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A comparison between the effect of Nano and traditional calcium fertilizer on growth and anatomical traits of two tomato cultivars

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Summary
This study was conducted in the field of vegetable crops, Department of Horticulture and Landscape Design, College of Agriculture and Forestry, University of Mosul, Mosul, Iraq, during the spring growth season of 2024.The study aimed to investigate the effect of two factors on growth and anatomical traits of two tomato. The first factor consisted of11 treatments (Nano and conventional calcium) using different concentrations and combinations: Results of the study can be summarized as follows: Queen cultivar plants significantly outperformed GS-12 cultivar plants in plant height, leaf chlorophyll content, leaf area, percentage of dry matter in leaves. The values of these traits reached 81.181 cm plant-1, 58.367 SPAD, and 28248.8 cm2 plant-1, 18.2818%, respectively, for each trait. Plants treated with (conventional calcium 200 mg L-1) and (Nano calcium 50 mg L-1 + conventional calcium 150 mg L-1) gave the highest significant value for leaf chlorophyll content, reaching 54.967 SPAD. While the treatment (Nano calcium 150 mg L-1 + conventional calcium 50 mg L-1) achieved the highest significant value in plant height, reaching 79,000 cm2 plant-1, and all fertilizer treatments were significantly superior compared to the no-spray treatment (comparison treatment) in the number of branches and stem diameter. The plants treated with (Nano calcium 400 mg L-1) gave the best significant value in leaf area, reaching 30,727 cm2 plant-1, with significant superiority over most other treatments. As for the treatment (Nano calcium 100 mg L-1 + conventional calcium 100 mg L-1), it gave the best significant value for the percentage of dry matter, reaching 18.9667%. In general, significant increases were achieved in the case of binary interaction in the vegetative growth traits, and they were as follows: In the trait of plant height, the best significant value was obtained when treated with (Nano calcium 150 mg L-1 + conventional calcium 50 mg L-1) interacted with the Queen variety, reaching 85.333 cm plant-1. In the chlorophyll content of leaves, the treatment (conventional calcium 200 mg L-1) interacted with the Queen variety gave the best significant value, reaching 65.667 SPAD. In the trait of the number of branches and leaf area, they were obtained when treated with (Nano calcium 400 mg L-1) interacted with the Queen variety, reaching 6.9000 branches plant-1 and 41685 cm2 leaves-1. In the percentage of dry matter in leaves, the treatment (conventional calcium 400 mg L-1) interacted with the Queen variety reached 19.8667%, and the treatment (Nano calcium 50 mg L-1 + conventional calcium 150 mg L-1) intercropped with the Queen variety gave the highest significant value in stem diameter, reaching 14.4667 mm stem-1. The treatment of plants with (Nano calcium 150 mg L-1 + conventional calcium 50 mg L-1) was characterized by the highest significant values in each of the percentage of total acidity that can be adjusted, reaching 0.136533%. No significant differences were recorded between the GS-12 variety and the Queen variety in the stomatal density and stomatal area. The use of the two treatments (Nano calcium 200 mg L-1) and (conventional calcium 200 mg L-1) led to a significant increase in

stomatal density, reaching 200.00 stomatal and significantly superior to the control treatment, while no significant differences were found in the control treatment. Significant differences in the gap area according to the fertilizer type compared to the comparison treatment.

Key Words: Tomato, Nano, Cultivars, Stomata.

INTRODUCTION

Tomato is one of the most important vegetable crops in terms of cultivated area and economic importance. It belongs to the Solanaceae family, which includes about 90 genera and about 2,000 species, known scientifically as Lycopersicum esculentum Mill. Tomatoes and their wild species originated in South America. All wild species, with the exception of two, grow in a narrow strip extending along the western coast of South America between southern Ecuador and northern Chile, passing through Peru, between the equator and 23° south latitude. The two excluded species inhabit the Galapagos Islands, which are located in the Pacific Ocean opposite the western coast of South America, where the other species are found. The beginning of the domestication of tomatoes was in Mexico, from which they moved to the Philippines, then to Europe in the sixteenth century, where they were first mentioned in Italy in 1554 AD, and from Europe they moved to North America (Tigchelaar 1986). Many studies have confirmed the importance of calcium in maintaining the water content and turgor pressure of cells. In plants, Wei et al. (2008) reported that calcium plays an effective and positive role in increasing plant tolerance to water stress conditions. This element regulates cell osmotic potential and maintains cell turgor under water stress conditions. It also regulates osmosis, maintains hormonal balance within cells, and maintains the stability and structure of cell membranes. It also inhibits the activity of many oxidative enzymes under water stress conditions. The word "Nano" means "dwarf" in ancient Greek. This term is also derived from the Greek word "midget," meaning minute, dwarf, or small. In science, the term "Nano" refers to materials whose particles measure one-billionth of a meter (101). This unit is used to express the diameters and scales of microscopic particles, atoms, or particles of composite materials (Raab et al., 2011). Jamaluddin et al. (2023) in a study conducted in Bangladesh on three tomato cultivars (Toh1220b, TM016 and Bahuboli) concluded that Bahuboli significantly outperformed the other two cultivars in number of branches and leaf chlorophyll content, while TOH1220b significantly outperformed in leaf area. Bekele et al. (2024) in a study conducted on four tomato cultivars in Ethiopia (Beshola), Melka salsa, Melka shola and Gelilema) concluded that Melka shola significantly outperformed the other cultivars in plant height. Porba et al. (2024) in a study conducted in Indonesia on three tomato cultivars (Cherry Golden), Mawar and Bareto) concluded that Cherry Golden significantly outperformed the other two cultivars in plant height. Haleema found (2020) in Pakistan, when using calcium chloride as a source of calcium sprayed on tomato plants at concentrations of 0, 0.25, 0.5 and 0.75%, there was a significant increase in the total acidity of the fruits when using the 0.75% concentration compared to other concentrations. Islam et al. (2023) in Bangladesh reported that spraying tomato plants with a source of calcium sulfate at a concentration of 10 mmol L-1 led to a significant increase in plant height and chlorophyll content in leaves compared to other concentrations (0, 5 and 15) mmol L-1. In a study conducted by Rajani et al. (2022) in India, using the foliar spray method with Nano calcium oxide as a source of calcium on tomato plants, thirteen concentrations of calcium oxide were used in this study. The results of the study showed that the concentration of 600 mg L-1 gave the highest results in the total acidity rate that can be corrected compared to Other concentrations: In a study conducted by Motlhalamme et al. (2023) in South Africa to study the effect of foliar spraying of calcium on tomato plants, Nano calcium carbonate was used as a source of calcium at four concentrations (0, 50, 150 and 250) mg L-1. The results showed that the

concentration of 150 mg L-1 was significantly superior to the rest of the concentrations in plant height, leaf area and number of aerial stems.

Materials and methods

The experiment was conducted in the vegetable field of the Department of Horticulture and Landscape Design, College of Agriculture and Forestry, University of Mosul, Mosul, Iraq, which is located at 36.35°N latitude and 43.15°E longitude, and 223 meters above sea level, during the spring growing season. The soil was prepared for cultivation by performing three-way plowing with a three-furrow plow, followed by leveling using a harrow. The experimental unit consisted of two ridges, each 1.25 meters wide and 2 meters long, with a 0.5-meter distance between beds. The distance between plants was 40 cm, and planting was done on both sides of the ridges, with 20 plants per experimental unit (60 plants per treatment). The experimental unit area was 6 m2. Seeds were sown in one of the plastic houses belonging to the Department of Horticulture and Landscape Design on March 1, 2024, in plastic trays containing 50 cells using peat moss as the growing medium. After sowing, the fungicide peltanol was added to the growing medium at a concentration of 0.5 cm3 L-1 as a preventive measure against fungal seedling blight. After 48 days from sowing, when the seedlings had three to four true leaves, they were transplanted to the permanent field in the morning, taking care to keep the peat moss around the root zone and maintain soil moisture. The study aimed to investigate the effect of two factors on growth and anatomical traits of tomato using Nano and conventional calcium fertilizers of tomato plants. The first factor consisted of calcium treatments (Nano and conventional) using different concentrations and combinations:

1. 0 mg L-1 (control treatment)
2. 200 mg L-1 Nano calcium
3. 400 mg L-1 Nano calcium
4. 200 mg L-1 conventional calcium
5. 400 mg L-1 conventional calcium
6. 100 mg L-1 Nano calcium + 100 mg L-1 conventional calcium
7. 75 mg L-1 Nano calcium + 25 mg L-1 conventional calcium
8. 25 mg L-1 Nano calcium + 75 mg L-1 conventional calcium
9. 200 mg L-1 Nano calcium + 200 mg L-1 conventional calcium
10. 150 mg L-1 Nano calcium + 50 mg L-1 conventional calcium
11. 50 mg L-1 Nano calcium + 150 mg L-1 conventional calcium

The second factor consisted of two tomato varieties: GS-12 and Queen. The plants were sprayed with Nano and conventional calcium at three growth stages: 15 days after transplanting, and then at 20-day intervals. The experiment consisted of 22 treatments (11 x 2) with three replicates, and was conducted using a randomized complete block design (RCBD). Results were analyzed statistically, and the means were compared using Duncan's multiple range test at a probability level of ≤ 0.05.

Studied Traits:

A- Vegetative Growth Indicators

1-Leaf Chlorophyll Content (SPAD): Leaf chlorophyll content was estimated using a Japanese-made SPAD-502 Chlorophyll Meter, with five readings per plant. The average was then calculated. 2- Plant

Height (cm/plant-1): Plant height was measured from soil level to the topmost leaf using a measuring tape. 4- Leaves area (cm2/plant-1): It was measured after separating six leaves from each plant, determining the separation area from the center of the plant, they were then weighed using an electronic balance, Six discs with a known area were then taken from each of the six leaves. The leaves and discs were weighed, and the leaf area of the plant was calculated using a ratio and proportion based on the wet weight of the discs and leaves, result was divided by 6 to obtain the area of a single leaf. The result was then multiplied by the number of leaves on the plant to obtain the laeves area of the plant (Muhammad, 1985). 5- Percentage of dry matter in leaves

B- Anatomical study: The anatomical study was conducted on both the control treatments and the two concentrations of 200 ml L-1 of Nano-calcium and conventional calcium for both varieties. The direct method was used in the anatomical study. 1- Stomatal density (stoma/mm-1) The number of stomata in the upper epidermis of a single leaf was calculated using a compound light microscope (OPTIKA) attached to an Optika B_388PLI digital camera. The number of stomata was estimated at 400x magnification. 2- Area Stomata (micron stoma-1) The area of a single stoma in the upper epidermis of a single leaflet was calculated using a compound light microscope (OPTIKA) attached to an Optika B_388PLI Digital Camera. Stomata area was estimated at 400x magnification.

RESULTS AND DISCUSSION

Leaf chlorophyll content (SPAD) The data presented in Table (1) indicate that, by fertilizer type, the two treatments (conventional calcium 200 mg L-1) and (Nano calcium 50 mg L-1 + conventional calcium 150 mg L-1) recorded the highest significant value in leaf chlorophyll content, reaching 54.967 SPAD for each, significantly superior to the treatments (Nano calcium 200 mg L-1) and (Nano calcium 25 mg L-1 + conventional calcium 75 mg L-1). The treatment (Nano calcium 200 mg L-1) produced the lowest value in leaf chlorophyll content, reaching 47.283 SPAD. As for the effect of cultivars, the results in the table indicate that Queen plants recorded a significant superiority over GS-12 plants in leaf chlorophyll SPAD content, with leaf chlorophyll content reaching 58.367 and 44.067 SPAD, respectively, for each cultivar. In the case of a two-way interaction between fertilizer type and cultivars, the results presented in the table show that the fertilizer treatment (conventional calcium 200 mg L-1) interacted with Queen plants significantly outperformed most interaction treatments in this trait. Leaf chlorophyll content for this superior interaction reached 65.667 SPAD, while leaf chlorophyll content decreased to its lowest level, reaching 40.767 SPAD in the interaction between 0 mg L-1 (the control treatment) and GS-12 plants.

- Plant Height (cm plant-1) The results of Table (2) indicate that the use of the fertilizer treatment (150 mg L-1 Nano-calcium + 50 mg L-1 conventional calcium) resulted in the highest plant height value of 79,000 cm plant-1, significantly superior to the 0 mg L-1 treatment (the control treatment) and the 400 mg L-1 conventional calcium treatment. However, there was no significant difference between the other treatments. The lowest value for this trait was 71.167 cm plant-1 in the 0 mg L-1 treatment (the control treatment). As for the effect of cultivars, the Queen cultivar significantly outperformed the GS-12 cultivar in plant height, reaching 81.181 cm plant-1 in the superior cultivar plant and 70,000 cm plant-1 in the GS-12 cultivar plant. In the two-way interaction between fertilizer type and varieties, the table results indicate that the spraying treatment (Nano calcium 150 mg L-1 + conventional calcium 50 mg L-1) interacted with the Queen variety recorded the highest value for plant height, reaching 85.333 cm plant-1, and it significantly outperformed all two-way interactions in the case of the GS-12 variety and some two-way interaction treatments in the case of the Queen variety. The lowest value in this trait was 65.667 cm plant-1 in the interaction between 0 mg L-1 (comparison treatment) and the plants of the GS-12 variety.- Leaves area (cm2 plant-1) The data presented in Table (3) indicate that the highest significant value for leaf area by fertilizer type was 30,727 cm2 plant-1 in the treatment (Nano calcium 400 mg L-1),

significantly outperforming all other fertilizer treatments except for the two treatments (conventional calcium 200 mg L-1) and (Nano calcium 200 mg L-1 + conventional calcium 200 mg L-1). The lowest value for this trait was 14,900 cm2 plant-1 in the 0 mg L-1 treatment (the control treatment). The results in the table indicate that Queen plants significantly outperformed GS-12 plants in leaf area, with leaf area reaching 28,248.8 and 24,119.8 cm2 plant-1, respectively, for each variety. The two-way interaction between the fertilizer type (Nano calcium 400 mg L-1) and the Queen variety was characterized by giving the highest significant value in leaf area, reaching 41685 cm2 plant-1, and with significant superiority over all other treatments related to this interaction. As for the 0 mg L-1 treatment (the comparison treatment), interacted with the GS-12 variety, it recorded the lowest value in this trait, reaching 17788 cm2 plant-1.- Percentage of Dry Matter in Leaves The results of Table (4) show that the percentage of dry matter in leaves increased to its highest value when using the fertilizer treatment (Nano calcium 100 mg L-1 + conventional calcium 100 mg L-1), significantly outperforming some fertilizer concentrations for both types (Nano and conventional). The percentage of dry matter decreased with the 0 mg L-1 treatment (the control treatment) to its lowest value of 16.8000%. Regarding the effect of cultivars, the results of the table show that the percentage of dry matter in Queen plants reached 18.2818%, significantly outperforming GS-12 plants, which had a leaf dry matter percentage of 17.1152%. As for the two-way interaction between the type of fertilizer (Nano-calcium and traditional) with the varieties, the results shown in the table show that the best value in this trait, 19.8667%, was recorded in the interaction treatment between (traditional calcium 400 mg L-1) with the Queen variety plants, and it was significantly superior to most of the treatments of this interaction. The results of the table indicate that the GS-12 variety plants interacted with the treatment of not spraying calcium 0 mg L-1 (the comparison treatment) recorded the lowest value in the percentage of dry matter in the leaves, which amounted to 16.0667%.- Stomata area (micron stomata-1) The results of Table (5) show no significant differences between the two study factors (fertilizer type, concentration, and varieties) and the two-way interaction between them.

- Stomatal Density (mm-1stoma) The results of Table (4) indicate that the two spray treatments at both concentrations (Nano calcium 200 mg L-1) and (conventional calcium 200 mg L-1) significantly outperformed the control treatment in stomatal density, which yielded the lowest value in stomatal number, reaching 126.67 mm stomas -1. The value of this trait reached 200.00 stomas mm-1 in the two significantly superior treatments. The results of the table indicate that stomatal density was not significantly affected in either of the two cultivars used in the study, Queen and GS-12. In the two-way interaction between fertilizer type and varieties, we conclude from the table results that the highest value in the stomatal density trait was when using the treatment (Nano calcium 200 mg L-1) interacting with the Queen variety, as the number of stomatal spaces reached 213.33 stomatal spaces mm-1, significantly superior to the Queen and GS-12 varieties only in the case of not using calcium (comparison treatment), and the lowest value in this trait reached 120.00 stomatal spaces mm-1 in the case of interacting with the treatment 200 mg L-1 with the GS-12 variety.

Table (1): Effect of Nano-calcium fertilizer, traditional, varieties and binary interaction on leaf chlorophyll content (SPAD).

Fertilizer Type and Concentration	Varieties		Mean Effect of Fertilizer Type and Concentration
	GS-12	**Queen**	
0 mg L-1 (control treatment)	40.767 f	58.200 ab	49.483 ab

200 mg L-1 Nano calcium	41.367 ef	53.200 b-d	47.283 b
400 mg L-1 Nano calcium	42.467 ef	58.867 ab	50.667 ab
200 mg L-1 conventional calcium	44.267 ef	65.667 a	54.967 a
400 mg L-1 conventional calcium	45.067 ef	57.700 ab	51.383 ab
100 mg L-1 Nano calcium + 100 mg L-1 conventional calcium	45.333 ef	59.133 ab	52.233 ab
75 mg L-1 Nano calcium + 25 mg L-1 conventional calcium	42.167 ef	59.000 ab	50.583 ab
25 mg L-1 Nano calcium + 75 mg L-1 conventional calcium	41.833 ef	53.667 b-d	47.750 b
200 mg L-1 Nano calcium + 200 mg L-1 conventional calcium	47.267 d-f	60.367 ab	53.817 a
150 mg L-1 Nano calcium + 50 mg L-1 conventional calcium	45.200 ef	55.300 bc	50.250 ab
50 mg L-1 Nano calcium + 150 mg L-1 conventional calcium	49.000 c-e	60.933 ab	54.967 a
Mean Effect of Varieties	44.067 b	58.367 a	

Means with the same letter not significantly different ($P \leq 0.05$, Duncan's test).

Table (2): The effect of Nano-calcium fertilizer and traditional fertilizer, varieties and binary interaction on plant height (cm plant-1).

Fertilizer Type and Concentration	Varieties		Mean Effect of Fertilizer Type and Concentration
	GS-12	**Queen**	
0 mg L-1 (control treatment)	65.667 f	76.667 b-d	71.167 c
200 mg L-1 Nano calcium	65.667 f	84.667 a	75.167 a-c

400 mg L-1 Nano calcium	73.000 de	80.000 a-c	76.500 a
200 mg L-1 conventional calcium	72.000 d-f	83.333 ab	77.667 a
400 mg L-1 conventional calcium	66.333 ef	76.667 b-d	71.500 bc
100 mg L-1 Nano calcium + 100 mg L-1 conventional calcium	68.000 ef	82.333 a-c	75.167 a-c
75 mg L-1 Nano calcium + 25 mg L-1 conventional calcium	70.667 d-f	81.333 a-c	76.000 ab
25 mg L-1 Nano calcium + 75 mg L-1 conventional calcium	72.333 d-f	83.000 ab	77.667 a
200 mg L-1 Nano calcium + 200 mg L-1 conventional calcium	76.000 cd	75.667 cd	75.833 a-c
150 mg L-1 Nano calcium + 50 mg L-1 conventional calcium	72.667 de	85.333 a	79.000 a
50 mg L-1 Nano calcium + 150 mg L-1 conventional calcium	67.667 ef	84.000 a	75.833 a-c
Mean Effect of Varieties	70.000 b	81.181 a	

Means with the same letter not significantly different (P ≤ 0.05, Duncan's test).

Table (3): The effect of Nano-calcium and traditional fertilizers, varieties and binary interaction on leaf area (cm2 plant-1).

Fertilizer Type and Concentration	Varieties		Mean Effect of Fertilizer Type and Concentration
	GS-12	**Queen**	
0 mg L-1 (control treatment)	17788 i	21011 g-i	19400 f
200 mg L-1 Nano calcium	22059 f-h	22180 e-h	22119 e
400 mg L-1 Nano calcium	19768 hi	41685 a	30727 a

200 mg L-1 conventional calcium	31912 c	27412 d	29662 ab
400 mg L-1 conventional calcium	25575 d-f	24638 d-g	25107 cd
100 mg L-1 Nano calcium + 100 mg L-1 conventional calcium	24493 d-g	31135 c	27814 bc
75 mg L-1 Nano calcium + 25 mg L-1 conventional calcium	26764 d	26296 de	26530 cd
25 mg L-1 Nano calcium + 75 mg L-1 conventional calcium	26646 d	24136 d-g	25391 cd
200 mg L-1 Nano calcium + 200 mg L-1 conventional calcium	24143 d-g	35846 b	29994 ab
150 mg L-1 Nano calcium + 50 mg L-1 conventional calcium	24167 d-g	23440 d-h	23784 de
50 mg L-1 Nano calcium + 150 mg L-1 conventional calcium	22003 f-h	32998 bc	27500 bc
Mean Effect of Varieties	24119.8 b	28248.8 a	

Means with the same letter not significantly different (P ≤ 0.05, Duncan's test).

Table (4): The effect of Nano-calcium and traditional fertilizers, varieties and binary interaction on the percentage of dry matter in leaves.

Fertilizer Type and Concentration	Varieties		Mean Effect of Fertilizer Type and Concentration
	GS-12	**Queen**	
0 mg L-1 (control treatment)	16.0667 ef	17.5333 b-f	16.8000 c
200 mg L-1 Nano calcium	16.6333 c-f	19.2333 ab	17.9333 a-c
400 mg L-1 Nano calcium	17.6333 b-f	18.1000 a-d	17.8667 a-c

200 mg L-1 conventional calcium	17.4667 b-f	17.4000 b-f	17.4333 bc
400 mg L-1 conventional calcium	16.6667 c-f	19.8667 a	18.2667 ab
100 mg L-1 Nano calcium + 100 mg L-1 conventional calcium	19.0000 ab	18.9333 ab	18.9667 a
75 mg L-1 Nano calcium + 25 mg L-1 conventional calcium	16.3000 d-f	19.0667 ab	17.6833 a-c
25 mg L-1 Nano calcium + 75 mg L-1 conventional calcium	17.4667 b-f	17.4667 b-f	17.4667 bc
200 mg L-1 Nano calcium + 200 mg L-1 conventional calcium	15.8333 f	18.2000 a-d	17.0167 bc
150 mg L-1 Nano calcium + 50 mg L-1 conventional calcium	18.5000 a-c	17.3333 b-f	17.9167 a-c
50 mg L-1 Nano calcium + 150 mg L-1 conventional calcium	16.7000 c-f	17.9667 a-e	17.3333 bc
Mean Effect of Varieties	17.1152 b	18.2818 a	

Means with the same letter not significantly different (P ≤ 0.05, Duncan's test).

Table (5): The effect of Nano-calcium fertilizer and traditional fertilizer, varieties and bilateral interference on the stomata area (micron stomata-1)

Fertilizer Type and Concentration	Varieties		Mean Effect of Fertilizer Type and Concentration
	GS-12	**Queen**	
0 mg L-1 (control treatment)	0.12750 a	0.14250 a	0.13500 a
200 mg L-1 Nano calcium	0.12667 a	0.12833 a	0.12750 a
200 mg L-1 conventional calcium	0.13583 a	0.12833 a	0.13208 a
Mean Effect of Varieties	0.133056 a	0.130000 a	

Means with the same letter not significantly different (P ≤ 0.05, Duncan's test).

Table (6): The effect of Nano-calcium fertilizer and traditional fertilizer, varieties and binary interaction on the gap density (mm stomata -1)

Fertilizer Type and Concentration	Varieties		Mean Effect of Fertilizer Type and Concentration
	GS-12	**Queen**	
0 mg L-1 (control treatment)	120.00 c	133.33 bc	126.67 b
200 mg L-1 Nano calcium	186.67 a-c	213.33 a	200.00 a
200 mg L-1 conventional calcium	200.00 ab	200.00 ab	200.00 a
Mean Effect of Varieties	168.89 a	182.22 a	

Means with the same letter not significantly different (P ≤ 0.05, Duncan's test).

Conclusions:

1. Most fertilizer treatments led to a significant increase in most vegetative growth traits. In general, a concentration of 200 mg L-1 for both Nano- and conventional calcium resulted in a significant increase in stomatal density (number of stomata). 2. Significant differences varied between the two studied cultivars, GS-12 and Queen, in vegetative growth and anatomical traits. Queen plants significantly outperformed GS-12 plants in all studied vegetative growth traits, such as plant height, leaf chlorophyll content, number of branches, leaf area, leaf dry matter percentage, and stem diameter. However, no significant differences were observed between the two cultivars in the studied anatomical traits, such as stomatal density and stomatal area.

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