

Effects of Exogenous Application of SiO₂ Nanoparticles Enhances Drought Stress Tolerance in Wild and Cultivated Chickpea Plants

Eray ŞİMŞEK¹, Elif TİRYAKİ², Büşra TATAŞ³, Sertan ÇEVİK⁴

¹ Department of Plant Protection, Faculty of Agriculture, Harran University, Şanlıurfa, Türkiye, ^{2,3,4} Department of Molecular Biology and Genetic, Faculty of Science and Letters, Harran University, Şanlıurfa, Türkiye.

¹<https://orcid.org/0000-0003-4984-4223>, ²<https://orcid.org/0009-0008-2110-3849>, ³<https://orcid.org/0009-0007-9069-5027>

⁴<https://orcid.org/0000-0003-1259-7863>

✉: eraysim@harran.edu.tr

ABSTRACT

Drought is one of the most significant stress factors that constrain plant growth. Many studies focused on methods to enhance the plant stress tolerance against drought. Recently, the focus has been on the exogenous applications and, in particular, the nanomaterials powered by advancements in the field of nanotechnology. Silicon appears to support some plants against different stress factors, including drought. Despite this, there is a remarkable lack of studies on the use of silicon for enhancing drought tolerance in wild and cultivated chickpeas. In this study, 150 mg L⁻¹ SiO₂ nanoparticle spraying was applied to two chickpea varieties, cultivated and wild, under drought stress. Changes were analyzed in morphological, physiological, and biochemical traits to find the change in plants' drought tolerance. Under drought stress, SiO₂ treatment increased antioxidant enzyme activities in both species. Similarly, nanoparticle treatment increased some growth characteristics of plants. Additionally, significant increases in leaf relative water content were detected in plants treated with SiO₂ under drought conditions. In this study, the effect of SiO₂ nanoparticle application on the stress tolerance of wild and cultivated chickpea plants has been studied. Basically, the results showed that exogenous application of SiO₂ NPs increases drought tolerance by stimulating water status and growth parameters, and by activities of antioxidant enzymes in both wild and cultivated species of chickpea.

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Dışardan Uygulanan SiO₂ Nanopartiküllerinin Yabani ve Kültür Nohut Bitkilerinde Kuraklık Stresi Toleransına Etkileri

ÖZET

Kuraklık, bitki büyümesini sınırlayan en önemli stres faktörlerinden biridir. Birçok çalışma, bitkilerin kuraklığa karşı toleransını artırma yöntemlerine odaklanmıştır. Son zamanlarda ise özellikle nanoteknoloji alanındaki ilerlemelerle güçlenen nanomalzemelerin bitkilere dıştan uygulamalarına odaklanılmıştır. Silikon, kuraklık dahil olmak üzere farklı stres faktörlerine karşı bazı bitkileri destekler gibi görünmektedir. Buna rağmen, yabani ve kültür nohutlarında kuraklık toleransını artırmak için silikon kullanımına dair kayda değer bir çalışma eksikliği vardır. Bu çalışmada, kültür ve yabani olmak üzere iki nohut türünde, kuraklık stresi altında 150 mg/L SiO₂ nanopartikül spreylemesi uygulanmıştır. Bitkilerin kuraklık toleransındaki değişimi belirlemek amacıyla morfolojik, fizyolojik ve biyokimyasal özelliklerdeki değişiklikler analiz edilmiştir. Kuraklık stresi altında, SiO₂ uygulaması her iki türde de antioksidan enzimlerin aktivitelerini artırmıştır. Benzer şekilde, nanopartikül uygulaması bitkilerin bazı büyüme özelliklerini de artırmıştır. Ayrıca, kurak koşullarda SiO₂ uygulanan bitkilerde yaprak oransal su içeriğinde önemli artışlar tespit edilmiştir. Bu çalışmada, SiO₂ nanopartikül uygulamasının yabani ve kültür nohut bitkilerinin stres toleransı üzerindeki etkisi incelenmiştir. Sonuçlar

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temel olarak, SiO₂ NP'lerin dışsal olarak uygulamasının, hem yabani hem de kültür nohut türlerinde su durumu ve büyüme parametrelerini uyararak ve antioksidan enzimlerin aktivitelerini artırarak kuraklığa toleransı artırdığını göstermiştir.

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INTRODUCTION

Chickpea (*Cicer arietinum* L.) is a legume plant that is generally grown under rain-fed conditions in arid and semi-arid regions (Millan et al., 2006). Important abiotic stress factors such as drought limit the expected yield of chickpea, and therefore, many researchers are studying chickpea breeding to manage drought-induced yield losses (Maqbool et al., 2017). Drought stress, triggered by global warming, is causing significant losses in agricultural productivity worldwide. This issue poses a major challenge to agricultural production, as it intensifies with rising temperatures, leading to reduced crop yields and threatening food security on a global scale (Chakraborty & Newton, 2011). Drought stress is one of the most significant challenges to chickpea farming in the changing climate conditions and ever-growing population of the world (Nadeem et al., 2019). It is responsible for an estimated 45-50% reduction in chickpea yields globally (Thudi et al., 2014).

From a plant perspective, drought disrupts photosynthesis due to changes caused in the ultrastructure of cell organelles. Furthermore, alterations in metabolites and enzymes essential for the synthesis and enhancement of photosynthetic products contribute to this physiological impact (Wahab et al., 2022). In the contemporary context, researchers are actively engaged in either developing new plant varieties or exploring applications that induce drought tolerance as part of efforts to address and mitigate the impact of drought (Gaur et al., 2019; Kandhol et al., 2022).

The use of nanoparticles (NPs) represents one of the most promising approaches developed so far in terms of enhancing plant tolerance against drought-caused damage (Kandhol et al., 2022). This strategy has been proven to be very effective in helping plants survive under drought conditions (Ahmed et al., 2021; Kandhol et al., 2022; Mohamed and Abdel-Hakeem, 2023). Nanoparticles have demonstrated potential to interact with plants and other organisms due to their distinctive nano-size, unique physical properties, and high surface area to volume ratio (Dura and Kepenkçi, 2022; Wang et al., 2023). Application of various types of nanoparticles on crop plants was reported to be effective in improving the plant health condition under osmotic stress conditions (Heikal et al., 2023; Mohamed and Abdel-Hakeem, 2023). Among various nanoparticles, the potential of silicon-based nanoparticles has recently been encouraged for assessment in terms of both biotic as well as abiotic stress agents (Du et al., 2022; Wang et al., 2022). Silicon NPs showed significant outcomes in improved growth, decreased water stress, and stimulation of a range of antioxidant enzymes, in addition to greater chlorophyll content and gas exchange attributes that ensured enhanced biomass and crop yield under stress interactions (Raza et al., 2023). Moreover, available empirical evidence suggests that silicon has positive impacts on plant growth. It supports plants in mitigating both biotic and abiotic stresses (Debona et al., 2017; Li et al., 2018). In this context, various studies have reported that silicon nanoparticles are capable of regulating the endogenous concentration of hormones, such as auxins, ABA, IAA, cytokinins, ethylene, gibberellins, and jasmonic acid. Altered phytohormone levels have significant impacts on plant growth, development, and productivity across various organs and tissues (Mukarram et al., 2022). In the same way, SiO₂ NPs control CAT, APX, SOD activities and the AsA-GSH cycle, thus providing enhanced efficiency to the defense system (Fatemi et al., 2021). Recent findings suggest that the use of SiO₂ NPs could exert a notable influence on the drought stress tolerance of maize seedlings. Notably, after SiO₂ NPs treatment, the increase in proline levels and reduction in total soluble sugars can significantly contribute to stress tolerance. The findings underscore the potential of SiO₂ NPs in facilitating osmotic adjustment, with specific emphasis on modulating proline accumulation as opposed to the accumulation of soluble sugars. Moreover, the utilization of SiO₂ nanoparticles decreases the malondialdehyde levels when compared to untreated vegetation subjected to drought conditions (Sharf-Eldin et al., 2023).

Research examining the effect of silicon NPs on chickpea plants against abiotic stress conditions has indicated the potential applicability of these nanoparticles in cultivation. Silica nanoparticles mitigate aluminum-induced injuries in *Cicer arietinum* by inhibiting cytotoxic agents and upregulating protective genes (Chandra et al., 2020). Particularly, considering cross-pollination and genetic exchanges between wild varieties and cultivated varieties

of chickpea plants, positive effects on stress tolerance have been observed (Coyne et al., 2020). Recent experiments indicate that applying conventional Zn+Si fertilizer to the leaves can mitigate the damage caused by osmotic stress in chickpeas. This protective effect is mainly due to the activation of adaptive mechanisms, such as the increased accumulation of compatible solutes and improved scavenging of reactive oxygen species (ROS) (Mohamadzadeh et al., 2023; Zahedi et al., 2023).

Although there are several studies about the effects of silicon on the physiology and growth of cultivated chickpeas, limited information on this substance under osmotic stress situations exists in the case of the wild relative (*Cicer reticulatum* L.) of the species. This study tests exogenous application of SiO₂ nanoparticles in two lines of chickpea cultivars, the cultivated variety, *Cicer arietinum*, and the wild type, *Cicer reticulatum*-under controlled drought stress. The objective is to determine the directional changes in osmotic stress tolerance within both species as a result of the application and subsequently conduct a comparative analysis. This literature review indicates that this is the first study to investigate and compare the effects of SiO₂ nanoparticles on drought-induced stress tolerance in both wild and cultivated chickpea plants.

MATERIAL and METHOD

Plant Material and Growth Conditions

Cicer arietinum ILC-482 and *Cicer reticulatum* AWC-611 seeds were used for the experiments. In order to surface sterilize the seeds, they were initially washed with 70% ethanol and surface sterilized with 1% NaOCl and washed with sterile water five times. Chickpea seeds were placed between sterile papers in petri dishes, with 10 seeds in each dish. Once seedlings germinated and radicles elongated to about 5 cm, they were transferred to pots prepared in a hydroponic setup in oriented Hoagland solution. Plants were grown in a growth room chamber kept at a constant temperature of 25±1°C. It was set on a long day photoperiod cycle of 16:8 hours light: dark with 300 µmol m⁻²s⁻¹ light density. There was a cultivation for 21 days over the environment set at a relative humidity of 65-70%.

SiO₂ NPs Treatment

In this study, commercially purchased SiO₂ NPs (Sigma Aldrich) were used, and the concentration of NPs (150 mg/L) was determined according to Yıldız (2018). After 21 days of growth, chickpea plants were sprayed with SiO₂ NPs every day for three days (three times total). Adjustments were made to ensure that each spray application delivered 20 mL of SiO₂ NP solution per plant. After a total of three spray applications, the SiO₂ NPs were uniformly distributed over the plant shoot tissues, resulting in a total deposition of 9 mg of SiO₂ NPs per plant. Untreated control groups were sprayed with pure water that did not contain nanoparticles. At this stage, the nutrient solution's surface was carefully covered to prevent the nanoparticles from mixing with the solution where the roots are located. Figure 1 presents representative images of the plants grown for the experiment.

Drought Stress Treatments

After being grown for a total of 3 weeks, SiO₂ NPs spraying was performed for three consecutive days at the same time each day. Twenty-four hours after the final spray application, the plants were transferred to a PEG 6000 solution to induce drought stress. The final concentration of PEG6000 (20%) was added to Hoagland solutions containing the roots of the plants. The concentration of PEG6000 was determined based on a study related to chickpeas by Kumar et al., (2019). Plants without PEG treatment served as drought control groups. The overall condition of the plants was monitored following PEG6000 application. When the plants in the drought groups lost turgor in their leaves, the experiment was terminated. After drought stress, plant biomass data and leaf relative water content were calculated, and plants were harvested and stored. Measurement methods are given in detail in subsequent sections. Plant leaves were rapidly harvested, ground with liquid nitrogen, and then stored at -80 °C until the day of analysis.

Determination of plant biomass

The length of the shoot was measured by using a ruler from the root collar to the tap. The fresh weights of the shoot and root were recorded, and then the samples were dried in an oven at 80°C and weighed again, with the results determined in grams.

Water status of the leaves

Harvested plant leaves were taken in equal numbers, weighed on a precision scale, and their fresh weights were recorded (FW). Subsequently, these leaves were immersed in pure water at 25°C for 4 hours to become turgid. After ensuring turgidity, excess water on the leaves was removed with the help of a tissue, and they were weighed

again to record turgid weights (TW). The leaf samples, whose weights were determined, were then dried in an oven at 65°C for 48 hours, and the dry weight was recorded as 'g' (DW). The relative water content of leaves (%) was calculated by ratioing the obtained fresh and dry weights using the following equation 1 (Smart & Bingham, 1974):

$$\text{Relative water content} = \frac{(FW - DW)}{(TW - DW)} \times 100 \quad (1)$$

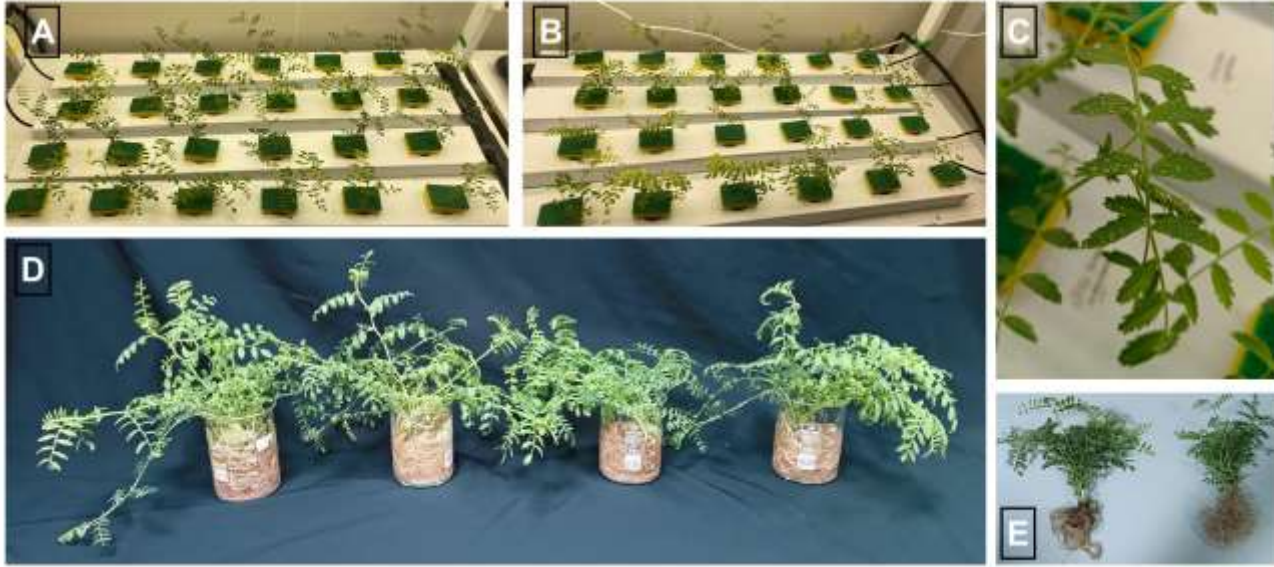


Figure 1. Growth and development of plants in a hydroponic system (A, B), close-up view of a plant leaf showing sprayed SiO₂ nanoparticle (C), Progression of plant growth for *C. reticulatum* and *C. arietinum* over time in the hydroponic setup, showing increased foliage and plant height (D). Root systems of the plants removed from the hydroponic setup (E).

Şekil 1. Hidroponik sistemde bitkilerin büyümesi ve gelişimi (A, B), SiO₂ nanopartikülü püskürtülmüş bir bitki yaprağının yakın plan görüntüsü (C), hidroponik sistemde zamanla artan yaprak yoğunluğu ve bitki boyunu gösteren *C. reticulatum* ve *C. arietinum* bitkilerinin büyüme ilerlemesi (D). Hidroponik sistemden çıkarılan bitkilerin kök sistemleri (E).

Estimation of free proline content from leaves

The free proline content was analysed based on the Bates et al. (1973) method with minor modifications. The procedure involved homogenizing 0.5 g of leaf tissue in 10 mL of 3% sulfosalicylic acid, followed by centrifugation. A 2 mL filtrate was combined with 2 mL of acetic acid and 2 mL of acid-ninhydrin. The mixture was boiled at 95°C for 1h. After the boiling, the reaction was stopped by placing the tubes on ice. Finally, toluene (4 mL) was added to the mixture, and the upper phase was measured at 520 nm using a spectrometer. The results were quantified in µmol proline/g FW by constructing a standard curve.

Lipid peroxidation levels of leaves

The malondialdehyde (MDA) level was determined to assess membrane damage and lipid peroxidation resulting from drought stress and/or SiO₂ NPs treatments. After the experiment, 0.2 g of fresh leaf tissues were rapidly frozen using liquid nitrogen and ground into a fine powder. Leaf powders were mixed in a trichloroacetic acid (5%, TCA) solution. The mixture was then subjected to centrifugation for 15 minutes at 12,000 g. Following centrifugation, the supernatant was carefully combined with equal volumes of 0.5% thiobarbituric acid (TBA) and 20% TCA. The mixture was then boiled at 95 °C for 25 minutes. After the centrifugation process, the final supernatant was spectrophotometrically read at 532 and 600 nm. MDA concentrations were quantified using an extinction coefficient of 155 mM⁻¹ cm⁻¹. The results were reported in nmol MDA per gram of fresh weight (Ohkawa et al., 1979).

Determination of Catalase Enzyme Activity

Catalase enzyme activity was assessed in accordance with the methodology described by Aebi (1984). Leaf tissues (one g) were mixed in 5 mL of potassium phosphate buffer (pH: 7 and 0.1 mM EDTA), with the addition of 100 mg

polyvinylpyrrolidone (PVP). The reaction was initiated by combining 2.8 mL of potassium phosphate buffer (pH: 7, without EDTA), 80 μ L of H₂O₂ (0.5M), and 120 μ L of enzyme extract. Catalase activity was determined by monitoring the reduction in absorbance within a 30-second interval at 240 nm. The results were quantified as H₂O₂ min⁻¹ g⁻¹ FW.

Statistical analysis

All statistical analyses were conducted with Minitab 18 software. The Shapiro-Wilk test was employed to test the normality and homogeneity of variance in the data. The examination of the impact of SiO₂ treatments on the studied analysis profile of chickpea plants was conducted through two-way analysis of variance (ANOVA) followed by Fisher's LSD post hoc multiple comparisons test. The data presented in this study were validated through a minimum of two independent experiments.

RESULTS and DISCUSSION

Effect of SiO₂ NPs on Chickpea Biomass

It was found that biomass parameters of *C. arietinum* were higher than those of *C. reticulatum*. PEG-induced drought stress diminished substantially both shoot and root fresh and dry weights in *C. arietinum*, while in the case of *C. reticulatum* these decreases were very slight. On the other hand, no remarkable change in shoot length as a result of drought stress was seen in either of these species. Figure 2A shows that under drought conditions, the fresh weights of plants treated with SiO₂ were significantly higher compared to those treated with H₂O, but with no major significant differences between SiO₂ and H₂O treatment under well-watered conditions in both *Cicer arietinum* and *Cicer reticulatum*. The SiO₂-treated plants under drought conditions in the case of *Cicer arietinum* show only a slightly higher shoot dry weight than the plants treated with H₂O. However, under well-watered conditions, there is no significant difference between SiO₂ and H₂O treatments. In the case of *Cicer reticulatum*, no significant differences between SiO₂ and H₂O treatments can be seen for shoot dry weight under both experimental conditions (well-watered and drought) (Figure 2B). Significantly higher values were observed in root fresh weight with the treatment of SiO₂ compared to the treatment of H₂O (Figure 2C). Both *Cicer arietinum* and *Cicer reticulatum*, under all conditions, show no significant difference in root dry weight between SiO₂ and H₂O treatments (Figure 2D). Both *Cicer arietinum* and *Cicer reticulatum*, under all conditions, do not show any significant difference in shoot lengths between SiO₂ and H₂O treatments (Figure 2E). While in the case of *Cicer arietinum*, in the SiO₂-treated plants, the relative water content is considerably higher than that of H₂O-treated plants under well-watered conditions; under drought conditions, the SiO₂ treatment is not significantly different from H₂O treatment. In contrast, in *Cicer reticulatum*, the relative water content is similar between SiO₂ and H₂O treatments when well-watered, while under drought, SiO₂-treated plants exhibit a significantly higher relative water content than H₂O-treated plants of this species (Figure 2F).

The negative effects of drought stress were more pronounced in *C. arietinum* compared to the wild type, however, foliar application of SiO₂ NPs had positive effects on growth parameters for both species under drought. Many studies have shown that drought stress disrupts the intercellular water balance, slows down cell division, reduces photosynthesis efficiency, and, as a result of all these processes, reduces growth and development (Seleiman et al., 2021). The obtained results indicated that exogenous SiO₂ NPs applications reduced these negative effects of drought, and as a result, growth parameters were stimulated in both species. Maintaining growth parameters under drought conditions is crucial for crop yield (Zhang et al., 2022). Several hypotheses have been put forward on how exogenous SiO₂ NPs applications protect plant yield under stress conditions. Among these hypotheses, it is one of the most reported results that photosynthesis rate increases with SiO₂ NPs application, and this stimulates growth parameters under stress conditions (Alharbi et al., 2022; Elshayb et al., 2021; Uddin et al., 2023; Zahedi et al., 2023). Kalal et al., (2022) showed that SiO₂ NPs treatment protected ps1 and ps2 complexes under drought conditions. Considering that these complexes are severely affected by radicals, it can be concluded that the amount of radicals decreased by SiO₂ treatment, and as a result, photosynthetic efficiency was preserved.

Several studies have shown that Si application increases water content under control and drought conditions. In these studies, it was generally stated that Si application decreases the transpiration rate and thus maintains water status, especially under stress conditions (Irfan et al., 2023). In this study, SiO₂ NPs application significantly increased leaf water content in both species. Maintaining water under drought conditions is part of the avoidance strategy and is vital for plants to tolerate drought (Seleiman et al., 2021). The contribution of SiO₂ NPs application to this situation is important for chickpea plants to tolerate drought.

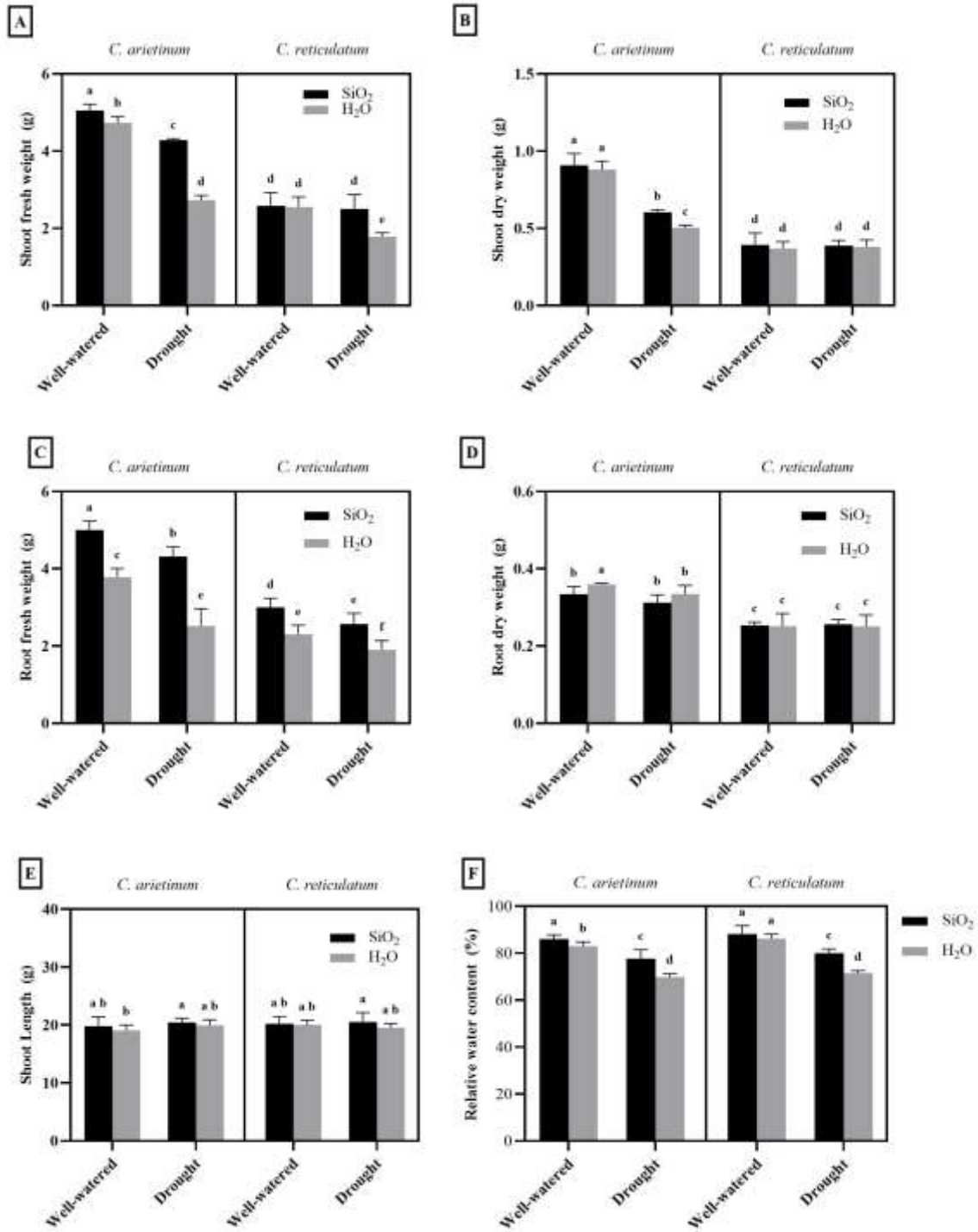


Figure 2. Effects of foliar application of SiO₂ NPs on shoot fresh weight (A), shoot dry weight (B), root fresh weight (C), root dry weight (D), shoot length (E), and relative water content (F) in *Cicer arietinum* and *Cicer reticulatum* genotypes under well-watered and drought conditions. Different letters indicate statistically significant differences (p≤0.05).

Şekil 2. İyi sulanan ve kuraklık koşulları altında *Cicer arietinum* ve *Cicer reticulatum* genotiplerinde SiO₂ NP'lerinin yapraktan uygulamasının sürgün yaş ağırlığı (A), sürgün kuru ağırlığı (B), kök yaş ağırlığı (C), kök kuru ağırlığı (D), sürgün uzunluğu (E) ve oransal su içeriği (F) üzerindeki etkileri. Farklı harfler istatistiksel farklılıkları göstermektedir (p≤0.05).

Effect of SiO₂ NPs on Lipid Peroxidation

In both species, the drought stress caused an increase in MDA content when compared with its well-watered groups. However, the increment was more pronounced in *C. arietinum* than of *C. reticulatum*. However, the SiO₂ NPs treatment decreased the MDA content in drought-conditioned plants of both species. Foliar spray of SiO₂ NPs has been reported to significantly decrease the MDA content in *Cicer arietinum* and *Cicer reticulatum* under drought conditions. Additionally, MDA levels in these samples were decreased by 34.46% and 27.33%, respectively, compared to those in the H₂O-treated group (Figure 3). These decreases in MDA levels were statistically significant ($p < 0.001$). From the results presented above, it follows that SiO₂ NPs worked against oxidative stress in chickpea genotypes under drought conditions. As for well-watered plants from both chickpea genotypes, application of SiO₂ NPs resulted in no statistically significant change in MDA levels (For *Cicer arietinum*, $p = 0.106$, and for *Cicer reticulatum*, $p = 0.189$). This implies that under well-watered conditions, foliar treatment with SiO₂ nanoparticles did not affect MDA content. It seems that the protective role of SiO₂ NPs is effective only under drought stress. This means that SiO₂ NPs are helpful in enhancing the drought tolerance capability of chickpea due to a reduction in oxidative damage, but it does not show negative effects on plants under normal watering conditions.

MDA is an end product of lipid peroxidation. Increased levels of free radicals typically lead to higher MDA production, making MDA levels a well-established marker of oxidative stress (Chauhan et al., 2022). In this study, the increase in MDA levels in both species under drought may be an indication that the plants were subjected to oxidative stress. However, the lower MDA level in wild chickpea *C. reticulatum* may be an indication that the wild species is less subjected to oxidative stress or copes better with oxidative stress under drought conditions compared to cultivated *C. arietinum*. The decrease in MDA content in both species with exogenous SiO₂ NPs application under drought conditions is important in terms of stress tolerance. MDA content is lower in plants with high antioxidant enzyme capacity under stress conditions (Feng et al., 2023). It has been shown in numerous studies that the antioxidant defense system is stimulated by SiO₂ NPs application, and the mechanism involved was described in detail by Huang et al., (2024). The fact that catalase activity was higher in the *C. reticulatum* than in the *C. arietinum* is consistent with the proposed hypothesis in the literature.

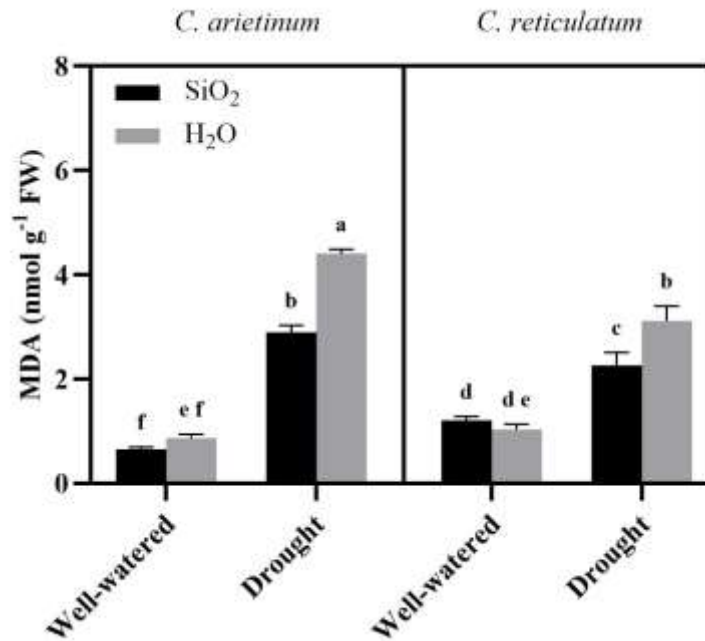


Figure 3. MDA content of all experimental groups. Different letters indicate statistically significant differences ($p \leq 0.05$).

Şekil 3. Tüm deney gruplarının MDA içeriği. Farklı harfler istatistiksel farklılıkları göstermektedir ($p \leq 0.05$).

Effect of SiO₂ NPs on Proline Content

Drought stress increased proline content in both species, while SiO₂ treatment lightly increased proline content in *C. arietinum* and slightly decreased it in *C. reticulatum*. More specifically, in well-watered conditions, *C. arietinum* did not show any significant differences in its proline content between SiO₂ and H₂O treatments ($p = 0.096$).

However, under drought conditions, SiO₂-treated *C. arietinum* was found significantly higher proline content compared to plants receiving H₂O treatment (p=0.003). Similarly, in well-watered conditions, *C. reticulatum* also did not have any significant difference in proline content between SiO₂ and H₂O treatments (p=0.251). However, under drought conditions, the SiO₂ treatment had a significantly lower proline content compared to that of the H₂O treatment in *C. reticulatum* (p<0.001). In the *Cicer arietinum* group, the average proline content of plants sprayed with H₂O and SiO₂ NPs under drought conditions was determined to be 11.38 and 12.55 µmol g⁻¹ FW, respectively. It was observed that SiO₂ NPs application increased proline content by 10.3% under drought conditions in this plant species. Conversely, in the *Cicer reticulatum* group, an opposite effect was observed. The average proline content of plants sprayed with H₂O and SiO₂ NPs under drought conditions was found to be 14.15 and 12.57 µmol g⁻¹ FW, respectively, indicating that SiO₂ application reduced proline content by 11.17% under drought conditions in this species. Interestingly, the findings showed that under drought conditions, SiO₂ treatment had opposite effects on the proline content in *C. arietinum* and *C. reticulatum* (Figure 4).

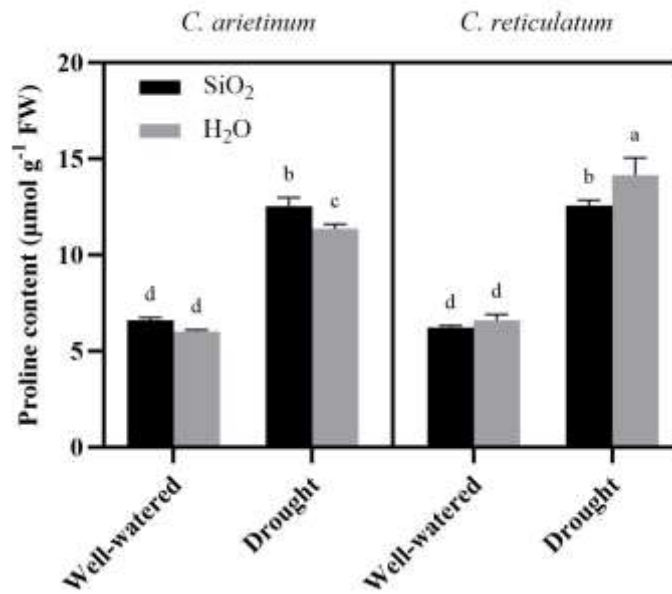


Figure 4. Effects of foliar application of SiO₂ NPs on proline content in *Cicer arietinum* and *Cicer reticulatum* genotypes under well-watered and drought conditions. Different letters indicate statistically significant differences (p≤0.05).

Şekil 4. İyi sulanan ve kurak koşullar altında *Cicer arietinum* ve *Cicer reticulatum* genotiplerinde SiO₂ NPlerin yapraktan uygulanmasının prolin içeriğine etkileri. Farklı harfler istatistiksel farklılıkları göstermektedir (p≤0.05).

The application of SiO₂-NPs significantly increased proline accumulation in the leaves of treated *C. arietinum* (domesticated chickpea) plants, underscoring their potential role in enhancing drought tolerance. In contrast, the proline content in *C. reticulatum* (wild chickpea) groups displayed an opposite trend. This divergence suggests that wild and domesticated chickpea genotypes may employ different strategies to cope with drought stress. While *C. arietinum* appears to rely on proline accumulation as a key adaptive mechanism, *C. reticulatum* may utilize alternative physiological or biochemical pathways to mitigate drought effects. These findings may highlight the importance of considering genetic variability when developing nanoparticle-based strategies for improving drought resilience in crops. Upon reviewing the literature, it becomes apparent that there are reports showing that the amount of proline decreased (Hajizadeh et al., 2022) and increased (Abd-El-Aty et al., 2024) as a result of SiO₂ NPs application. Further research is needed to elucidate the underlying mechanisms driving these differences and to optimize nanoparticle applications for diverse plant genotypes.

Wild relatives, unlike domesticated species, were not exposed to intense anthropogenic selection pressure focused on enhancing yield-related traits under optimal and controlled conditions (Quezada-Martinez et al., 2021). As a result, they may have retained a broader range of adaptive mechanisms to cope with environmental stresses, such as drought. This genetic diversity could explain why wild species often exhibit greater resilience to drought compared to their domesticated counterparts, which have been selectively bred for high productivity, sometimes

at the expense of stress tolerance. Understanding these differences is crucial for developing drought-resistant crop varieties, as wild relatives may serve as valuable genetic resources for improving the resilience of domesticated species in the face of increasing drought conditions due to climate change (Kapazoglou et al., 2023).

Proline, a key osmoprotectant, is known to accumulate in plant tissues under stress conditions, serving as a protective mechanism against cellular damage (Dikilitas et al., 2020). In this study, the observed species-dependent increase in proline content in nanoparticle-treated plants suggests that nanoparticles may stimulate the biosynthesis of proline or enhance the plant's ability to regulate osmotic balance under stress. This aligns with previous findings that nanoparticles can modulate physiological and biochemical pathways, improving stress resilience (Mohammadi et al., 2016; Zhang et al., 2020). Additionally, the relationship between proline levels and other stress-responsive mechanisms, such as antioxidant enzyme activity and photosynthetic efficiency, warrants deeper investigation. These findings underscore the potential of nanoparticles as a novel tool for improving crop resilience, but long-term studies are essential to evaluate their ecological and physiological impacts on plants and the environment.

Effect of SiO₂ NPs on Catalase Enzyme Activity

Catalase enzyme activity increased in both species under drought conditions, the increase being more pronounced in *C. reticulatum*. Exogenous SiO₂ NPs treatments also increased catalase enzyme activity in both species. In the *Cicer arietinum* group, the average catalase (CAT) enzyme activity of plants sprayed with H₂O and SiO₂ NPs under drought conditions was determined to be 2.05 and 3.50 $\mu\text{mol H}_2\text{O}_2 \text{ min}^{-1} \text{ g}^{-1} \text{ FW}$, respectively. It was observed that SiO₂ application increased CAT enzyme activity by 70.7% under drought conditions in this plant species ($p < 0.001$). Similarly, in the *Cicer reticulatum* group, a comparable effect was observed. The average CAT enzyme activity of plants sprayed with H₂O and SiO₂ NPs under drought conditions was found to be 2.82 and 5.62 $\mu\text{mol H}_2\text{O}_2 \text{ min}^{-1} \text{ g}^{-1} \text{ FW}$, respectively, indicating that SiO₂ application increased CAT enzyme activity by 99.3% under drought conditions in this species ($p < 0.001$). *C. arietinum* in well-watered conditions, CAT activity was found significantly different between the plants treated with either SiO₂ or H₂O ($p = 0.006$), while under drought conditions, SiO₂ treatment increased CAT activity significantly when compared with the treatment with H₂O ($p < 0.001$). Likewise, *C. reticulatum* as expected, under well-watered conditions, no significant difference in CAT activity is found between SiO₂ and H₂O treatments ($p = 0.159$). However, under drought conditions, SiO₂ treatment tends to significantly increase CAT activity compared to that by H₂O treatment at $p < 0.001$ (Figure 5). We could therefore conclude here that SiO₂ treatment causes a dramatic increase in CAT activity in both crop species under drought conditions. This result seems to reveal that SiO₂ causes some sort of protection from oxidative stress damage that results from drought.

The effects of nanoparticle application on drought tolerance vary between wild and domesticated species in terms of catalase enzyme activity. Wild species tend to exhibit a greater increase in catalase enzyme activity under drought stress, indicating their enhanced capacity to manage oxidative stress more effectively. This can be attributed to their evolutionary adaptation to environmental stress conditions over time. In contrast, domesticated species may show a more limited rise in catalase activity, as they have been selectively bred for high yield under optimal conditions rather than stress tolerance. Nanoparticle applications have the potential to improve drought tolerance in domesticated species by enhancing catalase activity. However, the natural adaptation mechanisms observed in wild species could serve as a valuable genetic resource for future breeding programs. These findings highlight the importance of nanoparticle technology and the genetic diversity of wild species to develop resilience against drought stress.

Other studies have also shown that nanoparticles enhance stress tolerance, primarily by influencing antioxidant systems (Wang et al., 2018). Nanoparticles modulate the activity of key antioxidant enzymes, such as catalase, superoxide dismutase, and peroxidase, which play essential roles in mitigating oxidative damage caused by drought, salinity, and heavy metal toxicity. By boosting the efficiency of these antioxidant systems, nanoparticles help maintain cellular homeostasis and reduce the accumulation of reactive oxygen species (ROS), thereby enhancing overall stress resilience. The antimicrobial activity of metal oxide-NPs or their forms synthesized with plant extract has also been reported to be mainly against environmental, food, and plant pathogens (Şahin et al., 2021; Şahin et al., 2022; Soylu et al., 2022). These findings highlight the potential of nanoparticles as a valuable tool for improving plant stress tolerance through their interaction with antioxidant defense mechanisms.

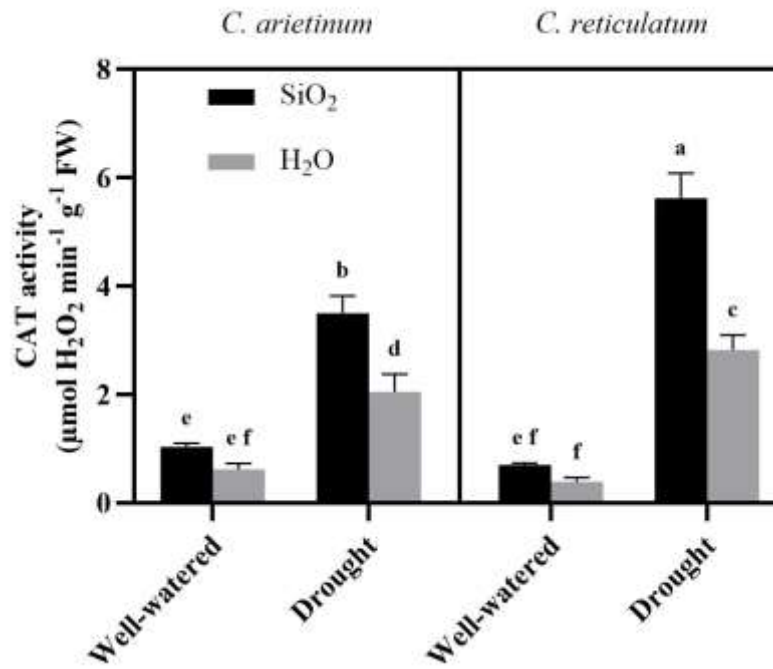


Figure 5. Effects of foliar application of SiO₂ NPs on CAT enzyme activity in *Cicer arietinum* and *Cicer reticulatum* genotypes under well-watered and drought conditions. Different letters indicate statistically significant differences ($p \leq 0.05$).

Şekil 5. *Cicer arietinum* ve *Cicer reticulatum* genotiplerinde iyi sulanmış ve kuraklık koşullarında SiO₂ NP'lerinin yaprak uygulamasının CAT enzim aktivitesi üzerindeki etkileri. Farklı harfler istatistiksel farklılıkları göstermektedir ($p \leq 0.05$).

CONCLUSIONS

Drought is considered to be the most important abiotic stress factor that reduces the yield of crops in agricultural areas. The ever-increasing human population has made it imperative to get more and more yield from agricultural areas. Therefore, the mechanisms necessary for plants in agricultural areas to cope with this stress have been intensively investigated. In order to contribute to these investigations, in the present study, the effects of exogenously applied SiO₂ NPs on two chickpea species with different drought tolerances under drought conditions were determined. In this study, exogenous SiO₂ NPs application similarly altered the parameters studied in both *C. arietinum* and *C. reticulatum*, except for proline content. Interestingly, under drought conditions, SiO₂ NPs treatment increased proline content in *C. arietinum* but decreased it in *C. reticulatum*. Under drought conditions, the increase in catalase (CAT) activity with SiO₂ application in both plant species suggests that this nanoparticle dose triggered the antioxidant defense system and induced noticeable improvements in plant resilience to drought stress. These results make it difficult to explain, but this results show that exogenous SiO₂ NPs application induces different responses depending on the tolerance level of the plant. However, the molecular response of this result needs to be studied in detail.

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Contribution Rate Statement Summary of Researchers

The authors declare that they have contributed equally to the article.

Conflicts of interest

The authors declare that there are no conflicts of interest.

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