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PHYSIOLOGICAL CHARACTERISTICS OF THE RED RICE WITH APPLICATION OF ASCORBIC ACID UNDER SALINITY STRESS CONDITIONS

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SUMMARY

Red rice (*Oryza glaberrima* L.) is a main food ingredient with some special characteristics and health benefits; therefore, enhancing its grain yield is necessary. However, the limited fertile land causes cultivation in the sub-optimal land, such as saline soil. Saline stress can cause damage to plant cells; hence, it is vital to apply exogenous antioxidants that can act as osmoprotectants. The presented study sought to determine the physiological characteristics of red rice under salinity stress conditions with ascorbic acid applications. The study commenced in a factorial separate plot design (SPD) with three features. The salinity levels (3-4 and >4-5 mho/cm) comprised the main plots, red rice cultivars (Inpari 24, Inpari 7, Pamelen, and MSP17) in the subplots, with the ascorbic acid concentrations (0, 500, 1000, and 1500 ppm) kept in the sub-sub-plots. The results showed that the studied red rice cultivars differed in responses to ascorbic acid concentrations under saline soil conditions. Cultivar MSP17 was the most tolerant genotype to salinity stress compared with the three other red rice cultivars based on physiological attributes. Applying ascorbic acid improved red rice genotypes' physiological characteristics (especially chlorophyll content and nutrient uptake) under saline stress conditions.

Keywords: Red rice (*O. glaberrima* L.), cultivars, salinity, ascorbic acid, physiological characteristics, grain yield

Key findings: Ascorbic acid is vital in improving the physiological characteristics of red rice (*O. glaberrima* L.) genotypes under salinity stress conditions.

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INTRODUCTION

Red rice (*Oryza glaberrima* L.) has numerous advantages compared to white rice, especially for various functions within the human body. Red rice contains carbohydrates, vitamin E, thiamin, vitamin B-6, magnesium, minerals, and fiber (Setyawaty *et al.*, 2020). It also has phenolic compounds, especially anthocyanin, which is an antioxidant preventing various diseases, such as heart ailments (Wang *et al.*, 2007), cancer (Wang and Stoner, 2008), diabetes (Ghosh and Konishi, 2007), and harmful cholesterol (Lee *et al*., 2008). Therefore, public awareness should intensify on red rice's health benefits to provide opportunities for its development on a broader scale. However, red rice production in fertile lands is decreasing due to land conversion; hence, rice cultivation extends to marginal and suboptimal lands, including saline areas (Lestari *et al*., 2020). Still, the red rice expansion in saline lands is unproductive due to salinity stress conditions.

Soil salinity induces osmotic and ionic stress and develops nutrient imbalance in crop plants (Hasanuzzaman *et al*., 2013). Plants grown in saline soils will experience two different physiological stresses. First, the toxic effect of specific ions, such as sodium and chloride, often found in salty soils, destroying the enzyme structure and other macromolecules, damaging cell organelles, interfering with photosynthesis and respiration, inhibiting protein synthesis, and promoting ion deficiency (Farhoudi *et al*., 2012). Second, plant exposure to a low osmotic potential under saline solutions will be at risk of physiological drought; these plants must maintain a low internal osmotic potential, which may cause an excess of ions, ultimately slowing growth in some crop plants (Kamran *et al*., 2019).

An excessive Na and Cl accumulation in the cytoplasm under saline soil conditions also impedes plant growth and development, causing tissue metabolic variations (Barus *et al*., 2015). Similarly, the excess of extracellular Na and Cl affects nitrogen assimilation because it directly inhibits the uptake of nitrates (NO_3) (Azzedine *et al*., 2011). The extreme buildup of Na^+ induces efflux of cytosolic K^+ and Ca^{2+}, which consequently lead to an imbalance in their cellular homeostasis, nutrient deficiency, oxidative stress, retarded growth, and cell death (Ahanger and Agarwal, 2017). The salinization levels radically affect plant photosynthesis due to chlorophyll malfunctioning (Jiang *et al*., 2012). Besides, a high salt solubility can also inhibit crops' water and nutrient absorption with an enhanced osmotic pressure.

Studying plant responses to salinity is crucial in the search for effective plant screening techniques. High salt stress conditions will kill the plants; however, moderate and low-stress conditions may influence plant growth rates and real symptoms associated with morphological, physiological, and biochemical variations (Seraz *et al*., 2008). Observations on crop cultivars did not differ in their inherent ability to modify the various physiological and biochemical processes in response to salt stress. Although diverse physiological and biochemical changes occur under stress environments, only a few reactions may be considerable and contribute to the salt tolerance mechanism.

Ascorbic acid use is one of the efforts to enhance plants' resilience to oxidative stress caused by a high salt content. Ascorbic acid, vitamin C, is a primary non-enzyme antioxidant in plants vital in mediating specific oxidative pressures caused by biotic and abiotic stress conditions (Sharma *et al*., 2019). The presented study sought to determine the physiological characteristics, especially chlorophyll content and nutrient uptake, in several red rice cultivars under salinity stress conditions by applying ascorbic acid.

MATERIALS AND METHODS

Plant material and procedure

In the pertinent study, the materials were seeds of four red rice (*O. glaberrima* L.) cultivars, i.e., Inpari 24, Inpari 7, Pamelen, and MSP 17. The seeds came from the Sukamandi Rice Center, West Java, Indonesia.

The saline soil procurement materialized from the Paluh Merbau Hamlet, Tanjung Rejo Village, Percut Sei Tuan District, Deli Serdang Regency, Medan, Indonesia. The experiment considered a factorial separate plot design (SPD) with three features. The main plots consisted of salinity levels (S_1 = 3-4 mho/cm and S_2 = >4-5 mho/cm), subplots contained the red rice cultivars (V_1 = Inpari 24, V_2 = Inpari 7, V_3 = Pamelen, and V_4 = MSP 17), and the sub-subplots comprised the ascorbic acid concentrations (A_0 = 0 ppm, A_1 = 500 ppm, A_2 = 1000 ppm, and A_3 = 1500 ppm). The data recorded for physiological parameters included chlorophyll a, b, and total chlorophyll and the uptake of nutrients (N, P, K, Na, and K/Na ratio).

Analysis of K uptake

Potassium K uptake determination used the $HClO_4$ + HNO_3 method with an atomic absorption spectrometer. The K uptake analysis was to weigh 0.5 g of plant sample < 0.5 mm into the digestion tube, then add 5 ml of HNO_3 p.a. and 0.5 ml of $HCLO_4$ p.a. and keep for one night. The next day, heating the sample in the digestion block at 100 °C took one hour, increasing the temperature to 150 °C. After the yellow steam ran out, the temperature rise of the digestion block to 200 °C followed (reaching complete digestion when white smoke came out and the remaining extract was approximately 0.5 ml). Removing the tube allowed it to cool. The extract dilution with ionized water continued to an exact volume of 50 ml and then shaken with a tube shaker until homogeneous. The employment of 1 ml of extract and standard series into a chemical tube comprised of 9 ml of 0.25% La solution and shaking using a shaker tube until it becomes homogeneous. The K measurement using a flame photometer had a standard series as a comparison.

Analysis of Na uptake

The analysis of Na uptake commenced in the laboratory after harvest to determine the uptake of Na in plants and the amount of nutrients in the soil. The plant nutrient uptake analysis had buds dried, weighed, and ground. The plant sample weighing 0.5 g proceeded digestion with H_2SO_4, $HClO_4$, and concentrated HNO_3, then heated until obtaining a clear extract. Measuring the extracts included an atomic absorption spectrophotometer (AAS), flame photometer, and spectrophotometer.

Analysis of chlorophyll content

Chlorophyll content analysis occurred at the end of the vegetative period. The methodology of Hendry and Grime (1993) engaged in calculating the amount of chlorophyll a, b, and the total chlorophyll. Chlorophyll extraction continued by grinding leaves using 80% acetone. The acquired supernatant’s transfer to a microcentrifuge tube ensued under cold conditions. The absorbance measurement by spectrophotometer had a wavelength of 645 nm for chlorophyll a and 663 nm for chlorophyll b. Chlorophyll a, b, and the total chlorophyll calculation followed the equations below.

$$\text{Chlorophyll a} = ([12.7 \times A663] - [2.69 \times A645]) / 10$$

$$\text{Chlorophyll b} = ([22.9 \times A645] - [4.68 \times A663]) / 10$$

$$\text{Total chlorophyll} = ([8.02 \times A663] + [20.2 \times A645]) / 10$$

Where:
A663 = absorbance of chlorophyll extract at 663 nm
A645 = absorbance of chlorophyll extract at 645 nm

Statistical analysis

All analyses of the recorded data for various parameters followed the F test at 5%. The treatment means’ further comparison and separation employed the Duncan Multiple Range (DMR) Test at a 5% probability level (Hanafiah, 2016).

RESULTS AND DISCUSSION

Chlorophyll content

Chlorophyll content revealed no interaction effects among the three factors, i.e., salinity levels, red rice cultivars, and different ascorbic acid concentrations on the chlorophyll content of red rice genotypes. However, each red rice cultivar manifested significantly varied responses with different ascorbic acid concentrations for chlorophyll content under saline stress conditions (Table 1).

The highest chlorophyll a and total chlorophyll contents resulted in the red rice cultivar MSP17 under the salinity level of 3-4 mho/cm with ascorbic acid application of 500 ppm. However, the lowest chlorophyll a and total chlorophyll content emerged in the rice cultivar Inpari 7 under the same salinity level (3-4 mho/cm) and ascorbic acid application (500 ppm). Meanwhile, the highest chlorophyll b content was apparent in cultivar Inpari 7 under salinity at >4-5 mho/cm, without ascorbic acid. The same cultivar gave the lowest chlorophyll b content at the salinity level of 3-4 mho/cm and an ascorbic acid application rate of 500 ppm.

Table 1. Chlorophyll content in four red rice cultivars under saline stress conditions with application of ascorbic acid.

Treatment combinations	Chlorophyll content (fruit/mm^2)		
	A	B	Total
$S_1V_1A_0$	18.04	7.71	24.92
$S_1V_1A_1$	13.73	5.85	19.27
$S_1V_1A_2$	14.70	6.45	20.54
$S_1V_1A_3$	23.61	10.48	33.05
$S_1V_2A_0$	17.63	6.71	24.12
$S_1V_2A_1$	12.76	5.10	18.35
$S_1V_2A_2$	14.70	7.59	22.05
$S_1V_2A_3$	16.43	7.20	23.58
$S_1V_3A_0$	21.44	8.04	29.25
$S_1V_3A_1$	15.97	5.72	21.52
$S_1V_3A_2$	16.48	6.34	23.11
$S_1V_3A_3$	20.39	9.66	33.27
$S_1V_4A_0$	17.33	7.92	25.34
$S_1V_4A_1$	26.12	12.70	38.23
$S_1V_4A_2$	16.27	10.56	25.86
$S_1V_4A_3$	21.37	9.41	30.70
$S_2V_1A_0$	22.13	9.30	31.05
$S_2V_1A_1$	15.23	7.08	21.16
$S_2V_1A_2$	14.98	6.52	23.09
$S_2V_1A_3$	16.36	9.01	28.00
$S_2V_2A_0$	12.83	18.16	28.04
$S_2V_2A_1$	19.41	9.31	30.51
$S_2V_2A_2$	14.94	8.47	23.77
$S_2V_2A_3$	20.98	8.10	29.23
$S_2V_3A_0$	19.42	8.61	26.34
$S_2V_3A_1$	19.68	10.99	30.77
$S_2V_3A_2$	22.27	8.50	30.51
$S_2V_3A_3$	18.87	7.44	25.36
$S_2V_4A_0$	23.19	10.36	32.35
$S_2V_4A_1$	21.98	12.81	34.49
$S_2V_4A_2$	20.22	7.69	28.07
$S_2V_4A_3$	19.43	8.29	28.16

The results further revealed considerable red rice cultivar response differences to varied salinity stress levels. Tolerant rice cultivars will pass through stress by a mechanism. According to Singh (2016), crop plants under salt-stress conditions either try to escape by avoiding the stress or employing various mechanisms to acclimatize to salinity. However, with the crops' varying responses when grown under diverse saline conditions, the salinity tolerance mechanism in the plants becomes even more complicated. Overall, the three types of plant responses generally occurred, i.e., a) the tolerance to osmotic stress, b) the Na^+ exclusion from leaf blades, and c) the tissue tolerance (Munns and Tester, 2008). In the presented study, the red rice cultivar MSP17 showed the most tolerance to salinity stress conditions, while the rice genotype Inpari 7 was the most sensitive compared with other rice cultivars. It could be due to the genetic potential of crop genotypes to accommodate salinity impacts. However, further testing is still necessary to prove it.

Generally, under saline conditions, ascorbic acid can enhance the chlorophyll content (a, b, and total chlorophyll) compared with the control treatment (with no ascorbic acid), which also revealed a low chlorophyll content. Under saline soil conditions, chlorophyll degradation might arise by salinity-induced superoxide radicals and H_2O_2, which may degrade the membranes of thylakoids and chloroplast (Subramanyam *et al*., 2019). However, the ascorbic acid can suppress the oxidative stress experienced by the plants. Munir and Aftab (2011) mentioned that ascorbic acid is crucial in protecting plants used in cell metabolism to synthesize hydroxyproline-containing proteins. Furthermore, higher soil salinity may increase reactive oxygen species (ROS) in cell chloroplasts distorting chlorophyll molecules. Vitamin C can detoxify the harmful effects of these ROS-free radicles, boosting chlorophyll contents and lowering the oxidative stress in crop plants (Farooq *et al*., 2013; Alhasnawi *et al*., 2015).

Nutrients' uptake

Based on the outcomes, red rice genotypes exhibited nonsignificant interaction effects among the salinity levels, red rice cultivars, and ascorbic acid application for nutrient uptake. However, considerable differences emerged in each rice cultivar's response with varying ascorbic acid concentrations under saline stress conditions for nutrient uptake in red rice (Table 2).

Rice cultivar Inpari 24 under salinity (3-4 mho/cm) and with no ascorbic acid treatment showed the highest value for N uptake, while the lowest N uptake resulted in the same cultivar under salinity (>4-5 mho/cm) and ascorbic acid at 1500 ppm application. Salt stress conditions will affect plants' N uptake, and the salinity level determines the N uptake (Barus *et al*., 2019). Salinity has been proven to alter N dynamics in soils, such as mineralization, nitrification, and denitrification (Zeng *et al*., 2015; Tao *et al*., 2020), thus influencing crops' N uptake and utilization. The plant's N uptake and utilization are essential to affect several physiological processes, such as photosynthesis and yield formation (Ma *et al*., 2022).

For the P uptake, rice cultivar MSP17 under a salinity level of >4-5 mho/cm and ascorbic acid rate of 1500 ppm showed the highest value, with the lowest P uptake obtained in the cultivar Inpari 24 with salinity stress under 3-4 mho/cm and ascorbic acid (1500 ppm). Soil salinity significantly affects and reduces mineral nutrient absorption (Shrivastava and Kumar, 2015), especially phosphorus, because phosphate ions bind to Ca^{2+} ions in saline soils and become unavailable to plants (Bano and Fatima, 2009). Thus, a conclusion can be that the high P uptake in plants is an adjustment to osmotic stress and morphological adaptation by absorbing more phosphorus to maintain the crop plant ionic balance.

Red rice cultivar Inpari 7 under salinity (3-4 mho/cm) and with no ascorbic acid showed the highest value for K uptake, and the

Table 2. Nutrient uptake of four red rice cultivars under saline stress conditions with application of ascorbic acid.

Treatment combinations	Nutrient uptake (mg/plant)				
	N	P	K	Na	K/Na ratio
$S_1V_1A_0$	3.02	0.25	3.21	0.34	9.51
$S_1V_1A_1$	2.72	0.25	3.00	0.31	9.58
$S_1V_1A_2$	2.90	0.27	3.10	0.39	8.10
$S_1V_1A_3$	2.79	0.22	3.20	0.39	8.68
$S_1V_2A_0$	2.79	0.28	3.29	0.44	7.47
$S_1V_2A_1$	2.69	0.26	3.06	0.36	8.83
$S_1V_2A_2$	2.71	0.27	3.19	0.46	7.15
$S_1V_2A_3$	2.62	0.25	2.93	0.44	7.03
$S_1V_3A_0$	2.67	0.26	3.13	0.41	7.72
$S_1V_3A_1$	2.68	0.39	3.05	0.35	10.63
$S_1V_3A_2$	2.53	0.23	2.87	0.43	6.62
$S_1V_3A_3$	2.72	0.27	3.19	0.37	8.86
$S_1V_4A_0$	2.75	0.29	3.12	0.55	5.78
$S_1V_4A_1$	2.68	0.27	2.82	0.39	7.28
$S_1V_4A_2$	2.68	0.39	3.13	0.43	7.54
$S_1V_4A_3$	2.74	0.28	2.97	0.37	8.74
$S_2V_1A_0$	2.55	0.25	2.80	0.39	7.25
$S_2V_1A_1$	2.74	0.26	2.95	0.49	6.01
$S_2V_1A_2$	2.49	0.25	2.91	0.44	6.76
$S_2V_1A_3$	2.42	0.26	2.76	0.39	7.49
$S_2V_2A_0$	2.47	0.28	3.00	0.43	6.93
$S_2V_2A_1$	2.77	0.25	2.87	0.44	6.75
$S_2V_2A_2$	2.72	0.24	3.06	0.40	7.98
$S_2V_2A_3$	2.65	0.26	2.98	0.31	17.38
$S_2V_3A_0$	2.54	0.26	2.75	0.51	5.39
$S_2V_3A_1$	2.64	0.26	3.16	0.41	8.05
$S_2V_3A_2$	2.50	0.23	2.95	0.37	8.09
$S_2V_3A_3$	2.60	0.24	2.76	0.41	6.85
$S_2V_4A_0$	2.59	0.26	3.13	0.35	9.48
$S_2V_4A_1$	2.82	0.28	3.27	0.28	45.19
$S_2V_4A_2$	2.63	0.29	3.16	0.29	45.20
$S_2V_4A_3$	2.54	0.42	3.24	0.27	65.55

lowest K uptake appeared in the cultivar Pamelen with salinity (>4-5 mho/cm) and no ascorbic acid. Higher salinity levels reduced the K uptake because salinity stress affects the stimulatory effects of K nutrients in various crop plants differently. Potassium is an essential cytoplasmic element because of its involvement in osmotic regulation and competitive influence on Na^+ to salinity stress. Latef and Miransari (2014) reported potassium is vital in the osmotic adjustment under saline soil conditions. They said it contributes to turgor-media responses, such as stomata, activating various enzymatic reactions and protein synthesis when tRNA binds to ribosomes.

For Na uptake, red rice cultivar MSP17 under salinity (3-4 mho/cm) without ascorbic acid application showed the highest value, while the lowest Na uptake occurred in the same cultivar with salinity stress (>4-5 mho/cm) and ascorbic acid at 1500 ppm. However, in the same rice cultivar, the Na uptake decreased when applied with ascorbic acid, where the low Na uptake indicated plants' mechanism to salinity stress. The higher the tolerance level of crop cultivars to salinity stress, the lower the plant Na^+ concentration (Jouyban, 2012). According to Kamran *et al*. (2019), salt tolerance correlates with the sequestration of Na^+ ions into vacuoles after they enter into leaf cells to maintain a low Na^+

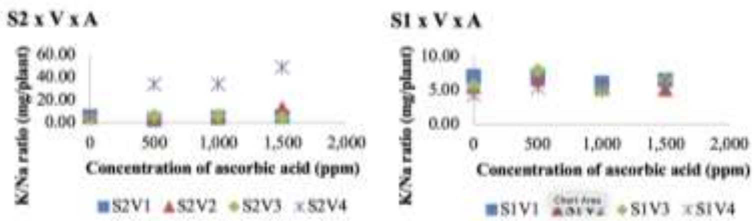

Figure 1. The K/Na ratio with the application of ascorbic acid under salinity levels a) 3-4 mho/cm, and b) >4-5 mho/cm.

concentration in the cytosol. Furthermore, once the excess Na^+ and Cl are in vacuoles, it significantly lowers the osmotic potential without any variation in the metabolic process and ultimately contributes to osmoregulation (Lee *et al*., 2013).

The K/Na ratio with ascorbic acid applications under various salinity levels appears in Figure 1. For the K/Na ratio, rice cultivar MSP17 under salinity stress (>4-5 mho/cm) plus ascorbic acid treatment (1500 ppm) revealed the maximum value, while the minimum K/Na ratio was evident in rice cultivar Pamelen under salinity stress of >4-5 mho/cm with no ascorbic acid applied. It showed that the red rice cultivar MSP17 with ascorbic acid application at 1500 ppm was superior, and one can conclude that cultivar MSP17 was the most tolerant to salinity stress conditions because it had the highest K/Na ratio. Abbasi *et al*. (2015) reported that maintaining a higher K^+/Na^+ ratio is essential for salt tolerance in crop plants. In this mechanism, the K^+/Na^+ ratio in the cytosol can remain by K^+ absorption maintenance, reducing K^+ efflux from cells, preventing Na^+ uptake, and enhancing Na^+ efflux from cells (Wakeel *et al*., 2011; Sakinah *et al*., 2022; Mukhina *et al*., 2024).

The effects of ascorbic acid application can be visible in red rice cultivars under higher salinity levels. The uptake of nutrients was higher when applying ascorbic acid at a salinity of >4-5 mho/cm than the low salinity level (3-4 mho/cm). The results indicated that ascorbic acid could increase the plant's tolerance to saline soil conditions as available nutrients improved with ascorbic acid. Previous studies reported that using ascorbic acid, as a non-enzymatic antioxidant and plant growth regulator, usually stimulates and boosts the nutrient uptake in different crop plants (Zhou *et al*., 2016) and lowers the toxic effects of soil salinity (Dolatabadian and Jouneghani, 2009). Besides, ascorbic acid usage decreases Na^+ contents in plants; however, it up-regulates the K^+ and Ca^{2+} contents under salt-stressed soil conditions (Alhasnawi *et al*., 2015). Hassan *et al.* (2021) also reported that exogenous application of ascorbic acid could lessen the adverse effect of salt stress on barley by improving morphophysiological characteristics and ion accumulation.

CONCLUSIONS

In studying the response of four different red rice cultivars, varied responses were evident to ascorbic acid application under saline soil conditions. Red rice cultivar MSP17 proved the most tolerant genotype to salinity stress conditions compared with three other rice cultivars. The ascorbic acid use also improved the physiological characteristics of red rice cultivars under the salinity stress, where the chlorophyll contents (a, b, and total) and nutrients (N, P, K, Na, and K/Na ratio) uptake were higher with ascorbic acid than the control treatment.

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