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RESEARCH ARTICLE

Effect of Calcium Nitrate Applications on Plant Development and Some Physiological Characteristics of Sunflower Seedlings Grown Under Drought Conditions

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ABSTRACT

Drought stress is one of the abiotic stresses that negatively affects plant growth, development and product yield. In recent years, nutrient solution applications to leaves have been frequently used to reduce the negative effects of drought stress. This study was conducted in 2024 at the Atatürk University, Plant Production Application and Research Center to determine the effect of calcium nitrate applications during the seedling period on the growth and some physiological characteristics of sunflower (*Helianthus annuus* L.) grown under drought stress. The study was based on two-factor completely randomized experimental design with three irrigation levels [full irrigation (100% (I_0), 70% (I_1) and 40% (I_2) of field capacity), two $Ca(NO_3)_2$ concentrations (15 mM and 30 mM)]. The study was carried out as a pot trial based on this experimental design. At the end of the trial period, plant growth parameters and some physiological measurements and analyzes were performed on sunflower plants and the differences between the treatments were evaluated. According to the research findings, significant differences were observed between the different treatments and irrigation levels. The application of $Ca(NO_3)_2$ significantly influenced plant growth parameters (such as plant height, stem diameter, fresh and dry weight) and physiological parameters [such as tissue relative water content (RWC)] in sunflower grown under varying irrigation levels. At the end of the study, it was determined that dry conditions negatively affected plant growth in sunflowers and reduced the RWC value. In conclusion; calcium nitrate applications reduced this negative effect of drought compared to the control application. It can be said that especially the results obtained from the application of 15 Mm CaN at 70% (I_1) irrigation level are relatively less affected by drought.

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1. Introduction

Sunflower (*Helianthus annuus* L.) is cultivated for snack purposes even in regions with short vegetation periods, such as the Eastern Anatolia Region and similar ecologies. This increases the importance of sunflowers in places where corn, the first plant that comes to mind when it comes to silage, cannot reach harvest maturity.

Sunflower is easier to cultivate than corn. It is drought resistant and can be grown in arid areas without irrigation (Arıoğlu, 2000). With its deep root system, sunflower can use groundwater with a depth of approximately 2 m. For this reason, sunflower can be considered an alternative to corn as a silage forage plant in periods and places where rainfall or water is low (Bremner et al., 1986). It is resistant to lower temperatures than corn; It is much less damaged by the last

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frosts of spring and the first frosts of autumn. The appropriate growth temperature in corn agriculture is 24-32°C, and the total temperature requirement during the development period varies between 2000-4000°C depending on the region and varieties (Kırtok, 1998). It also needs a relative humidity that does not fall below 60%. On the other hand, sunflower requires a total temperature of around 2600-2850°C during its growing period (Arıoğlu, 2000). In addition, the vegetation period of the sunflower plant until harvest maturity is quite short (90-120 days) (Ahmad et al., 2014). In addition to being a very valuable plant for silage, it is one of the four most important oilseeds in the world. In terms of vegetable oil production, sunflower ranks second after cotton and outperforms other non-traditional oilseed crops such as soybean and canola (Government of Pakistan, 2007).

Although Türkiye has suitable ecological conditions for sunflower cultivation, our sunflower cultivation areas cannot be increased (Meral, 2019). Although the sunflower plant is drought tolerant due to its taproot structure, the drought that occurs as a result of being a summer plant and not enough rainfall in this season reduces the yield per decare considerably (Kolsarıcı et al.,, 1995). It is known that drought has increased in recent years and is one of the main abiotic factors that negatively affects crop production worldwide (Reddy et al., 2004). Unlike other stress factors, drought stress does not appear suddenly, but develops gradually and increases in severity over time, causing damage (Larcher, 2003). Drought negatively affects plant growth and productivity by affecting water and nutrient supply to plants (Erdem et al., 2001; Jones & Qualset, 1984). Limited water in the plant prevents the accumulation of mineral elements in the tissues, negatively affecting normal root development, nutrient uptake and mobility in the soil (Luo et al., 2011). There are studies showing that the water potential, osmotic activity and gas exchange properties of sunflower are suppressed during drought (Tezara et al., 1999). Ashraf and O'Leary (1996) found that stomatal conductance and transpiration of sunflower plants grown under limited water stress were negatively affected. Studies aimed at reducing the negative effects of drought stress by spraying nutrient solutions on the leaves have been frequently used in recent years. Application of water-soluble fertilizers is foliar to increase nutrient availability (Al-Shammari et al., 2018, 2019).

Calcium nitrate fertilizer contains nitrate nitrogen and calcium, two essential nutrients for plants (Anonymous, 2024). It can be said that top fertilization is suitable for all types of soil and plants. Since the interaction of calcium and nitrate is favorable, there is no residue or salinity in the soil. It is generally used in foliar applications with drip and sprinkler irrigation systems on plants grown outdoors and in greenhouses. It has a white-colored granular structure and contains 15.5% N in the form of nitrate and 26.5% Ca, which is completely soluble in water. When given too little, it causes the plant to wilt, while when given too much, it causes crop maturation and harvest delays. Increasingly widespread agricultural drought limits irrigation opportunities in cultivation, and leading producers to seek alternative ways against the negative effects of drought stress. It has been determined that calcium has a reducing effect on drought stress in sugar beet, Arabidopsis thaliana, tea and corn plants (Hosseini et al., 2019; Huang et al., 2018; Malyukova et al., 2022; Naeem et al., 2018).

Numerous studies have shown that mineral nutrition plays an important role in increasing plant tolerance to abiotic stresses. However, despite the positive effect of calcium on plant growth and stress tolerance, studies on its application in sunflower are limited. The aim of this study is to investigate whether foliar application of calcium nitrate provides tolerance to drought stress in sunflower plants exposed to drought stress.

2. Materials and Methods

The study was conducted as a pot experiment in the greenhouses of the Atatürk University Plant Production Application and Research Center in 2024. The 'Bright Gold' variety of sunflower was used as the plant material in the research. The 2-liter pots were filled with the prepared soil mixture (garden soil (2): sand (1): peat mixture (1)) and 5 seeds were planted in each pot at a depth of 2-3 cm. After reaching the seedling stage, the plants were thinned to leave four uniform plants in each pot.

2.1. Calcium Nitrate and Water Restriction Practices

The study included nine different treatments: 100% irrigation (control), 70% irrigation, 40% irrigation, 15 (mM) $Ca(NO_3)_2$ + 100% irrigation, 15 (mM) $Ca(NO_3)_2$ + 70% irrigation, 15 (mM) $Ca(NO_3)_2$ + 40% irrigation, 30 (mM) $Ca(NO_3)_2$ + 100% irrigation, 30 (mM) $Ca(NO_3)_2$ + 70% irrigation, 30 (mM) $Ca(NO_3)_2$ + 40% irrigation. The experiment was conducted using a two-factor completely randomized experimental design, with three replications and four plants per replication. The 100% irrigation treatment (I_0) represents field capacity, while the 70% (I_1) and 40% (I_2) irrigation treatments represent water restriction levels relative to field capacity. Field capacity was calculated by weight percentage usingthe following formula (Güngör et al., 1996). 70% and 40% water restriction applications were calculated according to field capacity. Tap water was used in the study.

Water retention capacity = (Moisture level at field capacity (weight percentage) - Moisture level at wilting point (weight percentage))*Volume weight of the soil*Depth of the soil.

2.2. Physical Methods

Calcium nitrate and water restriction treatments were initiated when the first true leaves formed at the seedling stage.

Fertilizer applications were made by spraying the leaves using a hand sprayer.

Within the scope of the study, seedling height (cm) and the number of leaves were measured. Additionally, fresh and dry weights of stems and roots (g) determined. Samples were dried at 68°C until a stable weight was achieved. Leaf area was measured using a LICOR model LI-3100 (Lincoln, NE, USA). Chlorophyll content was assessed with a SPAD502 chlorophyll meter (Konica Minolta Sensing Inc., Japan).

The stem diameter (mm) of the above-ground parts of the plants was measured at the harvest stage using a digital caliper (Gullap et al., 2022).

Leaf relative water content (LRWC) was determined according to Kaya et al. (2003).

The experiment was designed using a two-factor completely randomized experimental design. All data obtained at the end of the research were analysed using the variance analysis test with the SPSS 18 package program, and means were compared using the Duncan multiple comparison test (N. Yıldız & Bircan, 1991).

3. Results and Discussion

Differences in the average stem diameter (mm), plant fresh and dry weight (g), as well as root fresh and dry weight (g) under water restriction and calcium nitrate treatments are presented in Table 1. It is known that water stress negatively affects nutrient uptake in plants and reduces productivity (Ahmad et al., 2014). As a matter of fact, in Table 1, the decrease in irrigation level led to a decrease in all parameters. The highest and lowest stem diameter values were obtained from the control application (4.84) and the lowest irrigation level (3.31 mm), respectively. In both 15 mM and 30 mM $Ca(NO_3)_2$ applications, the stem diameter under 70% irrigation applications was higher than in the control applications and was included in the same statistical group. While a 59.42% weight loss was observed in fresh plant weight between I_0 and I_2 irrigation levels in control applications, this value was 48.65% in 15 mM $Ca(NO_3)_2$ applications. The weight loss in 30 mM $Ca(NO_3)_2$ applications was determined to be 44.52%. These values suggest that calcium nitrate applications can reduce the yield loss in plants due to drought. In plant dry weight, the weight loss in control applications was 68.57%, the weight loss in 15 mM $Ca(NO_3)_2$ applications was 61.66%, and the weight loss in 30 mM $Ca(NO_3)_2$ applications was 72.35%. The highest root fresh weight was obtained from CaN_1-I_1 treatment (2.25 g), While the lowest fresh root weight was from CaN_2-I_2 treatment (0.54g). The highest values for root dry weight were obtained from CaN_1-I_0 (1.01) and CaN_1-I_1 (1.15 g) treatments (Table 1). It is known that drought stress reduces photosynthesis, suppresses plant growth and food production, and causes decreases in total biomass and yield (Sarıyer et al., 2023). In addition, it has been determined that leaf water content and relative humidity in plants decrease significantly (Anjum et al., 2011). Clapco et al. (2018) stated that sunflower seedling fresh weight was negatively affected by increasing drought doses.

Table 1. Effect of applications on plant development in sunflower.

Treatments	Stem diameter (mm)	Plant fresh weight (g)	Plant dry weight (g)	Root fresh weight (g)	Root dry weight (g)
I_0	4.84 a	13.16 a	5.25 a	2.03 ab	0.79 c
I_1	4.32 c	8.28 bc	3.15bc	1.95 ab	0.82 bc
I_2	3.31 d	5.34 d	1.65 d	1.17 cd	0.55 d
CaN_1-I_0	4.74 ab	12.60 a	5.53 a	2.08 ab	1.01 a
CaN_1-I_1	4.46 bc	9.47 b	3.99 b	2.25 a	1.15 a
CaN_1-I_2	3.57 d	6.47 cd	2.12 cd	1.80 abc	0.49 d
CaN_2-I_0	4.40 c	9.14 b 9,14	5.57 a	2.04 ab	0.98 ab
CaN_2-I_1	4.52 bc	7.78 bc	2.99 bc	1.40 bc	0.48 d
CaN_2-I_2	3.48 d	5.07 d	1.54 d	0.54 d	0.10 e

Means marked with different letters are statistically different.

The effects of calcium nitrate applications aimed at reducing the negative effects of different water restriction levels on seedling height, number of leaves, chlorophyll content, leaf area and tissue relative water content (RWC) in sunflower are presented in Table 2. The highest seedling height (52.00 cm and 52.67 cm) were obtained from control irrigation (I_0) and 15 mM $Ca(NO_3)_2$ with I_0 irrigation applications, both of which were in the same statistical group. The highest seedling height under I_2 irrigation application, which is the lowest irrigation level, was obtained from 15 mM $Ca(NO_3)_2$ application (42 cm). The number of leaves decreased with reduced irrigation levels in all treatments. The highest number of leaves (11 pieces per plant) was recorded with I_0 treatment, while the lowest number of leaves (6.67 pieces per plant) was observed with CaN_2-I_2 treatment. CaN_1-I_1 and CaN_1-I_2 treatments had higher leaf numbers compared to I_1 and I_2 irrigation levels of other treatments. Previous studies have determined that drought stress in sunflower, especially in the early development period

(4 to 8 leaves), causes a decrease in the number and area of leaves, a decrease in absorption during the maturity phase, and also a shorter life span of the plants (Göksoy et al., 2004). It is known that chlorophyll is the pigment that provides the green and healthy appearance of the plant. According to the results of our study, I_0 irrigation levels of all treatments and CaN_1-I_1 treatment had high chlorophyll content and were in the same statistical group. As expected, chlorophyll contents decreased with reduced irrigation levels. The stress caused by limited water in the plant develops due to the decrease in impulse growth, the expansion of cells in the root and shoot meristems, and the cessation of cell division. The suppression of cell expansion or division is parallel to the decrease in the rate of photosynthesis due to water deficiency (Anjum et al., 2011). Reducing irrigation levels decreased leaf area in all treatments. In the control application, there was a leaf area loss of 51.02 cm^2 between I_0 and I_2 irrigation levels, 32.05 cm^2 between 15 mM $Ca(NO_3)_2$ applications, and 37.62 cm^2 between 30 mM $Ca(NO_3)_2$ applications. Accordingly, it can be said that at least 15 mM $Ca(NO_3)_2$ applications of the leaf area are affected by water restriction applications. Researchers have stated that leaf area index is one of the most important growth indicators in sunflower. The reason for this is that sunflower reaches the highest leaf area and reaches maximum photosynthesis when it is not under any stress (Göksoy et al., 2004). Similarly, Pekcan et al. (2015) reported in their study that leaf area is the parameter most affected by drought stress in sunflower lines and that stress reduces leaf area up to 75%. In our study, it was determined that the highest RWC values were at I_0 levels, and as irrigation levels decreased, RWC values also decreased. Our results are parallel to other studies (Gür, 2019; S. Yıldız, 2017).

Table 2. Effect of applications on seedling height, number of leaves, chlorophyll value (SPAD), leaf area and tissue proportional water content in sunflower.

Treatments	Seedling height (cm)	Number of leaves (pcs per plant)	Chlorophyll SPAD	Leaf area (cm² per plant)	RWC (%)
I_0	52.00 a	11.0 a	40.00 a	194.83 a	67.00 bc
I_1	45.33 bc	8.67 cd	36.66 b	139.90 c	63.66 c
I_2	39.00 d	7.67 de	29.66 d	95.42 d	55.66 d
CaN_1-I_0	52.67 a	10.67 ab	39.44 a	187.82 ab	71.96 a
CaN_1-I_1	49.33 ab	9.33 bc	40.66 a	151.89,bc	56.48 d
CaN_1-I_2	42.00 cd	8.33 cd	33.28 c	127.62 cd	42.79 f
CaN_2-I_0	45.00 bc	9.67 abc	40.00 a	153.80 bc	70.33 ab
CaN_2-I_1	44.00 c	8.67 cd	36.33 b	135.11 cd	56.33 d
CaN_2-I_2	32.00 e	6.67 e	29.00 d	95.93 d	50.66 e

Means marked with different letters are statistically different.

4. Conclusion

Sunflower is a valuable silage alternative forage plant in regions where the corn plant has a risk of freezing. Although it is a drought-resistant plant due to its taproot structure, drought caused by the fact that it is a summer plant and there is insufficient rainfall in this season significantly reduces the yield per decare. It is known that mineral nutrition plays an important role in increasing tolerance to drought stress, which negatively affects plant production. In fact, it was determined in our research that different irrigation levels and calcium nitrate applications significantly affected plant development and some physiological characteristics in sunflower. Increasing drought levels negatively affected all parameters. However, it was determined that calcium nitrate applications reduced this negative effect caused by drought compared to the control application. It can be said that the results obtained from the application of 15 mM CaN $Ca(NO_3)_2$ at 70% (I_1) irrigation level were relatively less affected by drought. Testing the results of this research, which was carried out controlled greenhouse conditions, with different plants and varying fertilizer doses under field conditions will be useful for further evaluation in terms of yield and quality.

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Conflict of Interest

The author has no conflict of interest to declare.

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