# PHOSPHORUS APPLICATION IMPROVES THE PERFORMANCE OF SALT-STRESSED *PHASEOLUS VULGARIS* PLANTS

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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 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<sup>+</sup> 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<sup>+</sup>, and Ca<sup>2+</sup>, and the ratios of K<sup>+</sup>/Na<sup>+</sup>, Ca<sup>2+</sup>/Na<sup>+</sup> and K<sup>+</sup>+Ca<sup>2+</sup>/Na<sup>+</sup> were significantly increased by the two tested P treatments compared to the controls (the Ministry of Agriculture recommended P). The all tested treatments conferred, approximately, the same results. Therefore, results of this study recommend using P at the rate of 100 kg per feddan in addition to the recommended dose to optimize the common bean performance in saline soils.

**Keywords:** *Phaseolus vulgaris*, Salinity, Phosphorus, Plant performance, Antioxidant defense systems, Photosynthesis, Water relations.

#### 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 (**Broughton** *et al.*, 2003). 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 (**Isaac** *et al.*, 2011). 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 (**Wang** *et al.*, 2003). *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 (**Maas and Hoffman, 1977**).

Soil salinity is one of the major problems of agriculture, particularly in arid and semiarid regions, limiting plant growth and productivity (Bargaz et al., 2016). Salt stress adversely affects plant morphology and physiology through osmotic and ionic stresses, and changes biochemical responses in plants (Khan et al., 2013). It causes an overproduction of reactive oxygen species (ROS) such as superoxide (O2°-), hydrogen peroxide (H2O2) and hydroxyl (OH<sup>-</sup>) radicals. Chloroplasts are the major organelles that produce the ROS during photosynthesis (Asada, 1999; Hemida et al., 2017). The ROS cause damages for lipids, proteins and DNA (Yasar et al., 2006). They also cause chlorophyll degradation and membrane lipid peroxidation (Yildirim et al., 2008). Removal of the toxic ROS rapidly is important in any defense mechanism. This elimination occurs through antioxidant defense systems (Mishra et al., 2009). There are several reports underlining the intimate relationship between the activity of antioxidant systems and increased tolerance to environmental stresses (Bargaz et al., 2016; Hemida et al., **2017**). Differences in the accumulation patterns of Na<sup>+</sup> and K<sup>+</sup> are found under salinity stress. Salt tolerant species maintain a high K<sup>+</sup> content accompanied by a higher K<sup>+</sup>/Na<sup>+</sup> ratio (**Bargaz** et al., 2016; Hemida et al., 2017).

Soil application of phosphorus (P) can positively change the nutritional imbalance (Hu and Schmidhalter, 2005), optimizing the nutrient uptake capacity (Bargaz et al., 2016). Nutrient uptake limitation under adverse conditions, particularly P assimilation, is one of the most limiting factors decreasing biomass and grain yield in P. vulgaris (Vance, 2001). This nutrient deficiency is widespread with greater than 30% of the world's arable land affected by a P limitation (Vance et al., 2003). The interaction between salinity and P positively affects plant growth and yield (Bargaz et al., 2016). By increasing P application, there is an increased salt tolerance in plants (Cerda et al., 1977). Legumes are highly P-demanding crops; thus, P limitation negatively affects growth and symbiotic N<sub>2</sub> fixation (L'taief et al., 2012). 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 (Bargaz et al., 2016).

Accordingly, the present work was designed with the objective to evaluate the potential positive effects of P as soil amendment on the changes

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in the growth, 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 ( $EC_e = 7.80-7.86 \text{ dS m}^{-1}$ ).

#### 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 Demo 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 **Page** *et al.* (1982) and **Klute** (1986). Based on the determined EC<sub>e</sub> values in both seasons (7.86 and 7.80 dS m<sup>-1</sup>, respectively), the soil is classed as being saline according to **Dahnke and Whitney** (1988).

In addition to the Ministry of Agriculture recommended dose of 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

during son 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 l	oam						
рН	7.79	7.76						
$EC_e (dS m^{-1})$	7.86	7.80						
Organic matter (%)	0.81	0.84						
CEC* (cmol <sub>c</sub> kg <sup>-1</sup> )	5.54	5.60						
Field capacity (%)	32.6	32.8						
Available water (%)	28.4	28.8						
Available N (mg kg <sup>-1</sup>	111.7	122.8						
Available P (mg kg <sup>-1</sup> soil)	16.4	18.9						
Available K (mg kg <sup>-1</sup>	142.8	151.3						
Available Fe (mg kg <sup>-1</sup>	45.1	46.3						
Available Mn (mg kg <sup>-1</sup>	22.4	22.9						
Available Zn (mg kg <sup>-1</sup>	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  $\pm$  2 °C).

Commercial rhizobia inoculants were applied as peat slurry containing 107 Rhizobium  $\rm g^{-1}$ . 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 was consisted of five rows, 3 m long and 0.7 m wide (each unit =  $10.5 \, \rm m^2$ ), within row spacing was approximately 7.5 cm. Thinning of plants (two per hill) was performed prior to the first irrigation. During preparation (total recommended P) and plant growth (50% of N and K was added at the  $15^{\rm th}$  day after sowing and the other 50% was added 15 days later), the soil was supplemented in total with 200 kg of ammonium sulphate [20.5% (w/w) N], 200 kg of calcium superphosphate [15.5% (w/w) P<sub>2</sub>O<sub>5</sub>], and 100 kg of potassium sulphate [48% (w/w) K<sub>2</sub>O] as recommended for reclaimed saline soils.

The experimental design was complete randomized blocks with 3 levels of P, with three replicates per treatment. The experimental units were irrigated to that of reference crop evapotranspiration (ET<sub>0</sub>) values according to **Allen** *et al.* (1998). The all other recommended agricultural practices for common beans were carried out as recommended by **Abdelhamid** *et al.* (2013). 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: Length of plant shoot was measured and number of leaves plant<sup>-1</sup> was counted. Leaf area was measured using a leaf area meter (LI-COR 3100C, LI-COR, Inc., Lincoln, NE, USA). Fresh weight of shoot was assessed, and dry weight of shoot was 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<sup>2</sup>). 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 **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 Fv/Fm was calculated as; Fv/Fm = (Fm - Fo)/Fm (Maxwell and Johnson, 2000). Performance index of photosynthesis based on the equal absorption (PIABS) was calculated as reported by Clark *et al.* (2000).

## Determinations of N, P, K<sup>+</sup>, Ca<sup>2+</sup>, and Na<sup>+</sup> contents:

Content of N (%) was determined in powdery dried leaves by Orange-G dye colorimetric method according to **Hafez and Mikkelsen** (1981).

The wet digestion of 0.1 g of fine dried leaves was conducted using a sulphuric and perchloric acid mixture as mentioned by **Piper (1947).** The content of P (%) was colorimetrically determined using chlorostannusmolybdo-phosphoric blue color method in sulphuric acid system as described by **Jackson (1967)**. The content of  $Ca^{2+}$  (%) was determined using a Perkin-Elmer Model 3300 Atomic Absorption Spectrophotometer (**Chapman and Pratt, 1961**). The contents of  $K^+$  (%) and  $Na^+$  (%) were determined using a Perkin-Elmer Flame photometer (**Lachica** *et al.*, **1973**).

### 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 **Gomez and Gomez (1984)**. Duncan's multiple range test was applied to assess the least significant difference (LSD) of each treatment at a probability level of 95% ( $P \le 0.05$ ).

#### 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 on growth traits of common bean (*Phaseolus vulgaris* L., cv. "Bronco") plants grown under soil salinity stress

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

Mean values (n = 9) in each column for each year followed by a different lower-case letter are significantly different at  $p \le 0.05$  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 m<sup>2</sup>), dry seed weight per plot (10.5 m<sup>2</sup>) 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 on green pod and dry seed yields of common bean (*Phaseolus vulgaris* L., cv. "Bronco") plants grown under soil salinity stress.

		Parameters									
Treatment	Pod weight (g)	% of control	Pods No. plant <sup>-1</sup>	% of control	Pods weight plant <sup>-1</sup> (g)	% of control	Dry seed weight plant <sup>-1</sup> (g)	% of control	100-seed weight (g)	% of control	
2016 season											
Control	2.20b	ı	15.2b	-	31.5b	ı	10.6 <mark>b</mark>	-	16.6b	-	
P1	2.45 <b>a</b>	+ 11.4	19.1 <mark>a</mark>	+ 25.7	44.1 <mark>a</mark>	+ 40.0	11.8a	+ 11.3	18.4 <mark>a</mark>	+ 10.8	
P2	2.43 <b>a</b>	+ 10.5	18.9 <mark>a</mark>	+ 24.3	43.2 <b>a</b>	+ 37.1	11.9 <mark>a</mark>	+ 12.3	18.3 <mark>a</mark>	+ 10.2	
				2	017 season						
Control	2.24b	ı	15.5b	1	32.6b	ı	10.8 <mark>b</mark>	1	17.2b	-	
P1	2.50a	+ 11.6	19.4 <mark>a</mark>	+ 25.2	45.8 <mark>a</mark>	+ 40.5	12.1a	+ 12.0	19.1 <mark>a</mark>	+ 11.0	
P2	2.48 <mark>a</mark>	+ 10.7	19.3 <mark>a</mark>	+ 24.5	45.2a	+ 38.7	12.0a	+ 11.1	18.9 <mark>a</mark>	+ 9.9	

Mean values in each column for each year followed by a different lower-case letter are significantly different at  $p \le 0.05$  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 flourescence (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 on leaf photosynthetic pigments contents (mg g<sup>-1</sup> fresh weight) and chlorophyll fluorescence of common bean (*Phaseolus vulgaris* L., cv. "Bronco") plants grown under soil salinity stress

	Parameters											
Treatments	Total	% of	Total	% of	Fv/Fm	% of	PI	% of				
	chlorophylls	control	carotenoids	control	F V/FIII	control		control				
	2016 season											
Control	0.96b	-	0.32b	-	67.5 <mark>b</mark>	-	60.6 <mark>b</mark>	-				
P1	1.54a	+ 60.4	0.39a	+ 21.9	79.7 <mark>a</mark>	+ 18.1	71.6 <mark>a</mark>	+ 18.2				
P2	1.52a	+ 58.3	0.38a	+ 18.8	80.2a	+ 18.8	71.4 <mark>a</mark>	+ 17.8				
			2017 s	eason								
Control	0.99b	-	0.34b	1	68.2b	-	61.0 <mark>b</mark>	-				
P1	1.63a	+ 64.6	0.42a	+ 23.5	80.1a	+ 17.4	72.4 <mark>a</mark>	+ 18.7				
P2	1.61a	+ 62.6	0.43a	+ 26.5	81.3a	+ 19.2	72.2 <b>a</b>	+ 18.4				

Mean values (n = 9) in each column for each year followed by a different lower-case letter are significantly different at  $p \le 0.05$  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 on the contents of macro-nutrients (N, P, K<sup>+</sup> and Ca<sup>2+</sup>) and sodium (Na<sup>+</sup>) of common bean (*Phaseolus vulgaris* L., cv. "Bronco") plants grown under soil salinity stress

	Parameters										
Treatments	N (%)	% of control	P (%)	% of control	K <sup>+</sup> (%)	% of control	Ca <sup>2+</sup> (%)	% of control	Na <sup>+</sup> (%)	% of control	
	2016 season										
Control	2.64b	-	0.28b	-	2.55b	-	1.09b	-	0.64 <mark>a</mark>	-	
P1	3.05 <mark>a</mark>	+ 15.5	0.42 <mark>a</mark>	+ 50.0	2.91 <b>a</b>	+ 14.1	1.21 <b>a</b>	+ 11.0	0.48b	- 25.0	
P2	3.03 <mark>a</mark>	+ 14.8	0.46 <mark>a</mark>	+ 64.3	2.90a	+ 13.7	1.22 <mark>a</mark>	+ 11.9	0.45b	- 29.7	
				20	17 season						
Control	2.71b	-	0.27b	-	2.59b	-	1.03b	-	0.62 <mark>a</mark>	-	
P1	3.09a	+ 14.0	0.43 <mark>a</mark>	+ 59.3	3.03 <mark>a</mark>	+ 17.0	1.23 <mark>a</mark>	+ 19.4	0.44 <mark>b</mark>	- 29.0	
P2	3.12a	+ 15.1	0.44a	+ 63.0	3.00a	+ 15.8	1.24 <mark>a</mark>	+ 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  $p \le 0.05$  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<sup>+</sup> and Ca<sup>2+</sup> with Na<sup>+</sup> of salt-stressed-common bean plants:

Soil application with P significantly increased the ratios of K<sup>+</sup>/Na<sup>+</sup>, Ca<sup>2+</sup>/Na<sup>+</sup>, and K<sup>+</sup>+Ca<sup>2+</sup>/Na<sup>+</sup> 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 on nutrient relations with sodium (Na) ions in common bean (*Phaseolus vulgaris* L., cv. "Bronco") plants grown under soil salinity stress

	Parameters										
Treatments	K <sup>+</sup> /Na <sup>+</sup> ratio	% of control			K++Ca <sup>2+</sup> /Na+ ratio	% of control					
	2016 season										
Control	3.75b	-	1.61 <mark>b</mark>	-	5.37b	-					
P1	5.72 <b>a</b>	+ 52.5	2.38a	+ 47.8	8.12 <b>a</b>	+ 51.2					
P2	6.03 <mark>a</mark>	+ 60.8	2.55 <b>a</b>	+ 58.4	8.60a	+ 60.1					
			2017 seas	on							
Control	3.93b	ı	1.57 <b>b</b>	1	5.52 <b>b</b>	-					
P1	6.44 <mark>a</mark>	+ 63.9	2.62 <b>a</b>	+ 66.9	9.04a	+ 63.8					
P2	6.67 <mark>a</mark>	+ 69.7	2.75a	+ 75.2	9.41 <b>a</b>	+ 70.5					

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Mean values (n = 9) in each column for each year followed by a different lower-case letter are significantly different at  $p \le 0.05$  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 (Rady et al., 2013; Semida et al., 2014). 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 (Semida et al., 2016). To avoid these effects, plants adopt several strategies such as ion homeostasis, osmotic adjustment and enhancing the antioxidative defense system (Xiong and Zhu, 2002).

Several studies have shown to use soil amendments such as P, as exogenous support, to alleviate the plant cytotoxicity induced by salt stress (Cicek et al., 2010; Waraich et al., 2015; Bargaz et al., 2016). 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 (Waraich et al., 2015; Bargaz et al., 2016). 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<sup>-1</sup>) 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 (**Pandey** *et al.*, **2006**; **Cicek** *et al.*, **2010**; **Waraich** *et al.*, **2015**). 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 (Zapata and Zaharah, 2002), as well as for the acquisition, storage, and use of energy (Epstein and Bloom, 2004). 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 (Hudai et al., 2007), as well as basal stem diameter (Cicek et al., 2010), and that P application has a positive effect on the growth of some plant species (Verma et al., 1996; Pandey et al., 2006; Waraich et al., 2015). 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 (Cakmak et al., 1994). 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 (Dawood et al., 2014a; Rady et al., 2015). In this study, salinity stress caused a decrease in total chlorophylls and total carotenoids in common bean leaves. Bargaz et al. (2016) 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 (Rong-hua et al., 2006), and this should result in reducing plant net assimilation rate and relative growth rate (Rady et al., 2015), in addition to the inhibitory effect of the accumulated ions (Abdelhamid et al., 2010; Dawood et al., 2014b).

Chlorophyll and carotenoid synthesis are dependent upon mineral nutrition (**Daughtry** *et al.*, **2000**). Leaf green pigments depend on P content, since it facilitates the plant for stability in unfavorable conditions (**Bojovic and Stojanovic**, **2006**). However, the facilitation of biochemical characteristics and biosynthesis of pigment molecules depends on the uptake

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phosphorus application improves the performance... 53 of optimal P levels (Shubhra et al., 2004; Waraich et al., 2015). Optimal P conditions, in apricot seedlings, have been shown to increase total chlorophyll content and plant growth (Dutt et al., 2013). Previous studies have also reported that P application increases the biomass and carotenoid production of a blue-green alga Spirulina platensis (Celekli et al., 2009), whereas P deficiency decreases protein and chlorophyll contents (Liang et al., 2005). 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<sup>+</sup> (i.e., K<sup>+</sup>/Na<sup>+</sup>, Ca<sup>2+</sup>/Na<sup>+</sup> and K<sup>+</sup>+Ca<sup>2+</sup>/Na<sup>+</sup>; **Table 6**). In this connection, **Kiarostami** et al. (2010) suggested that increased accumulation of Na<sup>+</sup> and Cl<sup>-</sup> 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<sup>+</sup> ion uptake under salt stress was accompanied by a corresponding decline in K<sup>+</sup> content, showing an antagonism between K<sup>+</sup> and Na<sup>+</sup> (Cuin et al., 2009). The selectivity of high K<sup>+</sup>/Na<sup>+</sup> ratio in plants is considered an important mechanism and criterion selection for salt tolerance (Ashraf and Harris, 2004). Gharsa et al. (2008) have reported that better plant tolerance to salt stress is primarily due to better K<sup>+</sup> assimilation, resulting in higher K<sup>+</sup>/Na<sup>+</sup> ratio. In addition, maintenance of Ca<sup>2+</sup> acquisition and transport under salt stress is an important determinant of salt tolerance. The Ca<sup>2+</sup> 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 (Marschner, 1995).

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<sup>+</sup> contents in different plant cultivars (Noreen *et al.*, 2010; Lenis *et al.*, 2011). The increase in leaf Na<sup>+</sup> content may be due to increased concentrations of Na<sup>+</sup> in the growing medium ultimately resulting in the increased uptake of Na<sup>+</sup> by plant (Abdelhamid *et al.*, 2010). Our findings here portray through a decrease in Na<sup>+</sup> content by the increase in P supply. Moreover, Bargaz *et al.* (2016) have reported that Na<sup>+</sup> 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<sup>+</sup> (Munns and Tester, 2008). 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  $K^+$  (**Kuiper**, **1984**) or decline in  $K^+$  content occurs due to a decrease in sink size under salinity conditions.

Malik et al. (1999) have reported that synergistic relationship between P and other beneficial elements like K<sup>+</sup> and Ca<sup>2+</sup> 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<sup>+</sup>, and Ca<sup>2+</sup> contents, while reduced Na<sup>+</sup> content. P application increased the all tested nutrients contents and the K<sup>+</sup>/Na<sup>+</sup> and Ca<sup>2+</sup>/Na<sup>+</sup> ratios, and consequently K<sup>+</sup>+Ca<sup>2+</sup>/Na<sup>+</sup> ratio, indicating a salt tolerance of common bean is associated with an enhanced K<sup>+</sup>/Na<sup>+</sup> and Ca<sup>2+</sup>/Na<sup>+</sup> ratios discrimination trait (Bargaz et al., 2016). Precisely, the contents of K<sup>+</sup> and Ca<sup>2+</sup> 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, Grattan and Grieve (1993) attributed the reduction in P nutrient availability due to ionic strength effects which can reduce phosphate activity. In addition, Bargaz et al. (2016) have found that P content and seed yield per hectare are highly associated with one another in a linear way under salt stress.

In a 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.

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#### تطبيق الفوسفور يحسن أداء نباتات الفاصوليا المجهدة ملحيأ

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#### الملخص العربي

تهدف هذه الدراسة إلى تقييم التأثير المحسن لسوبر فوسفات الكالسيوم (صفر، ١٠٠، و ٢٠٠ كجم للفدان) كإضافات للتربة على صفات النمو، خصائص المحصول الأخضر والجاف، محتوى الورقة من صبغات البناء الضوئي، كفاءة عملية البناء الضوئي، محتوى الورقة من المغنيات في نباتات الفاصوليا (صنف برونكو) النامية تحت ظروف التربة الملحية. لتحقيق هدف الدراسة، أجريت تجربتي حقل في المزرعة التجريبية لكلية الزراعة، جامعة الفيوم أثناء الموسمين الصيفيين ٢٠١٦ و ٢٠١٧. أوضحت النتائج المتحصل عليها أن محتوى الصوديوم بالنباتات انخفض معنويًا، بينما زادت معنويًا جميع الصفات الأخرى مثل خصائص النمو (طول المجموع الخضري، عدد الأوراق/ نبات، مساحة الأوراق/ نبات، و الأوزان الطازجة والجافة للمجموع الخضري)، صفات محصول القرون الخضراء والبذور الجافة (متوسط وزن القرن، عدد القرون/ نبات، وزن القرون/ نبات، وزن البذور الجافة/ نبات، و وزن الـ١٠٠ بذرة)، محتوى الورقة من صبغات البناء الضوئي (الكلوروفيلات الكلية و الكاروتنويدات الكلية)، كفاءة عملية البناء الضوئي (أقصى محصول من وحدات الطاقة للنظام الضوئي الثاني لعملية البناء الضوئي، و دليل الأداء)، محتوى الأوراق من النيتروجين، الفوسفور، البوتاسيوم، و الكالسيوم، و نسبة كل من البوتاسيوم/الصوديوم، الكالسيوم/الصوديوم، و البوتاسيوم+الكالسيوم/ الصوديوم بتطبيق معاملتي الفوسفور مقارنة بالكنترول (المعدل الموصى به من قبل وزارة الزراعة). منحت معاملتي الفوسفور نفس النتائج تقريباً. لذلك توصى نتائج هذه الدراسة استخدام سوبر فوسفات الكالسيوم بمعدل ١٠٠ كجم للفدان، بالإضافة إلى المعدل الموصىي به، للوصول بنباتات الفاصوليا للأداء الأمثل في الأراضي الملحية.

الكلمات المفتاحية: الفاصوليا، الملوحة، المواد الهيومية، أداء النبات، أنظمة الدفاع المضادة للأكسدة، البناء الضوئي، العلاقات المائية.