

Full Length Research Paper

## Selection of chickpea cultivars with agronomic phosphorus (P) utilization characters as influenced by *Rhizobium* inoculation

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Evaluation of phosphorus (P) efficient chickpea cultivars with and without *Rhizobium* inoculation will provide considerable genetic resources for sustaining the yields and quality with reduced P application under the P deficient conditions on agricultural fields. For this aim, a pot experiment was conducted using a Calcareous Ustochrepts soil. Twenty different chickpea cultivars (*Cicer arietinum* L.) with and without bacterium (*Rhizobium ciceri*) inoculated were used for this study. Phosphorus fertilizer as  $H_3PO_3$  at the levels of 0 and 80 mg P  $kg^{-1}$  was applied to the pots. Nitrogenous fertilizer at the level of 60 mg N  $kg^{-1}$  as ammonium nitrate was applied to all pots for normal growth. After harvesting of chickpeas, total P concentrations in the plants were determined. As a result of the study, *rhizobium* inoculation and phosphorus application in combination increased growth rate and P utilization of chickpea cultivars as compared to the control. Whereas, significant variability among the chickpea cultivars for P utilization characters have also been observed depending on P treatment and *rhizobium* inoculation. Efficiency Index (EI) parameters were used to select chickpea cultivars with bacterium (*R. ciceri*) improved P utilization characters. This method provided characterization of cultivars as ER: efficient-responsive, ENR: efficient non-responsive, IR: inefficient responsive and INR: inefficient non-responsive. Based on this classification, chickpea cultivars of Aydın, Akçin-91, ILC-482 were called as ENR under non-inoculated conditions, however chickpea cultivars of Küsmen-99, Er-97, Diyar-95, Aydın, Akçin-91 were called as ENR under the rhizobial inoculated conditions. It has been established from the results that P treatments with and without *rhizobium* inoculation greatly affected the P Efficiency Index (EI) and P utilization performance of chickpea cultivars.

**Key words:** Chickpea cultivars, phosphorus, *Rhizobium*, inoculation.

### INTRODUCTION

Phosphorus (P) use efficiency and the resistance of plants to P deficiency stress are affected by many factors such as high clay and lime contents together with low soil moisture levels, especially on dry lands. On such soils, the efficiency of fertilizer P can be low, with only 10 to 30% of the P applied available for plant uptake in the year of application (McLaughlin et al., 1988; Lynch and Beebe, 1995). However, it has been reported that different physiological or morphological adaptation

mechanisms to low P conditions were developed by plant species to improve nutrient use efficiency under infertile soils (Romheld, 1998). For example, in the case of P deficiency, organic acid secretion of plants to rhizosphere has a very important role in adaptation of plants to P deficiency conditions. Thus, nutrient concentration and uptake by varied plant cultivars are the most important criteria for diagnosing the existing genetic specificity of plant nutrition (Hammond et al., 2004).

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Phosphorus efficient genotypes is also described in literature as the genotypes with high level of biomass or yield producing capacity in the soils have low soil P and/or low P fertilization (Gabelman and Gerloff, 1983; Tara and Nielsen, 2004). Evaluation of P efficient genotypes for adaptation to low soil P levels were also studied with many crops (Fagera and Costa, 2000; Li, 2005; Ltaief et al., 2012; Mourice and Tryphone, 2012). It has been reported that nodulated legumes require high levels of P for optimal symbiotic performance (Deng et al., 1998), and there was a closely relationship between P level and symbiotic mechanism in legumes (Bildirici and Yilmaz, 2005). In a field experiment carried out on a typical Ustochrept, inoculation of lentil seed with bacteria improved its yield besides improving P use efficiency (Singh et al., 2005). On the other hand, interactive effects of bacterium inoculation, biological nitrogen fixation and P utilization characters of chickpea cultivars or other legumes were reported by other researchers (Araujo and Teixeira, 2000; Vadez and Drevon, 2001; Togay et al., 2008). Chickpea genotypes with good adaptations to low or high P conditions will provide a better growing condition for their growth or the growth of subsequent crops. At this point, sometimes unsuitable P application levels may be the main yield or nodulation limiting factor. Thus, not only inoculation will be improved but also suitable P fertilizer level will be needed for nodulation and growth performance of legume crops. It is possible to increase chickpea yield by inoculation with *Rhizobium* strains under favorable P levels. Considered in this context, determination of chickpea genotypes with efficient phosphorus use efficiency and their impact on the following rotation plants could have a contribution to the optimal nodulation and growth. It has been indicated that legumes that use the P efficiently increased the mobility of soil P, and also positively affect the P uptake and growth of legume crops (Lynch and Beebe, 1995; Kamh et al., 1999). It has also been reported that legumes that use P efficiently had a positive effect on the yields of cereals that had a low P use efficiency (Horst et al., 2001). Hence, selection of P efficient chickpea cultivars, depending on *Rhizobial* inoculation or non-inoculation, can provide considerable genetic resources for sustaining the yields and quality with reduced P supply.

## MATERIALS AND METHODS

A pot experiment, based on a completely randomized design with three replications, was conducted using a Calcareous Ustochrepts soil. The polyvinyl chloride (PVC) pots holding 4000 g of air-dried soil were used for this study. In order to prevent soil-borne diseases, soils were solarized before used in the study. For this purpose, soils were incubated under plastic covers for 15 days. Twenty chickpea cultivars (*Cicer arietinum* L.) classified as resistance to blight (T: Tolerance, R: Resistance, S: Susceptible) have been chosen for this study, most of them are used by farmers. As a result of this classification, chickpea cultivars of Aydın-92 (T), Meksika (S), Diyar-95 (T), Sari-98 (T), Er-99 (R), Aziziye-94 (R),

Cevdet-98 (T), Gökçe (R), ILC-482 (R), İzmir-92 (T), Çağatay (T), Konya (S), Gülümser (T), Akçin-91 (T), Yerlisıra (T), Menemen-92 (T), Küsmen-99 (R), Eser-87 (T), Uzunlu-99 (T) and Damla-89 (T) were used under the bacterium inoculated and non-inoculated conditions. *Rhizobium* bacteria used in the study were obtained from Ankara Soil and Fertilizer Research Institute. Seeds, subjected to viability tests, were moistened using a 10% saccharose solution. Saccharose solution was about one tenth of the seed weight. For surface sterilization, seeds were placed in a 1% hypochlorite solution for three minutes and then rinsed three times using sterile water. Then bacteria (*Rhizobium ciceri*) (1% of seed weight) were added to the seeds in a shady area and mixed well in order to obtain a homogenous inoculation (Kaya et al., 2002). In the study, phosphorus fertilizer as  $H_3PO_3$  at the levels of 0 and 80 mg P  $kg^{-1}$  was applied to the pots. Nitrogenous fertilizer at the level of 60 mg N  $kg^{-1}$  as ammonium nitrate was applied to all pots. A basal dressing of other macro and micro nutrients were applied to all pots for normal plant growth. Chickpea plants were harvested at the seventh weeks after planting. Then, shoot dry matter yields were recorded and total P concentrations in aerial parts of the plants were also determined.

The analysis for P concentration in the top of chickpea plants was made by spectrophotometer after digestion (Barton, 1948). In the experimental soil, other routine analysis were made by the routine methods. The experimental soil was clay-loam in texture with 36, 34 and 30% clay, silt and sand, respectively, and there was no salt problem. It had also the following chemical properties: calcium carbonate content = 159 g  $kg^{-1}$ , pH (1:2.5) = 7.84, organic matter content = 1.7%, available phosphorus = 7.03 mg  $kg^{-1}$ , exchangeable potassium = 6.4 me 100  $g^{-1}$  and cation exchange capacity = 35.2 me 100  $g^{-1}$ . Experimental data were subjected to the statistical analysis of variance using MSTAT package program, and the means were separated by Duncan's multiple range test. Plant shoot dry matter yield (SDM) and total P content of the plants were used to calculate the Efficiency Index parameter ( $SDM^2 / \text{total P content}$ ) for classification of genotypes (adapted from Siddiqi and Glass (1981); Furlani et al. (2001). This classification method provided to characterization of cultivars as ER: efficient-responsive, ENR: efficient non-responsive, IR: inefficient responsive and INR: inefficient non-responsive.

## RESULTS AND DISCUSSION

### Dry matter yields of chickpea cultivars

Response of varied chickpea cultivars to P treatment and bacterium inoculation was investigated. Dry matter yield of chickpea cultivars varied significantly from 2.16 to 4.41 g  $pot^{-1}$  under non-inoculated condition, and from 2.09 to 3.84 g  $pot^{-1}$  under inoculated condition (Table 1).

Phosphorus application significantly increased dry matter yield of chickpeas under the inoculated and non-inoculated treatments. Similar studies have also revealed that P application influenced significantly the growth and yield components of chickpea (Dutta and Bandyopadhyay, 2009). On the other hand, interactions between bacterium inoculation x P application, P application x cultivars and bacterium inoculation x cultivars were also significant (Table 1). The highest dry matter yield of 4.91 g  $pot^{-1}$  was obtained from the chickpea cultivar of Meksika at P-80 level, whereas the lowest dry matter yield of 1.79 g  $pot^{-1}$  was determined from the cultivar of Yerlisıra at P-0 level. Chickpea

**Table 1.** Effect of inoculation and P application on dry matter yields ( $\text{g pot}^{-1}$ ) of chickpea cultivars.

Cultivars	- Inoculation			+ Inoculation			Cultivars $\times$ P		
	P0	P80	Mean	P0	P80	Mean	P0	P80	Mean
Aydın	3.58	2.95	3.26 <sup>b-g</sup>	3.09	3.34	3.21 <sup>b-g</sup>	3.34 <sup>b-f</sup>	3.15 <sup>b-g</sup>	3.24 <sup>bc</sup>
Meksika	4.70	5.24	4.97 <sup>a</sup>	3.10	4.58	3.84 <sup>b</sup>	3.91 <sup>b</sup>	4.91 <sup>a</sup>	4.41 <sup>a</sup>
Diyar-95	2.36	3.40	2.88 <sup>b-i</sup>	2.90	2.84	2.87 <sup>b-i</sup>	2.63 <sup>d-j</sup>	3.12 <sup>b-g</sup>	2.87 <sup>b-e</sup>
Sarı-98	2.72	3.83	3.27 <sup>b-g</sup>	2.68	4.03	3.35 <sup>b-f</sup>	2.70 <sup>c-j</sup>	3.93 <sup>b</sup>	3.31 <sup>bc</sup>
Er-97	2.88	3.45	3.16 <sup>b-h</sup>	4.02	3.12	3.57 <sup>bc</sup>	3.45 <sup>b-e</sup>	3.29 <sup>b-f</sup>	3.37 <sup>b</sup>
Aziziye-94	2.66	4.29	3.47 <sup>b-e</sup>	2.67	3.13	2.90 <sup>b-i</sup>	2.67 <sup>c-j</sup>	3.72 <sup>bc</sup>	3.19 <sup>bc</sup>
Cevdet	2.64	3.67	3.16 <sup>b-h</sup>	2.98	3.49	3.24 <sup>b-g</sup>	2.82 <sup>c-j</sup>	3.59 <sup>bcd</sup>	3.20 <sup>bc</sup>
Gökçe	2.25	2.39	2.32 <sup>f-j</sup>	2.31	3.62	2.97 <sup>b-i</sup>	2.29 <sup>f-j</sup>	3.01 <sup>b-h</sup>	2.64 <sup>b-e</sup>
ILC-482	2.71	3.15	2.93 <sup>b-i</sup>	1.50	3.02	2.26 <sup>f-j</sup>	2.11 <sup>g-j</sup>	3.09 <sup>b-g</sup>	2.59 <sup>b-e</sup>
İzmir	1.76	2.70	2.23 <sup>g-j</sup>	1.90	2.28	2.09 <sup>hij</sup>	1.83 <sup>j</sup>	2.50 <sup>d-j</sup>	2.16 <sup>e</sup>
Çağatay	2.93	3.78	3.35 <sup>b-f</sup>	3.08	3.23	3.16 <sup>b-h</sup>	3.01 <sup>b-h</sup>	3.51 <sup>b-e</sup>	3.25 <sup>bc</sup>
Konya	1.89	3.23	2.56 <sup>c-j</sup>	2.67	2.37	2.52 <sup>c-j</sup>	2.28 <sup>f-j</sup>	2.81 <sup>c-j</sup>	2.54 <sup>cde</sup>
Gülümser	1.65	2.32	1.99 <sup>i-j</sup>	2.13	2.69	2.41 <sup>e-j</sup>	1.89 <sup>j</sup>	2.51 <sup>d-j</sup>	2.20 <sup>e</sup>
Akçin-91	2.55	3.00	2.77 <sup>b-i</sup>	2.58	3.07	2.83 <sup>b-i</sup>	2.57 <sup>d-j</sup>	3.04 <sup>b-g</sup>	2.80 <sup>b-e</sup>
Yerlisıra	1.70	3.09	2.40 <sup>e-j</sup>	1.86	2.81	2.33 <sup>f-j</sup>	1.79 <sup>j</sup>	2.95 <sup>b-i</sup>	2.36 <sup>de</sup>
Menemen-92	2.17	4.10	3.13 <sup>b-h</sup>	2.80	3.79	3.29 <sup>b-g</sup>	2.49 <sup>e-j</sup>	3.95 <sup>b</sup>	3.21 <sup>bc</sup>
Küsmen-99	3.06	4.03	3.54 <sup>b-d</sup>	2.94	3.01	2.97 <sup>b-i</sup>	3.00 <sup>b-h</sup>	3.52 <sup>b-e</sup>	3.26 <sup>bc</sup>
Eser-87	2.01	2.93	2.47 <sup>d-j</sup>	1.90	2.71	2.30 <sup>f-j</sup>	1.96 <sup>hij</sup>	2.82 <sup>c-j</sup>	2.38 <sup>de</sup>
Uzunlu-99	2.46	4.02	3.24 <sup>b-g</sup>	2.73	3.04	2.88 <sup>b-i</sup>	2.60 <sup>d-j</sup>	3.53 <sup>b-e</sup>	3.06 <sup>bcd</sup>
Damla-89	1.65	1.73	1.69 <sup>j</sup>	3.51	3.21	3.36 <sup>b-f</sup>	2.59 <sup>d-j</sup>	2.47 <sup>e-j</sup>	2.53 <sup>cde</sup>
Mean	2.52 <sup>b</sup>	3.36 <sup>a</sup>		2.67 <sup>b</sup>	3.17 <sup>a</sup>				

F values: Cultivars (C): \*\*P<0.01, Bacterial inoculation (B): n.s.; Phosphorus application (P): \*\*P<0.01; C  $\times$  P: \*\*P<0.01; C  $\times$  B: \*\*P<0.01, B  $\times$  P: \*P<0.05.

cultivars also showed varied responses to P application based on inoculation treatment. For example, dry matter yield of cv. Gökçe increased from 2.25  $\text{g pot}^{-1}$  (P-0) to 2.30  $\text{g pot}^{-1}$  (P-80) under non-inoculated condition. However, it was varied from 2.31  $\text{g pot}^{-1}$  (P-0) to 3.62  $\text{g pot}^{-1}$  (P-80) under the inoculation condition. Similarly, chickpea cultivar of Sarı-98 showed a higher response to P application in combination with *rhizobium* inoculation than non-inoculation condition. The results can be explained by close relationships between chickpea cultivars and bacterium inoculation depending on P applications. The synergistic effects of bacterium inoculation and P application on the growth of chickpea cultivars have also been reported by others (Yağmur and Enin, 2005; Erman et al., 2009; Sital et al., 2011).

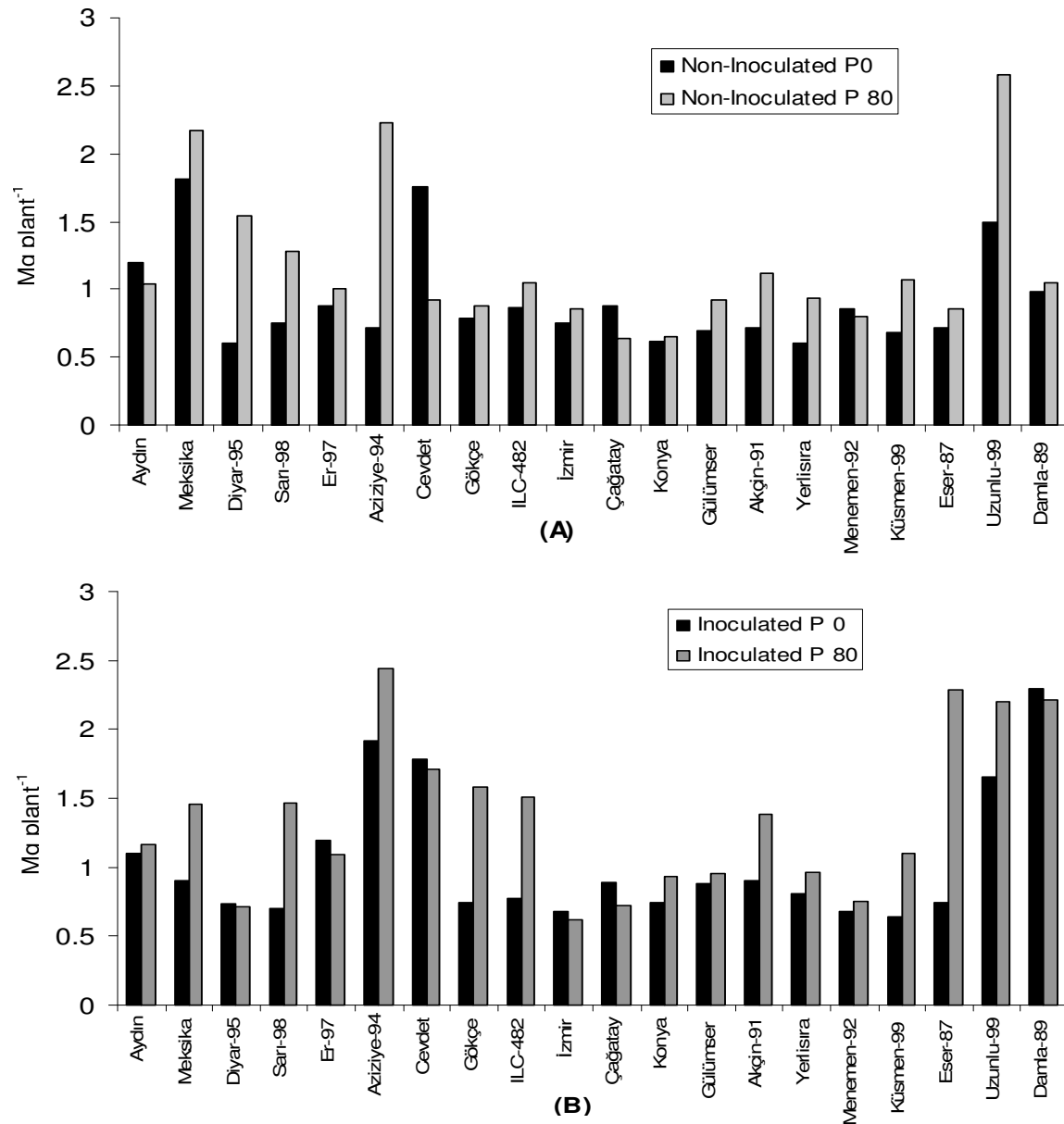
#### **Physiological efficiency of phosphorus (P) in chickpea cultivars**

Chickpea cultivars had significant differences for their P uptakes based on P application and bacterium inoculation (Figure 1). Phosphorus application significantly (P<0.01) increased total P content of chickpeas under the both non-inoculated and inoculated conditions as compared to the control treatments. Positive effect of P application on P uptake of chickpeas

was also reported by other researchers (Togay et al., 2008; Enania and Vyas, 1994). Bacterium inoculation significantly (P<0.05) increased the P content of chickpeas (Figure 1). Improved P uptake of chickpea plants after varied bacterial strains has also been observed in other studies (Gull et al., 2004). Chickpea cultivars as influenced by P application and bacterium inoculation have also showed significant (P<0.01) differences in P uptake. In the present study, according to the interactive effects of P application and bacterium inoculation on the absorbed P level, it could be seen that P content of chickpea cultivars was significantly varied. The highest P content was determined in cv. Aziziye-94 at P-80 level under inoculated treatment, whereas the lowest P content was determined in Yerlisıra cv at P-0 level under non-inoculated condition. Significant differences in N, P and K uptakes among chickpea cultivars and legumes have also been reported (Vadez and Drevon, 2001; Gallani et al., 2003).

#### **Agronomic phosphorus (P) utilization characters of chickpea cultivars**

Agronomic P Efficiency was determined, which was related to the response of a cultivar to supplied P level. As it is seen from Table 2, agronomic P efficiency



**Figure 1.** Total P content ( $\text{mg plant}^{-1}$ ) of chickpea cultivars as influenced by P application and bacterium inoculation; (A) without inoculation, (B) with inoculation.

changed depending on P fertilizer levels and chickpea cultivars under bacterium inoculated and non-inoculated conditions. Agronomic P efficiency ranged from 53 to 121% under non inoculated condition, whereas it ranged from 50 to 129% under inoculated condition. While the lowest value of agronomic P efficiency of 50% was found in chickpea cultivar of ILC-482, the highest value of agronomic P efficiency of 129% was found in the cultivar of Er-97. Efficiency Index (EI) parameters were used to select chickpea cultivars with improved P utilization characters under P application and bacterium inoculated conditions. Values of EI at P-0 rate were plotted against

the relative increases in SDM production ( $\text{SDM}_{\text{max}}/\text{SDM}_{\text{min}}$ ) in response to P-80 rate. Hence, Y-axis described EI parameter for P utilization at P-0 rate, whereas X-axis described relative increase in SDM in response to P-80 rate under non-inoculated condition. However, Y-axis described EI parameter for P utilization at P-0 rate, whereas X-axis described relative increase in SDM in response to P-80 rate under bacterium inoculated condition.

Based on these descriptions, under bacterium inoculated conditions, chickpea cultivars of Damla-89, Cevdet, Gökçe having higher SDM increases in response

**Table 2.** Agronomic P utilization characters of chickpea cultivars as influenced by P treatment and *rhizobium* inoculation.

Cultivars	Agronomic P Efficiency, % <sup>a</sup>		Efficiency Index, EI <sup>b</sup>	
	- Inoculation	+ Inoculation	- Inoculation	+ Inoculation
Aydın	121	92	9.5	9.1
Meksika	90	68	12.4	12.5
Diyar-95	69	102	7.8	11.4
Sarı-98	71	67	10.6	10.4
Er-97	83	129	10.6	11.2
Aziziye-94	62	85	8.2	3.9
Cevdet	72	85	7.5	6.0
Gökçe	94	64	6.7	7.6
ILC-482	86	50	8.9	4.5
İzmir	65	83	6.2	6.8
Çağatay	78	95	14.8	13.3
Konya	59	113	10.4	7.6
Gülümser	71	79	4.9	6.3
Akçin-91	85	84	8.2	7.0
Yerlisıra	55	66	7.5	7.4
Menemen-92	53	74	11.9	14.4
Küsmen-99	76	98	14.4	10.1
Eser-87	69	70	7.6	3.5
Uzunlu-99	61	90	5.2	4.3
Damla-89	95	109	2.8	5.0
Mean				

<sup>a</sup> Agronomic P Efficiency = Per cent value related to the response of a variety to supplied P level. <sup>b</sup> Efficiency Index (EI) =  $SDM^2 / \text{total P content}$ , and it provided to select chickpea variety with improved P utilization characters as ER (efficient responsive), ENR (efficient non-responsive), IR (inefficient responsive) and INR (inefficient non-responsive).

to P supply were called as IR, whereas the cultivars of İzmir, Gülümser, Eser-87, Uzunlu-99, Aziziye-94, ILC-482 having lower SDM increases in response to P supply were called as INR. Chickpea cultivars of Küsmen-99, Er-97, Diyar-95, Aydın, Akçin-91 were called as ENR above the average value of EI, and the cultivars of Menemen-92, Çağatay, Aydın, Sarı-98, Meksika were called as ER. On the other hand, under non inoculated conditions, chickpea cultivars of Cevdet, Uzunlu-99, Menemen-92 having higher SDM increases in response to P supply were called as IR, whereas the cultivars of Damla-89, Gülümser, İzmir, Yerli Sıra, Konya, Eser-87, Gökçe having lower SDM increases in response to P supply were called as INR (inefficient non-responsive). Chickpea cultivars of Aydın, Akçin-91, ILC-482 were called as ENR above the average value of EI, and the cultivars of Küsmen-99, Meksika, Diyar-95, Er-97, Çağatay, Sarı-98, Aziziye-94 were called as ER.

## Conclusion

As a concluding remark, P treatment and *rhizobium* inoculation in combination increased growth rate and P utilization of chickpea cultivars as compared to the

control. The interaction between P levels and *rhizobium* inoculation was highly significant. Whereas, significant variability among the chickpea cultivars for P utilization have also been observed depending on P treatment and inoculation. Thus, chickpea cultivars were classified according to their P utilization characters depending on *rhizobium* inoculation and non-inoculation conditions. In a P efficient cultivar, per cent P efficiency value was higher, which meant that the cultivar had lower response or non-response to the supplied P levels. As a result of this characterization; chickpea cultivars of Aydın, Akçin-91, ILC-482 were called as ENR under the non-inoculation condition, whereas chickpea cultivars of Küsmen-99, Er-97, Diyar-95, Aydın, Akçin-91 were called as ENR under the *rhizobium* inoculation condition. It has been established from the results that P treatments with and without *rhizobium* inoculation greatly affected the P Efficiency Index (EI) and P utilization performance of chickpea cultivars.

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