

IMPROVING MORPHO-PHYSIOLOGICAL ATTRIBUTES AND YIELD-RELATED TRAITS OF RICE SEEDLINGS BY APPLYING SALICYLIC ACID AND PROLINE UNDER DROUGHT

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Abstract. Rice provides a staple diet for over 1/3 of the global population. Drought reduces yield. The application of salicylic acid (SA) and proline can decrease this stress. The current study set out on rice seedlings and mature plants to investigate how SA and proline can reduce the negative impacts of drought. Five treatments were used: (i) control (C), with no water deficiency or utilization of salicylic acid (SA) and proline; (ii) Drought stress, maintained at 40% water holding capacity (WHC); (iii) 40% WHC combined with 1.5 mM salicylic acid; (iv) 40% WHC combined with 2 mM proline solution and (v) 40% WHC combined with 1.5 mM SA and 2 mM proline. Data of morpho-physiological and yield-related traits on rice seedlings and mature plants were recorded under drought stress, treatment of SA and proline enhanced growth and development attributes; however, co-application of micronutrients resulted in optimal performance. It was found that by dropping water loss and raising chlorophyll

concentrations, the combined application of salicylic acid and proline mitigates the effects of drought stress in rice.

Keywords: *treatment, abiotic stress, water holding capacity, growth, development attributes*

Introduction

The world's second-most-cultivated cereal crop, rice (*Oryza sativa* L.) provides the primary energy for almost half of the world's population (Muthayya et al., 2014). Most of the world's production, approximately 75-80%, is grown using flood irrigation systems (Mondal et al., 2024). Asia accounts for more than 90% of the world's rice production, and for one-third of the world's population, rice is the most adaptable food source (Faysal et al., 2022). The agricultural production system and food security are continuously threatened by climate change. One of the major environmental factors that have harmful effect on crop production is drought. For performing different physiological functions like growth, development and metabolism, water is needed. Moreover, plant biomass is also made up of 80-95 water (Broderson et al., 2019). Rice is a vital staple food for more than 50% of the world population and it is severely affected by drought, and there is a need to develop techniques which overcome this serious issue of rice production. There is a need to understand the mechanism of plants that they used under drought stress to overcome this problem. Different functions of physiological, biochemical and molecular pathways included regulation of hormones, defense through antioxidant and osmotic adjustment (Haghpanah et al., 2024). Salicylic acid and proline change these pathways of plant defense against drought and decrease the drought effect. So far, no techniques have been developed.

The world population is growing at a fast pace. Over eight billion people are fed by global agriculture today and by 2050, the population is predicted to rise to nine billion (Molotoks et al., 2021). To cover mankind's increasing food needs, it is imperative that the production of rice be increased significantly. However, due to biotic and abiotic stresses as well as careless usage, the amount of agricultural land available is steadily declining (Islam et al., 2023). Furthermore, the state of climate change as it exists now (Lackner et al., 2022) has resulted in increased temperatures, protracted dry periods and severe droughts that have a detrimental effect on rice output (Fahad et al., 2017). The drought is becoming a serious problem on a global scale. Globally, agriculture uses around 69% of freshwater resources (Claro et al., 2024). The world's leading crop, rice, is grown in regions where massive groundwater loss poses serious challenges to agriculture in the future. Roughly 40% of the 700 million world's population has been affected by drought (Promping and Tingsanchali, 2024).

Compared to other crops, rice is more sensitive to drought. Drought has a major impact on rice growth and development because it affects rice at various levels, such as physiological, metabolic and molecular (Zu et al., 2017). Drought stress causes crops' leaf area, cell size and intercellular volume to decrease (Jayaweera et al., 2016). Drought stress reduces soil moisture, leading to reduced water content in the leaves. This results in guard cells losing their turgor pressure, which in turn causes stomatal pores to shrink or close. Plant production is decreased by drought because it hinders development and photosynthesis (Larkunthod et al., 2018). It is typical to find a positive link between crop yield and photosynthesis. Plants use various mechanisms to survive or regrow in drought stress. Furthermore, plants exhibit a

variety of structural alterations. For example, drought restricts cell division, which results in a short stem, shortened internodal length, limited tillering capacity and a retarded root system. Abiotic stresses frequently result in a decrease in fresh and dry biomass (Sikuku et al., 2012). Relative water levels were formerly one of the most important factors in selecting drought tolerance (Bunnag and Pongthai, 2013). Therefore, drought stress has been a key problem for plant growth, as it impacts the lifespan and development of plants. Rice plants that are stressed by drought exhibit reduced root length and changed root architecture. In large-scale screening, rice's resistance to leaf rolling and mortality are useful characteristics for determining its potential for drought tolerance (Ghourri et al., 2022).

The mechanisms for minimizing water loss include reducing leaf area and stomatal closure, maximizing water uptake capacity through deep, dense root systems, altering the elasticity of the cell wall and maintaining osmotic adjustment (OA) (Saha et al., 2016). It is thought that one process involved in OA that might help plants become more tolerant of drought stress is the buildup of organic and inorganic compounds by plants under drought (Zivcak et al., 2016). Drought leads to the formation of reactive oxygen species (ROS) overproduction. Plant cells' oxidative and metabolic balance are disrupted by reactive oxygen species, which encourages membrane lipid peroxidation (Hussain et al., 2018). Numerous strategies have been devised to address the issue of drought. Obviously the most effective strategy to mitigate drought impact on agricultural production has been the switch to more resistant genotypes. However, plants cannot fully recover from stress damage due to a lack of genetic self-defense mechanisms. For this reason, scientists studying plants are looking for ways to improve their capacity to endure harsh environmental circumstances. It has recently been shown that osmoprotectants and plant growth regulators are essential to the integration of the responses that plants display when they are under stress.

A common phenolic phytohormone, salicylic acid (SA) is crucial to plants' defensive systems. It is essential to many of the physiological functions of the plant (Maruri-López et al., 2019). SA reduces the rice seed germination inhibition brought on by drought (Sohag et al., 2020). The addition of SA to crops under drought conditions results in a notable increase in the conductance of stomata, which in turn raises the rate of net CO₂ absorption and plant growth (Khan et al., 2015). Exogenous SA treatment causes a reduction of membrane lipid peroxidation under drought-stress (Urmi et al., 2023) in wheat while increasing plant height and dry mass. The use of SA greatly decreases ionic leakage and the buildup of harmful ions (Movahhedi-Dehnavi et al., 2019). Exogenous salicylic acid administration has been found to reduce oxidative stress in plants under drought stress by modifying the glyoxalase system (Khan et al., 2015). Increased proline accumulation has been noted in several plant species as a sign of stress resistance (Semida et al., 2019). Proline can reverse oxidative stress in addition to osmoprotection by activating the glyoxalase system and antioxidant defense (Abdelaal et al., 2020). The cellular redox buffering ability and subcellular structure are stabilized by proline (Shamsul et al., 2012). Many features of rice's external proline and SA-mediated resistance to drought are yet unknown. Based on this, the current work was carried out to examine the probable functions and processes of proline or SA facilitated drought stress tolerance in rice. Moreover, we investigated the effects of SA and proline, both

alone and in combination, on rice seedlings and yield related traits to reduce the negative effects of drought stress by enhancing morpho-physiological traits of rice.

Material and methods

The work was based on a previous study by Sattar et al. (2019). The Rice Research Institute Kala Shah Kaku, Pakistan, provided the approved rice variety Basmati-515, which was employed in this investigation. 500 nursery bags were filled with loamy soil. Each nursery bag contained five seeds of each genotype, which were then thinned to one seedling per bag following germination. For maturity traits, rice seedlings that were 28 days old were transplanted into big size pots. After 28 days of transplantation, the drought treatments were initiated. There were five treatments and two replications in the Completely Randomized Design (CRD) experiment. Each bag contained enough fertilizer. Drought treatment was started after 21 days and drought-treated plants were manually sprayed with salicylic acid and proline. Distilled water (DW) was sprayed on the control plants, compare (Urmi et al., 2023). At 36 days of the experiment and 15 days of drought initiation, morpho-physiological properties of seedlings were measured. While other yield related parameters were measured at maturity.

The treatments were as follows: (i) control (C), with no water deficiency or utilization of salicylic acid (SA) and proline; (ii) drought stress, maintained at 40% water holding capacity (WHC); (iii) 40% WHC combined with 1.5 millimoles (mM) salicylic acid; (iv) 40% WHC combined with 2 mM proline solution; and (v) 40% WHC combined with 1.5 mM SA and 2 mM proline (Urmi et al., 2023). From the transplanting day until data collection, 100% field capacity was maintained in the normal treatment. At seedling stage, 40% of the field capacity was retained under the stress of drought from the 21st to the 35th day, following an initial period of 100% field capacity. Foliar applications of SA and proline were performed between 18:00 and 19:00, using 10 ml per pot at intervals of 4 and 3 days, respectively, starting from the onset of drought stress and continuing for two weeks. While for measuring yield related characters, SA and/or proline were sprayed similarly as for seedlings traits following transplantation at days 15, 30, 45 and 60 on drought-treated plants, while the control plants were treated with deionized water, compare Urmi et al. (2023).

Data collection

Data of different morphological, physiological and yield-related parameters including root and shoot length, root fresh and dry weight, shoot fresh and dry weight, chlorophyll contents, stomatal conductance, relative water contents, flag leaf area, plant height, panicle length, number of tillers, panicle weight, number of grains per panicle and spikelet fertility were taken. For seedling parameters, the rice plants were uprooted to collect data on the length of the roots and shoots. After separating the main root from the shoot, the length of the shoot and the root were measured in centimeters using a scale. Fresh and dry weights of the roots and shoots were measured in grams. Samples were oven-dried at 75°C until a consistent weight was accomplished for the shoot and root dry weight. Next, dry weights were calculated in grams using an electric balance.

Three completely developed leaves were picked early in the morning on the seventh day following the drought induction and the fresh weight was noted. To remove dirt, these leaves samples were submerged in water for a whole night. Turgid weight (TW) was measured on the subsequent day, then the samples were dried at 70°C for 72 h. After recording the dry weight, the RWC (relative water content) was determined as described in *Equation 1* (Ghourri et al., 2022):

$$\text{RWC}\% = (\text{FW} - \text{DW}) / (\text{TW} - \text{DW}) \times 100 \quad (\text{Eq.1})$$

where relative water contents% = RWC%, fresh weight = FW, dry weight = DW and turgid weight = TW.

On the seventh day following the onset of the drought, the amount of chlorophyll was determined using the instrument SPAD-502 m (Spectrum Technologies, Inc., Plainfield, IL, U.S.A.). Yield-related data were collected at maturity. Flag leaf area was measured by Amin and Haque's (2009) formula. Plant height was measured with a meter rod in cm, and similarly panicle length was also measured. After harvesting the panicle of all treatments, panicle weight was calculated on an electric balance. At maturity, tillers of all treatments were counted manually. The number of grains of harvested panicles was counted manually. Spikelet fertility was computed by the formula presented in *Equation 2* (Ghosh et al., 2013):

$$\text{Spikelet fertility (\%)} = \text{number of filled spikelet} / \text{total number of spikelets} \times 100 (\text{Eq.2})$$

Statistical analysis

One way ANOVA was carried out to determine the differences among all the treatment groups. Fisher's analytical techniques were used to evaluate the data, and after significant ANOVA, Tukey's HSD (honestly significant difference) test was chosen to compare the means using Minitab 17 software. All statistical tests were carried out with a significance level of $\alpha = 0.05$. The data was displayed as mean, standard error, The observed data were plotted as a bar graph using Microsoft Excel.

Results

Statistical differences between treatment groups were evaluated using one-way ANOVA, and multiple comparisons were made using Tukey's HSD test. Exact p-values were provided where suitable and a significance level of $\alpha = 0.05$ was used. For better clarity of results, confidence intervals (95% CI) were provided for the significant parameters and results were presented as mean \pm standard error (SE). Bars sharing the same letter (a, b, c, etc.) were used to represent statistically significant differences ($p > 0.05$).

In the present investigation, a little increase in root length was seen in rice seedlings with a drought in respect to normal plants (*Fig. 1*). Under drought conditions, the root length was enhanced by 7.53% as compared to normal. It was also found that the application of SA and proline alone and combined also increased the root length of rice seedling but not too much.

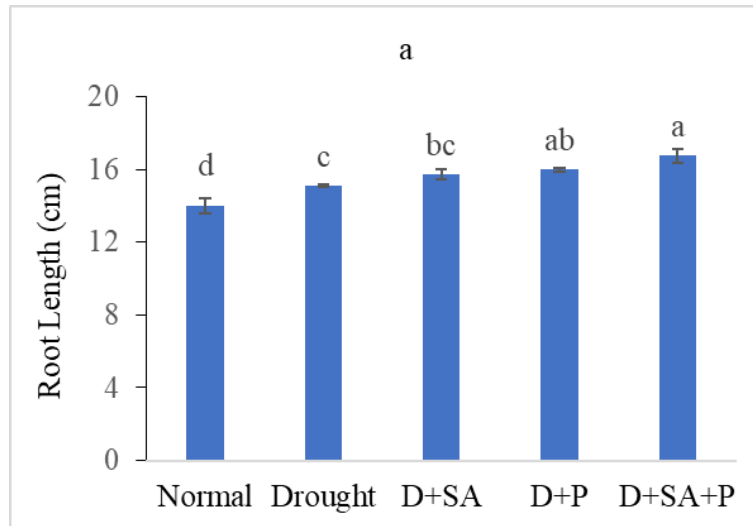


Figure 1. Evaluated the impact of drought stress, combined with salicylic acid, drought combined with proline, and drought combined with both salicylic acid and proline on *Oryza sativa* seedlings by measuring root length (RL)

When there is drought stress, shoot length decreased 24.70% but the alone application of salicylic acid increased 9.25% and proline enhanced 10.45% the SL (shoot length) as compared to conditions with drought, however, the simultaneous use of these two nutrients significantly increases 15.72% shoot length (Fig. 2).

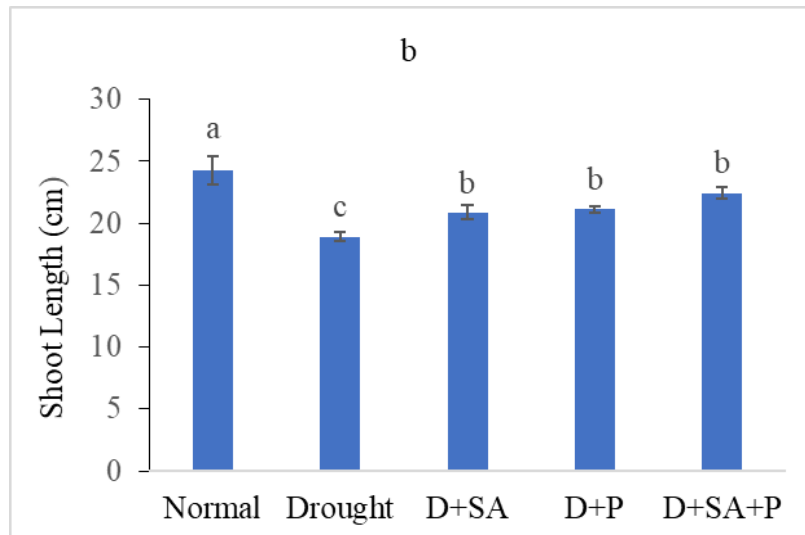


Figure 2. Evaluated the impact of drought, drought combined with salicylic acid, drought combined with proline, and drought combined with both salicylic acid and proline on *Oryza sativa* seedlings by measuring shoot length (RL)

As indicated by the results in Figures 3 and 4, the drought stress considerably reduced the seedlings' fresh and dry weight of roots when compared to the group of control plants. Root fresh weight and root dry weight were markedly decreased by drought stress. In comparison to the control plants, drought stress reduced RFW and

RDW by 40.43% and 60.71%, respectively, as paralleled to normal. In comparison to plants under drought stress, the exogenous administration of SA increased root fresh and dry weight by 18.84% and 42.86%, respectively, while the exogenous application of proline increased root fresh and dry weight by 16.83% and 49.25%, respectively. On the other hand, under drought combined dosage of SA + proline, increased RFW and dry weight by 26.96% and 58.28%, respectively.

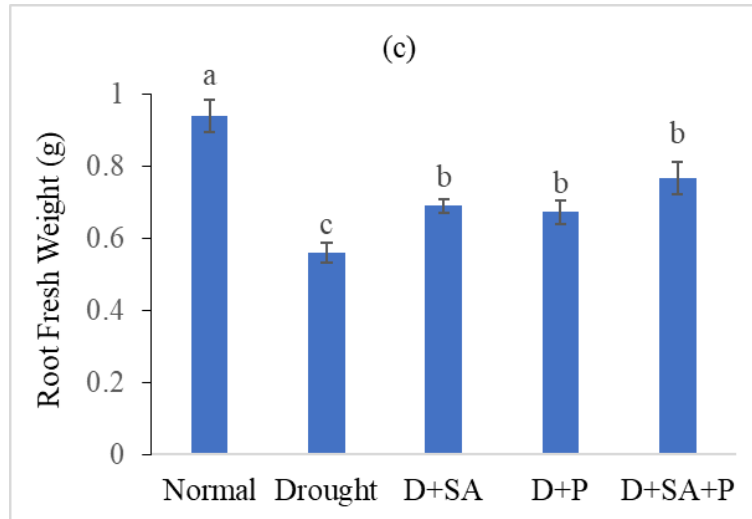


Figure 3. Evaluated the impact of drought, drought combined with salicylic acid, drought combined with proline, and drought combined with both salicylic acid and proline on *Oryza sativa* seedlings by measuring root fresh weight (RFW)

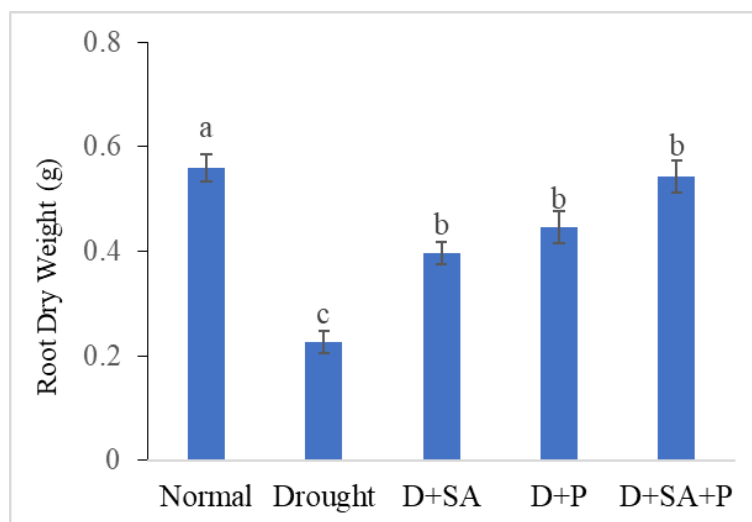


Figure 4. Evaluated the impact of drought, drought combined with salicylic acid, drought combined with proline, and drought combined with both salicylic acid and proline on *Oryza sativa* seedlings by measuring root dry weight (RDW)

Drought stress was found to cause a considerable reduction in the SFW and SDW (Figs. 5 and 6). Drought caused 32.89% and 40.42%, respectively, of reduction in SFW and SDW when compared to non-stress conditions. Application of SA alone yielded an

11.46% increase in shoot fresh weight while proline caused 13.85% improvement in SFW. However, combined foliar application of salicylic acid and proline caused 24.56% increase in shoot fresh weight. Similarly, SA gave 18.29% improvement in shoot dry weight, and proline caused 27.04% improvement in shoot dry weight. The joint application of SA and proline under drought stress increased 38.36% SDW.

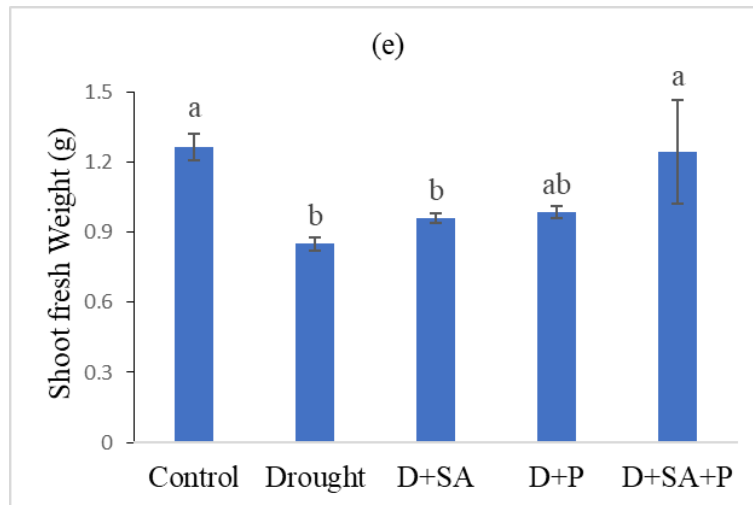


Figure 5. Evaluated the impact of drought, drought combined with salicylic acid, drought combined with proline, and drought combined with both salicylic acid and proline on *Oryza sativa* seedlings by measuring shoot fresh weight (RDW)

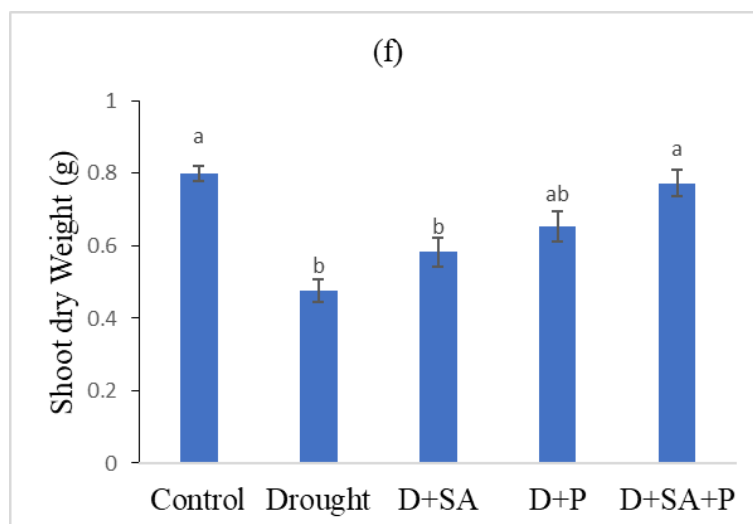


Figure 6. Evaluated the impact of drought, drought combined with salicylic acid, drought combined with proline, and drought combined with both salicylic acid and proline on *Oryza sativa* seedlings by measuring shoot dry weight (RDW)

Our findings showed that drought gave a 34.84% decrease in chlorophyll concentrations vs. the control (Fig. 7). Foliar utilization of salicylic acid and proline alone and combined caused 12.52%, 16.67% and 23.08% improvements in chlorophyll contents of drought plants.

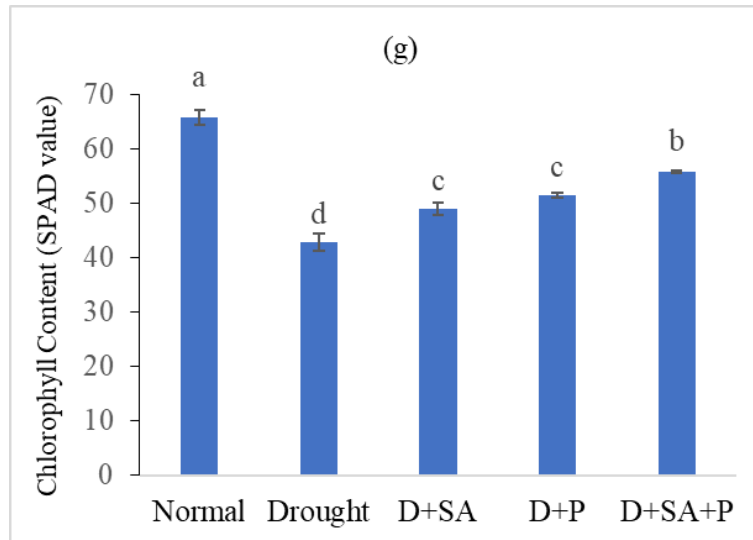


Figure 7. Evaluated the impact of drought, drought combined with salicylic acid, drought combined with proline, and drought combined with both salicylic acid and proline on *Oryza sativa* seedlings by measuring chlorophyll content (CC)

It was found that drought stress reduced stomatal conductance by 39.58% vs. the control. By contrast, the application of SA improved 23.01% of the SC while proline enhanced 32.32% in the same trait; however, the joint application of SA and proline under drought condition increased 36.86% of stomatal conductance (Fig. 8).

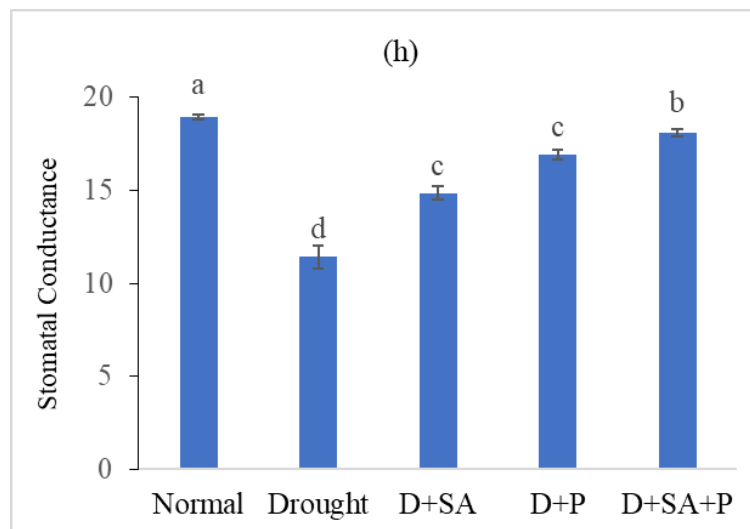


Figure 8. Evaluated the impact of drought, drought combined with salicylic acid, drought combined with proline, and drought combined with both salicylic acid and proline on *Oryza sativa* seedlings by measuring stomatal conductance (SC)

In comparison to the control treatment, rice seedlings underwent a 31.47% reduction in RWC due to drought stress. RWC increases by 13.98%, 20.28%, and 25.55% when rice plant seedlings are treated with SA, proline and SA + proline (Fig. 9).

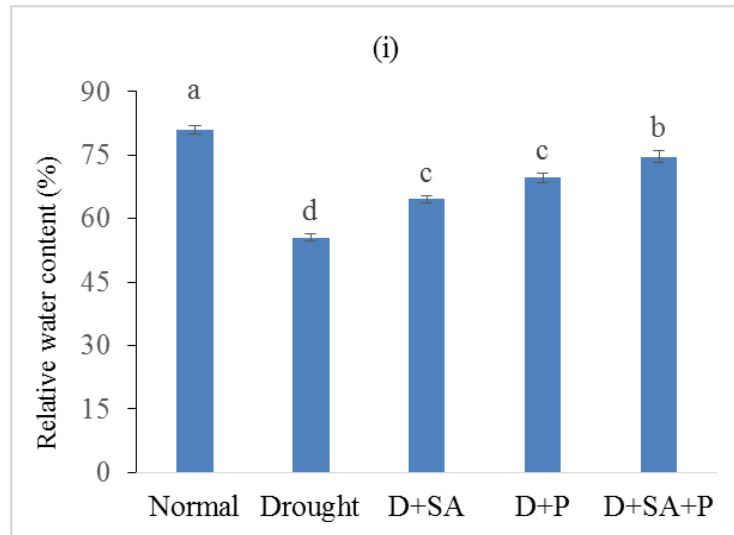


Figure 9. Evaluated the impact of drought, drought combined with salicylic acid, drought combined with proline, and drought combined with both salicylic acid and proline on *Oryza sativa* seedlings by measuring relative water contents (RWC)

From *Figure 10* it is clear that drought stress reduced the flag leaf area (FLA) of the rice plant. The drought condition caused a 15.31% reduction in FLA as compared to normal. It was found that foliar application of salicylic acid drought increased FLA by 5.91%, while proline enhanced it by 9.35%, while the combined application of SA and proline increased FLA by 12.38%.

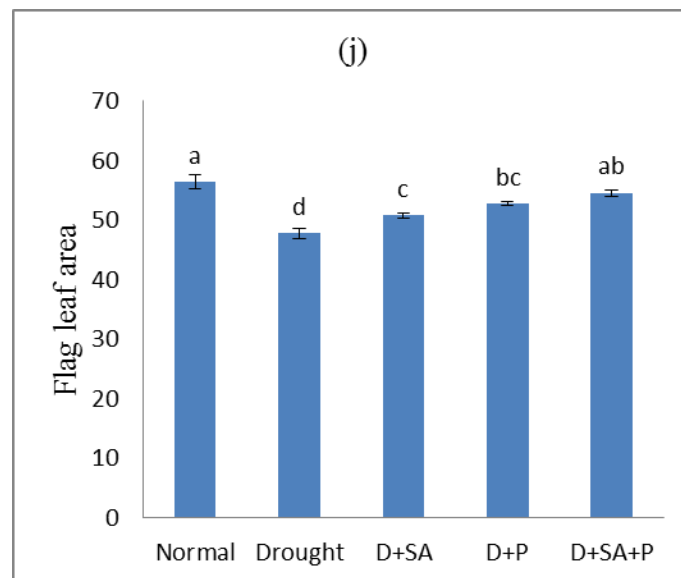


Figure 10. Evaluated the impact of drought, drought combined with salicylic acid, drought combined with proline, and drought combined with both salicylic acid and proline on *Oryza sativa* seedlings by measuring flag leaf area (FLA)

Drought stress led to a marked reduction in the pH value (Fig. 11) of 15.78% vs. non-stress conditions. The combined administration of proline and SA to rice under drought stress led to a 9.94% increase in pH as compared to drought-stressed rice alone.

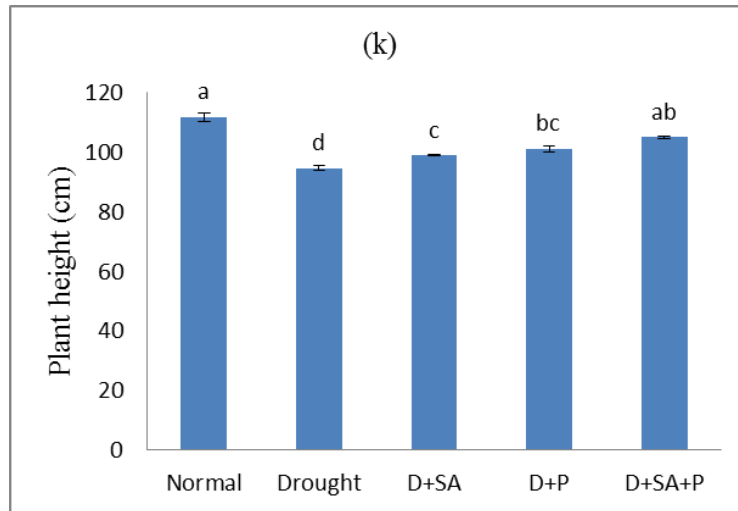


Figure 11. Evaluated the impact of drought, drought combined with salicylic acid, drought combined with proline, and drought combined with both salicylic acid and proline on *Oryza sativa* seedlings by measuring plant height (PH)

It was found that drought stress reduced 34.62% panicle length (Fig. 12). However, the administration of SA improved the panicle length by 11.44% while proline enhanced it by 15.99%; however, the joint application of SA and proline under water-limited conditions increased the trait by 22.12%.

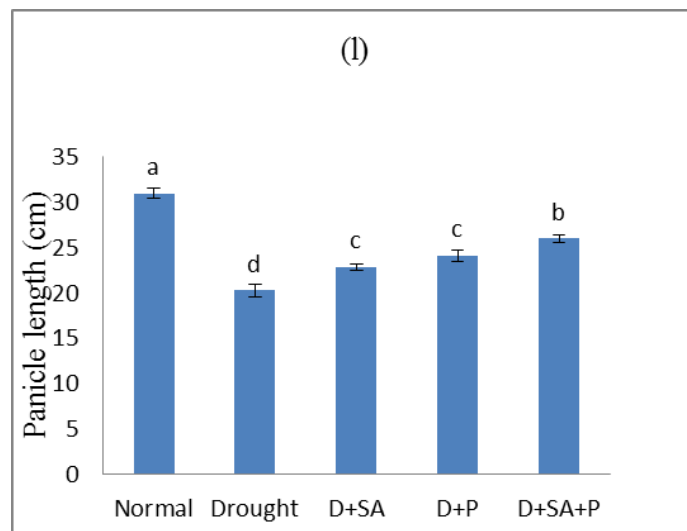


Figure 12. Evaluated the impact of drought, drought combined with salicylic acid, drought combined with proline, and drought combined with both salicylic acid and proline on *Oryza sativa* seedlings by measuring panicle length (PL)

As indicated by *Figure 13*, drought stress reduced NT by 47.83%. When SA and proline were applied separately to drought-inflicted rice plants, the NT was increased by 21.86% and 27.99%, respectively, and the joint application of SA and proline enhanced NT by 39.05%.

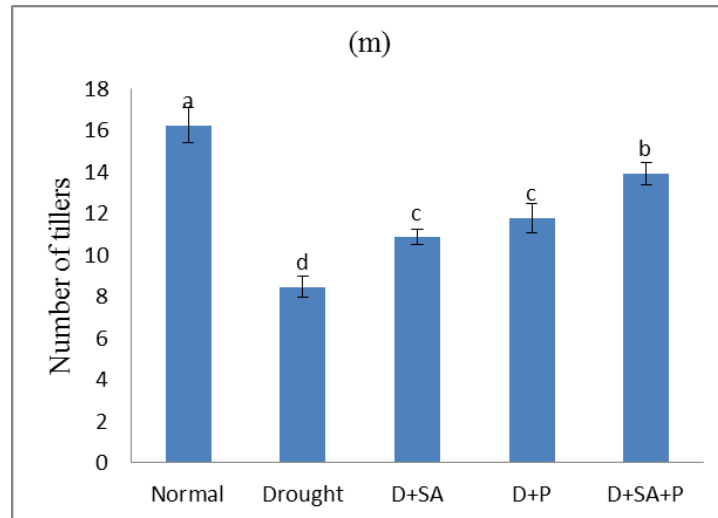


Figure 13. Evaluated the impact of drought, drought combined with salicylic acid, drought combined with proline, and drought combined with both salicylic acid and proline on *Oryza sativa* seedlings by measuring number of tillers (NT)

Panicle weight of the rice plant was also affected by drought conditions as illustrated by *Figure 14*. PW was reduced by 51.91% by drought stress. Foliar application of SA and proline under drought stress increased PW by 23.89% and 33.47%, respectively, while the combined application enhanced it by 46.67%.

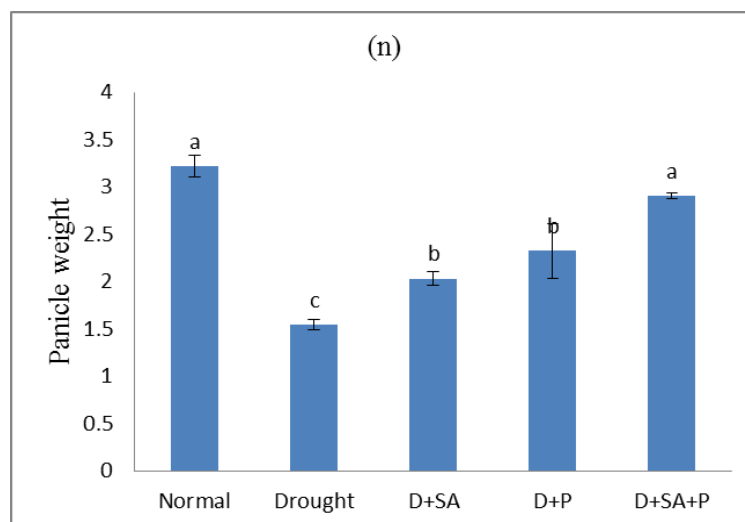


Figure 14. Evaluated the impact of drought, drought combined with salicylic acid, drought combined with proline, and drought combined with both salicylic acid and proline on *Oryza sativa* seedlings by measuring panicle weight (PW)

The number of grains per panicle (GN) and spikelet fertility (SF) is reduced by drought stress as presented in *Figures 15* and *16*. GN reduced 34.52% and SF 41.41% by drought stress. The application of SA enhanced GN by 14.28% and SF by 17.75% while proline administration increased GN and SF by 19.11% and 18.00%, respectively. Combined foliar application enhanced GN by 25.69% and SF by 28.94% under drought stress condition.

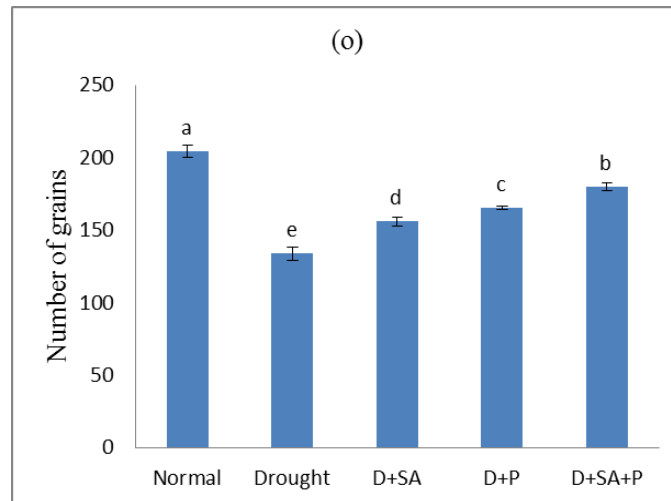


Figure 15. Evaluated the impact of drought, drought combined with salicylic acid, drought combined with proline, and drought combined with both salicylic acid and proline on *Oryza sativa* seedlings by measuring number of grains (NG)

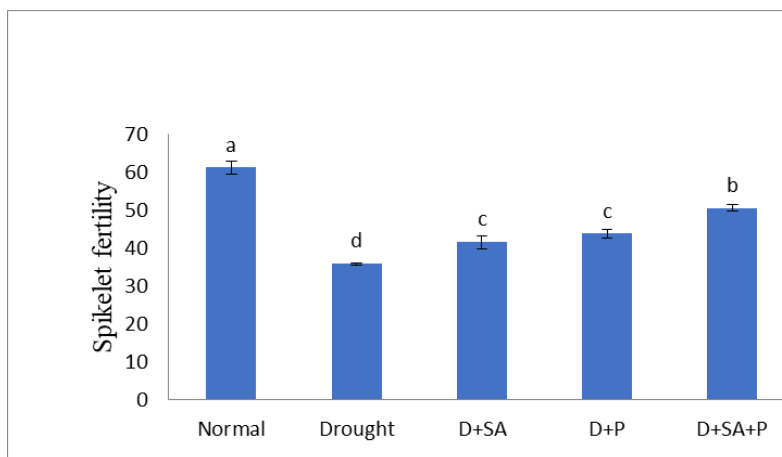


Figure 16. Evaluated the impact of drought, drought combined with salicylic acid, drought combined with proline, and drought combined with both salicylic acid and proline on *Oryza sativa* seedlings by measuring spikelet fertility (SF)

Discussion

In response to drought stress, rice plants' photosynthesis, stomatal conductance, starch metabolism and biochemical enzymatic activities are associated with a decline in growth and other metrics, for example seedling biomass and productivity (Quampah et al., 2011). Lack of water, particularly in rice, affects the morphological, physiological

and biochemical processes since its growth depends on an abundance of water. In this study, we focused on both seedlings and yield relevant parameters in drought-inflicted rice, which will be very useful in future because rice is a global staple food and drought is a serious issue due to climate change. In this study we focused on different yield, physiological and seedlings parameters, it is unclear how SA and proline interact molecularly and physiologically to help rice recover from drought-induced damage. Proline and SA have protective properties against a variety of stressors. It is yet unknown, though, how SA and proline work together to affect rice under drought stress. Thus, we looked at the protective functions of proline and/or SA in giving rice drought tolerance. Through the knowledge of different traits behavior under drought stress and interaction of SA and proline we can overcome the gap. Clearly state the research gap and emphasize how our work advances our understanding of plant stress physiology. Reduced osmotic activity during a period of drought is linked to a decrease in water availability in the cell's cytoplasm (Bukan et al., 2024). In addition to lowering plant weight, conditions of drought also reduce the effectiveness of photosystem II because they degrade photochemical proteins (Chen et al., 2016). This results in a drop in net photosynthesis. Additionally, during droughts, antioxidants are also generated, which in turn causes oxidative stress (Filek et al., 2015). This, in consequence, lowers root-shoot characteristics and reduces production.

In our investigation, we found that under drought conditions shoot length and root length as well as their fresh and dry weight was impacted. In present study RL increased with drought which was according to Kim et al. (2020) as they stated that under drought stress rice roots grow deeper. One consequence of drought is a decrease in biomass output (Farooq et al., 2009). As we found that shoot length and fresh and dry weight decrease with drought stress and foliar application of SA and proline enhance these traits under stress similarly Shan et al. (2024) also revealed that application of SA improved these traits. Lower productivity is ultimately the result of decreased intake and limited space for photosynthesis. We found that the development and biomass of rice seedlings decreased, but the detrimental consequences of the drought condition were mitigated by foliar application of SA and proline. Similarly, Urmi et al. (2023) found that the adverse effect of drought was mitigated by salicylic acid and proline. Drought stress interferes with leaf area, photosynthetic rates, developmental processes, and metabolic activities (Tani et al., 2019). Crop growth and biomass are decreased by drought stress because it prevents plant nutrient intake, cell division and elongation (Islam et al., 2018). Drought seems to reduce the amount of water absorbed and used, which probably hinders the intake of nutrients and as a result, lessens the difference in water potential between the crops' interior and exterior environments, which prevents crop development (Sokoto, 2014).

SA may increase the tolerance of plants under drought stress conditions and is a rescue growth regulator affecting many physiological and biochemical processes (Khalvandi et al., 2021). It has been mentioned by Wang et al. (2019) that SA can be a key player in overcoming drought stress by augmenting elements uptake, accelerating photosynthetic reactions, increasing enzymatic and nonenzymatic antioxidant actions, decreasing oxidative stress, sequestering reactive oxygen species (ROS), retaining water within cells, improving plasma membrane stability, as well as preserving the composition of hierarchy. Overall SA may be used as a protectant to modulate the reaction of plants under drought, which positively affects plant growth and yield properties of plants in drought-stressed habitats (Kimbembe et al., 2020). It has been

discovered that exogenous administration of proline or SA alleviated crop growth inhibition during drought stress (Semida et al., 2020; Tayyab et al., 2020). Exogenous administration of proline and/or SA may improve the water potential gradient and mineral element absorption, hence mitigating the negative effects of drought stress on crop development (Tayyab et al., 2020). Plant root development, cell expansion and cell elongation are all positively correlated with SA, or proline (Kibria et al., 2016; Sohag et al., 2020). According to Semida et al. (2020), using exogenous proline greatly increased the biomass production and shoot growth of onions. This may have been caused by proline's translocation to the roots and the triggering of defense responses in onions. Additionally, reports stated that exogenous SA treatment gives wheat drought resilience by raising the cell division of root cells inside the apical meristem (Loutfy et al., 2012). SA can boost plant stress tolerance and lower oxidative stress. It can also increase enzymatic (CAT, POX, and PPO) and nonenzymatic antioxidant activity (Mutlu et al., 2016). Proline treatment improved the root system's ability to get water at low water potentials and adjusted the osmotic pressure during drought stress (Meloni et al., 2001). Furthermore, proline accumulation rose noticeably in plants under drought stress and in cultivars that were tolerant rather than susceptible (Harley and Ferguson, 1990). Proline's beneficial effects may stem from its functions as an osmolyte and scavenger, which enhance growth characteristics and crop output during droughts. According to certain studies, osmoregulators improve certain plant characteristics related to growth and yield in situations including biotic stress, salt and drought (El-Nashaar et al., 2020). According to Caballero et al. (2005), proline can be utilized as a metabolic marker in connection to stress.

We observed that foliar application of salicylic acid improved the root length and shoot length under drought conditions. Previously, Bagautdinova et al. (2022), also found that SA is crucial for the promotion of root growth and other aspects of plant development. Lum et al. (2014) found that proline had a positive relation with root and shoot length under drought stress, which showed that proline could improve the root and shoot length in water limitation conditions, which is similar to our findings. We identified that foliar presentation of salicylic acid and proline enhance plant growth parameters, which is in consistence with the outcomes of Kunpratun et al. (2024) for SA. Abdelaal et al. (2020) found that the application of salicylic acid and proline improved the fresh and dry weight of the plant in barley as we also identified the application of SA and proline improved the shoot and RFW and RDW under drought.

Rice seedlings exposed to dry conditions contain lower chlorophyll levels. Numerous researchers (Chen et al., 2016; Chen et al., 2016; Embiale et al., 2016; Arjenaki et al., 2012) discovered that a drought was the cause of the decrease in chlorophyll concentrations in various crops. Osmotic stress deactivates chlorophyll. In the present study, we identified that foliar application of salicylic acid and proline increased the chlorophyll contents, similarly Umi et al. (2023) also exhibited that SA and proline mitigate the drought effect on chlorophyll contents in rice. Similarly, Kunpratun et al. (2024) also found salicylic acid utilization in drought stress cause improvement in chlorophyll contents. Abdelaal et al. (2020) also identified the application of SA and proline increased the chlorophyll contents in barley. Shan et al. (2023) also found that SA enhances the chlorophyll content in rice.

We found that the application of salicylic acid improved the stomatal conductance as Khan et al. (2015) found that when SA was added to drought-stressed crops, stomatal conductance significantly increased, which in turn raised the rate of net CO₂ absorption

and plant development. Proline and SA both contribute to the maintenance of leaf turgor, chlorophyll and carotenoid content and stomatal conductance, which is linked to drought resistance (Panda et al., 2021) and Umi et al. (2020) also applied salicylic acid and proline under drought stress and also found that their application improved the stomatal conductance, which is in accordance with our findings. The variances in a plant's turgor potential, water potential and osmotic adjustment (OA) are all characterized by the relative water contents, which are calculated as the highest combined assessment of plant water status (Bhushan et al., 2007). Due to increased cell osmotic potential, drought reduced plant RWC by 60 to 80% (Singh et al, 2015). In our study, we found that relative water contents decrease under drought conditions, and we could establish that SA and proline increase the relative water contents of plants. Abdelaal et al. (2020) also revealed the same outcomes regarding the RWC in barley. Moreover Umi et al. (2023) also discovered that proline and SA treatments help to lessen the delirious effects of lack of moisture on RWC. These findings imply that SA and/or proline assist the plant in adjusting water relations and membrane functions during periods of drought by inducing activation of the plant defense system (Urmi et al., 2023). It has been documented that proline and SA produce distinct metabolic reactions that impact several plant activities, such as water relations and membrane integrity (Merwad et al., 2018; Semida et al., 2020; Zia et al., 2021). Proline and SA are also said to support osmotic adjustment, preserve membrane integrity and maintain the structure of proteins and enzymes (Abdelaal et al., 2020). In the present study, we found that the foliar application of salicylic acid improved the plant height, which is in agreement with Movahhedi-Dehnavi et al. (2019) who stated that exogenous SA treatment reduced membrane lipid peroxidation in drought-stressed wheat while increasing plant height and dry mass (Urmi et al., 2023). Drought stress causes crops' leaf area, cell size and intercellular volume to decrease (Jayaweera et al., 2016). Drought stress appears to reduce water absorption and utilization, which likely impairs nutrient uptake and in turn, crop growth, as evidenced by lower tiller formation and poor yield (Urmi et al., 2023). This is because the water potential gradient between the external and internal environment of crops decreases (Sokoto, 2014). Dien et al. (2019) and Shukla et al. (2012) supported the idea that drought stress decreased rice seed yield by decreasing the number of tillers per hill, panicle length, and filled grains per panicle. The parameters of leaf gas exchange are disturbed by drought stress, which also results in the early senescence of plant parts, decreased assimilated translocation to growth regions, impaired phloem loading, increased ovule abortion, which leads to spikelet sterility, decreased full grain and ultimately decreased output (Islam et al., 2021).

In the present experiment, the number of days of flowering is improved by application of salicylic acid; Similarly, Liu et al. (2022) found that SA is crucial to plant development; for example, it induces flowering in plants. SA's function in enhancing grain production and flower formation led to a notable rise in yield characteristics. Application of SA increased the number of pods and 100-seed weights of faba beans under drought stress, which is consistent with our observations (Abdelaal, 2015). The present study revealed that the application of proline improved the yield-related parameters; Similarly, Hanif et al. (2022) also found that application of proline improved yield-related traits such as plant height, tillers, panicle length, grains per kernel.

This study provided useful information regarding the morpho-physiological parameters of rice under drought stress and the counteracting performance of salicylic acid and proline. As this experiment was performed in pot and controlled condition,

which is not presented complete condition of open environment. The second limitation of this experiment is that the open field application of foliar application of SA and proline is affected by hard-to-control environmental factors like wind, rain and the absorption rate of these nutrients. Therefore, in order to utilize the SA and proline in field condition, researchers have to make efforts under field conditions to check the effect of these in open field condition. As drought stress is the serious problem in this climatic change condition, this study can be useful and could be utilized in future. As this study illustrated that the foliar administration of salicylic acid and proline improved the drought tolerance of rice, other crops might be tested too, and strategies for field implementation can be developed.

Conclusion

According to this study, extended drought stress dramatically reduced the amount of soluble sugar, starch and nutrients that rice plants could absorb. Their growth, stomatal conductance, chlorophyll contents and relative water content were found to be reduced. Separately, the exogenous administration of proline and SA lessened the negative impacts of drought. Nevertheless, it was discovered that their combination application SA + proline was more successful than any of them alone. Our results of foliar administration of SA and Proline revealed that these nutrients could be useful in future for rice improvement. Our finding showed that these nutrients can overcome the negative of drought on morpho-physiological traits. Our findings imply that the administration of proline and/or SA shields rice from oxidative and osmotic stress of drought by adjusting the grain's physiological characteristics, biochemical parameters and activity of relevant antioxidant enzymes. Thus, this study could be useful in future for rice improvement under drought stress by improving yield. Before recommending SA and proline for commercial use, economic factors need to be taken into account, yet that approach looks promising from an ecological and economic point of view, contributing to food security under drought. In future this approach could be useful through the improvement of morpho-physiological traits.

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