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Phosphorus tolerance levels of different chickpea genotypes

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ABSTRACT

Phosphorus (P) is a macronutrient required by the plants in large quantities. This study assessed P-tolerance levels of different chickpea genotypes under greenhouse conditions. Nine genotypes ('Damla', 'Diyar. 95', 'ER. 98', 'ILC.482', 'İzmir', 'Çağatay', 'Gökçe', 'Gülümser' and 'Yaşa.05') were screened under seven P doses (i.e., 15, 30, 45, 75, 90, 100 and 120 mg P kg⁻¹ soil). The P-deficiency symptoms were graded, subsequently root and shoot biomass and P accumulation were recorded after harvesting the plants 55 days after sowing. Principal component analysis (PCA) was executed to group genotypes. Genotypes and P levels significantly differed for growth and nutrient acquisition traits. The highest shoot biomass was recorded under 90, 100 and 120 mg P kg⁻¹ soil, while plants grown under 15 mg kg⁻¹P recorded the lowest biomass. Similarly, the highest root biomass was noted for 45 and 90 mg P kg⁻¹ soil, while 15 and 30 mg P kg⁻¹ soil had the lowest root biomass. The highest root:shoot ratio (RSR) was observed for 15, 30 and 45 mg P kg⁻¹ soil, whereas 100 and 120 mg P kg⁻¹ soil recorded the lowest RSR. The 'Gökçe' and 'Çağatay' genotypes produced the highest shoot biomass, while the lowest shoot biomass production was recorded for 'Diyar. 95' genotype. The highest and the lowest root biomass and RSR were recorded for genotypes 'Diyar 95' and 'Gökçe', respectively. The highest P was accumulated by genotypes 'İzmir' and 'ILC.482', while 'Diyar. 95' accumulated the lowest amount of P. The PCA grouped genotypes in two different groups based on root biomass, shoot biomass, RSR and P accumulation. Genotype 'İzmir' was in the first group. Similarly, 'Çağatay', 'ER 98' and 'ILC.482' had similar P accumulation. Thus, the results provide valuable insights for the use of these genotypes in the future for breeding purpose.

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1. Introduction

Chickpea (*Cicer arietinum* L.) is the third most important grain legume globally. It provides low-cost and quality protein to millions of residents in the developing world (Merga and Haji, 2019; Muehlbauer and Sarker, 2017). Chickpea is the most commonly grown edible grain legume in Turkey. It plays an important role in human and animal nutrition due to rich nutritional values (Merga and Haji, 2019). Chickpea is a preferred for crop rotation due to its nitrogen fixing ability. While chickpea consumption per person in the world is 0.50 kg, a person in Turkey consumes 4.61 kg of chickpeas annually. These data reveal the importance

of chickpea for Turkey. Chickpea cultivation have increased by 32% and production has increased to 630 thousand tons during 2019, witnessing a 34% increase compared to 2017 (TÜİK, 2020). Although production of pulses is spread across the country, south-eastern Anatolia, south Marmara and central Anatolia regions host most of the pulses' production in the country.

Phosphorus (P) is a macronutrient and often limits plant growth under low availability (Raghothama and Karthikeyan, 2005). Similarly, it is the most costly nutrient compared to other macronutrients required by crop plants. Increasing population and hiking input prices, particularly of fertilizers have compelled the farmers to use fewer fertilizers. Thus, optimizing P dose will reduce input costs and lower the use of synthetic fertilizers. The available P in agricultural lands of Turkey is significantly lower than rest of the world. It has been reported that 29.5% of Turkish soils have "very low" available P, 28.5% have "low", 17.0% have "medium", 5.7% have high and 9.3% of the soil have "very high" amounts of available P (Eyüpoğlu, 1999). Thus, 58% of country's soils have low available P for crop production. Out of the total applied P, 10–20% is used by crop plants. The remaining turns into less useful

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forms due to Ca-P fixing in alkaline soils, and as Fe-P and/or Al-P in acidic soils, all of which are very difficult to dissolve (Manske et al., 2001). This is because the phosphate concentration in the soil solution is controlled by the solid phase of the soil (Raghothama and Karthikeyan, 2005). The concentration of inorganic P in the soil solution shows a close relationship with texture, pH, CaCO_3 , oxides and hydroxides, organic matter, soil moisture and soil temperature etc. (Eghball, 2002). It has been reported that under arid and semi-arid climatic conditions, P is retained by Ca-phosphates bonding to a great extent, and high pH, carbonate and low organic matter lead to P precipitation (Brosché and Strid, 2003; Mañas et al., 2011).

Inal (2001) conducted a pot experiment to determine the differences between durum and bread wheat genotypes for P uptake and activity. It was observed that application of 200 mg P/kg improved P intake and bread wheat genotypes benefited more effectively than durum genotypes (Inal, 2001). Srinivasarao et al. (2006) evaluated the impact of 0, 13.5 and 27 ppm P on growth, biomass accumulation and nutrient uptake of twenty chickpea genotypes. Dry matter yield was 1.57 g in P_0 , 2.04 g in $P_{13.5}$ and 3.69 g in P_{27} . The most effective genotypes were 'Phule G-5' (2.22 g), 'BG-256' (3.13 g) and 'HK 94-134' (2.54 g). In the same study, they reported that P removal from the soil by all genotypes was 1.12 g kg^{-1} under P_0 dose, 2.00 g kg^{-1} under $P_{13.5}$ and 2.73 g kg^{-1} under P_{27} .

Several plant species and their genotypes exhibit great variation for P accumulation (Kidd et al., 2016; Lyu et al., 2016; Waddell et al., 2017). Plants enhance P acquisition under its low availability through the evolution of various root functional traits (Lambers et al., 2008; Wen et al., 2020). These traits include increase in specific root length (Wen et al., 2017), increased mobilization of inorganic and organic P (Richardson et al., 2011) and association with arbuscular mycorrhizal fungi (Sawers et al., 2017). The available studies in Turkey have less focus on root traits and P-deficiency symptoms. Therefore, the current evaluated the P-deficiency symptoms and growth and nutrient acquisition traits of chickpea genotypes under various P doses. It was hypothesized that the genotypes will significantly differ for growth and nutrient acquisition traits. The result will help to select genotypes for future breeding programs focusing on breeding for low P-tolerance.

2. Materials and methods

2.1. Experimental material

Nine (9) chickpea genotypes ('Damla', 'Diyar. 95', 'ER. 98', 'ILC.482', 'İzmir', 'Çağatay', 'Gökçe', 'Gülümser' and 'Yaşa.05') were used in the study. The experiment was conducted at the greenhouse of Çukurova University, Faculty of Agriculture, Department of Soil Science and Plant Nutrition.

Soil brought from Eskişehir-Sultanönü region with low P concentration (2.3 mg P kg^{-1}) was used in the experiment. The nutrient concentration of the experimental soil was 423 mg K kg^{-1} , 12 mg S kg^{-1} , 0.1 mg Zn kg^{-1} , 2.92 mg Fe kg^{-1} , 3.36 mg Mn kg^{-1} and 0.54 mg Cu kg^{-1} . The pH of the soil was 8.04, contained 1% organic matter with clay texture and 14.9% lime contents.

The experiment was laid out according to randomized complete block design with split-plot arrangement. The P doses were kept as main factor, while genotypes were regarded as sub-factor. Seven different P doses (15, 30, 45, 75, 90, 100 and 120 mg P kg^{-1} soil) were used. Basic fertilizers, i.e., 100 mg kg^{-1} N in the form of $\text{Ca}(\text{NO}_3)_2$, 50 mg kg^{-1} K in the form of K_2SO_4 form, 2.5 mg kg^{-1} Zn in the form of $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ and 2.5 mg kg^{-1} Fe in the form of Fe-EDTA were applied in two splits during the growing period. The $\text{CaH}_4\text{O}_8\text{P}_2$ was used as P source in the experiment. All treatments had three replications and pots were irrigated frequently to avoid moisture stress.

2.2. Data collection

Plants were harvested at 55 days after sowing, divided into roots and shoots, and weighed after drying 70 °C for 48 h until constant weight. After the dried plant samples were ground in an agate mill, 0.2 g were weighed and burned according to the dry combustion method (Kacar and İnal, 2008). Phosphorus analysis was done according to Barton (1948) method. The shoot and root weight were measured and used to record the root:shoot ratio. The P-toxicity/deficiency symptoms were classified on 1–5 scale, where 1 represented intense symptoms, while 5 represented very low or no symptoms.

2.3. Statistical analysis

The collected data were tested for normality by Shapiro-Wilk normality test (Shapiro and Wilk, 1965). The data were normally distributed; therefore, statistical analysis was conducted with original data. Two-way analysis of variance (ANOVA) was used to test the significant differences among P availability regimes and genotypes (Steel et al., 1997). Least significant difference at 5% probability was used to separate the means where ANOVA indicated significant differences. The data relating to growth and nutrient acquisition traits were subjected to multivariate analysis. Principal component analysis with Varimax rotation was used for the easier interpretation of the data. The Pearson correlation matrix was computed to infer the relationship among different growth and nutrient acquisition traits.

3. Results

All of the growth and nutrient acquisition traits were significantly altered by P-doses and genotypes (Table 1). However, P doses by genotypes' interaction were non-significant for root and shoot biomass and P content (Table 1).

The highest shoot biomass was recorded under 90 (2.56 g plant^{-1}), 100 (2.56 g plant^{-1}) and 120 (2.63 g plant^{-1}) mg P kg^{-1} soil, while plants grown under 15 mg P kg^{-1} soil recorded the lowest (1.28 g plant^{-1}) shoot biomass (Table 2). Similarly, the highest root biomass was noted for 45 (1.15 g plant^{-1}) and 90 (1.17 g plant^{-1}) mg P kg^{-1} soil, while 15 (0.77 g plant^{-1}) and 30 (0.85 g plant^{-1}) mg P kg^{-1} soil had the lowest root biomass. The highest root:shoot ratio (RSR) was observed for 15 (0.62), 30 (0.59) and 45 (0.59) mg P kg^{-1} soil, whereas 100 (0.40) and 120 (0.40) mg P kg^{-1} soil recorded the lowest RSR. The highest P concentration and content were noted for 120 mg P kg^{-1} soil, whereas lowest P accumulation was noted for 15 mg P kg^{-1} soil (Table 2).

The most affected genotype from P-deficiency was 'Çağatay' with symptom degree of 1.5, whereas the least affected genotypes were 'ILC.482', 'Gökçe', 'Gülümser' and 'Diyar.95' genotypes with 4.0 symptom grade (Table 3). The 'Gökçe' and 'Çağatay' genotypes produced the highest biomass, while the lowest biomass production was recorded for 'Diyar. 95' genotype. The highest and the lowest root biomass and RSR were recorded for genotypes 'Diyar. 95' and 'Gökçe', respectively. The highest P was accumulated by genotypes 'İzmir' and 'ILC.482', while the genotypes 'Diyar. 95' and 'Gülümser' accumulated the lowest amount of P.

Different growth and nutrient acquisition traits exhibited significant positive/negative correlations with each other. Shoot biomass was negatively correlated with root biomass and RSR, whereas had positive correlation with P accumulation. Root biomass had positive and negative correlation with RSR and P accumulation, respectively (Table 4).

The PCA with varimax rotation and Kaiser normalization yield two principal components (PCs) with eigenvalues > 1 (Table 5).

Table 1
Analysis of variance of different growth and nutrient acquisition traits of different chickpea genotypes grown under various phosphorus doses.

Source	DF	Sum of squares	Mean squares	F value	P value
Dry matter of shoot					
Phosphorus regimes (P)	6	47.81	7.97	219.70	< 0.0001*
Genotypes (G)	8	3.96	0.49	13.65	< 0.0001*
P × G	48	1.76	0.04	1.01	0.46 ^{NS}
Dry matter of root					
Phosphorus regimes (P)	6	3.50	0.58	21.43	< 0.0001*
Genotypes (G)	8	1.27	0.16	5.82	< 0.0001*
P × G	48	1.39	0.03	1.06	0.39 ^{NS}
Root:shoot ratio					
Phosphorus regimes (P)	6	1.41	0.23	29.01	< 0.0001*
Genotypes (G)	8	1.10	0.14	16.99	< 0.0001*
P × G	48	0.55	0.01	1.42	0.06 ^{NS}
Phosphorus concentration (%)					
Phosphorus regimes (P)	6	0.33	0.06	139.25	< 0.0001*
Genotypes (G)	8	0.02	0.00	6.53	< 0.0001*
P × G	48	0.03	0.00	1.77	0.01*
Phosphorus content (mg plant⁻¹)					
Phosphorus regimes (P)	6	569.88	94.98	202.87	< 0.0001*
Genotypes (G)	8	13.39	1.67	3.57	0.00*
P × G	48	18.95	0.39	0.84	0.75 ^{NS}

Here, DF = degree of freedom, * = significant (p < 0.05), NS = non-significant (p > 0.05)

Table 2
The impact of different phosphorus doses on growth and nutrient acquisition traits of different chickpea genotypes.

Phosphorus levels	Dry matter of shoot (g plant ⁻¹)	Dry matter of root (g plant ⁻¹)	RSR	P concentration (%)	P content (mg plant ⁻¹)
15 mg kg ⁻¹	1.28 e	0.77c	0.62 a	0.12f	1.47 g
30 mg kg ⁻¹	1.48 d	0.85c	0.59 a	0.15 e	2.30f
45 mg kg ⁻¹	1.99c	1.15 a	0.59 a	0.18 d	3.59 e
75 mg kg ⁻¹	2.13b	1.06b	0.50b	0.20c	4.30 d
90 mg kg ⁻¹	2.56 a	1.17 a	0.47b	0.21c	5.33c
100 mg kg ⁻¹	2.56 a	1.02b	0.40c	0.23b	6.00b
120 mg kg ⁻¹	2.63 a	1.03b	0.40c	0.25 a	6.46 a
LSD 0.05	0.10	0.08	0.04	0.01	0.37

Here, RSR = root:shoot ratio, P = phosphorus, Means followed by the same letters within a column are statistically non-significant (p > 0.05).

Table 3
The impact of different genotypes on growth and nutrient acquisition traits.

Genotypes	Toxicity symptoms	Dry matter of shoot (g plant ⁻¹)	Dry matter of root (g plant ⁻¹)	RSR	P concentration (%)	P content (mg plant ⁻¹)
izmir	3.00	2.05 cd	1.09 ab	0.55b	0.21 a	4.45 ab
ILC.482	4.00	2.06 bcd	0.96c	0.50 bc	0.21 a	4.53 a
Damla	2.50	1.90 ef	1.10 ab	0.62 a	0.19 bc	3.94c
ER.98	3.50	2.17b	0.96c	0.45c	0.20 ab	4.48 a
Çağatay	1.50	2.30 a	1.00 bc	0.45c	0.19 bc	4.45 a
Diyar.95	4.00	1.87f	1.11 a	0.62 a	0.19 bc	3.84c
Gülümser	4.00	2.00 de	1.05 abc	0.55b	0.18 cd	3.89c
Yaşa.05	2.00	2.16 bc	0.98c	0.47c	0.18 cd	4.03 bc
Gökçe	4.00	2.29 a	0.84 d	0.38 d	0.18 cd	4.24 abc
LSD 0.05		0.12	0.10	0.06	0.02	0.46

Here, RSR = root:shoot ratio, P = phosphorus, Means followed by the same letters within a column are statistically non-significant (p > 0.05).

Table 4
Pearson correlation matrix of different growth and nutrient acquisition traits of different chickpea genotypes grown under various phosphorus doses.

Variables	Shoot dry biomass	Root dry biomass	RSR	P concentration	P content
Shoot dry biomass	1.00				
Root dry biomass	-0.81	1.00			
RSR	-0.96	0.93	1.00		
P concentration	-0.35	0.39	0.36	1.00	3
P content	0.60	-0.44	-0.57	0.53	1.00

Here, RSR = root:shoot ratio, P = phosphorus

Table 5
Eigenvalues and variability explained by different principal components of multivariate analysis.

	PC1	PC2	PC3	PC4	PC5
Eigenvalue	3.27	1.52	0.20	0.01	0.00
Variability (%)	65.31	30.46	4.00	0.17	0.06
Cumulative %	65.31	95.77	99.77	99.94	100.00

Table 6
Factor loadings of first two components of principal component analysis executed on growth and nutrient acquisition traits of different chickpea genotypes grown under various phosphorus doses.

Variables	PC1	PC2
Shoot dry biomass	-0.97	-0.01
Root dry biomass	0.93	0.13
RSR	1.00	0.04
P concentration	0.32	0.94
P content	-0.61	0.79

Here, RSR = root:shoot ratio, P = phosphorus,

The variability explained by 1st and 2nd PC was 65.31 and 30.46%, respectively. The first two PCs collectively explained 95.77% variability in the data set.

The factor loadings of first two PCs indicated that first PC was negatively influenced by P accumulation and whole plant biomass, while positively influenced by root biomass and RSR (Table 6). The second PC was positively influenced by P accumulation (Table 6).

The PCA grouped the genotypes in two different groups based on shoot biomass, root biomass and RSR, and P accumulation.

Genotypes ‘İzmir’ was in the root biomass and RSR group. Similarly, ‘Çağatay’, ‘ER. 98’ and ‘ILC.482’ had similar P accumulation (Fig. 1).

4. Discussion

The selection of plant species and varieties with better P use help to grow these successfully on the soil with low P mobility. The efficient genotypes can use P effectively with high P activity. Tested P doses and genotypes significantly differed for growth and nutrient acquisition traits as hypothesized. These variations can be explained with the inherent genetic potential of the genotypes for P accrual. Nonetheless, the evolution of different P acquisition traits such as in specific root length (Wen et al., 2017), increased mobilization of inorganic and organic P (Richardson et al., 2011) and association with arbuscular mycorrhizal fungi (Sawers et al., 2017) can be the other explanations. Several earlier studies have reported significant differences for P acquisition between species and genotypes belonging to the same species (Gunes et al., 2006; Hafner et al., 1993; Ozturk et al., 2005).

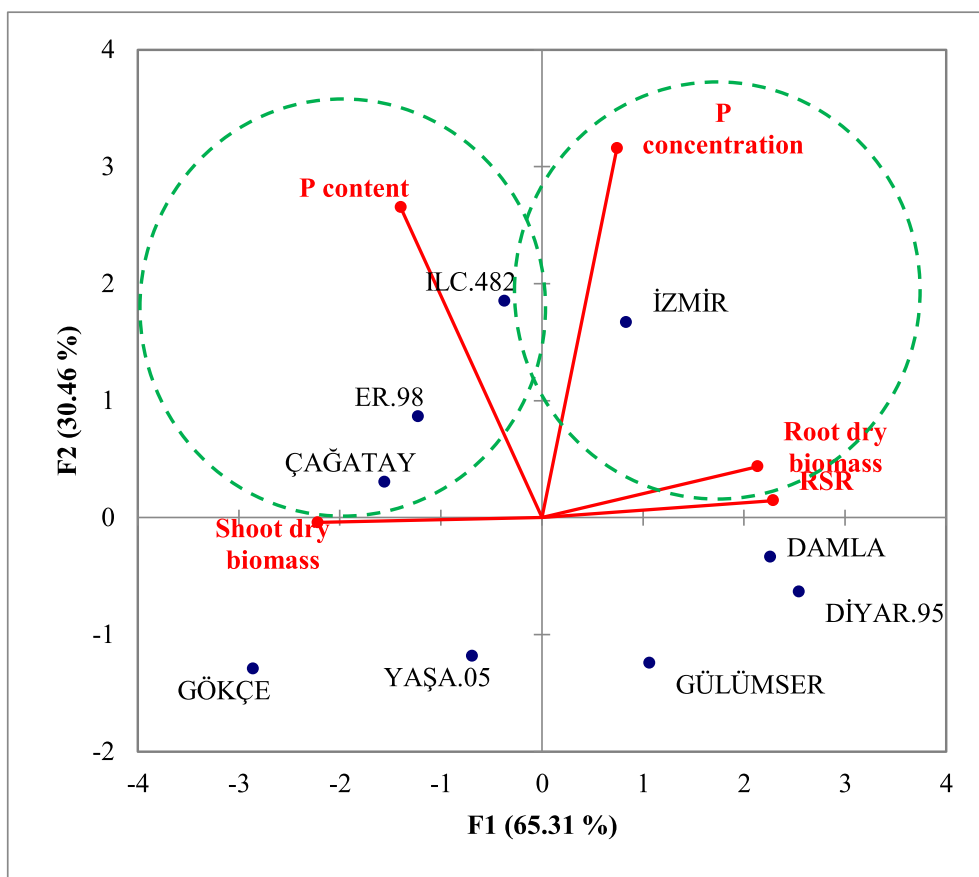


Fig. 1. Biplot of first two axis of first two components of principal component analysis executed on growth and nutrient acquisition traits of different chickpea genotypes grown under various phosphorus doses.

In the current study, P accumulation of 9 chickpea genotypes were investigated and all genotypes exhibited significant variations (Table 1 and 3). The 'Çağatay' genotype had a symptom grade of 1.5 and significantly affected by P-deficiency. However, no relationship was found between the symptom degrees of the genotypes and their efficiencies. It has also been reported earlier that there is no relationship between P-deficiency symptoms and P activity (Öztürk, 2001).

Dry matter production of chickpea genotypes increased significantly with increasing P availability. Similar increases were observed in root dry matter. It has been reported that root hairs are effective in the intake of nutrients with low mobility, such as P in the soil (Heuer et al., 2017). It is common for root hairs to grow in response to P-deficiency. Relatedly, P deficiency in *Arabidopsis* has been reported to reduce primary root growth and increase secondary root growth along with root hair growth and density (Svistoonoff et al., 2007). Among genotypes, 'Gökçe' had the highest dry matter yield. Due to increasing P-availability, increase in green parts and root dry matter production has been observed in wheat (Gunes et al., 2006), tomato and lentils (Toğay and Anlarsal, 2008).

Several studies have reported increase in root length and RSR is important in adaptation to P-deficiency (Anghinoni and Barber, 1980). It was determined that the average RSR of chickpea genotypes decreased with increasing P-availability. It has been reported that the dry matter yields of shoot under low P applications are a parameter that can be used for wheat selection (Fageria and Baligar, 1999; Osborne and Rengel, 2002; Ozturk et al., 2005).

Considering the average of genotypes, increasing P-availability led to a significant increase in shoot P concentrations of chickpea genotypes. The genotypes have significantly different P concentrations from each other. The effect of P application on P concentrations of chickpea genotypes was significant. It was observed that 'Gökçe' genotype had the lowest P concentration in shoot compared to the other genotypes. It has been shown that there is no relationship between shoot P concentration and P activity in corn (Da Silva and Gabelman, 1993) and wheat (Ozturk et al., 2005).

5. Conclusion

It was observed that P-deficiency symptoms emerging in the leaves were significantly different between tested genotypes. It was found that there was no statistically significant relationship between P-deficiency symptom and P accumulation. Among genotypes, 'Çağatay' had the highest P accumulation, which was followed by 'Gülümser'. In addition, the lack of relationship between symptom grade and P-concentration and P-accumulation warrants further investigation in terms of P activity mechanisms such as leaf elongation, leaf area, P-retranslocation capacity, P-absorption and acid phosphatase activity in roots.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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