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RESEARCH ARTICLE

Effects of Ascorbic, Citric, and Humic Acids on Maize Stem and Leaf Anatomy

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ABSTRACT

Anatomical changes in internal tissue of stem and leaf when seed and plant treated with acids to enhance growth and development in maize was studied during the spring seasons of 2019 and 2020. Randomized complete block design was used with three replications. Main plots received foliar nutrition treatments, including ascorbic acid (AA), citric acid (CA), and humic acid (HA) at concentrations of 100 mg L^{-1}, alongside HA at 1 ml L^{-1}, with distilled water as the control. Sub-plots underwent corresponding treatments for seed soaking. Results indicated variations in vascular bundle size among treatments, with foliar CA treatment showing superior results in both years, as well as seed soaking in CA and HA. Interaction effects were observed, notably in 2019 with the combination of foliar CA and seed soaking with distilled water, and in 2020 with HA. Effects on leaf epidermis were minimal, with slight distortions in stomatal shapes observed with AA and CA treatments compared to the control. AA and HA treatments led to larger ordinary epidermal cells with straighter cell walls than the control, along with an increase in cork and silica cell size in treated plants. This study contributes to understanding anatomical modifications in maize leaves and stems during the growing season, shedding light on the potential impacts of acid treatments on plant physiology.

Keywords: Foliar nutrition, Growth regulators, Plant anatomy, Seed soaking, *Zea mays* L

Introduction

Maize holds significant importance as a crop cultivated for its seed, silage feed, and its role in human and animal nutrition. In Iraq, there has been a noticeable increase in the area dedicated to maize cultivation. However, the cultivation of grain corn in this region is confronted with numerous environmental challenges, ultimately resulting in diminished productivity.[1,2] One critical factor contributing to this is the selection of inappropriate maize varieties, which can exacerbate issues such as moisture accumulation within the grain. Such accumulation poses a threat to the safe ripening of the crop at harvest time. Thus, the careful selection of maize varieties tailored to the specific environmental conditions of Iraq is paramount to ensure optimal productivity and harvest quality. Many challenges have emerged in the

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area, including the soil degradation and desertification, the scarcity of water, the harshness of climatic conditions, and global climate changes due to global warming. These have been exacerbated in the absence of proper agricultural planning, but they can be confronted through the use of techniques and technology in the application of modern agriculture, which is the basis through which these challenges can be faced, and this represents the research hypothesis.[3,4]

Soaking seeds in growth regulators before planting can be a beneficial practice in mitigating the negative effects of environmental stress on the growth, physiological and biochemical responses of crops.[3,5] Environmental stress factors such as salinity,[6] high temperatures,[7,8] and drought[9] can pose significant challenges to plant growth and overall crop productivity. Growth regulators typically affect germination time, steering to preferable growth and amended yield,[10] exceptionally in plants under the stress.[11,12]

Farmers keep trying to avoid chemical fertilizer by using some sustainable practices such as applications of biofertilizer or sea weed,[13–15] plant extracts and plant regulators[16–18] which led to enhance germination, growth and yield[19–21] significantly after seed soaking or spraying during vegetative stages even under stresses of drought and salt.[22,23]

Foliar ascorbic acid alleviated the negative effects of Cadmium stress in maize and improved photosynthetic processes, osmolytes, and antioxidant defense systems.[24] Ascorbate is contributing in translocating photosynthates from source to the sink, increase net photosynthesis rate, prevent auxin oxidation, reinforce plants' antioxidant potential, protection against oxidative stress which lead to improve growth and drought tolerance.[25] Ascorbic acid activates maize plant´s growth and improved water use efficiency, also enhancing biosynthesis of photosynthetic pigments and then improving yield. AA plays vital role in protecting plant tissues from harmful oxidative damage by acting as reductant drought stress.[26] Ascorbic acid alleviates the drought tolerance by improving photosynthetic pigments, osmo-protectants contents and antioxidant system, which lead to protect maize plant from damaging of drought stress specially in the early stages of growth. AA is a cofactor for control the phytohormones, enzymes, cell growth and development and can be more effective in mitigating the harmful effects of water deficit.[27]

Citric acid improves abiotic stress tolerance through better osmoregulation, induces antioxidant defense systems, promotes increased chlorophyll content and relieving heavy metal stress.[28] Citric acid is a low-molecular-weight organic acid exuded by the plant roots. Organic anions derived from this acid compete for phosphorus adsorption sites in clay minerals. CA use led to increase P availability of corn plants.[29] Growth, photosynthetic pigments, and biomass increased by enhancing antioxidant enzyme activity with the application of B. vietnamiensis and CA.[30] Citric acid foliar led to modification of pH and induction of macrobiotic activity of the rhizosphere or the capacity to form complexes with metallic ions or the mobilization of phosphorus. CA has an effect on the physiology leaves, especially in the increase of soluble protein and proteolytic activity.[31]

Humic acid (HA) is recognized as an important class of antioxidant compounds commonly found in natural water and soil. These compounds play a significant role in participating in extensive redox reactions within ecosystems. It is worth noting that HA can account for up to 80% of dissolved organics in natural water sources.[32,33] Humic acid as a foliar or pre-sowing seed treatment significantly increased the plant biomass, chlorophyll pigments and proline contents. Foliar spray was better in improving plant biomass, chlorophyll contents, accumulation of nutrients, however, in contrast, seed pre-treatment was more effective in altering leaf proline. HA led to enhance salinity tolerance through HA-induced increase in plant biomass, content of chlorophyll and mineral nutrients and activities of antioxidant enzyme.[34] Application of HA with SA could be an effective and low-cost approach to ensure seedling establishment and plant growth in fields affected by soil drought in the early season.[35] Addition of humic acid to the soil raised the possibility of maintaining the growth of maize in case of lack of water available. The plant growth stimulation by humic acids has been attributed to a hormone-like effect as promoting the root development and proliferation, resulting in a more efficient water and nutrient absorption.[36]

This study aimed to investigate the changes in internal tissues of the stem and leaf of maize when seeds and the whole plant were treated with ascorbic, citric, and humic acids. The application of these regulators induces changes in the anatomy of the plant's tissue. This change may play a crucial role in the vital processes occurring in the leaf, including photosynthesis, transpiration, respiration, and the transport processes facilitated by the vascular bundles in the stem. Consequently, any enhancements or improvements in the anatomical characteristics can positively impact overall plant growth and development.

Materials and methods

A field experiment was conducted over two spring seasons, in 2019 at the experimental fields of the College of Agricultural Engineering Sciences,

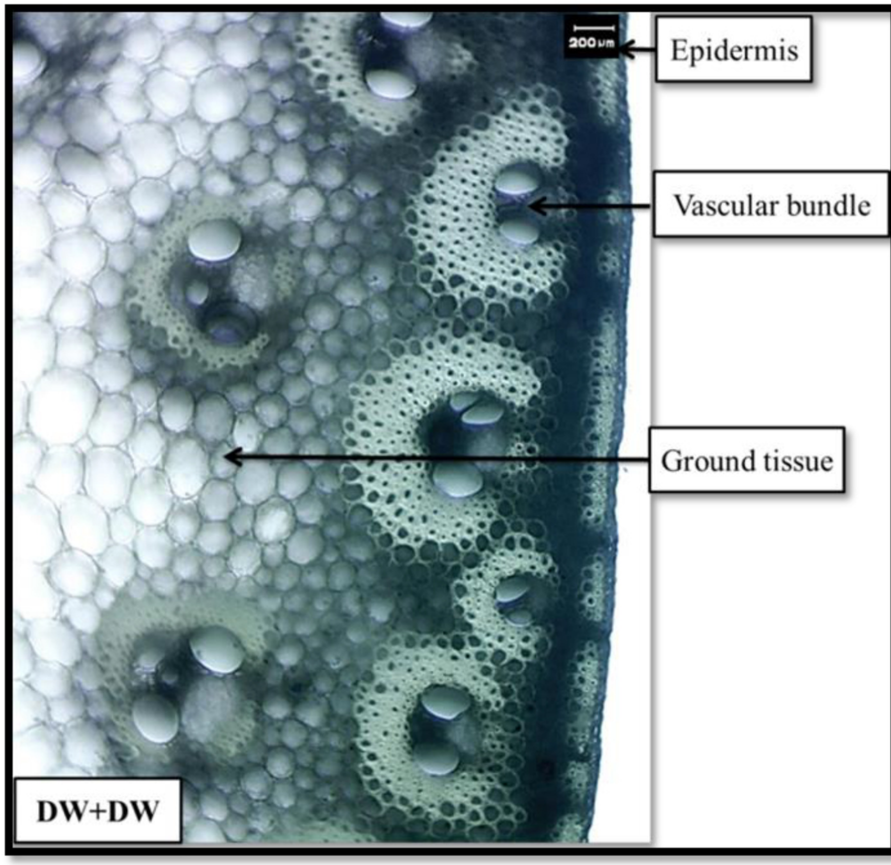

Fig. 1. Cross section of stem in Zea mays, DW: distilled water treatment.

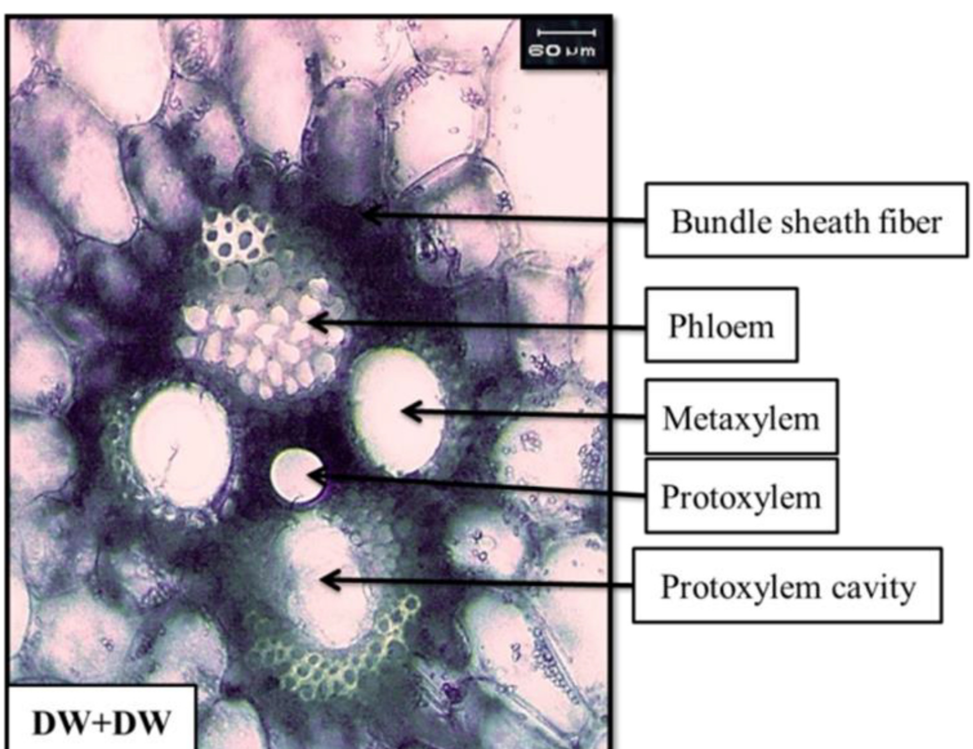

Fig. 2. Vascular bundle of stem in Zea mays, DW: distilled water treatment.

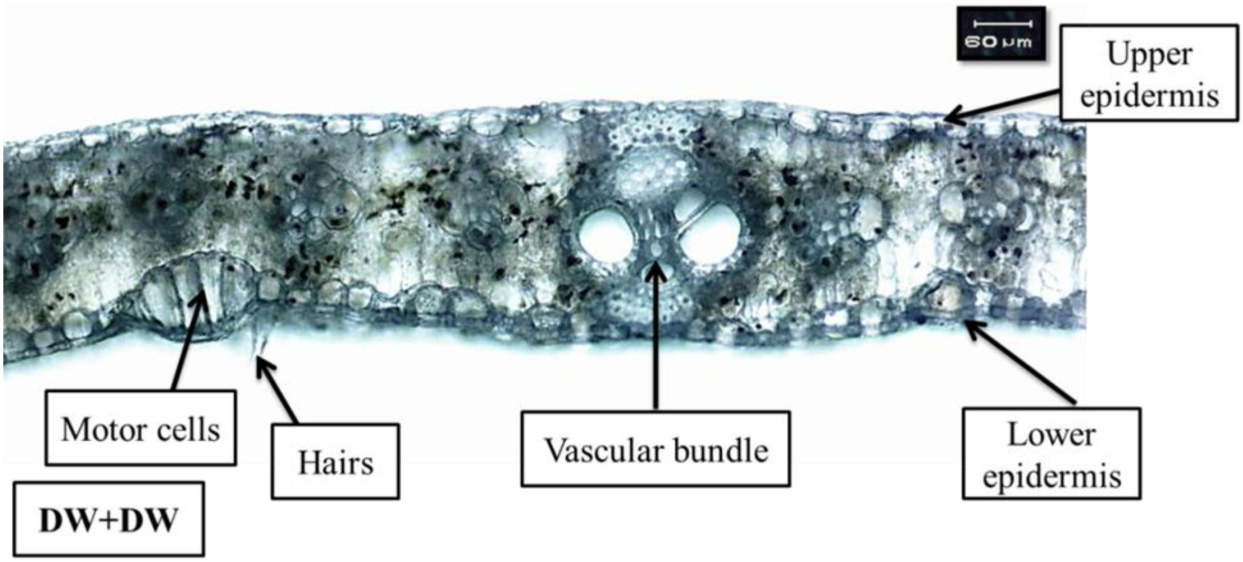

Fig. 3. Longitudinal section of leaf in Zea mays, DW: distilled water treatment.

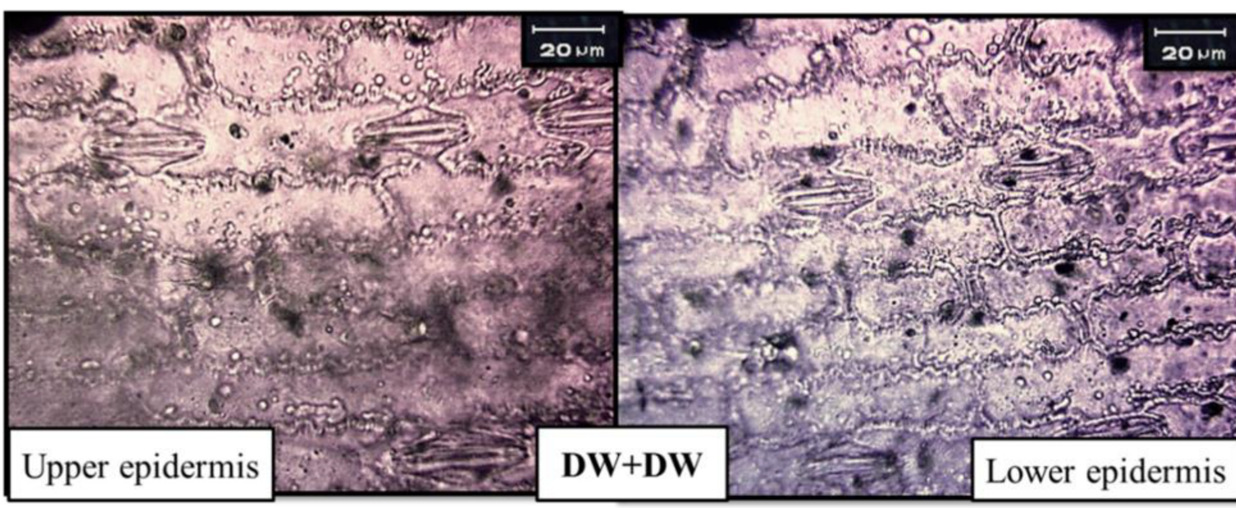

Fig. 4. Epidermis of leaf in Zea mays, DW: distilled water treatment.

University of Baghdad, and in 2020 at a private field in Babylon governorate. The relocation was due to COVID-19 restrictions, which prevented repetition at the original site. A randomized complete block design was applied in a split-plot arrangement with three replications.[37] The main plots involved foliar nutrition treatments with ascorbic acid (AA) and citric acid (CA) at 100 mg L^{-1} each, as well as humic acid (HA) at 1 ml L^{-1}, and a control with distilled water. Two application stages for the acids occurred when 6 and 10 true leaves appeared. Sub-plots included seed soaking with the same treatments, where seeds were soaked for 18 hours.[1,15]

Maize seeds of the Baghdad-3 cultivar were sourced from the Agricultural Research Directorate, Ministry of Agriculture. Soil and crop management followed Ministry of Agriculture recommendations.[38] Plant materials were collected from fresh stems and leaves and preserved in 70% ethanol. Cross-sections of stem and leaf samples were prepared[39] with modifications according to Al-Hadeethi.[40]

Stem and leaf samples were preserved in 70% ethanol, then cut into 4–5 cm pieces. Leaf epidermis was prepared,[41] and sections were washed with distilled water and treated with a 5% sodium hypochlorite solution for 5 minutes to remove chlorophyll pigment. Samples were mounted on glass slides and examined under an Olympus KRÜSS light microscope, with images captured using an AmScope camera. Characteristics studied

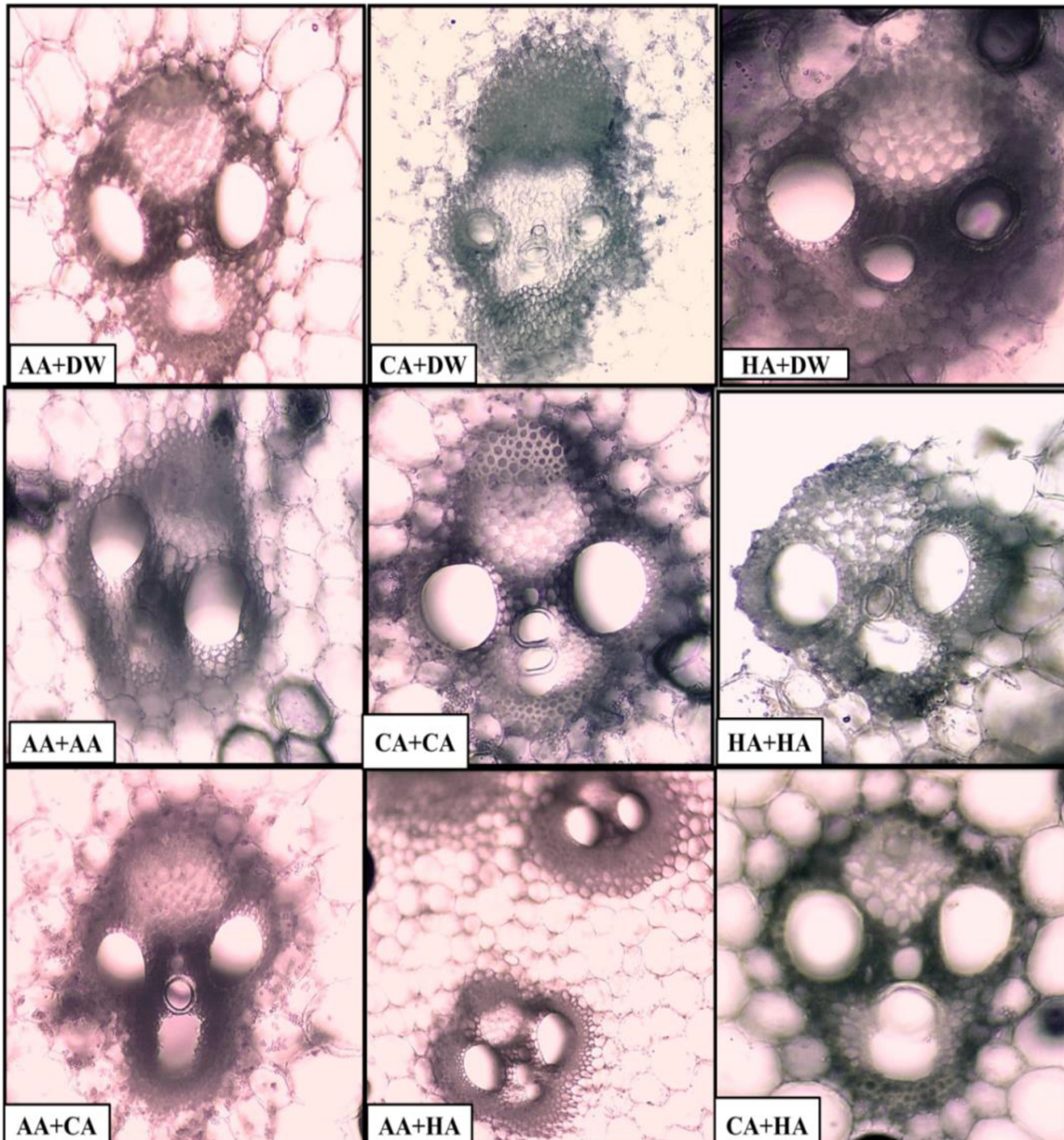

Fig. 5. Variations in shapes and sizes of the vascular bundles in the stem in *Zea mays*, DW: distilled water treatment, AA: Ascorbic acid treatment, HA: Humic acid treatment, CA: Citric acid treatment.

included the diameter of vascular bundles in the stem and the shapes of stomata in the leaf epidermis.

Data were analyzed statistically using the GenStat program. Means were compared using the least significant difference test at the probability level of 0.05 (LSD 5%).[42]

Results and discussion

The stem cross-section of the cultivar of *Zea mays* for the control treatment is illustrated in Fig. 1. The cross-section of the stem consists of one layer of epidermis with ovoid cells followed by 1–2 layers of hypodermal fibers in the ground tissue that consists

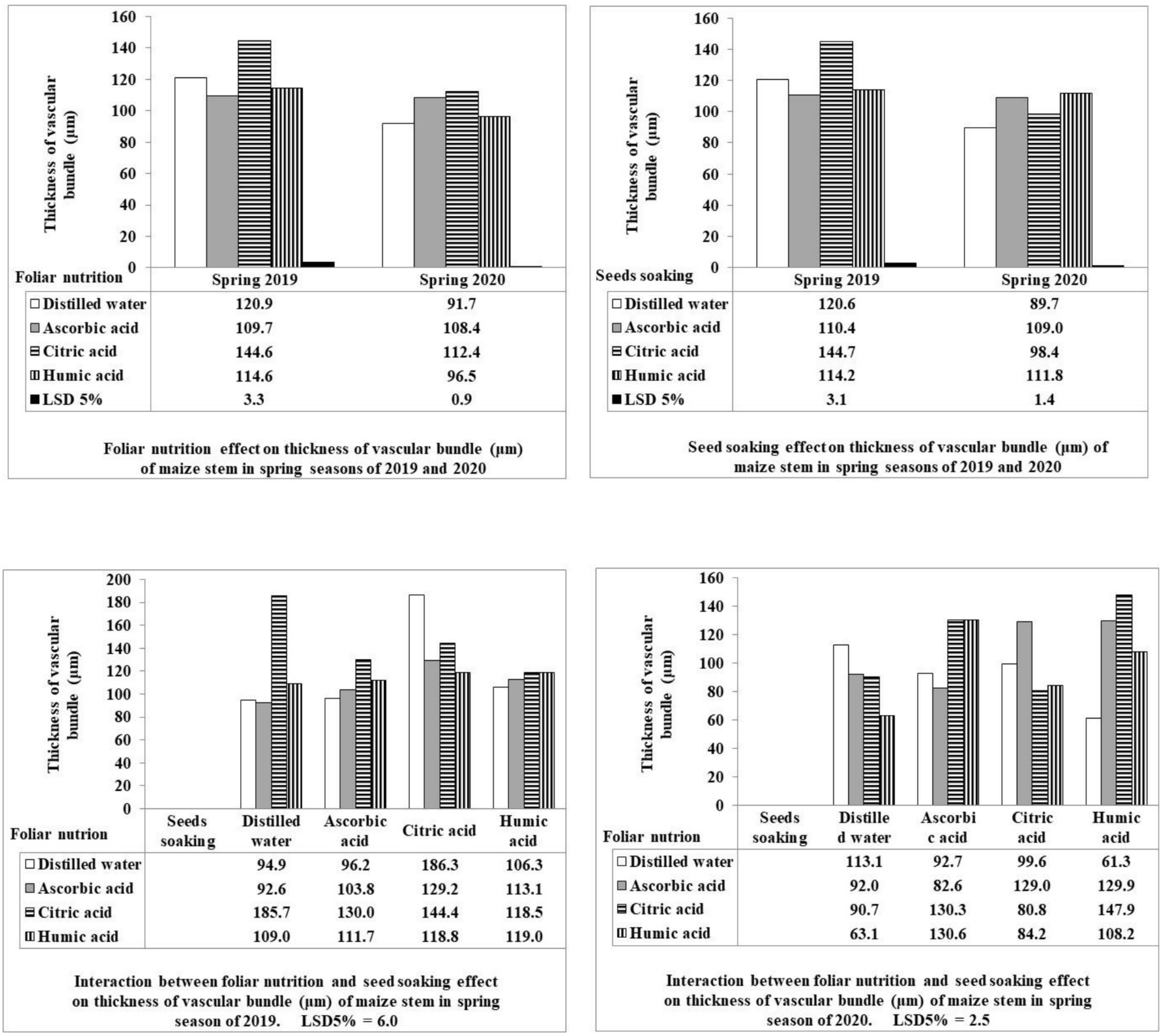

Fig. 6. Variations of diameter of vascular bundles in the stem of *Zea mays*.

of ordinary parenchyma cells and many vascular bundles scattered randomly. The bundle consists of the phloem and the xylem surrounded by the bundle sheath fibers, the xylem consists of metaxylem and protoxylem. The lacuna is located under the protoxylem and also known as the Schizo-Lysigenous Intercellular Space. This space expands with the extinction of adjacent cells[43,44] Fig. 2.

The leaf section consists of upper and lower epidermis surrounded the mesophyll with the vascular bundles[45] Fig. 3. Leaf-peeling appeared in the stomata, which usually consists of two guard cells which look thin from the center and dumbbell shaped from the ends and two subsidiary cells and short cells which is represented by cork and silica cells[46,47] Fig. 4.

The epidermis layer consists of compact ordinary epidermal cells covered with cuticle and there are stomata. It also contains large, and thin-walled cells at which the leaf folds, known as motor cells or Buliform cells. The mesophyll consists of chloranchyme cells that carry out the process of photosynthesis, in most plants with a single cotyledon, this tissue is not distinguished into a palisade tissue and another spongy tissue.[48]

Vascular bundles are found in a parallel system, usually as a result of parallel venation in monocots, in the leaf section the large bundles appear in the center and small bundles arranged laterally. The bundles are closed, with xylem located toward the upper epidermis, forming letters Y, V, and phloem

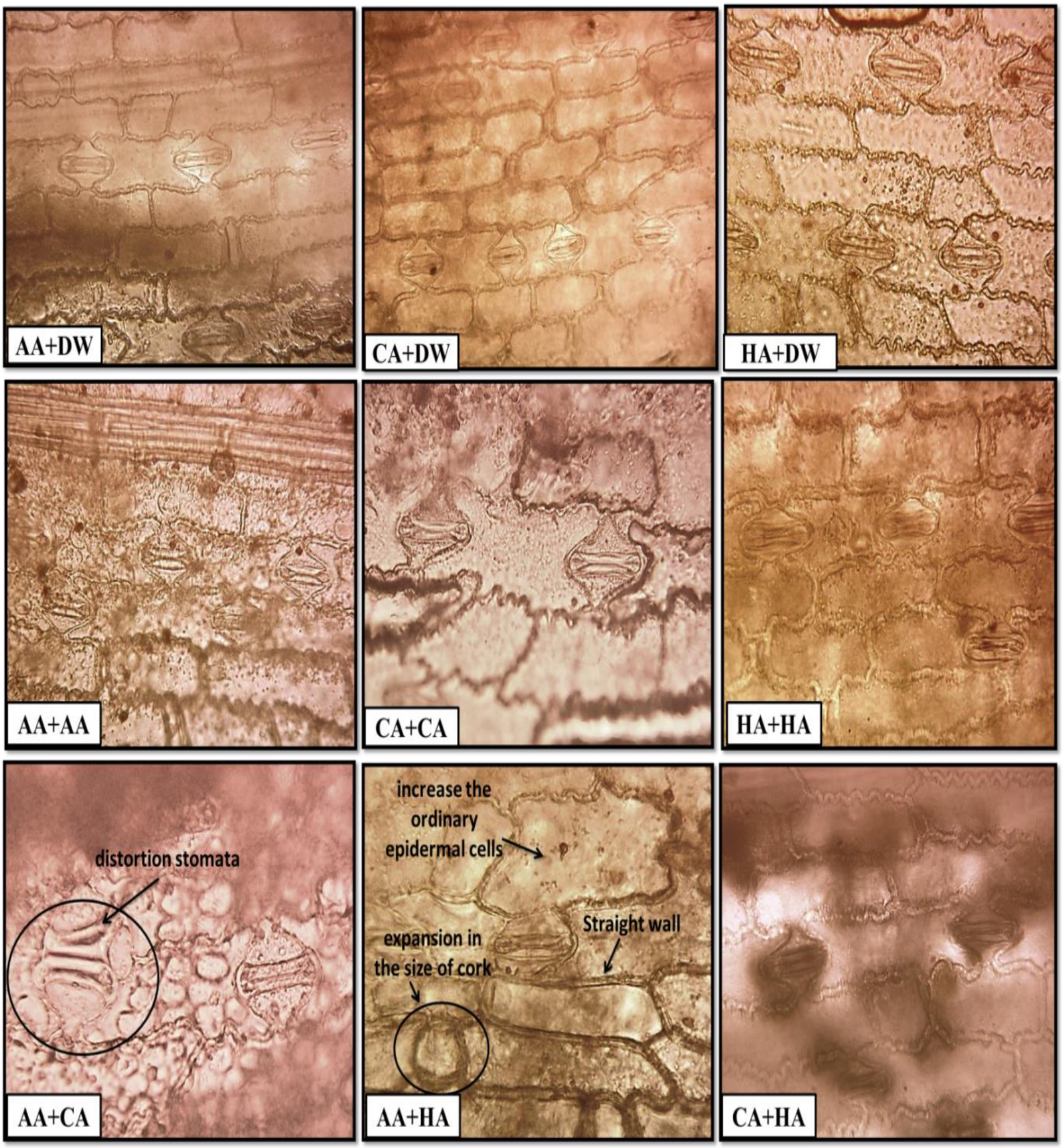

Fig. 7. Variations of upper epidermis in the leaf of *Zea mays*. DW: distilled water treatment, AA: Ascorbic acid treatment, HA: Humic acid treatment, CA: Citric acid treatment.

located toward the lower epidermis, sclerenchyma cells are observed around the bundle, especially around the main bundles, which act as a protective tissue for the bundles and support tissue for the leaf. The lower epidermis similar to the upper epidermis.[49,50]

The results of the experiment for the season 2019 showed variations in the diameters of the vascular bundles of the stem among the different treatments compared to the control treatment. The citric acid treatment outperformed the rest of the treatments, reaching 186.3 μm, and in 2020 also the citric acid treatment outperformed the rest of the treatments reaching 112.4 μm. Also, when soaking the seeds in acids, the citric acid outperformed, reaching 144.7 μm in 2019, and in 2020, and the humic acid outperformed reaching 111.8 μm. This may be due to the difference in the location of cultivation

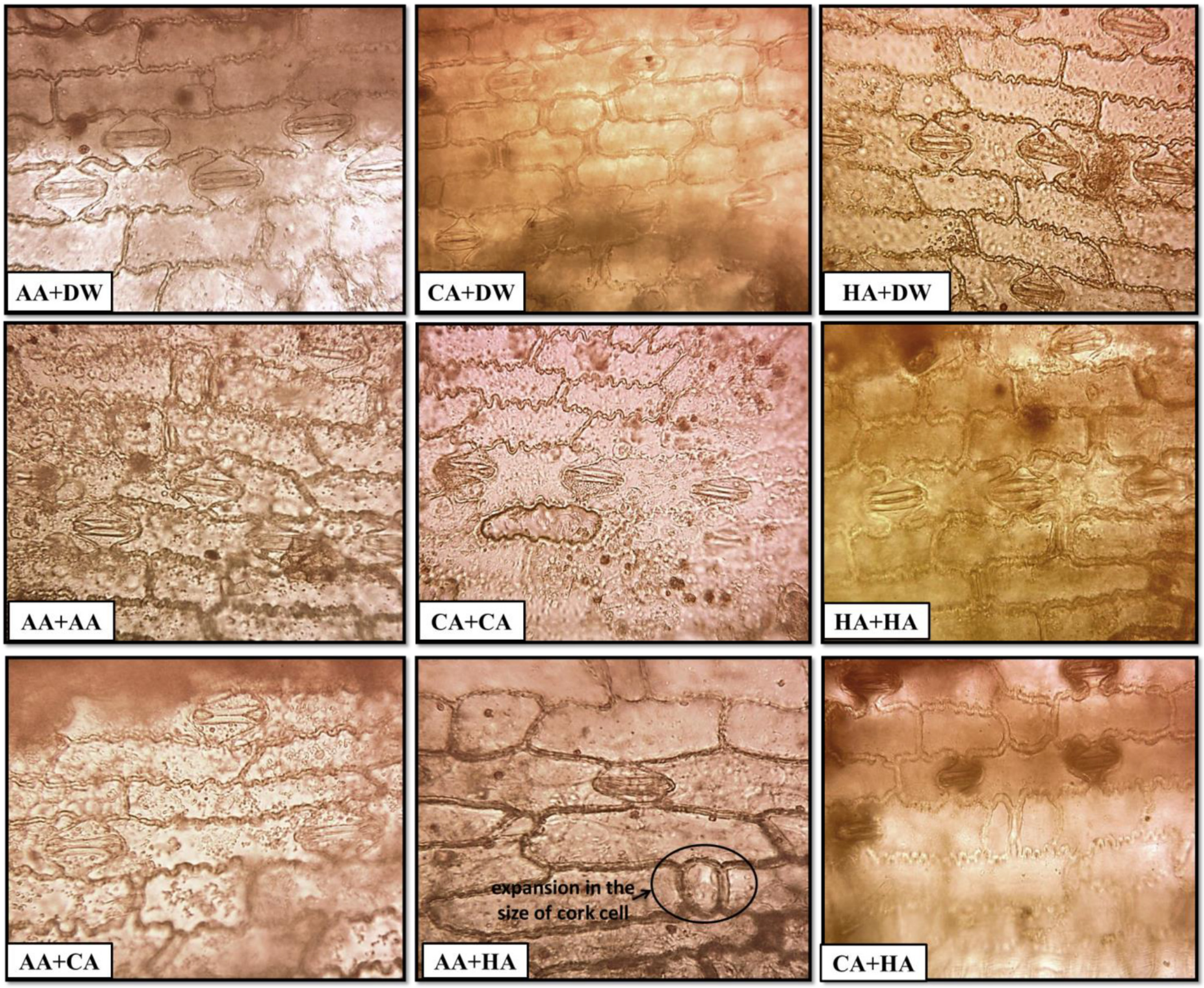

Fig. 8. The variations of lower epidermis in the leaf of *Zea mays*. DW: distilled water treatment, AA: Ascorbic acid treatment, HA: Humic acid treatment, CA: Citric acid treatment.

and the quality of the seeds, as mentioned in the methodology Figs. 5 and 6.

As for the interaction between the factors, in the spring season 2019, the treatment of spraying with citric acid and soaking with water outperformed the rest of the (treatments 185.7 μm), and for the spring season 2020, the treatment of spraying with citric acid and soaking with humic acid exceeded, as it reached 147.9 μm. This may be due to the difference in the location of cultivation and the quality of the seeds Figs. 5 and 6.

The results indicate that adding citric acid to water significantly increased the diameter of vascular bundles in the plant's stem compared to the control. However, it's important to note that the optimal pH range for plant growth is between 5.5 and 7.5, demonstrating the adaptability of some plants to varying environmental conditions. Consequently, excessive citric acid in a plant's water or soil can create an environment unsuitable for the plant as shown in Figs. 5 and 6.

The beneficial effects of citric acid, even in low doses, have been documented. A study conducted by the U.S. Department of Agriculture aimed at creating an insecticide derived from various plants containing citric acid revealed that direct application of 16% citric acid to plants had minimal impact, with occasional instances of discoloration. Despite this, the plants remained healthy, suggesting that citric acid could serve as a repellent.

In plants, citric acid plays a crucial role in the Krebs Cycle, where it is converted into citrate to generate phosphates, serving as a source of energy for cellular processes. However, the Krebs Cycle relies on the precise regulation of citric acid levels. Excess citric acid in the plant's water supply may disrupt this cycle or lead to an overabundance of phosphates, potentially impacting cellular function and plant health.

The results indicated that the other treatments increased the diameter of the bundle compared to the control. Citric acid was the first leader in the increase among all the treatments.

The treatments did not actually affect the epidermis of the plant leaf, except for minor effects on the upper epidermis when treated with ascorbic acid and citric acid, which led to a distortion in the shapes of some stomata compared to the control treatment. Ascorbic acid and humic acid treatment led to an increase in the size of ordinary epidermal cells and their walls became straight, while in the control treatment the walls were usually wavy, in addition to an increase and expansion in the size of cork and silica cells Fig. 7. The results of the study agree with the study on sorghum,[51] no changes were observed in the stem epidermis, except a few changes in the size of the guard cell and diffused the prismatic crystals in the cells and around the guard cells. Also, the results agree with an anatomical study which showed that cultivars had a significant effect on stem anatomical traits in the diameter of vascular bundles and xylem diameter of lupine crop.[52]

The effects of the solutions on the lower epidermis only appear in the treatment by ascorbic acid and humic acid; the treatment led to an increase in the size of cork and silica cells Fig. 8.

Conclusion

In summary, the current study demonstrates that the application of citric acid as a spray treatment showed notable effectiveness during both the 2019 and 2020 maize planting seasons in the Mid-North Iraqi region in spring. The treatments examined resulted in a significant enlargement of vascular bundles within the plant stem. This enlargement holds considerable potential for enhancing plant resilience against a broad spectrum of abiotic and biotic stresses.

The observed enhancement in vascular bundle size can be attributed to citric acid's pivotal roles in plasma membrane formation, lipid and protein breakdown, stimulation of photosynthesis, and its crucial involvement in the Krebs cycle for the generation of energy compounds, particularly ATP. Additionally, this treatment stimulated increased synthesis and accumulation of carbohydrates throughout the plant, contributing to overall plant vigor and resilience.

Moreover, the application of ascorbic acid and humic acid primarily impacted the lower epidermis, leading to the enlargement of cork and silica cells. This enlargement enhances leaf strength and rigidity, enabling the plant to withstand environmental and biological stresses more effectively. The composition of these cells, rich in cork and silica materials, serves as a protective shield, reinforcing the plant's structural integrity and resilience to various stressors.

Based on these findings, it is recommended to utilize soak and foliar application methods for maize seeds, particularly under stress conditions. This approach could effectively enhance plant health and resilience, ultimately leading to improved maize yields in the Mid-North Iraqi region during the spring planting season.

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Authors' declaration

- Conflicts of Interest: None.
- We hereby confirm that all the Figures in the manuscript are ours. Furthermore, any Figures and images that are not ours have been included with the necessary permission for re-publication, which is attached to the manuscript.
- No animal studies are present in the manuscript.
- No human studies are present in the manuscript.
- Ethical Clearance: The project was approved by the local ethical committee at the University of Baghdad.

Authors' contribution statement

The authors confirm contribution to the paper as follows: study conception and design: K. J. J., H. J. H.; data collection: K. J. J., H. J. H.; analysis and interpretation of results: H. J. H., H. M. A., Al. M., S. W.; draft manuscript preparation: K. J. J., H. J. H., H. M. A., Al. M., S. W., S. M. F., B. M., R. H. Z. reviewed the results and approved the final version of the manuscript.

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تأثير أحماض الاسكوربك والستريك والهيومك في تشريح ساق وأوراق الذرة الصفراء

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الخلاصة

درست التغيرات التشريحية في الأنسجة الداخلية للساق والورقة عند معاملة البذور والنبات بالأحماض لتعزيز النمو والتطور في الذرة الصفراء خلال فصلي الربيع لعامي 2019 و2020. تم تطبيق تصميم القطاعات الكاملة المعشاة بترتيب الالواح المنشقة بثلاثة مكررات. تضمنت الالواح الرئيسة معاملات التغذية الورقية (حامض الأسكوربيك (AA) وحامض الستريك (CA) بتركيز 100 ملغم لتر$^{-1}$، وكذلك حامض الهيوميك (HA) بتركيز 1 مل لتر $^{-1}$. تم استخدام الماء المقطر كمعاملة مقارنة. وتضمنت الالواح الثانوية معاملات نقع البذور بنفس المعاملات. كشفت نتائج تحليل المقطع العرضي للساق وجود اختلافات في حجم الحزم الوعائية بين المعاملات المختلفة بالمقارنة مع معاملة المقارنة، وأن التغذية الورقية باستخدام CA أظهرت نتائج متفوقة في كلا الموسمين. كما تفوقت البذور المنقوعة في CA و HA على المعاملات الاخرى. كان هناك تداخل معنوي بين التغذية الورقية بالـ CA ونقع البذور بالماء المقطر في عام 2019، أما في عام 2020 فتفوقت معاملة التداخل بين التغذية الورقية بالـ CA ونقع البذور بالـ HA على بقية المعاملات. ومن المهم أن نلاحظ أن المعاملات كان لها تأثير محدود على بشرة أوراق النبات، مع ملاحظة آثار طفيفة فقط. على سبيل المثال، تسببت المعاملات بالـ AA و CA في حدوث بعض التشوه في أشكال بعض الثغور في البشرة العلوية عند مقارنتها بمعاملة المقارنة. أدت المعاملات باستخدام الـ AA و HA إلى زيادة في حجم خلايا البشرة العادية، مع جدران خلايا أكثر استقامة، بينما في معاملة المقارنة، كانت جدران الخلايا متموجة فضلاً عن ذلك كان هناك زيادة وتوسع في حجم خلايا الفلين والسيليكا في النباتات المعاملة. تسهم هذه الدراسة في فهم التغيرات التشريحية التي تحدث في الأوراق والسيقان خلال فترات الإجهاد في موسم نمو الذرة الصفراء، وتسلط الضوء على التأثيرات المحتملة للمعاملات الحامضية على وظائف النبات.

الكلمات المفتاحية: التغذية الورقية، منظمات النمو، تشريح النبات، نقع البذور، الذرة الصفراء.