Phosphorus uptake relates to vegetative growth, grain yield, and grain quality in phosphorus-deprived rice genotypes

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Abstract

The response of rice towards phosphorus (P) deficiency, from vegetative growth to crop maturity, grain yield, and grain quality, has been less studied. Importance of acid phosphatases (ACP) under P deficiency is not well understood. In this study, P nonapplication (P_0) was compared to P application (P_{30}) at a rate of 30 kg ha^{-1} in seven rice genotypes grown under field conditions. Biomass, P uptake, and ACP activity of shoots and roots were measured 30 and 60 days after transplantation (DAT) while grain yield, grain size, and grain P content were measured at harvest, and grain quality after six months of storage following harvest. Thirty DAT, the biomass of shoots was mostly affected in Pusa44, but by 60 DAT, biomass of shoots and roots improved in CSR10/IET28816 compared to the other genotypes under P_0. P uptake was mostly affected in Pusa44 while it improved by 60 DAT in CSR10/IET28816 compared to the other genotypes under P_0. P uptake was positively related to root growth, and ACP activity in roots under P_0. At harvest, grain yield reduced in Pusa44/IET28075, and grain length reduced in Pusa44. In addition, the total P content of grains reduced in IET28066/IET28061/IET27641 under P_0. In stored grains, total antioxidant capacity decreased, and oxidative stress increased to a high extent in Pusa44 while it increased to a low extent in CSR10/IET28816 compared to the other genotypes under P_0. Results concluded that P uptake determined plant growth, grain yield, and grain quality under P_0. CSR10/IET28816 showed greater adaptation to P_0 compared to the other genotypes.

Keywords: acid phosphatase, antioxidant, phytic acid, root, shoot, seed

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Introduction

Phosphorus (P) is a crucial macronutrient and an essential component of various biomolecules such as nucleic acids, phospholipids, adenylates, phosphoproteins, and phosphorylated metabolites. In the soil, P exists in mineralized or organic forms but both forms are inaccessible to plants. Mineralized P is linked inorganically to minerals (Fe/Al/Ca) while organic P is linked by ester linkage to organic compounds of decaying biomatter. Organic P constitutes about 50-80% of total soil P, with phytic acid alone contributing to 60-80% of organic P (Aziz et al., 2014; Dissanayaka et al., 2021; Dissanayaka et al., 2018; Wang et al., 2021). Plants can only take up soluble P, but P is often linked with soil constituents, rendering it

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unavailable to plants. The application of large quantities of P fertilizers is a common strategy to overcome P deficiency, but 20-30% of applied P is taken up by plants while the remaining 70-80% is precipitated in the soil as insoluble forms, converted into organic P by soil microbes, or runs off into nearby aquatic and marine ecosystems, causing eutrophication. Breeding crops for enhanced yields under high fertilization regimes has led to crop varieties poorly adapted to low P conditions. Moreover, accessible global reserves of rock P (source of P fertilizers) are non-renewable and may largely be depleted by the end of this century. Therefore, plant adaptations to grow under low P conditions are required to be studied so that future crops can be built with such adaptations, reducing the need for these fertilizers (Aziz et al., 2014; Dissanayaka et al., 2021; Dissanayaka et al., 2018; Han et al., 2022).

Plants have been found to adopt two strategies to adapt to low P conditions: P acquisition and/or P remobilization. P acquisition involves increasing P uptake from the soil while P remobilization involves efficient use of P within plants, where plants may scavenge P from P containing compounds or remobilize P from older leaves to younger tissues or developing grains (Dissanayaka et al., 2021; Lambers, 2022; Stigter and Plaxton, 2015; Veneklaas et al., 2012). Phosphatases are hydrolytic enzymes that cleave the ester bond between the phosphate group and organic residue of organic phosphates releasing inorganic P from organic P compounds. Plants upregulate acid phosphatases when deprived of P, which cleaves P from soil organic P (Müller et al., 2015; Plaxton and Tran, 2011; Rose et al., 2013; Shane et al., 2014; Yang et al., 2012). However, the importance of phosphatases in plant adaptations to low P conditions is still not well understood.

Deficiency in macronutrients and micronutrients induces oxidative stress in plants, manifesting higher-than-usual levels of reactive oxygen species (ROS), increased lipid peroxidation and protein oxidation, loss of cell integrity, and eventually, cell death and tissue decay (Gill and Tuteja, 2010). Under P deficiency, plants produce oxidative stress at subcellular, cellular, tissue, and whole plant levels (Hernández and Munné-Bosch, 2015). Plants also increase antioxidants to counterbalance ROS (Liu et al., 2018; Parra-Almuna et al., 2018). Recent studies have indicated that common signalling networks may operate under abiotic stresses and nutritional homeostasis (Belgaroui et al., 2018), and salinity tolerant plants can be tolerant to low P (Miura et al., 2011). However, antioxidant activity under P deficiency is less studied than other environmental stresses. Deficiency in macronutrients (N, P, K) reduces yield and germination rate of seeds in Arabidopsis (Zhang et al., 2020), and alterations in seed components, including minerals, carbohydrates, proteins, and oils have been observed when plants are exposed to P deficiency(Singh et al., 2016). Nonetheless, grain quality in terms of oxidative stress and antioxidant levels affected by P deficiency is not well-reported.

Most of the investigations on low P have been carried out at the seedling stage and on plant materials grown in hydroponics under laboratory conditions. But plants face continuous stress from germination through tillering until maturity when grown in low-P conditions.

Materials and Methods

In the present study, we studied the response of seven rice genotypes to low P by growing them in fields and providing a recommended dose of P (30 kg ha^{-1}) for P application (P_{30}) and omitting P for the control (P_0). We measured growth, P uptake, and acid phosphatases of roots and shoots at the vegetative stage (30 and 60 days after transplantation) and assessed grain yield, grain size, grain P, total antioxidant capacity, ROS, and protein carbonyls of grains at harvest and after six months of grain storage. The genotypes included IET28066, IET28816, IET28061, IET27641, IET28075, CSR10, and Pusa44, where CSR10 is a salt-tolerant rice variety, released for cultivation in sodic and saline areas, and Pusa44 is a salinity-susceptible and P-starvation sensitive variety (Kumar et al., 2021).

Crop raising and plant material

The present study utilized seven rice genotypes: IET28066, IET28816, IET28061, IET27641, IET28075, CSR10, where CSR10 is a salt-tolerant

Table 1
Designation and cross combination of IET (initial evaluation trial) numbers

IET No.	Designation	Cross combination
IET28066	RP 5973-13-1-6-67-129-57	MTU 1010*2 / Swarna
IET28816	MTU 1329 (MTU 2513-24-2-2-1)	BPT 5204 / NLR 34449
IET28061	RP 5970-2-6-19-16-24-1	Improved Samba Mahsuri*2 / Swarna
IET27641	RP Bio 4919-B-B-NSR 86	KMR3 / *Oryza rufipogon*
IET28075	RP Bio 5477-NH-663	N 22 Mutant

rice variety, released for cultivation in sodic and saline areas, and Pusa44. Pusa44 is a salinity-susceptible and P-starvation sensitive variety (Kumar et al., 2021). Also, IET28066, IET28816, IET28063, IET27641, and IET28075 were elite lines recommended for Initial Variety Trial Low Phosphorus Tolerance (IVT-LPT) by the All India Coordinated Rice Improvement Project. Table 1 provides a description and cross of the IET genotypes. Meanwhile, Pusa44 and CSR10 were previously released varieties described in the Introduction section.

The genotypes were grown using split-plot design with three replications, with genotypes serving as subplots and treatments as main plots. The experiment took place during the kharif/wet season from June to October/November 2020 at the Research Farm of Punjab Agricultural University in Ludhiana, India (30° 56' N latitude; 75° 52' E longitude; 247 m altitude) situated in the Western Indo–Gangetic Plains (WIGPs). The area is characterized by a subtropical, semi-arid climate with an annual rainfall of 733 mm, of which approximately 80% occurs between June to September. The plants were transplanted using 30-day-old seedlings spaced at 20 cm x 15 cm during the last week of June. All recommended production and protection technologies were followed except P. Fertilizers were applied at a recommended dose of N: P: K in 105: 30: 30 kg ha^{-1} for P application (P_{30}) and N: P: K in 105:0:30 kg ha^{-1} for the P control (P_0). The initial soil analysis (0-15 cm depth) conducted at the beginning of the study revealed that the experimental site was a Typical Ustipsamment (Fatehpur sandy–loam) in texture and low in available N (225 kg ha^{-1}), but medium in available P (13.8 kg ha^{-1}), K (273 kg ha^{-1}), and soil organic carbon (0.42%). The soil pH (7.3) and electrical conductivity (0.25 dSm^{-1} at 25 ℃) were within the normal range. The genotypes were grown until maturity, after which they were harvested at physiological maturity and their seeds were stored for post-storage analysis.

Measurement of shoot and root biomass

For each plot, four plants were randomly selected, and their shoots and roots were separated. Roots were divided into four zones: top 5 cm, 5-10 cm, 10-15 cm, and 15-20 cm. The samples were then dried at 80 ^{o}C for 72 hours and weighed. Biomass was expressed in grams per plant.

Measurement of P uptake

Total P (TP) was measured following the methods outlined by Shane et al. (2014) and Kuppusamy et al. (2014). About 50 mg of the oven-dried tissue powder was digested in concentrated HNO_3:$HClO_4$ (3:1) at 170-180 ℃, and the P concentration was determined using an ammonium molybdate /ascorbic acid-based assay (Ames, 1966). P uptake was calculated using the following equation:

P uptake (mg P/plant) = (mg P per g dry weight x g dry weight per plant)

Measurement of cell wall and soluble acid phosphatases

Cell wall (CW) and soluble (S) acid phosphatases (ACP) were measured following the methods suggested by Shane et al. (2014). Tissue was homogenized in an ice-cold extraction buffer containing 25 mM HEPES-KOH pH 7.4, 10 mM MgCl2, 1 mM EDTA, 1 mM DTT, 1% Triton-X-100, and 1% PVP at 4 ℃ and then cold-centrifuged at 10,000 x g for 20 minutes. The resulting supernatant was used as the soluble fraction while the pellet was washed three times with ice cold

extraction buffer, and then dissolved in the same buffer supplemented with 1 M NaCl. The mixture was kept at 4 ℃ for 30 minutes and then cold-centrifuged at 10,000 x g for 20 minutes. The resulting supernatant was used as the cell wall fraction.

ACP was assayed from both fractions using para-nitrophenyl phosphate as substrate. Enzyme extract (extraction buffer as blank) was incubated in 0.1 M sodium acetate buffer pH 4.7 supplemented with 2.2 mM para-nitrophenyl phosphate at 37 ℃ for 30 minutes, and the reaction was stopped in 0.13 M NaOH. The released para-nitrophenol was read at 420 nm against the blank. The amount was calculated using standard p-nitrophenol (20-100 nmol). Activity was expressed in nkat g^{-1} fresh weight.

Measurement of grain yield, seed size, and seed P

To estimate grain yield, a net area of 6.0 m^2 (3.0 m x 2.0 m) was harvested from each plot, threshed, sun-dried, winnowed, cleaned, and weighed on an electronic balance. To ensure valid comparisons between treatments, moisture in the grains was estimated using a digital moisture meter (Kett's RICETER J handheld grain moisture meter). Grain yield was adjusted to 14% moisture content and expressed as tons per hectare. Seed size (length and breadth) were measured in millimetres for ten husked seeds selected randomly using a dial gauge (Baker, USA). Clean rice seeds were dehulled using a dehusker (Satake Corp. THU-35B, Hiroshima, Japan) to obtain dehulled seeds.

The P content of the dehulled seeds was measured in three forms: total P (TP), phytic acid P, and inorganic P (Pi). TP was measured using the method described earlier. Phytic acid P was estimated following the method outlined by Zemel and Shelef (Zemel and Shelef, 1982). About 0.1 g of seed sample was homogenized in 1 ml of 1.2% HCl and 10% Na_2SO_4, and then filtered. Next, 1 ml of 0.8% $FeCl_3.6H_2O$ was added to the filtrate, and the mixture was boiled in a water bath to precipitate the ferric-phytate salt. The mixture was then centrifuged to obtain the precipitate in the form of a pellet which was washed with 0.5 ml of 4% Na_2SO_4 in 0.07 N HCl. The pellet was then dissolved and digested in a mixture of nitric acid: perchloric acid (3:1) at 170-180 ℃, and the P concentration was determined using an ammonium molybdate/ascorbic acid-based assay (Ames, 1966). Inorganic P (Pi) was measured following the method described by Jeong et al. (Jeong et al., 2017). Pi content was extracted in 0.4 M HCl for 3 hours at room temperature. The extract was then centrifuged at 10,000 x g for 20 minutes, and the supernatant was diluted with double-distilled water. Pi was estimated using an ammonium molybdate/ascorbic acid-based assay (Ames, 1966).

Measurement of total antioxidant capacity, H_2O_2 and protein carbonyls

Dehusked and polished grains were used for all the following analyses. Dehusked grains were milled using a Satake TMO5C testing mill (Satake Laboratory Equipment) to obtain polished grains with approximately 10% degree of milling.

Total antioxidant capacity was measured using the method described by Prieto et al. (Prieto et al., 1999). Grains (0.25 g) were crushed in 80% methanol on ice, passed through cheese cloth, and cold-centrifuged at 10,000 x g for 20 minutes. The resulting supernatant was used in the estimation, where 0.2 ml of the supernatant was incubated with 2 ml of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate) at 95 ℃ for 90 minutes. After cooling to room temperature, the absorbance was taken at 695 nm against a reagent blank. The amount was expressed in ascorbate equivalents by running standard ascorbate (50-250 nmol).

H_2O_2 and protein carbonyls were measured following the methods described by Kaur et al. (Kaur et al., 2014a; Kaur et al., 2014b) . H_2O_2 was extracted by homogenizing grains (0.25 g) in 0.1% TCA on ice, and the extract was passed through cheese cloth and cold-centrifuged at 10,000 x g for 20 minutes. The resulting supernatant (appropriately diluted 1 ml) was incubated in 1.33 M KI, and 0.033 M potassium phosphate buffer pH 7 in the dark at room temperature for one hour. The mixture was then read at 390 nm against a reagent blank, and the amount was calculated using standard H_2O_2 (50-200 nmol).

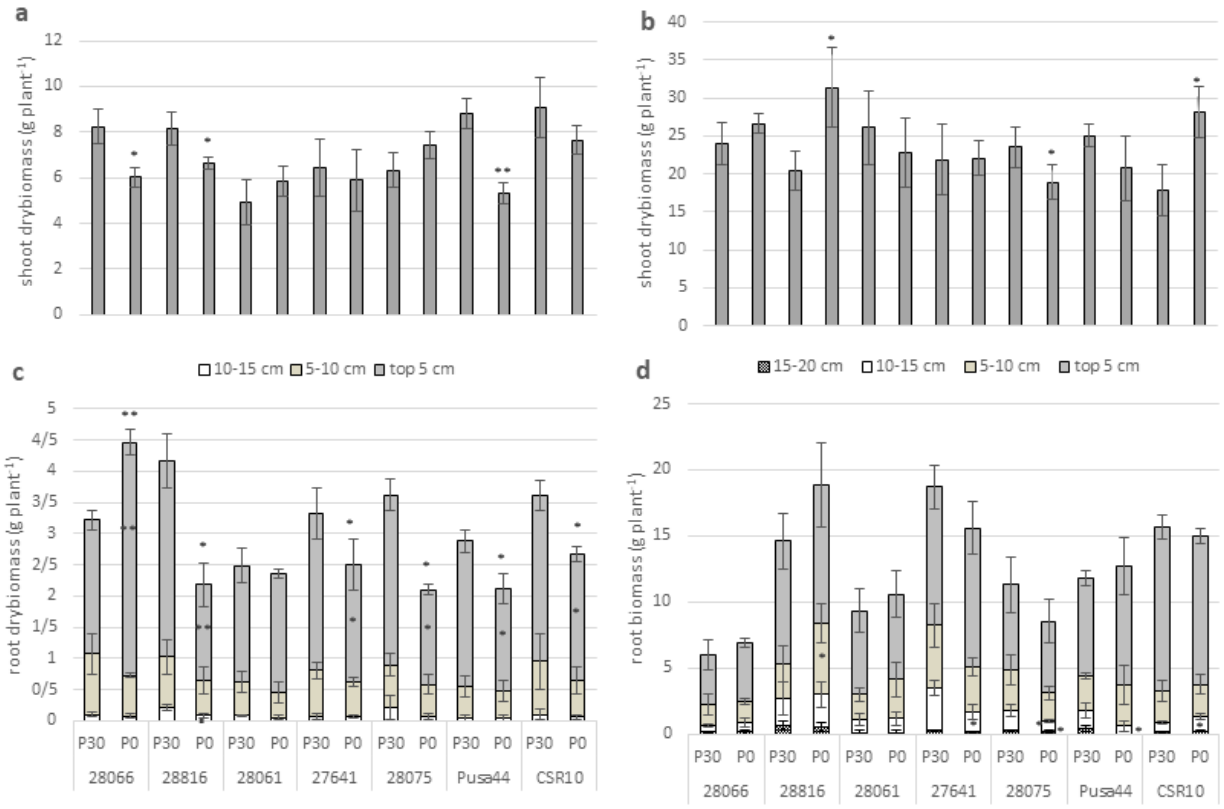

Fig. I. Shoot and root biomass of the genotypes under study

Protein carbonyls were extracted by homogenizing seeds (0.25 g) in 0.1 M potassium phosphate buffer pH 7 containing 1 mM EDTA, 1 mM PMSF, and 0.5 µg/ml aprotinin. The mixture was passed through cheese cloth and cold-centrifuged at 10,000 x g for 20 minutes. The resulting supernatant (about 0.1 ml) was reacted with 10 mM 2,4-dinitrophenyl hydrazine (DNPH) prepared in 2N HCl or 2N HCl (for control) in the dark for one hour and centrifuged after every 10-15 minutes. TCA was added to a final concentration of 5.2% to precipitate proteins, and the mixture was kept on ice for 10 minutes and then centrifuged at 10,000 × g for 20 minutes. The resulting pellet was washed once with 10% TCA and then recentrifuged at the same speed for 20 minutes. The pellet was then dissolved in 6 M guanidine HCl, kept at 37 ℃ for 15 minutes, and centrifuged to remove any precipitate. The content was read at 360 nm against a reagent blank and calculated using a molar extinction coefficient of hydrazone of 22,000 M^{-1} cm^{-1}.

Statistical Analysis

The mean ± standard deviation (S. D.) was calculated for the data. To compare the means between P_{30} and P_0, the Student's t-test was used at significant levels of $p \leq 0.05$ and $p \leq 0.01$. The t-test function was used in Excel-Formulas for this purpose.

Results

Shoot and root biomass

The shoot biomass of IET28066, IET28816, and Pusa44 decreased under P_0 at 30 days after transplantation (DAT) (Fig. I. a), and the shoot biomass of IET28075 decreased under P_0 at 60DAT (Fig. I. b). The fold reduction in shoot biomass was particularly high in Pusa44 (Fig. I. a). However, the shoot biomass increased at 60DAT under P_0 in IET28816 and CSR10. Under P_0, root biomass increased in IET28066 only while it reduced in IET28816, IET27641, IET28075, Pusa44, and CSR10. The reduction in root biomass was

observed in the top 5 cm zone of the root at 30

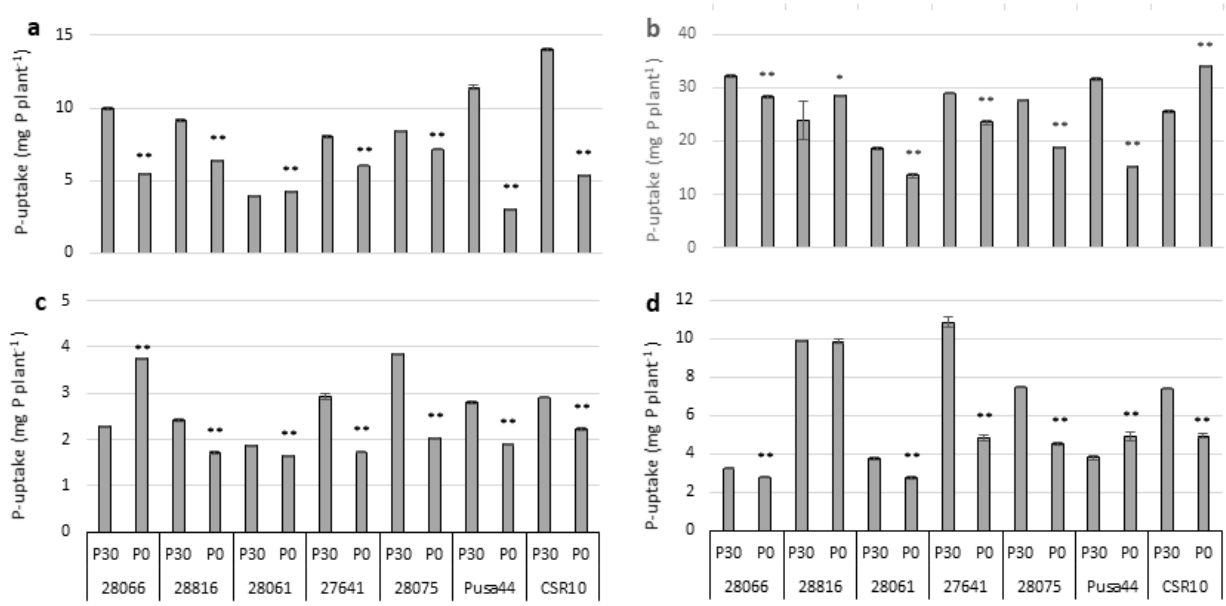

Fig. II. P uptake by shoots and roots in the genotypes under study

DAT (Fig. I. c). However, at 60DAT (Fig. I. d), the root biomass increased in the 5-10 cm root zone of IET28816 and in the 10-15 cm root zone of CSR10 while the root biomass decreased in the 10-15 cm root zone of IET27641 as well as in the 10-15 cm and 15-20 cm root zones of IET28075 and the 15-20 cm root zone of Pusa44 under P_0. Total root biomass remained unchanged in all genotypes at 60DAT under P_0. These findings suggest that CSR10/IET28816 were better adapted while Pusa44 was less adapted to P_0 compared to other genotypes. Although biomass was affected at 30DAT, it improved significantly by 60DAT in IET28816/CSR10 but not in IET28066 under P_0.

P uptake

P uptake by shoots was reduced in all genotypes except IET28061 at 30DAT (Fig. II. a), but it improved by 60DAT in IET28816 and CSR10 only while it did not improve in other genotypes under P_0 (Fig. II. b). P uptake by roots increased only in IET28066 while it decreased in other genotypes. The reduction in P uptake was low in IET28816/IET28061/CSR10 but high in other genotypes at 30DAT (Fig. II. c). At 60DAT (Fig. II. d), the reduction in P uptake remained high in the roots of IET27641/IET28075 under P_0. The results showed that the most deficient P levels were found in Pusa44 especially in shoots under P_0. P levels were sufficient by 60DAT in CSR10/IET28816 compared to other genotypes under P_0. The P uptake was found to be related to root growth under P_0.

CW-ACP and S-ACP

In shoots, at 30DAT (Fig. III. a), CW-ACP increased by a greater amount in IET27641/IET28075 /Pusa44/IET28061 than in CSR10 while it did not increase in IET28816/IET28066 under P_0. And at 60DAT (Fig. III. b), CW-ACP increased by a greater amount in IET28061/IET27641/IET28075/Pusa44 than in CSR10/IET28816 while it decreased in IET28066 under P_0. In roots, CW-ACP increased by a greater amount in IET28816 than in IET28061/IET27641/IET28075/Pusa44/CSR10 at 30DAT (Fig. III. c) and it increased only in IET28816/IET28061/CSR10 at 60DAT (Fig. III. d) under P_0. The fold increase of CW-ACP remained 1.1-fold at both 30 and 60DAT in the roots of CSR10 while it declined from 1.6-fold at 30DAT to 1.1-fold at 60DAT in the roots of IET28816 under P_0.

At 30DAT (Fig. IV. a), S-ACP increased only in the shoots of IET28075, and at 60DAT (Fig. IV. b), it increased by a greater amount in the shoots of

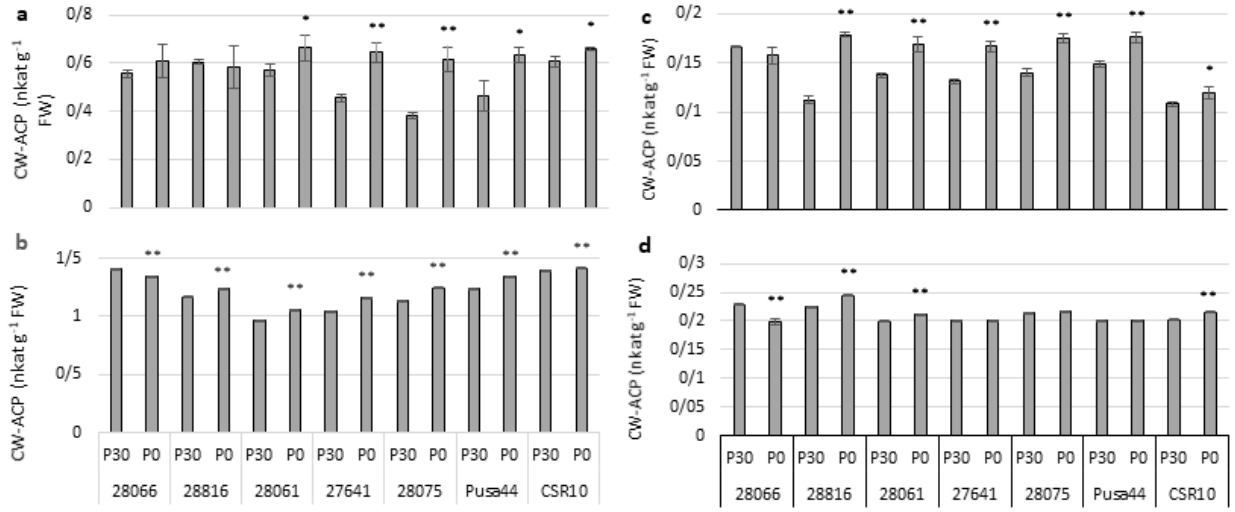

Fig. III. CW-ACP in shoots of the genotypes under study

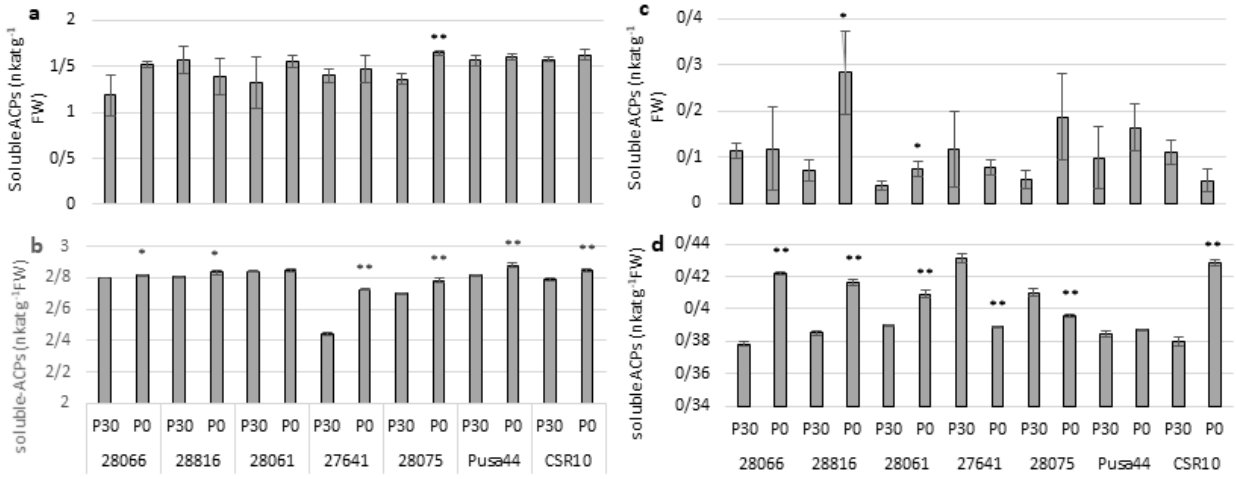

Fig. IV. S-ACP in shoots of the genotypes under study

IET27641/IET28075/Pusa44 than those of IET28066/IET28816/CSR10 while it remained unaltered in IET28061 under P_0. In roots, S-ACP increased only in IET28816 and IET28061 at 30DAT (Fig. IV. c), where the fold increase was much higher in IET28816 compared to IET28061 and at 60DAT (Fig. IV. d), S-ACP increased only in IET28066/IET28816/CSR10/IET28061 while it decreased in IET27641 and IET28075 under P_0.

Overall, the results suggested that ACPs might be involved in P remobilization in shoots while being involved in P uptake in roots under P0. The P uptake is related to ACPs in roots but not in shoots under P_0.

Grain parameters

At harvest, the grain yield was found to decrease in IET28075 and Pusa44, and the grain length reduced in Pusa44 under P_0 conditions (Table 2). In addition, the total P content decreased in the grains of IET28066, IET28061, and IET27641 while phytic acid P decreased in the grains of all genotypes, and inorganic P decreased in the grains of all genotypes except Pusa44 and IET28061 under P_0. These findings suggest that the plant's P uptake during the vegetative stage is related to grain yield and grain size, but it is only partially

Table 2
Grain parameters under P non application (P_0) compared to recommended P application at 30 Kg ha^{-1} (P_{30}) in rice genotypes

genotype	treatment	Grain yield t hac^{-1}	Grain size length (mm)	Grain size breadth (mm)	Grain-P (mg P g^{-1} seeds) Total-P	Grain-P (mg P g^{-1} seeds) Phytic acid-P
IET28066	P_{30}	6.16±0.4	9.09±0.61	1.99±0.06	6.11±0.02	5.30±0.05
	P_0	6.04±0.3	9.02±0.33	1.98±0.11	5.12±0.02**	2.78±0.05**
IET28816	P_{30}	5.66±0.15	8.20±0.58	1.83±0.14	5.52±0.01	4.31±0.03
	P_0	5.58±0.36	8.44±0.32	1.81±0.16	5.76±0.02**	3.10±0.04**
IET28061	P_{30}	5.35±0.41	7.84±0.52	1.83±0.15	6.05±0.02	5.45±0.05
	P_0	5.50±0.63	8.25±0.29*	1.78±0.08	5.64±0.04**	4.57±0.03**
IET27641	P_{30}	7.11±0.39	8.59±0.41	1.85±0.15	5.75±0.03	4.64±0.04
	P_0	7.29±1.41	8.72±0.39	1.89±0.1	4.77±0.02**	3.21±0.04**
IET28075	P_{30}	7.21±0.18	8.67±0.21	1.92±0.09	6.12±0.02	4.01±0.04
	P_0	6.68±0.36*	8.61±0.32	1.90±0.09	6.13±0.01	2.72±0.03**
Pusa44	P_{30}	8.46±0.29	9.36±0.26	1.97±0.08	5.60±0.03	3.45±0.04
	P_0	7.62±0.36*	9.04±0.38*	1.91±0.06	5.73±0.01**	3.32±0.02**
CSR10	P_{30}	7.47±0.35	9.02±0.26	1.91±0.07	5.49±0.02	2.63±0.02
	P_0	7.45±0.84	9.99±0.21**	1.97±0.06*	5.53±0.03	2.43±0.04**

Measurements were done at harvest. Values are Mean ± S. D. (n=3). Grain yield and grain size are given of husked grain while grain P is of dehusked grain. Significant difference between mean values of P_{30} and P_0 is shown as * at p≤0.05 and ** at p≤0.01 (Student t-test).

related to the P-content of grains under P_0 conditions.

Total antioxidant capacity, H_2O_2, and protein carbonyls in grains

According to the results, the total antioxidant capacity (TAC) decreased by a high amount in dehusked grains of Pusa44, IET28061, and IET28066 (Fig. V. a) and in polished grains of Pusa44, IET28061, IET28066, IET28075, and IET27641 (Fig. V. b) under P_0 conditions. However, TAC decreased by a small amount in polished grains only of CSR10 and it did not decrease in both polished and dehusked grains of IET28816 under P_0. H_2O_2 increased by a high amount in dehusked (Fig. V. c) and polished grains (Fig. V. d) of Pusa44, and dehusked grains of IET28061, and by a low amount in dehusked grains of IET28075 and in polished grains of IET27641, but not in other genotypes under P_0. Protein carbonyls increased by a low amount in dehusked (Fig. V. e) and polished grains (Fig. V. f) of CSR10, IET28816, and IET28061 compared to other genotypes under P_0.

These findings suggest that the total antioxidant capacity decreased while reactive oxygen species (ROS) and toxicity increased in grains under P_0. Grain antioxidant capacity was found to be related to P uptake by the plant during vegetative stage under P_0 conditions.

Discussion

Between 30% and 90% of seed P occurs in the form of phytate (White and Veneklaas, 2012). The present results found that phytic acid P varied from 44-90% of total P in the seeds of different genotypes. Phytic acid content is known to be affected by genotype, climatic factors, and location in rice and wheat (Brankovic et al., 2015; Singh et al., 2012). The present study observed a significant effect of genotype and P application on phytic acid P. Phytate concentration in seeds has been reported to be positively correlated with

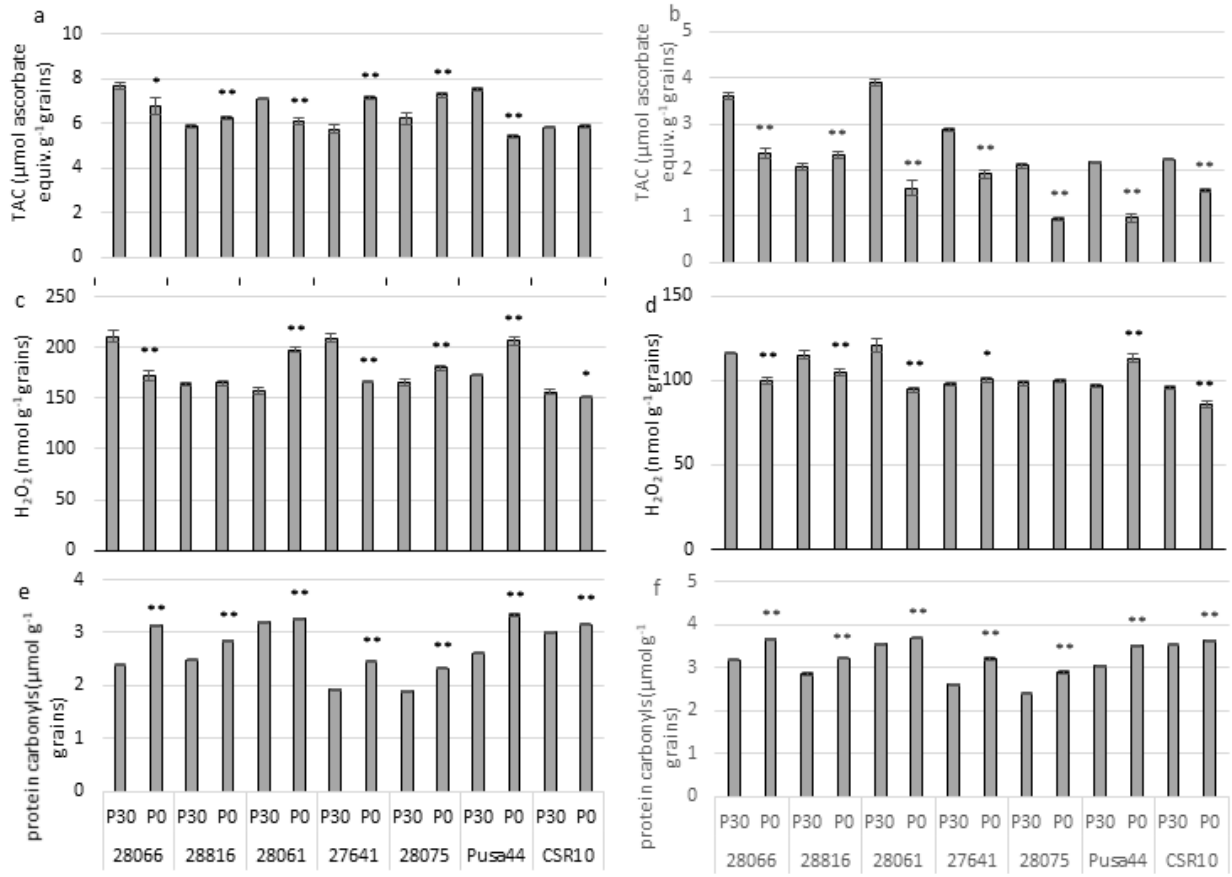

Fig. V. Total antioxidant capacity, H_2O_2, and protein carbonyls in grains of the genotypes under study

applied P levels in different plant species (Taliman et al., 2019). The present study found decreased phytic acid P in the seeds of all genotypes while total P decreased only in the seeds of three out of seven genotypes under P_0 conditions.

Enhanced root biomass under P_0 in IET28066, IET28816, and CSR10 suggests that these genotypes may have explored more soil for P under P_0. However, the exploration of root growth may have a negative effect, as the production and maintenance of the root system require energy. Root metabolic cost is an important parameter determining plant growth under low P availability (Dissanayaka et al., 2017; Dissanayaka et al., 2018; Lynch et al., 2005). In the present study, root biomass was enhanced only in zones while total root biomass remained unaltered under P_0 in CSR10 and IET28816. The effect of low P was more prominent on shoot biomass production compared to roots in rice (Kumar et al., 2021). Similarly, in the present study, genotypes varied largely in shoot biomass at 60 DAT of P_0 where tolerant genotypes CSR10/IET28816 maintained high shoot biomass compared to others. Pusa44 showed the highest reduction of shoot biomass at 30 DAT of P_0.

The present study showed that acid phosphatase of shoots might be involved in P remobilization, thus being increased more by genotypes poor in P-uptake. However, acid phosphatases of roots are related to P uptake and adaptation of the genotype towards P deficit. Tolerance to low P is related to enhanced P acquisition rather than to efficient P remobilization in rice lines contrasting in tolerance to P deficiency (Pariasca-Tanaka et al., 2009). In *Arabidopsis thaliana*, just over 50% of phosphatase genes were upregulated in shoots whereas about 80% were upregulated in roots under P deficiency(Misson et al., 2005; Morcuende et al., 2007). Kumar et al., 2021 found that increased acid phosphatase was more prominent in roots of P deficiency tolerant rice

genotypes compared to P deficiency susceptible genotypes. Timely regulation of acid phosphatases of roots under low P can also be important. Acid phosphatases of roots declined by 60DAT in less tolerant genotypes while maintained by tolerant genotypes under P_0, and so was the P uptake. Li et al. (Li et al., 2010)found that acid phosphatases of roots continued expression from 24 to 72 h in low P tolerant rice while less tolerant genotypes increased expression at 24 h only under P deficit.

Grains of rice plants grown under P_0 conditions showed high oxidative stress in the form of ROS and protein oxidation compared to plants grown under P_{30} conditions. Among genotypes, the antioxidant capacity of grains was related to P uptake by the plant at its vegetative stage under P_0 conditions. Therefore, rice plants might be stressed under P_0 conditions compared to P_{30} conditions during the growth period and thus produced oxidatively stressed seeds.

Conclusion

The present study concluded that the non-application of phosphorus affects vegetative growth, grain yield, and grain quality in rice. However, the selection of genotype can help to reduce such losses. CSR10 and IET28816 can be more adapted to phosphorus non application compared to other genotypes. Under phosphorus non application, P uptake by the plant determines vegetative growth, grain yield, and grain quality and partly determines grain P content. P uptake by the plant was related to root growth and acid phosphatases (soluble and cell wall) of roots under phosphorus non-application; therefore, these markers can be used to select genotypes with high P acquisition. Sustained P uptake by the plant may also be important for tolerance against low P conditions.

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References

Ames, B. N. 1966. [10] Assay of inorganic phosphate, total phosphate and phosphatases. In *Methods in enzymology*, 8:115-118: Elsevier. Number of 115-118 pp.

Aziz, T., M. Sabir, M. Farooq, M. A. Maqsood, H. R. Ahmad and E. A. Warraich. 2014. Phosphorus deficiency in plants: responses, adaptive mechanisms, and signaling. *Plant signaling: Understanding the molecular crosstalk,* 133-148.

Belgaroui, N., B. Lacombe, H. Rouached and M. Hanin. 2018. Phytase overexpression in Arabidopsis improves plant growth under osmotic stress and in combination with phosphate deficiency. *Scientific Reports,* 8, (1) 1137.

Brankovic, G., V. Dragičević, D. Dodig, M. Zoric and S. Denčić. 2015. Genotype x environment interaction for antioxidants and phytic acid contents in bread and durum wheat as influenced by climate. *Chilean journal of agricultural research,* 75, (2) 139-146.

Dissanayaka, D., M. Ghahremani, M. Siebers, J. Wasaki and W. C. Plaxton. 2021. Recent insights into the metabolic adaptations of phosphorus-deprived plants. *Journal of Experimental Botany,* 72, (2) 199-223.

Dissanayaka, D., H. Maruyama, S. Nishida, K. Tawaraya and J. Wasaki. 2017. Landrace of japonica rice, Akamai exhibits enhanced root growth and efficient leaf phosphorus remobilization in response to limited phosphorus availability. *Plant and Soil,* 414, 327-338.

Dissanayaka, D., W. C. Plaxton, H. Lambers, M. Siebers, B. Marambe and J. Wasaki. 2018. Molecular mechanisms underpinning phosphorus-use efficiency in rice. *Plant, Cell & Environment,* 41, (7) 1483-1496.

Gill, S. S. and N. Tuteja. 2010. Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. *Plant physiology and biochemistry,* 48, (12) 909-930.

Han, Y., P. J. White and L. Cheng. 2022. Mechanisms for improving phosphorus utilization efficiency in plants. *Annals of botany,* 129, (3) 247-258.

Hernández, I. and S. Munné-Bosch. 2015. Linking phosphorus availability with photo-oxidative stress in plants. *Journal of experimental botany,* 66, (10) 2889-2900.

Jeong, K., C. C. Julia, D. L. Waters, O. Pantoja, M. Wissuwa, S. Heuer, L. Liu and T. J. Rose. 2017. Remobilisation of phosphorus fractions in rice flag leaves during grain filling: implications for photosynthesis and grain yields. *PloS one,* 12, (11) e0187521.

Kaur, L., A. K. Gupta and V. K. Zhawar. 2014a. ABA improvement of antioxidant metabolism under water stress in two wheat cultivars contrasting in drought tolerance. *Indian Journal of Plant Physiology,* 19, 189-196.

Kaur, M., A. K. Gupta and V. K. Zhawar. 2014b. Antioxidant response and Lea genes expression under exogenous ABA and water deficit stress in wheat cultivars contrasting in drought tolerance. *Journal of Plant Biochemistry and Biotechnology,* 23, 18-30.

Kumar, S., Pallavi, C. Chugh, K. Seem, S. Kumar, K. Vinod and T. Mohapatra. 2021. Characterization of contrasting rice (Oryza sativa L.) genotypes reveals the Pi-efficient schema for phosphate starvation tolerance. *BMC plant biology,* 21, 1-26.

Lambers, H. 2022. Phosphorus acquisition and utilization in plants. *Annual Review of Plant Biology,* 73, 17-42.

Li, L., C. Liu and X. Lian. 2010. Gene expression profiles in rice roots under low phosphorus stress. *Plant molecular biology,* 72, 423-432.

Liu, L., D. Yang, B. Xing, H. Zhang and Z. Liang. 2018. Salvia castanea hairy roots are more tolerant to phosphate deficiency than Salvia miltiorrhiza hairy roots based on the secondary metabolism and antioxidant defenses. *Molecules,* 23, (5) 1132.

Lynch, J. P., M. D. Ho and L. Phosphorus. 2005. Rhizoeconomics: carbon costs of phosphorus acquisition. *Plant and soil,* 269, 45-56.

Misson, J., K. G. Raghothama, A. Jain, J. Jouhet, M. A. Block, R. Bligny, P. Ortet, A. Creff, S. Somerville and N. Rolland. 2005. A genome-wide transcriptional analysis using Arabidopsis thaliana Affymetrix gene chips determined plant responses to phosphate deprivation. *Proceedings of the National Academy of Sciences,* 102, (33) 11934-11939.

Miura, K., A. Sato, M. Ohta and J. Furukawa. 2011. Increased tolerance to salt stress in the phosphate-accumulating Arabidopsis mutants siz1 and pho2. *Planta,* 234, 1191-1199.

Morcuende, R., R. Bari, Y. Gibon, W. Zheng, B. D. Pant, O. Bläsing, B. Usadel, T. Czechowski, M. K. Udvardi and M. Stitt. 2007. Genome-wide reprogramming of metabolism and regulatory networks of Arabidopsis in response to phosphorus. *Plant, cell & environment,* 30, (1) 85-112.

Müller, J., V. Gödde, K. Niehaus and C. Zörb. 2015. Metabolic adaptations of white lupin roots and shoots under phosphorus deficiency. *Frontiers in plant science,* 6, 1014.

Pariasca-Tanaka, J., K. Satoh, T. Rose, R. Mauleon and M. Wissuwa. 2009. Stress response versus stress tolerance: a transcriptome analysis of two rice lines contrasting in tolerance to phosphorus deficiency. *Rice,* 2, 167-185.

Parra-Almuna, L., A. Diaz-Cortez, N. Ferrol and M. De La Luz Mora. 2018. Aluminium toxicity and phosphate deficiency activates antioxidant systems and up-regulates expression of phosphate transporters gene in ryegrass (Lolium perenne L.) plants. *Plant Physiology and Biochemistry,* 130, 445-454.

Plaxton, W. C. and H. T. Tran. 2011. Metabolic adaptations of phosphate-starved plants. *Plant physiology,* 156, (3) 1006-1015.

Prieto, P., M. Pineda and M. Aguilar. 1999. Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: specific application to the determination of vitamin E. *Analytical biochemistry,* 269, (2) 337-341.

Rose, T. J., S. M. Impa, M. T. Rose, J. Pariasca-Tanaka, A. Mori, S. Heuer, S. Johnson-Beebout and M. Wissuwa. 2013. Enhancing phosphorus and zinc acquisition efficiency in rice: a critical review of root traits and their potential utility in rice breeding. *Annals of botany,* 112, (2) 331-345.

Shane, M. W., K. Stigter, E. T. Fedosejevs and W. C. Plaxton. 2014. Senescence-inducible cell wall and intracellular purple acid phosphatases: implications for phosphorus remobilization in Hakea prostrata (Proteaceae) and Arabidopsis thaliana (Brassicaceae). *Journal of experimental botany,* 65, (20) 6097-6106.

Singh, S., A. K. Gupta and N. Kaur. 2012. Influence of drought and sowing time on protein composition, antinutrients, and mineral contents of wheat. *The Scientific World Journal,* 2012,

Singh, S. K., J. Y. Barnaby, V. R. Reddy and R. C. Sicher. 2016. Varying response of the concentration and yield of soybean seed mineral elements, carbohydrates, organic acids, amino acids, protein, and oil to phosphorus starvation and C enrichment. *Frontiers in Plant Science,* 7, 1967.

Stigter, K. A. and W. C. Plaxton. 2015. Molecular mechanisms of phosphorus metabolism and transport during leaf senescence. *Plants,* 4, (4) 773-798.

Taliman, N. A., Q. Dong, K. Echigo, V. Raboy and H. Saneoka. 2019. Effect of phosphorus fertilization on the growth, photosynthesis, nitrogen fixation, mineral accumulation, seed yield, and seed quality of a soybean low-phytate line. *Plants,* 8, (5) 119.

Veneklaas, E. J., H. Lambers, J. Bragg, P. M. Finnegan, C. E. Lovelock, W. C. Plaxton, C. A. Price, W. R. Scheible, M. W. Shane and P. J. White. 2012. Opportunities for improving phosphorus-use efficiency in crop plants. *New phytologist,* 195, (2) 306-320.

Wang, Y., F. Wang, H. Lu, Y. Liu and C. Mao. 2021. Phosphate uptake and transport in plants: an elaborate regulatory system. *Plant and Cell Physiology,* 62, (4) 564-572.

White, P. J. and E. J. Veneklaas. 2012. Nature and nurture: the importance of seed phosphorus content. *Plant and soil,* 357, 1-8.

Yang, L.-T., H.-X. Jiang, Y.-P. Qi and L.-S. Chen. 2012. Differential expression of genes involved in alternative glycolytic pathways, phosphorus scavenging and recycling in response to aluminum and phosphorus interactions in Citrus roots. *Molecular Biology Reports,* 39, 6353-6366.

Zemel, M. B. and L. A. Shelef. 1982. Phytic acid hydrolysis and soluble zinc and iron in whole wheat bread as affected by calcium containing additives. *Journal of Food Science,* 47, (2) 535-537.

Zhang, Z.-W., X.-Y. Yang, X.-J. Zheng, Y.-F. Fu, T. Lan, X.-Y. Tang, C.-Q. Wang, G.-D. Chen, J. Zeng and S. Yuan. 2020. Vitamin E is superior to vitamin c in delaying seedling senescence and improving resistance in Arabidopsis deficient in macro-elements. *International journal of molecular sciences,* 21, (19) 7429.