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Duration-dependent Seed Priming: A Strategy to Trigger Amylase and Protease-mediated Food Reserve Mobilization during Seed Germination and Seedling Growth of Pea (*Pisum sativum* L)

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ABSTRACT

Background: An *in vitro* study was carried out under controlled environmental conditions in the Laboratory of Plant Physiology, Department of Agronomy, School of Agriculture, Lovely Professional University, to assess the impact of duration-dependent seed priming on the mobilization of food reserve based on amylase and protease enzymes during seed germination and seedling growth of pea (*Pisum sativum* L).

Methods: The present study was laid out in a complete randomized design (CRD) comprising three priming durations and four seed priming agents. Wherein the duration of seed priming ranged from 5 to 15 hours, while the deionized water (T_1), magnesium nitrate 7.5 mM (T_2), calcium nitrate 7.5 mM (T_3), potassium nitrate 15 mM (T_4) and biopriming with *Rhizobium* (T_5) were used as a priming agent.

Result: Results indicated that among the priming durations, 10 hours was the most effective in terms of seed germination, speed and uniformity of germination, compared to 5 and 15 hours. Additionally, the activity of key enzymes *viz*., amylase and protease, was also markedly enhanced at 10 hours, reflecting the optimum activity. Among the seed priming agents, hydro-priming with deionized water was noticed to be the most effective for the evaluated parameters at 5 h priming duration, followed by magnesium nitrate (T_2)> potassium nitrate T_4 > biopriming with *Rhizobium* (T_5) and calcium nitrate (T_3). Conversely, potassium nitrate (15 mM) proved to be the most effective priming agent at 10 hours of priming, followed by magnesium nitrate (T_2) > calcium nitrate (T_3)> Hydro-priming with deionized (T_1) and biopriming with *Rhizobium* (T_5). However, similar trends with fewer magnitude were observed at 15 hours of seed priming, A per cent increase or decrease analysis over control clearly indicated that 10 hours of priming with potassium nitrate resulted in the maximum percent increase in seed germination (27.8%), germination index (GI, 38.8%), vigour index (VI, 49.6%), amylase activity (45.1%) and protease activity (44.8%) along with the reduction in time to 50% germination was (-16.7%). The findings of the results suggest that the use of 10 hours of seed priming with potassium nitrate (15 mM) significantly triggers the activity of hydrolytic enzymes, thereby promoting faster seed germination and vigorous seedling establishment in pea.

Key words: Amylase, Duration-dependent seed priming, Potassium Nitrate, Protease, Vigour index.

INTRODUCTION

Germination of seed is a vital and complex physico-chemical process regulated by a sequence of metabolic events that begins with the uptake of water, followed by radicle protrusion (Mildaziene *et al.,* 2022). The healthy establishment of seedlings over the field depends on the speed, uniformity and vigor of seeds, which influence the crop production under both normal and adverse environmental stress conditions (Adetunji *et al*., 2021). In terms of food and feed, pulse crops play a significant role in India and the world because of their rich protein and nutritional quality. Moreover, it improves soil fertility through nitrogen fixation mediated by the symbiotic association between rhizobium bacteria and root nodules (Chen and Zhou, 2024). The foundation of healthy seed germination and vigorous seedling establishment can be triggered by using the simple and effective techniques of seed priming. Basically, it is a controlled hydration up to a level that initiates the pre-germinative metabolic events without protrusion of radicle in the seeds, followed by drying up to the initial level of moisture (Jatana *et al.,* 2024 and Rhaman, 2025). Multiple investigations have reported that seed priming with several agents enhances the speed of seed germination,

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seedling vigor and healthy crop establishment, wherein the hydro-priming, osmopriming, hormonal priming, nutripriming and biopriming are the most popular (Waqas *et al.*, 2019). The duration of priming plays a vital role in initiating the biochemical response and subsequent processes in the seed through seed priming (Taer, 2024). A short period of seed priming may fail to initiate the metabolic response, while over hydration may cause membrane damage or reduce the seed viability. Therefore, optimizing the duration of seed priming in the respective crop is essential to maximize the benefits (Xing *et al.,* 2025). One of the key metabolic enzymes influenced by seed priming is amylase and protease plays a significant role in the mobilization of sugar and protein from cotyledons, which serve as a source of energy for rapid seed germination and seedling growth (Anwar *et al.*, 2021). Thereby, duration-dependent seed priming can be considered a strategy to trigger amylase and protease-mediated food reserve mobilization in early seedling establishment in pea.

MATERIALS AND METHODS

Experimental details

An experiment was carried out under controlled environmental conditions in the Laboratory of Plant Physiology, Department of Agronomy, School of Agriculture, Lovely Professional University in 2023-2024 to optimize the duration and suitability of the seed priming agent. Seeds of pea (*Pisum sativum* L.) variety PB-89 were procured from the Punjab Agricultural University, Ludhiana. The experiment was arranged in a complete randomized design (CRD) with three replications, along with six treatments, including an absolute control (T_0). To maintain controlled conditions in the laboratory, a seed germinator (Model No-NU-151) was used, set at 18°C and 60% relative humidity. To optimize the duration of seed priming and its suitability for maximizing the seed germination and healthy seedling establishment over the field, the durations (5, 10 and 15 hours) and priming agents (Hydro priming with deionized water (T_1), magnesium nitrate 7.5 mM (T_2), calcium nitrate 7.5 mM (T_3), potassium nitrate 15 mM (T_4) and biopriming with *Rhizobium* (T_5) were used.

Seed priming treatments

The process of seed priming treatment begins with the sterilization of seeds with $HgCl_2$ (0.2%), followed by the washing of seeds with distilled water. Three different lots of seeds were initially grouped for the 5, 10 and 15-hour durations and from there, a sufficient amount of seeds were placed in respective solutions of magnesium nitrate, calcium nitrate and potassium nitrate. However, a lot of seeds were treated with rhizobium culture thereafter; seeds were dried back up to their original seed weight under the fan.

Observations related to seed germination

As per the treatments, twenty-five primed seeds were scattered in a petri dish and placed in a pre-set seed germinator. The number of seeds that germinated was counted at 24 h intervals up to 6 days. The calculation of Seed germination %, germination Index (GI), CVG%, time of 50% germination (T50%), synchronization index, vigor index, mean germination time (MGT), mean germination rate (MGR) and uncertainty of seed germination were carried out using the following formulas given (Kader, 2005; Goodchild and Walker, 1971; Labouriau, 1983; Coolbear *et al.,* 1984; Primack, 1985; Abdul-Baki and Anderson, 1973; Czabator, 1962; Ranal and de Santana, 2006 and Labouriau and Valadares, 1976).

Total soluble sugar

The total soluble sugar content from pea seeds was analyzed as per the phenol-sulfuric acid-based method of Dubois *et al.* (1956). A hundred mg of seeds was ground and extracted with 80% ethanol at 80°C for 30 minutes. Samples were centrifuged and the supernatant was used for the analysis. 0.5 ml of an aliquot, 0.5 ml of (5%) phenol and 2.5 ml of H_2SO_4 (98%) were added, mixed thoroughly and incubated at 25°C for 25 minutes to stabilize the colour development. The absorbance was recorded at 490 nm and the total soluble sugar content was calculated using the standard graph of glucose.

Protein content

The total soluble protein was estimated from the seeds of pea using the method of Lowry *et al.* (1951). The sample was homogenized with phosphate buffer (pH 7). The supernatant was separated after centrifugation at 10,000 rpm for 10 minutes. An aliquot of 0.5 ml from the extracted sample was poured into test tubes and the volume was raised to 1 ml, followed by 5 ml of alkaline copper reagent and 0.5 ml of Folin-Ciocalteu reagent to each tube. Mixed it well using vortex mixture and recorded their absorbance at 660 nm after incubating at room temperature for half an hour.

Amylase activity

The activity of amylase in pea seeds during germination was measured according to Bernfield (1955). The enzyme extract was incubated with 1% starch solution at room temperature for 10 minutes. The reaction was stopped by adding the Dinitrosalicylic acid (DNS) reagent and the samples were boiled for five minutes to develop the colour. Absorbance of samples was recorded at 540 nm, while the maltose standard curve was used to calculate the amylase activity.

Protease activity

Protease activity in the seeds was assayed according to García-Carmona (1990) by homogenizing in Phosphate buffer (pH 7) and centrifuging at 10000 rpm for 10 minutes to obtain an aliquot of the enzyme. The reaction mixture, representing a 0.5 ml aliquot and casein (1%), was incubated at 37°C for 10 minutes. The reaction was stopped by adding TCA solution (1%). The absorbance of the

supernatant was measured at 280 nm, while tyrosine was used to make a standard curve for the calculation of protease activity.

Statistical analysis

Data recorded from the research work were subjected to statistical analysis to determine the significance of the research work. The analysis of variance was done as per Gomez and Gomez (1984), whereas the level of significance was estimated at p=0.05.

RESULTS AND DISCUSSION

Seed germination and its derived parameters

Duration-dependent seed priming, along with priming agents, was evaluated for the seed germination and vigorous establishment of pea seedlings (Table 1). It was depicted that the duration of seed priming with 10 h was recorded as the most suitable duration of seed priming, whereas seed germination %, germination index**,** coefficient of velocity of germination (CVG%), T50%, synchronization index, vigour index-I and vigour index-II were recorded comparatively better than the 5 and 15 h. However, within the priming duration, 10 h with potassium nitrate was recorded as one of the most promising combinations. Whereas the significantly highest value of germination % (100), GI (12.1), CVG% (33.3), synchronization index (0.224), VI-I (470) and VI-II (17530), while the minimum days for T50% was recorded (2.3), which was followed by Magnesium Nitrate (T_2)> Calcium Nitrate (T_3)> Hydro-priming with deionised water (T_1) and bio-priming with *Rhizobium* (T_5). Data depicted from Table 1 also revealed that the priming duration of 15 h recorded similar results with potassium nitrate, but the impact was less than 10 h of priming duration. In contrast, 5 h of priming duration, hydro priming with deionized water was recorded as the most promising treatment and recorded the significantly highest value of germination % (85.6), GI (9.3), CVG% (32.5), synchronization index (0.305), VI-I (345) and VI-II (13984) while T50% was recorded minimum (2.3). These results were supported by Jassal and Singh (2018) who reported that the rapid and vigorous seedling growth, including seed germination and CVG%, is possible at the optimum duration of seed priming, which differs from crop to crop (Mahmoodi *et al,* 2011; Jassal and Singh, 2018; Verma *et al.,* 2023). The seed priming with optimal duration, along with potassium nitrate, triggers the hydrolytic enzymes related to seed germination, enabling the maximum germination %, germination index, vigour index

Table 1: Influence of the treatments on the seed germination and their derived parameters of pea (*Pisum sativum* L).

Duration of seed priming/treatments	Seed germination %	GI	CVG%	T50%	Synchronization index	Vigor index-I	Vigor index-II
Five hours of seed priming							
T_0	70.0^{d}	6.9^{c}	27.6^{ab}	2.9^{a}	0.197^{bc}	225^{c}	7663^{c}
T_1	85.6^{a}	9.3^{a}	32.5^{a}	2.3^{a}	0.305^{a}	345^{a}	13984^{a}
T_2	82.2^{a}	8.4^{a}	29.3^{a}	2.7^{a}	0.225^{ab}	296^{a}	11649^{a}
T_3	72.2^{cd}	6.9^{c}	26.4^{b}	3.0^{a}	0.191^{c}	263^{b}	10616^{b}
T_4	81.1^{ab}	8.2^{a}	28.7^{a}	2.7^{a}	0.224^{ab}	288^{a}	11846^{a}
T_5	76.7^{bc}	7.6^{b}	29.1^{a}	2.8^{a}	0.234^{a}	254^{b}	9973^{b}
Ten hours of seed priming							
T_0	72.2^{d}	7.4^{d}	28.4^{d}	2.8^{a}	0.183^{c}	237^{d}	7790^{d}
T_1	87.8^{b}	10.1^{b}	31.4^{abc}	2.5^{ab}	0.200^{abc}	395^{b}	14673^{b}
T_2	98.9^{a}	11.6^{a}	32.0^{ab}	2.6^{abc}	0.210^{ab}	452^{a}	17370^{a}
T_3	91.1^{b}	10.1^{b}	29.6^{cd}	2.8^{a}	0.175^{c}	373^{b}	14146^{b}
T_4	100.0^{a}	12.1^{a}	33.3^{a}	2.4^{c}	0.224^{a}	470^{a}	17530^{a}
T_5	80.0^{c}	8.6^{c}	30.2^{bcd}	2.7^{ab}	0.195^{bc}	318^{c}	11450^{c}
Fifteen hours of seed priming							
T_0	72.2^{b}	6.3^{b}	25.0^{c}	3.5^{a}	0.171^{b}	211^{b}	6936^{d}
T_1	78.9^{ab}	8.0^{a}	29.8^{a}	2.7^{b}	0.214^{a}	264^{a}	12785^{a}
T_2	81.1^{a}	7.9^{a}	28.5^{ab}	2.8^{b}	0.213^{a}	273^{a}	13099^{a}
T_3	74.4^{ab}	6.7^{b}	25.6^{c}	3.2^{a}	0.231^{a}	248^{a}	8855^{c}
T_4	88.9^{a}	9.8^{a}	30.3^{a}	2.8^{b}	0.201^{ab}	306^{a}	14678^{a}
T_5	74.4^{ab}	6.7^{b}	26.6^{bc}	3.3^{a}	0.208^{a}	245^{a}	10305^{b}
CD of five hours duration (p<0.05)	5.22	0.38	2.10	NS	0.03	18.42	956.2
CD of ten hours duration (p<0.05)	6.98	0.76	2.29	0.28	0.03	37.61	864.7
CD of fifteen hours duration (p<0.05)	7.25	0.69	1.84	0.31	0.03	29.87	1149.3

Note: GI= Germination Index, CVG% = Coefficient of velocity of germination %, T50% = Time to 50% germination, T_0 = Control, T_1 = Deionized water, T_2 = Magnesium nitrate, T_3= Calcium nitrate, T_4= Potassium nitrate, T_5 = Rhizobium.

and reducing the time of 50% germination. These results are well correlated with the findings (Abdel-Baki *et al.,* 2018; Choudhury and Bordolui, 2022; Tiwari *et al.,* 2023), who reported that the optimum duration of seed priming with potassium nitrate not only influences the seed germination but also improves the vigour index (Table 1). Additionally, the negative relationship of germination % with MGT and uncertainty of germination (Fig 1 and 2), while a positive relationship of seed germination with MGR was noticed in the same treatments (Fig 3). Results are consistent with (Kaya, 2021; Zhang *et al.,* 2025) highlighted the significance of priming duration and seed priming agents.

Total soluble sugar and amylase activity

Data depicted in Fig 4 reveal that seed priming durations statistically (p=0.05) influenced total soluble sugar content and amylase activity in germinating pea seeds. The highest amount of total soluble sugar (39.2 mg g^{-1}) and amylase activity (174.6 µg g^{-1} h^{-1} fresh weight) was noticed at 10 h of potassium nitrate-based seed priming, followed by a moderate impact at 15 h of seed priming duration for total soluble sugar (37.4 mg g^{-1}) and amylase activity (162.7 µg g^{-1} h^{-1} fresh weight). Conversely, hydro-priming with deionized water for 5 h also showed a significant (p=0.05) improvement, resulting in total soluble sugar (29.4 mg g^{-1})

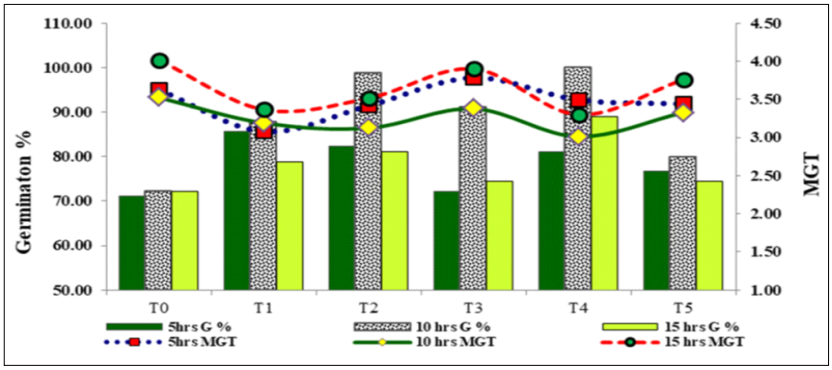

Fig 1: Influence of the treatments on germination (%) and mean germination time in pea.

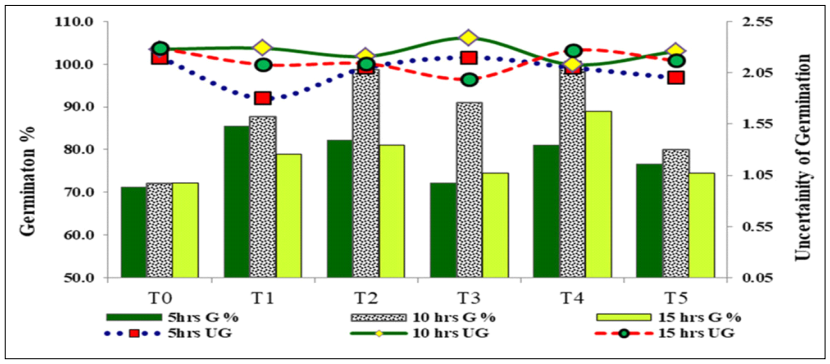

Fig 2: Influence of the treatments on germination (%) and uncertainty of germination in pea.

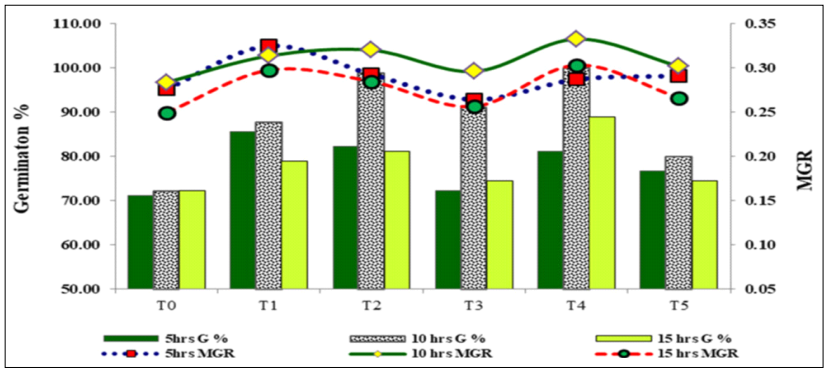

Fig 3: Influence of the treatments on germination (%) and mean germination rate in pea.

and amylase activity (149.3 µg g^{-1} h^{-1} fresh weight). In terms of percent increase over control is concerned, hydro priming with 5 h of priming resulted 36.2% increase in amylase activity (Fig 6), whereas potassium nitrate priming at 10h showed the highest increase (45.1%) of amylase activity (Fig 7). A similar trend, though with less magnitude, was also evident at 15 h of priming (Fig 8). The improvement in total soluble sugar and amylase activity, as noticed in the present study, indicates that seed priming treatments trigger the mobilization of food reserves available in the endosperm during seed germination. A statistically significant (p=0.05) increase in both parameters at 10 h of priming with potassium nitrate suggests that the duration of priming is optimum to accelerate the metabolic process in the seed during seed germination (Abdel-Baki *et al.,* 2018). The improved amount of soluble sugar and amylase activity during seed germination with potassium nitrate aligns with the previous work in pea and other crops (Tiwari and Tripathi, 2025; Abdel-Baki *et al.,* 2018; Basra *et al.,* 2006). The hydro-priming of 5 h duration also indicates a remarkable improvement in total soluble sugar and amylase activity, indicating the significance of triggering

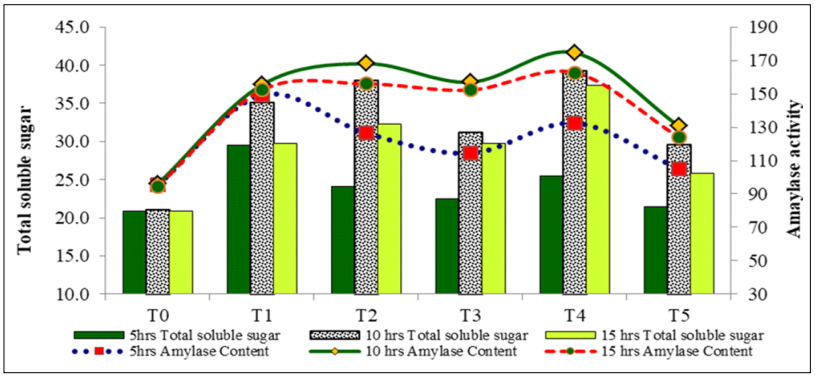

Fig 4: Influence of the treatments on total soluble sugar (mg g^{-1}) and amylase activity (µg g^{-1} h^{-1} fresh weight) in pea during seed germination.

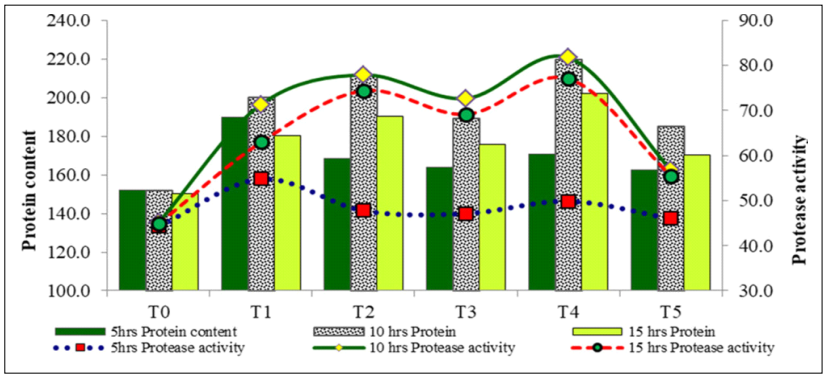

Fig 5: Influence of the treatments on protein (mg g^{-1}) and protease activity (mg tyrosine released min^{-1} g^{-1}) in pea during seed germination.

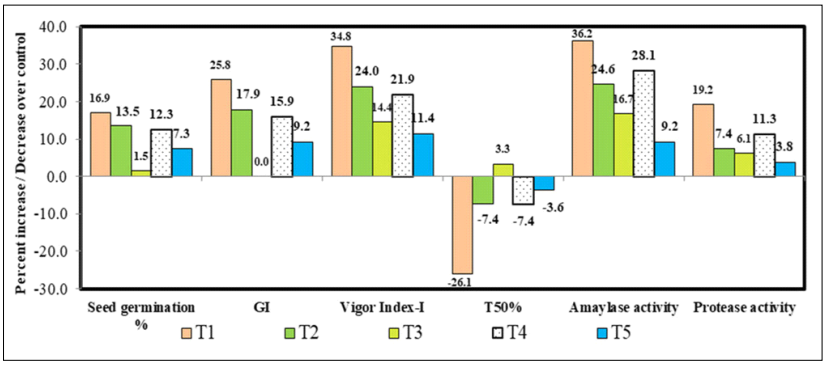

Fig 6: Influence of the treatments on the % increase /decrease over control at five hours of the seed priming.

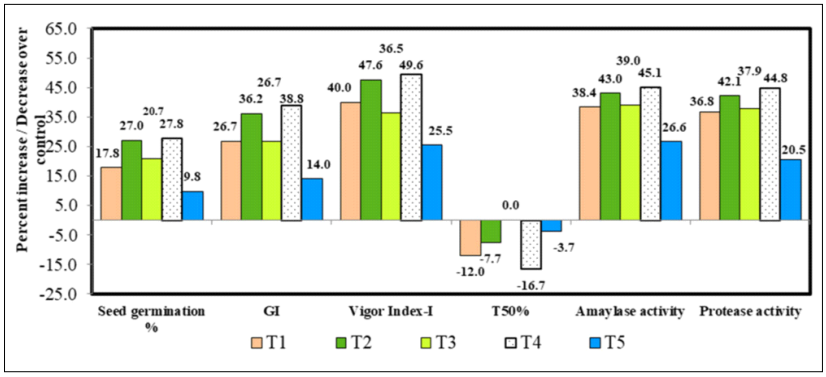

Fig 7: Influence of the treatments on the % increase/decrease over control at ten hours of the seed priming.

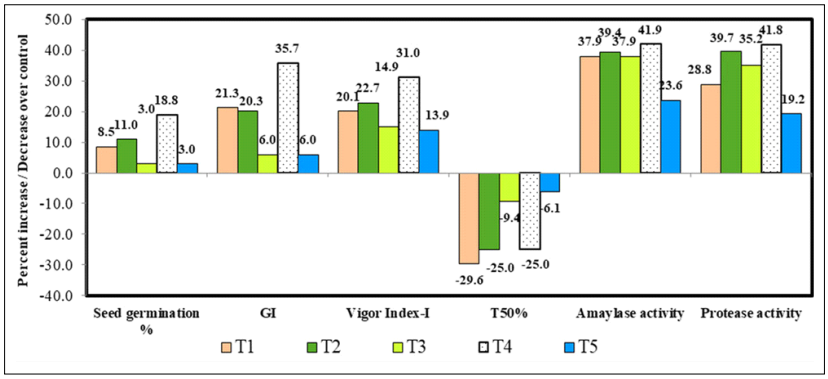

Fig 8: Influence of the treatments on the % increase/decrease over control at fifteen hours of the seed priming.

the enzyme activity and sugar metabolism, which is aligned with the earlier findings (Farooq *et al*., 2020; Vilakazi *et al.,* 2023) who emphasized that hydro priming with short duration enhances enzyme and sugar content during seed germination.

Total soluble protein and protease activity

Data depicted in Fig 5 reveal that seed priming durations statistically (p=0.05) also influenced total soluble protein content and protease activity in germinating pea seeds. The highest amount of total soluble protein (279.7 mg g^{-1}) and protease activity (81.7 mg tyrosine released $min^{-1}g^{-1}$) was noticed at 10 h of potassium nitrate-based seed priming, followed by a moderate response at 15 h of seed priming duration for total soluble protein (202.1 mg g^{-1}) and protease activity (77.1 mg tyrosine released min^{-1} g^{-1}). Conversely, hydro-priming with deionized water for 5 h also showed a significant (p=0.05) improvement, resulting in total soluble protein (189.7 mg g^{-1}) and protease activity (54.8 mg tyrosine released min^{-1} g^{-1}). In terms of percent increase over control is concerned, hydro priming with 5 h of priming resulted 19.2% increase in protease activity (Fig 6), whereas potassium nitrate priming at 10h showed the highest (44.8%) increaseof protease activity (Fig 7). A similar trend, though with less magnitude, was also evident at 15 h of priming (Fig 8). Results of the present finding corroborate earlier findings that seed priming with potassium nitrate enhances total soluble protein and mobilizes it by triggering the enzyme protease (Ali *et al.,* 2021). The elevated protease indicates catalytic action on storage proteins, which are useful for the synthesis of new enzymes and structural proteins during the germination period (Varier *et al.,* 2010; Mirza *et al.,* 2022). Moreover, it also contributes to accelerating the speed of imbibition and activity of enzymes (Cheng *et al.,* 2017). Hence, results confirm that 10 h of priming with potassium nitrate provides an optimum duration for rapid metabolic activation and efficient mobilization of food reserve towards the growing embryonic region.

CONCLUSION

The present study concludes that duration-dependent seed priming with potassium nitrate efficiently mobilizes the food reserve through improved amylase and protease activity in germinating pea seeds. Out of the tested priming durations, 10 h was the most effective, resulting in higher germination %, vigour and enzyme activity while reducing the time of 50% germination (T50%) as compared to 5 and 15 h of seed priming. However, hydro priming with deionized water was the most effective at 5 h of seed priming. Findings suggest that 10 h of potassium nitrate is the optimum duration to enable the pea seed to activate the metabolic process for the rapid mobilization of food reserves. Thereby, improving the performance of the seed and healthy seedling establishment. The overall study highlights that potassium nitrate priming

emerged as an effective and sustainable strategy to enhance seed vigor and early growth potential in pea.

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Disclaimers

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Conflict of interest

The authors declare that there are no conflicts of interest regarding the publication of this article. No funding or sponsorship influenced the design of the study, data collection, analysis, decision to publish, or preparation of the manuscript.

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