



RESEARCH ARTICLE

Unraveling the effect of salicylic acid on *Vigna radiata* L. under PEG- induced drought stress

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Abstract

The experiment was conducted on three mungbean genotypes to evaluate the effect of salicylic acid on polyethylene glycol (PEG)-induced drought stress. The drought stress imposes serious constraints on the plant growth, development, and yield attributes while the salicylic acid (SA) alleviates the effect of drought in all three genotypes. The highest level of drought (16% PEG induced) stress decreased plant water status, shoot length and root length, fresh weight and dry weight, nodule number and weight, floral bud per plant, number of pods per plant, and seed weight per plant. More negative values of OP (-MPa) were observed in root and nodules of MH 125 at moderate (8% PEG induced drought) level of stress, leaves of MH 215 genotype at highest level of stress. The seeds protein (12.17%), starch (5.45%), seed fiber (25.26%) decreased maximum in the genotype Asha among all the other genotypes while not much difference was reported in the phosphorus content under drought stress as compared to the control. The application of SA exogenously improves all the parameters in all the genotypes but the effect was pronounced more in the genotype MH 125 than other.

Keywords: Nodules, Mungbean, Salicylic acid, Polyethylene glycol, Floral bud.

Introduction

Mungbean (*Vigna radiata* L.), the most common crop grown mainly in tropical and sub-tropical areas plays an important role in nutritional values not only in developing countries but also in the globe. The demand for food, agricultural practices, industries, and energy has been increasing day by day resulting in water scarcity. The agriculture faces a major water shortage of good quality water for irrigation (IPCC, 2014). Drought caused the most severe and menacing problems in plants all over the world (Nadeem *et al.*, 2019; Dubey, 2021). Addressing global challenges such as water scarcity and salt stress is crucial to guarantee the continued viability of agricultural crops and sustainable food production in the long run (Abobatta, 2020; Khatun

et al., 2021). In plants, drought primarily damages osmotic balance by extending its ends with physiological and metabolic processes and thereby decreasing the water intake, which leads to a loss in growth and production by distorting cell turgor potential (Mohammad-Aghdam *et al.*, 2016; Ozturk *et al.*, 2021). The severity of drought can be measured by a global loss in crop production of about 30% by 2025 compared to a yield and more than 50%, especially in mungbean production (Gaur *et al.*, 2013). A drought stress response varies with plant species, genotype, age of plant, developmental stages, and drought severity (El-Nabarawy, 2016; Majeed *et al.*, 2016). Polyethylene glycol (PEG) is a non-penetrating polymer, osmotically active, non-toxic chemical used to create drought stress (Abeed *et al.*, 2021). Mungbean is well known for its capacity to moderately tolerate drought stress and perform well in low moisture content (Nazran *et al.*, 2019). Water deficit diminishes plant growth and, yield and effect more pronounced at the reproductive stage than the other stages (Yang *et al.*, 2019; Seleiman *et al.*, 2021). Water deficit in plants leads to oxidative stress which results in the production of significant quantities of reactive oxygen species (ROS) like superoxide radical (O_2^-) and hydrogen peroxide (H_2O_2). Salicylic acid (SA) serves as an intrinsic growth controller that controls various physiological processes, including germination, growth, root development, and photosynthesis (El-Nabarawy, 2016, Majeed *et al.*, 2016). Foliar administration of SA in water-deficit stressed plants reduces the adverse effect by

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reducing H_2O_2 accumulation and lipid production (Parveen *et al.*, 2021). SA is well known for the mitigation of adverse effects of stress and for playing a crucial role in altering physiological routes in plants (Shemi *et al.*, 2021, González-Villagra *et al.*, 2022).

Materials and Methods

Experimental Site and Treatment Combination

The present research was conducted on three mungbean genotypes, i.e., Asha, MH 125, and MH 215. The pots were placed under lit conditions using a completely randomized design (CRD) with five replications for each treatment. To avoid fungal contamination, undamaged seeds underwent sterilization with a 0.5% sodium hypochlorite solution and were subsequently washed 3 times with distilled water. Four seeds per pot were shown. The drought stress was using polyethylene glycol (PEG 8%) and (16%). The PEG solution was given after 35 days of sowing (DAS). After 3 days of PEG application, the plants were treated with foliar application of salicylic acid (mM). Wilson and Reisenauer's (1963) nutrient solution was given to the pots to provide nutrition as and when required.

The following treatment combinations were used in the study:

- Control,
- PEG (8%),
- PEG (8%) + SA
- PEG (16%)
- PEG (16%) + SA

The experimental observations were recorded for morpho-physiological and quality parameters under drought conditions by exogenous application of SA (Figure 1).

Growth Parameters

For each treatment, three plants were uprooted randomly, and the extra soil was removed with a soft brush. The root and shoot length of the plants were measured in centimeters.

The fresh weight (FW) and dry weight (DW) of all the genotypes were estimated by uprooting three plants per treatment randomly. The extra soil and moisture content was removed with tissue paper. The fresh weight of leaves, nodules, and roots were recorded for each part and dry weight was measured after oven dry weight (72 hours in an oven at 65°C).

Plant Water Status

Osmotic Potential (Ψ_s)

The osmotic potential of leaves, roots, and nodules was determined by an osmometer using a psychrometric technique. Apically fully expanded 3rd leaf was collected and quenched at ambient room temperature. A small piece of filter paper was quickly soaked in extracted exudate and

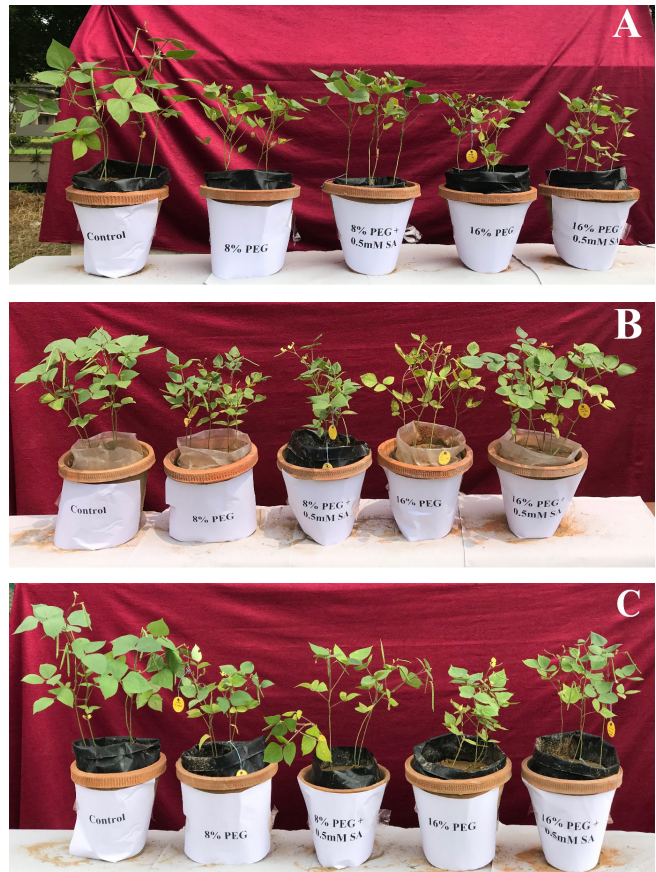


Figure 1: Effect of salicylic acid on the mungbean genotypes A) Asha, B) MH125, C) MH215 under PEG-induced drought stress.

inserted into the sample holder, to keep away from the contact of wet disc on the exterior surface. The sample was inserted in the device and closed the chamber by moving the knob. A beep tone was heard nearly after one minute and the osmotic potential reading ($mmol\ kg^{-1}$) was recorded from the screen. The osmometer was graded by using sodium chloride according to reference standards of osmolarity (Wescor Inc, USA), and the calculations were made:

$$1000\ mMkg^{-1} = 2.5\ MPa$$

$$2.5\ MPa = 25\ bars$$

Relative water content (RWC%)

In the tissues like roots, leaves, and nodules were calculated by using Turner's method (1986). The leaves, roots, and nodules were taken from three plants for each treatment. Then, the FW of all the samples were recorded and the samples were put into a petri dish filled with double-distilled water and incubated for 4 hours. The turgid weight (TW) was noted and then put all samples in an oven for 72 hours and then the DW of samples was noted.

$$RWC\ (\%) = [(F.W. - D.W.) / (T.W. - D.W.)] \times 100$$

Biochemical Parameters

The chlorophyll and carotenoid content were estimated according to the protocol of Wellburn (1994). The leaves

were placed in test tubes containing DMSO and left at room temperature for a duration of 5 hours. Subsequently, the absorbance was recorded at different wavelengths of 665 nm, 645 nm, and 480 nm was measured using a UV-vis spectrophotometer (UV-2450, Shimadzu), with DMSO serving as the blank reference.

Biochemical and Quality Parameters of Seeds

All biochemical (protein, starch, fiber, and phosphorus) parameters of the seeds were recorded using near-infrared (NIR Systems 6500 FOSS©) spectroscopy after harvesting

Sample preparations

Harvested samples were dried and kept at 60°C for at least three days (72 hours) before the estimation of all the parameters. The dried sample was allowed to be brought down the temperature (up to room temperature, 22 ± 3°C) and ground to fine powder, and sieve to ensure a uniform size of the particle. Cross-contamination should be avoided between samples to minimize error. The samples were then scanned using a NIR spectrometer (NIR Systems 6500 FOSS©). From the above samples the protein, starch, fiber, and phosphorus were determined using NIR techniques (Chadalavada *et al.*, 2022), and the experimental results, were exported data in an Excel, text, or PDF file.

Statistical Analysis

The data recorded from the results were analyzed statistically through a two-way analysis of variance (ANOVA). The data presented in the bar graphs represents the means of five replicates (Means ± SE). A post hoc Tukey's honestly significant difference (HSD) test was performed with a significance level of 5% to analyze the significant variations among various treatments and genotypes. Graphs were created using Origin software (Origin Pro 2023b), and the Pearson correlation coefficient was computed.

Results

Root and Shoot Length

Drought stress exerts a substantial adverse impact on the growth and development of crops. The data obtained from the present revealed that the shoot and root length of mungbean genotypes decreased with the increase in the level of drought stress in all the genotypes (Figure 2 A, and B). Not much difference in the shoot length was reported at the mild stress (8% PEG) treatment of drought while at severe stress (16% PEG), the shoot length decreased and was maximum in the genotype MH 125 (46.32%) followed by MH 215 (38.20%) and least in the genotype ASHA (33.85%) when compared to their respective control. Similarly, a decrease in root length was reported in all the mungbean genotypes while the foliar supplementation of salicylic acid to the leaves declined the impact of the drought and increased the root and shoot length under extreme drought. The

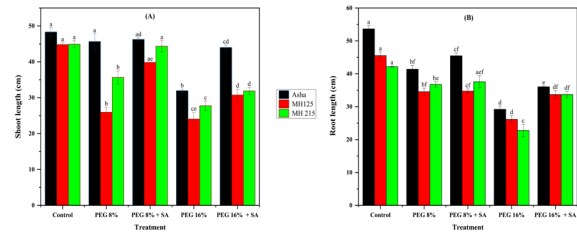


Figure 2: Impact of SA on shoot length (A) and root length (B) in mungbean genotypes under drought stress. Bar with different small alphabets letters is significant statistically among treatments and genotypes ($p < 0.05$).

decrease in root and shoot length of mungbean plants might be attributed to dehydration because under drought stress, proteins are denatured and ROS are produced, reducing the biomass of plants (Farooqi *et al.*, 2020; Kumar *et al.*, 2020; Yang *et al.*, 2021). The present study indicated that an increase in drought stress conditions in consistence with a reduction in the length of the root and shoot (Chavoushi *et al.*, 2020). The root length of plants under moderate drought stress conditions was greater than that of controls since roots were deep down for water, but the length of plant roots decreased under severe drought (Ahmad *et al.*, 2021). The outcomes derived from the experiment align with earlier findings in studies concerning mungbean (Sadeghipour, 2019; Camilo *et al.*, 2021; Zhang *et al.*, 2022), chickpea (Shamsi, 2010; Sujatha and Anuradha, 2019), sesame (Nasibeh *et al.*, 2020), purselane (Saheri *et al.*, 2020). The reason for the decrease in root shoot length might be that PEG disturbs osmotic potential in plants due to which less absorption of water takes place and reduces the shoot length of crop plants (Asati, 2023). However, the exogenous application of SA alleviates the toxic effect of drought stress. The findings of the present research advocated that the foliar application of SA mitigates the toxic effect caused by drought and increases the root-shoot length along with a fresh and dry weight of in all three genotypes at moderate and severe drought stress (Figures 2-4); which are in agreement with the effects of SA on *Carthamus tinctorius* under drought stress (Chavoushi *et al.*, 2020). The impact of SA treatment on plant growth in the presence of drought stress was alleviated through the promotion of photosynthesis and cell division (Pradhan *et al.*, 2016). The current findings align with those of Bijanzadeh *et al.*, (2019), who observed a noteworthy enhancement in growth parameters when supplementing with SA under conditions of drought stress. SA may induce antioxidant responses that increase germination and dry mass in water-stressed plants (Gaafar *et al.*, 2020).

Fresh Weight of Root and Leaves

The fresh weight of all three mungbean genotypes was reduced as a result of drought. All the genotypes experienced a decline in leaves fresh weight and it decreased from 30.8 to 61.74% at mild and severe (16%) stress in the genotype

Asha when compared to the control treatments. A similar decrease in the fresh weight of roots was also reported under mild and severe drought conditions. Among the three mungbean genotypes, MH 215 exhibited the greatest decrease in the root (58.57%), followed by Asha, and the least decrease was observed in MH 125 (54.23%), in comparison to their respective control plants while the foliar application of salicylic acid alleviates the impact of water deficit stress in all the genotypes. Whereas MH 125 experienced the maximum increase in fresh weight of leaves (44.69%) when compared to MH 215 (24.62%) (Figure 3 A, and B). The present research revealed that SA mitigates the toxic effect of drought and increases the root-shoot length and fresh and dry weight of all three genotypes under severe drought stress (Figure 3-4) which are in agreement with the effects of SA on *C. tinctorius* under drought stress (Chavoushi *et al.*, 2020). The effect of drought stress alleviated plant growth with SA treatment, attributed to the promotion of photosynthesis and cell division (Pradhan *et al.*, 2016). The present findings align with Bijanzadeh *et al.*, 2019, who reported a significant increase in growth parameters with SA supplementation under drought stress. SA may induce antioxidant responses that increase dry mass in drought-stressed plants (Gaafar *et al.*, 2020).

Dry Weight of Root, Leaves and Nodules

The present results revealed that drought affects the leaves roots, and nodules while the SA mollifies the impact of drought on the crop plants (Figure 4 A, B, and C). Dry weight decreased at severe stress conditions when compared to their respective control (Figure 4 A, B, and C). The leaves and roots of MH215 showed the highest decrease with (77.50%) and (63.08%), respectively when compared to their respective control in the dry weight at the highest levels of stress when compared to their respective control while Asha genotype experienced the maximum decline (-80%) in the nodules weight under stress conditions. Salicylic acid led to an increase in dry weight across all genotypes, with genotype MH215 exhibiting the highest enhancement in dry weight under severe stress conditions in comparison to drought stress conditions. The dry weight of the nodules, leaves, and roots dropped as the stress level rose during the drought. The results were consistent with early findings in Mungbean (Nazran *et al.*, 2019), grass pea (Talukdar, 2013) and nodules are also affected by the same were found in agreement with (Umamahesh *et al.*, 2018; Kumari and Chakraborty, 2019; Nazran *et al.*, 2019; Amanullah *et al.*, 2022). Significant changes in the growth, fresh and dry weight of shoot and root reported in mungbean that may be related to genotypes' varied susceptibilities to drought stress. The results are consistent with the findings of researchers (Kumar *et al.*, 2020).

Nodules Per Plant and Fresh Weight

The present result revealed that drought stress affects the nodule's number and weight compared to their respective

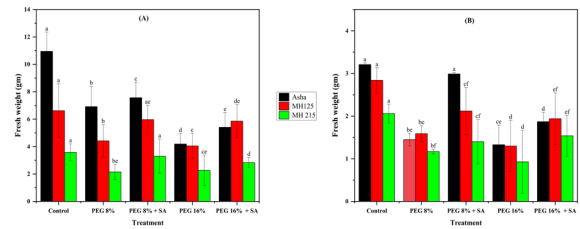


Figure 3: Effect of salicylic acid on leaves fresh weight (A) and root fresh weight (B) under drought stress in mungbean genotypes. Bar with different small alphabet letter is significant statistically among treatments and genotypes ($p < 0.05$).

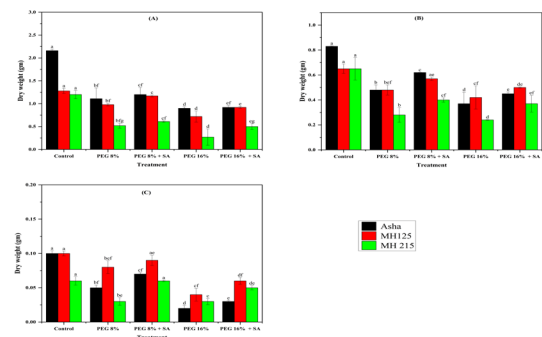


Figure 4: Effect of salicylic acid on dry weight (g) of leaves (A) root (B) and nodules (C) under drought stress conditions in mungbean genotypes. Bar with different small alphabet letters is significant statistically among treatments and genotypes ($p < 0.05$).

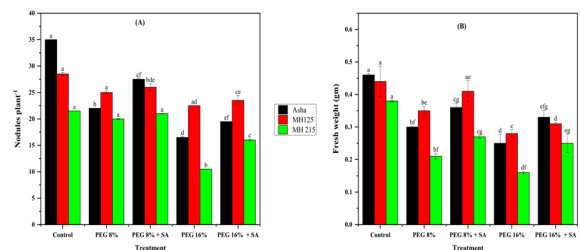


Figure 5: Effect of salicylic acid on nodules number (A) and nodules fresh weight plant^{-1} (B) under drought stress in mungbean genotypes. Bar with different small alphabet letters is significant statistically among treatments and varieties ($p < 0.05$).

control treatment. The genotype MH 215 experienced the most substantial reduction in nodule numbers compared to the control (Figure 5 A, B). Similar decreasing trends in the nodule weight were reported under increased stress in all the genotypes. All genotypes see a reduction in the effects of drought stress when exogenous SA is applied. The nodule number (52.38%) and nodule weight (56.25%) improved in the genotype MH 215 when compared to the severely (16% PEG) stressed plants. Similar results were recorded in different plants (Kumar *et al.*, 2016; Amanullah *et al.*, 2017). Drought changes the structure and weight of nodules in mungbean (Kumari and Chakraborty, 2019; Nazran *et al.*, 2019; Amanullah *et al.*, 2022), chickpea (Istanbul *et al.*, 2022),

soybean (Lumactud *et al.*, 2023) and in some plants, same results were also observed with salt stress (Sehrawat *et al.*, 2019). Drought-alleviating effects were recorded with foliar application of salicylic acid.

Osmotic Potential (Ψ_s) of Roots, Leaves, and Nodules

The findings from the current study indicate that drought stress significantly impacts the osmotic potential of roots, leaves, and nodules in comparison to their respective control treatments. The maximum impact of drought stress was reported in the roots of MH125 genotypes, nodules of Asha, and leaves of MH215 under mild stress. However, foliar supplementation of SA alleviates the determinate effect of drought stress conditions. It was reported that SA supplementation increases the Ψ_s of leaves, roots, and nodules with respective to stress condition alone. The Ψ_s became more negative in the MH125 genotype as compared to the other genotypes (Figure 6 A, B, and C). The maximum increase of 25, 13, and 28% was observed in the osmotic potential of leaves, roots, and nodules of all three genotypes as compared to the stress condition after SA application, respectively. Osmotic and water potential were found to be significantly decreased due to drought stress. Herrera *et al.*, 2022, reported lower osmotic potential in drought conditions, possibly affecting the water availability. A positive turgor pressure is essential for cell growth and stomatal conductance (Hernandez *et al.*, 2021). Turgor pressure can be re-established under drought stress by significantly increasing the osmotic potential (Vicente *et al.*, 2020; Waraich *et al.*, 2020). Seleiman *et al.*, (2021) demonstrate that plants can mitigate the effects of drought stress by enhancing leaf water potential through the accumulation of compatible solutes. These solutes aid in improving osmoregulation in crops when treated with salicylic acid. Kordi *et al.*, (2013) also documented similar findings in *Ocimum basilicum*, suggesting that these observations might be linked to alterations in total solute contents. This supports the notion that salicylic acid (SA) plays a more prominent role in stimulating the accumulation of soluble sugars in plant roots under drought-stress conditions. Hence, SA mitigates the toxic effect of drought stress by maintaining the RWC.

Relative Water Content

The results of this study imply that a water deficit condition exerts a significant adverse effect on the relative water content (RWC%) in the roots, leaves, and nodules as compared to their respective control treatment. Asha genotype experiences the maximum decline under extreme stress in the leaves (39.30%) and nodules (53.20%), whereas MH125 roots (39.49%) show the greatest decrease (Figure 7 A, B, and C). However, the salicylic acid improves the RWC% in leaves, roots, and nodules for all three genotypes under conditions of severe drought stress. The genotype MH 125 showed a maximum increase in RWC% as compared to

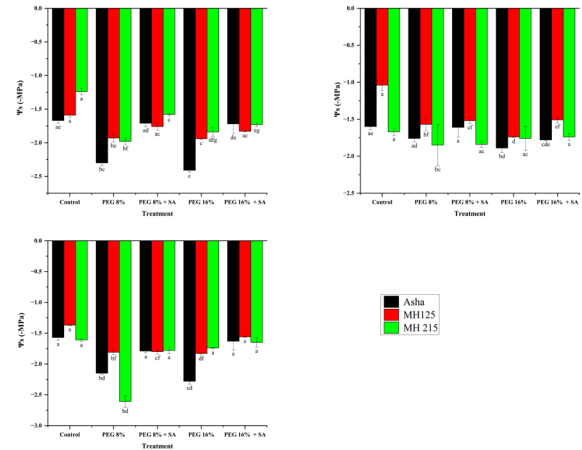


Figure 6: Effect of salicylic acid (SA) on leaves osmotic potential (A) roots osmotic potential (B) and nodules osmotic potential (C) under drought stress in mungbean genotypes. Bar with different small alphabet letters is significant statistically among treatments and varieties ($p < 0.05$).

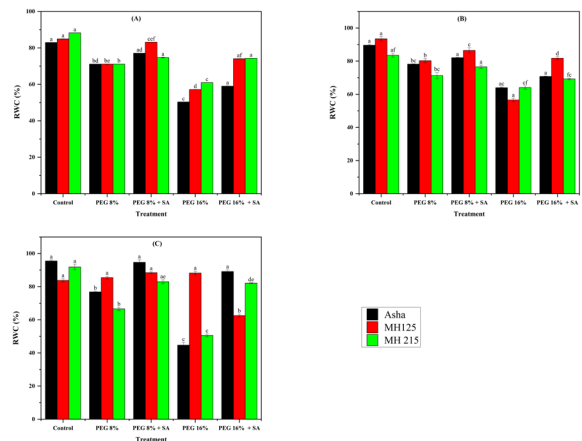


Figure 7: Effect of salicylic acid on relative water content (%) of leaves (A) roots (B) and nodules (C) under drought stress in mungbean genotypes. Bar with different small alphabets letter is significant statistically among treatments and varieties ($p < 0.05$).

Asha, and MH215 genotypes at the highest level of drought stress conditions. SA application alleviates the toxic effect of drought stress by modulating water status. The relative water content of a plant is determined by the combination of water intake from both the leaves and roots, driven by processes such as transpiration (Sadeghipour, 2020). The current study's findings indicate that, in comparison to control plants, all three mungbean genotypes' roots, nodules, and leaves had considerably lower relative water content under drought stress. Similar observations were recorded in *Mentha pulegium*, where drought stress decreases the RWC (Azad *et al.*, 2021). In consistent with our results, Singh *et al.*, (2021) found that SA treatment can enhance RWC by increasing compatible osmolytes which regulate osmotic regulation in wheat plants.

Chlorophyll 'a', Chlorophyll 'b', and Carotenoid Contents

The results revealed that chlorophyll and carotenoid content decreased in all three genotypes as compared to the control plants under drought. Among all genotypes, Asha genotype exhibited the most significant reduction of 38, 66, and 35% in chl 'a', chl 'b' and carotenoid content. The exogenous foliar application of SA increases the chlorophyll, 'b' and carotenoid content in all three genotypes of mungbean when compared to the drought stress condition i.e. mild and severe stress (Figure 8 A, B, and C). The maximum increase in chlorophyll and content was recorded in Asha genotype as compared to other genotypes studied. The photosynthetic pigments (chlorophyll and carotenoid content) declined with an increase in the drought stress. In line with our results, a similar observation has been documented in *C. tinctorius* under drought stress (Hashemabadi, 2019; Chavoushi *et al.*, 2020). A decrease in the soil's water potential affects both chl 'a' and chl 'b'; an essential component of the photosynthetic apparatus (Soufiani *et al.*, 2023). Due to drought stress, there was a decrease in the photosynthetic components, leading to unfavorable growth and plant development. The external application of SA alleviates the detrimental impact of drought stress by enhancing chlorophyll and photosynthetic pigments. Chavoushi *et al.*, (2020), reported that the use of SA resulted in elevated chlorophyll levels in safflower plants under drought stress, as opposed to those solely subjected to drought stress. A similar observation was made under drought stress conditions when SA was applied externally. SA acts as a protective measure for the photosynthetic apparatus against the harmful impacts of drought stress. By stabilizing Rubisco's structure, raising water potential, and lowering oxidative stress, this protection is achieved (Nazar *et al.*, 2015).

Root Length Stress Index and Shoot Height Stress Index

Under the drought stress caused by PEG, the lengths of the shoots and roots decreased. The result reveals that the drought stress conditions decreased the shoot length stress index and root length stress index in all the genotypes. The root length stress index and shoot height stress index decreased up to 37.36 and 34.93% in MH 215 and Asha, respectively, as compared to their respective control plants (Figure 9 A, B). PEG solution-provided osmotic stress conditions were evaluated using growth parameters as growth indices to reflect the relative value. For these growth metrics, different genotypes displayed varying responses (Rasheed *et al.*, 2021). Under the drought stress caused by PEG, the lengths of the shoots and roots decreased. At decreasing water potential, RLSI were significantly reduced, and as water potential declined more, the loss in seedling length happened gradually (Jincy *et al.*, 2021). In response to PEG-induced drought stress, both RLSI and PHSI declined (Geilfus, 2017; Moustafa *et al.*, 2020; Anitha *et al.*, 2022). However,

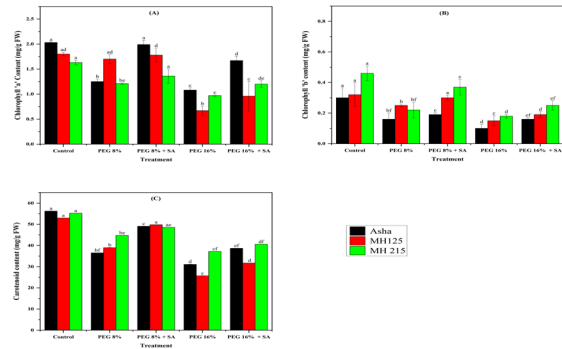


Figure 8: Effect of salicylic acid on chlorophyll 'a' (A) chlorophyll 'b' (B) and carotenoid (C) content under drought stress in mungbean genotypes. Bar with different small alphabet letter is significant statistically among treatments and genotypes ($p < 0.05$).

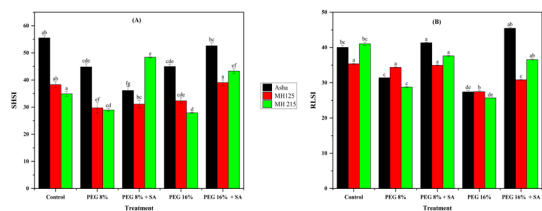


Figure 9: Effect of salicylic acid on shoot height stress index (A) root length stress index (B) under drought stress in mungbean genotypes. Bar with different small alphabet letters is significant statistically among treatments and varieties ($p < 0.05$).

exogenous supplementation of SA mollifies the toxic effect of drought stress conditions and improves the PHSI and RLSI decrease in response to PEG-induced drought stress.

Number of Floral Buds and Pods Plant⁻¹

Drought stress had a determinate impact at the harvesting stage of the plants and decreased the yield by decreasing the number of floral buds and pods plant⁻¹. The present study revealed that water deficit conditions reduced the number of floral buds and pods at both levels of drought stress, i.e., moderate and severe stress (Figure 10 A, B). The number of floral buds plant⁻¹ in the control condition was 21, 28 and 21 in the Asha, MH125 and MH215 genotypes, respectively. The number of floral buds decreased to 15, 22 and 20 in Asha, MH125 and MH215 genotypes, respectively at severe drought stress. However, the externally applied SA under drought-stressed plants, the number of floral buds increased to 16, 24 and 21 in the Asha, MH125, and MH215 genotypes as compared to severe drought-stressed plants (Figure 10 A, B). Similarly, the pod number in the control treatment was 17, 24 and 20 in the Asha, MH125, and MH215 genotypes, respectively. The number of pods per plant decreased with increased drought stress in all the genotypes. The maximum decrease was reported in the genotype Asha among all the other genotypes studied. However, the externally applied SA increased the number of pods per plant under drought stress conditions (Figure 10 A, B). Our results are in consistent with previous reports in mungbean (Hussain *et al.*, 2021) which is a

water-sensitive crop and its yield decreased when subjected to moisture-deficit conditions. The yield of mungbean is affected by a number of factors, which encompasses chlorophyll content, plant height, RWC, pods per plant, and the seeds per pod (Kanavi *et al.*, 2020; Bouzroud *et al.*, 2023). In the current research experiment, drought stress had an adverse impact on all three genotypes concerning various morpho-physiological and biochemical parameters, ultimately leading to a reduction in both yield and quality attributes. Drought elevates the rates of flower shedding and pod abortion (Lamin-Samu *et al.*, 2021) and also has a detrimental effect on the yield, primarily because of a reduction in the number of floral buds plant⁻¹ and the total buds plant⁻¹. However, in the present experimental results found that SA application increases the number of floral buds and pods per plant and improves the quality parameters of the seed. Similar results were obtained in *Syzygium cumini* where SA improves the quality attributes of Jamun seeds (Saurabh *et al.*, 2019).

Seed Protein and Starch Content

The current research revealed that insufficient water conditions had a negative impact on seed protein and starch contents (Figure 11 A, B). The protein content decreased to 12.17, 6.79, and 11.97% in Asha, MH 125, and MH 215 genotypes, respectively, at severe stress. A similar decreasing trend was repeated in starch content under drought stress in all the genotypes. However, the exogenously applied foliar SA improves the protein and starch content when compared to the stressed plant in all the genotypes. The SA improves the protein content by 5.51, 3.23, and 2.21% in Asha, MH 125, and MH 215, respectively when compared to drought-stressed plants. Similarly, starch content was decreased under drought-stress conditions in comparison to the control, the maximum decrease was reported in the genotype Asha (5.45%) followed by MH215 (4.86%) and least in MH215 (3.97%) under severe drought when compared to the control plants. Comparing drought-stressed plants to those treated with salicylic acid topically, the starch content increases. In Asha, MH125, and MH215, the foliar application of SA increases the starch content by 2.14, 1.89, and 0.89%, respectively, in comparison to the plants that are severely drought-stressed. Drought stress reduces the amount of endosperm and stored starchy resources, which lowers the weight of a hundred grains because of diminished sources, followed by the reservoir. Under drought, the starch content decreases in mungbean (Jha *et al.*, 2023), common bean (Gebeyehu *et al.*, 2011), rice (Fan *et al.*, 2019), lupin (Khalil and Ismael, 2010). Similar results were observed in the case of grain protein in legumes due to drought (Mohammadzadeh *et al.*, 2011; Farooq *et al.*, 2018). Drought reduces the yield of total protein, in mungbean and chickpeas (Mansourifar *et al.*, 2011; Yagoob and Yagoob, 2014).

Seed Fiber and Phosphorous Content

The outcomes of the current study demonstrated that water deficiency conditions had an invariable effect on seed fiber

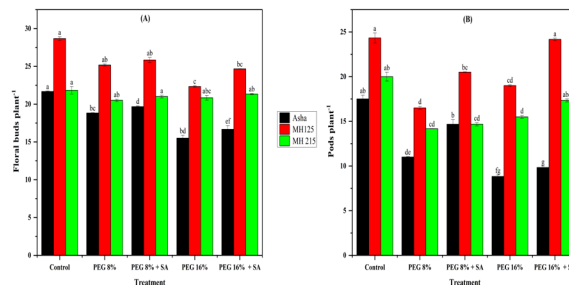


Figure 10: Effect of salicylic acid on number of floral buds plant⁻¹ (A) number of pods plant⁻¹ (B) under drought stress in mungbean genotypes. Bar with different small alphabets letter is significant statistically among treatments and varieties (p < 0.05).

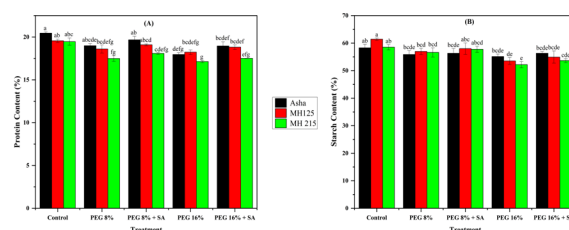


Figure 11: Effect of salicylic acid on seed protein content (A) and starch content (B) under drought stress conditions in mungbean genotypes. Bar with different small alphabets letter is significant statistically among treatments and genotypes (p < 0.05).

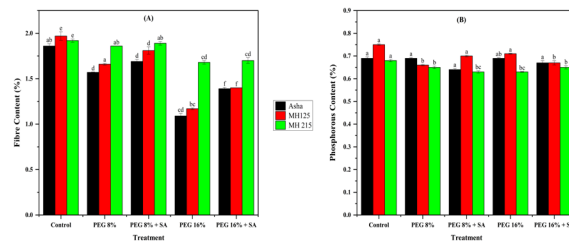
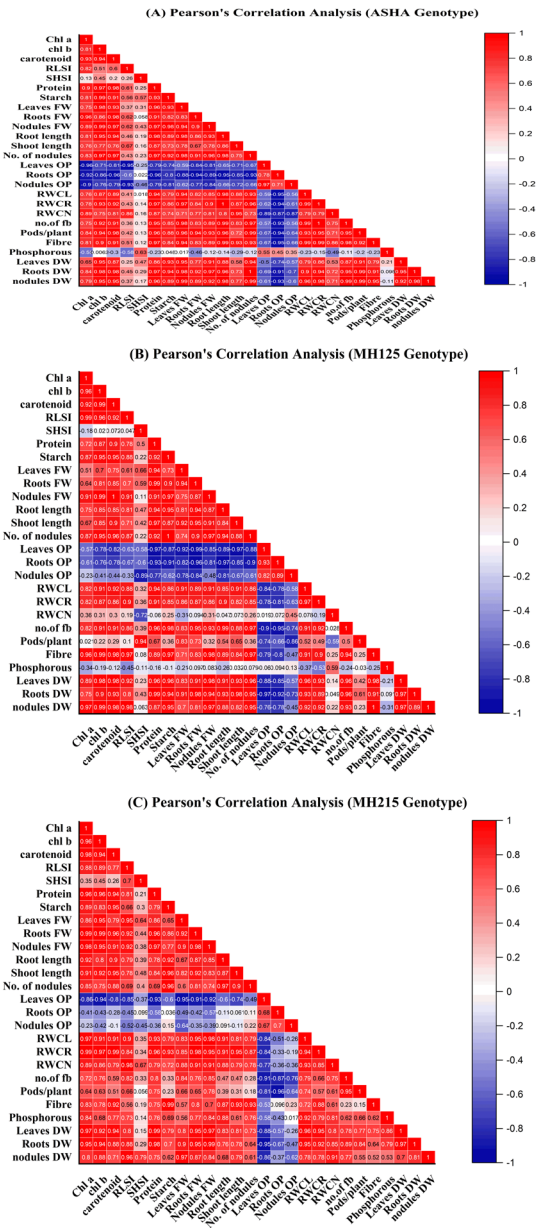


Figure 12: Effect of salicylic acid on seeds fiber content (A) and phosphorous content (B) under drought stress in mungbean genotypes. Bar with different small alphabets letter is significant statistically among treatments and varieties (p < 0.05).

and phosphorous contents (Figure 12 A, B). The present findings showed that seed fiber and phosphorus content was decreased under drought stress in all three genotypes. The fiber content decreased maximum in the genotype Asha (25.26%) and least in MH215 (12.50%) as compared with the control plant. A similar decreasing pattern was reported in the phosphorus content under drought stress. Our result reveals that phosphorus, a vital component of crops, is another mineral that is negatively impacted by drought stress. Similar findings for phosphorus were observed in common beans (Ghanbari *et al.*, 2015; Smith *et al.*, 2019). However, exogenous supplementation of SA improves the seed fiber and phosphorus content under drought stress in all three genotypes of mungbean. The maximum increase



(* $p < 0.05$, ** $p < 0.01$, *** and $p < 0.001$). Abbreviations used: Chl a: Chlorophyll a; Chl b: Chlorophyll b; RLSI: Root length stress index; SHSI: Shoot height stress index; Leaves FW: Leaves fresh weight; Roots FW: Roots fresh weight; Nodules FW: Nodules Fresh Weight; Leaves DW: Leaves dry weight; Roots DW: Roots Dry weight; Nodules DW: Nodules dry weight; No. of fb: Number of floral buds per plant; Pods/ plant: Pods per plant; Leaves OP: Leaves Osmotic potential; Roots OP: Roots Osmotic potential; Nodules OP: Nodules Osmotic potential; RWCL: Relative Water Content in leaves; RWCR: Relative Water Content in roots; RWLN: Relative Water Content in nodules.

Figure 13 (A-C): Correlation between different morpho-physiological, Biochemical and yield attributes of Asha (A), MH125 (B), and MH215 (C) under different drought stress treatments with SA application

was reported in the Asha genotype (21.58%) and the least in MH215 (1.19%) as compared to drought-stressed plants. Hence, SA improves the seed quality by alleviating the adverse effects of drought stress.

Correlation Analysis

Pearson’s correlation was used to analyze the correlation between different parameter studies in the present study (Figure 13 A-C). The correlation between various growth, physiological biochemical parameters of leaves, roots, and nodules, and quality parameters of seeds has been analyzed for three genotypes (Asha, MH125, and MH215), separately. In all three genotypes, chl a, chl b, carotenoid content, RLSI, SHSI, dry weight of roots leaves and nodules, root-shoot length, and relative water content of leaves and roots are positively correlated with each other and negatively correlated with leaves, roots, and nodules osmotic potential at different significant levels ($p < 0.001$, $p < 0.01$, $p < 0.05$) and *vice-versa*. In the MH125 variety, protein content is positively correlated with leaves, root, and nodules osmotic potential and negatively correlated with chl a, chl b, carotenoid content, RLSI, SHSI, dry weight of root, leaves and nodules, root-shoot length, and relative water content of leaves and roots. In contrast, in Asha and MH215 genotypes, starch content shows a positive correlation with protein content, leaves, roots and nodules osmotic potential, and fiber content at different significant levels. Similarly, in the Asha genotype, the number of floral buds shows a negative correlation with seed fiber and phosphorous content. However, in MH125 and MH215, the number of floral buds shows a negative correlation with seed fiber content and a positive correlation with seed phosphorous content at different significant levels.

Conclusion

PEG-induced drought stress affects in all three mungbean genotypes with varying severity that leads to various physiological, reproductive and quality parameters. More negative values of OP (-MPa) were observed in root and nodules of MH 125 at moderate (8% PEG induced drought) level of stress, leaves of genotype MH 215 at the highest level of stress. Seed protein, starch, seed fiber, and phosphorus content decreased more in the Asha genotypes than in the MH125 and MH215. The application of SA exogenously improves all the parameters in all the genotypes but the effect was pronounced more in the nodules of MH125 and the root of ASHA. Applying salicylic acid topically to mungbean genotypes enhances quality parameters such as FW, DW, RLSI, and SLSI, as well as water status and osmotic adjustment when drought stress is present. Improved morpho-physiological changes brought about by exogenous SA which enhanced yield attributes of mungbean under drought stress. Our findings suggested how well SA works to increase mungbean crop output in drought-stress situations.

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