Genotype-Dependent Alleviation of Water Deficit Stress in *Vicia faba* L. by Silicon Application

Samira Neyestani[1], Mansoureh Hoseini[1], Mohammad B. Bagherieh-Najjar[1*], Ahmad Abdolzdeh[1], Hamid Reza Sadeghipour[1], Fatemeh Sheikh[2]

1.Department of Biology, Faculty of Sciences, Golestan University, Gorgan, Iran
2.Golestan Agricultural and Natural Resources Research and Education Center, Gorgan, Iran

Abstract

Prolonged water stress can have detrimental effects on most plants, including *Vicia faba* L. (broad bean), which is an important legume. The use of exogenous coarse or nano-structured silicon on various plants has yielded conflicting results in terms of improving plant water relations and alleviating drought stress. Therefore, the objective of this study was to examine the effects of exogenously applied silicon and nano-silicon on the morpho-physiological responses of three recently introduced *Vicia faba* genotypes during the vegetative growth stage under water deficit stress. In a greenhouse experiment, three genotypes, two levels of irrigation regimes, and three levels of silicon were arranged in a completely randomized factorial design, with five replications. The results indicated that water deficit stress significantly reduced shoot height, as well as shoot and root fresh and dry weights, while also decreasing relative water content in all three genotypes. Conversely, chlorophyll, carotenoid, malondialdehyde (MDA) content, and soluble guaiacol peroxidase activity increased in response to water stress. Notably, the G62 genotype exhibited greater sensitivity to water stress compared to G20 and G61, based on guaiacol peroxidase activity and MDA content. Furthermore, the effects of silicon and nano-silicon on the morpho-physiological characteristics of the plants were found to be minimal, but were significantly influenced by the genotype and experimental conditions. These findings suggest that before the wide application of exogenous silicon in field trials, particularly in mitigating the adverse effects of water deficit during the vegetative stage of *Vicia faba*, prior testing of genotype-specific responses is necessary.

Keywords: Water deficit stress; Faba bean, Peroxidase; Silicone*; Vicia faba*

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Introduction

As one of the earliest crops to be domesticated in the Middle East, broad beans (*Vicia faba* L.) are now widely acknowledged as a primary source of protein and energy for both human and animal consumption on a global scale. These seeds consist of approximately 30% protein and are abundant in micronutrients (Warsame et al., 2018). Furthermore, the cultivation of broad beans offers numerous advantages in cereal-

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[*] *Corresponding Author*
E-mail Address: mb.bagherieh@gu.ac.ir
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based cropping systems, such as disrupting the life cycle of diseases and weeds. It also serves as a cover crop, aiding in the restoration of soil nitrogen levels and preventing soil erosion (Islam et al., 2024). According to the FAO report, the worldwide production of broad beans was estimated to be approximately 6 million tons in 2021, making it the sixth most cultivated legume crop globally (Fao, 2019). Nevertheless, like many other crops, the overall yield of broad beans is vulnerable to the impacts of global climate change.

Among the various abiotic factors that globally challenge crop production, water stress plays a crucial role. The uneven distribution of rainfall, combined with groundwater shortages, often leads to water stress in the environment (Virnodkar et al., 2020), ultimately resulting in a significant decrease in plant yield. Water deficit is one of the major limiting factors for the growth and production of crops in arid and semi-arid regions worldwide, posing a threat to human food security (Biswas et al., 2025). Regional climate change models identify the Mediterranean regions and the Middle East as "hotspots" of severe water stress, where crop failures and livestock deaths are common (Barlow et al., 2015).

Broad beans are particularly sensitive to water deficit, especially when cultivated under rain-fed systems in arid or semi-arid regions (Karwani et al., 2024). A meta-analysis revealed that a 65% reduction in water availability resulted in a 40% decrease in broad bean yield, with the sensitivity to water deficit depending on the cultivar and other environmental conditions (Daryanto et al., 2015). The most significant effects of water stress on plants include stomatal closure, cell injury caused by reactive oxygen species, a decline in cellular water content, and increased cellular temperature, all of which may hinder plant growth and limit the production of photosynthetic materials necessary for grain filling (Farooq et al., 2017). Ammar and colleagues (2015) examined the physiological and yield responses of various broad bean genotypes to drought stress and reported that water deficit reduced root and shoot length, chlorophyll content, and grain yield while increasing leaf free proline, leaf soluble proteins, and malondialdehyde content in a genotype-dependent manner (Ammar et al., 2015). Siddiqui and colleagues (2015) analyzed the tolerance of 10 broad bean genotypes to drought stress based on morpho-physiological and biochemical factors and found that although drought stress reduced all growth parameters of the examined genotypes, the detrimental effects of water deficit stress were less pronounced in several genotypes with higher levels of antioxidant activities.

Alghamdi and colleagues (2015) utilized amplified fragment length polymorphism analysis to demonstrate genetic variation among twenty broad bean genotypes with divergent drought tolerance, which was associated with different leaf area, leaf temperature, relative water content, and grain yield. Abid and co-workers (2020) reported that the exogenous application of 1 mM β-aminobutyric acid improved the drought tolerance of broad beans by activating several drought-tolerance-related genes such as *VfMYB*, *VfDHN*, and *VfHSP*, leading to increased leaf water content and photosynthesis, but decreased production of hydrogen peroxide, lipid peroxidation, and membrane leakage. In a pot trial, the application of superabsorbent nanocomposites was shown to enhance broad bean tolerance to drought stress by promoting broad bean growth and physiological characteristics, including increased levels of Chl b, carotenoids, catalase (CAT), and superoxide dismutase (SOD) (Kenawy et al., 2022). The above-mentioned reports confirm that drought tolerance in broad beans is a genotype-specific response and requires further detailed analysis for newly emerged genotypes.

Silicon is the second most abundant element in soil, comprising approximately 28% of the Earth's crust in the form of silicon dioxide (Tubana et al., 2016). When plants uptake silicon, it primarily accumulates in the cell walls of vascular tissues in the stem, leaf, pod, and epidermal cells. Silicon plays a crucial role in regulating plant physiological processes (Rizwan et al., 2015). It has been observed that silicon has beneficial effects on plant growth and enhances resistance to both biotic and abiotic stresses. For instance, exogenous silicon application has been shown to improve water stress tolerance in sorghum (Wang et al., 2021). Moreover, Sohrawardy et al. (2024) demonstrated that nanostructured silicon dioxide significantly enhances the growth characteristics of black pine (*Larix olgensis*). Roychoudhury (2020) reported that silicon nanoparticles had a more pronounced positive effect on the growth and physiology of maize (*Zea mays* L.) compared to coarse silicon. Karavidas and coworkers provided evidence that silicon application increases the 100-seed weight and yield of broad bean under saline conditions (Karavidas et al., 2022). It is important to note that the beneficial effects of silicon application may vary depending on the cultivar, e.g., Qados and Moftah (2015) demonstrated that the addition of 2 mM silicon or nanosilicon significantly increased the growth and water content of specific broad bean cultivars under salinity stress. Furthermore, silicon application has shown promise in enhancing broad bean responses to drought stress, as Desoky et al. (2021) found that foliar application of 1.5 mM nanosilicon improved plant responses to water deficit. This treatment has the potential to enhance seed and biomass yields in plants grown in arid environments (Desoky et al., 2021).

Given the increasing occurrence of water stress in broad bean cultivation, it is essential to conduct a comprehensive analysis of drought tolerance in different cultivars across various growth stages to improve plant performance and yield (Muktadir et al., 2020). In this study, our objective was to investigate the effects of silicon and nanosilicon on alleviating water stress and examine their impact on the morpho-physiological and biochemical characteristics of three relatively new broad bean genotypes, considering the genotype-specific interaction between drought stress and silicon in broad bean plants.

Materials and Methods

Plant Cultivation and Treatments

Three genotypes of broad bean (*Vicia faba* L.), *G20* (g-faba-20), *G61* (g-faba-61), and *G62* (g-faba-62), were used in this study. These genotypes were originally introduced as semi-water stress tolerant mother plants by the International Center for Agricultural Research in the Dry Areas (ICARDA) and were provided by the Seed and Plant Improvement Institute (SPII), Iran. The genotypes have the following codes: ILB1269 (*G20*), DT/B7/7380/ 0405-HBP/DS0/2000 (*G61*), and ILB1814 (*G62*). *G61* and *G62* have undergone three rounds of recurrent selection by the Gorgan Agricultural Organization in Iran to improve fungal resistance. The seeds were sown in plastic pots (ϕ = 30 cm) containing a 50/50 silty-loam soil with a pH of 7.8, an electrical conductivity of 460 $\mu S \cdot m^{-1}$, and a total nitrogen content of 0.12%.

The experiments were conducted using a factorial design with three factors (water stress, genotype, silicon) and five replications. The plants were grown in a growth chamber under cool white fluorescent light (45 μmol quanta $m^{-2}\ s^{-1}$) with a 16/8 light/dark photoperiod at 22 °C ± 2 for seed germination and seedling growth.

Water stress treatments were applied either every four days or every eight days. Preliminary experiments showed that irrigation every eight days led to a roughly 50% decrease in field capacity under our conditions. Three levels of silicon treatment were used, including a control group with no silicon, potassium silicate, and nano-silicon, each at a concentration of 2 mM. The solutions were mixed using an ultrasonic homogenizer (Bandlin, UV-3100) for 30 minutes before application. Silicon was applied to all plant leaves twice—once when the seedlings were 15 days old and again when they were 20 days old—using 5 mL of suspension solution each time. The plants were harvested 30 days after the treatments. After morphological analyses, they were transferred to -70 °C for biochemical assays.

Biochemical and Physiological Measurements

Chlorophyll content was measured according to the method of Arnon (1949) using the following formulas:

$$Chl_a = (12.78\ A_{663} - 2.69\ A_{645})$$

$$Chl_b = (22.98\ A_{645} - 4.68\ A_{663})$$

$$Chl_{tot} = (20.2 A_{645} + 8.02\ A_{663})$$

$$C = (1000\ A_{470} - 1.82\ Chl_a - 85.02\ Chl_b)\ /198$$

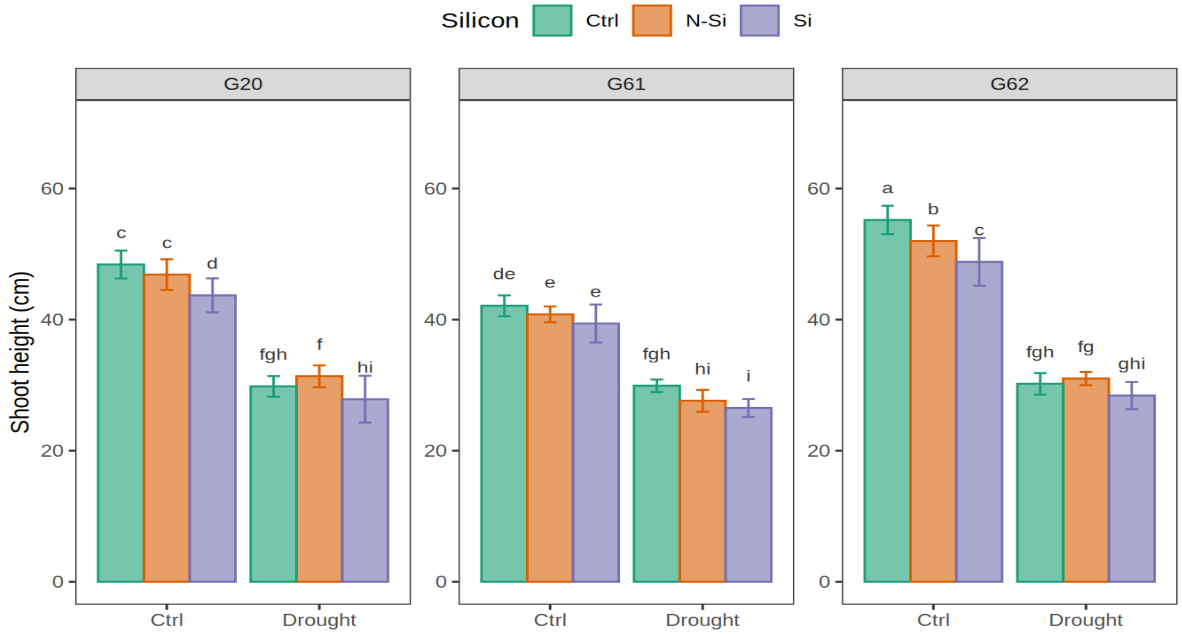

Fig. I. Effects of silicon treatment (Ctrl, control; N-Si, 2 mM nano-silicon; Si, 2 mM silicon) on three faba bean genotypes (G20, G61 and G62) shoot height, grown under water deficit condition (Ctrl, irrigation every 4 days; Drought, irrigation every 8 days). Plants were harvested 30 days after the last silicon treatment.

where Chl_a, Chl_b, and Chl_{tot} represent chlorophyll a, b, and total chlorophyll, respectively. C represents total carotenoids, and A represents light absorbance at the specified wavelengths.

Soluble guaiacol peroxidase was extracted following the method described by Kar and Mishra (1976) and measured in kinetic mode using a Shimadzu 3100 spectrophotometer (Japan) at 470 nm, as outlined by Chance and Mahley (1955). The reaction mixture consisted of 25 mM phosphate buffer (pH = 6.8), 2 mM H_2O_2, and 30 mM enzyme extract. Lipid peroxidation was measured as described by Prochazkova et al. (2001).

Statistical Analysis

Analysis of variance (ANOVA) and the LSD post hoc test, as well as figure creation, were performed using R software (version 4.1.2) (R Core Team, 2020).

Results

A three-way ANOVA was conducted to assess the impact of water stress, genotype, and silicon treatment on various morphological, biochemical, and physiological traits of broad bean (*Vicia faba* L.) plants during the vegetative growth phase. The absence of significant outliers, normal distribution of residuals, and homogeneity of variances was confirmed through box plots, Shapiro-Wilk's test, and Levene's test ($P \leq 0.05$), respectively (not shown).

ANOVA of the effects of the examined treatments on plant shoot height (Online Resource 1) reveals statistically significant main effects of irrigation [F (1, 102) = 1744.08, $p \leq 0.0001$], genotype [F (2, 102) = 64.67, $p \leq 0.0001$], and silicon [F (2, 102) = 26.348, $p \leq 0.0001$]. Additionally, there are two-way interactions between water stress and genotype [F (2, 102) = 33.36, $p \leq 0.00001$] and between drought stress and silicon treatment [F (2, 102) = 3.59, p = 0.003]. Mean comparisons (Fig. I) indicate that the applied water stress treatment, i.e., increasing the irrigation interval from 4 days to 8 days, significantly reduced the plant shoot height of all three examined genotypes. Although the *G62* genotype plants were considerably taller than both *G20* and *G61* genotypes in the absence of water stress (LSD, $p \leq 0.05$, n = 102), all three genotypes exhibited similar shoot heights under water stress treatment. Neither silicon nor nano-silicon treatments significantly affected plant

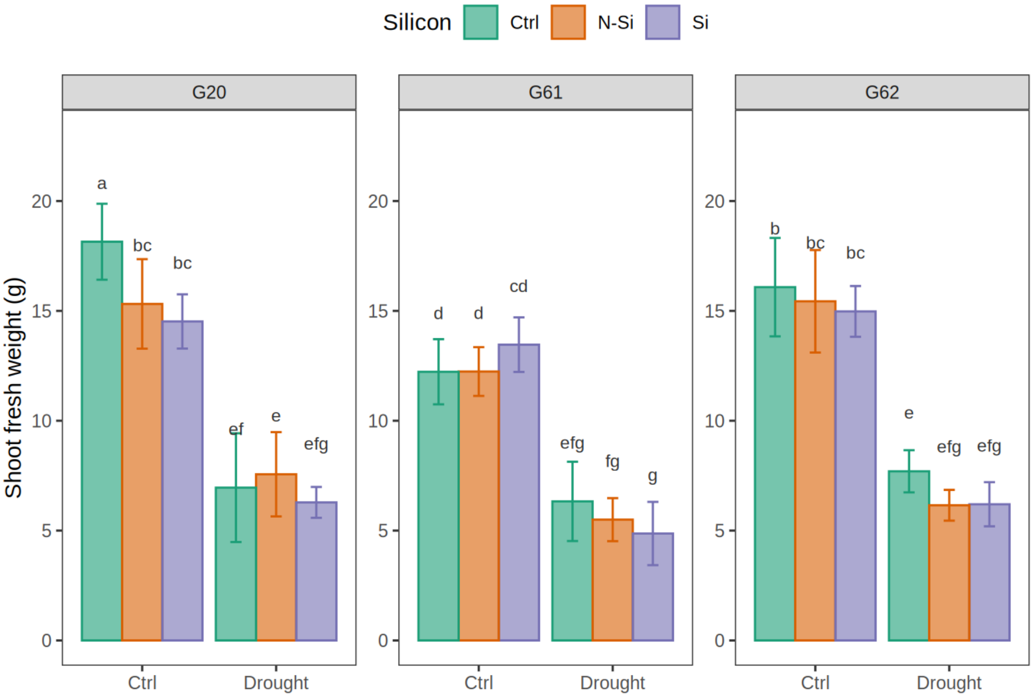

Fig. II. Effects of silicon treatment (Ctrl, control; N-Si, 2 mM nano-silicon; Si, 2 mM silicon) on three faba bean genotypes (G20, G61 and G62) shoot fresh weight, grown under water deficit condition (Ctrl, irrigation every 4 days; Drought, irrigation every 8 days). Plants were harvested 30 days after the last silicon treatment.

shoot height, regardless of the presence or absence of water stress (Fig. I).

The shoot fresh weight of the plants was significantly influenced by irrigation [F (1, 102) = 811.95, p < 0.0001], genotype [F (2, 102) = 21.56, p < 0.0001], and silicon [F (2, 102) = 7.88, p < 0.0006], as well as the interaction between irrigation and genotype [F (2, 102) = 3.86, p = 0.02] and the three-way interaction of irrigation, genotype, and silicon [F (2, 102) = 3.46, p = 0.01]. The mean comparison analysis reveals that shoot fresh weight decreased in all three genotypes under water stress. Only in *G20*, grown under control conditions, was shoot fresh weight reduced by silicon or nano-silicon treatments (LSD, p ≤ 0.05, n = 102; Fig. II). On the other hand, the shoot dry weight of the plants was only significantly affected by irrigation [F (1, 102) = 239.50, p < 0.0001] (Fig. III).

When considering the fresh weight of plant roots, the following factors had significant effects: irrigation [F (1, 102) = 550.45, p < 0.0001], irrigation: genotype interactions [F (2, 102) = 14.85, p < 0.00001], irrigation: silicon interactions [F (2, 102) = 6.18, p = 0.003], and genotype: silicon interactions [F (4, 102) = 4.41, p = 0.003]. Under control conditions, the root fresh weight of *G61* was higher, compared to the other genotypes. However, under water stress, the root fresh weight of all three genotypes was similar (Fig. IV). The effects of silicon or nano-silicon on plant responses to water stress were negligible.

The dry weight of plant roots was significantly influenced by irrigation [F (1, 102) = 208.39, p < 0.001], genotype [F (2, 102) = 22.35, p < 0.001], irrigation: genotype interactions [F (2, 102) = 14.00, p < 0.001], irrigation: silicon interactions [F (2, 102) = 5.59, p < 0.005], and genotype: silicon interactions [F (4, 102) = 3.10, p < 0.02]. As shown in Fig. V, under control conditions, the root dry weight of *G61* was higher compared to the other two genotypes. However, under water stress, all three genotypes exhibited similar root dry weights.

These findings suggest that the effects of silicon treatment on alleviating water deficit stress were

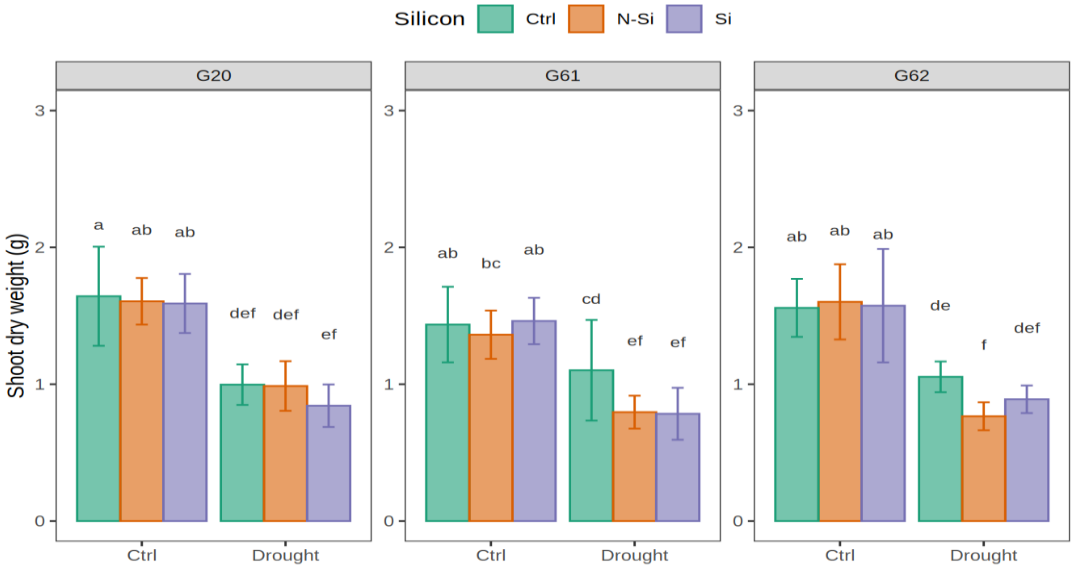

Fig. III. Effects of silicon treatment (Ctrl, control; N-Si, 2 mM nano-silicon; Si, 2 mM silicon) on three faba bean genotypes (G20, G61 and G62) shoot dry weight, grown under water deficit condition (Ctrl, irrigation every 4 days; Drought, irrigation every 8 days). Plants were harvested 30 days after the last silicon treatment.

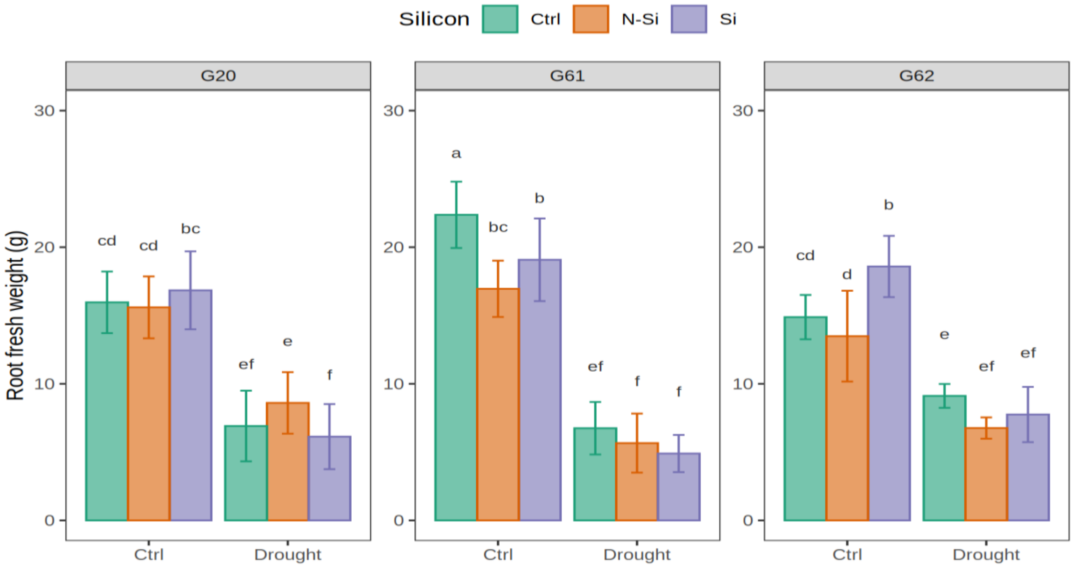

Fig. IV. Effects of silicon treatment (Ctrl, control; N-Si, 2 mM nano-silicon; Si, 2 mM silicon) on three faba bean genotypes (G20, G61 and G62) root fresh weight, grown under water deficit condition (Ctrl, irrigation every 4 days; Drought, irrigation every 8 days). Plants were harvested 30 days after the last silicon treatment.

limited to the plants' relative water content (Fig.VI).

The effects of water stress on the photosynthetic characteristics of broad bean seedlings were further analyzed by measuring chlorophyll and carotenoid content. The analysis of variance (Online Resource 1) revealed statistically significant main effects of irrigation [F (1, 102) = 73.86, p < 0.0001], genotype [F (2, 102) = 3.68, p < 0.03], and two-way effects of irrigation: genotype [F (2, 102) = 5.53, p < 0.005], as well as irrigation: silicon [F (2, 102) = 3.63, p < 0.03], on the chlorophyll content. Comparable results were observed in chlorophyll *b* and carotenoid contents (Online Resource 1). The water stress treatment, in the form of increased irrigation intervals, was associated with a significant increase in chlorophyll *a/b* and total carotenoids, which was more pronounced in the *G61* genotype (Figs. VII-IX).

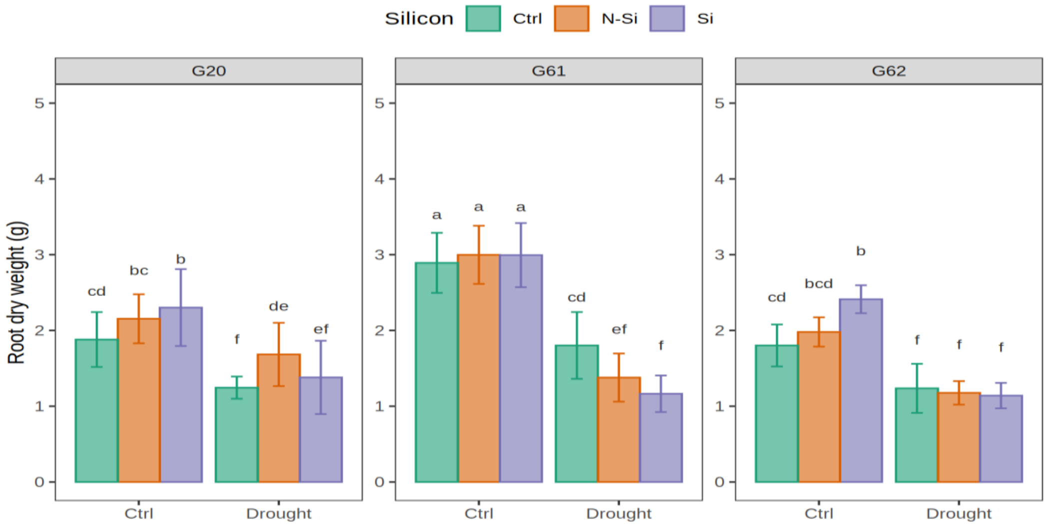

Fig. V. Effects of silicon treatment (Ctrl, control; N-Si, 2 mM nano-silicon; Si, 2 mM silicon) on three faba bean genotypes (G20, G61 and G62) root dry weight, grown under water deficit condition (Ctrl, irrigation every 4 days; Drought, irrigation every 8 days). Plants were harvested 30 days after the last silicon treatment.

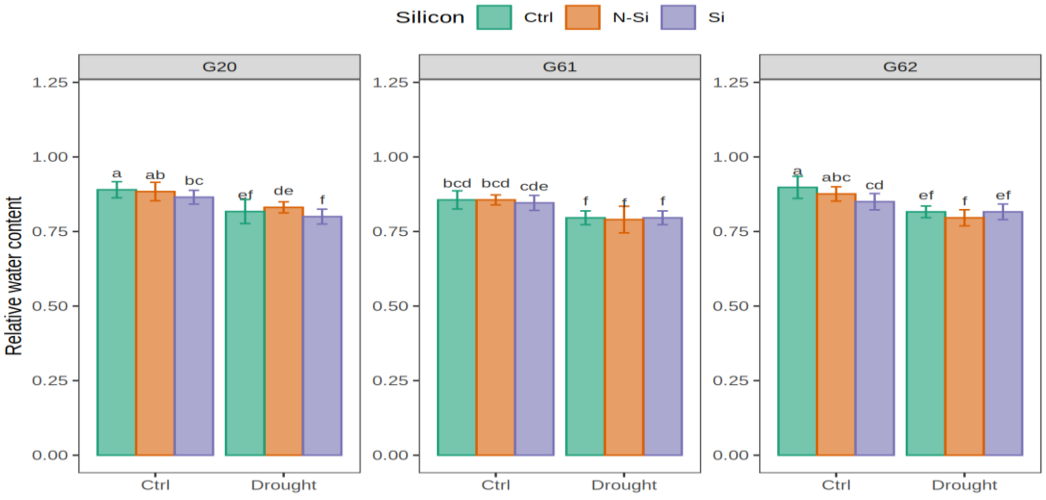

Fig. VI. Effects of silicon treatment (Ctrl, control; N-Si, 2 mM nano-silicon; Si, 2 mM silicon) on three faba bean genotypes (G20, G61 and G62) relative water content, grown under water deficit condition (Ctrl, irrigation every 4 days; Drought, irrigation every 8 days). Plants were harvested 30 days after the last silicon treatment.

Further biochemical analysis revealed that the amounts of carotenoids, the activity of guaiacol peroxidase (Fig. X), and malondialdehyde content (Fig. XI) were comparable between the three examined genotypes under control conditions and elevated in response to the applied water stress treatment. Under drought treatment, the elevation levels of carotenoids and guaiacol peroxidase in the *G62* genotype were lower than those in the *G20* and *G61* genotypes. However, the level of increase in malondialdehyde content in *G62* was higher than that in the other two genotypes. These observations may suggest that the *G62* genotype is more sensitive to water deficit than *G20* and *G61*.

Silicon treatment did not have a significant effect on carotenoid levels in the *G20* and *G61* genotypes under normal conditions or during drought stress.

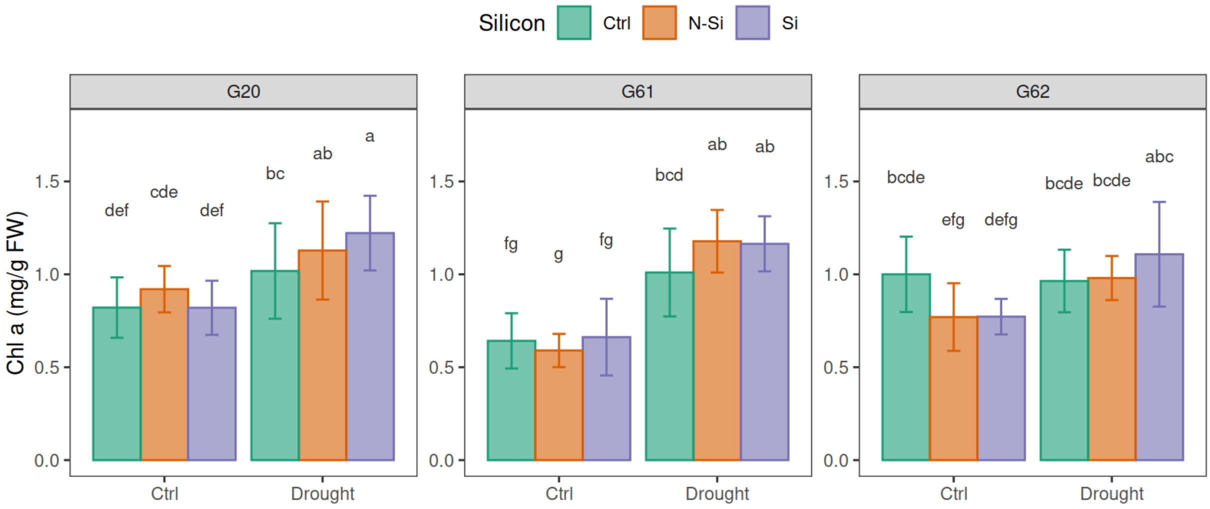

Fig. VII. Effects of silicon treatment (Ctrl, control; N-Si, 2 mM nano-silicon; Si, 2 mM silicon) on three faba bean genotypes (G20, G61 and G62) Chl a, grown under water deficit condition (Ctrl, irrigation every 4 days; Drought, irrigation every 8 days). Plants were harvested 30 days after the last silicon treatment.

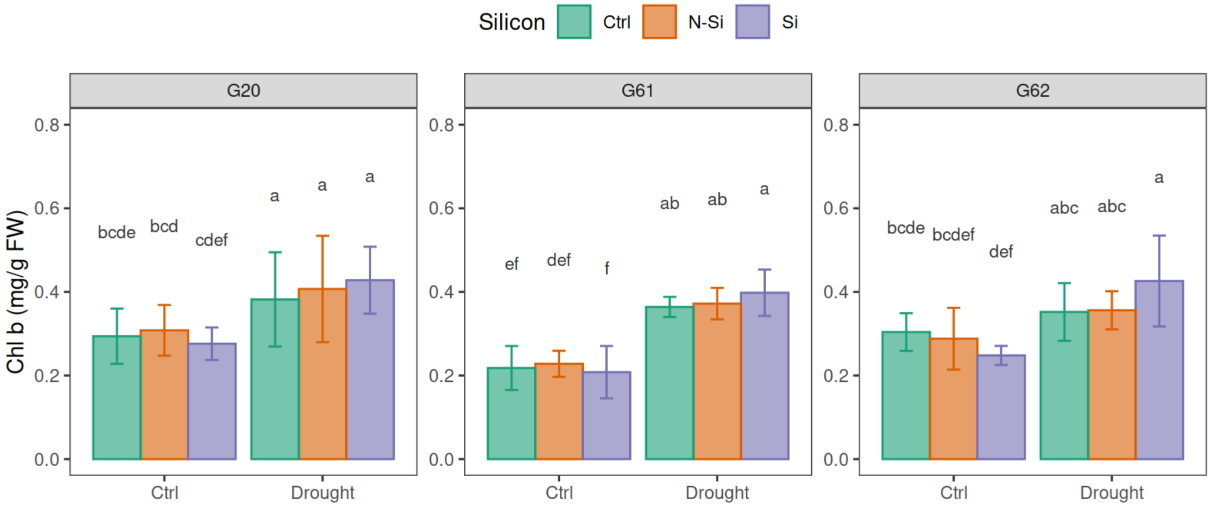

Fig. VIII. Effects of silicon treatment (Ctrl, control; N-Si, 2 mM nano-silicon; Si, 2 mM silicon) on three faba bean genotypes (G20, G61 and G62) Chl b, grown under water deficit condition (Ctrl, irrigation every 4 days; Drought, irrigation every 8 days). Plants were harvested 30 days after the last silicon treatment.

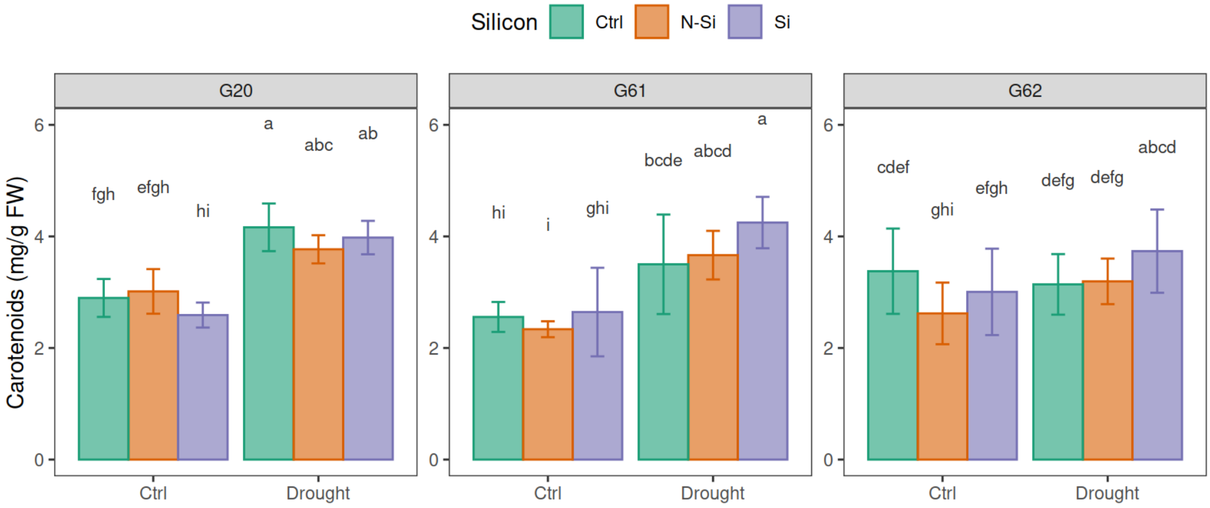

Fig. IX. Effects of silicon treatment (Ctrl, control; N-Si, 2 mM nano-silicon; Si, 2 mM silicon) on three faba bean genotypes (G20, G61 and G62) carotenoids, grown under water deficit condition (Ctrl, irrigation every 4 days; Drought, irrigation every 8 days). Plants were harvested 30 days after the last silicon treatment.

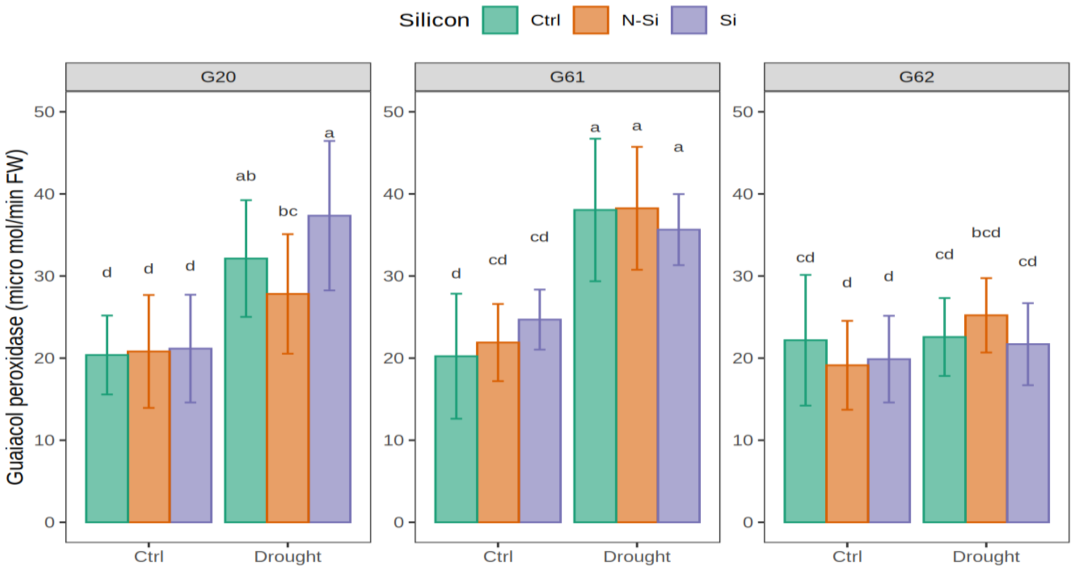

Fig. X. Effects of silicon treatment (Ctrl, control; N-Si, 2 mM nano-silicon; Si, 2 mM silicon) on three faba bean genotypes (G20, G61 and G62) guaiacol peroxidase, grown under water deficit condition (Ctrl, irrigation every 4 days; Drought, irrigation every 8 days). Plants were harvested 30 days after the last silicon treatment.

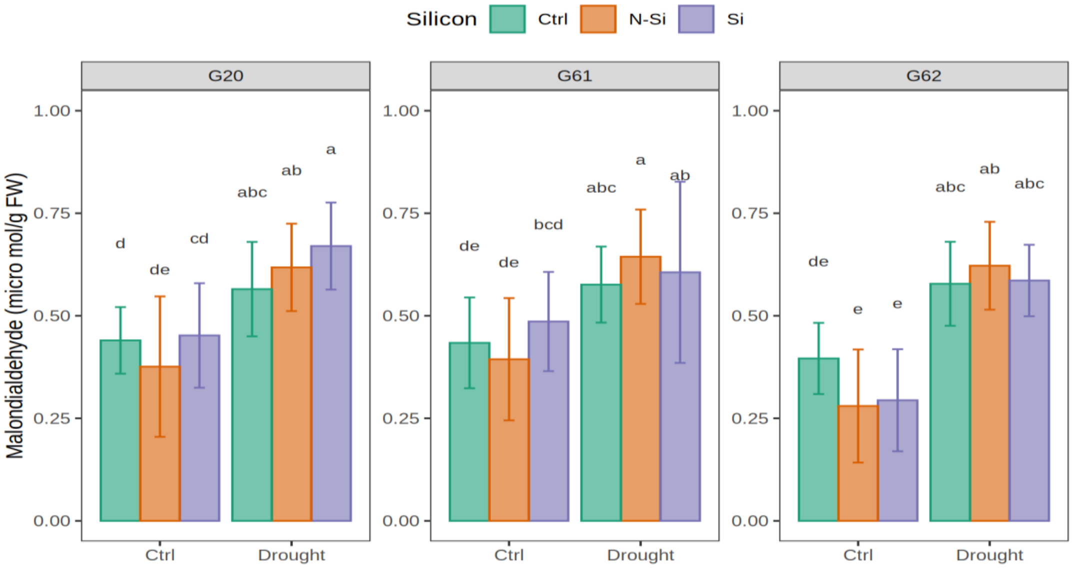

Fig. XI. Effects of silicon treatment (Ctrl, control; N-Si, 2 mM nano-silicon; Si, 2 mM silicon) on three faba bean genotypes (G20, G61 and G62) malondialdehyde, grown under water deficit condition (Ctrl, irrigation every 4 days; Drought, irrigation every 8 days). Plants were harvested 30 days after the last silicon treatment.

However, in the *G62* genotype, silicon treatment increased carotenoid content in well-watered plants. The effects of silicon or nano-silicon treatments on guaiacol peroxidase activity were minimal. The only significant increase in enzyme activity was observed in the *G20* genotype under drought stress with nano-silicon treatment. Silicon or nano-silicon did not have a statistically significant effect on malondialdehyde content in any of the tested genotypes.

Discussion

Terrestrial plants employ biochemical and physiological processes to safeguard their cells against dehydration during periods of water scarcity. In our experiments, the growth of all examined broad bean (*Vicia faba* L.) genotypes was affected by water stress imposed through delayed irrigation intervals. Similar reductions in shoot height under drought stress have also been observed in mung beans (Amanullah et al., 2022). Plant height can be influenced by both genetic characteristics and various environmental stressors, such as water deficit, which can impede cell division and elongation (Muktadir et al., 2020). Our data demonstrate that although all three examined genotypes were impacted by water deficit treatment, the detrimental effects of drought on shoot height were more pronounced in the G62 genotype compared to G20 or G61. However, this disparity was not observed in terms of shoot fresh/dry weight. Two plant hormones, namely abscisic acid (ABA) and cytokinin, are believed to play important roles in plant responses to drought stress. ABA rapidly accumulates in response to drought stress and plays a crucial role in regulating multiple processes, including the accumulation of protective compounds, closure of stomata, and shoot growth (Gomez-Cadenas et al., 2015).

Two lines of evidence suggest that cytokinin signaling components act as negative regulators of plant drought tolerance: (i) cytokinin-deficient plants, including CKX (cytokinin oxidase/dehydrogenase) overexpression lines and *ipt1,3,5,7* quadruple mutants, exhibit enhanced drought tolerance and slower shoot growth (Hussain et al., 2025); and (ii) the *Arabidopsis* type B cytokinin response regulators *ARR1, ARR10,* and *ARR12* have been shown to negatively regulate plant responses to dehydration (Nguyen et al., 2016). On the other hand, Gujjar et al. (2020)reported that stress-induced cytokinin synthesis increased the tolerance of rice to drought stress via the regulation of carbon and nitrogen assimilation.

Taking into consideration all the examined morphological traits, it appears that *G20*, *G61*, and *G62* do not exhibit significantly different genetic potential to withstand water deficit. Comparable sensitivity to water deficit has been reported in other broad bean genotypes (Siddiqui et al. 2015). Reductions in whole-plant dry weight can be attributed to decreased photosynthesis and diminished leaf area index (Muktadir et al., 2020). In conclusion, we can deduce that in plants exposed to water deficit, three phenomena can be observed:

1. Water deficit causes wilting and reduced plant height without significantly affecting dry mass.
2. A rebalancing between ABA and cytokinin, occurring during prolonged water stress, can further decrease plant height/weight due to the redistribution of photosynthetic products between the shoot and root. This type of effect may even result in increased chlorophyll/carotenoid contents per leaf fresh weight.
3. Prolonged and severe water deficit stress can impair plant metabolism and further diminish the photosynthetic apparatus and vegetative growth, ultimately resulting in reduced plant biomass and crop yield.

One of the effective mechanisms that plants use to cope with water stress is the production of osmoprotectants to maintain cellular osmotic potential (Abid et al., 2021). Our results demonstrated that water stress caused a slight but statistically significant decrease in leaf relative water content, indicating that the applied water deficit stress was effective at the cellular level. As a response to water deficit stress, the accumulation of osmoprotectants in cells may help maintain cellular water potential (Rady et al., 2021). Otherwise, it could result in reduced cell division, altered hormonal balance, impaired net photosynthesis, and eventual cell death.

Based on the findings of this experiment, it was observed that under the applied water deficit treatments, there was a decrease in vegetative growth rate but an increase in chlorophyll a and b, total carotenoids, and xanthophylls. This suggests that the treated plants were still in the early stages of adapting to water stress. In other words, the increase in chlorophyll content may be due to an artifact of chlorophyll measurement, which is based on the fresh weight of plants. When the fresh weight decreases, it can give the appearance of increased chlorophyll.

Taking a more realistic approach, we believe that the differential sensitivity of two events (cell expansion vs. chloroplast number per cell) in response to water deficit might play a role – cell expansion is more sensitive to drought than chloroplast number.

In general, the impact of water stress on chlorophyll content varies and depends on other environmental conditions and plant genotype. For example, some species have shown a decrease in chlorophyll a/b under mild water stress, while others have observed an increase in chlorophyll a/b (Muktadir et al., 2020). It has been reported that in wheat, soil water deficit might enhance the effect of atmospheric water deficit on solar-induced chlorophyll fluorescence (Liu et al., 2020). Therefore, it is tempting to speculate that in broad bean plants exposed to soil water deficit, a rapid loss of atmospheric moisture (vapor pressure deficit) may accelerate chlorophyll degradation, while increased atmospheric vapor pressure may inhibit chlorophyll degradation. Since the water deficit treatment in our experiments was applied in late autumn, increased atmospheric vapor pressure could have prevented chlorophyll degradation. However, we consider this possibility too speculative; thus, future experiments are needed to investigate the effect of heat and atmospheric water deficit on the physiological and growth responses of broad bean under soil water deficiency.

Antioxidant enzymes can counteract the accumulation of reactive oxygen species (ROS) caused by prolonged water deficiency, which can otherwise damage cells, including lipid peroxidation and cell membrane leakage (Abid et al., 2020). Our data reveal that the guaiacol peroxidase activity of *G20*, *G61*, and *G62* genotypes was similar under control conditions but increased up to two-fold only in G20 and G61 genotypes, suggesting that these lines may be more resistant to the applied drought treatment compared to G62.

Consistent with this idea, the level of malondialdehyde in *G62* plants under drought stress was almost twice that in well-watered plants. Under water deficit stress, malondialdehyde can be produced by the oxidation of unsaturated fatty acids with multiple double bonds found in the plasma membrane due to the accumulation of oxygen free radicals (Morales and Munné-Bosch, 2019). Overall, the observed low level of guaiacol peroxidase and high level of malondialdehyde in the *G62* genotype may imply that *G62* in the vegetative stage is more sensitive to water deficit than the other two genotypes.

Silicon (Si), the second most abundant element in Earth's crust, has been shown to exhibit controversial alleviating effects on a variety of plant species under drought stress (Bhardwaj and Kapoor, 2021; Malik et al., 2021; Wang et al., 2021). It has been reported that the application of Si improves a plant's ability to tolerate drought stress by enhancing photosynthesis, balancing nutrient absorption, and enhancing the plant's antioxidant defense system (Bhardwaj and Kapoor, 2021).

In our experiments, the effects of silicon or nano-silicon on alleviating the negative effects of water deficiency were almost negligible but varied among the different examined genotypes. For instance, marginal effects of silicon treatment were observed in increasing the shoot height of *G62* and the shoot fresh weight of *G20* genotypes under control conditions; and in the shoot dry weight of *G62* and root dry weight of G61 under drought stress.

Altogether, we may conclude that the mild effects of silicon or nano-silicon in ameliorating drought stress in broad bean plants are a genotype- and environment-dependent phenomenon that needs further attention.

Conclusion

Drought stress is a major concern in agriculture due to its deleterious effects on crop productivity. This study aimed to investigate the effects of externally applied silicon and nano-silicon on the morpho-physiological responses of three newly introduced broad bean (*Vicia faba*) genotypes during the vegetative growth stage water deficit stress. During the water deficit period, shoot height, as well as shoot and root fresh and dry weights, decreased, along with relative water content. However, chlorophyll, carotenoids, malon-dialdehyde (MDA) content, and guaiacol peroxidase activity increased in all three genotypes. Notably, the G62 genotype exhibited lower guaiacol peroxidase activity and higher MDA levels under drought stress, indicating greater sensitivity to water deficit compared to G20 and G61. It was observed that the impact of silicon on certain morpho-physiological characteristics was marginally positive, although it varied depending on genotype and environmental conditions. These findings suggest that before applying silicon or nano-silicon in field trials involving broad bean cultivation under water deficit stress, the genotype-dependent response of each *Vicia faba* variety should be carefully evaluated.

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References

Abid, G., R. N. Ouertani, S. H. Jebara, H. Boubakri, Y. Muhovski, E. Ghouili, S. Abdelkarim, O. Chaieb, Y. Hidri and S. Kadri. 2020. Alleviation of drought stress in faba bean (*Vicia faba* L.) by exogenous application of β-aminobutyric acid (BABA). *Physiology and molecular biology of plants,* 26, 1173-1186.

Abid, G., M. N. Saidi, R. N. Ouertani, Y. Muhovski, S. H. Jebara, E. Ghouili, K. Sassi, J.-P. Baudoin, M. E. Ayed and S. Elkahoui. 2021. Differential gene expression reveals candidate genes for osmotic stress response in faba bean (*Vicia faba* L.) involved in different molecular pathways. *Acta Physiologiae Plantarum,* 43, 1-20.

Alghamdi, S., A. Al-Shameri, H. Migdadi, M. Ammar, E. El-Harty, M. Khan and M. Farooq. 2015. Physiological and molecular characterization of faba bean (*Vicia faba* L.) genotypes for adaptation to drought stress. *Journal of Agronomy and Crop Science,* 201, (6) 401-409.

Amanullah, M. Yar, S. Khalid, M. S. Elshikh, H. M. Akram, Imran, A. R. M. Al-Tawaha, M. Ahmad, A. Muhammad and S. Ullah. 2022. Phenology, growth, productivity, and profitability of mungbean as affected by potassium and organic matter under water stress vs. no water stress conditions. *Journal of Plant Nutrition,* 45, (5) 629-650.

Ammar, M., F. Anwar, E. El-Harty, H. Migdadi, S. Abdel-Khalik, S. Al-Faifi, M. Farooq and S. Alghamdi. 2015. Physiological and yield responses of faba bean (*Vicia faba* L.) to drought stress in managed and open field environments. *Journal of Agronomy and Crop Science,* 201, (4) 280-287.

Arnon, D. I. 1949. Copper enzymes in isolated chloroplasts. Polyphenoloxidase in *Beta vulgari*s. *Plant physiology,* 24, (1) 1.

Barlow, K. M., B. P. Christy, G. J. O'leary, P. A. Riffkin and J. G. Nuttall. 2015. Simulating the impact of extreme heat and frost events on wheat crop production: A review. *Field crops research,* 171, 109-119.

Bhardwaj, S. and D. Kapoor. 2021. Fascinating regulatory mechanism of silicon for alleviating drought stress in plants. *Plant Physiology and Biochemistry,* 166, 1044-1053.

Biswas, A., S. Sarkar, S. Das, S. Dutta, M. R. Choudhury, A. Giri, B. Bera, K. Bag, B. Mukherjee and K. Banerjee. 2025. Water scarcity: A global hindrance to sustainable development and agricultural production–A critical review of the impacts and adaptation strategies. *Cambridge Prisms: Water,* 3, e4.

Chance, B. and A. Maehly. 1955. [136] Assay of catalases and peroxidases.

Daryanto, S., L. Wang and P.-A. Jacinthe. 2015. Global synthesis of drought effects on food legume production. *PloS one,* 10, (6) e0127401.

Desoky, E.-S. M., E. Mansour, E.-S. E. El-Sobky, M. I. Abdul-Hamid, T. F. Taha, H. A. Elakkad, S. M. Arnaout, R. S. Eid, K. A. El-Tarabily and M. A. Yasin. 2021. Physio-biochemical and agronomic responses of faba beans to exogenously applied nano-silicon under drought stress conditions. *Frontiers in plant science,* 12, 637783.

Fao. 2019. *The state of food and agriculture 2019: Moving forward on food loss and waste reduction*. UN

Farooq, M., N. Gogoi, S. Barthakur, B. Baroowa, N. Bharadwaj, S. S. Alghamdi and K. H. Siddique. 2017. Drought stress in grain legumes during reproduction and grain filling. *Journal of Agronomy and Crop Science,* 203, (2) 81-102.

Gomez-Cadenas, A., V. Vives, S. I Zandalinas, M. Manzi, A. M Sanchez-Perez, R. M Perez-Clemente and V. Arbona. 2015. Abscisic acid: a versatile phytohormone in plant signaling and beyond. *Current Protein and Peptide Science,* 16, (5) 413-434.

Gujjar, R. S., P. Banyen, W. Chuekong, P. Worakan, S. Roytrakul and K. Supaibulwatana. 2020. A synthetic cytokinin improves photosynthesis in rice under drought stress by modulating the abundance of proteins related to stomatal conductance, chlorophyll contents, and rubisco activity. *Plants,* 9, (9) 1106.

Hussain, S., J. Chang, J. Li, L. Chen, S. Ahmad, Z. Song, B. Zhang and X. Chen. 2025. Multifunctional Role of Cytokinin in Horticultural Crops. *International Journal of Molecular Sciences,* 26, (3) 1037.

Islam, S. S., S. Adhikary, M. Mostafa and M. M. Hossain. 2024. Vegetable Beans: Comprehensive Insights into Diversity, Production, Nutritional Benefits, Sustainable Cultivation and Future Prospects.

Kar, M. and D. Mishra. 1976. Catalase, peroxidase, and polyphenoloxidase activities during rice leaf senescence. *Plant physiology,* 57, (2) 315-319.

Karavidas, I., G. Ntatsi, V. Vougeleka, A. Karkanis, T. Ntanasi, C. Saitanis, E. Agathokleous, A. Ropokis, L. Sabatino and F. Tran. 2022. Agronomic practices to increase the yield and quality of common bean (*Phaseolus vulgaris* L.): a systematic review. *Agronomy,* 12, (2) 271.

Karwani, G., L. Mashamba, M. Akida and M. Teshale. 2024. Climate smart agriculture in Sub-Saharan Africa: Review of the potentials for maize and common beans smallholder farmers in semi-arid areas.

Kenawy, E.-R., M. Rashad, A. Hosny, S. Shendy, D. Gad and K. M. Saad-Allah. 2022. Enhancement of growth and physiological traits under drought stress in Faba bean (*Vicia faba* L.) using nanocomposite. *Journal of Plant Interactions,* 17, (1) 404-418.

Liu, L., Y. Teng, J. Wu, W. Zhao, S. Liu and Q. Shen. 2020. Soil water deficit promotes the effect of atmospheric water deficit on solar-induced chlorophyll fluorescence. *Science of the Total Environment,* 720, 137408.

Malik, M. A., A. H. Wani, S. H. Mir, I. U. Rehman, I. Tahir, P. Ahmad and I. Rashid. 2021. Elucidating the role of silicon in drought stress tolerance in plants. *Plant Physiology and Biochemistry,* 165, 187-195.

Morales, M. and S. Munné-Bosch. 2019. Malondialdehyde: facts and artifacts. *Plant physiology,* 180, (3) 1246-1250.

Muktadir, M. A., K. N. Adhikari, A. Merchant, K. Y. Belachew, A. Vandenberg, F. L. Stoddard and H. Khazaei. 2020. Physiological and biochemical basis of faba bean breeding for drought adaptation—A review. *Agronomy,* 10, (9) 1345.

Nguyen, K. H., C. V. Ha, R. Nishiyama, Y. Watanabe, M. A. Leyva-González, Y. Fujita, U. T. Tran, W. Li, M. Tanaka and M. Seki. 2016. Arabidopsis type B cytokinin response regulators ARR1, ARR10, and ARR12 negatively regulate plant responses to drought. *Proceedings of the National Academy of Sciences,* 113, (11) 3090-3095.

Prochazkova, D., R. Sairam, G. Srivastava and D. Singh. 2001. Oxidative stress and antioxidant activity as the basis of senescence in maize leaves. *Plant Science,* 161, (4) 765-771.

Qados, A. A. and A. E. Moftah. 2015. Influence of silicon and nano-silicon on germination, growth and yield of faba bean (*Vicia faba* L.) under salt stress conditions.

R Core Team. 2020. R: A language and environment for statistical computing, R Foundation for Statistical Computing. Vienna, Australia. https://www.R-project.org

Rady, M. M., S. H. Boriek, T. A. Abd El-Mageed, M. A. Seif El-Yazal, E. F. Ali, F. A. Hassan and A. Abdelkhalik. 2021. Exogenous gibberellic acid or dilute bee honey boosts drought stress tolerance in *Vicia faba* by rebalancing osmoprotectants, antioxid-ants, nutrients, and phytohormones. *Plants,* 10, (4) 748.

Rizwan, M., S. Ali, M. Ibrahim, M. Farid, M. Adrees, S. A. Bharwana, M. Zia-Ur-Rehman, M. F. Qayyum and F. Abbas. 2015. Mechanisms of silicon-mediated alleviation of drought and salt stress in plants: a review. *Environmental Science and Pollution Research,* 22, 15416-15431.

Roychoudhury, A. 2020. Silicon-nanoparticles in crop improvement and agriculture. *International Journal on Recent Advancement in Biotechnology & Nanotechnology,* 3, (1) 2582-1571.

Siddiqui, M. H., M. Y. Al-Khaishany, M. A. Al-Qutami, M. H. Al-Whaibi, A. Grover, H. M. Ali, M. S. Al-Wahibi and N. A. Bukhari. 2015. Response of different genotypes of faba bean plant to drought stress. *International Journal of Molecular Sciences,* 16, (5) 10214-10227.

Sohrawardy, H., S. H. Kasfy and T. Islam. 2024. Nanoselenium and nanosilicon for nutrition and disease protection of crop plants. *Nanofertilizer Delivery, Effects and Application Methods,* 227-249.

Tubana, B. S., T. Babu and L. E. Datnoff. 2016. A review of silicon in soils and plants and its role in US agriculture: history and future perspectives. *Soil science,* 181, (9/10) 393-411.

Virnodkar, S. S., V. K. Pachghare, V. Patil and S. K. Jha. 2020. Remote sensing and machine learning for crop water stress determination in various crops: a critical review. *Precision Agriculture,* 21, (5) 1121-1155.

Wang, M., R. Wang, L. a. J. Mur, J. Ruan, Q. Shen and S. Guo. 2021. Functions of silicon in plant drought stress responses. *Horticulture Research,* 8,

Warsame, A. O., D. M. O'sullivan and P. Tosi. 2018. Seed storage proteins of faba bean (*Vicia faba* L): Current status and prospects for genetic improvement. *Journal of Agricultural and Food chemistry,* 66, (48) 12617-12626.