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Seed Priming Improves Rice Seed Tolerance to Salinity Stress: Unveiling Through Multivariate Analysis

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Abstract

Salinity stress is a major constraint affecting rice establishment and productivity in many coastal and salt-affected regions of the world, as well as in Bangladesh. Seed priming has emerged as an effective technique to enhance seed germination, seedling vigor and growth, and stress tolerance. To address this challenge, the present study investigated the potential of four different seed-priming agents (non-, hydro-(H_2O), osmo-(Polyethylene glycol, 30%), nano-(Zinc EDTA (12%), and 170 ppm) applied to two rice varieties (Binadhan-10 and BINA dhan25) under four levels of salinity stress (0, 5, 8, and 11 dS m^{-1}), with the aim of enhancing germination, improving the seedling vigor index, and promoting early growth performance in a completely randomized design with four replications. Nano-priming with Zinc EDTA (12%, at 170 ppm) involves soaking seeds in a solution containing this concentration of zinc chelate, which can improve seedling vigor and stress resilience, especially under challenging conditions like salinity. The results indicated that salinity significantly reduced germination and seedling growth, whereas seed priming improved seed performance under stress conditions. Among the treatments, nano-priming showed the most pronounced improvement in germination and seedling vigor. Binadhan-10 exhibited a greater tolerance to salinity compared with BINA dhan25. Multivariate analyses, including principal component analysis, correlation analysis, and heatmap, revealed strong positive relationships among germination, vigor index, and seedling biomass traits. The findings demonstrate that seed priming, particularly nano-priming, can effectively enhance rice seed germination, the vigor index, and different seedling traits under saline conditions, providing a promising strategy for improving rice production in salt-affected areas in Bangladesh.

Keywords: abiotic stress tolerance; salinity stress; hydro-priming; osmo-priming; nano-priming; multivariate analysis

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1. Introduction

Rice cultivation plays a crucial role worldwide, especially in Asia, where it serves as the main food source for over half of the population [1]. Rice cultivation confronts obstacles including the effects of climate change, diminishing productivity increases, and environmental issues, despite the fact that it is essential for food security. Farmers are trying a variety of tactics, including automation, effective irrigation, and stress-tolerant cultivars, to solve these issues [2].

Salinity is a significant issue in arid and semi-arid regions, limiting crop yields by affecting plants at various physiological and biochemical levels. Globally, about 1381 million hectares of land are impacted by salinity [3]. In Bangladesh, this problem is concentrated along the coast of the Bay of Bengal, covering roughly 2.83 million hectares in the southern districts [4]. The affected area spans from a few kilometers to up to 180 km inland, accounting for about 20% of the country's total land and 30% of its cultivable area [5,6].

Nearly half of the world's population relies on rice as a main source of nutrition, making salinity stress a major threat to rice grain harvests [7]. During the reproductive periods of rice development, which occur in March and April when saline levels are at their highest, the problem is more acute. Traditional rice varieties have a tolerance threshold of 4–5 dS m^{-1} for salinity; however, recently produced salt-tolerant rice cultivars can tolerate 8–10 dS m^{-1} [8]. According to [9], salinity has a detrimental effect on the grain yield, photosynthesis, root growth, leaf features, and cell metabolism. Salinity alters several physiological, metabolic, and biochemical processes [10]. These changes can trigger oxidative stress and reduce plant growth, metabolism, and overall productivity [11]. Salinity also slows or inhibits seed germination and weakens early seedling establishment because of osmotic and salt stress [12]. High concentrations of Na^{+} and Cl^{-} ions can damage the developing embryo and lower the external osmotic potential around the seed, which limits the water uptake during germination [13]. As a result, seeds fail to absorb sufficient water to initiate normal metabolic activity. Seed priming offers a practical strategy to overcome these problems because it prepares the seed physiologically and improves its tolerance to salinity stress [14].

Seed priming is an affordable method that boosts crop performance by improving germination, seedling vigor, and stress resilience [15]. It works by carefully hydrating seeds to start pre-germination metabolic activity without allowing the radicle to emerge [16]. Common priming methods include solid matrix priming, osmo-priming, halo-priming, and hydro-priming [17]. These methods can result in greater plant development, synchronous germination, enhanced stress tolerance, and the more efficient use of water and nutrients [16]. Numerous field crops, including barley, wheat, and maize, have benefited from seed priming [18]. Changes in seed water content, cell cycle regulation, and oxidative stress management are among the physiological and biochemical alterations that occur during the process [16].

For obtaining practical agricultural yields, priming with nanoparticles (nano-priming) has shown greater promise than conventional priming techniques [19]. "Priming" refers to enhancing a plant's stress tolerance through moderate and repeated exposure to stress. Nano-priming applies this concept using nanoparticles (NPs) smaller than 100 nm [20]. Evidence suggests that applying nanoparticles during priming can enhance both seed germination and seedling development [20,21].

The problem of progressive salinization is especially critical during the Boro season, which is the main rice-growing period in Bangladesh. BRRI dhan28 and BRRI dhan29 were historically the most popular rice varieties to farmers in the Boro season in Bangladesh due to their high yield potential and broad adaptability [22,23]. However, these varieties are now being actively discouraged by the Government of Bangladesh because they require high amounts of irrigation, have longer growth durations, and are increasingly susceptible to climate-induced stresses, such as drought and salinity and a disease called rice blast [24]. As a result, there is a strategic shift towards the adoption of short-duration and stress-tolerant varieties such Binadhan-10 to maintain rice productivity under adverse environmental conditions. Among the emerging cultivars, Binadhan-10 stands out for its notable salinity tolerance and stable yield performance. In addition, BINA dhan25 has recently been identified as a promising short-duration, high-yielding, fine-grain variety with good adaptability and efficient resource utilization [25]. However, despite its agronomic advantages, the performance of BINA dhan25 under saline stress conditions remains insufficiently explored. While extensive research has been conducted on developing and evaluating salinity-tolerant rice varieties, there remains a distinct research gap in exploring the potential of seed-priming techniques to enhance salinity tolerance—particularly in newly released or non-traditional stress-adapted varieties such as BINA dhan25. Therefore, the present study is novel in that it systematically evaluates the effectiveness of different seed-priming agents in improving the germination, growth, and stress resilience of both Binadhan-10 and BINA dhan25 under saline conditions, aiming to identify practical, low-cost strategies for enhancing Boro rice productivity in salt-affected areas in Bangladesh.

2. Materials and Methods

2.1. Location, Treatments, and Sample

The experiment was designed to investigate the interactive effects of rice variety, seed-priming technique, and salinity level on various growth parameters of rice. It consisted of three factors: Factor A was the rice variety, which included two Boro varieties, namely, Binadhan-10 and BINA dhan25. Factor B was the type of seed-priming agent, with four levels—non-primed (control), hydro-primed, osmo-primed, and nano-primed. Factor C comprised four different salinity levels, specifically 0, 5, 8, and 11 dS m^{-1}, to simulate varying degrees of salt stress. The experiment was laid out in a completely randomized design (CRD). Each treatment combination was replicated four times, enabling a systematic and comprehensive evaluation of the responses of different rice varieties to various seed-priming methods under saline conditions.

The experiment was conducted at Seed Laboratory, Department of Agronomy, BAU (23°77′ N, 90°33′ E; 19 m elevation). Binadhan-10, released in 2012, is early-maturing, salt-tolerant (up to 12 dS m^{-1}), and high-yielding (7.5–8.5 t ha^{-1} under normal conditions and 5–6 t ha^{-1} in saline area). It features strong, erect stems, resistance to lodging, and low pest incidence. BINA dhan25, developed through mutation breeding from BRRI dhan29, matures in 138–148 days, yields 7.5–8.5 t ha^{-1}, and is known for its tall, narrow plant stature and long, thin grains. The seed lot used in this study was obtained from BINA and consisted of high-quality seeds with 98% purity, 98% germination, and 9.5% moisture content as determined by oven-dry method. After collection, seeds were kept in air-tight containers and preserved in a refrigerator at a temperature of 5 ± 1 °C till used.

2.2. Acronyms and Abbreviated Words Used in the Current Manuscript

We used acronyms and abbreviated forms of different traits related to this manuscript to reduce their similarity throughout the manuscript. (BAU = Bangladesh Agricultural University, CRD = completely randomized design, BINA = Bangladesh Institute of Nuclear Agriculture, AOSA = Association of Official Seed Analysts, V1 = Binadhan-10, V2 = BINA dhan25, P0 = Non-priming, P1 = Hydro-priming, P2 = Osmo-priming, P3 = Nano-priming; S0 = 0 dS m^{-1}, S1 = 5 dS m^{-1}, S2 = 8 dS m^{-1}, S3 = 11 dS m^{-1}. G = Germination (%), UGS = ungerminated seed, VI = Vigor index, NS = Normal Seedling, AS = Abnormal Seedling, SL = Shoot length (cm), RL = Root length (cm), SFW = Shoot fresh weight (g), SDW = Shoot dry weight (g), RFW = Root fresh weight (g) RDW = Root dry weight (g). PCA = Principal Component Analysis (PCA), CV = Co-efficient of variation.)

2.3. Priming Agents Used

Zn-EDTA (12% Zn) used in this study was a conventional chelated zinc fertilizer with an average particle size of about 100 μm. This water-soluble Zn–EDTA complex provides high chemical stability and consistent Zn^{2+} availability. Solutions were prepared by dissolving Zn-EDTA in distilled water with continuous stirring. Unlike nano-Zn formulations, Zn-EDTA does not require ultrasonication for dispersion and remains stable in aqueous media due to chelation. Details of the priming agents are presented in Table 1.

Table 1. Description of the priming methods.

Method/Chemical Formula	Agent	Concentration	Manufacturer
Non-priming	Control		
Hydro-priming (H_2O)	Distilled water		
Osmo-priming (PEG 4000)	Polyethylene glycol 4000	30%	MERCK, Bangalore, India
Nano-priming ($C_{10}H_{12}N_2O_8Zn{\cdot}2Na$)	Zinc EDTA (12%)	170 ppm	Geolife Agritech India Pvt. Ltd. Mumbai, India

2.4. Seed Preparation, Germination Media, and Aftercare

Seeds of both the rice varieties (Binadhan-10 and BINA dhan25) were separately soaked in different agent solutions (previously prepared using distilled water) as per treatments for 6 h at room temperature (25 ± 2 °C) (Figure 1). The ratio of seed weight to solution volume was 1:5 (g mL^{-1}). Then, seeds were removed from the agent solution, followed by washing several times with distilled water to remove the traces of chemicals. Seeds were then wiped lightly with blotting paper, and allowed to dry by forced air to bring them back to the original moisture content (calculated as 9.5% by oven-dry method). Dried seeds were put in brown envelopes with tags in sealed polythene bags and stored in a refrigerator at 5 ± 1 °C until they were placed for germination. Control treatment received no prior seed priming, but were kept in the refrigerator.

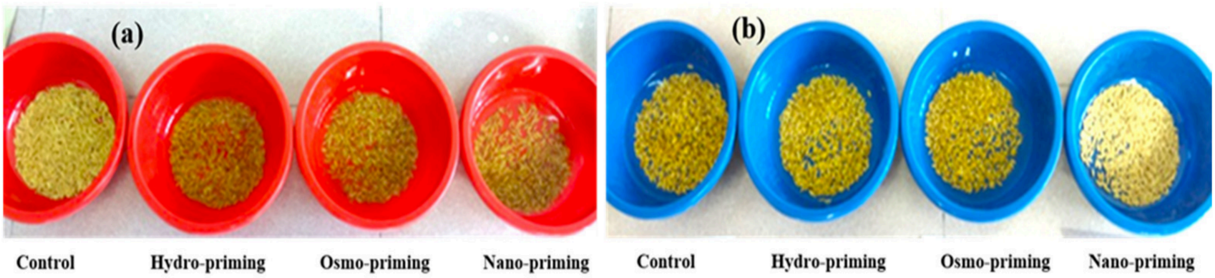

Figure 1. Priming of (**a**) Binadhan-10 and (**b**) BINA dhan25.

2.5. Salinity Level Preparation

Salinity stress was imposed using NaCl under in vitro conditions. Different salinity levels (0, 5, 8, and 11 dS m^{-1}) were prepared by dissolving calculated amounts of NaCl in distilled water to achieve the desired concentrations. Analytical-grade NaCl was gradually dissolved in 1 L of distilled water, and the electrical conductivity of the solution was monitored and adjusted to the desired level using a conductivity meter. Solutions of other concentrations were prepared similarly, with the required amounts of NaCl calculated to achieve the target electrical conductivity.

2.6. Seed Placement in Germination Media

Sterilized sand was used as germination media and plastic-made petri dishes of 90 mm diameter with 15 mm depth were used as containers. The moisture content of the media was maintained at around 80% of the field capacity by watering with distilled water as necessary. After 14 days storing at refrigerator, germination was tested using 400 seeds from the pure fraction, arranged in four replicates of 100 following International Seed Testing Association guidelines [26]. Every petri dish was monitored regularly. The observation was done to maintain proper moisture levels. No occurrence of insects or diseases was recorded. Watering was done on regular basis to maintain field capacity.

2.7. Data Collection and Measurement Procedures

2.7.1. Germination Percentage (GP)

Germinated seeds were counted daily at 10 am from the 4th to 14th day after sowing, considering normal seedlings emerging over the sand layer as germinated, and germination percentage (GP) was calculated following AOSA [27]:

$$\text{GP} = \frac{\text{No. of seeds that germinated}}{\text{No. of seeds sown}} \times 100$$

2.7.2. Vigor Index (VI)

On the 14th day after seed placement, ten seedlings per replicate were randomly selected for root and shoot length measurements, and the VI was calculated following AOSA [27] using the equation:

$$\text{Vigor Index (VI)} = \frac{\text{Seedling length (root + shoot length) (cm)} \times \text{Germination percentage}}{100} \quad (1)$$

2.7.3. Seedling Growth

On day 14, ten seedlings per replicate were assessed as normal or abnormal; SL, RL, SFW, RFW, SDW (oven-dried at 70 °C for 72 h), and RDW were measured.

2.8. Statistical Analysis

Treatment means were compared using Duncan's Multiple Range Test [28], with data analyzed in MSTAT-C (The current version is 8.1.2, which was released on 24 April 2025) and R (version 4.6.0).

3. Results

3.1. Varietal Effect

The germination, vigor index, and seedling growth parameters of Boro rice were significantly varied due to the varietal effect. Binadhan-10 showed a higher germination (96.41%) and vigor index (20.45), which were 3.16% and 3.69 units greater, respectively, than BINA dhan25. Similarly, Binadhan-10 showed a higher number of normal seedlings (87.75) and lower abnormal (8.66) and ungerminated seeds (3.58) compared to BINA dhan25, which had 78.75% normal, 14.5% abnormal, and 6.58% ungerminated seeds (Figure 2). In the case of the shoot and root length of rice seedlings, Binadhan-10 showed a higher shoot (11.81 cm) and root length (9.36 cm) compared to BINA dhan25 (10.17 cm and 7.81 cm). Similarly, the shoot and root fresh weights were higher in Binadhan-10 (0.0224, and 0.0062 g) than in BINA dhan25 (0.0145, and 0.0043 g). SDW and RDW also followed, respectively, the same trend, with Binadhan-10 showing 0.0069, and 0.0036 g, while BINA dhan25 had 0.0046, and 0.0025 g (Table 2).

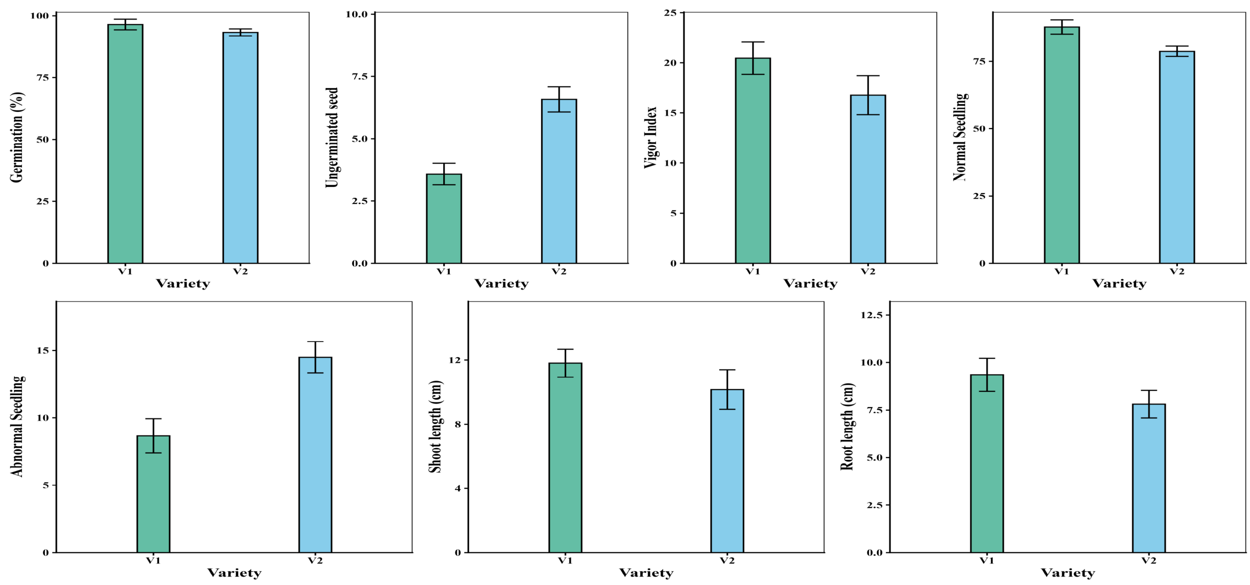

Figure 2. Variety impacts on seedling types. Here, V1 = BINA dhan10, and V2 = BINA dhan25.

Table 2. Impact of rice variety on Boro seedling growth.

Variety	G (%)	VI	SL (cm)	RL (cm)	SFW (g)	RFW (g)	SDW (g)	RDW (g)
V1	96.41 a	20.45 a	11.81 a	9.36 a	0.0224 a	0.0062 a	0.0069 a	0.0036 a
V2	93.25 b	16.76 b	10.17 b	7.81 b	0.0145 b	0.0043 b	0.0046 b	0.0025 b
Level of Sig.	**	**	**	**	**	**	**	**
CV (%)	4.00	10.11	9.71	12.43	17.52	15.64	12.24	12.26

** = significant at 1% level of probability. In a column, figures with same letter (s) or without letter do not differ significantly whereas figures with dissimilar letter differ significantly. All abbreviations are defined in Section 2.2.

3.2. Seed-Priming Impacts

G was not significantly affected by the priming methods. However, VI differed significantly, with the highest value (19.32) under nano-priming and the lowest (17.90) in the non-primed condition (Table 3). In the case of normal seedlings, abnormal seedlings, and ungerminated seedlings, the priming method had a non-significant effect on the rice seed (Figure 3). SL, RL, SFW, RFW, SDW, and RDW were significantly influenced by the priming methods. Nano-priming consistently produced the highest shoot (11.48 cm) and root length (8.72 cm), and the highest SFW and RFW (0.0198 g and 0.0063 g) and SDW and RDW (0.0063 g and 0.0035 g), compared to the other treatments (Table 3). The lowest values were generally observed in the non-priming and osmo-priming treatments.

Table 3. Response of Boro rice seedling growth to different seed priming treatments.

Priming	G (%)	VI	SL (cm)	RL (cm)	SFW (g)	RFW (g)	SDW (g)	RDW (g)
P0	93.83	17.90 b	10.81 b	8.26 b	0.0162 c	0.0057 ab	0.0055 ab	0.0030 ab
P1	94.50	18.61 ab	10.73 b	8.68 a	0.0197 a	0.0054 ab	0.0058 ab	0.0030 ab
P2	95.33	18.58 ab	10.66 b	8.69 a	0.0179 b	0.0047 b	0.0053 b	0.0027 b
P3	95.66	19.32 a	11.48 a	8.72 a	0.0198 a	0.0063 a	0.0063 a	0.0035 a
Level of Sig.	NS	**	*	*	**	**	**	**
CV (%)	4.00	10.11	9.71	12.43	17.52	15.64	12.24	12.26

NS = non-significant, ** = significant at 1% level of probability, * = significant at 5% level of probability. In a column, figures with same letter (s) or without letter do not differ significantly whereas figures with dissimilar letter differ significantly. All abbreviations are defined in Section 2.2.

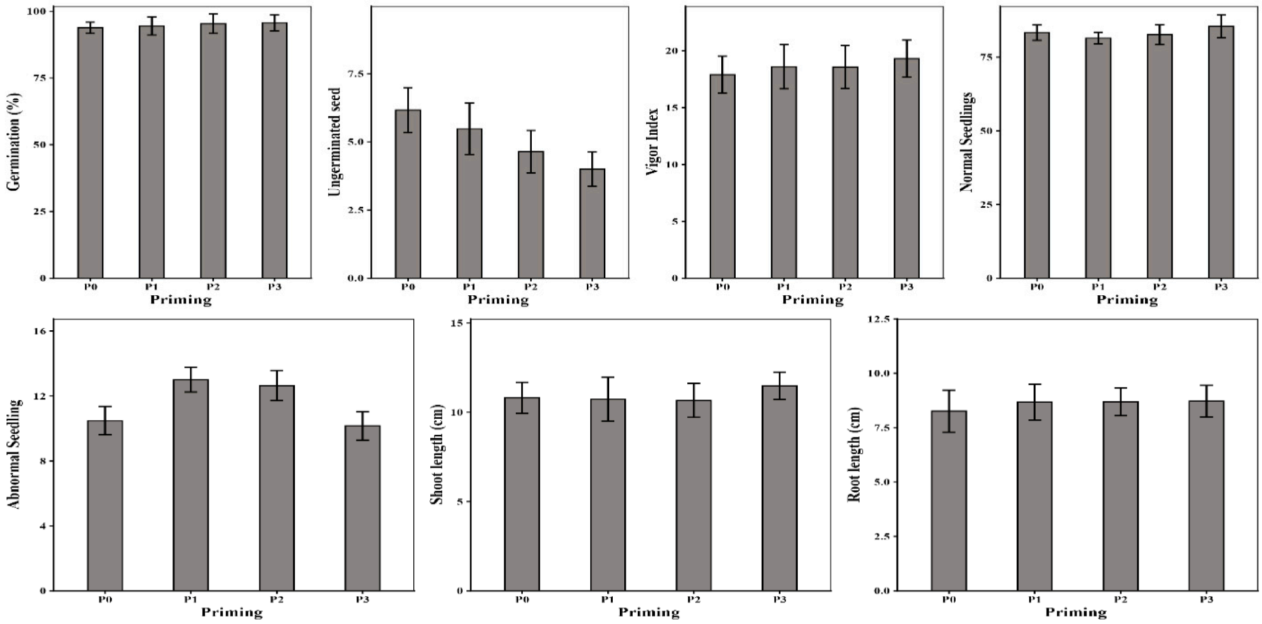

Figure 3. Priming methods impact on seedling types. All abbreviations are defined in Section 2.2.

3.3. Effect of Salinity Level

Salinity significantly affected the rice seed germination and vigor index. The highest germination (96.66%) and vigor index (21.50) were recorded at 0 dS m^{-1}, while the lowest values (93.16% and 15.24) were observed at 8 and 11 dS m^{-1}, respectively (Table 4). Salinity also significantly affected the seedling quality in rice. Normal seedlings decreased from 92.66% at 0 dS m^{-1} to 72.83% at 11 dS m^{-1}, while abnormal seedlings increased from 4% to 20.50%. Ungerminated seeds ranged from 3.33% (0 dS m^{-1}) to 6.83% (8 dS m^{-1}) (Figure 4). These results indicate that higher salinity levels reduce normal seedling development and increase abnormalities and ungerminated seeds. The salinity level significantly affected the SL, RL, SFW, RFW, SDW, and RDW of seedlings (Table 4). The highest shoot (12.48 cm) and root length (9.52 cm) were recorded at 0 dS m^{-1}, while the lowest (8.65 cm and 7.70 cm, respectively) occurred at 11 dS m^{-1}. The shoot and root fresh weights also declined with increasing salinity, from 0.0197 g and 0.0060 g at 0 dS m^{-1} to 0.0169 g and 0.0048 g at 11 dS m^{-1}. Similarly, the highest shoot and root dry weights (0.0063 g and 0.0034 g) were found at 0 dS m^{-1}, and the lowest (0.0051 g and 0.0028 g) at 11 dS m^{-1}. The decline in all parameters with increased salinity suggests that salt stress inhibits seedling growth, with the control (no salinity) showing superior performance across all traits.

Table 4. Response of Boro rice seedlings to different salinity levels.

Salinity	G (%)	VI	SL (cm)	RL (cm)	SFW (g)	RFW (g)	SDW (g)	RDW (g)
S0	96.66 a	21.50 a	12.48 a	9.52 a	0.0197 a	0.0060 a	0.0063 a	0.0034 a
S1	96.16 a	19.94 b	11.84 b	8.74 b	0.0192 a	0.0053 ab	0.0060 a	0.0033 ab
S2	93.16 b	17.73 c	10.69 c	8.39 c	0.0180 b	0.0049 b	0.0056 ab	0.0029 c
S3	93.33 b	15.24 d	8.65 d	7.70 d	0.0169 b	0.0048 b	0.0051 b	0.0028 bc
Level of Sig.	**	**	**	**	**	**	**	**
CV (%)	4.00	10.11	9.71	12.43	17.52	15.64	12.24	12.26

** = significant at 1% level of probability. In a column, figures with same letter (s) or without letter do not differ significantly whereas figures with dissimilar letter differ significantly. All abbreviations are defined in Section 2.2.

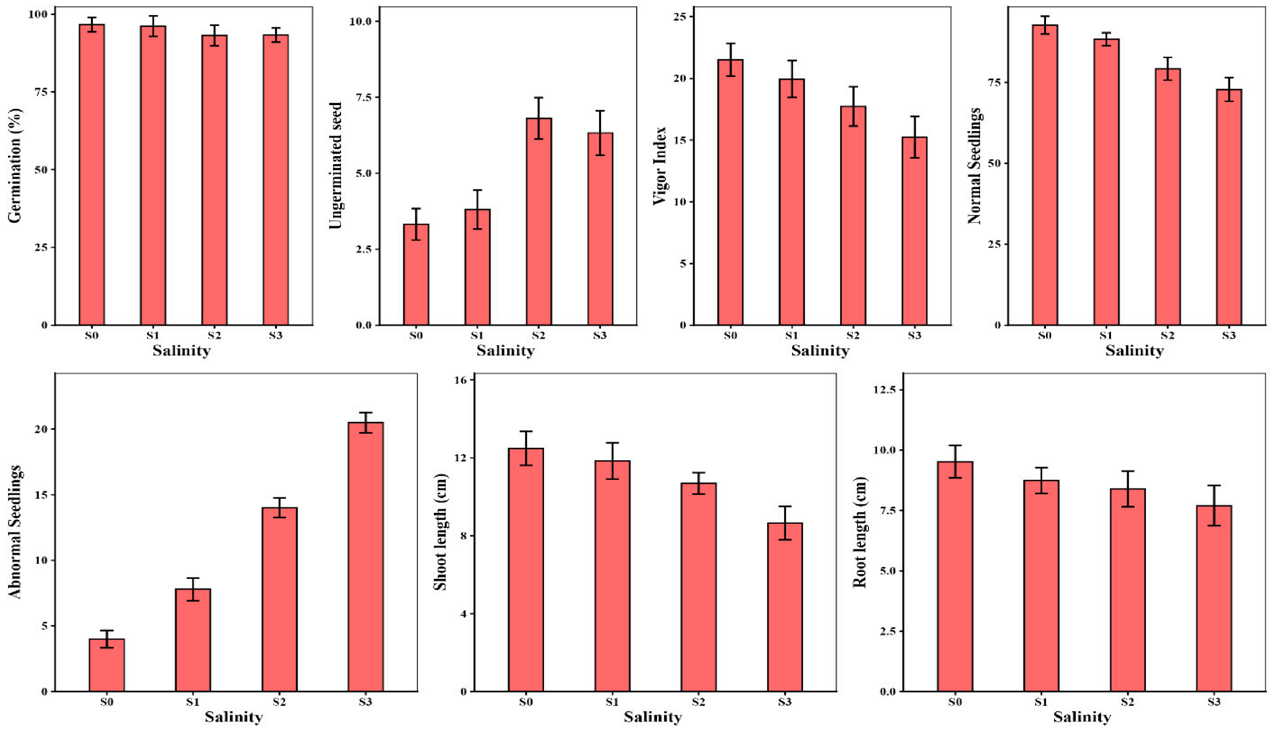

Figure 4. Response of seedling types to varying salinity levels. All abbreviations are defined in Section 2.2.

3.4. Variety–Priming Interaction's Impact on Boro Rice Seedlings

All studied seedling parameters were significantly influenced by the interaction between the variety and seed priming (Table 5). The highest G (97.73%), VI (21.43%), NS (91.66%), SL (12.77 cm), and RL (9.76 cm) were recorded in Binadhan-10 when treated with nano-priming, while the lowest values for these traits were mostly observed in BINA dhan25 with non-priming or osmo-priming. Similarly, SFW and RFW were the highest at V1P3 (0.0252 g and 0.0064 g) and lowest at V2P2 (0.0140 g and 0.0035 g), whereas SDW and RDW also followed the same trend. AS was most frequent in V2P2 (15.33%) and least frequent in V1P3 (5.66%). These results suggest that nano-priming, particularly with the salt-tolerant variety Binadhan-10, significantly enhances the G and seedling vigor under stress conditions (Table 5).

Table 5. Response of Boro Rice G, Vi, and Seedling Growth to Variety × Seed-Priming Interaction.

Variety × Priming	G (%)	UGS (%)	VI	NS	AS	SL (cm)	RL (cm)	SFW (g)	RFW (g)	SDW (g)	RDW (g)
V1P0	95.00 ab	5.00 ab	19.72 b	88.66 ab	6.33 b	11.89 b	8.74 c	0.0177 c	0.0063 a	0.0062 b	0.0030 bc
V1P1	95.66 ab	4.33 ab	20.35 ab	83.00 bc	12.66 a	11.58 b	9.61 b	0.0250 a	0.0063 a	0.0068 ab	0.0037 b
V1P2	97.66 a	2.33 b	20.29 ab	87.66 ab	10.00 ab	10.99 c	9.73 ab	0.0218 b	0.0058 ab	0.0070 ab	0.0033 b
V1P3	97.73 a	2.26 b	21.43 a	91.66 a	5.66 b	12.77 a	9.76 a	0.0252 a	0.0064 a	0.0075 a	0.0045 a
V2P0	92.66 b	7.33 a	16.08 c	78.00 c	14.66 a	9.73 e	7.78 de	0.0147 d	0.0050 bc	0.0049 c	0.0031 bc
V2P1	93.33 b	6.66 a	16.87 c	80.00 c	13.33 a	9.88 de	7.75 e	0.0148 d	0.0045 cd	0.0048 cd	0.0023 cd
V2P2	93.00 b	7.00 a	16.86 c	77.66 c	15.33 a	10.32 d	7.64 e	0.0140 d	0.0035 cd	0.0037 d	0.0022 d
V2P3	94.00 b	5.33 ab	17.22 c	79.33 c	14.66 a	10.19 de	8.08 d	0.0145 d	0.0043 d	0.0050 c	0.0024 cd
Level of Sig.	**	**	**	**	**	**	**	**	**	**	**
CV(%)	4.00	6.77	10.11	9.37	9.67	9.71	12.43	17.52	15.64	12.24	12.26

** = significant at 1% level of probability. In a column, figures with same letter (s) or without letter do not differ significantly whereas figures with dissimilar letter differ significantly. All abbreviations are defined in Section 2.2.

3.5. Variety–Salinity Level Interaction's Impact on Boro Rice Seedlings

The interaction of the variety and salinity level significantly influenced all measured seedling traits (Table 6). The highest G (99.00%), VI (23.74%), NS (97.66%), SL (13.50 cm), and RL (10.43 cm) were recorded in V1S0 (Binadhan-10 × 0 dS m^{-1}), while the lowest values were observed in V2S3 (BINA dhan25 × 11 dS m^{-1}), with G at 92.00%, VI at 14.01, and NS at 68.00%. Correspondingly, AS was the highest (24.00%) in V2S3 and lowest (1.33%) in V1S0. SFW and RFW were also the highest at V1S0 (0.0236 g and 0.0070 g) and lowest at V2S3 (0.0137 g and 0.0039 g). Similarly, SDW and RDW ranged from 0.0075 g and 0.0044 g at V1S0 to 0.0043 g and 0.0023 g at V2S3. These results highlight the superior performance of Binadhan-10 under non-saline conditions and the adverse effects of salinity, particularly on BINA dhan25 (Table 6).

Table 6. Response of Boro Rice G, Vi, and Seedling Growth to Variety × Salinity Level.

Variety × Salinity	G (%)	UGS (%)	VI	NS	AS	SL (cm)	RL (cm)	SFW (g)	RFW (g)	SDW (g)	RDW (g)
V1S0	99.00 a	1.00 b	23.74 a	97.66 a	1.33 e	13.50 a	10.43 a	0.0236 a	0.0070 a	0.0075 a	0.0044 ab
V1S1	98.33 a	1.66 b	22.24 a	93.66 ab	4.66 e	12.96 a	9.63 b	0.0232 a	0.0064 ab	0.0073 a	0.0043 a
V1S2	93.66 b	6.33 a	19.33 b	82.00 cde	11.66 bcd	11.39 b	9.22 c	0.0219 b	0.0057 bc	0.0067 ab	0.0029 cd
V1S3	94.66 b	5.33 a	16.47 c	77.66 de	17.00 b	9.37 e	8.17 e	0.0200 c	0.0058 bc	0.0059 bc	0.0033 bc
V2S0	94.33 b	5.66 a	19.26 b	87.66 bc	6.66 de	11.46 b	8.61 d	0.0157 d	0.0049 cd	0.0050 cd	0.0028 cd
V2S1	94.00 b	6.00 a	17.63 c	83.00 cd	11.00 cd	10.72 c	7.85 f	0.0143 de	0.0043 d	0.0046 d	0.0024 d
V2S2	92.66 b	7.33 a	16.13 c	76.33 e	16.33 bc	10.00 d	7.56 f	0.0142 de	0.0042 d	0.0045 d	0.0024 d
V2S3	92.00 b	7.33 a	14.01 d	68.00 f	24.00 a	7.93 f	7.24 g	0.0137 e	0.0039 d	0.0043 d	0.0023 d
Level of Sig.	**	**	**	**	**	**	**	**	**	**	**
CV (%)	4.00	6.77	10.11	9.37	9.67	9.71	12.43	17.52	15.64	12.24	12.26

** = significant at 1% level of probability. In a column, figures with same letter (s) or without letter do not differ significantly whereas figures with dissimilar letter differ significantly. All abbreviations are defined in Section 2.2.

3.6. Seed Priming–Salinity Level Interaction's Impact on Boro Rice Seedlings

The seed priming × salinity interaction significantly influenced all seedling traits (Table 7). Nano-priming under non-saline conditions (P3S0) consistently produced the best results, with the highest G (98.00%), VI (21.73%), NS (95.33%), and SFW and RFW (0.0233 g and 0.0063 g). In contrast, the poorest performance was noted under high salinity with no or ineffective priming methods such as P1S3 and P2S3 where the germination dropped to 90.66% and NS to 68.66%, alongside a rise in AS to 22.66%. SL and RL reached their maximum at P3S1 (13.06 cm) and P2S0 (10.10 cm), while the lowest values were recorded at P0S3 and P2S3. Similarly, the highest dry weights were obtained at P3S0, whereas P2S2 and P2S3 showed the lowest dry weights (Table 7).

3.7. Variety, Seed Priming, and Salinity Level Interaction's Effect on Boro Rice Seedlings

A significant three-way interaction among variety, seed priming, and salinity level was observed across all seedling traits (Table 8). The best performance was consistently noted in V1P3S0 (Binadhan-10 × nano-priming × 0 dS m^{-1}), which recorded the highest G (100%), VI (25.35), NS (100%), SL (14.83 cm), SFW (0.03 g), and SDW (0.0083 g). Root traits were similarly enhanced, with the highest RL (11.23 cm) at V1P2S0 (Binadhan-10 × osmo-priming × 0 dS m^{-1}), and peak RFW and RDW (0.0077 g and 0.0049 g) at V1P3S0. In contrast, the poorest performance occurred under high salinity with a sensitive variety and poor or no priming. For instance, the lowest G (86.67%) was recorded at V2P1S3 (BINA dhan25 × hydro-priming × 11 dS m^{-1}), while AS peaked at 25.33% under V2P0S3. The minimum SL and RL (7.00 cm and 6.78 cm), and lowest SDW and RDW (0.0027 g and 0.0013 g) were also observed in BINA dhan25 combinations under saline stress with suboptimal priming (Table 8).

Table 7. Response of Boro Rice G, Vi, and Seedling Growth to Seed Priming × Salinity Level Interaction.

Priming × Salinity	G (%)	UGS (%)	VI	NS	AS	SL (cm)	RL (cm)	SFW (g)	RFW (g)	SDW (g)	RDW (g)
P0S0	96.00 abc	4.00 cde	21.15 ab	91.33 ab	4.66 ef	12.87 ab	8.76 d	0.0155 gh	0.0058 ab	0.0065 ab	0.0032 b–e
P0S1	95.33 a–d	4.66 b–e	18.52 cde	90.00 ab	5.33 ef	11.39 de	7.97 ef	0.0153 gh	0.0060 ab	0.0050 bcd	0.0027 c–f
P0S2	91.33 de	8.66 ab	17.55 def	79.33 cde	12.00 cde	10.93 e	8.65 d	0.0182 def	0.0055 abc	0.0058 a–d	0.0030 b–f
P0S3	92.66 cde	7.33 abc	14.37 h	72.66 ef	20.00 ab	8.05 j	7.67 f	0.0157 gh	0.0053 a–d	0.0048 cd	0.0033 bcd
P1S0	97.33 ab	2.66 de	21.72 a	94.66 a	2.66 f	12.44 abc	9.62 b	0.0210 abc	0.0058 ab	0.0063 abc	0.0033 bcd
P1S1	96.66 abc	3.33 cde	19.62 bcd	88.00 abc	8.66 def	10.84 e	9.05 cd	0.0203 bcd	0.0057 ab	0.0062 a–d	0.0038 ab
P1S2	93.33 b–e	6.66 a–d	17.76 def	74.66 def	18.66 abc	10.75 ef	8.19 e	0.0210 abc	0.0053 a–d	0.0057 a–d	0.0022 ef
P1S3	90.66 e	9.33 a	15.23 gh	68.66 f	22.00 a	8.88 hi	7.87 ef	0.0173 efg	0.0047 bcd	0.0048 cd	0.0027 c–f
P2S0	95.33 a–d	4.66 b–e	21.31 ab	89.33 ab	6.00 ef	12.10 bcd	10.10 a	0.0188 cde	0.0058 ab	0.0052 bcd	0.0034 abc
P2S1	96.00 abc	4.00 cde	20.57 abc	88.00 abc	8.00 def	12.08 cd	9.25 bc	0.0200 bcd	0.0047 bcd	0.0065 ab	0.0023 def
P2S2	93.33 b–e	6.66 a–d	16.84 efg	79.33 cde	14.00 bcd	10.01 fg	7.86 ef	0.0143 h	0.0040 d	0.0047 d	0.0028 b–f
P2S3	96.66 abc	3.33 cde	15.60 fgh	74.00 ef	22.66 a	8.43 ij	7.55 f	0.0185 de	0.0042 cd	0.0050 bcd	0.0020 f
P3S0	98.00 a	2.00 e	21.73 a	95.33 a	2.66 f	12.52 abc	9.61 b	0.0233 a	0.0063 a	0.0070 a	0.0035 abc
P3S1	96.66 abc	3.33 cde	21.05 ab	87.33 abc	9.33 def	13.06 a	8.68 d	0.0213 ab	0.0050 a–d	0.0062 a–d	0.0045 a
P3S2	94.66 a–e	5.33 a–e	18.77 cde	83.33 bcd	11.33 cde	11.08 e	8.87 cd	0.0187 cde	0.0048 bcd	0.0062 a–d	0.0027 c–f
P3S3	93.33 bcde	5.33 a–e	15.75 fgh	76.00 def	17.33 abc	9.25 gh	7.74 f	0.0160 fgh	0.0052 a–d	0.0057 a–d	0.0032 b–e
Level of Sig.	**	**	**	**	**	**	**	**	**	**	**
CV(%)	4.00	6.77	10.11	9.37	9.67	9.71	12.43	17.52	15.64	12.24	12.26

** = significant at 1% level of probability. In a column, figures with same letter (s) or without letter do not differ significantly whereas figures with dissimilar letter differ significantly. All abbreviations are defined in Section 2.2.

Table 8. Interaction effect of variety, seed priming, and salinity level on germination, vigor index, and seedling growth of Boro rice.

Interaction	G (%)	UGS (%)	VI	NS	AS	SL (cm)	RL (cm)	SFW (g)	RFW (g)	SDW (g)	RDW (g)
V1P0S0	98.67 ab	1.33 ef	23.63 abc	98.67 a	0.00 g	14.21 abc	9.53 ef	0.0150 h–k	0.0067 abc	0.0073 a–d	0.0040 bc
V1P0S1	99.97 a	0.03 f	20.68 c–g	96.00 abc	4.00 fg	12.43 de	8.25 hij	0.0167 ghi	0.0070 ab	0.0057 c–g	0.0023 def
V1P0S2	89.33 ef	10.66 ab	19.31 f–k	82.67 d–j	6.66 efg	11.84 ef	9.74 de	0.0203 ef	0.0060 a–d	0.0067 a–f	0.0027 c–f
V1P0S3	92.00 c–f	8.000 a–d	15.25 m–p	77.33 g–k	14.66 a–f	9.11 klm	7.43 klm	0.0187 fg	0.0057 a–e	0.0050 e–h	0.0030 cde
V1P1S0	97.33 abc	2.66 def	23.16 a–d	97.33 ab	0.00 g	13.37 bcd	10.44 bc	0.0260 bc	0.0067 abc	0.0070 a–e	0.0040 bc
V1P1S1	97.33 abc	2.66 def	21.69 b–f	90.67 a–f	6.66 efg	11.99 ef	10.20 cd	0.0280 ab	0.0070 ab	0.0080 ab	0.0047 b
V1P1S2	93.33 b–e	6.66 b–e	19.67 e–j	70.67 jkl	22.66 ab	11.83 ef	9.19 efg	0.0253 bc	0.0060 a–d	0.0067 a–f	0.0030 cde
V1P1S3	94.67 a–e	5.33 b–f	16.86 j–o	73.33 h–l	21.33 abc	9.13 jklm	8.63 gh	0.0207 ef	0.0053 b–f	0.0053 d–g	0.0030 cde
V1P2S0	99.00 a	0.00 f	22.84 a–d	94.67 a–d	5.33 fg	11.61 ef	11.23 a	0.0233 cde	0.0070 ab	0.0073 a–d	0.0040 bc
V1P2S1	98.67 ab	1.33 ef	23.85 ab	94.67 a–d	4.00 fg	13.16 cd	11.00 ab	0.0240 cd	0.0057 a–e	0.0080 ab	0.0033 bcd
V1P2S2	94.67 a–e	5.33 b–f	18.02 g–m	85.33 b–h	9.33 d–g	10.20 hij	8.80 gh	0.0180 fgh	0.0047 c–f	0.0067 a–f	0.0033 bcd
V1P2S3	97.33 abc	2.66 def	16.47 k–p	76.00 h–k	21.33 abc	9.01 klm	7.90 jk	0.0220 de	0.0060 a–d	0.0060 b–g	0.0023 def
V1P3S0	100.00 a	0.00 f	25.35 a	100.00 a	0.00 g	14.83 a	10.51 bc	0.0300 a	0.0077 a	0.0083 a	0.0049 bc
V1P3S1	97.33 abc	2.66 def	22.74 a–e	93.33 a–e	4.00 fg	14.28 ab	9.07 fg	0.0280 ab	0.0060 a–d	0.0077 abc	0.0037 a
V1P3S2	97.33 abc	2.66 def	20.33 d–i	89.33 a–g	8.00 d–g	11.71 ef	9.17 efg	0.0240 cd	0.0060 a–d	0.0067 a–f	0.0027 c–f
V1P3S3	94.67 a–e	5.33 b–f	17.29 i–n	84.0 c–i	10.66 c–g	10.26 hi	8.70 gh	0.0187 fg	0.0060 a–d	0.0073 a–d	0.0047 b
V2P0S0	93.33 b–e	6.66 b–e	18.67 f–l	84.00 c–i	9.33 d–g	11.52 efg	7.99 ijk	0.0160 g–j	0.0050 b–f	0.0057 c–g	0.0023 def
V2P0S1	90.67 def	9.333 abc	16.36 k–p	84.00 c–i	6.66 efg	10.36 hi	7.69 jkl	0.0140 ijk	0.0050 b–f	0.0043 ghi	0.0030 cde
V2P0S2	93.33 b–e	6.66 b–e	15.79 l–p	76.00 h–l	17.33 a–e	10.03 hijk	7.55 kl	0.0160 g–j	0.0050 b–f	0.0050 e–h	0.0033 bcd
V2P0S3	93.33 b–e	6.66 b–e	13.49 p	68.00 kl	25.33 a	7.00 o	7.90 jk	0.0127 kl	0.0050 b–f	0.0047 f–i	0.0037 bcd
V2P1S0	97.33 abc	2.66 def	20.49 d–h	92.00 a–e	5.33 fg	11.51 efg	8.80 gh	0.0160 g–j	0.0050 b–f	0.0057 c–g	0.0027 c–f
V2P1S1	96.00 a–d	4.00 c–f	17.55 h–n	85.33 b–h	10.66 c–g	9.70 ijkl	7.90 jk	0.0127 kl	0.0043 d–g	0.0043 ghi	0.0030 cde
V2P1S2	93.33 b–e	6.66 b–e	15.85 l–p	78.67 f–k	14.66 a–f	9.68 ijkl	7.19 lmn	0.0167 ghi	0.0047 c–f	0.0047 f–i	0.0013 f
V2P1S3	86.67 f	13.33 a	13.60 p	64.00 l	22.66 ab	8.63 lmn	7.10 lmn	0.0140 ijk	0.0040 d–g	0.0043 ghi	0.0023 def
V2P2S0	90.67 def	9.333 abc	19.77 e–j	84.00 c–i	6.66 efg	12.59 de	8.96 fg	0.0143 ijk	0.0047 c–f	0.0030 hi	0.0033 bcd
V2P2S1	93.33 b–e	6.66 b–e	17.28 i–n	81.33 e–j	12.00 b–f	11.00 fgh	7.50 klm	0.0160 g–j	0.0037 efg	0.0050 e–h	0.0013 f
V2P2S2	92.00 c–f	8.00 a–d	15.66 l–p	73.33 h–l	18.66 a–d	9.82 ijk	6.92 mn	0.0107 l	0.0033 fg	0.0027 i	0.0023 def
V2P2S3	96.00 a–d	4.00 cdef	14.73 nop	72.00 i–l	24.00 a	7.86 no	7.19 lmn	0.0150 h–k	0.0023 g	0.0040 ghi	0.0017 ef
V2P3S0	96.00 a–d	4.00 cdef	18.11 g–m	90.67 a–f	5.33 fg	10.22 hi	8.70 gh	0.0167 ghi	0.0050 b–f	0.0057 c–g	0.0030 cde
V2P3S1	96.00 a–d	4.00 c–f	19.35 f–k	81.33 e–j	14.66 a–f	11.84 ef	8.30 hij	0.0147 ijk	0.0040 d–g	0.0047 f–i	0.0023 def
V2P3S2	92.00 c–f	8.00 a–d	17.20 j–o	77.33 g–k	14.66 a–f	10.46 ghi	8.57 ghi	0.0133 jkl	0.0037 efg	0.0057 c–g	0.0027 c–f
V2P3S3	92.00 c–f	5.33 b–f	14.21 op	68.00 kl	24.00 a	8.24 mn	6.78 n	0.0133 jkl	0.0043 d–g	0.0040 ghi	0.0017 ef
Level of Sig.	**	**	**	**	**	**	**	**	**	**	**
CV (%)	4	6.77	10.11	9.37	9.67	9.71	12.43	17.52	15.64	12.24	12.26

** = significant at 1% level of probability. In a column, figures with same letter (s) or without letter do not differ significantly whereas figures with dissimilar letter differ significantly. All abbreviations are defined in Section 2.2.

3.8. Principal Component Analysis (PCA), Correlation, and Heatmap Analyses

3.8.1. Principal Component Analysis (PCA)

The PCA explained 92% of the total variation (PCA1 = 86%, PCA2 = 6%). PCA1 clearly separated V1 from V2, indicating that the varietal response strongly influenced the seedling performance under priming conditions (Figure 5). Traits such as G (%), VI, SL, and RL, and SFW, RFW, SDW, and RDW were positively associated on the positive axis, suggesting a coordinated improvement in early vigor and biomass accumulation, likely due to the enhanced metabolic activity and resource mobilization. In contrast, V2 clustered on the negative side, reflecting a lower physiological efficiency. PCA2 contributed little additional variation. Overall, early vigor and biomass traits were key drivers of treatment differences.

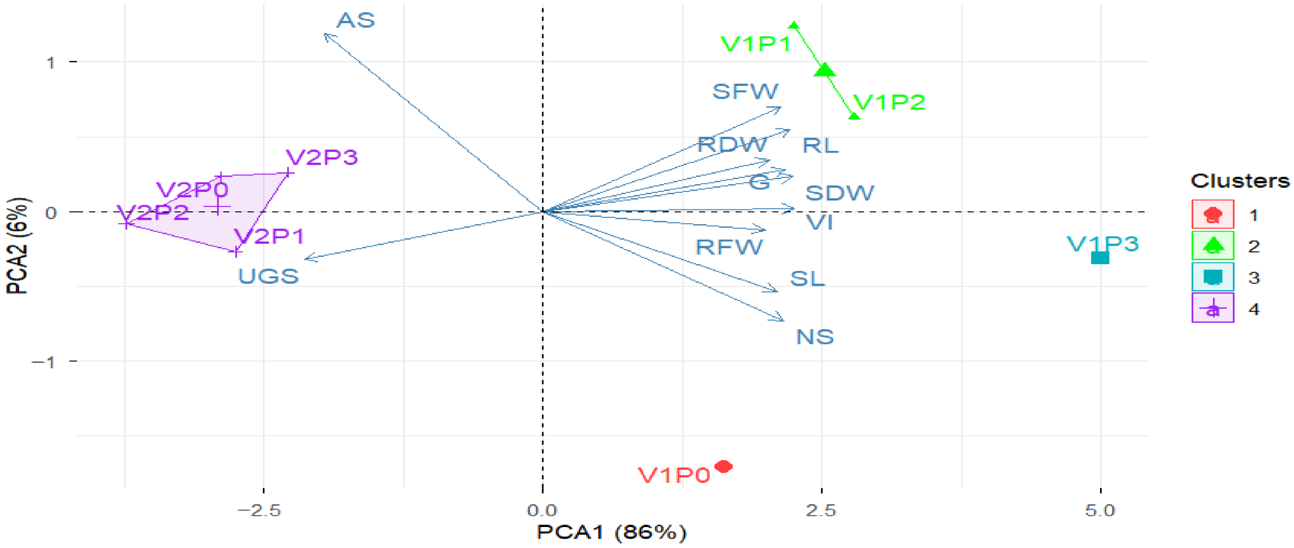

Figure 5. The Principal Component Analysis (PCA) of interaction effect of variety and seed priming on germination, vigor index, and seedling growth of Boro rice. All abbreviations are defined in Section 2.2.

Figure 6 depicts that the PCA accounted for 96% of the entire variation, with PCA1 contributing 88% and PCA2 8%. PCA1 distinctly separated V1 from V2, reflecting a strong varietal response to salinity stress. Traits such as G%, VI, SL, RL, SFW, RFW, SDW, RDW, and NS were positively associated, suggesting a coordinated improvement in early vigor and biomass, likely driven by the better osmotic adjustment and resource use under lower salinity. V1S0 and V1S1 were closely associated with these traits, reflecting superior performance. In contrast, V2 clustered on the negative side, indicating a lower efficiency. PCA2 provided minimal supplementary variation.

The PCA (Figure 7) indicated 76% of the overall variance, with PCA1 accounting for 66% and PCA2 for 10%. PCA1 mainly separated treatments based on the combined effects of seed priming and salinity stress. Growth and vigor traits clustered together, indicating strong positive associations in seedling performance, likely due to the better stress tolerance and efficient resource utilization. P0S0, P1S0, and P1S1 were closely aligned with these traits, reflecting improved establishment and biomass accumulation. In contrast, P2S2 and P2S3 were positioned oppositely, reflecting the reduced growth under higher salinity. Overall, a clear grouping indicates distinct physiological responses among treatments.

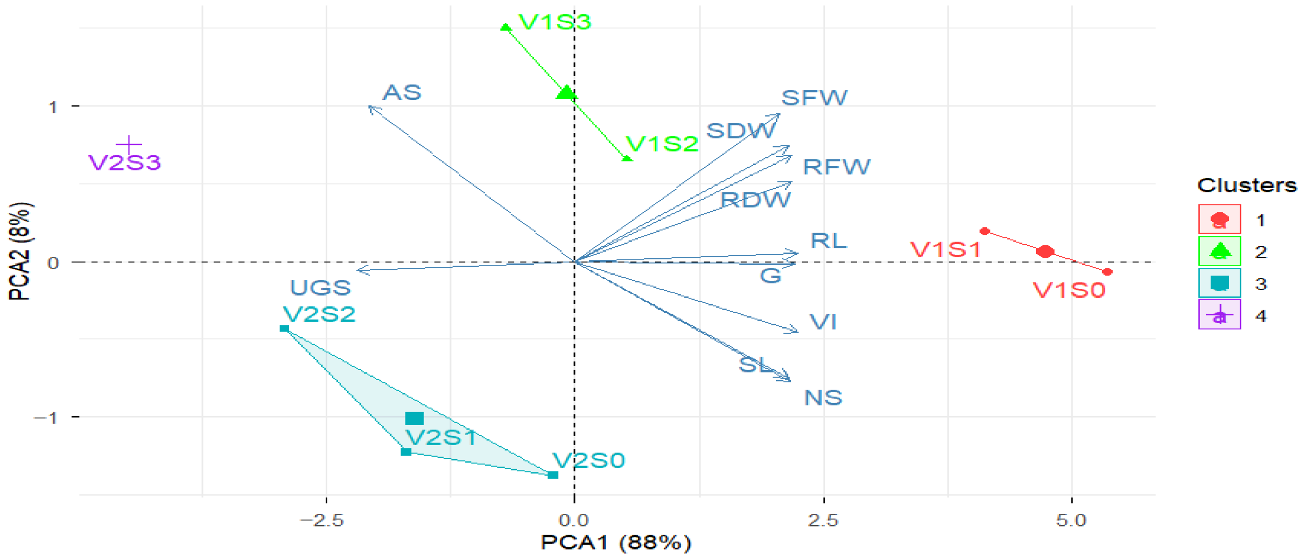

Figure 6. The Principal Component Analysis (PCA) of Interaction effect of variety and salinity level on germination, vigor index, and seedling growth of Boro rice. All abbreviations are defined in Section 2.2.

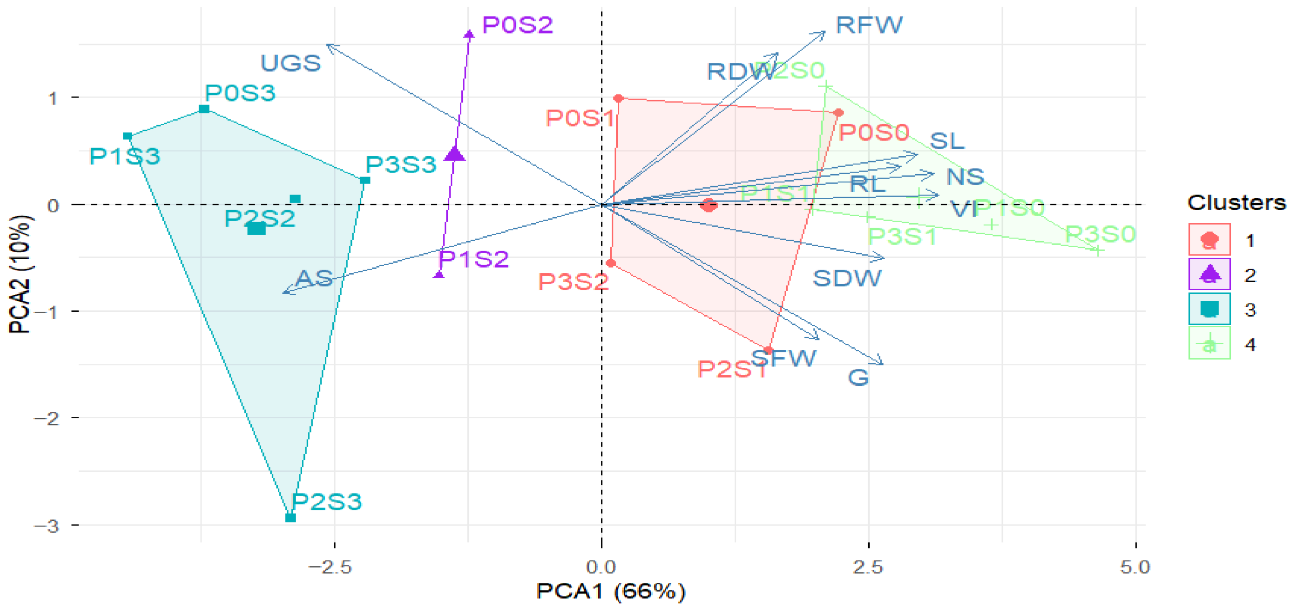

Figure 7. The Principal Component Analysis (PCA) of interaction effect of seed priming and salinity level on germination, vigor index, and seedling growth of Boro rice. All abbreviations are defined in Section 2.2.

The PCA biplot shows the interaction effects of variety, seed priming, and salinity (Figure 8). PCA1 explained 69% of the total variation, while PCA2 accounted for 10%, capturing a 79% cumulative variability and indicating reliable treatment separation. Cluster 1 (red) was positioned on the positive side of PCA1 and was strongly associated with SFW, RFW, SDW, RL, and NS, suggesting enhanced biomass production and better physiological efficiency, likely due to the improved stress adaptation and resource utilization. Cluster 2 (purple) showed a moderate association with G (%) and related traits, reflecting an intermediate performance. Cluster 3 (green) exhibited mixed, moderate responses, while Cluster 4 (cyan) on the negative PCA1 side indicated reduced growth and biomass under higher salinity conditions.

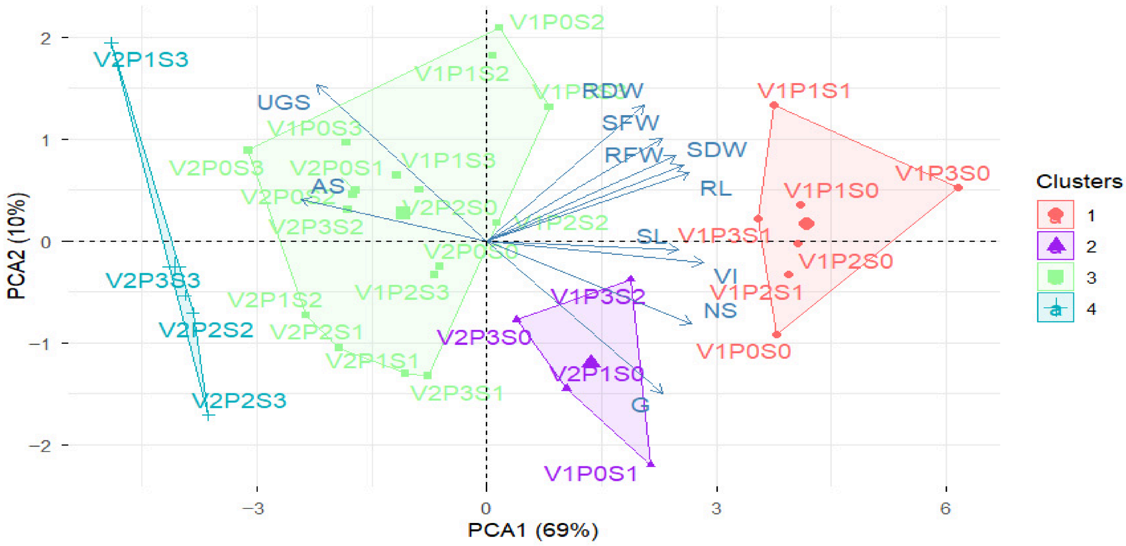

Figure 8. The Principal Component Analysis (PCA) of interaction effect of variety, seed priming, and salinity level on germination, vigor index, and seedling growth of Boro rice. All abbreviations are defined in Section 2.2.

3.8.2. Correlation Analysis

Figure 9 shows Pearson's correlations among G%, VI, SL, and RL, and SFW, RFW, SDW, and RDW under different varieties and priming treatments. Most traits were strongly and positively correlated, indicating a coordinated improvement in seedling performance. G% was highly correlated with VI (r ≈ 0.96), RL (r ≈ 0.96), and SDW (r ≈ 0.93), suggesting that better germination supports stronger growth and biomass, likely due to the enhanced metabolic activity and resource mobilization. VI also showed positive associations with SL, RL, and biomass traits. UGS was strongly negatively correlated with G% (r ≈ −0.99) and VI (r ≈ −0.92), indicating that seed failure reduces performance; effective seed priming improves growth under salinity.

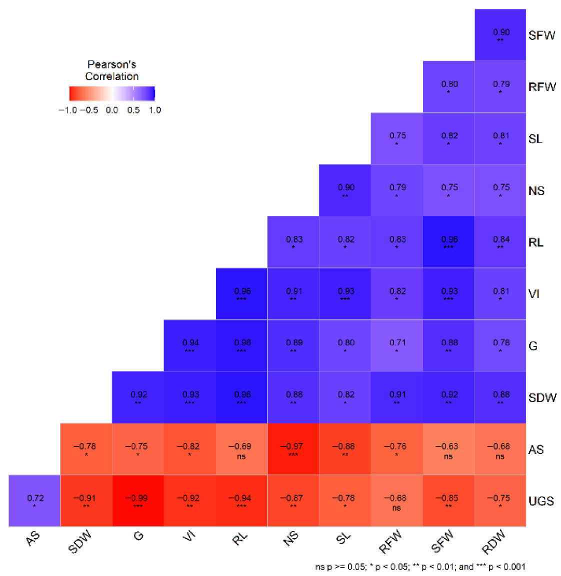

Figure 9. Correlation analysis of interaction effect of variety and seed priming on germination, vigor index, and seedling growth of Boro rice. All abbreviations are defined in Section 2.2.

Figure 10 illustrates Pearson's correlations among the G%, VI, and seedling growth of Boro rice across varieties and salinity levels. The majority of traits exhibited a strong and positive correlation, suggesting a coordinated development of seedlings. G% was positively related to SDW (r ≈ 0.92), SFW (r ≈ 0.90), and RFW (r ≈ 0.85), suggesting that better germination supports biomass accumulation, likely due to efficient resource use. VI also showed strong associations with RL (r ≈ 0.97) and SL. Overall, these relationships reflect the improved physiological performance under favorable conditions.

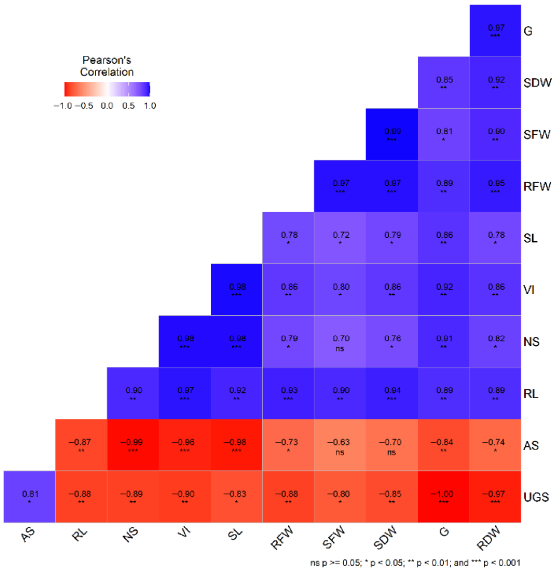

Figure 10. Correlation analysis of interaction effect of variety and salinity level on germination, vigor index, and seedling growth of Boro rice. All abbreviations are defined in Section 2.2.

A correlation analysis of the interaction effect of seed priming and salinity levels on the germination, vigor index, and seedling growth of Boro rice is presented in Figure 11. G% was positively related to RL (r ≈ 0.79), SDW (r ≈ 0.65), and SFW (r ≈ 0.53), indicating that better germination supports biomass, likely through improved metabolic activity. VI also showed strong associations with RL (r ≈ 0.88) and SL (r ≈ 0.88), reflecting enhanced seedling vigor. In contrast, UGS showed strong negative correlations with G% (r ≈ −0.99), VI (r ≈ −0.91), and RL (r ≈ −0.79), indicating that poor emergence reduces growth, likely due to the lower physiological efficiency.

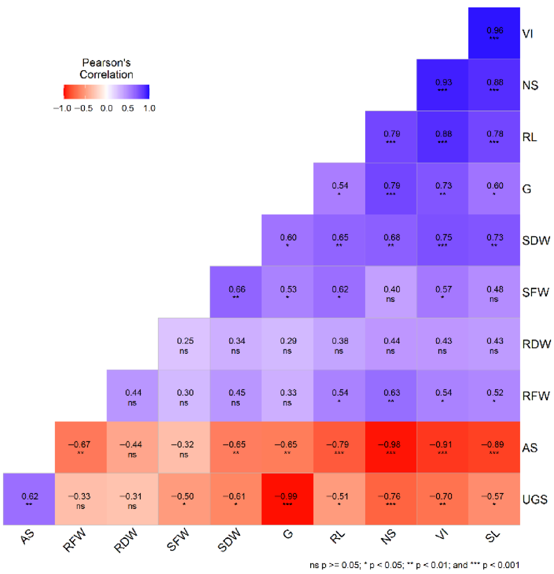

Figure 11. Correlation analysis of interaction effect of seed priming and salinity level on germination, vigor index, and seedling growth of Boro rice. All abbreviations are defined in Section 2.2.

The Pearson correlation (Figure 12) shows the relationships among germination (%), vigor index (VI), seedling growth, and biomass under different variety, seed priming, and salinity treatments. Germination (%) was strongly and positively associated with VI (r = 0.94), seedling length (r = 0.82), and root length (r = 0.73), indicating better early establishment and root development with higher vigor. Positive correlations among SFW, RFW, SDW, and RDW reflect coordinated biomass accumulation. In contrast, AS and UGS showed strong negative correlations with most growth traits, particularly G (r = −0.99) and VI (r = −0.70), confirming its inhibitory impact on seedling growth and establishment.

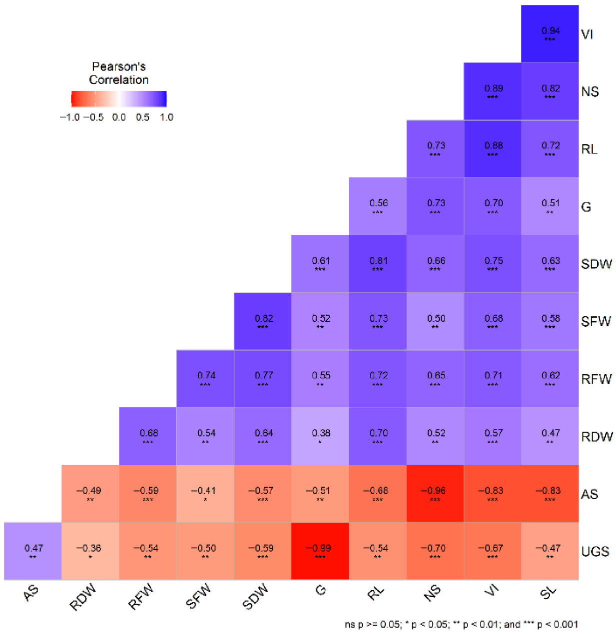

Figure 12. Correlation analysis of interaction effect of variety, seed priming, and salinity level on germination, vigor index, and seedling growth of Boro rice. All abbreviations are defined in Section 2.2.

3.8.3. Heatmap Analysis

The heatmap (Figure 13) shows clear differences in seedling responses between the two rice varieties under priming treatments. In Binadhan-10 (V1), hydro-, osmo-, and nano-priming were strongly associated with higher G, VI, SL, RL, and biomass traits (SFW, RFW, SDW, and RDW), indicating enhanced physiological activity and better seedling establishment. In contrast, UGS and AS showed negative relationships with these traits, reflecting the stress-induced inhibition of early growth. BINA dhan25 (V2) showed weaker or mostly negative responses, suggesting a lower physiological adaptability to priming. Overall, seed priming biologically improved early growth mainly in Binadhan-10(V1) through enhanced vigor and stress mitigation.

The heatmap exhibits specific varietal differences in response to salinity stress as observed in Figure 14. In Binadhan-10 (V1), the control (S_0) and moderate salinity (S2) supported higher germination, vigor, and seedling growth, indicating better tolerance at early stress levels. With increasing salinity (S2–S3), growth traits declined. In BINA dhan25 (V2), higher salinity was strongly associated with reduced growth and increased UGS and AS, reflecting greater sensitivity. Overall, Binadhan-10 showed comparatively better salinity tolerance during early seedling development.

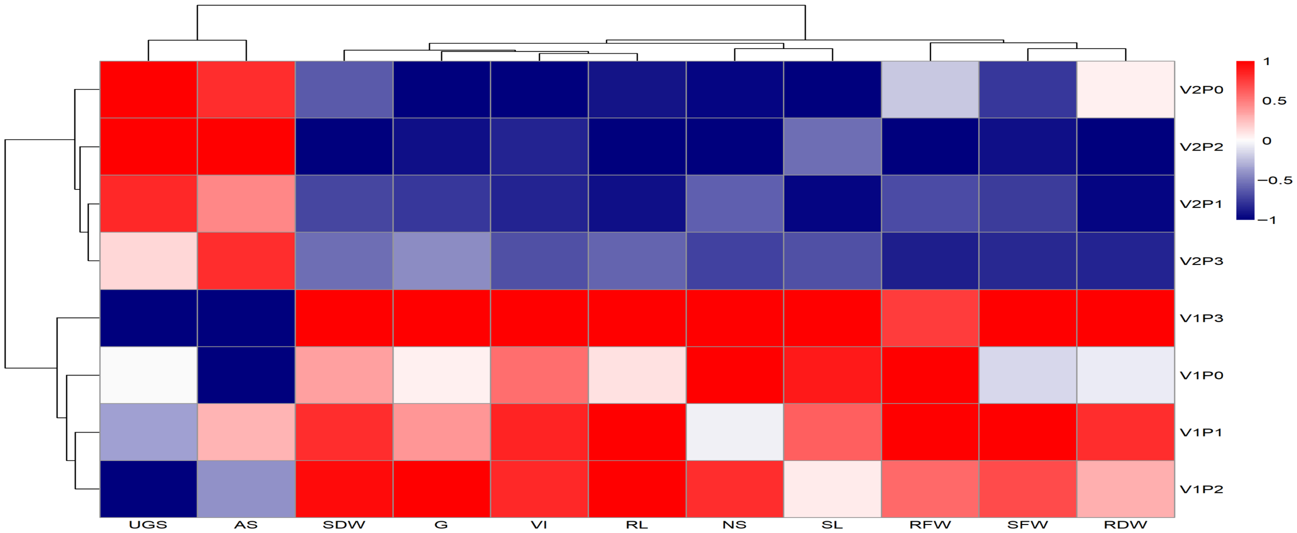

Figure 13. Heatmap analysis of interaction effect of variety and seed priming on germination, vigor index, and seedling growth of Boro rice. All abbreviations are defined in Section 2.2.

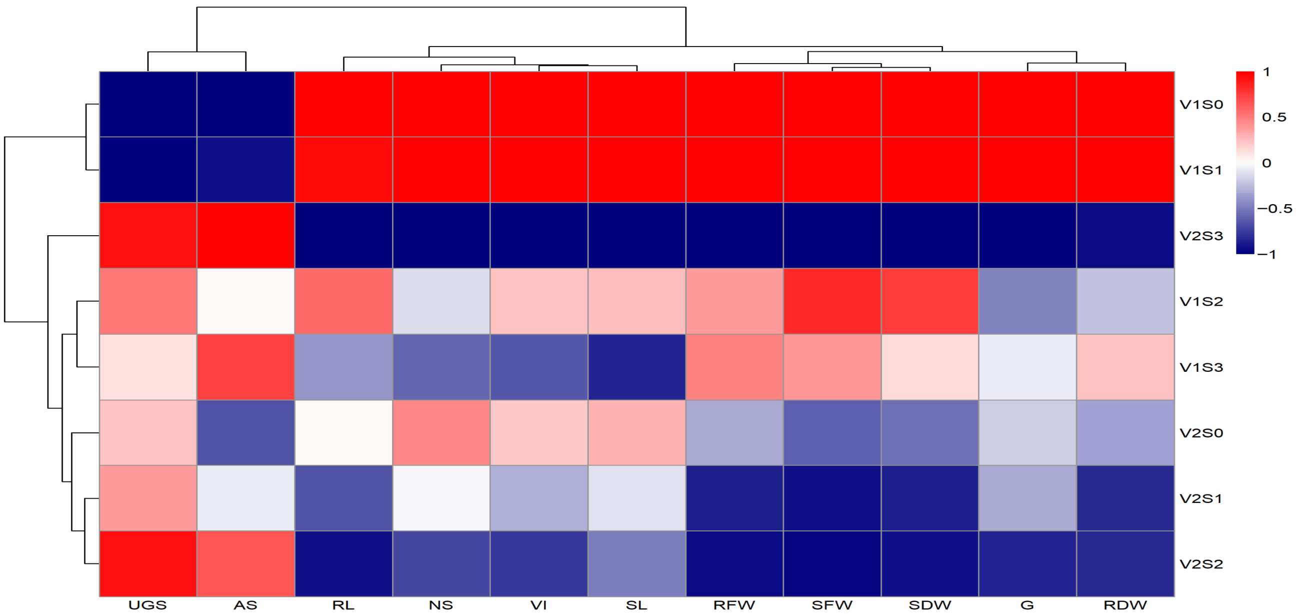

Figure 14. Heatmap analysis of interaction effect of variety and salinity level on germination, vigor index, and seedling growth of Boro rice. All abbreviations are defined in Section 2.2.

The heatmap presented in Figure 15 indicates that seed performance was significantly affected by both priming techniques and salinity levels. Under non-saline conditions (S0), hydro- and osmo-priming were positively associated with germination, vigor, and biomass traits, indicating improved early seedling growth. In contrast, higher salinity (S2–S3) was linked with increased UGS and AS and reduced growth traits, reflecting stress inhibition. Nano-priming maintained a relatively better performance under moderate salinity (S1). Overall, priming, especially hydro- and nano-priming, helped alleviate salinity stress effects in Boro rice.

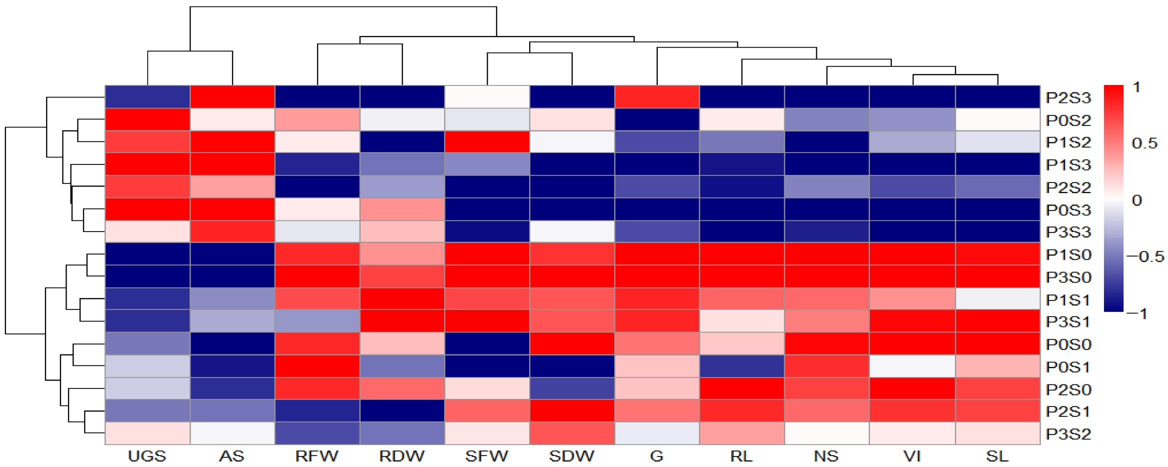

Figure 15. Heatmap analysis of interaction effect of seed priming and salinity level on germination, vigor index, and seedling growth of Boro rice. All abbreviations are defined in Section 2.2.

The heatmap (Figure 16) clearly shows that seed priming, especially hydro-priming (P1) and nano-priming (P3), enhanced G (%), VI, SL, RL, and biomass traits (SFW, RFW, SDW, and RDW) under non-saline to mild saline conditions (S0–S1). These benefits were more evident in Binadhan-10 (V1), suggesting better physiological resilience and early metabolic activation through priming. However, at higher salinity (S2–S3), the increased number of ungerminated and abnormal seedlings reflected the salt-induced stress that suppressed growth and biomass accumulation. The close association among germination, vigor, and biomass traits highlights their biological interdependence. Overall, suitable priming partially mitigates salinity stress, though varietal differences persist under severe salinity.

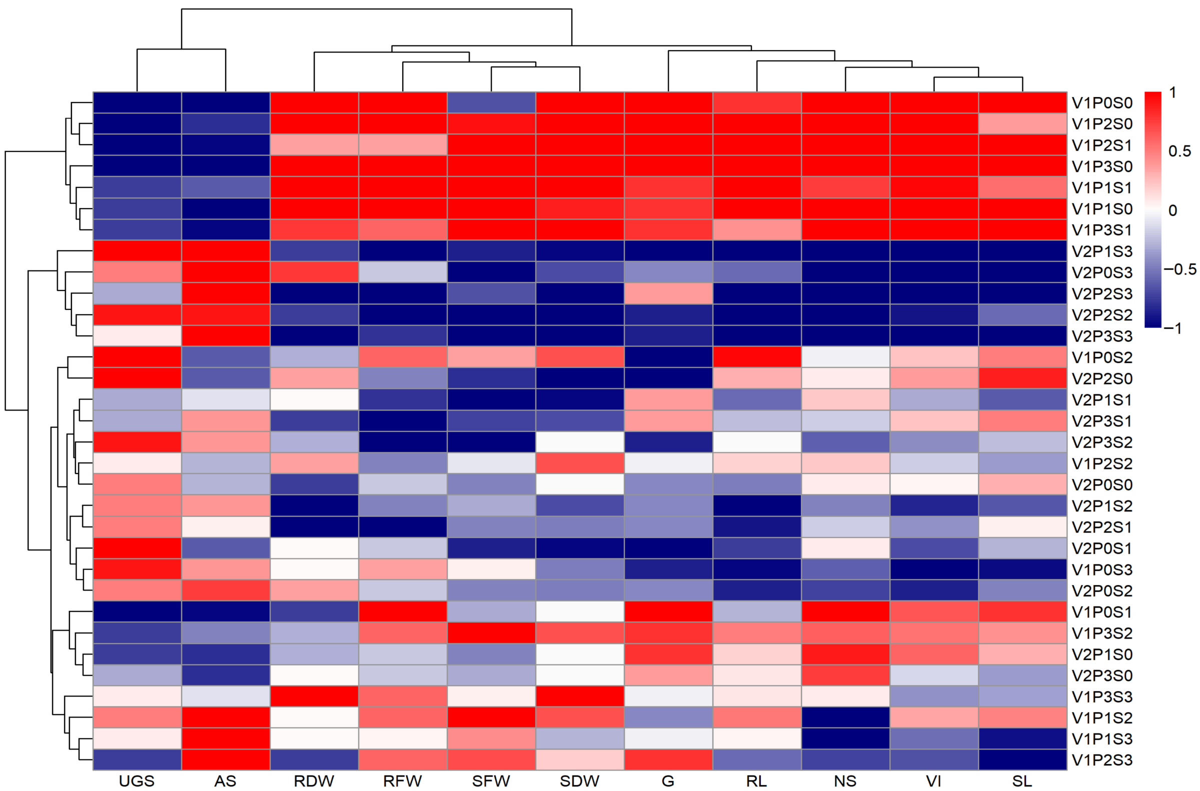

Figure 16. Heatmap analysis of interaction effect of variety, seed priming, and salinity level on germination, vigor index, and seedling growth of Boro rice. All abbreviations are defined in Section 2.2.

4. Discussion

In the study, the selected rice seeds received different levels of salinity stress from the seed germination to seedling growth stages. According to several investigations [29], seed priming increases a plant's ability to withstand stress. For rice seedlings subjected to salt stress, it was thus predicted that pre-sowing seed treatment might improve germination, raise the germination rate, and improve seedling vigor and development.

In both favorable and unfavorable circumstances, the rate, pattern, and strength of seed germination, as well as the growth and production of seedlings, are critical factors [30,31]. However, regions with varying levels of soil salt have a negative impact on seed germination and seedling development [32]. In this study, the variety showed significant variation on these parameters and Binadhan-10 outperformed BINA dhan25. This outcome might be caused by Binadhan-10's genetic makeup. Salt-tolerant varieties, like Binadhan-10, use mechanisms that control the ion balance, osmotic adjustment, and ROS detoxification to survive in saline environments, whereas susceptible varieties frequently experience the harmful effects of elevated salt concentrations [33]. Similarly, ref. [34] discovered that the germination % varied greatly amongst the cultivars.

Both Binadhan-10 and BINA dhan25 seeds were tested under four salinity levels (0, 5, 8, and 11 dS m^{-1}), with Binadhan-10 showing superior performance under saline conditions. With rising salinity, the germination, seedling growth, and vigor of both varieties steadily decreased. It is also noted that, compared to BINA dhan25, Binadhan-10 was capable of producing more at 11 dS m^{-1} because of its better intrinsic tolerance mechanism. These results indicate that the varietal response to environmental conditions varies significantly. Salinity decreases seedling growth and germination by inducing oxidative stress, ion toxicity, and osmotic stress, impairing the water uptake and overall vigor [35]. A delay in plant growth is one of the first effects of salt stress. For two reasons, salt in soil and water prevents plants from growing. Salinity slows water absorption, reducing growth (osmotic effect), and harms embryo viability, causing cell damage and enzyme structure alteration during germination [35,36].

Seed priming, a process to assist plants in becoming more tolerant to stress, is an alternative way to overcome these limitations [14]. Seed priming is a technique that can increase crop yield by ensuring consistent germination and accelerating germination. Among different priming techniques in this experiment, osmo-priming, hydro-priming, and nano-priming were used. It was observed that priming had a significant impact in overcoming this salinity stress and showed a steadiness in the case of the germination, seedling growth, and vigor of rice seeds of both varieties. Additionally, seed priming improved seed germination; primed seeds started off better than unprimed seeds. According to [37], the imbibition and lag phases of water absorption are reduced when the primed seeds are sowed. The embryo's expansion within the primed seed promotes water absorption, which accelerates germination [38]. As stated by [39], seed priming prepares the seed for radicle protrusion by promoting the metabolic processes that occur before germination.

The priming agents used in this study were capable of addressing the problems related to stress. Hydro-priming, or seed priming with distilled water, can help reduce the negative effects of salt stress on plant development by preserving the photosynthetic activity and boosting proline and sugar synthesis. In order to withstand salt stress, hydro-priming triggers adaptive processes. Numerous investigations have documented the enhanced photosystem performance and carbohydrate absorption after priming, which is consistent with the current study [40–42]. To enable partial hydration, seeds are treated with a lower potential osmotic solution, such as PEG, during the osmo-priming process. By exposing the seeds to possible osmotic solutions, stress and cross-tolerance are accelerated because more genes and proteins linked to stress are expressed. Under salinity stress, PEG priming increased osmotic adjustment, soluble sugar and organic acid concentrations, and the concentrations of K^+, Mg^{2+}, and Ca^{2+} ions, while decreasing the quantities of Cl^- or Na^+ ions [43].

Among the priming agents used in this experiment, nano-priming with Zinc EDTA (12%) showed outstanding results in the non-saline condition with 100% germination in Binadhan-10 and 96% germination in BINA dhan25 and also showed a steady performance in the saline condition. Nano-priming is thought to be a useful strategy for accelerating plant growth and development in both stressful and non-stressful environments. By reducing the detrimental effects of reactive oxygen species (ROS), this accelerates the activities of antioxidant enzymes, which lessen the stress in plants and promote better development and yield [44]. In the opinion of [45], zinc is a micronutrient that is crucial for reducing the negative effects of salt stress. In order to shield plants from oxidative damage caused by salt, zinc is a crucial part of many enzymes and protein stabilizers [46].

Zn seed priming is a beneficial agricultural practice that offers multiple advantages for crop performance and resilience. Firstly, Zn seed priming increases the rate of seedling emergence and promotes vigorous early growth, which, in turn, contributes to higher yields and improved crop quality. This is especially important for achieving uniform stands and better resource use efficiency. On a physiological level, Zn seed priming enhances the plant's antioxidant defense system by boosting the activity of key antioxidant enzymes, including superoxide dismutase (SOD), catalase (CAT), peroxidase (POD), and ascorbate peroxidase (APX). These enzymes play crucial roles in detoxifying reactive oxygen species (ROS), which accumulate in plants under various stress conditions such as drought or salinity [47]. By strengthening the antioxidant system, Zn priming helps protect cellular structures and maintain normal metabolic processes, thereby supporting the overall plant health. In addition to its effects on antioxidants, Zn priming stimulates the accumulation of proline and total soluble proteins within plant tissues. Proline is an important osmoprotectant that helps plants manage water stress by stabilizing proteins and membranes and scavenging free radicals. Total soluble proteins also contribute to osmoregulation and serve as reserves for plant growth and recovery during stress [47,48].

Nutritionally, Zn application helps maintain nutrient homeostasis in plants. It reduces the uptake and accumulation of harmful ions like sodium (Na^+) and chloride (Cl^-), which are typically elevated in saline soils and can cause toxicity. At the same time, Zn enhances potassium (K^+) uptake—an essential nutrient for maintaining cellular functions, enzyme activation, and osmotic balance. This nutrient balance is vital for optimum plant growth and resilience under saline conditions [47].

Furthermore, scientific studies have demonstrated that, during the seed-priming process, Zn^{2+} ions are absorbed and stored in the seed's aleurone layer—a tissue rich in proteins and nutrients. This stored Zn becomes available to the developing seedling, providing essential micronutrients during the early stages of growth. As a result, seedlings from Zn-primed seeds exhibit significantly improved development and biomass, with research showing up to a 25% increase in biomass under salt stress compared to non-primed controls [49].

Moreover, priming methods are being applied to enhance seed germination in both favorable and unfavorable circumstances. Priming induces physiological and biochemical changes in seeds during seed treatments, indicating a greater vigor index and well-developed shoot and root [50], allowing them to tolerate saline stress. A limitation of the present study is that the experiment was conducted under controlled indoor conditions. Nevertheless, further validation through multi-location field trials under diverse agro-ecological conditions is necessary to confirm the practical applicability of different seed-priming agents on rice seedling traits under saline areas in Bangladesh.

5. Conclusions

The current study unequivocally shows that, at various saline stress levels, seed priming greatly enhances the Boro rice germination, vigor index, and seedling growth. Binadhan-10 continuously beat BINA dhan25 of the two tested cultivars, demonstrating superior seedling performance and greater resistance to salt. The most successful priming method, especially in non-saline settings, was nano-priming with Zn-EDTA (170 ppm), which effectively lessened the negative effects of salinity stress. The highest germination percentage, vigor index, and seedling growth parameters were recorded at the nano-priming × no salinity (P3S0) interaction, while the poorest performance was observed under no priming × highest salinity (P0S3). The capacity of seeds to germinate and the nutritional level of the seedling are really tied to the vigor index, and they are influenced by a variety of elements, including the moisture, temperature, light, nutrition, pathogens, and more. Therefore, there is also a lot of room to test the outcome by using the same therapy at different times.

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