

## Priming impacts the germination and biochemical parameters of seeds in bitter gourd (*Momordica charantia* L.)

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### Abstract

Seeds of bitter gourd variety Preethi stored under ambient environment were subjected to various priming treatments after six months of storage aiming to improve germination. The eleven priming treatments employed were T<sub>1</sub>: KNO<sub>3</sub> 0.3 % for 2 h, T<sub>2</sub>: KNO<sub>3</sub> 0.015 % for 3 h, T<sub>3</sub>: KH<sub>2</sub>PO<sub>4</sub> 0.01 M for 24 h, T<sub>4</sub>: PEG 6000 -1.5 MPa for 24 h, T<sub>5</sub>: GA<sub>3</sub> 100 ppm for 24 h, T<sub>6</sub>: Solid matrix priming with Perlite for 48 h, T<sub>7</sub>: Solid matrix priming with Cocopeat for 48 h, T<sub>8</sub>: *Pseudomonas fluorescens* 1x10<sup>6</sup> cfu.ml<sup>-1</sup> for 24 h, T<sub>9</sub>: Hydration hot water at 50° C for 4 h, T<sub>10</sub>: Hydration with cold water at 28° C for 24 h and T<sub>11</sub>: Absolute control. The results indicated that solid matrix priming with perlite for 48 hours significantly enhanced germination parameters and seed biochemical indices in bitter gourd (*Momordica charantia*) seeds. Solid matrix priming with cocopeat for 48 hours and seed treatment with *Pseudomonas fluorescens* (1x10<sup>6</sup> cfu.ml<sup>-1</sup>) were also effective, though to a slightly lesser extent in a few instances than perlite-based priming. Osmopriming with PEG 6000 (-1.5 MPa for 24 h) was found to be detrimental and found to be inferior to untreated control.

**Keywords:** Cocopeat, Osmopriming, Perlite, Priming, *Pseudomonas fluorescens*

### Introduction

Bitter gourd (*Momordica charantia* L.) is a commonly cultivated vegetable known for its edible fruit and medicinal benefits. Although seeds are widely used for propagation, they have a shorter lifespan compared to other orthodox seeds (Peter et al., 1998). Cryo-storage at -196°C and cold storage are effective *ex-situ* conservation methods to ensure long-term seed storage. Despite being classified as an orthodox seed, the bitter gourd seed is sensitive to low temperatures. Research indicates significant declines in germination when the seeds are stored at low or sub-zero temperatures for more than six months (Sharmila et al., 2019). Furthermore, the thick seed coat surrounding the embryo affects germination by creating mechanical barriers that restrict the growth of the embryo.

Several methods to address the issue of poor or slow seed germination have been advocated, and one of them is seed priming (Pandita and Nagarajan, 2004). In gourds, seeds

subjected to priming with PEG 6000 -1.5 MPa (Shukla et al., 2018), 150 ppm KNO<sub>3</sub> *i.e.*, 0.015 per cent (KAU, 2016), KH<sub>2</sub>PO<sub>4</sub> 10<sup>-2</sup> M (Kaur et al., 2019), solid matrix priming with perlite and cocopeat (Kanwar and Mehta, 2017), hormonal priming with 100 ppm GA<sub>3</sub> (Kumar and Singh, 2013) and plant growth regulators, have been reported to foster high vigour resulting in improved crop growth and increased yield.

During the priming, seeds are partially hydrated leading to initiation of pre-germinative metabolic processes, ensuring that radical emergence is avoided (Pill, 1986). Primed seeds have been found to withstand biotic and abiotic stressors better (Sivasubramaniam et al., 2011). Priming helps minimize oxidative damage caused by sub-optimal temperatures and improves seed performance in various crop species (Chen and Sung, 2001).

Enzyme activation, seed coat softening, and seed dormancy break are all advantages of priming. Priming also helps in reducing the germination time (Nawaz et al., 2013). The

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repair mechanisms triggered by priming treatments could involve several physiological processes, such as enhanced protein and RNA synthesis (Khan et al., 1992), embryo development (Hegarty, 1970) and DNA replication (Jain and van Staden, 2007).

Based on the above-mentioned factors, the study aimed to assess the germination rate and biochemical parameters, such as total soluble sugar, total protein, total dehydrogenase activity, amylase activity, and catalase activity, of bitter gourd seeds subjected to different priming treatments after six months of storage under ambient conditions. The specific goal of the study was to delineate the most effective seed priming method to improve both germination and biochemical characteristics of bitter gourd seeds.

## Materials and Methods

The study was carried out in the laboratory of the Department of Seed Science and Technology at the College of Agriculture, Kerala Agricultural University, Vellanikkara, Thrissur, Kerala. It was designed as a Completely Randomized Design with three replications and eleven priming treatments. Seeds of the high-yielding bitter gourd variety Preethi were sourced within two weeks after harvest and dried to 6.84 per cent moisture content. Dry seeds of bitter gourd variety Preethi were packed in moisture proof bags (700-gauge polyethylene bags), sealed airtight, and

formulation of *P. fluorescens* ( $1 \times 10^6$  cfu.ml<sup>-1</sup>) was used @ 2.5 ml/kg seed. During hydropriming with hot water (T<sub>9</sub>), seeds were soaked in hot water maintained at  $50 \pm 1^\circ\text{C}$  for 4 h with the help of a water bath (Grant, LSB12), while during hydration with cold water (T<sub>10</sub>), the seeds were soaked in cold water (28°C) for 24 h. After the designated priming period in each case, the excess solution was poured off, and the seeds were placed on two layers of fresh blotting paper to absorb the remaining moisture. The seeds were then transferred to fresh blotter paper and shade-dried for two weeks.

For solid matrix priming with perlite (T<sub>6</sub>), two parts of perlite with a neutral pH, a specific gravity ranging from 2.2 to 2.4, and a moisture content of less than 1.0 per cent was carefully mixed with one part of water on weight-by-weight basis. The seeds were kept as a layer in between two layers of moist perlite in a tray and watered daily while ensuring drainage of excess water. The seeds were then removed, spread on two layers of fresh blotting paper and shade-dried for a period of two weeks. Similarly, in case of solid matrix priming with cocopeat (T<sub>7</sub>), two parts of cocopeat with a pH of 5.0, electrical conductivity of 0.6 dSm<sup>-1</sup>, bulk density of 0.11 and moisture content less than 18 per cent was combined with one part of water on weight-by-weight basis and used to prime the seeds as in solid matrix priming with perlite (T<sub>6</sub>).

Germination and biochemical parameters of the primed seeds were assessed before and after priming following standard procedures. Germination (%) was assessed in sand medium (ISTA, 2010). Four replicates of 100 seeds were germinated from each treatment, in trays containing fine sand (sieve size 0.80 mm) maintained in a germination room ( $25 \pm 2^\circ\text{C}$  and RH  $90 \pm 3$  per cent). The sand was moistened to 50 per cent of its water holding capacity. The seeds were planted on the levelled layer of moist sand at a depth of 2.50 to 3.00 cm and covered with 10-20 mm of uncompressed sand. Germination was recorded on the 4<sup>th</sup> and 14<sup>th</sup> days of the test. Germination (%) was calculated using the following formula:

$$\text{Germination (\%)} = \frac{\text{(No. of germinated normal seedlings / Total seeds)} \times 100}{1}$$

In order to assess the biochemical parameters, two replicates from each three replications of a priming treatment were used. Total soluble sugar (%) was determined through anthrone method (Trevelyan and Harrison, 1952). Total protein content (%) was calculated by the method developed by Lowry et al. (1951). The activity of the dehydrogenase enzyme was measured using the procedure described by Kittock and Law (1968) and represented as an OD value.  $\alpha$ -Amylase activity ( $\mu\text{mol min}^{-1}$ ) was quantified using the

Table 1. Details of priming treatments

Sl. No.	Treatment	Dosage	Solute per litre distilled water	Duration (h)
T <sub>1</sub>	KNO <sub>3</sub>	0.3 %	3 g	2
T <sub>2</sub>	KNO <sub>3</sub>	0.015 %	0.15 g	3
T <sub>3</sub>	KH <sub>2</sub> PO <sub>4</sub>	0.01 M	1.36 g	24
T <sub>4</sub>	PEG 6000	-1.5 MPa	367.67 g	24
T <sub>5</sub>	GA <sub>3</sub>	100 ppm	10 mg	24
T <sub>6</sub>	Solid matrix priming with Perlite			48
T <sub>7</sub>	Solid matrix priming with Cocopeat			48
T <sub>8</sub>	<i>Pseudomonas fluorescens</i> ( $1 \times 10^6$ cfu.ml <sup>-1</sup> )	2.5 ml/kg seed		24
T <sub>9</sub>	Hydration (hot water)	50°C		4
T <sub>10</sub>	Hydration (cold water)	28°C		24
T <sub>11</sub>	Absolute control	Untreated		

stored for a period of six month from 20 Nov, 2021 to 21 May, 2022. Following storage, the seeds were subjected to priming treatments as detailed in Table 1.

The required dosage of priming solutions of KNO<sub>3</sub> (T<sub>1</sub> and T<sub>2</sub>), KH<sub>2</sub>PO<sub>4</sub> (T<sub>3</sub>), PEG 6000 (T<sub>4</sub>) and GA<sub>3</sub> (T<sub>5</sub>) were prepared by dissolving the appropriate quantity of the chemical in distilled water. In order to bio-prime the seeds (T<sub>8</sub>), liquid

dinitrosalicylic acid (DNS) reagent method as described by Fossum and Whitaker (1974). Catalase activity ( $\mu\text{mol min}^{-1} \text{g}^{-1}$ ) was determined by measuring the absorbance of  $\text{H}_2\text{O}_2$  at 240 nm in the UV range (Aebi, 1984).

Statistical analysis of the data on various seed quality parameters was performed using GRAPES 1.0.0 (Gopinath et al., 2020) developed by Department of Agricultural Statistics, College of Agriculture, Kerala Agricultural University, Vellayani.

## Results and Discussion

Germination per cent of seed lot recorded before priming treatments (Table 2) was 80.71 per cent. The total soluble sugar and total protein content in the seeds prior to priming were 5.78 and 6.22 per cent respectively. The estimated total dehydrogenase activity in the seed before priming was 0.291 OD, while the activity of enzymes  $\alpha$ -Amylase and catalase activity in seeds before priming was  $7.81 \mu\text{mol min}^{-1}$  and  $22.39 \mu\text{mol min}^{-1} \text{g}^{-1}$  respectively.

It was evident that the priming treatments significantly influenced germination (%) and other biochemical indices of seeds of bitter gourd.  $T_6$  (Perlite for 48 h) with 95.08 per cent registered the highest germination, while germination in  $T_4$  (PEG 6000 -1.5 MPa for 24 h: 59.47 %) was the least. Germination in all treatments, except  $T_4$ ,  $T_2$  ( $\text{KNO}_3$  0.015 % for 3 h: 88.38 %),  $T_{11}$  (Absolute control: 80.38 %) and  $T_3$  ( $\text{KH}_2\text{PO}_4$  0.01 M for 24 h: 89.49 %) were on par with treatment  $T_6$ . Improved germination was observed in nine out of ten priming treatments and compared to the untreated seeds, while it tended to decrease by 26.32 per cent on osmopriming with PEG 6000 ( $T_4$ ). Conversely, it became

evident that priming with PEG 6000 -1.5 MPa for 24 h was detrimental in bitter gourd. Solid matrix priming with perlite or cocopeat, biopriming with *P. fluorescens*, hormonal priming, hydropriming with either hot water or cold water resulted in significant enhancement of germination over untreated seeds. Several reports point out that during early phases of germination, seed priming promotes the production of enzymes that are necessary for quick radicle protrusion and hypocotyl elongation which resulted quick germination (Bensen et al., 1990; Maske et al., 1997).

Significantly higher total soluble sugar was observed in the solid matrix priming treatment  $T_6$  (Perlite for 48 h: 7.70 %) followed by  $T_8$  (*P. fluorescens*  $1 \times 10^6$  cfu.ml $^{-1}$  for 24 h: 6.85 %),  $T_7$  (Cocopeat for 48 h: 6.67 %) and  $T_{10}$  (Hydration with cold water for 24 h: 6.58 %). It was evident that the treatments other than  $T_6$ ,  $T_8$  and  $T_7$  were significantly inferior in total soluble sugar content. Solid matrix priming with Perlite ( $T_6$ - Perlite for 48 h: 7.70 %) and bio-priming ( $T_8$ : *P. fluorescens*  $1 \times 10^6$  cfu.ml $^{-1}$  for 24 h: 7.58 %) resulted in significantly higher total protein content of seeds followed by  $T_7$  (Cocopeat for 48 h: 7.37 %) and  $T_5$  ( $\text{GA}_3$  100 ppm for 24 h: 7.25%).  $T_4$  (PEG 6000 -1.5 MPa for 24 h: 6.27 %) had registered the least total protein content in seeds and was found to be on par with  $T_2$  ( $\text{KNO}_3$  0.015 % for 3 h: 6.62 %),  $T_3$  ( $\text{KH}_2\text{PO}_4$  0.01M for 24 h: 6.28%),  $T_9$  (Hydration with hot water at 50°C for 4 h: 6.46 %) and  $T_{10}$  (Hydration with cold water for 24 h: 6.41 %).

The dehydrogenase activity in the seed lots primed was found to be significantly superior in treatments  $T_6$  (Perlite for 48 h: 0.409 OD) and  $T_7$  (Cocopeat for 48 h: 0.379 OD) and  $T_8$  (*P. fluorescens*  $1 \times 10^6$  cfu.ml $^{-1}$  for 24 h: 0.364 OD). Meanwhile, the enzyme activity in all the other treatments

Table 2. Variation in germination and biochemical parameters on priming in bitter gourd

Status of priming	Treatment	Germination (%)	Total soluble sugar (%)	Total protein (%)	Total dehydrogenase (OD)	$\alpha$ -Amylase activity ( $\mu\text{mol min}^{-1}$ )	Catalase activity ( $\mu\text{mol min}^{-1} \text{g}^{-1}$ )
Before priming		80.71	5.78	6.22	0.291	7.81	22.39
After priming		*Per cent change	*Per cent change	*Per cent change	*Per cent change	*Per cent change	*Per cent change
	$T_1$	91.16 <sup>abc</sup>	6.02 <sup>c</sup>	7.04 <sup>cd</sup>	0.327 <sup>c</sup>	8.47 <sup>abcd</sup>	23.53 <sup>bc</sup>
	$T_2$	88.38 <sup>cd</sup>	5.85 <sup>cd</sup>	6.62 <sup>dc</sup>	0.282 <sup>bc</sup>	7.67 <sup>dc</sup>	19.73 <sup>f</sup>
	$T_3$	89.49 <sup>bcd</sup>	5.69 <sup>cd</sup>	6.28 <sup>c</sup>	0.263 <sup>c</sup>	8.21 <sup>abcd</sup>	20.57 <sup>ef</sup>
	$T_4$	59.47 <sup>e</sup>	5.84 <sup>cd</sup>	6.27 <sup>c</sup>	0.274 <sup>c</sup>	6.15 <sup>f</sup>	19.49 <sup>f</sup>
	$T_5$	93.93 <sup>ab</sup>	5.94 <sup>cd</sup>	7.25 <sup>bc</sup>	0.246 <sup>c</sup>	9.21 <sup>a</sup>	25.28 <sup>a</sup>
	$T_6$	95.08 <sup>a</sup>	7.70 <sup>a</sup>	7.88 <sup>a</sup>	0.409 <sup>a</sup>	8.73 <sup>abc</sup>	25.13 <sup>a</sup>
	$T_7$	94.75 <sup>a</sup>	6.67 <sup>b</sup>	7.37 <sup>bc</sup>	0.379 <sup>a</sup>	8.30 <sup>abcd</sup>	24.32 <sup>ab</sup>
	$T_8$	95.05 <sup>a</sup>	6.85 <sup>b</sup>	7.58 <sup>ab</sup>	0.364 <sup>ab</sup>	8.86 <sup>ab</sup>	25.06 <sup>a</sup>
	$T_9$	90.60 <sup>abc</sup>	5.82 <sup>cd</sup>	6.46 <sup>c</sup>	0.279 <sup>c</sup>	7.11 <sup>ef</sup>	20.03 <sup>f</sup>
	$T_{10}$	91.16 <sup>abc</sup>	6.58 <sup>b</sup>	6.41 <sup>c</sup>	0.274 <sup>c</sup>	7.94 <sup>bcd</sup>	21.62 <sup>dc</sup>
	$T_{11}$	80.38 <sup>d</sup>	5.67 <sup>cd</sup>	6.29 <sup>c</sup>	0.290 <sup>bc</sup>	7.82 <sup>cdc</sup>	22.23 <sup>cd</sup>
	CD (0.05)	4.896	0.461	0.472	0.083	1.003	1.387

$T_1$ :  $\text{KNO}_3$  (0.3% for 2 h)

$T_2$ :  $\text{KNO}_3$  (0.015 % for 3 h)

$T_3$ :  $\text{KH}_2\text{PO}_4$  (0.01 M for 24 h)

$T_4$ : PEG 6000 (-1.5 MPa for 24 h)

$T_5$ :  $\text{GA}_3$  (100 ppm for 24 h)

$T_6$ : Solid matrix priming with Perlite (for 48 h)

$T_7$ : Solid matrix priming with cocopeat (for 48 h)

$T_8$ : *Pseudomonas fluorescens* ( $1 \times 10^6$  cfu.ml $^{-1}$  for 24 h)

$T_9$ : Hydration (hot water at 50°C for 4 h)

$T_{10}$ : Hydration (cold water soaking for 24 h)

$T_{11}$ : Absolute control

\*Per cent change: Per cent change in parameter after priming vs before priming

was significantly lower. The highest  $\alpha$ -amylase activity was observed in the treatment T<sub>5</sub> (GA<sub>3</sub> 100 ppm for 24 h: 9.21  $\mu\text{mol min}^{-1}$ ) followed by T<sub>8</sub> (*P. fluorescens* 1x10<sup>6</sup> cfu.ml<sup>-1</sup> for 24 h; 8.86  $\mu\text{mol min}^{-1}$ ), T<sub>6</sub> (Perlite for 48 h: 8.73  $\mu\text{mol min}^{-1}$ ) and T<sub>7</sub> (Cocopeat for 48 h: 8.30  $\mu\text{mol min}^{-1}$ ) and T<sub>3</sub> (KH<sub>2</sub>PO<sub>4</sub> 0.01 M for 24 h: 8.21  $\mu\text{mol min}^{-1}$ ). The activity of  $\alpha$ -Amylase enzyme was found to be significantly higher in these treatments. Significantly high activity of enzyme catalase was observed in the treatments T<sub>5</sub> (GA<sub>3</sub> 100 ppm for 24 h: 25.28  $\mu\text{mol min}^{-1} \text{g}^{-1}$ ), T<sub>6</sub> (Perlite for 48 h: 25.13  $\mu\text{mol min}^{-1} \text{g}^{-1}$ ), T<sub>8</sub> (*P. fluorescens* 1x10<sup>6</sup> cfu.ml<sup>-1</sup> for 24 h; 25.06  $\mu\text{mol min}^{-1} \text{g}^{-1}$ ) and T<sub>7</sub> (Cocopeat for 48 h: 24.32  $\mu\text{mol min}^{-1} \text{g}^{-1}$ ), while the activity of the enzyme was found to be significantly lower in other treatments.

The results indicated that seeds subjected to solid matrix priming with perlite (T<sub>6</sub>) or cocopeat (T<sub>7</sub>), as well as biopriming with *Pseudomonas fluorescens* (T<sub>8</sub>), exhibited significantly higher germination percentages, total soluble sugar and protein contents, and enzymatic activities of total dehydrogenase,  $\alpha$ -amylase, and catalase, compared to untreated seeds. The percentage increases in these parameters were also of a higher magnitude in the aforementioned priming treatments (T<sub>6</sub>, T<sub>7</sub>, and T<sub>8</sub>), whereas the improvements observed in treatments T<sub>4</sub> (PEG 6000 at -1.5 MPa for 24 h), T<sub>2</sub> (KNO<sub>3</sub> at 0.015 % for 3 h), and T<sub>3</sub> (KH<sub>2</sub>PO<sub>4</sub> at 0.01 M for 24 h) were extremely low.

Amylases are essential enzymes involved in breaking down the starch reserve in seeds, providing sugars to support the developing embryo. On the other hand, dehydrogenases, which belong to the oxidoreductase family, catalyses the removal of hydrogen from the substrate being oxidized. They affect a large number of processes, including seed germination. The activities of total dehydrogenase and malate dehydrogenase were higher in primed seed compared to the non-primed control (Pandita et al., 2010). Basra et al. (2005) suggested that increased  $\alpha$ -amylase activity in primed seeds leads to faster starch breakdown, quicker embryo growth, and more synchronized germination. It provides energy to the sprouting radicle of primed seeds. As a result, primed seeds germinated and grew faster than unprimed seeds (Afzal et al., 2006).

Seed priming induces metabolic changes in seeds before germination by regulating hydration but ensures that the moisture level does not induce radicle emergence. Enzymes such as dehydrogenases, peroxidase, and catalases play a crucial role in seedling emergence and germination. Following priming treatments, the activities of these enzymes were reported to be significantly higher compared to controls (Saha et al., 2022). Kibinza et al. (2011) noted that priming

enhanced catalase production, which aids in seed recovery during priming. This boost in enzyme activity may be linked to increased respiration, energy production, and nutrient delivery to the developing seeds. Similar findings of enhanced respiration, energy generation, and nutrient supply to germinating seeds were reported by Verma et al. (2003) in mustard and Pallavi et al. (2003) in sunflower.

Based on the overall results, solid matrix priming of bitter gourd seeds with perlite for 48 hours resulted in a significantly high enhancement of germination and biochemical parameters compared to untreated seeds. Solid matrix priming with cocopeat (48 h) and biopriming with *Pseudomonas fluorescens* (1x10<sup>6</sup> cfu.ml<sup>-1</sup> for 24 h) were very effective, though slightly less so than perlite - based priming.

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