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# Differential response of rice genotypes to mild and severe osmotic stress during seedling stage

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## ABSTRACT

*Though the response of plants exposed to severe drought stress has been studied extensively, little is known about how plants adapt their growth under mild drought stress conditions. In the present study seeds of sixteen genotypes were germinated under three treatments: non stress, 1% mannitol and 2% mannitol and the result revealed that average germination percentage was found to be low in both the treatments compared to non stress. However, better plant vigour on the basis of shot and root length was observed in 1% mannitol treated seeds compared to untreated and 2% mannitol treated seeds. Other traits like total protein content, catalase and peroxidase activity were also more at 1% mannitol compared to nonstress and 2% mannitol treatment. Among all the genotypes tested, AC 42994, AC 43030 and AC 43012 out performed in plant vigour as well as anti oxidant enzyme activities compared to other genotypes and tolerant check.*

***Key words:*** *Osmotic stress, mannitol, seedling stage, rice*

Drought imposes a major limitation on crop productivity (Boyer, 1982). For cereal crops, drought is the most important abiotic stress component reducing yield (Araus *et al*., 2002). Currently, about 75% of the world's freshwater supplies are utilized in agriculture and in the near future its availability will be a constraint due to expanding world population and unfavourable climate conditions (Wallace, 2000). Therefore, understanding plant's adaptation towards drought for increasing crop productivity under conditions of limiting water availability is a scientific requirement. Due to its immense importance in agriculture, the effects of drought on plant development have been studied extensively in the past decades which contribute to the understanding of physiological and molecular response to drought. On the basis of how plant responds to drought, the mechanism is categorised in to stress avoidance and stress tolerance (Verslues *et al*., 2006; Lawlor, 2013). Stress avoidance balance water uptake and water loss by accumulating osmolytes to lower the tissue water potential, enhancing root growth, restricting water loss by closing of stomata, inhibiting shoot growth and accelerating leaf senescence where as stress tolerance came into play when stress is too severe and stress avoidance mechanism is not sufficient to tackle with severe drought. Stress tolerance mechanism include detoxification of reactive oxygen species, accumulation of protective proteins like LEA proteins and heat shock proteins and solutes like proline which act as both osmolyte and osmoprotectant (Nakashima *et al*., 2009). Numerous studies have reported about the adaptations of plants to severe drought conditions (Akhtar *et al*., 2012; Golldack *et al*., 2014), either by withholding water until wilting or by letting cut leaves to dry to impose severe water deficits (Zhu *et al*., 2007; Zhang *et al*., 2008). However sudden appearance of drought is somewhat unnatural than the situations what actually happens in the field. In the field conditions, plants adapt to drought which gradually develop and became severe if there is no rainfall or irrigation for longer period (Baerenfaller *et al*., 2012; Des Marais *et al*., 2012). But in most of the cases, crop experience mild to moderate stress in the field conditions which is poorly understood compared to severe stress. According to Skirycz *et al*. (2011), better biomass gain yield has been encountered under moderate drought

stress rather than drought tolerance under severe stress which results in improved survival rate under lethal condition but with high yield penalty. Kasuga *et al*. (1999), stated that enhanced survival under severe drought does not ensure improved growth performance under mild drought conditions and leads to growth penalty due to constitutive activation of water saving mechanism like stomatal closure. Very little information is available about the behavioural pattern of morphogenetic and role of anti oxidative system under mild and severe stress. Present investigation put forwarded a comparative behavioural pattern of rice genotypes under non stress, mild and severe osmotic stress.

## MATERIALS AND METHODS

One hundred seeds of fourteen genotypes having vegetative stage drought tolerance along with two checks Brahmaninakhi (tolerant) and IR-20 (susceptible) were germinated under three different treatments *viz* NS - Non stress, $T_1$ - 1% mannitol (54 mM) and $T_2$ - 2% mannitol (108 mM) to check their germination percentage under osmotic stress. One hundred healthy seeds of each genotype were germinated on filter paper in petridish and for each treatment three replications were made. Seeds of each genotype were sown in nine petridishes which were divided into three treatments for NS, T1 and T2.

After three days of germination, number of germinated and non germinated seeds was counted for calculating percent of germination. After ten days of germination, when the susceptible check showed wilting symptoms in treated petridishes, root length and shoot length were measured.

Frozen leaf samples (1 g of fresh weight) were ground to a fine powder in liquid nitrogen and extracted with extraction buffer containing 50 mM potassium phosphate (pH 7.5). The protein concentration in leaf crude extracts was determined using bovine serum albumin as standard following Lowry *et al.* (1951).

Anti oxidative enzyme activity like catalase (EC.1.11.1.6) was estimated according to Beers and Sizer (1952). The absorbance was read at 240 nm. One unit of CAT activity was defined as the degradation of 1µM $H_2O_2$ in 1min at 240nm (å = 43.2$mM^{-1}$ $cm^{-1}$) (Margonis *et al.*, 2007).

Peroxidase (POX) activity was determined specifically with guaiacol at 470 nm following the method of (Choi *et al*., 2004).

The data was subjected to ANOVA over the two treatments to assist the variability among the genotypes. ANOVA of all the traits for each treatment were analyzed by using Cropstat ver 7 (2009).

## RESULTS AND DISCUSSION

To study the effect of mild and severe osmotic stress on seed germination as well as seedling growth, seeds were germinated under osmotic stress induced by applying mannitol which is useful for selection of emergency capacity under conditions of water deficit (Seong *et al*., 1988). Significant variation was observed between different genotypes and treatments (Table 1).

Average germination percentage was found to be low in both the treatments compared to nonstress. However better plant vigour was observed in 1% mannitol treated seeds compared to untreated and 2% mannitol treated seeds. Other traits like shoot length, root length, total protein content, catalase and peroxidase activity were found to increase at 1% mannitol compared to non stress and 2% mannitol treatment (Table 2).

Germination percentage, root length and shoot length were measured under normal and stress environments. Seeds germinated under normal conditions had higher germination percentage but lower root length and shoot length than those germinated under stress environments. Decrease in germination percentage in seeds treated with 1% and 2% mannitol was 6.11% and 6.39% respectively. Shoot length increased by15.64% in 1% mannitol treated seeds and

**Table 1.** Significance level of variance for various traits of rice accessions at Nonstress, 1% mannitol and 2% mannitol treatments as obtained by ANOVA

| Parameters | V | T | V × T |
|---|---|---|---|
| | *p*-value | *p*-value | *p*-value |
| Germination (%) | <0.001 | <0.001 | <0.001 |
| Shoot length | <0.001 | <0.001 | <0.003 |
| Root length | <0.001 | <0.001 | <0.001 |
| Protein | <0.001 | <0.001 | <0.001 |
| Catalase | <0.001 | <0.001 | <0.001 |
| Peroxidase | <0.001 | <0.001 | <0.001 |

**Table 2.** Mean, range and standard error of all the measured traits of 16 genotypes

| Traits | Treatments | Mean | SE± | Min. | Max. | Range | % increase or decrease |
|---|---|---|---|---|---|---|---|
| Germination (%) | NS | 92 | | 82.0 | 98.0 | 16. | |
| | T1 | 87 | | 64.0 | 95.0 | 31.0 | -5.4 |
| | T2 | 86 | 0.43 | 70.0 | 97.0 | 27.0 | -6.5 |
| Shoot length (cm) | NS | 7.44 | | 3.83 | 9.30 | 5.47 | |
| | T1 | 8.61 | | 7.33 | 10.40 | 3.07 | 15.7 |
| | T2 | 7.58 | 0.38 | 5.77 | 10.20 | 4.43 | 1.8 |
| Root length (cm) | NS | 6.79 | | 3.57 | 10.73 | 7.16 | |
| | T1 | 7.53 | | 2.80 | 10.57 | 7.77 | 10.89 |
| | T2 | 6.21 | 0.47 | 2.67 | 12.77 | 10.1 | -8.54 |
| Total protein content (mg/g fr wt) | NS | 13.48 | | 5.2 | 19.7 | 14.5 | |
| | T1 | 18.19 | | 13.2 | 23.7 | 10.5 | 34.9 |
| | T2 | 15.01 | 0.04 | 6.6 | 20.2 | 13.6 | 11.35 |
| Catalase (U/g fr wt) | NS | 0.65 | | 0.11 | 1.39 | 1.28 | |
| | T1 | 1.49 | | 0.45 | 1.99 | 1.54 | 129.2 |
| | T2 | 1.07 | 0.04 | 0.87 | 1.79 | 0.92 | 64.6 |
| Peroxidase (U/ g fr.wt) | NS | 2.98 | | 1.39 | 4.68 | 3.29 | |
| | T1 | 6.19 | | 1.01 | 8.59 | 7.58 | 107.7 |
| | T2 | 3.73 | 0.05 | 0.86 | 5.74 | 4.88 | 25.1 |

NS-Non stress, T1-1% mannitol, T2-2% mannitol

1.79% in 2% mannitol treated seeds over nonstress. Similarly, root length increased by 10.79% in 1% and 8.62% in 2% mannitol treated seeds than that of control. Total soluble protein content increased by 35% in 1% and 11.4% in 2% mannitol over nonstress.

In general, catalase had higher activity under stress condition. Catalase activity in seeds treated with 1% mannitol was 130.6% where as it is only 66% in 2% mannitol of that in untreated seeds. Similar trend was observed for peroxidase activity. In seeds treated with 1% mannitol, increase in peroxidase activity was 107.7% and 25.3% in 2% mannitol treated seeds than that in untreated seeds.

Among all the genotypes, AC 42997 and AC 43012 had more than 90 percent of germination in all the three treatments where as Khamtijoha had lowest values (64 - 84%). Tolerant check, Brahmaninakhi had 95% germination in NS, 94% in T1 and 79% in T2.

Shoot length invariably increased in all the genotypes under T1 and T2 but higher length was observed in T1 and the genotypes IC 568060, AC 42994 and AC 43012 had highest shoot length of 10.0.cm under T1. Root length though increased significantly under both the treatments, highest root length of 10.0 cm was observed in IC 568060, AC 42998, AC 43030 and AC 43012 under T1 (Table 3 and Fig 1).

Antioxidant enzymes like catalase and peroxidase also increased significantly under both the treatments T1 and T2 and the highest increase was observed in AC 42994, AC 43030 and Brahmannakhi (1.79-1.99 U g fr $wt^{-1}$) for catalase. Peroxidase was recorded highest in AC 42997 (8.59 U g fr $wt^{-1}$) followed by AC 43025, AC 43012 and Brahmannakhi (8.0 U g fr $wt^{-1}$) (Table 3 and Fig. 2).

Though germination percentage was low in AC 42994 and AC 43030, shoot length, root length and catalase activity were more under both the stress treatments compared to other genotypes. The tolerant check Brahmaninakhi showed higher values for all the traits under both the stress treatments compared to non stress showing its tolerance towards osmotic stress. However, IR-20, the susceptible check observed to have decreasing trend for all the traits under osmotic stress.

According to Skirycz *et al.* (2011a), in mannitol treated samples and the samples recovered from mannitol treatment had higher meristomoid division activity than in control samples under mild osmotic stress. In our investigation, enhanced root and shoot growth under mild stress (1% mannitol) was observed compared to severe osmotic stress and control which

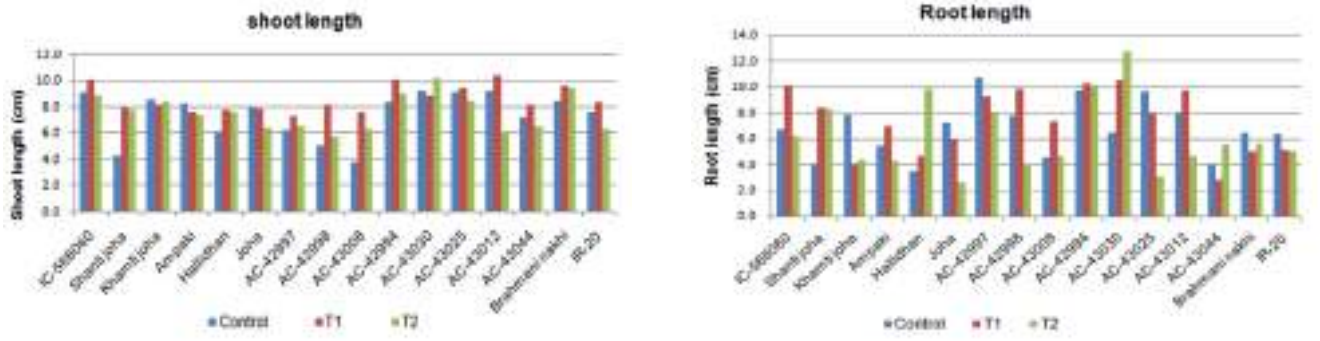


**Fig.1.** Shoot length and root length of 16 genotypes under nonstress (NS), 1% mannitol (T1) and 2% mannitol (T2) treatments.

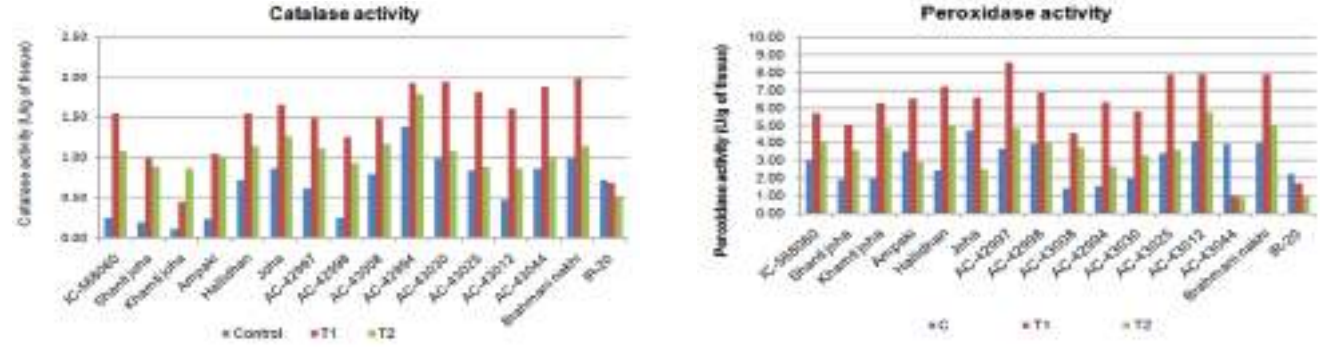


**Fig-2.** Catalase and peroxidise activity of 16 genotypes under nonstress (NS), 1% mannitol (T1) and 2% mannitol (T2) treatments.

is in consistent with the opinion of Skirycz *et al*. (2011b), that mild stress, in contrast to severe conditions, favor bolder plants maintaining more growth, photosynthesis and metabolism despite a water shortage. It might be due to some mitotic activity at the base of the leaf of plants having mild stress while in non stress leaves and severe stressed leaves the cell proliferation zone had completely disappeared leaving mitotic cycle and enter into differentiation state. However, up regulation of photosynthetic machinery, chlorophyll content and antioxidant and redox system has been reported across the growth zone which increases leaf growth rate by stimulating cell division (Avramova *et al.*, 2015). Also antioxidant activity was highest in meristematic growth zone and was enhanced by drought that could help in actively protecting the meristem from oxidative damage. It supports our findings where 1% mannitol treated plants had better plant vigour than control and 2% mannitol treated plants. According to Skirycz *et al.*, 2011b, lines that were having much better early vigour and growth in control environment were able to keep their high growth rate under stress condition that helps the plant to survive under stress. This might be contributed by the presence of favourable carbon status present in leaves under mild osmotic stress and drought stress (Hummel *et al.*, 2010, Skirycz *et al.*, 2010).

In summary, more extensive study is required to understand the physiological mechanism underlying improved growth rate under mild stress which will help to identify genotypes with higher biomass growth rate under stress conditions that ensures higher productivity rather than genotypes that gives high yield loss in turn

**Table 3. Morphological and biochemicals traits measured on sixteen genotypes under Non stress (NS), 1% mannitol (T1) and 2% mannitol (T2) conditions**

| Genotypes | Germination percentage | | | Shoot length (cm) | | | Root length (cm) | | | Total protein (mg/g fr wt.) | | | Catalase activity (U/ g fr wt) | | | Peroxidase activity (U/g fr wt) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | NS | T1 | T2 | NS | T1 | T2 | NS | T1 | T2 | NS | T1 | T2 | NS | T1 | T2 | NS | T1 | T2 |
| IC-568060 | 99 | 87 | 97 | 9.1 | 10.1 | 8.8 | 6.7 | 10.1 | 6.2 | 15.5 | 14.1 | 15.0 | 0.24 | 1.55 | 1.08 | 2.97 | 5.69 | 4.11 |
| Shanti joha | 98 | 89 | 87 | 4.3 | 8.0 | 7.8 | 4.1 | 8.4 | 8.3 | 9.1 | 23.7 | 10.6 | 0.20 | 1.01 | 0.89 | 1.92 | 5.05 | 3.62 |
| Khamti joha | 86 | 64 | 70 | 8.6 | 8.2 | 8.4 | 7.9 | 4.1 | 4.4 | 5.2 | 22.8 | 6.6 | 0.11 | 0.45 | 0.87 | 2.06 | 6.28 | 4.80 |
| Ampaki | 97 | 89 | 89 | 8.3 | 7.6 | 7.4 | 5.5 | 7.0 | 4.3 | 14.7 | 19.9 | 16.6 | 0.24 | 1.05 | 0.99 | 3.52 | 6.55 | 2.92 |
| Hallidhan | 90 | 86 | 81 | 6.2 | 7.8 | 7.6 | 3.6 | 4.7 | 9.8 | 18.8 | 20.8 | 10.6 | 0.73 | 1.55 | 1.14 | 2.38 | 7.19 | 4.08 |
| Joha | 82 | 85 | 83 | 8.0 | 7.9 | 6.4 | 7.3 | 6.1 | 2.7 | 16.5 | 17.7 | 19.1 | 0.86 | 1.67 | 1.27 | 4.68 | 6.60 | 2.47 |
| AC-42997 | 96 | 91 | 94 | 6.2 | 7.3 | 6.5 | 10.7 | 9.3 | 7.9 | 10.7 | 17.5 | 15.0 | 0.62 | 1.51 | 1.12 | 3.65 | 8.59 | 4.79 |
| AC-42998 | 90 | 86 | 80 | 5.1 | 8.2 | 5.8 | 7.8 | 9.9 | 4.0 | 8.9 | 15.2 | 14.5 | 0.26 | 1.25 | 0.94 | 3.94 | 6.92 | 4.0 |
| AC-43008 | 96 | 90 | 89 | 3.8 | 7.6 | 6.3 | 4.6 | 7.4 | 4.7 | 8.9 | 16.6 | 20.2 | 0.80 | 1.51 | 1.17 | 1.39 | 4.53 | 3.72 |
| AC-42994 | 82 | 79 | 88 | 8.4 | 10.1 | 9.1 | 9.7 | 10.3 | 10.1 | 19.7 | 15.2 | 18.4 | 1.39 | 1.92 | 1.79 | 1.52 | 6.36 | 2.66 |
| AC-43030 | 92 | 84 | 91 | 9.3 | 8.8 | 10.2 | 6.4 | 10.6 | 12.8 | 17.9 | 16.0 | 16.9 | 0.99 | 1.94 | 1.08 | 2.0 | 5.84 | 3.27 |
| AC-43025 | 95 | 93 | 95 | 9.2 | 9.4 | 8.5 | 9.7 | 7.9 | 3.1 | 14.2 | 20.1 | 15.1 | 0.84 | 1.82 | 0.89 | 3.40 | 7.90 | 3.64 |
| AC-43012 | 95 | 95 | 95 | 9.3 | 10.4 | 6.1 | 8.0 | 9.8 | 4.7 | 15.9 | 17.7 | 15.2 | 0.48 | 1.61 | 0.87 | 4.09 | 7.94 | 5.74 |
| AC-43044 | 93 | 89 | 90 | 7.2 | 8.2 | 6.5 | 4.0 | 2.8 | 5.6 | 14.2 | 13.2 | 13.5 | 0.87 | 1.88 | 0.99 | 3.94 | 1.01 | 0.86 |
| Brahmaninakhi | 92 | 86 | 75 | 8.5 | 9.6 | 9.5 | 6.4 | 5.0 | 5.7 | 11.0 | 19.1 | 17.7 | 0.99 | 1.99 | 1.14 | 4.02 | 7.91 | 5.04 |
| IR-20 | 95 | 94 | 79 | 7.6 | 8.4 | 6.4 | 6.4 | 7.2 | 5.0 | 14.4 | 21.4 | 15.4 | 0.73 | 1.08 | 0.94 | 2.22 | 4.68 | 3.02 |
| LSD 5% | 1.22 | | | 1.08 | | | 1.34 | | | 0.01 | | | 0.003 | | | 0.15 | | |

of high survival rate under severe stress during vegetative stage. This strategy will be useful not only in trait identification but also in selecting varieties with high yield coupled with other yield attributes.

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