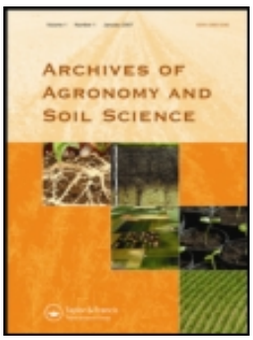

Archives of Agronomy and Soil Science

ISSN: 0365-0340 (Print) 1476-3567 (Online) Journal homepage: http://www.tandfonline.com/loi/gags20

Hydroponic experiment for identification of tolerance traits developed by rice Nagina 22 mutants to low-phosphorus in field condition

Madhusmita Panigrahy, D. Nageswara Rao, P. Yugandhar, N. Sravan Raju, P. Krishnamurthy, S.R. Voleti, G. Ashok Reddy, T. Mohapatra, S. Robin, A.K. Singh, Kuldeep Singh, M. Sheshshayee, R.P. Sharma & N. Sarla

To cite this article: Madhusmita Panigrahy, D. Nageswara Rao, P. Yugandhar, N. Sravan Raju, P. Krishnamurthy, S.R. Voleti, G. Ashok Reddy, T. Mohapatra, S. Robin, A.K. Singh, Kuldeep Singh, M. Sheshshayee, R.P. Sharma & N. Sarla (2014) Hydroponic experiment for identification of tolerance traits developed by rice Nagina 22 mutants to low-phosphorus in field condition, Archives of Agronomy and Soil Science, 60:4, 565-576, DOI: 10.1080/03650340.2013.821197

To link to this article: https://doi.org/10.1080/03650340.2013.821197

Archives of Agronomy and Soil Science, 2014
Vol. 60, No. 4, 565–576, http://dx.doi.org/10.1080/03650340.2013.821197

Hydroponic experiment for identification of tolerance traits developed by rice Nagina 22 mutants to low-phosphorus in field condition

Madhusmita Panigrahy[a], D. Nageswara Rao[a], P. Yugandhar[a], N. Sravan Raju[a], P. Krishnamurthy[a], S.R. Voleti[a], G. Ashok Reddy[a], T. Mohapatra[b], S. Robin[c], A.K. Singh[d], Kuldeep Singh[e], M. Sheshshayee[f], R.P. Sharma[b] and N. Sarla[a]*

[a]*Directorate of Rice Research, Rajendranagar, Hyderabad, Andhra Pradesh, India;* [b]*National Research Center on Plant Biotechnology, New Delhi, India;* [c]*Department of Rice, Tamilnadu Agricultural University, Coimbatore, India;* [d]*Genetics Division, Indian Agricultural Research Institute, New Delhi, India;* [e]*Department of Plant Breeding, Genetics and Biotechnology, Punjab Agricultural University, Ludhiana, India;* [f]*Department of Crop Physiology, University of Agricultural Sciences, Bangalore, India*

(*Received 25 August 2012; final version received 26 June 2013*)

Development of phosphate (P)-deficiency tolerant rice cultivars is constrained by lack of suitable, reproducible, and consistent seedling stage screening methods in breeding programs. This study reports the screening and characterization of M5 mutants derived from an ethyl methane sulfonate treated population of rice *cv.* Nagina 22 (N22) in low-P field (soil Olsen P 1.94–2.01 mg kg^{-1}; alkaline Vertisol; pH 7.94) for high yield. The present study showed that seedling growth responses such as increase in root weight, root length, root/shoot weight, and dry weight in P-deficient medium can be taken as indices of low-P tolerance in mature plants in field. Total phosphorus content in seedlings showed an inverse relationship with total phosphorus content and low-P tolerance in mature plants in the field. But, phosphorus content in seeds and acid phosphatase activity in the seedling stage were positively correlated with survival and seed set in low-P field. In low-P field, plant height correlated most with yield per plant, and the number of productive tillers in mature plants was highly correlated with tiller number at vegetative stage. These mutants (*NH776, NH710,* and *NH719*) have agronomic importance because of their ability to grow and give higher yield than N22 in P-deficient field.

Keywords: rice; Nagina 22; low-phosphorus; phosphate starvation; seedling trait

Introduction

Breeding rice for tolerance to phosphorus (P) deficiency is becoming increasingly important in India as the cost of P fertilizers rise and the profits to farmers decline. India depends largely on import of raw material or intermediates for domestic production of phosphate fertilizer. In intensive cropping systems, considerable part of the applied P enters the waterways through runoff and erosion (Filippelli 2008). In acidic and alkaline soils, P often becomes unavailable to plants as it forms insoluble complexes with iron, aluminum, or calcium. Therefore, improving P acquisition and use by crops is critical for economical and environment friendly crop agriculture. Another important strategy is the use of microorganisms that solubilize phosphate making P available to plants (Walpola &

*Corresponding author. Emails: nsarla@drricar.org; sarla_neelamraju@yahoo.com

Yoon 2012). However, improving tolerance of plants to low P remains an important breeding objective.

In response to low level of available P, plants have developed highly specialized morphological, physiological, and biochemical adaptations to acquire and utilize inorganic phosphate (Pi) from the environment. Plants develop more proliferated root system that is efficient in uptake of soil Pi (Lin et al. 2009; Rouached et al. 2010; Yang & Finnegan 2010) in response to low Pi availability. This includes increased influx rates, high root to shoot ratio, increased root mass, decrease in root diameter, and increase in absorptive surface area relative to root volume (Yuan & Liu 2008). Tiller number, number of roots, and shoot biomass per plant have been shown to be tightly linked with phosphorus deficiency tolerance (Raghothama 1999; Wissuwa & Ae 2001). Other features of adaptive value in low-Pi condition are mycorrhizal symbiosis and formation of specialized root clusters like proteoid roots (Ming et al. 2006). Pi starvation in plants leads to coordinated gene expression, which includes induction of many enzymes and genes. Some genes function to acquire and utilize Pi efficiently (Ramaekers et al. 2010) others are involved in regulating the expression of Pi starvation induced genes (Yuan & Liu 2008; Panigrahy et al. 2009). Recently, a gene called *PSTOL1* (phosphorus starvation tolerance 1) has been identified from Kasalath, an Indian variety and this gene helps rice to develop a larger root system that can take up more phosphorus (Gamuyao et al. 2012).

Development of P-deficiency tolerant rice cultivars is constrained by the lack of suitable screening methods that can be used reproducibly and consistently in breeding programs. Maintenance and preparation of a field with low phosphate needs much more attention than a normal field. Moreover, some researchers find field analysis of rice varieties for low-P tolerance less trust worthy because of the confounding soil and climatic factors. A screening method at the laboratory level in the seedling stage of rice for low-P tolerance, which can act as a surrogate for tedious field level testing for low-P tolerance, would be of great usefulness. Rice Nagina 22 (N22) is a deep-rooted, drought and heat tolerant, upland cultivar of indica rice, and has been used as a donor parent in studies on drought and heat tolerance (Markandeya et al. 2007; Jagadish et al. 2008). In this paper, we present the field analysis of 313 mutant lines (0.8% ethyl methane sulfonate treated mutants in M_3 generation). Four mutants which showed high seed set in low-P field, and four low-P susceptible mutant lines (which did not survive in the low-P field) were characterized in seedling stage under P-sufficient and P-deficient conditions in hydroponics. The focus was to correlate seedling traits in P-deficient condition with mature plant traits in low-P field. For further characterization, a subset of three mutants (two low-P-resistant and one low-P-susceptible) were analyzed for seed P content, uptake of P in initial 24 h after transfer to P-sufficient and P-deficient conditions, and phosphatase activity in seedling stage.

Materials and methods

Field experiment

A total of 313 EMS-induced mutant lines of rice Nagina 22 in M_3 generation were grown in highly P deficient soil (1.94–2.01 mg kg^{-1} Olsen P; alkaline Vertisol; pH = 7.94) in the wet season 2007 at fields of Directorate of Rice Research (DRR), Hyderabad. The farm is geographically situated at an altitude of 542.7 m above mean sea level at 17° 19′ N latitude and 78° 29′ E longitude. The P-deficient plot, 125 m^2, at DRR has not received P for the last 27 years, but has been receiving 100 kg N ha^{-1} and 60 kg K ha^{-1} each season. Mutants were further confirmed for their survival, plant phenotype, and yield in the P-deficient plot in M_4 and M_5 generation, during the dry

season of 2008 and the wet season of 2008. The plants were always planted with a distance of 15 × 20 cm^2 for a line with 22 plants per line (3.15 m long). A total of 15 mutants were short-listed out of which 4 mutants were selected among the highest yielding lines. The phenotype of the selected mutants was confirmed in M_6 generation during the dry season of 2009. The low-P susceptible lines were also transplanted in the wet season of 2009 at the same time in mid-July in normal irrigated P-sufficient plot (500 m^2 and 60 kg ha^{-1} of single super phosphate). Field data of the low-P tolerant mutants presented here are the means of observation for the dry seasons of 2008, 2009, and for the wet season of 2008. These mutants are named as Nagina 22-Hyderabad mutants and will be referred as *NH*.

Hydroponic experiment

Four P-deficiency tolerant (Pdt) mutants i.e., they set seed in P-deficiency, and four P-deficiency susceptible (Pds) mutants i.e., they do not set seed in P-deficiency were selected to test if their seedling response in P-deficient hydroponic conditions could predict the response observed in low-P field conditions at maturity. The four Pdt mutants were *NH776, NH635, NH710*, and *NH719*, and the four Pds mutants were *NH350, NH480, NH349, and NH368*.

Mutant lines of M5 generation were grown in hydroponic condition for 4 weeks in a medium containing half the strength of Hoagland's solution (+P) (Hoagland & Arnon 1950) (briefly: $MgSO_4.7H_2O$, $Ca(NO_3)_2.4\ H_2O$, KH_2PO_4, KNO_3, micronutrient solution, Fe-EDTA, pH adjusted to 5.7 at 27–30°C) for the analysis of seedling traits. The surface sterilized mutant seeds were germinated and grown in petri dishes containing water for 10 days. Seedlings were then transferred to either P-sufficient or P-deficient Hoagland's solution. In the P-deficient solution (–P), KH_2PO_4 was replaced with equimolar KCl.

Phenotypic evaluation and analysis of traits in field grown mature mutants and seedlings grown in hydroponics

Phenotypic observations were recorded in the field for yield and related traits from three middle plants of each row, for each mutant line, like plant height (PH)– length of the tallest tiller from soil surface till the tip of the panicle (cm), tiller number per plant (NT), number of productive tillers (NPT) – total number of panicles with seed set exceeding 15%, panicle length (PL) – length (cm) from neck to last spikelet of main panicle, yield per plant (Y/P) is total weight of filled grains (g) per plant, SPAD (soil plant analysis development) – value at the middle of the leaf lamina using a chlorophyll meter.

Observations were recorded for seedling traits from 4 weeks old seedlings grown in hydroponic condition (supplemented with P-sufficient or P-deficient medium) for 15 days. Each reading is a mean of five observations grown in three individual experiments. Maximum root length (RL) – length of the root from base to tip of the longest root, root weight (RW) – fresh weight of the roots, root/shoot ratio (RFW/SFW) – ratio of the root and shoot fresh weights, total dry weight (TDW) – dry weight of whole seedlings were determined.

Analysis of P *content in seeds and plants*

Dried plant tissues (only shoot in the case of field samples and both root and shoot in the case of seedling samples from hydroponics) or dehusked seed were ground, and 0.5 g of powdered tissue (1 g in case of seed) were used for digestion in a mixture of HNO_3, $HClO_4$, and H_2SO_4 (3:1:1). After digestion, P-content was obtained from the concentration of Pi in the medium,

which was determined using the phosphovanadate method (Hanson 1950).

$$\text{Amount of P} = \text{Concentration} \times \text{Weight} \quad (1)$$

$$\text{Total amount of P} = \text{Amount of P in shoot} + \text{Amount of P in root} \quad (2)$$

$$\text{Percentage of shoot or root P} = \frac{\text{Amount of P in root or shoot}}{\text{Total amount of P}} \times 100 \quad (3)$$

Trait correlation

Pearson correlations between six vegetative and mature plant morphological trait pairs in M5 population grown in low-P field were calculated at two probability levels ($p < 0.05$ and $p < 0.01$) in Microsoft Excel Stat using trait averages. The significant correlation is indicated with * or **, respectively.

Acid phosphatase activity

Enzyme extract was prepared by extracting 1 g of plant shoot tissue (10-day-old seedlings) in 10 ml of 100 mM citrate buffer, pH 5.2, and centrifuged at 9.3 g for 15 min at 4°C. From the supernatant, 0.1 ml was used as the enzyme source. The assay mixture contained 0.5 ml of 10 mM p-nitrophenol phosphate as substrate, 0.4 ml of citrate buffer, and 0.1 ml of enzyme (Johnson et al. 1973). The mixture was incubated at 37°C for 5 min. The reaction was terminated by adding 2 ml of 200 mM sodium carbonate. The absorbance of the solution was measured at 405 nm and the activity was expressed in nM of p-nitrophenol released min^{-1} g^{-1} fresh weight.

Super oxide dismutase (SOD) activity

Enzyme extract was prepared by extracting 1 g of leaf sample in 10 ml of 0.1 M phosphate buffer, pH 7.5, containing 1 mM EDTA, 5% sorbitol, 0.1% Triton X-100, and then centrifuged at 13.4 g for 20 min. From this, 0.1 ml of the supernatant was used as the enzyme source. All the operations were carried out at 4°C. Activity of super oxide dismutase enzyme was studied in the shoots of mutant seedlings after 24 h of P-deficiency.

The SOD enzyme activity was measured following Dhindsa et al. (1981). The 3 ml assay mixture contained 0.1 ml supernatant, 1.5 ml phosphate buffer (0.1 M pH 7.8), 0.1 ml sodium carbonate (1.5 M) 0.1 ml nitro blue tetrazolium (NBT, 2.25 mM), 0.2 ml methionine (200 mM), 0.1 ml riboflavin (60 μM), and 0.8 ml water. A non-irradiated complete reaction mixture served as a blank. The sample tubes were illuminated under 15 W fluorescent lamp for 10 min. The tubes lacking supernatant (enzyme source) but containing the assay mixture were illuminated and served as control. Tubes containing supernatant (enzyme source) were illuminated and served as experimental sample. Absorbance was recorded at 560 nm. One unit of enzyme activity was taken as the quantity of enzyme which reduced the absorbance to 50% in comparison with the control. The enzyme activity was expressed as:

$$\text{Enzyme activity} = \frac{(\text{Absorbance of control sample} - \text{absorbance of experimental sample})}{0.5 \times \text{absorbance of control sample}} \quad (4)$$

This value was divided by 10 to get the value min^{-1} as incubation was for 10 min. The value was then multiplied by 100 to get value per gram fresh weight, as aliquot was only 0.1 ml from the 10 ml supernatant derived from 1 g fresh weight tissue. The enzyme activity was thus expressed in unit min^{-1} g^{-1} fresh weight.

Results

Trait analysis, yield, and correlation in mutants grown in P *fertilized and low P field*

The frequency distribution for 6 field traits in 313 mutant lines showed approximately normal distribution, with the N22 plants being grouped in the highest value category except for yield (Figure 1). However, only 67% of mutant lines could complete the life cycle and set seeds in the P-deficient condition. Among all the traits, highest significant positive correlation was observed between number of tillers and number of productive tillers (0.9) (Table 1); and the least positive correlation was between SPAD value and the number of tillers (0.12). In the low-P field, plant height correlates the highest (0.23) with

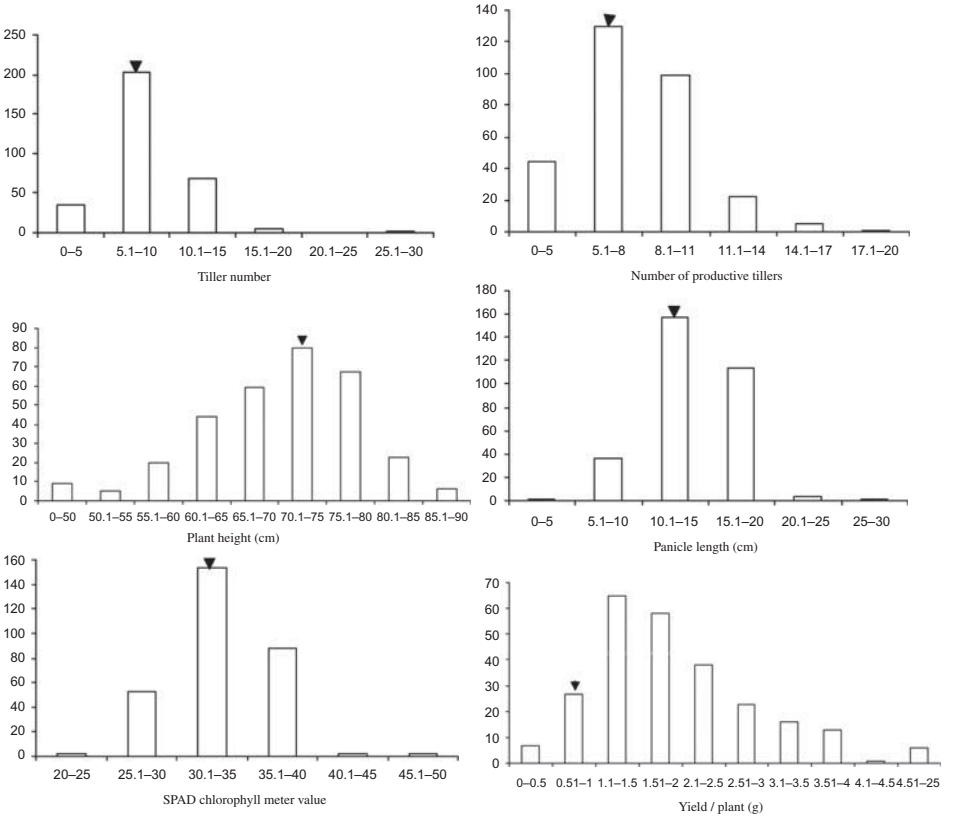

Figure 1. Frequency distribution of yield and related traits in the 313 M5 mutant populations in low-P field. Y-axis indicates the number of mutants in all the figures. Arrowheads indicate the value range of N22 plants. Tiller number (NT), total number of tillers per plant; number of productive tillers (NPT), total number of panicles with seed set exceeding 15%; plant height (PH), length of the tallest tiller from soil surface till the tip of the panicle (cm); panicle length (PL), length (cm) from neck to last spikelet of main panicle; SPAD value (SPAD), SPAD value at the middle of the leaf lamina; yield per plant (Y/P), total weight of filled grains (g) per plant.

Table 1. Correlation of measured plant traits in low-P field wet season – 2008.

	pH	NT	NPT	PL	Y/Pl	SPAD
pH	1					
NT	**0.336	1				
NPT	**0.382	**0.906	1			
PL	**0.608	**0.317	**0.338	1		
Y/Pl	**0.237	**0.165	*0.138	**0.213	1	
SPAD	*0.128	*0.120	0.079	**0.148	0.057	1

Notes: r = 0.098 at 0.05; r = 0.129 at 0.01 for n = 313. PH: plant height; NT: no. of tillers; NPT: no. of productive tillers; PL: panicle length; Y/Pl: Yield per plant; SPAD: SPAD chlorophyll meter reading.

yield per plant in these mutants. Correlation of SPAD value with number of productive tillers (0.07) and yield/plant (0.05) was not significant.

The height of the 4 Pdt mutants (*NH 719, NH 635, NH 710 and NH 776*) was 113, 100, 98 and 105 cm respectively. Days to 50% flowering in the same mutants were 76, 78, 82 and 72 respectively. The corresponding values for N22 were 98 cm for height and 73 days to 50% flowering. The grain yield of the four Pdt mutants (i.e. *NH776, NH635, NH710*, and *NH719*) in P-sufficient plot was 14.6, 17.5, 12.8, and 10.6 g plant^{-1}, respectively, compared to 12.5 g plant^{-1} in N22. Their yield in the P-deficient plot in DRR was 2.1, 0.5, 2.3, and 1.5 g plant^{-1}, respectively, compared to 1.9 g plant^{-1} in N22. *NH719* had lower yield than other mutants and N22 in P-sufficient plot, and *NH635* showed the least yield in P-deficient plot. The four Pds mutants (i.e.*NH350, NH480, NH349, and NH368*) did not survive in low-P field so there was no seed set. Their yield in P-sufficient plot was 11.3, 8.9, 14.3, and 11 g plant^{-1}, respectively, compared to 12.5 g plant^{-1} in N22.

Seedling phenotype under P*-deficiency condition in hydroponics*

Under low-P condition, increase in root length (RL) was the highest (i.e. 29%) in *NH776* (Figure 2a). All other lines showed decrease in RL with N22 having the maximum decrease (i.e. 20%). Increase in root weight was observed in all the Pdt mutants, but the highest was in *NH710* (Figure 2b). All the Pds lines showed minimum increase or decrease in RW, but N22 showed maximum decrease (i.e. 12%). Increase in root/shoot weight ratio was observed in two Pdt lines (*NH776* and *NH710*) and one Pds line (*NH350*), whereas in all the other lines there was minimal or no increase (Figure 2c). *NH719* showed decrease in RFW/SFW by 10.3%. Increase in TDW in response to P-deficiency stress was observed only in *NH776* and *NH710* (Figure 2d), whereas in all the other lines there was a decrease in TDW, with N22 line showing the highest decrease of 56.4%.

Phosphorus content in mutants and acid phosphatase activity

In the shoots of the low-P tolerant lines from the field, highest total P-content in the shoot was found in *NH776* (0.96 mg g^{-1} DW) followed by *NH710* (0.83 mg g^{-1} DW), which was similar to N22 (Figure 3a). *NH719* and *NH635* also had similar P-content as in N22. The P-content in the seed from P-sufficient plot was also analyzed in these Pdt lines in comparison with one Pds line and N22 (Figure 3b). Figure 4c shows that acid phosphatase activity in N22 and all the Pdt lines (*NH*776 and *NH*719) was significantly higher

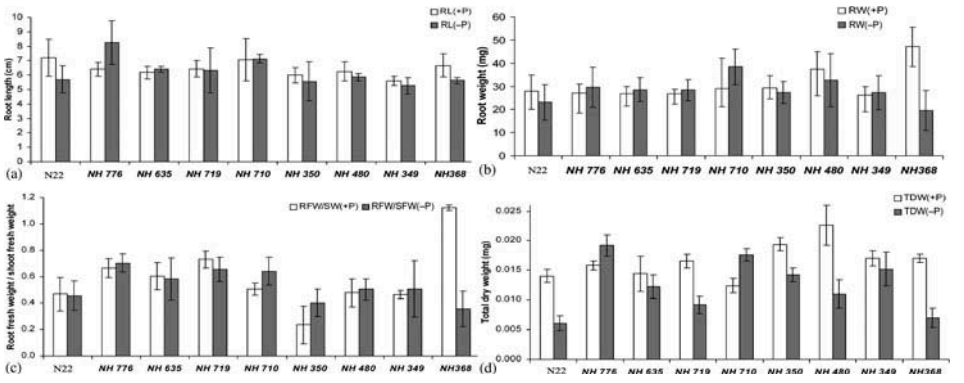

Figure 2. Phenotypic trait analysis of four low-P tolerant and four low-P susceptible lines in seedling stage: (a) root length (RL); (b) root weight (RW); (c) ratio of root fresh weight (RFW) and shoot fresh weight (SFW) (c) and (d) total dry weight (TDW). Four-week-old seedlings grown in hydroponic condition supplemented with P-sufficient (+P) or P-deficient medium (−P) for 15 days. *NH776, NH635, NH710*, and *NH719,* P-deficiency tolerant lines (Pdt); *NH350, NH480, NH349, and NH368,* P-deficiency susceptible line (Pds).

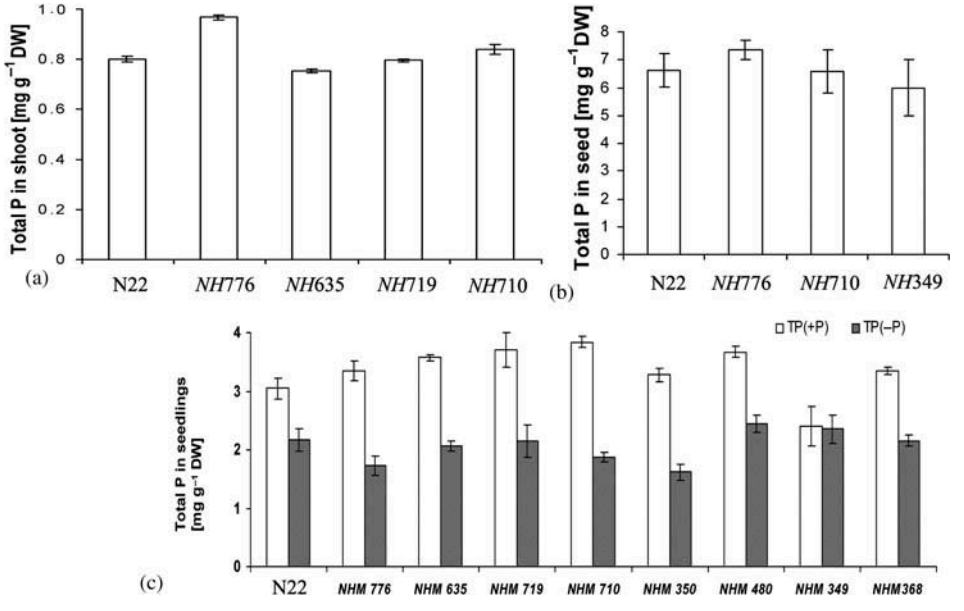

Figure 3. (a) Total P-content in mature shoot tissue, (b) dehusked seeds from plants grown in low-P field and (c) four-week-old seedlings grown in hydroponic condition supplemented with P-sufficient (+P) or P-deficient medium (−P) for 15 days. Data presented are a mean of five observations of plants grown in three experiments. *NH776, NH635, NH710*, and *NH719,* P-deficiency tolerant lines (Pdt); *NH350, NH480, NH349, and NH368,* P-deficiency susceptible line (Pds).

compared to that in Pds lines (*NH*368 and *NH*349). The P content in the seed from P-sufficient plot supported the above observation. Highest seed P content was found in *NH776* followed by *NH710,* whereas in the Pds line, *NH349* was lower. The P concentration in N22 and *NH*710 was also similar in P-sufficient plot.

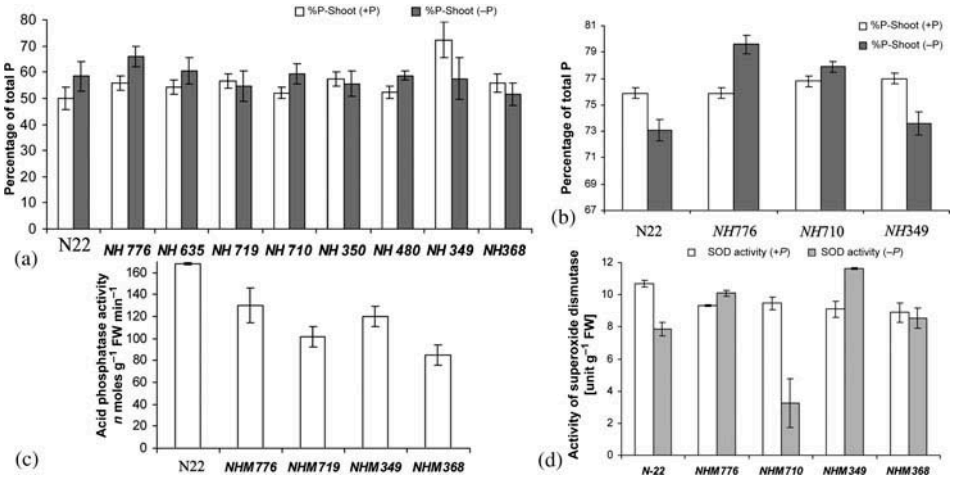

Figure 4. (a) Percentage of P in shoot after 7 days (b) percentage of P in shoot after 24 h (c) acid phosphatase activity, and (d) SOD activity in four-week-old seedlings grown in hydroponic condition supplemented with P-sufficient (+P) or P-deficient medium (−P) for 15 days. Data presented are a mean of three observations of plants grown in three experiments. *NH776, NH635, NH710*, and *NH719,* P-deficiency tolerant lines (Pdt); *NH350, NH480, NH349,* and *NH368,* P-deficiency susceptible line (Pds).

Figure 3c shows total phosphorus concentration in the seedlings of four selected Pdt, Pds, and N22 after growing them in (+P/−P) hydroponic conditions for 15 days. There is a decreasing trend in all the mutant lines from +P to −P. The values ranged from 2.4% (in *NH349*) to 51.2% (in *NH710*) under P-deficiency stress. Total-P content was the highest in *NH710*, under P-sufficient condition, whereas it was the highest in line *NH480* under P-deficient condition. Although *NH349* line had the lowest initial total-P content, it was maintained at a similar level after P-deficiency stress. The two Pdt lines *NH776* and *NH710* showed the highest decrease in tp (48.3% and 51.2%, respectively) in P-deficient medium.

P-*distribution in roots and shoots*

Figure 4a shows the result of P-allocation in shoots and roots separately after 7 days of seedling transfer to P-sufficient or P-deficient condition. Under P-sufficient condition, all the mutants and N22 except *NH349* showed nearly 50% distribution of P between root and shoot. Only *NH349* showed the highest percentage (i.e., 72.2%) of P in shoot. Under P-deficiency stress, N22 and all the mutants except *NH776* showed nearly 42% of P in roots and 58% of P in shoots (Figure 4a). *NH776* showed the highest percentage of P (i.e. 65%) in shoot under P-deficiency condition (Figure 4a). The P absorption in shoot was also analyzed at an earlier time point (i.e. 24 h) after the transfer to P-sufficient or P-deficient condition in N22 and only 3 mutants to see if there is any deviation in the early stage of transfer (Figure 4b). The observations of % P-content in shoot being the highest in *NH349* (77%) under P-sufficient and in *NH776* (79.6%) under P-deficiency conditions were also found at an earlier time point (after 24 h) analysis (Figure 4b).

SOD activity in shoots

Activity of super oxide dismutase enzyme was studied in the shoots of mutant seedlings of two Pdt lines (*NH776* and *NH719)* and two Pds lines (*NH368 and NH349)* after 24 h of

P-deficiency (Figure 4d). N22 showed slight decrease while *NH710* showed highest decrease in SOD enzyme activity during initial 24 h of P-deficiency. *NH*776 showed little increase while *NH349* showed highest increase, and in *NH*368 line there was no change in SOD activity.

Discussion

There are many reports describing *in vitro* screening assays of mutants at seedling stage for acid phosphatase activity (Lloyd et al. 2001; Zakhleniuk et al. 2001), metabolizing exogenous phosphate during phosphorus starvation (Chen et al. 2000), high inorganic phosphorus in seeds (Yu-hua et al. 2005), high phosphorus use efficiency (Guo et al. 2002), and phytate content in grain (Stangoulis et al. 2007). However, there are very few reports where both field screening and lab screening was carried out to develop criteria for predicting low-P tolerance. There are limited reports demonstrating that internal P utilization efficiency in low P solution at seedling stage can be a good screening index for low phosphorus tolerant rice as asserted by Li et al. (2005). In the present study, both field screening as well as laboratory analysis of mutants was carried out to select parameters at seedling stage which can be indicative of low-P tolerance in field in mature plants so that many germplasm accessions or large mapping populations can be screened easily. The present study showed that increase in root length, root weight, root/shoot fresh weight, and TDW in response to phosphorus deficiency in the seedling stage can be used as indicators of low-P tolerance in low-P field. In an earlier field experiment on maize it was shown that the shoot weight is a plant parameter most sensitive to P-deficiency (Fageria et al. 1988). Our study also again demonstrates similar results in rice.

The four Pdt mutants showed high seed set in P-deficient plot with three mutants (except *NH*635) showing higher yield than N22. Likewise, in P-sufficient plot, only *NH*719 gave lower yield than N22. Most of the mature plant traits in the present study (i.e., plant height, no. of tillers and productive tillers, panicle length, and yield/plant) show significant correlations with each other. Among all mature plant traits, tiller number at vegetative stage showed the highest correlation with number of productive tillers in the P-deficient field. This was supported by the observations from the following different studies. Tiller number under P-deficiency was an indicator of P-deficiency tolerance in rice (Wissuwa et al. 1998); in wheat also rate of tiller emergence and the emergence of leaves on the main stem was slowed down by phosphorus deficiency (Rodríguez et al. 1999) and phosphorus application enhances tiller number which is directly proportional to phosphorus level in rice (Alam et al. 2009). The correlation between SPAD chlorophyll value and yield per plant was not significant. In P-deficient condition, leaves become dark green in some lines as a result of increased anthocyanin and other pigments to protect chlorophyll functions. The mutant lines with higher total P-content (*NH776* and *NH710*) in the low-P field showed good seedling vigor in the seedling stage in P-deficient medium. On the other hand, the lines with lower total P-content in the low-P field (*NH719* and *NH635*) were not as vigorous in the seedling stage. However, *NH350,* a Pds line could also show good growth response (increase in RFW/SFW) in seedling stage in P-deficient condition. Total P-content in mutants of N22 in the seedling stage showed no significant correlation with total P-content of mature plant samples from low-P field. Thus, the total P-content in the seedling stage cannot be used to screen for low-P tolerance in the field condition. It is possible that some lines are adapted to absorb excess P in the seedling stage if there is P deficiency. But, according to the growth response of seedlings, increase in root length, root weight,

root/shoot fresh weight, and TDW are considered as screening parameters to obtain an indication of low-P tolerance in the field up to maturity.

The Pds line *NH349* was an efficient transporter of P from root to shoot as it maintained the highest shoot P-content under P-deficiency in the seedling stage. P-uptake in *NH349* in early (24 h) and late (7 days) time point and P transport to shoot was highly efficient in seedling stage in P- deficient medium, but in low-P field there was no seed set. Since, *NH349* could set seed only in P-sufficient but not in P-deficient condition, it can be assumed that the mobilization of shoot P into seeds was not efficient, which was evident by its low seed P-content from seeds of P-sufficient field. Su et al. (2006) also reported similar result, showing significant negative correlation between P-use efficiency and soil P-uptake. In low-P conditions, plants increase their level of intracellular and secreted acid phosphatase to catalyze the hydrolysis of inorganic phosphorus. Acid phosphatase activity was less in *NH349* compared to N22. Overexpression of a root-associated acid phosphatase increased extracellular organic phosphorus utilization in rice (Tian et al. 2012). These results indicate that high seed P-content, high acid phosphatase activity, and efficient P-uptake are essential to sustain yield in low-P field.

Recently, it was shown that PSTOL 1 gene (phosphorus-starvation tolerance 1) when over expressed in a japonica variety Nipponbare led to 60% yield enhancement in the transgenic line in a P-deficient plot (Gamuyao et al. 2012). The gene actively enhances growth of roots and thus increases P uptake. Our results show that there are mutants with good uptake and transport to shoot but do not result in growth or seed set. Thus, efficient use of phosphorus is more complex than just increase of uptake by roots. Its use for maximizing growth and seed set has to be ensured and several genes are likely to be involved. The low-P tolerant mutants of N22 have agronomic value in having higher or equivalent yield than the control in P-sufficient plot, and reasonably higher yield in low-P field. The low-P tolerant and susceptible mutants are also a useful genetic resource for functional genomic and epigenetic studies to discover genes and microRNAs involved in the complex processes that contribute to P homeostasis in rice.

Conclusion

Results of the present study showed that seedling traits like increase in root length, root weight, root/shoot fresh weight, and TDW in response to phosphorus deficiency can be used as indicators of low-P tolerance in mature plants in low-P field. This method of laboratory screening for low-P tolerance can be performed easily avoiding the tedious field screening and confounding climatic factors.

Total P-content in the seedling stage did not show significant correlation with total P-content of mature plant samples from low-P field and, therefore, cannot be used to screen for low-P tolerance in the field.

In the P-deficient field, number of productive tillers in mature plants was highly correlated with tiller number at vegetative stage among all other plant traits. Plant height of mature plants correlated most with yield per plant and on contrary, no significant correlation was observed between SPAD chlorophyll value and yield per plant.

In addition to efficient P-uptake in the seedling stage, mobilization of shoot P into seeds and hence high seed-P content, high acid phosphatase activity, and also high P-use efficiency is essential for sustainability and yield in low-P field.

The mutants of N22 used in the present study (i.e., *NH776, NH635, NH710*, and *NH719*) which had higher or equivalent yield in P-sufficient plot and reasonably higher yield in low-P field are of agronomic value, whereas the low-P susceptible mutants

(i.e., *NH350, NH480, NH349*, and *NH368*) are being used for functional genomic and epigenetic studies to understand P-signaling and homeostasis in rice.

Acknowledgments

The authors thank the Department of Biotechnology, Govt. of India (BT/PR9264/AGR/02/406(04)/2007) for financial support and Project Director, Dr B. C. Viraktamath for facilities. The valuable input of reviewers is highly acknowledged.

References

Alam MM, Ali MH, Ruhul Amin AKM, Hasanuzzaman M. 2009. Yield attributes, yield and harvest index of three irrigated rice varieties under different levels of phosphorus. Adv Biol Res. 3:132–139.

Chen DL, Delatorre CA, Bakker A, Abel S. 2000. Conditional identification of phosphate starvation- response mutants in *Arabidopsis thaliana*. Planta. 211:13–22.

Dhindsa RA, Plumb-Dhindsa P, Thorpe TA. 1981. Leaf senescence: correlated with increased permeability and lipid peroxidation and decreased levels of superoxide dismutase and catalase. J Exp Bot. 126:93–101.

Fageria NA, Wright RJ, Baligar VC. 1988. Rice cultivar evaluation for phosphorus use efficiency. Plant Soil. 111:105–109.

Filippelli GM. 2008. The global phosphorus cycle: past, present, and future. Elements. 4:89–95.

Gamuyao R, Chin HJ, Pariasca-Tanaka J, Pesaresi P, Catausan S, Dalid C, Slamet-Loedin I, Tecson-Mendoza EM, Wissuwa M, Heuer S. 2012. The protein kinase Pstol1 from traditional rice confers tolerance of phosphorus deficiency. Nature. 488:535–541.

Guo Y, Lin W, Shi Q, Liang Y, Chen F, He H, Liang K. 2002. Screening methodology for rice (*Oryza sativa*) genotypes with high phosphorus use efficiency at their seedling stage. Ying Yong Sheng Tai Xue Bao (Chinese J Appl Ecol). 13:1587–1591.

Hanson WC. 1950. The photometric determination of phosphorus in fertilizers using the phospho-vanado-molybdate complex. J Sci Food Agric. 1:172–173.

Hoagland DR, Arnon DI. 1950. The water culture method for growing plants without soil. Berkeley, CA: University of California Agricultural Experiment Station. Circular 347.

Jagadish SVK, Craufurd PQ, Wheeler TR. 2008. Phenotyping parents of mapping population of rice for heat tolerance during anthesis. Crop Sci. 48:1140–1146.

Johnson CB, Holloway BR, Smith H, Grierson D. 1973. Isoenzymes of acid phosphatase in germinating peas. Planta. 115:1–10.

Li Y, Luo A, Wang W, Yang C, Yang X. 2005. An approach to the screening index for low phosphorus tolerant rice genotype. Ying Yong Sheng Tai Xue Bao (Chinese J Appl Ecol). 16:119–124.

Lin WY, Lin SI, Chiou TJ. 2009. Molecular regulators of phosphate homeostasis in plants. J Exp Bot. 60:1427–1438.

Lloyd JC, Zakhleniuk OV, Raines CA. 2001. Identification of mutants in phosphorus metabolism. Ann Appl Biol. 138:111–115.

Markandeya G, Babu PR, Lachagari VBR, Reddy AMM, Wusirika R, Bennetzen JL. 2007. Identification of stress-responsive genes in an indica rice (*Oryza sativa* L.) using ESTs generated from drought-stressed seedlings. J Exp Bot. 58:253–265.

Ming F, Qun L, Wang W, Shanshan Z, Bin G, Daleng S. 2006. Cloning, expression and function of phosphate transporter encoded gene in *Oryza sativa* L. Sci China C Life Sci. 14:409–413.

Panigrahy M, Rao DN, Sarla N. 2009. Molecular mechanisms in response to phosphate starvation in rice. Biotech Adv. 27:389–397.

Raghothama KG. 1999. Phosphate acquisition. Ann Rev Physiol Mol Biol. 50:665–693.

Ramaekers L, Remans R, Rao IM, Blair MW, Vanderleyden J. 2010. Strategies for improving phosphorus acquisition efficiency of crop plants. Field Crops Res. 117:169–176.

Rodríguez D, Andrad FH, Goudriaan J. 1999. Effects of phosphorus nutrition on tiller emergence in wheat. Plant Soil. 209:283–295.

Rouached H, Arpat AB, Poirier Y. 2010. Regulation of phosphate starvation responses in plants: signaling players and cross-talks. Mol Plant. 3:288–299.

Stangoulis JCR, Huy B, Welch RM, Choi E, Graham RD. 2007. Quantative trait loci for phytate in rice grain and their relationship with grain concentration with grain micronutrient content. Euphytica. 154:289–294.

Su J, Xiao Y, Li M, Liu Q, Li B, Tong Y, Jia J, Li Z. 2006. Mapping QTLs for phosphorus-deficiency tolerance at wheat seedling stage. Plant Soil. 281:25–36.

Tian J, Chuang Wang C, Zhang Q, He H, Whelan J, Shou H. 2012. Overexpression of *OsPAP10a*, a root-associated acid phosphatase, increased extracellular organic phosphorus utilization in rice. J Integr Plant Biol. 54:631–639.

Walpola BC, Yoon MH. 2012. Prospectus of phosphate solubilizing microorganisms and phosphorus availability in agricultural soils. Afr J Microbiol Res. 6:6600–6605.

Wissuwa M, Ae N. 2001. Further characterisation of two QTLs that increase phosphorus uptake of rice (*Oryza sativa* L.) under phosphorus deficiency. Plant Soil. 237:275–286.

Wissuwa M, Yano M, Ae N. 1998. Mapping of QTLs for phosphorus-deficiency tolerance in rice (*Oryza sativa* L.). Theor Appl Genet. 97:777–783.

Yang XJ, Finnegan PM. 2010. Regulation of phosphate starvation responses in higher plants. Ann Bot. 105:513–526.

Yu-hua W, Xue-liang R, Qing-long L, Wen-yue C, Sheng-quan S, Dian-xing W, Qingyao S. 2005. Screening, selection and development of high inorganic phosphorus mutants in rice. Chin J Rice Sci. 19:47–51.

Yuan H, Liu D. 2008. Signaling components involved in plant responses to phosphate starvation. J Integr Plant Biol. 50:849–859.

Zakhleniuk OV, Raines CA, Lloyd JC. 2001. *pho3*: a phosphorus-deficient mutant of *A. thaliana* (L.) Heynh. Planta. 212:529–534.