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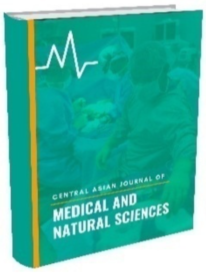

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Effect of NaCl and seed soaking in kinetin on growth and yield parameters of *Vicia faba* L

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Abstract: The experiment was carried out using plastic pots during the growing season 2019-2020 to find out the effect of NaCl (0, 50, and 100) mM and seeds soaking with a growth regulator (kinetin) at a concentration of 0, 50, and 100 $mg.L^{-1}$of faba bean (*Vicia faba* L.)plants and their interactions on some indicators of growth and yield. The experiment included three replications and was designed using a completely randomized design (CRD). The results showed that the effect of NaCl, especially at 100 mM, was significantly reduced in plant height rates, the number of branches, shoot dry weight, number of pods, pod length, and weight, the average weight of 100 seeds, chlorophyll content, protein percentage, Stress Tolerance Index, and Relative Water Content by 15.02%,50.00%,32.81%,44.50%,28.10%, 49.95%,6.83%, 16,82%, 26.36%, 32.82%, and 10.58% compared with control respectively. and that seeds soaking with kinetin, especially at 100 $mg.L^{-1}$ led to a significant increase in the indicators studied, by 11.87%, 89.00%,81.50%, 57.51%, 61.97%, 79.55%, 7.23% 17.66%, 20.78%, 81.51%, and 6.88% respectively, The results also showed a significant effect of the interaction between the study workers on all the studied traits.

Key words: Faba bean; kinetin; seeds soaking; NaCl; growth parameters

1. Introduction

One of the most damaging abiotic factors affecting crop growth and productivity is soil salinity, it was linked to severe economic effects leading to the decrease of large amounts of agricultural land and decreasing crop yields [1,2,3]. It was limiting crop growth and productivity all around the world [3,4]. particularly in arid and semi-arid regions[5]. Salinity hurts plant development and biomass production[4]. Osmotic stress is caused by salinity in plants, which has a significant effect on metabolic processes and causes ionic toxicity later on due to the over-absorption of Na and Cl ions in cells [6]. The negative osmosis potential of salinity limits the amount of water available to plant roots, In addition, ionic toxicity of Na and Cl reduces the germination dynamics of plant seeds[7]. Salinity inhibits plants' ability to absorb water, resulting in decreased development and metabolic processes equal to those produced by water stress[8]. Photosynthesis disruption and oxidative stress are caused by salinity-induced water insufficiency and nutritional deficiencies such as Ca^{2+}, K^{+}, Fe^{2+}, and Zn^{2+} shortages in plants [9]. Plants exposed to salinity suffer from increased oxidative stress as a result of the generation of excess reactive oxygen species (ROS) [10], Various cellular components, such as lipids, proteins, and nucleic acids, suffer oxidative damage, as a result, deactivating a range of different cellular processes in plants [11,12]. Several studies have found that salinity stress reduces growth and inhibits developmental processes in a variety of ways, including osmotic imbalance, cytotoxicity caused by excrescent Na^{+} and Cl^{-}, and nutritional irregularity. [13,14]. Different plants, such as the faba bean[15, 16] and wheat[17,18], have shown that salinity stress harms growth, physiological traits, and productivity. To reduce the harmful effects of salinity on plant growth; Several strategies have been implemented to improve plant performance under saline stress conditions, including the use of plant hormones as one of the most important factors in stimulating the plants' response to a different medium, stresses such as salinity stress, plant growth hormones have been found to play a role in regulating growth and development by mediating a wide range of stress responses [5]. exogenous plant growth hormones, such as cytokinins, have been found to increase agricultural crop yield potential in field conditions[19-21]. Water uptake, cell division, chlorophyll synthesis, and organ development are all known to be activated by cytokinins, resulting in rapid shoot tissue regeneration and proliferation[22,23]. Kinetin is a cytokinin that has been shown to significantly improve the growth of crop plants grown in salinity. many studies have been conducted on kinetin's ability to improve plant growth under salt stress by reducing sodium toxicity and increasing potassium levels inside the plant, in addition to kinetin's role in enzyme activation[24,25]. Through its interaction with other growth hormones, it has been reported to improve soybean salt tolerance[26]. Kinetin treatments reduced the detrimental impacts of salty water by improving root dry weights and NPK content in leaves, as well as enhancing POX and CAT activity and decreasing sodium levels in leaves. [7] .

Vicia faba L., which belongs to the Fabaceae family, is the world's third-largest legume crop and is grown as a cool-season legume in more than 60 countries[27]. It was one of the most important and widespread leguminous crops in the world, due to the importance of its green pods or its fresh or dry seeds and being a green crop for animals, as it is a major source of protein[28]. It was the largest pod crop with a high protein percentage ranging from 25 to 40%, providing it an important source of human food. It also has a high carbohydrate content, making it an essential component of the food supply of poor economies[29]. faba seeds are high in dietary fiber, nutrients, vitamins, lipids, aminobutyric acid, and phenolic compounds, all of which nourish humans and have a good impact on the antioxidant system and biological processes [30]. In addition to its role in increasing the nitrogenous compounds in the soil through its coexistence with the root nodule bacteria, thus improving the soil properties[31]. Saline soils significantly limit *Vicia faba* L. productivity in semi-arid areas [32] because of the faba bean plant's importance as a food crop in Iraq's economy on the one hand, and the growth of saline lands; this research aimed to see how salinity and kinetin, a type of plant hormone that regulates growth, influenced some of the bean plant's morphological and physiological traits.

2. Materials and methods

A pot experiment was conducted during the 2019-2020 growing season using a mixture of alluvial soil, The soil was air-dried, then crushed and sieved with a sieve with holes diameter of 2 mm, and it was filled into 5 kg plastic pots. The compound fertilizer NPK (17: 17: 17) was added by 1.6 g per pot. faba bean seeds were selected equal in size and shape, The seeds were washed with distilled water, then sterilized with 1% sodium hypochlorite solution for 3 minutes, then washed again with distilled water, and the seeds were soaked in a solution of kinetin at a concentration of 0, 50 and 100 $mg.L^{-1}$ for 12 hours at room temperature, distilled water was used for the control treatment. Then the seeds were air-dried and planted on 21/11/2019, with five seeds in each pot. Sodium chloride solution of 0, 50, and 100 mM was used as perfusion solutions.

The experiment was carried out according to a completely randomized design(CRD) with three replications. At the end of the agricultural season, measurements of vegetative growth indicators were taken, including plant height (cm), the number of branches, and the dry weight of the shoots after drying them in an electric oven at 65°C until the weight was stable. The plant's yield characteristics, such as the number of pods on the plant, the length and weight of the pod, and the weight of 100 seeds, were also calculated. Some physiological characteristics were also studied, as the total chlorophyll content was estimated by the Minolta (spad) chlorophyll measuring device, and the percentage of protein in the seeds was estimated according to the method [33] by calculating the percentage of nitrogen using a *Kjeldahl method* after digesting a known weight of the seeds, then the protein percentage was calculated according to the equation:

Protein % = Nitrogen % x 6.25.

The effect of salt stress on growth characteristics (shoot dry weight) was assessed using a Stress Tolerance Index (STI) computed as a percentage of the control [34], equation as follows :

$$STI\ (\%) = [(DW_{stressed}) / DW_{control}] \times 100$$

Where $DW_{stressed}$ and $DW_{control}$ are the mean values of the shoot dry weight in stressed and unstressed conditions, respectively.

Relative Water Content (RWC) was estimated by cut leaves weighed (Fresh Weight, FW), then soaked in distilled water for 24 hours inside a covered petri dish, and their Turgid Weights (TW) were measured. Leaf samples were placed in an oven at 80°C for three hours to get the Dry Weight(DW) [35], and using the following equation :

$$RWC\ (\%) = [(FM - DM)/(TM - DM)] \times 100$$

The results were statistically analyzed by adopting the averages of the coefficients using the least significant difference test (LSD) at the probability level of 0.05[36].

3. Results

The results in Table 1 showed that when the concentration of sodium chloride increased, the rate of plant height reduced dramatically; It decreased from 51.78 cm to 44.00 cm, with a decrease of 15.02%. kinetin played a role in decreasing the detrimental effects, with an increased rate of 11.87%, the plant height rate increased significantly, at a concentration of 100 $mg.L^{-1}$, the highest plant height was 50.34 cm compared to 0 of kinetin. the interaction between the two study factors on this trait was significant, and the highest value of plant height reached 54.67 cm at a concentration of 0 of NaCl and a concentration of 100 $mg.L^{-1}$ of kinetin.

Table 1- Influence of NaCl and Kinetin soaked seeds on plant height (cm).

Kin ($mg.L^{-1}$)	NaCl (mM)			Mean
	0	50	100	
0	49.00	44.33	41.67	45.00
50	51.67	48.33	45.67	48.56
100	54.67	51.67	44.67	50.33
Mean	51.78	48.11	44.00	
LSD 0.05	Kin = 0.63	NaCl =0.63	Interaction =1.09	

The negative effects of NaCl on plant growth led to a decrease in the number of plant branches, The results in Table 2 indicated that increasing the concentration of NaCl led to a significant decrease in the average number of branches per plant, with a decrease of 50.00%. When the seeds were soaked in kinetin solution, the number of branches increased significantly by 89.00 %. The interaction between the two study factors was insignificant, with the highest value for this trait at 4.67 branches per plant. at concentration 0 NaCl and concentration 100 mg. L^{-1} kinetin, At a concentration of 100 mM NaCl and no kinetin, the lowest value for each plant was 1.33 branches.

Table 2- Influence of NaCl and Kinetin-soaked seeds on the number of branches.

Kin ($mg.L^{-1}$)	NaCl (mM)			Mean
	0	50	100	
0	2.67	2.00	1.33	2.00
50	4.00	3.33	1.67	3.00
100	4.67	4.00	2.67	3.78
Mean	3.78	3.11	1.89	
LSD 0.05	Kin =0.57	NaCl =0.57	Interaction = N.S	

The data in Table 3 shows that increasing the NaCl concentration led to a significant reduction in the average shoot dry weight, with a decrease of 32.81%. When Kinetin's concentration was increased, it played a positive role in increasing the rate of this trait, The dry weight's average length increased significantly by 81.50 %. the interaction between the two factors of the study was significant, as the highest value for this trait was 17.75 g at 0 NaCl and 100 $mg.L^{-1}$ of kinetin compared with the lowest value for this trait 7.08 g at 100 mM NaCl and 0 kinetin concentration.

Table 3- Influence of NaCl and Kinetin soaked seeds on shoot dry weight (g).

Kin ($mg.L^{-1}$)	NaCl (mM)			Mean
	0	50	100	
0	9.85	8.21	7.08	8.38
50	14.27	10.21	8.70	11.06
100	17.75	15.54	12.35	15.21
Mean	13.96	11.32	9.38	
LSD 0.05	Kin =0.05	NaCl =0.05	Interaction =0.08	

The data in Table 4 showed that the number of pods was decreasing at a significant rate as the NaCl concentration was increased, with a decrease of 44.50%. The kinetin was found to play a positive role in increasing the number of pods produced, the mean of this trait increased by 57.51 %. The interaction between the two factors in the study was insignificant, with a maximum value of 4.67 for this trait at 0 NaCl and 100 mg. L^{-1} of kinetin compared with the lowest value for this trait at 100 mM NaCl and 0 kinetin.

Table 4- Influence of NaCl and Kinetin soaked seeds on the number of pods.

Kin (mg.L^{-1})	NaCl (mM)			Mean
	0	50	100	
0	3.33	2.33	1.33	2.33
50	4.00	3.67	2.67	3.44
100	4.67	3.67	2.67	3.67
Mean	4.00	3.22	2.22	
LSD 0.05	Kin =0.63	NaCl =0.63	Interaction = N.S	

The data in Table 5 shows that increasing the NaCl concentration led to a significant reduction in the average length of pods, with a decrease of 28.10%. When Kinetin's concentration was increased, it played a positive role in increasing the rate of this trait, The pod's average length increased significantly by 61.97 %. the interaction between the two factors of the study was significant, as the highest value for this trait was 15.67 cm at 0 NaCl and 100 mg.L^{-1} of kinetin compared with the lowest value for this trait 7.33 cm at 100 mM NaCl and 0 kinetin concentration

Table 5- Influence of NaCl and Kinetin soaked seeds on length of pods (cm).

Kin (mg.L^{-1})	NaCl (mM)			Mean
	0	50	100	
0	9.67	8.33	7.33	8.44
50	12.67	11.33	8.67	10.89
100	15.67	14.00	11.33	13.67
Mean	12.67	11.22	9.11	
LSD 0.05	Kin = 0.54	NaCl =0.54	Interaction =0.93	

The results in Table 6 showed that increasing the NaCl concentration caused a significant decrease in the rate of pod weight, with a decrease of 49.95%. while there was a significant increase in the average pod weight when increasing the concentration of kinetin with an increase of 79.55%. As for the interaction between the two factors of the study, it was significant and the highest value for this characteristic was 11.67 g at 0 NaCl and 100 mg. L^{-1} of kinetin compared to the lowest value for this characteristic of 3.33 g at a concentration of 100 mM NaCl and 0 of kinetin.

Table 6- Influence of NaCl and Kinetin soaked seeds on pod weight (g).

Kin (mg.L^{-1})	NaCl (mM)			Mean
	0	50	100	
0	6.67	4.67	3.33	4.89
50	9.67	7.67	5.00	7.44
100	11.67	9.00	5.67	8.78
Mean	9.33	7.11	4.67	
LSD 0.05	Kin = 0.50	NaCl =0.50		Interaction =0.87

The negative effect of NaCl on plant growth and yield components (Tables 5,6) resulted in a significant reduction in average seed weight; Table 7 shows that increasing the sodium chloride concentration reduced the average weight of 100 seeds significantly, with a decrease of 6.83%. The rate of this trait increased significantly when the kinetin concentration was increased with an increase of 7.23%. As for the interaction between the two factors of the study, it was insignificant, and the highest value for this characteristic was 74.67 g at 0 NaCl and 100 $mg.L^{-1}$.kinetin concentration compared to the lowest value for this characteristic 64.33 g at 100 mM NaCl and 0 kinetin concentrations.

Table 7- Influence of NaCl and Kinetin soaked seeds on the weight of 100 seeds (g).

Kin ($mg.L^{-1}$)	NaCl (mM)			Mean
	0	50	100	
0	71.00	67.67	64.33	67.67
50	74.00	71.00	69.67	71.56
100	74.67	72.33	70.67	72.56
Mean	73.22	70.33	68.22	
LSD 0.05	Kin =0.93	NaCl =0.93	Interaction = N.S	

Also, when the NaCl concentration was increased, the average total chlorophyll content in the plant decreased significantly, This is indicated by the results of Table 8 with a decrease of 16.82%. While there was a significant increase in the rate of this trait by 17.66% when the seeds were treated with kinetin, the highest rate for this trait was at the concentration of 100 mg. L^{-1} of Kinetin. As for the effect of the interaction between the two factors of the study, it was insignificant, and the highest value of chlorophyll content was 42.33 at 0 NaCl and 100 mg .L^{-1} kinetin.

Table 8- Influence of NaCl and Kinetin soaked seeds on chlorophyll content.

Kin ($mg.L^{-1}$)	NaCl (mM)			Mean
	0	50	100	
0	35.00	33.00	30.00	32.67
50	39.67	35.67	32.33	35.89
100	42.33	38.00	35.00	38.44
Mean	39.00	35.56	32.44	
LSD 0.05	Kin = 0.83	NaCl =0.83	Interaction = N.S	

The negative impact of NaCl on plant growth harms the protein percentage of seeds. The results in Table 9 indicated that there was a significant decrease in the percentage of protein by 26.36%. There was a significant increase in the rate of this trait when increasing the concentration of kinetin with an increase of 20.78%. As for the interaction between the two factors of the study, it had a significant effect on the average of this trait, and the highest value for this trait was 23.96% at 0 NaCl and 100 $mg.L^{-1}$ kinetin compared to the lowest value for this trait 14.08% at 100 mM NaCl and 0 kinetin concentrations.

Table 9- Influence of NaCl and Kinetin soaked seeds on protein percentage (%).

Kin ($mg.L^{-1}$)	NaCl (mM)			Mean
	0	50	100	
0	20.87	17.14	14.08	17.37
50	22.42	18.31	16.70	19.24
100	23.96	20.54	18.46	20.98
Mean	22.42	18.66	16.51	
LSD 0.05	Kin = 0.08	NaCl =0.08	Interaction =0.14	

The results in Table 10 showed that increasing the NaCl concentration caused a significant decrease in the rate of stress tolerance index, with a decrease of 32.82% compared to the control. while there was a significant increase in the average STI when increasing the concentration of kinetin with an increase of 81.51%. As for the interaction between the two factors of the study, it was significant and the highest value for this characteristic was 180.15% at 0 NaCl and 100 mg. L^{-1} of kinetin compared to the lowest value for this characteristic of 71.92% at a concentration of 100 mM NaCl and 0 of kinetin.

Table 10- Influence of NaCl and Kinetin soaked seeds on Stress Tolerance Index (%).

Kin ($mg.L^{-1}$)	NaCl (mM)			Mean
	0	50	100	
0	100.00	83.29	71.92	85.07
50	144.89	103.62	88.33	112.28
100	180.15	157.71	125.37	154.41
Mean	141.68	114.87	95.21	
LSD 0.05	Kin =0.70	NaCl =0.70	Interaction =1.22	

Also, when the NaCl concentration was increased, the average RWC decreased significantly, This is indicated by the results of Table 11 with a decrease of 10.58%. While there was a significant increase in the rate of this trait by 6.88% when the seeds were treated with kinetin, the highest rate for this trait was at the concentration of 100 mg. L^{-1} of Kinetin. As for the effect of the interaction between the two factors of the study, it was significant, and the highest value of RWCt was 73.27% at 0 NaCl and 50 mg .L^{-1} kinetin.

Table 11- Influence of NaCl and Kinetin soaked seeds on Relative Water Content (%).

Kin ($mg.L^{-1}$)	NaCl (mM)			Mean
	0	50	100	
0	69.66	67.04	59.84	65.51
50	73.27	71.02	65.91	70.06
100	71.93	71.75	66.38	70.02
Mean	71.62	69.93	64.04	
LSD 0.05	Kin =0.37	NaCl =0.37	Interaction =0.65	

4. Discussion

Because of the high osmosis of the soil solution the decrease in the water potential of its solution and the toxicity by specific ions, especially sodium and chloride[37], the rate of the studied growth indicators decreases when the concentration of sodium chloride in the growth medium is increased, which negatively affects the available water for the plant, thus disturbing the metabolic processes in it, and inhibiting the activities of meristematic tissues, cell division, and cell elongation, thus causing a weakness in the growth of the total root and vegetative [38,39]. Moreover, a decrease in photosynthetic efficiency due to an increase in reactive oxygen species (ROS), oxidizes the chloroplasts' internal structures and reduces the internal stroma, affecting the work of its enzymes and lowering the efficiency of the photosynthesis process[40]. These results are by[41], which showed that salinity harmed vegetative growth traits. These results are similar to the findings of[17, 18].

The effect of kinetin soaking in reducing salt toxicity and increasing potassium concentration is one of the positive effects of kinetin soaking on growth characteristics, which stimulates metabolism and activates enzymes, resulting in increased plant growth[42]. Its ability to stimulate cell division and root development, prevent leaf aging, and increase chlorophyll pigment content has a role in plant growth and development. [43]. as well as increased cell division in apical meristems and cambium[44]. These results are similar to the findings of[17], which found that soaking the seeds in kinetin resulted in significant enhancements in most of the parameters studied.

5. Conclusion

Finally, we found that soaking seeds with Kinetin resulted in a significant increase in growth indicators when exposed to salt stress, Kinetin has been shown to reduce salt stress by reducing the severity of the salinity effect.

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