotypes IR 83381-B-B-18-3, Sabour Ardhjal, Sabour Surbhit and BRR-0028 showed increasing as compared with those of control. BRR-0028 showed maximum increased in the photosynthetic rate with 13.03 % showing more tolerant to the drought stress. Similar result has also been reported in rice genotypes under drought stress [33, 34]. As photosynthesis plays a key role in plants production and adaptability, the increasing result in photosynthetic rate is strongly an indication of the genotypic variation in stress responses and differences in mode of their adaptation. Moreover, this increased in Photosynthetic rate might be due to photosynthetic resilience to drought as per variation with leaf age [35] or might generally be due the transcriptional control of photosynthetic genes by transcription factors (TFs) in response to abiotic stresses as recently reviewed by Saibo et al. [36], pointing out the role of several TFs in both stomatal and non-stomatal limitations of photosynthesis. The report also suggested that younger leaves tend to be more resistant to drought than older leaves, and this increased tolerance may be particularly relevant in plants where a severe reduction in the size of the leaf canopy occurs as a result of shedding of older leaves, because it allows a fast recovery following rehydration [37].

Table 1 showed a decreased in both transpiration rate and stomatal conductance in all the eight genotypes. Decreased in transpiration is more towards water saving under drought situation that might basically be due to the reduction in leaf size and area. The increased drought tolerance might be partly explained by the reduced transpiration rate. Relative water content (RWC), is considered a measure of plant water status, reflecting the metabolic activity in tissues and used as a most meaningful index for dehydration tolerance [6]. Wider range of responses for genotypes towards RWC under drought has been noticed in the result given in Table 1. Maximum decreased percent was noticed in the genotype IR-87759-5-2-1-3 whereby no significant change under the genotypes BRR-0028 and Sabour Surbhit showing more tolerance under drought stress.

Although components of plant water relations are affected by reduced availability of water, stomatal mechanisms strongly affected the relation thus altered the RWC, transpiration and photosynthetic rate in plants. Moreover, change in leaf temperature may be an important factor in controlling leaf water status under drought stress.
Table 1: Summary of the effect of drought on increase and decrease (%) on physiological traits (Photosynthesis, Transpiration, stomatal conductance, Relative water content), root morphological traits (Root: shoot, root dry mass, root mass fraction), Soluble protein (mg g⁻¹ dry wt) and Proline content (µg g⁻¹ fresh wt) and yield.

<table>
<thead>
<tr>
<th>S No.</th>
<th>Genotypes</th>
<th>% decreased and increased</th>
<th>Root Morphological Traits</th>
<th>Biochemical traits</th>
<th>Yield (Kg/ha)</th>
</tr>
</thead>
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<td>St. Cond.</td>
<td>RWC</td>
<td>R:S</td>
<td>RDM</td>
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</table>

*no change; Values with different alphabets are significantly different at P ≤ 0.05

Changes in biochemical traits under drought stress

Results of the present study would be of immense help in understanding the role of the components of drought stresses in plants responses. Drought altered various biochemical traits in eight genotypes when exposed to drought stress. The effect of drought stress on the generation of superoxide and hydrogen peroxide 60 days of growth is shown in Figure 1. Results indicated an increased superoxide anion (O²⁻) production and hydrogen peroxide (H₂O₂) content in all the genotypes under drought stress as compared to the control.

Due to imposition of drought stress, a significant increase in superoxide anion production was observed in genotypes BRR-0026, BRR-0028 and R Bhagwati (Figure 1). Similarly, maximum increased in the concentration of H₂O₂ under drought stress has been observed in genotypes Sabour Ardhjal, BRR-0028, MAS 946 and R. Bhagwati (Figure 1). The findings are in support that drought stress induces increases in levels of oxidative stress factors which may be caused by aggravation of membrane leakage and cellular damage as reported by different workers.

![Fig 1: Formation of H₂O₂ and O²⁻ in rice genotypes under drought stress. The data are mean of three replicates ± SD. Values are significantly different at P ≤ 0.05](image)

Plant has its own defence mechanism under and when exposed certain environmental stresses. The continuous overproduction of ROS in plants system has been taken care through various enzymatic and non-enzymatic antioxidants. Protection against oxidative stress is an important component in determining the survival of a plant under heat stress. The coordinated function of antioxidant enzymes such as SOD, APX, CAT and GR helps in processing of ROS and regeneration of redox ascorbate and glutathione metabolites, suggest an increase in antioxidant capacity of cells. The effect of drought stress on activity of POD, CAT and SOD is shown in Figure 2. As it is evident the activity of POD increased significantly in all the genotypes except in Sabour surbhit. Our results indicated a declined in POD (Figure 2 A) for genotype Sabour Surbhit, a non-significant increase in CAT (Figure 2 B), SOD (Figure 2 C) activity for the genotypes and BRR-0026, Sabour Ardhjal may not to be an effective scavenger of H₂O₂ and O²⁻ in our case. Findings indicated CAT and SOD might have relatively poor affinity for H₂O₂ and O²⁻ under drought for these genotypes. Overall, activities of all the antioxidant enzymes increased under drought stress in all the genotypes is an agreement findings with many of the workers report in various stress conditions.

Maintaining a higher level of antioxidative enzyme activities may contribute to drought induction by increasing the capacity against oxidative damage. The capability of antioxidant enzymes to scavenge ROS and reduce the damaging effects may correlate with the drought resistance of plants.

Alteration in total soluble protein and proline

It is suggested that the soluble protein and cellular protection enzymes, such as superoxide dismutase, peroxidise activity, and catalase in main stem leaves and fruit branching leaves play important physiological functions in the early growth stage under drought stress. The study suggests a strong reduction in soluble protein in rice genotypes under drought stress. Amongst the genotypes studied, BRR-0026 (23.06) and BRR-0028 (22.09) showed higher in soluble protein content as compared to the rest of genotypes (Table 1). The result is an agreement with the findings of Beena and Kumar et al. that water deficit stress tolerant rice genotypes increased their protein content, which may be due to synthesis of induced protein under stress situation. Plants under the stressful conditions accumulate arrays of metabolites in its system as defense mechanism such as amino acids. Proline, one of the amino acids that plays an essential role in plants when exposed to various adverse environments. It also contributes to stabilizing sub-cellular structures (e.g., membranes and proteins), scavenging free radicals and buffering cellular redox potential under stress conditions.
The phenomenon of proline accumulation and its increasing concentration is reported in various stresses like water deficit, salinity, low temperature, heavy metal exposure etc. Therefore, the present study also revealed an increased in proline content when and exposed to drought stress. Moreover, variation in the content also observed amongst the studied genotypes. Out of eight genotypes, Sabour Ardjhial recorded the highest proline content with 69.9 µg g\(^{-1}\) fresh wt (Table 1). Increased in 50% proline content over the control in rice sample was also reported by Roy et al.\[43\]. This increased in proline concentration has been marked as good index for stress defensive mechanism towards resistance in genotypes.

**Correlation matrix between the tolerant traits and yield under control and drought stress**

To identify the most desirable tolerant traits as screening criteria for high yield in drought situation, correlation between yield and effectual morphological, physiological and biochemical traits is presented in Table 2. At the 60 days of growth period, the root traits (RDM, RMF, R:S) were positively correlated. Amongst the root morphological traits R:S showed a strong contribution to yield in both the control (0.70*) and drought (0.81**) stress at p<0.05 and p<0.01 level respectively also by correlating with RWC at 0.92 level (Table 2). However, RMF and RDM have negative correlation with photosynthesis rate. The results also revealed a positive correlation amongst above ground physiological parameters. Photosynthetic rate was positively correlated with total chlorophyll content, transpiration rate and stomatal conductance. This correlation among the above ground physiological trait may be due to control of photosynthetic activity by stomatal and non stomatal mechanisms\[44, 45, 46\]. Stomata are the entrance of water loss and CO\(_2\) absorbability and stomatal closure is one of the first responses to drought stress which result in declined rate of photosynthesis and vice versa. Stomatal closure deprives the leaves of CO\(_2\) and photosynthetic carbon assimilation is decreased in favour of photorespiration. Considering the past literature as well as the current information on drought-induced photosynthetic responses, it is evident that stomata close progressively with increased drought stress.

It is well known that leaf water status always interacts with physiological patterns under drought stress. The findings showed non-significant negative correlation with transpiration rate and stomatal conductance, which probably be resulting in enhancing water holding capacity in leaves through stomatal closure. Stomatal closure deprives the leaves of CO\(_2\) and photosynthetic carbon assimilation is decreased in favour of photorespiration. Considering the past literature as well as the current information on drought-induced photosynthetic responses, it is evident that stomata close progressively with increased drought stress\[6\]. However, RWC has revealed a direct control over the photosynthesis and yield in rice genotypes under control as well as drought stress by showing a significant correlation at p<0.05 and p<0.01 level respectively. Higher percent of RWC always revealed a good index for the drought resistance in the rice genotypes. Therefore, the correlation pattern as in table 2 clearly showed the importance of RWC in grain yield in rice under drought stress. Similar report has also been given in rice by Kumar et al\[33\]. Transpiration and proline content in rice showed negative correlation with grain yield. The similar trait has also been reported in various other crops\[43, 47\].

***Fig 2: Effect of drought on antioxidant enzyme activities in rice genotypes. The data are mean of three replicates ± SD. Values are significantly different at P ≤ 0.05***
Correlation analyses also reveal that yield was positively and significantly correlated with chlorophyll stability in both the control and drought conditions with 0.719 and 0.804 at p<0.05 and p<0.01 level as reported earlier. Previous report has a cross talk on positive correlation of yield with chlorophyll contents in different crops [8, 9, 48]. But the study reveals a differential contribution of chlorophyll contents into the yield which might be highly genotypic and environmental specific [49, 50]. Chlorophyll stability has always been mentioned as one of the most important indicators for stress tolerance. The results shown in Table 2 clearly stated about close reliability and direct contribution of chlorophyll stability towards yield irrespective of chlorophyll contents as observed in genotypes, BRR-0028, BRR-0026, Sabour Surbhit, IR 83381-B-B-18-3. Earlier reports on different crops was also discussed by separate researchers that throws a light on low chlorophyll contents as not a sign for poor yield [51, 52]. From the discussion of the above results, it can be concluded that drought stress alters physiological plasticity in rice genotypes. However, plant responses are varied and genotype specific. Result revealed a strong indication to hypothesize that every efficient traits having tolerant to stress might possibly not be important traits that contribute to rice grain yield under drought situation. The reason for this may generally the involvement of differential gene expression and pathways for tolerance and grain yield. This study was conducted to support the future screening method to screen large amounts of plant material in the shortest time possible with minimum traits that directly contribute to yield under drought stress. Amongst all the efficient traits, result suggests chlorophyll stability, R:S and RWC for better and stable performance of grain yield and yield attribute morphological, physiological and biochemical traits. Moreover, genotypes Sabour Ardhjal, BRR-0028 and BRR-0026 showed better performance as compared to other rice genotypes which could be promising genotypes for future rice improvement program for higher yield under drought situation.

**References**


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<th>St.Cond.</th>
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*Significant (p<0.05); ** Significant (p<0.01)


