



Research Article

Morpho-physiological Responses of Rice to Salicylic Acid under Drought Stress

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ABSTRACT

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Salicylic acid (SA) is vital in controlling plant growth and development. Water deficiency is acute environmental stress to field crops causing low production of crops. Rice is one of the staple crops whose yields and quality are highly affected due to drought stress. Despite the importance of SA in crop production, little information has been reported concerning the effect of SA under drought stress. Therefore, the possible physiological and morphological responses of rice to SA were investigated under drought conditions. In this study, the external application of 2-hydroxybenzoic acid (Salicylic acid) was identified as a positive tool in decreasing the stress of the drought effect. The field experiment was arranged under a randomized complete block design (RCBD) containing three replications of SA and drought stress treatments. The SA treatments consisted of 0, 250, 500, 750 and 1000 μMm^{-2} concentrations and drought stresses were slight, moderate and severe. Following the treatments, higher leaf number, leaf area index, relative water content, leaf membrane stability index and pigment content were determined for the application of 750 μMm^{-2} of SA compared to the alternative treatments and management of plants. Exogenous application of SA during drought stress has growth-promoting and stress priming effects on rice plants, hence reducing yield limitation. The findings of the study imply that SA can be utilized as a protective agent to increase water use efficiency, osmotic management, and pigment content to reduce the negative effects of drought stress on rice growth and physiology, resulting in optimum yield.

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Introduction

Rice (*Oryza sativa* L.) is the staple food for a minimum of 62.8% of the total population, contributing to an average of 20% of the calorie intake of the planet and 30% of inhabitants in Asian countries (Ahmed, 2018). In Asia, more than 90% of this rice is consumed. In Bangladesh, rice is the staple food of concerning 160 million people and it covers 75% of the entire cropped spaces.

Salicylic acid (SA) acts as an endogenous phytohormone and functions as a signal molecule that controls plant physiological processes like plant growth and development, chemical changes, and different metabolic activities. Many types of research support numerous roles of SA in modulating the plant response to a variety of organic

phenomena and abiotic stresses (Pandey and Chakraborty, 2015; Brito et al., 2018). Salicylic acid is related to the tolerance of drought stress in plants. Many researchers reported SA to induce tolerance during abiotic and organic phenomenon stress together with different temperatures, salinity, drought, vital metal toxicity, diseases, and pathogens (Hayat and Ahmad, 2007). The substance metabolism, organic compound (Proline) metabolism, production of glycinebetaine (GB), and plant-water relations are regulated by SA in abiotic stress that harms the plants (Miura and Tada, 2014). Salicylic acid was found to regulate drought tolerance in wheat plants (Horváth et al., 2007) and act as a potential protectant in improving the growth and productivity of rice plants under drought stress (Hosain et al., 2020). Induction of defense-related genes and stress resistance related to

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the natural phenomenon was additionally shown as the crucial roles of SA (Kumar, 2014). Furthermore, exogenous application of SA was reported to result in a significant positive impact on plants that suffered water stress (Anwar et al., 2013).

Drought stress is one of the foremost devastating abiotic stresses negatively affecting the acute growth of plants and biological processes. Exposure of plants to drought stress associated with increased leaf temperature significantly diminishes leaf water potential, relative water contents (RWC), and transpiration rate (Halder and Burrage, 2003). Drought stress impacts the physiological processes of plants, for example, formation of secondary metabolites resulting in the assemblage of endogenous reactive oxygen species (ROS) and increased toxins (Farooq et al., 2009). Hence, drought stress not only reduces the yield or productivity of plants by hampering normal physiological processes (Hasanuzzaman et al., 2013, Hasanuzzaman et al., 2017) but also can result in ROS related lipid peroxidation, supermolecule degradation and polymer fragmentation, ultimately causing death to the plants (Foyer and Fletcher, 2001). Drought stress greatly reduced the plant growth and development during the vegetative stage of rice plants (Manikavelu et al., 2006). Rice is highly susceptible to drought stress during the reproductive stage leading to a significant reduction in grain yield (Palanog et al., 2014). Drought stresses such as mild and severe stress cause 53–92% and 48–94% yield reduction, respectively during the reproductive stage of rice (Lafitte et al., 2007) and 30–55% and 60% yield reduction, respectively during the grain filling stage of rice (Basnayake et al., 2006). Therefore, it is crucial to investigate the effect of SA on morphological and physiological response of rice in relation to drought conditions. A variety of reports so far have indicated the protecting role of SA below environmental stress conditions. However, the role of SA on the morphological and physiological responses of rice has rarely been evaluated. Therefore, the study was done to determine the impact of salicylic acid on the morpho-physiological responses in rice plants under drought conditions. To the best of our knowledge, this is the first SA application to find its effects related to the morpho-physiological response that will provide very useful information for further research in improving the performance of various rice varieties and related other crops.

Materials and Methods

Experimental site

The field experiment was carried out at the research farm of Sher-e-Bangla Agricultural University, Dhaka-1207, Bangladesh from November, 2017 to July, 2018.

The soil of the experimental plot belonged to the general soil type and shallow red-brown terrace soil with silty clay. Soil pH was 5.6 with an organic carbon content of 0.45%.

Treatments and experimental design

Rice variety (BRRI dhan28) was employed as the experimental crop in the present study. The experiment consisted of two factors *viz.* salicylic acid concentrations: control, 250 μMm^{-2} , 500 μMm^{-2} , 750 μMm^{-2} and 1000 μMm^{-2} and different drought stresses: control (irrigation without treatment), severe water stress (water withheld from panicle initiation stage to season end) and moderate drought stress (water withheld from flowering stage to season end). The experiment was performed based on a randomized complete block design (RCBD) with 15 treatment combinations and three replications. There were 45 plots of 6 m² (3 m × 2 m) in size and the distance maintained between the two-unit plots was 0.5 m and between the blocks was 1 m.

Crop husbandry

The seeds were sown in the seedbed @ 70 gm⁻² to have healthy seedlings. The 21 days aged seedlings were transplanted in the main field on January 12, 2018 with the plant to plant distance of 15 cm and row to row distance of 20 cm. Ten tons of cow dung, 215 kg of urea, 180 kg of TSP and 100 kg of MP ha⁻¹ were applied as the source of nitrogen, phosphorous and potassium (NPK) (BRRI, 2016). The whole amount of cow dung, TSP, MOP and 1/3rd of urea was applied before the final preparation of pots and rest of the urea was applied in two equal installments as top dressing at tillering and panicle initiation stages. Intercultural operations were performed for the normal growth and development of the plants. Plant protection measures were taken as and when necessary.

Application of salicylic acid (SA)

The study provided foliar spray of SA solution in three installments. First, 2nd and 3rd spray were done at 20, 30 and 40 days after transplanting (DAT), respectively.

Data collection

Plants (10) were chosen randomly from each of the plots. Data on various parameters such as number of leaves hill⁻¹, leaf area index (LAI), relative water content (%), leaf membrane stability index and chlorophyll content (μM) were collected at flowering and maturity stages during the study.

Relative water content (RWC)

Relative water content (%) of the leaf was measured at the flowering and maturity stage following the method built up by Barrs and Weatherly (1962) with the formula

of $[(FW-DW) / (TW-DW)] \times 100$. Here, FW= Fresh weight, DW= Dry weight, TW= Turgid weight

Leaf membrane stability index (%)

The 0.2 g of leaf strips from each treatment were taken and added with 10 ml of distilled water in a pair of test tubes. One set test tube was kept at 40^o C for 30 minutes in a water bath and the electrical conductivity of the samples was measured (C₁). Another test tube set was incubated at 100^o C for 15 minutes in the water bath and electrical conductivity (C₂) was measured. Membrane stability index (MSI) was calculated by the following formula as described by Singh et al. (2008).

$$MSI = [1-(C_1/C_2)] \times 100$$

C₁ = Electrical conductivity of test tube 1.

C₂ = Electrical conductivity of test tube 2.

Chlorophyll content ($\mu\text{M m}^{-2}$)

The absorbance of supernatant for chlorophyll 'a' and chlorophyll 'b' was determined at 647 nm and 664 nm, respectively by UV spectrophotometer. Absorbance values were used in the following expression to quantify chlorophyll content as reported by Coombs et al., (1987).

$$\text{Chlorophyll "a"} (\mu\text{M}) = 13.19 A_{664} - 2.57 A_{647}$$

$$\text{Chlorophyll "b"} (\mu\text{M}) = 22.10 A_{647} - 5.26 A_{664}$$

$$\text{Total Chlorophyll Content } (\mu\text{M}) = 7.93 A_{664} + 19.53 A_{647}$$

Statistical analysis

All the data collected were subjected to analysis of

variance technique (ANOVA) and subsequently, the least significant difference (LSD at 5%) was applied for comparing different treatment means using statistics 10 software as described by Gomez and Gomez (1984).

Results and Discussion

Effect of Salicylic Acid and Drought stress on the Number of leaves hill⁻¹ in rice plant

Effect of salicylic acid on the number of leaves

Emerging evidence showed that SA considerably reduced the effect of drought stress on rice plants regarding the numbers of leaves hill⁻¹. The leaf numbers of rice were multiplied with an increasing dose of SA up to a certain level (Amin et al., 2013). The numbers were shown to rise with the various growth stages. Lower concentrations of SA were reported to increase chemical process activity in *Ocimum basilicum* and *Origanum majorana*, whereas multiple numbers of leaves and leaf areas were observed (Gharib 2006). In this study, various doses of SA application significantly increased the number of leaves hill⁻¹ over the control. Results indicated that among all the SA concentrations (control, 250, 500, 750 and 1000 μMm^{-2}), 750 μMm^{-2} showed the utmost stress tolerance and gave the highest leaf numbers hill⁻¹ at flowering and maturity stages numbering approximately 95 and 63, respectively followed by 500 μMm^{-2} (Fig. 1). However, the minimum values of 75 and 50 were observed in control.

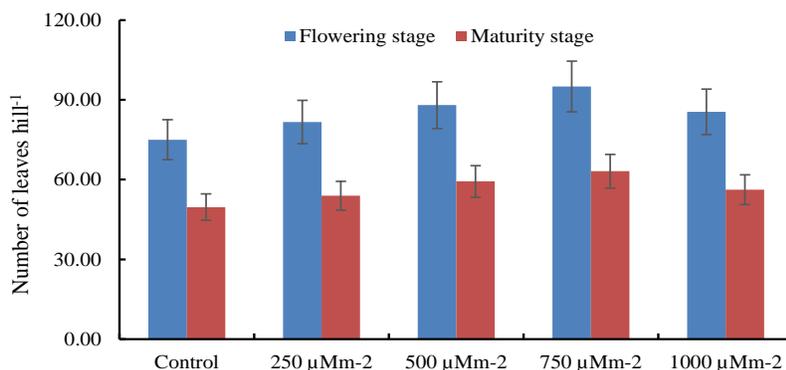


Figure 1. Effect of salicylic acid on number of leaves hill⁻¹ of rice at flowering and maturity stages of rice plants

Drought stress on leaf numbers

Drought stress greatly influences the leaf numbers hill⁻¹ of the rice plant. Results demonstrated that among the different levels of drought stresses, the number of leaves varied from 50 to 94 whereas severe drought stress produced the minimum number of leaves hill⁻¹ during flowering and maturity stages, respectively followed by moderate stress (Fig. 2). The maximum leaf numbers hill⁻¹ in control were observed to be 93.88 and

62.74, indicating that number declined steadily with the increase of drought stresses. The result is supported by a previous study (Riaz et al., 2013) where it was recommended that the shortage of water influences the metabolic processes of plants and thus manufacture a low quantity of assimilates that resulted in a small number of leaves in plants. The observation of our study points out that the scarcity of water is crucial at the flowering and maturity stages of rice.

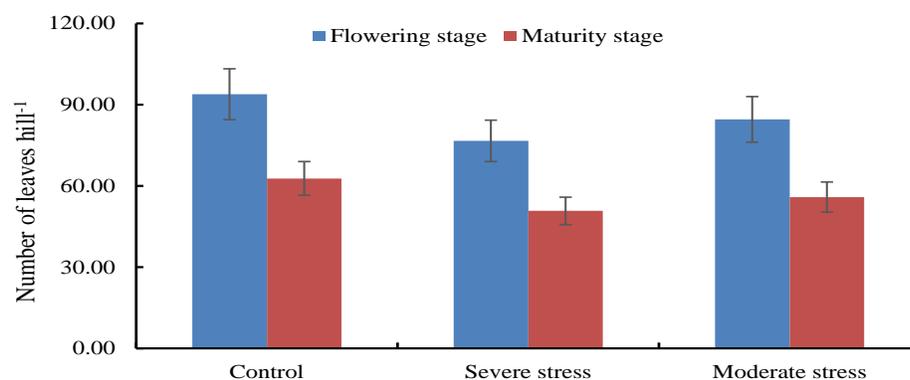


Figure 2. Effect of different levels of drought stress on number of leaves hill⁻¹ of rice at flowering stage and maturity stage

Combined effect of salicylic acid and drought stress on leaf numbers

The number of leaves of rice is generally influenced by the combined treatment of SA and drought stress in crops. In our study, the combined effect of SA and drought stresses resulted in rice plants having various leaf numbers hill⁻¹. The highest number of leaves hill⁻¹ was recorded for the SA treatment of 750 μMm^{-2} along with control, whereas the lowest number of leaves hill⁻¹ was recorded for the control (0 μMm^{-2} SA) with severe stress recorded at flowering and maturity stages, respectively (Table 1).

development (Amin et al., 2013; Waqas et al., 2017). In this study, we found a significant impact of SA treatment on increased LAI over control indicated that among the different SA concentrations, the highest dose of SA application (750 μMm^{-2}) showed the maximum stress tolerance and gave the highest number of LAI followed by 500 μMm^{-2} SA (Fig. 3). However, the minimum value of LAI was observed from the treatment of zero concentration of SA. The results might be due to the correct nutrient providing mechanism from soil to the plants intensity level and light holding capacity of SA, especially phenotypic characters of the rice variety.

Effect of Salicylic Acid and Drought Stress on Leaf Area Index (LAI)

Salicylic acid on leaf area index (LAI)

The leaf area index is an important parameter that influences photosynthesis and plant growth and

Table 1. Interaction effect of salicylic acid and different levels of drought stress on number of leaves, leaf area index, relative water content and membrane stability index of rice

Salicylic acid × Drought stress	Number of leaves hill ⁻¹		Leaf area index (LAI)	Relative Water Content (%)		Membrane stability index	
	Flowering stage	Maturity stage		Flowering stage	Maturity stage	Flowering stage	Maturity stage
Control	80.00 efg	55.00 efg	4.30 g	41.00 efg	55.33 fg	21.44 h	3.22 ef
0 μMm^{-2} ×severe	70.00 h	45.00 j	3.70 i	30.00 h	45.33 h	17.90 j	1.33 i
0 μMm^{-2} ×moderate	75.00 gh	49.00 hij	3.95 h	35.02 gh	50.33 gh	19.78 i	2.23 gh
250 μMm^{-2} ×control	90.00 cd	60.00 cd	4.55 c-f	50.33 cd	68.33 cde	23.07 d-g	3.98 cd
250 μMm^{-2} ×severe	74.00 gh	48.00 ij	3.98 h	40.00 efg	53.33 fgh	19.77 i	1.88 hi
250 μMm^{-2} ×moderate	81.00 efg	54.00 fg	4.28 g	45.00 def	58.13 fg	21.94 gh	2.90 ef
500 μMm^{-2} ×control	98.00 b	66.00 ab	4.85 b	63.00 ab	80.00 ab	26.57 bc	4.84 b
500 μMm^{-2} ×severe	79.00 fg	53.00 fgh	4.35 fg	40.67 efg	60.33 ef	22.86 e-h	2.65 fg
500 μMm^{-2} ×moderate	87.00 de	59.00 cde	4.63 b-e	52.67 cd	70.00 cd	24.37 d	3.48 de
750 μMm^{-2} ×control	106.12 a	69.80 a	5.14 a	67.36 a	87.51a	29.72 a	5.48 a
750 μMm^{-2} ×severe	84.29 def	57.03 def	4.47 efg	49.99 d	68.04 de	23.71def	3.01ef
750 μMm^{-2} ×moderate	94.62 bc	62.63 bc	4.73 bcd	58.67 bc	76.25 bc	25.79 c	4.05 cd
1000 μMm^{-2} ×control	95.26 bc	62.89 bc	4.79 bc	59.79 abc	72.99 bcd	27.66 b	4.64 bc
1000 μMm^{-2} ×severe	75.91gh	50.94 ghi	4.28 g	36.78 fgh	58.92 efg	22.19 fgh	2.69 f g
1000 μMm^{-2} ×moderate	85.26 def	54.74 efg	4.51 d-g	48.17 de	68.51 cde	24.16 de	3.37 def
LSD _(0.05)	6.30	3.93	0.19	7.69	7.74	1.23	0.56
CV (%)	5.12	4.81	3.07	11.08	8.24	3.64	11.63

Values followed by the same letter(s) do not differ significantly at 5% level of probability

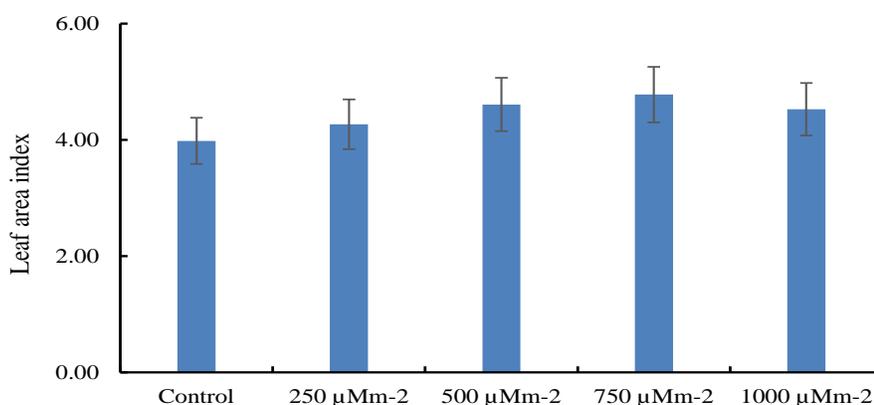


Figure 3. Effect of salicylic acid on leaf area index (LAI) of rice

Drought stress on Leaf Area Index (LAI)

Drought stress considerably influenced the leaf area index of the plant (Bideshki and Arvin, 2010). Results in this study showed that among the different levels of drought stresses, severe drought stress exhibited the lowest number of LAI compared to control and a moderate level of stress (Fig. 4). Cellular functions like

cellular division and cell elongation in crops were reported to reduce leaf part and crop development in drought conditions (Zhang et al., 2018; Bangar et al., 2019). Water stress in this study might inhibit the cellular division of meristematic tissues of rice.

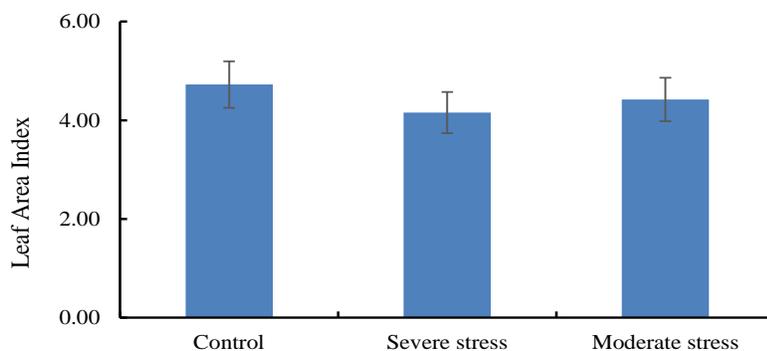


Figure 4. Effect of different levels of drought stresses on leaf area index (LAI) of rice

Relation of salicylic acid and drought stress on Leaf Area Index (LAI)

Under drought stresses, SA exerted a significant impact on the leaf area index (Table 1). The maximum leaf area index was found from the treatment of 750 μMm^{-2} of SA with control followed by the application of 750 μMm^{-2} and 1000 μMm^{-2} of SA. However, the minimum LAI was obtained from the application of zero (0) μMm^{-2} of SA with severe stress treatment (Table 1). Caser et al. (2019) reported that leaf growth decreases due to reduced evaporation levels and as a response to water stress by applying drought stress. The use of SA increases the leaf length of the plant due to the increased absorption of water and nutrients from fertilizers.

Effect of Salicylic Acid and Drought Stress on Relative Water Content (RWC)

Salicylic acid in relative water content (RWC)

Salicylic acid signifies the relative water content of the rice plant and is known to reduce drought stress

considerably (Kang et al., 2012; Li et al., 2019). He et al. (2005) found SA to enhance the metabolic process that created a lot of photosynthates increasing chemical activities and enhancing sap production within the leaf lamella. They identified SA to result in the maintenance of RWC in leaves and higher growth of plants. Results in our study showed that the external spray of SA significantly improved the RWC. Among the different SA concentrations, all doses increased the RWC over control, whereas the treatment with 750 μMm^{-2} of SA showed the highest RWC at flowering and maturity stages, respectively followed by 500 μMm^{-2} of SA (Fig. 5). However, the minimum value was observed in the application of 0 μMm^{-2} of SA. Foliar application of SA, in this case, might control the stomatal opening and decrease transpiration loss of water in drought stresses and thus it may enable the plants to keep up turgor and chemical process and to be productive underneath water deficit conditions.

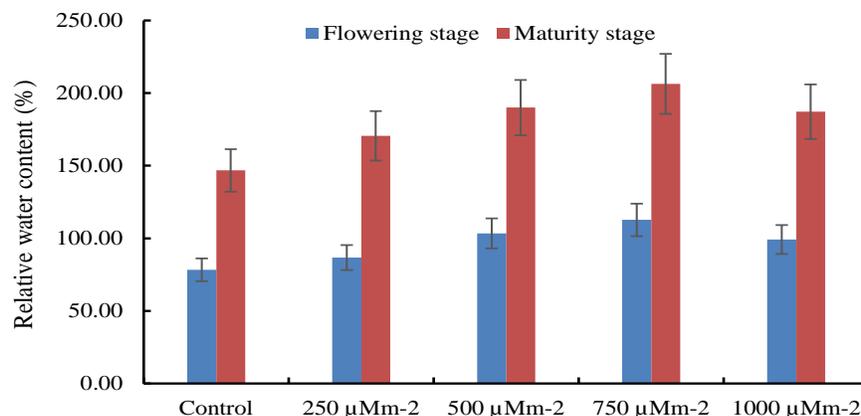


Figure 5. Effect of salicylic acid on relative water content (RWC) at flowering and maturity stage of rice

Drought Stress in relative water content (RWC)

Drought stress considerably reduces the RWC of plants and some water-stressed cereal crops were previously shown to have lower RWC than non-stressed ones (Chowdhury et al., 2017; Younis et al., 2018). The plants towards drought stress were shown to reduce the leaf water potential, RWC and transpiration rate (Bangar et al., 2019). In another study, RWC was found to decrease transpiration crosses water absorption underneath drought stress resulting in a decrease in the current physiological condition of a cell (Tas and Tas,

2007). In the present study, we found that among the different levels of drought stress, severe drought stress generated the minimum RWC at flowering and maturity stages, respectively compared to control and moderate stress (Fig. 6). Though plant water relations suffered from reduced accessibility of water that causes the stomatal opening and shutting difficulties and modification in leaf temperature might be a very important consideration in dominant leaf water standing under drought stress.

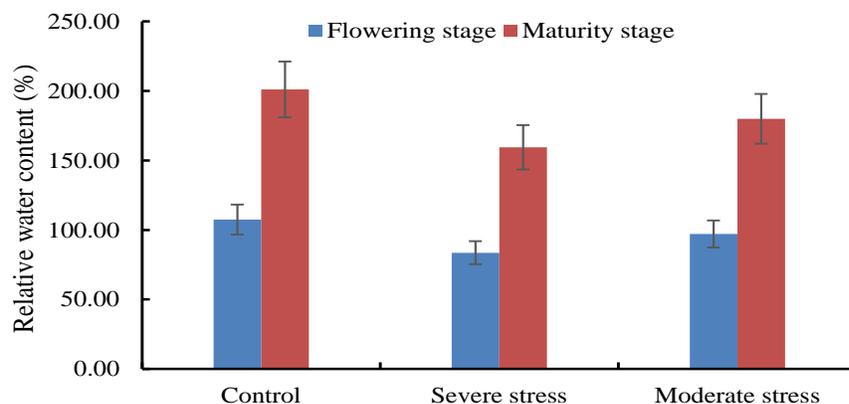


Figure 6. Effect of different levels of drought stresses on the relative water content (RWC) at flowering and maturity stage of rice

Combined effect of SA and drought stress in relative water content (RWC)

The joined effect of SA and drought stresses had a significant influence on the RWC of rice (Manzoor et al., 2015) (Table 1). The highest RWC of rice was recorded in the 750 μMm⁻² treatment of SA with control treatment, whereas the lowest RWC from 0 μMm⁻² SA with severe stress treatment at flowering and maturity stages, respectively.

Effect of Salicylic Acid and Drought Stress on Leaf Membrane Stability Index (LMSI)

Effect of salicylic acid on leaf membrane stability index (LMSI)

Salicylic acid greatly influenced the LMSI of the rice plants and reduced the drought stress considerably (Samea-Andabjadid et al., 2018). The application of SA in a previous study increased the building up of Ca⁺² that maintains membrane integrity (Khan et al., 2010). Salicylic Acid treatment in a study was reported

to ameliorate the effect of abiotic stress via an antioxidant system that is important to cut back aerobic injury and particle discharge from membranes (Yusuf et al., 2008). In this study, various doses of SA treatments significantly improved the LMSI compared to control.

Results indicated that among the different SA concentrations, the application of 750 μMm^{-2} of SA showed the maximum LMSI at flowering and maturity stages, respectively, whereas the lowest LMSI was found with the application of 250 μMm^{-2} of SA (Fig. 7).

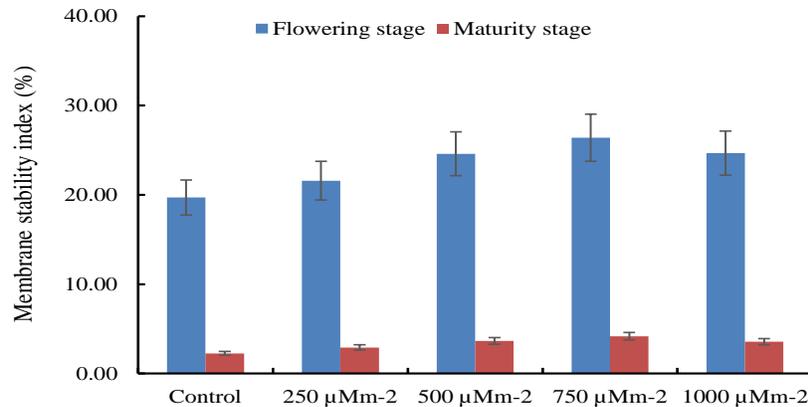


Figure 7. Effect of salicylic acid on the membrane stability index (MSI) at flowering and maturity stage of rice

Effect of drought stress on leaf membrane stability index (LMSI)

Drought stress markedly influenced the LMSI of the rice plant. In a previous study, it was shown that membrane porosity was considerably raised by an increased level of electrolyte discharge underneath drought stress (Bangar et al., 2019). Drought in a study was found to considerably affect the cell membrane

integrity marked by minimum values of MSI (Tas and Tas, 2007). The study here indicated that among the different levels of drought stresses, especially severe drought showed the lowest LMSI at flowering and maturity stages, respectively (Fig. 8) followed by moderate stress, whereas maximum MSI was found at both stages.

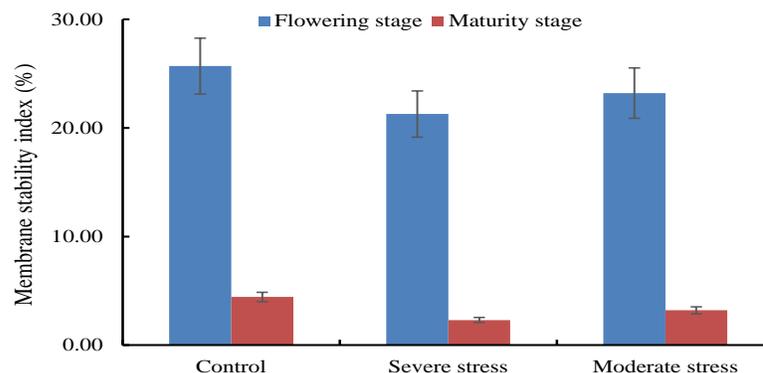


Figure 8. Effect of different levels of drought stress on the membrane stability index at flowering and maturity stage of rice

Combined effect of SA and drought stress on leaf membrane stability index (LMSI)

The combined effect of SA and drought stress in the present study greatly impacted the LMSI of rice. The highest leaf membrane stability index of rice was recorded from treatment with the concentration of 750 μMm^{-2} of SA with control, whereas the lowest was observed in 0 μMm^{-2} of SA with severe stress at flowering and maturity stages, respectively followed by 250 and 500 μMm^{-2} SA (Table 1).

Effect of Salicylic acid and Drought Stress on Leaf Chlorophyll Content

Salicylic acid on chlorophyll-a, chlorophyll-b and total chlorophyll content

Salicylic acid remarkably influenced the chlorophyll content of rice leaves. Foliar spray of SA in previous studies was identified in plants to contribute to drought tolerance and sustain productivity (Kakar et al., 2016). The application was additionally concerned with stomatal regulation and the dominant photosynthetic rate. Moreover, SA was reported

to lead to enhancing concentrations of chlorophyll-*a* and chlorophyll-*b* during both stress and non-stress conditions (Anosheh et al., 2012). Results in this study indicated that among the various SA concentrations, the treatment with the dose 750 μMm^{-2} of SA showed the utmost stress tolerance and provided the maximum chlorophyll-*a*, *b* and total chlorophyll content at flowering and maturity stages, respectively followed by 500, 1000 and 250 μMm^{-2} of SA (Table 2). However, the concentration of 0 μMm^{-2} SA exhibited the lowest chlorophyll-*a*, *b* and total chlorophyll content in both

cases. Our results indicate that SA spray may improve the performance of rice plants by protecting against the degradation of chlorophyll content. These observations support the previous studies indicating the increased chlorophyll content during drought stress due to the application of either SA, chlorocholin chloride or abscisic acid (Farooq and Bano, 2006). This sort of increase might be due to an improvement in keeping green attributes in response to plant growth regulators (PGRs).

Table 2. Effect of salicylic acid on leaf chlorophyll content of rice

Salicylic acid	Leaf chlorophyll- <i>a</i> (μM)		Leaf chlorophyll- <i>b</i> (μM)		Total leaf chlorophyll (μM)	
	Flowering stage	Maturity stage	Flowering stage	Maturity stage	Flowering stage	Maturity stage
Control	22.23 d	10.11 d	11.52 d	3.46 d	33.76 d	13.57 d
250 μMm^{-2}	23.47 c	12.01 c	13.03 c	4.12 cd	36.49 c	16.13 c
500 μMm^{-2}	25.47 b	14.01 b	14.22 b	5.00 b	39.69 b	19.01 b
750 μMm^{-2}	27.35 a	15.58 a	16.83 a	6.28 a	44.18 a	21.86 a
1000 μMm^{-2}	24.54 bc	13.51 b	14.34 b	4.44 bc	38.88 b	17.95 b
LSD _(0.05)	0.93	0.81	0.84	0.67	1.52	1.39
CV (%)	4.17	6.82	6.57	15.90	4.34	8.65

Values followed by the same letter(s) do not differ significantly at 5% level of probability

*Drought stress on chlorophyll-*a*, chlorophyll-*b* and total chlorophyll content*

Drought stress was proved to considerably decrease the chlorophyll content of rice leaf (Shivakrishna et al., 2018). Results in our study demonstrated that among the different levels of drought stresses, particularly severe drought stress produced the lowest content of chlorophyll *a*, *b* and total chlorophyll at flowering and maturity stages, respectively followed by moderate

drought stress (Table 3). In case of control, it generated the highest degree of chlorophyll-*a*, chlorophyll-*b* and total chlorophyll in both rice growth stages of flowering and maturity. Moreover, a restricted water system was reported to typically cause a decrease in the chlorophyll content (Paknejad et al., 2006). What is more, (Anosheh et al., 2012) found the drought stress to decrease chlorophyll *a* and *b* contents as well as carotenoids contents within the leaves of green gram.

Table 3. Effect of different levels of drought stress on leaf chlorophyll content of rice

Drought stress	Leaf chlorophyll- <i>a</i> (μM)		Leaf chlorophyll- <i>b</i> (μM)		Total leaf chlorophyll (μM)	
	Flowering stage	Maturity stage	Flowering stage	Maturity stage	Flowering stage	Maturity stage
Control	27.18 a	14.95 a	16.11 a	5.53 a	43.29 a	20.49 a
Severe drought stress	22.48 c	11.24 c	12.28 c	3.86 c	34.75 c	15.10 c
Moderate drought stress	24.18 b	12.93 b	13.58 b	4.58 b	37.76 b	17.52 b
LSD _(0.05)	0.79	0.68	0.70	0.57	1.28	1.17
CV (%)	4.17	6.82	6.57	15.90	4.34	8.65

Values followed by same letter(s) do not differ significantly at 5% level of probability

*Combine effect of SA and drought stress on chlorophyll-*a*, chlorophyll-*b* and total chlorophyll content*

In this study, the combined effect of SA and drought stresses showed a significant impact on the chlorophyll content of rice leaves. The maximal chlorophyll *a*, *b* and total chlorophyll contents were recorded from 750

μMm^{-2} of SA with control treatment followed by 500 and 1000 μMm^{-2} of SA with control, whereas the minimal from 0 μMm^{-2} of SA with severe stress treatment at flowering and maturity stages, respectively (Table 4).

Table 4. Interaction effect of salicylic acid and different levels of drought stress on leaf chlorophyll content of rice

Salicylic acid × Drought stress	Leaf chlorophyll-a (μM)		Leaf chlorophyll-b (μM)		Total leaf chlorophyll (μM)	
	Flowering stage	Maturity stage	Flowering stage	Maturity stage	Flowering stage	Maturity stage
Control	24.56 def	12.00 f	13.02 ef	4.00 cd	37.580 ef	16.00 ef
0 μMm^{-2} ×severe	20.01 i	8.32 h	10.0 2h	2.83 d	30.023 i	11.15 g
0 μMm^{-2} ×moderate	22.13 gh	10.00 g	11.53 fgh	3.55 d	33.667 gh	13.55 fg
250 μMm^{-2} ×control	26.00 cd	14.03 cd	15.10 cd	5.02 bc	41.100 cd	19.05 cd
250 μMm^{-2} ×severe	21.33 hi	10.00 g	11.02 gh	3.33 d	32.350 hi	13.33 g
250 μMm^{-2} ×moderate	23.07 fgh	12.00 f	12.97 ef	4.02 cd	36.033 fg	16.02 ef
500 μMm^{-2} ×control	28.07 b	16.02 b	17.00 b	6.00 b	45.067 b	22.02 b
500 μMm^{-2} ×severe	23.33 efg	12.00 f	13.00 ef	4.00 cd	36.333 fg	16.00 ef
500 μMm^{-2} ×moderate	25.00 de	14.00 cd	12.67efg	5.00 bc	37.667 ef	19.00 cd
750 μMm^{-2} ×control	30.12 a	17.55 a	19.32 a	7.60 a	49.446 a	25.14 a
750 μMm^{-2} ×severe	24.50 def	13.62 cde	14.67 d	5.10 bc	39.171 de	18.72 cd
750 μMm^{-2} ×moderate	27.42 bc	15.56 b	16.50 bc	6.15 b	43.921 b	21.71 b
1000 μMm^{-2} ×control	27.16 bc	15.16 bc	16.10 bcd	5.06 bc	43.257 bc	20.21 bc
1000 μMm^{-2} ×severe	23.21 e-h	12.26 ef	12.68 efg	4.06 cd	35.882 fg	16.31 def
1000 μMm^{-2} ×moderate	23.26 e-h	13.11 def	14.25 de	4.21 cd	37.507 ef	17.31 cde
LSD _(0.05)	1.49	1.29	1.33	1.07	2.43	2.22
CV (%)	4.17	6.82	6.57	15.90	4.34	8.65

Values followed by same letter(s) do not differ significantly at 5% level of probability

Conclusion

In this study, we assessed the possible functions of salicylic acid and drought stress on the growth and physiology of the rice plant. The results of the investigations recommend that among different doses of SA, 750 μMm^{-2} showed comparatively better performance in rice under drought stress conditions. Furthermore, the protecting action of salicylic acid might also be related to a reduction in transpiration rate and an enhancement of the chemical process that along with increased water use potency under drought conditions. These observations will provide an overview of SA under drought stress conditions in rice that might guide rice plants and related species.

Authors contribution

MTH was developed the concept and designed the experiments. MSR collected the sample performed the laboratory test. MSR and MHM contributed to recording the data. MSR evaluated the result, analyzed data statistically and contributed to writing the manuscript. MN and ASMF contributed to revising the manuscript critically for important intellectual content. All authors read the article and approved the final version to be published.

Competing interests

The authors have declared that no competing interests exist.

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