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ROLE OF MAGNESIUM NITRATE AND BORIC ACID ON GERMINATION AND SEEDLING GROWTH IN WHEAT (*TRITICUM AESTIVUM* L.) GENOTYPE HUW-468

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ABSTRACT

Plants require macronutrients and micronutrients to regulate their metabolic processes throughout growth and development. Magnesium and Boron are macro- and micronutrients that play unique roles inside plants. An experiment was done to determine the effectiveness of these two nutrients as seed priming agents. Wheat seeds from variety HUW-468 were soaked for 12 hours in solutions of Mg $(NO_3)_2$ (macro nutripriming, C1), H_3BO_3 (micro nutripriming, C2), Mg $(NO_3)_2$ + H_3BO_3 (combined nutripriming, C3), and distilled water (hydropriming, C4) for priming. The four sets are dehydrated using forced air and utilized for experiments. Along with germination percentage, plumule and radicle lengths, fresh and dry weights of radicle and plumule, alpha amylase activity, soluble sugar content, and seedling vigour (indices I and II) were also recorded at 4, 6, 8, and 10^{th} days after seed germination. Seeds primed with magnesium nitrate and boric acid (C3) had the maximum radicle length and fresh/dry weight. Macro and micro primed sets outperformed hydro primed and non-primed control sets across all criteria. The application of magnesium nitrate and boric acid has demonstrated significant effects on the germination and seedling growth parameters of wheat (*Triticum aestivum* L.) variety HUW-468. Both magnesium nitrate and boric acid contributed to enhanced seedling vigor, improved germination rates, and promoted better overall growth.
***Keywords*:** Magnesium Nitrate, Boric Acid, Seedling Growth, Seedling vigour.

Introduction

Many scientists are dedicated to increasing wheat yield and productivity. Crops require macronutrients and micronutrients for growth and development. Magnesium, a macronutrient, is a component of chlorophyll and is necessary for pigment production during photosynthesis. Magnesium modulates enzyme activity, potentially impacting yield. It connects two ribosome units and plays a role in protein translation. Boron, a micronutrient, influences pollen germination and sugar transport in plants. Seed priming is defined as the application of a chemical or physical substance to seed or seeding material. Seed priming involves regulated hydration, allowing seeds to absorb water prior to radicle protrusion (Bradford, 1986). Seed priming produces an atmosphere in which a seed can absorb enough moisture to speed up the germination process. The main goal of seed priming is to improve germination, hence shortening germination time and mitigating crop delayed sowing (Ashraf & Foolad, 2005). Farooq *et al.* (2008) found that at later stages, it enhances plant growth and vitality, allowing it to thrive even in extreme environments. It accelerates germination by allowing metabolic activities to proceed before germination. During seed priming, the osmotic pressure and duration of seed contact with the membrane allow for pre-germinative metabolic activities to occur up to the point of radicle emergence. Magnesium and boron, both essential macro and micronutrients, have a significant impact in wheat crop production. $Mg(NO_3)_2$-primed wheat seeds of HUW-206, HUW-234, and HP-1102 outperformed the control (Bose *et al.*, 2007). Several studies have demonstrated that several field crops like wheat, rice, maize, and mustard primed seeds showed improvement in germination physiology, vegetative growth, and yield (Sharma & Bose 2006). Priming wheat types boosted final percentage emergence and reduced time to 50% emergence compared to unsoaked seed (Murungu, 2004). Chakraborty and Dwivedi (2021) found that seed priming in wheat improved germination, early canopy development, and tillering compared to the untreated control. Verma *et al.* (2014) found that priming increased oat germination, seedling length, dry weight, vigour, and speed of germination. Toklu *et al.* (2015) found that PEG, IAA, and distilled water increased wheat seed germination, emergence, and growth rates. Priming the seed batch can boost germination rate and uniformity. According to Chakraborty and Dwivedi (2022), seed priming enhances wheat crop emergence and vigor, resulting in better plant stand. Seed priming improved wheat germination, maturity, and harvest, while also mitigating the negative effects of dry spells. Wheat biological yields were highest when seeds were sprouted, followed by priming with IAA, KCl, water, and $ZnSO_4$, and lowest when seeds were sown dry (control). Boron improves rice germination (Farooq *et al.*, 2011), as well as wheat seedling emergence, growth, and yield (Iqbal *et al.*, 2017). Every mineral nutrient has a distinct job to play in plant metabolism, and the current study discovered that magnesium and boron complete their roles appropriately when their salts are used as priming agents in seed priming techniques.

Materials and Methods

The experiment was done in the seed priming research laboratory of the Department of Plant Physiology at the Institute of Agricultural Sciences, Varanasi. The wheat seeds, variety HUW-468, were received from the same institute's Agronomy department. Seeds were sterilized with 0.1% $HgCl_2$ solution for 2-3 minutes before being properly washed with distilled water to ensure they were healthy and disease-free. The seeds were soaked for 12 hours in 7.5 mM Mg $(NO_3)_2$ and 8 mM H_3BO_3, or in a combination of both with distilled water (hydro primed). After 12 hours of soaking, the seeds were naturally dried to remove any moisture on their surface. Seeds were dried and placed in Petri-plates with germination paper. 10 ml of distilled water was then added to each plate. A control set (untreated) will be placed in Petri-plates for germination studies. Petri plates with four treatments, one control, and four replications are stored at room temperature (20±2 °C). After soaking, the seeds were dried beneath a fan until they returned to their original weight. Germination and seedling growth tests were conducted in petri-plates for up to 10 days under laboratory conditions. The treatments included non-primed seeds (C0), seeds primed with 7.5mM Mg $(NO_3)_2$ (C1), 8mM H_3BO_3 (C2), and a combination of 7.5mM Mg $(NO_3)_2$ (C3) and 8mM H_3BO_3 and distilled water (hydro primed; C4).

Seed germination and growth assessment

The following formula was used to determine seed germination percentages and other attributes at 24, 48, 72, 96, and 120 hours of germination: Germination percentage = Number of seeds germinated/ Total number of seeds sown × 100. Developing wheat seedlings' radicle and plumule lengths were measured on a cm scale. The fresh and dried weights of plumule and radicle were measured using the traditional method; the fresh weight was determined by using a weighing balance, whereas, to estimate the dry weights of plumule and radicle, the samples were kept for one hour at 100-110°C to kill the microbial infections. Thereafter, the temperature was set to 60±2 °C to ensure consistent sample weight. The formula for calculating seedling vigor I and II was as follows: Seedling vigour I: germination% × (shoot length + root length); Seedling vigour II: germination% × seedling dry weight. Biochemical parameters, including α-Amylase and soluble sugar, were calculated using Bernfield's (1955) and Dubois's (1956) methods, respectively, to validate the influence of magnesium and boron on wheat seedling growth. To estimate α-amylase activity in germinating wheat seedlings, 500 mg of wheat endosperm was homogenized in phosphate buffer (pH 6.9) and centrifuged to obtain the enzyme extract. The supernatant was mixed with a 19% starch solution and incubated at 20°C for 5 minutes. After incubation, DNS solution was added to halt the enzyme activity, and the mixture was heated, cooled, and diluted. Amylase activity was determined by measuring absorbance at 560 nm using a maltose standard curve. Statistical analysis was used to analyze all physicochemical and biochemical parameters at 4, 6, 8, and 10 days of germination in the laboratory.

Result and Discussion

Germination percentage is a key parameter that helps determine the viability and planting value of seeds. Specifically, it indicates the proportion of seeds in a sample that successfully germinate under optimal or controlled conditions within a specific time frame.

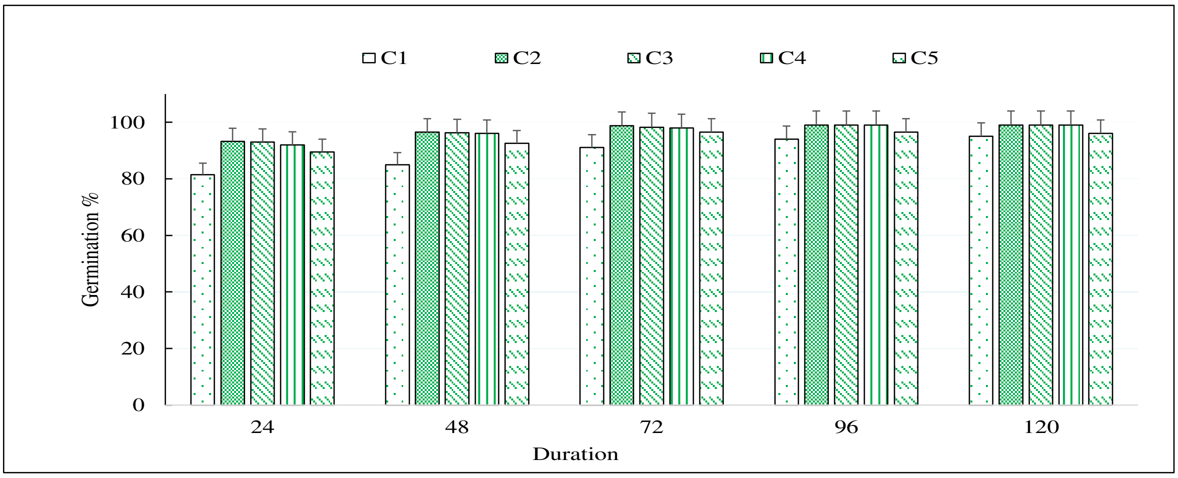

Fig. 1 : Germination Percentage

During 24 hours of seed germination, C1 had a higher germination percentage than C2, C3, C4, and C0. Germination percentages remained consistent between 48 and 72 hours. At 96 hours of germination, C3, C2, and C1 all showed 99 percent germination, whereas C0 and C4 showed 94 and 95 percent germination respectively. And during 120 hours of germination, C3, C2, and C1 all remained the same, with 99 percent germination, while C0 and C4 exhibited 95 and 96 percent, respectively (Figure 1).

Hydropriming and osmopriming wheat seeds can increase germination and emergence, leading to strong root growth and development (Ashraf *et al.*, 2005). Mondal and Bose (2019) identified boron as a crucial element that influences plant growth from germination to yield. Therefore, if the boron concentration reaches or exceeds the threshold level, it may affect germination and related processes due to its mechanism of action. Magnesium nitrate salt improves wheat seed germination rates. Using Mg $(NO_3)_2$ as a priming agent enhances germination by activating respiratory enzymes (Hexokinase, Phosphofructokinase, Enolase) and nitrogen metabolism (Decarboxylase, Nitrate reductase). It also improves plant height, fresh and dry weight, total nitrogen content, and nitrate reductase activity in roots and shoots (Anaytullah & Bose, 2007).

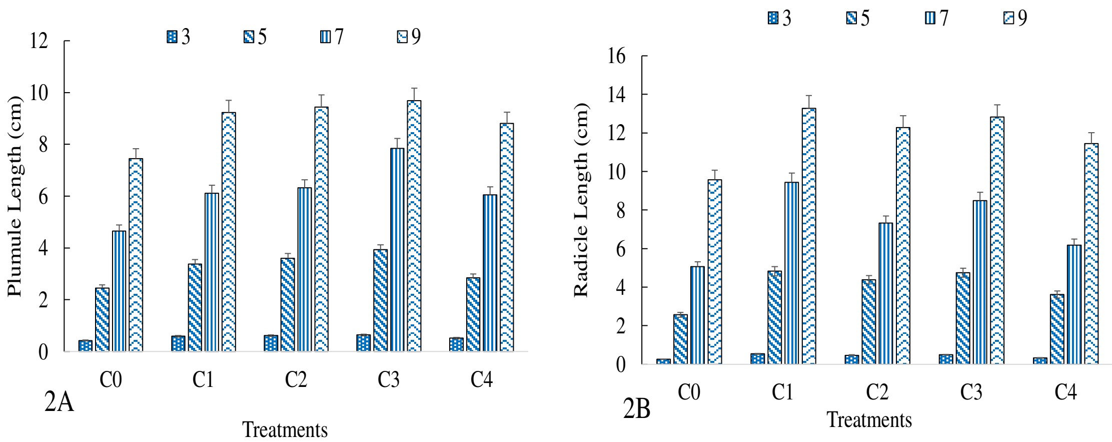

Fig. 2 A : Plumule Length**; 2B:** Radicle Length

Plumule and radicle length are vital components of seed evaluation parameters, as they provide direct insight into the vigor and potential performance of seedlings during the early stages of growth. The plumule length is longer in C1, followed by C2, C3, C4, and C0 at 4, 6, 8, and 10th days of germination. C3 has the longest radicle length, followed by C1, C2, C4, and C0 on days 4, 6, 8, and 10th of germination. Seeds primed with Mg $(NO_3)_2$ (C1) had longer plumules at each observation date, whereas seeds primed with

H_3BO_3 + Mg $(NO_3)_2$ (C3) only had longer radicles. C2 yielded superior results for plumule length after C1, while C1 produced good results for radicle length after C3. However, C3 yielded the second-best result for overall seedling length after C1 (Figure 2A and 2B). Studies by Sharma *et al.* (2006) found that magnesium nitrate-hardened seeds had the longest plumules and seedlings. Plumule length (the embryonic shoot) reflects the future growth potential of the shoot system, which is essential for photosynthesis and biomass accumulation. Radicle length (the embryonic root) indicates the root's ability to anchor the plant and absorb water and nutrients efficiently.

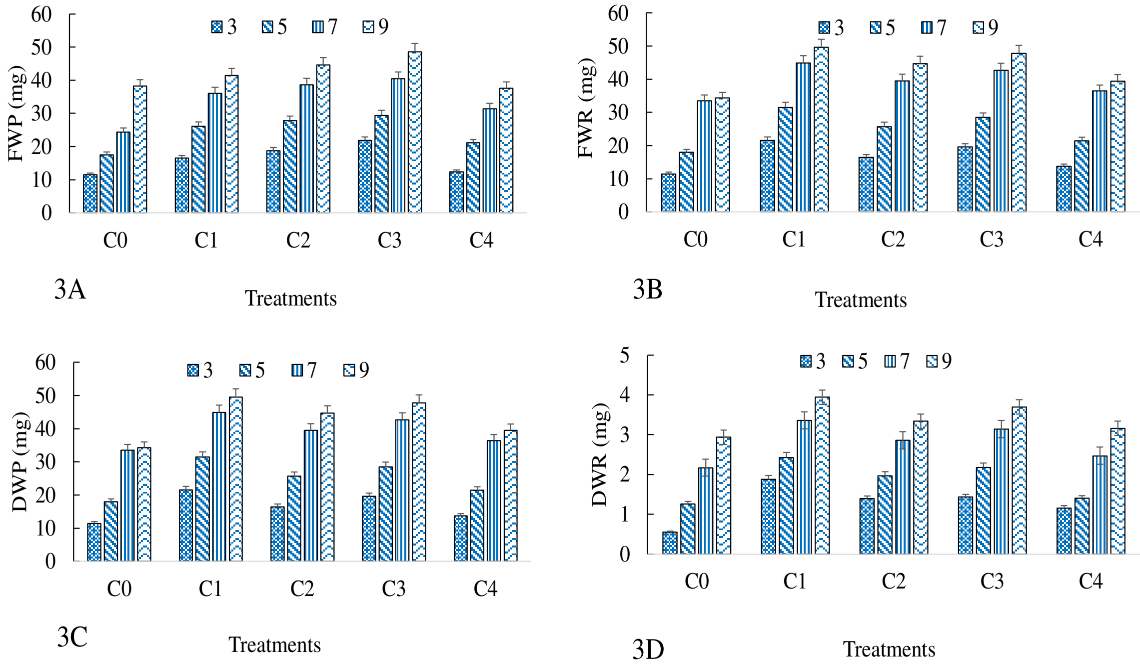

Fig. 1A: Fresh Weight of Plumule (FWP), **3B:** Fresh Weight of Radicle (FWR)
3C: Dry Weight of Plumule (DWR) **3D:** Dry Weight of Radicle (DWR)

The fresh weight of plumule and radicle is an important parameter in seed germination studies because it reflects the initial biomass accumulation and metabolic activity during early seedling development. Higher fresh weight of plumule (shoot) and radicle (root) indicates robust tissue development, which is crucial for strong and healthy seedlings. Figure 3A and 3B show fresh weights of plumules and radicles. Observations were made during germination on days 4, 6, 8, and 10th; seeds treated with magnesium nitrate (C1) performed the best regarding weight of fresh plumules, followed by C2, C3, C4, and C0. For fresh radicle weight, C3 performed best, followed by C1, C2, C4, and C0. Treatments produced varying results for various criteria; C1 set produced the greatest results for fresh plumule weight, whereas C3 set produced better results for fresh radicle weight. C1 had the best dry plumule weight results, followed by C2, C3, C4, and C0. The best results for dry weight of radicle were achieved in C3, followed by C1, C2, C4, and C0, respectively (Figure 3C). Pre-soaking nutrition solutions produce varying results based on criteria. For example, C1 yielded the greatest results for dry weight of plumule and C3 for dry weight of radicle. According to Sharma and Bose (2006), magnesium nitrate stiffened seeds, but seeds treated with a combination of magnesium nitrate and boric acid produced more fresh radicles than other treatments. According to Bose and Mishra (1992), plumule dry weight was higher in magnesium nitrate-hardened seeds compared to other nutrient treatments. Radicle dry weight was higher in seeds treated with a combination of magnesium nitrate and boric acid (Figure 3D). The dry weight of plumule and radicle is a crucial parameter in seed germination and early seedling evaluation because it reflects the actual biomass accumulation, excluding water content. Higher dry weight of plumule and radicle indicates vigorous seedlings, capable of better survival and establishment, especially in suboptimal conditions. It is often used as a reliable proxy for seedling health and developmental potential.

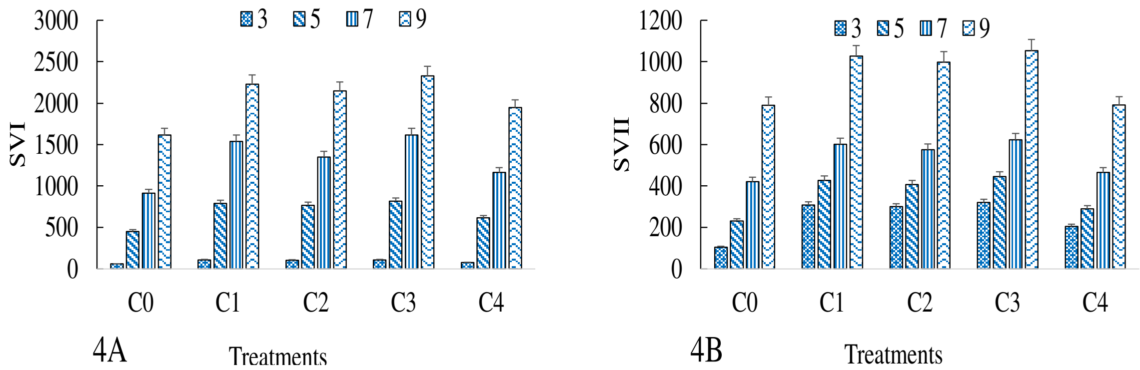

Fig. 2A: Seedling Vigour Index I; **Fig. 4B:** Seedling Vigour Index II

Seedling Vigour Index I (SVI-I) and Seedling Vigour Index II (SVI-II) are essential germination parameters used to evaluate the quality and vigor of seed lots. Index I and II values revealed that treatment C1 outperformed all other treatments, which can be attributed to the use of magnesium nitrate for seed priming. This treatment not only recorded the highest germination percentage but also the greatest total seedling length. Overall, seeds primed with magnesium nitrate (C1) exhibited superior performance, followed in descending order by those treated with a combination of magnesium nitrate and boric acid (C3), boric acid alone (C2), hydroprimed (C4), and finally the non-primed control (C0), based on their respective germination rates and total seedling growth (Figure 4A and 4B). A higher SVI-I indicates faster and stronger seedling development, which is critical for field emergence. Memon *et al.* (2013) found that boron-primed broccoli produced the greatest germination index and seedling vigour index. Iqbal *et al.* (2017) found that boron seed priming enhances seedling vigour, emergence, and grain biofortification in wheat. Using boron in seed priming can improve sugar mobilization from endosperm to the growing embryo, leading to improved germination and growth in terms of vigour index I and II.

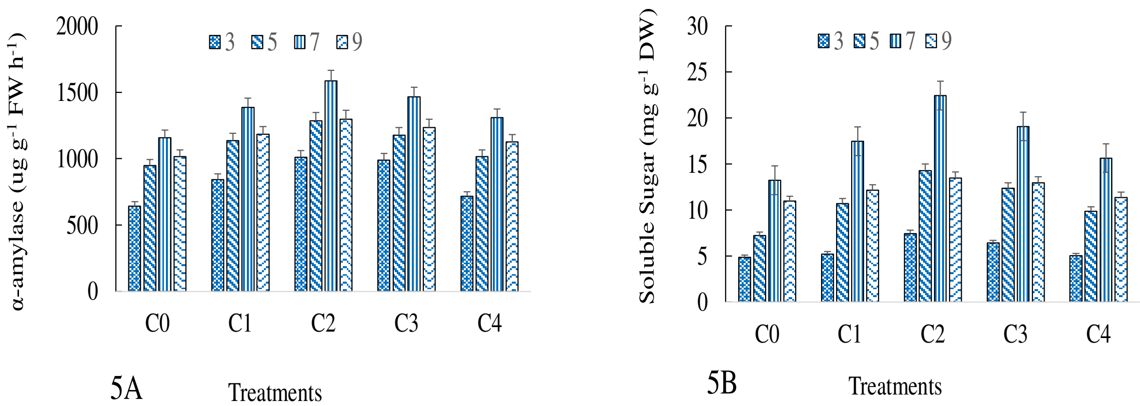

Fig. 3A: Soluble sugar; **5B:** α-amylase activity

Boron is an important micronutrient for plant growth. During priming, boron may enter the seed and improve germination. Solubilization and mobilization from endosperm to developing embryos/plantlets. Figure 5A shows the soluble sugar concentration in endosperm. Seeds primed with boric acid (C2) had the highest soluble sugar content, followed by a combination of magnesium nitrate and boric acid (C3), magnesium nitrate primed (C1), hydro primed (C4), and non-primed control (C0) at 4, 6, 8, and 10 days of germination. Soluble sugar level increased substantially from the 4th to the 8th day, then decreased until the 10th day. Plants use soluble sugar as a substrate to fuel biochemical and metabolic processes. Boric acid-primed seeds enhance this process. Figure 5B showed that boric acid primed sets (C2) had the highest α-amylase activity, indicating that boric acid enhances the activity. The study found that boron had a greater impact on α-amylase activity than magnesium or nitrate. α-amylase activity increased steadily from the 4th to the 8th day but decreased by the 10th day. Seeds primed with boric acid (C2) had the highest enzyme activity among the four treatments. Boron may improve solubilization and mobilization, which could be influenced by α-amylase activity. A study of the activity of α-amylase enzyme and soluble sugar revealed that boron may improved synthesis or activity of α-amylase in boric acid-primed wheat seeds leads to increased soluble sugar content in germinating seeds as the day of germination increases, according to this study. Anaytullah and Bose (2007) found that increasing α-amylase activity leads to higher soluble sugar content in wheat var. HUW-234 and HUW-468 after increasing days of germination in nitrate-primed and non-primed control seeds. Priming treatment boosts α-amylase activity in wheat kernels, resulting in a favorable connection with soluble sugar levels. Primed rice seeds (*Oryza sativa* L cv pumbyco) show a decrease in sucrose, maltose, and raffinose content, whereas glucose, fructose, and β-amylase activity increase more rapidly. Lee and Kim (2000) found a favorable correlation between α-amylase activity, germination rate, and total sugar concentration. Jangde *et al*. (2014) found that adding calcium salts to wheat endosperm increased α-amylase activity levels. According to Chakraborty and Bose (2023), seed priming either enhances the activity of α-amylase or promotes the production of new α-amylases. The study reveals that using boron as a priming agent can improve wheat seed germination by increasing the amylase enzyme activity which promotes carbohydrate solubilization in endosperms. Additionally, it promotes sugar mobilization in seedlings, resulting in higher vigor index in boron-primed seeds.

Conclusion

The results concluded that seeds primed with magnesium nitrate (C1) exhibited superior performance across all parameters, except for alpha-amylase activity and soluble sugar content, where boric acid primed seeds (C2) showed the most favorable outcomes. Interestingly, seeds treated with the combination of magnesium nitrate and boric acid (C3) demonstrated the best results in terms of radicle length, as well as both fresh and dry weights. Overall, it was observed that the sets primed with macro- and micronutrients, whether individually or in combination, consistently outperformed the hydro-primed and non-primed control sets across all observed parameters. This highlights the significant positive impact of priming treatments on seedling growth and development. The synergistic effects of these nutrients suggest that they play a crucial role in optimizing the early developmental stages of wheat, potentially leading to higher crop yields. Further studies are recommended to determine the optimal concentrations and application methods for these substances to maximize their beneficial impacts on wheat cultivation. The findings highlight the importance of micronutrient management in improving wheat productivity, especially under varying environmental conditions.

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