

AMELIORATIVE EFFECTS OF POTASSIUM ON THE NODULATION, LEGHEMOGLOBIN, AND FLAVONOID OF FABA BEAN (*VICIA FABA* L.) UNDER SALINITY STRESS

SAAID hadjer^{1*} and CHOUGUI saida¹

1. University of Frères Mentouri Constantine - Faculty of Natural and Life Sciences -Department of Biology and Plant Ecology-Laboratory of Ecology. Algeria

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Abstract

Description of the subject: High NaCl in soil and water irrigation impair K⁺ nutrition of plants, it is suggested that K⁺ deficiency at the cellular level might be a contributory factor to salt-induced oxidative stress and related cell damage. Therefore, improving K⁺ nutrition of plants under salt stress could be essential to minimizing oxidative cell damage.

Objective: The objective of the research was to evaluate the effect of potassium fertilizer on nodulation and some biochemical parameter of broad bean (*Vicia faba* L. cultivar Hista) under salt stress.

Methods: The experiment was conducted under greenhouse, broad bean seedlings were irrigation with NaCl (S0:0, S1:25, S2:50, S3:150 mM), treated with potassium acetate CH₃CO₂K (A0:0, A1:25, A2:50 mM). After 50 days of growing, we have measured nodulation and some biochemical parameters.

Results: The results showed that the application of potassium acetate is removed deleterious effects of salinity more appreciably at 50 mM in salinity levels (25, 50 mM NaCl). As the number of nodules and their dry weight, content of leghemoglobin in nodules, and total flavonoids in roots increased, with reduced accumulation of proline and soluble sugars in roots.

Conclusion: The nodulation of broad bean cultivar showed tolerance to salinity within 50 days of growing. The application of potassium acetate in concentration (50 mM) ameliorated the salinity resistance better than concentration (25 mM) during this period.

Keywords: Flavonoïd; leghemoglobin; nodule; potassium; salinity; *Vicia faba* L.

LES EFFETS AMÉLIORANTS DU POTASSIUM SUR LA NODULATION, LA LÉGHÉMOGLOBINE, ET LE FLAVONOÏDE DE LA FÈVE (*VICIA FABA* L.) SOUS STRESS SALIN

Résumé

Description du sujet : la teneur élevée en NaCl dans le sol et l'eau de l'irrigation nuisent à la nutrition K⁺ des plantes, il est suggéré qu'une carence en K⁺ au niveau cellulaire pourrait être un facteur contributif au stress oxydatif induit par le sel et aux dommages cellulaires associés. Par conséquent, l'amélioration de la nutrition K⁺ des plantes sous stress salin pourrait être essentielle pour minimiser les dommages cellulaires oxydatifs.

Objectifs: L'objectif de la recherche était d'évaluer l'effet de l'engrais potassique sur la nodulation et quelques paramètres biochimiques de la fève (*Vicia faba* L. cultivar Hista) sous stress salin.

Méthodes: L'expérience a été conduite sous serre, les semis de la fève ont été irrigués avec NaCl (S0:0, S1:25, S2:50, S3:150 mM). Traité avec acétate de potassium CH₃CO₂K (A0:0, A1:25, A2:50 mM). Après 50 jours de culture, nous avons mesuré la nodulation et quelques paramètres biochimiques.

Résultats: Les résultats ont démontré que l'application d'acétate de potassium élimine les effets délétères de la salinité plus significative à 50 mM dans les niveaux de salinité (25, 50 mM NaCl). À mesure que le nombre de nodules et leur poids sec, la teneur de léghémoglobine dans les nodules, et les flavonoïdes totales dans racines augmentait, avec une accumulation réduite de proline et de sucres solubles dans les racines.

Conclusion : La nodulation de cultivar de la fève a montré une tolérance à la salinité pendant 50 jours de la culture. L'application d'acétate potassium en concentration (50 mM) a amélioré la résistance à la salinité mieux que la concentration (25 mM) pendant cette période.

Mots clés : Flavonoïde ; léghémoglobine; nodule; potassium; salinité ; *Vicia faba* L.

* Auteur correspondant: SAAID Hadjer, E-mail: saidhadjer 84@gmail.com

INTRODUCTION

Leguminous plants are the major source of proteins in human and animal nutrition mainly in developing countries [1]. Many legumes interact symbiotically with nitrogen-fixing soil bacteria, collectively called rhizobia; rhizobium-legume symbioses involve the formation of root nodule [2]. The formation of root nodule is initiated with a chemical signal exchange between host plant and rhizobia, specific flavonoids are secreted by the host roots, play a role in chemo-attraction of rhizobia toward the root, induce the transcription of bacterial nodulation genes (nod genes). The induction of these nod genes leads to the synthesis of specific lipo-oligosaccharides (called Nod factors) that can induce various root responses, e.g. root hair deformation, depolarization of the root hair membrane potential, induction of nodulin gene expression, and formation of nodule primordia [3 and 4]. Biological nitrogen fixation process in the nodules, is catalyzed by the nitrogenase enzyme system which is sensitive to oxygen, where the legumes produce protein of leghemoglobin to carry away any oxygen that would inhibit nitrogenase activity [5]. Salinity is a major concern for irrigated agriculture in arid and semi-arid regions of the world [6]. High salt content, especially high Na^+ accumulation significantly reduces growth and productivity of glycophytes, plant species belong to this category [7]. In legumes, being a glycophytes, salinity effects overall plant growth and development [8], by disturbing nutritional homeostasis, vital physiological functions and complex interaction of hormones, osmotic effects and specific ion toxicity [9]. Soil salinity reduces survival and growth of rhizobia in the soil [10], and reduces the nodulation of legumes by inhibiting the very early symbiotic events, and impairment of active nodule functioning [11], reduced numbers and weight of root nodules [12], reduction in cytosolic protein production specifically leghemoglobin by nodules [13], and secretion of flavonoid by root legume is reduced by salt stress [14].

On the other hand, there are solutions to remove the harmful effect of salinity on the plant among these solutions are fertilization and special potassium fertilization. Potassium is the most versatile nutrient in plants, highly mobile and involved in numerous regulation mechanisms, for example N metabolism, sugar transport, water utilization,

stress resistance and osmotic adjustment [15]. Sodium-potassium relationship may be synergistic or antagonistic depending on the ratio between them [16]. The synergistic effects of sodium and potassium they appear when the sodium concentration is low, where Mary and Emmanuel [17], observed that the application of 2–4 mg Na^+ kg^{-1} soil with 32–64 mg K^+ kg^{-1} soil increased fruit yield of tomato by about 100%, and increased number and area of leaves increased the growth, number of flowers. As for the antagonistic effects of sodium and potassium appear when the presence high concentration of Na^+ in the nutrient medium, it hinders potassium absorption by the plant [18]. So that sodium competes with the essential nutrient ion K^+ in the process of transport across the cell membrane during uptake, making it difficult for plants to obtain the K^+ , again high level of Na^+ can cause imbalance in the uptake and utilization of other cations [19]. Maintaining growth of the plant under salt stress is related to its ability to maintain cellular turgor at low osmotic potential and maintaining low cytosolic Na^+ contents with high K^+/Na^+ ratios [20]. It also creates organic compounds generically named compatible solutes or osmolytes, among them is proline and soluble sugars [21].

As for the leguminous plants potassium is considered necessary for proper development and functional longevity of root nodules as it helps in the translocation of sufficient amount of photosynthesis to roots nodules, and total nitrogen accumulation in the plant increased with increasing K^+ supply [22]. Potassium being the most abundant intracellular ratios, makes a major contribution to the turgor pressure of the cells, playing important roles in bacterial osmo-adaptation, pH regulation, gene expression [23], and activates more than 60 enzyme systems, including the nitrogenase enzyme which is essential for N_2 –fixation, and had a significant effect on nodule number and nodule dry weight [24]. The objective of the present research work was to investigate the effect of salinity and K^+ fertilizer application on nodulation and leghemoglobin in nodules, total flavonoid in roots, proline and soluble sugars in roots of (Hista) Spanish cultivar of broad bean (*Vicia faba* L.).

MATERIAL AND METHODS

1. Plant materials and growth conditions

The research was carried out under greenhouse conditions from November 2017 to January 2018 in agricultural land in the region of Jijel – Algeria. “Hista1” Spanish cultivar of broad bean was selected for this study. Broad bean seedlings irrigated separately in the soil with NaCl solution of (S0:0, S1:25, S2:50, S3:150) mM three times a week, and they were treated separately in the soil with potassium acetate CH₃CO₂K (A0:0, A1:25, A2:50) mM for once a week. Plants were harvested after 50 days (beginning of flowering) for sampling (Table1).

Table 1: Distribution of levels the salinity (NaCl) and potassium acetate (CH₃CO₂K).

Salinity	Potassium acetate		
	A0	A1	A2
S0	S0A0	S0A1	S0A2
S1	S1A0	S1A1	S1A2
S2	S2A0	S2A1	S2A2
S3	S3A0	S3A1	S3A2

2. The applied analytical study

2.1. Analyses of soil

Soil texture was determined using hydrometric method Bouyoucos [25]. Soil pH and electrical conductivity (EC) were analyzed using a saturated paste extract, deionized water was added to ground and sieved soil and mixed uniformly until a saturated paste was obtained. The EC value was measured using an EC meter (Model WTW/720). The pH was measured using a pH meter (model HANNA 211). Soluble Na⁺ and K⁺ were estimated by Jackson [26]. To 5 g of soil sample, 50 ml of ammonium acetate solution was added and kept for shaking on a reciprocating shaker for 15 min; filter the solution by filter paper. Na⁺ and K⁺ concentration calculated by use of the flame photometer (Jenway PFP7) with the help of K⁺ and Na⁺ standard solutions and calibration curve. The ppm reading unit (ppm=mg/l) has been converted to meq/L according to the following formula: $Na^+ \text{ meq/L} = \text{mg/l} / \text{Equivalent weight of the element } Na^+$; $K^+ \text{ meq/L} = \text{mg/l} / \text{Equivalent weight of the element } K^+$

Carbonate (CO₃⁻²) and bicarbonate (HCO₃⁻) soil, and soluble (Ca⁺², Mg⁺²) were estimated by Richards [27]. Pipette 10-15 mL soil saturation extract in to a wide-mouthed porcelain crucible or a 150-mL Erlenmeyer flask, add 1 drop phenolphthalein indicator, as it was noticed that no pink color appeared due

to the absence of carbonate. Then we moved to the second stage to identify the bicarbonate with the same extract by adding 2 drops 0.1% methyl orange indicator, and then titrating with sulfuric acid until the color is orange, indicating the presence of bicarbonate, the reading T was taken. Bicarbonate in the soil was calculated by the following formula: $HCO_3^- \text{ (meq/L)} = T - 2Y \times N / Wt \times 100$. T = the volume of acid used to titration the bicarbonate, Y = the volume of acid used to titration the carbonate, N = Normality of H₂SO₄ solution, Wt = Weight of air-dry soil (g) Soluble Ca⁺² and Mg⁺² are obtained by extracting the soil by water and measurement of their concentrations in the extract by titration with EDTA. As the concentration of Ca⁺² was determined first, then Ca⁺² and Mg⁺² are estimated together. Then we subtract the first from the second to get the Mg⁺² content according to the following equations: $Ca^{+2} \text{ meq/L} = V(EDTA) \times N(EDTA) \times (1000/V \text{ liquid})$; $Ca^{+2} + Mg^{+2} \text{ meq/L} = V(EDTA) \times N(EDTA) \times (1000/V \text{ liquid})$; $Mg^{+2} \text{ meq/L} = (Ca^{+2} + Mg^{+2}) - Ca^{+2}$ (Table 2).

Table2: The results of the physico-chemical analyzes of soil.

Parameters	Results	
	value	units
pH	7,8	-
EC	0,35	dS/m
K ⁺	1,65	meq/L
Na ⁺	0,47	meq/L
CO ₃ ⁻²	-	meq/L
HCO ₃ ⁻	2,1	meq/L
Ca ⁺²	2,6	meq/L
Mg ⁺²	1,8	meq/L
Sand	62,5	%
Clay	25	%
Silt	12.5	%

2.2. Number of nodules

The number of nodules was calculated after removing the roots from the soil.

2.3. Dry weight of nodule

The nodules were separated in roots and washed with tap water to remove the adhering sand, kept in paper bags and dried in a drying oven at 80°C until the weight was stable. The oven dried samples were taken and their dry weights were recorded in mg.

2.4. Determination of Leghemoglobin in nodule

Leghemoglobin concentration was determined by the cyanmethemoglobin method by Wilson and Reisenau [28].

50 to 100 mg nodules were collected and it was crushed in 9 volumes of Drabkin's solution in a microfuge tube with a glass rod, and then the tube was centrifuged at 12 000 for 15min. Supernatant was filtered through a 0.2 μm syringe filter. The filtrate was taken in a micro cuvette and its absorbance is noted in a spectrophotometer at wavelength 540 nm. Leghemoglobine content was calculated from the standard curve of hem and using the straight equation, the following equation was obtained: *Leghemoglobin (mg/ g MF of nodule) = DO₅₄₀ - 0.072/0.0056*.

2.5. Determination of roots total flavonoids

The extraction of the flavonoids was carried out according to the Zheng and Wang [29] Protocol, total flavonoids were determined in roots using the method of Ordoñez *et al.* [30]. To 0.5 ml of ethanolic extract, 0.5 ml of 2% AlCl_3 ethanol solution was added, after 1h at room temperature filtered, then the absorbance was measured at 420 nm. Total flavonoids content was calculated as quercetin equivalent (QE) from a calibration curve and using the straight equation, the following equation was obtained: *Total flavonoids (mg QE /g MF) = DO₄₂₀- 0.018/0.034*.

2.6. Determination of roots proline

Proline content was measured by the modified method of Bates *et al.* [31]. Samples 50 mg fresh weight from the roots was each extracted with 2 ml of 40% methanol. 1 ml extract was mixed with 1 ml of a mixture of glacial acetic acid and orthophosphoric acid (6 M) (3: 2, v/v) and 25 mg ninhydrin. After one hour of incubation at 100 °C, the tubes were cooled and 5 ml toluene were added, the absorbance of the upper phase was spectrophotometric ally determined at 528 nm, the determination of proline content is carried out according to standard curve and using the straight equation, the following equation was obtained: *Proline (ug. g-1 MF) = DO₅₂₈- 0.0158/0.0205*.

2.7. Determination of roots soluble sugar

The soluble sugars in roots are determined by the phenol-sulfuric acid method described by Dubois *et al.* [32]. The extraction of the carbohydrate is made by the 80% ethanol. 100 mg of the samples were ground in 5ml ethanol 80%.

the tube is heated to evaporate alcohol to each tube 10 ml of water was added distilled is the solution to be analyzed .1ml solution to be assayed which is added phenol solution of 1ml of 5% and 5 ml of sulfuric acid the tubes are shaken and placed in 5°C for 45 min and 30 min in the dark reading is performed at 485 nm using the formula for the straight line and the standard curve for glucose, we obtained the formula for calculating the soluble sugar concentration: *Soluble sugar (ug. g⁻¹ MF) = DO₄₈₅- 0.0096/0.0155*.

3. Data analysis

Data were analyzed for analysis of variance (ANOVA) with XLSTAT version 2014, and presented as mean of 3 replicates \pm SE, and significance was checked at $p \leq 0.05$.

RESULTS

1. Effect of salinity and potassium on nodulation

The impact of both salinity stress and potassium acetate on nodulation of broad bean cultivar is presented in (table 3). Where the results showed that the nodules number and dry weight of nodules decreased slightly in the salinity concentration (S1=25 mM NaCl) by percent 9.54%, 7.14% compared with control respectively, at (S2=50 mM NaCl) the decrease in nodules number and dry weight of nodules compared to the control was 27.13% and 25% respectively, while the highest decrease was observed at the highest salinity level (S3=150 mM NaCl) by percent 69.84% and 32.14% in nodules number and nodules dry weight of the compared to the control. However in control conditions, potassium acetate increased nodules number and dry weight of nodules with the highest increase in concentration (A2=50 mM $\text{CH}_3\text{CO}_2\text{K}$) by percent of 65.83% for nodules number and 46.42% for dry weight of nodules compared with control. It also had the same effect under saline conditions, where the number of nodule and dry weight of nodule increased with increasing potassium application in each salinity levels (S1, S2, S3), the positive effect of potassium was clear in A2 than that of A1 by percent of 96.65% in concentration S3A2 for number of nodules compared with salt treated plants S3, and by percent of 38.46% in concentration S1A2 for dry weight of nodules compared with salt treated plants S1 (Table 3).

Table 3: Nodules number and nodules dry weight of broad bean cultivar responses at potassium acetate fertilizer (A0=0, A1=25, A2=50 mM CH₃C₂K) under different salinity levels (S0=0, S1=25, S2=50, S3=150 mM NaCl).

Levels of salinity and potassium	Nodulation parameters	
	Nodule number (number/plant)	Nodule dry weight (mg/plant)
S0A0	66.33±1.71***	0.28 ±0.03**
S0A1	84.0±3.33***	0.33±0.027***
S0A2	110.0±4.66***	0.41±0.095***
S1A0	60.0±1.33***	0.26±0.035**
S1A1	67.66±2.01***	0.29 ±0.025***
S1A2	75.33±1.87**	0.36±0.041***
S2A0	48,33±1,77***	0.21±0.071 ^{ns}
S2A1	57.0±2.00***	0.23±0.055*
S2A2	64.66±1.95***	0.27±0.058***
S3A3	20.0±1.12***	0.14±0.047**
S3A1	27.0±1.53***	0.16 ±0.052*
S3A2	39.33±1.63**	0.19 ±0.056**

Values presented are means ± SE (standard error), *, **, ***Significant at $p = 0.05$, $p \leq 0.01$, $p \leq 0.001$ respectively, ns = not significant

2. Effect of salinity and potassium on total flavonoids and leghemoglobin

The treatment effect of salinity and potassium acetate on total flavonoids in roots and leghemoglobin in nodules is shown in (Table 4). Whereas the levels of salinity (S1=25, S2=50, S3=150) reduced total flavonoids content in roots and the concentration of leghemoglobin in nodules, the highest decline observed in S3 by percent 57.71% and 55.30% respectively compared to the control. Conversely, the levels

of potassium (A1=25, A2=50 Mm CH₃CO₂K) increased the content of total flavonoids and leghemoglobin both in control conditions and presence of salinity, where the highest increase with presence of salinity was the concentration S3A2 by percent 71.88% at total flavonoids, and 35.54% at leghemoglobin compared with salt treated plants S3, and with concentration S0A2 by percent 32.20 % at total flavonoid, and 28.27 % at leghemoglobin in control conditions (Table 4).

Table 4: Biochemical responses of broad bean cultivar at potassium acetate fertilizer (A0=0, A1=25, A2=50 mM CH₃C₂K) under different salinity levels (S0=0, S1=25, S2=50, S3=150 mM NaCl).

Levels of salinity and potassium	Biochemical parameters			
	Leghemoglobine (mg/g MF)	Total flavonoids (mg QE/g MF)	Proline (ug. g-1 MF)	Soluble sugars (ug. g-1 MF)
S0A0	37.14±1.83**	12.70±2.19***	9.22 ±0.83***	12.11±2.37***
S0A1	42.85±2.39***	14.32±2.13***	8.52±0.79**	11.75±2.01***
S0A2	47.64±2.31***	16.79±2.05***	8.41±0.40**	11.02 ±1.83***
S1A0	31.78±2.08**	11.08±1.12***	12.33±0.98***	15.28±1.91***
S1A1	35.38±1.99**	12.58±1.61***	10.51±0.67***	13.32±2.88***
S1A2	39.20±2.12***	13.87±1.84***	9.36±0.95***	11.36±2.45***
S2A0	27.50±1.91*	9.46±2.12*	18.98±1.34*	26.07±2.17 ^{ns}
S2A1	29.64±2.28*	11.14±1.48**	15.71±1.77***	19.32±2.00**
S2A2	34.75 ±1.89**	12.65±2.56***	10.53 ±1.75***	12.67±2.07***
S3A0	16.60±1.95*	5.37±2.01*	27.57±1.79***	39.21±2.09***
S3A1	18.39±1.98 ^{ns}	6.64±1.54**	25.83±2.03***	35.46±2.93*
S3A2	22.50±1.95***	9.23±1.81**	21.33 ±1.9*	27.53±2.74**

Values presented are means ± SE (standard error), *, **, ***Significant at $p = 0.05$, $p \leq 0.01$, $p \leq 0.001$ respectively, ns = not significant

3. Effect of salinity and potassium on proline content and soluble sugars content in root

The results in (table 4), indicated that (S1=25, S2=50, S3=150) of salt caused increased in proline and soluble sugars content in roots, where it reached the highest increase in the S3=150 by percent of 199.02 %, 223.78 %

respectively as compared with control. As for the addition of potassium under saline conditions led to a decline of proline and soluble sugars in each salinity levels (S1, S2, S3), the concentration of potassium (A2=50 Mm CH₃CO₂K) was most effective in reducing the accumulation of proline and soluble sugars,

the highest decline observed in S2A2 by percent of 44.52 % for proline and 51.50 % for soluble sugars compared with salt treated plants S2. In control conditions, the concentration of proline and soluble sugars did not change significantly compared with control by percent 7.59 % in S0A1 and 8.78 % in S0A2 for proline, and 2.92 % in S0A1 and 9 % in S0A2 for soluble sugars (Table 4).

DISCUSSION

Potassium is an important fertilizer involved in numerous biochemical and physiological processes vital to plant growth, development, yield, quality of plants, it also controls in certain cases the osmotic adjustment in plants under various stressed conditions [33]. Under saline conditions potassium has a positive role in plant growth, because this element plays an essential role in stomata movement, photosynthesis and regulation of osmotic pressure for plant, energy status, charge balance, protein synthesis and homeostasis [34]. Therefore, we studied the efficiency of potassium acetate fertilizer ($\text{CH}_3\text{CO}_2\text{K}$) under salinity stress conditions on nodulation and biochemical parameter of broad bean. In our work, high concentrations of NaCl (150 mM) significantly decreased the nodulation of the broad bean cultivar, measured in reduction in the number and dry weight of nodules, this could be due to the adverse effects on the process of nodule initiation, an event in rhizobium-legume symbiosis which is very sensitive to osmotic stress [35]. Similar results obtained by Babber *et al.* [36], who reported that the nodulation of Chickpea (*Cicer arietinum* L.) was reduced by salinity levels. Use of potassium acetate as fertilizer, improved of number and dry weight of nodules in both presence of salt stress and in control conditions, the increase in the number and dry weight of nodules is due to the role of potassium in the stimulation and transfer of carbohydrate synthesis enzymes, as well as in the transfer of carbohydrates to the root nodes [37], these findings are in agreement with those found by Sanghmitra *et al.* [38], where they observed an increase in number and dry weight of nodule as potassium levels increased in lentils (*Lens culinaris* L.).

As observed for leghemoglobin content in nodules was also significantly influenced by irrigation water salinity, this due to aging and irreversible oxidation of legheamoglobin [39].

Likewise, the total flavonoids content in roots has been negatively affected by high salinity concentrations, is related from adverse effect of salt toxicity on roots and soil pH, and salinity stress alters roots morphology and roots hair traits [40], this is because of the responsible roots for the secretion of flavonoids in legume, which are have a key role in nodulation [41]. Our findings are accordance with those findings of Nabizadeh *et al.* [42], who reported that leghemoglobin content in nodules decreased as the salinity levels increased. Dardanelli *et al.* [43], note the low concentration of roots flavonoids with increasing levels of salinity. On the other hand, an improvement of both leghemoglobin and flavonoid concentration was observed by addition of potassium acetate, this is due to the role of potassium in mitigates the negative effects of salt stress by enhancing the translocation and bringing water balance maintenance, it also K^+ is actively implicated in several basic biochemical and physiological functions such as stoma movement, enzyme activation, protein synthesis, osmoregulation, and in reduction of excess uptake of ions like Na^+ [44 and 45].

While the concentration of both proline and soluble sugars in roots increased with increasing concentrations of salinity, the reason this increasing is due to proline contributes to osmotic adjustment, stabilization and protection of membranes integrity and macromolecules from the damaging effects of salinity and as hydroxyl radical scavenger [46]. Kishor *et al.* [47], explained that the reason for proline accumulation in the plant under salinity conditions is due to increased activity of proline biosynthesis enzymes such as ornithine aminotransferase and pyrroline-5-carboxylate reductase, as well as due to inhibition of proline degradation enzymes, proline oxidase and proline dehydrogenase, as the role of soluble sugars in osmotic adjustment and maintained turgor for growth under salinity [48]. Kerepsi and Galiba [49], reported that carbohydrates changes in life of wheat under saline condition are important, because of their relationship with such physiological processes as photosynthesis, translocation and respiration.

Nevertheless, the addition of K^+ fertilizer to saline-plants allowed a decrease in the concentration of both proline and soluble sugar in roots. This is because K^+ activates enzymes that work to regulate the balance of nutrients,

it also contributed to increasing plant control over closing and opening stomata, which increases maintaining the amount of moisture in the tissues and maintaining a low osmotic effort, this was reflected in a decrease in the concentration of both proline and soluble sugars [50]. Trotel- Aziz et al. [51], have proposed that proline accumulated under stress conditions is rapidly consumed when stress conditions were alleviated. These results have been confirmed by the results of Kabir et al. [52], on (*Vigna radiata* L.) they mention that there is a decrease in proline and soluble sugars content in leaves when treated with 40 and 60 Mm of potassium sulfate under saline condition.

CONCLUSION

The study of the effect of potassium acetate on nodulation of broad bean cultivar, as well as biochemical parameters (leghemoglobin, total flavonoids, proline and soluble sugars) in saline condition allowed us to evaluate the ameliorative effect that potassium plays on salinity stress. Our results showed that application of potassium acetate had a significant positive effect on the nodulation (the number of nodule and their dry weight), leghemoglobin content in nodule, and total flavonoids content in roots, in both control and salt-stressed plants. The effect of potassium acetate was much more pronounced in concentration 50 mM than in 25 mM. This was demonstrated by decline of stress indicators proline and soluble sugars.

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