



IMPACT OF IRRIGATION INTERVALS AND ASCORBIC ACID CONCENTRATIONS ON SOME GROWTH AND ANATOMICAL CHARACTERISTICS OF THE SOYBEAN CROP

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Article info	Abstract
Received: 2024-12-20 Accepted: 2025-02-26 Published: 2025-06-30	The study was conducted at the College of Agriculture's research station in the Al-Bu'itha area. It aimed to determine how drought stress affects the growth and anatomical features of the soybean crop and how ascorbic acid lessens the negative effects of low water availability. The research applied a split-plot arrangement with three replications using a randomized complete block design (RCBD). It involved irrigating the main plots at intervals of 3, 6, 9, and 12 days, and applying ascorbic acid spray concentrations of 0, 1000, and 2000 mg L ⁻¹ to the sub-plots. The findings demonstrated that plant height and leaf area considerably decreased with higher irrigation intervals while the highest concentration of ascorbic acid (2000 mg L ⁻¹) gave the maximum values for the two traits. Optical microscopy was used to examine anatomical changes under drought stress conditions in the stems and leaves of the soybean [<i>Glycine max</i> (L.) Merr. cv. Rami] plants, and numerical analysis assessed the importance of these alterations. Some differences in anatomical characteristics were found in the stressed soybean plants, including the size and average number of stomata and epidermal cells, as well as characteristics relating to the stem, leaf, and stomata indexes.
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Keywords: Glycine max, Anatomical structure, Irrigation interval, Ascorbic acid.

تأثير فترات الري وتراكيز حمض الأسكوربيك على بعض صفات النمو والتشريح لحصول فول الصويا

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الخلاصة

نفذت الدراسة في محطة أبحاث كلية الزراعة في منطقة البوعيثة، وهدفت الدراسة الى معرفة مدى تأثير اجهاد الجفاف على نمو وصفات محصول فول الصويا التشريحية وكيف يعمل حامض الأسكوربيك على تقليل الآثار السلبية لقلة توفر الماء. استخدم البحث ترتيب القطع المنشقة بثلاثة مكررات باستخدام تصميم القطاعات الكاملة العشوائية (RCBD)، والتي تضمنت القطع الرئيسية فواصل الارواء وهي 3، 6، 9 و 12 يومًا، في حين كانت القطع الثانوية تركيز الرش بحامض الأسكوربيك (0، 1000، 2000) ملغم لتر⁻¹. وأظهرت النتائج أن زيادة فاصلة الارواء أدت إلى انخفاض كبير في ارتفاع النبات ومساحة الأوراق، كما أعطى التركيز العالي من حامض الأسكوربيك (2000 ملغم لتر⁻¹) أعلى ارتفاع للنبات ومساحة أوراق. تحت اجهاد الجفاف، تم استخدام المجهر الضوئي لفحص التغيرات التشريحية في ساق وأوراق نباتات فول الصويا لصنف (Rami) وتم استخدام التحليل العددي لتقييم أهمية هذه التغيرات. تم اكتشاف أن بعض الصفات التشريحية الرئيسية تختلف في نباتات فول الصويا المجهد، بما في ذلك حجم ومتوسط عدد الثغور وخلايا البشرة، بالإضافة إلى الصفات المرتبطة بمؤشر الساق والأوراق والثغور.

كلمات مفتاحية: فول الصويا، البنية التشريحية، فاصلة الارواء، حامض الاسكوربيك.

Introduction

The soybean is considered a major economic crop because its seeds have a high content of unsaturated fatty acids such as linoleic and linolenic acids as well as the majority of essential amino acids and several vital vitamins. The seeds also have a good percentage of protein at 30–50% and oil at 14–24% (18). Numerous severe environmental stresses harm a plant's development, metabolism, and production with drought stress being the greatest threat (5 and 6). Drought stress is a phenomenon that affects plants when there is insufficient water available for their roots or during extremely high transpiration conditions. The combination of these two circumstances is common in arid and semi-arid regions.

Drought stress creates highly complicated reactions in plants, one of the most significant and easily noticed being the anatomical changes brought on by a lack of

water. These changes can be directly adopted in agriculture and addressed because they provide true indicators of the internal reactions in plants to external environmental stimuli (15). Throughout a decrease in cell size or an increase in vascular tissues and cell wall thickness, water stress in plant cells determines the transmission of its effects across plant tissues, which are represented by anatomical characteristic (7). Anatomical changes brought on by a lack of water shield the plant from these impacts which make the required modifications.

Among the most effective strategies for addressing biological challenges, regulating physiological and biochemical processes, eliminating free radicals, regulating photosynthesis and blooming, regulating the generation of reactive oxygen species (ROS), and delaying the aging of plants is by spraying ascorbic acid (4). Since ascorbic acid is a non-enzymatic antioxidant and plays a variety of roles in cell growth, expansion, and delaying the onset of aging, it holds a significant physiological position in plants. It stimulates the mechanism for electron transportation and protects the chloroplast from oxidation. The spraying of ascorbic acid on plants helps in strengthening the potency of their numerous antioxidant enzymes (12).

The purpose of this study was to determine how drought stress affects certain anatomical and growth traits in the soybean crop and the impact of ascorbic acid in mitigating the negative effects of low water availability.

Materials and Methods

Crop cultivation or experimental designing and sowing of the crop: This field experiment was conducted in the summer of 2023 at the research station of the College of Agriculture, University of Anbar, in the Al-Bu'itha region. It used a split-plot arrangement with three replications based on the randomized complete block design (RCBD). The main plots were irrigated at intervals of 3, 6, 9, and 12 days while plants in the sub-plots were sprayed with ascorbic acid concentrations of 0, 1000, and 2000 mg L⁻¹. A variety of Rami soybean seeds were planted on June 3, 2023 by insertion into holes to a depth of two to three centimeters.

Immediately after sowing, the plots were lightly irrigated to prevent the seeds from washing away. After the experimental field reached the four-leaf stage, irrigation was administered based on the number of days in each irrigation interval. Ascorbic acid of 1 and 2 grams was dissolved in one liter of water to produce concentrations of 1000 and 2000 mg L⁻¹. To ensure total saturation of the leaves, reduce the surface tension of the water, and increase the spray solution's capacity to penetrate the leaf's outer layer, 0.15 mg L⁻¹ of diffuse material was added as a spreading agent.

Data collection: Plant height: Five plants were picked from the center of the experimental units and their heights measured from the top of the soil to their maximum heights, and the averages computed.

Leaf area was identified during the pod formation stage using the (20) equation:
$$LA = 0.624 + (0.723) (L.W)$$

Individual leaf areas were multiplied by the total number of leaves on a plant to derive the total leaf area in dm².

Preparation of the anatomical sections: Fresh samples of the plants collected from the field were cleaned and left in a FAA (formaldehyde: acetic acid: alcohol) solution for 24 h. Ethyl alcohol at 70% concentration was then used to wash the samples and kept at the same concentration until use. Transverse sections of the stem and surface epidermis of the leaves scraped by hand were used for all observations. Following the scraping process, the skin was placed in a sterile petri dish filled with water to eliminate any leftover materials and tissue markers. The epidermis was then placed in a 5% sodium hypochlorite solution for six minutes to eliminate chlorophyll pigment. Following their preparation for microscopic analysis and investigation, the samples were inspected using a compound microscope (Kruss compound microscope), according to (13).

Statistical analysis: Using the computer and the Genstat statistical analysis program, the findings were compared using least significant difference (LSD) at the 5% probability level.

Results and Discussion

Plant height (cm): As seen in Table 1, average plant height increased significantly in the 3-day-interval irrigation treatment to reach a maximum of 128 cm compared to the lowest at 78.22 cm for the 12-day interval period. This decrease is attributed to the high moisture content and heat stress experienced during the early growth phases which cause the production of free radicals (ROS) that inhibit cell division and elongation. This outcome is consistent with both (6 and 17).

Also, plants sprayed with high ascorbic acid concentrations had an average height of 109.50 cm, compared to 103.33 cm for the comparison treatment. This is attributed to the role of vitamin C in stimulating enzymes for producing gibberellin which increases growth indicators such as stem epidermis thickness (Table 3) and the number of vascular bundles carrying nutrients and water, both of which positively correlate with the plant's increased length. This outcome is consistent with (11).

Plants sprayed with high ascorbic acid concentration of 2000 mg L⁻¹ and irrigated every three days recorded the highest average height of 133.33 cm, while those sprayed with distilled water only and irrigated every twelve days had the lowest at 76.67 cm. These results indicate that the interaction between the two factors had a significant impact on the average for this trait.

Table 1: The impact of ascorbic acid spraying, irrigation intervals, and their interaction on soybean plant height (cm).

Ascorbic acid concentration (mg. l ⁻¹)	Irrigation intervals (days)				Mean ascorbic acid
	3	6	9	12	
0	123.00	116.67	97.00	76.67	103.33
1000	127.67	118.33	105.00	78.00	107.25
2000	133.33	119.33	105.33	80.00	109.50
Mean intervals	128.00	118.11	102.44	78.22	
LSD	Irrigation intervals		Ascorbic acid		Interaction
0.05	2.68		1.56		3.41

Leaf area (dm^2): The results in Table 2 demonstrate the significant variations in average leaf areas. Plants irrigated every three days had the highest average for this trait at 112.74 dm^2 , while those irrigated every 12 days had significantly lower average leaf area at 88.28 dm^2 . The growth regulator auxin, which stimulates cell division in the leaf sheaths, may be the reason for the higher leaf area. On the contrary, for the 12-day irrigation plants the amount of auxin decreases as more indoleacetic acid (IAA) oxidase is produced, which breaks down auxins and decreases leaf area (14). Moreover, high acid concentrations and short irrigation intervals increased the lengths and widths of the upper and lower stomata (Tables 4 - 7).

Table 2 also shows the considerable increase in leaf area (102.22 dm^2) for plants sprayed with a high concentration of ascorbic acid (2000 mg L^{-1}) compared to the 93.09 dm^2 for the distilled water only treatment. This is because ascorbic acid has a beneficial effect in promoting cell division and growth from the increased production of photosynthesis, which is positively reflected in the greater leaf area. This outcome is in line with (16) who noted that applying this acid to plants increased their leaf areas.

Table 2: The impact of ascorbic acid spraying, irrigation intervals, and their interaction on soybean plant leaf area (dm^2).

Ascorbic acid concentration (mg. l^{-1})	Irrigation intervals (days)				Mean ascorbic acid
	3	6	9	12	
0	110.05	95.33	83.45	83.52	93.09
1000	113.28	100.49	95.89	89.08	99.69
2000	114.88	102.93	98.85	92.22	102.22
Mean intervals	112.74	99.58	92.73	88.28	
LSD	Irrigation intervals		Ascorbic acid		Interaction
0.05	2.040		1.912		3.511

Thickness of stem epidermis (μm): Table 3 shows the notable average variations in this characteristic across irrigation intervals with the stem epidermal thickness increasing significantly to $3.30 \mu\text{m}$ for the 3-day interval irrigated plants compared to $2.11 \mu\text{m}$ for the 12-day interval plants. However, the lack of water affects all essential plant processes, including photosynthesis, and consequently the nutrients required for cell growth, both longitudinally and laterally. This has an adverse effect on the vascular bundles and the thickness of the stem epidermis, as also noted by (1 and 2).

This characteristic was significantly affected by spraying ascorbic acid at a concentration of 2000 mg L^{-1} on the soybean plants, which produced the greatest average thickness of $2.85 \mu\text{m}$ as against the lowest of $2.41 \mu\text{m}$ for the control. The superiority of the high ascorbic acid concentration treatment may be attributed to the activation of enzymes and stimulation of biochemical processes that increase photosynthesis efficiency and, consequently, cell division and the thickness of the stem epidermis, making it more resistant to biotic stress. The first irrigation treatment and ascorbic acid spraying both resulted increase in the number of vascular bundles, which is reflected in the thickness of the stem epidermis, as shown in Figures 1 - 5. This result is consistent with (16).

Table 3: The impact of ascorbic acid spraying, irrigation intervals, and their interaction on stem epidermis thickness of the soybean plant (μm).

Ascorbic acid concentration (mg. l^{-1})	Irrigation intervals (days)				Mean ascorbic acid
	3	6	9	12	
0	3.10	2.30	2.20	2.03	2.41
1000	3.34	3.02	2.35	2.11	2.70
2000	3.46	3.11	2.63	2.19	2.85
Mean intervals	3.30	2.81	2.39	2.11	
LSD	Irrigation intervals		Ascorbic acid		Interaction
0.05	0.123		0.045		0.133

Stomata lengths on the upper leaf epidermis (μm): Table 4 shows the significant increase in stomata length on the upper epidermis of the leaves for the plants irrigated every three days. This treatment produced the highest average length for this trait at $31.79 \mu\text{m}$ compared to the lowest at $26.29 \mu\text{m}$ for those watered every twelve days. The higher stomata length is due to the role of water in opening and closing the stomata and increasing their expansion. These results are consistent with (19), who found that water stress decreased the length of the stomata.

Notable variations in stomata length also occurred from different ascorbic acid spray concentrations. Plants sprayed with 2000 mg L^{-1} ascorbic acid had the highest average length of the upper stomata in their leaves ($30.81 \mu\text{m}$), while the distilled water treatment produced the lowest ($28.46 \mu\text{m}$). Along with enhancing the water gradient between the stomata and the surrounding cells and, consequently, osmosis and water potential, ascorbic acid also has the critical function of opening and closing the stomata and increasing their expansion, as observed in the anatomical sections of the leaves (Figures 7 - 9). These outcomes agree with (3).

Table 4: Effect of ascorbic acid spraying, irrigation intervals, and their interaction on stomata lengths in the upper leaf epidermis of the soybean plant (μm).

Ascorbic acid concentration (mg. l^{-1})	Irrigation intervals (days)				Mean ascorbic acid
	3	6	9	12	
0	30.74	28.26	30.29	24.57	28.46
1000	32.20	31.56	29.65	26.79	30.05
2000	32.42	32.01	31.30	27.52	30.81
Mean intervals	31.79	30.61	30.41	26.29	
LSD	Irrigation intervals		Ascorbic acid		Interaction
0.05	0.765		0.911		NS

Stomata lengths on the lower leaf epidermis (μm): Table 5 shows the significant differences in the study factors and their interactions. The length of the stomata on the lower leaf epidermis increased significantly for plants irrigated every three days, producing the highest average of $30.65 \mu\text{m}$ as against the lowest at $23.77 \mu\text{m}$ for the 12-day irrigation interval treatment. The reason could be that low water levels release the abscisic acid (ABA) hormone which decreases the amount of green space needed for photosynthesis and is reflected in the length of the stomata on the leaves. Water levels are also important for stomata opening and closing processes. These findings are

in line with (8) that the lengths of other plants' stomata shortened in response to water stress.

Table 5 also shows that plants sprayed with a high concentration of ascorbic acid had the longest average stomata lengths measuring 28.50 μm , while the control treatment involving only distilled water spraying had the shortest at 25.78 μm . Ascorbic acid helps the plant maintain its water balance, raising the amount of carbon metabolism products in the leaves and opening protein channels to allow negative ion flows within the cells, thereby extending and expanding the stomata. This is in accordance with the findings of (7) that applying ascorbic acid to other plants extended their stomata (Figures 7 – 9). The study factors interaction had a notable impact on the averages of this trait. Plants sprayed with a concentration of 2000 mg L^{-1} ascorbic acid and irrigated every three days had the highest average at 31.62 μm , while plants sprayed with diluted concentrations at 12-day intervals had the lowest at 22.63 μm .

Table 5: The impact of ascorbic acid spraying, irrigation intervals, and their interaction on stomata lengths in the lower leaf epidermis of the soybean plant (μm).

Ascorbic acid concentration (mg. l^{-1})	Irrigation intervals (days)				Mean ascorbic acid
	3	6	9	12	
0	29.33	26.41	24.75	22.63	25.78
1000	30.99	27.88	27.68	24.30	27.71
2000	31.62	29.25	28.75	24.38	28.50
Mean intervals	30.65	27.85	27.06	23.77	
LSD	Irrigation intervals		Ascorbic acid		Interaction
0.05	0.65		0.33		0.78

Stomata widths on the upper leaf epidermis (μm): The findings in Table 6 demonstrate that 3-day irrigation intervals produced the greatest statistically significant variation in the upper epidermis stomata width, measuring 21.95 μm . Conversely, the width declined to 12.95 μm when the plants were irrigated every 12 days. The increased water content in the guard cells promotes plant absorption, division, growth and subsequently widens the stomata. The reduction in water content results in a variation in the water gradient, which influences the plant's general growth processes, including cell elongation and growth. This is negatively reflected in the width of the leaf epidermis' stomata. This outcome is compatible with the findings of (8), who found that a lack of water results in fewer stomata.

Ascorbic acid spraying contributes to the expansion of stomata width. Plants treated with 2000 mg L^{-1} ascorbic acid showed a noteworthy advantage in this regard, exhibiting the highest average stomata width of 19.23 μm , compared to the lowest of 17.30 μm for the control treatment. The beneficial effects of ascorbic acid on cell division and growth may contribute to this as shown by Table 2's depiction of the area of the leaves and the consequent increase in the width of their stomata. This corresponds to the findings of (7). Additionally, Figures 7, 10, and 11 illustrate how the four irrigation intervals and acid spraying changed the stomata forms.

Table 6: The impact of ascorbic acid spraying, irrigation intervals, and their interaction on stomata width in the upper leaf epidermis of the soybean plant (μm).

Ascorbic acid concentration (mg. l^{-1})	Irrigation intervals (days)				Mean ascorbic acid
	3	6	9	12	
0	20.64	20.31	15.85	12.40	17.30
1000	22.09	20.52	16.74	12.40	17.94
2000	23.13	21.48	18.25	14.04	19.23
Mean intervals	21.95	20.77	16.95	12.95	
LSD	Irrigation intervals		Ascorbic acid		Interaction
0.05	0.753		0.567		NS

Stomata widths on the lower leaf epidermis (μm): Table 7 shows that the lower stomata's width significantly increased when watering occurred every three days producing the highest average at $21.41\mu\text{m}$. Conversely, for 12-day irrigation intervals, the stomata's width decreased, reaching $12.12\mu\text{m}$. These outcomes agreed with the findings of (8 and 10).

According to the results in the same table, plants sprayed with 2000 mg L^{-1} ascorbic acid produced the highest average for this characteristic ($17.68\mu\text{m}$), exceeding the other concentrations (Figures 7, 10 and 11). On the other hand, the lowest average of $15.89\mu\text{m}$ was obtained when plants were sprayed with distilled water alone. The elevation observed in the width of the stomata after treatment with distilled water is due to this vitamin's role in increasing the relative water content of the leaves. This outcome agrees with the findings of (7, 12, 13 and 14).

Table 7: The impact of ascorbic acid spraying, irrigation intervals, and their interaction on stomata width in the lower leaf epidermis of the soybean plant (μm).

Ascorbic acid concentration (mg. l^{-1})	Irrigation intervals (days)				Mean ascorbic acid
	3	6	9	12	
0	19.71	18.18	14.60	11.09	15.89
1000	20.10	18.59	15.96	12.08	16.68
2000	21.41	19.61	16.52	13.19	17.68
Mean intervals	21.41	19.61	16.52	13.19	
LSD	Irrigation intervals		Ascorbic acid		Interaction
0.05	0.39		0.367		NS

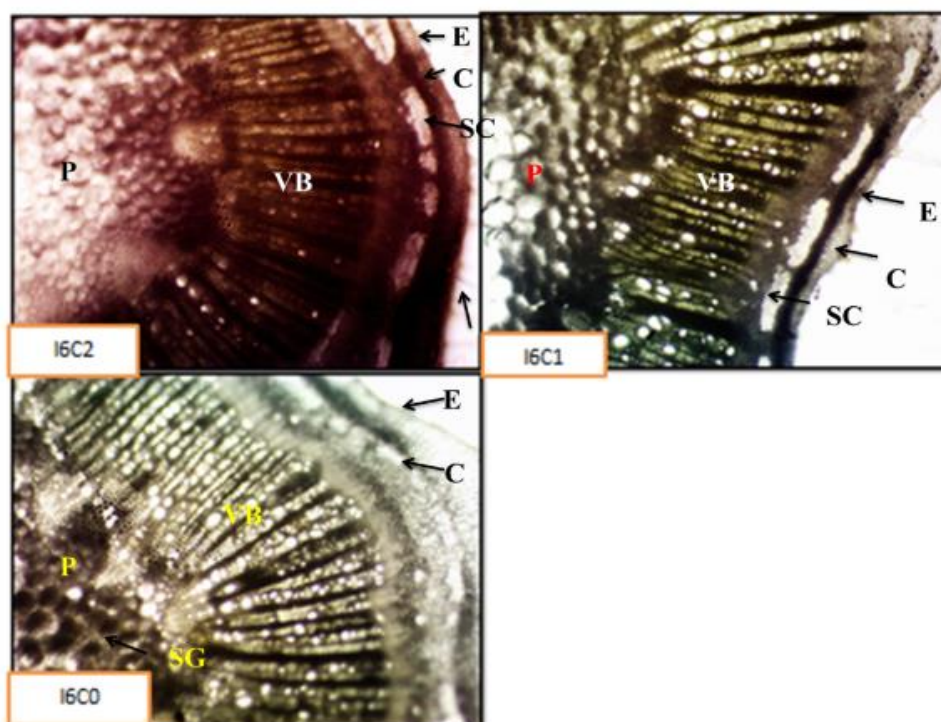


Figure 1: Cross-section of stressed and unstressed soybean stems at 10X magnification revealing the fission intercellular space within the stem cortex. This space accumulates surplus materials that the plant does not require, including active substances, metabolic byproducts, and other materials generated from the plant's vital processes or absorbed by the root system from the soil.

I6: 6-day irrigation interval; C0, C1, and C2: irrigation with distilled water, 1000 mg. l⁻¹, and 2000 mg. l⁻¹ ascorbic acid concentrations, respectively. E: epidermis; C: cortex; SC: sclerenchyma layer; VB: vascular bundle; P: pith; SG: starch grains.

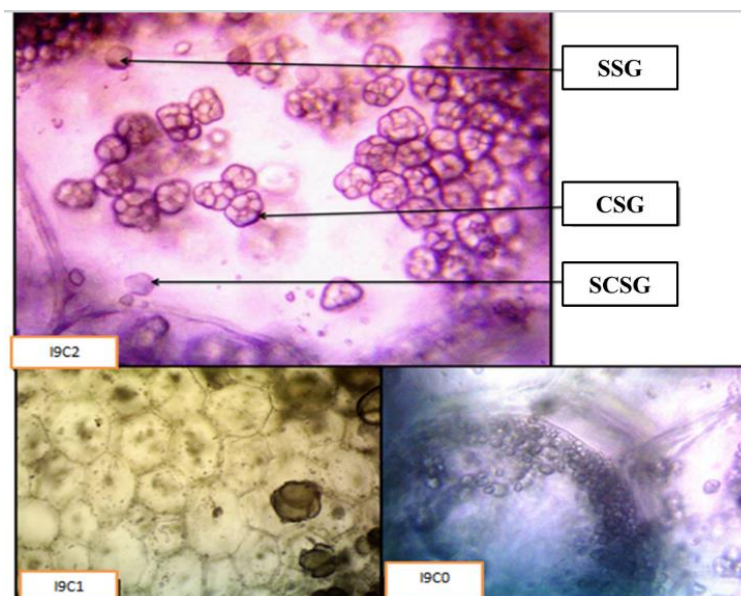


Figure 2: Cross-section of the stem's pith region at 40X magnification showing the morphology, distribution, and density of starch within the pith cells. Significant variations exist in the distribution and density of starch grains in the cells with different treatments. The figure reveals three forms of the grains: simple (SSG), compound (CSG), and semi-complex (SCSG).

I9: 9-day irrigation interval; C0, C1, and C2: irrigation with distilled water, 1000 mg. l⁻¹, and 2000 mg. l⁻¹ ascorbic acid concentrations, respectively.

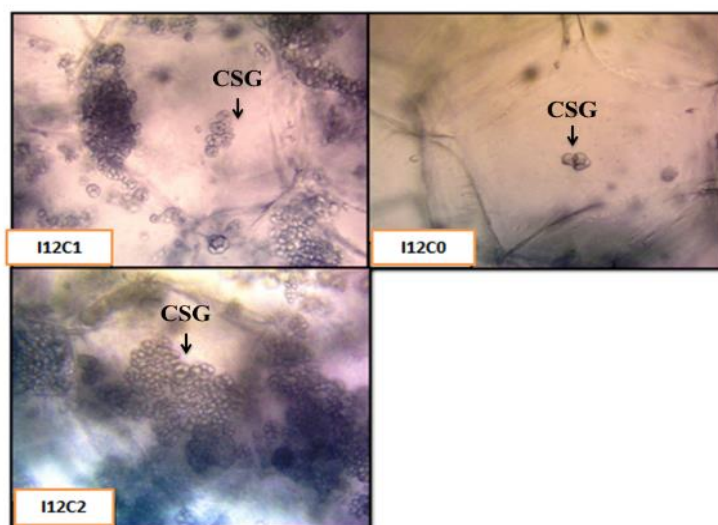


Figure 3: Cross-section of the stem's pith region at 40X magnification showing the morphology, distribution, and density of starch within the pith cells at varying concentrations.

CSG: compound starch grains; I12: 12-day irrigation interval; C0, C1, and C2: irrigation with distilled water, 1000 mg. l⁻¹, and 2000 mg. l⁻¹ ascorbic acid concentrations, respectively.

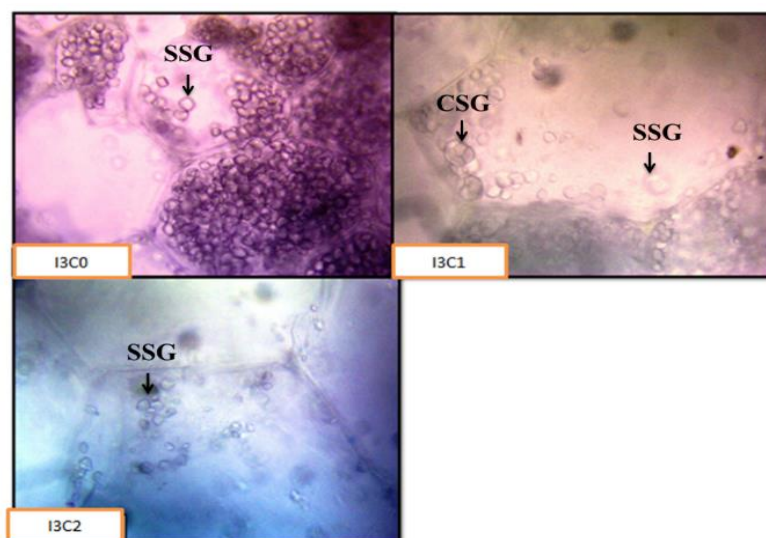


Figure 4: Cross-section of the stem's pith region at 40X magnification showing the morphology, distribution, and density of starch within the pith cells at varying concentrations.

SSG and CSG: simple and compound starch grains, respectively; I3: 3-day irrigation interval; C0, C1, and C2: irrigation with distilled water, 1000 mg. l⁻¹, and 2000 mg. l⁻¹ ascorbic acid concentrations, respectively.

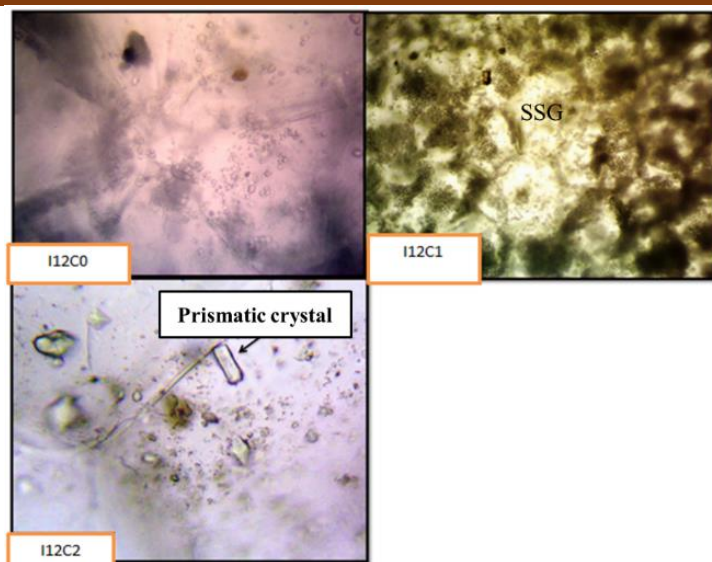


Figure. 5: Cross-section of the stem's pith region at 40X magnification showing the shapes, distribution, density of starchy material in the pith cells, and diffuse crystals. Diffuse crystals are calcium oxalates that the plant does not need and removes in the form of different shaped crystals either prismatic, druse, or needle.

I12: 12-day irrigation interval; C0, C1, and C2: irrigation with distilled water, 1000 mg. l⁻¹, and 2000 mg. l⁻¹ ascorbic acid concentrations, respectively.

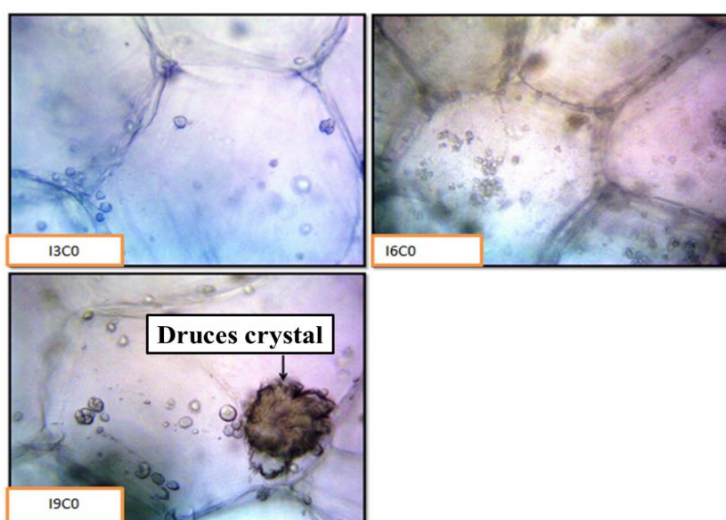


Figure 6: Cross-section of the stem's pith region showing the shapes, distribution, and density of starch in the pith cells at different concentrations, and druse crystals.

I9, I6, and I3: 9, 6, and 3-day irrigation intervals. C0, C1, and C2: irrigation with distilled water, 1000 mg. l⁻¹, and 2000 mg. l⁻¹ ascorbic acid concentrations, respectively.

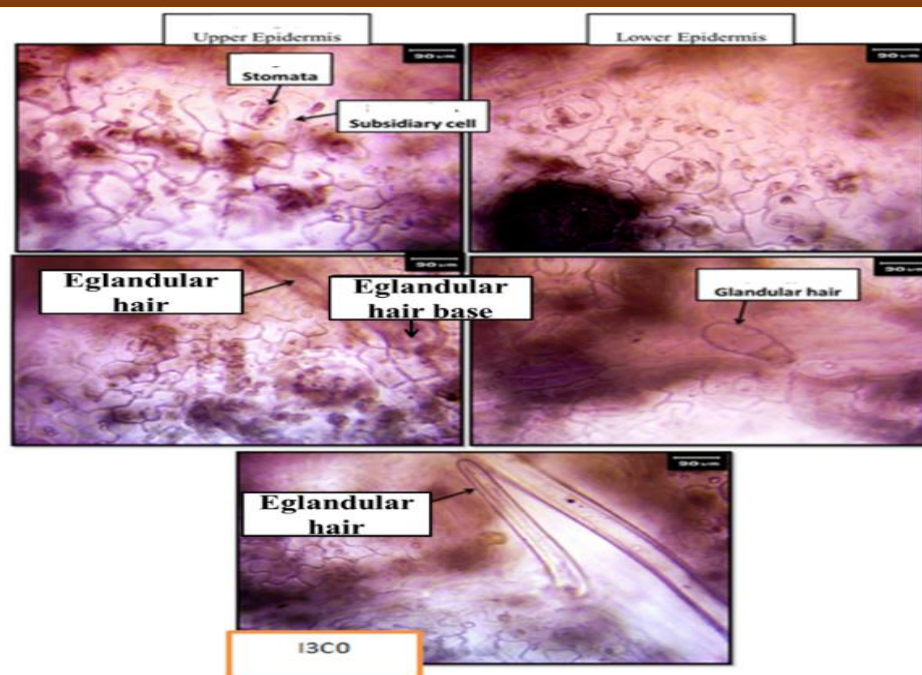


Figure 7: The upper and lower epidermis of soybean leaves at 40X magnification showing the distribution of glandular hairs and eglanular hairs. The stomatal pattern is of the paracytic type, in which the stomatal complex consists of two accessory cells on either side of the guard cells parallel to the longitudinal axis of the stoma. The hairs are of two types: glandular, consisting of a multicellular glandular head and a short, unicellular neck, and eglanular of the unicellular, uniseriate type, with a multicellular base.

I3: 3-day irrigation interval; C0: distilled water irrigation.

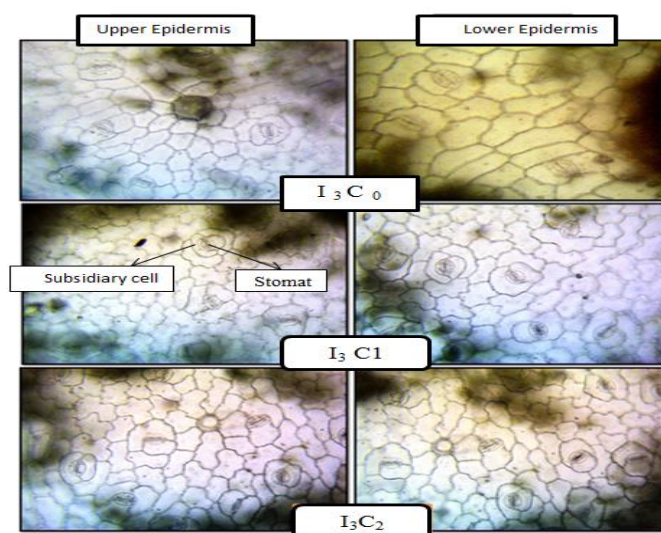


Figure 8: Leaf epidermis at 40x magnification displaying the stomata's forms and locations in the upper and bottom layers of the green leaves.

I3: 3-day irrigation interval. C0, C1, and C2: irrigation with distilled water, 1000 mg. l⁻¹, and 2000 mg. l⁻¹ ascorbic acid concentrations, respectively.

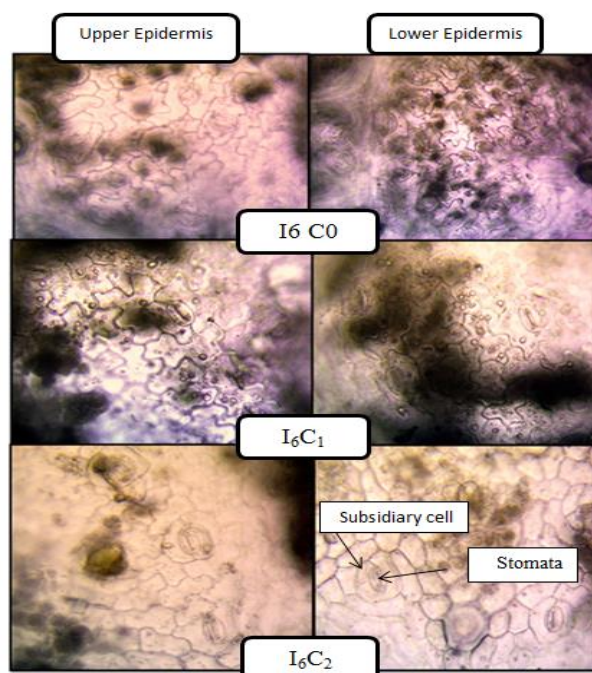


Figure 9: Leaf epidermis at 40x magnification displaying the stomata's forms and locations in the upper and bottom layers of the green leaves.

I6: 6-day irrigation. C0, C1, and C2: irrigation with distilled water, 1000 mg. l⁻¹, and 2000 mg. l⁻¹ ascorbic acid concentrations, respectively.

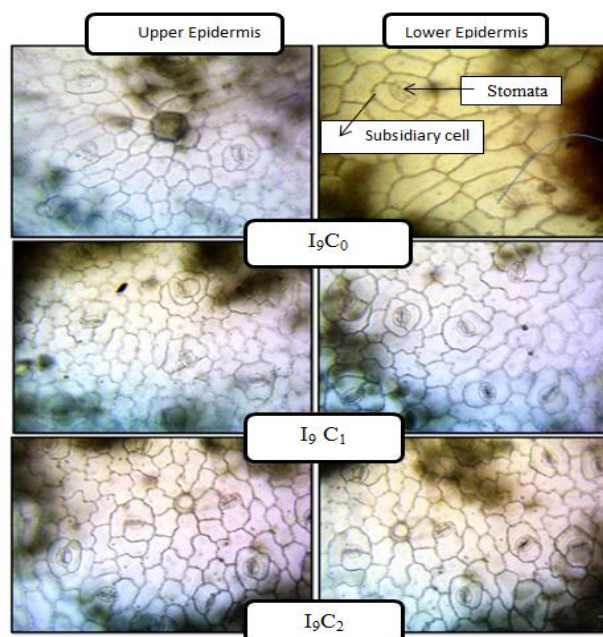


Figure 10: Leaf epidermis at 40x magnification displaying the stomata's forms and locations in the upper and bottom layers of the green leaves.

I9: 9-day irrigation interval. C0, C1, and C2: irrigation with distilled water, 1000 mg. l⁻¹, and 2000 mg. l⁻¹ ascorbic acid concentrations, respectively.

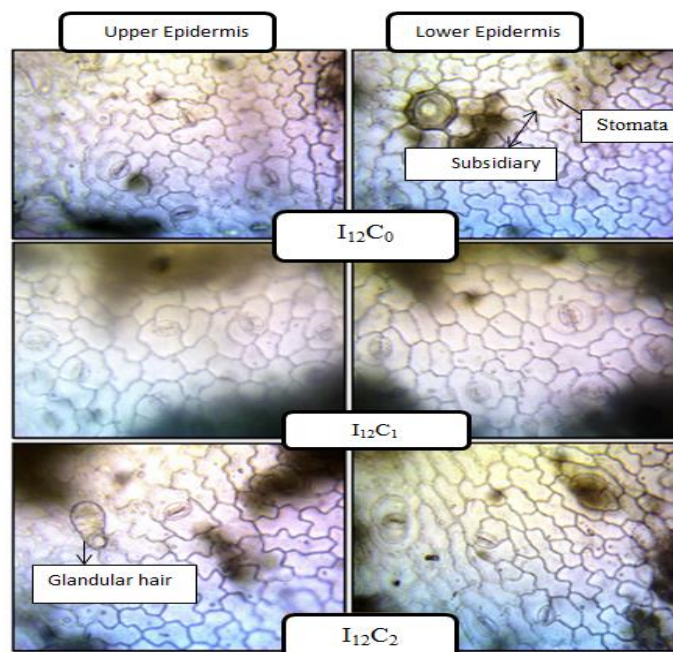


Figure 11: Leaf epidermis at 40x magnification displaying the stomata's forms and locations in the upper and bottom layers of the green leaves.

I12: 12-day irrigation interval. **C0, C1, and C2:** irrigation with distilled water, 1000 mg. l⁻¹, and 2000 mg. l⁻¹ ascorbic acid concentrations, respectively.

Conclusions

Plants irrigated every three days showed notable improvements in all anatomical parameters. By intensifying the activation of most physiological processes, including photosynthesis, this irrigation level raised the amount of nutrients and had a direct impact on growth parameters. On the other hand, lengthening the irrigation period generated adverse impacts on growth and anatomical features. The high concentration of ascorbic acid affected the height and leaf areas of the plants by increasing cell division due to the high auxin concentration, as well as the thickness of the epidermis and the lengths and widths of the stomata.

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The authors declare that they have no conflict of interest.

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