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Amplification of Phosphorus Utilization Among Symbiotic N2-Fixing Recombinant Inbred Lines of Common Bean (*Phaseolus vulgaris* L.) Under Fertigation Technique

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Abstract: Six common bean recombinant inbred lines (RIL) of the cross of BAT477 and DOR364 were inoculated with Rhizobium tropici CIAT 899 (originating from International Center of Tropical Agriculture, Colombia) and grown in sandy soil using three levels of phosphorus (0, 45, 90 kg ha^{-1}) injected through fertigation technique. The greatest records were noticed in RILs 75, 83 and 34, respectively. Vegetative growth characteristics were considerably amplified due to increasing phosphorus levels under fertigation technique. The RIL 75 gave high significant values for yield, and its components followed by RIL 104 and RIL 34 compared with the other three RILs of common bean plants. The high rate of P fertigation leads to higher mean values of seed, straw and biomass yield (Mg ha^{-1}) except for phosphorus use efficiency. The highest mean values of seed, straw and biomass yield were 6.78, 7.93 and 14.71 Mg ha^{-1} occurred with RIL 75 injected 90 kg P_2O_5 ha^{-1} through fertigation technique, respectively. While the fertigated RIL 75 had the highest fertigated phosphorus use efficiency (321.65kg biomass $kg^{-1}P_2O_5$) with the rate of 45kg P_2O_5 under fertigation technique. Lastly, the statistical test showed that there was a variation between different genotypes as a response of high and low P fertigation supply. A correlation between N and P concentrations in nodules was high ($r^2 = 0.959$) for RIL 75 under high P level and ($r^2 = 0.634$) for RIL 104 under low P level.
Key words: Fertigation; N_2-fixation; Nodulation; *Phaseolus vulgaris* L.; Phosphorus nutrition; Recombinent lines.

Introduction

Legumes in general represent 27% of the world's primary crop production, with grain legumes alone providing 33% of the dietary protein nitrogen needs for humans[1].

Common bean (*Phaseolus vulgaris* L.) is one of the most important legumes for vitamins and proteins production, the crop production of common bean is harshly incomplete due to phosphorus (P) shortagespecially in arid and semi-arid regions[2].

Phosphorus and nitrogen are considered vital nutrients in plant production; meanwhile, the whole extent of the requirement for these nutrients in the physiological processes leading to crop growth seems not to be always fully appreciated. There is a quantitative relationship between accumulation by plants of each of these elements and crop yield. Deficiency in either element may retard plant growth[3]. Consequently, it is not possible without N to synthesize the necessary proteins, enzymes, RNA and DNA required for almost all plant cells for their initial development, sustained growth and functioning supporting other tissues of the plant[4].

Phosphorus is considered the second most imperative element in the plant nutrition. It plays an essential role in all major metabolic processes in plant including signal transduction, photosynthesis, energy transfer, macro-molecular synthesis processes e.g. DNA replication, RNA transcription, protein synthesis and cellular respiration[5] and N fixation in leguminous plants . Although both inorganic and organic forms of P is abundant in soils, it is a necessary and a large limiting factor for plant growth at the same time most of it is not in the available form for root uptake[6].

As known, symbiotic nitrogen fixation (SNF) is a luxurious operation in relation to producing of ATP-energy that determined by the accessibility of P[7]. So, SNF is considered a high P dependant process and any P deficiency can retard legume SNF according to[8]. In addition, P insufficiency causes severe shortage in nodule processing. Common bean has been considered a species with fixation, in contrast to other grain that low in biological N_2 legumes[1], which is partly due to the sensitivity of the varieties to the short interval of vegetative fixation, environmental and to nutritional stresses[9]. Therefore, improving the genetic adaptability of common bean in low P content soils is essential[1].

Evidence of fixation at different levels of genotypic variability of available P under cultivars able to sustain biological N_2 inlimited soil-P indicates a possibility of selecting bean fixation[10], but such genetic variation is likely to be affected by cultivar phonology[11]. Although, the genotypic variability under limited P supply of vegetative growth has been verified on common bean [12], the influence of plant's stage of life remains unclear on the term of P efficiency.

Recently, reclaiming of calcareous and/or sandy soils has received superior consideration in Egypt, newly reclaimed desert soils[13]. Generally, management of water and fertilizer application in sandy soils is a major challenge due to the poor nutritional status of these soils[14]. Therefore, Fertigation systems technology were used under the new reclaimed soil conditions of Egypt to improve water and nutrient managements[15, 16, 17].

Fertigation-a modern agro-technique provides an excellent opportunity to maximize yield and minimize environmental pollution[18] by increasing fertilizer use efficiency, minimizing fertilizer application and increasing return on the fertilizer invested. In fertigation, timing, amounts and concentration of fertilizers applied are easily controlled. Furthermore, this technique includes better flexibility in time of fertilizer application as compared with the soil application method. Nutrients soluble in water are less leached by excess or more-irrigation when fertilizers are injected in small doses with plant demands[19].

Results from P nutrition experiments with drip irrigation indicate, that phosphorus fertigation improved the ability of root nodules for atmospheric N_2 fixation by common bean recombinant inbred lines and sustained the soil fertility[20].

The main goal of this research was to study the response of several RILs of common bean to three levels of fertigated phosphorus under Egyptian newly reclaimed sandy soil conditions.

Material and Methods

Site Characterization and Experimental Design

A fertigation trial was laid out in a sandy-textured soil (*Entisol-Typic Torripsamments*) at Nubaria district western of Nile Delta, Egypt during October 2013-April2014 season. The research study is situated in an arid climate region (latitude of 30°30'N and longitude of 30°20'E). Its climate wasdescribed as moderate, air temperature during the growing season was around20.2°C, and the average precipitation was 15.9 mm.Asplit split plot design with three replicates was laid out; main plots were assigned with three levels of phosphoric acid (H_3PO_4); P_0 without addition; P_1 (45 kg ha^{-1}) and P_2 (90 kg ha^{-1}). While, the sub-plots were occupied with six genotypes of common bean plant. Each plot measured of 10 m length and 10 m width, and surrounded with a 2m border band.

Irrigation-water and soil analyses

Irrigation water samples were collected and analyzed for main cations and anions, electrical conductivity (EC) and pH harmonizing to[21]. This water is good for irrigation and categorized as C2S1 according to[22], where pH value was7.5, electrical conductivity (EC) was 0.42dSm^{-1} and the sodium adsorption ratio (SAR) was 2.60.

At 0–30 cm depth, surface-soil sample was taken from the research field, air-dried, ground, and passed through a 2mm sieve. Soil analysis was carried out according to standard procedures such as; particle-size distribution using the pipette method[23], soil-pH (saturated soil paste); electrical conductivity (EC) determined in soil paste extract according to[24]; field capacity of soil[23], calcium carbonate[25], Available nitrogen, phosphorus and potassiumwere determined according to[23].

Table 1: Physico-chemical parameters of the investigated soil.

Soil physical properties	**Value**
Particle-size distribution / %	
Sand	88.10
Silt	9.10
Clay	3.00
Soil texture	Sandy (*Entisol-TypicTorripsamments*)
Field capacity (V/V)	0.20
Permanent wilting point	0.10
Soil chemical properties	
pH	8.0
EC (dS m^{-1})	0.40
$CaCO_3$%	6.68
Available nutrients in soil (mg kg^{-1})	
Nitrogen	18.25
Phosphorus	3.00
Potassium	70.56

Available nitrogen was extracted using KCl (2.0 M) and available phosphorus was determined using the Olsen method ($NaHCO_3$ [0.5 M] at pH 8.5) [26] and calorimetrically determined after stannous chloride. Available potassium was extracted using ammonium acetate (1.0 M at pH 7.0). Some physical and chemical properties of the experimental soil are listed in Table 1.

Drip irrigation set up

The main source of irrigation water was ground water and the drip irrigation system consisted of GRlines with built-in drippers set 0.50 m apart with 4 liters hour^{-1} flow capacity at 1.5 bar working pressure and the positioning between lateral lines was 0.50 m.

Cultivation

Six RILs of common bean seeds were cultivated with the spacing of 0.5m between the ridges and 0.25m between the plants in a ridge, Figure 1.

The combined fertilizer of 10N - 0P- 36K was injected from the 8thweek until the end of fertigation program according to the Egyptian Ministry of Agriculture recommendations (N,P_2O_5 and K_2O kg ha^{-1}). The combined fertilizer was manufactured by Fertilizers Development Center, El-Delta Fertilizers Plant, Egypt.

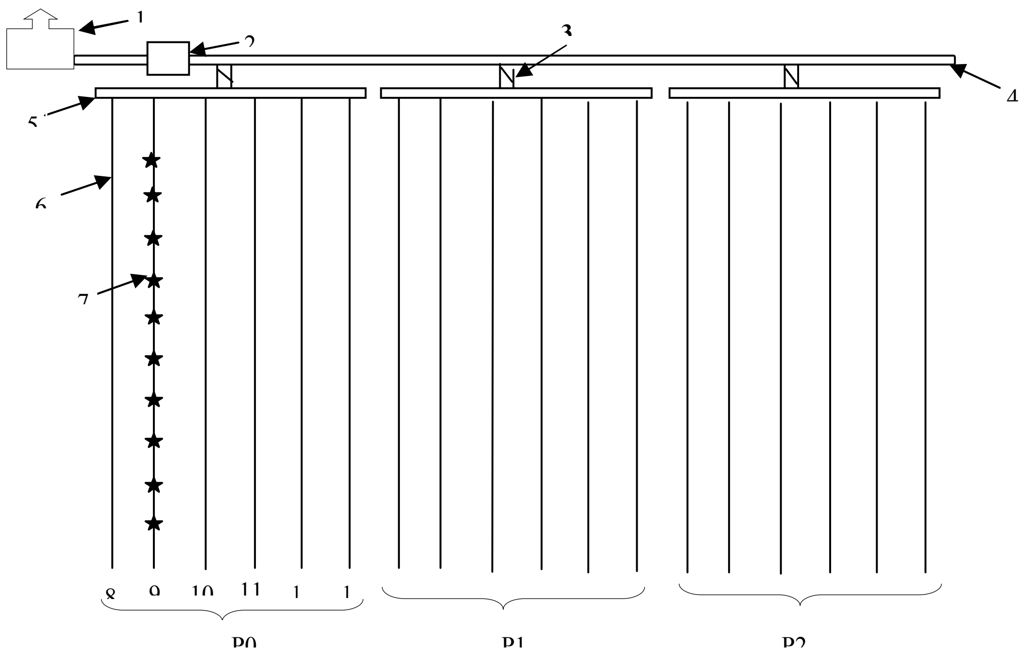
Plant sampling and chemical analysis

At physiological maturity, five samples of common bean plants were harvested from each plot.Morphological parameters such as plant height (cm), number of branches, leaf area (m^2), fresh and dry weights for plant components (g $plant^{-1}$) were recorded. After 60 planting days, nodules status were measured i.e., nodules number and fresh and dry weight of nodules (g $plant^{-1}$). Further, seed and straw yield biomass (Mg ha^{-1}) were recorded. In order to determine N and P content of shoots, nodules and seeds, the samples were prepared and oven dried at 70◦C. Weight of 0.2 g was digested by the mixture of sulfuric (H_2SO_4) and perchloric ($HClO_4$) acids (1:1). Total nitrogen was estimated by macro-Kjeldahl method according to [23]. While, total phosphorus was determined calorimetrically at wavelength 430 nm using a spectrophotometer (Spekol) according to the outlined procedures of [27]. Also, the protein content in harvested seeds was calculated by multiplying nitrogen percentage by value of 6.25 as described by [28].

Phosphorus use efficiency was calculated according to [29];

Phosphorus Use Efficiency = $(Y_P-Y_0)/F_P$.

Where, Y_P; Crop yield with applied P (Mg ha^{-1}), Y_0; crop yield (Mg ha^{-1}) in a control treatment with no P and F_P; amount of applied P fertilizer (kg ha^{-1}).



1. Irrigation + fertilizer tank	**6. Liness**	**11. RIL 75**
2. Filter.	**7. Dripers**	**12. RIL 147**
3. Valve.	**8. RIL 83**	**13. RIL 34**
4. Main line.	**9. RIL104**	
5. Submain lines	**10. RIL 115**	

Fig. 1: Graphical layout of drip irrigation system of one replicate of the experiment.

Statistical analysis

Obtained data were statistically analyzed using descriptive statistics and analysis of variance (ANOVA). Originally, the influence of phosphorus fertigation and six genotypes of common bean plant as well as their interactions on a two-way ANOVA were assessed using CoStat (CoHort, USA, 1998–2004, Version 6.303) according to the [30]. Moreover, Least-significant-differences test (LSD) at the confidence level of 5% conducted on means of treatments to measure the considered significantly different according to the procedures of [31].

Results and Discussion

This experiment focused on the effect of two P rates injected through fertigation technique on six different genotypes of common bean under Egyptian sandy soil conditions. The six common bean genotypes screened for nodulation, growth parameters, protein, N and P concentration in shoots, nodules and seeds. Also, the statistical correlation relation between N and P concentrations in nodules and the recorded yield and its components under high and low P supply were evaluated.

Growth characters

Data in Table 2 indicated that the P fertigation positively increased the vegetative growth of all investigated genotypes since the highest rate of P fertigation (90 kg P_2O_5 ha^{-1}) produced the top mean values of all studied plant growth traits as compared with the control.

Table 2: Vegetative growth parameters for common bean as affected by different P fertigation rates and genotypes under Egyptian sandy soil conditions

Treatments	Plant height (cm)	No. of branches	L. A. (m^2)	Fresh weight (g $plant^{-1}$)			
				roots	stems	Leaves	Total
P fertigation rates (kg ha^{-1})							
0	35.72b	4.97	0.452c	2.55	8.9b	66.32b	77.8b
45	37.04b	4.78	0.583b	2.61	8.94b	66.32b	77.9b
90	45.83a	4.64	0.584a	2.96	10.38a	64.9a	78.2a
Genotypes							
RIL 83	33.44b	4.11	0.565c	2.46b	7.63b	45.64c	55.7e
RIL 104	41.06ab	5.00	0.585b	2.59b	6.74b	58.97bc	68.3c
RIL 115	32.29b	5.06	0.502d	2.36b	9.72b	59.40bc	71.5bc
RIL 147	49.58 a	4.61	0.573b	3.09ab	12.55a	65.01b	80.9b
RIL 34	49.58 a	5.17	0.439d	3.45a	12.75a	101.53a	117.5a
RIL 75	31.36b	4.83	0.607a	2.27b	7.05b	49.24c	58.6d
According to the Duncan's multipletest, the same letter within the treatments indicate that the mean values are not different significantly ($p < 0.05$).							

However, the response of leaf area showed similar results at both low P rate (45 kg P_2O_5 ha^{-1}) and high P rate (90 kg P_2O_5 ha^{-1}). Figure 2 shows dry weight (g $plant^{-1}$) of shoots (A), roots (B) and shoot to root dry weight ratio (C) of six genotypes of common bean in response to two different levels of P (45 and 90 kg P_2O_5 ha^{-1}).

As shown in Table 2, obtained results showed that all studied parameters of plant growth significantly influenced by genotypes of bean with the exception of branches number. The top mean value of plant height was 49.58 cm occurred with RILs 34 and 147 in contrast with the others. In addition, the extreme mean value of branches number was 5.17 occurred with RIL 34 followed by RIL 115 (5.06), meanwhile the minimum average value of this trait was 4.11 branches per plant occurred with RIL 83 under fertigation technique.

The RIL 34 had an excessive vegetative growth, with high fresh roots (3.45 g $plant^{-1}$), stems (12.75 g $plant^{-1}$), leaves (101.53 g $plant^{-1}$) and total fresh weight (117.5 g $plant^{-1}$) than other investigated genotypes. While, the RIL 75 recorded the highest leaf area (0.607m^2).

Data reveal an upsurge in biomass of shoot with the high P level by more than 40% for RIL 75 and about 15% for RIL 83 as compared to the other RILs (Figure 2A). On the other hand, Figure 2B shows a similar manner in response to high and low P rate. RIL 147 shows a significant high value of root dry weight (3.77 g $plant^{-1}$) and RIL 115 shows the lowest significant value (2.89 g $plant^{-1}$) under the high P level as compared to the other RILs. Whereas, there was no influence for progressing the P supply on the root dry weight as reported by [32]. Thus, there was a significant decrease in the dry weight of shoot to root ratio under high P rate for RILs 83, 34,104 and 147 more than for RILs 75 and 115 (Figure 2C). Genotypes that having a great root system or a fabulous shoot/root ratio and with a superior exposure to P sufficient solution that could be look forward to supplementary advantage and genotypes with high P uptake rates per unit of root length would also be favorite [33].

Nodulation status

A variety of processes controlled nodulation, both internal (auto regulation of nodulation, ethylene) and external (heat, acidic soils, drought and nitrate).

In Figure 3B, RILs 83 shows that there is an upsurge in biomass of nodules with the higher rate of P by more than 45%, about 28% for RILs 115,147, 34 and 104 and about 10% for RIL 75 in contrast to the untreated plants. The P supply, RILs and combined effects of P supply and RILs had a significant impact on the nodule biomass and nodule numbers. A glance at Figure 3, it is easily to note that nodules number differed among genotypes under fertigation technique.

Figure 3A shows that the higher level of P fertigation had a significant increase in nodule numbers. Nodule numbers were more than twofold higher as compared to the untreated RIL 83, more than (42%) for RIL 34 and about (15%) for RILs 104, 115 and 147 compared to untreated plants. Data pointed out that the biomass of nodule per unit plant biomass was stirred as a result of an upsurge in soil-P supply, representative that the influence of external P on the nodulation was unambiguous and independent on the growth of host plant as suggested by [32]. The obtained data reported notably that biomass of common bean shoot was associated to the rate of root nodulation. This confirmed the adequacy of rhizobial symbiosis in six RILs. Further, regression analysis pointed out that the rate of nodulation, therefore, shoots biomass; associated to an available Olsen-P in soil. These genotypes have the capability to diminish the quantity of applied nitrogen by fixing atmospheric N_2 by their root nodules. This is stimulating because N fertilizer is costly and this soil under study has struggled in nutrients supply. Consequently, these RILs seem as appropriate to this newly reclaimed sandy soil under fertigation technique [20].

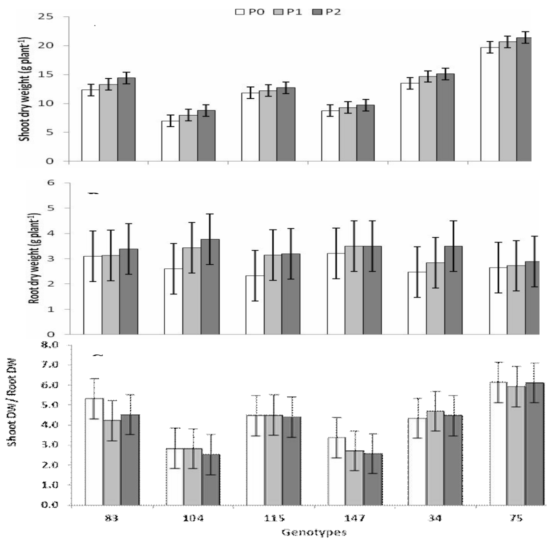


Figure 2: Dry weight (g plant^{-1}) of shoot (A), root (B) and shoot to root DW ratio (C) for common bean as affected by genotypes and different P fertigation rates, Data are means and error bars represent standard deviation (SD) of 5 replicates harvested at 120 days after planting.

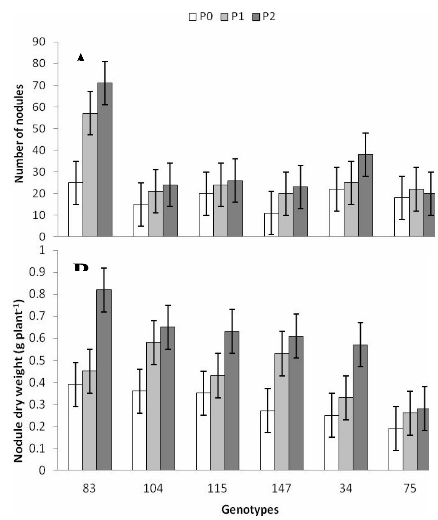


Figure 3: Dry weight (g plant^{-1}) of nodules (A) and number of nodules (B) for common bean as affected by genotypes and different P fertigation rates, data are means and error bars represent standard deviation (SD) of 5 replicates harvested at 120 days after

Phosphorus content in shoot, nodules and seeds

Data in Figure 4 show P content (%) in shoots (A), in nodules (B) and in seeds (C) of six different genotypes of common bean as affected by two different levels of P fertigation (45 and 90 kg P_2O_5 ha^{-1}). RILs showed a similar trend of phosphorus content in shoots, nodules and seeds of all genotypes in response to high and low levels of phosphorus fertigation. RIL 83 revealed a markedly high phosphorus content (0.35 %) in shoots under high P level, RIL 75 in nodules (0.49 %) and RIL 34 in seeds (0.82 %) than the other genotypes. In case of RIL 147 showed a significant high value (0.43 %) in nodules under low P level rather than under high P level. In contrast, P content in shoots was significantly declined in RIL115 under low P level which gives 0.18% in comparison to the other genotypes. Under low P nutrition some genotypes vary in their ability to nodulate and to fix N_2. On the other hand, tolerant genotypes showed a better uptake efficiency and preferential allocation of this nutrient towards the nodules [34].

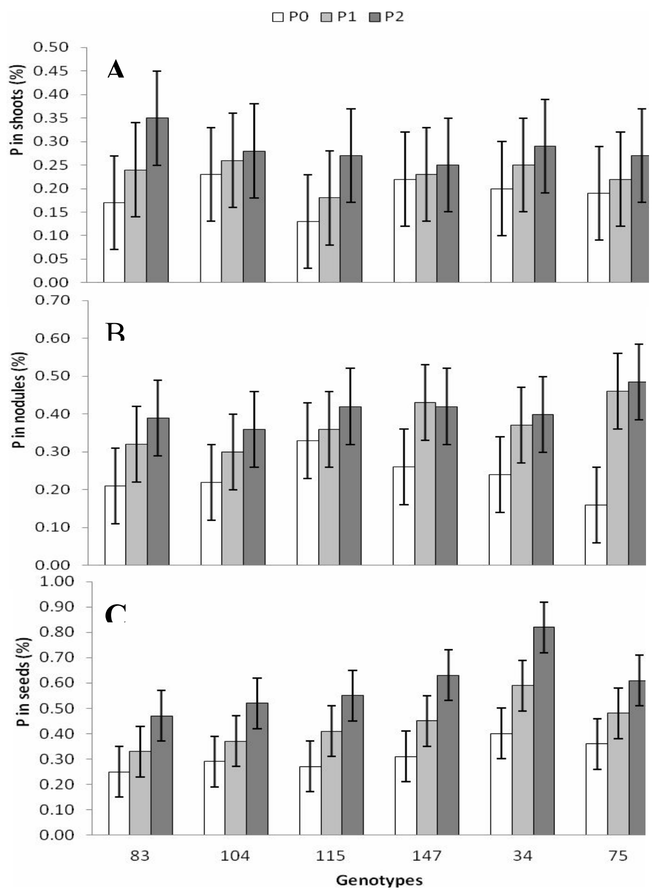


Figure 4: Phosphorus content (%) in shoots (A), nodules (B) and seeds (C) for common bean as affected by genotypes and different P fertigation rates, data are means and error bars represent standard deviation (SD) of 5 replicates harvested at 120 days after planting.

The development of nodule depends mostly on their P supply that gave the highest nodule development allocated more P to its nodules [35]. [36] found that frequent P injection through drip irrigation water preserved a time averaged P concentration in the soil solution which was appreciably higher than the equilibrium P concentration of prevailing Ca-P minerals. Under the super saturated P concentration, P uptake increased and consequently yield upraised.

Nitrogen content in shoot, nodules and seeds

In all genotypes the high P supply significantly amplified N content in shoots, nodules and seeds as compared with the supply of low P and the control (without P).

Data in Figure 5 showed N content (%) in shoots (A), in nodules (B) and in seeds (C) of six genotypes of common bean in response to two different levels of P (45 and 90 kg P_2O_5 ha^{-1}). In Figure 5 (A, B and C), fertigated RILs 75, 104 and 34 achieved a higher significant values of N concentration in shoots, nodules and seeds under high P supply which were 3.22, 3.03 and 3.00 %), (4.97, 4.68 and 4.48 % and 4.42, 4.06 and 3.80 %, respectively.

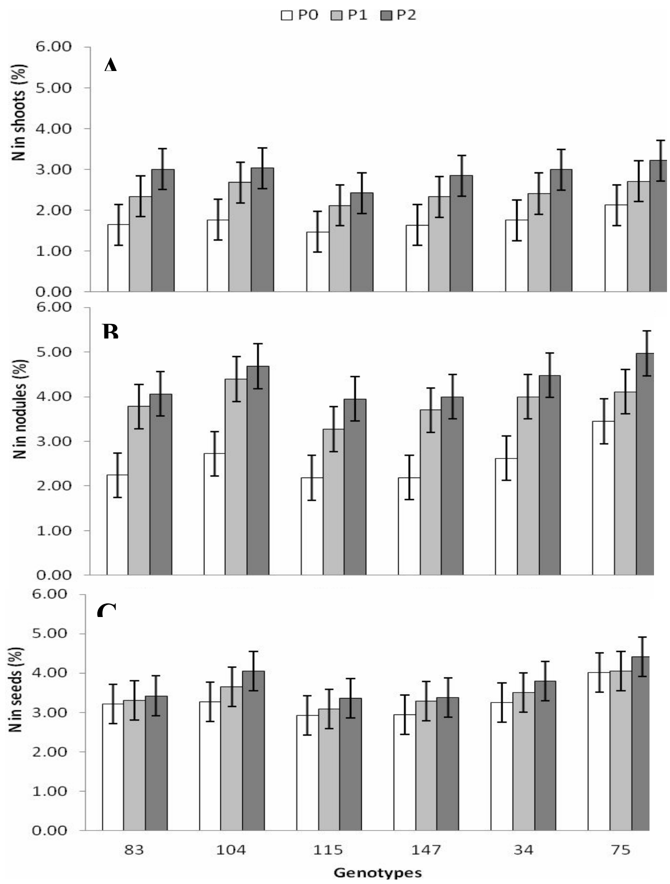


Figure 5: Nitrogen content (%) in shoots (A), nodules (B) and seeds (C) for common bean as affected by genotypes and different P fertigation rates, data are means and error bars represent standard deviation (SD) of 5 replicates harvested at 120 days after planting.

Protein content

Formerly, protein content followed the same trend obtained in nitrogen content in shoot, nodules and seeds (Figure 5) were also increased by using high P level under fertigation technique. Meanwhile, Figure 6 shows that protein content gave the highest values for RILs 75, 104 and 34 gave a high significant values of protein content in shoots, nodules and seeds under high P supply which were 20.13, 18.94 and 18.75%, 31.06, 29.25and 28.00 % and 27.60, 25.36 and 23.77 %, respectively.

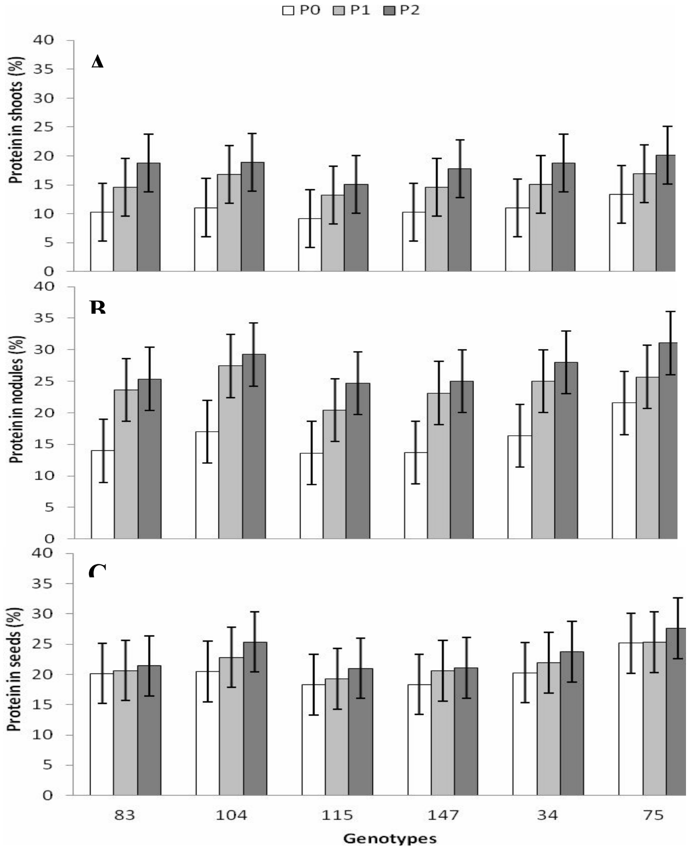


Figure 6: Protein content in shoots (A), nodules (B) and seeds (C) for common bean as affected by genotypes and different P fertigation rates, data are means and error bars represent standard deviation (SD) of 5 replicates harvested at 120 days after planting

Phosphorus and nitrogen relationship in nodules

The correlation between N and P concentration in nodules was high (r^2= 0.959, 0.675 and 0.513) for RILs 75, 115 and 83, respectively under high P level, (r^2= 0.634 and 0.549) for RILs 104 and 34 respectively under low P level and it was moderate (r^2= 0.345, 0.339 and 0.205) for RILs 115, 104 and 34 respectively under high P level.

On the other side, the correlation between P and N concentration in nodules was very low (r^2= 0.043 and 0.023) for RILs 34 and 147, respectively under high P level and (r^2= 0.006 and 0.010) for RILs 83 and 75, respectively under low level of P as shown in Figure 7. Addition of P inputs and PGPR caused considerable increase in growth parameters and P and N uptake in plants which would ultimately amplify the yield and yield components of common bean as reported by [37].

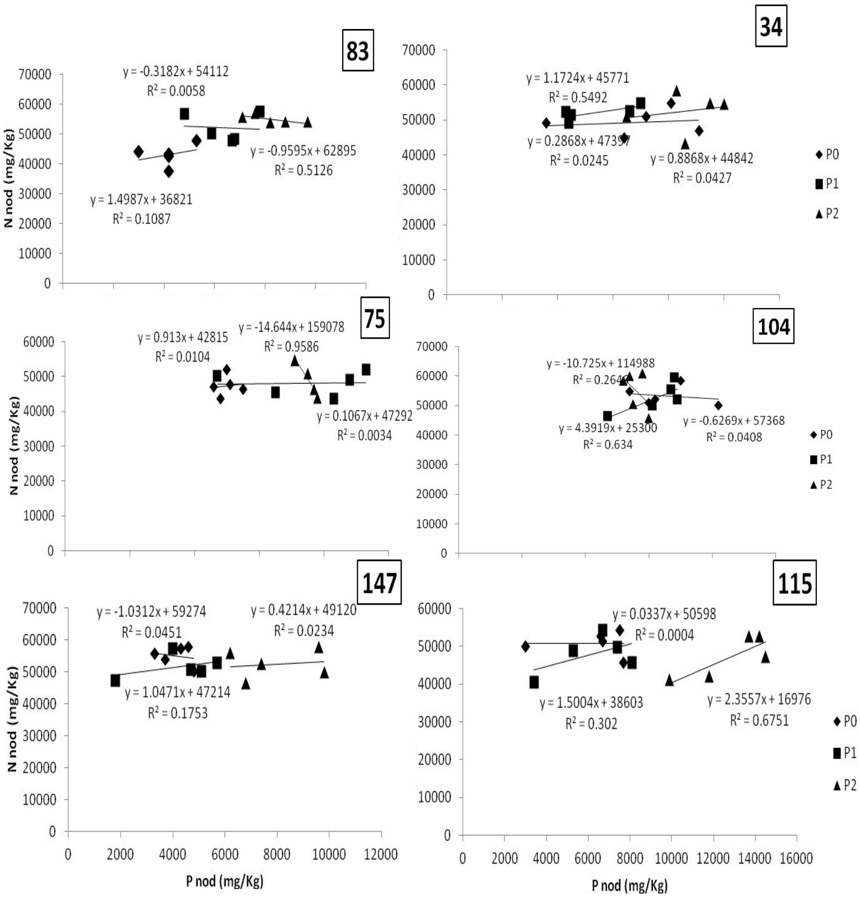


Figure 7: Effect of different P fertigation rate and genotypes on the regression parameter of N in nodules as a function of P in nodules in six common bean genotypes. data are individual values of plants harvested at 120 days after sowing.

Fertilizer-use efficiency

Fertilizer-use efficiency was significantly superior in all the treatments as compared to the control. The highest mean values of fertilizer use efficiency was 19.56 kg yield occurred with RILs 83fertigated with 45 kg P_2O_5 ha^{-1}, while the lowest mean values of the same trait was found with un-fertigated phosphoric acid, respectively.

Based on the presented data in Table 3, it can be noticed that RIL 83 achieved highly significant values for yield and its components and it is followed by RILs 75 and RILs 34 compared with the other three RILs of common beans. This increase in yield may be referred to the fact that phosphorus is a constituent of many compounds in plants, i.e. phospholipids, nucleotides and Co-enzymes[38]. Further, [36] showed that plant response to P injected through drip fertigation system under calcareous soil conditions was better than the conventional method of broadcast P fertilization. The positive yield response can be attributed to increase nodules per plant and individual seed weight due to P nutrition. Similarly, [39]reported that 100-seed weight which is important determinants of grain yield increased with P application, caused considerable progress in the yield of seeds.

Yield and yield components

The test of variance (ANOVA) revealed significant (*P*=0.05) effects of genotypes and P fertigation rates for almost all studied traits of common bean yield as shown in Table 3.

Under fertigation technique, the higher the rate of P the higher the mean values of seed, straw and biomass (Mg ha^{-1}). Obtained results in the same Table demonstrated that, the highest mean values of seed, straw and biomass yields were 6.78, 7.93 and 14.71Mg ha^{-1} occurred with RILs 75 which injected with 90 kg P_2O_5 ha^{-1} through drip irrigation system, respectively. Meanwhile, the lowest mean values of the same traits were 2.88, 3.16 and 6.04Mg ha^{-1} occurred with untreated RILs 83 with phosphoric acid, respectively.

Conclusion

Based on the findings of our work, we can conclude that there is a good relation between nodulation status of roots and shoot biomass of the common bean. This confirmed the adequacy of rhizobial symbiosis in six RILs under the technique of fertigation. Moreover, seed and its components significantly responded with the higher P supplied through fertigation technique in all evaluated RILs. Overall, the fertigated RIL 75 had the highest phosphorus use efficiency with the rate of 45kg P_2O_5 using the fertigation technique under the new reclaimed sandy soil conditions of Egypt. This, in turn, decreases the potential of phosphorus hazards environment.

Acknowledgments

Our great acknowledge to the National Research Centre for simplifying the responsibilities during these relations, providing the necessary place to conduct the field experiment and the laboratory analysis.

Table 3: Means of yield (Mg ha^{-1}) of common bean crop and phosphorus utilizationefficiency (PUE) as influenced by integrated with genotypes and different P fertigation rates under Egyptian sandy soil conditions.

Genotypes	P fertigation rates (kg ha^{-1})	100 seed weight (g)	Yield (Mg ha^{-1})*			P-Utilization Efficiency (Kg yieldkg^{-1} P_2O_5)
			Seed	Straw	Biomass	
RIL 83	0	18.28d	2.88c	3.16d	6.04d	----
	45	18.48d	3.19c	3.73c	6.92cd	19.56a
	90	18.58cd	3.26cb	4.00c	7.26c	13.55b
RIL 104	0	20.88b	4.65ab	5.27b	9.92ab	----
	45	20.90b	4.80ab	5.15b	9.95ab	0.66g
	90	20.98b	5.18ab	5.70ab	10.88ab	10.66c
RIL 115	0	20.58ab	4.34ab	4.77bc	9.11b	----
	45	20.78b	4.41ab	4.97bc	9.38ab	6.00e
	90	20.88b	4.56ab	5.04bc	9.60ab	5.44ef
RIL 147	0	18.69c	3.13c	3.44c	6.57cd	----
	45	18.78cd	3.29cb	3.48c	6.77cd	4.44f
	90	18.88cd	3.89b	3.43c	7.32c	8.33d
RIL 34	0	18.22d	4.19ab	4.62bc	8.81bc	----
	45	19.25cd	4.43ab	4.87bc	9.30ab	10.88c
	90	19.69cd	4.57ab	5.20b	9.77ab	10.66c
RIL 75	0	21.12ab	6.48a	7.13a	13.61a	----
	45	21.18a	6.59a	7.88a	14.47a	19.11a
	90	21.20a	6.78a	7.93a	14.71a	12.22b
According to the Duncan's multiple test, the same letter within the treatments indicate that the mean values are not different significantly (*P*< 0.05). **Mg; Mega = ton.						

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