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Enhancing of Early Seedling Vigour (ESV) parameters in Lentils through integrated priming with silicic and humic acid

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Abstract

Seed priming has emerged as an innovative and economical technique to elevate seed quality, fostering uniform, swift, and robust germination under both stress and non-stress conditions. This study endeavors to scrutinize the effects of organic (silicic acid, SA) and inorganic (humic acid, HA) acids, alongside their synergistic combinations, on seed quality parameters in three distinct lentil (Lens culinaris) genotypes: IPL-316 (tolerant), PSL-9, and PDL-1 (sensitive). Critical parameters assessed encompass germination percentage, root and shoot length, seed vigor indices I and II, and dry weight under meticulously controlled laboratory conditions. The priming agents were standardized across a spectrum of concentrations and durations. Sterilized seeds were immersed in silicic acid (1, 2, 3, 4, and 5 mM), humic acid (100, 200, 300, 400, 600, 800, and 1000 ppm), and their combinations over varying durations (2 to 18 hours), including control and hydropriming treatments. Following treatment, seeds were air-dried and subjected to growth assessments. The findings reveal that priming significantly bolsters early-stage plant growth across all three lentil genotypes, with the combined application of silicic and humic acids yielding remarkable enhancements in all seed quality parameters, intricately influenced by genotype and treatment combination.

Keywords: Seed priming, Silicic acid, Humic acid, Lentil (*Lens culinaris*), Germination percentage, Seed vigor indices, Root and shoot length, Dry weight, Integrated priming.

Introduction

Lentil (*Lens culinaris* Medik) is a cherished annual cool-season grain legume in India, cultivated across 1.45 million hectares, yielding 1.46 million tonnes—accounting for 26.5% of the world's annual production (GOI, 2021). The lower Gangetic plain (LGP) contributes significantly, encompassing 10.3% (0.16 million ha) of the nation's lentil growing expanse and 9.6% (0.15 million tonnes) of its annual yield. The rice fallows of this fertile region harbor approximately 150–200 mm of carryover soil moisture (Bandyopadhyay et al., 2018), offering an ideal environment for cultivating the water-efficient lentil. Yet, farmers typically plant lentils in the pre-winter months (late November to early December) post-harvest of long-duration puddled-transplanted rice, resulting in delayed sowing. Optimal growth requires temperatures between 18 to 30°C, with cooler conditions essential during early to mid-growth stages, while warm climes are vital for maturation (Sinsawat et al., 2004). Elevated temperatures exceeding 32/20°C (max/min) during flowering and pod-filling can devastate production potential (Bourgault et al., 2018).

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Priming seeds through controlled hydration induces mild stress, enhancing plants' resilience (Paparella et al., 2015). This eco-friendly method cultivates epigenetic memory, strengthens salt tolerance, promotes germination, plant development, and physiological adaptability, fortifying crops to withstand environmental adversities (Thakur et al., 2020; Guo et al., 2022). Exposure of seeds to mild stress imprints epigenetic memory in the primed seeds and strengthens plants to encounter future stresses (Marcos et al., 2018). This eco-friendly technique also activates signaling molecules to enhance the inherent salt tolerance potential in plants that helps in recovery from salt-induced damages (Guo et al., 2022; Khaitov et al. 2024). Seed priming facilitates seed germination and stand establishment of seedlings, boosts plant development, regulates physiological, biochemical, and molecular responses in plants, promotes nutrient uptake, and strengthens tolerance to stress factors.

Seed priming involves the gentle soaking of seeds in a solution characterized by low osmotic potential for a designated duration, followed by a return to their original humidity (Ceritoglu et al., 2023). This transformative process triggers a cascade of biochemical reactions within the seed, activating antioxidant defense systems while concurrently facilitating germination (Mauch-Mani et al., 2017). The overarching aims of seed priming encompass the enhancement of germination traits, the promotion of seedling vigor and nutrient absorption, the reduction of susceptibility to environmental stresses, and the augmentation of crop yields (Paul et al., 2022). Sirisuntornlak et al. (2021) found that treatment with 1 mM silicon significantly boosted nitrogen uptake in maize. Likewise, Özyazıcı et al. (2023) revealed that priming seeds with 0.25 mM salicylic acid improved both germination metrics and seedling development in switchgrass (*Panicum virgatum* L.). Raza et al. (2024) demonstrated that priming seeds with 6 mg L^{-1} selenium effectively enhanced quinoa yields under drought conditions. Notably, Mazhar et al. (2023) reported that priming with 75 ppm iron oxide nanoparticles elevated superoxide dismutase (SOD) and catalase (CAT) activities by 13% and 38%, respectively. Freitas and da Silva (2024) concluded that seed priming treatments bolster stress memory in plants.

Seed priming is a meticulous triadic process commencing with the imbibition of seeds in a carefully selected priming agent for a predetermined duration, a practice refined through the alchemy of trial and error. This initial phase paves the way for the activation phase, where a symphony of metabolic events unfurls at the cellular level spurring protein synthesis, catalyzing enzymes, fortifying the antioxidant machinery, and heralding the formation of new mitochondria alongside DNA repair. The final phase, known as rehydration, activates cell division and fuels the synthesis of nucleic acids and ATP, thereby amplifying cellular energy (Devika et al., 2021).

While research indicates that seedlings nurtured from primed seeds demonstrate enhancements in water content, improved regulation of the cell cycle, adept management of oxidative stress, and efficient reserve food mobilization, the success of seed priming remains intricately tied to both the plant species and the method employed (Raj and Raj, 2019; Johnson and Puthur, 2021). Notably, our findings underscore that the application of silicic and humic acids in lentils not only elevates seed quality parameters but also bolsters seed potential amid adverse conditions. This study is pioneering, illustrating the crucial role these agents play in fostering plant establishment and vigor during early growth phases.

Material and Methods

Seed priming

The current study has been conducted in the hallowed confines of the Seed Biochemistry Laboratory within the Division of Seed Science and Technology at the Indian Agricultural Research Institute (ICAR-IARI), New Delhi, India. Three distinct varieties of lentil seeds were procured from the Division of Genetics, hailing from the abiotic stress laboratory: IPL-316 (tolerant), PSL-9, and PDL-1 (sensitive). Two different priming agents, silicic and humic acid, were employed at varying concentrations and durations. Five concentrations of silicic acid1, 2, 3, 4, and 5 mM were utilized alongside control and hydropriming conditions. Additionally, six concentrations of humic acid 100, 200, 400, 600, 800, and 1000 ppm were also tested, complemented by control and hydropriming (Figure 1). A total of eleven combinations of humic and silicic acid were meticulously standardized across differing durations and concentrations (Table 1). Initially, the seeds were sterilized using a 1% sodium hypochlorite solution, after which they were immersed in the priming agent solution (1:1 w/v) at 20°C, tailored for specific durations prior to achieving 2 mm of radical emergence.

Table 1. Experiment design and standardization

Silicic acid	Humic acid	Combination of humic and silicic acids
Durations = 2, 4, 6, 8, 10, 12, 15, 16, 18 hours		
T1 = SA @ 1 mM	T1 = HA @ 100 ppm	T_1 = HA + SA @ 100 ppm+1 mM
T2 = SA @ 2 mM	T2 = HA @ 200 ppm	T_2 = HA + SA @ 200 ppm+2 mM
T3 = SA @ 3 mM	T3 = HA @ 400 ppm	T_3 = HA + SA @ 300 ppm + 3 mM
T4 = SA @ 4 mM	T4 = HA @ 600 ppm	T_4 = HA + SA @ 400 ppm + 4 mM
T5 = SA @ 5 mM	T5 = HA @ 800 ppm	T_5 = HA + SA @ 600 ppm + 5 mM
T6 = Control	T6 = HA @ 1000 ppm	T_6 = HA + SA @ 100 ppm + 3 mM
T7 = Hydropriming	T7 = Control	T_7 = HA + SA @ 200 ppm + 3 mM
-	T8 = Hydropriming	T_8 = HA + SA @ 400 ppm + 3 mM
-	-	T_9 = HA + SA @ 600 ppm + 1 mM
-	-	T_{10} = HA + SA @ 600 ppm + 2 mM
-	-	T_{11} = HA + SA @ 600 ppm + 3 mM
-	-	T_{12} = HA + SA @ 600 ppm + 4 mM
-	-	T_{13} = Control
-	-	T_{14} = Hydropriming
After standardization		
T1 = Control T2 = SA @ 3 mM for 18 hr T3 = HA @ 600 ppm for 18 hr T4 = HA+SA @100 ppm + 1 mM for 16 hr T5 = Hydropriming @ 18 hr		

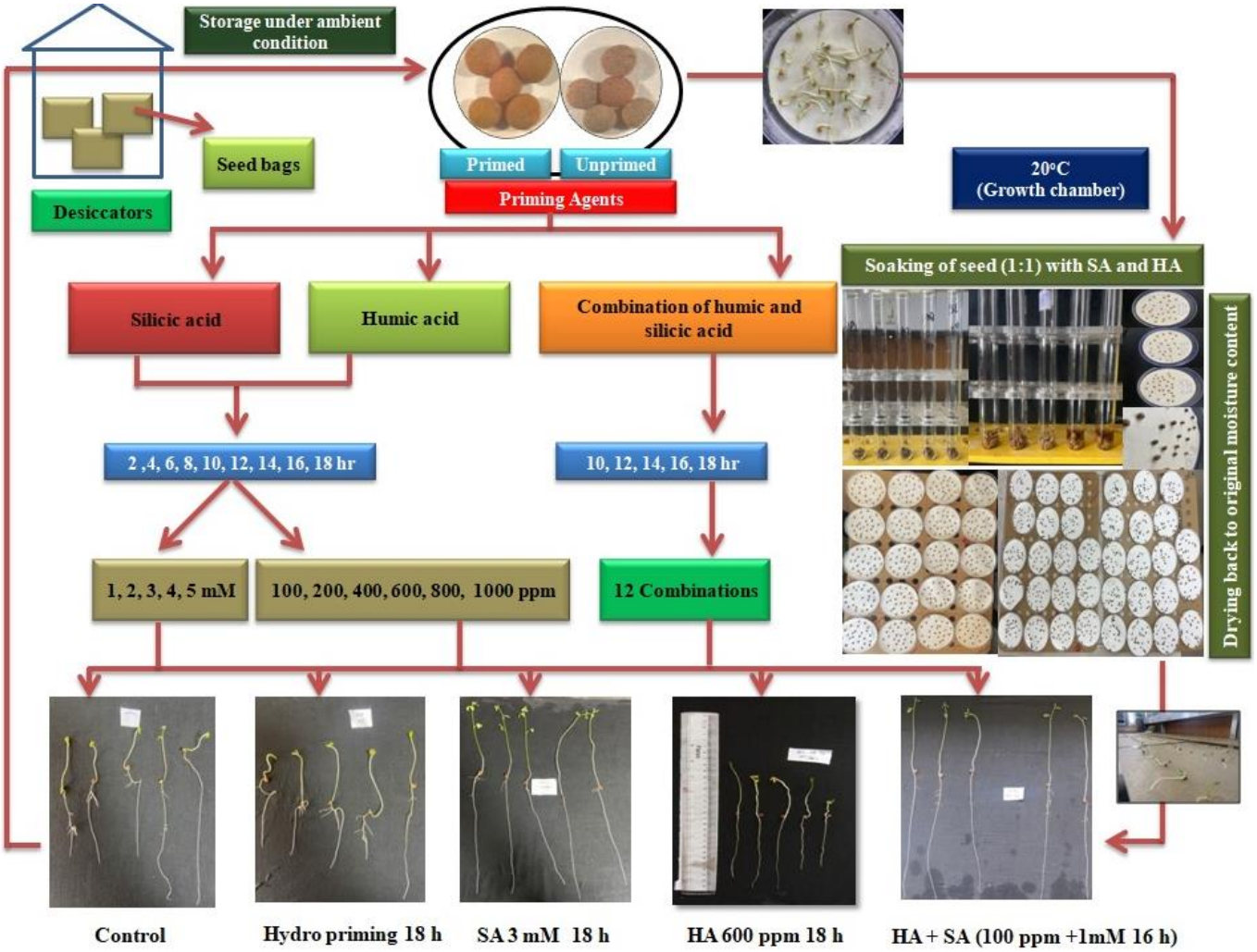

Figure 1. The experimental methodology

Germination

In the experiment, a total of 50 seeds per three replicates were utilized. Following the priming process, the seeds were gently dried to restore their original moisture content. Germination commenced using the top-paper method. After the initial count, the seeds were then transitioned to the between-paper method for further development (ISTA, 2022).

The culmination of the study was marked by a comprehensive evaluation of the seedlings, conducted on the designated final count day. This assessment encompassed the classification of the seedlings into categories: normal, abnormal, hard, dead, and those exhibiting signs of disease. Each seedling's fate was meticulously recorded, reflecting the intricate interplay of environmental influences and inherent genetic potential. Through this careful observation, the experiment sought to unveil the delicate balance between life and adversity, illuminating the nuances of seed germination and seedling development. Ultimately, the outcomes would contribute valuable insights to the realm of botany and agricultural science.

Root and Shoot length (cm)

On the final day of measurement, the lengths of the root and shoot were meticulously recorded using a calibrated metal scale. A selection of ten seedlings was made based on their morphological characteristics. A vibrant red cloth was employed to gently cradle the seedlings, ensuring the fabric remained moist and free from drying. Each seedling's length was measured by hand with precision, capturing the essence of their growth. Following this careful measurement, the selected seedlings were placed in an oven, prepared to undergo the process of drying after recording their fresh weight (ISTA, 2022).

Dry weight (gm)

The chosen seedling, having undergone a gentle drying process in the oven at 42°C for three days, was carefully extracted from the beaker. The dry weight was meticulously measured using a precise weighing scale. According to ISTA (2022), a greater dry weight serves as a clear and direct indicator of superior seed quality.

Seed Vigour Index

The seed vigour index is meticulously calculated following the methodologies established by Abdul-Baki and Anderson (1973). Specifically, Seed Vigour Index-I emerges from the product of the germination percentage and the total length of seedlings measured in centimeters. Meanwhile, Seed Vigour Index-II is derived by multiplying the germination percentage by the dry weight of the seedlings, expressed in grams, in accordance with the guidelines set forth by ISTA (2022).

Root Scanning

The seedling underwent meticulous scrutiny by the root scanning machine (REGENT LA2400 Scanner expertly calibrated for image analysis). From the final count, five carefully selected seedlings were gathered for observation. The process of root scanning encompassed a multitude of parameters, including area, width, length, surface area, primary area, volume, the count of tips, forks, crosses, and nodules. Each parameter revealed the intricate tapestry of the seedling's underground architecture, laying bare the delicate balance between nature's design and the relentless pursuit of growth. In this harmonious convergence of technology and biology, the seedlings emerged not merely as botanical entities, but as testaments to the resilience and complexity of life itself, grasping at the soil from which they sprang, imbued with the promise of flourishing above the earth.

Results

Germination energy

The findings unveil a remarkable journey of germination, demonstrating that the pinnacle of success an astounding 97% germination rate was attained through the treatment with silicic acid (T_3) at a concentration of 3mM for an enduring 18 hours. Following closely, T_5 at 5mM produced a commendable 91%. In stark contrast, the control and hydropriming treatments languished at significantly lower rates of 89% and 87% respectively before standardization (Figure 2). Similarly enchanting was the outcome in the realm of humic acid, where T_4 at 600 ppm achieved an immaculate 100%. The T_2 treatment at 200 ppm, after 18 hours, yielded a satisfying 95%, while control and hydropriming treatments were again reminiscent of lower achievements at 95% and 97% (Figure 2). In the interplay of combined treatments, T_1 (HA+SA at 100ppm+1mM for 16 hours) reached a noteworthy 96%, trailed by T_2 at 94% (200ppm+2mM for 16 hours). Yet again, control and hydropriming resulted in lesser performances at 92% and 88%. Post-standardization revealed T4 with HA+SA leading at 94%, with T_3 and T_2 both sustaining robust 92% rates. The least favorable result, a modest 88%, was noted in the control treatment. The impacts of salinity stress were addressed in prior research (Figure 2).

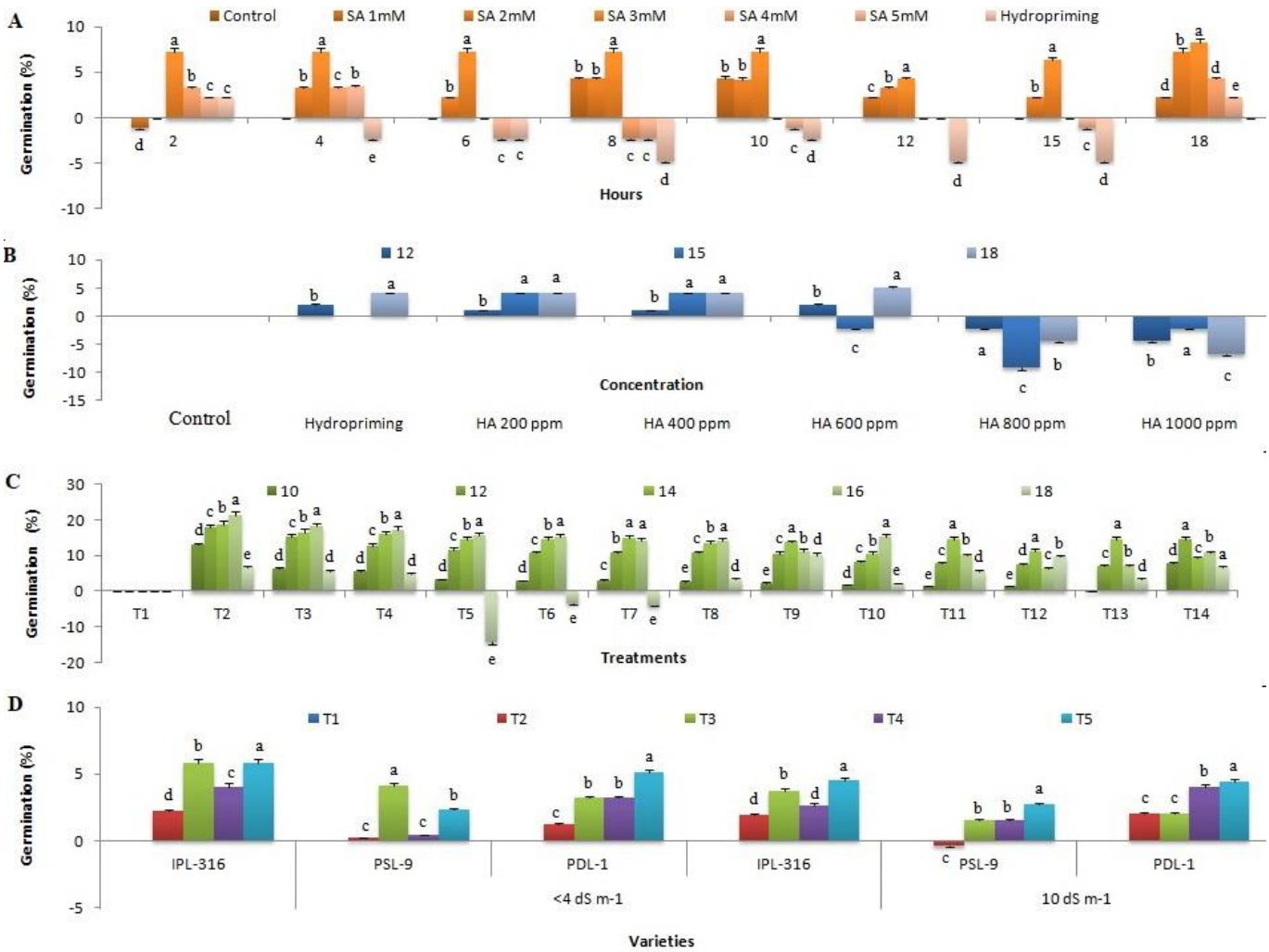

Figure 2. Effect of different treatments on the germination percentage at different durations and concentrations (A); Different concentrations and durations of silicic acid. (B); Different concentrations and durations of humic acids. (C): Combination of humic and silicic acid. (D);After standardization under normal and salinity stress condition.

Seed vigour index-I

Among the various treatments of silicic acid, the apex of seed vigour index-I was recorded in T_3 (SA @ 3mM for 18 hours) with a remarkable value of 3596. Close behind was T_2 (SA @ 2mM for 18 hours), boasting an index of 3491. In stark contrast, the lowest indices emerged from the control group (2821 and 3063) and various hydropriming treatments (Figure 3). Turning to humic acid, T_4 (HA @ 600 ppm for 18 hours) yielded the highest vigour index of 1891, followed closely by T_3 (HA @ 600 ppm for 18 hours) at 1767. The control and hydropriming treatments languished at the bottom, recording 1571 and 1474, respectively (Figure 3). In a harmonious blend of humic and silicic acids, T_1 (HA+SA @ 100 ppm + 1mM for 16 hours) reached the zenith with an impressive vigour index of 3852, followed by T_4 (HA+SA @ 400 ppm + 4mM for 16 hours) at 3681. The control and hydropriming conditions again trailed, resulting in 2866 and 2945. Post-standardization, T_4 claimed the highest vigour index of 2787, with T_3 and T_2 at 2561 and 2479, while the control and hydropriming treatments persisted in their lower realm, recording 2345 and 2446 (Figure 3).

Seed Vigour Index-II

The pinnacle of seed vigor index-II was attained with treatment T_3, wherein silicic acid (SA) was administered at 3 mM for an 18-hour duration, yielding a score of 6.23. This success was closely followed by treatment T_2, utilizing 2 mM of SA for the same period. Meanwhile, the control and hydropriming treatments languished with the lowest indices of 4.91 and 5.47, respectively (Figure 4). In the realm of humic acid application, treatment T_3 emerged triumphant, employing humic acid (HA) at 600 ppm for 18 hours, succeeded by treatment T_2 with HA at 400 ppm. The control and hydropriming treatments, once again, displayed the least vigor, with indices of 0.85 and 0.96. Within the realm of combined treatments, prominence was observed in treatment T_1, which melded HA and SA at 100 ppm and 1 mM for 16 hours, reaching a value of 7.19. Following closely, treatment T_2 combined HA and SA at 200 ppm and 2 mM (Figure 4).

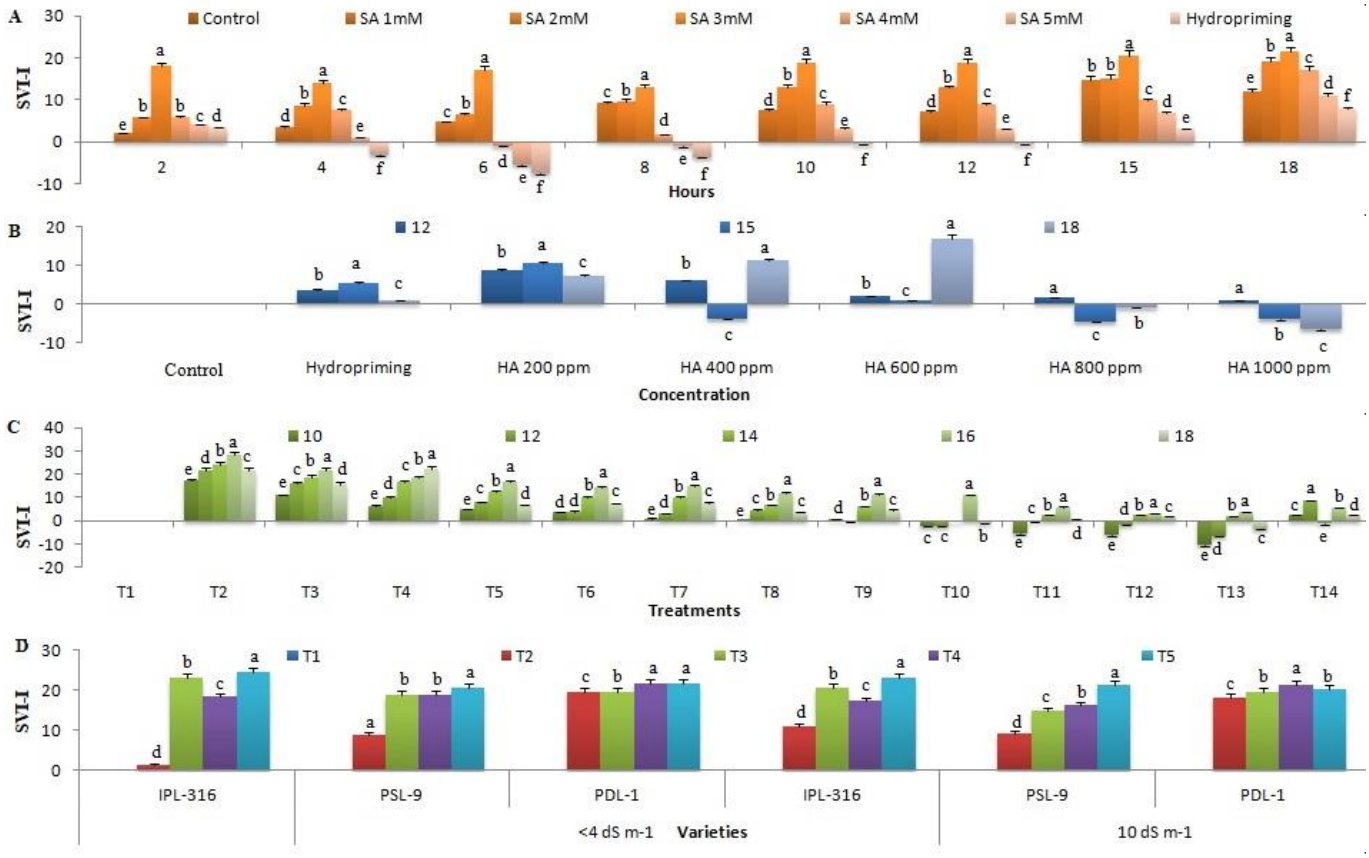

Figure 3. Effect of different treatments on the seed vigour index-I at different durations and concentrations (A); Different concentrations and durations of silicic acid. (B); Different concentrations and durations of humic acids. (C): Combination of humic and silicic acid. (D);After standardization under normal and salinity stress condition.

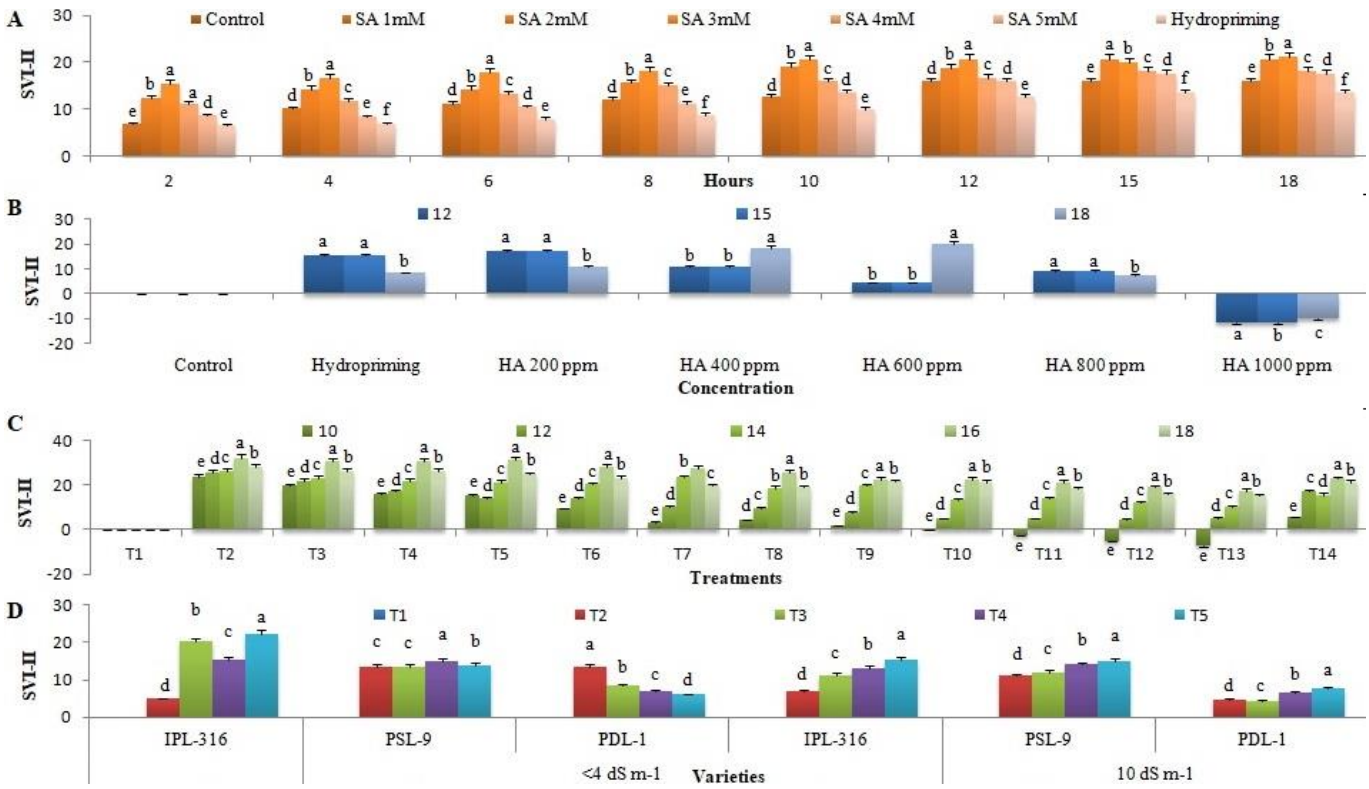

Figure 4. Effect of different treatments on the seed vigour index-II at different durations and concentrations (A); Different concentrations and durations of silicic acid. (B); Different concentrations and durations of humic acids. (C): Combination of humic and silicic acid. (D);After standardization under normal and salinity stress condition.

The control and hydropriming methods continued to show the lowest vigor, with scores of 4.89 and 6.33. Ultimately, the zenith of seed vigor index-II, across all trials, was 6.94, achieved through the blend of HA and SA at 100 ppm and 1 mM for 16 hours in treatment T_3. Treatments T_3 and T_2 followed this benchmark. The least vigorous indices, yet again, arose from the control and hydropriming endeavors, with values of 6.05 and 6.46 (Figure 4).

Root scanning

The study reveals that the treatment (T_4), which combined humic and silicic acid at 100ppm and 1mM for 16 hours, showed the most significantly different root scanning parameters, followed by treatments T_3 and T_2 (Table 2). The lowest significant results were observed in the control treatment (T_1) and hydropriming (T_5). Similarly, the correlation (Figure 5) and heat map (Figure 6) studies indicated positive correlations among all the parameters. The highest significant results for all root scanning parameters, including length, surface area, projected area, volume, average diameter, number of tips, forks, and crossings, were observed in treatment (T_4), followed by the other treatments (Figure 7).

Table 2. Effect of the seed priming on the different root scanning parameters

	Root scanning							
	Length (cm)	Surface area (cm)	Projected area (cm)	Volume (cm^2)	Avg. Diameter (mm)	No. of Tips	No. of forks	No. of cross
T1	118.123 ^{d}	25.397 ^{d}	8.084 ^{d}	0.436 ^{d}	0.684 ^{d}	325 ^{d}	140 ^{d}	7 ^{d}
T2	140.081 ^{bc}	28.538 ^{bc}	9.084 ^{bc}	0.463 ^{bc}	0.648 ^{ab}	376 ^{ab}	147 ^{bc}	18 ^{ab}
T3	146.587 ^{ab}	29.973 ^{ab}	9.541 ^{ab}	0.488 ^{ab}	0.651 ^{ab}	361 ^{ab}	157 ^{ab}	17 ^{ab}
T4	170.523 ^{a}	35.131 ^{a}	11.183 ^{a}	0.656 ^{a}	0.656 ^{a}	425 ^{a}	270 ^{a}	24 ^{a}
T5	107.587 ^{cd}	29.639 ^{bc}	9.434 ^{cd}	0.417 ^{d}	0.563 ^{cd}	345 ^{c}	114 ^{cd}	15 ^{cd}
	P=0.05							
CD (Treatment)					0.561			
CD (Parameter)					0.754			
CD (T*P)					0.985			

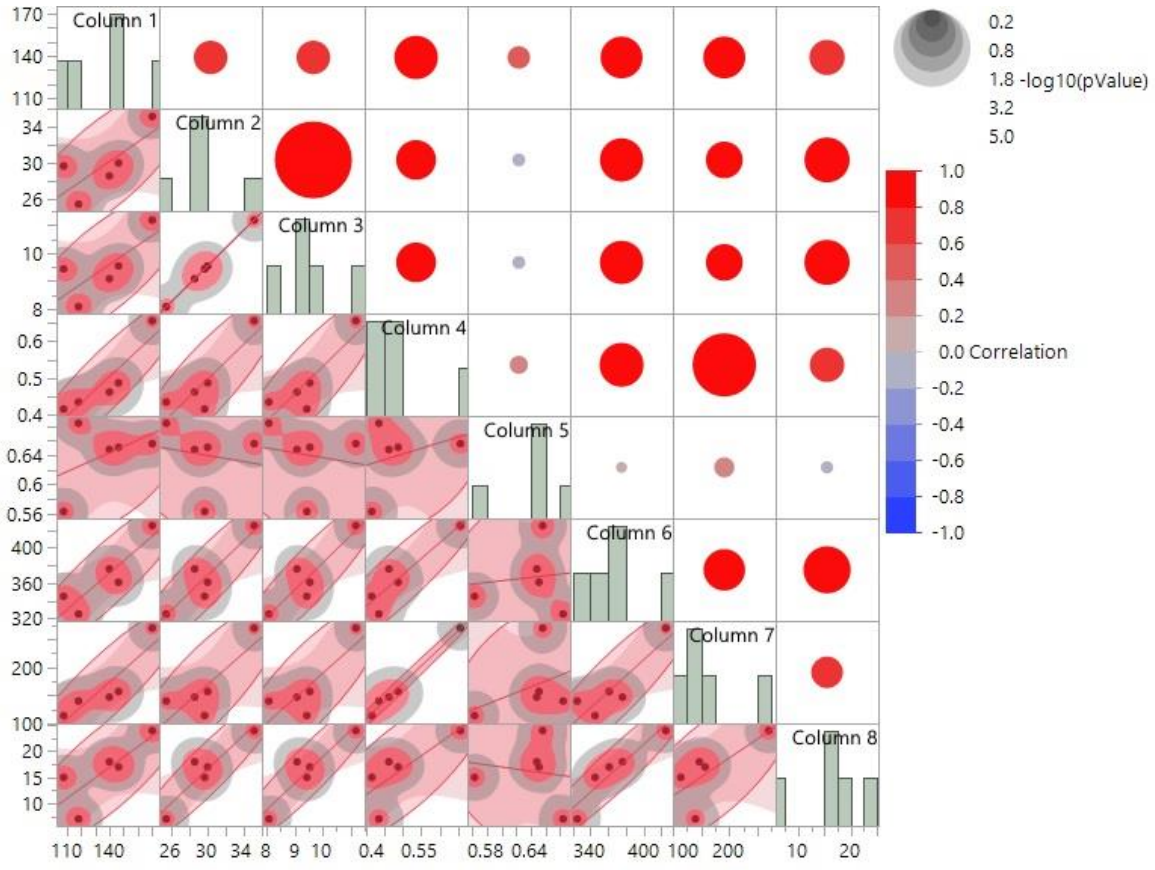

Figure 5. The correlation studies among all the root scanning parameters whereas, the column 1= Length (cm), column 2= surface area (cm2), column 3= projected area (cm), column 4=volume (cm2), column 5= average diameter (mm), column 6= number of tips, column 7= number of forks, column 8= number of cross.

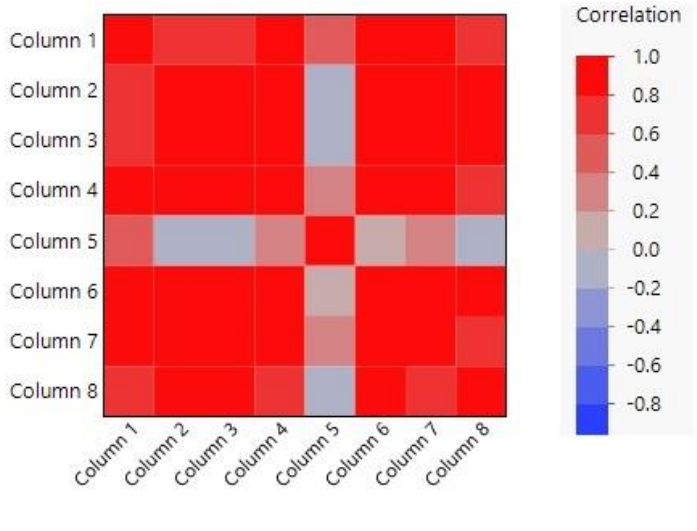

Figure 6. The heat map among all the root scanning parameters whereas, the column 1= Length (cm), column 2= surface area (cm2), column 3= projected area (cm), column 4=volume (cm2), column 5= average diameter (mm), column 6= number of tips, column 7= number of forks, column 8= number of cross.

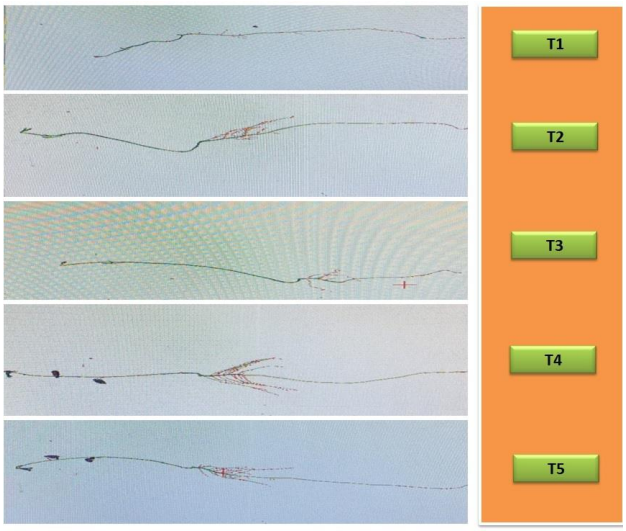

Figure 7. The scanned images of all the treatments whereas, T1= control, T2= SA @ 3mM for 18 hr, T3= HA@ 600ppm for 18 hr, T4= Combination of HA+SA (100pp+1mM for 16 hr) and T5= Hydropriming @ 18 hr.

Discussion

Seeds of pulses swiftly succumb to a decline in vigor and viability when stored under ambient conditions, especially in tropical climes where humidity reigns. For instance, lentil seeds, freshly harvested with an initial germination rate of 75%, may dwindle to a mere 50-60% within a year's storage at ambient temperatures, accompanied by a marked deterioration in seed quality measures, namely seed vigor indices I and II. Beyond the mere loss of viability, seed vigor diminishes significantly, leading to lethargic germination, stunted seedling growth, and reduced dry weight in seedlings. This degradation in seed quality wreaks havoc on field establishment and is exacerbated under conditions of water stress. To counteract these deleterious effects, seed priming emerges as a proposed panacea, mitigating the adversities of seed aging on both germination and seedling development. Priming treatments are believed to awaken enzymes, stimulate protein synthesis, mend cellular membranes, and bolster antioxidant defenses (Mohamed et al., 2018; Karim et al., 2020).

The present study that priming with humic acid (HA) and silicic acid (SA) profoundly enhanced crucial physiological traits, such as germination percentage, seed vigor indices, and root scanning parameters, thereby invigorating both germination and growth in moderately aged seeds. Notably, hydro priming failed to elicit a comparable boost in physiological traits, underscoring the critical role of reactivating antioxidant defenses for the rejuvenation of aged lentil seeds. The results from HA and SA priming underscore the pivotal influence of priming duration in modulating germination and vigor attributes by adjusting seed moisture content to allow only the preliminary resumption of metabolic activities vital for seed repair. Yet, excessively prolonged priming may inflict seed damage due to the advancement of germination beyond the repair phase, potentially impairing seeds during subsequent drying (Aghamir et al., 2016). These findings highlight the necessity of optimizing the priming duration to maximize the efficacy of seed priming techniques in aged seeds.

Research into lentil responses to salt stress reveals that seed priming with silicic and humic acids enhances seed quality parameters and invigorates seedlings (Ruan et al., 2002). This study delved into the impact of such priming on germination percentage (GP), seed vigor index-I (SVI-I), seed vigor index-II (SVI-II), and root scanning across various lentil varieties. The findings demonstrate that seed priming significantly improves these parameters for all lentil types under salinity stress, while also mitigating antioxidant damage induced by the stress. Seed priming is renowned for mending membrane damage caused by seed storage or abiotic stress (Asgedom and Becker, 2001). Prior studies indicate that seed priming induces biochemical transformations, such as activating enzymes linked to cellular metabolism, halting inhibition of metabolism, ending dormancy, and ensuring water uptake, thereby facilitating germination (Ajouri et al., 2004; Bahrani and Pourreza, 2012).

Seed priming, a preparatory technique before sowing, involves immersing seeds in various substances to enhance germination and the initial growth of seedlings. Among these substances, silicic acid and humic acid, along with their amalgamations, have become esteemed as priming agents celebrated for their beneficial impact on plant growth and development (Ghosh et al., 2024). Soluble silicic acid, a mineral element integral to plant growth, has consistently demonstrated its ability to increase seed germination, promote plant development, and fortify resilience against biotic and abiotic stressors. Illustrative studies indicate that priming with silicic acid enhanced seedling growth and germination rates in wheat and lentil plants under saline conditions (Chourasiya et al., 2021; Rao et al., 2023). Similarly, silicic acid priming has been reported to improve early growth and salt stress resilience in pea, wheat, and rice seedlings (Dhiman et al., 2021).

Humic acid, an intricate organic substance born from the natural decay of plant and animal remnants, serves extensively as a transformative soil enhancer and fertilizer. Among its manifold benefits are the enrichment of soil structure, the augmentation of nutrient accessibility, and the fostering of plant growth. Notably, the application of humic acid through seed priming has shown to elevate germination rates and bolster seedling development in maize plants enduring drought conditions (Hussain et al., 2023). Likewise, reports indicate that humic acid priming not only augments seed germination but also supports early growth in lentil seedlings (Poomani et al., 2023). The synergy of humic acid and silicic acid as seed priming agents has proven to advance germination rates, amplify seedling development, and enhance nutrient absorption in wheat cultivated under salty environments (Rao et al., 2024). Additionally, it was revealed that the combined use of humic and silicic acids significantly promotes seed germination and early development in cucumber seedlings (Richmond and Sussman 2003).

The present study unveiled significant variations in seedling characteristics across different priming practices. Among the diverse applications and control plants, hydropriming and priming with 3 mM silicic acid and 600 ppm humic acid emerged as the most effective. Chemical priming accelerated germination by promoting rapid water absorption by the seeds. These priming practices initiate germination by activating the seed's biochemical machinery, fostering enzyme production, cell wall expansion, and breaking dormancy. Silicic acid, a vital bioactive element, plays an essential role in enhancing leaf morphology, root penetration, stress tolerance, plant growth, resistance to pathogens, and nutrient uptake. It also creates silicic acid deposits in the roots, diminishing apo plastic flow and absorption of toxic minerals, thereby reducing water loss through transpiration. The promotion of lateral root formation by priming applications is a significant outcome for seedling development. Lateral roots form critical components of the plant's comprehensive root system, inclusive of all underground organs, and play a vital role in water and nutrient absorption.

Conclusion

In essence, the practice of priming with silicic and humic acids is paramount in elevating seed quality characteristics. Research reveals that these priming techniques bolster germination rates, nurture seedling growth, and enhance root development. Moreover, the application of silicic and humic acids, whether alone or in tandem, amplifies antioxidant enzyme activity, thereby mitigating oxidative harm and lipid peroxidation. Their combined use reveals a synergistic effect, significantly improving seed and seedling resilience under saline stress conditions. However, further exploration, especially at the molecular level, is essential to unravel the biochemical dynamics at varying degrees of salinity stress. Long-term field trials are also advocated to evaluate the sustainability and practical efficacy of these priming methods in agriculture. Ultimately, these techniques emerge as promising and sustainable strategies for advancing seed quality and plant vigor in saline environments. Continued research into their deployment across diverse crops and ecological settings will be crucial in refining their agricultural applications.

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