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

Abstract

The ten rice varietiesviz. NAUR-1, Purna, GNR-6, NVSR-2227, NVSR-3093, NVSR-2285, NVSR-2278, NVSR-2852, NVSR-2601,and NVSR-2927 were grown in *kharif* seasonfor observing their efficiency in terms of yield under a deficit of water at tillering stage. The effects of short-termwater 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, glycinebetaine, 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 inPurna as compared to other varieties. The abscisic acid as well as proline and glycinebetaine content recorded maximum in Purna which might help in keeping hydration in cellsduring water deficit stress. Thus, with short-termwater deficit stress at tillering stage, the varietyPurnawould be better in terms of breeding for tolerant variety as well as yield among ten rice varieties.

Keywords: Rice,Stress,Tillers,Varieties,Water,

Introduction

Cereals have a major role in feeding billions of peopleand 50% to 80% of daily calorie intakeis 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 reproductivestages.The arenchymatous tissue is a specializedstructurein ricethat makes its growth and developmentduring the submerged condition(Henry et al., 2016). Direct-seeded rice generally grows onupland/low water regimefaces water deficit stress, changes its structure and developdeep andthick rootsresulting in the improvement of hydraulic properties of roots therebyextractingwater from deeper soil profiles (K.Ji et al., 2012). Thesedeveloped anatomy under drought stress support higher yield inupland ricethan lowland rice. Manydevelopmental processes of plants such asthe number of tillers, panicle initiation, and anthesisadversely affected bydrought stress(R. Serraj et al., 2011). Drought severity activates the production of Reactive Oxygen Species (ROS)mainly in chloroplasts andmitochondria which reduce the membrane stability index and promote leakage of ions due to membrane disruption (Pandey and Shukla, 2015). The ROS viz. Singlet oxygen, superoxide anion radicals, hydroxyl radicals, and hydrogen peroxide (H_2O_2) are produced and initiate indirect disruption of metabolism (Kant, 2020). The defense mechanism for ROSscavengingis 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, 2027; Kant, 2023).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 osmolytesretain water molecules in their structure and theybecomeshields of the osmoprotectantsinplants during severe water/salinity stress(Anjum, S. A. et al., 2017).

Prolineis one of the best osmolyteswhich support the vitality of the plant during water deficit. It is a neutral imino acid at pH-7, hasrigid structure, and is highly soluble in water. It stabilizes protein structures and inhibits protein unfolding during water stress(Szabados, L. &Savoure A. 2010). The enzymes of the metabolic pathway are alleviated by proline particularly ribulose 1, 5 bisphosphate carboxylase that initiatesthe Calvin cycle (Kaur and Asthir, 2015). 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. Prolineis considered an effective hydroxyl radical scavenger and promoter of enzymatic antioxidants and its biosynthesis in chloroplasts reached up to80% during stress as comparedto 5% in normal which facilitatesthe 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(Bandurska et al. 2017). Proline accumulation increases cellular osmolarity drives the influx of water or reduces its efflux resulting in an increase inpressure potential for cell expansion (Bhaskara et al., 2015). Another biomolecule that protects plants during water stress is glycine betainewhich acts as a compatible solute and improves the tissue water status. It is synthesizedfrom choline and glycinewhen a plant is exposed to drought and salinity.It improves Ca^{2+}-ATPase and Hill reaction activities in the thylakoid membrane thereby promotingphotosynthesis and translocation of sucrose. It is a quaternaryamine with zwitterionic natureand can protect biological membranes from ROS as well as improveantioxidantdefense system under stress (Nawaz and Wang, 2020).The biosynthetic genes of proline andglycine betainehave beenwidely used in transgenic production to improve abiotic stress tolerance in plants(Schafleitner et al. 2007, Swamy and Kumar, 2013). Therefore, the present study has been focused on creating short-termwater deficit stress at the tillering stage of rice for the determination of betteryield among different varieties.

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 ± 1^{0}C and 80%, respectively. Theseeds of ten ricevarieties (NAUR-1, Purna, GNR-6, NVSR-2227, NVSR-3093, NVSR-2285, NVSR-2278, NVSR-2852, NVSR-2601, NVSR-2927) subspecies *Indica* groupsown 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 withcarbendazim 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_2O_5 - K_2O (100-30-00 kg ha^{-1}).The water deficit stress was created atthe tilleringstagewith field capacity of 60%. The morphophysiological andbiochemical traits were studied after the creation of stress.Plantheight was measured in centimetresfrom 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 countedand 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 leakagewas measured following the method of R. Sivakumar*etal.* (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 electricalconductivity (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/C2×100

Relative Water Content (RWC)was calculated following the method described by R. K. Sairam (1994).The thirdleaf (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 takingout the leaf discs from the water, and wipedout 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 keptthe cover in a hot air oven at 80°C for 48 hours. Recorded the dry weight (Dw) after removing from the Oven.RWC = Fw-Dw/Tw-Dw× 100.

Harvest index (%) =Economic yield/Biological yield×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 wereground 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 absorbanceof the reddish pink up- per phase was recorded at 520 nm against a toluene blank.

The glycine betaine (GB) content was determinedfollowing the method of Bates et al.(1973) with minor modifications. Finely ground drymaterial (0.5 g) was mixed with 20 ml ofdeionized water and maintained with mechanicalagitation for 48 h at 25^{0}C; subsequently,the mixture was centrifuged at10,000 rpm for 10 min using arefrigerated centrifuge. The supernatant was collectedand stored in the freezer until the analysis.Samples were thawed and diluted (1:1 v/v)with 2N of sulphuric acid (H_2SO_4). An aliquotof 0.5 ml was collected and incubated for 1 hin ice; after which, 0.2 mL of cold potassiumiodide-iodine (KI-I2) was added and mixedgently. The samples were stored at 0 to 4^{0}Cfor 16 h; after which, they were centrifuged at10,000 rpm for 15 min at 0^{0}C. The supernatantwas discarded while ensuring not to shake it;during this time, the samples were maintainedin ice to allow separation of the periodidecomplexes from the acidmedium. The periodidecrystals that formed were dissolved in 9ml of 1,2-dichloroethane, and the mixturewas left to sit for 2 h; after which, theabsorbance was read at365 nm. For the standard GB curve, a stockcomprising 1 mg/ml diluted in 1 N $H_2SO_4$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.

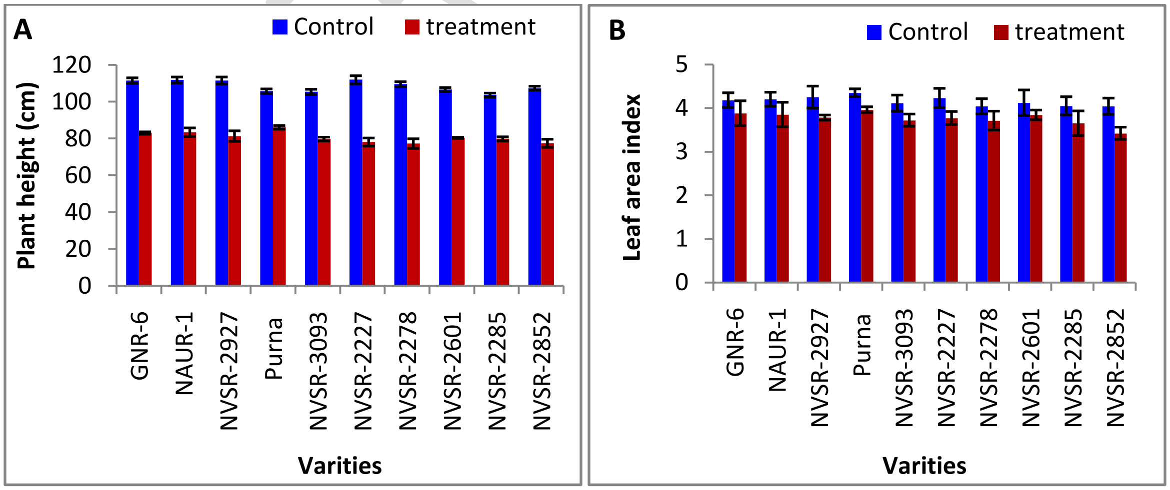
The Ascorbic acid content in the leaves was estimated following the method described by Shivkumar et al. (2015).

Statistical analysis

The data obtained were converted to the mean value for each parameter with threerepetitions under a complete randomized design with factorial concept and statistically analyzedasdescribed by Pans and Sukahtme (1978) factorial concept of theexperiment.

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 rainfedconditions 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.



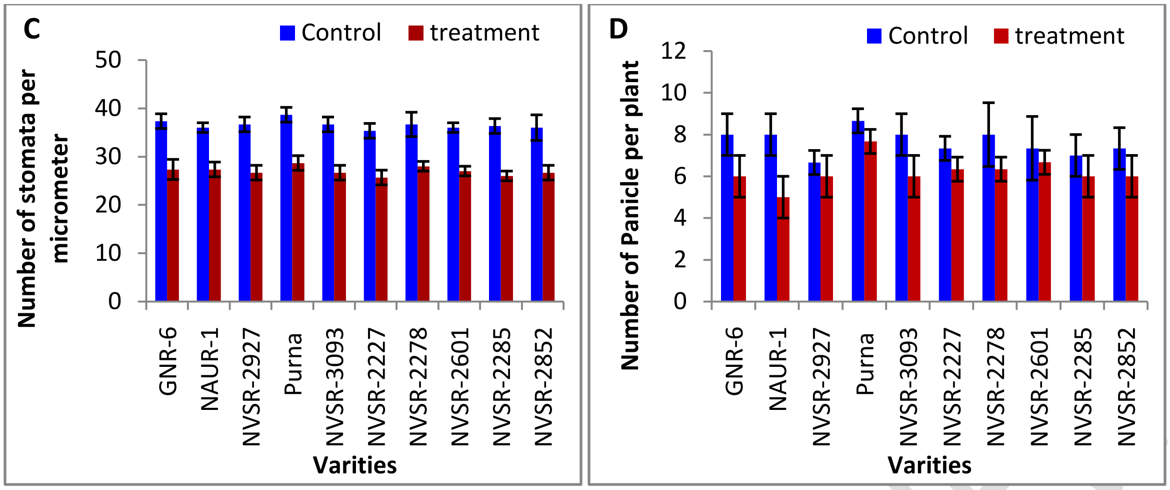
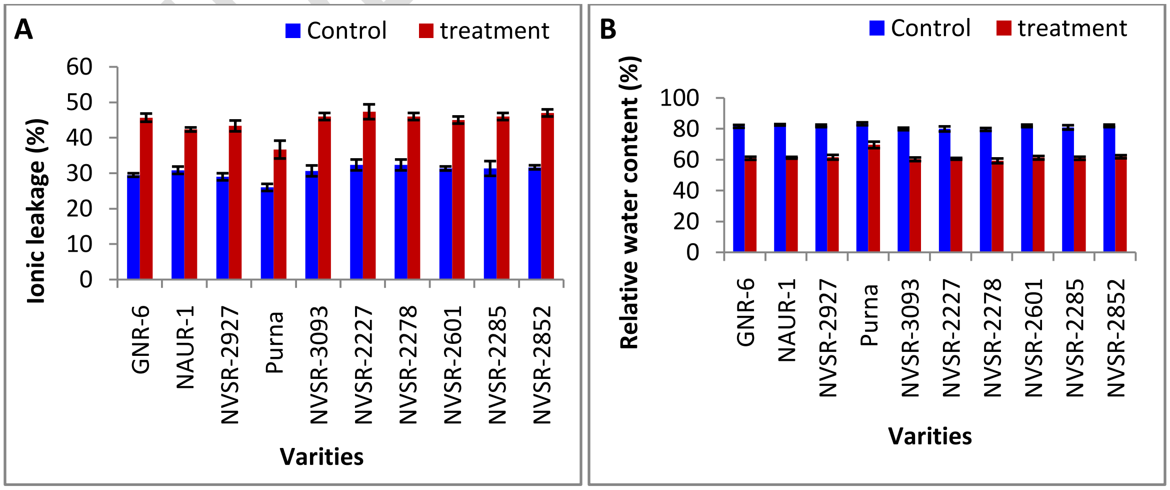


Fig. 1. (A) Plant height as affected by water deficit stress at the tillering stage and control. ± Bars indicate SD (n = 10) **(B)**Leaf area index as affected by water deficit stress at the tillering stage and control. ± Bars indicate SD (n = 10) (**C**) Number of stomata per micrometer of leaf area 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 varietyPurnawas 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. (2010) 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 (T. R. Sinclair, 2011).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 (R. Tuberosa, 2012).



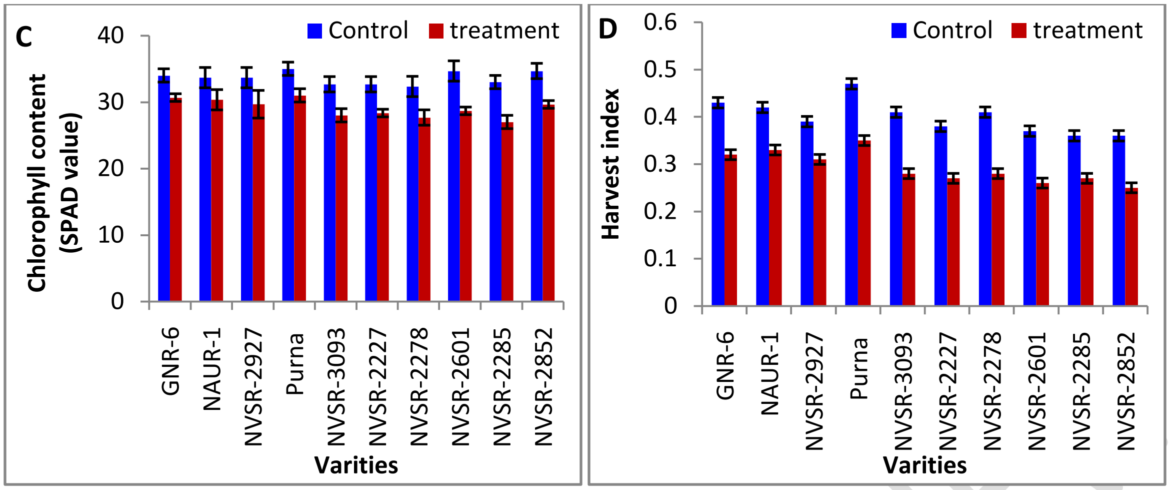
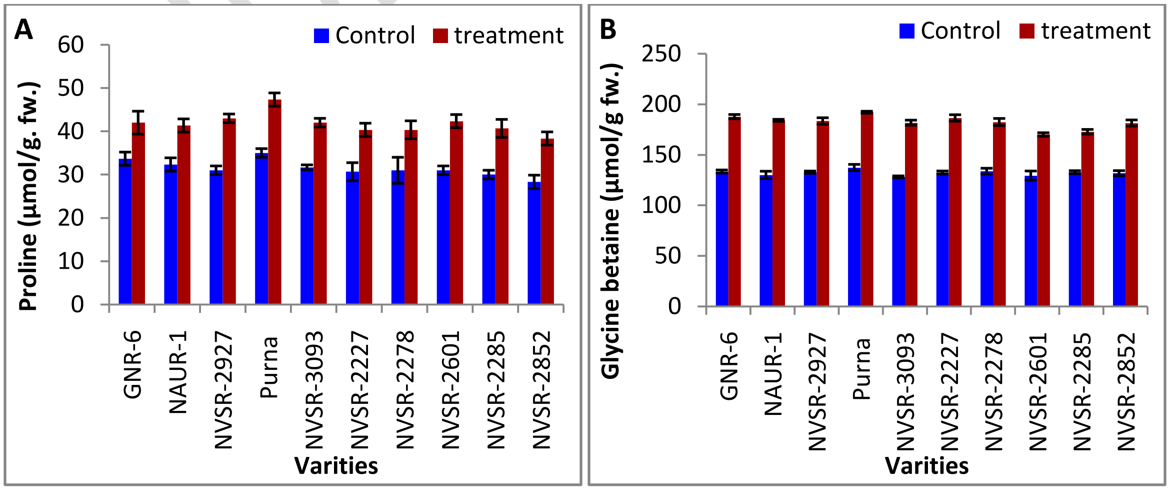


Fig. 2. (A) Ionic leakage as affected by water deficit stress at the tillering stage and control. ± Bars indicate SD (n = 10) **(B)**Relative water content as affected by water deficit stress at the tillering stage and control. ± Bars indicate SD (n = 10) **(C)** Chlorophyll content as affected by water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

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 respiration as well as ATP production in rice (Kadam et al., 2017). 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 (Upadhyaya and Panda, 2019). 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 (Singh et al., 2012). 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(Wei et al., 2017). Water deficit in the short term negatively affected the RWCwhich created an osmotic imbalance, lipidperoxidation, and membrane damage, and in severecases, necrosisspots have been also reported in rice(Zhu et al., 2020; Panda et al., 2021).

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 ofphotosynthesis and transpiration continued with lower stomatal resistance and higher osmoprotectant under water-limitedconditions are some of the most important traits to select genotypes for different environmental conditions(Kamoshita et al., 2008).The acclimatization of genotypes in response to the changing environments is the Genotype-environment interactions (G × E).These two sources of variation might be considered for varietal evaluation for breeding and cultivation across environments (Yan et al., 2003). In the present study, Purna showed better among 10 varietiesin 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 Purnacompared to otherninevarieties while ascorbic acid was higher in all 10 varieties under control. These two osmolytes-Proline and Glycine betainehave a greater impact on the hydration capacity of cellswhich leadsto higher yieldsunder greenhouseconditionsin comparison to the other nine varieties. Accumulating osmolytesmaintain turgidity and exposethe leaves to get photosynthetic active radiation and assimilate translocation. Prolineis a well-knownvital osmoprotectant increased in rice under water deficit conditions and it helps the plant to continue stomatal conductance and maintain leaf turgidity (Kumar et al., 2016).



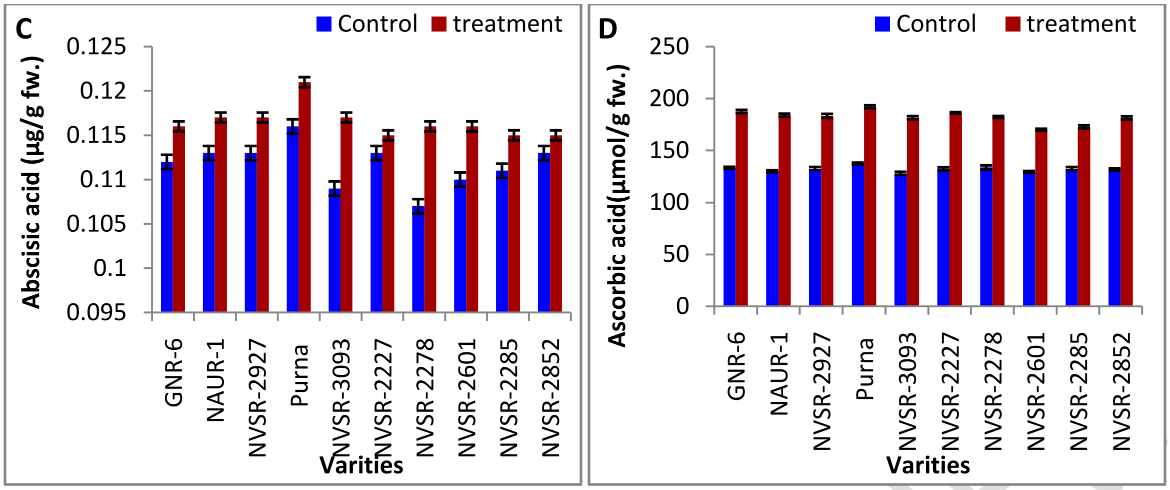


Fig. 3. (A) Proline content as affected by water deficit stress at the tillering stage and control. ± Bars indicate SD (n = 10) **(B)**Glycine betaine content as affected by water deficit stress at the tillering stage and control. ± Bars indicate SD (n = 10) **(C)** Abscisic acid content as affected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

Proline is one of the important iminoacidsthat act in cellular processes in two ways -a component of protein and an osmoprotectant under drought. Hence,the content of proline would be animportant tool for screening plants under drought and might be taken as a biochemical marker for the analysis of rice genotypes (Mishra et al., 2019).The increased proline content in Purna resulted in hydration in cells, thusincreasing relative water content which continued the metabolism under short-term water deficitstress (Fig 3A). The ABA and osmolytes viz. proline, glycine betaine, etc. reduced theoxidative damage and ionic leakage under drought stressin plants (Song, J.-X. et al., 2017). Water deficit stress increased ABA in all 10 varieties but it was higher in Purnawhichmight influenced the stomata closure and activatedthe 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(Fahad, S. et al., 2017).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 (Raza et al., 2014)sunflower (Hussain et al., 2008), etc., and increased yield compared to otherGB-deficient plants. The increased GB content remarkably enhances theosmotic balance, enzymes, and genes associated with drought resistance (Hussain et al., 2013). Hence, the maintained hydration and osmoticum in the cell led to the survival and yield of the plant under short-term water deficit stress.

Conclusion

The abiotic stress particularly water deficit in plants under rainfedconditions 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-tolerantvarieties can be produced through a breeding program with the help of Purna.

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