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Research Article

EFFECT OF HALOPRIMING AND HYDROPRIMING ON THE GERMINATION AND SEEDLING TRAIT OF SOYBEAN (GLYCINE MAX)

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Abstract

Low germination rates and environmental stress frequently effect the crucial stages of soybean (*Glycine max*) germination and early seedling development which have a substantial impact on crop output. The effectiveness of various priming techniques like hydropriming and halopriming on ten soybean genotypes was assessed. Germination characteristics including germination percentage (GP), germination index (GI), germination energy (GE) and germination rate index (GRI) were examined using a completely randomized design. The results showed that KNO_3 50 mM was the most efficient priming concentration increasing germination by up to 63.33% in high performing genotypes such as UAM-SB-162 and 1-S. Treatment effects on GP, GI, and GE were significant while higher salt concentrations (100–150 mM). On the other hands, significantly reduced germination in all types. AUST-94-2 and 2-S were among the genotypes that responded substantially to halopriming while PKN-38-2-1 and other genotypes fared better with hydropriming or untreated controls. The study shows how significant it is to modify hydropriming and halopriming techniques based on genetic backgrounds in order to maximize stand establishment especially in settings that are prone to stress. To create useful priming standards for future should investigate the physiological processes behind genotypic variations and confirm these findings in field environment.

Keywords: *Glycine max, seed priming, halopriming, germination enhancement, genotypic variability, KNO_3, $CaCl_2$.*

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1. INTRODUCTION

As a major source of plant-based protein and edible oil for human consumption and animal feed soybean (*Glycine max*) stands are among the most commercially important legume crops in the world (Ravisankar *et al.*, 2021). In addition to their nutritional benefits soybeans are essential to sustainable agriculture because they can fix atmospheric nitrogen through a symbiotic connection with Bradyrhizobium bacteria increasing soil fertility and lowering reliance on synthetic fertilizers (Meena *et al.*, 2018). Soyabean production is agronomic importance especially during the critical germination and early seedling establishment period. Globally, Brazil and the USA lead soybean production, while Pakistan ranks very low and remains heavily import-dependent due to poor domestic stand establishment and environmental stressors. My research addresses these production gaps by utilizing KNO_3 and hydropriming to significantly enhance germination rates, providing a cost-

effective strategy to improve crop uniformity and yield potential in Pakistan's challenging climate (Pagano *et al.,* 2016). Uneven seed emergence low germination rates and vulnerability to abiotic stressors like salinity and drought reduce yield. These drawbacks highlight the necessity of an efficient seed augmentation method to raise the rate of germination (Oyebamiji *et al.,* 2024).

Seed priming is method in which soaking of seed in solution before radicle emergence and promoting metabolic activities and increase uniform seed germination (Farooq *et al*., 2019). Moreover, hydropriming and halopriming enhances seed germination and tolerance against various abiotic stresses (Hameed *et al*., 2025). Hydropriming facilitates uniform water uptake, reactive enzyme processes and repairs cellular damage leading to faster and more synchronized germination (Khalid *et al*., 2019). On the other hand, halopriming makes seeds more resistant to salinity and drought stress by improving osmotic adjustment and antioxidant defense mechanisms in addition to increasing germination in typical circumstances (Khalid *et al*., 2019). Priming has several physiological advantages such as boosting antioxidant enzyme activity accumulating suitable solutes for osmotic adjustment and activating metabolic repair pathways all of which promote seed vigor and stress tolerance (Bhanuprakash *et al*., 2016).

Priming of soybean seeds has been demonstrated to significantly decrease the average germination time, increases germination and increase the seedling growth trait including root and shoot length Mangena *et al*., 2020). Priming time, salt, concentration and genetic variability across soyabean cultivars are some of the variables that affect priming effectiveness (Lewandowska *et al*., 2020).

Soybean is an oil seed with high lipid content, large seed size large seed size and their longer hydration time significantly hinders the utilization of water during imbibition (Xiong *et al*., 2021). As opposed to cereals, soybean cover a three stage water uptake sequence with a distinct lag period period when metabolic reactivation must occur before germination. Because any change in water potential might result in partial metabolic activation or membrane damage the imbibition phase is especially susceptible to external stressors (Kermode *et al*., 1990). Conventional methods of addressing these issues, including higher seeding rates or chemical treatments frequently turn out to be unfeasible from an economic stand point for smallholder farmers and may have adverse environmental effects (Sims *et al*., 2018).

The result of recent field trials with other crops indicate that in order to practically use priming strategies, the optimization must be more careful (Singh *et al.,* 2015). For instance, insufficient priming activates not the necessary metabolic pathways, while excess leads to precocious radicle protrusion and diminished seedling vigour (Xing *et al.,* 2025). Preliminary research on soybeans in particular suggests that variations in seed coat permeability and biochemical makeup may cause the ideal priming time to fluctuate considerably between cultivars. Additionally, little is known about how priming effects and planting soil conditions interact especially in heavy clay soils where oxygen limitation may contribute to priming treatments (Bernard *et al.,* 2022).

In light of these research gaps the purpose of this study is to assess how hydropriming and halopriming affect soybean germination and early seedling characteristics (Tania *et al.,* 2020). In particular, the study evaluates the effects of priming on seedling growth parameters such as root and shoot development, fresh and dry weight and vigor index, and compares germination percentage, germination rate under various priming treatments. It also determines the best

priming technique for improving the establishment of soybean stands (Teshome *et al.,* 2018). In order to improve crop uniformity, yield potential and agricultural sustainability the results offer important insights into how to best optimize seed priming strategies for soybean production especially in areas vulnerable to abiotic pressures.

2. MATERIALS AND METHODS

The experiment was conducted in Seed and Plant Testing lab at MNS- University of Agriculture, Multan, Pakistan. Experimental material consisted of 10 genotypes of Soybean collected from United State Department of Agriculture (USDA) and National Agriculture Research Centre (NARC) Islamabad. The seeds of soybean genotypes were sown in petri dish with double layers of filter paper using complete randomized design under factorial arrangement with three replications and seven priming treatments. Seeds were primed for 4 hours in hydropriming and halopriming solutions using a 1:5 (w/v) seed-to-solution ratio at 25C 2+C with continuous aeration via an aspiration pump, followed by rinsing with distilled water and a 24-hour dry-back procedure at 25C to return seeds to their original moisture content. The seeds were germinated in Petri dishes within a growth chamber at 25C under a 12-hour photoperiod for 10 days to record daily germination metrics. Data were recorded for following traits.

- Germination percentage
- Germination index
- Germination energy
- Mean germination time
- Germination rate index

Germination percentage:

Following formula was used to calculated germination percentage.

$$\text{Germination percentage} = \frac{\text{Number of germinated seeds}}{\text{Total seeds}} \times 100$$

Germination index:

Germination index was calculated by using following formula (AOSA, 1983).

$$\text{Germination Index} = \frac{\text{Number of germinated seeds}}{\text{Day of first count}} + \cdots \frac{\text{Number of germinated seeds}}{\text{Days of final count}}$$

Germination rate index:

Germination rate index was calculated by using following formula (Khan *at el*., 2004).

$$\text{Germination rate index} = \frac{\text{Germination index}}{\text{Germination percentage}}$$

Germination energy:

Germination energy was calculated by followed this formula.

$$\text{Germination energy} = \frac{\text{Number of germinated seeds at 4 DAS}}{\text{Total number of seeds tested}} \times 100$$

Mean germination time:

Mean germination time was calculated by followed this formula.

$$MGT = \sum \frac{(\frac{n}{d})}{N}$$

n=represents the number of seeds that germinated on a specific day.

d=is the number of days from the start of the experiment.

N=is the total number of seeds that germinated by the end of the experiment

3. RESULTS

The ANOVA results show that all calculated seed germination parameters germination percentage, mean germination time, germination index, germination energy and germination rate index were significantly affected by the different treatments $p < 0.0001$. This means that priming treatments such as hydropriming, KNO_3 and $CaCl_2$ had a strong influence on seed germination performance. The high F-values and low p-values indicate clear differences between treatments with KNO_3 50mM consistently showing the best results especially in germination percentage and germination rate index while $CaCl_2$ 150mM had the poorest

Table 1: Analysis of Variance Table for Germination %

Source	DF	SS	MS	F	P
Treatment	7	21895.6	3127.94	3753.52	0.0000
Error	72	60.0	0.83		
Total	79	21955.6			
Grand Mean	19.250	**CV** 4.74			

Table 2: Analysis of Variance Table for Mean Germination Time

Source	DF	SS	MS	F	P
Treatment	7	324.884	46.4119	4091.86	0.0000
Error	72	0.817	0.0113		
Total	79	325.700			
Grand Mean	2.1395	**CV** 4.98			

Table 3: Analysis of Variance Table for Germination Index

Source	DF	SS	MS	F	P
Treatment	7	291.335	41.6193	4513.41	0.0000
Error	72	0.664	0.0092		
Total	79	291.999			
Grand Mean	4.9969	**CV** 1.92			

Table 4: Analysis of Variance Table for Germination Energy

Source	DF	SS	MS	F	P
Treatment	7	0.12452	0.01779	418.55	0.0000
Error	72	0.00306	0.00004		
Total	79	0.12758			
Grand Mean	0.0945	**CV** 6.90			

Table 5: Analysis of Variance Table for Germination Rate Index

Source	DF	SS	MS	F	P
Treatment	7	11786.6	1683.80	1102.52	0.0000
Error	72	110.0	1.53		
Total	79	11896.6			
Grand Mean	13.875	**CV** 8.91			

performance often leading to no germination shown in table 1,2,3,4 and 5).

The high F-values also shows that the treatments had a much larger effect compared to random variation. The low coefficient of variation values for most parameters ranging from 1.92% to 8.91% indicate that the data was consistent and reliable. Overall, the study confirms that seed priming treatments, particularly KNO_3 at 50mM, can significantly improve germination metrics compared to the control, while higher concentrations of $CaCl_2$ may inhibit germination.

Among the varieties UAM-SB-273 performed slightly better than other varieties such as mean germination time and germination energy, particularly when treated with hydropriming or KNO_3 50mM. $CaCl_2$ 150mM completely inhibited

germination in most cases while control seeds showed poor performance compared to primed seeds. Overall, KNO_3 50mM priming significantly enhanced germination with UAM-SB-273 emerging as one of the most responsive varieties

The graph compares the germination performance of UAM-SB-162 seeds under different treatments. KNO_3 50mM was the most effective treatment providing 60% germination and the highest germination index. Hydropriming and control showed moderate results while higher KNO_3 and $CaCl_2$ concentrations performed poorly with $CaCl_2$ 150mM completely slow down (stop) seed germination (figure 1).

The germination performance of the 4-S variety under various treatments shows that KNO_3 50mM was the most effective giving 50% germination and the highest germination energy 36.67% while $CaCl_2$ 50mM also performed well with 33.33% germination. The control group and hydropriming show lower germination rates 10% (figure 2).

The 2-S variety under different priming treatments showing that KNO_3 50mM was the most effective treatment achieving 50% germination along with the highest germination index 5.89 and energy 36.67%. $CaCl_2$ 50mM also improved germination 33.33% while higher concentrations of both compounds like 100mM and 150mM positively reduced germination (figure 3).

The KNO_3 50mM was the most effective treatment achieving 63.33% germination with high germination energy 50% and a strong germination index 7.25. While KNO_3 100mM and $CaCl_2$ 150mM completely inhibited germination 0% and other treatments including hydropriming (16.67%) performed poorly (figure 4).

The KNO_3 50mM was the most effective treatment giving 50% germination with moderate germination energy and the highest germination index. KNO_3 100mM and $CaCl_2$ 150mM completely slow down seed germination 0% while other treatments like hydropriming (16.67%) showed limited improvement over the control (10%) (figure 5).

The hydropriming was the strongest treatment for UAM-SB-313 providing 20% germination the highest among all treatments with the best germination index and energy 20%. While KNO_3 100mM and $CaCl_2$ 150mM completely inhibited germination to 0% on the other hand chemical treatments performed poorly or similarly to the control to 10% (figure 6).

The KNO_3 50mM was optimal for AUST-94-2 yielding 56.67% germination with high vigor (germination index to 5.95 while $CaCl_2$ 50mM showed moderate improvement to 40%. All higher concentrations of both compounds failed completely 0% germination and hydropriming 16.67% performed only slightly better than the control to 10% (figure 7).

For PG-RA-9 hydropriming and KNO_3 150mM provide the best results to 16.67% seed germination each though all treatments performed poorly overall. KNO_3 100mM and $CaCl_2$ 150mM completely inhibited seed germination to 0% while other treatments matched or fell below the control 13.33% (figure 8).

UAM-SB-273 hydropriming was the most effective treatment providing 20% seed germination the highest among all treatments along with the best germination index and energy to 20%. All other treatments including chemical priming performed poorly or completely failed to 0% at 100mM/150mM matching or falling below the control to 10% (figure 9).

For PKN-38-2-1 hydropriming yielded the best results with 20% germination, the highest germination index and energy to 20% outperforming all other treatments. While KNO_3 150mM and the control provide moderate seed germination to 16.67%, KNO_3 100mM and $CaCl_2$ 150mM completely inhibited seed germination to 0% (figure 10).

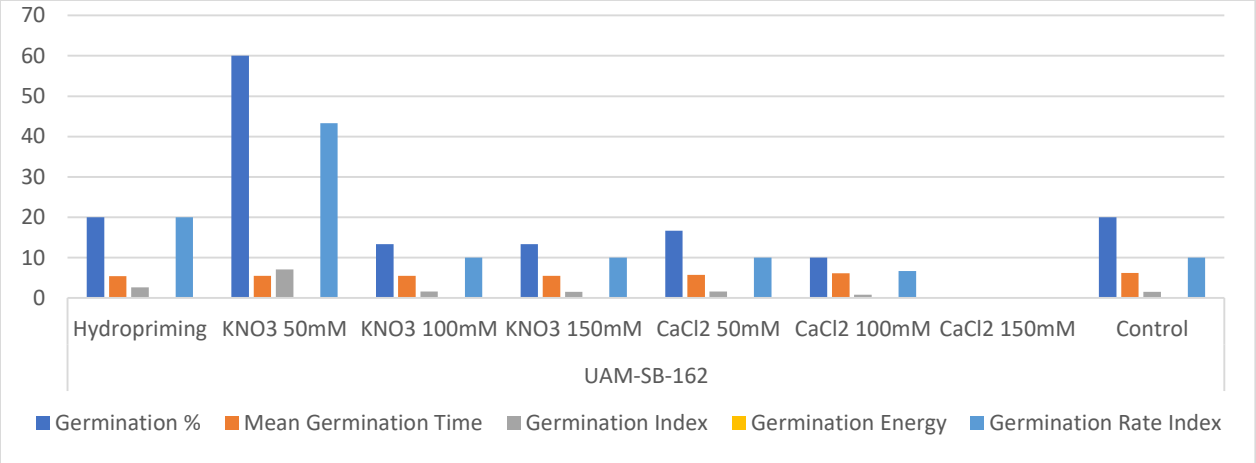

Figure 1: UAM-SB-162 genotype

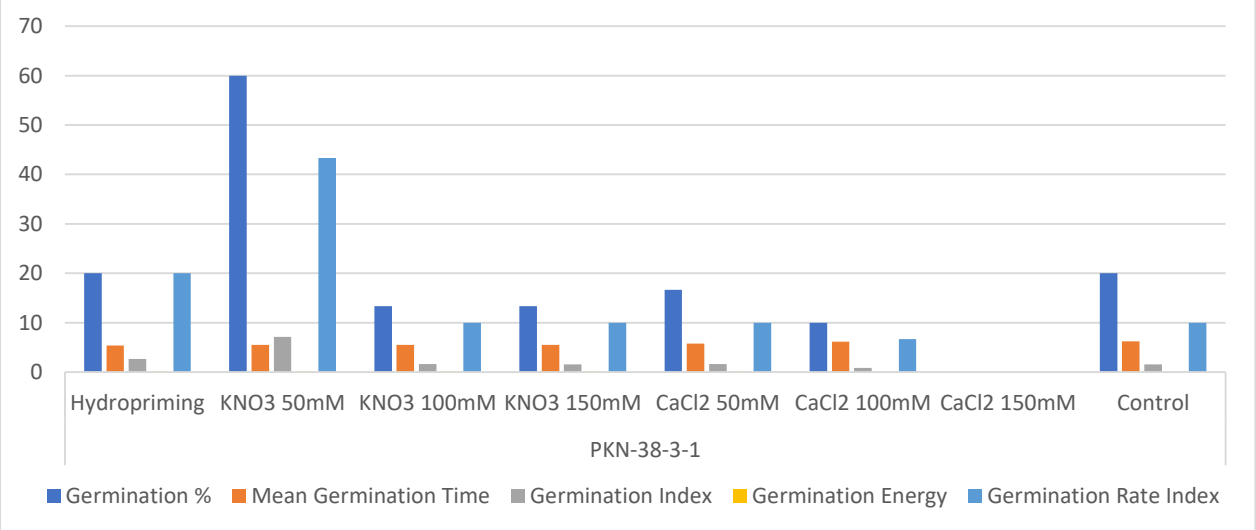

Figure 2: 4-S genotype

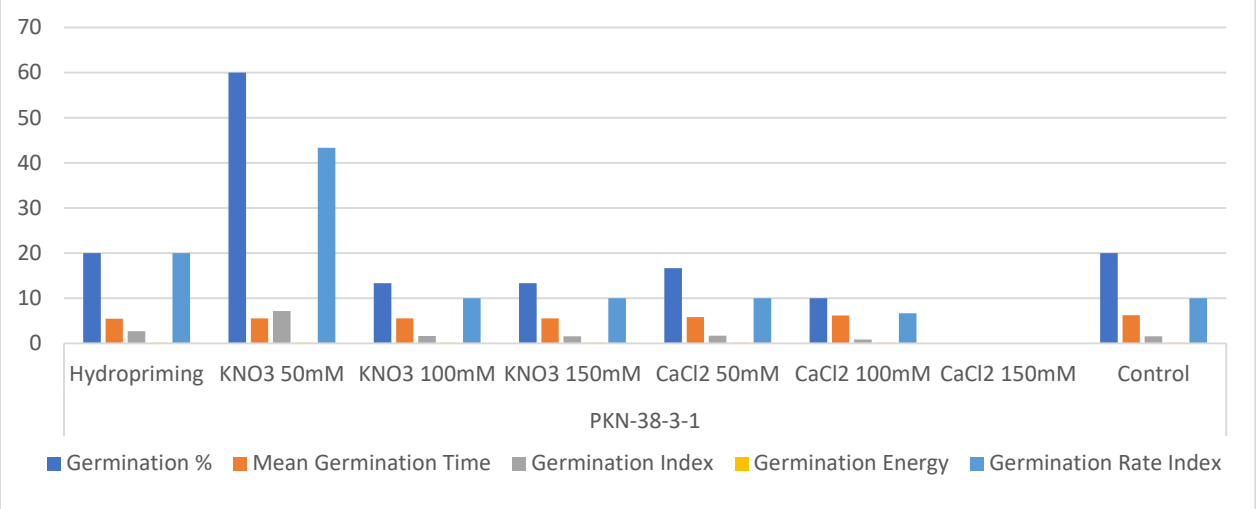

Figure 3: 2-S genotype

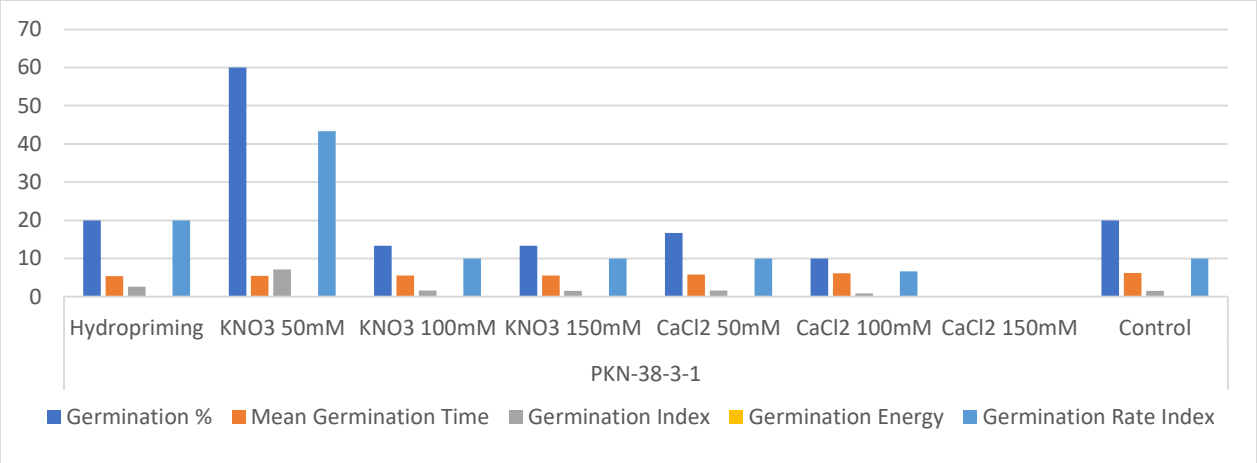

Figure 4: 1-S genotype

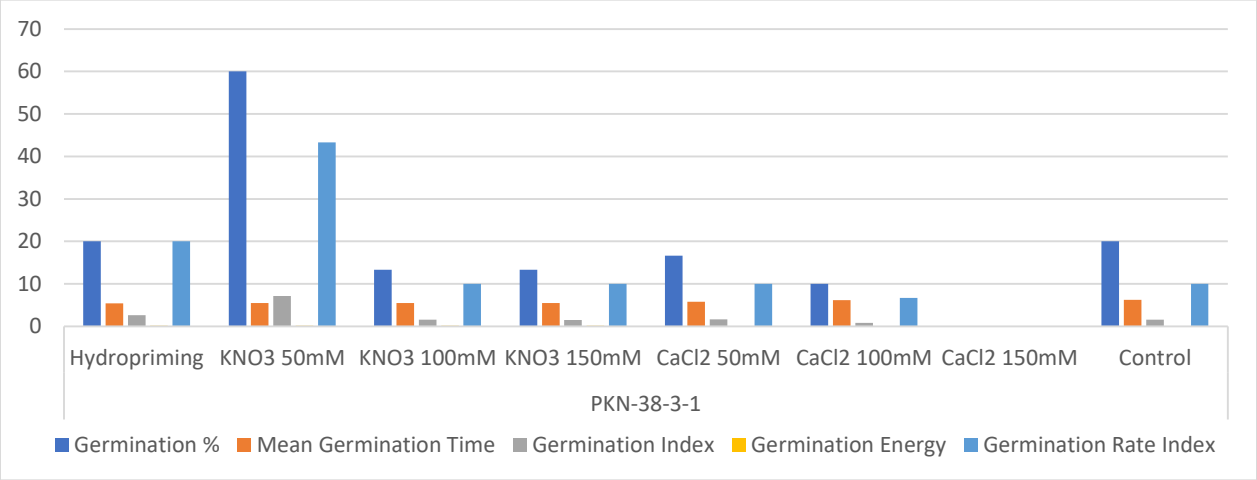

Figure 5: 3-S genotype

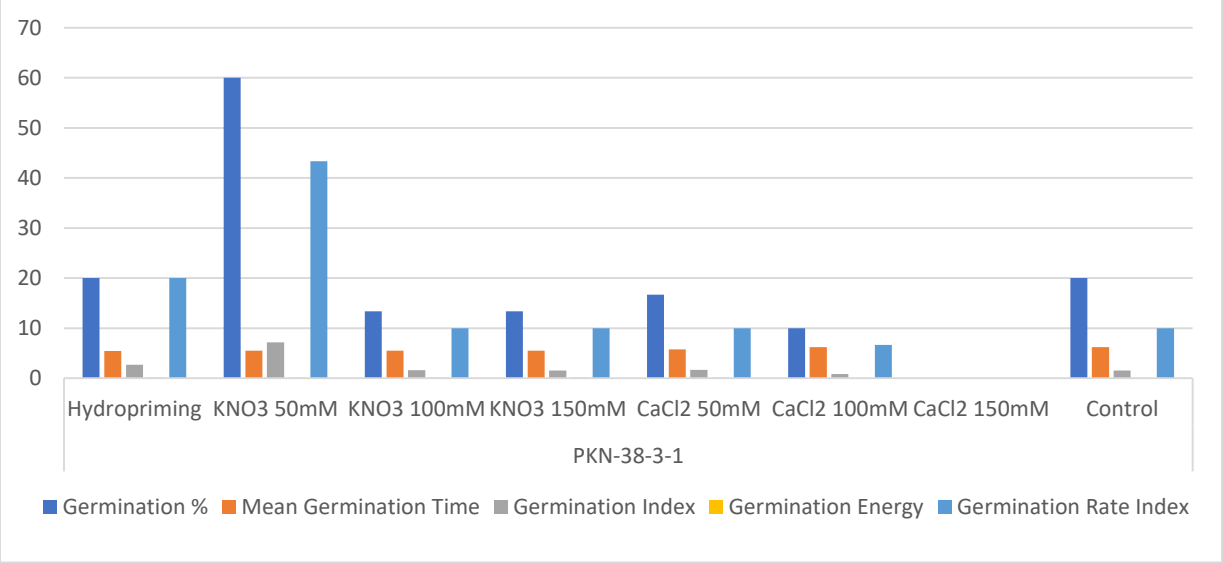

Figure 6: UAM-SB-313

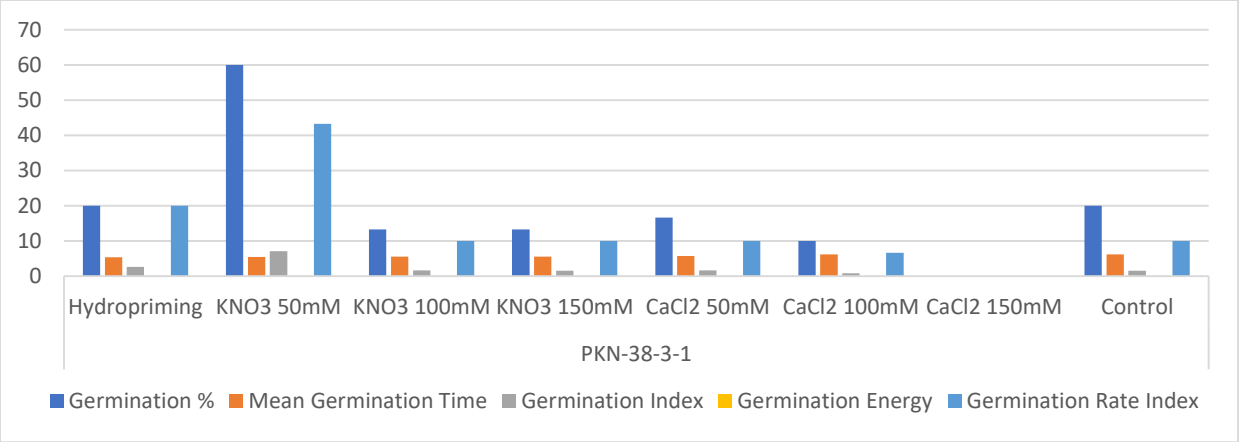

Figure 7: AUST-94-2

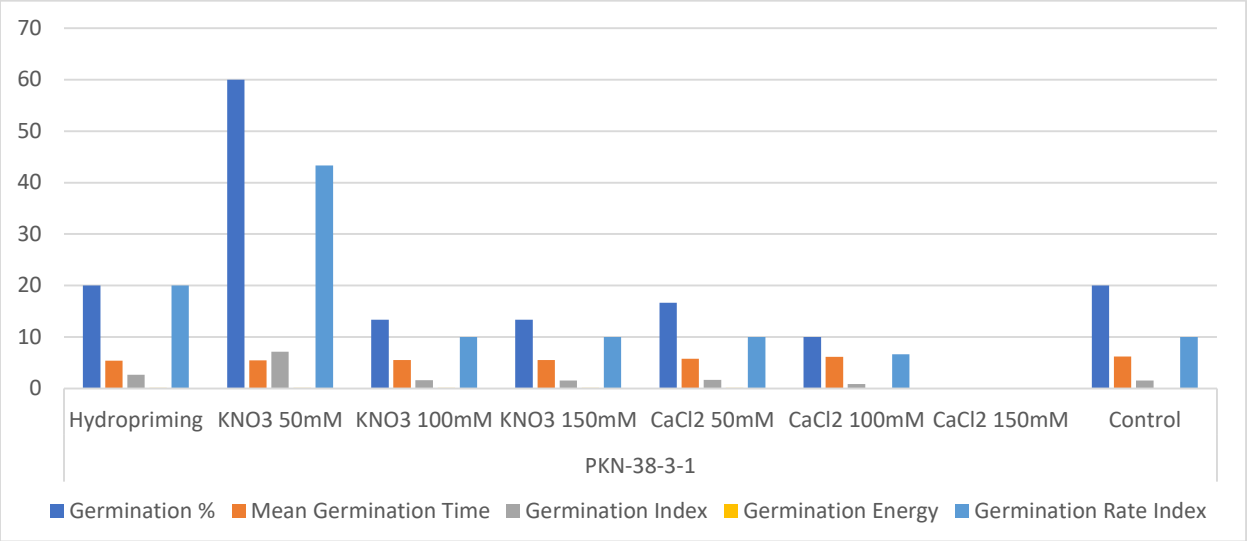

Figure 8: PG-RA-9

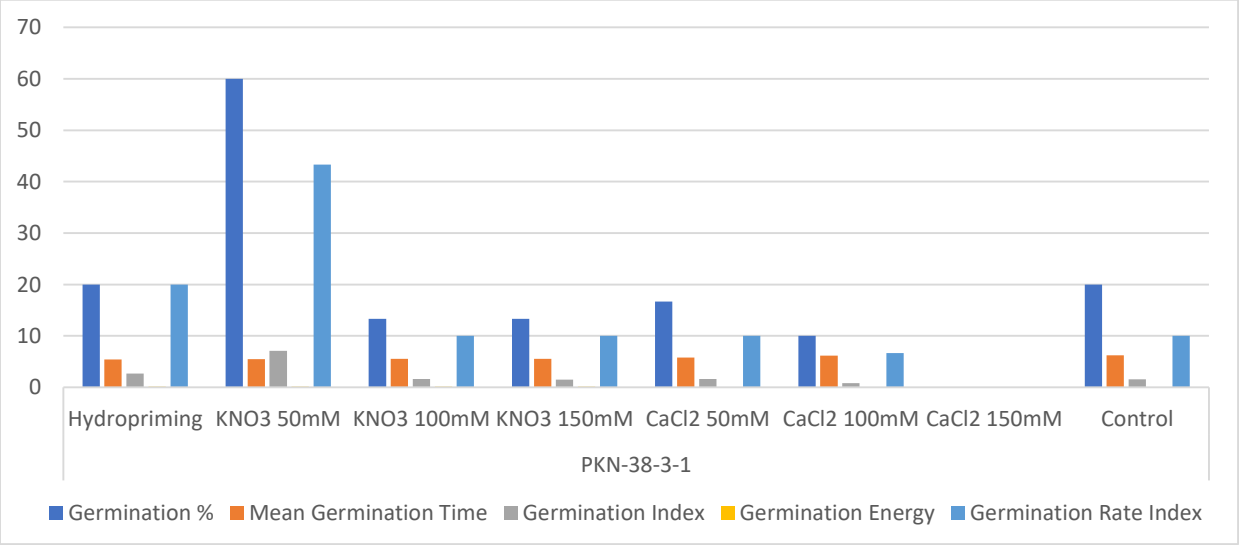

Figure 9: UAM-SB-273

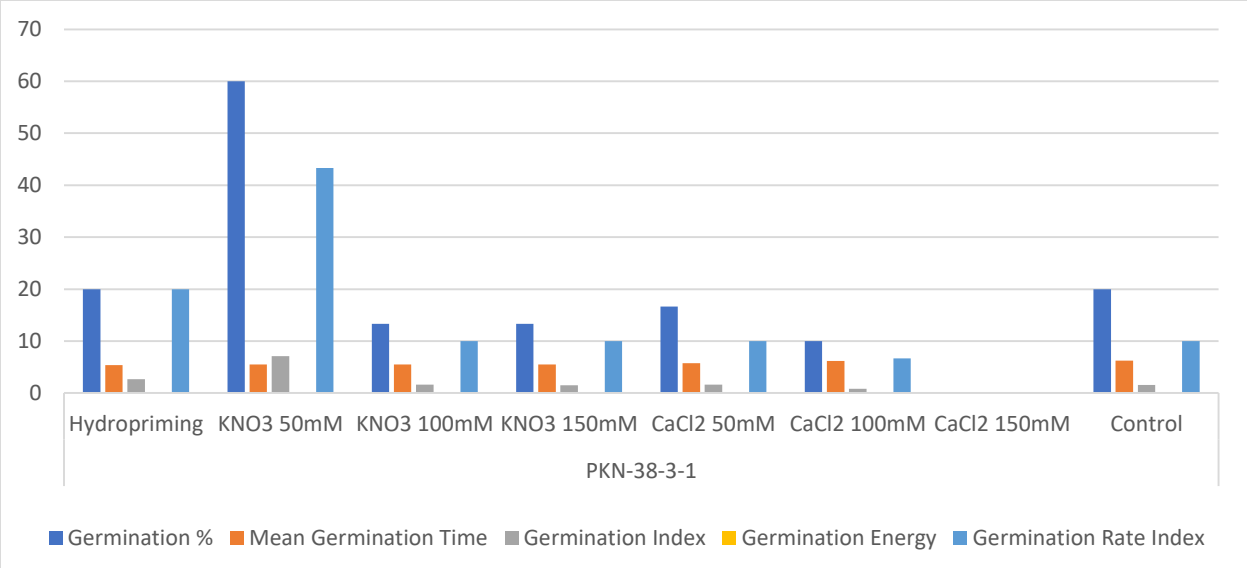

Figure 10: PKN-38-2-1

4. DISCUSSION

The effects of hydropriming and halopriming on germination parameters across ten soybean genotypes were thoroughly assessed in this study. The complicated interplay between priming techniques and genetic background in soybean germination physiology is highlighted by our findings which show significant genotypic heterogeneity in response to seed priming regimens (Fu *et al.*, 2024).

The most notable finding was that the effectiveness of halopriming treatments varied with concentration (Nawaz *et al.*, 2011). Higher concentrations consistently decreased germination across all varieties while KNO_3 50mM and $CaCl_2$ 50mM demonstrated encouraging outcomes in a number of genotypes (Shahi-Gharahlar *et al.*, 2010).

Our results imply that thorough genotype-specific tuning is necessary for soybean seed priming from a practical standpoint (Pissolato *et al.*, 2024). Variety-specific priming instructions would be helpful to farmers especially the warning not to exceed 50 mM salt concentrations. Some genotypes may respond better to hydropriming or even untreated planting than to chemical priming, including PKN-38-2-1 and UAM-SB-273 (Latif *et al.*, 2025).

The AUST-94-2 and 2-S responded favorably to halopriming showing much better osmotic adjustment capacity while PKN-38-2-1 and UAM-SB-273 shows great performance with simple hydropriming or even controls environment. These differences may link to morphological and physiological variations in seed coat permeability, lipid composition or stress response mechanisms among all genotypes (Radchuk *et al.*, 2014). The complete seed germination inhibition at higher salt concentrations particularly $CaCl_2$ 150 mM underscores the delicate balance required in priming solutions where excessive solute concentrations can outweigh potential benefits (Chen *et al.*, 2022).

An agricultural view these findings suggest that low concentration KNO_3 priming such as 50 mM could serve as a standard treatment for many soybean varieties while hydropriming may represent a safer alternative for more sensitive genotypes (Alam *et al.*, 2023). This is mainly beneficial for small farmers who may have less resources for extensive seed testing as improper priming could potentially reduce as

a stand establishment rather than improve it (Almekinders *et al.*, 2007).

The germination rate index and mean germination time shows particularly strong responses to priming indicating these parameters may act as more sensitive indicators of treatment efficacy than the germination percentage (Yari *et al.*, 2010). While some treatments showed similar germination percentages and their effects on germination coordinating and speed differed remarkably (Fernández-Pascual *et al.*, 2021). With better crop performance this has practical implications for field conditions where rapid uniform emergence often correlates (Finch-Savage *et al.*, 2016).

First the controlled laboratory conditions may not fully show field stresses particularly soil related factors like variable moisture and temperature (Poorter *et al.*, 2016). Second the study examined only initial germination parameters leaving questions about how priming affects after growth stages and ultimate yield (McDonald *et al.*, 2000). Third the physiological mechanisms highlighting the observed genotypic differences remain unclear (Hirel *et al.*, 2007). Potential factors include variations in seed coat structure and reserve mobilization efficiency or stress response pathways that could influence priming effectiveness (Srivastava *et al.*, 2021).

Future studies should highlight into (1) the physiological processes that underlie genotypic variations in priming response, specifically lipid metabolism and seed coat characteristics; (2) field testing of the best laboratory-identified treatments (Ping *et al.*, 2025) and (3) possible overlaps between priming and other seed technologies for stressful conditions. Furthermore, compared to merely raising seeding rates economic evaluations should assess if the slight benefits in certain genotypes brought about by priming outweigh the additional work and inputs (Farooq *et al.*, 2019). The KNO_3 concentrations generally work best different varieties require different approaches and higher concentrations often have negative effects. These findings provide a solid foundation for developing optimized priming strategies that can help farmers gain better stand foundation and potentially for higher yields. Successful implementation will require additional research to understand the underlying mechanisms validate results in field conditions and develop practical cost effective application methods suitable for different farming systems.

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