Oryza Vol. 59 Issue 2 2022 (221-231)
DOI https://doi.org/10.35709/ory.2022.59.2.12

Identification and characterization of drought tolerant rice genotypes using physiological and biochemical traits

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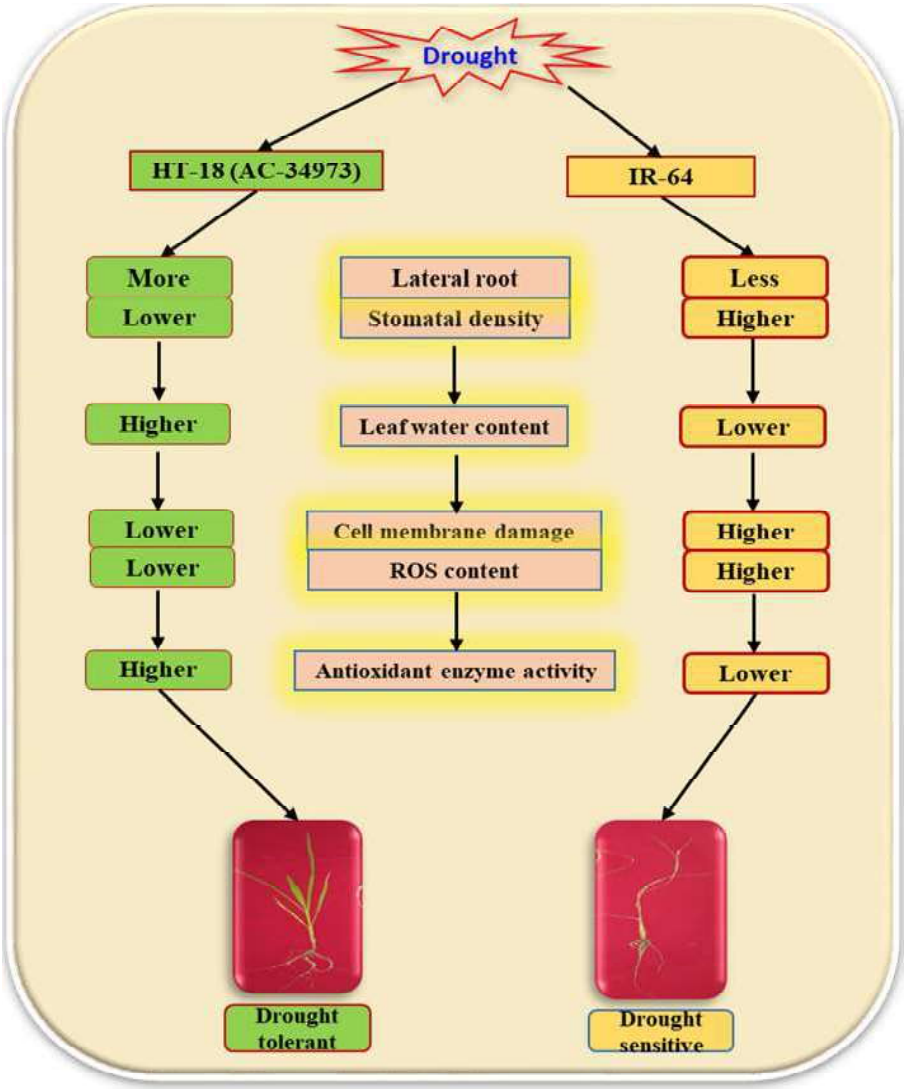
Received : 5 June 2022 Accepted: 28 June 2022 Published : 29 June 2022

Graphical abstract:



ABSTRACT

The present study was conducted to evaluate the performance of seven rice genotypes using morphological, physiological and biochemical parameters, under induced drought (water stress) conditions at seedling level using PEG6000 in Hoagland's medium. At the end of the stress period sampling was done to record the root and shoot lengths and various physiological parameters viz., total chlorophyll content, cell membrane stability index (MSI), relative water content were estimated. Proline and Malondialdehyde (MDA) content were also estimated as biochemical parameters. The results obtained from the study revealed the existence of significant variation in the seven genotypes studied for different physiological and biochemical parameters. Out of the seven genotypes studied, HT-18 (AC-34973) had performed better than the tolerant check CR-143-2-2 and showed better root and shoot growth, maintained higher total chlorophyll content (2.6 mg/gm FW), relative water content (61.3%) and membrane stability index (MSI) (52.9%), it has also shown higher proline content (20.52 ?moles/gm FW) and lesser MDA content (0.068) under stress. To assess the membrane integrity under osmotic stress, roots from all the genotypes grown in hydroponic culture with 20% PEG were stained with Evan's blue, where the stress effect is directly reflected on the intensity of Evans blue uptake by the cell. Because of more membrane damage, the roots of the susceptible genotype, IR-64, had taken up more stain than the roots of tolerant genotype HT-18. The present study has identified HT-18 as seedling level drought tolerant genotype.

Key words: *Drought, vegetative stage, MDA, MSI, proline, rice*

INTRODUCTION

Drought is the most devastating stress affecting crop production worldwide. Climate change is expected to increase the occurrence and severity of droughts, posing ever more serious constraints to global rice production (Wassmann et al., 2009). Drought can come at any phase of the rice crop in any year in rainfed areas. Modern rice cultivars are particularly prone to drought stress at the seedling, vegetative, and reproductive stages, and even minor drought stress can result in a large yield drop (O'Toole, 1982; Torres and Henry, 2016). Drought has an impact on establishment of crop and seedling survival rates at the seedling stage. Drought lowers leaf production and tillering during the vegetative stage, affecting the number of panicles per plant and a loss of output. Drought reduces the quantity of grains per panicle, increases grain sterility, and reduces grain weight at the reproductive stage (Pantuwan et al., 2002).

Drought stress at the reproductive stage is known to induce a large yield drop (Hsiao, 1973), although vegetative stage drought was previously thought to have a very little influence on grain yield in rice (Boonjung and Fukai, 1996). It should be highlighted, however, that these conclusions are based on the impacts of drought stress on the rice plant, not on the type of drought stress that occurs most commonly in farmers' fields. Because of the late arrival of monsoon rains or extended gaps between first rains, vegetative-stage dryness has become a key factor in lowering rice output in shallow rainfed areas in recent years (Bunnag and Pongthai, 2013). In shallow rainfed parts of South and Southeast Asia, notably in eastern India, the frequency and intensity of vegetative-stage drought stress has risen in recent years. Farmers fail to amass enough water in the field early in the season to prepare land and begin transplanting due to lack of first rainfall. As a result, in years with lower early rainfall, significant portions of shallow rainfed ecosystems are left untransplanted. Slow development, decreased tillering, and in some cases mortality of early transplanted seedlings owing to vegetative-stage drought stress produce large output losses even when farmers are able to transplant.

Drought tolerance phenotyping at the seedling stage is a frequent practise in wheat, barley, triticale, maize, and rice (Moud and Maghsoudi, 2008; Gonzalez and Ayrbe, 2011; Grzesiak et al., 2012; Krishnamurthy et al., 2016). It is a quick, cost-effective, and dependable way to assess a plant's performance (Krishnamurthy et al., 2016). There are reports on the relationship between drought tolerance at the seedling stage and reproductive stage in rice and wheat, demonstrating the necessity of drought tolerance screening at the seedling stage (Singh et al., 1999; Dodig et al., 2015). Seedling survival, dry weight, root shoot

ratio and root length, relative water content, and seed reserve mobilization are the features that have been employed for drought tolerance screening of germplasm (Soltani et al.,2006; Hameed et al., 2010).

Although some information on screening techniques is available (Verulkar and Verma, 2014), parameters for the level of drought stress to be induced have not been particularly specifically established for vegetative-stage drought stress screening. These guidelines will assist scientists in determining the best watering time for vegetative-stage drought stress screening trials. The discovery and development of rice varieties that are tolerant to vegetative-stage drought will be aided by developing methods for successful vegetative-stage drought stress screening. Only a few types have been identified as having great production potential as well as drought tolerance at both phases. Breeders can select lines that combine tolerance to vegetative- and reproductive-stage drought stress in high-yielding genetic backgrounds by selecting for yield and yield-associated traits at the vegetative and reproductive stages in standardized drought screens, in addition to high yield potential under well-watered conditions.

Rice (*Oryza sativa* L.) is the chief source of nutrition for more than half of the world's population (Singh et al., 2012). In underdeveloped nations, it accounts for 27% of dietary calories and 20% of dietary protein (Singh and Singh, 2007). It is grown in at least 114 poor nations, and it provides income and work to more than 100 million Asian households (Singh et al., 2015). Approximately 16.2 million hectares of India's total 20.7 million ha of rainfed rice area are in eastern India (Singh and Singh, 2000), with 6.3 million ha of highland and 7.3 million ha of lowland being very drought prone (Pandey and Bhandari, 2009). The eastern Indo-Gangetic Plain is one of the world's most important rice-producing regions, yet it is also one of the most drought-prone (Huke and Huke, 1997). Over-all losses to rice production in Chhattisgarh, Odisha and Jharkhand have been reported to be as high as 40% in severe drought years, totaling US$ 650 million (Pandey et al., 2005). In order to ease the growing food crisis, rice cultivars that can withstand drought stress at both the growth stages and deliver economic yields must be identified or developed. With this in mind, the current work used morpho-physiological and biochemical characterization under drought stress to discover drought resistant rice genotypes in the vegetative stage.

MATERIALS AND METHODS

Plant material

Seven diverse rice genotypes *viz*., CR-143-2-2 (tolerant check), Satyabhama, Sahbhagi Dhan, HT-18 (AC-34973), HT-72 (AC-35076), DBT-917 and IR-64 (susceptible check) were selected for the present study.

Growth conditions

Seeds of all the genotypes were surface sterilized in 0.5% bavistin for 15mins and then thoroughly washed in distilled water for 4-5 times, after washing seeds were soaked in distilled water for overnight for imbibition. Next day seeds were put for germination in sterile pertiplates containing blotting paper moistened with distilled water. 5 days after germination, seedlings were transferred to ½ strength Hoagland's solution in plastic trays for seedling establishment. 21 days after seedling growth, osmotic stress (drought) was imposed using 20% PEG 6000 in hydroponic system for 7 days. After the stress treatment leaf samples were collected for physiological and biochemical analysis along with the control (without PEG) samples.

Morpho-physiological and biochemical analysis to assess the stress effect

To measure the stress effect on genotypes, various morphological, physiological and biochemical traits were measured after imposing osmotic stress. Data was recorded for different physiological and morphological traits using standardized methods as described in literature and with the help of appropriate instruments.

Morphological traits

Root and shoot lengths

At the end of the stress period, root and shoot lengths (cm) were measured in both control and stressed seedlings to assess the impact of water stress on growth parameters.

Evan's blue staining for membrane integrity assay

The roots from all the genotypes which were grown in hydroponic culture with PEG solution were stained with Evan's blue to check the membrane integrity of root cells under osmotic stress. The cells when subjected to

stress, loss of membrane stability enhances the uptake of the dye and results in accumulation of blue protoplasmic stain. The stress effect is directly reflected on the intensity of Evans blue uptake by the cell (Baker and Mock,1994).

Physiological traits

Relative water content (RWC)

Leaf RWC was measured by recording the turgid weight of 0.5 g fresh leaf sample by keeping in water for 4h, subsequently drying it in hot air oven till constant weight was achieved (Weatherly, 1950). A precision analytical balance (HR- 60) was used for all weight measurements. The relative water content of a leaf was calculated by using the formula given below,

$$RWC\ (\%) = \frac{FW - DW}{TW - DW} \times 100$$

Membrane stability index (MSI)

MSI was estimated as per protocol given by Sairam et al. (1997). For estimation of membrane stability index, 50 mg leaf material, in two sets, was taken in test tubes containing 10 ml of double distilled water. One set was heated at 40 ºC for 30 min in a water bath, and the electrical conductivity of the solution was recorded by using conductivity meter (CL-250) on a conductivity bridge (C1). Second set was boiled at 100 ºC on a boiling water bath for 10 min, and its conductivity was measured on a conductivity bridge (C2). Membrane stability index was calculated by using the formula,

$$MSI\ (\%) = [1 - (C1/C2)] \times 100$$

Total chlorophyll (TC) content

Total chlorophyll content was estimated by extracting 0.05 g of leaf material in 10 ml dimethyl sulfoxide (DMSO) (Hiscox and Israelstam, 1979). The optical densities of the samples were recorded by using UV-VIS Spectrophotometer (UV-2600, SHIMADLU). Chlorophyll content was expressed as mg/g fresh weight and calculated using the formula,

$$TC = (20.2 \times OD_{645} + 8.02 \times OD_{663}) \times V / 1000 \times W$$

OD_{645} = absorbance value at 645nm

OD_{663} = absorbance value at 663nm

W= weight of sample in mg

V = Volume of solvent used (ml)

Biochemical traits

Measurement of malondialdehyde accumulation by thiobarbituric acid reactive substances (TBARS) assay

Malondialdehyde (MDA) is a lipid peroxidation breakdown product that may be utilised as a lipid peroxidation indicator. The MDA content was measured using the thiobarbituric acid (TBA) reaction, which was modified slightly from Zhou Q. (2001). 0.5g of leaf tissue was collected and homogenised in 2 ml of 0.1 percent trichloroacetic acid (w/v) (TCA). After centrifuging the homogenate for 5 minutes at 10000 rpm, 1 ml of a solution comprising (4 percent (w/v) TCA+0.5 percent (w/v) TBA was added to 0.5 ml of supernatant. The mixture was heated to 95°C for 1 hour, then cooled to ambient temperature before being centrifuged for 5 minutes at 10000 rpm. The clear solution's absorbance was measured at 532 nm and the absorbance at 600 nm was subtracted to adjust for non-specific turbidity.

Proline estimation

Proline was estimated in both control and stressed samples seedlings. About 0.5gm plant material was ground in 10ml of 3% aqueous sulfosalicylic acid homogenized and filtrated via Whatman No.2 then about 2ml of extract was taken and to this 2ml acid - Ninhydrin + 2 ml acetic acid glacial was added and samples were heated at 100 °C for 1hrs later samples were allowed to cool on ice bath to stop the reaction. The reaction mixture was then extracted with 4ml toluene mixed vigorously using test tube stirrer for 15-20 seconds. Chromophore containing toluene was aspirated from aqueous phase, solution was warmed to room temperature and absorbance was recorded at 520nm with toluene as blank. Proline content was calculated using the formula,

$$\text{Proline Conc.}\ \left(\frac{\mu\text{moles}}{\text{gm FW}}\right) = \frac{\mu\text{g}\ \frac{\text{proline}}{\text{ml}} \times \text{toluene in ml}}{\frac{115.5\ \frac{\mu\text{g}}{\mu\text{mole}}}{\frac{\text{gram sample}}{5}}}$$

Superoxide radical (O_2^-) content

The production of Superoxide radical (O_2^-) was determined by the oxygenated hydroxylamine method

of Wang and Luo, 1990. The reaction mixture consisting of 0.5 ml potassium phosphate buffer (pH 7.8) and 0.5 ml of enzyme extract was incubated for 30 minutes at 25 °C. One millilitre of 3-aminobenzenesulfonic acid (58 mM) and an equal volume of 7 mM of 1-naphthyl amine was added to the reaction mixture and again incubated for 20 minutes. The absorbance was taken at 530 nm, and the amount of O_2^- production was calculated from the standard curve of $NaNO_2$.

Hydrogen peroxide (H_2O_2) content

Hydrogen peroxide (H_2O_2) was determined by the method of Alexieva et al., 2001. Fifty milligram of fresh leaf sample macerated with liquid nitrogen using pre-chilled mortar and pestle, and further homogenized with 10 ml of 0.1% TCA (w/v). The product was centrifuged for 15 minutes at 1000 rpm under 4°C. The supernatant was stored at -80°C refrigerator and used for enzyme activity assays. The reaction mixture consisted of 0.5 ml 100 mM potassium phosphate buffer, 2 ml KI (1M) (W/V), and 0.5 ml of leaf enzyme extract. The mixture was incubated for 1 hour at room temperature under dark conditions, and the absorbance was taken at 390 nm. The amount of H_2O_2 was calculated from the standard curve obtained from the known concentrations of H_2O_2.

ROS (Reactive Oxygen Species) scavenging enzymes activity

Fifty milligrams of fresh leave samples were homogenized with 3 ml of extraction buffer [100 mM potassium phosphate buffer (pH 7.8), 0.1 mM EDTA, and 1% Polyvinylpyrrolidone (w/v)] in an ice water bath. The homogenized material was centrifuged at 13000g for 10 minutes at 4°C. The supernatant was stored at -80°C refrigerator and used for enzyme activity assays. Activity of Super oxide dismutase (SOD) was determined by measuring the photochemical reduction of nitro-blue tetrazolium chloride (NBT) following the procedure of Giannopolitis and Ries, 1977. One unit of SOD was determined as the amount of enzyme causing 50% inhibition of NBT by photo-reduction at 560 nm absorbance.

Catalase activity was measured by the method described by Beers and Sizer, 1952. To 0.1 ml of enzyme extract, 0.9 ml of reaction mixture containing 0.1 ml of H_2O_2, 0.7 ml of 50 mM potassium phosphate buffer (pH 7.0), and 0.1 ml of EDTA was added. Rate of CAT activity was determined from the degradation of H_2O_2 per minute at 240 nm absorbance.

Statistical analysis

The means and standard error for expression values were calculated for three replicates using MS Excel.

RESULTS AND DISCUSSION

Rice requires water not just for growth and development, but also for increased harvests. Rice is thought to be poorly suited to low water circumstances as it was developed in semi-aquatic habitats. Rice droughts may occur in both irrigated and non-irrigated lowland systems, and they can impair the crop's early juvenile, reproductive, and grain development phases. The majority of drought research to date has focused on the effects of stress on panicle initiation and anthesis, as well as spikelet sterility. One of the key issues in rice research is identifying rice varieties and breeding lines with promising levels of drought resistance for use as donors in breeding and gene discovery. Drought stress at seedling level leads to significant reduction ($p<0.05$) in RWC, MSI, chlorophyll content, and significant increase ($p<0.05$) in lipid peroxidation and ROS scavenging enzymes.

Morphological analysis to assess the stress effect

To quantify the stress effect on genotypes at the end of the stress period, root and shoot lengths were measured with numerical scale. Under drought stress, root length is an essential feature of plant kinds, and

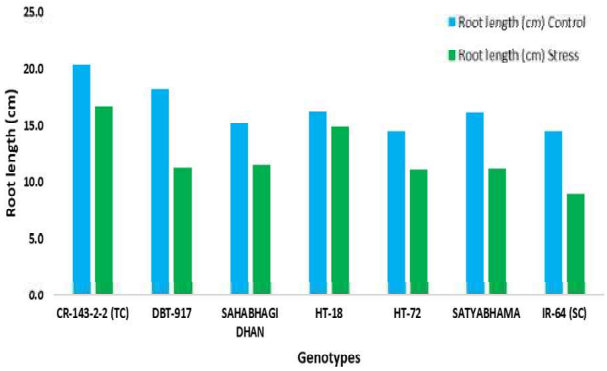


Fig. 1. Root length of different rice genotypes under control and osmotic stress

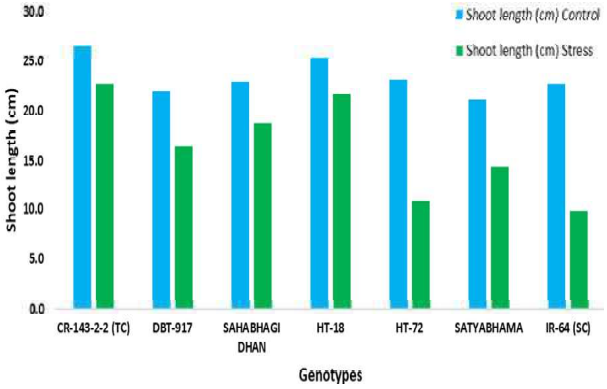


Fig. 2. Shoot length of different rice genotypes under control and osmotic stress.

roots play a key role in plant survival during drought times (Hoogenboom et al., 1987). Drought resilience is often better in varieties with longer root development. Under control conditions, the root lengths ranged between 14.5 to 20.5 cm whereas, under osmotic stress condition a significant variation in the root length was observed (Fig. 1). Tolerant check had 16.7 cm root length which was comparable with the genotype HT-18 with 14.9cm, and in other genotypes it ranged between 10-11.5 cm and susceptible check IR-64 had a root length of 8.9cm (Fig. 1). When it comes to the shoot length, HT-18 had shown 21.7cm as compared to tolerant check CR 143-2-2 with 22.8cm and others ranged between 10-16.4cm under stress situations and under control condition there was no significant variation observed, shoot length ranged between 22-26.4cm (Fig. 2). The results are in agreement with Sahoo et al. (2019) who found drought stress decreased both root and shoot lengths in 5 rice varieties. Similar kind of results were also observed by Khan et al., 2001 in maize genotypes.

Evan's blue staining for membrane integrity assay

The roots from all the genotypes grown in hydroponic culture with 20% PEG were stained with Evan's blue to check the membrane integrity under osmotic stress. The roots of susceptible genotype, IR-64 had taken up more stain compared to HT-18 roots as a result of more membrane damage. The stress effect is directly reflected on the intensity of Evans blue uptake by the cell (Fig. 3). Similar results were reported by Preethi et al., 2020 in both wheat and rice genotypes subjected to drought stress.

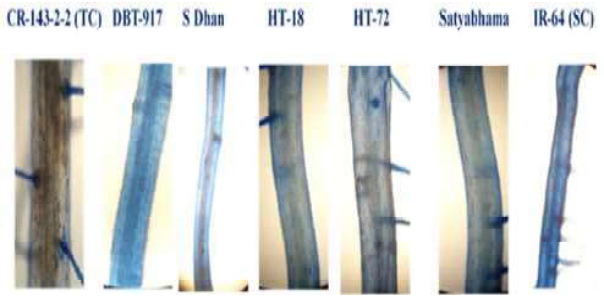


Fig. 3. Evan's blue staining for membrane integrity of rice genotypes under osmotic stress condition.

Physiological analysis to assess the stress effect

Relative Water Content (RWC)

The link between plants and water may be represented in a variety of ways, including the water potential of the leaf and relative water content (RWC) (Farooq et al., 2009). RWC is a key aspect of plant water relations and is regarded as the most comprehensive assessment of plant water status, representing fluctuations in water potential and turgor potential (Gupta et al., 2020). Between the stress and control conditions, there was a substantial variation in RWC among genotypes. In osmotic stress, the water deficit stress tolerant rice genotype HT-18 had a higher RWC of 73 percent, which is comparable to the tolerant check CR-143-RWC 22's of 73 percent, and the rest of the genotypes had RWCs ranging from 40-57.5 percent, compared to the susceptible genotype's RWC of 36.9 percent (Fig. 4). RWC of all genotypes ranged from 89 to 91 percent under control circumstances, with no significant

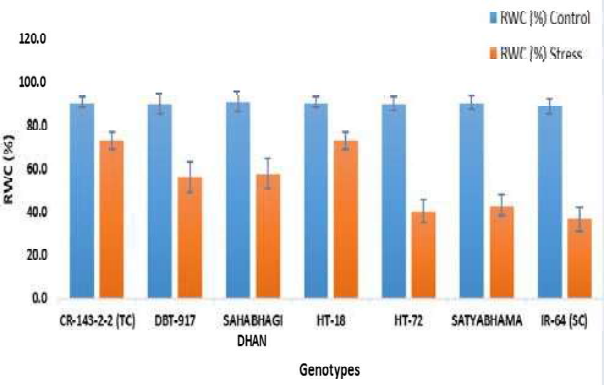


Fig. 4. Relative Water Content (RWC) of different rice genotypes under control and osmotic stress.

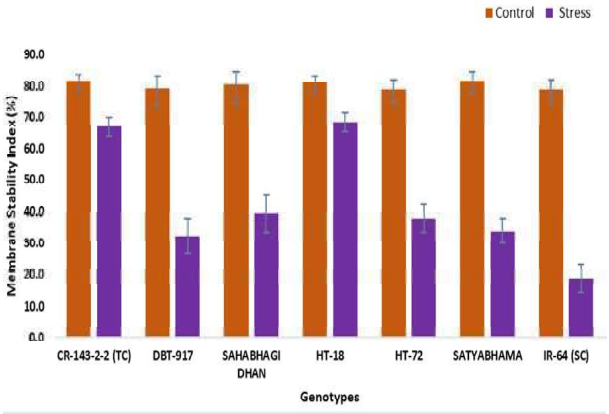


Fig. 5. Membrane Stability Index (MSI) of different rice genotypes under control and osmotic stress.

variance. Dien et al., 2019 found that genotypes with a lower drop in RWC have superior tissue tolerance potential under drought circumstances.

Membrane stability index (MSI)

Change in the membrane integrity is considered as a primary injury under stress. This is caused by osmotic effects of drought injury; leading to disorganization of cell membrane and leading to electrolyte leakage and is determined based on this parameter. The MSI was significantly higher in HT-18 *i.e.*, 68.2% as compared to other genotypes which ranged between 34-37% under stress, but under control condition all the genotypes performed same with MSI of 79-81.5% (Fig. 5). Bangar et al., 2019 reported that the ability of the genotype to maintain higher MSI is one of the acquired traits of tolerance under moisture stress condition.

Total chlorophyll content

The decrease in chlorophyll pigment is mainly due to chloroplast photo-oxidation, ultrastructure degradation and increment in chlorophyllase activity (Mafakheri et al., 2010; Kabiri et al., 2014). Higher genotypic differences in chlorophyll content were observed under stress condition and it ranged between 1.5 to 3.29 mg/gm FW. The tolerant check has shown total chlorophyll of 3.14 mg, HT-18 with 3.29 mg/gm FW and susceptible check IR-64 with 1.5 mg/gm FW. The total chlorophyll content observed under normal condition was 3.3 to 4.06 mg/gm FW (Fig. 6). Chutia and Borah, 2012 in there investigation, observed the significant decrease in the Chlorophyll-a and Chlorophyll-b and total chlorophyll content in the rice plants of unirrigated upland situations.

Biochemical analysis

Measurement of malondialdehyde accumulation by thiobarbituric acid reactive substances (TBARS) assay

The concentration of malondialdehyde (MDA) generated by the thiobarbituric acid (TBA) reaction in the drought stressed and control leaves of all genotypes was used to evaluate membrane lipid peroxidation. The production of MDA is an indicator of lipid peroxidation (Celik et al., 2017). There was significant reduction in membrane lipid peroxidation in HT-18 with MDA content of 0.055 compared to susceptible check IR-64 with MDA content of 0.139 (Fig. 7).

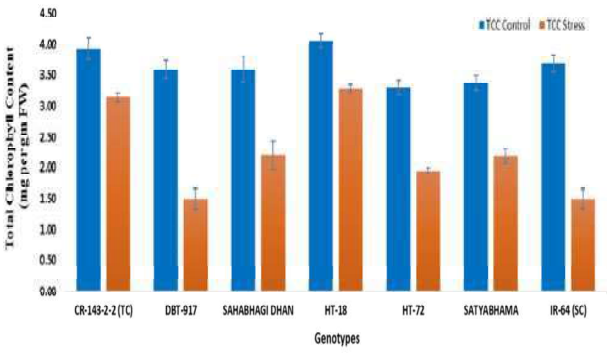


Fig. 6. Total Chlorophyll Content (TCC) of different rice genotypes under control and osmotic stress.

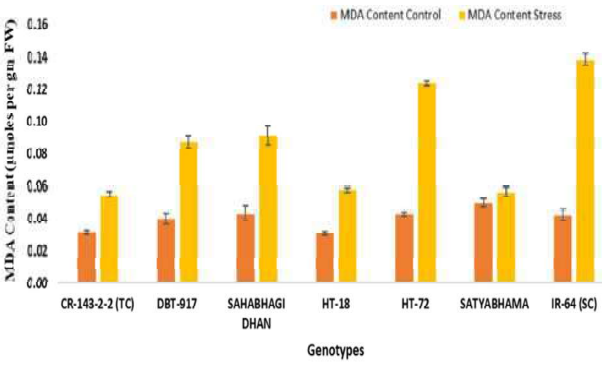


Fig. 7. Malondialdehyde (MDA) content of different rice genotypes under control and osmotic stress.

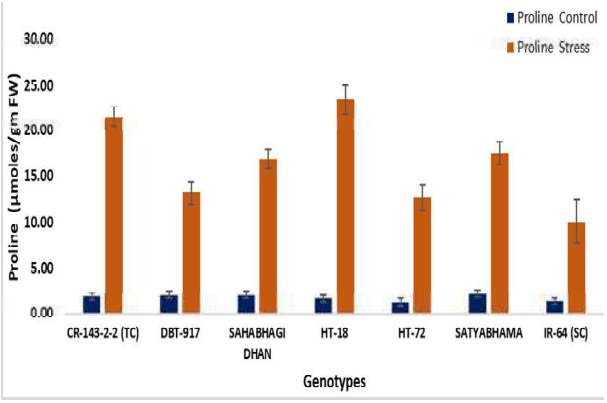


Fig. 8. Proline content of different rice genotypes under control and osmotic stress

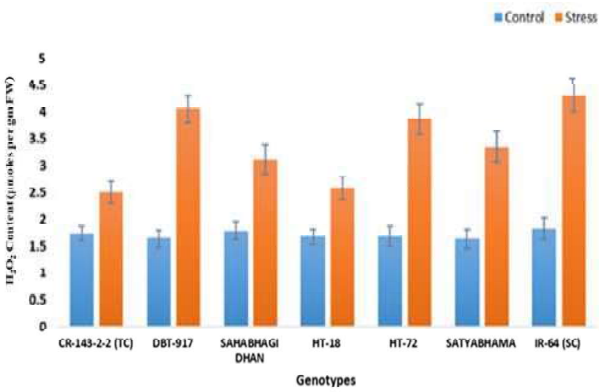


Fig. 9(B). Variation in Hydrogen Peroxide content (H_2O_2) in different rice genotypes under control and osmotic stress.

Proline estimation

Plants strive to maintain cell turgor during drought stress by accumulating organic and inorganic solutes that reduce the osmotic potential. Plants get osmotic adaptations through accumulating osmoprotectants such as proline, glycine betaine, and soluble sugar (Hayat et al., 2012). Compared to well-watered settings, proline accumulation rises in all rice cultivars during drought (Mishra et al., 2018). Between stress and control circumstances, there was a substantial variation in proline accumulation among genotypes. In osmotic stress condition, higher value of proline was observed in HT-18 with 23.45 moles/gm FW and in tolerant check it was 21.55 moles/gm FW, the susceptible genotype had 10.08 moles/gm FW (Fig. 8).

ROS content

Excessive production of ROS *i.e.*, O_2^- and H_2O_2 because of water loss causes oxidative damage and lipid peroxidation under drought conditions (Mittler, 2002). Drought stress significantly increased ($p<0.05$) O_2^- and H_2O_2 in all the genotypes. The value of O_2^- ranged between 0.0031-0.045 molg^{-1} fw under stress condition and HT-18 had shown similar O_2^- value (0.031 molg^{-1} fw) with that of tolerant check (CR143-2-2) (Fig. 9A). The values for H_2O_2 ranged between 2.51 to 4.31 mg/FW and HT-18 has showed lowest content of H_2O_2 (2.59 mg/FW) (Fig. 9B). Tolerant genotypes have antioxidant defense mechanisms to scavenge ROS production and mitigate oxidative damage (Gill and Tuteja, 2010). Similar kind of results were also obtained by Lu et al., 2010; Kumar et al., 2014; Dudziak et al.,

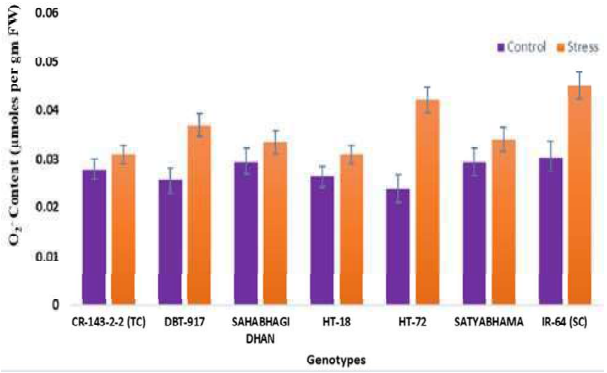


Fig. 9(A). Variation in super oxide radicle content (O_2^-) in different rice genotypes under control and osmotic stress.

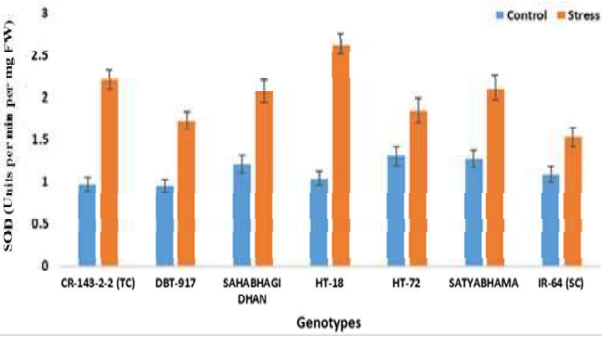


Fig. 10(A). Variation in Super Oxide Dismutase (SOD) enzyme activity in different rice genotypes under control and osmotic stress.

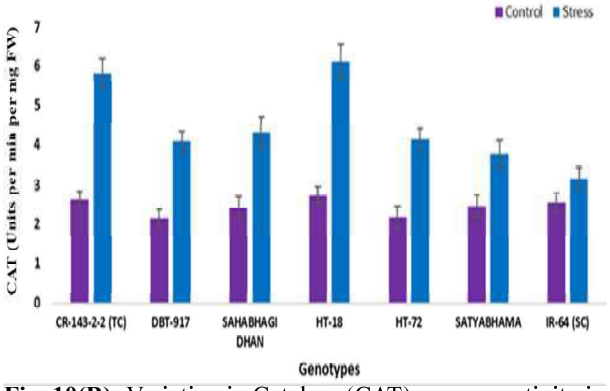


Fig. 10(B). Variation in Catalase (CAT) enzyme activity in different rice genotypes under control and osmotic stress.

2019.

ROS scavenging enzymes activity

Plants have an antioxidant defense mechanism to protect the cells from oxidative stress by producing antioxidant enzymes under DS conditions (Chutipaijit, 2016). Drought stress significantly increased ($p<0.05$) both SOD and Catalase activity in HT-18 genotype. (Fig. 10 A and B). Activity of both the enzymes SOD and CAT was increased in tolerant genotype HT-18 to mitigate the toxic effects of O_2^- and H_2O_2 respectively. Samota et al., 2017 reported the similar findings from their study.

CONCLUSION

Approximately 1100 to 1250 mm water is required for the proper cultivation of rice which is more than 50 percent of all the fresh water used in agriculture. With increase in population and the forecasted global food demand, cultivating rice by the conventional method is increasingly becoming uneconomical. So, priority must be given to save water and also sustaining productivity under water limited conditions. Therefore, the emphasis in this study was to improve the vegetative level drought tolerance of rice and to identify genotypes with better tolerance at cellular level for water deficit condition. The present study evaluated the performance of seven rice genotypes using morphological and physiological growth parameters, under induced drought conditions. All the genotypes which were studied, indicated the presence of exploitable genetic variation in terms of physiological and biochemical traits. Of the genotypes evaluated, HT-18 (AC-34973) was identified as seedling level drought tolerant genotype whose performance was better than CR-143-2-2 which is a tolerant check used in the present study.

Conflict of interest: The authors declare that they have no conflict of interest.

REFERENCES

Alexieva IV, Sergiev S, Mapelli E Karanov (2001). The effect of drought and ultraviolet radiation on growth and stress markers in pea and wheat. Plant Cell and Environment 24(12): 1337-1344

Baker CJ and Mock NM (1994). An improved method for monitoring cell death in cell suspension and leaf disc assay using Evan's blue. Plant Cell Tissue Organ Cult. 39(1): 7-12

Bangar P, Chaudhury A, Tiwari B, Kumar S, Kumari R and Bhat KV (2019). Morphophysiological and biochemical response of mungbean [*Vigna radiata* (L.) Wilczek] varieties at different developmental stages under drought stress. Turkish Journal of Biology 43: 58-69

Beers RF and Sizer IW (1952). A spectrophotometric method for measuring the breakdown of hydrogen peroxide by catalase. J. Biol. Chem. 195(1): 133-40

Boonjung H and Fukai S (1996). Effects of soil water deficit at different growth stages on rice growth and yield under upland conditions. 2. Phenology, biomass production and yield. Field Crops Research 48(1): 47-55

Bunnag S and Pongthai P (2013). Selection of rice (*Oryza sativa* L.) cultivars tolerant to drought stress at the vegetative stage under field conditions. American Journal of Plant Sciences 04(09): 1701-1708

Çelik Ö, Ayan A and Atak Ç (2017). Enzymatic and non-enzymatic comparison of two different industrial tomato (*Solanum lycopersicum*) varieties against drought stress. Botanical Studies 58

Challam C, Ghosh T, Rai M and Tyagi W (2015). Allele mining across DREB1A and DREB1B in diverse rice genotypes suggest a highly conserved pathway inducible by low temperature. J. Genet. 94: 231-238

Chutia J and Borah S (2012). Water stress effects on leaf growth and chlorophyll content but not the grain yield in traditional rice (*Oryza sativa* Linn.) genotypes of Assam, India II. Protein and Proline Status in Seedlings under PEG Induced Water Stress. American Journal of Plant Sciences 3(7): 971-980 doi: 10.4236/ajps.2012.37115

Chutipaijit S (2016). Changes in physiological and antioxidant activity of Indica rice seedlings in response to mannitol-induced osmotic stress. Chilean Journal of Agricultural Research 76: 455-462

Dien DC, Mochizuki T and Yamakawa T (2019). Effect of various drought stresses and subsequent recovery on proline, total soluble sugar and starch metabolisms in rice (*Oryza sativa* L.) varieties. Plant Production Science 22: 530-545

Dodig D, Zoric M, JovicM, Kandic V, Stanisavljevic R and Surlan-Momirovic G (2015). Wheat seedling growth responses to water deficiency and how it correlates with adult plant tolerance to drought. J Agric Sci. 153: 466

Dudziak K, Zapalska M, Börner A, Szczerba H, Kowalczyk K and Nowak M (2019). Analysis of wheat gene expression related to the oxidative stress response and signal transduction under short-term osmotic stress. Scientific Reports 9: 1-14

Farooq M, Wahid A, Kobayashi N, Fujita D and Basra SMA (2009). Plant drought stress: Effects, mechanisms and management. Agronomy for Sustainable Development 29: 185-212

Giannopolitis CN and Ries KS (1977). Superoxide dismutases II. Purification and quantitative relationship with water-soluble protein in seedlings. Plant Physiol. 59(2): 315-318

Gill SS and Tuteja N (2010). Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. Plant Physiology and Biochemistry 48: 909-930

Gonzalez A and Ayerbe L (2011). Response of coleoptiles to water deficit: growth, turgor maintenance and osmotic adjustment in barley plants (*Hordeum vulgare* L.). Agric. Sci. 2: 159

Grzesiak MT, Marcinska I,Janowiak F, Rzepka A and Hura T (2012). The relationship between seedling growth and grain yield under drought conditions in maize and triticale genotypes. Acta Physiol. Plant. 34: 1757

Gupta A, Rico-Medina A and Caño-Delgado AI (2020). The physiology of plant responses to drought. Science 368: 266-269

Hameed A, Goher M and Iqbal N (2010). Evaluation of seedling survivability and growth response as selection criteria for breeding drought tolerance in wheat. Cereal Res Commun 38: 193

Hayat S, Hayat Q, Alyemeni MN, Wani AS, Pichtel J and Ahmad A (2012). Role of proline under changing environments: a review. Plant Signal Behav. 7(11): 1456-66

Hiscox JD and Israelstam GF (1979). A method for the extraction of chlorophyll from leaf tissue without maceration. Can. J. Bot. 57: 1332-1334

Hoogenboom G, Huck MG and Peterson CM (1987). Root growth rate of soybean as affected by drought stress. Agronomy Journal 79(4)

Hsiao TC (1973). Plant responses to water stress. Ann. Rev. Plant Physiol. 24: 519-570

Huke RE and Huke EH (1997). Rice Area by Type of Culture: South, Southeast, and East Asia. International Rice Research Institute, Los Banos, Philippines

Kabiri R, Nasibi F and Farahbakhsh H (2014). Effect of exogenous salicylic acid on some physiological parameters and alleviation of drought stress in Nigella sativa plant under hydroponic culture. Plant Protection Science 50: 43-51

Khan AA, McNeilly T and Azhar FM (2001). Stress tolerance in crop plants. (Review). Int. J. Agri. Biol. 3: 250-55

Khan MH and Panda SK (2008). Alterations in root lipid peroxidation and antioxidative responses in two rice cultivars under NaCl-salinity stress. Acta Physiologiae Plantarum 30: 81-89

Krishnamurthy SL, Sharma PC, Sharma SK, Batra V, Kumar V and Rao LVS (2016). Effect of salinity and stress indices of morphological and physiological traits at the seedling stage in rice. Indian J Exp Biol, 54, 843.

Kumar M, Lee SC, Kim JY, Kim SJ, Aye SS and Kim SR (2014). Over-expression of dehydrin gene, *OsDhn1*, improves drought and salt stress tolerance through scavenging of reactive oxygen species in rice (*Oryza sativa* L.). Journal of Plant Biology 57: 383-393

Lu YY, Deng XP and Kwak SS (2010). Over expression of CuZn superoxide dismutase (CuZn SOD) and ascorbate peroxidase (APX) in transgenic sweet potato enhances tolerance and recovery from drought stress. African Journal of Biotechnology 9: 8378-8391

Mafakheri A, Siosemardeh A, Bahramnejad B, Struik PC and Sohrabi E (2010). Effect of drought stress on yield, proline and chlorophyll contents in three chickpea cultivars. Australian Journal of Crop Science 4: 580-585

Mishra SS, Behera PK, Kumar V, Lenka SK and Panda D

(2018). Physiological characterization and allelic diversity of selected drought tolerant traditional rice (*Oryza sativa* L.) landraces of Koraput, India. Physiol. Mol. Biol. Plants 24(6): 1035-1046

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

Mittler R (2002). Oxidative stress, antioxidants and stress tolerance. Trends in Plant Science 7: 405-410

Moud AM and Maghsoudi K (2008). Application of coleoptile growth response method to differentiate osmoregulation capability of wheat (*Triticum aestivum* L.) cultivars. Res. J. Agron. 2: 36

O'toole JC (1982). Adaptation of rice environments. Drought resistance in crops with emphasis on rice; Paddy field: Manila, Philippines pp. 195-213

Pandey S and Bhandari H (2009). Drought: economic costs and research implications. In: Seeraj R, Bennet J and Hardy B.(eds.), Drought frontiers in rice: crop improvement for increased rainfed production. World Scientific publishing. Singapore pp. 3-17

Pandey S, Bhandari H, Sharan R, Naik D, Taunk SK and Sastri ASRAS (2005). Economic costs of drought and rainfed rice farmers' coping mechanisms in Eastern India. Final Project Report. International Rice Research Institute

Pantuwan G, Fukai S, Cooper M, Rajatasereekul S, O' Toole JC (2002). Yield response of rice (*Oryza sativa* L.) genotypes to drought under rainfed lowland: 3. Plant factors contributing to drought resistance. Field Crops Research 73: 181-200

Preethi V, Ramu SV, Xinyou Y, Paul CS, Udayakumar M and Sheshshayee S (2020). Acquired traits contribute more to drought tolerance in wheat than in rice. Plant Phenomics pp. 1-16

Sahoo S, Saha B, Awasthi JP, Omisun T, Borgohain P, Hussain S, Panigrahi J and Panda SK (2019). Physiological introspection into differential drought tolerance in rice cultivars of North East India. Acta Physiologiae Plantarum pp. 41

Sairam RK, Deshmukh PS and Shukla DS (1997). Tolerance to drought and temperature stress in relation to increased antioxidant enzyme activity in wheat. J. Agron. Crop Sci. 178: 171-177

Samota MK, Sasi M, Awana M, Yadav OP, Amitha Mithra SV, Tyagi A, Kumar S and Singh A (2017). Elicitor-induced biochemical and molecular manifestations to improve drought tolerance in rice (*Oryza sativa* L.) through seed-priming. Frontiers in Plant Science 6: 8: 934

Singh AK and Singh Lal (2007). Role of thermal time in rice phenology. Envi. and Ecol. 25 (1): 46-49

Singh AK, Chandra N and Bharti RC. 2012. Effects of genotype and planting time on phenology and performance of rice (*Oryza sativa* L.). Vegetos 25(1): 151-156

Singh BB, Mai- kodomi Y and Terao T (1999). A simple screening method for drought tolerance in cowpea. Indian J. Genet. Plant Breed. 59: 211

Singh D, Singh AK, Singh A, Patel AK and Baghel MS (2015). Impact assessment of short duration paddy variety Birsa Vikas Dhan-109 in Sidhi district of Madhya Pradesh. Journal of Agri. Search 2(1): 53-56

Singh S and Singh TN (2000). Morphological, chemical and environmental factor affecting leaf rolling in rice during water stress. Indian J. Plant Physiol. 5: 136-141

Soltani A, Gholipoor M and Zeinali E (2006). Seed reserve mobilization and seedling growth of wheat as affected by drought and salinity. Environ. Exp. Bot. 55: 195

Torres RO and Henry A (2016). Yield stability of selected rice breeding lines and donors traditional rice landraces from Koraput, India. Rice Sci. 24(4)

Verulkar SB and Verma SK (2014). Screening protocols in breeding for drought tolerance. Agricultural Research 3: 2-40

Wang AG and Luo GH (1990). Quantitative relation between the reaction of hydroxylamine and superoxide anion radicals in plants. Plant Physiology Communication 6: 55-57

Wassmann R, Jagadish SVK, Sumfleth K, Pathak H, Howell G, Ismail A et al. (2009). Regional vulnerability of climate change impacts on Asian rice production and scope for adaptation. Advances in agronomy, 102: 91-133

Weatherley PE (1950). Studies in the water relations of the cotton plant I. The field measurements of water deficit in leaves. New Phytologist 49: 81-97

Zhou Q (2001). The measurement of malondialdehyde in plants. In: Zhou Q. (ed.): Methods in Plant Physiology. China Agricultural Press, Beijing 173-174