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Anatomical and physiological traits of broad bean (*Vicia faba* L.) seedling affected by salicylic acid and salt stress

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ABSTRACT

A laboratory experiment was carried out at the College of Agriculture University of Baghdad in 2017. The aim was to improve the anatomical and physiological traits of broad bean seedling under salt stress by soaking it in salicylic acid. The concentrations of salicylic acid were 0, 10, and 20 mg L^{-1} and the electrical conductivity levels were 0, 3, and 6 dS m^{-1}. The complete randomized design was used with four replications. The increasing of salicylic acid concentration up to 10 mg L^{-1} led to increasing the stem cortex thickness, stem vascular bundles thickness, and root cortex thickness significantly by (34.9,36.7,and 55 µm) respectively, while the treatment of 20 mg L^{-1} led to decreasing these traits by (28.2, 27.8, and 48.1 µm), compared to control treatment (33.8, 35.9, and 53.8 µm), respectively, and the interaction of studied factors led to increasing those traits up to 10 mg L^{-1} and then decreased up to 20 mg L^{-1} of salicylic acid under each level of electrical conductivity. Therefore, it is recommended to soak the broad been seeds with 10 mg L^{-1} salicylic acid to improve the anatomical traits of seedlings and increase their tolerance to salt stress up to 6 dS m^{-1}.

Key words: Stem and root anatomy, Broad bean, Salicylic acid (SA), Salt stress, Epidermis, Cortex, Vascular bundles.

INTRODUCTION

Salinity is one of the factors which reduce plants growth and productivity. An increase in the NaCl concentration results in a decrease in the early seedling growth characteristic germination rates (Kusvuran, 2015). Salicylic acid occurs in plants naturally and plays an important role in regulating the growth and development (Khodary, 2004). Encouraging or inhibiting some of the physiological processes is related to the salicylic acid concentration and plants species (Senaratna *et al*., 2000). Gomaa *et al*. (2015) found that foliar application with 75 ppm SA in lupine led to increasing the stem diameter due to the increases in stem wall thickness and hollow pith diameter, and that increase belonged to the increase in epidermis thickness, cortex, fiber strands, tissues of phloem and xylem in addition to parenchymatous area thickness of the pith, and in vessel diameter. Maddah *et al*. (2007) found that spraying salicylic acid with 0.1 mM led to increasing the stomata number, but watering plants with 1.5 mM of salicylic acid damages the tissues of parenchyma in leaves, the tissue of sclerenchyma in stems, and xylem in the root. Application of salicylic acid led to significant increase in protein content of chickpen leaves in complete drought stress condition (Farjam *et al*., 2015). Moussa and Hassan (2016) concluded the decreases in carotenoids, chlorophyll (a + b), contents of total protein, and activity of photosynthetic as a result to grown broad bean plants at 13 dS m^{-1}. Rui *et al*. (2009) recorded the effect of using different concentrations of NaCl on decreasing the upper and lower endodermis thickness and increasing upper and lower cuticle thickness. The salinity is expanding through wide areas of Iraq. Currently, it represents one of the major problems that restrict the extension of the agricultural lands or the rise in the agricultural production of many crops. The study of the morphological feature appears by the plant as a response to salicylic acid and salt stress may not be sufficient to diagnosis its effect. Therefore, it is necessary to distinguish other anatomical factors.

MATERIALS AND METHODS

A laboratory experiment was carried out at the laboratory of Seed Technology, College of Agriculture, University of Baghdad in 2017. The first factor was the concentration of salicylic acid (0, 10, and 20 mg L^{-1}) and the second factor was the electrical conductivity (0, 3, and 6 dS m^{-1}). The complete randomized design was used with four replications. The seed of Italian broad bean "Aquadulge" was soaked in the salicylic acid solution for 24 hours. After that, the treated seeds were sown in rectangle pots (17×12×4 cm^3) which contained sandy soil as a bed. Pots were incubated in germinator at 25ºC, 80% RH according to recommendations of ISTA (ISTA, 2013). Seeds were watered every three days with 200 ml during germination period (14 days) with each level of electrical conductivity that prepared

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by NaCl and about the salt threshold of broad bean (1.6 dS m^{-1}). After the gemination period, fresh materials of seedling stem and root were fixed in formalin acetic acid (FAA) at 24-48 h and changed the solution to put samples in the ethanol (70%). Fresh plant samples of seedling stems and roots were sectioned using hand sectioning method of Al-Hadeethi (2016) as stems and roots of a selected seedlings were cut into small pieces of a length ranged 4-6 cm. Segments were sectioned into thin pieces by a razor blade and treated with 0.5% sodium hypochlorite for 10 min to remove the chlorophyll pigments. Finally, the samples were placed on the slides and mounted the cover slides by D.P.X. and fixed by Olympus KRÜSS light microscope then photographed using Olympus Am scope camera. Traits were studied on seedling to measure stem epidermis thickness, stem cortex thickness, stem vascular bundle thickness, stem diameter, root epidermis thickness, root cortex thickness, root vascular bundle diameter, root diameter.

Data were analyzed statistically at $P<0.05$. Genstat software was used. Test of least significant differences at 5% level of probability (LSD 5%) was used to compare the calculated averages of traits (Steel and Torrie, 1980).

RESULTS AND DISCUSSION

General description of stem and root cross-sections: Fig 1 shows that the quadrilateral stem consists of a single layer of epidermis consisting, the cells oval, covered by the cuticle layer. The cortex consists of two layers of cells, the first layer is near the epidermis known collenchyma cells consist of three rows, so several rows of them are grouped in the corner of stem, after the collenchyma layer can show several layers of the parenchyma cells separate between as the ordinary intercellular space, followed the cortex the vascular tissue that consists from vascular bundles, each bundle is connected to the other by a group of cells called the interfascicular cambium.

Fig 2 shows the vascular bundle consists as the follows; vascular bundle cup, beneath of it the phloem elements, followed by xylem elements and between the xylem and phloem there is the fascicular cambium layer, so it appears as four small vascular bundles in the corner of the stem. From fig 1, pith is in the center of the stem and has a cavity in the center of pith due to the dissolution of many pith cells. All the results above described in corresponding with Evert (2006).

Fig 3 shows that the root is oval shape and consists of a single layer of epidermis and the cortex consists of many layer of parenchyma cells separate between as the intercellular space, followed by the cortex the pericycle that consist from 2-4 layers of perivascular fiber and inside, it's located the vascular bundles, each bundle is covered by the vascular bundle cup from the above of phloem elements and the xylem elements located under the phloem, pith located in the center of root contain from parenchyma cells fill up the center of root (Fig 4). All the results above that described were corresponded with (Fahn, 1979 and Evert, 2006).

Stem epidermal thickness: An increase of salicylic acid concentration and electrical conductivity level led to decreasing in epidermis thickness significantly at each level, and the performance of epidermis thickness under the interaction of studied factors was like its performance under their main effects (Table 1).

Stem cortex thickness: Table 2 shows that the increase of salicylic acid concentration to 10 mg L^{-1} led to increasing

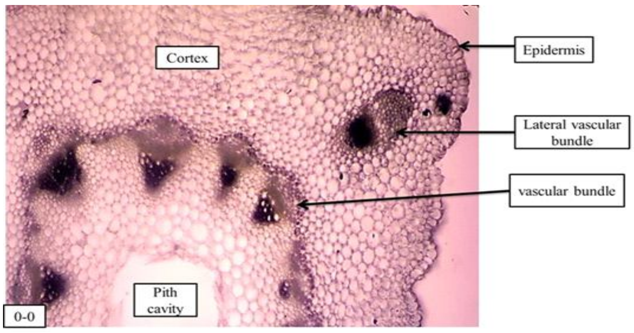

Fig 1: A cross-section of control treatment (0×0) stem in broad bean

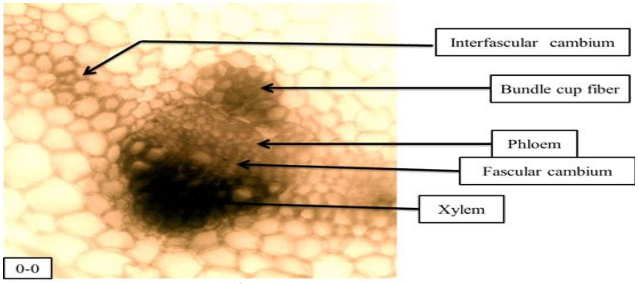

Fig 2: A cross-section of control treatment (0×0) stem vascular bundle in broad bean

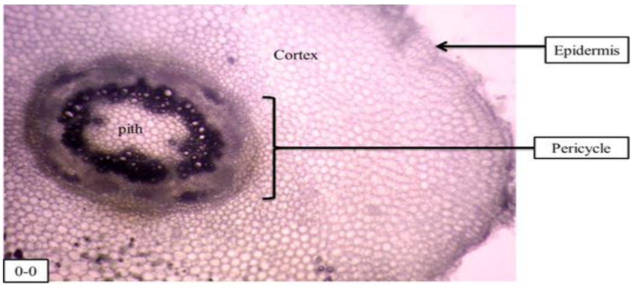

Fig 3: A cross-section of control treatment (0×0) root in broad bean

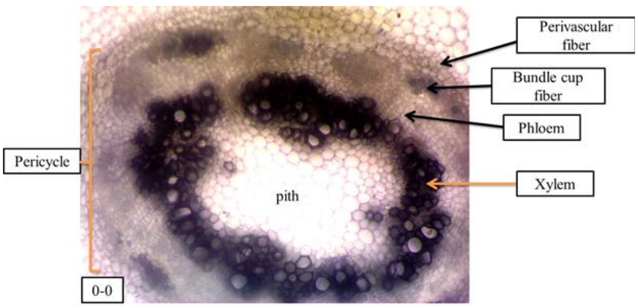

Fig 4: A cross-section of control treatment (0×0) root vascular bundles in broad bean

the thickness of the cortex (34.9 µm) significantly, while the treatment of 20 mg L^{-1} led to decreasing the thickness of the cortex (28.2 µm) compared to control treatment. The increasing of electrical conductivity led to decreasing in the stem cortex thickness significantly. Also, their interaction led to increasing the stem cortex thickness up to 10 mg L^{-1} of salicylic acid under each level of electrical conductivity and then decreased up to 20 mg L^{-1} of salicylic acid under each level of electrical conductivity.

Stem vascular bundles thickness: Table 3 shows that the increase in salicylic acid concentration to 10 mg L^{-1} led to increasing the thickness of the vascular bundles of the stem (36.7 µm) significantly, while the treatment of 20 mg L^{-1} led to decreasing the thickness of the vascular bundles of the stem (27.8 µm) compared to control treatment. The electrical conductivity increase led to decreasing the thickness of the vascular bundles of the stem significantly. Also, their interaction led to increasing stem vascular bundles thickness up to 10 mg L^{-1} of salicylic acid under each level of electrical conductivity and then decreased up to 20 mg L^{-1} of salicylic acid under each level of electrical conductivity.

Stem diameter: Table 4 shows that the increase of salicylic acid concentration and electrical conductivity level led to decreasing of the diameter of the stem significantly at each level, and the performance of stem diameter under the interaction of studied factors was like its performance under their main effects.

Root epidermal thickness: An increase of salicylic acid concentration and electrical conductivity level led to a decrease of root epidermal thickness significantly at each level, and the performance of root epidermal thickness under the interaction of studied factors was like its performance under their main effects (Table 5).

Root cortex thickness: Table 6 shows that the increase of salicylic acid concentration to 10 mg L^{-1} led to increasing the thickness of the root cortex (55 µm) significantly, while the treatment of 20 mg L^{-1} led to decreasing the thickness of the root cortex (48.1 µm) compared to control treatment. The increasing of electrical conductivity led to decreasing of root cortex thickness significantly. Also, their interaction led to increasing root cortex thickness up to 10 mg L^{-1} of salicylic acid under each level of electrical conductivity and then decreased up to 20 mg L^{-1} of salicylic acid under each level of electrical conductivity.

Root vascular bundles thickness: Salicylic acid concentration, electrical conductivity, and their interaction had a significant effect on root vascular bundles thickness.

Table 1: Effect of salicylic acid concentration, electrical conductivity, and their interaction on stem epidermal thickness (µm) in broad bean.

Electrical	**Concentration of salicylic acid (mg L^{-1})**			**Average**
conductivity (dS m^{-1})	**0**	**10**	**20**	
0	5.9	6.1	3.1	5
3	4.4	3.5	2.9	3.6
6	4	3.1	3	3.3
Average	4.8	4.2	3	

LSD 5% for salicylic acid concentration, electrical conductivity, and their interaction were 0.1, 0.1, and 0.3, respectively.

Table 2: Effect of salicylic acid concentration, electrical conductivity, and their interaction on stem cortex thickness (µm) in broad bean.

Electrical	**Concentration of salicylic acid (mg L^{-1})**			**Average**
conductivity (dS m^{-1})	**0**	**10**	**20**	
0	43.5	44.6	28.9	39
3	30.5	30.8	28.4	29.9
6	27.5	29.4	27.2	28
Average	33.8	34.9	28.2	

LSD 5% for salicylic acid concentration, electrical conductivity, and their interaction were 0.7, 0.7, and 1.3, respectively.

Table 3: Effect of salicylic acid concentration, electrical conductivity, and their interaction on stem vascular bundles thickness (µm) in broad bean.

Electrical	**Concentration of salicylic acid (mg L^{-1})**			**Average**
conductivity (dS m^{-1})	**0**	**10**	**20**	
0	44.4	45.5	29.4	39.8
3	36.2	33.7	27.8	32.6
6	27.1	31	26.2	28.1
Average	35.9	36.7	27.8	

LSD 5% for salicylic acid concentration, electrical conductivity, and their interaction were 0.4, 0.4, and 0.8, respectively.

Table 4: Effect of salicylic acid concentration, electrical conductivity, and their interaction on stem diameter (μm) in broad bean.

Electrical conductivity (dS m^{-1})	**Concentration of salicylic acid (mg L^{-1})**			**Average**
	0	**10**	**20**	
0	415	411.8	252.3	359.7
3	321.7	299.7	242.7	288
6	283.3	278.3	221	260.9
Average	340	329.9	238.7	

LSD 5% for salicylic acid concentration, electrical conductivity, and their interaction were 4.8, 4.8, and 8.3, respectively.

Table 5: Effect of salicylic acid concentration, electrical conductivity, and their interaction on root epidermal thickness (μm) in broad bean.

Electrical conductivity (dS m^{-1})	**Concentration of salicylic acid (mg L^{-1})**			**Average**
	0	**10**	**20**	
0	6.2	6.2	3.1	5.2
3	4.2	3.4	2.9	3.5
6	3.9	3.2	2.9	3.3
Average	4.8	4.3	3	

LSD 5% for salicylic acid concentration, electrical conductivity, and their interaction were 0.2, 0.2, and 0.4, respectively.

Table 6: Effect of salicylic acid concentration, electrical conductivity, and their interaction on root cortex thickness (μm) in broad bean.

Electrical conductivity (dS m^{-1})	**Concentration of salicylic acid (mg L^{-1})**			**Average**
	0	**10**	**20**	
0	63.5	64.7	48.7	59
3	50.5	50.8	48.4	49.9
6	47.5	49.4	47.1	48
Average	53.8	55	48.1	

LSD 5% for salicylic acid concentration, electrical conductivity, and their interaction were 0.7, 0.7, and 1.3, respectively.

Table 7 shows that the increase of salicylic acid concentration and electrical conductivity level led to decreasing of root vascular bundles thickness significantly at each level, and the performance of root vascular bundles thickness under the interaction of studied factors was like its performance under their main effects.

Root diameter: The increase of salicylic acid concentration and electrical conductivity level led to decreasing of root diameter significantly at each level (Table 8), and the performance of root diameter under the interaction of studied factors was like its performance under their main effects.

The concentration of salicylic acid 10 mg L^{-1} led to decreasing the stem epidermis thickness, stem diameter, root epidermis thickness, root vascular bundle diameter, and root diameter, but it increased stem cortex thickness, stem vascular bundles thickness, and root cortex thickness. Therefore, this increase may provide the required support for the stem and root growth positively, in comparison with not to soak the seeds with salicylic acid or soak them with a concentration of 20 mg L^{-1}. This is probably due to the salicylic acid role to promote plants to increase plant efficiency to transport the water and nutrient under wide range of environmental conditions (Metcalfe and Chalk, 1979).These results were agreed with Singh *et al*., (2015) who suggested to use salicylic acid to improve the antioxidant system and protect the nitrate reductase (NR) activity of two field pea genotypes by seed hardening through optimum concentration salicylic acid (SA) 1.0 mM under salinity stress condition.

The increase of electrical conductivity of irrigation water led to decrease all traits, and that's maybe because of increasing of NaCl solution concentration that withdrew the water out of the cells, shrinkage the stem tissues, and reducing tissues thickness as result of that. Plants exposed to saline environment suffer from ion excess or water deficit and oxidative stress linked to the production of reactive oxygen species (ROS), which cause damage to lipids, proteins and nucleic acids (Hernandez, *et al.*, 2000). Also many studies reported that using high salinity led to physiological (Shannon, 1997) and anatomical changes like lack of stomata number (Hwang and Chen, 1995 and Çavuşoğlu *et al*., 2007), leaves thickness (Shennan *et al*., 1987 and Çavuşoğlu *et al*., 2008), vascular bundles thickness and epidermis cell number (Çavusoglu *et al*., 2007), inhibition in diameter and number of xylem vessels introduced by Kiliç *et al* (2007), so salinity stress induced the production of new protein bands which not occur in the control plants (Dawood and El-Awadi ,2015). The interaction treatments of salicylic acid 10 mg L^{-1} either

Table 7: Effect of salicylic acid concentration, electrical conductivity, and their interaction on root vascular bundles diameter (µm) in broad bean

Electrical conductivity ($dS\ m^{-1}$)	Concentration of salicylic acid ($mg\ L^{-1}$) 0	10	20	Average
0	56.5	57.5	29.1	47.7
3	43.3	38	28.5	36.6
6	39.5	31.8	27	32.8
Average	46.4	42.4	28.2	

LSD 5% for salicylic acid concentration, electrical conductivity, and their interaction were 1.2, 1.2, and 2.1, respectively.

Table 8: Effect of salicylic acid concentration, electrical conductivity, and their interaction on root diameter (µm) in broad bean

Electrical conductivity ($dS\ m^{-1}$)	Concentration of salicylic acid ($mg\ L^{-1}$) 0	10	20	Average
0	428.3	416	274.3	372.9
3	332.3	312.7	265.3	303.4
6	295.3	288.3	244.7	276.1
Average	352	339	261.4	

LSD 5% for salicylic acid concentration, electrical conductivity, and their interaction were 7.3, 7.3, and 12.6, respectively.

with salt stress or not (0, 3, and 6 $dS\ m^{-1}$) led to decreasing the stem epidermis thickness, stem diameter, root epidermisthickness, root vascular bundle diameter, and root diameter, but it increased stem cortex thickness, stem vascular bundles thickness, and root cortex thickness. Similar results were obtained by Senaratna et al. (2000) that the encouraging or inhibiting some of physiological processes by salicylic acid is related to the salicylic acid concentration and plants species.

CONCLUSION

Based on the results, it means that salicylic acid has a role to improve some of the anatomical traits of stem and root of broad bean seedling even under salt stress conditions. Therefore, it can be concluded that the soaking the seeds of broad bean with salicylic acid at a concentration of 10 $mg\ L^{-1}$ to improve some of the anatomical traits and increasing the tolerance of broad bean against saline stress up to 6 $dS\ m^{-1}$.

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