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EFFECTS OF SEED PRIMING ON GERMINABILITY AND BIOCHEMICAL PARAMETERS OF *PRAECITRULLUS FISTULOSUS* (STOCKS) PANGALO

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Abstract

The physiological dormancy of freshly harvested seeds of *Praecitrullus fistulosus* naturally overcomes to some extent if the seeds are stored before germination. Therefore, to avoid storage period, the priming treatments *viz.*, GA_3 (150, 500, 1000 µg/ml), cytokinin (150, 500, 1000 µg/ml Kn), ethylene (150, 500,1000 µl/l ethrel), KNO_3 (150, 500 µg/ml), HNO_3 (150, 500 µl/l), and water (hydropriming) were tested at 25°C at intervals of 12 and 24 hours, respectively. Conventional seed priming can provide unpredictable outcomes for morpho-physiological characters, as what works for one species or variety may not be successful for another. As a result, it is necessary to investigate the metabolic components that affect seed physiology and germination. Therefore, in the present study, seed metabolic efficiency (SME) and various biochemical parameters were observed, such as total soluble sugars, total starch, total soluble protein, total free amino acids, and α-amylase content. It was observed that seeds primed with 500 μgml^{-1} KNO_3 for 12 h and 24 h respectively, recorded maximum seed metabolic efficiency. Priming in GA_3 and KNO_3 showed significant improvement in the biochemical parameters which was manifested in better seed metabolic efficiency. Further, correlation analysis validated that SME exhibited a significant positive correlation with the content of total soluble sugar, total free amino acids, and α- amylase activity and negatively correlated with total starch and total soluble protein content. With appropriate priming treatments, it is possible to improve germinability, seed metabolic efficiency, and biochemical attributes of freshly harvested seed lots. This finding suggests that implementing these correlations could potentially alleviate dormancy states in related species within the same family.

Introduction

Seed dormancy is a complex trait and defines plant fitness. It causes a delay of germination until the arrival of a favourable growth season (Footitt and Finch-Savage 2017). In general, it is an undesirable characteristic in agricultural crops, where rapid and uniform germination is required. In cucurbits, seed dormancy is related to seed coat impermeability or hard seed coat (Nerson 2007). Apart from this, it has been found that round gourd family contains cucurbitacins, which are group of oxygenated tetracyclic triterpenes (Kim *et al*. 2020) are also responsible for inducing seed dormancy in this family (Chen *et al*. 2005). In a dormant seed, germination might proceed successfully provided interference factors are ameliorated by either natural or artificial means (Yildiz *et al*. 2017). It is well accepted fact that priming improves germinability, reduces seedling emergence and improves seed establishment. It is a pre-sowing technique that allows controlled seed rehydration to trigger the metabolic processes normally activated during the early phase of germination called pre-germinative metabolism which is a method to improve the rate and uniformity of germination in the seed lot (Mondal and Bose 2021).

Priming helps to improve germination even in dormant seeds. In ash gourd seeds, less germination was attributed due to the hard seed coat and observed maximum germination value when primed in KNO_3 solution (Rahman *et al.* 2014). Likewise, priming with KNO_3 enhanced

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germination percentage and reduced the mean time to germination in watermelon seeds (Demir and Mavi 2004). In sponge and snake gourds, chemical (GA_3@100 ppm, KNO_3 @ 0.5 %) treatments were adopted along with water soaking and dry heat treatments to overcome the dormancy (Rani *et al.* 2021). These works draw attention to overcoming dormancy by adopting these aforementioned treatments.

Consequently, the present study was investigated on round gourd (*Praecitrullus fistulosus* (Stocks) Pangalo) which is an important vegetable crop of plains of north India and exhibits seed dormancy immediately after harvest of seed. It is either sown in the spring season (February-March) or in the rainy season (June-July). For seed production crop is sown in February- March and harvested in June. However, the freshly harvested cucurbit seeds exhibit dormancy and the germination percentage is reduced as per Indian Minimum Seed Certificate standards (IMSCS). The minimum standard prescribed for germination percentage in cucurbits is 60% (IMSCS 2013), which indicates that it is difficult to have a seed lot with a higher percentage of germination in these crops. It has been observed that germination in seed lots of *Praecitrullus* improves if such seeds are stored prior to germination rather than when subjected to germination soon after harvesting. By standardizing the priming technique and duration for *Praecitrullus* seeds, the dormancy of freshly harvested seeds can be overcome and avoiding the wait for the natural process of dormancy breaking.

Materials and Methods

The study was conducted on round gourd (*Praecitrullus fistulosus*) cv. Punjab Tinda-I was chosen because it is the latest variety of PAU recommended for cultivation in Punjab and is quite popular among farmers due to its earliness and tenderness. The crop was sown in the spring season (mid-February sown), and the seeds were harvested in mid-June in the years 2018 and 2019, respectively in the field area of the Department of Vegetable Science, Punjab Agricultural University, Ludhiana. The seeds were washed, air dried and divided into two lots. The drawn seed lot was surface sterilized with mercuric chloride (0.1%) for one minute followed by rinsing twice in distilled water and then subjected to priming treatment in an appropriate solution. One seed lot was subjected to the priming and germination studies immediately. The second seed lot was stored in poly bags for 15, 30, 45 and 60 DAH (days after harvest) at 25°C. At the end of the storage period, a batch of seeds was subjected to the priming treatments and grown.

Priming was done by soaking the seeds in respective priming solutions for 12 hts and 24 hrs respectively. The following priming solutions were used: GA_3 (150, 500, 1000 µg/ml gibberellic acid), cytokinin (150, 500,1000 µg/ml Kn), ethylene (150, 500, 1000 µl/l ethrel), KNO_3 (150, 500 µg/ml), HNO_3 (150, 500µl/l), water (hydropriming). These concentrations provide a range that allows to observe the potential dose-dependent responses. Higher concentrations of these chemicals could inhibit germination or damage the seeds, prompting to limit the upper concentration for these particular solutions (Abdel-Baki *et al.* 2018, Timilsina *et al.* 2022). Fifty seeds were soaked per 50 ml of priming solution. At the end of the soaking duration, the seeds were rinsed in distilled water and blotted dry. To avoid unintended reactions of chemicals from the priming solutions, rinsing was done with distilled water. The primed seeds were divided into two lots, one seed lot was subjected to biochemical estimations and the second lot was subjected to germination studies. Biochemical parameters *viz.*, total soluble sugars, total starch (Dubois *et al.* 1956), total soluble proteins (Lowry *et al.* 1951), total free amino acids (Lee and Takahashi 1956), and α-amylase activity (Murata *et al.* 1968) were estimated. Prior to germination studies, seed fresh weight and seed dry weight (SDW) were recorded. For dry weight determination, the seeds were oven-dried at 60°C till constant weight was reached (3 days) (Bewley *et al.* 2013). For

germination studies, ten seeds were distributed per Petri dish (10 cm) lined with two layers of Whatman filter paper (No.1) and moistened with distilled water (8.0 ml). The Whatman filter paper was re-moistened with water as required. At the end of 15 days, germinated seeds were separated into shoot, root, and residual seeds. Shoot dry weight (SHW), root dry weight (RTW) and remaining seed dry weight (RSW) were recorded. Seed metabolic efficiency was calculated as per Rao and Sinha (1993).

$$SME = (SHW + RTW)/RESP$$

The amount of seed material respired (RESP) was calculated as:

$$RESP = SDW - (SHW + RTW + RSW)$$

The experiment was laid out as a split-plot design with three replications where storage durations were assigned as the main plot and seed priming treatments as the subplot (Singh *et al.* 1998). The data obtained for the trait SME were subjected to analysis of variance (ANOVA) to identify significant differences among treatments with CPCS1 statistical software. Treatment-wise and storage duration-wise comparison was done by Duncan's New Multiple Range Test (DMRT). A paired t-test was performed in data analysis to compare the means of SME with all biochemical indices. Pearson correlation coefficient was used to estimate the strength and direction of the linear relationship among variables using the "Metan version 1.18.0" package of R software. The degree of correlation is categorized as weak (0-0.3), moderate (0.3-0.7), and strong (0.7-1.0).

Results and Discussion

As per the analysis of variance using a split-plot design with storage durations as the main plot and priming treatments as the sub-plot. The results showed a significant variation when different storage durations were employed on the seed lots. However, priming treatments and interaction with storage duration showed insignificant outcomes on biochemical parameters (Table 1). The length of the storage period independently had a substantial impact on these parameters. This suggested that storage duration is a critical factor influencing the biochemical changes in seeds, rather than the priming treatments or their interaction with storage time. However, priming treatments showed statistically significant results for unstored seeds substantiated by post-hoc test (Figs 1-3). Further, the analysis performed by CRD for the seed metabolic efficiency (SME) trait showed significant results at a 0.05 significance level (Table 2). Analysis of germination data indicated that the 60% germination threshold was surpassed for seeds stored for 30 DAH and those primed with KNO_3 at 0 DAH. However, this barrier was naturally exceeded as storage time extended beyond 30 DAH. Given the coherence with previous germination pattern analyses already performed (Singh *et al*. 2023), it was logical to perform further biochemical estimations on seeds at 0 DAH and 60 DAH to better understand the underlying biochemical changes.

As 60% germination barrier was crossed when seeds were stored up to 60 DAH. Therefore, biochemical estimations were carried out for seed lot stored for 60 DAH versus freshly harvested seeds (0 DAH). Although the 30 DAH seed lot also surpassed the 60% germination threshold (Singh *et al*. 202**3**), the decision to focus on the 60 DAH seed lot was based on the more significant biochemical changes observed over a longer storage period. The extended storage time at 60 DAH provides a more comprehensive understanding of the metabolic adjustments and their impact on seed vigor compared to freshly harvested seeds (0 DAH). In the seeds stored for 60 DAH, when compared with freshly harvested seeds, there was a significant decline in starch content with a concomitant increase in the activity of α-amylase and total soluble sugar (TSS) content (Fig. 1). The priming treatments *viz.*, KNO_3 and GA_3 yielded significant results demonstrating a low starch content in primed seeds over the control which suggested that

Table 1. Split-plot analysis of variance for biochemical parameters.

Source of Variation	D.F.	TSS	Starch	α-Amylase	Proteins	Amino acids
Replication	2	0.091	0.037	0.0076	0.032	0.001
Treatments	55	0.079	0.031	0.0066	0.033	0.000
Factor A	1	4.037**	1.448**	0.0125**	1.682**	0.020**
Error(a)	53	0.141	0.204	0.0029	0.069	0.004
Factor B	27	0.011 ^{NS}	0.004 ^{NS}	0.0015 ^{NS}	0.003 ^{NS}	0.004 ^{NS}
A x B	27	0.002 ^{NS}	0.006 ^{NS}	0.0042 ^{NS}	0.003 ^{NS}	0.002 ^{NS}
Error(b)	2862	0.205	0.237	0.0022	0.102	0.004

** = Significant at $p < 0.01$, and ^{NS} = Non-significant

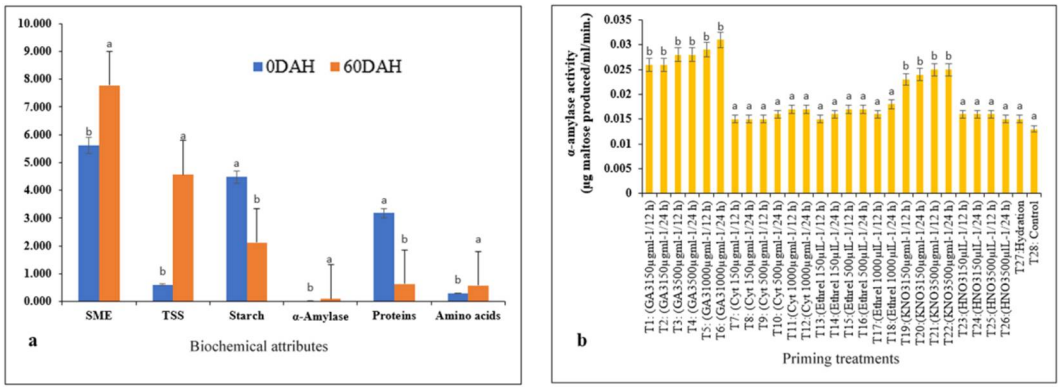

Fig. 1. Data analyzed for seed metabolic efficiency (SME) and biochemical attributes. (a). Effect of priming on α-amylase activity of freshly harvested seeds (b). Means that are followed by different letters are significantly different ($P \leq 0.05$) according to Duncan's New Multiple Range Test (DMRT).

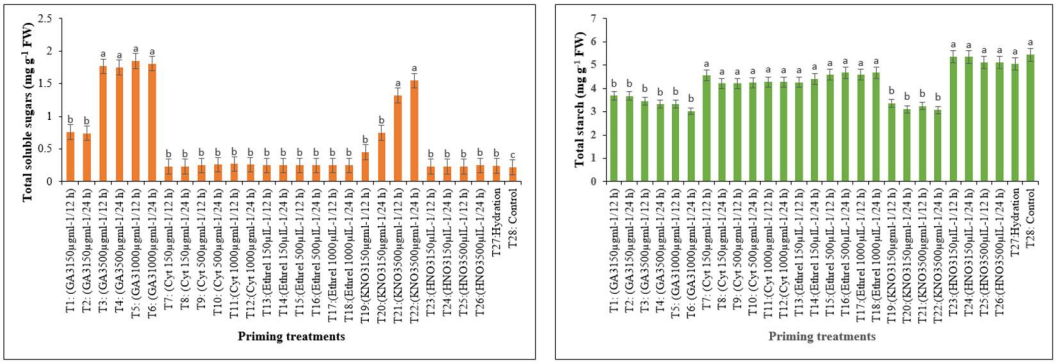

Fig. 2. Effect of priming on total soluble sugars (left-side) and total starch content (right-side) of freshly harvested seeds. Means that are followed by different letters are significantly different ($P \leq 0.05$) according to Duncan's New Multiple Range Test (DMRT).

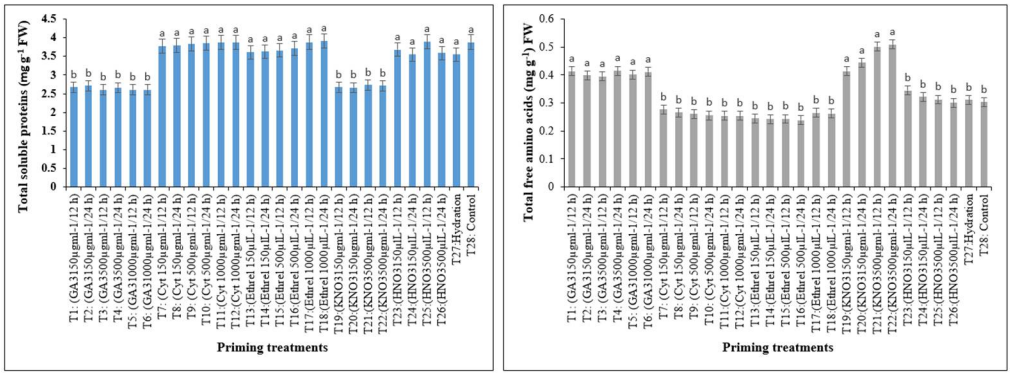

Fig. 3. Effect of priming on total soluble proteins (left-side) and total free amino acids (right-side) of freshly harvested seeds. Means that are followed by different letters are significantly different (P≤0.05) according to Duncan's New Multiple Range Test (DMRT).

these treatments were more effective in reducing the starch content of freshly harvested seeds compared to other treatments. Other treatments do not contribute to the breakdown of the starch reserves in freshly harvested seeds, allowing the seeds to maintain their nutrient reserves and dormancy status (Fig. 2). Likewise, KNO_3 and GA_3 primed seeds showed maximum activity of α-amylase and high content of TSS (Figs 1-2). The possible reason could be that priming stimulates initial synthesis and activation of hydrolytic enzymes such as alpha-amylase that produce the energy required for seedling germination, emergence, and growth through hydrolysis of seed food storage (Varier *et al.* 2010). Starch is a primary product of photosynthesis and an important storage carbohydrate.

It undergoes degradation by the hydrolytic enzymes, mainly α-amylase, releasing sugars along with amino acids which are essential for embryo growth (Aguirre *et al.* 2018). The main role of GA is the activation of genes encoding enzymes involved in seed germination, especially alpha-amylase enzyme by increasing the mRNAs encoding this enzyme (Gonzalez-Bento *et al.* 2004). The present findings are corroborated by studies on tomato (Ali *et al.* 2020) and chicory (Dehkordi *et al.* 2012) seeds when primed with KNO_3 showed enhanced total soluble sugar content and in rice (Watanabe *et al.* 2018) by application of GA_3.

The amount of total soluble protein decreased and total free amino acids increased in the stored seeds (60 DAH) as compared to 0 DAH seeds (Fig. 1) Among all the priming treatments, priming with GA_3 and KNO_3 led to a decrease in total soluble protein content and an increase in total free amino acids content over unprimed control in freshly harvested seeds (0 DAH) (Fig. 3). Similarly, in *Nigella sativa* seeds (Fallah *et al.* 2018), priming with KNO_3 enhanced soluble protein content. In addition to this, the KNO_3 priming also raised the rate and percentage of emergence and the seedling length. A plausible explanation could be that KNO_3 might be involved in influencing the permeability of the membranes which ultimately lead to the activation of enzymes involved in protein synthesis and carbohydrate metabolism (Cetinbas and Koyuncu 2006).

A paired samples test was conducted to determine if there was a significant difference between the two related groups. The paired samples test showed that the mean difference between the SME and TSS variables was 4.11. The t-value for the paired samples test was 12.38 and the p-value for the two-tailed test was 0.0005, indicating that this difference was highly significant ($p < 0.01$ level of significance). For SME and starch variables the mean value observed was 3.40. Based on the results, the t-statistic ($t = 7.3529$) was highly significant for these variables. Similar

Table 2. Effect of priming treatments on seed metabolic efficiency (SME) in freshly harvested (0 DAH), and stored seeds viz., 30 DAH and 60 DAH under lab conditions.

Seed Metabolic Efficiency (SME)						
Durations / Treatments	0 DAH D_0	15 DAH D_1	30 DAH D_2	45 DAH D_3	60 DAH D_4	Mean
T_1: ($GA_3$150 µg/ml/12 h)	7.43±0.22	07.58±0.15	07.58±0.15	08.90±0.22	08.96±0.15	8.25
T_2: ($GA_3$150 µg/ml/24 h)	7.49±0.32	07.98±0.33	07.98±0.33	08.87±0.25	09.08±0.11	8.47
T_3: ($GA_3$500 µg/ml/12 h)	7.25±0.33	07.27±0.14	07.27±0.14	08.82±0.33	09.11±0.32	8.12
T_4: ($GA_3$500 µg/ml/24 h)	8.44±0.14	08.48±0.27	08.48±0.27	08.90±0.41	09.63±0.27	8.87
T_5: ($GA_3$1000 µg/ml /12 h)	8.05±0.22	08.08±0.16	08.08±0.16	08.36±0.22	09.78±0.11	8.57
T_6: ($GA_3$1000 µg/ml/24 h)	8.32±0.34	08.39±0.11	08.39±0.11	08.50±0.28	06.04±0.12	7.83
T_7: (Cyt150 µg/ml/12 h)	3.60±0.44	03.61±0.16	03.61±0.16	04.85±0.16	06.17±0.27	4.56
T_8: (Cyt150 µg/ml/24 h)	2.66±0.46	02.67±0.18	02.67±0.18	04.95±0.25	07.00±0.14	4.32
T_9: (Cyt500 µg/ml/12 h)	3.50±0.31	03.51±0.15	03.51±0.15	05.55±0.33	07.18±0.25	4.94
T_{10}: (Cyt500 µg/ml/24 h)	2.69±0.25	02.70±0.37	02.70±0.37	05.45±0.28	07.42±0.22	4.57
T_{11}:(Cyt1000 µg/ml/12 h)	2.50±0.36	02.53±0.33	02.53±0.33	04.19±0.41	07.64±0.15	4.22
T_{12}:(Cyt1000 µg/ml/24 h)	3.16±0.28	03.25±0.11	03.71±0.11	04.29±0.55	04.66±0.34	3.74
T_{13}:(Ethrel 150 µl/l/12 h)	2.33±0.34	02.39±1.12	02.39±1.12	03.77±0.12	04.69±0.25	3.31
T_{14}:(Ethrel 150 µl/l/24 h)	2.12±0.26	02.15±0.22	02.15±0.22	03.41±0.12	05.35±0.19	3.27
T_{15}:(Ethrel 500 µl/l12 h)	2.31±0.28	02.38±0.41	02.38±0.41	02.94±0.33	05.39±0.31	3.27
T_{16}:(Ethrel 500 µl/l/24 h)	2.05±0.36	02.03±0.17	02.03±0.17	03.18±0.41	05.39±0.26	3.16
T_{17}:(Ethrel 1000 µl/l/12 h)	2.70±0.17	02.77±0.31	02.77±0.31	02.64±0.25	05.56±0.13	3.44
T_{18}:(Ethrel 1000 µl/l/24 h)	2.55±0.35	02.63±0.11	02.63±0.11	02.11±0.13	05.59±0.12	3.24
T_{19}:($KNO_3$150 µg/ml/12 h)	8.59±0.28	08.62±0.41	08.62±0.41	09.43±0.15	10.00±0.31	9.17
T_{20}:($KNO_3$150 µg/ml/24 h)	9.11±0.34	09.16±0.23	09.16±0.23	09.04±0.24	10.55±0.12	9.48
T_{21}:($KNO_3$500 µg/ml/12 h)	9.10±0.56	09.07±0.22	09.07±0.22	09.29±0.51	10.72±0.22	9.54
T_{22}:($KNO_3$500 µg/ml/24 h)	9.34±0.26	09.38±0.16	09.38±0.16	09.39±0.12	10.79±0.24	9.74
T_{23}:($HNO_3$150 µll/12 h)	8.37±0.35	08.46±0.13	08.46±0.13	08.96±0.33	10.08±0.11	8.99
T_{24}:($HNO_3$150 µll/24 h)	8.22±0.27	08.27±0.12	08.27±0.12	09.07±0.13	10.17±0.11	8.95
T_{25}:($HNO_3$500 µll /12 h)	8.43±0.25	08.46±0.42	08.66±0.42	08.78±0.24	09.56±0.22	8.87
T_{26}:($HNO_3$500 µl1 /24 h)	8.51±0.41	08.55±0.44	08.55±0.44	08.93±0.19	09.97±0.41	9.00
T_{27}:Hydration	6.24±0.35	06.28±0.32	06.28±0.32	08.23±0.21	07.71±0.26	7.13
T_{28}: Control	2.23±0.26	02.41±0.18	02.41±0.18	02.15±0.19	03.74±0.15	2.68
Mean	5.62	5.67	5.68	6.53	7.78	
CD at 5% (D)	0.127					
CD at 5% (T)	0.242					
CD at 5% (DXT)	0.483					

Data presented as means ± standard deviations, where D indicates Days and T indicates Treatment whereas D × T indicates interaction between the variables

results were obtained for the pairs *viz.*, SME-protein (t = 9.7027) and SME-amino acids (t = 17.4643). The paired samples test results demonstrate significant differences between SME and other biochemical parameters. The high t-values and extremely low p-values ($p < 0.01$) across all

pairs indicate that these differences are highly significant. This suggested that the biochemical changes observed between the different parameters are substantial due to specific treatment effects i.e., priming and duration conditions rather than random chance. Also, it reflected that metabolic reserves had a significant relationship with the metabolic efficiency of the seed investigated under dormant (immediately after harvesting i.e., 0 DAH) and non-dormant conditions (60 DAH).

Table 3. Paired samples test (t-statistic) on biochemical parameters of round gourd seeds.

Pair	Pair Member	Pair differences			t-value	d.f.	p-value
		Mean	Std. deviation	Std. error mean			
1.	SME - TSS	4.11	2.49	0.33	12.38	55	0.0005*
2.	SME - Starch	3.40	3.46	0.46	7.35	55	0.0005*
3.	SME - α-amylase	6.66	2.76	0.37	18.05	55	0.0004*
4.	SME - Proteins	4.79	3.70	0.49	9.70	55	0.0003*
5.	SME – Amino acids	6.27	2.69	0.36	17.46	55	0.0003*

*Result is significant at 0.01 level (2-tailed).

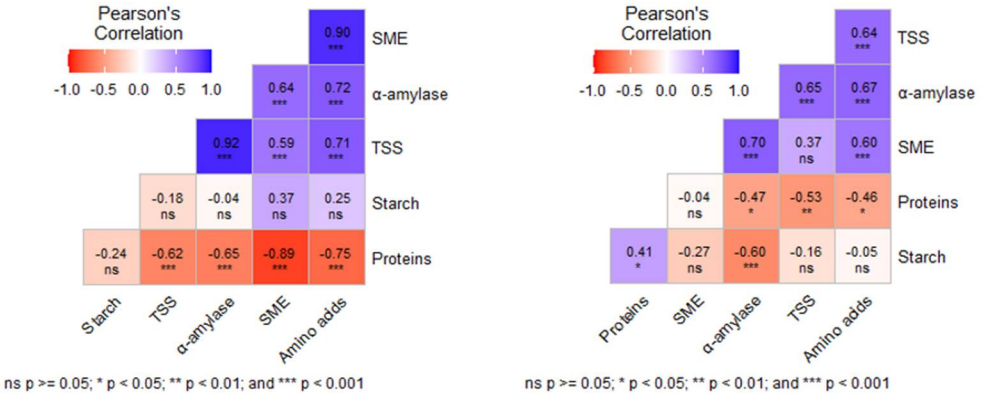

Fig. 4. Correlation heat-maps: Pearson correlation coefficient analysis among various biochemical indices and seed metabolic efficiency (SME) under different priming treatments × durations *viz.*, 0 DAH (left-side) and 60 DAH (right-side).

Further, the seeds stored for 60 days after harvest showed a higher value of seed metabolic efficiency (SME) than the freshly harvested seeds and 30 DAH stored seeds (Table 1). The priming treatments enhance SME and hasten the germination process. However, the priming with 500 µgml^{-1} KNO_3 for 12 hrs and 24 hrs (T_{21} and T_{22}, respectively) treatment gave the maximum value of seed metabolic efficiency over all other treatments. The seed metabolic efficiency was negatively correlated with total starch and total soluble protein content whereas exhibited a significant positive correlation with total soluble sugar content, total free amino acids, and α-amylase activity (Fig. 4). The correlation analysis suggested that seeds with higher metabolic efficiency are better at mobilizing and utilizing their stored biochemical reserves. Efficient starch metabolism (negative correlation with starch) results in higher levels of soluble sugars (positive

correlation) due to active conversion by enzymes like α-amylase (positive correlation). Likewise, efficient protein metabolism (negative correlation with soluble proteins) results in higher levels of free amino acids (positive correlation), reflecting active protein turnover and synthesis. This balance of efficient resource mobilization and utilization is crucial for the successful germination and early growth of seeds (Ali and Elozeiri 2017, Fu *et al.* 2024).

With appropriate priming treatments, it is possible to improve germination percentage, seed metabolic efficiency, and biochemical attributes of freshly harvested seed lots. Among all the priming treatments, priming with KNO_3 @ 500 µg/ml for 12 hrs and 24 hrs (T_{21} and T_{22}, respectively) was the only treatment that gave maximum seed metabolic efficiency. Priming in GA_3 and KNO_3 showed significant improvement in the biochemical parameters which was manifested in better seed metabolic efficiency. Future research could explore specific priming protocols tailored to different seed types and environmental conditions, aiming to maximize biochemical attributes that promote robust seedling establishment and overall crop productivity.

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