



# Effects of Water Deficit Stress at the Tillering Stage of Direct Seeded Rice (*Oryza sativa* L.)

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## Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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## ABSTRACT

The ten rice varieties viz. NAUR-1, Purna, GNR-6, NVSR-2227, NVSR-3093, NVSR-2285, NVSR-2278, NVSR-2852, NVSR-2601, and NVSR-2927 were grown in kharif season for observing their efficiency in terms of yield under a deficit of water at tillering stage. The effects of short-term water stress on various morpho-physiological and biochemical traits viz. leaf area index, number of panicles, chlorophyll content, number of stomata, relative water content, abscisic acid, proline, glycine betaine, ascorbic acid, and harvest index content were studied and they were observed higher in Purna as compared to other nine varieties. However, Plant height and Ionic leakage were recorded lower in Purna as compared to other varieties. The abscisic acid as well as proline and glycine betaine content recorded maximum in Purna which might help in keeping hydration in cells

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during water deficit stress. Thus, with short-term water deficit stress at tillering stage, the variety Purna would be better in terms of breeding for tolerant variety as well as yield among ten rice varieties.

*Keywords: Rice; stress; tillers; varieties; water.*

## 1. INTRODUCTION

Cereals have a major role in feeding billions of people and 50% to 80% of daily calorie intake is provided by rice, especially in Asian countries. Among cereals, rice consumes almost 80% of the total irrigation freshwater resources, and its roots are submerged during the vegetative and reproductive stages. The aerenchymatous tissue is a specialized structure in rice that makes its growth and development during the submerged condition [1]. Direct-seeded rice generally grows on upland/low water regime faces water deficit stress, changes its structure and develop deep and thick roots resulting in the improvement of hydraulic properties of roots thereby extracting water from deeper soil profiles [2]. These developed anatomy under drought stress support higher yield in upland rice than lowland rice. Many developmental processes of plants such as the number of tillers, panicle initiation, and anthesis adversely affected by drought stress [3]. Drought severity activates the production of Reactive Oxygen Species (ROS) mainly in chloroplasts and mitochondria which reduce the membrane stability index and promote leakage of ions due to membrane disruption Pandey and Shukla, [4]. The ROS viz. Singlet oxygen, superoxide anion radicals, hydroxyl radicals, and hydrogen peroxide ( $H_2O_2$ ) are produced and initiate indirect disruption of metabolism [5]. The defense mechanism for ROS scavenging is developed in plants to detoxify and suppress the production of ROS by synthesis of different enzymatic and non-enzymatic antioxidants. The drought-tolerant genotype of rice dramatically increases antioxidant enzymes viz. superoxide dismutase, peroxidase, and catalase which suppress the production of ROS in the cell Mishra and Panda, [6] Kant, [5]. The adverse effects of ROS reduced by the synthesis of compatible solutes/osmolytes such as proline, glycine betaine, sugars (trehalose), and polyols (sorbitol, mannitol, myo-inositol, and pinitol) which exalted hydration in the cell. These osmolytes retain water molecules in their structure and they become shields of the osmoprotectants in plants during severe water/salinity stress [7].

Proline is one of the best osmolytes which support the vitality of the plant during water deficit. It is a neutral imino acid at pH-7, has rigid structure, and is highly soluble in water. It stabilizes protein structures and inhibits protein unfolding during water stress [8]. The enzymes of the metabolic pathway are alleviated by proline particularly ribulose 1, 5 bisphosphate carboxylase that initiates the Calvin cycle [9]. The oxidative stress is the main factor for membrane disruption and ionic leakage, and this occurs when the synthesis of reactive oxygen species (ROS) exceeds their scavenging in the cell. Proline is considered an effective hydroxyl radical scavenger and promoter of enzymatic antioxidants and its biosynthesis in chloroplasts reached up to 80% during stress as compared to 5% in normal which facilitates the electron flow between photosynthetic excitation centers and maintains a low NADPH: NADP ratio in cell. Thus, it protects the plant from photoinhibition and damage to the photosynthetic apparatus because an extra electron in the cell is the root cause of ROS production [10]. Proline accumulation increases cellular osmolarity drives the influx of water or reduces its efflux resulting in an increase in pressure potential for cell expansion [11]. Another biomolecule that protects plants during water stress is glycine betaine which acts as a compatible solute and improves the tissue water status. It is synthesized from choline and glycine when a plant is exposed to drought and salinity. It improves  $Ca^{2+}$ -ATPase and Hill reaction activities in the thylakoid membrane thereby promoting photosynthesis and translocation of sucrose. It is a quaternary amine with zwitterionic nature and can protect biological membranes from ROS as well as improve antioxidant defense system under stress [12]. The biosynthetic genes of proline and glycine betaine have been widely used in transgenic production to improve abiotic stress tolerance in plants [13], Swamy and Kumar, [14]. Therefore, the present study has been focused on creating short-term water deficit stress at the tillering stage of rice for the determination of better yield among different varieties.

## 2. MATERIALS AND METHODS

The experiment was carried out at the greenhouse located at N. M. College of Agriculture, Navsari Agricultural University, Navsari, Gujarat, India. The Navsari Agricultural University, Navsari campus is geographically located at 20° 57' N latitude and 72° 54' E longitude at an altitude of 16 m above the mean sea level. The average temperature and relative humidity recorded  $31\pm 1^{\circ}\text{C}$  and 80%, respectively. The seeds of ten rice varieties (NAUR-1, Purna, GNR-6, NVSR-2227, NVSR-3093, NVSR-2285, NVSR-2278, NVSR-2852, NVSR-2601, NVSR-2927) subspecies *Indica* group sown in the pot filled with soil and FYM in the ratio of 2:1 in each pot after soaking in water for 12 hours and treatment with carbendazim 50% WP by immersing for 10 minutes. Both mixtures of soil and FYM were weighed and it was 7.5 Kg per pot. Fertilizer application to the test crop was done adopting the recommended fertilizer dose (RDF) for the Navsari region *i.e.*, N - P<sub>2</sub>O<sub>5</sub> - K<sub>2</sub>O (100-30-00 kg ha<sup>-1</sup>). The water deficit stress was created at the tillering stage with field capacity of 60%. The morphophysiological and biochemical traits were studied after the creation of stress. Plant height was measured in centimetres from ground level to the tallest leaf of the plant at different stages of crop growth and the average mean height was recorded. The leaf area index (LAI) was calculated by dividing the leaf area per plant by the land area occupied by the plant. The number of panicles per plant was counted and the average was worked out. Stomatal Frequency was measured with a microscope and expressed as the number of stomata per micrometer square. Chlorophyll content was measured with a SPAD meter.

Ionic leakage was measured following the method of R. Sivakumar *et al.* (2015). Weighed two sets of 200 mg of leaf samples and put them into two test tubes containing 10 ml double distilled water. Kept one test tube at 40°C in a water bath for 30 minutes. Thereafter the electrical conductivity (EC) of water containing the sample using a Conductivity Bridge and noted (C1). The other test tube was incubated at 100°C in the boiling water bath for 15 minutes and measured the EC (C2). Ionic leakage (100) =  $C1/C2 \times 100$

Relative Water Content (RWC) was calculated following the method described by R. K. Sairam (1994). The third leaf (physiologically functional)

from the top is selected for RWC estimation. Floated the leaf discs in water for four hours to attain full turgid, and after taking out the leaf discs from the water, and wiped out the water droplets sticking on the leaf surface by using filter paper. Immediately recorded the turgid weight (Tw) after an hour of floating. Transferred the leaf discs to a butter paper cover and then kept the cover in a hot air oven at 80°C for 48 hours. Recorded the dry weight (Dw) after removing from the Oven.  $RWC = \frac{Fw - Dw}{Tw - Dw} \times 100$ .

Harvest index (%) =  $\frac{\text{Economic yield}}{\text{Biological yield}} \times 100$ . (Biological yield = Grain yield + Straw yield).

The proline content in the leaves was estimated following the method described by Shivkumar *et al.* (2015). The 0.1 g of rice leaves were ground with 5 ml of 3% sulfosalicylic acid, and the mixture was then filtered. 2 ml of the filtered mixture in a test tube, 2 ml of acid-ninhydrin and 2 ml of glacial acetic acid were added. The mixture was mixed with a Vortex mixer and boiled at 100°C for 1 h. The mixture was then frozen in ice and combined with 4 ml of toluene, mixed, and then left to stand for 5-10 min. The absorbance of the reddish pink up- per phase was recorded at 520 nm against a toluene blank.

The glycine betaine (GB) content was determined following the method of Bates *et al.* (1973) with minor modifications. Finely ground dry material (0.5 g) was mixed with 20 ml of deionized water and maintained with mechanical agitation for 48 h at 25°C; subsequently, the mixture was centrifuged at 10,000 rpm for 10 min using a refrigerated centrifuge. The supernatant was collected and stored in the freezer until the analysis. Samples were thawed and diluted (1:1 v/v) with 2N of sulphuric acid (H<sub>2</sub>SO<sub>4</sub>). An aliquot of 0.5 ml was collected and incubated for 1 h in ice; after which, 0.2 mL of cold potassium iodide-iodine (KI-I<sub>2</sub>) was added and mixed gently. The samples were stored at 0 to 4°C for 16 h; after which, they were centrifuged at 10,000 rpm for 15 min at 0°C. The supernatant was discarded while ensuring not to shake it; during this time, the samples were maintained in ice to allow separation of the periodide complexes from the acid medium. The periodide crystals that formed were dissolved in 9 ml of 1, 2-dichloroethane, and the mixture was left to sit for 2 h; after which, the absorbance was read at 365 nm. For the standard GB curve, a stock comprising 1 mg/ml diluted in 1 N H<sub>2</sub>SO<sub>4</sub> was prepared; concentrations of 50-200 mg/ml were used.

Abscisic acid (ABA) content is determined by following the method described by Plessis et al. (2011). 100 mg of the leaves were ground and mixed with 2 ml of extraction solvent (acetone, water, acetic acid, 80/19/1, v/v/v). The supernatant was recovered by centrifugation and the pellet was rinsed with 1 ml of extraction solvent. The extraction solvent was evaporated and the residue was resuspended in 0.5 ml of HPLC solvent (acetonitrile, water, acetic acid, 50/50/0.05, v/v/v) and ABA was quantified.

The Ascorbic acid content in the leaves was estimated following the method described by Shivkumar et al. (2015).

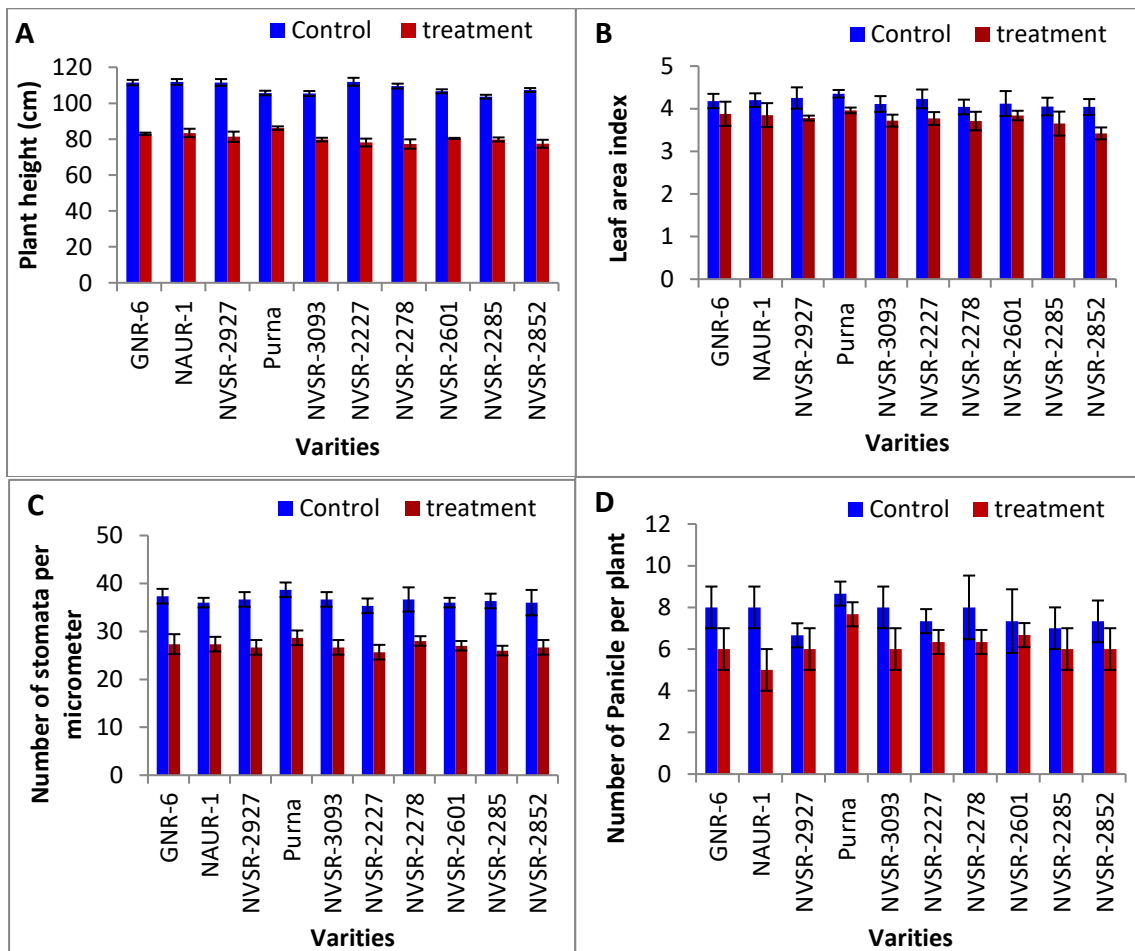
### 2.1 Statistical Analysis

The data obtained were converted to the mean value for each parameter with three repetitions

under a complete randomized design with factorial concept and statistically analyzed as described by Pans and Sukahtme (1978) factorial concept of the experiment.

### 3. RESULTS AND DISCUSSION

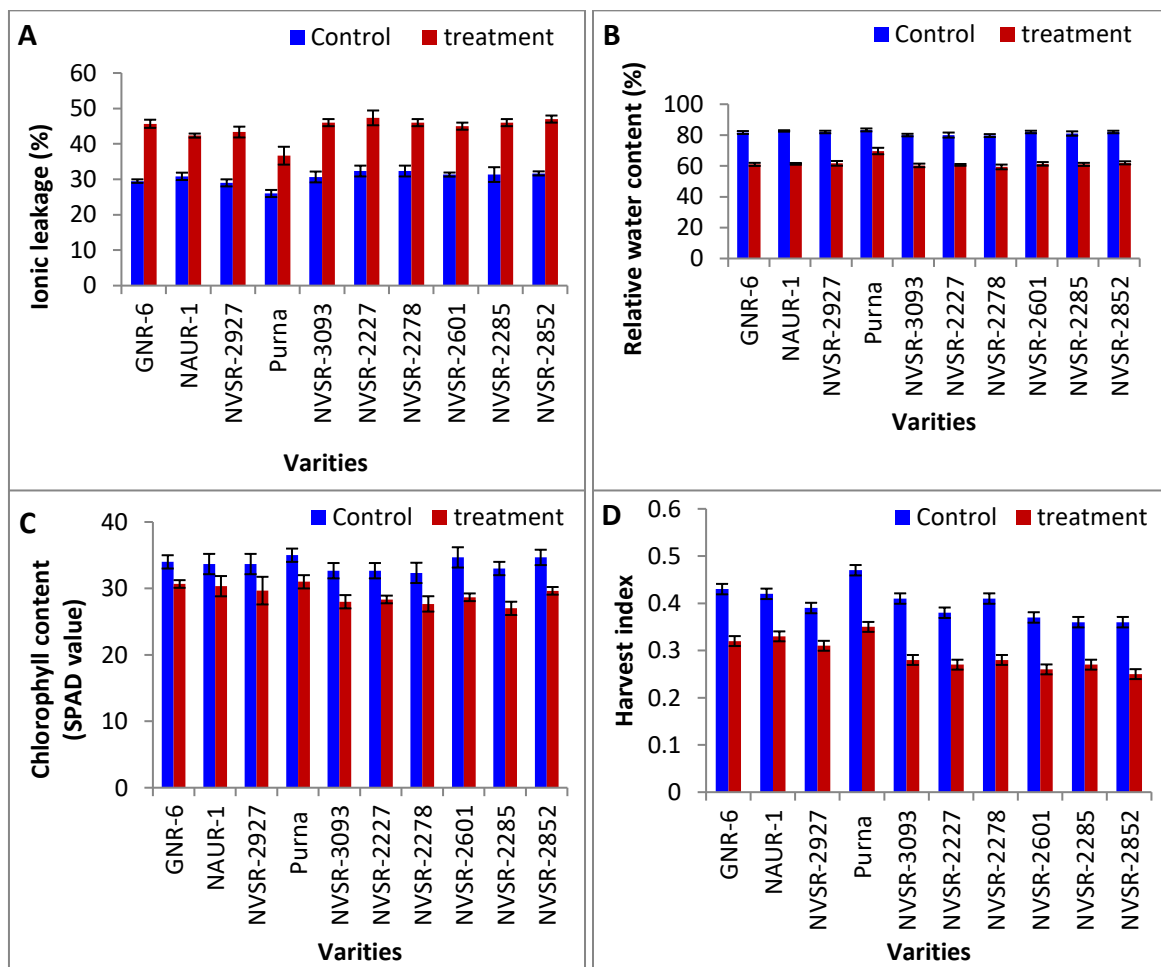
Drought stress at the typical stage of rice led to a reduction in maturity period and yield. Cultivation of direct-seeded rice under rainfed conditions usually faces a water deficit during its growth stage. Growing of rice genotypes having the tolerance capacity of drought would be the beneficial approach for getting comparatively higher yields under short-term water limitations at a specific stage. Hence, 10 varieties have been grown and their performance under water deficit stress at the tillering stage was analysed.



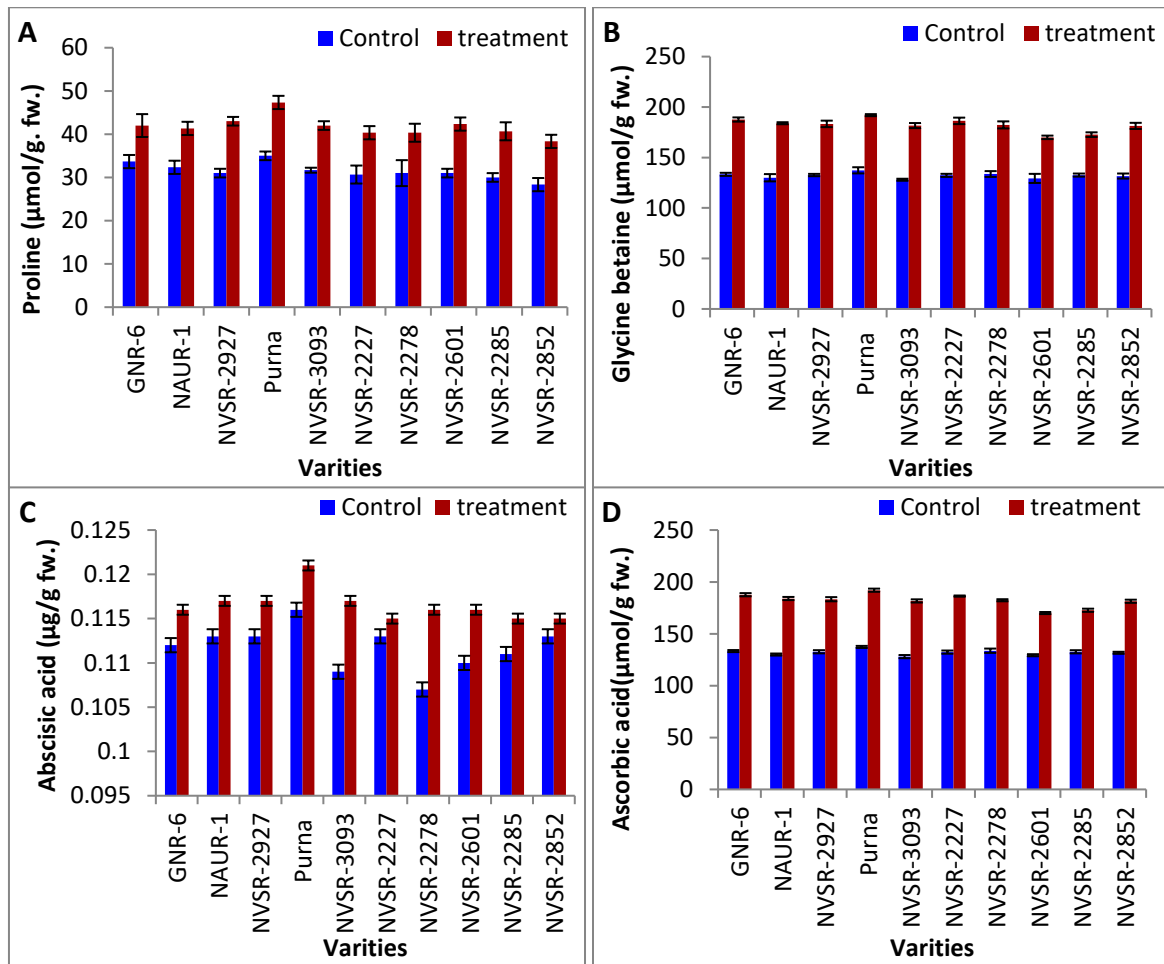
**Fig. 1. (A) Plant height as affected by water deficit stress at the tillering stage and control** ± Bars indicate SD (n = 10) (B) Leaf area index as affected by water deficit stress at the tillering stage and control. ± Bars indicate SD (n = 10) (C) Number of stomata per micrometer of leaf area as affected by water deficit stress at tillering stage and control. ± Bars indicate SD (n = 10) (D) Number of panicles per plant as affected by water deficit stress at the tillering stage and control. ± Bars indicate SD (n = 10)

The results of 10 varieties of their four morphological traits showed that the variety Purna was lower plant stature (Fig. 1A), higher leaf area index (Fig. 1B), higher number of stomata per micrometer (Fig. 1C), and higher number of panicles per plant (Fig. 1D). These are the traits which support the plants their performance under stage specific stress. Fleury et al. [15] reported that genotypic variance in wheat increase tolerance capacity of drought. It has been indicated that breeding strategy with genotypic selection might increase the tolerance against drought [16]. Morphological traits might have the greater influence on drought tolerance and phenotyping of crops in the genomic era provided higher impact on yield and ecosystem [17].

The ionic leakage was observed lower in Purna among 10 treated varieties under water deficit conditions in comparison to control (Fig. 2 A) and relative water content (Fig. 2 B) Chlorophyll content (Fig. 2 C) was recorded (69.66%) and (31 SPAD value) respectively, in Purna and they were higher among 10 treated varieties while in control relative water content (RWC) maintained at >80% and chlorophyll content was higher in all 10 varieties compared to treated plants. The percentage harvest index showed that Purna performed better under stage-specific water stress among 10 varieties while in control harvest index was higher in all cases compared to treated plants (Fig. 2 D). The water deficit stress delayed panicle initiation and increased ionic leakage led to membrane damage, decreased



**Fig. 2. (A) Ionic leakage as affected by water deficit stress at the tillering stage and control** ± Bars indicate SD (n = 10) (B) Relative water content as affected by water deficit stress at the tillering stage and control. ± Bars indicate SD (n = 10) (C) Chlorophyll content as affected by water deficit stress at the tillering stage and control. ± Bars indicate SD (n = 10) (D) Harvest index as affected by water deficit stress at the tillering stage and control. ± Bars indicate SD (n = 10)



**Fig. 3. (A) Proline content as affected by water deficit stress at the tillering stage and control**  $\pm$  Bars indicate SD ( $n = 10$ ) **(B) Glycine betaine content as affected by water deficit stress at the tillering stage and control.**  $\pm$  Bars indicate SD ( $n = 10$ ) **(C) Abscisic acid content as affected by water deficit stress at the tillering stage and control.**  $\pm$  Bars indicate SD ( $n = 10$ ) **(D) Ascorbic acid content as affected by water deficit stress at the tillering stage and control.**  $\pm$  Bars indicate SD ( $n = 10$ )

respiration as well as ATP production in rice [18]. Low water potential under drought is a common phenomenon and is genotypically regulated as per the tolerance of the plant. It affects the anatomy of leaves and their ultrastructure is highly influenced under low relative water content leading to a lower number of stomata per micrometer of leaf [19]. Reduced leaf area and chlorophyll content, leaf rolling, stomatal closure, and cutinized layer on the leaf surface induce early flowering and a decline harvest index [20]. It has been reported that a short period of drought in rice reduced panicle length and grain filling duration resulting in a loss in grain yield as well as harvest index. A reduced number of stomata and higher ionic leakage followed by lower stomatal conductance hinders the exchange of gases, resulting in poor seed setting

and reduced grain size [21]. Water deficit in the short term negatively affected the RWC which created an osmotic imbalance, lipid peroxidation, and membrane damage, and in severe cases, necrosis spots have been also reported in rice Zhu et al., [22] Panda et al., [23].

Osmotic imbalance under low RWC is genotypically regulated in plants. Some plants have their physiological and biochemical mechanism to synthesize osmolytes and protect from water stress. The simultaneous processes of photosynthesis and transpiration continued with lower stomatal resistance and higher osmoprotectant under water-limited conditions are some of the most important traits to select genotypes for different environmental conditions [24]. The acclimatization of genotypes in

response to the changing environments is the Genotype-environment interactions ( $G \times E$ ). These two sources of variation might be considered for varietal evaluation for breeding and cultivation across environments [25]. In the present study, Purna showed better among 10 varieties in the synthesis of Proline, Glycine betaine which induced by increased amount of abscisic acid (Fig. 3). The increased amount of ascorbic acid was significant impact on stress mitigation in case of selected 10 varieties (Fig. 3D). The content of Proline, Glycine betaine, abscisic acid and ascorbic acid were higher in Purna compared to other nine varieties while ascorbic acid was higher in all 10 varieties under control. These two osmolytes- Proline and Glycine betaine have a greater impact on the hydration capacity of cells which leads to higher yields under greenhouse conditions in comparison to the other nine varieties. Accumulating osmolytes maintain turgidity and expose the leaves to get photosynthetic active radiation and assimilate translocation. Proline is a well-known vital osmoprotectant increased in rice under water deficit conditions and it helps the plant to continue stomatal conductance and maintain leaf turgidity [26].

Proline is one of the important imino acids that act in cellular processes in two ways - a component of protein and an osmoprotectant under drought. Hence, the content of proline would be an important tool for screening plants under drought and might be taken as a biochemical marker for the analysis of rice genotypes [27]. The increased proline content in Purna resulted in hydration in cells, thus increasing relative water content which continued the metabolism under short-term water deficit stress (Fig 3A). The ABA and osmolytes viz. proline, glycine betaine, etc. reduced the oxidative damage and ionic leakage under drought stress in plants [28]. Water deficit stress increased ABA in all 10 varieties but it was higher in Purna which might influenced the stomata closure and activated the defense mechanisms and synthesis of osmolytes in plant cells. It has been reported that an increased concentration of ABA induces the synthesis of primary and secondary metabolites and enhances biomass production [29]. The Glycine betaine acts as a compatible solute and it has a major role in improving the water stress tolerance in plants. It has been well documented that Glycine betaine (GB) improved drought tolerance in various crops viz. wheat [30] sunflower [31]

etc., and increased yield compared to other GB-deficient plants. The increased GB content remarkably enhances the osmotic balance, enzymes, and genes associated with drought resistance [32-34]. Hence, the maintained hydration and osmoticum in the cell led to the survival and yield of the plant under short-term water deficit stress. [35-37].

#### 4. CONCLUSION

The abiotic stress particularly water deficit in plants under rainfed conditions is the major challenge for marginal farmers for production and yield of crops. The selection of drought-tolerant varieties might reduce severe loss caused by water stress. The variety Purna has been found better under short-term water deficit among 10 varieties of rice. Hence, drought-tolerant varieties can be produced through a breeding program with the help of Purna.

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#### COMPETING INTERESTS

Authors have declared that no competing interests exist.

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