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Chemical seed priming for enhancing the germination in soybean (*Glycine max* L.) under osmotic stress

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Abstract
Abiotic stress particularly drought, significantly hampers soybean productivity by affecting seed germination and early seedling development. Seed priming with chemical agents offers a potential strategy to enhance stress tolerance during germination. This study examines the effectiveness of chemical and hydropriming seed priming treatments in improving germination and stress tolerance in soybean (*Glycine max* L.) variety MAUS-158 under osmotic stress induced by polyethylene glycol (PEG-6000). The seed priming agents tested were calcium chloride ($CaCl_2$), chitosan and mannitol. The optimal concentration of PEG-6000 for inducing osmotic stress was determined to be 15%, resulting in a 50% inhibition of germination. Priming durations and concentrations of selected chemicals were optimized based on germination percentage and seedling vigor. The 1% $CaCl_2$ and 6% mannitol with 6 hour priming durations, and 0.2% chitosan with a 1 hour priming duration were found to be the most effective for enhancing the germination percentage from 70±5.7 to 80±5.7 under normal or osmotic stress conditions. Alone hydropriming for 6 hours duration also showed significant improvement in germination percentage under osmotic stress tolerance. The chemical seed priming unravelled that priming enhanced seed performance under both normal and osmotic stress condition.

Keywords: Seed priming, hydropriming, osmotic stress, polyethylene glycol

1. Introduction

The soybean, a legume crop from the Leguminosae family, is native to East Asia and is often referred to as the 'Wonder Crop' and the 'Gold of the Century'. Soybean, an oilseed crops rich in essential amino acids, minerals, and vitamins, making a significant source of dietary protein. It contain 40% protein, 30% carbohydrates, 20% oil, 9% water, and 5% ash content (Anonymous, 2005) [2]. Soybean was introduced to India from China in the tenth century AD through the Himalayan routes and by traders from Indonesia via Burma (now Myanmar). Currently, India ranks fifth globally in soybean production, following the USA, Brazil, Argentina, and China (FAO, 2023) [7]. Soybean (*Glycine max* L. Merril) is a crucial seed legume, contributing to 25% of the world's edible oil and two-thirds of the world's protein concentrate used in livestock feed (Hartman *et al.*, 2011) [8]. Soybean meal is a valuable ingredient in poultry and fish feeds due to its high protein and amino acid content (Kumar *et al.*, 2022) [12].

India accounts for 10% of the global soybean area but only 4% of global production, with low productivity of 1.1 t/ha compared to the global average of 2.2 t/ha (FAO, 2022) [6]. Major soybean-producing states in India are Madhya Pradesh, Maharashtra, and Rajasthan, contributing over 90% of the national output. In the 2024-25 season, India is expected to produce 12.58 million metric tons of soybeans, with Maharashtra alone contributing around 7.33 million metric tons due to favorable rainfall and improved agronomic practices. (Data from ICAR-IISR, SOPA & Investing.com, 2021-2024) [9, 21, 10]. Soybean cultivation faces challenges such as declining market prices, leading farmers to switch to more profitable crops like corn and sugarcane, raising concerns about the sustainability of soybean farming in the regions (Reuters, 2024) [17]. Additionally, soybean productivity is affected by biotic and abiotic stresses, with drought stress being a significant factor causing up to 40% loss in productivity globally (Fahad *et al.*, 2017) [5]. Plants employ various mechanisms to cope with stress, including producing osmolytes or osmoprotectants as compatible solutes to combat osmotic stresses caused by drought (Alam *et al.*, 2020) [1].

Germination and seedling emergence are crucial stages in the plant life cycle. Inadequate seedling emergence and improper stand establishment are major constraints in crop production in regions with low rainfall. Farmers often lack resources for proper seedbed preparation, putting them at a higher risk compared to progressive farmers. Good establishment enhances competitiveness against weeds, improves drought tolerance, increases yield, and eliminates the need for costly re-sowing. Priming is a widely accepted method to improve germination, reduce emergence time, and enhance stand establishment. Seed priming involves partially hydrating seeds to initiate germination processes, leading to rapid germination under normal or stress conditions. Research on seed priming highlights its importance in achieving a successful crop stand (Singh *et al.*, 2015) [20].

Seed priming is a simple, cost-effective, and sustainable method that can be easily implemented in the field (Lal, 2018) [13]. Seed priming is the pre-sowing partial hydration of seeds without allowing radicle emergence to improve germination rate and stress tolerance of germinating seeds, and even to improve seedling establishment. Various priming methods, including hydro-priming, osmo-priming, chemical priming, hormonal priming, biological priming, redox priming, and solid matrix priming, have been reported to improve seed germination under osmotic stress and promote drought tolerance in seedlings (Li and Liu, 2016) [14]. Priming is significantly influenced by factors like temperature, aeration, light exposure, duration of priming, and seed traits (Waqas *et al.*, 2019) [22].

There are numerous reports on the effects of seed priming on various plants. For example, a study found that priming soybean seeds led to improved seedling emergence and increased yield (Arif *et al.*, 2008) [3]. Crop plants are exposed to various abiotic stresses throughout their growth cycle, which significantly decrease productivity and pose a threat to global food security. Recent studies indicate that plants can be preconditioned with chemical compounds to enhance their tolerance to different abiotic stresses. Chemical priming is an emerging area in plant stress physiology and crop stress management (Savvides *et al.*, 2016) [18].

2. Material and Methods

2.1 Plant Material

Seeds of soybean variety MAUS-158 collected from Soybean Research Station, Vasantrao Naik Marathwada Krishi Vidyapeeth, Parbhani.

2.2 Chemicals

All chemicals used in this investigation were of analytical grade and molecular grade procured from standard manufacturer like Himedia, Sigma Aldrich, SRL Mumbai, Merck, Loba Chemmie Pvt. Ltd., Mumbai.

2.3 Methods

2.3.1 Optimization of Time and Chemical Concentration

The priming parameters i.e. time and duration for enhancing osmotic stress tolerance in soybean (*Glycine max* L.) variety MAUS-158 were optimised by selecting healthy and uniform-sized seeds. The seeds were surface sterilized using 1% sodium hypochlorite for five minutes then rinsed with distilled water three times and treated with 0.5% Bavistin for five minutes. The osmotic stress was induced using PEG-6000. The concentration of PEG was optimised using 5%, 10%, and 15% PEG-6000 solutions incubated in Petri plates with moistened Whatman filter paper in triplicates. The PEG concentration i.e. 15% causing approximately 50% reduction in germination percentage compared to control was chosen for stress imposition in subsequent trials. To determine the optimal hydropriming duration, seeds were soaked in distilled water for different durations (30 minutes, 60 minutes, 90 minutes, 2 hours, 4 hours, and 6 hours) under dark conditions. They were then rinsed, dried on tissue paper, and dehydrated under shade to regain their original moisture content, confirmed by monitoring seed weight. For chemical priming, seeds were soaked in various concentrations of $CaCl_2$ (0.5%, 1%, 1.5%, 2%, and 3%), Mannitol (2%, 4%, 6%, and 8%), and Chitosan (0.1%, 0.2%, and 0.25%) for specific durations. After priming, both primed and non-primed seeds were sown in Petri plates under two conditions: non-stress and with osmotic stress (15% PEG concentration) to evaluate the effects of different priming methods on germination and early growth under abiotic stress.

2.4 Statistical analysis

Microsoft Excel 2013 was used for data processing and tabulation, XLSTAT 2021 software was used to for one-way analysis of variance (ANOVA) by the online statistical analysis tools (OPSTAT).

3. Results and Discussion

Soybean (*Glycine max* L.) variety MAUS-158 was selected for this study to evaluate the effects of seed priming with $CaCl_2$, Chitosan, and Mannitol under PEG 6000-induced osmotic stress. A 15% PEG-6000 solution (-3 bar), which reduced germination by approximately 50%, was used to simulate stress. Priming durations and concentrations were chosen based on earlier studies (Jadhav *et al.*, 2017 [11]; Mangena, 2020 [15]; Sheteiwy *et al.*, 2018) [19]. A 6-hour priming duration was optimal for soybean (Miladinov *et al.*, 2020) [16], except for Chitosan, where 6 hours inhibited germination; thus, a 1-hour treatment was used (Costales *et al.*, 2020) [4].

3.1 Optimization effect of PEG concentration, priming duration and concentration of chemical ($CaCl_2$, Chitosan, and Mannitol) seed priming on germination.

Table 1: Optimization of PEG concentration Based on germination percentage of MAUS-158 with Osmotic Stress of PEG

Sr. No.	PEG Concentration	Germination Percentage			
		R_1	R_2	R_3	Mean ± SE
1	5%	9	8	8	83.33±3.3
2	10%	8	7	6	70±5.7
3	15%	4	5	5	46.66±3.3
4	control	9	9	8	86.66±3.3
				CD	1.352
				CV	9.867

The PEG Concentration of 15% was found best for inhibition of seed Germination less than 50%, Thus for inducing osmotic stress 15% PEG concentration was used (Table.1).

Table 2: Priming duration for MAUS-158 was optimised using hydropriming for 30 min, 60 min, 90 min, 2 hr, 4hr and 6hr

Sr. No	Treatments	Non-Stress condition [A]				Osmotic stress with 15% PEG [B]			
		Replication			Germination Percentage	Replication			Germination Percentage
		R_1	R_2	R_3	Mean ± SE	R_1	R_2	R_3	Mean ± SE
1	Non-Primed	6	8	7	70±5.7	5	5	6	53.33±3.3
2	Hydropriming-30 min	4	7	5	53.33±8.8	6	5	4	50±5.7
	60 min	7	7	8	73.33±3.3	6	7	5	60±5.7
	90 min	6	5	7	60±5.7	5	6	4	50±5.7
	2 hr	6	5	6	56.66±3.3	5	6	5	53. 33±3.3
	4 hr	8	8	7	76.66±3.3	6	6	5	56.66±3.3
	6 hr	9	7	8	80±5.7	7	6	6	63.33±3.3
	CD				16.82				N/A
	CV				14.167				14.244

The highest germination percentage was observed at 6 hr hydropriming, followed by 4 hr in MAUS-158 under non-stress as well as osmotic stress conditions (Table 2). The seedling vigour i.e. plant height and root formation were recorded at 7 days after sowing. The best results were observed for the 6-hour hydropriming treatment.

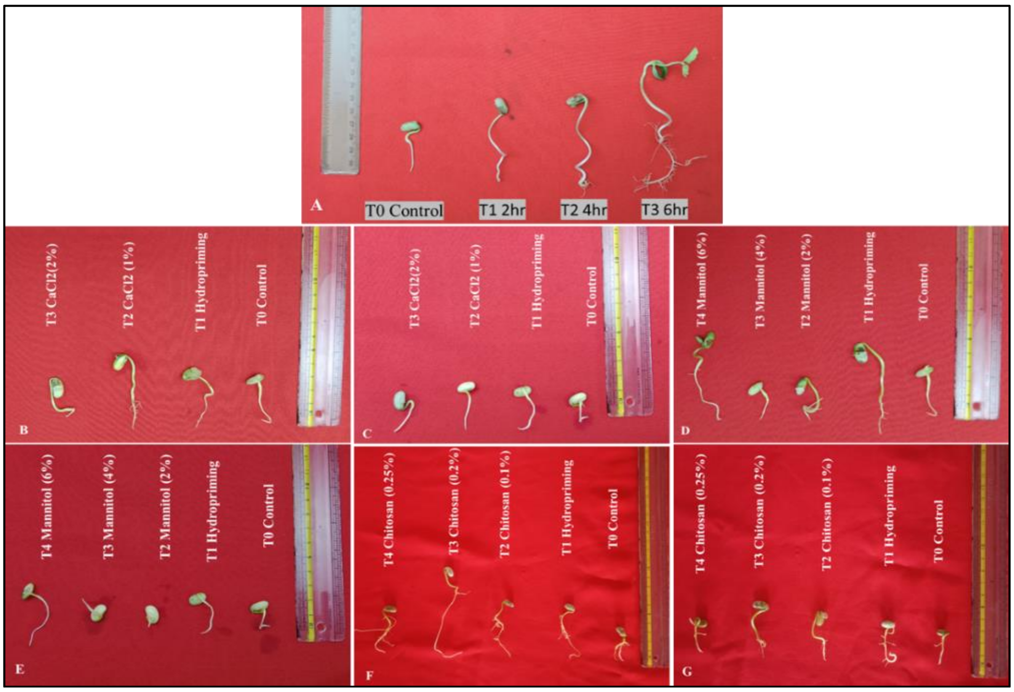

Fig 1: Effect of Seed Priming with Hydropriming T_1 (2 hr), T_2 (4hr) and T_3 (6 hr) (A), $CaCl_2$ Non-stress condition (B), $CaCl_2$ with Osmotic stress (C), Mannitol Non-stress condition (D), Mannitol with Osmotic stress (E), Chitosan Non-stress condition (F) and Chitosan with Osmotic stress condition (G) at 7 days old seedlings of Soybean variety MAUS-158 under Control [A] and Osmotic Stress [B]

Table 3: Optimization of $CaCl_2$, Mannitol and Chitosan Concentration for seed priming

Treatments	Germination Percentage (%) Mean ± SE	
	Non-Stress Condition [A]	Osmotic Stress (15% PEG [B]
Non-Primed	70±5.7	53.33±3.3
Hydropriming	80±5.7	73.33±3.3
$CaCl_2$ (0.5%)	56.66±6.6	46.66±3.3
$CaCl_2$ (1%)	76.66±3.3	80±5.7
$CaCl_2$ (1.5%)	73.33±3.3	56.66±3.3
$CaCl_2$ (2%)	70±5.7	63.33±8.8
$CaCl_2$ (3%)	63.33±8.8	46.66±3.3
Mannitol (2%)	56.66±3.3	53.33±8.8
Mannitol (4%)	80±5.7	56.66±3.3
Mannitol (6%)	83.33±3.3	63.33±3.3
Mannitol (8%)	60±5.7	50±3.3
Chitosan (0.1%)	70±5.7	56.66±3.3
Chitosan (0.2%)	80±5.7	63.33±3.3
Chitosan (0.25%)	63.33±3.3	46.66±3.3
CD	1.578	1.421
CV	13.363	14.548

The experiment aimed to determine the best concentrations of $CaCl_2$, Mannitol, and Chitosan to improve soybean germination and early seedling growth under non-stress and osmotic stress conditions induced by 15% PEG-6000 (Fig 1). Among the $CaCl_2$ treatments, 1% $CaCl_2$ with 6-hour priming significantly improved germination (76.66% under non-stress conditions and 80% under stress), as well as plant height and root development. For Mannitol, a 6% concentration with 6-hour priming resulted in the highest germination rate (83.33% under non-stress conditions and 63.33% under stress) and better seedling vigor. Initially, Chitosan priming at 0.25-0.75% for 6 hours did not promote germination. However, when the priming duration was reduced to 1 hour and concentrations were adjusted to 0.10%, 0.20%, and 0.25%, germination significantly improved. Among these, 0.20% Chitosan with 1-hour priming showed optimal performance with 80% germination under non-stress conditions and 63.33% under stress, along with healthy seedling development. Therefore, the optimized conditions for each priming agent were: 1%

$CaCl_2$ (6 hr), 6% Mannitol (6 hr), and 0.20% Chitosan (1 hr). These results emphasize the importance of adjusting priming conditions to enhance soybean stress tolerance and early growth (Table 3).

4. Conclusion

The study successfully optimized seed priming conditions to enhance germination and early seedling growth of soybean (*Glycine max* L.) variety MAUS-158 under PEG 6000-induced osmotic stress. A 15% PEG concentration (-3 bar) was used to simulate osmotic stress. Hydropriming for 6 hours was found to be the optimal duration for enhancing germination. Among the chemical priming agents tested, 1% $CaCl_2$ (6 hr), 6% Mannitol (6 hr), and 0.20% Chitosan (1 hr) significantly improved germination under both non-stress and stress conditions. Particularly, 1% $CaCl_2$ and 6% Mannitol showed the highest germination and seedling vigor under stress, while a shorter duration was more effective for Chitosan. These results indicated that seed priming with optimized chemical concentrations and durations can alleviate osmotic stress effects, enhance germination, and promote early seedling establishment, thereby supporting stress-resilient soybean cultivation practices.

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