BOTANY SECTION

Alleviation of salt stress in *Vicia faba* plants by foliar application of Potassium fertilizer

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Abstract: Salinity stress increases Na^+ and Cl^- ions in the soil. These harmful ions inhibited the overall plant growth by several mechanisms. Interestingly, application of potassium as an essential element required for plant growth can alleviate the detrimental effect of salinity. The present study focused on the effect of potassium (K_2HPO_4) as a fertilizer on the vegetative growth of salinized faba plants due to its effect on regulation and stabilization of stomatal function, protein synthesis and stress resistance. Potassium application and foliar fertilizer was used to enhance the photosynthetic pigment content, leaf area, stomata numbers, transpiration rate, carbohydrate content, protein synthesis and uptake of some minerals in the stressed *Vicia faba* plants with different salinity levels of NaCl. Results indicate also, that salinity retarded the level of endogenous hormone ABA but the application of potassium fertilizer could regulate it.

Keywords: *ABA, alleviate, foliar fertilizer, K_2HPO_4, vital.*

Abbreviations: Abscisic acid (ABA); calcium (Ca); chlorophyll a (Chl. a); chlorophyll b (Chl. b); insoluble (Insol.); lower epidermis (L.E.); magnesium (Mg); phosphorous (P); potassium (K); soluble (Sol.); stomatal frequency (Stomatal freq.); upper epidermis (U.E.)

Introduction

Egypt to date depends much on agriculture especially the cultivation of broad bean. Broad bean (*Vicia faba cv. Giza 40*) is an important legume vegetable during the winter season. Faba bean is an important source of protein for human and animal consumption.

Salinity is one of the adverse environmental factors limiting the productivity of crop plants because most of the crop plants are sensitive to salinity. It was estimated that about 20% (45 million ha) of irrigated land, producing one-third of the world's food is affected by salinity. Thus, it is a serious threat to agricultural productivity, especially in arid and semi-arid regions (Anju et al., 2018). Moreover, salinization of soils occurs primarily due to agricultural practices that include poor water management, high evaporation, and exposure to seawater (Kouam et al., 2017).

Numerous strategies to mitigate the adverse effects of salt stress on plants have been studied, but the management of fertilization favoring the acquisition of nutrients by plants under saline conditions has considered as the most promising (Silva, 2011; Prazers, 2015). For example, potassium (K) which has been used on the crop field since the nineteenth century (Cakmak, 2005).

ABA is an important growth regulator in many manifestations of plant growth and development and is animated for stress resistance. Also, it controls the cell,s water balance (Li and Li, 2017).

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Bonifácio, et al. (2018) reported that the most direct methods to maintain normal levels of K in the plant under saline conditions would be to increase the concentration of this nutrient in the root zone up to a certain level by increasing the dose of potassium, which would possibly, cause a higher absorption of K in relation to Na, presenting lower Na/K ratios in the leaves and, consequently, a nutritional balance more adequate to the plants.

Therefore, this study was carried out to test potassium applied foliar spraying efficacy against the drastic effect of toxic NaCl on growth, development, and physiology of broad bean.

Materials and Methods

Materials

The study was conducted during winter 2017. *Vicia faba L.* (Broad bean, Giza 40), seeds were obtained from agriculture research center, Giza, Egypt. These seeds were grown in plastic pots containing 2 kg of air-dried (sand: clay 1/ 1 v/ v). Five seeds were sowed in each pot, after 10 days of germination, plants were thinned to three plants per three replication for each.

Salinity levels

Five salinity levels (0.0, 75,125,175 and 225 mM NaCl) were used for the experiment. Plants were subjected to salt 20 days after sowing.

Potassium level and application

100 ppm of K_2HPO_4 was foliar sprayed two days before salt application and irrigated twice throughout the experiment which continued for three weeks after application.

Parameters

The plants were harvested to estimate the following parameters Leaf area was measured by the disk method (Watson and Watson, 1953) and given as dm2 plant-1. Transpiration rate was measured as described by Bozcuk (1975) and calculated as g plant-1 day-1. The stomatal frequency on the upper and lower epidermis was determined using a square ocular micrometer. The endogenous ABA contents were extracted following the method of Wurst et al. (1984).

The pigment contents were calculated according to Metzner et al. (1965). Carbohydrate content was measured using anthrone-sulfuric acid method according to (Fales, 1951). Free amino acids were determined according to the method of Moore and Stein (1948). Proline was determined according to the method of Bates et al. (1973) while, soluble protein fraction was determined according to the method of Lowery et al. (1951). Sodium and potassium were determined by flame photometer method (Williams and Twine, 1960), calcium and magnesium were determined according to the method of (Schwarzenbach and Biedermann,1948) and phosphorous was determined according to (Woods and Mellon, 1958).

Results

Data given in table 1 showed a progressive decrease in each of the transpiration rate, leaf area, and stomatal frequency with the increase of NaCl levels. The opposite cut held in ABA, where it accumulates under higher salinity levels. Nevertheless, K fertilization which enhanced the reduction in all the previous criteria and accumulation in ABA as it compared with the corresponding salinized plants.

Table 1. Effect of salinization and treatment with K_2HPO_4 (100ppm) on the transpiration rate [gcm^{-2} day^{-1}) , stomatal frequency (number of stomata per mm^2, L.E and U.E), leaf area (dm^2 $plant^{-1}$) and ABA content (μg g^{-1} dry weight) of faba bean plants.

K_2HPO_4 ppm	NaCl mM	Transpiration rate	Leaf area	ABA	Stomatal freq. L.E	Stomatal freq. U.E
	0	17.3	5.4	6.26	210	80
	75	15.9	5.6	6.31	200	72*
0.0	125	12.3**	3.7**	8.26*	185*	64**
	175	11.5**	2.8**	9.40**	123**	50**
	225	11.1**	1.4**	10.20**	90**	37**
	0	19.5	7.1**	4.14*	218	92**
	75	18.1	6.3	5.31	222	85
100	125	17.2	5.9	5.90	212	74
	175	15.7	4.5	6.41	201	60**
	225	12.6**	2.7**	7.10	133**	49**
L.S.D	5%	2.3	1.12	1.66	24.7	7.8
L.S.D	1%	3.2	1.53	2.30	33.8	10.7

Significant difference to control: *P = 0.05; **P = 0.01

The data in table 2 revealed that the contents of pigment fractions were retarded markedly under the higher treatments of salinity. The opposite cut held in case of K fertilization in all salinity levels.

Table 2. Effect of salinization and treatment with K_2HPO_4 (100ppm) on pigment content (mg g^{-1} dry weight) of faba bean plants.

K_2HPO_4 ppm	NaCl mM	Chl.a	Chl.b	Carotenoids	Total pigments
	0	9.70	4.66	3.85	18.21
	75	9.21	4.31	3.01*	16.53
0.0	125	8.98	4.00	2.76**	15.74
	175	8.10*	3.61**	1.98**	13.69**
	225	6.31**	3.02**	1.62**	10.95**
	0	11.2*	5.69**	3.99	20.88
	75	10.8	5.31	3.87	19.98
100	125	9.8	4.91	3.25	17.96
	175	9.1	4.33	2.61**	16.04
	225	7.5**	3.92*	2.15**	13.57**
L.S.D	5%	1.22	0.73	0.71	2.82
L.S.D	1%	1.70	1.00	0.97	3.90

Significant difference to control: *P = 0.05; **P = 0.01

In table 3 salinity stress led to reduction in the content of soluble and insoluble fractions. In turn under higher levels of salinity, carbohydrate contents were reduced in both organs however, at mild conc. (75 mM) remained unaffected. The spraying of K_2HPO_4 induced an accumulation of these fractions whatever the level of stress used and the plant organ analyzed in comparison with those of stressed reference control plants.

Table 3. Effect of salinization and treatment with K_2HPO_4 (100ppm) on soluble, insoluble and total carbohydrates (mg g^{-1} dry weight) of shoot and root of faba bean plants.

K_2HPO_4 ppm	NaCl mM	Shoot Sol.	Shoot Insol.	Shoot Total	Root Sol.	Root Insol.	Root Total
	0	174.3	210.5	384.8	147.6	233.4	381.0
	75	170.2	206.3	376.5	135.4**	227.1	362.5**
0.0	125	166.4**	201.2**	367.6**	129.2**	218.3**	347.5**
	175	158.7**	197.5**	356.2**	115.1**	209.5**	324.6**
	225	133.4**	180.6**	314.0**	98.3**	188.4**	286.7**
	0	180.6*	217.1*	397.7**	153.4*	242.1*	395.5**
	75	178.2	212.9	391.1	148.3	235.2	383.5
100	125	172.1	208.6	380.7	139.5**	226.8	366.3**
	175	168.3*	201.5**	369.8**	127.2**	219.1**	346.3**
	225	155.4**	196.4**	351.8**	117.1**	197.5**	314.6**
L.S.D	5%	5.3	6.33	8.6	4.70	6.8	9.8
L.S.D	1%	7.3	8.70	11.8	6.44	9.3	13.4

Significant difference to control: *P = 0.05; **P = 0.01

From the results presented in table 4 it can be seen that the content of soluble proteins and total free amino acids in *Vicia faba* plants are decreased highly significantly by the rise of salinization level in the two main organs in comparison with the absolute control plants. Induction of K_2HPO_4 as a fertilizing agent resulted in stimulation of insoluble proteins and total free amino acids content in either shoot or root system as they compared with the symmetrical stressed plants.

Table 4. Effect of salinization and treatment with K_2HPO_4 (100ppm) on soluble saccharides [mg g^{-1} dry weight], soluble protein [mg g^{-1} dry weight], amino acids [mg g^{-1} dry weight] and proline content [mg g^{-1} dry weight] of shoot and root of faba bean plants.

K_2HPO_4 ppm	NaCl mM	Sol. Protein Shoot	Sol. Protein Root	Free amino acids Shoot	Free amino acids Root	Proline Shoot	Proline Root
	0	45.8	25.1	12.7	16.3	6.11	7.81
	75	39.9*	26.3	14.8**	18.9**	6.34	9.52**
0.0	125	34.3**	20.6**	15.3**	22.4**	7.81**	10.14**
	175	29.8**	20.4**	15.9**	23.1**	9.12**	11.3**
	225	22.4**	15.3**	16.4**	23.8**	10.01**	11.9**
	0	51.3	32.4**	14.3*	17.5*	6.02	7.21
	75	47.8	26.8	13.6	16.1	5.91	7.91
100	125	40.3	22.3	12.9	16.8	5.99	8.35
	175	35.7**	22.5	14.3*	18.7**	7.21*	9.13**
	225	29.5**	18.5**	15.6**	20.0**	8.31**	10.2**
L.S.D	5%	5.70	3.10	1.40	1.07	0.83	0.65
L.S.D	1%	7.8	4.20	2.0	1.50	1.14	0.90

Significant difference to control: *P = 0.05; **P = 0.01

With respect to proline content accumulated highly significantly under various levels of salinity stress in each experimental organs. On the other hand, K fertilizer exhibited retardation in proline accumulation in both tested organs as they compared with the stressed corresponding levels.

Table 5. Effect of salinization and treatment with K_2HPO_4 (100ppm on mineral contents (mg g^{-1} dry weight) of shoot and root of faba bean plants.

K_2HPO_4 ppm	NaCl mM	Shoot						Root					
		Na	K	Ca	Mg	P	K/Na	Na	K	Ca	Mg	P	K/Na
0.0	0	8.6	19.6	22.2	10.4	6.5	2.27	11.1	23.2	31.3	6.3	6.81	2.09
	75	13.8*	15.4*	20.1	9.4	6.6	1.21**	14.3	22.7	32.5	5.4	6.91	1.59**
	125	19.9**	13.9**	19.4	9.0*	7.2**	0.698**	20.7**	20.2*	32.0	4.8*	6.22	0.976**
	175	25.4**	11.6**	16.1**	7.3**	6.4	0.457**	29.1**	17.5**	29.5*	4.0**	4.43**	0.601**
	225	32.5**	10.6**	14.5**	6.2**	5.61**	0.326**	38.8**	15.4**	25.0**	3.7**	4.01**	0.397**
100	0	8.1	22.9*	25.3*	10.9	11.7**	2.83**	10.3	27.4**	37.4**	7.6*	12.3**	2.66**
	75	10.3	22.1	24.3	10.5	11.01**	2.15**	11.5	25.7	36.0**	7.01	12.01**	2.24**
	125	14.2**	24.3**	26.7**	11.2	10.5**	1.71**	16.2*	28.2**	39.2**	6.43	12.6**	1.74**
	175	20.4**	27.6**	28.6**	10.6	10.13**	1.35**	22.1**	29.3**	40.0**	5.33	11.8**	1.33**
	225	27.6**	28.1**	30.1**	9.8	9.31**	1.02**	26.7**	33.1**	39.1**	4.61*	11.3**	1.24**
L.S.D	5%	3.90	3.3	3.07	1.38	0.24	0.08	4.05	2.70	1.33	1.22	1.05	0.06
L.S.D	1%	5.34	4.5	4.21	1.90	0.33	0.11	5.55	3.70	1.82	1.70	1.44	0.08

Significant difference to control: *P = 0.05; **P = 0.01

Data in table 5 concerning to sodium content in both shoot and root illustrated that there is a highly significant increase in Na^+ as compared with absolute control plants. While foliar spraying and addition of K_2HPO_4 induced retardation in Na^+ content in different parts of faba plants. Generally, the contents of each K, Ca, Mg, P and K^+/Na^+ ratio retarded highly significantly with the rise of salinity level. While fertilization with K_2HPO_4 considerably enhanced the accumulation of these elements in the two experimental organs as they compared to the plants subjected to salinity levels only.

Discussion

The toxic effect of salt stress includes several aspects. Uptake of excessive Na^+ and Cl^- interfere with many intracellular metabolic processes. High salt concentration in the soil causes osmotic stress, which limits water uptake from soil (Li and Li, 2017). The research showed that there is a reduction in leaf area, transpiration rate, stomatal frequency, and photosynthetic pigment.

Under salinity conditions, the levels of ABA increase where it triggers changes in ion fluxes leading to stomata closure and inhibiting stomatal opening. Totally these effects minimize water loss and allow plants to cope with salinity condition episodes (Araújo et al., 2011). Salt stress can lead to cell dehydration and/or the loss of cell turgor (Jia et al., 2002).

The present study determined changes in endogenous hormone levels in relation to K application methods. As the result is in accordance with that of Chen et al. (2017) on cotton where ABA decline strongly.

Chlorophyll contents was significantly decreased with the increase in salinity levels. Consistent with these results, Khalid and Cai (2011); Celik and Atak (2012) were found an inhibition in chlorophyll biosynthesis in sorghum due to salt stress. This inhibition may be attributed to the formation of proteolytic enzymes such as chlorophyllase, which is responsible for chlorophyll degradation.

However, Potassium plays a mitigating role in various abiotic stresses such as salinity stress. It was found that K controls photosynthesis through sunlight interception. The leaf surface area and sunlight interception were both reduced dramatically when the K was below the level required by the plant (Pettigrew, 2008).

The current data showed that there was a reduction in the soluble protein and carbohydrate fractions especially under higher levels of NaCl and this may be due to the sensitivity of the plant to salinity. This strategy would agree with (Ashraf and Waheed 1993).

Application of K was shown to improve the organic osmolyte synthesis, especially the rapid rate of cell recovery under osmotic stress was regulated by the higher accumulation of K^+, Cl^-, and Na^+ in the epidermal root cells of *Arabidopsis* (Shabala and Lew, 2002).

The accumulation of proline in response to salt stress has an important role in osmotic adjustment and can reduce the water potential to maintain the water content in leaves. Although in high external salt concentrations this occurs at the expense of plant growth, it may allow the plant to resist salt stress or even to osmotic pressure (Stavridou et al. 2016).

Generally, Na levels increase, while Ca and K levels decrease in plant cells and tissues with increasing external Na concentration. Because Na toxicity begins when NaCl accumulates due to toxic concentrations in the different organs, the maintenance of K and Na homeostasis is important for the activities of many cytosolic enzymes and for the maintenance of membrane integrity (Lu et al., 2017).

Na^+ competes with K^+ for intracellular uptake because they are transported by common proteins. However, Na^+ cannot replace K^+ in enforcing physiological function (Li, 2017). High concentrations of Cl^- and Na^+ in leaf tissue are toxic. So, the K^+/Na^+ ratio has been significantly affected by increasing salinity level.

It can be inferred that potassium fertilization increased the availability of the nutrient minerals as since potassium is directly involved in the nutrients absorption through the process of phloem loading as a counter ion to H^+ and so promoting the mineral content of plant foliage (Abou El-Khair and Mohsen, 2016). Thus, the application of K increase the K^+ content in plant cells and reduce the Na^+ concentration, which increases the K^+/Na^+ ratio. The reduction of Mg and P by increasing salinity in the plant tissue may be attributed to the presence of Na^+ and Cl^- as Na^+ interference or eventual ion-pair formation and subsequent precipitation. Thus, the decrease in Mg content may also have contributed to the reduction in photosynthetic pigment content. As well as Cl^- which reduces P absorption (Gomes et al., 2011).

Conclusions

It is concluded from the present investigation that salt stress have imparted destructive effects on the growth of Vicia faba plants, however, the use of potassium as a fertilizing agent under salt stress condition has been found to be very much effective to alleviate salt-induced damages.

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