

Genotype-dependent responses of different rice varieties exposed to alkaline stress

Md Rokibul Hasan^{1,3}, Sheikh Farah Diba², Md. Mostafizur Rahman¹, Md. Rezaul Karim^{1*}

¹Department of Botany, University of Rajshahi, Rajshahi 6205, Bangladesh

²International Rice Research Institute (IRRI), Dhaka 1212, Bangladesh

³Biology Program, University of Louisiana at Monroe, Monroe, LA 71209, USA

*Correspondence

mrkarimbot@ru.ac.bd

Article history

Received: 22 February 2026

Accepted: 21 April 2026

Published: 10 June 2026

Keywords

Abiotic stress, alkaline stress, chlorophyll, morphology, pH, stress tolerance



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ABSTRACT

Alkaline stress is an important abiotic constraint limiting rice productivity in many agricultural regions worldwide. Elevated soil pH negatively affects nutrient availability, chlorophyll biosynthesis, photosynthesis, and overall plant growth. The present study investigated the morphological responses of six rice genotypes (BR-4, BRRI-7, BRRI-40, BRRI-55, BRRI-67, and BRRI-78) grown under control and alkaline stress (pH 7.8) conditions for six weeks under greenhouse conditions to identify genotype-dependent variation in alkaline stress tolerance. In this study, alkaline stress significantly affected plant performance in several rice genotypes, although the magnitude of stress-induced reduction differed among genotypes. Significant decreases in chlorophyll score and growth-related parameters were observed in sensitive genotypes, indicating severe physiological disruption under alkaline conditions. In contrast, BRRI-67 and BRRI-78 maintained comparatively higher chlorophyll retention and biomass accumulation under stress conditions, suggesting improved stress adaptation mechanisms. Statistical analysis revealed significant treatment effects in multiple physiological parameters. The observed variation among rice genotypes demonstrates the importance of genetic background in regulating alkaline stress tolerance. Tolerant genotypes likely maintained better nutrient acquisition, chlorophyll stability, and physiological homeostasis under alkaline environments. The findings may provide useful germplasm for mechanistic studies on alkaline stress tolerance and may contribute to breeding programs aimed at improving rice productivity in alkaline soils.

Introduction

Rice (*Oryza sativa* L.) is one of the most important staple food crops globally and provides the primary source of calories for billions of people. However, rice productivity is increasingly threatened by various abiotic stresses including drought, salinity, alkalinity, and nutrient imbalance. Among these stresses, alkaline stress has emerged as a major agricultural problem

affecting crop production in many regions of the world. Alkaline soils are characterized by elevated pH levels and excessive accumulation of sodium bicarbonate and sodium carbonate. High pH conditions reduce nutrient solubility and interfere with nutrient uptake, particularly iron, zinc, phosphorus, and manganese leading to severe nutritional disorders in plants (Marschner, 2012). Reduced nutrient acquisition



ultimately affects chlorophyll biosynthesis, photosynthetic performance, and cellular metabolism.

One of the most visible symptoms of alkaline stress is chlorosis caused by iron deficiency-induced disruption of chlorophyll biosynthesis. Under alkaline conditions, iron becomes insoluble and unavailable for plant uptake, leading to impaired chloroplast function and reduced photosynthetic activity (Fageria et al., 2002). As a result, plants exposed to alkaline environments frequently exhibit reduced growth, lower biomass accumulation, and impaired physiological performance. In addition to nutrient imbalance, alkaline stress also induces osmotic stress and oxidative damage. Excessive accumulation of reactive oxygen species (ROS) under stress conditions can damage proteins, lipids, nucleic acids, and cellular membranes (Mittler, 2002).

Rice is generally considered sensitive to high pH stress conditions compared with many halophytic species. However, substantial genotypic variation exists within rice germplasm regarding tolerance to alkaline environments. Stress-tolerant genotypes may maintain better nutrient acquisition efficiency, chlorophyll stability, ionic balance, and photosynthetic performance under alkaline conditions (Horie et al., 2012). Therefore, identifying tolerant rice genotypes is essential for breeding and crop improvement programs. Moreover, knowledge of genotype-dependent physiological variation among Bangladeshi rice genotypes under alkaline conditions remains limited. Therefore, This work is aimed at evaluating the effects of alkaline stress on chlorophyll-related and growth-associated physiological parameters, determine genotype-dependent variation in alkaline stress tolerance, and identify rice genotypes with improved physiological adaptation under alkaline conditions.

Materials and methods

Plant materials and growth conditions

Six rice genotypes including BR-4, BRRI-7, BRRI-40, BRRI-55, BRRI-67, and BRRI-78 were

collected from Bangladesh Rice Research Institute (BRRI). Seeds were germinated in tray for 3 days before transferring to the soil pot. Seedlings were cultivated in two treatment groups: control and alkaline having pH 7.8 (7.5g CaCO₃/pot) under controlled greenhouse conditions for 6 weeks. The experiment was conducted under greenhouse conditions using a completely randomized design with control and alkaline stress treatments. Each treatment contained three biological replications. Greenhouse conditions were maintained under natural photoperiod with adequate irrigation and temperature conditions suitable for rice growth.

Measurement of chlorophyll score

Chlorophyll score was measured using a portable SPAD chlorophyll meter. Fully expanded leaves were selected for chlorophyll determination. Measurements were recorded from multiple leaves per plant and averaged for analysis.

Measurement of growth-related parameters

Shoot height, shoot fresh weight, root length, and root fresh weight were measured after harvesting the plants. Shoot height was determined using a ruler by measuring the distance from the soil surface to the tip of the longest leaf. Shoots and roots were carefully separated and shoot fresh weight as well as root fresh weight were immediately recorded using an analytical balance. Root systems were gently washed to remove soil particles prior to measurement, and root length was measured from the root base to the tip of the longest root using a ruler.

Statistical analysis

Data were analyzed using Student's *t*-test to compare control and alkaline stress treatments within each genotype. Mean values and standard deviations were calculated using R statistical software. Statistical significant differences were determined at $P < 0.05$.

Results

Visual growth performance under alkaline stress

Representative images of six rice genotypes grown under control and alkaline stress conditions demonstrated substantial variation in stress-induced growth inhibition (Fig. 1). Plants exposed to alkaline conditions (pH 7.8) exhibited visible reductions in vigor, leaf greenness, shoot growth, and root development compared with control plants. However, the severity of stress symptoms differed markedly among genotypes. Among the tested rice genotypes, BRR-67 and BRR-78 maintained comparatively better growth performance and greener foliage under alkaline conditions, whereas BR-4 and BRR-55 showed greater chlorosis and reduced overall growth.

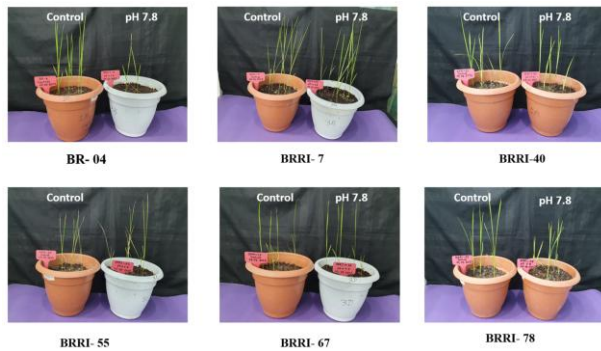


Fig. 1. Representative growth performance of six rice genotypes (BR-04, BRR-7, BRR-40, BRR-55, BRR-67, and BRR-78) grown under control and alkaline stress conditions (pH 7.8). Plants were cultivated in pots under greenhouse conditions for 6 weeks.

Changes in above-ground parameters

Chlorophyll score was significantly affected by alkaline stress in multiple rice genotypes (Fig. 2). Under alkaline conditions, substantial reductions in chlorophyll score were observed in BR-4 and BRR-55, indicating increased chlorophyll degradation and impaired photosynthetic performance. In contrast, BRR-67 maintained relatively stable chlorophyll values under stress conditions, suggesting improved chlorophyll retention and better adaptation to alkaline environments. BRR-78 also exhibited moderate tolerance with comparatively higher chlorophyll scores than the sensitive genotypes (Fig. 2).

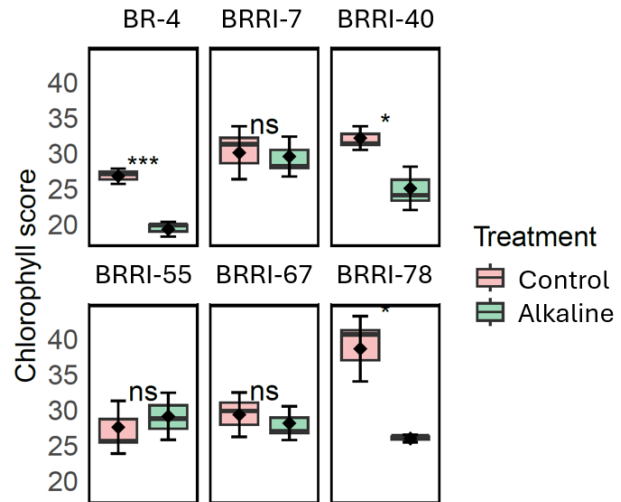


Fig. 2. Representative growth performance of six rice genotypes (BR-04, BRR-7, BRR-40, BRR-55, BRR-67, and BRR-78) grown under control and alkaline stress conditions (pH 7.8). Plants were cultivated in pots under greenhouse conditions for 6 weeks.

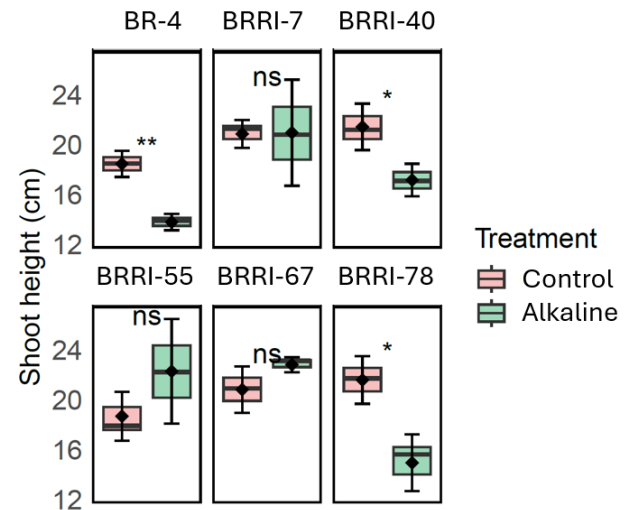


Fig. 3. Chlorophyll scores in the leaves of six rice genotypes (BR-4, BRR-7, BRR-40, BRR-55, BRR-67, and BRR-78) grown under control and alkaline stress conditions for 6 weeks. Data are presented as mean \pm standard deviation. Asterisks indicate significant differences between control and alkaline treatments within each genotype based on Student's *t*-test ($P < 0.05$, $**P < 0.001$), while "ns" indicates non-significant differences. The results demonstrate genotype-dependent variation in chlorophyll retention and tolerance under alkaline stress conditions.

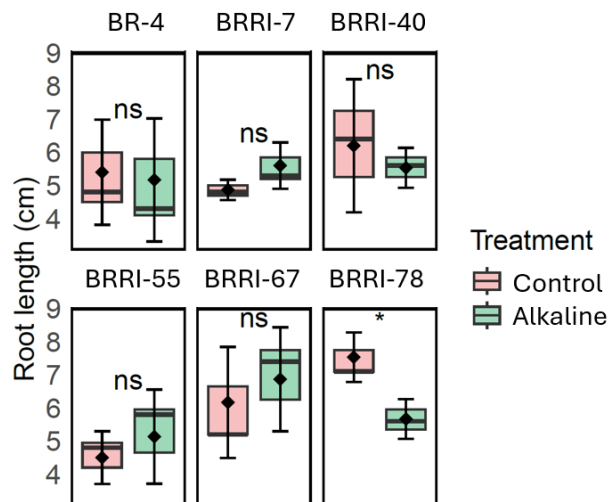
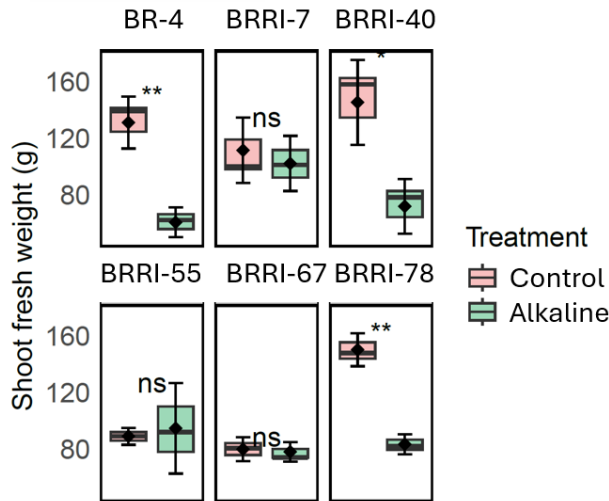


Fig. 5. Shoot fresh weight in six rice genotypes (BR-4, BRRI-7, BRRI-40, BRRI-55, BRRI-67, and BRRI-78) grown under control and alkaline stress conditions for 6 weeks. Data are presented as mean \pm standard deviation. Asterisks indicate significant differences between control and alkaline treatments within each genotype based on Student's *t*-test ($P < 0.05$, $**P < 0.001$), while "ns" indicates non-significant differences.

Alkaline stress negatively affected shoot height in all rice genotypes; however, the extent of reduction varied significantly among genotypes (Fig. 3). BR-4 exhibited the greatest reduction in shoot growth under alkaline conditions, whereas BRRI-67 and BRRI-78 maintained comparatively greater shoot elongation. Furthermore, shoot fresh weight declined significantly in several genotypes under alkaline stress conditions (Fig. 3). Sensitive genotypes displayed pronounced reductions in biomass accumulation, indicating impaired physiological performance and reduced metabolic activity. BRRI-67 showed the highest biomass retention among the tested genotypes, followed by BRRI-78 (Fig. 3).

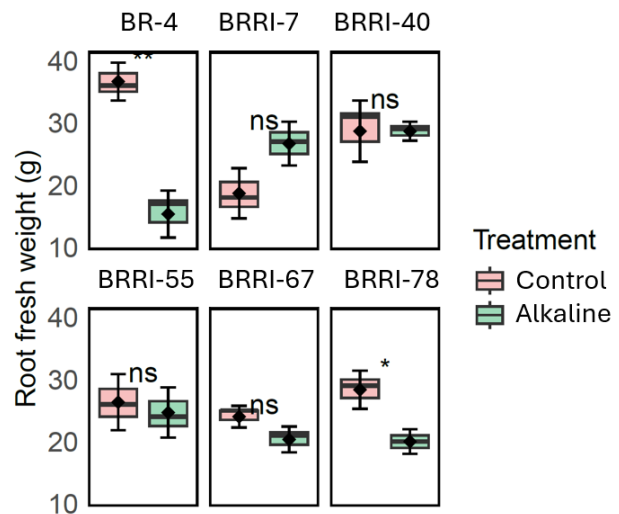


Fig. 6. Root fresh weight in six rice genotypes (BR-4, BRRI-7, BRRI-40, BRRI-55, BRRI-67, and BRRI-78) grown under control and alkaline stress conditions for 6 weeks. Data are presented as mean \pm standard deviation. Asterisks indicate significant differences between control and alkaline treatments within each genotype based on Student's *t*-test ($P < 0.05$, $**P < 0.001$), while "ns" indicates non-significant differences.

Changes in below-ground parameters

Root length measurements revealed substantial genotype-dependent variation under alkaline stress conditions (Figure 5). Root growth was severely inhibited in sensitive genotypes, whereas tolerant genotypes maintained relatively stable root development. BRRI-67 exhibited comparatively greater root length

under alkaline conditions, indicating improved root system adaptation and nutrient foraging capacity (Fig. 5). Also, root fresh weight was significantly affected by alkaline stress (Fig. 6). Sensitive genotypes exhibited substantial reductions in root biomass accumulation, whereas BRRI-67 and BRRI-78 maintained comparatively higher root fresh weight (Fig. 6).

Overall genotypic ranking under alkaline stress

The combined analysis of chlorophyll score, shoot height, shoot fresh weight, root length, and root fresh weight demonstrated clear variation in alkaline stress tolerance among the tested rice genotypes (Table 1). Based on overall physiological performance, the rice genotypes were ranked as follows: BRRI-67 > BRRI-78 > BRRI-40 > BRRI-7 > BRRI-55 > BR-4. BRRI-67 was identified as the most tolerant genotype because it maintained comparatively stable chlorophyll score, shoot growth, and root performance under alkaline stress conditions. BRRI-78 also exhibited comparatively high physiological stability and biomass retention under stress conditions. In contrast, BR-4 displayed the greatest physiological reduction and was categorized as highly sensitive to alkaline stress. The ranking analysis further confirmed that substantial genotype-dependent variation exists among rice genotypes in response to alkaline stress conditions (Table 1).

Discussion

Alkaline stress is increasingly recognized as one of the major abiotic factors limiting crop productivity in calcareous and high-pH soils. Elevated soil pH disrupts nutrient solubility and restricts the uptake of essential micronutrients, particularly iron, zinc, manganese, and phosphorus. These nutrient limitations impair chlorophyll biosynthesis, photosynthetic activity, membrane integrity, and metabolic homeostasis (Marschner, 2012). In the present study, substantial genotype-dependent variation was observed among the six tested genotypes under alkaline stress conditions, indicating differential physiological adaptation capacities. The reduction in SPAD chlorophyll values observed in

several genotypes under alkaline stress indicates impairment of chlorophyll synthesis and photosynthetic machinery. Chlorophyll degradation is commonly associated with iron unavailability under alkaline conditions because iron becomes insoluble at elevated soil pH levels (Taiz et al., 2018). Iron deficiency frequently induces interveinal chlorosis and reduced photosynthetic efficiency, ultimately limiting plant growth and biomass accumulation.

Table 1. Ranking of different rice genotypes based on overall physiological performance and tolerance under alkaline stress conditions.

| Rank | Genotype | Stress tolerance | Observation |
|------|----------|---------------------|---|
| 1 | BRRI-67 | Highly tolerant | Maintained comparatively stable chlorophyll score, shoot growth, and root performance under alkaline stress |
| 2 | BRRI-78 | Tolerant | Showed relatively high physiological stability and biomass retention under stress |
| 3 | BRRI-40 | Moderately tolerant | Moderate reduction in chlorophyll and growth-related parameters |
| 4 | BRRI-7 | Intermediate | Showed moderate stress response with partial reduction in physiological traits |
| 5 | BRRI-55 | Sensitive | Greater reduction in growth and chlorophyll-related traits under alkaline conditions |
| 6 | BR-4 | Highly sensitive | Exhibited the strongest reduction in physiological performance under stress |

Among the genetic lines, genotypes BRRI-67 and BRRI-78 maintained comparatively higher SPAD values and displayed improved physiological stability under stress conditions. This observation suggests that these genotypes possess enhanced alkaline tolerance mechanisms. Several physiological mechanisms may contribute to alkaline tolerance, including improved nutrient uptake efficiency, enhanced root proton extrusion, better ionic homeostasis,



efficient antioxidant defense systems, and improved osmotic adjustment. Previous studies have demonstrated that tolerant plants can maintain higher root ferric-chelate reductase activity and enhanced rhizosphere acidification under alkaline environments, thereby improving nutrient acquisition efficiency (Yang et al., 2008). Maintenance of chlorophyll content under stress conditions is frequently associated with sustained photosynthetic performance and improved carbon assimilation. Therefore, the relatively stable SPAD values observed in tolerant genotypes in the present study may indicate better preservation of chloroplast structure and function. The significant variation among genotypes also indicates that genetic background strongly influences alkaline stress adaptation. Such variability is important for crop improvement programs because tolerant germplasm can be utilized in breeding strategies aimed at developing stress-resilient cultivars. Genotypic screening under stress conditions provides valuable information regarding adaptive physiological responses and stress-associated metabolic adjustments.

In addition to chlorophyll-related responses, alkaline stress likely affected several downstream physiological and biochemical processes. High pH stress is known to induce oxidative stress through excessive accumulation of reactive oxygen species (ROS), which can damage cellular membranes, proteins, and nucleic acids (Guo et al., 2010). Tolerant genotypes may possess stronger antioxidant defense systems that mitigate oxidative injury and maintain cellular stability under stress. Another possible explanation for the improved tolerance observed in certain genotypes is enhanced osmotic regulation. Stress-tolerant plants often accumulate compatible solutes such as proline, soluble sugars, and amino acids that contribute to osmotic adjustment and protection of cellular structures. Maintenance of cellular water balance under alkaline conditions may therefore contribute to the improved physiological performance observed in tolerant genotypes.

The present findings are consistent with previous reports indicating that alkaline stress severely reduces chlorophyll content and photosynthetic performance in sensitive plants. For example, Guo et al. (2010) reported substantial metabolic and ionic disruptions in maize seedlings exposed to alkaline conditions. Similarly, Yang et al. (2008) demonstrated that stress-tolerant plants maintain improved ionic balance and osmotic adjustment under alkaline environments. The SPAD-based assessment used in the present study provides a rapid and non-destructive approach for evaluating stress-induced chlorophyll changes. SPAD measurements are widely used as indicators of nutrient status and photosynthetic potential in plant stress physiology studies (Ling et al., 2011). The integration of SPAD analysis with morphological and biochemical assessments can provide comprehensive insights into plant adaptation mechanisms. Although the present study successfully identified genotype-dependent variation under alkaline stress, additional molecular and biochemical analyses would further strengthen understanding of tolerance mechanisms. Future studies should investigate antioxidant enzyme activities, nutrient accumulation patterns, transcriptomic responses, and root-associated microbial interactions under alkaline environments. Such analyses would provide deeper mechanistic insights into stress adaptation pathways.

The combined analysis of chlorophyll score, shoot height, shoot fresh weight, root length, and root fresh weight demonstrated clear genotype-dependent variation under alkaline stress conditions. Sensitive genotypes displayed substantial reductions in multiple physiological parameters, indicating impaired adaptation under elevated pH environments. In contrast, tolerant genotypes maintained relatively stable physiological performance. BRRI-67 and BRRI-78 consistently performed better across multiple measured parameters, suggesting enhanced stress adaptation mechanisms. These genotypes likely maintained improved nutrient acquisition and chlorophyll stability under alkaline conditions. Conversely, BR-4 and BRRI-55



showed greater reductions in physiological performance and appeared comparatively sensitive to alkaline stress. The variation observed among the tested rice genotypes demonstrates the importance of physiological screening for identifying tolerant germplasm. Such tolerant genotypes may be useful in breeding programs aimed at improving rice productivity in alkaline soils.

Conclusion

The present study demonstrated significant genotype-dependent variation in response to alkaline stress. Alkaline stress reduced chlorophyll-related physiological performance in several genotypes, whereas genotypes E and F exhibited comparatively greater tolerance. The findings provide useful insights into physiological adaptation mechanisms under alkaline conditions and may contribute to future breeding strategies aimed at developing stress-resilient crop cultivars.

Acknowledgements

We thank Bangladesh Rice Research Institute (IRRI) for providing rice seeds.

Conflict of interest

The authors declare they have no conflicts of interest.

Author contributions

M.R.H. conducted the study, performed data analysis, and prepared the original manuscript draft. S.F.D. contributed to data collection and validation. M.M.R. assisted with methodology development, data interpretation, and manuscript revision. M.R.K. supervised the research, contributed to study design and data interpretation, and critically revised the manuscript.

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