# Effect of Gypsum Application on Salinity Stress Mitigation at Different Growth Stages of Boro Rice cv. BRRI dhan47

#### Abstract

Salinity is a critical abiotic stress in Bangladesh, severely limiting rice growth and yield due to the crop's high sensitivity to saline conditions. While gypsum application is known to mitigate salinity stress, its effects on different growth stages of rice under varying salinity levels remain inadequately explored. To address this gap, a study was conducted in the net house of the Department of Agronomy, Bangladesh Agricultural University, Mymensingh (24.75°N, 90.5°E), during the Boro season (November 2023 to April 2024). The experiment employed a Completely Randomized Design (CRD) with four salinity levels (0, 4, 8, and 12 dS m<sup>-1</sup>) and three gypsum levels (0, 1, and 2 g gypsum kg<sup>-1</sup> soil) applied across three growth stages: tillering, panicle initiation, and flowering. Results demonstrated that high salinity (120 mM NaCl) significantly reduced plant height (9.63%), tiller number (7.8%), leaf area index (19.32%), and grain yield (24.19%) compared to the control. In contrast, gypsum application at 2 g kg<sup>-1</sup> soil effectively mitigated salinity stress, enhancing leaf area index (5%), root length (2%), and grain yield (29.55%) over the control. Moreover, it recovered almost 8% grain yield under highly saline conditions. The findings highlight gypsum's potential as a practical and effective soil amendment for improving rice performance in saline environments. Future research should investigate the long-term effects of gypsum application under field conditions and explore its integration with complementary agronomic practices for sustainable rice production.

*Keywords:* Salinity, Gypsum, Completely Randomized Design (CRD), Tillering, Panicle Initiation, Flowering

#### 1. Introduction

Rice, the staple food for over half of the world's population, is cultivated in more than 100 countries, with Asia contributing 90% of the global production [1]. In Bangladesh, where per capita rice consumption is among the highest globally, rice plays a critical role in ensuring nutritional security as it is the primary source of dietary energy [2]. Approximately 75% of the country's total cropped area and over 80% of irrigated land are dedicated to rice cultivation [3].

However, rice cultivation faces significant challenges from abiotic stresses such as drought, salinity, and extreme temperatures, all of which threaten global food security [4,5]. Among these, salinity is one of the most significant constraints to rice production [6]. Saline soils, characterized by high concentrations of sodium cations and soluble chloride and sulfate anions, exhibit electrical conductivity (EC) levels exceeding 4 dS m<sup>-1</sup>, an Exchangeable Sodium Percentage (ESP) of less than 15, and relatively low pH compared to sodic soils [3].

In Bangladesh, salinity stress is particularly acute in the southern coastal regions, where approximately 1.056 million hectares of coastal land—out of a total of 1.689 million hectares—are affected by varying degrees of soil salinity, with some areas recording EC levels as high as 18.5 dS m<sup>-1</sup>. This impacts 49 Upazilas across 19 districts [7]. Salinity reduces agricultural productivity by inducing osmotic stress, restricting water availability, and impairing plant growth. These effects are particularly detrimental during key growth stages, influencing processes such as seed germination, photosynthesis, nutrient uptake, and hormonal regulation, ultimately reducing both seed quality and quantity [8].

Salinity also disrupts ion homeostasis, impairing the uptake of essential nutrients like potassium (K) while promoting toxic sodium (Na) accumulation. This imbalance negatively impacts chlorophyll content, leaf morphology, photosynthetic efficiency, and overall plant vigor [9,10]. As a moderately salt-sensitive crop, rice exhibits reduced grain yield traits, including grain weight, seed set rate, panicle length, and grain number per panicle, when soil salinity exceeds the critical threshold of 3.0 dS m<sup>-1</sup> [11]. For instance, an electrical conductivity (EC) level of 6.0 dS m<sup>-1</sup> can reduce rice grain yield by as much as 50% [12].

To cope with salinity stress, rice plants employ physiological, biochemical, and genetic mechanisms, such as the biosynthesis and accumulation of osmolytes, ion homeostasis and compartmentation, reactive oxygen species (ROS) detoxification, and programmed cell death [13]. However, these natural mechanisms are often insufficient under high salinity conditions, necessitating effective agronomic interventions.

Agronomic practices, including the application of organic amendments such as duckweed, dhaincha, mustard seed meal, rice straw compost, and sawdust, as well as inorganic amendments like gypsum and silicon, have been shown to alleviate salinity stress in rice [14,15,16,17]. Among

these, gypsum (CaSO<sub>4</sub>·2H<sub>2</sub>O) has gained significant attention for its ability to mitigate salinity stress. Gypsum works by exchanging sodium ions (Na<sup>+</sup>) with calcium ions (Ca<sup>2+</sup>), thereby enhancing the Ca<sup>2+</sup>/Na<sup>+</sup> ratio, while also supplying sulfur, which promotes the production of phytohormones, amino acids, and osmoprotectants vital for salinity stress tolerance [18]. Additionally, gypsum application improves soil properties by reducing pH, electrical conductivity, and the sodium adsorption ratio, which, in turn, enhances root length, paddy yield, and overall crop performance under saline conditions [19].

Previous studies have extensively examined the role of gypsum application in mitigating salinity stress in rice. However, there is limited understanding of how gypsum influences different growth stages of rice under varying levels of salinity. To address this gap, the present study aims to evaluate the effects of different salinity levels on various growth stages of rice and to investigate the efficacy of gypsum in alleviating salinity stress across these stages.

### 2. Materials and Methods

#### 2.1 Experimental site and soil

The experiment was conducted in the net house of the Department of Agronomy, Bangladesh Agricultural University, Mymensingh (24.75°N, 90.5°E), during the Boro season, spanning from November 2023 to April 2024. Soil samples for the experimental pots were collected from the Agronomy Field Laboratory of Bangladesh Agricultural University at a depth of 0–15 cm. The morphological, physical, and chemical properties of the soil are presented in Tables 1, 2, and 3.

A. Physical Characteristics of Soil	Results	Methods
Sand (%) (0.0-0.02 mm)	20	Hydrometer [20]
Silt (%) (0.02-0.002 mm)	67	
Clay (%) (<0.002 mm)	13	
Soil textural class	Silt loam	
Particle density (g/cc)	2.60	[21].
Bulk density (g/cc)	1.35	[22].
Porosity (%)	46.67	

## Table 1. Physical properties of initial soil

<b>B.</b> Chemical Characteristics of Soil	Results	Methods
pH	6.20	Glass Electrode pH meter [23].
Organic carbon (%)	1.67	Wet oxidation [24].
Total Nitrogen (%)	0.101	Semi-micro Kjeldahl [25].
Available Phosphorus (P) (ppm)	26.00	Olsen [26].
Exchangeable Potassium (K) (me%) %)	0.14	Ammonium acetate Extraction [27].
Available Sulfur (S) (ppm)	13.90	CaCl <sub>2</sub> Extraction [28].
Available Zinc (Zn) (ppm)	0.92	[29].
EC (dS $m^{-1}$ )	0.348	[30].

Table 2. Chemical properties of initial soil

## 2.2 Design and treatments of the study

The experiment was conducted in plastic pots with a depth of 15 cm and a diameter of 13.5 cm, arranged following a Completely Randomized Design (CRD) with three replications. A total of 90 pots were used in the study.

The experiment consisted of three factors:

## Factor A. Growth Stage

- 1. Tillering stage (T)
- 2. Panicle Initiation stage (P)
- 3. Flowering stage (F)

## **Factor B. Salinity Levels**

- 1.  $S_0$  (control): No salinity
- 2. S<sub>1</sub>: 40 mM NaCl (4 dS m<sup>-1</sup>)
- 3. S<sub>2</sub>: 80 mM NaCl (8 dS m<sup>-1</sup>)
- 4. S<sub>3</sub>: 120 mM NaCl (12 dS m<sup>-1</sup>)

## Factor C. Gypsum levels

1. G<sub>0</sub> (control): No gypsum

- 2. G<sub>1</sub>: 1 g gypsum kg<sup>-1</sup> soil
- 3.  $G_2$ : 2 g gypsum kg<sup>-1</sup> soil

#### 2.3 Management of the crop

BRRI dhan47, a salt-tolerant rice variety developed by BRRI, was selected as the test crop for this study. According to BRRI, this variety can tolerate salinity levels of 12–14 dS m<sup>-1</sup> at the seedling stage and 6 dS m<sup>-1</sup> at the maturity stage [31]. Each pot was filled with 9.5 kg of soil, leveled, and fertilized following the guidelines of the Fertilizer Recommendation Guide [32]. The fertilizers were calculated and applied including 1.7 g pot<sup>-1</sup> urea, 0.4 g pot<sup>-1</sup> TSP, 0.71 g pot<sup>-1</sup> MoP, 0.25 g pot<sup>-1</sup> gypsum, and 0.035 g pot<sup>-1</sup> zinc sulfate to supply N, P, K, S, and Zn, respectively. All fertilizers, except urea, were incorporated into the soil during the final soil preparation. Urea was applied in three equal splits at 10, 30, and 50 days after transplanting (DAT) of rice [32]. Intercultural operations, including weeding, thinning, gap filling, and irrigation, were carried out as needed to ensure optimal growth of the crop.

## 2.4 Incorporation of Salinity and Gypsum

Commercial-grade sodium chloride (NaCl) was used to induce salinity in the soil of the pots. For the salinity treatments, solutions with electrical conductivities of 4 dS m<sup>-1</sup>, 8 dS m<sup>-1</sup>, and 12 dS m<sup>-1</sup> were prepared by dissolving 2.3376 g, 4.6752 g, and 7.0128 g of NaCl, respectively, in 1 L of water. These solutions, corresponding to salinity treatments  $S_1$  (4 dS m<sup>-1</sup>),  $S_2$  (8 dS m<sup>-1</sup>), and  $S_3$  (12 dS m<sup>-1</sup>), were evenly applied to the pots at specific growth stages: tillering (T), panicle initiation (P), and flowering (F). For the gypsum treatments, 9.5 g (G<sub>1</sub>) and 19 g (G<sub>2</sub>) of gypsum were applied to the respective pots immediately after the NaCl solutions were added.

## 2.5 Harvesting and Data Collection

The crops were harvested pot-wise at full maturity on April 30, 2024. Yield measurements were carefully conducted, with the grain yield adjusted to a 14% moisture basis, ensuring accurate standardization. The straw yield was evaluated under sun-dried conditions, following the procedure described by Nasim et al. [33]. Subsequently, the weights of grain and straw were converted into tons per hectare (t ha<sup>-1</sup>) for consistency in reporting. The harvest index (%) was calculated using the formula proposed by Ripta et al. [34]:

Harvest Index (%) = (Grain yield / Biological yield) \* 100

Data collection encompassed a range of growth parameters, including plant height, effective tillers per hill, root length, leaf area index (LAI), and root-to-shoot (RS) ratio. These parameters were recorded at various critical growth stages, namely tillering, panicle initiation, and flowering stages, to capture dynamic changes during crop development.

The leaf area was measured using an automatic leaf area meter (Type AAN-7, Hayashi DamKo Co., Japan). The leaf area index (LAI) was subsequently calculated as the ratio of total leaf area to ground area, using the formula described by Rana et al. [35]:

LAI = Total Leaf Area (cm<sup>2</sup>) / Ground Area (cm<sup>2</sup>)

Similarly, the root-to-shoot (RS) ratio was determined by dividing the dry weight of roots (g) by the dry weight of the shoot, following the method outlined by Huang et al. [36]. After harvesting, yield-contributing parameters, such as the number of spikelets per panicle and the 1000-grain weight, were evaluated.

### 2.6 Statistical analysis

The experimental data were analyzed using R programming software (version 4.2.2) based on a three-factor Completely Randomized Design (CRD). Analysis of variance (ANOVA) was performed to determine the significance of treatment effects, and Tukey's Honest Significant Difference (HSD) test was used for pairwise comparisons at the 5% significance level, following the methodology outlined by Gomez and Gomez [37]. Graphical representations of the results were created using the ggplot2 package in R. Principal Component Analysis (PCA) was performed under the factoextra package in the R programming environment.

## **3. Results and Discussion**

## 3.1 Physiological and Morphological Responses of Rice to Salinity and Gypsum Levels Across Growth Stages

Statistically significant differences in different growth parameters were observed across different salinity and gypsum levels, as well as their interactions (Table 4).

### 3.1.1 Plant height

Plant height ranged from 103.1 cm at the tillering stage to 104.91 cm at the flowering stage, indicating a slight increase in growth (Table 4). This trend aligns with the findings of Wei et al. [38], who reported a consistent increase in plant height as growth progressed.

Salinity had a notable impact, with plant height decreasing by 9.63% under  $S_3$  (120 mM NaCl) compared to the control ( $S_0$ ). These results are consistent with the findings of Xu et al. [39], who reported that elevated salinity levels disrupted osmotic potential, ionic equilibrium, and nutrient uptake, ultimately leading to reduced plant growth. Conversely, gypsum application exhibited a positive effect, with plant height increasing by 3% under  $G_2$  (2 g gypsum kg<sup>-1</sup> soil) compared to  $G_0$  (no gypsum).

## 3.1.2 Effective tillers hill<sup>-1</sup>

The number of effective tillers per hill increased by 8.5% at the flowering stage compared to the panicle initiation stage, reflecting the natural progression of growth (Table 4). However, salinity stress had a significant negative impact, with the S<sub>3</sub> treatment (120 mM NaCl) causing a 7.8% decline in tiller number compared to the control (S<sub>0</sub>). These findings are in line with Mojakkir et al. [40], who reported a 43% decline in tiller number under salinity stress. The reduction can be attributed to ion toxicity, particularly from sodium (Na<sup>+</sup>) and chloride (Cl<sup>-</sup>), which disrupt cellular processes such as division and elongation—key mechanisms for tiller formation, as described by Lashari et al. [41]. In contrast, gypsum application at G<sub>2</sub> (2 g gypsum kg<sup>-1</sup> soil) enhanced tiller production by approximately 3% over G<sub>0</sub> (no gypsum). This might be since gypsum likely

mitigated salinity effects by removing excessive salt ions, thereby improving growth traits as supported by Alim et al. [42].

## 3.1.3 Leaf Area Index (LAI), root length, and root-shoot ratio

All three parameters—LAI, root length, and root-shoot (RS) ratio—significantly increased with the progression of growth stages (Table 4). However, high salinity levels (S<sub>3</sub>) caused a 19.32%, 8.67%, and 14.28% reduction in LAI, root length, and RS ratio, respectively. The reduction in LAI was attributed to salinity-induced negative effects on leaf photosynthetic characteristics, which reduced photosynthesis, transpiration rates, and stomatal conductance, as suggested by Wei et al. [43]. Furthermore, Studies by Lv et al. [44], Riaz et al. [45], and Coca et al. [46] indicated that excess sodium ions disrupted the Na<sup>+</sup>/K<sup>+</sup> balance, impaired essential physiological processes, caused root cell damage, and, along with increased reactive oxygen species (ROS) production under salinity, exacerbated cellular damage and inhibited root growth.

In contrast, gypsum application at 2 g kg<sup>-1</sup> soil (G<sub>2</sub>) improved these parameters by 5%, 2%, and 4%, respectively. This improvement was likely due to gypsum's ability to facilitate the exchange of Na<sup>+</sup> with Ca<sup>2+</sup>, thereby increasing the Ca<sup>2+</sup>/Na<sup>+</sup> ratio in the soil which was crucial for maintaining root development and overall plant health, as highlighted by Bello et al. [47].

Treatment	Plant height	Effective tillers	LAI	Root Length	Root-shoot ratio
Growth					
Tillering (T)	103.1b	9.16c	1.79c	32.55	0.42
Panicle Initiation (P)	103.59b	9.84b	2.31b	37.13	0.5
Flowering (F)	104.91a	10.01a	3.01a	41.43	0.64
CV (%)	0.3	2.89	1.6	0.45	0.69
Sig.	**	***	***	***	***
SE (±)	4.47	0.04	0.006	0.03	0.003
Salinity					
$S_0$	109.41a	13.58a	2.64a	38.65	0.56
$\mathbf{S}_1$	106.36b	10.27b	2.42b	37.69	0.53
$\mathbf{S}_2$	100.83c	8.18c	2.29c	36.5	0.51
$S_3$	98.87d	6.66d	2.13d	35.3	0.48
CV (%)	0.3	2.89	1.6	0.45	0.69
Sig.	***	***	***	***	***
SE (±)	0.06	0.05	0.007	0.03	0.006
Gypsum					
G <sub>0</sub>	103.04a	8.27c	2.31c	36.71	0.51
G1	103.33b	9.09b	2.37b	37.06	0.52
G <sub>2</sub>	105.23a	11.65a	2.43a	37.36	0.53
CV (%)	0.3	2.89	0.007	0.45	0.69
Sig.	***	***	***	***	***
SE (±)	0.05	0.05	0.006	0.02	0.006
Interaction					
Growth*Salinity	***	***	***	***	***
Growth*Gypsum	***	***	***	NS	NS
Salinity*Gypsum	***	***	NS	NS	NS
rowth*Salinity*Gypsum	***	***	***	**	NS

Table 4. Morphological and growth traits of rice

In a column, figures with the same letter (s) or without a letter do not differ significantly. \*\*\* = Significant at 0.1% level of probability, \*\* = Significant at 1% level of probability, NS = Non-significant.

# 3.2 Yield Contributing Characters of Rice as Influenced by Various Salinity and Gypsum Levels

Statistically significant differences were observed in panicle length, the number of fertile spikelets, and 1000-grain weight under varying salinity and gypsum treatments (Table 5). High salinity stress (S<sub>3</sub>) led to reductions of 5.45%, 20.4%, and 5.51% in panicle length, the number of fertile spikelets, and 1000-grain weight, respectively, aligning with the findings of Ismail and Horie [48]. Salinity-induced Na<sup>+</sup> toxicity resulted in nutritional imbalances and a decline in photosynthesis, which restricted plant growth and development, as reflected by reduced plant height, tiller number, biomass accumulation, and relative growth rate, as reported by Meng et al. [49] and Xu et al. [38]. Conversely, gypsum application significantly improved these yield parameters. Panicle length and the number of fertile spikelets increased by 5.74% and 12.09%, respectively, under G<sub>2</sub> (2 g gypsum kg<sup>-1</sup> soil) compared to G<sub>0</sub> (no gypsum). The beneficial effects of gypsum were attributed to its ability to replace Na<sup>+</sup> with Ca<sup>2+</sup> in the soil exchange complex, improving soil structure, reducing salinity stress, and facilitating the leaching of sodium ions below the root zone with irrigation water, thereby creating a more favorable environment for plant growth and yield enhancement, as suggested by Khan et al. [50].

Treatment	Plant height	Effective tillers	Root Length	Root-shoot ratio
Growth				
Tillering (T)	103.1b	9.16c	32.55	0.42
Panicle Initiation (P)	103.59b	9.84b	37.13	0.5
Flowering (F)	104.91a	10.01a	41.43	0.64
CV (%)	0.3	2.89	0.45	0.69
Sig.	**	***	***	***
SE (±)	4.47	0.04	0.03	0.003
Salinity				
$\mathbf{S}_0$	109.41a	13.58a	38.65	0.56
$\mathbf{S}_1$	106.36b	10.27b	37.69	0.53
$S_2$	100.83c	8.18c	36.5	0.51
$S_3$	98.87d	6.66d	35.3	0.48
CV (%)	0.3	2.89	0.45	0.69
Sig.	***	***	***	***
SE (±)	0.06	0.05	0.03	0.006
Gypsum				
$G_0$	103.04a	8.27c	36.71	0.51
$G_1$	103.33b	9.09b	37.06	0.52
G <sub>2</sub>	105.23a	11.65a	37.36	0.53
CV (%)	0.3	2.89	0.45	0.69
Sig.	***	***	***	***
SE (±)	0.05	0.05	0.02	0.006
Interaction				
Growth*Salinity	***	***	***	***
Growth*Gypsum	***	***	NS	NS
Salinity*Gypsum	***	***	NS	NS
Growth*Salinity*Gypsum	***	***	**	NS

Table 5. Yield contributing characteristics of rice

In a column, figures with the same letter (s) or without a letter do not differ significantly. \*\*\* = Significant at 0.1% level of probability, \*\* = Significant at 1% level of probability, NS = Non-significant.

## 3.3 Yield parameters of rice as influenced by various salinity and gypsum levels

Statistically significant differences were observed in grain yield under varying salinity and gypsum treatments, while straw yield did not exhibit any significant variation as displayed in Figure 1. Grain yield decreased by 24.19% under high salinity stress compared to no salinity (S<sub>0</sub>). These findings aligns with the results of Irin et al. [14], who reported that salinity stress reduced grain yield due to its adverse effects on plant growth, relative water content, and membrane stability. Moreover, the reduction in grain yield was closely associated with a decline in yield-contributing traits, particularly under abiotic stresses, as suggested by Nutan et al. [51]. This trend was further corroborated by the PCA analysis, as illustrated in Figure 2.

In contrast, the application of gypsum significantly ameliorated the negative effects of salinity stress, increasing grain yield by 29.55% over the control treatment (G<sub>0</sub>). The beneficial effects of gypsum can be attributed to its calcium and sulfur content, which promoted plant growth, emergence, and yield under saline-sodic conditions, as supported by Qayyum et al. [52] and Rehman et al. [53]. Specifically, gypsum application improved soil structure and reduced exchangeable Na<sup>+</sup> concentrations by boosting Ca<sup>2+</sup> ions on clay surfaces, as described by Abdul Qadir et al. [54]. Furthermore, Hamoud et al. [55] demonstrated that gypsum application under salinity stress enhanced grain weight, total yield, and nutrient uptake while reducing leaf Na<sup>+</sup> content due to the presence of sulfur in its composition.



Figure 1. Yield parameters of rice as influenced by various salinity and gypsum levels: (a) Grain yield under salinity levels, (b) Grain yield under gypsum levels, (c) Straw yield under salinity levels, (d) Straw yield under gypsum levels. Data are presented as mean  $\pm$  SE (n = 3).



Figure 2. PCA of growth and yield parameters of rice

#### 4. Conclusion

This study highlights the detrimental effects of salinity stress on the growth and yield of Boro rice cv. BRRI dhan47, particularly under high salinity levels. Salinity significantly reduced key growth parameters, including plant height, tiller number, and LAI, as well as yield-contributing traits like panicle length and grain weight. However, gypsum application proved to be an effective agronomic strategy for mitigating salinity stress. The application of 2 g gypsum kg<sup>-1</sup> soil improved physiological traits such as root length and LAI, while significantly enhancing grain yield by 29.55%. These findings support the use of gypsum as a practical and cost-effective soil amendment to mitigate salinity stress in rice cultivation. Future research should explore the long-term effects of gypsum application, its interactions with organic amendments, and its potential to improve the resilience of other rice varieties and crops in saline environments.

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