

## MAIZE ROOT ARCHITECTURE RESPONSE TO PHOSPHORUS AVAILABILITY IN ROOTING MEDIUM

AYSHA KIRAN<sup>1</sup>, QUMQUM NOSHAD<sup>2</sup>, MUHAMMAD AJAIB<sup>2</sup>, AHMAD NAEEM SHAHZAD<sup>3</sup>, SANA PERVEZ<sup>4</sup>, IRFANA LALARUKH<sup>5</sup>, MUHAMMAD FAHEEM SIDDIQUI<sup>6</sup> AND ABDUL WAKEEL<sup>4\*</sup>

<sup>1</sup>Department of Botany, University of Agriculture Faisalabad, Pakistan

<sup>2</sup>Department of Botany, Mirpur University of Science & Technology (MUST), Mirpur-10250 (AJK), Pakistan

<sup>3</sup>Department of Agronomy, Baha ud Din Zakariya University, Multan

<sup>4</sup>Institute of Soil and Environmental Sciences, University of Agriculture Faisalabad, Pakistan

<sup>5</sup>Department of Botany, Government College Woman University, Faisalabad

<sup>6</sup>Department of Botany, University of Karachi, Karachi 75270, Pakistan

\*Corresponding author's email: [abdul.wakeel@uaf.edu.pk](mailto:abdul.wakeel@uaf.edu.pk)

### Abstract

Phosphorus (P) is precipitated with calcium (Ca) in calcareous soil very quickly, thus very immobile in the soil with restricted availability to plants for uptake by plant roots. Therefore, Pakistani soils are limiting maize growth due to P deficiency. Root architectural alteration can influence P accessibility and uptake by maize plants. Constitutive and P-deficiency-induced alterations in the root architecture of maize were investigated through a rhizobox study carried out in a net house during the maize growing season under natural climatic conditions. In the first treatment, P was deficient, whereas in the second treatment, the recommended amount of P fertilizer was applied to maize with four replications for precise results. Maize varieties named Faisalabad Maize, S-2002, Maize-2018, DTC-46, and EV-77 were sown in sand-filled rhizobox. Plants were harvested after seven days of germination, and plant shoots and roots were measured. Maize roots were scanned to digitalize the image to study root system architecture using Image-J software. Primary root length, number, and density of the lateral root of each seedling were measured at low and sufficient P. Primary root length decreased to a low level of P in the rooting medium. Root architectural variation in response to P availability differs among maize varieties because Maize-2018 and Faisalabad maize showed improved responses to P availability regarding root architectural traits. Interestingly, LRN showed a significant increase and correlation with the growth parameters of all cultivars under P-deficient conditions. Therefore, it is concluded that varieties with more lateral roots can tolerate P-deficient conditions in a better way, and such characteristics may be incorporated into future breeding programmes.

**Key words:** Root architecture, Nutrients, Stress, Maize, Growth.

### Introduction

Phosphorus (P) deficiency reduced crop production from 30 to 40 % (Uexkull & Mutert, 1995) because of restricted access of P to plants owing to its precipitation with calcium (Ca) in high pH soils, such as Pakistani soils. Various strategies are used to enhance the availability of P such as by using microbial strains (Attar *et al.*, 2022), polymer enriched harmones coated chemical fertilizers (Yasmeen *et al.*, 2021), etc. It contributes to various functions in plants *viz.*, production of energy, construction of nucleic acids, photosynthesis, glycolysis, respiration, membrane constancy, activation or inactivation of enzymes, oxidation-reduction reactions, metabolism of carbohydrate and fixation of nitrogen (Vance, 2008; Abel *et al.*, 2002). Plant residues left over plant roots in the soil, P fertilizers fixed at top surface are major source of P to crop plants. Therefore, the availability of P is more at top surface as compared to deep soil layers. The genotypes or cultivars with deep root systems are prone to P deficiency due to low P concentration in most soils. Whereas P availability and uptake is more for those plants which have sufficient adventitious roots to explore the topsoil rich in total P content (Lynch & Brown, 2001; Ranjha *et al.*, 2007). However, in case of inadequate availability of P, it is necessary to identify the root characters that allow the varieties to produce more lateral roots at low-P availability. Plant root hairs also play a vital role in nutrient uptake such as phosphorus. Depletion of P in the region of roots shows that depletion zone size in the region of the root increases by

increasing the root hair length. Species with larger root hairs have better P acquisition (Wissuwa *et al.*, 2005). It is reported that worldwide agricultural soils will be depleted by between 4 - 19 kg ha<sup>-1</sup> per year, with average losses of P due to erosion by water contributing over 50% of total P losses. Concentration of phosphate in the soil solution is well below the critical level required for better crop production (Alewell *et al.*, 2020; Khan & Inamullah, 2021).

Root system architecture means spatial arrangement of various root architectural traits. Plants need to adopt such an efficient mechanism that retrieves even a low amount of phosphorus. Modification in root architecture having good phosphorus uptake ability is beneficial for better crop production. Under low P, modification in root structure is associated with the composition and concentration of phytohormone (Chiou & Lin, 2011). In the case of P deficiency, hormones like auxin, sugars, and ethylene have an important role in root architecture modification (Peret *et al.*, 2011).

Rooting depth, lateral root density, root hairs, the position of roots, and the mode of root structure in the soil are involved in the shape of the root (Hodge *et al.*, 2009). Primary root growth is reduced in response to low P in plants such as *Arabidopsis* (Perez-Torres *et al.*, 2008; Tyburski *et al.*, 2012). Lateral roots help in the uptake of P by their widespread parts in the soil (Zhu *et al.*, 2005). It is reported that in *Arabidopsis*, lateral root growth is favored by P deficiency through the decrease in primary root length and enhancing lateral root density and length (Williamson

*et al.*, 2001). The availability of P is greatest at the upper soil surface and reduces with soil depth, therefore an increase in lateral roots may help to improve P uptake.

Therefore, the present study was designed to identify the genotypic variation in maize for root architectural traits. Furthermore, the root architectural response to the P deficient condition was also observed.

## Material and Methods

A study was conducted in a rain protected net house/wire house in rhizobox especially prepared for root architectural studies like used by Kiran *et al.*, (2019). There were two treatments of phosphorus P<sub>0</sub> (no phosphorus) and P<sub>1</sub> (recommended phosphorus) which were replicated four times. Rhizoboxes were filled with washed sand and seeds of maize varieties (Faisalabad maize, S-2002, Maize-2018, DTC-46 and EV-77) were sown in rhizoboxes with 30×30 cm dimension (Photo 1). Rhizobox was put in the tubs containing nutrient solution at the bottom for capillary uptake in the rhizobox sand. The nutrient solution was added according to crop requirements. The solution used for maize contained 5 mM N, 0.2 mM P, 0.6 mM Mg, 7.5 mM Ca, 1.2 mM K, 0.2 mM Fe, 1 μM B, 2 μM Mn, 0.5 μM Zn, 0.3 μM Cu and 0.005 μM Mo. The crop was harvested seven days after planting (Wakeel *et al.*, 2011).



Photo. 1. The plants were grown in rhizobox developed especially for root architectural studies. The filling material was sand with continuous supply of nutrients solution from bottom via capillary action.

Plants shoot and roots were separated by sharp knife and maize shoots and roots were scanned using digital camera. The scanned roots were then uploaded to Image-J and various root architectural traits were determined using Image-J software as mentioned earlier. The primary root

length and number of lateral roots were determined to calculate the lateral root density following the equation referred to Kiran *et al.*, (2019).

$$\text{Lateral root density} = \frac{\text{No. of lateral roots}}{\text{Primary root length (cm)}}$$

Furthermore, fresh shoot weight and fresh root weight were determined. Later, shoots and roots were oven dried at 100°C in a hot air forced oven. Dried shoot and roots samples were digested using di-acid wet digestions. After sample preparation for UV-visible spectrophotometer (Shimadzu UV-1201), P was determined using P standard curve. The method provided by Chapman & Pratt, (1961) was followed using vanadate- molybdate as color development reagent. Phosphorus uptake was calculated after determination of P concentration and multiplying with dry weight of root and shoot separately.

## Results

Variations and modifications in root architecture influence the P uptake by plants through increased lateral root growth. The study aimed to identify the genotypic variation in maize regarding root architectural characteristics in response to P availability.

**Plant biomass production:** Genetic variation for plant biomass was observed in maize seedlings grown in rhizoboxes under P deficient and sufficient condition (Table 1). Significant varietal and P treatment effect was observed for plant biomass among selected maize varieties, while combined effect of P and varieties found non-significant. Maximum fresh shoot weight was recorded in Maize-2018 and Faisalabad maize i.e., 0.60 g. Minimum fresh shoot weight i.e., 0.42 g was observed in EV-77 in treatment when no phosphorus was applied. Different varieties of maize have shown different responses to phosphorus availability. Plants after harvesting were immediately weighed then oven-dried to record their dry weight. Data were recorded from all the treatments and subjected to analysis of variance under factorial design. From the results, it was observed that phosphorus and genotypic effect were found significant while interaction effect found non-significant. Results regarding the dry shoot weight indicated that there was no difference among treatments by P application except EV-77 where dry shoot weight was increased with P application. Maximum dry shoot weight was recorded in Maize-2018 and Faisalabad maize i.e., 0.05 g. Minimum dry shoot weight i.e., 0.025 g was observed in EV-77 in treatment when no phosphorus was applied.

**Table 1. Effect of P deficiency and P sufficiency on dry mass of shoot and root, number of lateral roots and primary root length.**

Varieties	Dry root mass (mg)		Dry shoot mass (mg)		Number of lateral roots		Primary root length (cm)	
	P <sub>0</sub>	P <sub>1</sub>	P <sub>0</sub>	P <sub>1</sub>	P <sub>0</sub>	P <sub>1</sub>	P <sub>0</sub>	P <sub>1</sub>
Faisalabad maize	47 ab	45 ab	45 a	50 a	81 a	57 b	11.4 c	16.1 ab
S-2002	50 a	48 ab	46 a	48 a	51 bc	39 d	16.7 a	14.7 ab
Maize-2018	42 b	47 ab	49 a	50 a	85 a	48 c	15.6 ab	16.2 ab
DTC-46	33 c	32 c	29 cd	33 bc	32 d	39 d	15.1 ab	16.8 a
EV-77	27 c	28 c	25 d	36 b	38 d	32 d	13.5 bc	16.2 ab

A significant increase in fresh root weight in all varieties of maize when phosphorus was applied as compared to the control showed a consistent effect of P fertilizers without discriminating varietal differences. Maximum fresh root weight was recorded in Maize-2018 i.e., 0.82 g. Minimum fresh root weight i.e., 0.56 g was observed in S-2002 in treatment when no phosphorus was applied. For dry root weight, significant effect of varieties was observed while effect of P treatment and interactive effect of both factors was statistically non-significant. Highest dry root weight was observed in S-2002 while minimum was in EV-77 under P contrasting conditions (Table 1).

**Root architectural traits:** Root system of seedlings was harvested non-destructively from rhizoboxes, and images were taken by digital camera. Primary root length, lateral root number and lateral root density were recorded by subjecting root images to Image-J software.

**Primary root length:** The primary root length of all the treatments was recorded by using image-J software and was subjected to analysis of variance under factorial design. The results showed that the genotypic effect was non-significant, but phosphorus and the interaction of both factors were found significant (Table 1). Results regarding primary root length indicated that there was no difference among treatments by P application except in Faisalabad maize where primary root length was increased with P application. Maximum primary root length was recorded in S-2002 in P<sub>0</sub> treatment i.e., 16.746 cm. While minimum primary root length was observed in Faisalabad maize i.e., 11.440 cm as compared to other varieties.

**Number of lateral roots:** Each lateral root was given a number on the image of root system and total number of lateral roots were counted for each seedling. The results showed that P treatment, varieties and interaction of both factors was significantly affecting the lateral root number (Table 1). Increase in lateral root number was observed for Faisalabad, S-2002 and Maize 2018 under P deficient condition as compared to P availability while no significant difference was observed for DTC-46 and EV-47.

**Lateral root density:** Significantly higher lateral root density was observed in Faisalabad, S-2002 and Maize-2018 under P deficient condition as compared to P sufficient condition. DTC-46 and EV-47 showed no significant difference for lateral root density under contrasting P conditions. Maximum lateral root density was observed in Faisalabad maize under P deficient condition while minimum was of EV-77 (Fig. 1).

**P concentration in shoot and root:** Phosphorus concentration of all the treatments was recorded. P concentration in root and shoot did not significantly differ under P contrasting conditions among maize varieties (Fig. 2). Results regarding P concentration in shoot indicate that there was no significant difference among treatments.

Maximum P concentration observed in shoots of Maize-2018. P concentration in root and shoot did not significantly differ under P contrasting conditions among maize varieties. P concentration in root indicated that there was no significant difference among treatments by P application except Maize-2018 where P concentration in root was increased with no P application and maximum P concentration in root was recorded in Maize-2018 i.e., 2403.48 mg kg<sup>-1</sup> while minimum P concentration in shoot was observed in S-2002 i.e., 1436.44 mg kg<sup>-1</sup> (Fig. 2).

#### **Phosphorus translocation and uptake by plant:**

Phosphorus translocation from root to shoot was calculated. Results regarding phosphorus translocation from root to shoot indicated that there was no significant difference among treatments by P application except DTC-46 and EV-77 where P translocation from root to shoot was increased by P application. Maximum phosphorus translocation from root to shoot was observed in Maize-2018 i.e., 2.990 while minimum phosphorus translocation from root to shoot was observed in EV-77 i.e., 1.550 as compared to other varieties (Fig. 3). Results regarding phosphorus uptake by plant indicated that phosphorus uptake by the plant was increased in P<sub>0</sub> treatment as compared to P<sub>1</sub> treatment when recommended phosphorus was applied. Maximum phosphorus uptake by the plant was observed in Maize-2018 i.e., 0.380 mg/plant while minimum phosphorus uptake by plant was observed in EV-77 i.e., 0.172 mg/plant (Fig. 3).

#### **Discussion**

Root architectural traits do vary in different crop species and genotypes as well. The current study has also revealed that root architectural variation among maize varieties is useful information for further studies. Natural variation in root architectural traits lead to identifying the best root characteristics helpful for enhanced crop production adapting various environmental conditions in root vicinity (Zhang *et al.*, 2012; Alewell *et al.*, 2020). It has been further added that plants are able to modify their root system architecture for better P uptake under varying soil pH conditions. Under P deficient conditions in root medium, lateral root density was increased in general (Fig. 1) as low P availability induces the plant root for further growth to explore P in the rooting medium (Kano *et al.*, 2011). However great genotypic variation for lateral root density with maximum value of 7.40 roots cm<sup>-1</sup> noted in Faisalabad maize. whereas the lowest lateral root density of ~2 root cm<sup>-1</sup> in EV-77. The most interesting finding was the positive association between P acquisition and lateral root density (Fig. 4; R<sup>2</sup> = 0.317). Furthermore, fresh shoot weight was also related to lateral root numbers in the treatment where P was not applied in contrast to the treatment where sufficient P was provided to maize plants (the data is not presented). Vance (2008) also found similar results showing the correlation between root branching and shoot growth.

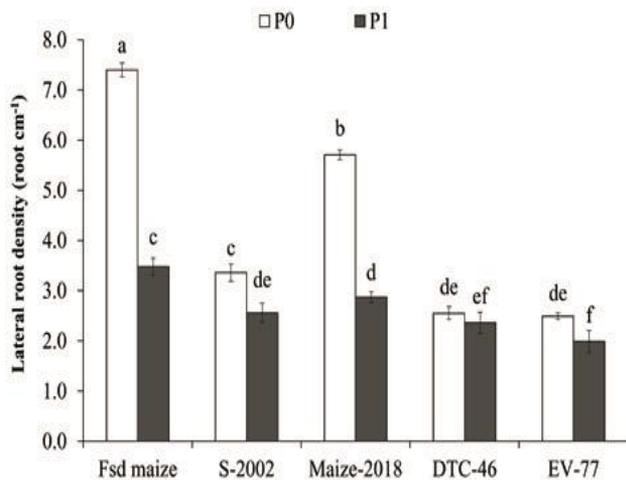


Fig. 1. Effect of P availability on lateral root density (root cm<sup>-1</sup>) in various maize varieties. P<sub>0</sub> (deficient phosphorus), P<sub>1</sub> (recommended phosphorus). Mean of four replicates presented in columns are not significantly different if share the similar alphabets, at  $p < 0.05$  according to LSD test. Bars at column top denote the standard error. (Fsd maize= Faisalabad maize).

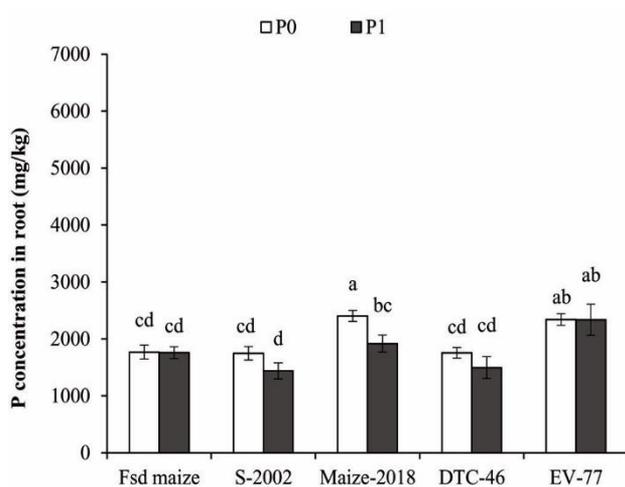
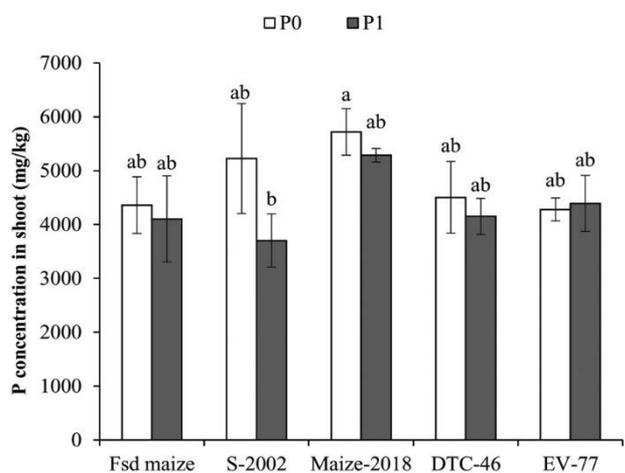


Fig. 2. Shoot and root P concentration as affected by P fertilization in five maize varieties. P<sub>0</sub> (no phosphorus), P<sub>1</sub> (recommended phosphorus). Columns show the mean of four replications, whereas bars show standard error. Columns sharing the similar letter (s) do not differ significantly at  $p < 0.05$  according to LSD test.

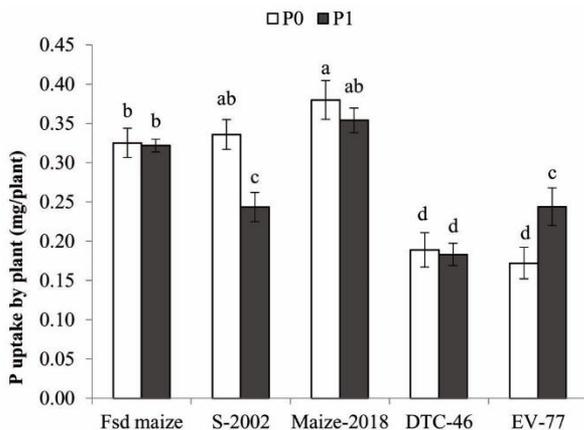
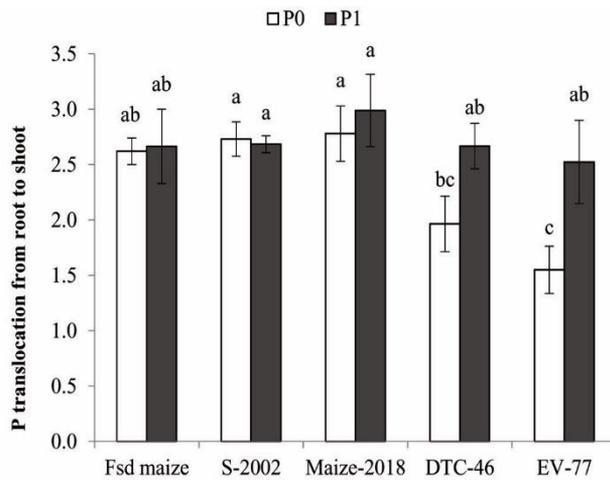


Fig. 3. P translocation from root to shoot and P uptake by various maize varieties. P<sub>0</sub> (no phosphorus), P<sub>1</sub> (recommended phosphorus). Columns show the mean of four replications, whereas bars show standard error. Columns sharing the similar letter (s) do not differ significantly at  $p < 0.05$  according to LSD test.

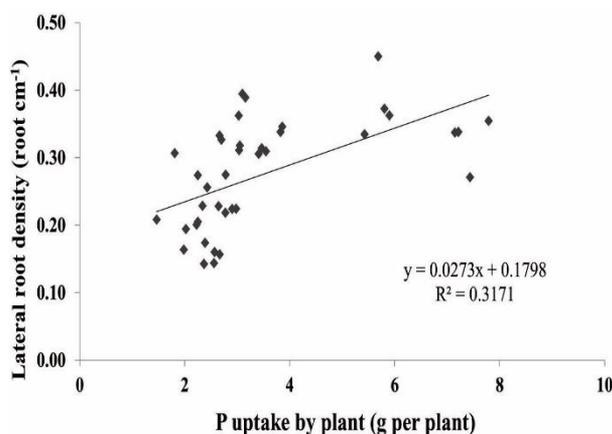


Fig. 4. Correlation between lateral root density and P uptake by plant. P uptake was determined by multiplying P concentration with dry mass, whereas root density was determined using image-J software after scanning the roots using digital camera.

Phosphorus deficiency enhances the later root growth in Arabidopsis as revealed by Williamson *et al.*, (2001) as reported in this study on maize where lateral root number was increased whereas primary root length was decreased (Table 1). It led to enhanced lateral root density both in Arabidopsis

and maize (Williamson *et al.* 2001; Table 1). Plant root hairs and increased number of lateral roots promote the phosphorus uptake by enhancing the depletion zone thus exploring more soil (Holz *et al.*, 2018; Vissenberg *et al.*, 2020). Spatial rhizosphere expansion at the account of more root hairs increases the nutrient cycling and rhizosphere interaction to a larger soil volume leading enhanced plant production under nutrient deficient conditions (Holz *et al.*, 2018). Crop species with greater number of root hairs lead to more P uptake by plants as explained by Nielsen *et al.* (1994). A greater number of lateral roots are induced due to P deficiency as plants can sense and respond for P absorption by plant roots. In line with results of this study Desnos (2008) also reported that primary root length is reduced whereas the root hair density and lateral root length was enhanced. Trybulski *et al.*, (2012) reported his study on Arabidopsis and stated that P deficiency reduced the primary root length. Similarly, Lynch and Brown (2008) have reported increased root branching topsoil exploration for P through enhanced root hairs and root to shoot ratio. Furthermore, it was also stated that special cluster like structures were developed in some species only.

High phosphorus concentration in root and shoot at low P availability might be due to root architectural variation in maize varieties (Fig. 2). Increased number of lateral roots increased the phosphorus uptake. Zhu *et al.*, (2005) also stated that lateral roots help in the uptake of P through searching the soil.

Along with plant growth improvement, high levels of P in root and shoot under P deficiency is evident for enhanced P exploration through better root system architecture due to release of exudates possibly (Holz *et al.*, 2018; Khan and Inamullah, 2021). Total P uptake was also increased due to an increased number of lateral roots, further emphasizing the role of root adaptability through better root system architecture under P deficient conditions (Figs. 3 & 5). Cluster root development and increased number of seminal roots are also helpful for better P uptake under P deficient conditions, however not in all crop species and genotypes. Lambers *et al.*, (2006) reported that white lupin developed thick root clusters for increased P uptake under P limiting conditions.

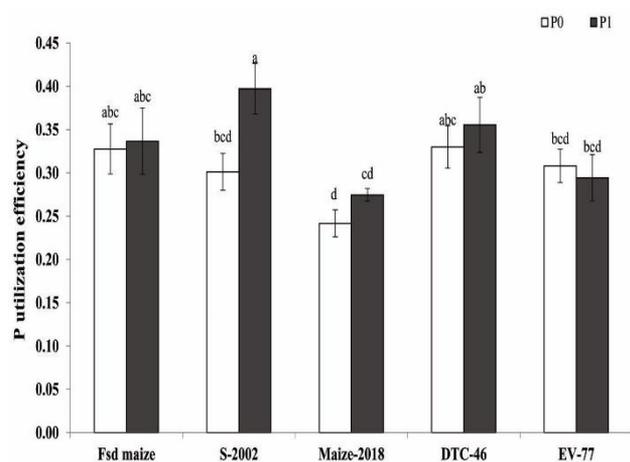


Fig. 5. Effect of P availability on P utilization efficiency in various maize varieties. P<sub>0</sub> (no phosphorus), P<sub>1</sub> (recommended phosphorus). Columns show the mean of four replications, whereas bars show standard error. Columns sharing the similar letter (s) do not differ significantly at  $p < 0.05$  according to LSD test.

## Conclusion

Phosphorus content in rooting medium led to root architectural adaptability along with genotypic variation in maize. Furthermore, P deficient conditions reduced the primary length of the roots with significant increase in lateral root number and lateral root length. Among maize genotypes Maize-2018 and Faisalabad-maize exhibited better root adaptability to P deficient conditions considering root system architecture.

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