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ORIGINAL ARTICLE

Impact of Dormancy-Modulating Chemical Treatments on Seed Storage Stability and Protein Profiling in Mungbean (*Vigna radiata* L.)

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ABSTRACT

This study evaluates the effects of different dormancy-modulating chemical treatments on seed storability, physiological stability, and protein profile integrity in mungbean (*Vigna radiata* L.) genotype IPM 2-3. Treatments included gibberellic acid (GA_3, 80 ppm), abscisic acid (ABA, 50 ppm), hydrogen peroxide (H_2O_2, 10 mM), maleic hydrazide (MH, 1000 ppm), potassium nitrate (KNO_3, 1 mM), indole-3-acetic acid (IAA, 100 ppm), and an untreated control. Seed viability and electrical conductivity (EC) were assessed at two-, four-, and six-months post-harvest to examine long-term impacts on membrane integrity and physiological health. The untreated control (T_0) maintained the highest viability (82.33% at two months, decreasing to 62.67% at six months) with the lowest EC values (0.213 dS/m/gm at two months, decreasing to 0.073 dS/m/gm at six months). Among treated seeds, GA_3 sustained high viability (79.33% initially, 59% at six months) and moderate EC values, suggesting enhanced storability. In contrast, MH treatment showed the lowest viability (42% at six months) and highest EC (0.303 dS/m/gm initially), indicating significant membrane degradation and compromised physiological health. SDS-PAGE protein profiling revealed that T_0, GA_3, and ABA treatments had strong protein bands at 60 kDa and 50 kDa, correlating with high viability and lower EC. In contrast, MH-treated seeds showed weak bands at 55 kDa and 45 kDa, indicating substantial protein degradation. Statistical analysis confirmed a negative correlation between seed viability and EC and a positive correlation between viability and protein band intensity. Overall, GA_3 and ABA treatments support balanced dormancy modulation and storability, while MH adversely affects seed quality, providing valuable insights for optimizing mungbean seed storage practices.

KEY WORDS: Mungbean, Seed dormancy, Seed viability, Electrical conductivity, Protein profiling, Chemical treatments, SDS-PAGE

INTRODUCTION

Mungbean (*Vigna radiata* L.), also known as green gram, is a crucial legume in Indian agriculture. It is recognized for its versatility as a vegetable, pulse, fodder, and green manure crop. Belonging to the Fabaceae family, mungbean is widely cultivated due to its adaptability to various soils and climates and nutritional value. The crop provides a significant source of protein (22-24%), minerals like calcium and potassium, and essential vitamins, making it an integral part of the diet, especially in vegetarian populations (Hou *et al.,* 2019).

India is the leading producer of mungbean globally, accounting for a substantial portion of total pulse production. However, the country's pulse output has not kept pace with increasing demand, necessitating imports. One of the major challenges facing mungbean production is pre-harvest sprouting (PHS), which occurs due to excess moisture absorbed by seeds during maturity. This issue is exacerbated by unpredictable rainfall, particularly in regions where mungbean is grown under rainfed conditions. PHS affects seed viability and storability, which are critical for maintaining seed quality during storage.

Seed dormancy is a natural physiological mechanism that delays germination under unfavorable conditions. In mungbean, dormancy can be beneficial as it prevents seeds from sprouting prematurely before harvest (Stevens *et al.,* 2020). However, the absence or reduction of dormancy can increase susceptibility to PHS, leading to

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yield losses and reduced seed quality. As dormancy plays a pivotal role in controlling germination timing, investigating non-traditional methods to induce dormancy in mungbean is essential for enhancing seed storage stability and maintaining yield.

Chemical treatments such as maleic hydrazide have been explored for inducing seed dormancy and controlling sprouting in various crops. These treatments can regulate physiological traits such as seed viability and seed membrane integrity, which are indicators of seed vigour and storability (Shelar *et al.,* 2014). The tetrazolium test and electrical conductivity (EC) test are commonly used to assess seed viability and membrane permeability, respectively. High electrical conductivity indicates increased solute leakage, which is associated with poor storability and lower seed quality (Mandizvo *et al.,* 2019).

Protein synthesis is another critical factor influencing seed dormancy and germination. Studies have shown that certain proteins, such as 23 kDa proteins, are upregulated during the release of dormancy, indicating their involvement in the transition from dormancy to active germination. Protein profiling through techniques like SDS-polyacrylamide gel electrophoresis (SDS-PAGE) allows for the identification of specific proteins associated with dormancy and sprouting resistance in mungbean genotypes (Corbineau *et al.,* 1991; Garello *et al.,* 2000).

This study aims to explore the impact of dormancy-modulating chemical treatments on seed storage stability, focusing on traits such as seed viability percentage and electrical conductivity. Additionally, protein profiling will be conducted to identify the biochemical changes that occur during dormancy induction and sprouting resistance. By examining these physiological and biochemical traits, the study seeks to provide insights into improving mungbean seed quality and reducing pre-harvest sprouting through targeted chemical treatments.

MATERIAL AND METHOD

This study was carried out to assess the impact of dormancy-modulating chemical treatments on seed storage stability and protein profiling in mungbean (*V. radiata* L.). The research took place at the Student Instructional Farm, Rabindranath Tagore University, Raisen, Madhya Pradesh, India (latitude: 23.250° N, longitude: 77.420° E, elevation: 427 meters). The region experiences a semi-arid climate with hot summers, cool winters, and approximately 80% of the annual rainfall occurring during the monsoon season. The experimental soil had a pH of 7.8, cation exchange capacity (CEC) of 0.41, and an exchangeable sodium percentage (ESP) of 7.15, being rich in potash but deficient in organic carbon, nitrogen, and phosphorus.

One released mungbean genotype, IPM 2-3, obtained from ICAR-IIPR, Kanpur, was selected for its consistent agronomic traits and known susceptibility to pre-harvest sprouting. Field trials were conducted during the kharif season of 2022-23, followed by laboratory analyses in the 2022-23 and 2023-24 seasons. The primary objective was to evaluate the effects of various chemical treatments on seed viability, electrical conductivity (EC), and protein profiling to determine their influence on seed storage stability and dormancy induction.

A total of six dormancy-modulating chemical treatments were tested alongside a control. Foliar applications were administered at two critical growth stages, 45 days after sowing (DAS) and 60 DAS. The treatments included: T_0– control (water spray), T_1– gibberellic acid (GA_3) at 80 ppm, T_2– abscisic acid (ABA) at 50 ppm, T_3– hydrogen peroxide (H_2O_2) at 10 mM, T_4– maleic hydrazide (MH) at 1000 ppm, T_5– potassium nitrate (KNO_3) at 1 mM, and T_6– indole-3-acetic acid (IAA) at 100 ppm. These treatments were designed to explore their effectiveness in inducing dormancy, reducing pre-harvest sprouting, and maintaining seed quality under simulated rainfall conditions. Data was recorded for the different traits and parameters.

Seed Viability (%)

Seed viability will be estimated using the T_Z (2,3,5-Triphenyl Tetrazolium Chloride) test recommended by Lakon, 1949. A total 100 seeds from each treatment will be selected and soaked in water at 30°C for 16 hours. After soaking, the seeds will be removed from the water, and their seed coats will be carefully peeled off using forceps. The peeled seeds will then be rinsed with fresh water.

A 1% tetrazolium solution will be prepared by dissolving 1 gram of triphenyl tetrazolium chloride powder in 100 ml of distilled water. The solution will be placed in Petri dishes, and the peeled seeds will be soaked in it for 3-4 hours. After this period, the seeds will be examined based on their staining patterns, and viability will be calculated as a percentage.

Electrical Conductivity (dS/m/gm)

From each treatment, three replicates of 25 seeds were randomly selected and soaked in 75 ml of distilled water for 24 hours at 25°C. Before analysis, the seed and solution mixture were gently swirled for 10 to 15 seconds. Following the method outlined by Loeffler et. al., (1988), the electrical conductivity of the solution was measured using a conductivity meter with a cell constant of 1. The results are expressed in deciSiemens per meter per gram (dS/m/g).

SDS-PAGE Electrophoresis for Protein Profiling

In the present study of protein profiling in mungbean,

the polymorphism of total seed proteins and isozymes was analyzed following the ISTA guidelines using SDS-PAGE (Sodium Dodecylsulphate-Polyacrylamide Gel Electrophoresis). A modified version of the Laemmli, 1970 method was employed to separate the protein subunits.

A 12% separating gel and a 5% stacking gel were prepared using standard acrylamide solutions. Protein samples were diluted to 350-400 µg/ml in a sample buffer (1M Tris-HCl, β-mercaptoethanol, SDS, bromophenol blue, and glycerol), boiled for 3-5 minutes, centrifuged, and loaded into the wells.

Electrophoresis was run in a vertical mini-slab gel electrophoresis system with a current of 25 mA for 2–3 hours. After electrophoresis, the gel was fixed in 7% acetic acid and stained using Coomassie Brilliant Blue solution for 1 hour, followed by destaining with acetic acid and methanol to remove excess stain. Protein subunits were analyzed based on banding patterns relative to standard molecular weights.

Table 1: Analysis of variance for the percentage of seed viability and electrical conductivity (dS/m/gm) during different storability period after harvesting in mungbean genotypes IPM 2-3.

Source of variation	d.f.	Mean Sum of Squares					
		Seed Viability (%)			Electrical Conductivity (dS/m/gm)		
		Two Months After Harvesting	Four Months After Harvesting	Six Months After Harvesting	Two Months After Harvesting	Four Months After Harvesting	Six Months After Harvesting
Treatment	6	176.3333**	179.3333**	145.9683**	0.0047**	0.0048**	0.0038**
Residual	14	4.7143	5.1905	6.1429	0.0001	0.0002	0.00009
Total	20						

** Sig. at 1% level

Table 2: Effects of foliar application of dormancy-modulating chemicals treatments on percentage of seed viability and electrical conductivity (dS/m/gm) during different storability period after harvesting in mungbean genotypes IPM 2-3.

Treatment	Seed Viability (%)			Electrical Conductivity (dS/m/gm)		
	Two Months After Harvesting	Four Months After Harvesting	Six Months After Harvesting	Two Months After Harvesting	Four Months After Harvesting	Six Months After Harvesting
Control (T_0)	82.33	72.67	62.67	0.213	0.150	0.073
Gibberellic acid (GA3) @80ppm(T_1)	79.33	69.00	59.00	0.263	0.193	0.137
Abscisic acid (ABA) @50ppm(T_2)	77.67	67.00	57.33	0.217	0.147	0.090
Hydrogen peroxide (H_2O_2) @10 mM(T_3)	73.67	63.67	53.67	0.217	0.147	0.083
Maleic hydrazide (MH) @1000ppm(T_4)	59.33	49.67	42.00	0.303	0.233	0.160
Potassium nitrate (KNO_3) @1 mM(T_5)	73.00	60.67	51.00	0.190	0.120	0.070
Indole-3-acetic acid (IAA) @100ppm(T_6)	79.67	70.00	60.00	0.203	0.127	0.073
Mean	75.000	64.667	55.095	0.230	0.160	0.098
Sem ±	1.254	1.315	1.431	0.007	0.009	0.006
SEd	1.773	1.860	2.024	0.010	0.013	0.008
CD (LSD) 5%	3.802	3.990	4.340	0.021	0.027	0.017
CD (LSD) 1%	5.277	5.538	6.024	0.030	0.038	0.023
C.V. (%)	2.895	3.523	4.499	5.208	9.673	9.697

RESULTS AND DISCUSSION

Seed Viability and Electrical Conductivity Analysis

The seed viability and electrical conductivity (EC) analyses provide essential insights into the effects of dormancy-modulating chemicals on mungbean seed storability. Seed viability reflects the seed's potential for germination, while EC indicates membrane integrity by measuring solute leakage, critical for assessing physiological stability under various treatments (Tables 1&2).

Seed Viability Across Treatments

The findings indicate that various chemical treatments had a marked effect on seed viability, consistent with prior studies showing that certain treatments can extend storage life and maintain quality by stabilizing physiological conditions (Table 1) (Ghasemnezhad & Honermeier, 2009; Walters *et al.,* 2010; Adetunji *et al.,* 2021). The control treatment (T_0) showed the highest viability rates over storage periods, with values decreasing gradually from 82.33% at two months to 62.67% after six months. Treatment with gibberellic acid (GA_3, T_1) followed closely, starting at 79.33% at two months and reducing to 59% after six months (Table 2). This trend suggests that GA_3 has some effect on maintaining seed health over time, although not as robust as the untreated control (Mutinda *et al.,* 2017). While GA_3 demonstrated moderate support for storability, studies suggest its efficacy could vary across species (Pulatkan *et al.,* 2022). Abscisic acid (ABA, T_2) demonstrated moderate effects, with a viability of 77.67% at two months, declining to 57.33% by the sixth month, with the findings consistent with ABA's role in maintaining membrane stability (Finkelstein *et al.,* 2002).

However, seeds treated with maleic hydrazide (MH, T_4) showed the lowest viability (59.33% initially, reducing sharply to 42% at six months) (Table 2), supporting research that links MH application may lead to a decline in seed vigour, possibly due to stress mechanisms that contribute to dormancy but decrease storability due to potential stress mechanisms (Subbarao *et al.,* 2012; Gadhave *et al.,* 2017). Treatments like hydrogen peroxide (H_2O_2, T_3), potassium nitrate (KNO_3, T_5), and indole-3-acetic acid (IAA, T_6) resulted in moderate viabilities, indicating balanced effects on seed health without significant dormancy induction (Nazir *et al.,* 2024; Kanjevac *et al.,* 2022).

Electrical Conductivity Across Treatments

The EC values generally inversely correlated with seed viability; treatments that exhibited higher seed viability, like T_0 and T_1, displayed lower EC values, suggesting better membrane stability and reduced solute leakage for instance, T_0 showed a gradual decline in EC from 0.213 dS/m/gm at two months to 0.073 dS/m/gm at six months (Table 2), consistent with previous findings that link low EC values with storability (Surki *et al.,* 2012). In contrast, MH-treated seeds (T_4) presented the highest EC values, starting from 0.303 dS/m/gm at two months, indicating compromised membrane integrity (Table 2). Elevated EC values for MH-treated seeds, along with reduced viability, support reports that MH increases physiological stress and decreases storage longevity (Magar *et al.,* 2024). The increased EC in MH-treated seeds aligns with its dormancy-inducing effects and a consequent reduction in seed vigour. Other treatments like ABA, H_2O_2, and IAA displayed moderate EC values, suggesting these chemicals support storability with relatively stable membrane integrity (Table 2).

These findings emphasize that treatments such as GA_3 and ABA support seed longevity while MH has detrimental effects on seed health, likely due to mechanisms that increase dormancy-related stress.

Simulated Electrophoresis Protein Profiling

Simulated SDS-PAGE protein profiling highlighted biochemical changes in seed storage proteins across treatments (Table 3 & Fig. 1). Previous research confirms that protein band intensities in electrophoresis reflect protein stability, with higher intensities indicative of better integrity and reduced degradation (Ochuodho *et al.,* 2006).

The control treatment displayed three protein bands at 60 kDa, 50 kDa, and 40 kDa with intensities of 80%, 60%, and 30% respectively, in line with prior research that links strong protein profiles to higher storability (Table 3 & Fig. 1). This profile, with minimal degradation, aligns with the high viability and low EC observed in the untreated seeds, showing that seed protein integrity remains intact under control conditions.

GA_3 @ 80 ppm-treated seeds exhibited similar protein bands, though with slightly lower intensities (70%, 50%, 25%) at 60 kDa, 50 kDa, and 35 kDa (Table 3 & Fig. 1). The decrease in band intensity suggests mild protein degradation, though GA3 effectively maintained protein stability. The moderate stress imposed by GA_3 is consistent with its role as a growth promoter, resulting in relatively high seed viability and EC values (Ochuodho *et al.,* 2006; Mansoor *et al.,* 2021).

ABA @ 50 ppm treatment produced strong bands at 60 kDa, 45 kDa, and 35 kDa, with higher intensities (75%, 65%, and 35%) (Table 3 & Fig. 1). The reduced degradation compared to other treatments suggests ABA's role in enhancing seed longevity by maintaining protein integrity. The protein profiles of ABA-treated seeds, showing moderate viability and low EC, indicate ABA's

Table 3: Protein Band Intensity and Molecular Weights Across Treatments in Mungbean genotype: IPM 2-3.

Treatment	Molecular Weight (kDa)			Band Intensity (%)			
	Two Months After Harvesting	Four Months After Harvesting	Six Months After Harvesting	Two Months After Harvesting	Four Months After Harvesting	Six Months After Harvesting	Number of Bands
Control (T_0)	60	50	40	80%	60%	30%	3
Gibberellic acid (GA3) @ 80 ppm (T_1)	60	50	35	70%	50%	25%	3
Abscisic acid (ABA) @ 50 ppm (T_2)	60	45	35	75%	65%	35%	3
Hydrogen peroxide (H_2O_2) @ 10 mM (T_3)	60	50	40	60%	55%	40%	3
Maleic hydrazide (MH) @ 1000 ppm (T_4)	55	45	35	50%	40%	20%	3
Potassium nitrate (KNO_3) @ 1 mM (T_5)	60	50	40	65%	55%	30%	3
Indole-3-acetic acid (IAA) @ 100 ppm (T_6)	60	50	40	70%	60%	25%	3

effectiveness in modulating dormancy. This supports research on ABA's role in preserving protein integrity and enhancing storability in legume seeds (Rodríguez *et al.,* 2009).

The protein profile for seeds treated with H_2O_2 at 10 mM showed bands at 60 kDa, 50 kDa, and 40 kDa with intensities of 60%, 55%, and 40%, respectively (Table 3 & Fig. 1). While H_2O_2 induced moderate protein degradation–reflected in lower band intensities compared to the control (T_0)–it maintained a relatively stable protein profile with moderate viability and EC, suggesting that H_2O_2 moderately supports storability and viability. Seeds treated with KNO_3 at 1 mM displayed bands at 60 kDa, 50 kDa, and 40 kDa with intensities of 65%, 55%, and 30%, maintaining a stable protein profile similar to the control (Table 3 & Fig. 1). Known for breaking dormancy, KNO_3 supported seed viability and storability without significantly inducing dormancy. The stable band patterns observed for both H_2O_2 and KNO_3 treatments align with their roles in inducing mild dormancy without considerable degradation (Bian *et al.,* 2013; Pereira *et al.,* 2021).

MH @ 1000 ppm-treated seeds exhibited weak bands at 55 kDa, 45 kDa, and 35 kDa, with the lowest intensities (50%, 40%, and 20%) (Table 3 & Fig. 1). This profile suggests significant protein degradation, corresponding to high EC values and low viability, implying that MH induces considerable stress and dormancy, compromising seed quality. These findings align with research on MH's adverse effects on storability (Singh *et al.,* 2018).

Seeds treated with IAA at 100 ppm, maintained protein bands at 60 kDa, 50 kDa, and 40 kDa, with band intensities of 70%, 60%, and 25%, respectively. This treatment preserved protein structure with minimal degradation, consistent with IAA's role in promoting growth while maintaining seed quality and viability. The strong protein bands observed with IAA treatment support previous findings that IAA enhances seed health without compromising storage stability (Mansoor *et al.,* 2021)

Interpretation of Protein Banding Patterns and Treatment Effects

Protein profiling across treatments showed that higher seed viability was associated with stronger protein bands, indicating reduced degradation. This correlation between viability and band stability aligns with recent studies emphasizing the importance of protein integrity for seed health (Ochuodho *et al.,* 2006; Rodríguez *et al.,* 2009). Treatments like T_0, T_1, and T_3, which maintained high viability, showed stable band patterns, highlighting the protective role of proteins in viability (Table 2 & 3). Lower viability and higher EC values, as seen with MH (T_4), corresponded with weaker bands and increased degradation. The protein profiles demonstrate that while high-molecular-weight proteins (60 kDa) are crucial for seed viability and longevity (Table 2 and 3), the reduction in band intensity for lower molecular weights in MH treatment suggests these proteins are linked to dormancy induction and degradation during stress (Ochuodho *et al.,* 2006; Rodríguez *et al.;* 2009, Singh *et al.,* 2018; Mansoor *et al.,* 2021).

Correlation among Seed Viability, Electrical Conductivity and Protein Profiling

A strong negative correlation between seed viability and EC values emerged across treatments, where higher seed viability was consistently associated with lower EC values (Table 1 and 2), indicative of healthier membrane integrity and reduced solute leakage (Gupta *et al.,* 2017; Magar *et al.,* 2024). Additionally, a positive correlation was observed between protein band intensity and seed viability, with treatments showing higher intensities correlating with better seed storability (Table 3). MH-treated seeds exhibited the weakest protein profiles, highest EC, and lowest viability, supporting the hypothesis that MH induces dormancy at the expense of seed vigour. These findings are consistent with reports of MH's adverse effects on seed health (Singh *et al.,* 2018). Meanwhile, GA_3 and ABA treatments displayed moderate to high seed viability and protein stability, supporting research that identifies these agents as promising for balancing dormancy and storability (Mansoor *et al.,* 2021; Ochuodho *et al.,* 2006; Rodríguez *et al.,* 2009).

These insights enhance the understanding of chemical treatments in dormancy modulation and seed storability, suggesting that GA_3 and ABA preserve protein integrity and storability effectively, while MH compromises seed quality.

CONCLUSION

Treatments with IAA and ABA show promising potential in enhancing seed storability by preserving membrane integrity and reducing protein degradation, as evidenced by lower EC values and strong protein band intensities. Similarly, GA_3 was effective in maintaining moderate seed viability and protein integrity. In contrast, MH induced dormancy but at the cost of seed quality, leading to reduced viability, increased EC, and notable protein degradation, indicating its unsuitability for long-term storage. Overall, the study provides valuable insights

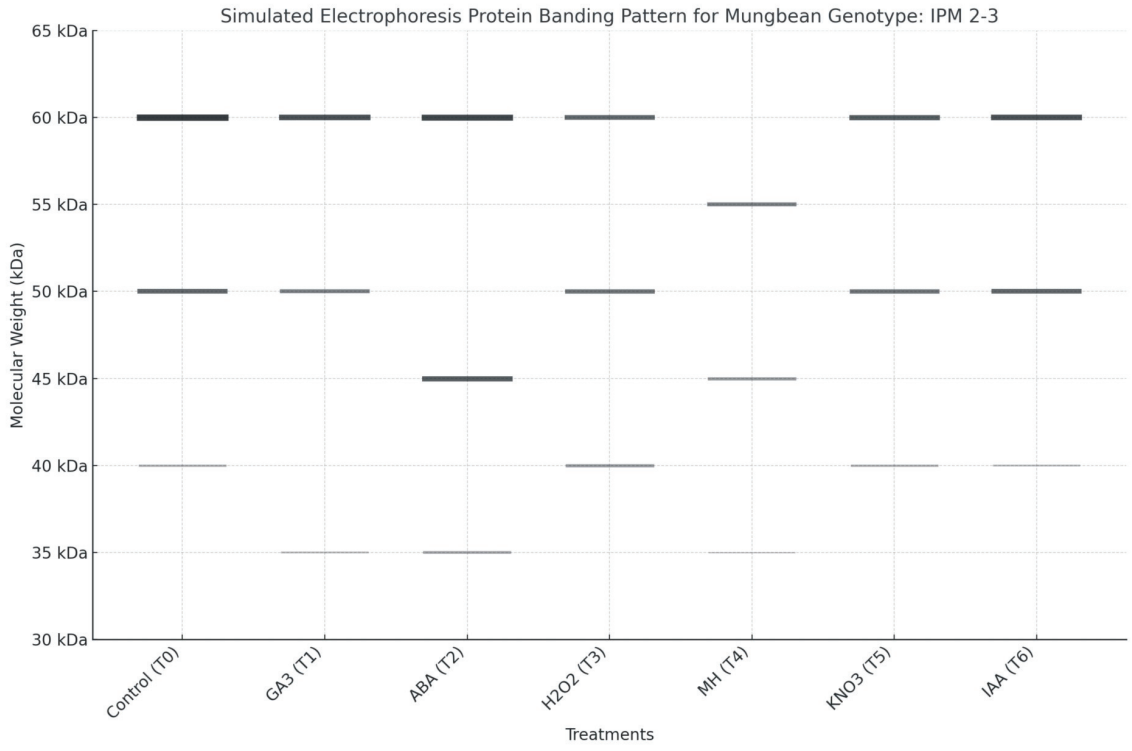

Fig. 1. Simulated electrophoresis protein banding pattern for the mungbean genotype IPM 2-3.

Here are the molecular weights of the protein bands are shown on the y-axis, while different treatments are displayed on the x-axis. The thickness and intensity of each band correspond to the percentage of band intensity over time (2, 4, and 6 months after harvesting). Darker and thicker bands represent higher intensities, indicating a stronger protein expression in those conditions.

into selecting chemical treatments that balance dormancy modulation with enhanced seed vigour and longevity, supporting the use of IAA, ABA, and GA3 as viable options for improved mungbean seed preservation.

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Conflict of Interest

The authors declare no conflicts of interest.

Authors' Contribution

All listed authors have made significant, direct, and intellectual contributions to this research and have approved the final version for publication.

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Data Availability

All data generated or analyzed during this study are included within the manuscript.

Ethics Statement

This research did not involve any studies with human participants or animals, and no ethical issues are associated with the work presented in this paper.

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