THE ROLE OF PLANT GROWTH PROMOTING RHIZOBACTERIA (PGPR) AND PHOSPHORUS FERTILIZATION IN IMPROVING PHENOLOGY AND PHYSIOLOGY OF BEAN (*PHASEOLUS VULGARIS* L.)

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Abstract. The field experiment was conducted during 2017 and 2018 at the experimental area of the Faculty of Agriculture, Eskisehir Osmangazi University, Eskisehir, Turkey. It was designed as factorial arrangement in the complete randomized block design with three replications. Three phosphorus doses (0, 30 and 60 kg ha^{-1} P_2O_5) were investigated with different biofertilizers (Control, Bontera (*Bacillus amyloliquefociens, Bacillus pumilus, Bacillus subtilis, Bacillus licheniformis, Bacillus megaterium, Trichoderma harzianum, Trichoderma kanigi*), Bactoboost (*Bacillus subtilis, Bacillus magaterium, Loctococcus* spp.), Koklendirici (*Bacillus subtilis, Bacillus magaterium, Loctococcus* spp.) Lifebac NP (*Bacillus subtilis, Bacillus magaterium*), NSAH (15% organic matter, 6% organic carbon, 13% humic + fulvic acid), Rhizobia (*Rhizobium leguminosorum*). Emergence time, flowering time, maturity time, chlorophyll content, nodule number, nodule fresh weight, nodule dry weight, leaf area index, normalized difference vegetation index, and grain yield were investigated during the research. All of the investigated characteristics were higher in the first year than in the second year due to high temperature in the second year. Temperature stress negatively affected phenological and physiological characters during the research. Increasing phosphorus doses raised maturity time, chlorophyll content, leaf area index, NDVI and grain yield. In the research biofertilizer positively affected all of the investigated characters except for nodulation potential.
Keywords: *NDVI, leaf area index, chlorophyll content, biofertilizer*

Introduction

Phosphorus is the second most important macronutrient after nitrogen that is required by the plants (Sarwar et al., 2016). Chemical fertilizers are needed to get good crop yields but their abuse and overuse can be harmful for the environment and their cost cannot make economic and profitable agricultural products (Bobade et al., 1992). Simultaneous inoculation with *Rhizobium* and other plant growth-promoting bacteria has shown potential to enhance plant growth, nodulation and nitrogen fixation of several legumes. (Remans et al., 2007). N_2-fixing and P-solubilizing bacteria may be important for plant nutrition by increasing N and P uptake of the plants, and playing a significant role as plant growth-promoting rhizobacteria (PGPR) in the biofertilization of crops (Yazdani et al., 2009). PGPR is thought to stimulate plant growth through any of the following mechanisms: (1) by altering the hormone balance in the host plant; (2) by increasing mineral nutrient solubilization; and (3) antagonism towards plant pathogens. In addition to the improvement of plant growth, PGPR is directly involved in increased uptake of nitrogen, synthesis of phytohormones, solubilization of minerals such as phosphorus and

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production of siderophores that chelate iron and make it available to the plant root (Glick, 1995). Rhizobacterial-based technologies have been investigated for their use as alternatives to synthetic fertilizers for sustainable crop production (Patel and Minocheherhomji, 2018). Economic and environmental benefits can include increased income from high yields, reduced fertilizer costs and reduced emission of the greenhouse gas, N_2O as well as reduced leaching of NO_3^-, N to ground water (Yazdani et al., 2009). Wang et al. (2016) reported that PGPR can improve seed germination. Fatnassi et al. (2015) found that PGPR increased nodule numbers and nodule dry weight by 50% for Vicia faba. Elkoca et al. (2010) reported that single, dual and triple inoculation with *Bacillus subtilis, B. megaterium* and *Rhizobium leguminosarum* bv. *phaseoli* increased chlorophyll contents, nodule dry weight, and nutrient uptake of *Phaseolus vulgaris* L. Inoculation with *Bacillus* spp. and *Rhizobium* or *Bradyrhizobium* spp. developed the nodulation and plant growth of common bean (*Phaseolus vulgaris* L.) and soybean (*Glycine max* (L.) Merr*)* (Srinivasan et al., 1997; Camacho et al., 2001; Bai et al., 2003).

Plant growth-promoting rhizobacteria (PGPR) actively participated in the transformation of phosphate in the soil and made phosphorus available to the plant. (Bechtaoui et al., 2020) The use of biofertilizers along with chemical fertilizers may serve as an effective approach for enhancing the crop nutrient requirements, thereby leading to sustainable crop production. (Israr et al., 2016). Plant growth-promoting rhizobacteria (PGPR) exert a beneficial effect on plant growth in several ways, e.g. phosphate-solubilizing ability can increase the availability of phosphorus (P) in the soil from the residual soil P, produce growth-promoting substances and improve N-fixation, and thereby increase the overall growth and physiology of the crop (Singh et al., 2018).

We aimed to elucidate the effects of PGPR and different phosphorus doses on phenological and physiological characters of bean under field experiments.

Materials and methods

The field experiment was conducted during 2017 and 2018 at the experimental area of the Faculty of Agriculture, Eskisehir Osmangazi University, Eskisehir, Turkey (39°48’ N; 30°31’ E, 798 m above sea level). Climatic data for long-term and experimental years are shown in *Figure 1*.

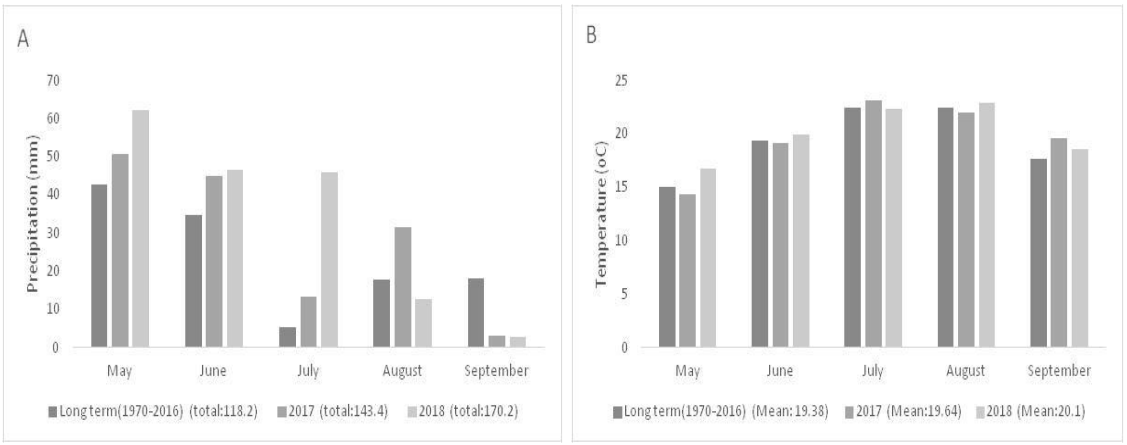

Figure 1. *Climatic data of the research area*

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Long-term annual total precipitation is 104.1 mm and it was 143.4 and 170.2 mm in the experimental years, respectively. The annual average temperature was 19.64 °C in 2017 and 20.1 °C in 2018. Physical and chemical properties of the soil in the experimental areas are presented *Table 1*.

Table 1. *Soil physical and chemical properties of the experimental area*

Depth (cm)	pH	Lime (%)	Organic matter (%)	P_2O_5 kg ha^{-1}	K_2O (kg ha^{-1})	N (%)	Ca (mg/kg)	Mg (mg/kg)	Cu (ppm)	Mn (ppm)	Fe (ppm)	Zn (ppm)
0-30	7.83	5.40	0.79	40.55	1810	0.03	4197	876.30	0.95	3.16	1.56	0.66
0-30	7.71	7.56	1.65	170.75	2450	0.08	2061	482.8	0.82	2.94	2.84	0.32

The experiment was designed as factorial arrangement in the complete randomized block design with three replications. Three phosphorus doses (0, 30 and 60 kg ha^{-1} P_2O_5) were investigated with different biofertilizers (Control, Bontera (Bacillus amyloliquefociens, Bacillus pumilus, Bacillus subtilis, Bacillus licheniformis, Bacillus megaterium, Trichoderma harzianum, Trichoderma kanigi), Bactoboost (Bacillus subtilis, Bacillus magaterium, Loctococcus spp.), Koklendirici (Bacillus subtilis, Bacillus magaterium, Loctococcus spp.) Lifebac NP (Bacillus subtilis, Bacillus magaterium), NSAH (15% organic matter, 6% organic carbon, 13% humic + fulvic acid), Rhizobia (Rhizobium leguminosorum). Bean varieties Topcu were used as research materials. Each plot was 7.2 m^2 (4 m x 1.8 m) and bean was sown with 45 cm row spacing and seeding rate was 26 seeds m^{-2}. The sowing time was 04 May and 04 May in 2017 and 2018, respectively. Triple super phosphate containing 43-45% P_2O_5 was used as phosphorus fertilizer. Ammonium sulfate fertilizer (21%) was applied to all of the plots at 25 kg ha^{-1} N at sowing time, emergence time was when 50% of plots were emerging, flowering time was when 50% of plots were in flowering; maturation time was observed when 90% of the plots were mature. Chlorophyll content (spad) was evaluated on 5 randomly selected plants in each plot at the time of flowering with the Minolta Spad 502 Plus chlorophyll meter. The nodule number, nodule fresh and dry weight (g) were determined in 5 plants taken from all parcels to determine the nodulation potential in June when flowering started. Leaf area index was measured at the beginning of the podding stage with a portable field meter Delta-T SunScan in the middle of the two rows. Normalized difference vegetation index was measured in the middle of each plot by a hand-held optical sensor with Greenskeer^{TM} at the beginning of the podding stage. Each plot was harvested, blended and grain yield (kg ha^{-1}) was estimated.

The variance analysis was based on General Linear Model using the Statview package (SAS Institute). Means were compared by Least Significant Differences (LSD) test.

Results and discussion

The effects of years and bacteria were significant for all of the investigated characters but differences between phosphorus doses were insignificant for some investigated properties such as flowering time, nodule number and nodule dry weight (*Tables 2* and *3*).

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Table 2. *Effects of different phosphorus doses and bacteria on some traits of bean*

	Emergence time (day)	Flowering time (day)	Maturity time (day)	Chlorophyll content (spad)	Nodule number
2017	15.96 A	39.67 A	116.25 A	44.29 A	24.03 A
2018	15.32 B	38.16 B	106.60 B	37.74 B	15.28 B
Mean	15.64	38.91	111.42	41.01	19.65
0 kg ha^{-1} P_2O_5	15.69 A	38.96	110.77 B	38.00 C	19.51
30 kg ha^{-1} P_2O_5	15.79 A	38.91	111.71 A	40.44 B	17.72
60 kg ha^{-1} P_2O_5	15.44 B	38.88	111.78 A	44.61 A	21.74
Mean	15.64	38.91	111.42	41.01	19.65
Control	15.18 C	38.79 B	112.53 AB	38.68 D	32.64 A
Bontera	15.27 C	38.23 C	109.78 E	36.33 E	14.80 C
Bactoboost	15.76 B	39.04 B	110.72 D	40.03 CD	24.67 AB
Koklendirici	15.80 AB	38.95 B	111.64 C	42.69 B	17.37 BC
Lifebac NP	15.67 B	38.83 B	110.31 D	43.26 B	18.07 BC
NSAH	15.73 B	38.93 B	112.74 A	40.62 C	17.24 BC
Rhizobia	16.08 A	39.63 A	112.23 B	45.50 A	12.81 C
Mean	15.64	38.91	111.42	41.01	19.65
General mean	15.64	38.91	111.42	41.01	19.65
Year	**	**	**	**	**
Phosphorus doses	**	ns	**	**	ns
Bacteria	**	**	**	**	**
Year x phosph.	ns	ns	**	*	ns
Year x bacteria	**	**	**	**	ns
Phosp. x bacteria	**	**	**	**	ns
Year x phosp. x bac.	**	**	**	**	ns

ns: non-significant, *: $p \leq 0.05$, **: $p \leq 0.01$

While emergence time and flowering time were higher in 60 kg ha^{-1} P_2O_5 plots in 2017 rhizobia and bacteria showed lower values in the same plots in 30 kg ha^{-1} in the same year (*Fig. 2A, B*). While chlorophyll content and leaf area index were higher in 60 kg ha^{-1} P_2O_5 plots in 2017 for Lifebac NP, the same bacteria showed lower values in the same plots in 2018 (*Figs. 3B, 4A*). While NDVI was higher in 60 kg ha^{-1} P_2O_5 plots in 2017 for control, in the second year control plots showed lower values in the same doses (*Fig. 4B*). Nodule fresh weight and nodule dry weight showed superior performance in control plots in 2018 but same plots showed lower values in 2017 (*Fig. 5 A, B*). While maturity time and grain yield showed superior performance in control plots in 2017, same plots showed lower values in 2018 (*Figs. 3A, 6*). Therefore, year x rhizobia x nitrogen fertilization interaction was significant.

All of the investigated characters were higher in the first year than in the second year (*Tables 2* and *3*). The temperature was higher in the second year than in the first year in our research. In the second year, higher temperatures in May when experiments were established caused earlier emergence. In addition, higher June temperatures in the flowering stage caused earlier flowering in the second year (*Fig. 1*). Chlorophyll content was 44.29 spads in 2017 but it was 37.74 spads in 2018 (*Table 2*). High temperature caused lower photosynthetic activity and therefore chlorophyll content

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decreased (Xu et al., 1995). High temperatures in the second year may be caused by low chlorophyll content. Nodule number, nodule fresh weight and nodule dry weight were lower in 2018 (*Tables 2* and *3*). High temperatures negatively affected nodule formation and nitrogenase activity (Yavas and Unay, 2018). Temperature stress before flowering causes nodules degeneration (Gaur et al., 2015). The higher temperatures in the second year had negative effects on the nodule number, nodule fresh weight and nodule dry weight. Leaf area index was 3.18 in 2017 but it was 2.33 in 2018 (*Table 3*). Oner and Sezer (2007) reported that the leaf area index increased with increasing light intensity and low temperatures. Low temperatures in the first year may be caused by higher leaf area index. NDVI is defined as the ratio of radiation reflected from healthy vegetation to radiation reflected from all other sources. There is a positive relationship between NDVI and leaf area index and grain yield (Tahir et al., 2020). Grain yield was lower due to total high temperature especially grain-filled period in the second experimental year (*Table 3*). High temperatures reduced the total leaf area and net assimilation amount and therefore plant growth also reduced (Rodríguez et al., 2005; Ashraf and Hafeez, 2004).

Table 3. *Effects of different phosphorus doses and bacteria on some traits of bean*

	Nodule fresh weight (g)	**Nodule dry weight (g)**	**Leaf area index**	**Normalized difference vegetation index**	**Grain yield (kg ha^{-1})**
2017	0.29 A	0.18 A	3.18 A	0.69 A	2110 A
2018	0.24 B	0.15 B	2.33 B	0.59 B	1490 B
Mean	0.26	0.16	2.75	0.64	1800
0 kg ha^{-1} P_2O_5	0.24 B	0.17	2.47 C	0.62 C	1650 C
30 kg ha^{-1} P_2O_5	0.29 A	0.16	2.82 B	0.63 B	1810 B
60 kg ha^{-1} P_2O_5	0.26 B	0.15	2.98 A	0.67 A	1950 A
Mean	0.26	0.16	2.75	0.64	1800
Control	0.36 A	0.22 A	2.60 C	0.62 D	1930 B
Bontera	0.28 B	0.18 B	2.49 C	0.62 D	1490 D
Bactoboost	0.26 B	0.15 C	2.71 BC	0.64 C	1580 C
Koklendirici	0.21 C	0.13 C	2.86 AB	0.65 B	1930 B
Lifebac NP	0.21 C	0.14 C	3.03 A	0.63 C	1630 C
NSAH	0.26 B	0.15 BC	2.71 BC	0.65 B	2100 A
Rhizobia	0.27 B	0.16 BC	2.91 AB	0.67 A	1930 B
Mean	0.26	0.16	2.75	0.64	1800
General mean	0.26	0.16	2.75	0.64	1800
Year	**	**	**	**	**
Phosphorus doses	**	ns	**	**	**
Bacteria	**	**	**	**	**
Year x phosphorus	**	**	**	**	**
Year x bacteria	**	**	**	**	**
Phosp. x bacteria	**	**	**	**	**
Year x phosp. x bac.	**	**	**	**	**

ns: non-significant, *: $p \leq 0.05$, **: $p \leq 0.01$

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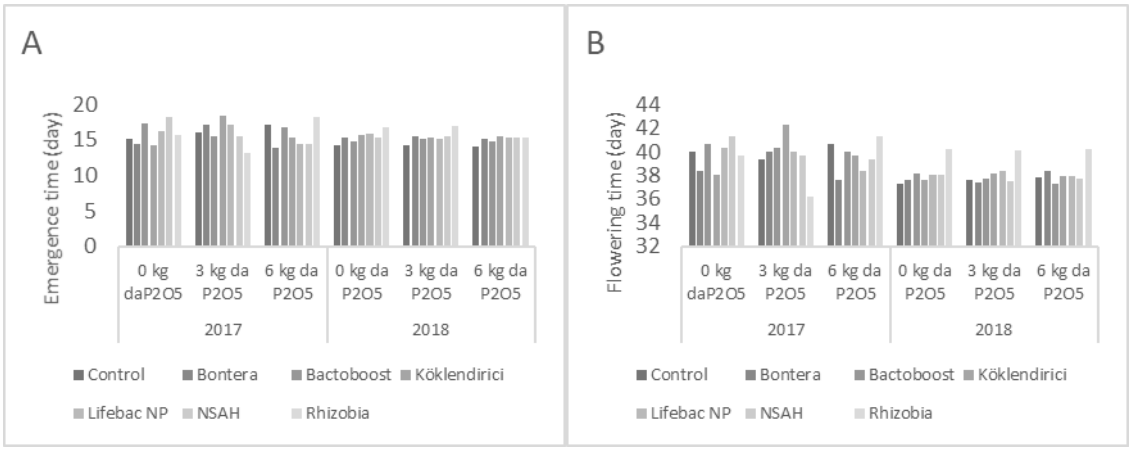

Figure 2. *The interaction between year, phosphorus doses and bacteria on emergence time (A) and flowering time (B) of bean [LSD 1%: 0.657 (A);1%: 1.146 (B)]*

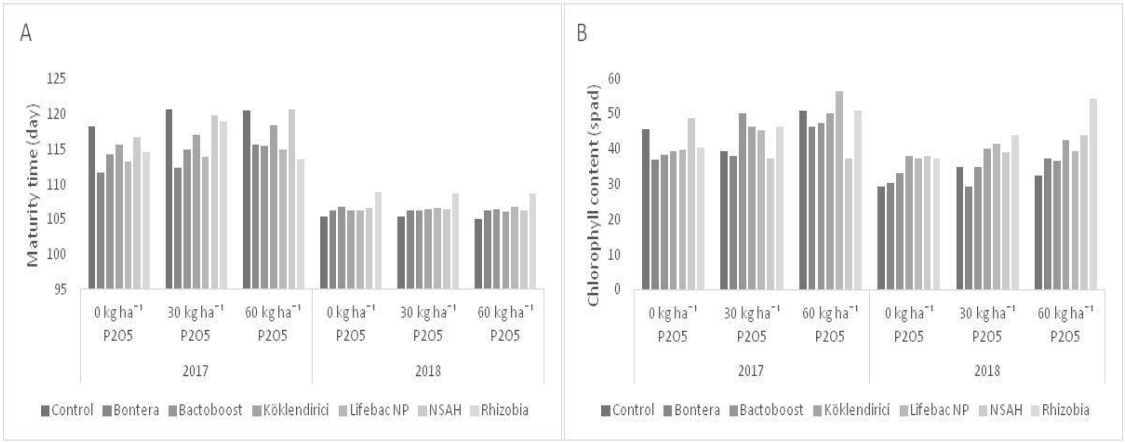

Figure 3. *The interaction between year, phosphorus doses and bacteria on maturity time (A) and chlorophyll content (B) of bean [LSD 1%: 1.040 (A);1%: 3.597 (B)]*

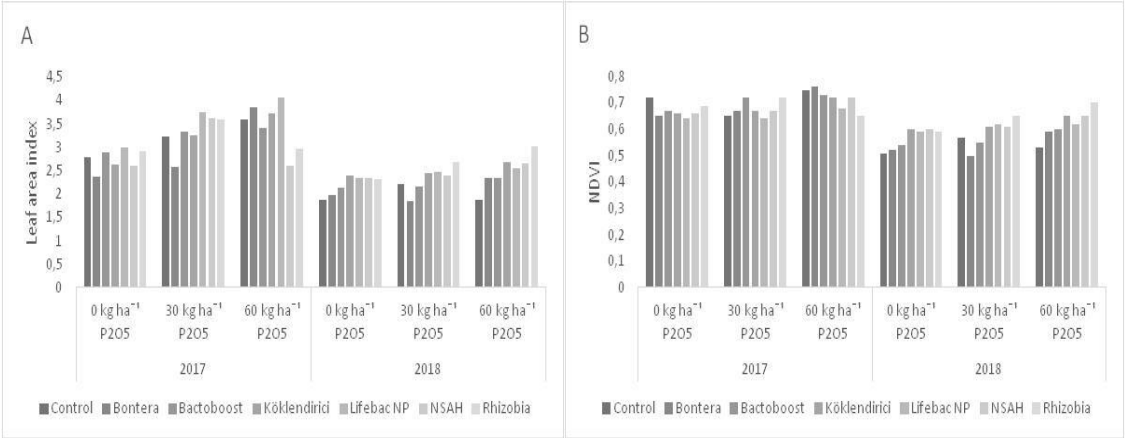

Figure 4. *The interaction between year, phosphorus doses and bacteria on leaf area index (A) and NDVI (B) of bean [LSD 1%: 0.476 (A);1%: 0.002 (B)]*

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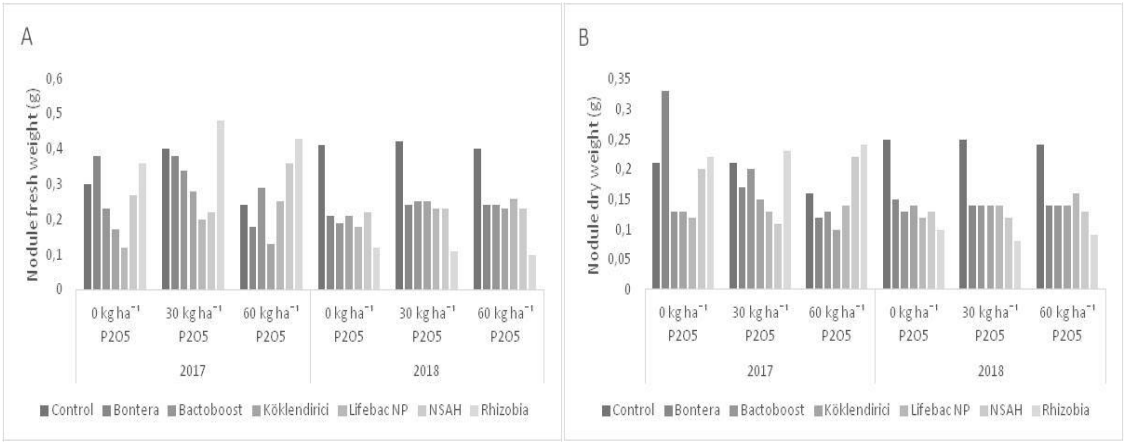

Figure 5. *The interaction between year, phosphorus doses and bacteria on nodule fresh weight (A) and nodule dry weight (B) of bean [LSD 1%: 0.068 (A);1%: 0.068 (B)]*

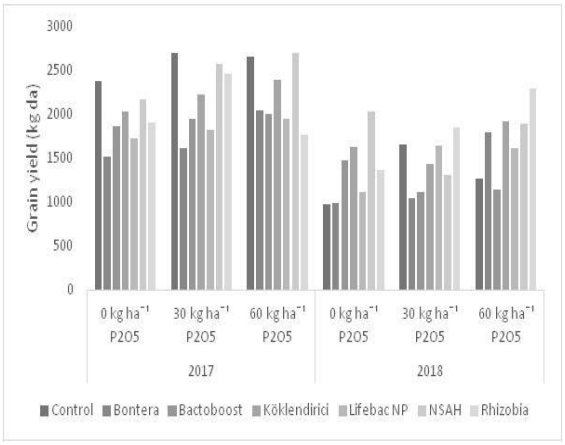

Figure 6. *The interaction between year, phosphorus doses and bacteria on grain yield of bean [LSD 1%: 14.15]*

Increasing phosphorus doses decreased the emergence time but increased maturity time (*Table 2*). 0 kg ha^{-1} and 30 kg ha^{-1} phosphorus doses show similarity regarding emergence time. 30 kg ha^{-1} and 60 kg ha^{-1} phosphorus doses show no difference for maturity time. The highest chlorophyll content was obtained when 60 kg ha^{-1} P_2O_5 was applied (*Table 2*). Chlorophyll content increased with increasing phosphorus doses. Mtua (2015) reported that phosphorus doses increased chlorophyll content for bean. Phosphorus fertilization increased nodule number but it was not significant statistically. 0 kg ha^{-1} and 60 kg ha^{-1} phosphorus doses show similarities for nodule fresh weight. The highest nodule fresh weight was obtained from 30 kg ha^{-1} P_2O_5. Yilmaz (2010) indicated that phosphorus fertilization increased nodule number. The highest leaf area index was obtained from 60 kg ha^{-1} P_2O_5 (*Table 3*). Turuko and Mohammed (2014) indicated that leaf area index increased with increasing phosphorus doses for bean. Many researchers reported that phosphorous fertilization increased grain yield in bean (Baydemir, 2013; Turuko and Mohammed, 2014; Mtua, 2015).

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The latest exit time was determined in the rhizobi parcels and the earliest exit time was determined in the control parcels, but this was followed by the bontera. Control plots and bontera are in the same statistical group. The earliest flowering time was in Bontera but the latest was in rhizobia. Flowering time is similar for control plots, Bactoboost, Koklendirici, Lifebac NP and NSAH content. The latest maturity time was determined in NSAH plots but this was followed by the rhizobia and the earliest maturity time was determined in bontera plots. Control plots are in the same group with NSAH and Rhizobia plots, Life NP and Bactoboost plots are statistically in the same group for maturity time. Bontera biofertilizer provided earlier emergence, flowering and maturity in our research. While the highest chlorophyll content is in rhizobia plots, it was the lowest in Bontera plots but this was followed by the control plots. Control plots and Bactoboost applications, Koklendirici and Lifebac NP applications, and Bactoboost and NSAH applications are statistically in the same group. Baset Mia et al. (2010) and Ahamd et al. (2014) reported that biofertilizer increased chlorophyll content. The highest nodule number, nodule fresh weight and nodule dry weight were determined for the control plots (*Tables 2* and *3*). Nodule number is similar to the control and Bactoboost plots. Also Koklendirici, Lifebac Np and NSAH are in the same statistical group. Biofertilizer had no effect on nodulation potential. The highest nodule wet weight was observed in control plots. Bontera, Bactoboost, NSAH and Rhizobia showed similar values. Nodule dry weight was observed in the highest control plots. These are followed by Bontera, NSAH and rhizobia plots. The highest leaf area index was determined in Lifebac NP plots and the lowest was determined in Bontera plots but this was followed by the control plots. Metwali et al. (2015) indicated that PGPR increased leaf area index. The highest Normalized Difference Vegetation Index (NDVI) was determined for rhizobia plots and lower value was observed in control plots (*Table 3*). Biofertilizer positively affected NDVI. While grain yield was 1930 kg ha in control plots, it was 2100 kg ha in NSAH plots (*Table 3*). NSAH increased grain yield but other biofertilizers showed no positive effect on grain yield in our research. After NSAH plots, the highest yields were obtained from the plots using rhizobia and Koklendirici in the same statistical group. They were followed by Lifebac NP and Bactoboost plots in the same statistical group. Many researchers reported that grain yield was increased with PGPR application (Zahir et al., 2007; Ardekani et al., 2008; Akhtar et al., 2013; Naseri et al., 2013; Fatetorbay et al., 2014; Talat, 2019).

Conclusions

Considering the climatic data of the years when the experiment was established although the amount of precipitation was higher in the second year compared to the first year, lower values were observed in all the characteristics examined. The reason for this was irrigation in the experimental areas. The second year the temperature was higher. All of the investigated characters were higher in the first year than in the second year due to high temperature in the second year. Temperature stress negatively affected phenological and physiological characters in our research. Increasing phosphorus doses increased maturity time, chlorophyll content, leaf area index, NDVI and grain yield. When the phenological and physiological characteristics were examined, the most appropriate phosphorus doses were found to be 60 kg ha^{-1}. Effective biofertilizers are promising tools to maintain agricultural resources to improve soil fertility and plant growth. Biofertilizers positively affected all of the investigated characters except for

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nodulation potential in our research. While good results were obtained from Rhizobia bacteria in terms of the investigated characters, the highest grain yield was determined in NSAH plots. The effect of PGPR depends on the type and number of bacteria, plant-bacteria combination, plant genotype, harvest date, soil type, soil organic matter and environmental conditions. Some unpredictable conditions in field experiments, sometimes prevents correct results. Therefore, field experiments should be increased.

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