

Original Research Article

Mitigation of Water Stress Damage in Mungbean [*Vigna radiata* L.] by Foliar Application of Proline

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Abstract: The aim of this study was to assess the impact of various levels of water stress, foliar application of proline and their interaction on the performance of mung bean [*Vigna radiata* L.]. A field experiment was carried out during the summer of 2025, in Najaf Governorate [Iraq], under a randomized complete block design with a split plot experimental design and three replications. Relevant main plot treatments were water depletion levels of 50, 60 and 70% of available water, with the subplots having four proline AtF treatments of 0, 50, 100 and 150 mg L⁻¹ foliar. In addition to leaf proline content and peroxidase [POD] activity, plant height, branch count, leaf area, pods plant⁻¹, seeds pod⁻¹, dry weight of the plants and yield of their seeds were measured. The results showed that increasing available water depletion from 50 to 70% caused a significant reduction in most growth traits and yield components; seed yield decreased from 1.64 to 0.69 t ha⁻¹, equivalent to a reduction of approximately 57.9%, while plant dry weight decreased from 58.25 to 40.18 g plant⁻¹. In contrast, foliar application of proline improved a number of the studied traits, and the concentration of 100 mg L⁻¹ was the most effective in terms of mean seed yield, which reached 1.43 t ha⁻¹ compared with 1.18 t ha⁻¹ in the control treatment, representing an increase of approximately 21.2%. The interaction between the two factors also showed that the response of mung bean to proline depended on the severity of water stress, with the 100 mg L⁻¹ treatment recording the highest yield of 1.89 t ha⁻¹ at 50% depletion, whereas the highest yield under severe stress was achieved by spraying at a concentration of 150 mg L⁻¹ and reached 0.77 t ha⁻¹. Increasing stress severity and proline treatments were accompanied by clear changes in proline accumulation and peroxidase activity, indicating the contribution of osmotic regulation and antioxidant defense mechanisms to the plant response to water deficit. The study concludes that foliar application of proline can serve as a supplementary physiological tool for alleviating water stress damage and improving mung bean performance.

Keywords: Mung bean, *Vigna radiata*, proline, water stress, available water depletion, peroxidase, seed yield, osmotic regulation.

INTRODUCTION

Mung bean, scientifically known as *Vigna radiata* L., is one of the most important leguminous crops widely cultivated in many regions of the world. Its strategic importance lies in its high nutritional value as a rich source of proteins, essential amino acids, and vitamins, in addition to its pivotal role in sustainable agriculture through improving soil fertility by fixing atmospheric nitrogen [20]. However, the expansion of this crop's cultivation and productivity faces increasing environmental challenges, foremost among which is water stress, which is classified as one of the most severe abiotic stresses threatening global food security and causing substantial losses to the agricultural sector [13].

A reduction in soil water content causes profound physiological and biochemical disturbances in mung bean plants. Plants respond to the stress by shutting their stomata to minimise transpiration and substantially reduce the amounts of gas being exchanged and the rates of photosynthesis. Moreover, water stress induces an increase of the concentration of reactive oxygen species [ROS], which induces an oxidative stress that damages chloroplast pigments, damages cellular membranes, damages proteins and DNA, and that therefore negatively affects vegetative growth indicators causing a

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decrease in productivity [1]. Plants are forced to adapt in order to deal with these cold-stress environments, especially by the accumulation of low-molecular-weight organic compounds within the body, such as amino acids like proline, known as 'osmotic adjustment'. Proline performs other functions, such as acting as a scavenger for free radicals [ROS scavenger] and a stabiliser of protein and enzyme structure which contribute to protection of cellular membranes from drought induced oxidative damage [11].

In recent years, the management of modern agriculture has commenced to turn to apply physiological techniques that are both environmental-friendly and effective to improve plants' water stress tolerance. One of the most relevant of these approaches is foliar use of biological agents such as exogenous proline. Recent studies have demonstrated that the exogenous application of proline through foliar spraying can stimulate and support endogenous defense mechanisms in stressed plants, improving the efficiency of the antioxidant system, maintaining leaf relative water content, and protecting the photosynthetic apparatus, thereby reducing damage caused by water deficit and improving growth and yield performance [1].

Despite the evident importance of proline in alleviating environmental stresses, determining the optimal foliar application concentrations and specific application times for mung bean plants under varying levels of water scarcity still requires further scientific investigation. Based on the foregoing, this research aims to evaluate the effects of water stress on the morphological, physiological, and yield traits of mung bean plants and to explore the efficacy of different levels of foliar-applied proline as an effective physiological mechanism for alleviating water stress damage, with the aim of recommending the best treatments that ensure sustainable crop productivity under conditions of water scarcity.

MATERIALS AND METHODS

Experimental Site and Design

This summer field experiment was conducted in the fields of Najaf governorate, Iraq in lat. 32°00'00" N and long. 44°19'00" E, at elevations of 32 m a.s.l. The accessible water content for this silty loam soil was 0.17 cm³ cm⁻³, field capacity 0.32 cm³ cm⁻³ and permanent wilting threshold 0.15 cm³ cm⁻³. For the experiment, a randomized complete block design [RCBD] with three replications for each kind was done on a split plot design. Irrigation regimes were given to the main plots, whereas foliar spray treatments were applied to the subplots.

Treatments: The main plots included three irrigation levels based on the depletion of available soil water:

- I₁: 50% depletion [mild stress]
- I₂: 60% depletion [moderate stress]
- I₃: 70% depletion [severe stress]

The subplots received the following foliar application treatments:

- P₀: Control treatment [spraying with distilled water]
- P₁: Proline at a concentration of 50 mg L⁻¹
- P₂: Proline at a concentration of 100 mg L⁻¹
- P₃: Proline at a concentration of 150 mg L⁻¹

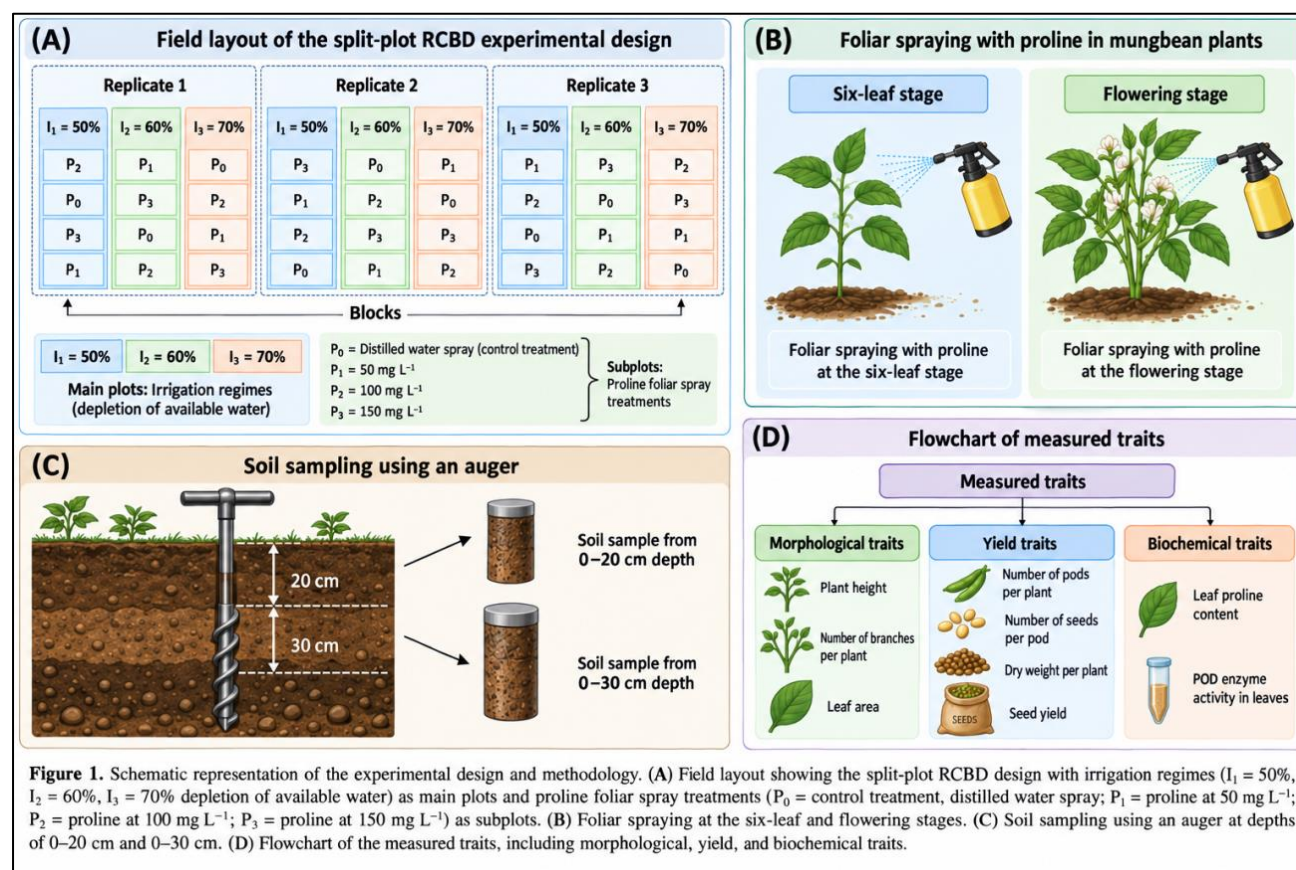
Foliar applications were performed at the six-leaf stage and again at the onset of leaf flowering to ensure that the treatment effect did not diminish.

Crop Management

The mung bean seeds [*Vigna radiata* L., cultivar Al-Khadrawi] were scattered on 1 July 2025, 0.25 m apart between hills. Each experimental unit was 3 × 2 m² and there were four ridges, each one 3 m in length. The germinating seedlings were thinned after one week, leaving only one per each hill; and hills without seedling were replanted. The fertilizer amount applied, weed control and land preparation of all treatments were the same as that applied to the control group. On one hand, a 75 kg P₂O₅ ha⁻¹ application of triple superphosphate fertilizer, which is 46% P₂O₅, was mixed with the soil before planting, while on the other hand, 40 kg N ha⁻¹ of nitrogen fertilizer, in the form of urea, was applied twice: once at planting and again during the flowering stage [2].

Irrigation Scheduling and Soil Moisture Determination

Irrigation was done by a system of irrigation pipes created with plastic pipes and pumps. Before stress they were watered up until the soil water supply was depleted by 50% on all plots. Depletion times of 50%, 60% and 70% and a target that the seedlings should be watered 6-leaves stage were used. Soil moisture for each stage of vegetative development, blooming, and pod-filling was taken gravimetrically [after oven drying to constant weight] to 0-20 cm of soil and to 0-30 cm for the latter two stages, using an auger to collect soil samples. These samples then were heated at 105 °C. In order to find the irrigation depth, the procedure outlined in [32] was followed.



Measurements: Ten randomly selected plants from each plot were used to measure

- Affecting the mature plant size in cm.
- The amount of limbs that a plant has
- Area of leaves [in cm²] when the plant is half blossoming
- Pod density per plant was greater by 75%.
- The number of seeds found in each pod.
- The plant's dry weight [g plant⁻¹] when it blooms
- Dry matter contents of corn grain were assumed to be 14% on a dry matter basis. The moisture content of corn grain for the purpose of yield was assumed to be 14% dry matter.

Biochemical analyses included:

- Leaf proline content [30]
- Peroxidase [POD] activity [31]

Statistical Analysis: A split plot RCBD design [analysis of variance] ANOVA was made with software [GenStat Version 20]. The LSD test [33; 5% probability level] was used.

RESULTS AND DISCUSSION

1-Plant Height

The results in Table 1 showed a significant effect of water stress levels, foliar application of proline, and their interaction on the height of mung bean plants. Mean plant height under the irrigation treatments was 65.16, 57.27, and 42.34 cm at available water depletion levels of 50, 60, and 70%, respectively. This indicates a reduction in plant height of approximately 35.0% when moving from 50 to 70% depletion of available water. The gradual decline in plant height with increasing available water depletion from 50 to 70% can be explained by the reduction in cell elongation and division caused by water deficit as a result of reduced turgor pressure [Turgor Pressure] and the accumulation of reactive oxygen species [ROS] [3].

Regarding proline, mean plant height increased from 52.31 cm in P_0 to 54.62 and 57.47 cm in P_1 and P_2 , respectively, and then decreased to 55.27 cm in P_3 . Thus, the highest mean value was recorded at P_2 [100] mg L⁻¹, reaching

57.47 cm. The increase in plant height resulting from proline application up to 100 mg L⁻¹ may be attributed to the ability of exogenous proline to improve osmotic balance, maintain the relative water content of cells, and protect membranes and proteins against oxidative damage. Proline acts as a protective osmotic solute and is also associated with antioxidant regulation and a reduction in the effects of reactive oxygen species [ROS] [14]. Recent studies have also shown that foliar application of proline can alleviate the negative effects of water stress and maintain plant growth under water deficit conditions [8]. However, the decrease in the mean at 150 mg L⁻¹ compared with 100 mg L⁻¹ may indicate that the response to proline is not necessarily linear with increasing concentration, as there may be an optimal dose beyond which the benefit may not continue or an inhibitory effect may occur. Previous studies have indicated that low and appropriate concentrations of proline can improve stress tolerance, whereas high concentrations may be harmful to some species and under certain conditions [11].

Table 1: Effect of foliar-applied proline under different water stress levels on plant height [cm] in mungbean [*Vigna radiata* L.]

Foliar treatment	I ₁ [50% AW depletion]	I ₂ [60% AW depletion]	I ₃ [70% AW depletion]	Mean
P ₀	63.0	54.71	39.23	52.31
P ₁	65.12	56.14	42.61	54.62
P ₂	68.2	60.21	44.02	57.47
P ₃	64.33	58.0	43.5	55.27
Mean	65.1625	57.265	42.34	
LSD 0.05	Irrigation = 2.02	Foliar = 2.41	Interaction = 4.12	

2-Number of Branches

The results in Table 2 showed significant differences among water stress treatments, the proline treatment, and their interaction. Increasing available water depletion from 50 to 70% reduced the number of branches from 10.58 to 5.97 branches plant⁻¹, a reduction of approximately 43.5%. The large reduction in the number of branches resulting from increased water stress severity may be attributed to the fact that, when exposed to water deficit, the plant reallocates its resources towards maintaining vital parts and reducing vegetative growth, particularly the formation of new branches. Water deficit also reduces photosynthesis and carbohydrate availability, which limits the formation and growth of lateral buds [5], or it may inhibit the growth hormones responsible for branching [9].

In contrast, proline spraying gradually increased the number of branches; the mean increased from 6.86 branches plant⁻¹ in P₀ to 7.80, 8.53, and 9.54 branches plant⁻¹ in P₁, P₂, and P₃, respectively. The highest value was 9.54 branches plant⁻¹ in P₃, and the lowest was 6.86 branches plant⁻¹ in P₀. This agrees with the results of studies on mung bean showing that water stress negatively affects vegetative growth traits [5]. The increase may be attributed to the association of this improvement with the ability of proline to improve tissue water status and enhance photosynthetic efficiency, thereby providing the energy required for branch formation [3]. A recent review explained that exogenous proline can improve growth and tolerance under abiotic stresses by improving photosynthesis, antioxidant activity, and the regulation of osmotic solutes [13]. The results in the table indicate significant differences for the interaction between water stress treatments and the proline spraying treatment.

Table 2: Effect of foliar-applied proline under different water stress levels on number of branches per plant in mungbean [*Vigna radiata* L.]

Foliar treatment	I ₁ [50% AW depletion]	I ₂ [60% AW depletion]	I ₃ [70% AW depletion]	Mean
P ₀	8.6	6.5	5.5	6.86
P ₁	10.4	7.2	5.8	7.8
P ₂	11.0	8.5	6.1	8.53
P ₃	12.33	9.8	6.5	9.54
Mean	10.58	8.00	5.97	
LSD 0.05	Irrigation = 0.82	Foliar = 1.13	Interaction = 1.92	

3-Leaf Area [cm² plant⁻¹]

Leaf area [cm² plant⁻¹]: The results in the table showed a significant effect of water stress levels, foliar application of proline, and their interaction on leaf area. Mean leaf area gradually decreased with increasing available water depletion, with the 50% water depletion treatment [I₁] recording the highest mean of 5254.75 cm² plant⁻¹, which decreased to 4531.25 cm² plant⁻¹ at 60% depletion [I₂] and then to 3564 cm² plant⁻¹ at 70% depletion of available water [I₃]. Thus, the reduction at the most severe stress level reached approximately 32.2% compared with treatment I₁. This reduction can be explained by the fact that water stress lowers water potential and cell turgor, limiting cell elongation, division, and leaf expansion. It also causes stomatal closure and reduces stomatal conductance and the rate of CO₂ fixation, thereby decreasing the amount of carbohydrates available for the formation of new leaf tissues [26]. This is directly supported by the findings reported in [6] for mung bean, where water stress reduced leaf area by approximately 35.4%, accompanied by a 51.8% reduction in the photosynthetic rate.

In contrast, foliar application of proline caused a significant, gradual increase in leaf area. The mean was 4023.66 cm² plant⁻¹ in plants not sprayed with proline [P0] and then increased to 4325, 4594.33, and 4857 cm² plant⁻¹ when sprayed at concentrations of 50, 100, and 150 mg L⁻¹, respectively. Thus, the concentration of 150 mg L⁻¹ increased leaf area by approximately 20.7% compared with the unsprayed treatment. This response may be attributed to the multiple physiological roles of proline as a compatible solute that contributes to lowering osmotic potential and maintaining turgor pressure within cells, in addition to protecting proteins, membranes, and the photosynthetic apparatus and reducing damage caused by reactive oxygen species, allowing cell division, elongation, and leaf expansion to continue under conditions of reduced water availability. This mechanism is supported by evidence indicating the role of proline in osmotic regulation, maintaining membrane stability, and enhancing the antioxidant defense system under stress conditions [21], in addition to its contribution to maintaining leaf expansion under stress conditions regarding the interaction, treatment P3 × I1, i.e., 150 mg L⁻¹ proline with 50% depletion of available water, recorded the highest leaf area of 5586 cm² plant⁻¹, whereas the treatment without proline application under the most severe water stress level, P0 × I3, recorded the lowest value of 3150 cm² plant⁻¹, with a difference of approximately 77.3% in favor of the former treatment, and this can be explained by the ability of proline to improve osmotic regulation and retain water within tissues, support chlorophyll and membrane stability, and reduce oxidative stress. Recent studies have shown the importance of these mechanisms in maintaining mung bean growth under water deficit [27], [6], and [4].

Table 3: Effect of foliar-applied proline under different water stress levels on leaf area [cm² plant⁻¹] in mungbean [*Vigna radiata* L.].

Foliar treatment	I ₁ [50% AW depletion]	I ₂ [60% AW depletion]	I ₃ [70% AW depletion]	Mean
P ₀	4850	4070	3150	4023.66
P ₁	5150	4450	3370	4325
P ₂	5433	4670	3680	4594.33
P ₃	5586	4930	4050	4857
Mean	5254.75	4531.25	3564	
LSD 0.05	Irrigation = 185.0	Foliar = 220.2	Interaction = 312.26	

4-Number of Pods per Plant

The results in Table 4 showed a significant effect of water stress levels, foliar application of proline, and their interaction on the number of pods per plant. Mean pod number decreased from 82.97 pods plant⁻¹ at 50% depletion of available water to 50.16 pods plant⁻¹ at 70% depletion, whereas the difference between 50 and 60% depletion was not significant. The reduction in pod number with increasing water stress severity may be attributed to reduced plant water status and cell turgor and to stomatal closure, with the resulting decline in photosynthesis and the supply of carbon assimilates required for continued reproductive growth and pod formation. Studies on mung bean have indicated that water deficit reduces pod number and yield components, with a clear association between pod number, plant water status, and leaf area [25] and [4].

Foliar application of proline resulted in a significant and gradual increase in pod number, with the mean increasing from 55.25 pods plant⁻¹ in the control treatment to 93.30 pods plant⁻¹ at a concentration of 100 mg L⁻¹. This increase can be explained by the physiological role of proline in improving osmotic balance, maintaining cell water status, protecting membranes and proteins, and reducing oxidative damage, which helps the plant maintain its physiological activity during flowering and pod set. The study in [24] showed that exogenous proline treatment improved plant growth in mung bean, which was reflected in yield components. Previous studies have also shown that exogenous proline can enhance plant tolerance to abiotic stresses through several interrelated mechanisms, including osmotic balance, antioxidant defense, and protection of cellular structures [13].

The interaction between water stress and proline showed that the response of pod number to proline varied with the water level. Treatment I₁P₂ recorded the highest pod number of 110.90 pods plant⁻¹, whereas I₃P₀ recorded the lowest number of 40.33 pods plant⁻¹. Proline at a concentration of 100 mg L⁻¹ also increased pod number at 70% water depletion from 40.33 to 60.67 pods plant⁻¹, indicating the ability of proline to alleviate part of the negative effects of severe water stress.

Table 4: Effect of foliar-applied proline under different water stress levels on number of pods per plant in mungbean [*Vigna radiata* L.].

Foliar treatment	I ₁ [50% AW depletion]	I ₂ [60% AW depletion]	I ₃ [70% AW depletion]	Mean
P ₀	60.44	65.00	40.33	55.25
P ₁	72.33	70.00	48.67	63.66
P ₂	110.9	108.33	60.67	93.3
P ₃	88.21	83.33	51	74.18
Mean	82.97	81.66	50.16	
LSD 0.05	Irrigation = 1.45	Foliar = 1.72	Interaction = 2.88	

5-Number of Seeds per Pod

The effects of water stress, foliar application of proline with water stress interaction on the number of seeds per pod was significant as revealed in the result obtained. However, as the severity of water stress increased, the number of seeds per pod decreased, with mean seed numbers of 8.55 seeds pod⁻¹, 7.25 seeds pod⁻¹ and 6.02 seeds pod⁻¹ for water stress levels I₁, I₂ and I₃, respectively, which represents a reduction of approximately 29.59% from I₁ to I₃. This reduction reflects the sensitivity of mung bean under water deficit stress during flower and pod formation, and seed filling phases and how this affects the water status and plant photosynthetic potential to provide flower and pod vegetative organs the dry matter and carbohydrates needed to sustain continued seed formation. Recent studies on mung bean have shown that water stress reduces the efficiency of physiological processes and production-related traits, including the number of seeds per pod, as a result of reduced photosynthetic efficiency and declining plant water status [28] and [15]. In contrast, proline spraying significantly increased the number of seeds per pod, with the mean increasing from 6.10 seeds pod⁻¹ in P₀ to 6.90, 8.66, and 7.43 seeds pod⁻¹ in P₁, P₂, and P₃, respectively. Level P₂ was the highest, exceeding the unsprayed treatment P₀ by approximately 41.97%. This response can be explained by the physiological role of proline as an important osmoprotectant that contributes to maintaining osmotic balance and cell water content, protecting membranes and proteins, and reducing oxidative damage, in addition to its contribution to maintaining photosynthetic efficiency under water deficit conditions. A recent review concluded that exogenous proline can improve plant resistance to stress by regulating osmotic status, activating the antioxidant system, and maintaining membrane integrity and ionic balance [13]. The importance of proline becomes more evident through the significant interaction between water stress and foliar application. Treatment I₁P₂ recorded the highest value of 10.00 seeds pod⁻¹, whereas treatment I₃P₀ recorded the lowest value of 5.30 seeds pod⁻¹. The effect of proline was also more pronounced under better water conditions; P₂ increased seed number from 7.00 to 10.00 seeds pod⁻¹ at I₁, whereas its effect was smaller at I₃, where the number increased from 5.30 to 6.70 seeds pod⁻¹. The interaction between plant response to proline and water availability is significant because the plant response to the effect of proline varies as a function of the amount of water available. Proline seems to be more beneficial for plant physiological and reproductive functions under moderate water deficit but it was more limiting for seed formation under severe water limitation. This finding is consistent with the results obtained in [15] where it was observed that the level of proline accumulation in mung bean correlates with their retention of water status, physiological performance and productivity in water deficit conditions.

Table 5: Effect of foliar-applied proline under different water stress levels on number of seeds per pod in mungbean [*Vigna radiata* L.].

Foliar treatment	I ₁ [50% AW depletion]	I ₂ [60% AW depletion]	I ₃ [70% AW depletion]	Mean
P ₀	7	6	5.3	6.1
P ₁	8.4	6.5	5.8	6.9
P ₂	10	9.3	6.7	8.66
P ₃	8.8	7.2	6.3	7.43
Mean	8.55	7.25	6.02	
LSD 0.05	Irrigation = 0.48	Foliar = 0.57	Interaction = 0.94	

6-Plant Dry Weight [g plant⁻¹]

An effect of water stress, foliar application of proline and interaction of their effects on dry weight of plants was revealed as observed in Table [6]. The mean dry weight decreased from 58.25 g plant⁻¹ [I₁] to 52.60 g plant⁻¹ [I₂] and 40.18 g plant⁻¹ [I₃] representing a total reduction of approximately 31.03% between I₁ and I₃. This reduction is the result of water deficit's effects in restricting vegetative growth and dry matter production which include reduced water and nutrient uptake with decreasing water content, stomatal closure and reduction in carbon dioxide entry and photosynthetic rate, consequently reducing dry matter production and accumulation. Recent research on mung bean also supports the conclusion that due to their water stress induced effect on water relations, gas exchange and photosynthetic efficiency, the impact of water stress is related to the amount of stress and the plant's physiological characteristics [28, 27, and 12].

On the effect of proline, it produced 48.73 g plant⁻¹ for P₀ and 49.80, 52.50, and 50.33 g plant⁻¹ at P₁, P₂ and P₃ respectively; P₂ had the highest treatment value which was approximately equal to P₀ by 7.73%. This increase could be due to the role of the proline, in controlling the hydration state of the cell, integrity of membranes and proteins, and reducing the oxidation so that a continued cell physiology and dry matter production occurs in times of water stress. The review in [13] explained that exogenous proline plays multiple roles in stress tolerance, including osmotic adjustment, protection of membranes and proteins, and activation of antioxidant systems, which explains its ability to improve growth under unfavorable conditions. This result also agrees with [19 and 23], which found that exogenous treatments promoting drought tolerance improved mung bean dry weight under water deficit by maintaining photosynthetic pigments and water relations and enhancing antioxidant defense. The interaction between the two factors showed that the response to proline varied with stress severity. Treatment I₁P₂ recorded the highest dry weight of 62.40 g plant⁻¹ compared with 54.80 g plant⁻¹ in I₁P₀, whereas the values under severe stress I₃ were 41.20 and 39.30 g plant⁻¹ in P₂ and P₀, respectively. Thus, the effect of P₂ was more pronounced at stress level I₁, whereas the differences among proline treatments narrowed at I₃, indicating that,

at the highest level, water deficit severity became a limiting factor for dry matter accumulation that partially exceeded the compensatory capacity of proline treatment. This result is consistent with the concept that the ability of mung bean to tolerate drought depends on maintaining water status and photosynthetic activity, and that increasing stress severity causes a gradual reduction in growth even in plants with effective defense mechanisms [22] and [15].

Table 6: Effect of foliar-applied proline under different water stress levels on plant dry weight [g plant⁻¹] in mungbean [*Vigna radiata* L.].

Foliar treatment	I ₁ [50% AW depletion]	I ₂ [60% AW depletion]	I ₃ [70% AW depletion]	Mean
P ₀	54.8	52.1	39.3	48.73333
P ₁	55.7	53.1	40.6	49.8
P ₂	62.4	53.9	41.2	52.5
P ₃	60.1	51.3	39.6	50.33333
Mean	58.25	52.6	40.175	
LSD 0.05	Irrigation = 0.62	Foliar = 0.74	Interaction = 1.28	

7-Seed Yield [t ha⁻¹]

The results in Table [7] showed that mung bean seed yield was significantly affected by water stress levels, foliar application of proline, and their interaction. The effect of water stress was more pronounced for this trait than for the preceding traits, with mean seed yield decreasing from 1.64 t ha⁻¹ at I₁ to 1.49 t ha⁻¹ at I₂ and then to 0.69 t ha⁻¹ at I₃, representing a reduction of approximately 57.93% between I₁ and I₃. This reduction confirms that seed yield is the trait most sensitive to worsening water deficit because water shortage affects not only vegetative growth but also the reproductive stages and the formation and filling of pods and seeds. Consequently, the effects of reduced seed number and dry matter accumulate and are directly reflected in final yield. Recent studies on mung bean have shown that water deficit reduces yield by affecting physiological processes and yield components, particularly seed number, pod number, and seed weight [15] and [28].

A recent study also showed that water stress in mung bean can reduce growth, dry weight, and yield components as a result of reduced stomatal conductance and photosynthesis and disrupted water and nutrient uptake, in addition to its effect on seed filling [19]. Regarding proline, treatments P₁, P₂, and P₃ increased mean yield to 1.21, 1.43, and 1.28 t ha⁻¹ compared with 1.18 t ha⁻¹ in P₀. P₂ was the highest, with an increase of approximately 21.19% over the control treatment. This can be explained by the fact that exogenous proline supply may have enhanced the ability of the plant to maintain water balance, cells, and photosynthesis and reduce oxidative damage, which was ultimately reflected in the maintenance of yield components and yield. This mechanism is supported by the findings in [13 and 18], which indicated that exogenous proline contributes to increased stress tolerance through osmotic adjustment, membrane protection, antioxidant activation, and maintenance of plant physiological activity. Recent studies on mung bean also confirm that improving water status and maintaining photosynthetic activity during stress positively affect yield formation and its components [10]. The interaction between water stress and proline was significant, which is important for interpretation; treatment I₁P₂ recorded the highest seed yield of 1.89 t ha⁻¹ compared with 1.50 t ha⁻¹ in I₁P₀, representing an increase of approximately 26.00%, and P₂ also maintained its superiority at I₂, recording 1.68 t ha⁻¹ compared with 1.42 t ha⁻¹ in P₀. However, the response pattern changed under severe stress I₃, where I₃P₃ recorded the highest yield of 0.77 t ha⁻¹ compared with 0.72 t ha⁻¹ in I₃P₂ and 0.62 t ha⁻¹ in I₃P₀. The increase in P₃ compared with P₀ was approximately 24.19%. This means that the level achieving the highest yield under less stressful conditions, namely P₂, was not necessarily the same level recording the highest value under more severe water stress, confirming that the response of mung bean to proline depends on the severity of water deficit. A general explanation for this pattern is that more severe stress produces increased physiological demands for water retention and protection of the reproductive tissues and thus could require the physiological dose of proline to vary with stress severity. Proline is also specifically involved in important functions in reproductive tissues, as indicated by recent reviews on the involvement of proline in maintaining the integrity of reproductive components, regulation of water status, and protection of cellular systems during situations of stress [13]. This finding is relevant for practice because mung bean is a rather vulnerable crop to water stress at the period related with pod development and filling. Treatment promoting stress tolerance should therefore be used in relation to the intensity and occurrence of water stress and not be the same treatment level for all water stress.

Table 7: Effect of foliar-applied proline under different water stress levels on seed yield [t ha⁻¹] in mungbean [*Vigna radiata* L.].

Foliar treatment	I ₁ [50% AW depletion]	I ₂ [60% AW depletion]	I ₃ [70% AW depletion]	Mean
P ₀	1.50	1.42	0.62	1.18
P ₁	1.58	1.39	0.66	1.21
P ₂	1.89	1.68	0.72	1.43
P ₃	1.59	1.50	0.77	1.28
Mean	1.64	1.49	0.69	
LSD 0.05	Irrigation = 0.14	Foliar = 0.09	Interaction = 0.16	

8-Leaf Proline Content $\mu\text{mol g}^{-1}$ FW

Table 8. Leaf proline content [$\mu\text{mol g}^{-1}$ FW]: The results showed that proline content in mung bean leaves was clearly affected by water stress levels, foliar application of proline, and their interaction. Increasing available water depletion from 50 to 70% increased mean proline content from 2.65 to 3.18 and then to 3.94 $\mu\text{mol g}^{-1}$ FW in treatments I1, I2, and I3, respectively, representing an increase of approximately 48.6% in I3 compared with I1. The differences among stress levels exceeded the LSD value of 0.24. This increase can be explained as an adaptive response of mung bean to reduced water potential, as proline acts as a compatible solute that contributes to osmotic lowering and the maintenance of cell turgor and membrane and protein stability, in addition to its role in limiting damage caused by reactive oxygen species. This result closely agrees with the findings in [4] for mung bean, where more drought-tolerant cultivars were associated with greater proline accumulation and better maintenance of water content and membrane stability. The study in [29] also found that exposing mung bean seedlings to water deficit stimulated proline accumulation and the antioxidant system, while [28] showed that low soil moisture clearly increased proline content in different mung bean genotypes as a result of its role in osmotic regulation

Regarding the effect of proline spraying, the mean increased from 2.70 $\mu\text{mol g}^{-1}$ FW in the unsprayed treatment P0 to 3.13, 3.73, and 3.45 in P1, P2, and P3, respectively. The 100 mg L^{-1} treatment [P2] was the highest, with an increase of approximately 38.3% compared with the control treatment, whereas the value decreased numerically when the concentration increased to 150 mg L^{-1} ; nevertheless, the difference between P2 and P3 was only 0.28. The better response at 100 mg L^{-1} may reflect the plant reaching an appropriate concentration that supports osmotic balance and water retention and maintains cellular functions, whereas increasing exogenous proline beyond the appropriate level may not achieve a parallel increase in endogenous proline because of feedback regulation of its synthesis and utilization pathways. In relation to the physiology interpretation of the present study [16], a recent study on the exogenous proline application under drought stressed conditions demonstrated that the exogenous application of proline contributed to an increase in its endogenous content, stabilization of the water status, and decrease in the oxidative damages accumulation within the seeds [16].

Regarding the interaction between the two factors, the highest numerical value [4.45 micromole g^{-1} FW] was obtained for the treatment [P2 $\times$ I3], which was a combination of 100 mg L^{-1} proline and 70% low available water, the other treatment [P0 $\times$ I1] was inferior with a value of [2.10 micromole g^{-1} FW] with a gap of closely 111.9%. An increase in stress severity caused the formation of prolines within the body of the plant and spraying at 100 mg L^{-1} resulted in an increase in production. This behaviour is consistent with the view that proline accumulation in mung bean represents an integral drought stress response network, osmotic regulation, water conservation, and antioxidant defence which is independent of the level of exogenous proline application [4] and [17].

Table 8: Effect of foliar-applied proline under different water stress levels on leaf proline content [$\mu\text{mol g}^{-1}$ FW] in mungbean [*Vigna radiata* L.].

Foliar treatment	I ₁ [50% AW depletion]	I ₂ [60% AW depletion]	I ₃ [70% AW depletion]	Mean
P ₀	2.10	2.65	3.35	2.70
P ₁	2.55	3.05	3.80	3.13
P ₂	3.1	3.65	4.45	3.73
P ₃	2.85	3.35	4.15	3.45
Mean	2.65	3.18	3.94	
LSD 0.05	Irrigation = 0.24	Foliar = 0.29	Interaction = 0.48	

9-Peroxidase Activity

Table 9. Increased water stress severity increased peroxidase activity of leaves of mung bean. The average values shown as per the original table are presented to be 37.83 at i3=50%, 43.13 at i3=60%, and 46.38 at i3=70% which compares to an increase of around 22.6% when subjecting to the highest stress compared with the lowest. This indicates activation of the enzymatic defense system to counteract the oxidative stress accompanying water deficit; drought increases the production of reactive oxygen species, including H_2O_2 , and peroxidase [POD] participates in removing hydrogen peroxide and limiting the oxidation of cellular components. This is supported by recent findings in mung bean; the study in [4] found that more drought-tolerant genotypes showed higher peroxidase activity, along with SOD and CAT, and greater proline accumulation. The study in [29] also showed that drought stimulated SOD, POD, and CAT enzymes in mung bean to counteract the increase in H_2O_2 and lipid peroxidation, while [22] showed that tolerant mung bean genotypes have a more efficient antioxidant system under water stress.

Proline treatments also increased peroxidase activity, with the mean reaching 36.67 in P0 and then increasing to 40.67, 46.67, and 45.78 at 50, 100, and 150 mg L^{-1} , respectively. Thus, the 100 mg L^{-1} treatment achieved an increase of approximately 27.3% compared with no spraying; however, the difference between P2 and P3 was 0.89, indicating no significant difference between these two concentrations. The effect of proline may be attributed to its dual role as an osmotic regulator and a modulator of redox status, as it helps stabilize membranes and proteins and may support the ability

of the antioxidant system to remove ROS. This interpretation agrees with the recent study in [16], which showed that exogenous proline application under drought increased POD, CAT, and SOD activities and reduced H₂O₂ accumulation, noting that this study was conducted on maize and is used here to explain the general mechanism of proline, whereas mung bean-specific evidence confirms the association of drought tolerance with increases in both proline and antioxidant enzymes

The interaction showed a notable nonlinear response; treatment P2 × I3 recorded the highest peroxidase activity of 53.0 compared with 31.5 in P0 × I1, representing an increase of approximately 68.3%, whereas the 150 mg L⁻¹ treatment increased from 43.83 at I1 to 50.5 at I2 and then decreased to 43.0 at I3. This indicates that the enzyme response to proline depends on stress severity rather than concentration alone. The decrease in POD at the highest proline concentration under severe stress may be explained by exogenous proline having reduced the oxidative load or H₂O₂ concentration to an extent that reduced the need for continued high peroxidase activity, or by 150 mg L⁻¹ having exceeded the optimal concentration for this response. Therefore, lower POD activity in this case does not necessarily indicate reduced plant tolerance. This cautious interpretation is supported by published differences in the direction of the POD response in mung bean, with some studies recording an increase under drought, whereas [7] found a decrease in its activity under different drought conditions, confirming that the direction of the response is influenced by stress severity and duration, physiological age, genotypes, and ROS status in the tissues.

Table 9: Effect of foliar-applied proline under different water stress levels on peroxidase [POD] activity in mungbean [*Vigna radiata* L.].

Foliar treatment	I ₁ [50% AW depletion]	I ₂ [60% AW depletion]	I ₃ [70% AW depletion]	Mean
P ₀	31.5	36.0	42.5	36.67
P ₁	35.0	40.0	47.0	40.67
P ₂	41.0	46.0	53.0	46.67
P ₃	43.83	50.5	43.0	45.78
Mean	37.83	43.13	46.38	
LSD 0.05	Irrigation = 2.02	Foliar = 2.41	Interaction = 4.05	

CONCLUSIONS

The results of the study showed that water stress had a clear effect in reducing mung bean growth, physiological performance, and the studied yield traits, as increasing water stress severity reduced the number of seeds per pod, plant dry weight, and seed yield. This is attributed to the fact that water deficit limits water and nutrient uptake and reduces photosynthetic efficiency and dry matter accumulation, negatively affecting the formation of yield components and final yield. In contrast, foliar application of proline had a positive effect on improving the studied traits and limiting the negative effects of water stress, as it contributed to maintaining plant water status and enhancing the ability of the plant to tolerate water deficit, which positively affected growth, seed formation, and yield. The interaction between water stress and proline also showed that the plant response to proline treatments varied with stress severity, indicating that proline efficacy is associated with the level of water availability and that its use can serve as a supplementary physiological means of alleviating the harmful effects of water stress and improving mung bean performance and productivity under water deficit conditions.

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