



## MORPHOPHYSIOLOGICAL AND YIELD RESPONSES OF RED RICE TO SALINITY WITH APPLICATION OF RICE HUSK ASH AND ASCORBIC ACID

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### SUMMARY

In rice production, salinity is a major limitation requiring sustainable mitigation strategies to get the optimum yield. Practical approaches, such as the use of rice husk ash (RHA) as a silica-rich soil amendment and ascorbic acid as an antioxidant, are essential to enhance plant tolerance under saline conditions. This study aimed to evaluate the effects of salinity, RHA, and ascorbic acid on the morphophysiological and yield-related traits of the red rice cultivar Pamelan, carried out in 2024 at the Growth Center LLDIKTI I, Medan, North Sumatra, Indonesia. The experiment layout was in a factorial split-plot design with salinity levels (0, 4, and 8 dS/m), RHA dosages (0, 20, 40, and 60 g/plant), and ascorbic acid concentrations (0, 500, and 1000 ppm). Salinity stress levels significantly reduced rice growth and yield components, while chlorophyll content remained relatively stable. The application of RHA at 60 g/plant significantly improved vegetative growth, while the recorded highest grain weight per panicle was 40 g/plant. The treatment of ascorbic acid notably increased proline content, with the highest accumulation observed at 1000 ppm. Overall, RHA and ascorbic acid proved an effective ameliorant for reducing salinity-induced yield losses in red rice.

**Keywords:** Red rice, salinity stress, rice husk ash, ascorbic acid, morphophysiological traits, grain yield

**Key findings:** Salinity considerably reduced vegetative growth and development and grain yield of red rice. However, RHA substantially improved vegetative performance and grain formation, while ascorbic acid aided in proline-based stress adjustment. Chlorophyll remained stable across treatments, indicating maintained photosynthetic integrity.

Communicating Editor: Dr. Turkan Hasanova Allahverdi

Manuscript received: Septe December 29, 2025; Accepted: April 24, 2026.

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**Citation:** Barus WA, Tarigan DM, Munar A, Lestami A (2026). Morphophysiological and yield responses of red rice to salinity with application of rice husk ash and ascorbic acid. *SABRAO J. Breed. Genet.* 58 (3) 1356-1366. <http://doi.org/10.54910/sabrao2026.58.3.38>.

## INTRODUCTION

Salinity is one of the main limiting factors in global rice production, especially in tropical and subtropical regions. The world's irrigated land of more than 20% has undergone degradation due to salt accumulation, with a remarkable decline in productivity (Shrivastava and Kumar, 2014). These conditions reached further exacerbation by the current climate change, seawater intrusion, and unsustainable cultivation practices (Ghirardelli *et al.*, 2024). In Indonesia, the salinity threat is increasingly evident in coastal areas, such as North Sumatra, needing technological innovations to support food security (Oelviani *et al.*, 2023).

Red rice has the highest levels of antioxidants, anthocyanins, minerals, and dietary fibers and has become recognized as a strategic commodity for supporting functional foods and food security (Haruni *et al.*, 2024). However, red rice cultivars appeared generally more sensitive to abiotic stress conditions, including salinity, and their development potential is still inadequate in marginal lands (United States Department of Agriculture, 2016). Therefore, a thorough biological approach is urgently vital to enhance the physiological resistance of rice plants to salinity stress conditions, particularly through soil amendments and physiological enhancements such as rice husk ash and ascorbic acid.

Rice husk ash (RHA) is a silica-rich soil conditioner that can improve soil structure and aeration and considerably reduce the Na<sup>+</sup> ion toxicity by increasing the cation exchange capacity (Islabão *et al.*, 2014). Silica is well known to strengthen cell walls, increase cuticle thickness, and reduce excessive transpiration rates in plants affected by salinity stress (Manimaran *et al.*, 2025). Past studies stated that RHA can reduce salt stress by enhancing ion balance, improving root development, and enhancing the plant's ability to maintain higher K<sup>+</sup>/Na<sup>+</sup> ratios under saline stress conditions (Lestari and Rachmawati, 2020). Moreover, the addition of silica has many reports of promoting better osmotic adjustment and sustaining photosynthetic activity, ultimately contributing to improved growth and yield in salt-affected crops (Khan *et al.*, 2019).

Ascorbic acid is an important antioxidant that plays a positive role in neutralizing reactive oxygen species (ROS) in plants facing salinity stress conditions (Rahman *et al.*, 2020). Exogenous application of ascorbic acid has successful reports of improving the physiological processes, such as chlorophyll stability (Rauf *et al.*, 2024), antioxidant enzyme activity (Xu and Huang, 2017), as well as preventing the ion imbalance and osmotic stress conditions (Kanwal *et al.*, 2024). However, no such comprehensive studies have come out on the effects of ascorbic acid in combination with silica-based ameliorants in red rice under salinity stress conditions.

Previous studies have separately evaluated the effects of salinity and organic amendments on rice growth and yield, and the integrated studies on salinity, RHA, and ascorbic acid in red rice are still very few. Furthermore, the physiological tolerance mechanisms involving chlorophyll content and proline accumulation have not reached an in-depth description of the red rice cultivar Pamelen, a variety released by the Indonesian Ministry of Agriculture in 2019. It serves as a functional red rice alternative with improved eating quality (softer and more palatable than conventional red rice), moderately tolerant to biotic stresses, but relatively sensitive to abiotic stresses. Thus, a considerable gap exists regarding the interactive response of these three factors in rice crops. Therefore, the pioneering study aimed to recognize the combined effects of salinity levels, rice husk ash, and ascorbic acid on the morphophysiological traits and grain yield of the red rice cultivar Pamelen. These results will provide a sound basis for the development of salinity-tolerant rice cultivation technology under saline soil conditions.

## MATERIALS AND METHODS

### Experimental material and procedure

The materials used comprised the red rice cultivar Pamelen (an inbred variety, primarily categorized as upland rice but also adaptable to irrigated conditions), ascorbic acid, rice husk

ash, NPK fertilizer, saline soil, distilled water, and chemicals needed for sample analysis in the laboratory. The research commenced in 2024 at the Growth Center LLDIKTI I, Medan, North Sumatra, Indonesia. The experiment layout was in a factorial split-plot design with three factors and replications. Levels of salinity (S) were the main plots comprising 0, 4, and 8 dS/m. Dosages of rice husk ash (R) were the subplots consisting of 0, 20, 40, and 60 g/plant. The ascorbic acid (A) concentrations were the subplots at 0, 500, and 1000 ppm.

Sowing red rice cultivar Pamelen seeds proceeded to use trays with one seed per hole. The 18-day seedlings succeeded in their transplanting into polybags (35 cm × 35 cm), each filled with approximately 5 kg of saline soil. The saline soil used as a planting medium came from three locations in Paluh Merbau Village, Percut Sei Tuan District, North Sumatra, representing different salinity levels. The initial soil salinity underwent determination based on electrical conductivity (EC) values. RHA obtained from rice husks came from a local rice mill before processing through controlled burning under open-air conditions until complete combustion to produce ash. Then, its application took place once before planting by homogeneously mixing it with the saline soil. Ascorbic acid solution preparations used distilled water before applying four times with foliar spray at 14, 28, 42, and 56 days after transplanting (DAT). Plant maintenance includes fertilization, watering, and pest control.

Fertilization continued three times, consisting of basal, first, and second topdressing applications. The basal fertilization 7 DAT consisted of 0.75 g polybag<sup>-1</sup> urea, 1.00 g polybag<sup>-1</sup> SP-36, and 0.50 g polybag<sup>-1</sup> KCl. The first topdressing (21 DAT) consisted of 1.50 g polybag<sup>-1</sup> urea, while the second topdressing (42 DAT) was 0.75 g polybag<sup>-1</sup> urea. Watering occurred once daily, either in the morning or afternoon, using a watering can equipped with a perforated nozzle to produce a gentle water flow. Carefully controlling the amount of water applied maintained soil

moisture near field capacity without interfering with the imposed salinity treatments. Pest control proceeded through routine monitoring and manual removal.

### **Leaf chlorophyll content analysis**

The study determined leaf chlorophyll content using the acetone extraction method. Fresh leaf samples (0.1 g), as collected from fully expanded upper leaves eight weeks after planting, reached homogenization in 80% (v/v) acetone and centrifugation at 10,000 rpm for 10 min. The absorbance of the supernatant measurement was at 645 and 663 nm using a UV-Vis spectrophotometer. Total chlorophyll content calculation was according to the equations proposed by Arnon (1949) and expressed as mg g<sup>-1</sup> fresh weight.

### **Proline content analysis**

Proline content analysis followed the ninhydrin-based colorimetric method. Fresh leaf tissue (0.5 g), collected from fully expanded upper leaves eight weeks after planting, entailed extraction with 3% (w/v) sulfosalicylic acid before filtration. The extract underwent a reaction with acid ninhydrin and glacial acetic acid, then incubation at 100 °C for 1 h. The reaction's termination employed an ice bath, with the chromophore extracted with toluene. Absorbance measurement at 520 nm used a spectrophotometer, with the quantification of the proline concentration using a standard curve and expressed as µmol g<sup>-1</sup> fresh weight.

### **Data recorded and analysis**

The data recorded comprised various parameters, including plant height, flag leaf area, leaf chlorophyll and proline content, the number of productive tillers, and grain weight per panicle. All the data underwent the analysis of variance. The significant mean differences sustained further comparison and separation with the Duncan's multiple range test (DMRT) at the 5% level of probability.

## RESULTS AND DISCUSSION

Analysis of variance showed salinity has a significant effect on most morphological parameters and yield. Plant height, flag leaf area, the number of productive tillers, and grain weight per panicle succeeded in their recording with significantly reduced values under the highest salinity stress conditions (Table 1). These results were consistent with the salinity stress effects, which cause growth inhibition due to osmotic pressure and the accumulation of toxic  $\text{Na}^+$  ions in plant tissues (Balasubramaniam *et al.*, 2023). Physiologically, salinity also increased the proline content, which is an adaptive response of plants in coping with abiotic stress conditions through osmoregulation mechanisms. However, salinity did not affect the chlorophyll content, indicating that ion-related physiological variations may require a longer adaptation period than morphological responses (Talla *et al.*, 2016).

### Plant height

Salinity stress conditions significantly affect the rice plant's height, with the plant height gradually decreasing as salinity increased (Table 2). Under non-saline conditions (S0), plant height reached 93.97 cm, which appeared markedly higher than at 4 dS/m (S1) with 64.99 cm and 8 dS/m (S2) with 61.30 cm. This reduction in plant height reflects typically salinity-induced growth inhibition associated with decreased cell turgor, impaired water uptake, and elevated osmotic pressure that limits stem elongation. Past studies reported the same reduction in plant height under salinity stress that has been attributed to osmotic stress and ionic toxicity, disrupting cellular homeostasis and metabolic activity in rice (Balasubramaniam *et al.*, 2023). Akter *et al.* (2014) also reported a substantial decline in plant height at the salinity level of 8 dS/m in rice crops, consistent with the pattern observed in this study.

The rice husk ash (RHA) application substantially improved plant height, and the highest mean value emerged with 60 g/plant RHA (R3), reaching 77.37 cm compared with 0

g/plant RHA (R0) with 71.76 cm. This improvement in plant height mainly showed an association with the silica content of RHA, which enhanced soil physical properties, water availability, and plant tissue strength under saline conditions. Previous studies revealed silica improved the ionic selectivity by maintaining a favorable  $\text{K}^+/\text{Na}^+$  ratio and enhancing  $\text{Na}^+$  exclusion from shoots through increased activity of ion transporters such as HKT1;5 in crop plants (Manimaran *et al.*, 2025). Additionally, reports on silica application have stated to improve the cuticle thickness, reduce water loss, and increase the plant height by up to 15%–22% in rice under salinity stress conditions (El-Okkiah *et al.*, 2022).

### Flag leaf area

Flag leaf area expressed significant effects from salinity levels, RHA, and their interactions (Table 3). The highest flag leaf area was evident at 0 dS/m salinity and 60 g/plant RHA (S0R3), reaching 166.32 cm<sup>2</sup>. Meanwhile, the lowest occurred at 4 dS/m salinity and 0 g/plant RHA (S1R0) with 54.64 cm<sup>2</sup>, revealing that salinity stress markedly reduced leaf expansion, whereas RHA mitigated this effect. This mitigation shows an association with the silica content in RHA. It improves soil physical properties, enhances water retention, and reduces  $\text{Na}^+$  toxicity by improving ion selectivity and maintaining  $\text{K}^+/\text{Na}^+$  balance, thereby preserving cell turgor and supporting leaf cell expansion under saline conditions (Jin *et al.*, 2024). The significant  $\text{S} \times \text{R}$  interaction suggested the effectiveness of RHA further increased under saline conditions, as reflected by the progressive rise in mean leaf area obtained in the 0 g/plant (77.91 cm<sup>2</sup>) and 60 g/plant (114.45 cm<sup>2</sup>). Salinity-induced reductions in leaf area signified a common link to ionic stress, membrane damage, and inhibition of cell enlargement due to excessive  $\text{Na}^+$  and  $\text{Cl}^-$  accumulation in leaf tissues of crop plants (Liu *et al.*, 2024). These past studies highlighted that leaf area was one of the most sensitive morphological traits under salinity stress conditions, supporting the pronounced decline observed in this study.

**Table 1.** Analysis of variance for morphological, physiological, and yield traits in red rice.

Source of variation	Plant height	Flag leaf area	Chlorophyll	Proline	Number of productive tillers	Grain weight per panicle
Salinity (S)	*	**	NS	*	*	*
Rice husk ash (R)	*	**	NS	*	*	*
Ascorbic acid (A)	NS	NS	NS	*	NS	NS
S x R	NS	**	NS	*	NS	NS
S x A	NS	NS	NS	*	NS	NS
R x A	NS	NS	NS	*	*	NS
S x R x A	NS	NS	NS	*	NS	NS

Note: \*\* =  $p < 0.01$ , \* =  $p < 0.05$ , NS = Nonsignificant.

**Table 2.** Plant height and flag leaf area of red rice under salinity levels and rice husk ash treatments.

Treatments	Means	
	Plant height (cm)	Flag leaf area (cm <sup>2</sup> )
Salinity (S)		
0 dS/m (S0)	93.97	140.47
4 dS/m (S1)	64.99	72.76
8 dS/m (S2)	61.30	78.71
DMRT 5%	R2=14.22, R3=14.51	R2=12.91, R3=13.17
Rice husk ash (R)		
0 g/plant (R0)	71.76	77.91
20 g/plant (R1)	72.95	92.16
40 g/plant (R2)	71.60	104.74
60 g/plant (R3)	77.37	114.45
DMRT 5%	R2=10.66, R3=11.20, R4=11.52	R2=9.07, R3=9.53, R4=9.81
Salinity (S) x Rice husk ash (R)		
0 dS/m + 0 g/plant (S0R0)	92.09	109.59
0 dS/m + 20 g/plant (S0R1)	92.19	128.57
0 dS/m + 40 g/plant (S0R2)	93.44	157.42
0 dS/m + 60 g/plant (S0R3)	98.14	166.32
4 dS/m + 0 g/plant (S1R0)	63.01	54.64
4 dS/m + 20 g/plant (S1R1)	66.56	71.44
4 dS/m + 40 g/plant (S1R2)	61.71	84.97
4 dS/m + 60 g/plant (S1R3)	68.70	79.99
8 dS/m + 0 g/plant (S2R0)	60.18	69.52
8 dS/m + 20 g/plant (S2R1)	60.10	76.45
8 dS/m + 40 g/plant (S2R2)	59.66	71.82
8 dS/m + 60 g/plant (S2R3)	65.27	97.04
DMRT 5%	NS	R2=9.07, R3=9.53, R4=9.81, R5=9.99, R6=10.14, R7=10.24, R8=10.30, R9=10.36, R10=10.42, R11=10.48, R12=10.54

Note: DMRT = Duncan's multiple range test, NS= Nonsignificant.

With the RHA application, the flag leaf area increase revealed the positive role of silica as a soil and physiological ameliorant under salinity stress conditions. The silica helps maintain leaf tissue integrity by reducing Na<sup>+</sup> toxicity, improving K<sup>+</sup>/Na<sup>+</sup> homeostasis, and

sustaining cell turgor necessary for leaf growth and expansion. Previous studies reported silica application enhanced the epidermal strength, reduced the transpiration, and improved the water use efficiency, leading to a larger leaf area in rice grown under saline stress

**Table 3.** Chlorophyll content of red rice under salinity, rice husk ash, and ascorbic acid treatments.

Treatments	0 ppm (A <sub>0</sub> )	500 ppm (A <sub>1</sub> ) .....mg/g.....	1000 ppm (A <sub>2</sub> )
0 dS/m + 0 g/plant (S0R0)	77.42	85.25	87.73
0 dS/m + 20 g/plant (S0R1)	87.99	87.63	86.93
0 dS/m + 40 g/plant (S0R2)	85.11	89.54	88.74
0 dS/m + 60 g/plant (S0R3)	87.29	87.98	90.48
4 dS/m + 0 g/plant (S1R0)	89.29	90.21	81.96
4 dS/m + 20 g/plant (S1R1)	87.05	87.29	90.07
4 dS/m + 40 g/plant (S1R2)	89.38	89.31	89.70
4 dS/m + 60 g/plant (S1R3)	87.15	89.44	88.89
8 dS/m + 0 g/plant (S2R0)	91.38	88.17	89.15
8 dS/m + 20 g/plant (S2R1)	92.05	83.90	86.03
8 dS/m + 40 g/plant (S2R2)	90.32	89.56	86.99
8 dS/m + 60 g/plant (S2R3)	88.55	89.46	91.33

conditions (Khan *et al.*, 2019). Moreover, the enhanced flag leaf area has considerably shown to have an association with improved grain filling and yield, as the flag leaf is the primary source of photosynthates during the reproductive stage (Rahman *et al.*, 2014).

### Chlorophyll

The levels of salinity, RHA, ascorbic acid, and their interactions have a nonsignificant effect on chlorophyll content (Table 3). This result suggests the salinity levels applied in this study were not severe enough to induce chlorophyll degradation. The minor variations among the treatments (77-92 mg/g) revealed the red rice cultivar Pamelen maintained photosynthetic capacity despite reduced morphological growth. Stable chlorophyll content also suggested that the applied salinity levels were below the threshold for severe chloroplast damage, and the plants may have also activated endogenous protective mechanisms to preserve chlorophyll pigments (Zhao *et al.*, 2019). Under salinity stress conditions, chlorophyll maintenance reflects the ability of rice plants to preserve thylakoid membrane integrity under moderate ionic stress. Chlorophyll degradation generally occurs when excessive Na<sup>+</sup> and Cl<sup>-</sup> accumulation damages the photosynthetic proteins, such as the D1 protein of photosystem II (Hameed *et al.*, 2021). Tarigan *et al.* (2025) further reported several rice genotypes maintained stable chlorophyll

content under moderate salinity levels, even though the vegetative growth received notable effects.

The lack of significant positive effect of RHA and ascorbic acid on chlorophyll content detailed that their role under the tested conditions was largely indirect. Silica primarily enhanced the structural strength and water-use efficiency rather than directly increasing the chlorophyll concentration (Rastogi *et al.*, 2021), while ascorbic acid emerged as most effective in protecting chlorophyll under severe oxidative stress conditions (Rauf *et al.*, 2024). The stable chlorophyll content across various treatments further suggested oxidative damage was less, with the internal redox balance sufficiently maintained in the red rice cultivar Pamelen under the applied salinity levels (Johnson *et al.*, 2024).

### Proline

Salinity levels, rice husk ash (RHA), ascorbic acid, and their interactions significantly affect the proline content in red rice (Table 4). Proline accumulation was the highest under moderate salinity of 4 dS/m (S1), followed by 0 dS/m (S0) and 8 dS/m (S2), exhibiting that osmotic adjustment was most active at stress levels, still permitting physiological adaptation. Proline is a key osmoprotectant and plays a viable role in maintaining cellular osmotic balance and stabilizing proteins under salinity stress conditions (Hayat *et al.*, 2012). Koc *et al.* (2024) reported moderate salinity levels

**Table 4.** Proline content of red rice under salinity, rice husk ash, and ascorbic acid treatments.

Treatments	0 ppm (A <sub>0</sub> )	500 ppm (A <sub>1</sub> )	1000 ppm (A <sub>2</sub> )	Means	DMRT 5%
	.....µmol/g.....				
0 dS/m (S <sub>0</sub> )	0 g/plant (R <sub>0</sub> )	0.90	0.55	0.69	1.26
	20 g/plant (R <sub>1</sub> )	1.12	1.41	1.95	
	40 g/plant (R <sub>2</sub> )	4.36	0.30	0.97	
	60 g/plant (R <sub>3</sub> )	0.52	1.16	1.15 <sup>j</sup>	
4 dS/m (S <sub>1</sub> )	0 g/plant (R <sub>0</sub> )	0.25	3.14	3.12	2.32
	20 g/plant (R <sub>1</sub> )	3.13	2.96	2.54	
	40 g/plant (R <sub>2</sub> )	1.95	2.41	3.34	
	60 g/plant (R <sub>3</sub> )	1.88	1.50	1.57	
8 dS/m (S <sub>2</sub> )	0 g/plant (R <sub>0</sub> )	0.27	1.28	1.18	0.85
	20 g/plant (R <sub>1</sub> )	1.49	0.36	0.46	
	40 g/plant (R <sub>2</sub> )	0.42	1.05	1.41	
	60 g/plant (R <sub>3</sub> )	0.26	0.72	1.25	
DMRT 5%	R <sub>2</sub> =0.17, R <sub>3</sub> =0.17, R <sub>4</sub> =0.18, R <sub>5</sub> =0.18, R <sub>6</sub> =0.19, R <sub>7</sub> =0.19, R <sub>8</sub> =0.19, R <sub>9</sub> =0.19, R <sub>10</sub> =0.19, R <sub>11</sub> =0.20, R <sub>12</sub> =0.20				
Mean	1.38	1.40	1.64		
DMRT 5%	R <sub>2</sub> =0.17, R <sub>3</sub> =0.17				

Note: DMRT = Duncan's multiple range test.

often induced a higher proline synthesis than higher salinity, where metabolic activity becomes constrained.

Considerable interactions among different levels of salinity, RHA, and ascorbic acid resulted in marked variations in proline accumulation. Several variants' combinations under 4 dS/m (S<sub>1</sub>) exhibited the highest proline levels, whereas accumulation declined under a high salinity of 8 dS/m (S<sub>2</sub>), suggesting reduced adaptive capacity at the elevated salinity stress conditions. Mushtaq *et al.* (2025) demonstrated that excessive salinity suppressed proline biosynthesis due to a disruption of nitrogen metabolism. Similar response patterns have occurred in rice exposed to severe salinity, where osmoprotectant production was insufficient (Nguyen *et al.*, 2021).

The treatments of RHA and ascorbic acid further modulated proline responses depending on the salinity levels. Silica from RHA likely reduced Na<sup>+</sup> toxicity and improved root physiological functions, indirectly supporting osmoprotectant accumulation. Islabão *et al.* (2014) reported that silica enhanced plant salinity tolerance by improving water relations and restricting sodium uptake. Ascorbic acid strengthened the antioxidant

defense system, facilitating coordinated osmotic and oxidative stress responses, including enhanced proline accumulation in crop plants (Akram *et al.*, 2017).

### The number of productive tillers

The productive tillers incurred significant effects from different levels of salinity, RHA, and the R × A interactions. The 0 dS/m (S<sub>0</sub>) produced the highest number of productive tillers (8.28), whereas the 4 dS/m (S<sub>1</sub>) and 8 dS/m (S<sub>2</sub>) treatments showed a marked decline for the said trait (Table 5). This reduction reflects the integrated effect of decreased number of tillers and impaired panicle development under salinity stress conditions. Salinity disrupts the photosynthate allocation, suppresses meristem activity, and increases the Na<sup>+</sup> and Cl<sup>-</sup> toxicity in developing shoots, thereby limiting the conversion of tillers into productive panicles (Parida and Das, 2005). Salinity stress modifies rice tiller formation primarily by reducing tillering capacity, with primary and secondary tillers being more sensitive than the main stem (Zhang *et al.*, 2024). This reduction has an association with impaired nutrient uptake and ionic imbalance under saline conditions, which

**Table 5.** Number of productive tillers and grain weight per panicle of red rice under salinity, rice husk ash, and ascorbic acid treatments.

Treatments	Means		
	Number of productive tillers		Grain weight per panicle (g)
<b>Salinity (S)</b>			
0 dS/m (S0)	8.28		28.96
4 dS/m (S1)	4.07		24.04
8 dS/m (S2)	4.68		22.45
DMRT 5%	R2=3.26, R3=3.32		R2=8.44, R3=8.61
<b>Rice husk ash (R)</b>			
0 g/plant (R0)	5.26		23.62
20 g/plant (R1)	5.48		25.37
40 g/plant (R2)	5.69		26.15
60 g/plant (R3)	6.28		25.45
DMRT 5%	R2=1.96, R3=2.06, R4=2.12		R2=5.10, R3=5.36, R4=5.51
<b>Rice husk ash (R) x Ascorbic acid (A)</b>			
0 g/plant + 0 ppm (R0A0)	4.56		23.26
0 g/plant + 500 ppm (R0A1)	5.56		23.50
0 g/plant + 1000 ppm (R0A2)	5.67		24.09
20 g/plant + 0 ppm (R1A0)	5.67		25.56
20 g/plant + 500 ppm (R1A1)	5.22		25.17
20 g/plant + 1000 ppm (R1A2)	5.56		25.38
40 g/plant + 0 ppm (R2A0)	5.78		26.07
40 g/plant + 500 ppm (R2A1)	6.00		27.82
40 g/plant + 1000 ppm (R2A2)	5.28		24.56
60 g/plant + 0 ppm (R3A0)	5.61		25.01
60 g/plant + 500 ppm (R3A1)	6.11		24.72
60 g/plant + 1000 ppm (R3A2)	7.11		26.62
DMRT 5%	R2=1.66, R3=1.75, R4=1.80, R5=1.84, R6=1.87, R7=1.90, R8=1.91, R9=1.93, R10=1.94, R11=1.97, R12=1.98		NS

Note: DMRT = Duncan’s multiple range test, NS= Nonsignificant.

decrease soil fertility and limit the availability of essential nutrients for growth. Moreover, salinity disrupts the transport of water and nutrients between the main stem and tillers through vascular bundles at the tiller nodes, which is crucial for tiller development and survival. Consequently, these limitations lead to reduced tiller formation or even tiller mortality due to nutrient deficiency (Yang *et al.*, 2022).

Rice husk ash raised the number of productive tillers progressively, ranging from 0 g/plant (5.26) to 60 g/plant (6.28), highlighting the remarkable role of silica in improving root function, water-use efficiency, and stem strength. Silica enhanced the structural integrity and reduced ionic toxicity in reproductive tissues, supporting tiller survival

and panicle formation (Shen *et al.*, 2022). The highest number of productive tillers was evident under the 60 g/plant RHA and 1000 ppm ascorbic acid (R3A2) combination (7.11), indicating synergistic combined effects of the RHA and ascorbic acid. Ascorbic acid complements silica-mediated stress alleviation by scavenging reactive oxygen species and maintaining meristem viability, thereby sustaining reproductive development under salinity stress conditions (Aziz *et al.*, 2025).

### Grain weight per panicle

Grain weight per panicle gained remarkable changes from salinity and RHA, with a nonsignificant influence of ascorbic acid (Table

5). The highest grain weight appeared under non-saline conditions (0 dS/m) at 28.96 g, while moderate and highest salinity levels reduced the grain weight to 24.04 g (4 dS/m) and 22.45 g (8 dS/m), respectively. This reduction appeared to be primarily associated with limited photosynthate availability due to impaired vegetative growth under salinity stress conditions. Salinity disrupts photosynthesis, damages stomatal function, and heightens the toxic ion accumulation, thereby restricting assimilate supply to developing grains (Riaz *et al.*, 2019). Moreover, salinity inhibits the starch-synthesizing enzymes during grain filling, resulting in incomplete grain formation (Wei *et al.*, 2024).

Rice husk ash increased the grain weight from 0 g/plant (23.62 g) to 40 g/plant (26.15 g), revealing that improved soil conditions and enhanced silica availability positively influenced rice grain filling. Silica strengthened the stems and panicles, improved water uptake, and enhanced the photosynthetic efficiency, thereby enhancing assimilate translocation to grains (Jin *et al.*, 2024). Lavinsky *et al.* (2016) similarly reported silica application under soil saline conditions improved grain weight and yield by maintaining the root's function and reducing Na<sup>+</sup> accumulation in reproductive tissues. These confirmed the role of RHA as a soil ameliorant and physiological enhancer in grain filling.

## CONCLUSIONS

Salinity stress significantly reduced growth and development and yield components of red rice. The application of rice husk ash (RHA) at 60 g/plant substantially improved vegetative growth, while the highest grain weight per panicle was noteworthy at 40 g/plant. The treatment of ascorbic acid notably increased proline content, with the highest accumulation observed at 1000 ppm. Proline rose with salinity, while chlorophyll content remained relatively stable across all variants. The RHA influence was consistently greater than that of ascorbic acid, although their interaction

provided additional physiological benefits under salinity stress conditions (4–8 dS/m). The results disclosed that RHA and ascorbic acid proved to be an effective agronomic input for mitigating salinity-induced yield losses in red rice.

## ACKNOWLEDGMENTS

The authors gratefully acknowledge the Ministry of Higher Education, Science, and Technology for funding the research through the Fundamental Research—Regular Scheme 2024, and the Universitas Muhammadiyah Sumatera Utara, Medan, Indonesia, for facilitating the presented research.

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