

Influence of low phosphorus concentration on leaf photosynthetic characteristics and antioxidant response of rice genotypes

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Abstract

Influence of different phosphorus concentrations was studied in four rice varieties (Akhanphou, MTU1010, RP BIO 226, and Swarna) differing in their tolerance to low phosphorus. There was an increase in shoot and root dry mass with the increase in phosphorus concentration. At the low phosphorus concentration at both tillering and reproductive stages, Swarna, followed by Akhanphou, recorded maximum biomass for both roots and shoots, while the minimum was observed in RP BIO 226. Reduction in photosynthetic rate, stomatal conductance, transpiration rate, and internal CO₂ concentration at low phosphorus concentrations were observed at both tillering and reproductive stages in all the genotypes. In low phosphorus, maximum photosynthetic rate was found in Swarna followed by Akhanphou. Phosphorus deficiency did not alter the maximum efficiency of PSII photochemistry, however, there was a reduction in effective PSII quantum yield, electron transport rate, and coefficient of photochemical quenching, while the coefficient of nonphotochemical quenching was higher in the low phosphorus-treated plants. Prolonged exposure to excessive energy and failure to utilize the energy in carbon-reduction cycle induced the generation of reactive oxygen species, which affected PSII as indicated by the fluorescence traits. The reduction was less severe in case of Swarna and Akhanphou. The activities of superoxide dismutase, peroxidase, and catalase increased in roots under low phosphorus concentration indicating that photoprotective mechanisms have been initiated in rice plants in response to phosphorus deficiency. Comparatively, Swarna and Akhanphou exhibited a higher biomass, higher photosynthetic rate, and better reactive oxygen species-scavenging ability which conferred tolerance under low phosphorus conditions.

Additional key words: antioxidants; chlorophyll fluorescence; gas exchange; phosphorus deficiency; rice.

Introduction

Rice (*Oryza sativa* L.) is the most important cereal crop in the world and is the staple food for more than 50% of the population. Global food security is at stake since the demand for rice is exceeding its production. The rapid population growth and economic development have been posing a growing pressure for increased rice production. Main limiting factor considered for the low crop yields across the world is lack of certain macronutrients or their imbalanced use (Sanchez 2000). Phosphorus (P) is essential for plants and all other forms of life as it is an

important constituent of molecules such as RNA, DNA, and cell membranes (Elser 2012). Among the macronutrients, phosphorus is one of the essential inputs for rice production. Plant function and productivity are dependent on availability of P (Hell and Hillebrand 2001, Epstein and Bloom 2005). Phosphorus deficiency is a major abiotic stress and the most important nutritional constraints to rice growth across the globe (Ismail *et al.* 2007). Soils generally contain suboptimal concentrations of nutrients (Marschner 1995). P deficiency arises either in soils where

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Abbreviations: Chl – chlorophyll; CAT – catalase; C_i – intercellular CO₂ concentration; DM – dry mass; E – transpiration rate; ETR – electron transport rate; FM – fresh mass; F_v/F_m – maximum efficiency of PSII photochemistry; g_s – stomatal conductance; Pi – inorganic phosphorus; P_{leaf} – leaf phosphorus concentration; P_N – net photosynthetic rate; POD – peroxidase; q_N – coefficient of nonphotochemical quenching; q_P – coefficient of photochemical quenching; ROS – reactive oxygen species; SOD – superoxide dismutase; φ_{PSII} – effective PSII quantum yield.

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there is low absolute P content or where P is strongly bound to soil particles forming insoluble complexes resulting in its lesser mobility.

Phosphorus deficiency in plants strongly limits a tiller number, yield, biomass accumulation, reduces plant leaf area, induces photoinhibition and damage to PSII, and alters the biochemical metabolic pathways (Chaudhary *et al.* 2008). It also affects carbon metabolism and various aspects of photosynthesis (Xu *et al.* 2007), hence decreasing the photosynthetic efficiency. Partitioning and transport of photosynthates are also inhibited. Rice is a C₃ plant and reports revealed that the growth of C₃ species was more affected by inorganic phosphorus (Pi) supply and the photosynthetic rates are affected by the concentrations of Pi in the leaf (Stitt and Quick 1989). Low Pi concentration in the cytosol has a negative effect on the Calvin cycle (Heldt *et al.* 1977). Reports revealed that P deficiency reduced the photosynthetic efficiency in soybean (Lauer *et al.* 1989), barley, and spinach (Foyer and Spencer 1986) as well as in wheat (Rodríguez *et al.* 1998).

Phosphate deficiency was shown to decrease photosynthetic oxygen evolution by leaves, the efficiency of PSII photochemistry and quantum efficiency of PSII, and electron transport. Reactive oxygen species (ROS) are

Materials and methods

Growth conditions: A pot culture experiment was performed at ICAR-Indian Institute of Rice Research, Hyderabad, during a wet season (July to November) with the aim to investigate the influence of different phosphorus concentrations on four rice genotypes (Akhanphou, MTU1010, RP BIO 226, and Swarna). Phosphorus-deficient soil was collected from phosphate-deficient plot created at ICAR-Indian Institute of Rice Research, where rice was grown in both wet and dry seasons continuously for over 10 years without the addition of phosphate fertilizer. The soil had a pH of 7.6. The available nitrogen was 220 kg ha⁻¹, phosphorus of 1.8 kg ha⁻¹, and potassium of 402 kg ha⁻¹. Seeds were surface-sterilized and sown on the same day (16 July) in earthen pots with volume of 12.5 L. After 7 d of growth, the seedlings were transplanted in pots of a volume of 15 L filled with soil collected from phosphorus-deficient plot and recommended doses of nitrogen and potash (100 and 40 kg ha⁻¹, respectively) were added. Graded concentrations of phosphorus [0, 15, 30, 45, and 60 kg(P₂O₅) ha⁻¹] were added by the addition of appropriate amount of single superphosphate (SSP). The highest SSP treatment of 60 kg ha⁻¹ was considered as the control. Each genotype was planted in four separate pots for each phosphorus concentration. Two seedlings were allowed to grow per pot. In all the pots, 4 cm ponding of water was maintained. Need-based irrigation was provided. The pots were maintained under natural conditions in a net house. During the experimental period (sowing to harvest), the minimum and maximum temperature was 21.2 and 30.7°C, respectively. Minimum

and maximum relative humidity was 57.5 and 84.1%, respectively. The average rainfall was 120 mm. Physiological observations were recorded at the same phenological stages (tillering stage and four days after anthesis). The data pertaining to days to 50% flowering and days to maturity for the rice cultivars is given in Table 1S (*supplement available online*).

released from normal physiological processes and are important in regulation of signal transduction pathways by modulating ion channel activity (Pei *et al.* 2000, Mustilli *et al.* 2002, Neill *et al.* 2002). But when the plants are exposed to P deficiency, there is an increased ROS production, *i.e.*, O², O²⁻, H₂O₂, and OH⁻, which are cytotoxic and seriously disrupt normal metabolism through oxidative damage of lipids, proteins, and nucleic acids (McKersie and Leshem 1994) leading to oxidative stress. In order to eliminate effectively stress-induced accumulation of toxic reactive oxygen intermediates, plants employ antioxidant defence systems (Gill and Tuteja 2010) consisting of low-molecular-mass antioxidants (ascorbate, carotenoids, α-tocopherol, and flavonoids) and protective enzymes [superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT)]. In the root tissues, the activities of scavenging enzymes, *i.e.*, SOD, POD, and CAT were found to be affected by phosphate deficiency (Juszczuk *et al.* 2001).

The present investigation was conducted to investigate the influence of low phosphorus concentrations on the leaf photosynthetic characteristics and antioxidant response of selected rice varieties differing in their tolerance to suboptimal contents of P.

Shoot and root dry mass: At tillering stage and at reproductive stage, three plants from each treatment and from each variety were uprooted and separated into roots and shoots, dried at 80°C for 48 h, and the dry mass (DM) was recorded and expressed in g per plant.

Leaf gas-exchange: Photosynthetic traits [net photosynthetic rate (P_N), transpiration rate (E), stomatal conductance (g_s), and intercellular CO₂ concentration (C_i)] were measured using an infrared gas analyser (IRGA) LI6400XT photosynthesis measurement system attached to leaf chamber fluorometer (LCF Model 6400-1, LICOR, USA), which was used as an artificial light source. During measurements the PAR was kept at 1,200 μmol(photon) m⁻² s⁻¹ and CO₂ concentration at 387 ± 6 ppm. Gas-exchange measurements were made at the tillering stage (50 d after sowing) on a matured leaf and 4 d after anthesis (reproductive stage) on flag leaf in triplicate. Plants growing at zero P concentration could not flower and in general, their growth was very poor.

Chlorophyll (Chl) fluorescence was measured 50 d after

emergence with a portable fluorometer (*PAM-210*, Walz, Effeltrich, Germany) at maximum tillering stage and the following fluorescence parameters were calculated: the maximum efficiency of PSII photochemistry (F_v/F_m), the effective PSII quantum yield (ϕ_{PSII}), the electron transport rate (ETR), coefficient of photochemical quenching (q_P), and coefficient of nonphotochemical quenching (q_N) in dark-adapted leaf (Maxwell and Johnson 2000).

Assay of antioxidant enzyme activities (SOD, POD, and CAT): The activity of important ROS-scavenging enzymes were estimated four days after anthesis at the reproductive stage. Plants grown at 0 P did not flower and many plants could not survive beyond the vegetative stage. Hence the activities of enzymes were estimated only in plants which received 15, 30, 45, and 60 kg(P₂O₅) ha⁻¹. Activities were assayed spectrophotometrically and absorbances were recorded by double beam UV-VIS spectrophotometer (*Spectrascan UV 2600*, Chemito, India).

Preparation of enzyme extract: Enzymes (SOD, POD, and CAT) were extracted by first freezing the weighed amount of leaf or root samples (0.2 g) in liquid nitrogen to prevent proteolytic activity followed by grinding with 5 ml of extraction buffer (0.1 M phosphate buffer, pH 7.5, containing 0.5 mM EDTA). The enzyme extract was centrifuged for 20 min at 15,000 rpm and the supernatant was used.

Superoxide dismutase (SOD) activity (EC 1.15.1.1): The SOD activity was measured according to Dhindsa *et al.* (1981). The 3 ml of reaction mixture consisted of methionine (200 mM), nitroblue tetrazolium chloride (NBT) (2.25 mM), EDTA (3.0 mM), riboflavin (60 µM), sodium carbonate (1.5 M), phosphate buffer (100 mM, pH 7.8). The absorbance was recorded at 560 nm and the enzyme activity was expressed in [unit min⁻¹ g⁻¹(FM)]. One unit of activity is the amount of enzyme required to inhibit 50% initial reduction of NBT under light.

Peroxidase (POD) activity (EC 1.11.1.7): Peroxidase assay was carried out according to Castillo *et al.* (1984). The 3 ml of assay mixture consisted of 1.0 ml phosphate buffer (pH 6.1), 0.5 ml of guaiacol, 0.5 ml of H₂O₂, 0.1 ml of enzyme extract, and 0.9 ml of water. Increase in the

absorbance due to formation of tetraguaicol was recorded at 470 nm up to 3 min and it was expressed in µmol(guaiacol reduced) min⁻¹ g⁻¹(FM).

Catalase (CAT) activity (EC 1.11.1.6): The same leaf extract, prepared for SOD assay was used for CAT assay. The CAT activity was measured according to Aebi (1984). The assay mixture of 3 ml consisted of 0.05 ml of leaf extract, 1.5 ml of phosphate buffer (100 mM buffer, pH 7.0), 0.5 ml of H₂O₂, and 0.95 ml of distilled water. Decrease in the absorbance was recorded at 240 nm up to 3 min and it was expressed in µmol(H₂O₂ oxidised) min⁻¹ g⁻¹(FM).

Phosphorus concentration in leaves and roots at the tillering stage was estimated by following vanado-molybdate phosphoric yellow colour method (Jackson 1967). Fine-powdered sample (leaf or root) of 0.5 g was taken and digested with 5 ml of triacid mixture (HNO₃ + HClO₄ + H₂SO₄, 10:4:1) until milky colour was obtained. After this, the content was passed through *Whatman no. 42* filter paper into a 50-ml volumetric flask and the volume was made up to 50 ml with distilled water. From this, 5 ml was taken into 25-ml volumetric flask to which 5 ml of Barton's reagent was added and volume was made up to 25 ml with distilled water. After 15–30 min, absorbance was recorded at 420 nm in double beam UV-VIS spectrophotometer (*Spectrascan UV 2600*, Chemito, India). Phosphorus concentration was calculated by taking KH₂PO₄ as standard and it was expressed in mg g⁻¹(DM).

A regression analysis was done between the P concentration of leaf and photosynthetic rate at tillering stage.

Grain yield: At physiological maturity, panicles from each treatments were harvested, sun dried, threshed, cleaned, and mass of grains was recorded and expressed in g per plant.

Statistical analysis: Two way analysis of variance (ANOVA) was performed using an open source software *R* (*R Core Team* 2012) with *Agricolae* package (de Mendiburu 2012). Statistical significance of the parameter means were determined by performing Fisher's LSD test to test the statistical significance.

Results

Shoot and root dry mass: Phosphorus application led to a significant increase in the shoot and root DM at both tillering and reproductive stages. Maximum shoot and root DM were noted at 60 kg(P) ha⁻¹ at both stages, while minimum DM were obtained without P at the tillering stage and at 15 kg(P) ha⁻¹ at the reproductive stage. Among the varieties, maximum shoot DM at 0 P at the tillering and 15 kg(P) ha⁻¹ at reproductive stages was found in Swarna followed by Akhanphou, and minimum in RP BIO 226 at

both the stages (Table 2S, *supplement available online*). At 60 kg(P) ha⁻¹, at the tillering and reproductive stage, maximum shoot DM was in Swarna and Akhanphou, respectively, and minimum in MTU1010 at the tillering stage and in RP BIO 226 at the reproductive stage.

Similarly, the root DM reached maximum under low P in Swarna at both the stages at 0 P and 15 kg(P) ha⁻¹ and at the tillering stage at 60 kg(P) ha⁻¹, and in Akhanphou at the reproductive stage (Table 3S, *supplement available*

online). The difference between remaining varieties were under 0 P in the tillering stage. Minimum root DM was found in RP BIO 226 at the reproductive stage under lower P [15 kg(P) ha⁻¹] and in control at both the stages. The maximum reduction in shoot and root biomass at the reproductive stage was observed in RP BIO 226 (80 and 98%, respectively). Minimum reduction in shoot biomass was noted in MTU1010 (65.0%) and Swarna (65.7%) which were on par and maximum reduction was observed in root biomass was in Swarna (75%).

Leaf gas exchange: A significant reduction in gas-exchange traits, such as P_N , g_s , E , and C_i at low P (0 and 15 kg ha⁻¹) in comparison with the control plants, which received recommended concentration of P (60 kg ha⁻¹) was observed. Interaction between the P concentrations and the genotypes was also significant with respect to P_N . At the tillering stage (Fig. 1A), under zero P and control [60 kg(P) ha⁻¹], P_N was the highest in Swarna. At the reproductive stage (Fig. 1B), the highest and lowest P_N were found in Akhanphou and MTU1010 under low P (15 kg ha⁻¹) and 60 kg(P) ha⁻¹. Minimum reduction in P_N from control to low P at both the stages was evident in Swarna. At the tillering stage and reproductive stage, the mean maximum g_s and E was recorded at 45 kg(P) ha⁻¹ and minimum at low P [0 P at tillering stage and 15 kg(P) ha⁻¹ at reproductive stage]. At the tillering stage, under low P (0), the highest and least g_s was noted in Swarna and Akhanphou, respectively (Fig. 1C). In control, maximum g_s was observed in Akhanphou and minimum in MTU1010. At the reproductive stage, maximum g_s under low P (15 kg ha⁻¹) and control was found in MTU1010 (Fig. 1D). The interaction between varieties and P concentrations was not significant. The relationship between leaf P concentration and leaf photosynthetic efficiency was studied by simple linear regression analysis (Fig. 2). A strong positive association was observed between P concentration and P_N

for all the tested varieties.

Varietal differences for mean E and interaction between varieties and P concentrations were not significant at the tillering stage, but were significant at the reproductive stage. At the tillering stage, at low P (0) and control, maximum E was seen in Swarna and lower in Akhanphou (Fig. 3A). At the reproductive stage low P (15 kg ha⁻¹) and control, MTU1010 showed the maximum value, while minimum at low P (15 kg ha⁻¹) was observed in RP BIO 226 (Fig. 3B).

At the tillering (Fig. 3C) and reproductive stages (Fig. 3D), maximum C_i was found in control and at 45 kg(P) ha⁻¹, whereas minimum was at low P (0 and 15 kg ha⁻¹, respectively). Differences between the varieties as well and interaction between treatment and varieties was found to be insignificant at both stages.

Chl fluorescence emission is linked to the photosynthetic apparatus and such measurements can be used to monitor and quantify the response of plants to biotic and abiotic stresses. Phosphorus deficiency had no significant effect on the maximum efficiency of PSII photochemistry (F_v/F_m) in all the tested varieties (Fig. 4A). However, significant reduction in the quantum yield of PSII (ϕ_{PSII}) was observed under 0 P and 15 kg(P) ha⁻¹ compared with the control plants which received 60 kg(P) ha⁻¹. Significant differences were observed between the genotypes and the reduction was comparatively higher in RP BIO 226 and MTU 1010 than that of Swarna and Akhanphou. However, the difference observed between the remaining treatments was insignificant (Fig. 4B). The apparent electron transport rate (ETR) was significantly reduced under 0 P treatment. Significant increase in ETR value was observed in plants which received 15 kg(P) ha⁻¹ and the difference between the remaining treatments was not significant indicating that provision of low P (15 kg ha⁻¹) was sufficient to restore the ETR in rice. Significant differences

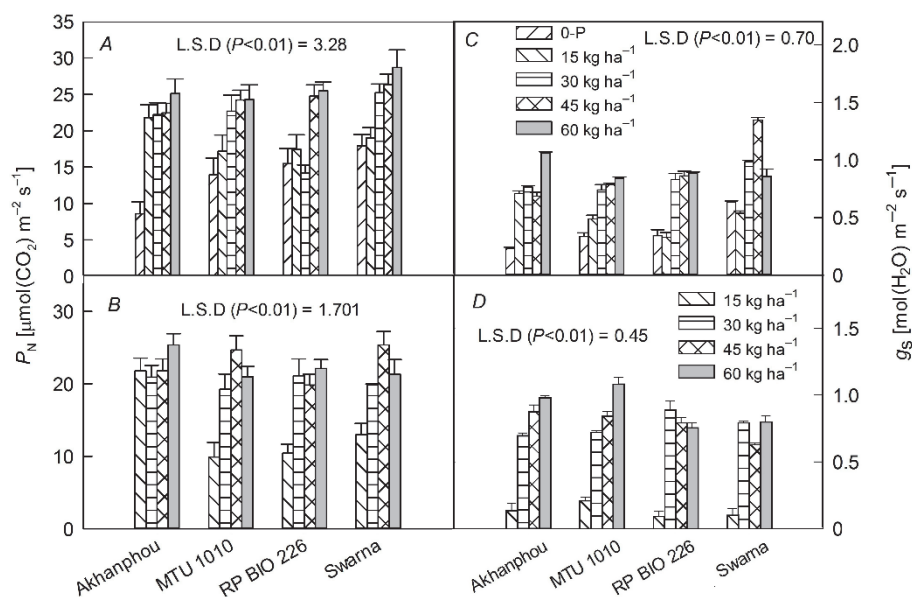


Fig. 1. Influence of phosphorus concentration on (A) net photosynthetic rate (P_N) at tillering (50 days after sowing), (B) at reproductive stage (four days after anthesis), (C) stomatal conductance (g_s) at tillering, and (D) at reproductive stage in four rice varieties. Each bar represents the mean of three replications \pm SD.

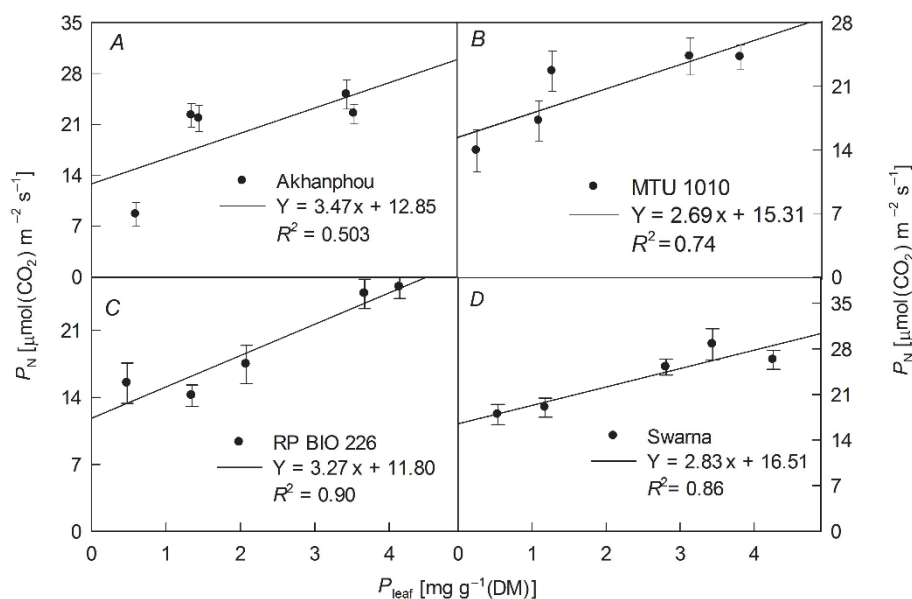


Fig. 2. Relationship between leaf phosphorus concentration (P_{leaf}) and rate of photosynthesis (P_N) in (A) Akhanphou, (B) MTU1010, (C) RP BIO 226, and (D) Swarna at tillering. Each value represents the mean of three replications \pm SD.

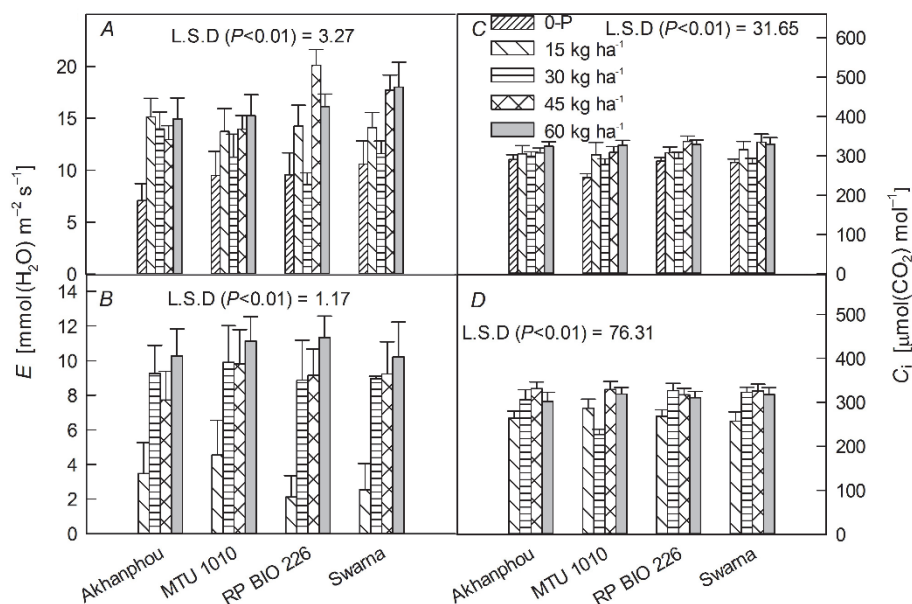


Fig. 3. Influence of phosphorus concentration on (A) transpiration (E) at tillering, (B) at reproductive stage, (C) intercellular CO_2 concentration (C_i) at tillering, and (D) at reproductive stage in four rice varieties. Each bar represents the mean of three replications \pm SD.

were observed between the tested genotypes. Although the ETR was reduced under 0 P treatment, the reduction was higher in RP BIO 226 and MTU 1010 than that in Swarna and Akhanphou (Fig. 4C). The coefficient of photochemical quenching (q_p) was not affected by low P treatments. The differences observed in the q_p between the varieties or between different P treatments was insignificant (Fig. 5A). However, the coefficient of nonphotochemical quenching (q_N) was higher under the low P (0 and 15 kg ha⁻¹)-treated plants (Fig. 5B). The q_N was lower at 30, 40, and 60 kg(P) ha⁻¹. No significant differences were noticed between the tested genotypes for their response to P application.

Antioxidant enzyme activity: Activities of some important enzymes, such as SOD, CAT, and POD, involved in

the metabolism of ROS, were measured in leaf and root tissues at the reproductive stage only. Plants grown under 0 P could not survive beyond the vegetative stage.

Significant increase in the activities of root SOD, POD, and CAT activity under low P condition (15 kg ha⁻¹) suggested that photoprotective mechanisms were initiated in rice in response to P deficiency. Compared to the leaves, the antioxidant activities were higher in roots with low P. With increasing P concentration, there was a gradient reduction in all the antioxidant enzyme activities in roots, while they conversely increased in leaves.

Under low P, the highest root SOD activity was noted in MTU1010 (5.8 folds higher than that of control) and the least activity was observed in RP BIO 226 (2.19 folds higher than that of control) (Table 1). RP BIO 226 exhibited an elevated POD activity compared to other

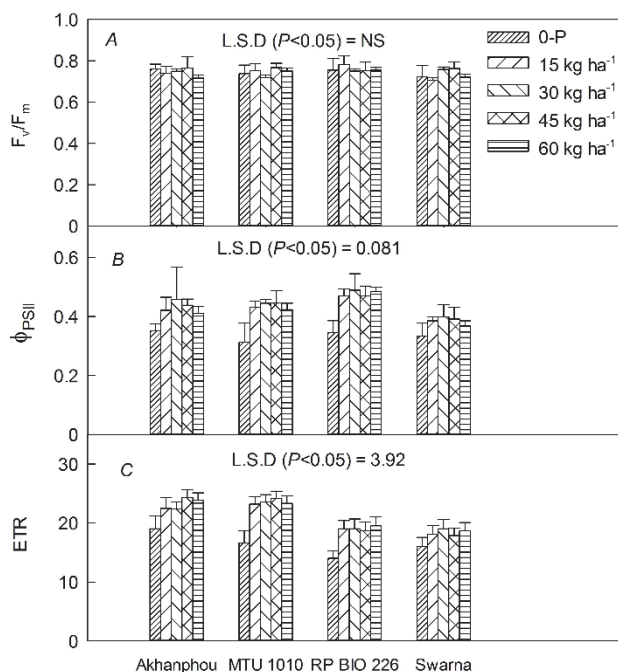


Fig. 4. Influence of phosphorus application rate on (A) maximum efficiency of PSII photochemistry (F_v/F_m), (B) effective PSII quantum yield (ϕ_{PSII}), and (C) electron transport rate (ETR). The fluorescence traits were measured at tillering stage (50 days after sowing). Each bar represents the mean of three replications \pm SD.

varieties (Table 2) under low P, which was 2.4 folds higher than that of control, and the least activity was observed in Swarna (1.36 folds higher than that of control). The highest root CAT activity was noted in Swarna (3.8 folds higher) and the least one in RP BIO 226 (1.2 folds higher than that of control) (Table 3). Under low P, Swarna showed the highest leaf SOD and leaf POD activity, but the least leaf CAT activity. MTU1010 recorded maximum leaf CAT and minimum leaf SOD activity.

Discussion

Increase in shoot and root DM with application of P clearly supported the fact that P has a crucial role in improving plant development and hence the parameters could be also used as a screening parameters under low P. The reduction in shoot biomass under low P occurred because of reduced net photosynthesis and the reduction in root biomass was attributed to the reduction in absolute root growth under P deficiency in rice (Wissuwa *et al.* 2005). Higher values for biomass of shoot and root at low P concentration indicate P tolerance (Panigrahy *et al.* 2014). Our data revealed higher shoot mass under low P contents in Swarna and Akhanphou which indicated that these genotypes are relatively tolerant to low P; conversely the lowest shoot and root DM at low P content indicated the susceptible nature of RP BIO 226. The grain yield was recorded after the harvest and the data was presented in Table 5S (*supplement available online*). The plants, which received 0 kg(P)

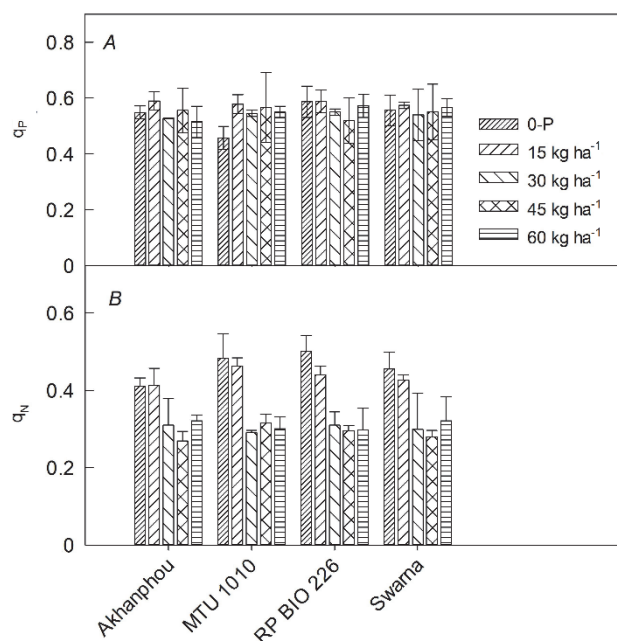


Fig. 5. Influence of phosphorus application rate on (A) coefficient of photochemical quenching (q_p) and (B) coefficient of nonphotochemical quenching (q_N). The fluorescence traits were measured at tillering stage (50 days after sowing). Each bar represents the mean of three replications \pm SD.

Leaf and root P concentrations: Increase in P contents resulted in an increase in P concentration in both leaves and roots. At 0 kg(P) ha⁻¹, maximum leaf and root P concentrations were noted in both Akhanphou and Swarna (Table 4S, *supplement available online*) at the tillering stage.

ha⁻¹, did not survive until the reproductive stage. At the lowest P concentration (15 kg ha⁻¹), Swarna produced the highest grain yield followed by Akhanphou and MTU1010, indicating that these varieties are tolerant to low P. The increase in P concentration resulted in an increase of the grain yield in all the tested varieties (Table 5S).

Phosphorus deficiency reduces the P_N and g_s (Ghannoum and Conroy 2007), thereby restricting the plant growth. Leaf area, mesophyll conductance (g_m), g_s , and ETR the main determinants of carbon assimilation (Tardieu 2005, Flexas *et al.* 2008, Galle *et al.* 2009), and leaf area and g_s also determine transpiration by plants (Tardieu 2005). The reduction of P_N in our experiment under P deficiency might occur because of reduction in the rate of ribulose-1,5-bisphosphate (RuBP) regeneration rather than due to the effect on Rubisco activation (Rao

Table 1. Influence of phosphorus application rate on superoxide dismutase (SOD) activity [units min⁻¹ g⁻¹(FM)] activity in leaves and roots at reproductive stage. Each value represents mean of three replications. T – treatment; V – varieties.

Variety	SOD (leaf)						SOD (root)					
	P application rate [kg ha ⁻¹]											
	0	15	30	45	60	Mean	0	15	30	45	60	Mean
Akhanphou	-	2.97	4.27	5.19	6.65	3.82	-	4.97	2.47	2.96	2.12	2.50
MTU 1010	-	2.79	2.67	5.73	6.42	3.52	-	6.73	2.70	1.55	1.16	2.43
RP BIO 226	-	3.50	5.61	6.89	4.87	4.17	-	4.34	3.20	1.88	1.98	2.28
Swarna	-	3.63	4.73	6.00	4.99	3.87	-	4.74	3.02	4.71	2.09	2.91
Mean	-	3.22	4.32	5.95	5.73		-	5.20	2.85	2.77	1.84	
LSD (T)			0.62	(P<0.05)				0.54	(P<0.05)			
LSD (V)			0.56	(P<0.05)				0.49	(P<0.05)			
LSD (T×V)			1.25	(P<0.05)				1.09	(P<0.05)			

Table 2. Influence of phosphorus application rate on peroxidase (POD) [μmol(guaicol reduced) min⁻¹ g⁻¹(FM)] activity in leaves and roots at reproductive stage. Each value represents mean of three replications. T – treatment; V – varieties.

Variety	POD (leaf)						POD (root)					
	P application rate [kg ha ⁻¹]											
	0	15	30	45	60	Mean	0	15	30	45	60	Mean
Akhanphou	-	10.7	16.4	27.9	33.6	17.72	-	41.8	32.3	24.4	23.9	24.48
MTU 1010	-	19.7	44.1	43.6	24.7	26.42	-	36.2	42.6	20.5	20	23.86
RP BIO 226	-	18.7	49.8	39.3	32.7	28.1	-	43.2	21.3	24.3	17.4	21.24
Swarna	-	19.8	33.2	44.3	34.3	26.32	-	32	24	27.3	23.5	21.36
Mean	-	17.22	35.87	38.77	31.32		-	38.3	30.05	24.125	21.2	
LSD (T)			1.24	(P<0.05)				1.0889	(P<0.05)			
LSD (V)			1.11	(P<0.05)				0.9739	(P<0.05)			
LSD (T×V)			2.49	(P<0.05)				2.1778	(P<0.05)			

Table 3. Influence of P application rate on catalase (CAT) [μmol(H₂O₂ oxidized) min⁻¹ g⁻¹(FM)] activity in leaves and roots at reproductive stage. Each value represents mean of three replications. T – treatment; V – varieties.

Variety	CAT (leaf)						CAT (root)					
	P application rate [kg ha ⁻¹]											
	0	15	30	45	60	Mean	0	15	30	45	60	Mean
Akhanphou	-	13.40	11.80	9.90	12.90	9.60	-	3.10	2.70	1.20	1.00	1.60
MTU 1010	-	15.20	12.10	6.40	15.10	9.76	-	3.00	1.40	2.30	1.10	1.56
RP BIO 226	-	12.60	13.70	6.90	14.00	9.44	-	1.80	1.20	0.80	1.50	1.06
Swarna	-	11.00	9.30	8.80	11.20	8.06	-	3.50	1.80	1.80	0.90	1.60
Mean		13.05	11.73	8.00	13.30			2.85	1.78	1.53	1.13	
LSD (T)			1.44	(P<0.05)				0.81	(P<0.05)			
LSD (V)			1.29	(P<0.05)				0.72	(P<0.05)			
LSD (T×V)			2.89	(P<0.05)				1.61	(P<0.05)			

and Terry 1989) and also reduced triose phosphate export from chloroplast stroma to cytoplasm in exchange for Pi via the Pi translocator (Stitt and Quick 1989). Plants, which received suboptimal amounts of phosphorus, show reduced capacity for electron transport resulting in impaired production of NADPH and ATP (Lauer *et al.* 1989, Fredeen *et al.* 1989). P deficiency also resulted in the reduction of g_s , E , and C_i in all the genotypes which may be attributed to the stomatal limitation. Low P_N may be attributed to stomatal limitation as there was a reduction

in both g_s as well as C_i under low P, that also effected the E at both the stages. On an average, Swarna and Akhanphou had relatively higher P_N under low P which help the plant cope under low P. Similar genotypic differences in rice cultivars, differing in their low P tolerance, was reported by Li *et al.* (2006).

Based on detailed fluorescence study, Ripley *et al.* (2004) reported that *Sorghum* plants, which received adequate phosphorus nutrition, show higher PSII performance due to increased number of reaction centres;

these photosystems trapped energy more efficiently, showed higher electron transport efficiency, and dissipated less energy as heat or fluorescence. Under P deficiency, the F_v/F_m was not significantly affected in rice plants (Xu *et al.* 2007), *Phaseolus* plants (Lima *et al.* 1999), *Pharbitis nil*, and *Parthenocissus tricuspidata* (Xing and Wu 2014). In our study, also no significant reduction in F_v/F_m was discernible in the plants, which received low P, indicating that the PSII reaction centres were unaffected. In sugar beet plants grown hydroponically with different Pi supply, a significant (32%) reduction was observed in light-saturated photosynthesis. However, low P treatments had not altered significantly the thylakoid membrane composition and function with little effect on quantum yield and fluorescence traits (Abadia *et al.* 1987). Xu *et al.* (2007) reported that in rice plants prolonged (> 30 d) exposure to phosphorus deficiency significantly reduced ϕ_{PSII} , ETR, and q_p , but increased NPQ. We also observed a reduction of ϕ_{PSII} and ETR under 0 P treatment. The increase in NPQ indicates that the excitation energy can be dissipated as heat before reaching the reaction centres and nonphotochemical quenching is an indicator of an essential regulation and photoprotection mechanism against high-light stress (Xu *et al.* 2007, Lambrev *et al.* 2012).

ROS are the natural byproducts of metabolism and have important roles in cell signaling and homeostasis. During abiotic stress, a dramatic increase in ROS contents was observed with concomitant increase in activities of antioxidant enzymes, such as SOD, POD, and CAT. Under low P conditions, antioxidant enzyme activities increased to overcome the stress condition (Ons *et al.* 2012). SOD, CAT, and POD are considered as the key enzymes involved in the scavenging of ROS and thus maintaining homeostasis in the plant cells (Guo *et al.* 2005). A higher POD and CAT activity under low P stress revealed that the plants were undergoing stress. Results indicated a high activity of SOD, POD, and CAT under low P (15 kg ha^{-1}) as compared with control (60 kg ha^{-1}) in the rice roots, whereas there was an increase in the activities

in leaves (Guo *et al.* 2012). Increased SOD activity in roots under low P conditions indicated that free radicals generated due to stress were neutralized, resulting in high accumulation of H_2O_2 as SOD is involved in the dismutation of superoxide free radical into H_2O_2 . Generation of H_2O_2 due to high SOD activity led to a higher POD and CAT activity as H_2O_2 is considered as an optimal substrate for POD and CAT and they are known to work efficiently when the H_2O_2 concentration is high, thus protecting the cells from oxidative damage by ROS (Willekens *et al.* 1997). Similar increase in SOD and POD activity in the roots of rice seedlings under P-deficient conditions were reported by Guo *et al.* (2005) and Fu *et al.* (2014), in seedlings of tomato (Wan *et al.* 2010), *Hordeum maritimum L.* plants (Ons *et al.* 2012), and *Brassica napus* (Chen 2015). Varieties with higher SOD activity at prolonged P stress and lower CAT indicate that these varieties are inefficient in scavenging ROS compared to those varieties having lower SOD and higher CAT activity, because higher SOD and low CAT activity results in the accumulation of H_2O_2 , which inhibits 50% of the photosynthesis (Kaiser *et al.* 1979). Swarna followed by Akhanphou had the lower SOD activity in roots accompanied with a high root CAT activity; thus they were efficient in scavenging ROS under low P when compared to remaining varieties. Our data of photosynthesis in this experiment supported this statement. Higher POD activity is also a good indication for neutralization of the ROS effect. RP BIO 226 had higher POD activity, but POD alone was not sufficient to confer tolerance to low P and hence, it was inefficient and susceptible under low P. Therefore, it is possible that tolerance to low P might be related to efficient ROS scavenging enzymes which enabled the tolerant genotype to overcome the stress and support growth. In conclusion, Swarna and Akhanphou performed better than other varieties which was indicated by a higher shoot and root biomass, a higher photosynthetic rate, and an efficient antioxidant enzyme system under low P.

References

- Abadia J., Rao I.M., Terry N.: Changes in leaf phosphate status have only small effects on the photochemical apparatus of sugar beet leaves. – *Plant Sci.* **50**: 49-55, 1987.
- Aebi H.: Catalase *in vitro*. – *Methods Enzymol.* **105**: 121-126, 1984.
- Castillo F.J., Penel C., Greppin H.: Peroxidase release induced by ozone in *Sedum album* leaves. – *Plant Physiol.* **74**: 846-851, 1984.
- Chaudhary M.I., Adu-Gyamfi J.J., Saneoka H. *et al.*: The effect of phosphorus deficiency on nutrient uptake, nitrogen fixation and photosynthetic rate in mashbean, mungbean and soybean. – *Acta Physiol. Plant.* **30**: 537-544, 2008.
- Chen S., Zhao H., Ding G., Xu F.: Genotypic differences in antioxidant response to phosphorus deficiency in *Brassica napus*. – *Plant Soil* **391**: 19-32, 2015.
- de Mendiburu F.: *Agricolae: Statistical Procedures for Agricultural Research.* – R package version 1.1-2. <http://CRAN.R-project.org/package=agricolae>. 2012.
- Dhindsa R.S., Plumb-Dhindsa P., Thorpe T.A.: Leaf senescence: Correlated with increased permeability and lipid peroxidation, and decreased levels of superoxide dismutase and catalase. – *J. Exp. Bot.* **32**: 93-101, 1981.
- Elser J. J.: Phosphorus: A limiting nutrient for humanity? – *Curr. Opin. Biotech.* **23**: 833-839, 2012.
- Epstein E., Bloom A.J.: *Mineral Nutrition of Plants: Principles and Perspectives*, 2nd ed. Pp. 380. Sinauer Associates, Inc., Sunderland 2005.
- Flexas J., Ribas-Carbó M., Diaz-Espej A. *et al.*: Mesophyll conductance to CO_2 : current knowledge and future prospects. – *Plant Cell Environ.* **31**: 602-621, 2008.
- Foyer C., Spencer C.: The relationship between phosphate status and photosynthesis in leaves: Effects on intracellular orthophosphate distribution, photosynthesis and assimilate partitioning. – *Planta* **167**: 369-375, 1986.

- Fredeen A.L., Rao I.M., Terry N.: Influence of phosphorus nutrition on growth and carbon partitioning in *Glycine max.* – *Plant Physiol.* **89**: 225-230, 1989.
- Fu Z.Q., Yang X.J., Shen H.: The physiological mechanism of enhanced oxidizing capacity of rice (*Oryza sativa* L.) roots induced by phosphorus deficiency. – *Acta Physiol. Plant.* **36**: 179-190, 2014.
- Galle A., Florez-Sarasa I., Tomas M.: The role of mesophyll conductance during water stress and recovery in tobacco (*Nicotiana glauca*): acclimation or limitation? – *J. Exp. Bot.* **60**: 2379-2390, 2009.
- Ghannoum O., Conroy J.P.: Phosphorus deficiency inhibits growth in parallel with photosynthesis in a C₃ (*Panicum laxum*) but not two C₄ (*P. Coloratum* and *Cenchrus ciliaris*) grasses. – *Funct. Plant Biol.* **34**: 72-81, 2007.
- Gill S.S., Tuteja N.: Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. – *Plant Physiol. Bioch.* **48**: 909-930, 2010.
- Guo T.R., Yao P.C., Zhang Z.D., Wang M.: Involvement of antioxidative defense system in rice growing seedlings exposed to aluminum toxicity and phosphorus deficiency. – *Rice Sci.* **19**: 207-212, 2012.
- Guo D.P., Guo Y.P., Zhao J.P. *et al.*: Photosynthetic rate and chlorophyll fluorescence in leaves of stem mustard (*Brassica juncea* var. *tsatsai*) after turnip mosaic virus infection. – *Plant Sci.* **168**: 57-63, 2005.
- Heldt H.W., Chon C.J., Maronde D. *et al.*: Role of orthophosphate and other factors in the regulation of starch formation in leaves and isolated chloroplasts. – *Plant Physiol.* **59**: 1146-1155, 1977.
- Hell R., Hillebrand H.: Plant concepts for mineral acquisition and allocation. – *Curr. Opin. Biotech.* **12**: 161-168, 2001.
- Ismail A.M., Heuer S., Thomson M.J., Wissuwa M.: Genetic and genomic approaches to develop rice germplasm for problem soils. – *Plant Mol. Biol.* **65**: 547-570, 2007.
- Jackson M.L.: *Soil Chemical Analysis*. Pp. 498. Oxford IBH Publ. House, Bombay 1967.
- Juszczuk I.M., Wagner A.M., Rychter A.M.: Regulation of alternative oxidase activity during phosphate deficiency in bean roots (*Phaseolus vulgaris*). – *Physiol. Plantarum* **113**: 185-192, 2001.
- Kaiser W.M.: Reversible inhibition of the Calvin cycle and activation of oxidative pentose phosphate cycle in isolated intact chloroplasts by hydrogen peroxide. – *Planta* **145**: 377-382, 1979.
- Lambrev L.H., Miloslavina Y., Jahns P., Holzwarth A.R.: On the relationship between non-photochemical quenching and photoprotection of Photosystem II. – *BBA-Bioenergetics* **1817**: 760-769, 2012.
- Lauer M.J., Pallardy S.G., Belvins D.G., Randall D.D.: Whole leaf carbon exchange characteristics of phosphate deficient soybeans (*Glycine max* L.). – *Plant Physiol.* **91**: 848-854, 1989.
- Li Z.F., Luo A.C., Hassan M.J., Wei X.H.: Effect of phosphorus deficiency on leaf photosynthesis and carbohydrates partitioning in two rice genotypes with contrasting low P susceptibility. – *Rice Science* **13**: 283-290, 2006.
- Lima J.D., Mosquim P.R., da Matta F.M.: Leaf gas exchange and chlorophyll fluorescence parameters in *Phaseolus vulgaris* as affected by nitrogen and phosphorus deficiency. – *Photosynthetica* **37**: 113-121, 1999.
- Marschner H.: *Mineral Nutrition of Higher Plants*. 2nd ed. Pp. 889. Academic press, San Diego 1995.
- Maxwell K., Johnson G.N.: Chlorophyll fluorescence – a practical guide. – *J. Exp. Bot.* **51**: 659-668, 2000.
- McKersie B.D., Leshem Y.Y.: *Stress and Stress Coping in Cultivated Plants*. Pp. 256. Kluwer Academic Publishers, Dordrecht-Boston-London 1994.
- Mustilli A.C., Merlot S., Vavasseur A. *et al.*: *Arabidopsis* OST1 protein kinase mediates the regulation of stomatal aperture by abscisic acid and acts upstream of reactive oxygen species production. – *Plant Cell* **14**: 3089-3099, 2002.
- Neill S.J., Desikan R., Clarke A.: Hydrogen peroxide and nitric oxide as signaling molecules in plants. – *J. Exp. Bot.* **53**: 1237-1247, 2002.
- Panigrahy M., Nageswara Rao D., Yugandhar P. *et al.*: Hydroponic experiment for identification of tolerance traits developed by rice Nagina 22 mutants to low-phosphorus in field condition. – *Arch. Agron. Soil Sci.* **60**: 565-576, 2014.
- Pei Z.M., Murata Y., Benning G. *et al.*: Calcium channels activated by hydrogen peroxide mediate abscisic acid signalling in guard cells. – *Nature* **406**: 731-734, 2000.
- R Core Team: R: A language and environment for statistical computing. R Foundation for Statistical Computing, <http://www.R-project.org>, Vienna 2012
- Rao I.M., Terry N.: Leaf phosphate status, photosynthesis, and carbon partitioning in sugar beet: I. Changes in growth, gas exchange, and calvin cycle enzymes. – *Plant Physiol.* **90**: 814-819, 1989.
- Ripley B.S., Redfern S.P., Dames J.: Quantification of the photosynthetic performance of phosphorus-deficient Sorghum by means of chlorophyll-a fluorescence kinetics. – *S. Afr. J. Sci.* **100**: 615-618, 2004.
- Rodríguez D., Keltjens W.G., Goudriaan J.: Plant leaf area expansion and assimilate production in wheat (*Triticum aestivum* L.) growing under low phosphorus conditions. – *Plant Soil* **200**: 227-240, 1998.
- Sanchez P.A.: Linking climate change research with food security and poverty reduction in the tropics. – *Agr. Ecosyst. Environ.* **82**: 371-383, 2000.
- Stitt M., Quick W.P.: Photosynthetic carbon partitioning: its regulation and possibilities for manipulation. – *Physiol. Plantarum* **77**: 633-641, 1989.
- Tardieu F.: [Plant tolerance to water deficit: physical limits and possibilities for progress.] – *C. R. Geosci.* **337**: 57-67, 2005. [In French]
- Wan Y.H., Zhu L.Y., Yang S.J. *et al.*: Effects of P deficiency on protective enzyme activity and membrane lipid peroxidation in different tomato genotypes. – *Acta Hort.* **856**: 113-120, 2010.
- Willekens H., Chamnongpol S., Davey M. *et al.*: Catalase is a sink for H₂O₂ and is indispensable for stress defence in C₃ plants. – *EMBO J.* **16**: 4806-4816, 1997.
- Wissuwa M., Gamat G., Ismail A.M.: Is root growth under phosphorus deficiency affected by source or sink limitations. – *J. Exp. Bot.* **56**: 1943-1950, 2005.
- Xing D., Wu Y.: Effect of phosphorus deficiency on photosynthetic inorganic carbon assimilation of three climber plant species. – *Bot. Stud.* **55**: 60, 2014.
- Xu H.X., Weng X.Y., Yan Y.: Effect of phosphorus deficiency on the photosynthetic characteristics of rice plants. – *Russ. J. Plant Physiol+* **54**: 741-748, 2007.
- Zribi O.T., Libidi N., Slama I. *et al.*: Alleviation of phosphorus deficiency stress by moderate salinity in the halophyte *Hordeum maritimum* L. – *Plant Growth Regul.* **66**: 75-85, 2012.