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Effects of Seed Priming on Germination of *Nigella sativa* L. and Comparison of Germination Performance with Yield Parameters in Field Conditions

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A B S T R A C T

The utilization of *Nigella sativa* L., commonly known as black cumin, in traditional and medicinal practices is well-documented, attributing to its wide-ranging biological activities. Given its significance, this study explores the efficacy of various seed priming treatments (control, distilled water, potassium humate, and gibberellic acid) under laboratory and field conditions to enhance the early growth stages of *Nigella sativa* L. Priming treatments aimed to improve germination rates, plant height, and other growth parameters, potentially translating into increased agricultural productivity. According to results plant height ranged from 52.38 to 58.91 cm, number of branches between 3.76 and 3.98 (branch plant^{-1}). The number of capsules varied from 4.93 to 6.81 (capsule plant^{-1}), capsule seed weight was between 0.19 and 0.22 (gram capsule^{-1}), and the thousand-seed weight ranged from 2.26 to 2.39 grams. The germination rate ranged from 78% to 93%, germination index 4.22-5.83 and mean germination time was observed from 4.27 to 4.82 days. Our findings reveal significant effects of priming on germination parameters and plant height, this offering insights into the potential of these treatments to optimize crop growth. However, the transition of these benefits to field conditions, particularly yield-related parameters, appeared limited, suggesting the complex nature of growth enhancement strategies and their implications for agricultural practices.

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Introduction

Medicinal plants, especially those with diverse biological activities, have gained significant interest in the scientific and medical communities. Among these, *Nigella sativa* L., commonly known as black seed or black cumin belonging to the family Ranunculaceae, is particularly noteworthy due to its extensive use in culinary and therapeutic contexts (Mohamed et al., 2014; Kiralan et al., 2016). The use of *Nigella sativa* L. for medicinal purpose has gained global attention due to its effectiveness in treating various illnesses. This interest has been partly fueled by historical narratives, notably the pronouncements attributed to the Prophet Muhammad, who is declared that black seeds are a panacea for all ailments except death (Islam et al., 2019).

In traditional medicine, *Nigella sativa* L. seeds have historically used to treat conditions such as melancholy, fatigue and persistent headaches. The Roasted *Nigella sativa* seeds combined with honey or butter has been recommended for the alleviation of colic and coughs, and these seeds are acknowledged for their lactogenic and antiseptic properties, particularly in the treatment of ocular infections. The emergence of the coronavirus disease (COVID-19), initially identified in Wuhan, China, on December 31, 2019, has intensified the search for effective treatments. Because, the potential of *Nigella sativa* extract to inhibit the replication of coronavirus (CoV) and affect the expression of the TRP gene during CoV infection has been investigated (Ulasli et al., 2014). The findings indicate that *Nigella sativa* extract has a significant effect on viral load and TRP-gene expression following CoV infection. The efficacy of black cumin in therapeutic contexts and its commercial viability are closely tied to both its yield (including raw products, oils, seeds, and bioactive compounds) and its quality (Yimam et al., 2015).

The genus *Nigella* encompasses approximately 20 species, 14 of them present in the flora of Turkiye (Nemtinov et al., 2022). The cultivation of this plant is widespread globally, including in Southern Europe, Syria, Pakistan, India, Egypt, Saudi Arabia, Iran and other countries (Onuah & Singh 2020). In our country, it is primarily cultivated in the Thrace, Northern Anatolia, and Mediterranean regions. *Nigella* cultivation is extensively practiced in various province of Turkiye, specially Burdur, Afyon, Isparta, Amasya, Mersin, Istanbul, Gaziantep, and Kahramanmaras (Inan et al., 2022). Seed priming is an economical and effective strategy to enhance the early growth of crops, resulting more uniform emergence. This practice, which benefits yield in various field crops including oilseeds, involves the use of different osmotic for seed treatment, each offering unique characteristics and levels of effectiveness (Farooq et al., 2008; Rehman et al., 2011). The range of substances used for seed priming includes plant growth promoters such as salicylic acid, cytokinin, and gibberellic acid (GA3), organic solutes, polyethylene glycol (PEG), inorganic salts like KNO_3, $CaCl_2$, KCl, NaCl, and more recently, natural extracts from moringa leaves (Afzal et al., 2012). Typically, priming seeds leads to faster and more uniform germination, improved stand establishment, and numerous subsequent benefits. Specifically, the application of KNO_3 has been shown to not only hasten germination and enhance seedling growth but also to shorten emergence time, improve field emergence, and increase both yield and protein content when combined with hydro-priming and osmo-priming with NaCl in sunflowers (Rehman, 2014).

Several studies have indicated that various priming methods positively affect seed germination and seedling development (Paparella et al., 2015; Sharma et al., 2014; Khalaki et al., 2020). The primary objective of this study is to determine whether the performance enhancement observed under laboratory conditions can also be achieved under field conditions. For this purpose, seeds of *Nigella sativa* L. (black cumin) treated with priming processes were cultivated under field conditions, and their performance was evaluated. The cultivation of *Nigella sativa* seeds under field conditions and the examination of their performance through this study have the potential to fill a gap in the field and can therefore be considered a new and significant contribution to the literature.

Material and Method

This study investigates the effects of different priming treatments on the *Nigella sativa* L. plants under both laboratory and field conditions. For the laboratory phase, seeds underwent germination tests by being placed on quadruple layers of moistened filter paper at a constant temperature of 22 °C in a dark incubator. Germination was determined by the emergence of a 1 mm radicle, monitored over a period of 10 days, and during experiment no microbial contamination was detected (Kamal et al., 2010). For priming, gibberellic acid at 75 ppm, potassium humate at 100mg/L, distilled water and a control (no treatment) were used. The seeds were soaked with priming treatments in darkness for 12 hours. Subsequently, the dried seeds were utilized for both germination studies and field experiments. The concentrations of gibberellic acid and potassium humate, as well as the soaking duration, were determined based on preliminary studies and by consulting the literature of various researchers (Azarnia et al., 2016; Mridha et al., 2021).

After the laboratory priming treatments, primed seeds were sown in the field. This phase of the research was conducted at the Field Crops Department of the Adnan Menderes University Faculty of Agriculture's Research, Application, and Production Farm during the 2022-2023 growing season.

The climate data for the period during which the experiment was conducted, 2022-2023, are presented in Figure 1. The red area indicates the range over which the *Nigella sativa* plant was cultivated under field conditions.

During 12 months period, a total of 544 mm of precipitation was recorded, with 498 mm of this precipitation has occurred during the experiment period between December 2022 and June 2023. This indicates that the experiment was conducted during a significantly rainy part of the year, capturing a large portion of the annual precipitation. The average temperature varied from 7.18 °C to 24.62 °C, while the average humidity ranged between 64.6% and 89.5%. These conditions shows that the experiment period experienced temperate to warm weather with high humidity levels (Figure 1).

In the study, four distinct priming treatments were applied: control (no treatment), distilled water, potassium humate and gibberellic acid. The experimental design was structured as a randomized complete block design with four replicates. The seeding rate was standardized at 2.5 kg/da.

The experimental plots were designed with six rows, each 4 meters long, with rows spaced 20 cm. To mitigate edge effects and ensure uniformity in data collection, observations and measurements were conducted on the central four rows of each plot. Specifically, the outermost rows at both ends of the plot, as well as 0.5 m from the beginning and end of these four rows, were excluded from the harvest area. Consequently, the total area of each plot was calculated to be 4.8 m², with a designated harvest area of 2.4 m².

The fertilization protocol involved the application of 6 kg/da of nitrogen (N) and 4 kg/da of phosphorus (P). The nitrogenous fertilizer was applied in two equal parts: half as basal fertilizer before sowing, and the remaining half just before the onset of the stem elongation phase (February). Harvesting was carried out towards the end of June, after the capsules had dried and the seeds had matured.

Germination Rate (%): = (Number of germinated seeds/Total number of seeds) × 100 (Scott et al., 1984).

Germination Index: Defined as the number of germinated seeds divided by the days to the first count plus the number of germinated seeds divided by the days to the last count (Benech Arnold et al., 1991).

Mean germination time (Day): It is calculated as $MGT=\Sigma(n\times d)/N$, where n is the number of seeds germinated per day, d is the number of days from the start of the test, and N is the total number of seeds germinated by the end of the experiment (Ellis &Roberts, 1981).

Plant Height (cm): Before harvesting, the height of 20 randomly selected plants from each plot was measured from the soil level to the top point of the plant, and the average height was calculated.

Branch Number (number/plant): The number of branches directly attached to the main stem of 20 randomly selected plants from each plot was counted, and the average number of branches per plant was determined.

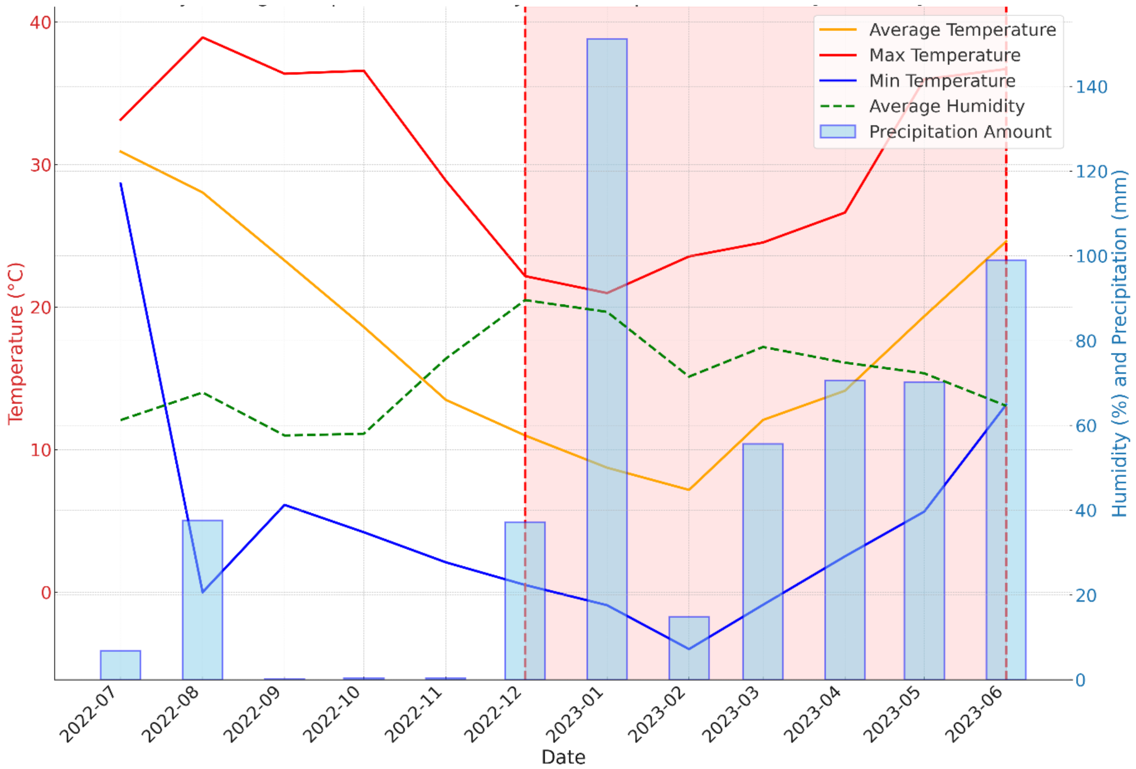

Figure 1. The climatic data during the field study period

Number of Capsules (number/plant): The number of capsules on 20 randomly selected plants from each plot was recorded, and the average number of capsules per plant was calculated.

Number of Seeds (number /capsule): The number of seeds within 20 randomly selected capsules from each plot was counted, and the average number of seeds per capsule was determined.

Seed Weight (g/capsule): The seeds from 20 randomly selected capsules from each harvest plot were weighed using a precision scale with a sensitivity of 0.001 g, and the average seed weight per capsule was calculated.

Thousand seed weight(g): (The average weight was calculated by counting 100 seeds from each plot four times, weighing them on a precision scale with a sensitivity of 0.001, and then multiplying the found average weight by 10.

Yield (kg/da): The capsules harvested from all the plants within the harvest area of each plot were weighed to determine the yield per plot. These values were then converted to kg/da based on the plot area.

The obtained data were analyzed separately and differences were determined using the analysis of variance (one-way ANOVA). Variance analyses were performed using JMP Pro v.16 software. Statistically significant factors were grouped using the LSD (Least Significant Difference) test. Factors that were not significant in the LSD test were compared based on mean tables.

Results and Discussion

In this study, the effect of four priming treatments (Control, Distilled Water, Potassium Humate and Gibberellic Acid) on various plant growth parameters was investigated. The analysis was conducted using ANOVA to determine if there were significant differences in plant height (cm), number of branches (number/plant), number of capsules (g/capsule), seed weight (g/capsule), thousand seed weight(g), yield (kg/da), germination rate (%), germination index (GI) and mean germination time (days) within the treatments (Table 1).

The results indicated significant differences in plant height ($p<0.05$), suggesting that the priming treatment influences the overall height of the plants. According to results of plant height suggest that specific priming agents can enhance growth compared to the control group. The GR (%) metric also showed significant differences ($p<0.05$), pointing to the influence of priming treatments on the germination rate of the plants. Furthermore, highly significant differences were found in the GI ($p<0.001$) and MGT ($p<0.001$), indicating that priming treatments significantly affect germination behavior. These results underscore the importance of priming in enhancing germination efficiency and speed, with some treatments markedly improving these aspects compared to the control.

Other studies about seed priming clearly shown that priming treatments significantly improve various germination metrics such as germination ratio, the germination index and mean germination time (Shim et al., 2008; Ziaf et al., 2017; Ghimire et al., 2021). In this regard It is similar with our findings and priming not only enhanced germination speed (as indicated by lower mean germination time) but also improved the overall germination index, suggesting a more synchronized and efficient germination process. Conversely, the number of branches (number/plant), number of capsules (g/capsule), seed weight (g/capsule), thousand seed weight(g) and yield (kg/da) did not show significant differences across treatments. This suggests that while priming affects certain growth parameters and germination characteristics, it may not significantly alter aspects such as branching, capsule formation, and yield parameters under the priming effect.

Table 1. Significance levels in variance analysis for the various priming applications in *Nigella sativa L.* plant

Variable	MS	p-Value
Plant height (cm)	38.332*	0.0104
Number of branches (branches/plant)	0.148 ns	0.3741
Number of capsules (capsule/plant)	3.008 ns	0.0812
Seed weight in capsule (g)	0.001 ns	0.7378
Thousand seed weight (g)	0.021 ns	0.7867
Yield (kg/da)	80.474 ns	0.5229
Germination rate (%)	150.667*	0.0465
Germination index (GI)	1.762***	0.0006
Mean germination time (day)	0.213***	0.0000

*** Highly significant $p<0.001$, ** Very significant ($p<0.01$), * Significant ($p<0.05$), ns: not significant ($p\geq0.05$)

Table 2. Effect of different priming applications on *Nigella sativa* L. plant germination, growth and yield parameters.

Priming Group	Means								
	PH	NB	NC	SWC	TSW	YD	GR	GI	MGT
Control	52.38 B	3.76	6.81	0.20	2.26	33.66	78 B	4.22 C	4.82 A
Distilled Water	58.69 A	3.98	5.31	0.22	2.39	29.66	85 AB	4.85 B	4.62 B
Potassium Humate	57.94 A	3.53	4.93	0.19	2.23	24.44	93 A	5.83 A	4.27 C
Gibberellic Acid	58.91 A	3.88	6.31	0.22	2.3	34.10	86 AB	4.83 BC	4.62 B
LSD	3.93	-	-	-	-	-	9.98	0.58	0.11

PH: Plant height, NB: Number of branches, NC: Number of capsules; SWC: Seed Weight per capsule; TSW: 1000 seed weight; GR: germination rate, GI: Germination index, MGT: mean germination time.

The statistical analysis indicate that the treatments involving distilled water, potassium humate, and gibberellic acid significantly enhanced plant height compared to the control group. Furthermore, these treatments were statistically categorized within the same group, indicating a similar level of efficacy within them (Table 2).

Gibberellic acid (GA) plays a crucial role in regulating plant height by affecting cell and internode elongation. This growth-promoting phytohormone has a widespread impact on various growth processes, consistently increasing plant height across numerous species (Shah et al., 2023). Although it is well-documented that applying GA3 exogenously during the vegetation period can enhance growth and plant height (Shahzad et al., 2021), here is no clear studies have yet demonstrated its effects on plant height when applied through seed priming. It is well known that seed priming techniques, including those using GA3, potassium humate, and hydro-priming (distilled water), primarily improve early germination and seedling growth (shoots and roots) (Mridha et al., 2021; Patil et al., 2010; Lemmens et al., 2019). Based on these studies and our findings, we can conclude that seed priming with GA3, potassium humate, and distilled water (hydro-priming) effectively enhances plant height by promoting early seedling growth.

Similarly, statistically significant differences were observed in germination characteristics such as the germination rate, germination Index and mean germination time, with potassium humate as the most effective priming method for enhancing germination performance.

The germination index (GI) measures the effectiveness of the germination process, incorporating both the speed and the uniformity of germination and mean germination time (MGT) indicates the average time required for seeds to initiate germination.

Potassium Humate treatment resulted in the highest GI with 5.83 and 93% germination rate, indicating it significantly enhances the germination process compared to other treatments. Distilled water treatment showed a moderate improvement in GI 4.85 over the control but was significantly lower than the potassium humate treatment, indicating a positive but less effect on germination effectiveness. The Control group had the lowest GI with 4.22 and germination rate of 78%.

The Control group had the longest MGT 4.82 days, suggesting that seeds without any priming treatment germinated more delayed than those receiving treatments. Distilled Water treated seeds had a slightly faster MGT 4.62 day, indicating a less delay in germination time compared to the control. Potassium humate-treated seeds experienced a faster MGT 4.27 day, suggesting that while this treatment significantly improves germination quality and also it has faster germination more than the other treatments.

The analysis indicated that priming treatments, particularly with potassium humate, substantially influence seed germination parameters. Notably, potassium humate was linked to enhancements in both the rates and effectiveness of germination. These results imply that priming seeds with potassium humate can significantly improve germination outcomes. Nevertheless, it was observed that these priming treatments did not exert a significant impact on field performance metrics. Despite the observed improvements in germination speed and performance under controlled experimental conditions, such enhancements did not translate to improved yield-related parameters in field conditions. This discrepancy shows that while priming can optimize germination parameters, its benefits may not directly extend to agricultural productivity in real-world settings.

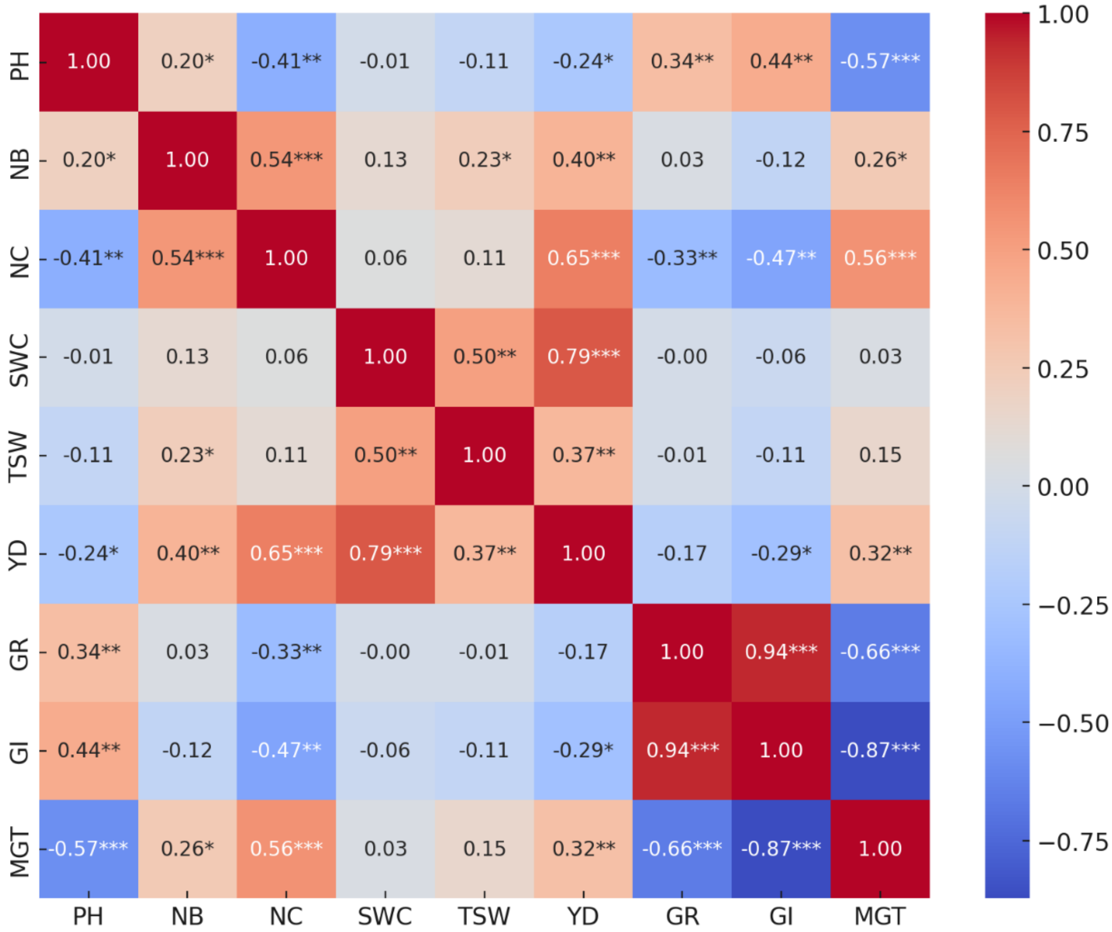

Figure 2. Correlation analysis of the measured traits.

Seed priming with various agents, including gibberellic acid (GA3) has been shown to significantly improve the germination percentage, rate, and seedling vigor in crops such as lentil (*Lens culinaris* L.) (Azarnia et al., 2016) and anise (*Pimpinella anisum* L.) (Mahdavi, 2016).

Recent studies indicates that the technique of priming seeds with potassium humate has a substantial positive impact on multiple aspects of germination and plant growth. Significantly, this treatment enhances the rate and efficiency of germination, while decreasing the average germination time of seeds (Arun et al., 2022). Seeds treated with potassium humate exhibit enhanced vigor, as well as longer and heavier roots and shoots, even in difficult conditions (Hussein & Ibraheem, 2023).

Patil et al., (2010) and Canavar et al., (2023) have contributed with research investigating the effects of potassium humate on germination and associated characteristics. These studies explore the multifaceted impact of potassium humate on the germination process. Awaad et al. (2020) report that potassium humate has positive effects on plant height. Also, Debeaujon et al., (2000) and Kim et al., (2008) showed the effects of gibberellic acid on germination in their studies. In our study, the results indicate, except plant height, the effects of priming applications were not statistically significant under field conditions.

To better understand the relationship between germination characteristics and field performance, we conducted a correlation analysis (Figure 2). According to correlation analysis, there's a moderate positive correlation (0.438) between PH and GI, suggesting that taller plants tend to have a higher germination index. A moderate negative correlation (-0.574) was found between PH and MGT, indicating that taller plants tend to have shorter mean germination times. There is a moderate positive correlation (0.562) between NC and MGT, suggesting that plants with more capsules tend to have longer mean germination times.

In conclusion, our correlation analysis revealed that germination characteristics exhibit a positive correlation with plant height. This association suggests that an increase in the germination index and a decrease in the mean germination time positively affect plant height. Conversely, plant height was found to correlate negatively with the number of capsules produced by the plant. This inverse relationship implies that taller plants may adversely affect the number of capsules, potentially it may impact yield negatively.

Therefore, according to the data we have obtained, while priming applications have favorable effects on germination characteristics, they do not appear to enhance yield-related traits in field conditions. Moreover, the tendency of priming treatments to promote taller growth, and consequently a reduced capsule count, may suggest a possible detrimental effect on overall yield. This outcome underscores the need for a nuanced approach to the application of priming treatments, balancing the benefits observed during the early stages of plant development with the ultimate goal of maximizing yield.

Conclusion

The study concluded that while seed priming with gibberellic acid and potassium humate significantly enhances germination rates and plant height of *Nigella sativa* L., these improvements do not translate into increased yield under field conditions. Priming treatments were effective in optimizing early growth stages but showed no substantial effect on yield-related parameters, indicating a discrepancy between germination enhancement and agricultural productivity in real-world settings. The correlation analysis further elucidated the relationship between germination characteristics and plant growth parameters, suggesting a nuanced approach to seed priming's necessity.

Early development in plants often aids in the cultivation of healthier and stronger plants, yet claiming that it directly leads to yield increases may not always hold true. Early growth allows plants to grow faster and complete their development stages more efficiently, which could potentially enhance productivity. However, a plant's yield is dependent on numerous factors, and early development alone might not suffice.

The importance of early growth benefits with the goal of maximizing yield, necessitating further research to understand the complex interactions between priming treatments and field performance.

Future researches those aims the improving crop yields through seed priming should explore diverse priming agents, optimize priming times and durations, and investigate how environmental factors influence priming efficacy. Longitudinal field studies and the integration of priming with other agricultural practices could help to develop strategies that not only enhance early growth but also significantly boost yields.

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