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RESPONSE OF MUNG BEAN (*VIGNA RADIATA* L.) SEED TREATED WITH GIBBERELLIN UNDER WATER-STRESS CONDITIONS

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SUMMARY

The latest experiment on mung bean (*Vigna radiata* L.) materialized during the spring of 2023 in the laboratories of the Department of Horticulture and Landscape Gardening, College of Agriculture, University of Kufa, Iraq. The study aimed to improve mung bean seed germination under water-stress conditions. It employed a completely randomized design (CRD) by organizing two factors with three replications. The first factor included water-stress treatments by adding Polyethylene glycol solution (PEG 6000) to the mung bean seeds, with three levels (0, -8, and -12 bar). The second factor included seed soaking for 24 h in three different concentrations of gibberellin acid (0, 125, and 250 mg L⁻¹). The results revealed that water stress significantly affected the properties of mung bean seedlings. The -8 bar water stress reduced the average seed germination ratio of mung bean (85.89%), root and plumule lengths (2.84 and 3.50 cm), seedling dry weight (0.010 mg), and strength (551.96). The gibberellin concentration of 250 mg L⁻¹ showed superiority in all traits. The interaction of stress treatments significantly affected the studied traits. The mung bean seed achieved a germination percentage of 100% when exposed to G3 treatment (250 ppm) compared with the treatment PEG-0, giving the lowest mean (93.67%). Therefore, the study concluded that gibberellin could better help in mung bean seed germination under drought conditions and increase the number of seedlings.

Keywords: Mung bean (*Vigna radiata* L.), PEG 6000, water-stress conditions, gibberellin levels, seed germination, growth traits

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Key findings: Water stress significantly affected the seedlings of mung beans (*V. radiata* L.). The PEG 6000 (-8 Bar) created the water-stress condition considerably, lowering the mung bean seed germination and seedling ratio, root and plumule lengths, seedling dry weight, and strength. However, gibberellin (250 mg L⁻¹) showed a significant superiority for all the above germination and growth traits in mung beans.

INTRODUCTION

The practice of mung bean (*Vigna radiata* L.) seed-soaking technology has emerged, involving seed immersion in water or nutritional solutions and vitamins for a designated duration before planting (Umair *et al.*, 2010). The considered approach is a widely recognized technique essentially regulating seed germination and growth traits. However, inducing a pre-adaptive response to various stresses, such as water scarcity, can augment its efficacy, thereby mitigating the subsequent impairment caused by these stressors in bean fields (Duran *et al.*, 2018). The stress experienced by the plant manifests in several morphological, physiological, anatomical, and biochemical variations, eventually leading to a loss in plant size, reduction in leaf area, and production decline.

Furthermore, this approach also facilitates the production of healthy and genetically uniform seedlings in mung beans, leading to the favorable and prompt establishment of the crop in the field. Additionally, it frequently results in a substantial crop yield, typically without a notable reduction in seed production compared with alternative methods (Zhang *et al.*, 2018). Plants derived from non-soaked seeds possess characteristics that make them easily applicable and cost-effective, rendering them valuable for utilization in dry and semi-arid regions. Consequently, farming communities employ these plants in their agricultural fields, particularly in diverse legume crops, such as mung beans.

Studies of water stress mainly depend upon exposing the barley plants to environments of relatively low humidity by employing some chemical and organic composites to reduce water potentials like Mannitol or Polyethylene glycol (PEG) that work depending on increasing the viscosity of

the water (Skribanek and Tomcsanyi, 2008; Abdul Mohsin and Farhood, 2023). PEG is the most used material in plant tolerance of water-stress tests because it works on reducing water potential. Moreover, it also weakens the *Vigna radiata* plant's capacity to absorb water to the level stimulating natural water stress circumstances (Jisha and Puthur, 2014).

Several studies confirmed mung bean seed sensitivity to water stress during germination, causing a significant reduction in germination and seedling growth ratios. Mung bean seedlings incur water stress effects in labs by using three different levels of water stress, instigating a severe decrease in the germination ratio and root and plumule lengths of the seedlings (Al-Sabagh, 2019). It is evident from contemporary scientific investigations that applying water stress in laboratory settings, such as tissue culture, can succeed by subjecting mung bean plants to environments characterized by low humidity or exposing plant roots to reduced water conditions (Kundu *et al.*, 2017).

Water stress is relieved by controlling irrigation frequency and by employing certain organic chemical compounds that are known to reduce drought stress. Important examples of such substances are polyethylene glycol (PEG), sorbitol, and mannitol, which are widely utilized in mung beans (*V. radiata* L.) (Kumari *et al.*, 2016). Still, since the PEG component is a chemical of great significance, its use in the study of drought is given higher prominence. The material in issue is non-ionic and inert, yet it has a high attraction for water. Moreover, it is harmless to agricultural plants (Lei *et al.*, 2021). Its use in several biochemical and industrial applications follows. The molecule is available in molecular weights of 4000, 600, and 800 g mol⁻¹ among others. Nevertheless, the most common molecular weight of 6000 PEG g mol⁻¹ made it one of the most often used chemicals in green grammes under

drought stress (Anitha *et al.*, 2021). The investigation of plants' ability to withstand water stress commonly proceeds by introducing specific concentrations of water stress to the nutrient media. This approach operates on the principle of water movement from the medium due to the osmotic potential disparity between the internal and external media. The solution concentration is crucial in diminishing the plants' capacity to uptake water, thereby simulating natural water-stress conditions in kidney beans (*Phaseolus vulgaris*) (Wani *et al.*, 2010).

Polyethylene glycol (PEG), alternatively referred to as an osmotic agent, is a non-ionic water polymer that envelops the seed, hence diminishing the rapid infiltration of water into the tissues. Guo *et al.* (2013) research employed PEG compounds to replicate the osmotic pressure conditions. Using this approach assessed the resilience of seedlings under stress by impeding the moisture absorption rate. Plant hormones are crucial in germination, growth, plant improvement, and crop yield, requiring seed germination an effective enzyme system to perform metabolism processes during the germination stage of mung bean seedlings (Kaur *et al.*, 2023). It showed that this enzyme system falls under plant hormones. Gibberellin acid is one of these hormones that cause a boost in the germination process by stimulating hydrolysis enzymes necessary for food degeneration and cell division like alpha-amylase and beta-amylase, in addition to several other enzymes like the protein and ribonucleic (Sponsel, 2003). The presented research probed to reduce the harmful effects of water stress and improve the seed germination of the mung bean under suboptimal conditions.

MATERIALS AND METHODS

The recent experiment on mung bean (*V. radiata* L.) occurred in 2023 at the laboratories of the Department of Horticulture and Landscape Gardening, College of Agriculture, University of Kufa, Iraq. A completely randomized design (CRD) included three replications. The first factor was exposing

mung bean seeds to three levels of PEG 6000 (0, -8, and -12 bar) for developing the water-stress conditions. Preparing the solutions followed the method of Michel and Kaufmann (1973). Selecting undamaged and unpolluted seeds ensured their size was identical, received washing with regular water to remove dust, and then sanitized with 1% sodium hypochlorite liquid for three minutes (Ashraf and McNeilly, 1990). The second factor included seed soaking with three different concentrations of gibberellin liquid (0, 125, and 250 ppm), with the mung bean seeds soaked in the concentrations for 24 h in Petri dishes and dried with a blotting paper. The 25 seeds, arranged in 27 dishes (27 experimental units), had equal dimensions on a particular type of paper according to a folding method. The seeds advanced in the germination room had a 25 °C ± temperature of eight hours of light and 16 hours of darkness, employing a standard neon lamp and 95% humidity (ISTA, 2005).

Studied traits

Standard germination

After seven days of placing the mung bean seeds in the germination room, the conducted test continued by counting the total number of natural seedlings, then calculating the number according to the following equation to get the germination percentage (ISTA, 2005).

$$\text{standard germination test} = \frac{(\text{number of natural seedlings})}{(\text{number of total seeds})} \times 100$$

Root and plumule length (cm)

After carrying out the standard germination test, 10 randomly selected seedlings sustained measuring for the root length using the ruler after cutting the root from the seed and the plumule (ISTA, 2005).

Seedling dry weight (mg)

Ten natural seedlings resulting from the standard test became samples, removing each

seed's seed coat. The seedlings reached placement in a paper bag with holes and then into an electric oven at 65 °C for 24 h. A sensitive electric scale weighed the seedlings to obtain the average seedling dried weight (ISTA, 2005).

Seedling strength

The seedling strength calculation used the following equation (Murthi *et al.*, 2004):

$$\text{Seedling strength} = \text{germination ratio}\% \times (\text{root length} + \text{plumule length})$$

Root ratio to the plumule

The root ratio to the plumule computation had the following formula (ISTA, 2005): Dry weight of the seedlings' root / Dry weight of the plumule.

Statistical analysis

All the recorded data for various parameters underwent analysis by the Genstat program according to the employed design. The average treatment values comparison and separation were according to the least significant differences (LSD_{0.05}) test (Steel and Torrie, 1980).

RESULTS AND DISCUSSION

Standard germination

The results showed a significant influence of the polyethylene glycol (PEG) and gibberellin concentrations and their interaction on the germination percentage of *Vigna radiata* L (Table 1). The PEG concentrations (PEG -8 and PEG -12 bars) cause a significant decline in mung bean germination. The PEG -12 bar treatment reduced the germination percentage to 64.55% compared with the control treatment, which gave an average and highest germination (97.22%). It could be because the water stress triggered a decline in seed saturation of water by increasing water viscosity. It may also be due to inhibiting

alpha-amylase and beta-amylase enzymes inside the seeds with water content decline, as these enzymes play an essential role in seed germination (Yan *et al.*, 2006; Farooq *et al.*, 2009).

Varying gibberellin concentrations also differed in their influence on the average mung bean seed germination. Gibberellin (250 ppm) treatment showed a germination rate of 86.44% in comparison to gibberellin (125 ppm) treatment, which gave the lowest germination ratio (77.11%). The reason is due to the role of gibberellin in the hydrolysis of enzymes like alpha-amylase and other enzymes responsible for germination. Those enzymes are pivotal in decomposing the materials in the endosperm, such as proteins, lipids, and carbohydrates, to elemental materials transformed into cotyledons. In addition, these enzymes also enhance the amino acids, which also have a positive impact on mung bean seed germination. The presented results also conformed to past findings about the humic acid's role in improving the growth characteristics of corn (Abdulameer and Ahmed, 2019).

Significant interactions were noteworthy among the PEG treatments and gibberellin concentrations (Table 1). It indicates the divergence of gibberellin's behavior toward PEG concentrations. The results further revealed that the germination percentage declines when PEG-0 (0 bar) concentration increases to PEG-2 (-12 bar). As a result, the mung bean seeds achieved 100% germination percentage by exposing the seeds to PEG-0 treatment versus the treatment of gibberellin (250 ppm), whereas PEG-3 (-12 bar) treatment with gibberellin (125 ppm) provided the lowest germination percentage (60.67%).

Root length

Results demonstrated a remarkable effect of gibberellin's stimulation of PEG and mung bean seeds on root length (Table 2). The PEG concentrations (-8, -12 bar) caused a considerable decline in the average root length up to 6.35 cm compared to the PEG control

Table 1. Role of gibberellin concentrations in mungbean seed germination (%) under water stress conditions.

Gibberellin concentrations (mg L ⁻¹)	Polyethylene glycol (bar)			Means (%)
	PEG0 (0 bar)	PEG1 (-8 bar)	PEG2 (-12 bar)	
G1 (0 ppm)	93.67	77.00	60.67	77.11
G2 (125 ppm)	98.00	90	64.33	84.11
G3 (250 ppm)	100	90.67	68.67	86.44
Means (%)	97.22	85.89	64.55	

LSD_{0.05} Polyethylene glycol = 1.02, Gibberellin concentrations = 2.38, P × G interaction = 3.65

Table 2. Role of gibberellin concentrations in mung bean's root length (cm) under water-tension conditions.

Gibberellin concentrations (mg L ⁻¹)	Polyethylene glycol (bar)			Means (cm)
	PEG0 (0 bar)	PEG1 (-8 bar)	PEG2 (-12 bar)	
G1 (0 ppm)	4.93	2.15	1.12	2.73
G2 (125 ppm)	7.00	3.00	1.20	3.73
G3 (250 ppm)	7.13	3.37	2.03	4.17
Means (cm)	6.35	2.84	1.45	

LSD_{0.05} Polyethylene glycol = 0.29, Gibberellin concentrations = 0.51, P × G interaction = 0.93

treatment, giving the lowest average length (1.45 cm). The decline may refer to the role of PEG, which reduces the cell division responsible for root elongation (Nida and Hamza, 2018). Alternatively, it could be directly the influence of PEG in inducing an enzyme dysfunction that hampers the water uptake, impeding the photosynthetic process and, subsequently, diminishing the mitotic activity.

Outcomes revealed a significant difference among gibberellin concentrations. The gibberellin concentration (250 ppm) gave the highest average root length (4.17 cm) compared with the control treatment (2.73 cm) (Table 2). The gibberellin acid influences and increases cellular expansion and division. Gibberellin boosts the meristem region and raises the number of dividing cells (Attia and Jadoua, 1999). Alternatively, the observed phenomenon might be due to the influence of gibberellins, which disrupt the process of promoting stem elongation primarily by stimulating cellular division and altering cell wall extensibility.

Notable interaction was also evident among the PEG treatments and gibberellin concentrations (Table 2), indicating the divergence of gibberellin's behavior toward PEG concentrations. Based on this, the root

length declines by increasing the PEG concentration (-12 bar). The PEG operates based on the mechanism of osmotic potential, which removes water from the surrounding medium due to the difference in osmotic potential between the internal and external environments. The effectiveness of PEG in reducing water absorption by plants is also dependent on the concentration of the solution, which simulates the natural response of plants to water stress.

Plumule length

The results enunciated that PEG treatments, gibberellin concentrations, and their interactions significantly affect the plumule length (Table 3). The PEG concentrations (-8, -12 bar) reduced the average plumule length to 4.61 cm and 1.27 cm, respectively, compared with the PEG control treatment (0 bar) (7.8 cm). A reason is the PEG's role in reducing cellular division, which weakens plant growth due to moisture stress conditions. Alternatively, this phenomenon could be because of the impact of polyethylene glycol (PEG) on the disruption of enzyme functions, reducing water intake. As a result, it hinders the photosynthetic process, consequently leading to a decrease in mitotic activity.

Table 3. Role of gibberellin concentrations in mung bean's plumule length under water stress conditions.

Gibberellin concentrations (mg L ⁻¹)	Polyethylene glycol (bar)			Means (cm)
	PEG2 (0 bar)	PEG1 (-8 bar)	PEG0 (-12 bar)	
G1 (0 ppm)	5.34	2.66	1.23	3.07
G2 (125 ppm)	7.33	4.01	1.50	4.28
G3 (250 ppm)	10.11	3.85	1.61	5.19
Means (cm)	7.59	3.50	1.44	

LSD_{0.05} Polyethylene glycol = 0.44, Gibberellin concentrations = 0.71, P × G interaction = 0.44

Table 4. Role of gibberellin concentrations in the dry weight of mung bean seedlings (g) under water stress conditions.

Gibberellin concentrations (mg L ⁻¹)	Polyethylene glycol (bar)			Means (g)
	PEG0 (0 bar)	PEG1 (-8 bar)	PEG2 (-12 bar)	
G1 (0 ppm)	0.031	0.007	0.006	0.014
G2 (125 ppm)	0.048	0.012	0.008	0.022
G3 (250 ppm)	0.063	0.012	0.010	0.028
Means (g)	0.047	0.010	0.008	

LSD_{0.05} Polyethylene glycol = 0.001, Gibberellin concentrations = 0.002, P × G interaction = 0.005

Gibberellin concentrations showed a substantial difference in plumule length (Table 3). The gibberellin (250 ppm) emerged with the maximum average for plumule length (5.22 cm) compared with the lowest value obtained in the control treatment (4.62 cm). The root length may cause the plumule length for the same treatment (Table 2). However, it is plausible to attribute the observed phenomena to the impact of gibberellins, which immensely interfere with the mechanism of facilitating stem elongation through promoting cellular proliferation and modification of cell wall extensibility.

Meaningful interactions manifested among the PEG treatments and gibberellin concentrations (Table 3). It asserts that gibberellin concentrations behave completely differently toward PEG concentrations. It was also visible that the plumule's length declines as the PEG concentration increases. Therefore, gibberellin (250 ppm) and PEG - (0 bar), PEG-1 (-8 bar), and PEG-3 (-12 bar) treatments showed the highest average of plumule length (9.07, 5.80, and 1.49 cm, respectively), whereas PEG-2 (-12 bar) with G-0 exhibited the lowest average of plumule length (1.10 cm).

Seedling dry weight

Results showed that PEG treatments, gibberellin concentrations, and their interaction significantly affected the dry weight of seedlings (Table 4). The PEG concentrations (-8, -12 bar) decreased the average seedling dry weight (0.010 and 0.008 g, respectively), compared with the PEG control treatment (0 bar) that reached 0.47 g. The reason behind the decline of this trait was due to its ratio decline in germination percentage (Table 1), thus reducing the root and plumule length and affecting the seedling dry weight (Tables 2 and 3). It reveals the exposure of mung bean seeds to water stress, which slowed down the growth during the early stages of seedling germination, sprouting with weak seedlings compared with seeds unexposed to water stress, negatively affecting the seedling dry weight (Al-Sabagh, 2019). Alternatively, this effect may be because of the influence of the PEG on the perturbation of enzyme functionality, resulting in a decrease in water consumption. Consequently, the hindrance of the photosynthetic process results in a subsequent decline in mitotic activity.

The treatment of mung bean seeds stimulation by gibberellin concentrations also showed superiority over the control treatment (Table 4). The highest gibberellin concentration (250 ppm) provided increased seedling dry weight (0.028 g) compared with the control treatment (0.014 g). This treatment superiority might be due to its dominance in root and plumule lengths (Tables 2 and 3), with the said effect also reflected in the seedling dry weight. Alternatively, gibberellins widely interfere with encouraging stem elongation by stimulating cellular division and altering cell wall extensibility and may be responsible for the observed occurrence (Baliah *et al.*, 2018; Marwiyah *et al.*, 2021; Mahmoud *et al.*, 2023).

The interaction among the PEG treatments and gibberellin acid concentrations revealed significant differences in seedling dry weights (Table 4). The interaction between PEG-0 with gibberellin (250 ppm) treatments gave the utmost average of seedling dry weight (0.063 g). However, the seedling dry weight declined when the PEG concentration increased to -12 bar. On the other hand, the combination of PEG-2 (-12) with the control treatment G-0 supplied the lowest average of this trait (0.006 g), which might be because of the gibberellin acid's role in reducing the harmful effects of the water-stress condition.

Seedling strength

Results further showed that PEG and Gibberellin concentrations and their interactions considerably affected the seedling strength (Table 5). PEG concentrations (-8, -12 bar) declined the average seedling strength to 551.96 and 188.74, respectively, compared with the PEG control treatment, showing enhanced seedling strength (1,363.44). The decline in seedling strength resulted from the higher PEG concentrations, which affected the biological processes of seeds during germination, also causing a lower average germination percentage (Table 1). Thus, the root and plumule lengths also reduced (Tables 2 and 3), eventually reflected in seedling strength. Alternately, these phenomena refer to the effect of polyethylene glycol (PEG) on the disruption of enzyme functions, reducing

water intake. Consequently, it hampered the photosynthetic process, which, in turn, reduced mitotic activity.

Outcomes revealed the relevant effects of gibberellin acid concentrations on the mung bean seedling strength (Table 5). The seeds soaked in gibberellin (250 ppm) showed superiority over all other concentrations in seedling strength (876.19) compared with the control treatment (491.64). This superiority resulted from the dominance of the said treatment in other traits like germination ratio, root length, and plumule length (Tables 1, 2, and 3). The observed phenomena could also refer to the vital role of gibberellins, which mainly disrupt the process of encouraging stem elongation by stimulating cellular division and altering the cell wall extensibility (Sasidharan *et al.*, 2011).

The interaction among the PEG and gibberellin concentrations also revealed significant differences in seedling strength (Table 5), indicating the divergence of gibberellin treatment behavior toward PEG treatments. It is also evident that seedling strength declines when the PEG concentration increases to PEG (-12 bar). However, exposing the mung bean seeds to PEG0 (0 bar) and gibberellin concentration (250 ppm), they exhibited the maximum average of seedling strength (1,724.00), whereas PEG (-12 bar) with gibberellin concentration (125 ppm) showed the lowest average for the said trait (142.57).

Root and plumule ratio

Results showed significant effects of PEG treatments and gibberellin concentrations on the root and plumule ratio (Table 6). The PEG concentration (-8, -12 bar) lowered the average root and plumule ratio, reaching 0.96 and 1.06, respectively, compared with the control treatment (PEG 0 bar), reaching 0.75. It is a conditioning trait, allowing for continuous root growth and helping their penetration and divaricates under stress; hence, it is notably the principal property measured under stress conditions. These phenomena could also be due to the polyethylene glycol affecting the disruption of

Table 5. Role of gibberellin concentrations in seedling strength of mung beans under water stress conditions.

Gibberellin concentrations (mg L ⁻¹)	Polyethylene glycol (bar)			Means
	PEG0 (0 bar)	PEG1 (-8 bar)	PEG2 (-12 bar)	
G1 (0 ppm)	961.99	370.37	142.57	491.64
G2 (125 ppm)	1404.34	630.9	173.69	736.31
G3 (250 ppm)	1724	654.63	249.95	876.19
Means	1363.44	551.96	188.74	

LSD_{0.05} Polyethylene glycol = 72.15, Gibberellin concentrations = 112.55, P × G interaction = 144.83

Table 6. The role of gibberellin concentrations in mung bean roots and plumule ratio under water stress conditions.

Gibberellin concentrations (mg L ⁻¹)	Polyethylene glycol (bar)			Means
	PEG0 (0 bar)	PEG1 (-8 bar)	PEG2 (-12 bar)	
G1 (0 ppm)	1.10	1.31	1.44	1.28
G2 (125 ppm)	0.55	0.83	0.71	0.69
G3 (250 ppm)	0.62	0.75	1.04	0.80
Means	0.75	0.96	1.06	

LSD_{0.05} Polyethylene glycol = 0.13, Gibberellin concentrations = 0.19, P × G interaction = N.S

enzyme functions, leading to a decrease in water intake. Consequently, it slows down the photosynthetic process, reducing the mitotic activity.

Moreover, the above results refer to the differences among various gibberellin concentrations that give the highest average ratio of root and plumule (1.28) with gibberellin concentration (125 ppm), while the lowest average (0.80) with gibberellin concentration (250 ppm). Gibberellins are plant hormone types that seemed responsible for the observed occurrence. Gibberellins have mainly disrupted the process of encouraging stem elongation by stimulating cellular division and altering the cell wall's extensibility (Keikha *et al.*, 2017; Farhood *et al.*, 2020).

CONCLUSIONS

The mung bean (*V. radiata* L.) seed soaking with gibberellin acid behaved differently in the direction of the PEG water stress. The seed soaking with gibberellin concentration (250 ppm) was more responsive than other concentrations under water-stress conditions

because the said treatment excelled in mung bean germination and other growth-related traits studied for tolerance to water stress.

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