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# Inducing Drought Tolerance in Rice Plants by Presoaking with Salicylic Acid

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**Abstract:** Rice needs more water, and therefore any water stress results in a reduction in overall yield and productivity. The purpose of this study was to examine the effects of salicylic acid (SA) 0.5 mM on the morphological and physiological traits of rice plants during the tillering stage under drought stress. Rice seeds presoaked with SA showed better growth in 7 days old rice seedlings in terms of germination percentage, root length, shoot length and fresh weight compared to the control. Drought conditions were applied to rice plants after 35 days of transplantation in two groups, SA treated and SA untreated, for 7 and 14 days of drought stress conditions, respectively. All experiments were conducted in a totally randomised manner with three replicates per treatment. Leaf area and relative water content (RWC) reduction were significantly prevented in salicylic acid-treated plants under drought conditions. Our data suggest that, using exogenous SA as a presoaking treatment on rice seeds improved drought tolerance in the later stages of rice plants in coping with drought stress by preserving water content in rice leaves. This method is low-cost and accessible to farmers for protecting plants during the seedling and tillering stages, which also affect rice yield at the end.

**Keywords:** Abiotic Stress; Drought; Rice; Salicylic acid; Presoaking.

## Introduction

Abiotic environmental circumstances, such as drought stress, are key factors in limiting agricultural efficiency, negatively impacting plant output capabilities (Loutfy et al., 2020). Food security is currently a worldwide concern, and rice is one of three main cereal grains that contribute significantly to global food security. Rice plants are particularly sensitive to water stress owing to their short root length, scant cuticular wax, and rapid rate of stomatal closure, therefore producing drought-tolerant variants is crucial (Sahebi et al., 2018). Drought disrupts several cell cycle activities such as cell replication, cell expansion, and chromosome segregation, which eventually affects the pace of seed germination and subsequent root and shoot systems that aid in plant development by absorbing water from the environment. Proper photosynthesis is regulated by the texture of the leaf. Roots, shoots, and leaves adapt to a variety of drought-resistance mechanisms (Jaleel et al., 2008). To cope with droughts, plants have developed acclimation and adaptation processes, such as stress avoidance, escape, and protoplast dehydration tolerance (Soltys-Kalina et al., 2016).

Salicylic acid is one of the growth regulators in plants, which acts as a signaling molecule in controlling various metabolic activities of the cell under stressed as well as non-stressed conditions. SA's influence on plant physiological systems varies according to species, stage of growth, SA dosage, and external conditions

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(Abd El-Mageed et al., 2016). According to Sharma et al. (2017) SA significantly boosted drought resistance and might be utilised to increase and stabilize crop yield under stressful circumstances. Traditional, genetic, breeding and recombinant technologies are some of the physiological methods for boosting rice emergence and drought resistance. However, these procedures are costly, laborious, and unavailable to inexperienced farmers (Ali et al., 2021). Farooq et al. (2008) found that SA boosted maize emergence in their study, while Pouramir-Dashtmian et al. (2014) examined that cooling stress increased antioxidative enzymes in rice. Different growth stages suffer irreparable, non-recoverable harm from a severe drought. Making plants drought-resistant is therefore required (Zhang et al., 2018). The impact of SA varies depending on the species, methods of application, SA concentration, and cultivation circumstances (Sousa et al., 2021). It is still unclear how the SA molecule, particularly in rice, controls the entire defence and resistance process (Nadarajah et al., 2021). According to a study, pretreating rice with SA made it drought-tolerant later on in its life cycle (Verma et al., 2022a). The purpose of this study was to investigate the effect of SA on morpho-physiological attributes in Swarna MTU 7029, a variety of rice after 7-14 days of drought stress conditions as compared to untreated rice plants.

## Materials and Methods

### Plant Material

During the rainy season, the research was carried out in the Udai Pratap Botanical Garden and Laboratory in Varanasi, Uttar Pradesh, India. “Swarna (MTU 7029)”, a variety of rice seeds were received from the Institute of Agricultural Sciences (IAS), Banaras Hindu University, Varanasi, Uttar Pradesh, India

### Seed Germination and Experimental Design

The seeds were uniformly sorted by choosing seeds of homogenous and similar sizes and hues. The seeds were sterilized by dipping them in 5% sodium hypochlorite for 3 minutes, then rinsing them three times with distilled water to eliminate chlorine residues and drying them on blotting paper. After sterilisation, for 24 hours in the dark, the seeds were immersed in two beakers, one with distilled water (untreated) and the other with 0.5 mM salicylic acid (treated). To assess seedling growth metrics, pre-soaked seeds were placed in separate petri plates with three replicates. In addition, the pre-soaked seeds were placed separately in two pots for seedling development. After three weeks (at the fourth leaf stage), seedlings were transplanted in four pots (two for each untreated and treated) with three replications and labeled as control (C; well-watered without SA), drought without SA (D; water-stressed), drought with SA (D+SA; water-stressed with SA), and SA control (SA; well watered treated with SA). Seeds were planted in three hills in each pot, with three plants in each hill.

### Measurement of Growth Parameters

Separate petri dishes (10 cm in diameter and 1.5 cm in height) were prepared for germination by bedding with a double layer of Whatman filter paper no-1. Both treated and untreated pre-soaked seeds were germinated (30 seeds in each) in vitro at 25±2°C temperature and 50-70% humidity. Each petri dish was watered daily with 20 ml of distilled water for 7 days. Growth parameters were evaluated after 7 days by the following determinants:

Germination percentage: It was calculated by the formula of

$$\text{Germination percentage (\%)} = (n/N) * 100$$

where $n$ is the number of germinated seeds (7 days); and $N$ is the total number of seeds.

Root Length and Shoot Length: The root and shoot length of 7 days old treated and untreated seedlings were measured with steel rule and estimated in centimeters.

Fresh Weight and Dry weight: Fresh weight of 7 days old treated and untreated seedlings were weighed on an electronic weighing balance and the dry weight was taken after drying all seedlings in the oven at 65°C for 48 hours.

### Measurement of Morphological and Physiological Parameters

Experimental conditions (water and drought) were provided after 35 days of transplantation. Thereafter, morphological and physiological parameters were measured accordingly after 7 days and 14 days of experimental condition.

*Leaf Area*: Leaf width and length were measured in fully matured leaves at the widest portion and maximum length of the leaf using the steel rule. The area of the leaf was calculated by the equation

$$A = K (L*W)$$

where $A$ is the area, $K$ is the adjustment factor, $L$ is the length, and $W$ is the width.

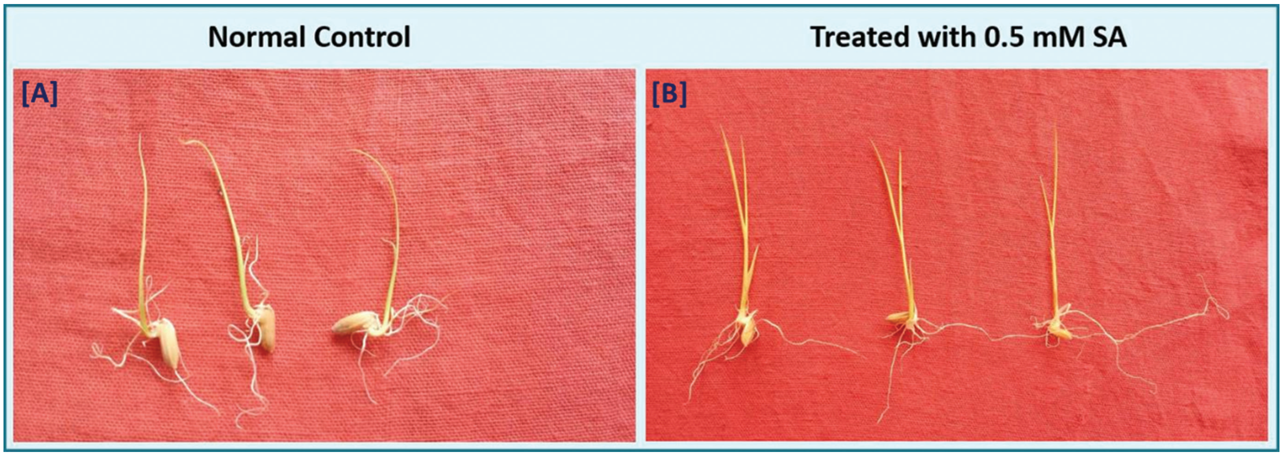


**Figure 1: Seven days old rice seedling growth in response to salicylic acid: (A) Control, (B) 0.5 mM treated salicylic acid.**

### Relative Water Content (RWC)

Leaves samples were taken early at 6:30 am. Extra water drops were surface dried with filter paper and weighed immediately on a digital weighing balance. Thereafter, leaves were dipped in a tray containing distilled water for 12 hours and weighed to calculate the turgid weight. Further leaves were oven-dried for 48 hours at 80°C and weighed to obtain dry weight. RWC was estimated by using the following formula

$$RWC\ (\%) = [(\text{Fresh weight} - \text{Dry weight})/(\text{Turgid weight} - \text{Dry weight})] * 100$$

### Statistical Analysis

The experiments were performed in triplicate. A one-way ANOVA (analysis of variance) was accomplished for data analysis. Means were compared using Duncan's multiple comparison test for post hoc analysis ($P \leq 0.05$) by using the SPSS statistical analysis software, version 16.0 (SPSS Inc., Chicago, IL, USA).

## Results and Discussion

### Effect of SA on Seedling Growth in Rice

The major germination parameters, such as germination percentage, root length, shoot length, fresh weight and dry weight were analysed in both groups; control and SA treated, after 7 days of seedling growth (Figure 1). The germination percentage, root length, shoot length, and fresh weight were significantly enhanced, and dry weight was reduced in 0.5 mM SA treated seedlings compared to untreated controls. Germination percentage, root length, shoot length, and fresh weight were improved by approximately 14%, 73.4%, 33.6%, and 21.6% respectively, while dry weight was declined by 13.4% (Table 1).

SA is a phytohormone found in plants and plays an important role in defense mechanisms under abiotic stress (Maruri-López et al., 2019). Pre-soaking rice seeds with 0.5 mM SA, increased the germination percentage, root length, shoot length, and fresh weight of seedlings compared with untreated controls. However, the dry weight of the seedlings was decreased more in SA treated seedlings. This demonstrated that SA treated rice seedlings contained more water than untreated seedlings. The higher germination percentage revealed that SA treatment improved the viability of seeds. SA induced the early growth of seedlings by activating various enzymes like proteases, lipases, and amylases (Ali et al., 2021). SA may break proteins, lipids, and starch to provide food reserve for the early emergence of

**Table 1: Comparatives of seedling growth parameters between control and salicylic acid treatment of seeds**

| *Parameters* | *Control (Mean ± SD)* | *Treated (Mean ± SD)* | *P-value* |
|---|---|---|---|
| Germination (%) | 55.75 ± 1.77 | 69.83 ± 2.13 | <0.001 |
| Root Length (cm) | 3.42 ± 1.28 | 5.93 ± 1.01 | <0.001 |
| Shoot Length (cm) | 3.48 ± 0.38 | 4.65 ± 0.34 | <0.001 |
| Fresh weight (g) | 0.037 ± 0.004 | 0.045 ± 0.006 | <0.001 |
| Dry weight (g) | 0.015 ± 0.001 | 0.013 ± 0.001 | 0.001 |

SD = Standard deviation; *P* = Significance value (<0.05); cm = Centimeter; g = Grams; % = Percentage.

seedling and their growth. Better seedling growth may be the result of greater alpha amylase activity during seed germination. Such an enzyme's synthesis could be prompted by salicylic acid. Particularly in rice, amylase activity is strongly enhanced during germination (Wang et al., 2016). In *V. faba*, seeds primed with SA caused the radicle and plumule to emerge sooner than seeds that weren't primed; this might be because the metabolic process started much earlier than the radicle and plumule developed (Anaya et al., 2018).

**Effect of SA on Morphological and Physiological Characters in Rice Plants Under Drought Stress**

Leaf area and relative water content (RWC) reduced significantly under drought conditions. These losses were lesser in salicylic acid treated plants under drought conditions (Figure 2). The leaf area of untreated plants under drought conditions was reduced by 24.8% and 50.3%, respectively, at 7 days and 14 days while in the treated plants, it was reduced by 16% and 36.4%, respectively. RWC of untreated plants under drought conditions was reduced by 28.7% and 37.8% while in the treated plants, it was reduced by 16.2% and 26.3% at 7 days and 14 days, respectively.

Drought-induced various morphological changes like leaf area and leaf rolling. Reduced leaf area has an impact on the nutritional components necessary for plants to grow healthily (Ghassemi-golezani et al., 2018). The leaf area reduces under drought stress which lowers the photosynthetic pigments followed by the decline in photosynthetic products under stress. It limits the energy to the plants for cell division and elongation and affects the overall plant's growth and development. In our study, we observed that the treatment of seeds with SA alters these problems and improved crop growth and quality. A similar result was reported by Ghaderi and their coworkers in the tolerant variety of strawberries (Ghaderi et al., 2015). Many plant species showed reduced RWC in stressed plants, which has been linked to a decline in plant vigour (Ghassemi-golezani et al., 2018). We also observed a diminished loss of RWC percentage in SA-treated plants in comparison to untreated plants under drought. This observation clarified that SA treatment up to a certain period of drought conditions maintained water potential in leaves under drought stress. When stress continued for a longer period (14 days of drought), the percentage of RWC decreased in both untreated and treated rice plants but the proportion of reduction in RWC under 14 days of drought is lower in SA treated plants than in untreated rice plants. After seven and fourteen days of drought stress, salicylic acid enhanced the enzymatic activities in SA-treated rice plants (Verma et al., 2022b). Cell division is impacted by protoplasmic dehydration under water stress, which could lead to a reduction in leaf area (Ahmad et al., 2021). Under 7 and 14 days of drought stress, SA may have stopped rice plants from dehydrating to some extent. Our findings were favoured by a study, in which authors explained that the SA develops adaptation in plants towards drought stress by minimising transpiration rate and light interception (Pandey and Shukla, 2015).

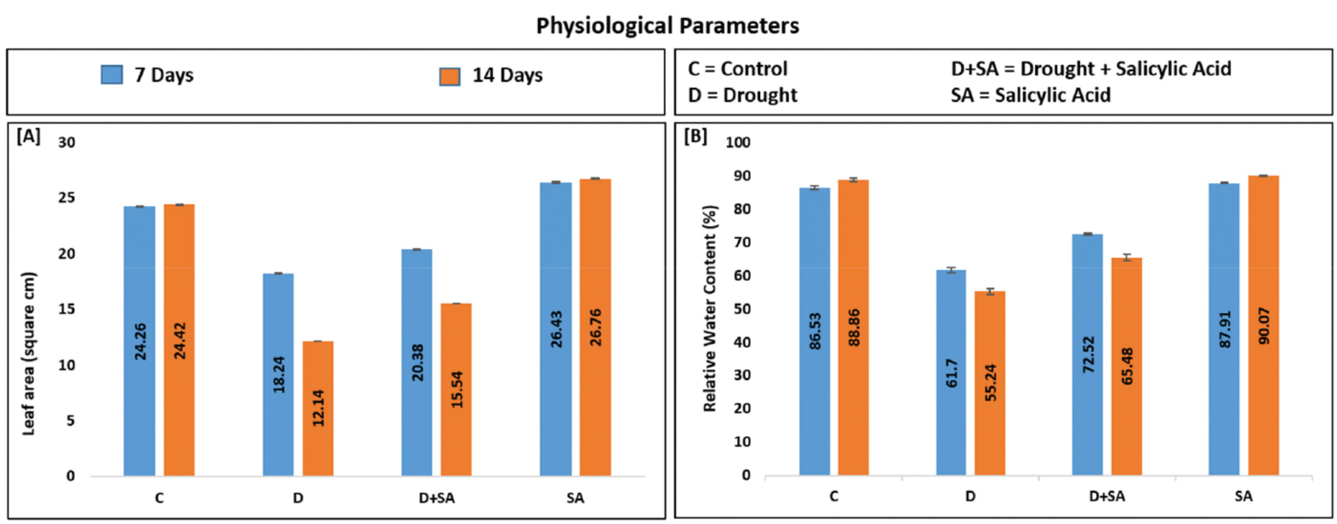


**Figure 2: Effect of salicylic acid (SA) on (A) leaf area; and (B) relative water content. Data are presented as mean ± standard deviation. C – control; D – drought; D+SA – drought with salicylic acid treatment; SA – salicylic acid treatment; % – percentage.**

## Conclusion

Based on the findings, it is possible to infer that 0.5 mM SA had a significant favourable influence on germination parameters in 7-day-old rice seedlings, as well as leaf area and relative water content in 56-day-old rice under drought stress conditions for 7 and 14 days. Pre-soak treatment with SA had a long-term influence on the tillering stage of rice plants during drought, which is beneficial for farmers who want to prepare their rice plants before they are exposed to drought.

## Research Significance

The results of this study may contribute to enhancing the effectiveness of scientific research. Our analysis indicates that SA administered exogenously to rice seeds is effective in enhancing rice characteristics over the long run under stressful circumstances. The outcome of this study serves to understand the variation in morphological and physiological characteristics of rice under drought stress, which is necessary to produce tolerant varieties of rice in today's changing environmental conditions.

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## Conflict of interest

The authors declare no competing interest.

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