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NACL AND IRON INTERACTION ON SEED GERMINATION OF FOUR BROAD BEANS VARIETIES (*VICIA FABA* L.)

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Abstract: This work aimed to determine the interaction iron-NaCl effect on germination characteristics (germination percent, germination rate, germination capacity, germination stress index, dry matter stress index, salinity tolerance index and seed vigor) of four varieties bean's plant *Vicia faba* L. The measurement was designed in the form of splitting segments with four replicates by treating each variety with four NaCl concentration (0, 50, 150, 200 mmol/L), In addition, the seeds were soaked in iron sulfate solution 0.6 µmol/L. The results showed that the salinity had a significant negative effect on the parameters studied during the germination phases and depended on the different salinity levels compared to the control. The iron element appeared a positive effect on these parameters. In addition, the salinity resistance in the presence of iron element of the four varieties in the following order; broad, Aguadulce then Malti and Histal with the same order. Moreover, the two parameters germination stress index and salinity tolerance index showed the best results in salinity resistance in the presence of iron element on the rest of the studied parameters.

Keywords: salt stress, iron, germination, *Vicia faba* L.

INTRODUCTION

The agriculture modern is facing major challenges to achieve a 70% production ceiling in order to meet the food needs of the world population (Tester et langride, 2010; FAO,2009). Salinity, due to the accumulation of salts (magnesium salts, sodium salts, sodium chloride ...) in agricultural land, which makes it unsuitable soil for agriculture, is one of the main obstacles to the realization of purpose sought (Reynolds et *al*,2005).

Sodium chloride is one of the most common salts in soils and is the most effective and reduces the growth of agricultural production (Reynolds et *al*,2005); salt soil effect lead to reduced cell division, small cell size, affecting the area of plant leaves, leading to lack plant photosynthesis, low germination rate and root shortening, seedling growth and prevents the absorption of nutrients necessary for plant growth (Ghoulam et *al*., 2002; Hakim et *al*. 2010; Romero-Aranda et *al*., 2001; Lallu and Dixit,2005).

The plant resistance salinity capacity due to two methods; the first one results from the osmotic pressure and the second by eliminating the toxic element effect (Munns,2002; Ozaslan&Parlak, 2008). An increase in the salinity concentration in the soil solution leads to an increase in osmotic pressure, which necessitates an increased effort of the plants to absorb the nutrients, which negatively affected their growth and production (Khodadad,2012; Ozaslan and Parlak, 2008).

At the germination phase, the ability of the embryo to absorb water is reduced resulting from low soil water potential owing to high concentrations and the accumulation of (Na^+ Cl^-) around the seeds (Munns et *al*., 2008). This effect is due to the rise of the water from the lower layers to the upper layers of the soil and after evaporation causes an increase in the concentration of these salts in the surface layer around the seeds. (Jamil et *al*,2006).

The increase in crops production is a necessity to achieve self-sufficiency, especially in the field of vegetables and legumes. The legume family is one of the most widely used and diversified food groups for example beans, haricot and lentils(Martin et *al*,1994).

The broad bean is one of the most important vegetable protein sources, is achieved to (26% to 43%), carbohydrate (45% to 48%), many world population, especially poor countries, for a human and animal. It also has an important role in soil fertilization as they supply nitrogen to the soil with root nodes.

Iron is an essential micro-element, it is absorbed in the iron anion form, which is incorporated in the structure and enzymes activity involved in the metabolic processes, as well as in oxido-reduction reactions, in particular catalase, peroxidase and cytochrome oxidase. It contributes to the photosystems structure, mitochondrial enzymes, and phytoferritin. The iron deficiency symptoms are represented in leaves yellowing and burning edges.

The reason for this study determines response salinity tolerance in the availability of iron element on four varieties of broad bean at the germination phase by measuring various parameters under determined conditions.

MATERIALS AND METHODS

It has been selected four varieties of broad bean seeds (V1 - *Malti*, V2 - *Histale*, V3 - *Broad* and V4 – *Aguadulce*). The $Fe_2SO_47H_2O$ solution 6µg/L and the sodium chloride with four concentrations 0, 25, 50 and 150 mmol/L.

The experiments were carried out in great Petri plates. 20 seeds were sown in a Petri plate and germinated on papers imbibed with distilled water or in

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sodium chloride solutions, each has been treated with four salinity levels (S_0, S_1, S_2, S_3) Appropriate the concentrations 0, 25, 50 and 150 mmol/L respectively (in the presence F_1 or in the absence F_0 iron element treatment). The filter papers have been changed every two days to avoid salt accumulation.

The experiment consists of four varieties of bean seed, which designed as a random sampling method with four replicates, under the appropriate temperature is set (18 to 20°C) and in dark conditions. The following parameters have been calculated:

- Germination Percent GP %

$GP = \frac{n}{N} x\ 100$ n: Number of seeds germinated, N: Total number of seeds (Fateme *et al.*, 2016)

5)

-Germination rate: GR%

$GR = \frac{G1*Day1+G2*Day2+\cdots.+Gn*Dayn}{GP} x\ 100$ Gn: the number of germinated seeds in the n day (Kader,200-+

- Germination capacity: GC %

$GC = \frac{\text{Total germinated seeds}}{\text{Total seeds tested}} x\ 100$(Kiran & Bargali, 2016)

- Index of germination stress GSI

GSI (%) = [P.I of stressed seeds / P.I control seeds] x 100 P.I: promotion index nd2 (1) +nd4 (0.75) +nd6 (0.5) +nd8 (0.25); nd2: Germinated seeds in the n day (Shamim *et al.*, 2009).

- Dry matter stress tolerance index (DMSI)

DMSI = (Dry matter of stressed plant / Dry matter of control plants) x 100 (Shamim et *al*.,2009)

- Salt tolerance index STI

$STI = \frac{\text{Seedling Dry Weight (Root+Shoot)atx}}{\text{Seedling Dry Weight (Root+Shoot)atc}}$ (Fateme et *al*., 2016)

- Seed vigor SV

SV = *[seedling length (cm) × germination percentage]* (Janet *al*.,2016)

RESULTS

Germination Percent GP %

The results in the table (1) shows there is a significant difference in the effect of the germination percent of all varieties, the third variety outperforms on the other varieties worth (94.22). The second variety exceeds the first and the fourth with the value of (85.26). Salinity has a negative effect on the three levels compared to the control. For the interaction salinity-iron element, the germination percent is exceeded in the presence of iron compared to its absence.

Table 1.

Effect of imbibition of seeds of *Viciafaba* L. in iron on the germination Percent under the effect of sodium chloride salinity

varieties	Element	Salinity levels mmol/L				V*E
		S_0	S_1	S_2	S_3	
V_1	Fe_0	90.83	87.83	87.83	78.31	84.95
	Fe_1	92.96	91.28	91.28	78.71	85.18
V_2	Fe_0	87.28	88.57	88.57	71.80	82.89
	Fe_1	91.28	87.43	87.43	80.71	87.63
V_3	Fe_0	93.75	87.50	87.50	95.00	93.44
	Fe_1	98.75	91.25	91.25	93.75	95.00
V_4	Fe_0	91.25	81.25	81.25	72.50	83.44
	Fe_1	76.25	83.75	83.75	77.50	79.38
LSD		11.46				5.73
S		90.29	87.36	87.27	81.04	
LSD		4.05				V
V*S	V_1	91.90	89.55	80.31	78.51	85.07
	V_2	89.28	88.00	87.51	76.26	85.26
	V_3	96.25	89.38	96.88	94.38	94.22
	V_4	83.75	82.50	84.38	75.00	81.41
LSD		8.10				4.05
S*E						E
	Fe_0	90.78	86.29	88.25	79.40	86.18
	Fe_1	89.81	88.43	86.29	82.67	86.80
LSD		5.73				2.87

Germination rate: GR%

The results in Table (2) showed an increase in germination rate of the first variety on the other varieties and are estimated at 86.69, 74,59,52,81 and 60.13 respectively. The high concentration of salinity (S_2 and S_3) has an increased germination rate. There was also an increase in the germination rate in the presence of iron (71.23) compared to their absence (65.88) on all varieties and salinity levels. The interaction salinity -iron element, the germination rate in the presence of iron exceeded their absence.

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Table 2.
Effect of imbibition of seeds of *Vicia faba* L. in iron on the germination rate under the effect of sodium chloride salinity

Varieties	Element	Salinity levels mmol/L				V*E
		S_0	S_1	S_2	S_3	
V_1	Fe_0	82.38	85.00	84.25	82.00	84.95
	Fe_1	91.00	90.00	101.50	95.00	85.18
V_2	Fe_0	67.81	69.75	68.75	62.25	82.89
	Fe_1	81.38	74.25	83.00	78.25	87.63
V_3	Fe_0	52.63	50.50	50.25	55.50	93.44
	Fe_1	53.00	49.50	47.75	45.25	95.00
V_4	Fe_0	60.69	61.25	56.50	75.25	83.44
	Fe_1	59.56	58.75	60.25	69.25	79.38
LSD		18.25				9.12
S		67.47	67.38	69.03	70.34	
LSD		6.45				V
V*S	V_1	77.88	87.50	92.88	88.50	86.69
	V_2	80.25	72.00	75.88	70.25	74.59
	V_3	61.88	50.00	49.00	50.38	52.81
	V_4	49.88	60.00	58.38	72.25	60.13
LSD		12.90				6.45
S*E						E
	Fe_0	63.19	66.63	64.94	68.75	65.88
	Fe_1	71.75	68.13	73.13	71.94	71.23
LSD		9.12				4.56

Germination capacity: GC %

Table (3) represents the varieties effect on the germination capacity, which appears the superiority of the variety V_3 (95.00) on other varieties, no significant differences between the other varieties V_1, V_2 and V4.

The negative effect of salinity on germination capacity, particularly at the first three salinity concentrations compared to the control.

Concerning the interaction iron - varieties on the germination capacity, the results represented that the superiority of the variety V_3 on all the other varieties In the presence or absence of iron. An increase in germination capacity in the presence (88.82) of iron compared to its absence (88.10) in all varieties and salinity levels.

Table 3.
Effect of imbibition of seeds of *Vicia faba* L. in iron on the germination capacity under the effect of sodium chloride salinity

varieties	Element	Salinity levels mmol/L				V*E
		S_0	S_1	S_2	S_3	
V_1	Fe_0	90.83	88.83	86.88	80.40	86.73
	Fe_1	92.96	93.32	82.92	81.83	87.76
V_2	Fe_0	90.81	88.57	83.92	71.80	83.78
	Fe_1	93.32	87.43	91.10	80.71	88.14
V_3	Fe_0	96.25	87.50	97.50	98.75	95.00
	Fe_1	98.75	91.25	96.25	93.75	95.00
V_4	Fe_0	91.25	85.00	88.75	82.50	86.88
	Fe_1	82.50	87.50	82.50	85.00	84.38
LSD		11.63				5.82
S		92.08	88.67	88.73	84.34	
LSD		4.11				V
V*S	V_1	91.90	91.07	84.90	81.12	87.24
	V_2	92.07	88.00	87.51	76.26	85.96
	V_3	97.50	89.38	96.88	96.25	95.00
	V_4	86.88	86.25	85.63	83.75	85.63
LSD		8.23				4.11
S*E						E
	Fe_0	92.29	87.47	89.26	83.36	88.10
	Fe_1	91.88	89.88	88.19	85.32	88.82
LSD		5.82				2.91

Index of germination stress GSI

Table (4) shows the effect of salinity on the germination stress index in the presence of the iron element, the V_2 (136.31) was significantly higher than V_3 and V_4.

The results showed non-significant differences, S1 (120.81) was higher than the other salinity levels, whereas the results of the interaction salinity-iron showed a significant superiority of the S_1 level of V_2 and V_3. As well as a significant difference of V_1 and V_4.

Table 4.

Effect of imbibition of seeds of *Vicia faba* L. in iron on the Index of germination stress under the effect of sodium chloride salinity

	Varieties	Salinity levels mmol/L				V
		S0	S1	S2	S3	
V*s	v_1	98.00	145.00	106.00	108.50	114.38
	v_2	145.00	134.50	75.00	190.75	136.31
	v_3	88.25	103.75	110.50	80.25	95.69
	v_4	96.75	100.00	90.00	96.75	95.88
Lsd	55.91					27.95
S		107.00	120.81	95.38	119.06	
lsd		27.95				

Dry matter stress tolerance index (DMSI)

No great change significantly with the increase in salt stress concentrations in the four varieties (Table 5),

The data analysis of variance of dry matter stress tolerance index revealed the minimum DMSI values were recorded at S_3 (101.56) whereas the maximum values for DMSI were observed under S_1 (110.94) application in all varieties. Highly significant differences among different broad bean varieties, the V_4 maintained the highest value for DMSI (123.38); which was the minimum in V_1 (94.38).

Table 5.

Effect of imbibition of seeds of *Vicia faba* L. in iron on the Dry matter stress tolerance index under the effect of sodium chloride salinity

Var	Varieties	Salinity levels mmol/L				V
		S0	S1	S2	S3	
V*s	v_1	103.50	80.75	97.75	95.50	94.38
	v_2	80.75	105.00	97.25	114.00	99.25
	v_3	135.00	88.25	111.50	103.75	109.63
	v_4	111.75	169.75	119.00	93.00	123.38
lsd	43.76					21.88
S		107.75	110.94	106.38	101.56	
Lsd		21.88				

Salt tolerance index STI

The salt tolerance index at the seedling stage also showed a little genotypic variation. V_4 (98.38) had the highest salt tolerance index while the V_2 (92.56) is the lowest. Therefore, the effects of different salt concentrations on salt tolerance indices of cultivars were of importance. the salt concentrations increased the salt tolerance indices. The lowest value of salt tolerance index was determined at S_0 (88.94) and the largest S_3 (104.31) (Table6).

Table 6.

Effect of imbibition of seeds of Vicia faba L. in iron on the Salt tolerance index under the effect of sodium chloride salinity

Var	Varieties	Salinity levels mmol/L				V
		S0	S1	S2	S3	
V*s	v_1	96.50	93.25	109.75	90.50	97.50
	v_2	93.25	63.50	85.50	128.00	92.56
	v_3	69.50	108.25	90.00	112.00	94.94
	v_4	96.50	118.50	91.75	86.75	98.38
lsd	29.87					14.93

S		88.94	95.88	94.25	104.31	
lsd		14.93				

Seeds vigor SV

The measurement of the average shoot and root length was determined at the end germination stage. After drying the seedlings at 104 °C for 24 hours, the dry weight was determined. Interaction effect of seed iron treatment and salts stress significantly affected the seeds vigor (SV) in the four varieties. the genotype significantly effect on the SV, V_3 have high SV (499.03) followed by V_4, V_2 and V_1 (397.59, 271.19, 255.94) respectively (table7). The SV seed higher in all varieties of seeds treated by iron compound compared to non-treated. The effect of salinity on SV represents high value in S_2 compared to the control and less value in S_1 and S_3.

Table 7.

Effect of imbibition of seeds of *Viciafaba* L. in iron on seeds Vigor under the effect of sodium chloride salinity

Varieties	Element	Salinity levels mmol/L				V*E
		S_0	S_1	S_2	S_3	
V_1	Fe_0	261.25	296.00	238.50	247.50	260.81
	Fe_1	277.00	261.25	223.25	242.75	251.06
V_2	Fe_0	269.25	267.25	302.75	214.25	263.38
	Fe_1	261.25	278.50	305.75	270.50	279.00
V_3	Fe_0	482.75	419.25	549.25	497.25	487.13
	Fe_1	581.25	400.75	552.50	509.25	510.94
V_4	Fe_0	427.50	331.25	430.00	353.25	385.50
	Fe_1	361.50	480.00	455.00	342.25	409.69
LSD		125.06				62.53
S		365.22	341.78	382.13	334.63	
LSD		44.22				V
V*S	V_1	269.13	278.63	230.88	245.13	255.94
	V_2	265.25	272.88	304.25	242.38	271.19
	V_3	532.00	410.00	550.88	503.25	499.03
	V_4	394.50	405.63	442.50	347.75	397.59
LSD		88.43				44.22
S*E						E
	Fe_0	360.19	328.44	380.13	328.06	349.20
	Fe_1	370.25	355.13	384.13	341.19	362.67
LSD		62.53				31.27

DISCUSSION

The increased concentration for any saline types has negative effects on seed germination and seedling growth so that the seeds cannot absorb the water necessary to germinate, due to the increase in the concentration of salts in the medium around the seeds, resulting in high osmotic pressure inhibiting the germination process.

Also, the salinity has a negative effect on the all parameters studied, these results are compatible with Pearson and Bauder (2003), their results confirm the negative effect by raising the osmotic pressure of the medium around the seeds or by the presence of toxic ions in the solution, which affected the embryo germination.

Generally, salinity levels have once been negatively affected, and again positively, as low levels play a catalytic role in accelerating germination, particularly sodium chloride salt (Ganatsas&Tsakaldimi, 2007).

Other effects of salinity include the effect on the activity of a number of germination enzymes, such as the Amylase and Invertase (Almustouri et *al*.2001).

Sodium salts in general and sodium chloride, in particular, are the most efficient elements of salinity, which concentrate on reducing the rate and speed of germination, the length of roots and the vegetative part, dry and fresh weights and the leaf surface. Due to the effect of salinity on a number of important biological processes, including photosynthesis, protein and carbohydrate synthesis, nutrient uptake and transition **(**Elsahookie, 2013**)**.

The increase of salinity in the middle of the growth of the plants decreases the germination percentage with the time necessary to finish the germination. The salts increase the osmotic pressure of the growth medium, thus reducing the amount of water available to be absorbed by the seeds causing inhibition or retardation of germination (Othman et *al*.2006).

For iron, most of its addition has had a positive impact, this agrees with Naghuib et al. (2005) and Farooqi et al (2012), that microelements such as iron, zinc, and manganese are among the mineral constituents of different enzymes and are associated with the metabolism of sugars, photosynthesis and protein synthesis. Where iron has important functions in food metabolism of the plant such as activation of catalase enzymes associated with the enzyme

Supermide dismutase and photosynthesis and chlorophyll content.

Iron (Fe) is involved in the transport of electrons in many ubiquitous metabolic processes such as respiration and photosynthesis and is required as a co-factor of numerous enzymes. Inside seeds, Fe is essential for embryo development (Stacey et *al.* 2002) but might also become toxic at high concentrations.

Iron is an essential micronutrient for all organisms. It is involved in several vital plant functions, including photosynthesis, respiration and chlorophyll biosynthesis. It is a component in heme, Fe-sulfur cluster, and other Fe binding sites. The Fe in different soil types is available in the form of insoluble Fe (III) makes it a scarce element for plant's uptake (Römheld & Marschner, 1986).

All the varieties showed significant resistance to salinity at germination speed. The positive effect was greater in the presence of iron for most results, these results are consistent with the findings of many scientists Matter et Al-Yazal (2002) to determine the response of *Ambrosia maritima* to paper fertilization with some minor elements (Iron, zinc, and manganese) in the form of clavicle and concentrations (0.5, 1.5 and 2.0%). All the concentrations resulted have a significant effect on the plant length, number of plant branches, plant diameter, fresh and dry weight.

Interaction of the iron element with salinity levels shows that the presence of the iron element has a positive effect on these parameters, and the effect of salinity was close and this is what noted by (Gincle,1982), That the impact of salt increase positive to growth and production due to low absorption of toxic salts (NaCl). As a result of increased plasmolysis of absorption cells and reduced permeability of cytoplasmic membranes to ions. Other researchers (Cayuela et *al*. 1995) interpreted this effect to encourage some physiological changes such as increased sugar and organic acids in leaves and seedling roots, which were associated with increased tolerance to salinity. The four varieties differed in their resistance to salts in the presence of the element of iron and increased tolerance to salinity compared to the witness and this is confirmed by many, (Misra & Bansa,1992), a study on Rosemary plant to determine the effect of leaves fertilization spraying with some minor elements (iron, manganese, and zinc), each of them individually or their mixtures with a concentration of (50 mg / L) of each element led to a significant increase in plant height and number of main branches as well as fresh weight and dry weight of grass. The seeds of bean varieties showed varying resistance to salinity levels during germination stages with a slight improvement in the presence of the iron element, as confirmed by Jacoub(1995). The effect of salinity has repercussions on the growth and lengthening of plant members because the increase of the osmotic tension of the soil solution around the root reduces the absorption of water and increases the absorption of salts Which in turn lead to inhibition of enzymatic activity in the growth and expansion of elongation of cells and then weakness in growth.

CONCLUSIONS

From previous results it is clear that the first variety, V 3 Broad recorded the greatest salinity resistance in the presence of iron followed by the third variety, followed by the variety V 4 Aguadulce).

Salinity with these different concentrations have an inhibiting effect on the germination process of bean seeds, and this effect continues until the beginning stage, which is the end of the germination phase.

It was also found that the iron element contributed to salinity resistance when interact with it at all the levels studied. We also expect better results for this element in other concentrations, both for salinity or for iron.

AUTHORS CONTRIBUTION

Laouedj H.: performed the experiment, collect the results, analyzed it, reached the goals; Chougui S.: design of the study under the supervision of the experiment; Chemam Amara D.: computational and statistical study; Kherraz K.: translated the article; Alia Z.: correct writing and expression errors.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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