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ORIGINAL RESEARCH

Effect of Growth Regulators and Nano Materials to Cope with Salinity on Anatomical Characteristics of Pea Plant

Hala G. El-Araby[1*], Sahar F.M. El-Hefnawy[2], Mohammed A. Nassar[3], Ahmed Mohamed El--Taher[4]

[1]Biological and Environmental Science Department, Faculty of Home Economics, Al-Azhar University, Egypt.
[2]Biological and Environmental Science Department, Faculty of Home Economics, Al-Azhar University, Egypt.
[3]Agriculture Botany Department, Faculty of Agriculture, Al-Azhar University, Egypt. 4 Agriculture Botany Department, Faculty of Agriculture, Tanta University, Egypt
[4]Deptarment of Agricultural Botany, Faculty of Agriculture Al-Azhar University Cairo, Egypt

*Corresponding author:
h3arby@yahoo.com

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ABSTRACT: Abiotic stresses, particularly salinity, severely hinder crop productivity by disrupting physiological processes and reducing yields. Pea *(Pisum sativum* L.), a vital crop, is highly sensitive to salinity, making it crucial to explore strategies that enhance its tolerance to such stresses. This study investigates the effects of Ascorbic Acid (AsA), 5-Aminolevulinic Acid (ALA), and Nano-Selenium (N-Se) on the anatomical characteristics of pea plants subjected to severe salinity stress (120 mM NaCl). Transverse sections of the fourth internode and leaf blade were analyzed, focusing on stem and leaf structure. The results showed that foliar application of AsA (100 ppm) significantly improved anatomical traits, such as stem diameter, cortex thickness, and vascular bundle dimensions, compared to the control and other treatments. ALA (50 ppm) also improved anatomical features, albeit to a lesser extent, while N-Se (20 ppm) exhibited the lowest enhancement. Leaf tissue analysis revealed that AsA improved leaflet structure, increasing epidermis thickness and vascular bundle dimensions under salinity stress. The application of AsA, ALA, and N-Se mitigated the negative effects of salinity, likely due to their roles in enhancing stress tolerance, reducing oxidative damage, and improving nutrient uptake. This study highlights the potential of these bio-stimulants to improve the anatomical resilience of pea plants under salinity stress, contributing to better crop performance in saline environments.

KEYWORDS: Abiotic stresses, nano-materials, anatomical, anatomical characters; plant growth regulators; field pea

1. Introduction

Plants encounter two major types of environmental stresses: abiotic stresses and biotic stresses. Abiotic stresses, including salinity, drought, flooding, radiation, heavy metals, and extreme temperatures, significantly reduce the productivity of major crops worldwide (Nehra et al., 2024; Oyebamiji et al., 2024). On the other hand, biotic stresses result from attacks by pathogens such as oomycetes, bacteria, fungi, nematodes, and herbivores, which further exacerbate the decline in crop yields (Robles-Zazueta et al., 2024; Ali et al., 2024). Among abiotic stresses, salinity presents a particularly severe challenge, causing

substantial inhibition of mitotic activity and stunting plant growth (Khan et al., 2024; Shah et al., 2021; El-Mashad and Kamel, 2001). Plants subjected to salt stress can survive and grow through adaptive processes that mitigate some of the stress-induced metabolic disturbances. Prolonged exposure to salinity induces ionic stress, leading to the premature senescence of leaves, thereby reducing photosynthetic efficiency (Zhou et al., 2024; Carillo et al., 2011). Ion toxicity under salt stress disrupts enzymatic activities vital to biological processes, while adaptive changes at transcriptional and translational levels help maintain photosynthetic responses under suboptimal conditions (Abideen et al., 2022; Tuteja, 2007).

Pea (*Pisum sativum* L.), a member of the Fabaceae family, holds significant agronomic and nutritional value due to its high protein content and extensive use as both human food and animal fodder (Windsor et al., 2024; Murtaza et al., 2007). However, pea production is severely impacted by various stresses, particularly salinity, which adversely affects growth and yield (El-Beltagi et al., 2024). Ascorbic acid (AsA) plays a crucial role in plant stress tolerance by functioning as an enzyme co-factor, antioxidant, and electron donor/acceptor in photosynthetic and plasma membrane-associated processes, thus helping to maintain plant growth and tissue turgor under stress conditions (Mishra et al., 2023; Hussein et al., 2019; Akram et al., 2017).

Additionally, 5-Aminolevulinic acid (ALA), a non-protein amino acid, has been shown to enhance plant tolerance to various stresses (Yang et al., 2017; Zhang, et al., 2024; Ye et al., 2024), although the detailed physiological and biochemical mechanisms underlying this protection remain incompletely understood. The application of ALA has been reported to restore photosynthetic efficiency and promote plant resilience under adverse conditions (Rhaman et al., 2021; Wang et al., 2022). Recently, nano-fertilizers, such as Nano-Selenium (N-Se), have emerged as novel tools for improving plant growth and stress tolerance (Malik et al., 2024; Nedjimi et al., 2024; Samynathan et al., 2023). While the biological effects of N-Se are still being explored, initial studies suggest its potential for mitigating the negative impacts of abiotic stresses (Elsheery et al., 2020a, b; Cushen et al., 2012).

Given the increasing salinity problems in agricultural systems and the growing interest in innovative growth regulators and nanomaterials, further research is needed to explore the potential of these compounds in enhancing plant resilience. This study focuses on the effects of AsA, ALA, and N-Se on the anatomical characteristics of pea plants under salinity stress, aiming to provide insights into their roles in modulating stress responses and promoting growth under challenging environmental conditions.

2. Materials and methods

2.1. Plant material

The sand culture technique with sub-irrigation was conducted during the winter seasons of 2019 and 2020 in the greenhouse of the Agricultural Botany Department, Faculty of Agriculture, Tanta University, Egypt, using the pea cultivar *Pisum sativum* L. (Master B).

2.2. Sand culture technique of equipment

The sand culture technique used in this study was based on the method described by Hewitt, (1966). White fine sand, with particles approximately 1.0 mm in diameter, was sieved and then purified by soaking in 5% HCl for 3 days. After this, the sand was thoroughly washed for an additional 3 days with a continuous stream of tap water until the total soluble salts in the sand were reduced to about 150 ppm (0.23 dS/m).

Pea seeds were sterilized by immersing them successively for two minutes in a 0.001% mercuric chloride solution followed by 70% ethanol. The seeds were then washed thoroughly with sterile tap water. The sterilized seeds were germinated in dark polyethylene bags (25 cm in diameter) filled with 5.44 kg of washed sand. The sand in each bag was leached with a sufficient amount of the desired saline solution, supplemented with a nutrient solution, to remove hydrogen ions adsorbed onto the fine sand particles (Hewitt, 1966).

Table 1. Volumes of Macro Nutrients Added to Prepare One Liter of Nutrient Solution

Macro nutrients	Volume (ml) added to form one liter of nutrient solution
$Ca(NO_3)^2.4H_2O$ molar solution	5
KNO_3 molar solution	5
$MgSO_4$. $7H_2O$ molar solution	2
KH_2PO_4 molar solution	1
Distilled water	Before 1000 mL

A half-strength Hoagland solution (Hoagland and Arnon, 1950) was used as the nutrient medium. The final nutrient solution was prepared as follows: volumes of macro nutrients were added according to Table 1, and trace elements were included based on the concentrations provided in Table 2. Iron was added to the nutrient solution in the form of iron citrate to achieve a final concentration of 0.5% (w/v).

Table 2. Concentrations of Micro Nutrients in the Nutrient Solution

Micro nutrients	Concentration (g/L)
$MnCl2.4H_2O$	1.81
$ZnSO.7H_2O$	0.22
$CuSO4.5H_2O$	0.08
$H_2MO_4.H_2O$	0.02
H_3BO_3	2.86

2.3. Salinity levels:

Three salinity levels were tested: 0.0 mM (control), 60 mM, and 120 mM. The salt mixture, consisting of $MgSO_4$, $CaSO_4$, NaCl, $MgCl_2$, and $CaCO_3$ in a ratio of 10:1:78:2:9, was prepared according to the method suggested by Strongonov (1964). The nutrient solution, adjusted to pH 5.8, was mixed with the salt solution and used for sub-irrigation throughout the experiment.

Plastic dishes, 35 cm in diameter, were used to contain 1 liter of the nutrient solution for each treatment. The treatments were replicated four times, with ten bags per replication.

2.4. Seedling treatments

Seedlings with two true leaves were subjected to stress and then sprayed until dripping with one of the following treatments: Ascorbic acid (AsA) at concentrations of 50 ppm and 100 ppm, 5-Aminolevulinic acid (ALA) at 25 ppm and 50 ppm, or Nano-Selenium (N-Se) at 10 ppm and 20 ppm. The purpose was to evaluate their effects on

alleviating salt stress. Spraying was repeated after the emergence of the fourth true leaf. Ascorbic acid (AsA) was sourced from Sigma, Tanta El-Gharbia Governorate.

2.5. Sampling and biometric observations

Plant samples were collected during the vegetative growth period, 15 days after the second foliar treatment, to conduct biochemical studies. This timing was chosen to align with the physiological stage of six-leaf development before flowering. Nine pots were kept without foliar treatments: the first group received no salinity treatment (3 replicates), the second group was treated with a 60 mM salt mixture (3 replicates), and the third group received a 120 mM salt mixture (3 replicates). Plants were harvested 15 days after the foliar application, and growth parameters were recorded. To minimize root loss, each plant was carefully extracted from the sand using a gentle water stream. The samples were then transferred immediately to a cool, dry container and taken to the laboratory for further measurement.

2.6. Anatomical characteristics

To evaluate the effects of ALA, AsA, and Nano-Se on alleviating salinity stress in pea plants, a comparative microscopic study was conducted on anatomical features. Specimens, including 1 cm sections from leaves emerging from the fourth internode and the fourth internode of the stem, were collected during the winter season of 2019 from pea plants that were 40 days old.

The samples were fixed in a solution of 5 ml glacial acetic acid, 10 ml formalin, and 85 ml 70% ethyl alcohol (FAA solution) for at least 48 hours. After fixation, the samples were washed in 50% ethyl alcohol and dehydrated through a series of butyl alcohol concentrations (70%, 80%, 90%, absolute I, and absolute II). Subsequently, the samples were infiltrated with xylene I and xylene II overnight and then embedded in paraffin wax at 56-58°C.

Transverse sections, 15 microns thick, were cut using a rotary microtome (Model 820). The paraffin sections were mounted on slides with albumin, stained with a safranin-light green combination, and mounted in Canada balsam (Nassar and El-Sahhar, 1998). The slides were examined microscopically, and photomicrographs were taken. Measurements were recorded, and the average of 10 readings from 3 slides was calculated and analyzed comparatively.

2.7. Statistical analysis

The data obtained from the experiments were analyzed using appropriate statistical methods to determine the effects of various treatments on plant growth and anatomical characteristics. Statistix 8.1 (Analytical Software) was used for the data analysis, and the figures were plotted using Microsoft Excel (2013). The significance level was set at $p < 0.05$ for all statistical tests. Results were presented as means ± standard deviations, and statistical significance was indicated by asterisks or other markers in the figures and tables.

3. Results and discussion

3.1. Anatomical characteristics

3.1.1. Main stem structure

The anatomical observations of pea plants focused on the medium portion of the fourth internode of the main stem and the leaf blade developed on the same internode, particularly under severe salinity stress (120 mM). Transverse sections of these tissues were analyzed, with measurements provided in

micrometers (µ), as detailed in Table 3 and illustrated in Figures 1 and 2.

Under high salinity conditions, foliar application of Ascorbic Acid (AsA) at 100 ppm resulted in a significant increase in stem diameter compared to the control. Specifically, the thicknesses of the epidermis and cortex, the length of the vascular bundle, and the width of the vascular bundle were recorded as 23.26 ± 0.59 µm, 199.60 ± 0.54 µm, 313.20 ± 0.02 µm, and 218.38 ± 0.19 µm, respectively. In comparison, application of 50 ppm of 5-Aminolevulinic Acid (ALA) resulted in values of 21.95 ± 0.55 µm, 192.74 ± 0.56 µm, 298.26 ± 0.02 µm, and 210.84 ± 0.14 µm. Nano-Selenium (N-Se) at 20 ppm showed the lowest values, with measurements of 17.36 ± 0.61 µm for epidermis and cortex thickness, 145.26 ± 0.63 µm for the length of the vascular bundle, 276.28 ± 0.05 µm for the width of the vascular bundle, and 190.37 ± 0.13 µm.

Table 3. Measurements in micrometers of certain anatomical characters in transverse section through the medium portion of the forth internode on the main stem of pea plant (40 day old) treated with AsA, ALA and Nano-Se foliar application under saline condition (120mM).

Salinity level (mM)	Treats.	Conc. Ppm	Epiderm diameter (µm)	Cortex diameter (µm)	Length of vascular bundle (µm)	Width of vascular bundle (µm)
Control (0)	0	0	20.07±1.13	188.15±041	325.38±3.98	243.43±5.53
	AsA	50	24.43±1.30	190.35±0.86	336.15±3.44	249.58±5.41
		100	28.83±0.48	196.78±0.21	350.31±1.50	298.33±4.16
	ALA	25	23.95±1.28	190.11±0.52	330.81±1.97	247.76±4.18
		50	26.44±0.55	194.52±0.67	343.20±1.52	269.75±4.14
	Nano selenium	10	24.59±0.60	193.02±0.57	337.00±1.64	258.95±4.71
		20	21.76±0.56	189.19±0.65	327.07±1.70	245.83±4.64
Mean			24.30	191.73	335.70	259.09
120Mm	0	0	16.60±0.70	134.06±0.61	271.59±0.09	184.53±0.58
	AsA	50	19.51±0.66	147.96±1.75	279.97±0.05	195.78±0.64
		100	23.26±0.59	199.60±0.54	313.20±0.02	218.38±0.19
	ALA	25	18.25±0.64	147.02±1.16	278.26±0.06	192.75±0.27
		50	21.95±0.55	192.74±0.56	298.26±0.02	210.84±0.14
	Nano selenium	10	20.49±0.62	168.03±0.55	293.44±0.04	202.95±0.16
		20	17.36±0.61	145.26±0.63	276.28±0.05	190.37±0.13
Mean			19.63	162.10	287.29	199.37
L.S.D. (0.05)						
Salinity			0.685*	1.563*	2.236*	3.257*
Treat			1.282*	2.925*	4.184*	6.094*
Salinity xTreat			1.813*	4.136*	5.917*	8.618*

Note: Treats–treatments, Conc–concentrations, LSD–least significant difference, AsA–Ascorbic Acid, ALA – 5-Aminolevulinic Acid (ALA), * indicated significant difference at $p < 0.05$, ± indicated standard error (n = 3).

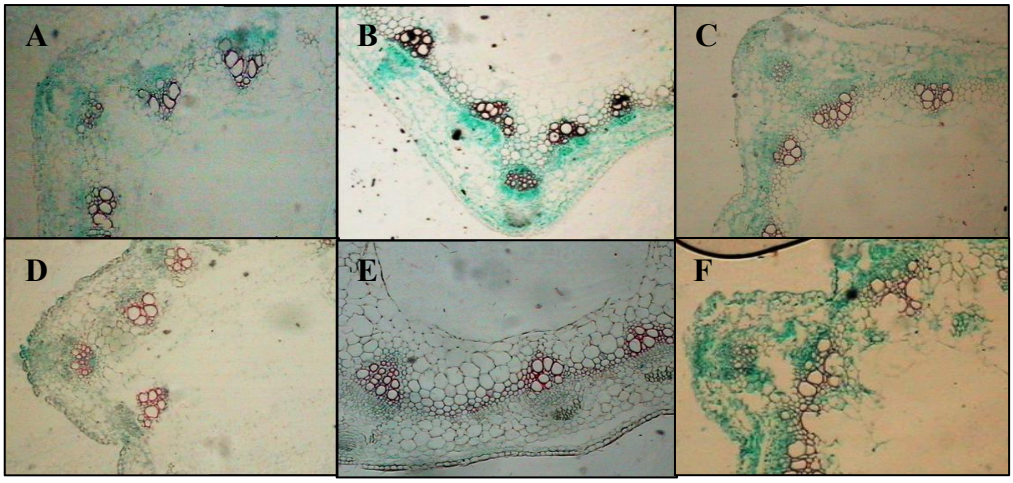

Figure 1. Transverse section through medium portion of the fourth internode of the main stem of pea plant (40 day old) grown under salinity. A-Control (plant grown under non-salinized condition), B-Plants grown under 120mM of salinity level, C-Plants treated with ALA 25ppm under non-salinized condition., D-Plants treated with ALA 25ppm under 120mM of salinity level, E-Plants treated with ALA 50ppm under non-salinized condition, F-Plants treated with ALA 50ppm under 120mM of salinity level.

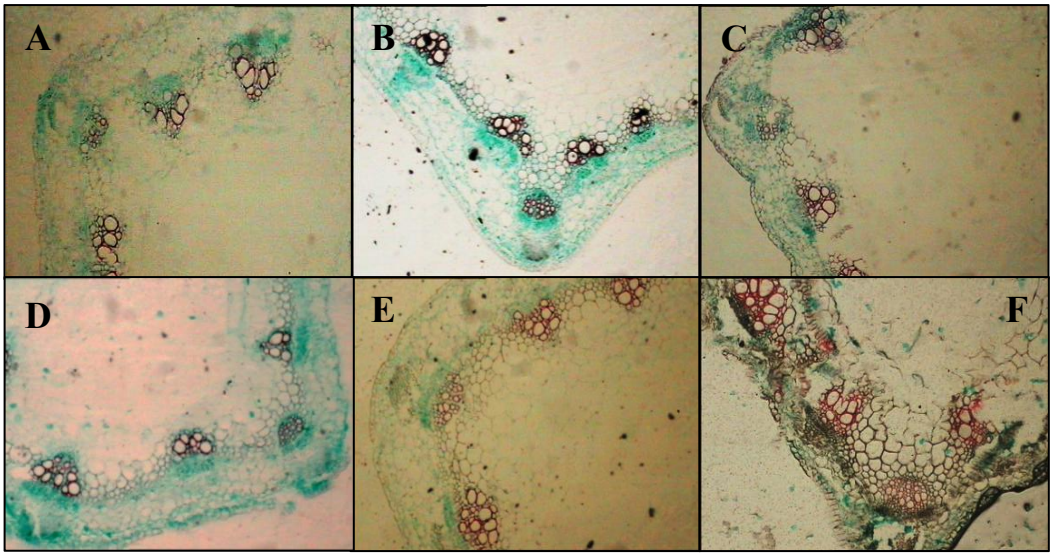

Figure 2. Transverse section through medium portion of the fourth internode of the main stem of pea plant (40 day old) grown under salinity.A-Control (plant grown under non-salinized condition), B-Plants grown under 120mM of salinity level, C-Plants treated with ALA 25ppm under non-salinized condition., D-Plants treated with ALA 25ppm under 120mM of salinity level, E-Plants treated with ALA 50ppm under non-salinized condition, F-Plants treated with ALA 50ppm under 120mM of salinity level.

The improvement in anatomical characteristics observed with AsA, ALA, and N-Se treatments indicates their effectiveness in mitigating the detrimental effects of salinity stress. Ascorbic Acid, in particular, appeared to mitigate the adverse impacts of salinity on stem anatomy (Hamid et al., 2024), enhancing parameters such as stem diameter, cortex thickness, vascular tissue thickness, and the number of xylem vessels per bundle.

These findings align with those reported by El-Kobisy et al. (2005) and Nassar et al. (2019), who observed similar beneficial effects of AsA on the anatomical structure of pea and mung bean plants, respectively. They noted that AsA treatment promoted significant increases in tissue thickness, consistent with the current study's results.

The observed decrease in anatomical characters under stress can be attributed to the harmful effects of salinity on cell division, expansion, and nutrient uptake (Loudari et al., 2022). Salinity stress is known to disrupt physiological processes, leading to reduced cell growth and altered tissue structure (Hameed et al., 2021; Makbul et al., 2011). This disruption impairs the plant's ability to maintain normal anatomical features, contributing to reduced stem diameter and other structural anomalies. The beneficial effects of AsA, ALA, and N-Se on stem diameter, cortex thickness, and vascular tissues are likely due to their roles in enhancing stress tolerance and mitigating damage caused by salinity. AsA, for example, is a known antioxidant that helps reduce oxidative stress and protect cellular components under adverse conditions (Khan et al., 2024; Hussein et al., 2019). Similarly, ALA has been reported to enhance plant stress responses by influencing metabolic pathways and improving stress tolerance (Wu et al., 2019; Anwar et al., 2018; Xiong et al., 2018). N-Se, as a nano-fertilizer, may contribute to stress alleviation through improved nutrient availability and reduced ion toxicity (Sharma et al., 2021; Elsheery et al., 2020).

Overall, the integration of these bio-stimulants demonstrates a promising approach to improving the anatomical resilience of pea plants under salinity stress, highlighting their potential utility in enhancing crop performance in challenging environmental conditions.

3.2. Leaflet structure

The study revealed that increasing concentrations of salt mixture significantly decreased leaf thickness and vessel diameter in pea plants (Table 4). This finding is consistent with Akcin et al. (2015), who observed that drought stress led to smaller epidermal cells, thicker cuticles, and increased numbers of smaller mesophyll cell layers in sugar beet leaves. Under 120 mM of salt mixture, foliar application of Ascorbic Acid (AsA) at 100 ppm resulted in improved leaflet tissue structure, with measurements of 313.20 µm for upper epidermis thickness, 14.18 µm for lower epidermis thickness, 56.81 µm for palisade tissue thickness, 68.53 µm for spongy tissue thickness, 159.12 µm for midrib zone thickness, 111.36 µm for the length of the vascular bundle, and 57.50 µm for the width of the vascular bundle. In comparison, 50 ppm of 5-Aminolevulinic Acid (ALA) yielded values of 298.26 µm for upper epidermis, 12.45 µm for lower epidermis, 55.10 µm for palisade tissue, 62.43 µm for spongy tissue, 147.86 µm for midrib zone, 105.88 µm for vascular bundle length, and 54.48 µm for vascular bundle width. Nano-Selenium (N-Se) at 20 ppm provided the lowest measurements: 276.28 µm for upper epidermis, 8.75 µm for lower epidermis, 44.97 µm for palisade tissue, 51.83 µm for spongy tissue, 136.15 µm for midrib zone, 95.66 µm for vascular bundle length, and 48.14 µm for vascular bundle width.

AsA treatment, both in the presence and absence of NaCl stress, notably enhanced leaf thickness, as depicted in Table 10 and Figure 17. Under saline stress, there was a decrease in cortex thickness but an increase in xylem thickness. Abdelaal (2015) similarly reported reductions in stem diameter, cortex thickness, and the number of xylem vessels per bundle in faba bean plants under abiotic stress. The reduction in leaf thickness and vessel diameter under increasing NaCl concentrations aligns with previous observations of stress-induced reductions in epidermal cell size and mesophyll cell layers (Akcin et al., 2015).

Table 4. Measurements in micron of certain anatomical characters in through the medium portion of the forth leaflet including the midrib 0.5 cm length of pea plant (40 day old) treated with AsA, ALA and Nano-Se foliar application under saline condition (120mM).

Salinity level mM	Treats.	Conc. (ppm)	Upper epidermis (µm)	lower epidermis (µm)	Palisade tissue (µm)	Spongy tissue (µm)	Midrib zoon (µm)	The length of vascular bundle (µm)	The width of vascular bundle (µm)
Control (0)	0	0	325.38±0.12	9.24±0.01	59.03±0.80	70.60±1.20	333.31±1.52	103.78±0.02	56.86±0.11
	AsA	50	336.15±0.40	11.98±0.08	69.73±0.82	72.93±0.10	369.84±1.22	114.80±0.05	62.06±0.05
		100	350.31±0.35	15.09±0.05	72.08±0.58	81.23±0.11	418.24±0.18	142.48±0.001	77.53±0.09
	ALA	25	330.81±0.57	11.63±0.05	68.25±0.61	71.57±0.59	358.73±0.64	110.71±0.03	59.75±0.14
		50	343.20±0.37	13.88±0.05	71.06±0.63	78.38±0.23	389.56±0.58	136.79±0.01	75.18±0.05
	Nano seleniu m	10	337.00±0.44	12.40±0.04	70.00±0.72	76.96±0.34	370.28±0.64	128.63±0.05	73.89±0.06
		20	327.07±0.43	11.10±0.04	63.34±0.76	70.92±0.39	338.29±0.79	107.17±0.05	58.06±0.03
Mean			335.70	12.19	67.64	74.66	368.32	120.62	66.19
120Mm	0	0	271.59±0.08	7.75±0.02	43.08±0.02	50.60±0.03	133.31±0.05	93.78±0.01	46.86±0.05
	AsA	50	279.97±0.06	10.61±0.02	47.19±0.08	53.90±0.02	139.00±0.13	97.48±0.14	49.04±0.22
		100	313.20±0.05	14.18±0.03	56.81±0.03	68.53±0.04	159.12±0.02	111.36±0.08	57.50±0.03
	ALA	25	278.26±0.06	9.16±0.02	46.45±0.02	52.61±0.10	137.67±0.12	96.11±0.12	48.31±0.05
		50	298.26±0.06	12.45±0.07	55.10±0.01	62.43±0.05	147.86±0.03	105.88±0.06	54.48±0.06
	Nano seleniu m	10	293.44±0.06	10.98±0.05	53.47±0.07	57.72±0.09	143.91±0.14	101.19±0.06	52.86±0.07
		20	276.28±0.11	8.75±0.12	44.97±0.08	51.83±0.09	136.15±0.14	95.66±0.14	48.14±0.16
Mean			287.29	10.55	49.58	56.80	142.43	100.21	51.03
L.S.D. (0.05)									
Salinity			2.236*	0.051*	0.877*	0.381*	1.044*	0.373*	0.398*
Treat			4.184*	0.095*	1.641*	0.712*	1.954*	0.697*	0.744*
Salinity xTreat			5.917*	0.134*	2.321*	1.007*	2.763*	0.986*	1.053*

Note: Treats–treatments, Conc–concentrations, LSD–least significant difference, AsA–Ascorbic Acid, ALA – 5-Aminolevulinic Acid (ALA), * indicated significant difference at $p < 0.05$, ± indicated standard error (n = 3).

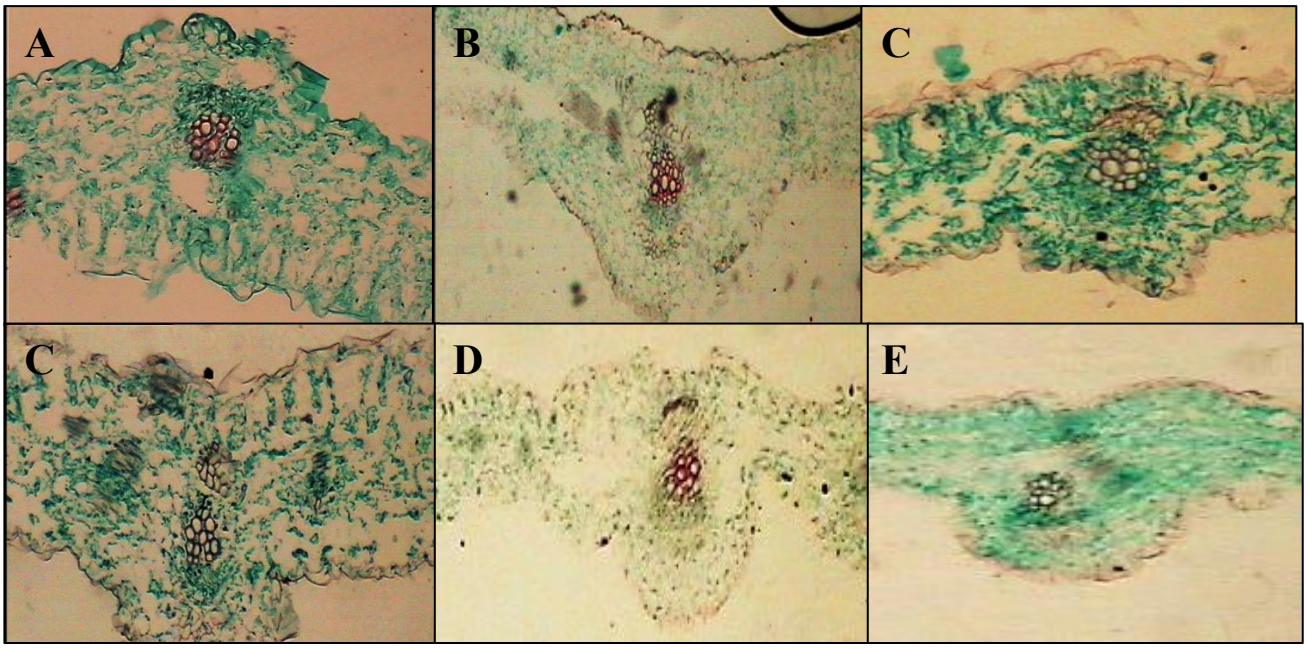

Figure 3. Transverse section through medium portion of the leaflet of the fourth leaf of pea plant (40 day old) grown under salinity. A-Control (plant grown under non-salinized condition), B-Plants grown under 120mM of salinity level, C-Plants treated with ALA 25ppm under non-salinized condition., D-Plants treated with ALA 25ppm under 120mM of salinity level, E-Plants treated with ALA 50ppm under non-salinized condition, F-Plants treated with ALA 50ppm under 120mM of salinity level.

The beneficial effects of AsA on leaf structure can be attributed to its role in cell division and cell wall expansion (Pignocchi and Foyer, 2003). AsA's interaction with salinity appears to enhance the structural integrity of leaf tissues by increasing xylem row numbers and vessel bundles (Agami, 2014). This study is among the first to explore the impact of AsA on the anatomical structure of pea leaves under salinity stress. The findings highlight that salinity stress diminishes leaflet lamina and xylem thickness, potentially due to shrinkage in palisade and spongy tissue thickness and reductions in bundle dimensions. These observations corroborate with Boghdady (2009) in mung beans and Petrov et al. (2012) in barley, who reported similar stress-induced anatomical alterations.Salinity stress is known to impair plant growth by causing osmotic inhibition of water absorption and increased energy expenditure for ionic and osmotic balance, which in turn reduces growth energy (Mekki and Orabi, 2007). This stress often leads to decreased leaf expansion rates and reduced photosynthesis, contributing to lower crop yields (Rucker et al., 1995). EL-Awadi et al. (2014) found that AsA treatment increased plant height and leaf area by enhancing water uptake efficiency and promoting cell division and enlargement.

Additionally, ALA has been reported to repair chloroplast lamellar structures and enhance water absorption by increasing proline content, which supports photosynthetic function (Ali et al., 2013; Ye et al., 2016). ALA's effects on pea seedlings exposed to salinity stress include suppression

of Na+ toxicity, improved water retention, enhanced antioxidant metabolism, and increased accumulation of osmotic regulators. These effects contribute to overall stress protection and enhanced plant growth.

Future research should focus on elucidating the molecular and metabolic pathways through which ALA mitigates stress in various plant species. Field trials are also recommended to evaluate the practical efficacy of ALA in improving plant tolerance to salinity under natural conditions. The data suggest that exogenous ALA treatment significantly improves growth parameters compared to NaCl stress alone, potentially through enhanced water uptake and maintenance of cellular homeostasis (Wahid et al., 2020).

4. Conclusion

This study demonstrates the effectiveness of Ascorbic Acid (AsA), 5-Aminolevulinic Acid (ALA), and Nano-Selenium (N-Se) in mitigating the adverse effects of salinity stress on the anatomical structure of pea plants. The results reveal that AsA, particularly at 100 ppm, significantly improved stem and leaflet anatomical parameters, including stem diameter, cortex thickness, and vascular bundle dimensions, compared to the control and other treatments. ALA also showed beneficial effects, though to a lesser extent, while N-Se was less effective under high salinity conditions. Salinity stress led to notable reductions in leaf and stem thickness and vessel diameter, confirming its detrimental impact on plant anatomy. However, the application of AsA, ALA, and N-Se mitigated these effects by enhancing tissue structure and stress tolerance. AsA, in particular, was effective in improving leaf and stem characteristics, likely due to its role in reducing oxidative stress and promoting cell growth. These findings highlight the potential of bio-stimulants in enhancing the resilience of pea plants to salinity stress, offering valuable insights for developing strategies to improve crop performance under challenging environmental conditions. Further research should explore the underlying mechanisms of these bio-stimulants and their applicability in field settings.

Author Contribution: Conceptualization: Hala G. El-Araby and Ahmed Mohamed El-Taher; Methodology: Hala G. El-Araby; Investigation: Hala G. El-Araby; Formal Analysis: Sahar F.M. El-Hefnawy, Mohammed A. Nassar, and Ahmed Mohamed El-Taher; Writing—Original Draft Preparation: Hala G. El-Araby; Writing—Review and Editing: Sahar F.M. El-Hefnawy, Mohammed A. Nassar, and Ahmed Mohamed El-Taher; Visualization: Sahar F.M. El-Hefnawy; Data Curation: Sahar F.M. El-Hefnawy and Mohammed A. Nassar; Project Administration: Ahmed Mohamed El-Taher; Supervision: Ahmed Mohamed El-Taher and Hala G. El-Araby. All authors have read and agreed to the published version of the manuscript.

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