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Research Article

Drought stress-induced modification of morpho-anatomical and yield attributes of mung bean associated with the application of silicon and *Moringa* leaf extract

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Abstract

Mung bean (*Vigna radiata*) is the rich source of fiber and essential nutrients. They play a vital role in sustainable agriculture due to their ability to fix nitrogen in the soil and enhance soil fertility. Drought is characterized by limited water resources and severe arid climatic conditions, notably impair crop growth and yield. In the current experiment, two genotypes, Azri-M 2006 and NM-92, were studied against drought stress that was applied as 2 days and 4 days irrigation gap per week. Foliar application of magnesium-silicate (20 ppm and 30 ppm concentrations) and *Moringa* leaf extract (30% v/v solution) was applied as treatments. The results from the experiment morphology anatomical and yield components were recorded according to the prescribed methods. The result revealed that drought stress reduced the growth of plant. Foliar application of 30 ppm silicon against drought stress showed a highly significant ($p<0.001$) result compared with control group.

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Morphology parameters, including shoot and root length, shoot and root fresh weight, root dry weight, leaf area, leaf number, the anatomical structure included (stem epidermis, cortex, and stem vascular bundles,) and also yield components (pod length, and seed numbers). In contrast, MLE (30%) showed a significant impact ($p<0.01$) on leaf lamina thickness (Leaf anatomical parameters; midrib xylem and phloem, number of stomata on the adaxial and abaxial surface) and yield components included (100-grain weight, grains weight per plant, and numbers of pods,). The overall impact of 30 ppm Si was 39.9% more positive on Azri-M2006 than the NM-92 against the drought stress. The 30-ppm silicon and 30% MLE showed 90% similar results in all studied parameters. This study confirms that 30% MLE could be recommended to farmers to improve productivity under arid conditions than the silicon.

Keywords: anatomy; drought; morphology; *Moringa* leaf extract; mung bean; silicon; yield

Introduction

Around 20,000 distinct species on the planet, the third largest family of terrestrial flowering plants are legumes. Except for Antarctica, every continent on the planet is home to legumes, which may be found in a wide variety of environments from the desert to the plains to the high mountains. Asia is well recognized for its production of legumes. There are more than 150 species in the genus *Vigna*. 22 species can be found in India, 16 in South East Asia, and numerous others in Africa (Lorenz *et al.*, 2022).

One of the most significant food legume crops is the mung bean (*Vigna radiata* L.)*,* which accounts for 90% of current global production. Mung bean is most ingress Kharif pulse crop of Pakistan. According to Ayub Agriculture Research Institution Faisalabad (ARIF), it is primarily grown in Sind province and southern Punjab. Punjab provinces the major mung bean growing area of Pakistan that alone accounts 80% region for production. Punjab is autonomous and famous for mung bean production and consumption (Ali, 2019).

Mung bean cultivation occurs during the dry season, which spans from March to May. However, many countries often face issues with water scarcity and insufficient soil moisture. Despite this, studies have shown that mung bean growth, development, and yield are significantly hampered by water deficit stress during various critical stages of its growth cycle. Specifically, water deficit stress during the early stage, late vegetative stage, and flowering stage has been found to have detrimental effects on mung bean crops. These adverse conditions can lead to a reduction in grain yield by approximately 25% during the early stage, 39% during the late vegetative stage, and a substantial 59% reduction during the flowering stage (Islam *et al*., 2023).

Mung bean is a very beneficial legume crop due to its high nutritional value and multiple uses in food, feed, and industrial applications. It a potential crop for sustainable agriculture because it can fix nitrogen, improve soil health and fertility, and reduce greenhouse gas emissions (Singh *et al.,* 2020). Effective nutrient management plays a crucial role in enhancing seed yield in pulse crop cultivation, with particular importance in the case of grain legumes like green gram. It's worth noting that green gram, being a leguminous crop, exhibits a substantial demand for proper nutrients and sufficient water to support its optimal plant growth, as highlighted by (Singh *et al.,* 2016). Mung beans plays important role in activities included antioxidant, anti-inflammatory and antitumor (Tang *et al.,* 2014). Fresh and dry mung bean seeds used for various purposes seeds as a whole or may be processed to bread and porridges etc. It is a fat free protein rich and in additional it contains minerals and vitamins that's are very essential for human heath (Usman *et al.,* 2014).

Drought stress significantly impacts legume crops, including reduced growth, biomass accumulation, and yield. The reason is that legumes have a high-water demand, and water stress can limit their ability to take up nutrients and carry out critical physiological processes. Drought stress led to reduced root growth and biomass accumulation in legume crops such as soybean *(Glycine max),* common bean (*Phaseolus vulgaris*), and chickpea (*Cicer arietinum*) (Aref *et al*., 2019; Khazaei *et al*., 2019). Hydric stress decreases the number of seeds produced per plant and decreases seed size, quality and yield (Moradi *et al*., 2019; Mousavi *et al*., 2018). The ability of plants to withstand drought is influenced by the severity and length of the stress period, and it also varies among different plant species. Therefore, it is essential to identify and select mung bean genotypes that exhibit drought tolerance in order to maintain viable yields when faced with drought conditions (Nasir and Toth, 2022).

Drought stress can be mitigated using several approaches; soil and water management, foliar external application of salts and natural bio-regulators to mitigate water stress (Zaman *et al.,* 2019). Silicon has several favorable impacts on plant growth and development due to its distinctive function. Silicon fertilization adjusts plants' turgor status and improves stomatal conductance that does not affect any physical alteration. Si treatment can prevent plant damage when grown under stressful, including water, salt heat weed, insect, and disease. In addition, Silicate application considered most essential element for growing and sustaining crop production during the summer season. Magnesium silicate develops and increase the membrane functioning of plant cell and integrity. It upturns the absorption and transportation of the nutrients during dry condition (Ali *et al*., 2018).

Foliar application of silicone increases the plant's normal growth. The application of magnesium silicate provides resistance against the lodging and provides erectness to leaves. It also improves photosynthesis by allowing better light transmittance through canopies. It also improves the number of stomata and stomatal density. Generally, magnesium silicate improves the growth plant and enhances the ounce yield in crops. It was also noted that magnesium silicate alleviates the toxic effect of Zn in summer crops. Si- accumulating crop species are positively impacted by the application of silicate. It increases its growth and yield. Si's impacts on plants under biotic or abiotic stress may be connected to the beneficial benefits of silicate (Sarto *et al.,* 2017).

Moringa oleifera is a very efficient bio-regulator used as fertilizer. *Moringa* leaf juice (MLE), which contains Zeatin, a naturally occurring plant hormone, impacts seed germination and promotes crop growth and productivity (Busani *et al*., 2011). Potassium, iron, calcium, ascorbate, and amino acids are the major constituents of MLE. *Moringa* leaf juice has the ability to act as a natural organic compound it effects the metabolic process and signaling process.it enhanced the signaling effect in leaf and also help to increases the chlorophyll content (Nouman *et al*., 2014).

The application of MEL speeds up its growth and increases its life span. MEL application provides richness to shoots and thickness to leaves. It also increases the number of roots and biomass. It provides strength against pathogens, diseases, and pests. It generally increases crop production by up to 20-35%. In addition, it also develops resistance against abiotic stresses, including salinity, drought, and heavy metals (Yasmeen *et al*., 2018). The foliar application of *Moringa* on plants boosts plant height, grain no., protein, carbohydrate, oil, leaf chlorophyll contents, and productivity (Noor *et al.,* 2016). MLE increased the thickness of leaves, stomatal density, index, size of stomata, and plant yield (Latef *et al*., 2017).

Moringa plays a pivotal role in enhancing plant growth and resilience in challenging environmental conditions. It effectively boosts various key factors such as relative water content, water use efficiency, mineral content, gas exchange traits, and yield attributes. Additionally, *Moringa* serves a crucial purpose as a natural biopesticide, effectively combating plant pathogens and contributing significantly to disease management and overall plant sustenance (Yamshi *et al*., 2023).

This study is different from the past studies because this research is based on the use of low cost and eco-friendly fertilizer to mitigate the drought stress.

This research aims to check how silicon and *Moringa* leaf extract benefits mung-bean plants in low water availability and how silicon and MLE alter the plant's anatomy that grows against drought. This study provide baseline for the researchers to use of natural organic bio-regulator to mitigate the drought stress rather than the synthetic salts including silicon.

Materials and Methods

Plant material and treatments

Pure quality seeds of genotype Azri-M2006 and NM-92 of mung bean were obtained from the local market of Bahawalnagar. The experimental procedure was performed at the research area of Islamia University of Bahawalpur, Pakistan, under a control level of drought stress with 2 days and 4 days irrigation gap per week. The experiment was randomized completely block design with two factorial factors, and three replicates. The experiment was pot experiment, each pot containing 10 plants seed per pot. Each pot was filled with 8 kg soil, with 3:2 sandy soil. There were 8 treatments including control group. Treatments including foliar application of $SiMgO_3$, concentration applied at two levels 30 ppm and 20 ppm against 2 days and 4 days drought stress. The foliar application of *Moringa* leaf extract concentration applied at one level (30% v/v) against two levels of drought stress (two- and four-days interval per week). Receding of germinating plants was done after 2 weeks of complete germination, to maintain five plants for each pot. Morphological data was recorded at somatic stage, anatomical was determined during developmental stage, and harvesting was done at ripe stage.

Observations and measurements of morphological, anatomical, and yield components in mung beans

Morphological parameters recorded at the vegetative stage, shoot length(cm), root length(cm), shoot biomass(g), root biomass(g), number of leaves and leaf area (cm^2) from each replicate of each treatment. Leaf area was measured by using Cochran (1950) method formula:

Leaf area (cm^2) = length (cm) × width (cm) × 0.75

At the reproductive stage, mung bean plants' cut pieces (stem, root, and leaf) were preserved in formalin acetic acid (FAA) solution. Root, stem, and leaf sections were prepared from each treatment, dehydrated, and mounted on slides. Micrographs were taken at 4×, 10×, and 40× magnifications and analyzed using Image J software to measure the anatomical parameters.

Another set of replicates remained for the final yield evaluation. When the stressed plants reached maturity, the number of flowers, yield productivity percentage, number of pods and average yield (g)/ plant, and 100-grain weight (g) were recorded.

Statistical analysis

The data was analyzed using the Analysis of variance (ANOVA), and treatment means were compared by the least significant difference (LSD) test at a 0.05 probability level. Graphical presentation of data was done by using Excel (2016).

Results

Effect of silicon and MEL on Vigna radiata morphology against drought stress

The ANOVA results revealed that the effect was highly significant at ($p<0.001$) among all the applied treatments (30 ppm, 20 ppm and 30% MEL) on both mung bean varieties against the drought stress. The interaction between treatments and shoot length, shoot biomass, root biomass, leaf area and the number of leaves was significant at ($p<0.01$) In contrast, the interaction was non-significant for root length at ($p<0.05$). The plants treated with foliar application of 30 ppm silicone against the drought stress showed the highest shoot length, shoot fresh and dry weight, root fresh and dry weight, leaf area and the number of leaves, while the lowest values were observed in plants that faced 4 days drought stress without any external foliar application as compared with control. While comparing all treatments, 30 ppm Si and 30% MEL showed overlapping results than 20 ppm Si to mitigate the hydric stress as shown in Figure 1a (a to c) Figure 1b (d to f) and Figure 1c (f, g).

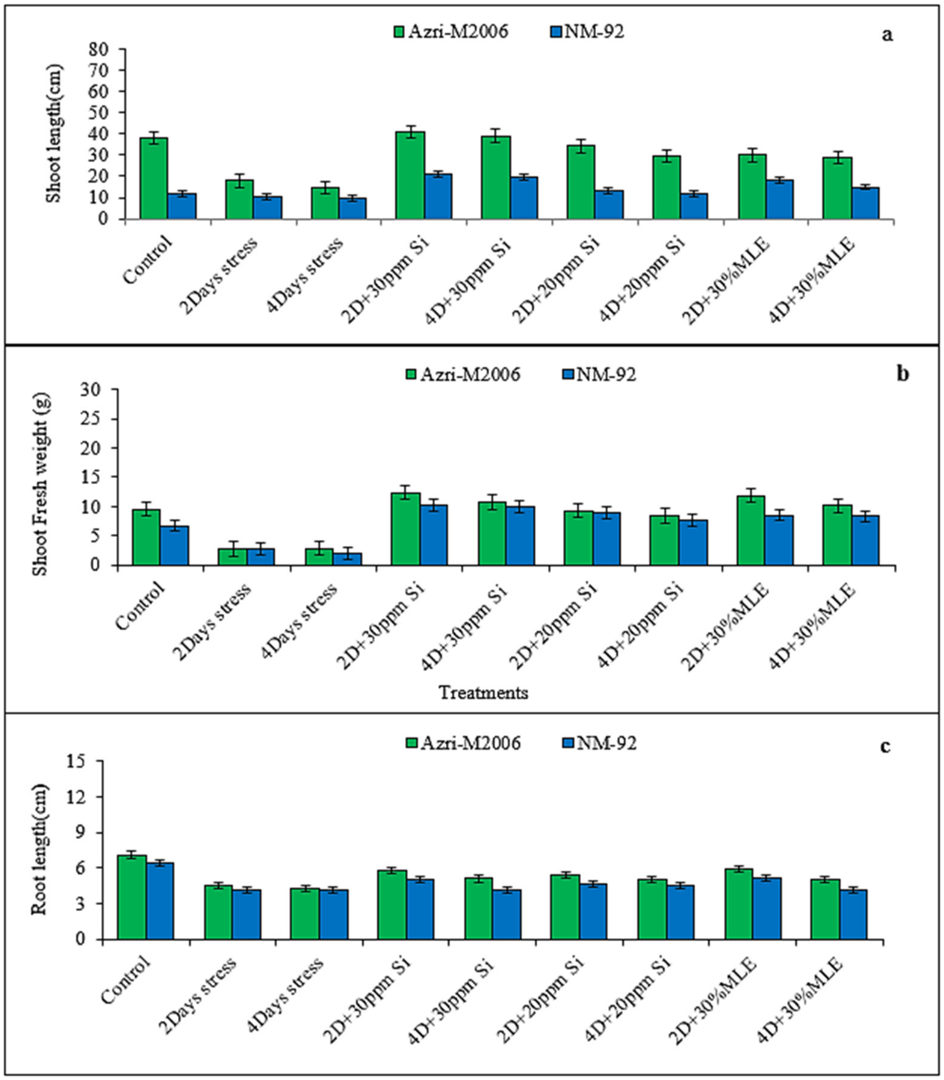


Figure 1a. Influence of silicon application (30 ppm, 20 ppm) and 30% *Moringa* leaf extract on two varieties of mung bean morphology against drought stress conditions

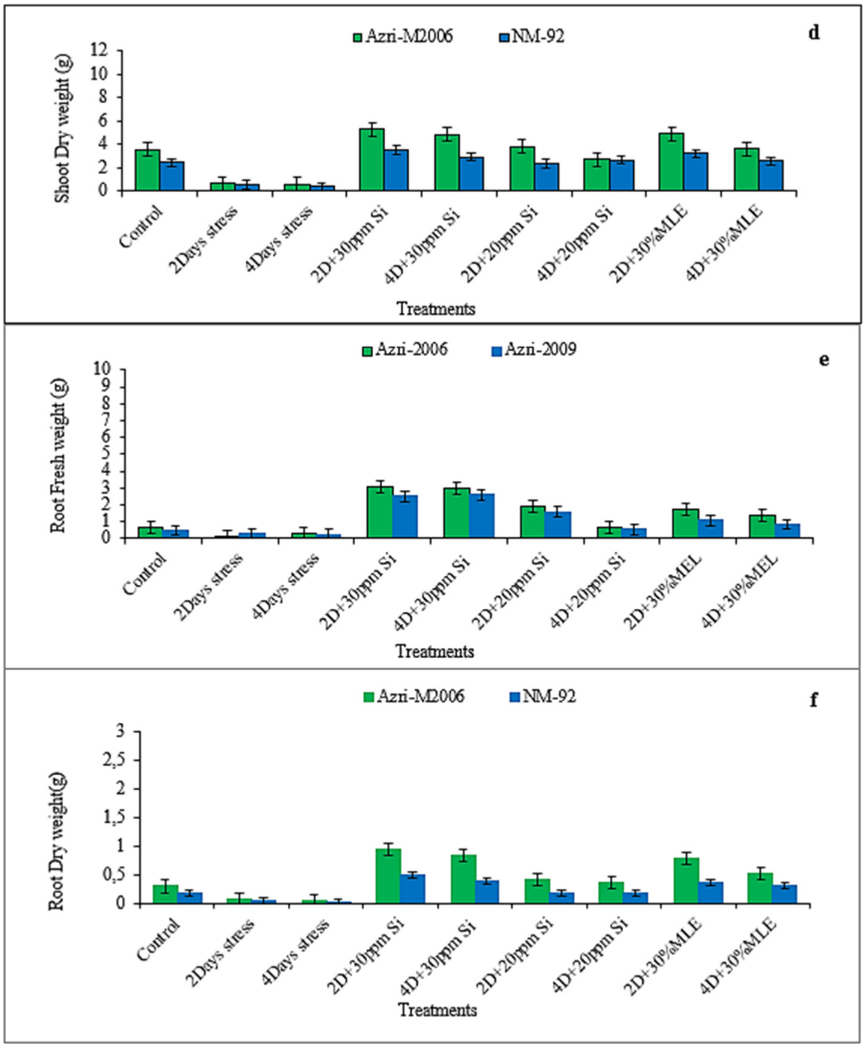


Figure 1b. Influence of silicon application (30 ppm, 20 ppm) and 30% *Moringa* leaf extract on two varieties of mung bean morphology against drought stress conditions

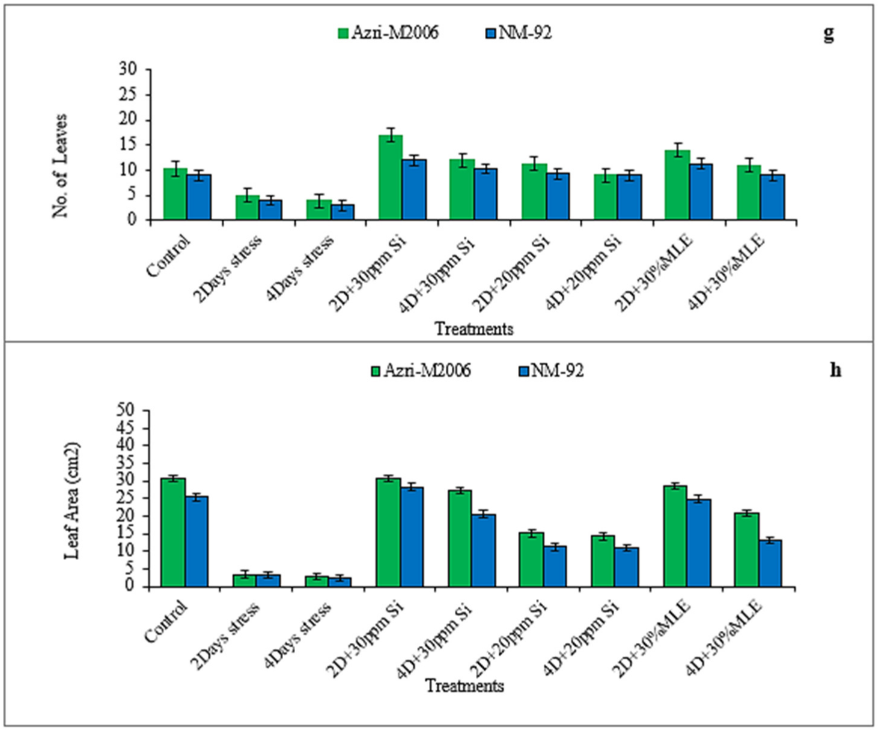
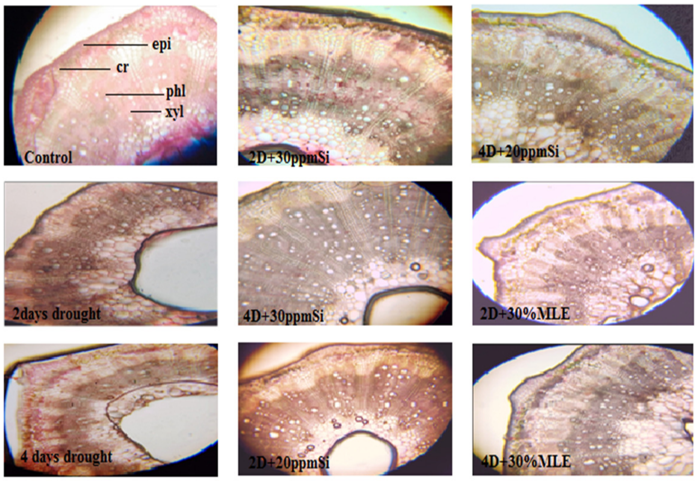


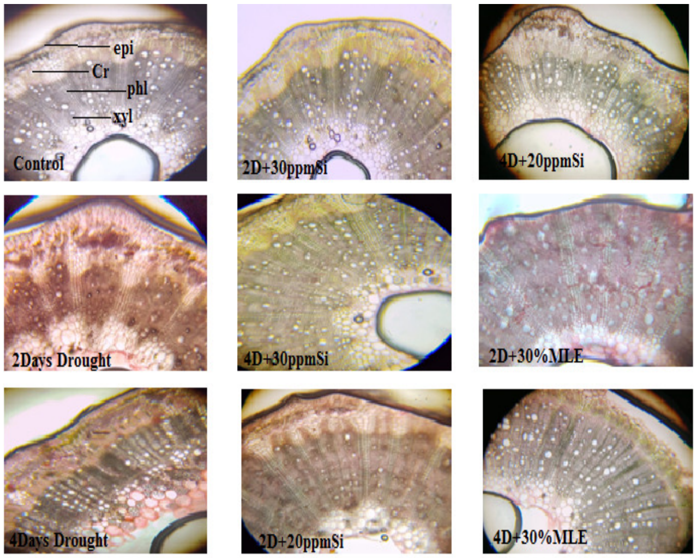
Figure 1c. Influence of silicon application (30 ppm, 20 ppm) and 30% *Moringa* leaf extract on two varieties of mung bean morphology against drought stress conditions

Effect of silicon and MEL on Vigna radiata Anatomy against drought stress

The ANOVA results revealed that the effect was highly significant at (p<0.001) among all the applied treatments (30 ppm, 20 ppm and 30% MEL) on both mung bean varieties against the drought stress. The statistical results showed that interaction between all treatments and the Internal structure of the mung stem was non-significant for the stem epidermis and stem cortex. Still, it was statistically significant for xylem and phloem. The highest thickness of the epidermis, cortex cells and vascular bundles length was measured in plants that treated with 30 ppm silicone against the drought stress. Minimum thickness was observed in internal stem cells. Minimum thickness was observed in internal stem cells that faced 4 days of hydric stress, as shown in (Figure 2). The interaction between the treatments and leaf midrib xylem and phloem cell was also significant. To concerning midrib xylem and phloem length, plants treated with 30% MEL against drought stress displayed the greatest length, whereas plants treated with 4 days of drought stress had the smallest length (Figure 3). The plants treated with 30% MLE against the drought stress showed increased stomatal number on the adaxial surface while the lowest number was observed in control plants in comparison with that treated with 4 days drought stress while; the plants treated with 30% MLE against the drought stress showed highest no. of stomata on abaxial leaf surface but lower than the control plants while the lowest no. of stomata was observed in plants that treated with 4 days drought stress. Plants that were treated with 30 ppm Si showed higher stomatal No. than 20 ppm (Figure 4 ,5).

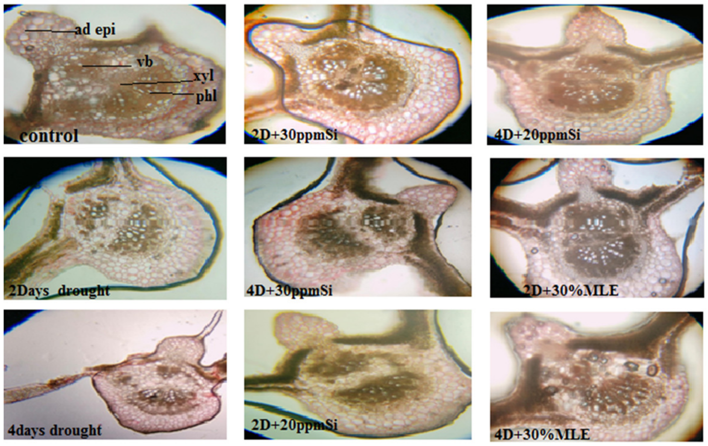


T.S Stem Azri-M2006

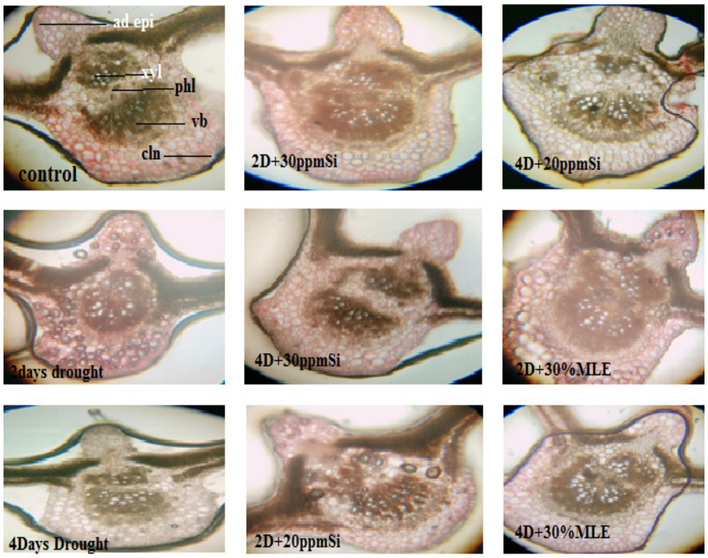


T.S Stem NM-92

Figure 2. Influence of silicon application (30 ppm, 20 ppm) and 30% *Moringa* leaf extract on two varieties of mung bean anatomy against drought stress conditions cr (cortex), epi(epidermis), phl (phloem), and xyl (xylem)

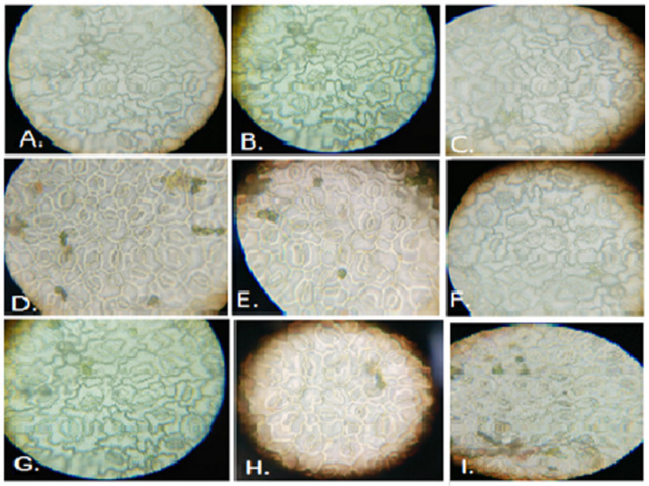


T.S leaf Midrib Azri-2006

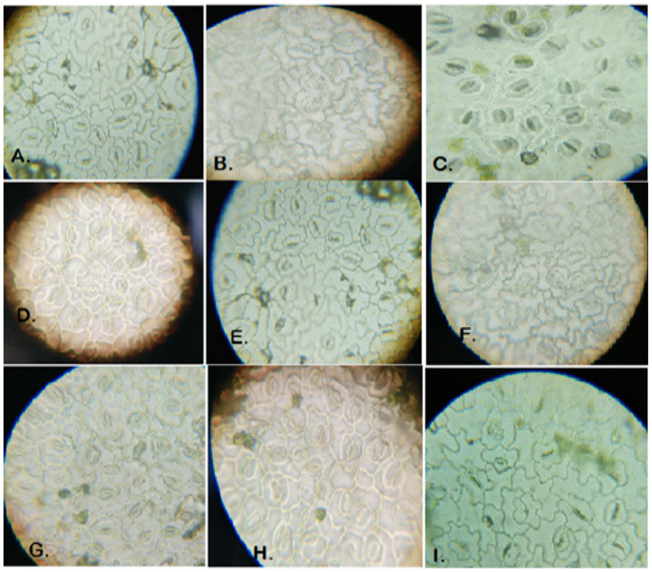


T.S leaf Midrib NM-92

Figure 3. Influence of silicon application (30 ppm, 20 ppm) and 30% *Moringa* leaf extract on two varieties of mung bean anatomy against drought stress conditions ad epi (adaxial epidermis), xyl (xylem), phl (phloem), vb(vascular bundle), and cln (collenchyma)



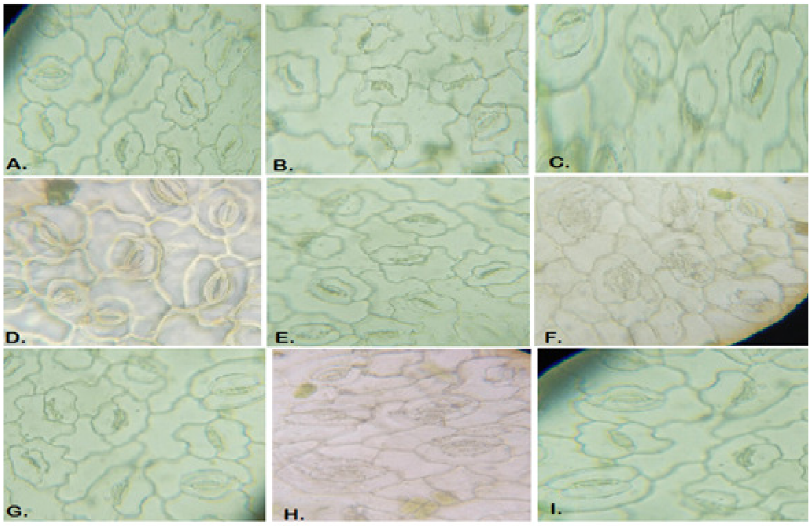
Abaxial Azri-M2006



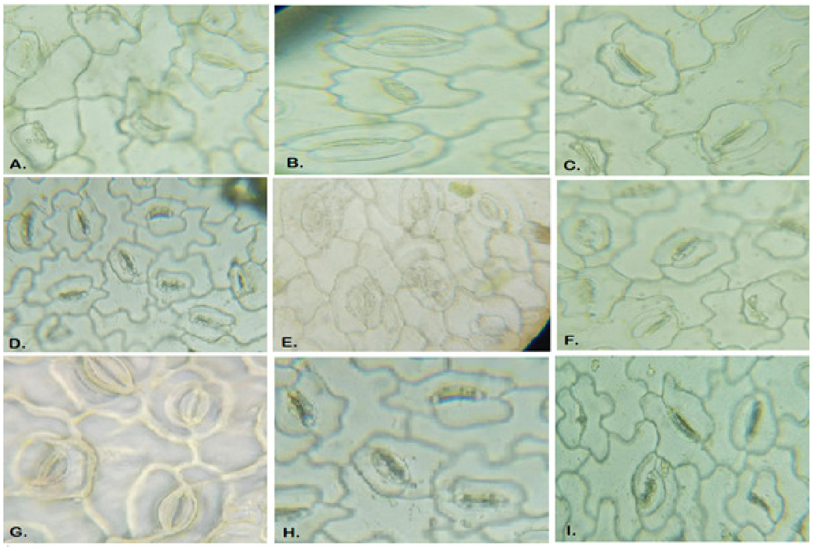
Abaxial NM-92

Figure 4. Influence of silicon application (30 ppm, 20 ppm) and 30% *Moringa* leaf extract on two varieties of mung bean anatomy against drought stress conditions

A(control), B (2D+30 ppm Si), C (4D+20 ppm Si), D (2 days drought), E (4D+30 ppm Si), F (2D+30% MLE), G (4 days drought), H (4D+20 ppm Si) and I (4D+30% MLE)



Adaxial Azri-2006



Adaxial NM-92

Figure 5. Influence of silicon application (30 ppm, 20 ppm) and 30% *Moringa* leaf extract on two varieties of mung bean anatomy against drought stress conditions
A (control), B (2D+30 ppm Si), C (4D+20 ppm Si), D (2 days drought), E (4D+30 ppm Si), F (2D+30% MLE), G (4 days drought), H (4D+20 ppm Si) and I (4D+30% MLE)

Effect of silicon and MEL on Vigna radiata yield against drought stress

The data revealed that the interaction between the treatment Pod length, seed numbers, seed weight per plant, number of pods in both genotypes was non-significant at (P>0.05) but 100-grain weight was statistically significant. Comparison among all the treatments and yield parameters revealed that 30% MEL showed a remarkable effect on yield against the hydric stress than 30 ppm and 20 ppm silicone, as shown in Figure 6a (a to c) and Figure 6b (d, e)

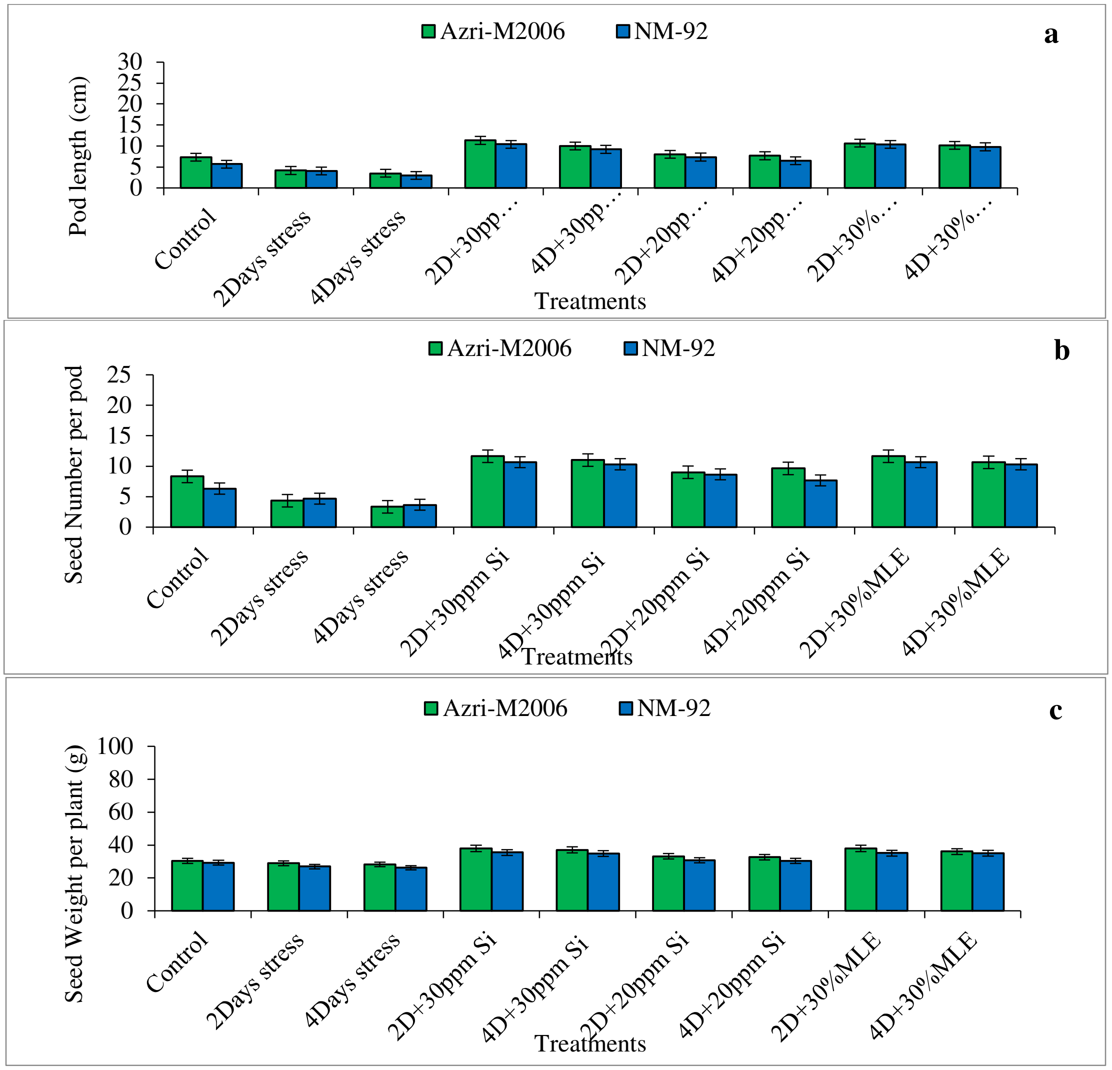


Figure 6a. Influence of silicon application (30 ppm, 20 ppm) and 30% *Moringa* leaf extract on two varieties of mung bean yield against drought stress conditions

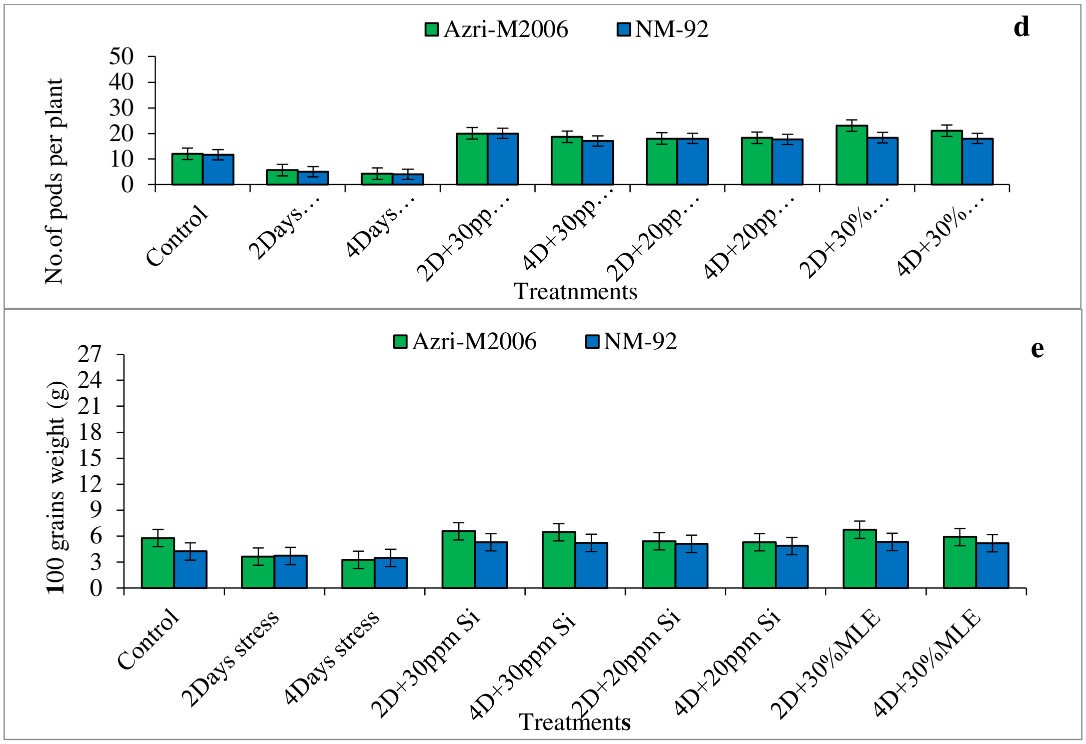


Figure 6b. Influence of silicon application (30 ppm, 20 ppm) and 30% *Moringa* leaf extract on two varieties of mung bean yield against drought stress conditions

Discussion

The plants treated with foliar application of 30 ppm silicone against the drought stress showed the highest shoot length, shoot fresh and dry weight, root fresh and dry weight, leaf area and number of leaves, while the lowest values were observed in plants that faced 4 days drought stress without any external foliar application as compared with control. While comparing all treatments, 30 ppm Si and 30% MEL showed overlapping results than 20 ppm Si to mitigate the hydric stress as the previous study (Ahmad *et al.*, 2012) proved that silicon plays a beneficial role in plants by improving their strength against wilt and water stress. Silicon, a which affects the cell walls, and forms a defending layer that reduces transpiration through the outer cells.

The anatomical finding of current studies confirmed the previous study of (Soe *et al.,* 2019), who reported that the typic average value of stem epidermis of *Vigna radiate* was 15.0-25.0 µm in length, the cortex layers 25.0-250.0 µm thick, Vascular bundles embedded in the ground tissue and arranged in a continuous circular ring, xylem 30.0-500.0 µm thick and layers of phloem 22.5-200 µm thick. In drought conditions, the internal thickness of the stem was reduced to the normal condition in both mung bean varieties. The treatments 30 ppm and 30% MEL positively impacted stem anatomy against drought stress. Shen *et al.* (2010) showed that silicon help plant tolerates drought stress by increasing the metabolic process that increases plant growth. Sivanesan and Park (2014) also reported that silicone fertilizer's application enhances the crops' productivity and alternates plants' anatomy. The water shortage condition adversely affected the number of stomata because drought stress reduces the transpiration resulting in the closure of stomata. In the present study, the highest number of stomata on both the adaxial and abaxial surfaces was observed in control plants treated with regular water. However, four days of drought stress observed the least number of stomata on both the upper and lower epidermis in both *Vigna radiata* L. varieties. Comparing the past works with the current experimental information, it evidences those photosynthetic responses also induces by hydric stress. It was evident by Samarah (2009) that stomata shrink gradually with increased of water stress.

In the present study, the yield components of mung bean were reduced due to different levels of water stress and somehow improved by the different applied treatments. Hao *et al.* (2011) stated that irrigation stress might reduce the mung bean seed yield and yield components. Sarkar *et al.* (2021) reported that drought adversely impacts the various aspects of plant yield, including reducing the number of pods, diminishing the quantity of seeds, and decreasing the size of individual seeds. Ahmad *et al.* (2016) revealed that a decrease in irrigation had significantly delayed all the studied parameters of different genotypes of mung bean, including Plant height, dry and fresh weight of root and shoot, 100 seed weight, biological yield, leaf area seed yield, and harvest index.

Conclusions

Drought stress adversely affects the growth of plants. Statistical comparisons showed significant results among the treatments and varieties at ($P<0.05$). It was also found that against the drought stress, magnesium silicate as a source of silicon and *Moringa* leaf juice showed a very positive impact to mitigate the drought stress in both varieties of Mung bean. The AzriM-2006 showed 39.9% more positive impact than NM-92 except in a few parameters. The NM-92 adversely effected by the drought stress than Azri-2006. Applying silicon and MLE improves mung bean varieties' morphology, anatomy, and yield against the drought stress. The higher concentration of 30 ppm of silicon improves more growth and development of plants more than the lower concentration of 20 ppm. *Moringa* leaf extract also improved mung bean morphology anatomy and yield. Foliar application of silicon and *Moringa* alleviates the effect of drought at the individual level. Although, mung beans both varieties were naturally drought resistance but 'Azri-M2006' and 'NM-92' showed more growth with the foliar application of silicon and *Moringa* leaf extract. The comparison of both treatments also with control plants it was concluded that the foliar application of silicon and *Moringa* showed 90% same results on growth improvement against the drought stress. So, it is recommended that the *Moringa* is a natural bio-regulator, low-cost fertilizer to overcome the drought stress than the expensive synthetic magnesium silicate salt. This research will provide baseline for the future studies to overcome the drought stress by using the natural bio-regulator zeatin.

Authors' Contributions

MA, AH, ZG, conducted research study and wrote manuscript. SS, AH, MG, participated in data collection. AR critically reviewed and improved the manuscript. JMA, MIA, MNS, ABSR, MIA, WFS, TAS provided funding.

All authors read and approved the final manuscript.

Ethical approval (for researches involving animals or humans)

Not applicable.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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