

Impacts of Phosphorus as Soil Application on Growth, Yield and some Chemical Constitutes of Common Bean Plants Grown under Saline Soil Conditions

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Abstract. This study aimed to assess the impact of calcium superphosphate (P; 0, 100, and 200 kg per feddan) as soil amendments, in addition to the recommended P, on the growth traits, green and dry yields characteristics, leaf photosynthetic pigments, chlorophyll fluorescence, and leaf contents of nutrients of common bean (*Phaseolus vulgaris* L., cv. “Bronco”) plants grown under saline soil conditions. Two field trials were conducted at the Experimental Farm of Faculty of Agriculture, Fayoum University during the 2016 and 2017 summer seasons. The obtained results showed that, Na⁺ content was significantly declined, while the all other tested parameters such as growth characteristics (i.e., shoot length, number of leaves per plant, area of leaves per plant, and shoot fresh and dry weights), yield characteristics of green pods and dry seeds (i.e., average pod weight, number of pods per plant, pods weight per plant, dry seed weight per plant and 100-seed weight), leaf photosynthetic pigments (i.e., total chlorophylls, total carotenoids) contents and leaf chlorophyll fluorescence (i.e., Fv/Fm and PI), leaf contents of N, P, K⁺, and Ca²⁺, and the ratios of K⁺/Na⁺, Ca²⁺/Na⁺ and K⁺+Ca²⁺/Na⁺ were significantly increased by the two tested P treatments compared to the controls (without more P than the recommended). The all tested treatments conferred, approximately, the same results. Therefore, results of this study recommend using P at 100 kg per feddan above the recommended dose to optimize the common bean performance in saline soils.

Introduction

Food legumes are considered as an important component in promoting sustainable agriculture and human dietary nutrition, worldwide. Legumes are a health-promoting source of protein, especially the common bean (*Phaseolus vulgaris* L.) that constitutes 50% of the total grain legumes consumed globally [1]. Legume cultivation is beneficial to non-legume crops through multiple agro-ecological services such as biological nitrogen fixation, improvement of soil fertility and N-rich green manure [2]. However, the economical, nutritional and ecological services provided by legumes are often compromised by sensitivity to environmental stresses whose increased frequency can reduce major crop production by more than half [3]. *Phaseolus vulgaris* (L.) is one of the most important Fabaceae vegetables produced for human nutrition, particularly in the Middle Eastern, including Egypt. It is classified as a salt-sensitive plant [4].

Soil salinity is one of the major problems of agriculture, particularly in arid and semiarid regions, limiting plant growth and productivity [5]. Salt stress adversely affects plant morphology and physiology through osmotic and ionic stresses, and changes biochemical responses in plants [6]. It causes an overproduction of reactive oxygen species (ROS) such as superoxide (O₂⁻), hydrogen peroxide (H₂O₂) and hydroxyl (OH⁻) radicals. Chloroplasts are the major organelles that produce the ROS during photosynthesis [7,8]. The ROS cause damages for lipids, proteins and DNA [9]. They also cause chlorophyll degradation and membrane lipid peroxidation [10]. Removal of the toxic ROS rapidly is important in any defense mechanism. This elimination occurs through

antioxidant defense systems [11]. There are several reports underlining the intimate relationship between the activity of antioxidant systems and increased tolerance to environmental stresses [5,8]. Differences in the accumulation patterns of Na^+ and K^+ are found under salinity stress. Salt tolerant species maintain a high K^+ content accompanied by a higher K^+/Na^+ ratio [5,8].

Soil application of phosphorus (P) can positively change the nutritional imbalance [12], optimizing the nutrient uptake capacity [5]. Nutrient uptake limitation under adverse conditions, particularly P assimilation, is one of the most limiting factors decreasing biomass and grain yield in *P. vulgaris* [13]. This nutrient deficiency is widespread with greater than 30% of the world's arable land affected by a P limitation [14]. The interaction between salinity and P positively affects plant growth and yield [5]. By increasing P application, there is an increased salt tolerance in plants [15]. Legumes are highly P-demanding crops; thus, P limitation negatively affects growth and symbiotic N_2 fixation [16]. It has been hypothesized that legumes respond positively to increasing P supply under soil salinity and P-deficiency conditions. Increasing P supply can buffer legume performances against the soil salinity effects, particularly decreases in essential nutrients' absorptive capacity and subsequent yield instability. The magnitude of these growth limitations are reduced through a number of P-induced physiological changes such as stimulation of biosynthesis and accumulation of nitrogenous osmolytes, antioxidant reactions and affected photosynthetic activity and growth and yield of salt-stressed plants[5].

Accordingly, the present work was designed with the objective to evaluate the potential positive effects of P as soil amendment on the changes in the growth and green and dry yields characteristics, leaf photosynthetic pigments, chlorophyll fluorescence, and leaf contents of nutrients of *Phaseolus vulgaris* L. plants exposed to soil salinity stress ($\text{EC}_e = 7.80\text{--}7.86 \text{ dS m}^{-1}$).

Materials and Methods

Experimental site, soil analyses, materials and treatments:

Two field experiments were conducted during the summer seasons of 2016 and 2017 at the Experimental Farm of the Faculty of Agriculture, Fayoum University, Southeast Fayoum (29° 17'N; 30° 53'E), Egypt. Assessments of the main soil chemical and physical characteristics (**Table 1**) were performed according to the procedures of [17,18]. Based on the determined EC_e values in both seasons (7.86 and 7.80 dS m^{-1} , respectively), the soil is classed as being saline according to [19].

In addition to the recommended dose of phosphorus (P) fertilizer, P was used also in the form of calcium superphosphate [15.5% (w/w) P_2O_5] at three levels (i.e., 0, 100 or 200 kg per feddan) as soil addition treatments. The selected levels of P for the two main field experiments were based on a pot preliminary study (data not shown).

Table (1): Physical and chemical properties of the experimental soil during soil preparation for sowing in two seasons

Parameter	2016 season	2017 season
Clay	41.0	40.5
Silt	35.5	35.0
Sand	23.5	24.5
Soil texture	Clay loam	
pH	7.79	7.76
EC _e (dS m ⁻¹)	7.86	7.80
Organic matter(%)	0.81	0.84
CEC* (cmol _c kg ⁻¹)	5.54	5.60
Field capacity (%)	32.6	32.8
Available water (%)	28.4	28.8
Available N (mg kg ⁻¹ soil)	111.7	122.8
Available P (mg kg ⁻¹ soil)	16.4	18.9
Available K (mg kg ⁻¹ soil)	142.8	151.3
Available Fe (mg kg ⁻¹ soil)	45.1	46.3
Available Mn (mg kg ⁻¹ soil)	22.4	22.9
Available Zn (mg kg ⁻¹ soil)	11.0	11.6

*CEC; cation exchange capacity.

Healthy common bean (*Phaseolus vulgaris* L., cv. Bronco) seeds were obtained from The Horticulture Research Institute, Agricultural Research Centre, Giza, Egypt, and were sown on 27 Feb. 2016, and on 26 Feb. 2017. Seeds were selected for uniformity by choosing those of equal size and same color. They were washed with distilled water, sterilized in 1% (v/v) sodium hypochlorite for approximately 2 min, and washed thoroughly again with distilled water. The sterilized seeds were left to dry at room temperature (22 ± 2 °C).

Commercial rhizobia inoculants were applied as peat slurry containing 107 Rhizobium g⁻¹. Uniform, air-dried seeds were field sown on two different adjacent locations; one for 2016 season and the other for 2017 season, in the same Farm. Each location was divided into 15 experimental units allocated for 5 treatments (3 replicates per each) including the control. The recommended seed rate of 35–40 kg per feddan for common beans was used. Each experimental unit consisted of five rows, 3 m long and 0.7 m wide (each unit = 10.5 m²), within row spacing was approximately 7.5 cm. Thinning of plants (two per hill) was performed prior to the first irrigation. During preparation and plant growth, the soil was supplemented in total with ammonium sulphate [20.5% (w/w) N], calcium superphosphate [15.5% (w/w) P₂O₅] and potassium sulphate [48% (w/w) K₂O]. The supplemented amounts were at a corresponding of 200, 200 and 100 kg per feddan, respectively as recommended for reclaimed saline soils.

The experimental design was complete randomized blocks with 3 levels of each of KH and P, with three replicates per treatment. Irrigation water was added to 100% of the reference crop evapotranspiration (ET_o), values from the Fayoum Meteo Station according to [20]. Seven irrigations were applied in each season, with total water rates of 2,750.4 m³ ha⁻¹ and 2,829.6 m³ ha⁻¹ in 2016 and 2017, respectively. The evapotranspiration rate from a reference surface, not short of water, is called the reference crop evapotranspiration or reference evapotranspiration and is denoted as ET_o. The reference surface is a hypothetical grass reference crop with specific characteristics. The use of other denominations such as potential ET is strongly discouraged due to ambiguities in their definitions. The only factors affecting ET_o are climatic parameters. Consequently, ET_o is a climatic parameter and can be computed from weather data. ET_o expresses the evaporating power of the atmosphere at a specific location and time of the year and does not consider the crop characteristics and soil factors. The crop evapotranspiration under standard conditions, denoted as ET_c, is the evapotranspiration from disease-free, well fertilized

crops, grown in large fields, under optimum soil water conditions, and achieving full production under the given climatic conditions. ET_c will be between 1 to 9 mm/day from cool to warm average temperature. The amount of water required to compensate the evapotranspiration loss from the cropped field is defined as crop water requirement. Although the values for crop evapotranspiration and crop water requirement are identical, crop water requirement refers to the amount of water that needs to be supplied, while crop evapotranspiration refers to the amount of water that is lost through evapotranspiration. The irrigation water requirement basically represents the difference between the crop water requirement and effective precipitation. The irrigation water requirement also includes additional water for leaching of salts and to compensate for non-uniformity of water application. All other recommended agricultural practices for common beans were carried out as recommended by [21]. Three experimental sites were chosen in each season. Soil analyses were carried out according to [22]. The results from physical and chemical analyses of the soils are shown in Table I. Electrical conductivity (EC) was measured using a conductivity meter and an extract of each soil paste. Soil EC values were 1.84, 6.03, and 8.97 dS m^{-1} at sites 1, 2, and 3, respectively. These EC values classed the soils as being non-saline, moderately saline, or strongly saline at sites 1, 2, and 3, respectively, according to [19]. Treatments of P were added at two equal doses; at 25 and 40 days after sowing (DAS).

Measurements of vegetative growth traits:

Fifty-day-old bean plants ($n = 9$) were removed and shoots were separated from plants, and the following vegetative growth attributes were recorded: Lengths of plants shoots were measured and number of leaves $plant^{-1}$ was counted. Leaves area was measured using a leaf area meter (LI-COR 3100C, LI-COR, Inc., Lincoln, NE, USA). Fresh weights of shoots were assessed, and dry weights of shoots were recorded after placing them in an oven at 70 °C until a constant weight.

Yield characteristics assessments (green pods and dry seeds):

At the marketable green pod stage of both experiments, green pods from randomly 5 rows (approximately 200 plants) from each treatment were collected, counted and weighed individually and per experimental plot (10.5 m^2). At the end of both experiments, dry pods from the other 10 rows (approximately 400 plants) from each treatment were collected, seeds were extracted from pods, air-dried and weighed.

Determination of leaf photosynthetic pigments contents:

Total chlorophylls and total carotenoids were extracted by homogenization of leaf sample (0.2 g) in 80% acetone (50 ml). After filtration, the absorbance of the clear extract was measured at 663, 646 and 470 nm [23].

Determination of chlorophyll fluorescence:

Chlorophyll fluorescence was measured on two different sunny days using a portable fluorometer (Handy PEA, Hansatech Instruments Ltd, Kings Lynn, UK). One leaf (the same age) was chosen per plant from three plants in each experimental plot of each treatment. Fluorescence measurements included: Maximum quantum yield of PS II F_v/F_m was calculated as; $F_v/F_m = (F_m - F_o)/F_m$ [24]. Performance index of photosynthesis based on the equal absorption (PIABS) was calculated as reported by [25].

Determinations of N, P, K^+ , Ca^{2+} , and Na^+ contents:

Content of N (%) was determined in powdery dried material of plants by Orange-G dye colorimetric method according to [26].

The wet digestion of 0.1 g of fine dried material of plants was conducted using a sulphuric and perchloric acid mixture as mentioned by [27]. The content of P (%) was colorimetrically determined using chlorostannum-molybdo-phosphoric blue color method in sulphuric acid system as described by [22]. The content of Ca^{2+} (%) was determined using a Perkin-Elmer Model 3300 Atomic Absorption Spectrophotometer [28]. The contents of K^+ (%) and Na^+ (%) were determined using a Perkin-Elmer Flame photometer [29].

Calculations of K^+/Na^+ , Ca^{2+}/Na^+ and $K^+ + Ca^{2+}/Na^+$ ratios

The ratios of K^+/Na^+ , Ca^{2+}/Na^+ and $K^+ + Ca^{2+}/Na^+$ were calculated from the determined contents of K, Ca and Na.

Statistical analysis:

All values (in 9 samples per treatment; $n = 9$) of the measured parameters for the common bean plants were subjected to statistical analysis following the standard procedures described by [30]. Duncan's multiple range test was applied to assess the least significant difference (LSD) of each treatment at a probability level of 95% ($P \leq 0.05$).

Results

Effect of soil application with P on growth of salt-stressed-common bean plants:

Soil treatment with P significantly increased the all tested growth characteristics (i.e., shoot length, number of leaves per plant, area of leaves per plant, and shoot fresh and dry weights) of salt-stressed common bean plants compared to the controls (**Table 2**). The two tested P treatments showed no significant differences. Results of the two seasons showed the same trend. P at 100 kg per feddan is found to be preferred addition above its recommended dose.

Table (2): Effect of soil application with phosphorus or potassium humate on growth traits of common bean (*Phaseolus vulgaris* L., cv. "Bronco") plants grown under soil salinity stress

Treatments	Parameters									
	Shoot length (cm)	% of control	No. of leaves plant ⁻¹	% of control	Leaf area plant ⁻¹ (dm ²)	% of control	Shoot fresh weight (g)	% of control	Shoot dry weight (g)	% of control
2016 season										
Control	25.4b	-	7.31b	-	9.51b	-	25.0b	-	5.74b	-
P1	27.0a	+ 6.3	7.59a	+ 3.8	10.34a	+ 8.7	29.0a	+ 16.0	6.76a	+ 17.8
P2	27.6a	+ 8.7	7.59a	+ 3.8	10.39a	+ 9.3	28.9a	+ 15.6	6.81a	+ 18.6
2017 season										
Control	26.1b	-	7.28b	-	9.58b	-	25.8b	-	5.87b	-
P1	27.8a	+ 6.5	7.60a	+ 4.4	10.49a	+ 9.5	29.4a	+ 14.0	6.90a	+ 17.5
P2	27.9a	+ 6.9	7.52a	+ 3.3	10.54a	+ 10.0	29.6a	+ 14.7	6.88a	+ 17.2

Mean values ($n = 9$) in each column for each year followed by a different lower-case letter are significantly different at $p \leq 0.05$ by Duncan's multiple range test. Control means plots without any treatments except for addition of the recommended doses of NPK, P1 means 100 kg calcium superphosphate per feddan + the recommended doses of NPK, and P2 means 200 kg calcium superphosphate per feddan + the recommended doses of NPK.

Effect of soil application with P on yields of salt-stressed-common bean plants:

Soil treatment with P significantly increased the all tested green pods and dry seed yields characteristics [i.e., average pod weight, number of pods per plant, pods weight per plot (10.5 m²), dry seed weight per plot (10.5 m²) and 100-seed weight] of salt-stressed common bean plants compared to the controls (**Table 3**). The two tested P treatments showed no significant differences. Results of the two seasons conferred the same trend. P at 100 kg per feddan is found to be preferred addition above its recommended dose.

Table (3): Effect of soil application with phosphorus or potassium humate on green pod and dry seed yields of common bean (*Phaseolus vulgaris* L., cv. “Bronco”) plants grown under soil salinity stress

Treatment	Parameters									
	Pod weight (g)	% of control	Pods No. plant ⁻¹	% of control	Pods weight plant ⁻¹ (g)	% of control	Dry seed weight plant ⁻¹ (g)	% of control	100-seed weight (g)	% of control
2016 season										
Control	2.20b	-	15.2b	-	31.5b	-	10.6b	-	16.6b	-
P1	2.45a	+ 11.4	19.1a	+ 25.7	44.1a	+ 40.0	11.8a	+ 11.3	18.4a	+ 10.8
P2	2.43a	+ 10.5	18.9a	+ 24.3	43.2a	+ 37.1	11.9a	+ 12.3	18.3a	+ 10.2
2017 season										
Control	2.24b	-	15.5b	-	32.6b	-	10.8b	-	17.2b	-
P1	2.50a	+ 11.6	19.4a	+ 25.2	45.8a	+ 40.5	12.1a	+ 12.0	19.1a	+ 11.0
P2	2.48a	+ 10.7	19.3a	+ 24.5	45.2a	+ 38.7	12.0a	+ 11.1	18.9a	+ 9.9

Mean values in each column for each year followed by a different lower-case letter are significantly different at $p \leq 0.05$ by Duncan's multiple range test. Control means plots without any treatments except for addition of the recommended doses of NPK, P1 means 100 kg calcium superphosphate per feddan + the recommended doses of NPK, and P2 means 200 kg calcium superphosphate per feddan + the recommended doses of NPK.

Effect of soil application with P on the contents of leaf photosynthetic pigments and chlorophyll fluorescence of salt-stressed-common bean plants:

Soil application with P significantly increased leaf photosynthetic pigments contents and chlorophyll fluorescence (i.e., total chlorophylls, total carotenoids, Fv/Fm and PI) of salt-stressed common bean plants compared to the controls (Table 4). The two tested P treatments represented no significant differences. Results of the two seasons represented the same trend. P at 100 kg per feddan is found to be preferred addition above its recommended dose.

Table (4): Effect of soil application with phosphorus or potassium humate on leaf photosynthetic pigments contents (mg g⁻¹ fresh weight) and chlorophyll fluorescence of common bean (*Phaseolus vulgaris* L., cv. “Bronco”) plants grown under soil salinity stress

Treatments	Parameters							
	Total chlorophylls	% of control	Total carotenoids	% of control	Fv/Fm	% of control	PI	% of control
2016 season								
Control	0.96b	-	0.32b	-	67.5b	-	60.6b	-
P1	1.54a	+ 60.4	0.39a	+ 21.9	79.7a	+ 18.1	71.6a	+ 18.2
P2	1.52a	+ 58.3	0.38a	+ 18.8	80.2a	+ 18.8	71.4a	+ 17.8
2017 season								
Control	0.99b	-	0.34b	-	68.2b	-	61.0b	-
P1	1.63a	+ 64.6	0.42a	+ 23.5	80.1a	+ 17.4	72.4a	+ 18.7
P2	1.61a	+ 62.6	0.43a	+ 26.5	81.3a	+ 19.2	72.2a	+ 18.4

Mean values (n = 9) in each column for each year followed by a different lower-case letter are significantly different at $p \leq 0.05$ by Duncan's multiple range test. Control means plots without any treatments except for addition of the recommended doses of NPK, P1 means 100 kg calcium superphosphate per feddan + the recommended doses of NPK, and P2 means 200 kg calcium superphosphate per feddan + the recommended doses of NPK.

Effect of soil application with P on leaf contents of nutrients and sodium of salt-stressed-common bean plants:

Soil application with P significantly increased leaf contents of nitrogen (N), phosphorus (P), potassium (K^+), and calcium (Ca^{2+}), while significantly reduced leaf sodium (Na^+) content of salt-stressed common bean plants compared to the controls (**Table 5**). The two tested P treatments showed no significant differences. Results of the two seasons showed the same trend. P at 100 kg per feddan is found to be preferred addition above its recommended dose.

Table (5): Effect of soil application with phosphorus or potassium humate on the contents of macro-nutrients (N, P, K^+ and Ca^{2+}) and sodium (Na^+) of common bean (*Phaseolus vulgaris* L., cv. “Bronco”) plants grown under soil salinity stress

Treatments	Parameters									
	N (%)	% of control	P (%)	% of control	K^+ (%)	% of control	Ca^{2+} (%)	% of control 1	Na^+ (%)	% of control
2016 season										
Control	2.64b	-	0.28b	-	2.55b	-	1.09b	-	0.64a	-
P1	3.05a	+ 15.5	0.42a	+ 50.0	2.91a	+ 14.1	1.21a	+ 11.0	0.48b	- 25.0
P2	3.03a	+ 14.8	0.46a	+ 64.3	2.90a	+ 13.7	1.22a	+ 11.9	0.45b	- 29.7
2017 season										
Control	2.71b	-	0.27b	-	2.59b	-	1.03b	-	0.62a	-
P1	3.09a	+ 14.0	0.43a	+ 59.3	3.03a	+ 17.0	1.23a	+ 19.4	0.44b	- 29.0
P2	3.12a	+ 15.1	0.44a	+ 63.0	3.00a	+ 15.8	1.24a	+ 20.4	0.42b	- 32.3

Mean values ($n = 9$) in each column for each year followed by a different lower-case letter are significantly different at $p \leq 0.05$ by Duncan's multiple range test. Control means plots without any treatments except for addition of the recommended doses of NPK, P1 means 100 kg calcium superphosphate per feddan + the recommended doses of NPK, and P2 means 200 kg calcium superphosphate per feddan + the recommended doses of NPK.

Effect of soil application with P on antagonistic relations of K^+ and Ca^{2+} with Na^+ of salt-stressed-common bean plants:

Soil application with P significantly increased the ratios of K^+/Na^+ , Ca^{2+}/Na^+ , and K^+Ca^{2+}/Na^+ in salt-stressed common bean plants compared to the controls (**Table 6**). The two treatments of P showed no significant differences for the all tested ratios. Results of the two seasons showed the same trend. P at 100 kg per feddan is found to be preferred addition above its recommended dose.

Table (6): Effect of soil application with phosphorus or potassium humate on nutrient relations with sodium (Na) ions in common bean (*Phaseolus vulgaris* L., cv. “Bronco”) plants grown under soil salinity stress

Treatments	Parameters					
	K^+/Na^+ ratio	% of control	Ca^{2+}/Na^+ ratio	% of control	K^+Ca^{2+}/Na^+ ratio	% of control
2016 season						
Control	3.75b	-	1.61b	-	5.37b	-
P1	5.72a	+ 52.5	2.38a	+ 47.8	8.12a	+ 51.2
P2	6.03a	+ 60.8	2.55a	+ 58.4	8.60a	+ 60.1
2017 season						
Control	3.93b	-	1.57b	-	5.52b	-
P1	6.44a	+ 63.9	2.62a	+ 66.9	9.04a	+ 63.8
P2	6.67a	+ 69.7	2.75a	+ 75.2	9.41a	+ 70.5

Mean values ($n = 9$) in each column for each year followed by a different lower-case letter are significantly different at $p \leq 0.05$ by Duncan's multiple range test. Control means plots without any treatments except for addition of the recommended doses of NPK, P1 means 100 kg calcium superphosphate per feddan + the recommended doses of NPK, and P2 means 200 kg calcium superphosphate per feddan + the recommended doses of NPK.

Discussion

In arid and semi-arid regions (dry environments), agricultural sector faces a massive problem due to salinity. Salinity occurred in growing media in such regions could be caused by one or more of the following reasons: (1) poor irrigation water which contains considerable amounts of salts, (2) accumulation of salts in the top layer of the soil due to over-irrigation, (3) proximity to the sea, (4) capillarity rise of salts from underground water into the root zone due to excessive evaporation, (5) low rainfall, (6) high evaporation rate, and (6) poor water management [31,32]. These soil salinization causes expose plants to osmotic stress. Salt stress adversely affects plant performance due to stimulating the overproduction of reactive oxygen species (ROS) through various organelles and enzymes [33]. To avoid these effects, plants adopt several strategies such as ion homeostasis, osmotic adjustment and enhancing the antioxidative defense system [34].

Several studies have shown to use soil amendments such as P, as exogenous support, to alleviate the plant cytotoxicity induced by salt stress [5,35,36]. These applications have proved to enhance the natural antioxidative defense systems of plants, offering the opportunity for in-field protection against the dangerous salt stress. Exploring suitable stress alleviant applied for soil is one of the plant biologist tasks. In recent decades, fertilizers applied for growing media, including P have been found to be effective in mitigating the salt induced damages in plants [5,36]. These protectants and amendments donated the capacity, in different degrees, to improve the plant's growth and productivity, as well as stress tolerance under salinity.

Soil addition of P significantly improved growth characteristics and yields of bean plants grown under saline soil (7.80–7.86 dS m⁻¹) conditions (**Tables 2 and 3**). Application of P as soil addition alleviated the harmful effects of salt stress on growth and yields of some crops [35,36,37]. Growth parameters of common bean plants grown under soil salinity stress responded positively to P in our study. The loss of growth and consequently in yield components under salt stress may be attributed to the decreases in photosynthetic pigments (**Table 4**) and disturbance in the nutrients' balance (**Tables 5 and 6**). However, P added for soil caused significant increases in growth and yields of salt-stressed common bean plants compared with the controls (**Tables 2 and 3**). Application of P fertilizer is necessary to ensure optimum plant production and quality [38], as well as for the acquisition, storage, and use of energy [39]. The present study demonstrated the positive relationship between P application and plant growth, which is supported by previous findings that P application increases plant height and root collar diameter [40], as well as basal stem diameter [36], and that P application has a positive effect on the growth of some plant species [36,37,41]. It is known that leaf development depends on a high degree of P concentration in the tissue because P plays an important role in the synthesis of sucrose and starch in photosynthesis, which increases plant dry weight [42]. Sufficient P makes efforts to increase dry matter accumulation by increasing the photosynthesis product of root and shoot, and consequently the increase in yields components.

Salt stress partially inhibited photosynthesis by a reduction in leaf photosynthetic pigments and chlorophyll fluorescence (Fv/Fm and PI); **Table 4**. However, soil P application increased these attributes, protecting photosynthetic machinery from salt-induced ROS by acting as a free radical scavenger. Leaf chlorophyll, as a biochemical attribute, is among the most important physiological indicators reflecting the stress of the plant in part due to its reliance on water and nutritional availability [43,44]. In this study, salinity stress caused a decrease in total chlorophylls and total carotenoids in common bean leaves. [5] have reported strong evidence that total chlorophyll in the leaves of common bean and plant dry weight, and total chlorophyll and seed yield per hectare at harvest are highly associated with one another in a linear way under saline conditions. In addition, total carotenoids in the leaves of common bean and seed yield per hectare are highly associated with one another in a linear way under salt stress. The reduction in chlorophyll in the stressed plants might be due to the disorganization of thylakoid membranes, more degradation than synthesis of chlorophyll via the formation of proteolytic enzymes such as chlorophyllase, which is responsible for the chlorophyll degradation and damaging to the photosynthetic apparatus [45], and this should result in reducing plant net assimilation rate and relative growth rate [44], in addition to the inhibitory effect of the accumulated ions [45,46].

Chlorophyll and carotenoid synthesis are dependent upon mineral nutrition [47]. Leaf green pigments depend on P content, since it facilitates the plant for stability in unfavorable conditions [48]. However, the facilitation of biochemical characteristics and biosynthesis of pigment molecules depends on the uptake of optimal P levels [36,49]. Optimal P conditions, in apricot seedlings, have been shown to increase total chlorophyll content and plant growth [50]. Previous studies have also reported that P application increases the biomass and carotenoid production of a blue-green alga *Spirulina platensis* [51], whereas P deficiency decreases protein and chlorophyll contents [52]. The Fv/Fm and PI are used as a noninvasive method to determine the functional state of photosynthetic machinery. These physiological attributes were reduced significantly by salt stress, while P application significantly improved these attributes in leaves of salt-stressed plants (**Table 4**).

P application to soil mitigates the adverse effects of salt stress, leading to an increase in the contents of nutrients (**Table 5**) and their relations with Na^+ (i.e., K^+/Na^+ , $\text{Ca}^{2+}/\text{Na}^+$ and $\text{K}^+ + \text{Ca}^{2+}/\text{Na}^+$; **Table 6**). In this connection, [53] suggested that increased accumulation of Na^+ and Cl^- ions in the tissues under salt stress inhibits biochemical processes related to photosynthesis through direct toxicity, leading to low water potential. The promotion of Na^+ ion uptake under salt stress was accompanied by a corresponding decline in K^+ content, showing an antagonism between K^+ and Na^+ [54]. The selectivity of high K^+/Na^+ ratio in plants is considered an important mechanism and criterion selection for salt tolerance [55] have reported that better plant tolerance to salt stress is primarily due to better K^+ assimilation, resulting in higher K^+/Na^+ ratio. In addition, maintenance of Ca^{2+} acquisition and transport under salt stress is an important determinant of salt tolerance. The Ca^{2+} is known to play an important role in maintaining the structural and functional integrity of cell membranes, stabilizing the cell walls and regulating the ion transport, as well as the selectivity and activation of cell wall enzymes [57].

Differences in nutrient concentrations have revealed clear biochemical differences in plants in their response to salinity and P treatment. Previous researches have shown that varying levels of salinity significantly increased Na^+ contents in different plant cultivars [58,59]. The increase in leaf Na^+ content may be due to increased concentrations of Na^+ in the growing medium ultimately resulting in the increased uptake of Na^+ by plant [45]. Our findings here portray through a decrease in Na^+ content by the increase in P supply. Moreover, [5] have reported that Na^+ in the leaves of common bean and seed yield per hectare are highly associated with one another in a linear way under salt stress. This may be attributed to the positive role of P in improved plant growth (**Table 2**), increased contents of photosynthetic pigments (**Table 4**), and increased nutrient contents, especially P (**Table 5**), consequently increasing the plant adaptive capacity to salinity by exclusion of Na^+ [60]. Soil salinity significantly reduced K^+ content in common bean leaves due to that salinity could be related to a gradient competition and resulting in selective uptake between K^+ and Na^+ , which causes an increase in uptake of Na^+ at the cost of K^+ [61] or decline in K^+ content occurs due to a decrease in sink size under salinity conditions.

[62] have reported that synergistic relationship between P and other beneficial elements like K^+ and Ca^{2+} might have initiated an osmotic effect and thus can be held responsible for salt tolerance to some degree. Our results confirmed these results where P application increased N, P, K^+ , and Ca^{2+} contents, while reduced Na^+ content. P application increased the all tested nutrients contents and the K^+/Na^+ and $\text{Ca}^{2+}/\text{Na}^+$ ratios, and consequently $\text{K}^+ + \text{Ca}^{2+}/\text{Na}^+$ ratio, indicating a salt tolerance of common bean is associated with an enhanced K^+/Na^+ and $\text{Ca}^{2+}/\text{Na}^+$ ratios discrimination trait [5]. Precisely, the contents of K^+ and Ca^{2+} were significantly increased in common bean plants extra supplemented with P, which may underline a mechanism behind the sensitivity of common bean plant that is likely associated with low nutrient uptake capacity. The contents of P and N were increased significantly under salinity stress with the application of P. The application of P increased P content rather than N. The nutrient P is stored in vacuoles, yet the mobility of P may be decreased by the presence of salinity, consequently an inhibition of export from this storage in particular to other parts of the plant. In addition, [63] attributed the reduction in P nutrient availability due to ionic strength effects which can reduce phosphate activity. In addition, [5] have found that P content and seed yield per hectare are highly associated with one another in a linear way under salt stress.

Conclusion

Application of P to saline soils has been shown to enhance plant salt stress-defense responses, to act directly and/or indirectly at improving total plant performances (growth and yields) under salt stress via increasing the photosynthetic efficiency (Fv/Fm and PI). Thus, P may provide an effective strategy to alleviate the adverse effects of salt stress through increased N-utilization, resulting in less damage to photosynthesis and greater protection of dangerous effects of salt stress. Therefore, the application of P may act to alleviate the severity of the effects of salt stress on *Phaseolus vulgaris* plants grown on saline soils.

References

- [1] Broughton, W.J., Hernander, G., Blair, B., Beebe, S., Gepts, P., and Vanderleyden, J. (2003): Beans (*Phaseolus* spp.) – model food legumes. *Plant Soil*, 252: 55–128.
- [2] Isaac, M.E., Harmand, J.M. and Drevon, J.J. (2011): Growth and nitrogen acquisition strategies of *Acacia senegal* seedlings under exponential phosphorus additions. *J. Plant Physiol.*, 168: 776–781.
- [3] Wang, X.W., Vinocur, B., and Altman, A. (2003): Plant responses to drought, salinity and extreme temperatures: towards genetic engineering for stress tolerance. *Planta*, 218: 1–14.
- [4] Maas, E.V., and Hoffman, G.J. (1977): Crop salt tolerance–Current assessment. *Journal of the Irrigation and Drainage Division – PUBDB*, 103(2): 115–134.
- [5] Bargaz, A., Nassar, R.M.A., Rady, M.M., Gaballah, M.S., Thompson, S.M., Brestic, M., Schmidhalter, U., and Abdelhamid, M.T. (2016): Improved salinity tolerance by phosphorus fertilizer in two *Phaseolus vulgaris* recombinant inbred lines contrasting in their P-efficiency. *J. Agron. Crop Sci.*, 202: 497–507.
- [6] Khan, M.I., R., Mughal, A., Iqbal, N., and Khan, N.A. (2013): Potentiality of sulphur containing compounds in salt stress tolerance. In: Parvaiz, A., Azooz, M. M., Prasad, M. N. V. (Eds.). *Ecophysiology and responses of plants under salt stress*. Chapter 17, p: 443–472, Springer.
- [7] Asada, K. (1999): The water-water cycle in chloroplasts: Scavenging of active oxygens and dissipation of excess photons. *Annu. Rev. Plant Physiol. Plant Mol. Biol.*, 50: 601–639.
- [8] Hemida, Kh.A., Eloufey, A.Z.A., Seif El-Yazal, M.A., and Rady, M.M. (2017): Integrated effect of potassium humate and α -tocopherol applications on soil characteristics and performance of *Phaseolus vulgaris* plants grown on a saline soil. *Arch. Agron. Soil Sci.*, 63: 1556–1571.
- [9] Yasar, F., Kusvuran, S., and Ellialtıođlu, S. (2006): Determination of anti-oxidant activities in some melon (*Cucumis melo* L.) varieties and cultivars under salt stress. *J. Hortic. Sci. Biotechnol.*, 81: 627–630.
- [10] Yildirim, B., Yaser, F., Ozpay, T., TurkOzu, D., Terzio lu, O., and Tamkoc, A. (2008): Variations in response to salt stress among field pea genotypes (*Pisum sativum* sp. arvense L.). *J. Anim. Vet. Adv.*, 7: 907–910.
- [11] Mishra, M., Mishra, P. K., Kumar, U., and Prakash, V. (2009): NaCl phytotoxicity induces oxidative stress and response of antioxidant system in *Cicer arietinum* L. cv. Abrodbi. *Bot. Res. Intl.*, 2: 74–82.
- [12] Hu, Y., and Schmidhalter, U. (2005): Drought and salinity: a comparison of their effects on the mineral nutrition of plants. *J. Plant Nutr. Soil Sci.*, 168: 541–549.
- [13] Vance, C.P. (2001): Symbiotic nitrogen fixation and phosphorus acquisition. *Plant nutrition in a world of declining renewable resources*. *Plant Physiol.*, 127: 390–397.

-
- [14] Vance, C.P., Uhde-Stone, C., and Allan, D.L. (2003): Phosphorus acquisition and use: critical adaptations by plants for securing a nonrenewable resource. *New Phytol.*, 157: 423–447.
- [15] Cerda, A., Bingham, F.T., and Hoffman, G. (1977): Interactive effect of salinity and phosphorus on sesame. *Soil Sci. Soc. Amer. J.*, 41: 915–918.
- [16] L'taief, B., Bouaziz, S., Mainassara, Z., Ralf, H., Molina, C., Beebe, S., Winter, P., Kahl, G., Drevon, J.J., and Lachaâl, M., (2012): Genotypic variability for tolerance to salinity and phosphorus deficiency among N₂-dependent recombinant inbred lines of Common Bean (*Phaseolus vulgaris*). *Afr. J. Microbiol. Res.*, 6: 4205–4213.
- [17] Page, A.I., Miller, R.H., and Keeney, D.R. (1982): *Methods of Soil Analysis. Part 2: Chemical and Microbiological Properties*. 2nd Ed. American Society of Agronomy, Madison, Wisconsin, USA.
- [18] Klute, A. (1986): *Methods of Soil Analysis. Part 1: Physical and Mineralogical Methods*. 2nd Ed. Wisconsin, USA: American Society of Agronomy Madison.
- [19] Dahnke, W.C., and Whitney, D.A. (1988): Measurement of soil salinity. In: Dahnke, W. C. (Ed.). *Recommended Chemical Soil Test Procedures for the North Central Region*. North Central Regional Publication 221. North Dakota Agric. Exp. St. Bull., 499: 32–34.
- [20] Allen, R.G., Pereira, L.S., Raes, D., and Smith, M. (1998): Crop evapotranspiration guidelines for computing crop water requirements. *Irrig. Drain.*, Paper 56, FAO, Rome, pp. 300.
- [21] Abdelhamid, M.T., Rady, M.M., Osman, A.Sh., and Abdalla, M.S. (2013): Exogenous application of proline alleviates salt induced oxidative stress in *Phaseolus vulgaris* L. plants. *J. Hortic. Sci. Biotechnol.*, 88: 439–446.
- [22] Jackson, M.L. (1967): *Soil Chemical Analysis*. Prentice Hall of India Pvt. Ltd, New Delhi, India, pp: 144–197, 326–338.
- [23] Welburn, A.R., and Lichtenthaler, H. (1984): Formulae and program to determine total carotenoids and chlorophylls a and b leaf extracts in different solvents. In: *Advances in photosynthesis research* (Sybesma, C., Ed.), (2): 9–12.
- [24] Maxwell, K., and Johnson, G. N. (2000): Chlorophyll fluorescence—a practical guide. *J. Exp. Bot.*, 51: 659–668.
- [25] Clark, A.J., Landolt, W., Bucher, J.B., and Strasser, R.J. (2000): Beech (*Fagus sylvatica*) response to ozone exposure assessed with a chlorophyll a fluorescence performance index. *Environ. Pollut.*, 109: 501–507.
- [26] Hafez, A. R., and Mikkelsen, D.S. (1981): Colorimetric determination of nitrogen for evaluating the nutritional status of rice. *Commun. Soil Sci. Plant Anal.*, 12: 61–69.
- [27] Piper, C. S. (1947): *Soil and plant analysis*. Inter. Sci. Inc. Nc. USA.
- [28] Chapman, H.D., and Pratt, P.F. (1961): *Methods of Analysis for Soil, Plants and Water*. University of California, Division of Agricultural Science, Berkeley, CA, USA, pp: 56–63.
- [29] Lachica, M., Aguilar, A., and Yanez, J. (1973): Analisis Foliar. Métodos Utilizados en la Estación Experimental del Zaidin, 32. *Anales de Edafología y Agrobiología*, p: 1033–1047.
- [30] Gomez, K.A., and Gomez, A.A. (1984): *Statistical Analysis Procedures for Agricultural Research*. John Wiley and Sons, New York, NY, USA, pp: 25–30.
- [31] Rady, M.M., Varma, B.C., and Howladar, S.M. (2013): Common bean (*Phaseolus vulgaris* L.) seedlings overcome NaCl stress as a result of presoaking in *Moringa oleifera* leaf extract. *Sci. Hortic.*, 162: 63–70.
- [32] Semida, W.M., Taha, R.S., Abdelhamid, M.T., and Rady, M.M. (2014): Foliar-applied α -tocopherol enhances salt-tolerance in *Vicia faba* L. plants grown under saline conditions. *S. Afr. J. Bot.*, 95: 24–31.

-
- [33] Semida, W.M., Abd El-Mageed, T.A., Howladar, S.M., and Rady, M.M. (2016): Foliar-applied α -tocopherol enhances salt-tolerance in onion plants by improving antioxidant defence system. *Aust. J. Crop Sci.*, 10(7): 1835–2707.
- [34] Xiong, L., and Zhu, J.K. (2002): Molecular and genetic aspects of plant responses to osmotic stress. *Plant Cell Environ.*, 25: 131–139.
- [35] Cicek, E., Yilmaz, F., and Yilmaz, M. (2010): Effect of N and NPK fertilizers on early field performance of narrow-leaved ash, *Fraxinus angustifolia*. *J. Environ. Biol.*, 31(1–2):109–114. PMID: 20648820
- [36] Waraich, E.A., Ahmad, Z., Ahmad, R., Saifullah, and Ashraf, M.Y. (2015): Foliar applied phosphorous enhanced growth, chlorophyll contents, gas exchange attributes and PUE in wheat (*Triticum aestivum* L.). *J. Plant Nutr.*, 38(12):1929–1943.
- [37] Pandey, S.T., Singh, P., and Pandey, P. (2006): Site specific nutrient management for *Withania somnifera* at subtropical belt of Uttaranchal. *Intl. J. Agric. Sci.*, 2:626–628.
- [38] Zapata, F., and Zaharah, A.R. (2002): Phosphate availability from phosphate rock and sewage sludge as influenced by addition of water soluble phosphate fertilizers. *Nutr. Cycl. Agroecosyst.*, 2002(1); 63:43–48.
- [39] Epstein, E., and Bloom, A.J. (2004): Mineral nutrition of plants: Principles and perspectives (Second Edition). Sunderland, MA: Sinauer Associates, Inc.; 2004. 402p.
- [40] Hudai, S.M.S., Sujaudhin, M., Shafinat, S., and Uddin, M.S. (2007): Effects of phosphorus and potassium addition on growth and nodulation of *Dalbergia sissoo* in the nursery. *J. For. Res.*, 18(4):279–282.
- [41] Verma, R.K., Khatri, P.K., Bagde, M., Pathak, H.D., and Totet, N.G. (1996): Effect of biofertilizer and phosphorous on growth of *Dalbergia sissoo*. *Ind. J. For.*, 19(3):244–246.
- [42] Cakmak, I., Hengeler, C., Marschner, H. (1994): Partitioning of shoot and root dry matter and carbohydrates in bean plants suffering from phosphorus, potassium and magnesium deficiency. *J. Exp. Bot.*, 45(9): 1245–1250.
- [43] Dawood, M.G., Abdelhamid, M.T., and Schmidhalter, U. (2014a): Potassium fertiliser enhances the salt-tolerance of common bean (*Phaseolus vulgaris* L.). *J. Hort. Sci. Biotech.*, 89: 185–192.
- [44] Rady, M.M., Sadak, M.Sh., El-Lethy, S.R., Abd Elhamid, E.M., and Abdelhamid, M.T. (2015): Exogenous α -tocopherol has a beneficial effect on *Glycine max* (L.) plants irrigated with diluted sea water. *J. Hortic. Sci. Biotechnol.*, 90(2): 195–202.
- [45] Abdelhamid, M.T., Shokr, M., and Bekheta, M.A. (2010): Growth, root characteristics, and leaf nutrients accumulation of four faba bean (*Vicia faba* L.) cultivars differing in their broomrape tolerance and the soil properties in relation to salinity. *Commun. Soil Sci. Plant Anal.*, 41: 2713–2728.
- [46] Dawood, M.G., Taie, H.A.A., Nassar, R.M.A., Abdelhamid, M.T., and Schmidhalter, U. (2014b): The changes induced in the physiological, biochemical and anatomical structure of *Vicia faba* by the exogenous application of proline under seawater stress. *S. Afr. J. Bot.*, 93: 54–63.
- [47] Daughtry, C.S.T, Walthall, C.L., Kim, M.S., Brown de Colstoun, E., and McMurtrey, J.E. (2000): Estimating corn leaf chlorophyll concentration from leaf and canopy reflectance. *Remote Sensing of Environment*, 74(2): 229–239.
- [48] Bojovic, B., and Stojanovic, J. (2006): Some wheat leaf characteristics in dependence of fertilization. *Kragujevac J. Sci.*, 28: 139–146.
- [49] Shubhra, Dayal, J., Goswami, C.L., and Munjal, R. (2004): Influence of phosphorus application on water relations, biochemical parameters and gum content in cluster bean under water deficit. *Biol. Plant.*, 48(3): 445–448.

-
- [50] Dutt, S., Sharma, S.D., and Kumar, P. (2013): Inoculation of apricot seedlings with indigenous arbuscular mycorrhizal fungi in optimum phosphorus fertilization for quality growth attributes. *J. Plant Nutr.*, 36(1): 15–31.
- [51] Celekli, A., Yavuzatmaca, M., and Bozkurt, H. (2009): Modeling of biomass production by *Spirulina platensis* as function of phosphate concentrations and pH regimes. *Biores. Technol.*, 100(14): 3625–3629.
- [52] Liang, X.L., Lin, Y.C., Nian, H., and Xie, L.X. (2005): The effect of low phosphorus stress on main physiological traits of different maize genotypes. *Acta Agron. Sin.*, 31(5): 667–669.
- [53] Kiarostami, K.H., Mohseni, R., and Saboora, A. (2010): Biochemical changes of *Rosmarinus officinalis* under salt stress. *J. Stress Physiol. Biochem.*, 6: 114–122.
- [54] Cuin, T.A., Tian, Y., Betts, S.A., Chalmandrier, R., and Shabala, S. (2009): Ionic relations and osmotic adjustment in durum and bread wheat under saline conditions. *Funct. Plant Biol.*, 36: 1110–1119.
- [55] Ashraf, M., and Harris, P.J.C. (2004): Potential biochemical indicators of salinity tolerance in plants. *Plant Sci.*, 166: 3–16.
- [56] Gharsa, M.A., Parre, E., Debez, A., Bordenava, M., Richard, L., Leport, L., Bouchereau, A., Savoure, A., and Abdelly, C. (2008): Comparative salt tolerance analysis between *Arabidopsis thaliana* and *Thellungiella halophila*, with special emphasis on K^+/Na^+ selectivity and proline accumulation. *J. Plant Physiol.*, 165: 588–599.
- [57] Marschner, H. (1995): Mineral Nutrition of Higher Plants. 2nd Ed. New York, NY, USA: Academic Press Publication, p: 559–579.
- [58] Noreen, Z., Ashraf, M., and Akram, N.A. (2010): Salt-induced regulation of some key antioxidant enzymes and physio-biochemical phenomena in five diverse cultivars of turnip (*Brassica rapa* L.). *J. Agron. Crop Sci.*, 196: 273–285.
- [59] Lenis, J.M., Ellersieck, M., Blevins, D.G., Sleper, D.A., Nguyen, H.T., Dunn, D., Lee, J.D., and Shannon, J.G. (2011): Differences in ion accumulation and salt tolerance among glycine accessions. *J. Agron. Crop Sci.*, 197: 302–310.
- [60] Munns, R., and Tester, M. (2008): Mechanisms of salinity tolerance. *Annu. Rev. Plant Biol.*, 59: 651–681.
- [61] Kuiper, P.J.C. (1984): Functioning of plant cell membrane under saline conditions: membrane lipid composition and ATPases. In: R.C. Staples, and G.H. Toenniessen, eds. *Salinity Tolerance in Plant: Strategies for Crop Improvement*, pp. 77–91. John Wiley and Sons, Inc., New York, NY, USA.
- [62] Malik, R.S., Gupta, A.P., Haneklaus, S., and El-Bassam, N. (1999): Role of phosphorus (P) in inducing salt tolerance in sunflower. *Landbauforsch. Völk.*, 49: 169–176.
- [63] Grattan, S.R., and Grieve, C.M. (1993): Mineral nutrient acquisition and response by plants in saline environment. In: M. Pessarakali, ed. *Handbook of Plant and Crop Stress*, pp. 203–266. Marcel Dekker, Inc., New York, NY, USA.