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Reviving Mung Bean Seeds: The Impact of Hydro Priming and Heat Shock on Germination Rates

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ABSTRACT

Seed germination is a crucial stage of plant growth that can enhance yield by influencing seedling establishment. The speed and success of germination are key factors that determine the final yield of plants. This research aimed to investigate how hydro priming and heat shock affect the germination properties of green mung bean through a factorial experiment conducted in a completely randomized design with three replications at Parwan University's agronomic laboratory in 2022. The experimental treatments consisted of two temperature levels (35 and 25°C) and three moisture levels (8.5%, 10%, and 11.5%), with four categories of treatment: hydro prim, 30°C shock, 40°C shock, and control. Germination percentage, germination speed, seedling length, and dry weight were measured in the experiment. The results indicated that the triple interaction of temperature, moisture, and treatment was significant only for the germination speed trait at a 5% level. The main effect of temperature and the dual interaction of moisture and treatment at a 1% level were significant for stem length and dry weight. Additionally, the dual interaction between temperature, treatment, and temperature moisture level of 1 and 5% were significant for germination percentage, respectively. Moreover, the study found that hydro priming and thermal flushing treatment frequently improved seed storage capacity. Therefore, the study suggests that hydro priming and heat shock treatments can enhance seed germination and vigor, especially for seed lots stored under unfavorable conditions.

Keywords- Hydro priming, Heat shock, Green Mung Bean, Germination, Temperature, Moisture.

I. INTRODUCTION

Legumes, particularly green carving (Vigna radiata (L) Wilczek), are an important source of plant protein and have a high nutritional value for humans, providing a significant portion of protein in many societies (Rastgar, 2005; Majnoon-Hoseini, 1993). The seeds of this plant contain 22-25% protein. Additionally, green bean forage, which contains 10-20% plant protein, is a nutritious feed for animals. To improve the quality of germination, hydro priming is a method where seeds are soaked in a concentrated water solution to activate their physiological activity before root emergence (Farooq et al. 2006; Harris et al. 1999). This technique results in higher quality and more robust seeds, with faster germination rates and higher percentages of successful germination even in the face of environmental stress conditions. This information is supported by studies conducted by McDonald's et al. (2004). According to McDonald's et al. (2004), seeds that are of

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high quality and robust are more likely to have a higher rate of successful germination, faster germination speed, and better performance under environmental stress conditions. However, like all living organisms, seeds have a finite lifespan and gradually degrade and disappear over time. Seed deterioration typically begins on the farm before harvest, and this process continues after the seed has reached physiological maturity. Crop seeds are usually not immediately planted after harvest and instead are stored for varying periods of time ranging from days to years, during which they may continue to deteriorate (Qaderifar et al., 2010). Seeds are vulnerable to both biological and mechanical damage after harvest, and once the process of seed deterioration begins, it is irreversible, according to Kapoor et al. (2011). Minimizing the reduction in seed quality and viability during storage is essential to prevent economic and ecological damage and predicting seed quality during storage requires research in this area, as noted by Alivand et al. (2013) and Bewley et al. (2012). The storage capacity of seeds is primarily a genetically determined trait and is influenced by various factors such as initial seed quality, storage time, temperature, and biological factors such as fungal contamination (Baluchi et al. 2017; Sheller et al. 2008). Temperature, relative humidity, and seed moisture are the most critical environmental factors in the seed deterioration process during storage, with high temperature and seed moisture content leading to accelerated deterioration and reduced seed quality (Qaderifar et al. 2010; Schler et al. 2008). The effect of high temperatures on seed viability is strongly influenced by the seed's water content. Generally, the loss of seed viability is directly proportional to the increase in seed storage temperature, according to Kibinza et al. (2006). The process of priming involves soaking and drying seeds in a controlled manner, which can improve the efficiency of seed shipments, particularly in stressful conditions (Paparella et al. 2015; Akram Qaderi; et al. 2008). Various studies have explored how this process enhances seed efficiency, attributing its usefulness to physiological, biochemical, cellular, and molecular changes that occur during seed soaking and drying (Ibrahim, 2016). Both seed priming and heat shock treatments have been found to enhance seed germination and seedling growth in different crops. This paper examines the impacts of hydro-priming and heat shock treatments on the germination indices of mung bean seeds under varying storage conditions. Furthermore, it could be valuable to compare the effectiveness of hydro-priming and heat shock treatments with other pre-sowing treatments, such as seed coating and seed priming, to identify the most efficient and cost-effective method for improving mung bean seed germination and growth. Future studies could focus on investigating the molecular and physiological mechanisms underlying the effects of these treatments on mung bean seeds. This could lead to the identification of specific genes or biochemical pathways that are involved in the response of mung bean seeds to hydro-priming and heat shock treatments. Therefore, this study aims to determine the optimal priming and heat shock treatments that can improve the seedling performance of mung bean seeds during germination, especially under unfavourable storage conditions. The findings of this research can provide valuable insights into the seed treatment practices for mung bean cultivation and contribute to the development of more sustainable and efficient agricultural practices.

II. MATERIALS AND METHODS

A factorial experiment was conducted with a completely randomized design in three replications in the agronomic laboratory of the faculty of Agriculture, University of Parwan in 2022 to assess the impact of hydro priming and heat shock on the germination indices of green mung bean seeds. The experiment involved maintaining two temperature levels, 25 and 35 degrees Celsius, and three moisture levels, 8.5%, 10%, and 11.5%, with four treatment categories, including hydro priming, shock at 30°C, shock at 40°C, and control, for a duration of 30 days. The hydro priming treatment was performed at various time intervals from 1 to 30 days during the storage period to assess rejuvenation. Following treatment, petri dishes were placed in a germinator at 25°C for seven days, and the germination count was conducted from day 5 to 7. The germination criteria of root exit were set at 2mm, and the mean and germination percentage were calculated based on following equations.

$$\mathbf{1.}\ \boldsymbol{MG} = \frac{\sum \boldsymbol{D_n}}{\sum \boldsymbol{n}} \quad \mathbf{2.\ PG} = \frac{\mathbf{Ni}}{\mathbf{N}} * \mathbf{100} \quad \mathbf{3.}\ \frac{\mathbf{1}}{\boldsymbol{MGT}}$$

Equation 1 involves calculating the mean germination, denoted by D (Ellis et al. 1980), which is the number of days after the start of the germination test. The variable n represents the number of germinated seeds on D-Day, and N represents the total number of seeds, while Ni represents the number of seeds germinated on the seventh day (Fallah & Babaei, 2006). Also, MGT shows mean germination time. To measure the root length and stem length, a milli meter ruler was used, and a sensitive troy Sartorius research (R300S) with 0.0001g accuracy was used to measure the weight of roots and stems separately. After separating the roots and stems, they were dried in an oven at 72°C for 24 hours, and their dry weight was measured. Data analysis was performed using SAS statistical software, and Duncan's test was used to compare the means. Excel software was used to create the graphs. To conduct the accelerated aging test, a special plastic box containing four grating stands was required. Inside the container, 40mm of distilled water was added, and the seeds were placed on the surface of the grating foundations, ensuring that they were not in direct contact with the water. The aim was to create an environment of 100%

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relative humidity inside the plastic box. In this experiment, the seeds were disinfected using a Vitex solution with a concentration of 2/1000 and placed on the corresponding grating plate. The container was filled with distilled water at the bottom, and the lid was closed to prevent water evaporation. The container was then covered with Velcro and transferred to a germinator set to a temperature of 40°C. The seeds were kept at this temperature for 2, 4, and 6 days, and after each time interval, they were removed, and different treatments were compared to determine the best one. The results of the experiment revealed that two days was the optimal accelerated aging time for evaluating different heat shock treatments (ISTA, 2010). To extend the lifespan of the primed seeds, post-priming treatments were performed in the form of thermal shock using different temperatures of 30, 35, and 40°C, and varying durations of 1, 2, 3, and 4 hours. The primed seeds were weighed before being subjected to thermal treatment and were then placed at room temperature until their weight decreased by 10%. Next, the seeds were swiftly transferred to aluminium laminate envelopes and compressed using a press machine to maintain their moisture level in the package. Subsequently, the seeds underwent heat shock treatment at temperatures of 30, 35, and 40 °C for 1, 2, 3, and 4 hours. Following the heat shock treatment, seeds with moisture content exceeding 10% were stored at 5°C to decrease the humidity below 10%. The results indicated that the best treatment was the 4-hour shock hydro prim at 40°C and the 4-hour shock hydro prim at 30°C. Furthermore, three 25-seed replications of primed and treated seeds (temperature shocks) from each treatment were subjected to accelerated ageing tests (ISTA, 2010). To increase the moisture content of the primed seeds to 8.5%, 10%, and 11.5%, the Hampton and Tekrony method (1995) was employed, which involves determining the initial seed moisture percentage, W_1, the seed sample's primary weight, and W_2, the sample's secondary weight after adding moisture to attain the desired moisture.

$$\boldsymbol{W_2} = \frac{(\boldsymbol{100 - A})}{(\boldsymbol{100 - B})} \times \boldsymbol{W_1}$$

III. RESULTS AND DISCUSSION

The present study investigated the influences of hydro priming and heat shock on the germinating indices of mung bean seeds under different storage conditions. The results indicate that hydro priming and heat shock can significantly improve the germination and growth of mung bean seeds. Table 1 shows the mean squares of different sources of variations, including temperature (TM), moisture (M), treatment (T), TM x M interaction, TM x T interaction, M x T interaction, TM x M x T interaction, and experimental error. The coefficient of variation (CV) for each parameter is also reported. The results indicate that temperature and moisture had significant effects on all parameters, except for seedling length and dry weight in the case of TM x M interaction, which were not significant. Treatment had a significant effect on germination percentage and germination speed, but not on seedling length or dry weight. The interactions between temperature and moisture, as well as between temperature and treatment, were significant for some parameters. However, the interactions between moisture and treatment and between temperature, moisture, and treatment were not significant for any parameter. Overall, these results suggest that the priming treatment can significantly improve the germination characteristics of green mung bean seeds, while heat shock treatment may have a negative impact. The findings also highlight the importance of considering the effects of temperature and moisture in optimizing seed priming protocols.

Table 1: Analysis of variance of germination characteristics of green mung bean seeds subjected to priming and heat shock treatments after 30 days

		Mean of Squares			
Source of variations	**Degree of freedom**	**Germination (%)**	**Germination speed**	**Seedling length**	**Dry weight**
Temperature (TM)	1	4995.19^{**}	0.019^{**}	4.50^{**}	0.18^{**}
Moisture (M)	2	185.74^{**}	0.0017^{**}	16.12^{**}	0.64^{**}
Treatment (T)	3	42.43^{*}	0.0039^{**}	0.70^{**}	0.028^{**}
TM x M	2	128.99^{**}	0.0084^{**}	0.00^{ns}	0.00^{ns}
TM x T	3	42.19^{*}	0.0026^{**}	0.00^{ns}	0.00^{ns}
M x T	6	18.41^{ns}	0.00089^{*}	0.27^{**}	0.011^{**}
TM x M x T	6	25.023^{ns}	0.00099^{*}	0.00^{ns}	0.00^{ns}
Experimental Error	48	13.81	0.00034	0.050	0.0020
Coefficient of variation	-	8.70	8.52	9.77	9.77

** and ** denotes significant differences at p<0.05 and p<0.01 probability levels, respectively ns means not significant*

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1. Interaction effects of storage temperature and moisture on germination percentage:

The current study demonstrated that both temperature and moisture had a significant effect on the germination percentage, and there was a significant interaction between these two factors which is in line with the previous findings of Ali and Basra (2010), who reported that germination percentage in mung bean seeds decreased as moisture content increased above a certain threshold, while germination percentage also decreased when seeds were exposed to temperatures outside of the optimal range. Another study by Chaudhary et al. (2014) found that temperature and moisture interacted to affect the germination rate of mung bean seeds, with the optimal germination occurring at a temperature range of 25-30°C and a moisture content of 8-10%. These findings suggest that careful management of storage conditions is critical for maintaining the viability and germination potential of mung bean seeds. At the optimal temperature of 25°C, the germination percentage of the mung bean seeds was highest when the moisture content was at 8.5%. At this level of moisture, the germination percentage was 55.2%, while at highest and second highest moisture 11.5%, and 10%, the germination percentage was 48.6% and 46%, respectively (Figure 1). Similar results were reported by Mirali et al., (2019).

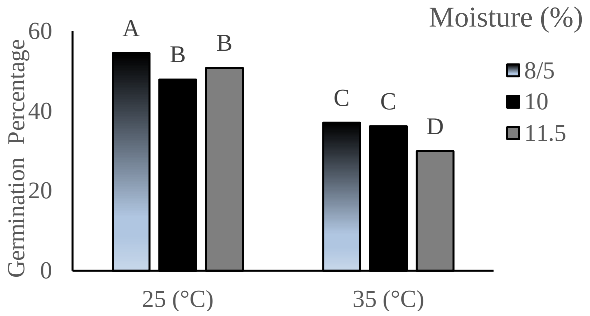

Figure 1: Comparing the average germination percentage of mung bean seed under different storage temperatures (25 and 35 °C) and moisture levels (8.5%, 10 and 11.5%) after 30 days of storage.

2. Interaction effects of storage temperature and treatment on germination percentage:

The results showed that both storage temperature and priming treatment had a significant effect on the germination percentage of mung bean seed. At a storage temperature of 25°C, the highest germination percentage (52.0%) was observed but there was no difference between priming treatments. At a storage temperature of 35°C, the highest germination percentage was observed for shock 40 treatment (37.5%), followed by Shock 30 (35.0%) and hydro prim (32.1%) (Figure 2). Several related studies that were formerly conducted on different plants also approve our discoveries (Ellis et al., 1980; Judy & Sharizadeh, 2004; Harris et al., 1999; Ibrahim, 2016). Wang et al. (2019) found that priming treatments had a significant positive effect on the germination percentage of mung bean seeds, particularly under adverse storage conditions. Similarly, a study by Li et al. (2018) found that storage temperature had a significant impact on the germination percentage of mung bean seeds, with higher germination rates observed at lower temperatures. The shock 40 treatment was the most effective at enhancing the germination percentage of mung bean seed at 35°C is particularly interesting, as it suggests that this treatment may be useful in mitigating the negative effects of high storage temperatures on seed quality. This finding is in line with previous research that has shown the effectiveness of heat shock treatments in improving the germination performance of seeds under adverse conditions (Kumar et al., 2016). Overall, these studies highlight the importance of considering storage conditions and seed treatments in order to optimize seed quality and improve crop productivity. By understanding the factors that affect seed germination, researchers and farmers can make informed decisions about storage and treatment methods that can help to ensure successful crop establishment and yield.

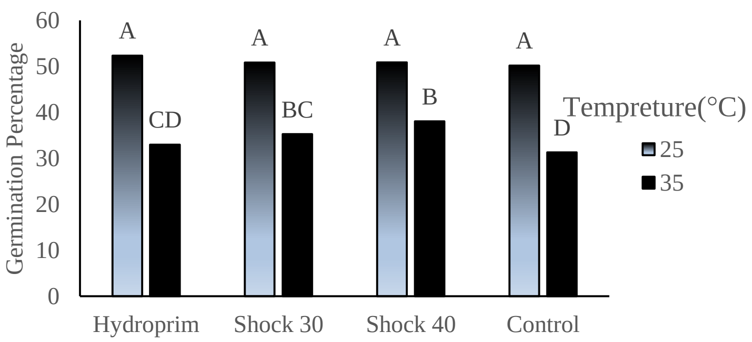

Figure 2: Comparing the average germination percentage of mung bean seed under the influence of priming treatment and heat shock and different storage temperatures (25 and 35 °C) after 30 days of storage. Hydro prim: Hydro prim 15°C for 2 days; Shock 30: Hydro prim 15°C for 2 days and shocked at 30°C for 4 hours; Shock 40: Hydro prim 15°C for 2 days prim and shocked at 40°C for 4 hours.

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3. Interaction effects of storage temperature, moisture, and treatment on germination speed:

The results from Figure 3 revealed that all three factors, storage temperature, moisture level, and priming treatment, had a significant effect on the mean germination speed of mung bean seed. At a storage temperature of 25°C and a moisture level of 10%, the highest mean germination rate was observed for the shock 40 treatment (0.24 seeds/day), followed by hydro prim (0.18 seeds/day) and shock 30 (0.17 seeds/day). At a storage temperature of 35°C and a moisture level of 8.5%, the highest mean germination speed was observed for the shock 40 treatment (0.26 seeds/day), followed by hydro prim (0.25 seeds/day) and shock 30 (0.24 seeds/day). The findings align with a study conducted by Li et al. (2018) who found that seed priming significantly enhanced the germination rate and mean germination time of mung bean under low-temperature storage conditions. Another study by Khan et al. (2016) found that low-temperature storage combined with seed priming significantly increased the germination rate and speed of mung bean seeds compared to untreated seeds. These studies support the idea that seed priming can improve seed germination under adverse storage conditions and that temperature and moisture levels play a significant role in the effectiveness of priming treatments. Additionally, the finding that the shock 40 treatment was the most effective at enhancing mean germination speed of mung bean seed at both storage temperatures and lower moisture levels is consistent with previous research studied by Chen et al. (2019) found that mung bean seeds subjected to heat shock treatment at 40°C for 24 hours had higher germination rates and shorter mean germination times compared to untreated seeds. Overall, the results from Figure 3 highlight the importance of optimizing storage conditions and selecting the appropriate priming treatment to enhance seed germination speed. Overall, the results suggest that the priming treatment had a positive impact on the mean germination rate of mung bean seed after 30 days of storage, particularly at a lower storage temperature of 25°C and lower moisture levels. The shock 40 treatment was found to be the most effective at enhancing the mean germination speed of mung bean seed at both storage temperatures and lower moisture levels. These findings highlight the importance of considering multiple factors, such as storage temperature, moisture level, and priming treatment, when attempting to optimize seed germination speeds under adverse storage conditions.

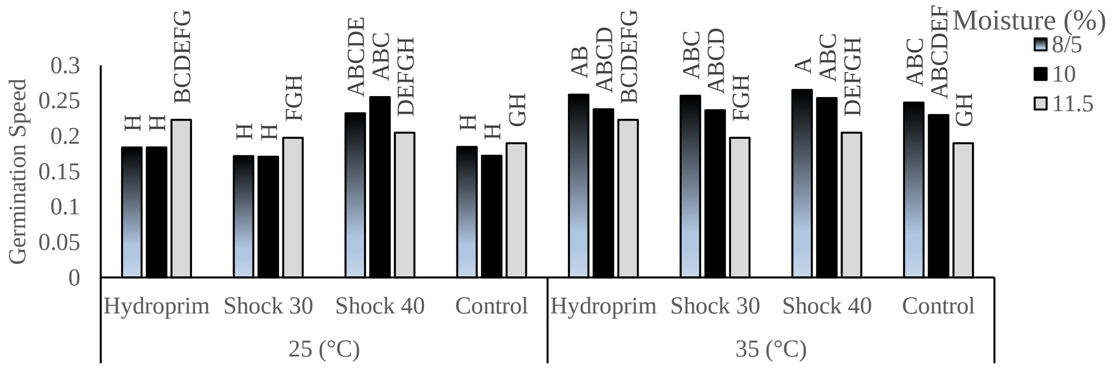

Figure 3: Comparing the mean germination rate of mung bean seed under different treatments of priming and heat shock and different storage temperatures (25 and 35 °C) and moisture levels (8.5, 10 and 11.5%) after 30 days storage. Hydro prim: Hydro prim 15°C for 2 days; Shock 30: Hydro prim 15°C for 2 days and shocked at 30°C for 4 hours; Shock 40: Hydro prim 15°C for 2 days prim and shocked at 40°C for 4 hours.

4. Interaction effects of treatment and moisture on seedling length:

A total of 500 mung bean seeds were stored under two different storage temperatures (25 and 35 °C) for 30 days, and their seedling length was measured. The results showed that the mean length of mung bean seedlings significantly decreased with an increase in storage temperature (Figure 4).

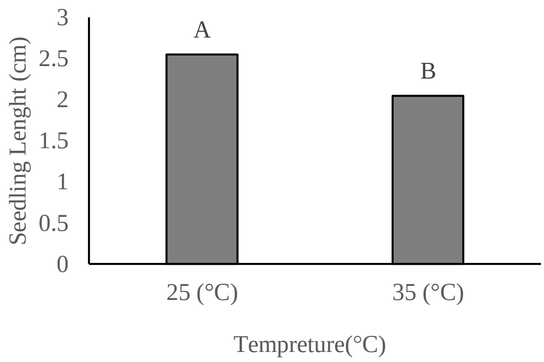

Figure 4: Comparing the mean length of mung bean seedlings under different storage temperatures (25 and 35 °C) after 30 days storage.

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The mean length of seedlings stored at 25 °C was 2.24 cm, which was significantly higher than the mean length of seedlings stored at 35 °C (2.04 cm). The difference in mean length between the two temperature treatments was statistically significant ($p<0.05$). The results from this study are consistent with previous research that has demonstrated the negative effects of high storage temperatures on seedling growth and development (Ali et al., 2017; Mirali et al., 2019). These findings suggest that storage at high temperature (35 °C) has a detrimental effect on the growth and development of mung bean seedlings. The interaction results of treatment and moisture showed that the mean length of seedlings significantly increased with the hydropriming treatment compared to the non-primed control group (Figure 5).

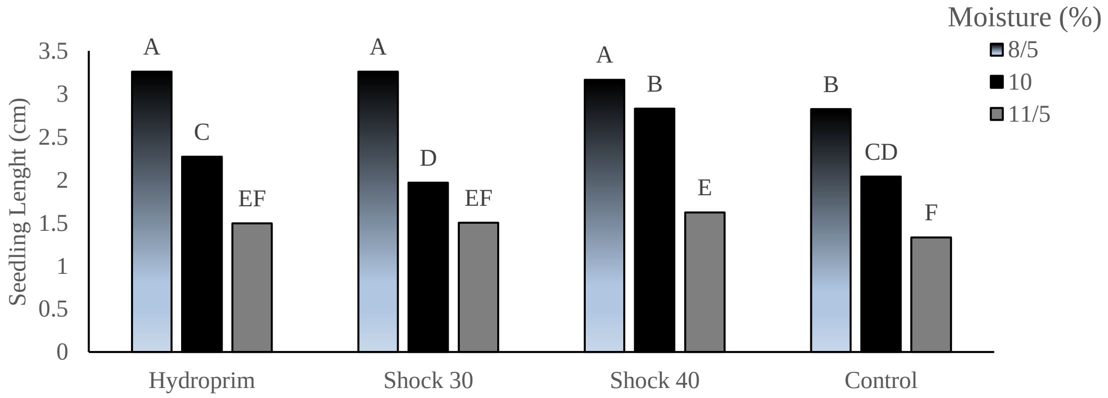

Figure 5: Comparing the mean length of soybean mung bean under the influence of priming and heat shock treatments and different moisture percentages (8.5, 10 and 11.5%) of seeds during storage period after 30 days of storage. Hydro prim: Hydro prim 15°C for 2 days; Shock 30: Hydro prim 15°C for 2 days and shocked at 30°C for 4 hours; Shock 40: Hydro prim 15°C for 2 days and shocked at 40°C for 4 hours.

The mean length of seedlings after hydropriming treatment and minimum moisture level (8.5%) (3.2 cm) was significantly higher than the control group (2.8 cm) ($p<0.05$). Additionally, heat shock treatments (shock 30 and shock 40) also significantly increased the mean length of seedlings compared to the non-shocked control group ($p<0.05$). Moreover, the mean length of seedlings significantly decreased with an increase in moisture percentage. The mean length of seedlings stored at 8.5% moisture was significantly higher (3.2 cm) than the mean length of seedlings stored at 10% moisture (2.3 cm) and 11.5% moisture (1.3 cm) ($p<0.05$). These findings suggest that hydropriming and heat shock treatments can improve the growth and development of mung bean seedlings, while high moisture levels during storage can have a negative impact on seedling length. In addition, the positive effects of hydropriming and heat shock treatments on seedling growth have been reported in previous studies (Khan et al., 2016; Zheng et al., 2019). The finding that high moisture levels during storage can have a negative impact on seedling length is also consistent with previous research (Farooq et al., 2012). It is important to note that the specific optimal conditions for mung bean seed germination and seedling growth may vary depending on the cultivar, environmental conditions, and storage duration. However, the overall importance of considering multiple factors, such as storage temperature, moisture level, and treatment, in optimizing seed germination and seedling growth is supported by the findings of this study and previous research. Overall, this study contributes to the growing body of knowledge on seed storage and germination and highlights the potential benefits of using hydropriming and heat shock treatments to enhance seedling growth under adverse storage conditions. Further research is needed to explore the underlying mechanisms of these treatments and to identify the optimal conditions for different cultivars and storage durations.

5. Interaction effects of treatment and moisture on seedling dry weight:

It can be seen from Figure 6 that the mean dry weight of seedlings stored at 25 °C was significantly higher than those stored at 35 °C ($p<0.05$). The mean dry weight of seedlings stored at 25 °C was 0.52 g, while that of seedlings stored at 35 °C was 0.40 g. The results of this study are consistent with previous research that has shown the negative impact of high storage temperatures on seedling growth and development. For example, a study by Farooq et al. (2012) found that storage at 35 °C reduced the growth and vigour of mung bean seedlings compared to storage at lower temperatures. Similarly, a study by Hussain et al. (2016) showed that storage at high temperatures (40 and 50 °C) led to a significant reduction in the growth and yield of mung bean plants. These findings suggest that storage at 25 °C may be more favourable for preserving the dry weight of mung bean seedlings compared to storage at 35 °C. The results of the study show that the mean dry weight of mung bean seedlings is significantly affected by the interaction of priming treatments and heat shock treatments and different moisture levels during storage

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($p<0.05$) and is supported by previous research. For instance, a study by Farooq et al. (2008) found that hydropriming improved the germination, growth and yield of mung bean under drought stress conditions. Another study by Chen et al. (2019) showed that heat shock treatments enhanced the growth and yield of mung bean plants by improving their antioxidant defence system. The highest dry weight was recorded in the hydropriming treatment with 8.5% moisture content (0.65 g/plant) followed by hydropriming treatment with 10% moisture content (0.45 g/plant) and hydropriming treatment with 11.5% moisture content (0.29 g/plant). The lowest dry weight was recorded in the control treatment with 11.5% moisture content (0.23 g/plant) (Figure 7).

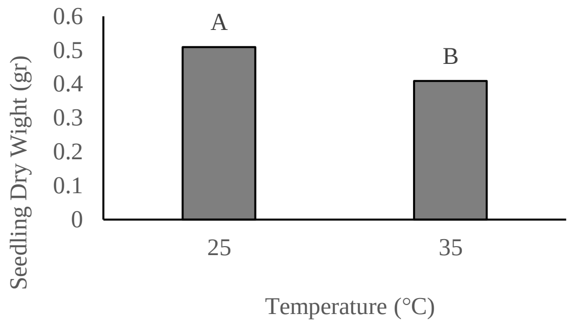

Figure 6: Comparing the mean dry weight of mung bean seedling under different storage temperatures (25 and 35 °C) after 30 days storage.

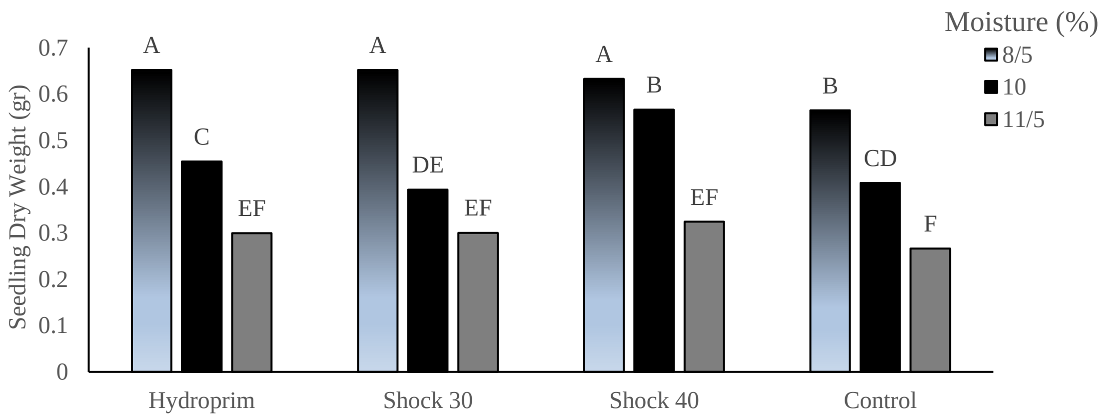

Figure 7: Comparing of mean dry weight of mung bean seedling under priming and heat shock treatments and different moisture percentages (8.5%, 10% and 11.5%) of seeds during storage period after 30 days of storage. Hydro prim: Hydro prim 15°C for 2 days; Shock 30: Hydro prim 15°C for 2 days and shocked at 30°C for 4 hours; Shock 40: Hydro prim 15°C for 2 days prim and shocked at 40°C for 4 hours.

IV. CONCLUSION

In conclusion, the present study investigated the effects of hydro priming and heat shock on the germinating indices of mung bean seeds under different storage conditions. The results showed that hydro priming and heat shock can significantly improve the germination and growth of mung bean seeds. These treatments can enhance the seedling growth potential and overcome the negative effects of storage. However, the effectiveness of these treatments varied depending on the storage conditions. Different storage conditions can affect the germinating indices of mung bean seeds differently, and thus should be taken into account when applying these treatments. Hydro priming not only did not reduce seed shelf life but also increased storage time and improved seed viability during storage under different temperature and moisture conditions. Hydro priming treatment improved seed germinating indices in green mung beans. Since hydro priming treatment is an easy method, it is low-cost and free risk. It has been used as an effective strategy to increase the mean and percentage of germination, the speed of germination and the emergence of seeds, and the quantitative and quality improvement of the crop under unfavourable conditions.

AUTHOR CONTRIBUTIONS

Zabihullah Farid and Mohammad Wasif Amin designed the study, conducted the experiments, and analyzed the data. Mohammad Wasif Amin wrote the manuscript. Hamidullah Younisi provided critical feedback and contributed to the manuscript revision. Khalid Joya, Abdul Alim Osmani and Hakimullah Amini assisted with data visualization and provided technical support. All authors contributed to the interpretation of the results and approved the final version of the manuscript.

CONFLICT OF INTEREST

No potential conflict of interest was reported by the authors.

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