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GENOTYPIC AND SPECIES VARIABILITY IN CARBOXYLATE EXUDATION OF WHEAT (*Triticum aestivum* L.) AND MAIZE (*Zea mays* L.) IN PHOSPHORUS DEFICIENCY

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Under low phosphorus (P) availability, plants change their root physiology and release more protons and carboxylates to acquire P from residual sources. This ability differs among genotypes. In this situation, P efficient genotypes may be a conceivable way of decreasing the P demand and using applied P efficiently. This study was planned with the objective of screening P efficient wheat and maize genotypes on the basis of carboxylate and proton release under P deficient condition. Five genotypes each of wheat and maize were grown in solution culture with normal and deficient P treatments under CRD (completely randomized design) factorial. At harvest carboxylates, proton and P were analyzed. Results revealed that wheat genotype, SARC-1 and maize genotype, Pioneer-32F10 was better in carboxylate exudation and more release of carboxylates and proton was recorded in these genotypes comparative to other genotypes. Both genotypes also showed better P and phosphorus use efficiency in P deficient treatment compared to normal P treatment. In the case of species, maize roots released more fraction of malate and wheat roots released more fraction of citrate. This genetic difference can be used for the selection of P efficient genotypes for P deficient soils through the breeding process in the future.
Keywords: Carboxylates, pH, Deficient P, Genotypes, Phosphorus use efficiency.

INTRODUCTION

Total phosphate in agricultural soils is high but the low availability of soluble phosphate for plant root uptake is a major issue in both acidic and alkaline soils (Shen *et al.,* 2011). Low phosphorus (P) availability to plant roots can reduce the yield of crops in almost 30% of the world's arable land (Vance *et al*., 2003; Kochian, 2012). A chemical fertilizer application typically distresses the soil health and affects the production cost approach, as chemical P fertilizer recovery is low in soils. In this situation, identification and selection of P efficient genotypes that can improve P use efficiency may be an effective solution (Li *et al.*,2010).

Many plants adopt different strategies to survive under this condition such as the release of protons and carboxylates (citrate, malate), increase in root to shoot ratio, root hairs, and root surface area. These adaptive mechanisms make the plant a 'P efficient plant'. However, these abilities vary within plant genotype and species to adopt these P efficient mechanisms which help to convert unavailable P to available forms (Sadana *et al.,* 2002).Factors underlying the differential capacities of plant genotypes to access soil nutrients composition and concentration of root exudates (Jones *et al.,* 2004) results in changing the chemistry and biology of the rhizosphere. For example, the rhizosphere acidification is more prominent in P-efficient *Phaseolus vulgaris* genotypes than in P-inefficient ones, whereas, no difference in rhizosphere acidification between *Triticum. Aestivum* and *Hordeum. vulgare* genotypes with different P efficiency (Rengel and Marschner, 2005). This variation also occurs among crops due to genetic differences. Under low P, most plant acquires P by changing morphology and physiology of roots (Lambers *et al.,* 2006; Shen *et al.,* 2011). Such kind of changes may include an increased the formation of root hairs (Zhang *et al.,* 2014), increase total root length, and root to shoot ratio (Shen *et al.,* 2011), increased expression of Pi transporter, increased acid phosphatase, and organic acid excretion, a decrease of pH in rhizosphere (Lambers *et al.,* 2006).

Release of protons, organic acids and phosphatases were higher in P non-sensitive genotype (*JX*17) as compared to P-sensitive genotypes (*ZYQ*8) of rice in P stress environment (Ming *et al.,* 2002). Carboxylates concentration differs considerably among maize genotypes. Malate, citrate and trans-aconitase acids account for about 80% of total carboxylates exuded in all genotypes (Gaume *et al.,* 2001). Thus, the amount and composition of root exuded carboxylates varies with nutritional status, plant species, and cultivars (Zhao and Wu, 2014).

Low Input Sustainable Agriculture (LISA) has increased attentiveness among researchers for the selection of crop genotypes that are easy-going to mineral stress in the soils. In

a low nutrient environment, the ability of plant genotype to absorb, use, accumulate, and translocate mineral elements is important. These characteristic variations among genotypes are accountable for failure or survival in a low nutrient environment (Ahmad *et al.,* 1998). A commonly established goal is to develop such agricultural cultivars that yield well in soils with low concentrations of extractable P (Lynch, 2007). Developing and selection of P-efficient genotypes may be a conceivable way of decreasing the P demand and loss of P. Many studies have revealed that variances in stress tolerance of low P occur in genotypes within crop species (Richardson *et al.,* 2011; Simpson *et al.,* 2011; Cordell and White, 2015). Keeping in view these points, this study was planned with objective to screen P efficient wheat and maize genotypes on the basis of carboxylate and proton release (pH) with better P use efficiency under P deficiency. Difference in carboxylates release by maize and wheat was studied in this research.

MATERIALS AND METHODS

***Growth conditions and planting material*:** The experiment was carried out in a glasshouse of the Institute of Soil and Environmental Sciences, University of Agriculture, Faisalabad, Pakistan. Seeds of five wheat (Kohinoor-83, B4-5711, SARC-1, SARC-2, and SARC-3) and five maize (Cargel-6525, Syngenta-8711, Pioneer-33H15, Monsanto-6525, and Pioneer-32F10) genotypes treated with sodium hypochlorite (1%) were used as planting material. Two P treatments, T1: normal P treatment (Control) received complete nutrients of half strength Hoagland solution and T2: P deficient treatment where P was omitted from the nutrient solution was used. In P deficient treatment, the source of P, KH_2PO_4 was replaced by KNO_3 in order to maintain the potassium concentration in the treatment.

***Nursery raising, transplantation, and treatment application*:** Seeds of selected genotypes were sown in polyethylene coated iron trays with 2-inch washed sand layer. For seed germination and seedling establishment, moisture was sustained with distilled water. Fifteen days old seedlings were shifted to (6 liters for maize and 3 liters for wheat) foam plugged holes in polystyrene sheets floating over ½ strength Hoagland's solution (Hoagland and Arnon, 1950) in plastic tubs with 3 replications. Plastic tubs were aerated using aeration pumps for about 8 h every day. Solution pH was daily maintained at 6-6.5 using NaOH and HCl. The solution was changed every week. After growing plants for 7 days in normal ½ strength Hoagland solution according to lower demand of plant for P and slow initiation of deficiency responses by plant, described by Nagy *et al*. (2006), the P deficiency period was set to 15 days**.**

Sampling and measurements:

***Root exudates collection*:** Root exudates were collected after fifteen days of treatment application. Two hours after the onset of the light period, plants were transferred to 250 ml vials containing 0.2 mM $CaCl_2$, in which root exudates were collected for 6 h. Immediately after the root exudates collection in 250 ml vial, the pH of this extract was measured. This extract was used for carboxylate determination on High performance liquid chromatography (HPLC). For plant dry biomass measurement, plants were harvested dried in an oven at 65 ± 5 °C and measured by analytical balance

Analysis of carboxylates

***Mobile phase preparation*:** For mobile phase preparation (0.1% Phosphoric acid), 1ml of HPLC grade phosphoric acid was taken and mixed into 999 ml of double deionized water and this was then filtered through syringe membrane filter assembly.

***Sample preparation*:** For the determination of carboxylates on HPLC, samples were prepared by using mobile. For this purpose, 1ml of root exudate extract was taken in centrifuge tubes and then 7 ml of mobile phase was added in this centrifuge tube. These samples were centrifuged at 1600 rpm for 25 minutes at 20 ºC temperature. After centrifugation, the supernatant was taken and filtered through a 0.2 µm syringe filter in 1ml cryo vials for HPLC analysis.

***Analysis on HPLC*:** The carboxylates in the rhizosphere extracts were analyzed by HPLC (LC-10AT pump, plus manual sampler and UV-VIS-SPD-10AV detector, Shimadzu, Japan) using Shim-Pack CLC-ODS (C-18) reverse-phase column (Cawthray, 2003) with flow rate 1mL/min at room temperature. The working standards included malic, citric and oxalic acids. These organic acid standards were purchased from Merck. Firstly, working standards were run on HPLC and their retention times were determined by chromatogram. Then samples were run on HPLC.

***Qualitative Analysis*:** Qualitative determinations of carboxylates were done by comparing the retention times of the samples on chromatogram against retention times of the standards. When retention times corresponded with those of carboxylate standards, the identity was confirmed by paralleling the absorption spectra of the samples at 215 nm with the spectra of the corresponding carboxylates.

***Quantitative Analysis*:** Quantitative determination in each sample was done by comparing peak heights of standards with the peak heights of samples. Carboxylates were expressed per unit root dry mass.

***Phosphorus determination*:** Dried samples were ground with a mechanical grinder to powder form and stored in plastic bags. Dried, finely ground root and shoot were separately digested.

P determination in plants was based upon the light absorbance at wavelength 880 nm by standard or sample placed in the light path.

***Phosphorus use efficiency (PUE)*:** For determining P use efficiency, shoot/root dry matter was divided by shoot/root P concentration (Siddiqi and Glass, 1981).

***Statistical analysis*:** Data were compared between the groups with normal and deficient P applications. The statistical analysis of the data was performed with software Package SPSS Statistics 19.0 (Hussain *et al*., 2018). The data were accomplished by the standard analysis of variance (TWO WAY ANOVA) (Steel *et al.*, 1997) and mean values were compared by Duncan's least significant difference (LSD) at 5, 1 and 0.1% level of significance (Duncan,1955). Excel 2010 was used for graphical presentation. Multiple comparison tests (descriptive) were used to compare the genotypes.

RESULTS

Wheat genotypes

***Phosphorus concentrations and phosphorus use efficiency*:** Root has direct contact with soil and plays a key role in mineral uptake. Phosphorus concentration in root was significantly different within all wheat genotypes (Table 1). In this experiment, in P deficient treatment, reduction in root P was recorded in all genotypes compared to normal P treatment. SARC-2 showed the highest percent of reduction (11.1%) in shoot P concentration while the minimum reduction (2.5%) in root P concentration was detected in SARC-1 as compared to its P concentration in P normal treatment.

The variation among genotypes for shoot P uptake was observed among different wheat genotypes (Table 1). The response of P application on shoot P concentration was significantly different in all wheat genotypes except Kohinoor-83 and SARC-1. Under P deficiency, all genotypes exhibited a reduction in shoot P concentration and their P concentration was different from each other. Shoot P concentration was decreased and the maximum decrease (15.9%) in shoot P concentration was recorded in SARC-1 with respect to the P concentration of SARC-1 in P normal treatment. Reduction of 10.2% was shown by Kohinoor-83 which was the minimum among genotypes while B4-5711 (12.9%), SARC-2 (11.6%) and SARC-3 (12.5%) also showed a reduction in P concentration as compared to normal P treatment, respectively.

Phosphorus use efficiency showed a significant difference in wheat genotypes and it was non-significant between normal and deficient P (Table 1). In normal P treatment, the difference in PUE was observed by different genotypes. Phosphorus use efficiency was higher for B4-5711 and the lowest PUE was shown by SARC-3. In P deficient treatment, B4-5711, SARC-1, and SARC-2 showed an increase in their PUE while Kohinoor-83 and SARC-3 showed reduced PUE comparative to normal P treatment. The increase in PUE by SARC-1 was 16.7% presenting the highest increase among all genotypes. The lowest increase was given by B4-5711 (7.5%). Kohinoor-83 and SARC-3 showed a reduction of 0.24% and 21.3%, respectively compared to PUE of these genotypes in normal P treatment.

***pH in various wheat genotypes*:** In this study, all genotypes showed variation in their proton release in the root system (Table 2). Under P normal application, the SARC-1, and SARC-3, and Kohinoor, B4-5711 and SARC-2 showed the non-significant difference in root medium pH. Phosphorus deficiency activates the carboxylate release in the root system to create homeostasis in a plant system. These carboxylates caused an increase in the release of protons than normal P condition. In P deficient treatment, SARC-3 showed significantly variable results than rest genotypes. More protons release reduced pH due to P deficiency. The maximum and the minimum reduction in pH were recorded in SARC-1 (11.2%) and Kohinoor (2.7%) relative to normal P. Other wheat genotypes, B4-5711, SARC-2, and SARC-3 decreased the pH by 4.2%, 6.5%, and 5.4%, respectively, compared with normal P.

***Carboxylate release in various wheat genotypes*:** All the wheat genotypes showed significantly different responses in the release of citrate in P normal and deficient conditions

Table 1. Shoot P, Root P and Phosphorus use efficiency (PUE) of Wheat genotypes

Genotypes	Shoot P (%)		Root P(%)		PUE (g^2 RDW/mg P)	
	P(N)	P(D)	P(N)	P(D)	P(N)	P(D)
Kohinoor-83	0.21±0.001c	0.19±0.003e	0.16±0.001d	0.15±0.002e	0.32±0.013b	0.32±0.035b
B4-5711	0.18±0.002f	0.16±0.002g	0.19±0.003b	0.18±0.003c	0.38±0.018a	0.40±0.019a
SARC-1	0.22±0.001bc	0.18±0.002f	0.20±0.003a	0.20±0.005ab	0.20±0.003dc	0.23±0.015cd
SARC-2	0.23±0.005a	0.20±0.002d	0.18±0.002c	0.16±0.001d	0.25±0.002d	0.27±0.015c
SARC-3	0.22±0.002b	0.19±0.002e	0.14±0.003f	0.13±0.002g	0.14±0.004e	0.11±0.020e
Phosphorus	S (0.1 %)		S (0.1 %)		NS	
Genotypes	S (0.1 %)		S (0.1 %)		S (0.1 %)	
Interaction	NS		NS		NS	

Data refers to mean ± SE (n = 3). Values having same letters are not different significantly ($p \leq 0.05$) by LSD test with multiple comparisons. F-values of the Two-way ANOVAs of Genotypes, P levels, and their interaction is signposted: ns, not significant; S(0.1 %) significant at $p \leq 0.001$; S (1 %) significant at $p \leq 0.01$; S(5 %)significant at $p \leq 0.05$. P(N) represents normal P and P(D) represents deficient P.

Table 2. Carboxylate release and rhizosphere pH of Wheat genotypes

Genotypes	Citrate (µM/g RDW)		Oxalate (µM/g RDW)		Malate (µM/g RDW)		pH	
	P(N)	P(D)	P(N)	P(D)	P(N)	P(D)	P(N)	P(D)
Kohinoor-83	103±13.90i	199±0.76f	84±1.41e	283±1.31b	121±1.76f	249±2.13c	6.23±0.07d	6.07±0.03d
B4-5711	463±2.64d	593±3.86c	164±2.57df	0.00±0.00j	13.99±1.83h	181±1.49d	6.40±0.06b-d	6.13±0.03d
SARC-1	759±2.26b	1532±3.22a	74±2.71	287±2.40a	173±1.79e	353±0.68a	7.13±0.23a	6.33±0.07cd
SARC-2	238±0.65e	191±5.05g	62±2.91g	201±1.71c	283±2.49b	19±1.07g	6.63±0.19bc	6.20±0.06d
SARC-3	105±8.74j	162±0.60h	39±2.59i	53±2.77h	NDi	NDi	7.10±0.12a	6.71±0.01b
Phosphorus	S (0.1 %)		S (0.1 %)		S (0.1 %)		S (0.1 %)	
Genotypes	S (0.1 %)		S (0.1 %)		S (0.1 %)		S (0.1 %)	
Interaction	S (0.1 %)		S (0.1 %)		S (0.1 %)		NS	

*ND: Not detectable; Data refers to mean ± SE (n = 3). Values having same letters are not different significantly ($p \leq 0.05$) by LSD test with multiple comparisons. F-values of the Two-way ANOVAs of Genotypes, P levels, and their interaction is signposted: ns, not significant; S(0.1 %) significant at $p \leq 0.001$; S (1 %) significant at $p \leq 0.01$; S(5 %) significant at $p \leq 0.05$. P(N) represents normal P and P(D) represents deficient P.

Table 3. Shoot P, Root P and Phosphorus use efficiency (PUE) of Maize genotypes

Genotypes	Shoot P (%)		Root P(%)		PUE (g^2 RDW/mgP)	
	P(N)	P(D)	P(N)	P(D)	P(N)	P(D)
Cargel-6525	0.25±0.004a	0.13±0.012cd	0.25±0.003bc	0.18±0.007ef	0.53±0.010de	0.61±0.016bc
Syngenta-8711	0.25±0.002a	0.14±0.002c	0.21±0.004de	0.18±0.007ef	0.48±0.001ef	0.63±0.007bc
Pioneer-33H15	0.22±0.005b	0.12±0.004d	0.28±0.005a	0.26±0.033ab	0.67±0.011d	0.81±0.042a
Monsanto-6525	0.26±0.002a	0.14±0.004c	0.20±0.009de	0.14±0.004f	0.44±0.004f	0.57±0.011bc
Pioneer-32F10	0.25±0.004a	0.13±0.002cd	0.22±0.004b-d	0.22±0.003cd	0.33±0.002g	0.58±0.027bc
Phosphorus	S (0.1 %)		S (0.1 %)		S (0.1 %)	
Genotype	S (0.1 %)		S (0.1 %)		S (0.1 %)	
Interaction	NS		NS		S (5 %)	

Data refers to mean ± SE (n = 3). Values having same letters are not different significantly ($p \leq 0.05$) by LSD test with multiple comparisons. F-values of the Two-way ANOVAs of Genotypes, P levels, and their interaction is signposted: ns, not significant; S (0.1 %) significant at $p \leq 0.001$; S (1 %) significant at $p \leq 0.01$; S(5 %) significant at $p \leq 0.05$. P(N) represents normal P and P(D) represents deficient P.

(Table 2). All wheat genotypes showed the ability to release citrate in both P normal and deficient treatment. In contrast with normal P treatment, the deficient P gave a positive response to citrate release in root especially in Kohinoor-83, B4-5711, SARC-1, and SARC-3. In deficient P condition, the release of citrate by these genotypes was increased except in B4-5711 and this behavior revealed the P tolerance potential of genotypes towards low P. The highest increase in citrate concentration was noticed in SARC-1 (101%) under deficient P condition than normal P.

Genotypic difference in the release of oxalate is evident from the data in both P normal and deficient treatment (Table 2). In treatment where normal P was applied, oxalate concentration in wheat genotypes was ranged from 39 to 164 µM g^{-1} root DW. All the genotypes showed a release of oxalate in control treatment. The maximum oxalate release capacity was shown by B4-5711 while the minimum oxalate concentration was noticed in SARC-3. In a treatment where P was deficient, genotypes of wheat showed an increase in oxalate release with respect to the release of oxalate in normal P treatment except B4-5711 in which oxalate was not detectable. Among the other four genotypes, SARC-1 proved better as it exhibited an increase of 290% in oxalate concentration with respect to oxalate released in normal P treatment while genotype SARC-3 showed the minimum potential (34%) to release oxalate in P deficient condition as compared to control.

Malate exudation was significantly affected by genotype, P treatments and their interaction (Table 2). Among wheat genotypes, SARC-2 showed a significantly higher result for the release of malate in normal P treatment. Although all genotypes released malate in normal P treatment except SARC-3 genotype, in which release of malate by root was not in the detectable range. Results have shown that all genotypes had significantly variable potential to release malate in response to P deficiency. In a P deficient treatment, different genotypes responded to P deficiency by enhancing malate exudation. Out of five, three genotypes showed that behavior. Kohinoor-83 (105.2%), B4-5711 (119%) and SARC-1 (104.4%) released more malate in P deficient treatment compared to normal P treatment. Malate concentration was not detectable in SARC-3 in P deficient treatment.

Maize genotypes

***Phosphorus concentrations and phosphorus use efficiency*:** Genotypes and P treatment affected root P concentration

significantly and the interaction effect was not significant (Table 3). At the deficient P, genotypes exhibited a reduction in root P concentration. The maximum decrease in root P concentration was recorded in Monsanto-6525 and that a decrease in root P concentration was 29% with respect to its root P concentration in P normal treatment. A reduction of 2.82% was shown by Pioneer-32F10 which was the minimum among genotypes while Cargel-6525 (28.9%), Syngenta-8711(14.15%) and Pioneer-33H15 (9.59%) showed a decrease in root P concentration as compared to normal P treatment.

Shoot P concentration was affected significantly by genotype and P treatments while the interaction effect was not significant (Table 3). At P deficient treatment, shoot P concentration decreased overall compared to normal P treatment. However, the percent decrease was different in all genotypes. Pioneer-32F10 showed the highest percent (49%) of reduction in shoot P concentration and the minimum reduction in P concentration was noticed in Syngenta-8711(45.6%) as compared to its P concentration in normal P treatment.

Phosphorus use efficiency for all genotypes in P deficient and normal P treatment is presented in Table 3. Treatment, genotypic and interaction effect was significant. Pioneer-33H15 was more efficient in using P as compared to other genotypes. Phosphorus use efficiency demoed by Pioneer-32F10 was less than other genotypes. In P deficient treatment, all the genotypes used P efficiently and depicted a sharp increase in PUE compared to their efficiencies in normal P treatment. The maximum PUE was calculated in Pioneer-33H15 and the minimum was in Monsanto-6525.

***pH in various maize genotypes*:** Root medium pH was significantly affected by genotypes except in genotypes Syngenta-8711 and Pioneer-33H15 that shared the same letter and were not statistically different. The effect of P treatments and interaction was highly significant (Table 4). In normal P treatment, pH was not variable among genotypes. While in P deficient condition, results differed significantly among genotypes. All genotypes showed a reduction in pH in P deficient treatment as compared to normal P treatment. In P deficient condition, reduction in pH was higher in Pioneer-32F10 (19.7%) while the minimum reduction was observed by Cargel-6525 (1.5%) as compared pH of the same genotypes in normal P treatment. Reduction in pH of 10.1%, 7.9%, and 11.4% were noticed by Syngenta-8711, Pioneer-33H15, and Monsanto-6525, respectively as compared to pH for these genotypes in normal P treatment.

***Carboxylate release in various maize genotypes*:** Phosphorus treatment and genotype, as well as their interaction, showed a significant effect on citrate release (Table 4). In normal P treatment, all genotypes released citrate, though their potential to release citrate was variable. In normal P treatment, the highest citrate release was noticed in Pioneer-33H15. Pioneer-32F10 released the minimum amount of citrate among all genotypes. In P deficient treatment, an increase in citrate release was recorded by Cargel-6525 (5.45%), Syngenta-8711 (127.4 %) and Pioneer-32F10 (2771%) and the maximum release of citrate was recorded by Pioneer-32F10 as compared to normal P treatment. Pioneer-33H15 and Monsanto-6525 reduced citrate release by 62% and 4%, respectively when exposed to P deficiency. Monsanto-6525 revealed the lowest potential to release citrate in P deficient condition.

The effect of P treatment and genotypes on oxalate release by plant root was significant (Table 4). Among all maize genotypes, only two genotypes Monsanto-6525 and Pioneer-32F10 revealed their potential of oxalate exudation at both normal P as well as P deficient treatment. The other three genotypes Cargel-6525, Syngenta-8711, and Pioneer-33H15 were not efficient enough to exude oxalate in normal P as well as P deficient treatments. Under P deficiency, Monsanto-6525 and Pioneer-32F10 both showed an increase in their exudation of oxalate. Monsanto-6525 showed 16.54% and Pioneer-32F10 showed 32.20% more oxalate released in P deficient treatment comparative to normal P treatment that represents the potential of these genotypes to cope to low P environment.

Table 4. Carboxylate releaseand rhizosphere pH of Maize genotypes

Genotypes	Citrate(µM/g RDW)		Oxalate(µM/gRDW)		Malate(µM/g RDW)		pH	
	P(N)	P(D)	P(N)	P(D)	P(N)	P(D)	P(N)	P(D)
Cargel-6525	0.56±0.02cd	0.59±0.02cd	NDe	NDe	105±8.74j	162±0.60h	6.47±0.03ab	6.43±0.04a-c
Syngenta-8711	2.52±0.02c	5.73±0.03b	NDe	NDe	103±13.09i	198±0.76f	6.58±0.06a	5.92±0.01d
Pioneer-33H15	6.39±0.02b	2.41±0.03cd	NDe	NDe	462±2.64d	593±3.86c	6.58±0.01a	6.06±0.08d
Monsanto-6525	0.49±0.02cd	0.47±0.03cd	58.88±0.79d	68.62±3.47c	238±0.65e	190±5.05g	6.36±0.03bc	5.63±0.09e
Pioneer-32F10	0.38±0.01d	10.91±0.07a	86.81±5.46b	116.5±3.44a	759±2.26b	1532±3.22a	6.27±0.07c	5.03±0.12f
Phosphorus	S (0.1 %)		S (0.1 %)		S (0.1 %)		S (0.1 %)	
Genotypes	S (0.1 %)		S (0.1 %)		S (0.1 %)		S (0.1 %)	
Interaction	S (0.1 %)		S (0.1 %)		S (0.1 %)		S (0.1 %)	

*ND: Not detectable; Data refers to mean ± SE (n = 3). Values having same letters are not different significantly ($p \leq 0.05$) by LSD test with multiple comparisons. F-values of the Two-way ANOVAs of Genotypes, P levels, and their interaction is signposted: ns, not significant; S(0.1 %) significant at $p \leq 0.001$; S (1 %) significant at $p \leq 0.01$; S(5 %) significant at $p \leq 0.05$. P(N) represents normal P and P(D) represents deficient P.

Maize genotypes showed different responses in the release of malate in P deficient treatment as well as in P normal treatment (Table 4). From the statisticalresults, it was evaluated that treatment effect and genotypic response to P deficiency for the release of malate was significant. In P normal treatment, Pioneer-32F10 showed a significantly higher malate release compared to all other genotypes. In P deficient treatment, malate release by Pioneer-32F10 was the highest among all genotypes. An increase in malate release by Pioneer-32F10 was the highest (101.8%) in P deficient condition as compared to normal P among genotypes. Syngenta-8711, Pioneer-33H15, Pioneer-32F10 and Cargel-6525 increased malate release 93.45%, 28.15%, 101.8% and 54.26%, respectively in P deficient treatment that depicted adoption of these genotypes to low P condition. However, Monsanto-6525 gave a decrease of 20.02% in malate release by roots in P deficient treatment as compared to normal P.

DISCUSSION

Shoot P concentration at deficient P was lower as compared to P normal in all genotypes. These differences varied from one genotype to another genotype. This indicates that wheat genotypes that accumulated more P in their shoots from a deficient growth medium were more tolerant to P deficiency (Yaseen and Malhi, 2009). The reason for this behavior is that under low P condition, plants start using internal P sources to overcome the P deficiency which may reduce the plant P contents. Haynes *et al.* (1991) also observed similar responses. He reported that higher shoot P concentration in P efficient genotypes was related to improved P uptake efficiency in terms of higher R/S ratio at low P relative to P sensitive genotypes (Balemi and Schenk, 2009).

In this experiment, P deficiency showed a decrease in root P in all genotypes. This decrease in root P was significantly different in maize genotypes. Phosphorus efficient genotypes normally gave the minimum reduction in its root P concentration. These P efficient genotypes also maintain relatively higher P content in root as compared to shoot so that the root system can be improved. So, these improved root systems can efficiently uptake nutrients (Akhtar *et al*., 2008; Yi-Kai *et al*., 2013).

Phosphorus use efficiency increased in almost all wheat and maize genotypes under P deficient treatment. An important factor that caused an increase in PUE might be due to the intrinsic characteristics of different genotypes. Marked differences in the P utilization efficiency among genotypes of both wheat and maize were noticed. Significant variation in the ability of wheat and maize genotypes to take up and utilize P under low P conditions was observed. Similarly, Osborne and Rangel (2002); Chen *et al*. (2009); Do Vale and Fritsche-Neto (2013) also reported variation in maize and wheat genotypes in the P use efficiency in the nutrient solution.

The most important response of plants to P deficient condition is the release of carboxylates by roots in this study. A significant variation in P deficient as well as in normal P was observed in the root released carboxylates among genotypes of both maize and wheat. In some genotypes, carboxylate release was not in detectable range, while some genotypes respond to P deficient condition by the release of carboxylates. Genotypes that respond to P deficiency by the release of carboxylates are considered efficient genotypes under P deficiency. The reason behind this increased carboxylate exudation is the change in tri-carboxylic acid cycle (TCA). The TCA cycle in P efficient plants proceeds very differently from the TCA cycle in P-deficient plants. Enhanced synthesis of citrate occurred but conversion of citrate to iso-citrate is inhibited due to decreasing activity of the enzyme involved in this conversion. This results in an increased accumulation of citrate in the cell, that is then released into the rhizosphere (Neumann and Martinoia, 2002; Kihara *et al*., 2003). Our results are also in line with the findings of Shen *et al*. (2002) who reported increased secretion of carboxylates by roots in P efficient common bean genotypes that resulted in enhanced P uptake as compared to the P inefficient genotypes of less P solubilizing activity. Similar findings were reported by Ming *et al.* (2002). They reported that carboxylate release was higher in P non-sensitive genotype (JX17) as compared to P-sensitive genotypes (ZYQ8) of rice in a P stress environment.

In this study, it was found that the concentration of carboxylates release varies within species and genotypes (Fig. 1, Table 5) (Zhao and Wu, 2014). Genotypic variation in the carboxylate release was observed in soybean genotypes. Phosphorus efficient soybean genotypes exuded more malic acid, which ultimately caused improvement in P nutrition of plant (Liao *et al*., 2006). Genotypic variation in carboxylates exudation by roots of green gram and maize genotypes were

Table 5. Classification of wheat and maize genotypes on the basis of % increase in total carboxylate in deficient P compared to normal P

Wheat Genotypes	Total carboxylate increase (%)	Classification	Maize Genotypes	Total carboxylate increase (%)	Classification
Kohinoor-83	80.72	2	Cargel-6525	54.00	3
B4- 5711	20.81	4	Syngenta-8711	94.29	2
SARC-1	116.02	1	Pioneer-33H15	26.92	4
SARC-2	-29.37	5	Monsanto-6525	-12.76	5
SARC-3	48.99	3	Pioneer-32F10	96.05	1

noted in nutrient solution culture with P concentrations ranged from 0-100 µM (Singh and Pandy, 2003). An increased carboxylate exudation with a decreasing supply of P was noticed, indicating that P deprivation had roused carboxylate release (Abrahao *et al*., 2014).

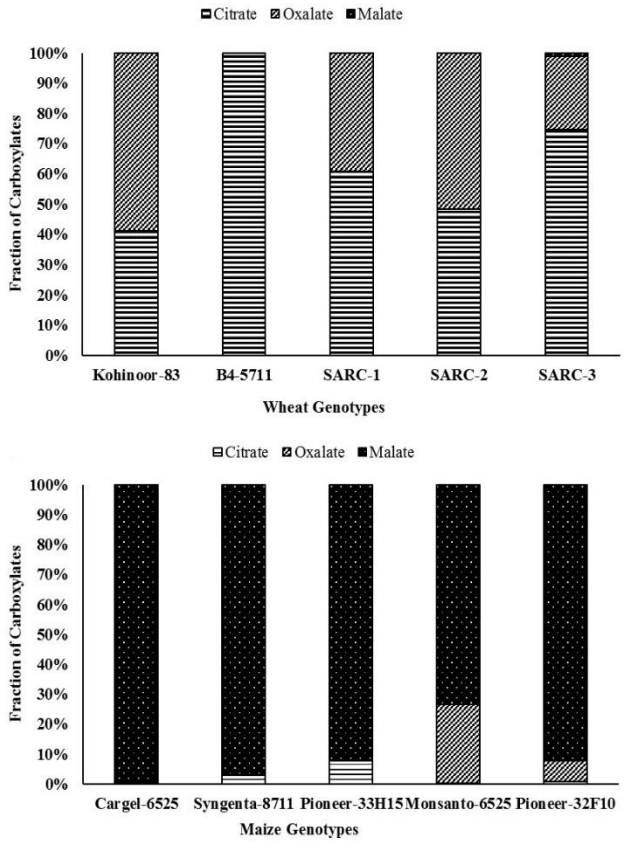

Figure 1. Fractions of Citrate, Malate and Oxalate in Wheat and Maize under deficient P.

In this study, the amount and composition of carboxylate exudation were different in wheat genotypes compared with maize genotypes. The percent increase in carboxylates release in maize genotypes was more as compared to wheat genotypes in deficient P compared to normal P (Table 5). This difference might occur due to the difference in plant metabolism. If all the factors that can influence root exudation like root architecture, type of collection medium, mechanical impedance, plant sterility, and age are not considered as the reason for exudation, (Aulakh *et al*., 2001; Skene, 2003; Tu *et al*., 2004; Johansson *et al*., 2009; Toyama *et al*., 2011) C4 plants may release a high amount of total organic carbon per unit mass of plant roots compared to C3 plant (Yoshitomi and Shann, 2001; Baudoin *et al*., 2003). Maize released more fraction of malate and wheat exuded more citrate fraction (Fig. 1). C4 and C3 metabolism caused changes in the composition of carboxylates. Maize, as a C4 crop produce and accumulate more malate while in C3 metabolism, the TCA cycle proceeds directly and in P deficiency more citrate is produced due to the downregulation of enzymes responsible for conversion of citrate to iso-citrate. Our results are supported by many researchers previously (Haase *et al*., 2007; Johansson *et al*., 2009; Hajlaoui *et al*., 2010; Nabais *et al*., 2011) as they reported that C4 (maize) showed more carboxylate release than C3 (wheat). Wheat, being a C3 crop, released fewer carboxylates as compared to maize that has C4 metabolism.

The total release of carboxylates was associated with PUE (Fig. 2). An increase in total carboxylate in P deficient treatment compared to P normal treatment is directly related to the increase in PUE in maize genotypes. However, in wheat, it was not correlated to that degree as in maize (Fig. 2). Both wheat and maize genotypes showed a reduction in pH in deficient P treatment as compared to normal P treatment. Genotypes that showed more reduction in pH can be considered P efficient genotypes. This lowering of pH might be due to the increased proton release by the roots of P efficient genotypes.

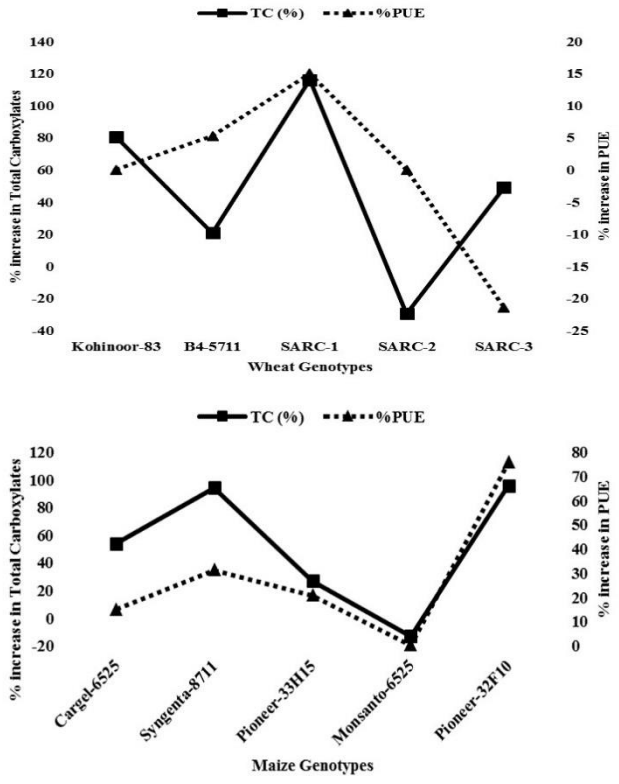

Figure 2. Increase in Total Carboxylates (TC) release in relation to Change in PUE.

This proton release occurs due to the release of carboxylates to maintain charge neutrality. When carboxylates are released by roots, protons help to maintain charge neutrality inside the

plant (Hinsinger *et al*., 2003). Our results are supported by Keerthisinghe *et al*. (1998) and Neumann *et al*. (1999), who reported that acidification of the rhizosphere, might be due to the release of organic acids from roots, resulting in increased proton release. On the basis of percent increase in total carboxylates in P deficient condition compared to normal P, we categorized the tolerance approach of wheat and maize (Table 5) genotypes against deficient P as wheat genotypes SARC-1 > Kohinoor-83 > SARC-3 > B4-5711 > SARC-2 and maize genotypes, Pioneer-32F10 > Syngenta-8711 > Cargel-6525 > Pioneer-33H15 > Monsanto-6525.

***Conclusion*:** In this hydroponic study, plant physiological responses, carboxylate release, proton release, and P use efficiency were significantly enhanced under P deficient condition compared to normal P. However, genotypes differ in their response to deficient P condition. Species variation was also observed as maize plant roots released more malate and protons compared to wheat. From all the above results evaluation and discussion, it is concluded wheat genotype SARC-1 and maize genotypes Pioneer-32F10 showed better adaptability and are efficient and responsive to the deficient P environment. This useful genetic difference that exists among wheat and maize genotypes can be well used for the identification and selection of high yielding P efficient genotypes for P deficient soils through the breeding process in the future.

REFERENCES

Abrahao, A., H. Lambers, A.C.H.F. Sawaya, P. Mazzafera and R.S. Oliveira. 2014. Convergence of a specialized root trait in plants from nutrient-impoverished soils: phosphorus acquisition strategy in a nonmycorrhizal cactus. Oecologia: Physiol. Eco. 76:345-355.

Ahmad, Z., M.A. Gill, A.M. Shah, T. Mahmood, Hamud-ur-Rehman and M. Yaseen.1998. Differential Growth Behavior of Cotton Varieties at Adequate and Deficient Levels of Nitrogen and Phosphorus. Pak. J. Biol. Sci. 1:342-345.

Akhtar, M.S., Y. Oki and T. Adachi. 2008. Phosphorus and biomass distribution, and P-efficiency by diverse Brassica cultivars exposed to adequate- and P-stress environment. J. Facu. Environ. Sci. Tech. 13:111-119.

Aulakh, M.S., R. Wassmann, C. Bueno, J. Kreuzwieser and H. Rennenberg. 2001. Characterization of root exudates at different growth stages of ten rice (*Oryza sativa* L.) cultivars. Plant Biol. 3:139-148.

Balemi, T. and M.K. Schenk. 2009. Genotypic variation of potato for phosphorus efficiency and quantification of P uptake in terms of root characteristics. J. Plant Nutr. Soil Sci.172: 669-677.

Baudoin, E., E. Benizri and A. Guckert. 2003. Impact of artificial root exudates on the bacterial community structure in bulk soil and maize rhizosphere. Soil Biol. Biochem. 35:1183-1192.

Cawthray, G.R. 2003. An improved reversed-phase liquid chromatographic method for the analysis of low-molecular mass organic acids in plant root exudates. J. Chromato. A. 1011:233-240.

Chen, J., L. Xu, Y. Cai and J. Xu. 2009. Identification of QTLs for phosphorus utilization efficiency in maize (*Zea mays* L.) across P levels. Euphytica 167:245-252.

Cordell, D. and S. White. 2015. Tracking phosphorus security: indicators of phosphorus vulnerability in the global food system. Food Secu. 7:337-350.

DoVale, J.C. and R. Fritsche-Neto. 2013. Genetic control of traits associated with phosphorus use efficiency in maize by REML/BLUP. Rev. Ciênc. Agron. 44:554-563.

Duncan, D. B. 1955. Multiple range and multiple F-Test. Biometrics.11:1-42.

Gaume, A., A. Mächler, C. De León, L. Narro and E. Frossard. 2001. Low-P tolerance by maize (*Zea mays* L.) genotypes: significance of root growth, and organic acids and acid phosphatase root exudation. Plant Soil 228:253-264.

Haase, S., G. Neuman. A. Kania. Y. Kuzyakov. V. Römheld and E. Kandeler. 2007. Elevation of atmospheric CO_2 and N-nutritional status modify nodulation, nodule-carbon supply, and root exudation of *Phaseolus vulgaris* L. Soil Biol. Biochem. 39:2208-2221.

Hajlaoui, H., N. El Ayeb, J.P. Garrec and M. Denden. 2010. Differential effects of salt stress on osmotic adjustment and solutes allocation on the basis of root and leaf tissue senescence of two silage maize (*Zea mays*L.) varieties. Ind. Crop Prod. 31:122-130.

Haynes, B.R., T. Koide and G. Elliott. 1991. Phosphorus uptake and utilization in wild and cultivated oats (*Avena* spp.). J. Plant Nutr. 14:105-118.

Hinsinger, P., C. Plassard, C. Tang and B. Jaillard. 2003. Origins of root mediated pH changes in the rhizosphere and their responses to environmental constraints: a review. Plant Soil. 248:43-59.

Hoagland, D.R. and D.I. Arnon. 1950. The water culture method for growing plants without soil. Circ. 347.Univ. Calif.Agric. Exp. Station, Berkley.

Hussain, S., X. Cao, C. Zhong, L. Zhu, M A. Khaskheli, S. Fiaz, J. Zhang and Q. Jin. 2018. Sodium chloride stress during early growth stages altered physiological and growth characteristics of rice. Chilean J. Agri. Rese. 78:34-48.

Johansson, E.M., P.M.A. Fransson, R.D. Finlay and P.A.W.V. Hees. 2009. Quantitative analysis of soluble exudates produced by ectomycorrhizal roots as a response to ambient and elevated CO2. Soil Biol. Biochem. 41:1111-1116.

Jones, D.L., A. Hodge and Y. Kuzyakov. 2004. Plant and mycorrhizal regulation of rhizodeposition. New Phytol. 163:459-480.

Keerthisinghe, G., P.J. Hocking, P.R. Ryan and E. Delhaize. 1998. Effect of phosphorus supply on the formation and function of proteoid roots of white lupin (*Lupins albus*L.). Plant Cell Environ. 21:467-478.

Kihara, T., T. Wada, Y. Suzuki, T. Hara and H. Koyama. 2003. Alteration of citrate metabolism in cluster roots of white lupin. Plant Cell Physiol. 44:901-908.

Kochian, L.V. 2012. Rooting for more phosphorus. Nature. 488:466-467.

Lambers, H., M.W. Shane, M.D. Cramer, S.J. Pearse and E.J. Veneklaas. 2006. Root structure and functioning for efficient acquisition of phosphorus: matching morphological and physiological traits. Annal. Bot. 98:693-713.

Li, H., J., Shen, F. Zhang, P. Marschner, G. Cawthray and Z. Rengel. 2010. Phosphorus uptake and rhizosphere properties of intercropped and monocropped maize, faba bean, and white lupin in acidic soil Biol. Fertil. Soils 46:79-91.

Liao, H., H. Wan, J. Shaff, X. Wang, X. Yan and L.V. Kochian. 2006. Phosphorus and aluminum interactions in soybean in relation to aluminum tolerance. Exudation of specific organic acids from different regions of the intact root system. Plant Physiol. 141:674-684.

Lynch, J.P. 2007. Roots in the second green revolution; turner review. Aust. J. Bot. 55:493-512.

Ming, F., G.H. Mi, F.S. Zhang and L.H. Zhu. 2002. Differential response of rice plants to low-phosphorus stress and its physiological adaptive mechanism, J. Plant Nutr. 25:1213-1224.

Nabais, C., G. Labuto, S. Gonçalves, E. Buscardo, D. Semensatto, A.R.A. Nogueira and H. Freitas. 2011. Effect of root age on the allocation of metals, amino acids and sugars in different cell fractions of the perennial grass *Paspalum notatum* (bahiagrass). Plant Physiol. Biochem. 49:1442-1447.

Nagy, R., M.J.V. Vasconcelos, S. Zhao, J. McElver, W. Bruce, N. Amrhein, K.G. Raghothama and M. Bucher. 2006. Differential regulation of five Pht1 phosphate transporters from maize (*Zea mays*L.). Plant Biol. 8:186-197.

Neumann, G. and E. Martinoia. 2002. Cluster roots-an underground adaptation for survival in extreme environments. Trends Plant Sci. 7:162-167.

Neumann, G., A. Massonneau, E. Martinoia and V. Römheld. 1999. Physiological adaptations to phosphorus deficiency during proteoid root development in white lupin. Planta 208:373-382.

Osborne, L. D. and Z. Rengel. 2002. Screening cereals for genotypic variation in efficiency of phosphorus uptake and utilization. Aust. J. Agric. Res. 53:295-303.

Rengel, Z. and P. Marschner. 2005. Nutrient availability and management in the rhizosphere: exploiting genotypic differences. New Phytol. 168:305-312.

Richardson, A.E., J.P. Lynch, P.R. Ryan, E. Delhaize, F.A. Smith, S.E. Smith, P.R. Harvey, M.H. Ryan, E.J. Veneklaas, H. Lambers, A. Oberson, R.A. Culvenor and R.J. Simpson. 2011. Plant and microbial strategies to improve the phosphorus efficiency of agriculture. Plant Soil 349:121-156.

Sadana, U.S., L. Kusum and N. Claassen. 2002. Manganese efficiency of wheat cultivars as related to root growth and internal manganese requirement. J. Plant Nutr. 25:2677-2688.

Shen, H., X. Yan, M. Zhao, S. Zheng and X. Wang. 2002. Exudation of organic acids in common bean as related to mobilization of aluminum- and iron-bound phosphates. Environ Exp. Bot. 48:1-9.

Shen, J., L. Yuan, J. Zhang, H. Li, Z. Bai and X. Chen. 2011. Phosphorus dynamics: from soil to plant. Plant Physiol. 156:997-1005.

Siddiqi, M.Y. and A.D.M. Glass. 1981. Utilization index: A modified approach to the estimation and comparison of nutrient utilization efficiency in plants. J. Plant Nutr. 4:289-302.

Simpson, R.J., A. Oberson, R.A. Culvenor, M.H. Ryan, E.J. Veneklaas, H. Lambers, J.P. Lynch, P.R. Ryan, E. Delhaize, F.A. Smith, S.E. Smith, P.R. Harvey and A.E. Richardson. 2011. Strategies and agronomic interventions to improve the phosphorus-use efficiency of farming systems. Plant Soil 349:89-120.

Singh, B. and R. Pandey. 2003. Differences in Root Exudation Among Phosphorus-Starved Genotypes of Maize and Green Gram and Its Relationship with Phosphorus Uptake, J. Plant Nutr. 26:2391-2401.

Skene, K.R. 2003. The evolution of physiology and development in the cluste root. Plant Soil 248:21-30.

Steel, R.G.D., J.H. Torrie and D.A. Dicky. 1997. Principles and Procedures of Statistics-A Biometrical Approach. 3rd Ed. McGraw Hill Book International Co., Singapore.

Toyama, T., T. Furukawa, N. Maeda, D. Inoue, K. Sei, K. Mori, S. Kikuchi and M. Ike. 2011. Accelerated biodegradation of pyrene and benzo[a]pyrene in the *Phragmites australis* rhizosphere by bacteria–root exudate interactions. Water Res. 45:1629-1638.

Tu, S., L. Ma and T. Luongo. 2004. Root exudates and arsenic accumulation in arsenic hyperaccumulating *Pteris vittata* and non-hyper accumulating *Nephro lepisexaltata*. Plant Soil 258:9-19.

Vance, C.P., C. Uhde-Stone and D.L. Allan. 2003. Phosphorus acquisition and use: critical adaptations by plants for securing a nonrenewable resource. New Phytol. 157:423-447.

Yaseen, M. and S.S. Malhi. 2009. Differential Growth Performance of 15 Wheat Genotypes for Grain Yield and

Phosphorus Uptake on Low Phosphorus Soil Without and With Applied Phosphorus Fertilizer. J. Plant Nutr. 32:1015-1043.

Yi-kai, Z., C. Fan-jun, C. Xiao-chao, L. Li-zhi, G. Kun, Y. Li-xing, Z. Fu-suo and M.I. Guo-hua. 2013. Genetic Improvement of Root Growth Contributes to Efficient Phosphorus Acquisition in maize (*Zea mays* L.). J. Integ. Agri. 12:1098-1111.

Yoshitomi, K.J. and J.R. Shann. 2001. Corn (*Zea mays* L.) root exudates and their impact on 14C-pyrene mineralization. Soil Biol. Biochem. 33:1769-1776.

Zhang, Z., H. Liao and W.J. Lucas. 2014. Molecular mechanisms underlying phosphate sensing, signaling, and adaptation in plants. *J. Integr. Plant Biol.* 56:192-220.

Zhao, K. and Y. Wu. 2014. Rhizosphere calcareous soil P-extraction at the expense of organic carbon from root-exuded organic acids induced by phosphorus deficiency in several plant species. Soil Sci. J. Plant Nutr. 60:640-650.

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