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ScienceDirect

Rice Science, 2016, 23(4): 184–195



# Characterization and Selection of Phosphorus Deficiency Tolerant Rice Genotypes in Sri Lanka



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**Abstract:** Phosphorus (P) deficiency in soil is a major constrain for rice production. An important set of rice genotypes (landraces, old improved and new improved varieties) were screened for P deficiency tolerance in two major cropping seasons of Sri Lanka, in 2012. The Ultisol soil, which was collected from a plot cultivated with rice without fertilizer application for past 40 years ( $P_0$ ) at the Rice Research and Development Institute (RRDI), Bathalagoda, Sri Lanka, was used as the potting medium for greenhouse trials. Two field trials were conducted in the same plots at RRDI. Both  $P_0$  and  $P_{30}$  (30 mg/kg  $P_2O_5$ ) conditions were used in the two greenhouse trials. At the early vegetative (three weeks after transplanting), late vegetative (six weeks after transplanting) and flowering stages, plant height and number of tillers per plant were recorded. At the flowering stage, shoots were harvested and shoot dry weight, shoot P concentration, shoot P uptake and P utilization efficiency were measured. All data were statistically analyzed using analysis of variance, regression and cluster procedures. The measured parameters were significantly different between  $P_0$  and  $P_{30}$  conditions ( $P < 0.05$ ). Higher shoot dry weight was reported by the rice genotypes H4 and Marss under  $P_0$  conditions. The regression analysis between shoot dry weight and P utilization efficiency revealed that the studied rice genotypes could be categorized to three P deficiency tolerance classes. A total of 13 genotypes could be considered as highly tolerant and 4 genotypes as sensitive for P deficiency. These results could be used to select parental genotypes for breeding and genetic studies and also to select interesting varieties or landraces for organic rice production.

**Key words:** phosphorus; tolerance; rice; genotype; phosphorus utilization efficiency; landrace

Rice is the staple food for more than 50% of the world population (Maclean et al, 2002), and it provides 21% of the energy and 15% of the protein requirements of human beings (Kennedy et al, 2002). Rice farming is the major livelihood for many people (Leff et al, 2004). Cost effective practices of rice farming is essential to maintain significant profit gains for the growers and to have affordable market prices for the

consumers.

Biotic stress and abiotic stress affect the growth, reproduction and hence the productivity of rice. Biotic stress is mainly caused by the insect pests and diseases (Pathak and Khan, 1994), and abiotic stress is mainly caused by drought (Pandey et al, 2007), salinity (Grattan et al, 2002), submergence (Septiningsih et al, 2015), nutrient deficiency (Dobermann and Fairhurst,

**Received:** 9 July 2015; **Accepted:** 19 October 2015

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Peer review under responsibility of China National Rice Research Institute

<http://dx.doi.org/10.1016/j.rsci.2015.10.001>

2000) and iron toxicity (Fageria et al, 2008).

Phosphorus (P) is one of the essential macronutrients for growth and development of plants (Marschner, 2012). P deficiency symptoms include growth retardation, formation of small and curly leaves, occurrence of reduced numbers of flowers and tillers (Dobermann and Fairhurst, 2000), massive promotion of lateral and hairy root development (Raghothama, 1999) and dark green to purple coloration of leaves due to increased synthesis of anthocyanin (Dobermann and Fairhurst, 2000).

One third of the cultivable lands in the world lack required level of P in the soil for optimum plant growth and development (MacDonald et al, 2011). Most of the Sri Lankan rice growing soils lack adequate levels of P (Kumaragamage and Indraratne, 2011). To circumvent this problem, P is applied extensively as an artificial fertilizer. Global P fertilizer demand in 2014 was  $43.9 \times 10^7$  t (FAO, 2014). In Sri Lanka, application of the excessive amounts of P fertilizer could be seen and nearly  $1.2 \times 10^5$  t of P fertilizer worth of 0.3 billion US Dollars are required annually. P fertilizer is imported to the country and the amount of expenditure on P purchases is equivalent to 1.5% of GDP (Economic and Social Statistics of Sri Lanka, Sri Lanka, 2013). At micro-economic scale, P fertilizer is very expensive which is leading to shrink the profit margin causing many negative socio-economic consequences. Because of the non-renewable nature of P reserves and the rapid rate of depletion due to extensive consumption, the price of P fertilizer will continue to increase, making the problem worse in the future (Cordell et al, 2009). Fertilizer application is not practiced based on the recommended levels especially in developing countries like Sri Lanka. Growers tend to apply more fertilizer seeking a visual appeal in their crops. Consequently, the excessive amounts get washed away from agricultural lands and getting accumulated in water bodies, causing water pollution and eutrophication (Bennett et al, 2001).

To better answer the P crisis in rice farming, the development of P efficient rice genotypes, which are adapted to low P soils, would be a promising solution (Cordell et al, 2009; Rose et al, 2011). The currently recommended rice cultivars in Sri Lanka cannot be grown profitably under low P conditions. Although these cultivars have higher harvesting indices, they usually possess low nutrient use efficiency. However, there are other rice genotypes namely landraces and accessions with promising capabilities of nutrient use

efficiency (Herath et al, 1982). However, these favorable traits with reference to the specific nutrients such as P have not been properly studied and the local rice germplasm has not been screened for P deficiency tolerance (PDT). In many countries, screening of rice genotypes for PDT have been conducted successfully and P efficient genotypes can be used as parents in the breeding programs (Wissuwa et al, 1998; Chin et al, 2011). The genomic regions (i.e. QTL/quantitative trait loci) have been identified in the studied rice genotypes and they can be used in marker assisted selection (i.e. molecular breeding) to expedite the process of cultivar improvement (Chin et al, 2011). However, the information obtained from the studies conducted in other countries cannot be applied directly in the local breeding programs due to the variability of locally available germplasm compared to the germplasms used in those studies. If the important rice genotypes in Sri Lanka could be screened for PDT, then genetic characterization can be carried out to validate the presence of previously identified alleles and to detect possible novel alleles causing PDT. This genomic information can be used in marker assisted breeding of rice in the future. Therefore, as the first step, the present study was conducted to screen a set of important rice genotypes in Sri Lanka for PDT.

## MATERIALS AND METHODS

### Rice materials

The rice genotypes (varieties, cultivars, accessions and landraces, here in after commonly referred to as genotypes) (Table 1) were obtained from the Rice Research and Development Institute (RRDI), Bathalagoda, Sri Lanka.

### Growing seasons

The agricultural practices in Sri Lanka are coincided with two major growing seasons, *Yala* and *Maha*, primarily based on the two different seasons of monsoonal rains. *Yala* season runs from early April to late August fostered primarily by southwest monsoonal rains. The other growing season *Maha* runs from late September to early March fostered primarily by northeast monsoonal rains [Department of Agriculture (DOA), Sri Lanka, 2006a]. The greenhouse and field trials were conducted in *Yala* and *Maha* seasons in 2012. The mean daily temperature, sunshine hours and the total amount of precipitation received in each season are given in Supplemental Table 1.

**Table 1. Rice genotypes screened for phosphorous deficiency tolerance.**

Genotype	Type	Condition	Important characteristic
Suwandel	L	Upland	White pericarp, exquisite aroma, special milky taste (ideal choice for festive occasions)
Murungakayan	L	Upland	Resistant to stem borer and blast, susceptible to lodging
Sudubalawee	L	Upland	Resistant to gall midge (biotype I), brown planthopper, blast and bacterial blight
Suduheenati	L	Upland	Antioxidant properties, resistant to blast
Hondarawala	L	Upland	Resistant to blast and brown planthopper
Marss	L	Upland	Red pericarp, good eating quality, moderately resistant to blast and bacterial blight
Kokuwellai	L	Upland	Wide adaptability
Kaluheenati	L	Upland	High nutritional and medicinal values
Pokkali	L	Upland	Resistant to brown planthopper and tolerant to salinity
Rathuheenati	L	Upland	High medicinal values and resistant to brown planthopper
Rathel	L	Upland	High nutritional and medicinal values
Sudurusamba	L	Upland	Resistant to brown planthopper and blast
H10	OI	Lowland	Red pericarp
H4	OI	Lowland	Red pericarp, wide adaptability and resistant to blast
H7	OI	Lowland	Good grain quality
At 353	NI	Lowland	Red pericarp, suitable for parboiling, moderately resistant to blast and bacterial blight, tolerant to salinity
At 306	NI	Lowland	Red pericarp, similar with Basmati like grain quality with long seeds, moderately resistant to blast and bacterial blight, and tolerant to salinity
At 362	NI	Lowland	Red pericarp, moderately resistant to brown planthopper, blast and bacterial blight
At 354	NI	Lowland	White pericarp, tolerant to salinity and resistant to lodging
Bg 403	NI	Lowland	White pericarp, resistant to blast and bacterial blight
Bg 250	NI	Lowland	White pericarp, ultra-short in maturity and resistant or moderately resistant to brown planthopper and blast
Bg 358	NI	Lowland	High yielding, small grains, resistant to brown planthopper, blast, bacterial blight and moderately tolerant to iron toxicity
Bg 357	NI	Lowland	High yielding, resistant to brown planthopper, gall midge (biotypes I and II), moderately resistant to thrips, resistant to blast and moderately resistant to iron toxicity
Bg 300	NI	Lowland	High yielding, early maturity, higher adaptability, resistant to gall midge (biotype I), brown planthopper, blast and bacterial blight
Bg 450	NI	Lowland	Resistant to gall midge (biotype I)
Bg 94-1	NI	Lowland	White pericarp, high yielding and suitable for parboiling
Bg 352	NI	Lowland	White pericarp, intermediate sized bold type grains, high yielding, early maturity, wide adaptability and resistant to blast and brown planthopper
Bg 379-2	NI	Lowland	Resistant to brown planthopper, bacterial blight and iron toxicity
Bw 364	NI	Lowland	Red pericarp, resistance to gall midge (biotype I) and iron toxicity
Ld 356	NI	Lowland	Short round grains, moderately tolerant to iron toxicity, resistant to seed spotting and gall midge (biotype I)

L, Landrace; OI, Old improved landrace before 1970; NI, New improved landrace after 1970.

Data are from the Database of Rice Varieties, Department of Agriculture, Sri Lanka (2006b).

## Screening for P deficiency tolerance

### Greenhouse trial

In *Yala* season, the greenhouse trial was conducted at RRDI, Sri Lanka, and in *Maha* season, the greenhouse trial was conducted at University of Peradeniya, Sri Lanka. However, the same Ultisol soil collected from RRDI was used for the two greenhouse screening trials. This particular soil was characterized for the very low availability of P (1 mg/kg) and macronutrients (0.02% total nitrogen and 11 mg/kg exchangeable potassium). In addition, the Ultisol soil had the electrical conductivity of 54.8  $\mu$ S/cm, pH of 5.8 and organic matter content of 0.61% (Kumaragamage and Indraratne, 2011; Sirisena and Wanninayake, 2014). A total of 5 kg air-dried and sieved (mesh size of 2 mm) soil was used to fill each plastic pot. Six seedlings of each genotype were transplanted to each pot and only four plants were kept after one week. In total, 11 and

30 rice genotypes were screened in *Yala* and *Maha* seasons, respectively. The basal application of fertilizer comprised 30 mg urea, 50 mg K<sub>2</sub>O and 5 mg ZnSO<sub>4</sub> based on the recommendations provided by DOA, Sri Lanka. Two levels of P were maintained in the greenhouse trials, no application of P fertilizer (designated as P<sub>0</sub>) and application of P fertilizer as recommended by DOA (30 mg P<sub>2</sub>O<sub>5</sub> per 1 kg Ultisol soil, designated as P<sub>30</sub>). Four replicates of each rice genotype were used with the two levels of P in a layout of the completely randomized design. The other management practices such as watering, pest and disease management were conducted according to the recommendations provided by DOA, Sri Lanka.

### Field trial

A unique field space available at RRDI, Sri Lanka was used for the trial. This particular block of field has been maintained without fertilizer in last 40 years.

Seedlings were transplanted according to a straight row method. Randomized complete block design was used with four plots (i.e. replicates). A total of 11 and 20 rice genotypes were screened in *Yala* and *Maha* seasons, respectively.

### Data collection

#### Growth parameters

Plant height was measured from the base of the plant to the tip of the top leaf. Plant height and number of tillers per plant were collected at three growth stages, early vegetative stage (three weeks after transplanting), late vegetative stage (six weeks after transplanting) and flowering stage (immediately after the emergence of first panicle).

Harvested shoots in each pot were collected at the flowering stage to investigate shoot dry weight (SDW). Shoots were washed with distilled water and oven dried at 60 °C until a constant dry weight (g/pot).

#### P efficiency indicators

Oven-dried shoots were crushed into fine particles. A total of 0.2 g powdered shoots was taken and digested with a mixture of HNO<sub>3</sub>, HClO<sub>4</sub> and H<sub>2</sub>SO<sub>4</sub> in a ratio of 1:3:1. Shoot P concentration (SPC) was measured as the amount of P (mg) in 1 g shoot dry matter using the phosphovanadate method (Hanson, 1950).

Shoot P uptake (SPU) (mg/pot) was calculated collectively for whole four plants grown in the pot according to Fageria et al (1988a) and Gunes et al (2006).

$$SPU = SDW \times SPC$$

P utilization efficiency (PUE) was calculated as the biomass produced per unit P accumulated in shoot tissue (Hedley et al, 1994; Rose and Wissuwa, 2012).

$$PUE = SDW / SPU = 1 / SPC$$

### Data analysis

All the tested parameters were analyzed using Proc GLM in SAS (SAS Institute, Cary, NC, USA). The correlation between parameters was calculated using CORR procedure in SAS. Regression analyses between SDW and PUE under P<sub>0</sub> and P<sub>30</sub> conditions in *Maha* season were separately conducted. Principal component analysis (PCA) followed by clustering using the principal components (PCs) were done separately for the parameters measured under P<sub>0</sub> and P<sub>30</sub> conditions using the statistical package Minitab 16 (Minitab Inc. USA). The dendrograms were constructed using the algorithms of complete linkage and Euclidean distance.

## RESULTS

### Plant height

Plant heights of rice genotypes were compared under two P regimes in greenhouse and field conditions in *Yala* and *Maha* seasons, Sri Lanka in 2012. In *Yala* season, average plant height at the early vegetative stage was significantly higher under P<sub>30</sub> condition (33.20 cm) compared to that under P<sub>0</sub> condition (28.25 cm) in the greenhouse ( $P < 0.05$ ) (Table 2). However, at the late vegetative stage, the trend was reversed, in which, average plant height of the genotypes under P<sub>0</sub> was significantly higher (58.11 cm) than that under P<sub>30</sub> conditions (46.93 cm) ( $P < 0.05$ ). At the flowering stage, 9 out of the 11 genotypes grown in greenhouse under P<sub>0</sub> conditions were higher than the same genotypes grown under P<sub>30</sub> conditions. At the vegetative stages, plants grown under P<sub>0</sub> field conditions were significantly higher compared to those under greenhouse conditions.

In *Maha* season, at the early vegetative stage, except the genotypes Suduheenati, Murungakayan, Ld 356, At 354 and Sudurusamba, the other genotypes grown in greenhouse under P<sub>0</sub> conditions were higher than the plants grown in greenhouse under P<sub>30</sub> conditions (Table 2). At the late vegetative stage, all plants under P<sub>0</sub> conditions were higher than those under P<sub>30</sub> conditions. Average plant heights at early and late vegetative stages were significantly higher (35.77 and 44.42 cm, respectively) under P<sub>0</sub> condition compared to those under P<sub>30</sub> condition (33.98 and 39.22 cm) in the greenhouse. However, at the flowering stage, the trend was reversed, in which average plant height of the genotypes under P<sub>30</sub> was significantly higher (76.35 cm) than that (73.89 cm) under P<sub>0</sub> conditions ( $P < 0.05$ ).

### Number of tillers per plant

In *Yala* season, greenhouse conditions, average number of tillers per plant at early and late vegetative stages were significantly higher under P<sub>0</sub> conditions (2.6 and 3.1, respectively) than those under P<sub>30</sub> conditions (1.8 and 2.3, respectively) ( $P < 0.05$ ) (Table 3). At the flowering stage, the average number of tillers per plant under P<sub>30</sub> (4.2) was significantly less than that (4.5) under P<sub>0</sub> conditions ( $P < 0.05$ ).

Genotypes in *Maha* season exhibited reduced overall tillering ability compared to that in *Yala* season. In *Maha* season all genotypes responded to applied P by exhibiting higher average number of

**Table 2. Plant height of the tested rice genotypes.**

Season	Genotype	Early vegetative stage			Late vegetative stage			Flowering stage		
		P <sub>0</sub> GH	P <sub>0</sub> F	P <sub>30</sub> GH	P <sub>0</sub> GH	P <sub>0</sub> F	P <sub>30</sub> GH	P <sub>0</sub> GH	P <sub>0</sub> F	P <sub>30</sub> GH
<i>Yala</i>	H4	34.69 a	65.01 a	41.00 a	67.69 a	70.43 b	58.12 a	81.16 a	73.31 d	80.13 a
	Suduheenati	34.38 a	70.75 a	38.43 b	74.63 a	79.37 a	56.94 a	97.50 a	85.63 b	75.50 a
	H10	34.88 a	65.13 a	41.06 a	68.17 a	85.56 a	59.62 a	83.43 a	87.19 b	76.68 a
	Murungakayan	35.19 a	60.50 a	36.50 b	72.48 a	67.50 b	51.06 a	88.31 a	69.25 a	71.83 a
	H7	35.50 a	66.62 a	40.12 a	67.94 a	89.00 a	55.25 a	89.84 a	95.13 a	84.94 a
	Bg 403	17.63 b	56.81 b	27.34 d	42.02 b	69.31 b	39.62 b	55.53 c	72.19 d	58.50 a
	Bg 358	23.44 b	57.68 b	28.88 d	51.75 b	70.75 b	41.80 b	67.39 b	79.31 c	55.23 a
	Bg 379-2	22.06 b	52.00 b	26.88 d	38.35 b	57.06 c	35.19 b	46.14 c	64.31 d	47.79 a
	Bg 352	25.81 b	52.00 b	25.69 d	53.00 b	67.25 b	36.69 b	63.53 b	69.63 d	51.19 a
	Bg 357	24.88 b	43.50 c	31.75 c	48.69 b	44.55 d	42.00 b	58.10 b	46.75 e	53.53 a
	At 354	22.25 b	40.75 c	27.50 d	54.46 b	42.75 d	39.94 b	61.66 b	45.50 e	52.80 a
	<i>Maha</i>	H4	46.54 a	43.13 a	44.54 a	65.08 a	59.63 a	56.13 a	102.58 a	70.69 c
Marss		30.38 c	35.75 b	29.42 b	41.63 b	44.69 b	38.25 b	76.00 c	61.00 c	75.90 c
Suduheenati		42.88 a	43.63 a	43.71 a	53.08 a	62.94 a	44.83 a	91.25 b	71.50 c	89.25 a
H10		50.25 a	43.38 a	42.00 a	69.21 a	56.81 a	48.67 a	109.17 a	94.00 a	91.42 a
Rathel		35.08 c	–	30.92 b	52.63 a	–	41.13 b	94.73 b	–	82.91 b
Kaluheenati		46.50 a	–	45.63 a	61.46 a	–	54.88 a	90.82 b	–	91.80 a
Murungakayan		43.96 a	45.31 a	46.42 a	53.54 a	62.75 a	53.42 a	85.00 b	80.63 b	92.00 a
Kokuwellai		31.83 c	–	27.79 b	44.38 b	–	35.67 b	73.50 c	–	66.17 c
H7		46.33 a	43.13 a	42.71 a	59.58 a	60.88 a	50.29 a	92.83 b	94.81 a	92.92 a
Sudubalawee		46.71 a	44.63 a	45.71 a	55.38 a	63.63 a	47.33 a	94.58 b	102.69 a	93.58 a
Bg 94-1		31.33 c	31.13 b	29.08 b	37.54 b	44.50 b	33.42 b	53.92 e	66.69 c	59.50 d
Bg 403		29.63 c	32.50 b	27.75 b	36.46 b	43.31 b	30.88 b	64.92 d	63.19 c	59.58 d
At 362		33.75 c	35.06 b	32.21 b	41.75 b	50.13 b	37.83 b	77.58 c	71.06 c	74.58 c
Pokkali		43.38 a	–	42.58 a	53.79 a	–	48.17 a	93.83 b	–	95.83 a
Bg 358		27.42 c	32.13 b	22.88 c	30.42 b	46.94 b	28.38 b	61.42 d	66.00 c	66.92 c
Bg 450		23.88 c	30.75 b	22.50 c	25.71 c	44.69 b	25.18 b	65.45 d	52.44 d	63.18 c
Sudurusamba		31.25 c	–	31.79 b	35.92 b	–	35.92 b	60.75 d	–	83.83 b
Bg 379-2		28.33 c	30.94 a	24.92 b	38.50 b	41.19 b	27.38 b	42.58 f	52.88 d	52.25 e
Hondarawala		46.46 a	45.19 a	45.58 a	51.00 a	69.44 a	56.63 a	74.33 c	96.56 a	95.08 a
Bg 352		31.35 c	31.38 b	28.88 b	36.63 b	43.13 b	29.92 b	63.42 d	68.94 c	64.67 c
Bg 250		29.92 c	–	29.42 b	41.63 b	–	32.79 b	68.25 d	–	68.33 c
Bw 364		31.54 c	–	28.00 b	37.67 b	–	33.46 b	64.75 d	–	62.58 c
At 353		31.54 c	33.31 b	26.00 b	34.92 b	48.81 b	29.17 b	66.25 d	79.69 b	69.00 c
Suwandel		37.58 b	–	36.00 b	44.38 b	–	39.42 b	73.67 c	–	87.55 a
Ld 356		31.58 c	–	32.71 b	40.92 b	–	36.71 b	62.17 d	–	73.92 c
Rathuheenati		46.92 a	–	46.67 a	56.30 a	–	46.50 a	77.09 c	–	83.10 b
Bg 357		27.88 c	30.44 b	26.96 b	33.25 b	43.06 b	29.83 b	58.08 d	61.19 c	58.83 d
At 306		31.50 c	36.63 b	29.46 b	36.96 b	47.06 b	35.50 b	60.50 d	62.44 c	69.63 c
At 354		25.21 c	33.81 b	25.75 b	28.42 c	48.00 b	32.50 b	54.08 e	70.94 c	61.92 c
Bg 300		32.04 c	35.69 b	31.29 b	34.58 b	47.00 b	36.29 b	63.08 d	73.50 c	70.36 c

GH, Greenhouse; F, Field.

Means denoted by the same letters within each column for the same season are not significantly different at  $P < 0.05$ . The dashes (-) indicate that data are not available for these genotypes under the phosphorus levels and seasons mentioned.

tillers per plant under P<sub>30</sub> (3.1) than P<sub>0</sub> (1.6) ( $P < 0.05$ ) (Table 3).

### Shoot dry weight

In *Yala* season, mean SDW was higher under P<sub>30</sub> soils (22.37 g/pot) compared to those under P<sub>0</sub> soils in the greenhouse (20.34 g/pot) and the field (16.74 g/pot) (Table 4). Likewise, in *Maha* season, the mean SDW was higher (21.04 g/pot) under P<sub>30</sub> soils compared to those under P<sub>0</sub> soils in the greenhouse (16.33 g/pot) and the field (18.30 g/hill) ( $P < 0.05$ ). In both *Yala* and *Maha* seasons, the highest SDW was shown by the genotype H4, whereas, the lowest SDW was

observed by the genotype Bg 357 under greenhouse conditions. In addition, the genotype Marss showed extremely higher SDW under field and P<sub>0</sub> conditions in *Maha* season and Suduheenati also showed higher SDW in both seasons.

### Shoot phosphorus concentration

In *Yala* season, average SPC was higher (0.76 mg/g) under P<sub>30</sub> soils compared to those under P<sub>0</sub> soils in the greenhouse (0.59 mg/g) and the field (0.47 mg/g) (Table 4). Equally, in *Maha* season, average SPC was higher (0.79 mg/g) under P<sub>30</sub> soils compared to those under P<sub>0</sub> soil in the greenhouse (0.65 mg/g) and the

**Table 3. Mean number of tillers per plant of the tested rice genotypes.**

Season	Genotype	Early vegetative stage			Late vegetative stage			Flowering stage			
		P <sub>0</sub> GH	P <sub>0</sub> F	P <sub>30</sub> GH	P <sub>0</sub> GH	P <sub>0</sub> F	P <sub>30</sub> GH	P <sub>0</sub> GH	P <sub>0</sub> F	P <sub>30</sub> GH	
<i>Yala</i>	H4	3.3 b	2.1 a	2.3 a	3.6 b	2.6 b	2.9 b	4.4 c	2.7 b	3.7 c	
	Suduheenati	1.6 c	1.7 c	1.1 d	3.1 b	2.0 c	1.3 c	5.2 a	2.2 b	4.3 c	
	H10	2.1 c	1.7 c	2.0 b	2.3 c	1.9 c	2.3 b	2.7 f	2.0 b	4.2 c	
	Murungakayan	2.0 c	1.7 b	1.6 c	2.5 c	2.0 c	1.6 c	5.1 b	2.3 b	4.7 c	
	H7	2.3 c	2.2 a	1.9 b	3.2 b	2.2 b	2.2 b	4.7 b	2.6 b	3.8 c	
	Bg 403	2.0 c	2.3 a	2.5 a	2.7 c	2.7 a	3.9 a	4.6 c	3.2 a	6.4 a	
	Bg 358	2.3 c	1.7 c	1.4 d	3.1 b	2.1 c	2.2 b	4.9 b	2.1 b	4.1 c	
	Bg 379-2	1.8 c	1.8 b	2.2 a	2.1 c	2.3 b	2.5 b	4.1 d	2.5 b	3.3 c	
	Bg 352	3.4 b	1.7 b	1.3 d	3.5 b	2.1 b	1.4 c	5.0 b	2.2 b	3.3 c	
	Bg 357	3.1 b	1.9 b	1.8 b	3.4 b	1.9 c	2.7 b	3.8 e	2.1 b	2.8 d	
	At 354	4.2 a	1.9 b	2.2 a	4.6 a	2.0 c	2.8 b	4.9 b	2.2 b	5.8 b	
	<i>Maha</i>	H4	1.1 b	2.2 a	1.2 d	2.1 b	2.7 a	3.1 d	2.1 b	2.8 a	3.1 d
		Marss	1.0 c	2.3 a	2.0 b	1.7 c	2.7 a	4.5 b	1.7 c	2.9 a	4.5 b
Suduheenati		1.0 c	1.6 d	1.3 d	1.3 d	1.9 c	3.6 c	1.3 d	2.0 c	3.6 c	
H10		1.2 b	1.7 c	1.5 c	2.2 b	2.0 c	2.8 d	2.2 b	2.1 c	2.8 d	
Rathel		1.0 c	-	1.4 c	1.5 d	-	2.7 d	1.5 d	-	2.7 d	
Kaluheenati		1.0 c	-	1.8 b	1.8 c	-	2.6 d	1.8 c	-	2.6 d	
Murungakayan		1.0 c	1.7 c	1.1 d	1.0 e	2.0 c	2.8 d	1.0 e	2.1 c	2.8 d	
Kokuwellai		1.2 b	-	1.9 b	3.3 a	-	4.3 b	3.3 a	-	4.3 b	
H7		1.0 c	2.2 a	1.6 b	1.6 c	2.2 b	2.2 d	1.6 c	2.5 b	2.2 d	
Sudubalawee		1.0 c	1.6 d	1.4 c	1.0 e	1.6 d	2.6 d	1.0 e	1.7 d	2.6 d	
Bg 94-1		1.5 a	2.5 a	2.8 a	3.1 a	2.6 a	5.3 a	3.1 a	2.7 b	5.3 a	
Bg 403		1.0 c	2.3 a	1.7 b	1.9 c	3.0 a	4.1 b	1.9 c	3.0 a	4.1 b	
At 362		1.0 c	1.4 e	1.2 d	1.7 c	1.7 d	2.9 d	1.7 c	1.8 d	2.9 d	
Pokkali		1.0 c	-	1.0 d	1.0 e	-	2.0 d	1.0 e	-	2.0 d	
Bg 358		1.0 c	1.7 c	1.3 d	1.1 e	2.0 c	3.5 c	1.1 e	2.1 c	3.5 c	
Bg 450		1.0 c	1.8 b	1.7 b	1.3 d	2.4 b	3.4 c	1.3 d	2.5 b	3.4 c	
Sudurusamba		1.0 c	-	1.1 d	1.3 d	-	2.8 d	1.3 d	-	2.8 d	
Bg 379-2		1.0 c	1.9 b	1.5 c	1.8 c	2.4 b	3.3 c	1.8 c	2.5 b	3.3 c	
Hondarawala		1.0 c	1.6 d	1.1 d	1.3 d	1.8 c	2.7 d	1.3 d	1.9 c	2.7 d	
Bg 352		1.0 c	1.8 b	1.3 d	1.8 c	2.1 b	2.6 d	1.8 c	2.2 c	2.6 d	
Bg 250		1.0 c	-	1.2 d	1.9 c	-	3.2 c	1.9 c	-	3.2 c	
Bw 364		1.0 c	-	1.6 b	1.6 c	-	4.0 b	1.6 c	-	4.0 b	
At 353		1.0 c	1.9 b	1.2 d	1.1 e	2.2 b	2.1 d	1.1 e	2.4 b	2.1 d	
Suwandel		1.0 c	-	1.0 d	1.1 e	-	2.3 d	1.1 e	-	2.3 d	
Ld 356		1.0 c	-	1.1 d	1.4 d	-	2.8 d	1.4 d	-	2.8 d	
Rathuheenati		1.0 c	-	1.2 d	1.3 d	-	2.3 d	1.3 d	-	2.3 d	
Bg 357		1.0 c	1.7 c	1.4 c	1.0 e	1.9 c	3.4 c	1.0 e	2.0 c	3.4 c	
At 306		1.0 c	1.5 d	1.7 b	1.3 d	1.8 c	3.3 c	1.3 d	1.8 c	3.3 c	
At 354		1.2 b	1.9 b	1.6 c	1.1 e	2.1 c	4.0 b	1.1 e	2.2 c	4.0 b	
Bg 300		1.0 c	1.8 b	1.2 d	1.4 d	1.8 c	2.7 d	1.4 d	1.9 c	2.7 d	

GH, Greenhouse; F, Field.

Means denoted by the same letters within each column for the same season are not significantly different at  $P < 0.05$ . The dashes (-) indicate that data are not available for these genotypes under the phosphorus levels and seasons mentioned.

field (0.64 mg/g) ( $P < 0.05$ ). In *Yala* season, the highest SPC was shown by Suduheenati and the lowest SPC were shown by H10 and Bg 357, and in *Maha* season, the highest SPC was shown by At 306, whereas the lowest SPC was shown by Sudurusamba.

### Shoot phosphorus uptake

In *Yala* season, SPU was higher (16.88 mg/pot) under P<sub>30</sub> soils compared to those under P<sub>0</sub> soils in the greenhouse (13.09 mg/pot) and the field (7.74 mg/hill) (Table 4). Similarly, in *Maha* season, SPU was higher (16.09 mg/pot) under P<sub>30</sub> soils compared to those under P<sub>0</sub> soils in the greenhouse (10.30 mg/pot) and

the field (15.24 mg/hill) ( $P < 0.05$ ). In *Yala* season, the highest SPU was shown by Bg 358, and the lowest SPU was shown by Bg 357 and in *Maha* season, the highest SPU was shown by Marss, whereas, the lowest SPU were shown by Suwandel and At 353.

### Phosphorus utilization efficiency

In *Yala* season, PUE was higher under P<sub>0</sub> soils in both greenhouse (1.71 g/mg) and field conditions (2.26 g/mg) compared that under P<sub>30</sub> soils in the greenhouse (1.38 g/mg) (Table 4). Similarly, in *Maha* season, PUE was higher under P<sub>0</sub> soils in both greenhouse (1.64 g/mg) and field conditions (1.65 g/mg)

**Table 4. Shoot dry weight (SDW), shoot phosphorus concentration (SPC) and shoot phosphorus uptake (SPU), phosphorus utilization efficiency (PUE) of the tested rice genotypes under studied conditions.**

Season	Genotype	SDW (g/pot)			SPC (mg/g)			SPU (mg/pot)			PUE (g/mg)		
		P <sub>0</sub> GH	P <sub>0</sub> F	P <sub>30</sub> GH	P <sub>0</sub> GH	P <sub>0</sub> F	P <sub>30</sub> GH	P <sub>0</sub> GH	P <sub>0</sub> F	P <sub>30</sub> GH	P <sub>0</sub> GH	P <sub>0</sub> F	P <sub>30</sub> GH
<i>Yala</i>	H4	29.80 a	11.95 c	33.93 a	0.65 b	0.68 a	0.50 e	15.70 a	8.12 b	21.80 a	1.50 c	1.50 c	2.00 a
	Suduheenati	18.15 c	21.18 a	19.50 c	0.75 a	0.45 c	1.04 a	10.10 d	9.53 b	22.90 a	1.30 d	2.20 b	1.00 b
	H10	14.20 d	9.83 c	17.55 c	0.47 d	0.60 b	0.85 c	8.10 e	5.89 c	19.80 b	2.10 a	1.70 c	1.20 b
	Murungakayan	20.40 c	11.20 c	24.90 b	0.53 c	0.38 d	0.88 c	12.00 c	4.25 d	15.00 d	1.90 b	2.60 b	1.10 b
	H7	24.10 b	11.88 c	21.00 c	0.64 b	0.29 e	0.59 d	16.50 a	3.44 d	17.20 c	1.60 b	3.40 a	1.70 a
	Bg 403	24.90 b	21.28 a	27.20 b	0.59 b	0.55 b	0.55 d	16.20 a	11.70 a	17.10 c	1.70 b	1.80 c	1.80 a
	Bg 358	13.00 d	16.08 b	15.10 c	0.69 b	0.45 c	0.79 c	18.60 a	7.23 b	12.00 e	1.40 c	2.20 b	1.30 b
	Bg 379-2	26.50 b	18.75 b	28.20 b	0.68 b	0.52 b	0.61 d	13.70 b	9.75 b	17.90 c	1.50 c	1.90 c	1.60 a
	Bg 352	22.80 b	15.40 b	25.00 b	0.55 c	0.48 c	0.91 b	14.70 b	7.39 b	12.50 e	1.80 b	2.10 b	1.10 b
	Bg 357	10.40 d	23.60 a	12.50 d	0.47 d	0.29 e	0.82 c	7.80 e	6.84 c	19.30 b	2.10 a	3.40 a	1.20 b
At 354	19.50 c	23.00 a	21.20 c	0.52 c	0.48 c	0.85 c	10.60 d	11.04 a	10.20 e	1.90 b	2.10 b	1.20 b	
<i>Maha</i>	H4	32.00 a	18.56 b	25.93 a	0.50 c	0.65 c	0.52 d	16.00 b	16.10 c	13.48 c	2.00 b	1.50 c	1.90 b
	Marss	24.93 b	38.12 a	26.87 a	0.77 b	0.61 c	0.50 d	19.20 a	31.00 a	13.44 c	2.00 b	1.60 c	1.20 c
	Suduheenati	23.61 b	20.34 b	28.27 a	0.49 c	0.81 a	0.85 b	11.57 c	22.00 b	24.03 a	2.00 b	1.20 d	1.20 c
	H10	24.01 b	14.59 c	16.66 c	0.56 c	0.59 c	0.70 c	13.45 c	11.50 d	11.66 d	1.80 c	1.70 c	1.40 c
	Rathel	23.32 b	–	25.32 a	0.70 b	–	0.46 d	16.32 b	–	11.65 d	2.10 b	–	1.00 d
	Kaluheenati	22.05 b	–	25.11 a	0.59 c	–	0.83 b	13.01 c	–	20.84 a	1.40 d	–	1.30 c
	Murungakayan	21.03 b	25.23 b	28.07 a	0.50 c	0.51 d	0.60 c	10.52 c	17.10 c	16.84 b	2.00 b	2.00 b	1.70 b
	Kokuwellai	20.05 b	–	21.59 b	0.51 c	–	0.75 c	10.23 c	–	16.19 b	1.70 c	–	1.10 d
	H7	18.44 b	17.41 b	18.25 b	0.72 b	0.85 a	0.76 c	13.28 c	19.70 b	13.87 c	1.40 d	1.20 d	1.30 c
	Sudubalawee	18.43 b	14.43 c	21.87 b	0.59 c	0.43 d	0.90 b	10.87 c	8.30 f	19.68 a	0.80 d	2.30 a	0.80 e
	Bg 94-1	17.40 c	15.51 c	20.62 b	0.79 b	0.51 c	0.65 c	13.75 c	10.40 e	13.40 c	2.00 b	2.00 b	1.30 c
	Bg 403	17.49 c	19.13 b	27.08 a	0.52 c	0.50 c	0.83 b	9.09 d	12.80 d	22.48 a	1.90 c	2.00 b	1.20 c
	At 362	17.09 c	20.31 b	28.06 a	0.70 b	0.40 d	0.54 d	11.96 c	10.80 e	15.15 b	1.00 d	2.50 a	1.00 d
	Pokkali	15.80 d	–	19.62 b	0.70 b	–	0.76 c	11.06 c	–	14.91 b	2.40 a	–	1.10 d
	Bg 358	15.20 d	16.19 c	22.53 b	0.72 b	0.48 d	0.53 d	10.94 c	10.40 e	11.94 d	1.40 d	2.10 b	1.90 b
	Bg 450	13.87 d	20.93 b	24.37 a	0.64 b	0.81 a	0.75 c	8.88 d	12.60 d	18.28 b	1.30 d	1.20 d	2.00 a
	Sudurusamba	14.51 d	–	20.28 b	0.41 c	–	0.88 b	5.95 e	–	17.85 b	1.30 d	–	1.00 d
	Bg 379-2	14.72 d	23.79 b	20.65 b	0.70 b	0.68 c	0.82 b	10.30 c	21.60 b	16.93 b	1.40 d	1.50 c	1.20 c
	Hondarawala	13.22 d	15.57 c	24.20 a	0.52 c	0.49 d	0.56 d	6.87 e	10.20 e	13.55 c	1.70 c	2.00 b	1.20 c
	Bg 352	12.38 d	16.28 c	16.13 c	0.48 c	0.79 a	0.94 b	5.94 e	17.10 c	15.16 b	2.10 b	1.30 d	1.10 d
	Bg 250	13.18 d	–	14.62 c	0.76 b	–	1.03 b	10.02 c	–	15.06 b	1.30 d	–	1.50 c
	Bw 364	13.14 d	–	21.38 b	0.52 c	–	0.93 b	6.83 e	–	19.88 a	1.60 c	–	1.30 c
	At 353	11.43 d	15.19 c	15.17 c	0.49 c	0.76 b	0.63 c	5.60 e	15.40 c	9.56 e	1.40 d	1.30 d	1.90 b
	Suwandel	10.63 d	–	15.71 c	0.48 c	–	0.98 b	5.10 e	–	15.40 b	1.80 c	–	1.00 d
	Ld 356	11.34 d	–	22.70 b	0.56 c	–	0.98 b	6.35 e	–	22.25 a	1.40 d	–	2.20 a
	Rathuheenati	11.29 d	–	17.45 c	0.51 c	–	0.86 b	5.76 e	–	15.01 b	1.90 c	–	1.10 d
	Bg 357	7.70 e	14.51 c	17.60 c	1.09 a	0.83 a	0.95 b	8.39 d	15.40 c	16.72 b	0.90 d	1.20 d	1.10 d
At 306	8.72 e	11.61 d	12.07 d	1.25 a	0.74 b	1.26 a	10.90 c	16.00 c	15.21 b	2.00 b	1.40 c	1.60 c	
At 354	11.19 d	14.76 c	18.83 b	0.81 b	0.75 b	0.98 b	9.06 d	11.50 d	18.45 b	1.20 d	1.30 d	1.00 d	
Bg 300	11.63 d	13.50 c	14.20 c	1.01 a	0.64 c	0.98 b	11.75 c	14.80 c	13.92 c	1.90 c	1.60 c	1.80 b	

GH, Greenhouse, F, Field.

Means denoted by the same letters within each column are not significantly different at  $P < 0.05$ . The dashes (-) indicate that data are not available for these genotypes under the phosphorus levels and seasons mentioned.

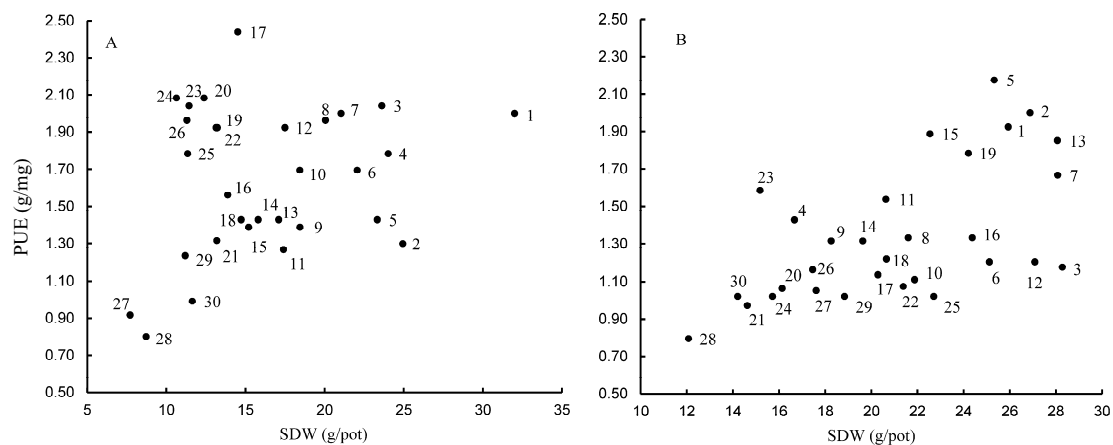
compared to that under P<sub>30</sub> soils in the greenhouse (1.35 g/mg) ( $P < 0.05$ ). In *Yala* season, in P<sub>30</sub> conditions, the highest PUE were resulted by H4 and Bg 403 and the lowest PUE was shown by Suduheenati, and in *Maha* season, in P<sub>30</sub> conditions, the highest PUE was shown by Bg 450 and Ld 356, whereas the lowest PUE was given by Sudubalawee.

A summary of the rice genotypes that exhibited the highest and lowest trait values for Plant height, number of tillers per plant, SDW, SPC, SPU and PUE are given in the Supplemental Table 2. For plant height, number of tillers per plant, SDW, SPU and PUE, the highest ranked (top five) genotypes could be

considered as the tolerant genotypes and the lowest ranked (bottom five) genotypes could be considered as the sensitive genotypes for P deficiency. For SPC, the highest ranked (top five) genotypes could be considered as the sensitive genotypes and the lowest ranked (bottom five) genotypes could be considered as the tolerant genotypes for P deficiency.

### Principal component and cluster analyses

The parameters measured under P<sub>0</sub> and P<sub>30</sub> conditions for the 30 rice genotypes tested in *Maha* season in 2012 were subjected to PCA. Two dendrograms (separately for P<sub>0</sub> and P<sub>30</sub>) were developed based on



**Fig. 1. Classification of 30 rice genotypes for shoot dry weight (SDW) and phosphorus utilization efficiency (PUE) under  $P_0$  (A) and  $P_{30}$  (B) soil conditions in Maha season, 2012.**

SDW, Shoot dry weight; PUE, Phosphorus utilization efficiency; 1, H4; 2, Marss; 3, Suduheenati; 4, H10; 5, Rathel; 6, Kaluheenati; 7, Murungakayan; 8, Kokuwellai; 9, H7; 10, Sudubalawee; 11, Bg 94-1; 12, Bg 403; 13, At 362; 14, Pokkali; 15, Bg 358; 16, Bg 450; 17, Sudurusamba; 18, Bg 379-2; 19, Hondarawala; 20, Bg 352; 21, Bg 250; 22, Bw 364; 23, At 353; 24, Suwandel; 25, Ld 356; 26, Rathuheenati; 27, Bg 357; 28, At 306; 29, At 354; 30, Bg 300.

the first four PCs. Under  $P_0$  conditions, five clusters were obtained at about 55% of similarity (Supplemental Fig. 1). Under  $P_{30}$  conditions, four clusters were obtained at about 55% of similarity and the previously clustered genotypes under  $P_0$  got clustered in mixtures (Supplemental Fig. 2). The eigen value and percentage variance explained by each of the four main PCs derived from PCA are separately given for  $P_0$  and  $P_{30}$  levels in Supplemental Table 3.

When PUE was plotted against SDW for the genotypes under  $P_0$  and  $P_{30}$  conditions separately (Fig. 1), an interesting pattern of grouping was identified. When independent culling levels (16 g/pot for SDW and 1.25 g/mg for PUE) were applied, H4, H10, Marss, Rathel, Kaluheenati, At 362, H7, Bg 94-1, Bg 403, Sudubalawee, Murungakayan, Suduheenati and Kokuwellai were grouped together and the genotype Pokkali was also closely located to that group. The genotype H4 was apparently separated from the rest (Fig. 1-A). It is also interesting to note that the tolerant genotypes identified in Fig. 1-A were also clustered as a group in Supplemental Fig. 1. However, in Supplemental Fig. 1, these tolerant genotypes were in three different clusters and only Bg 403 is separately clustered. The most sensitive genotypes (in comparison to the set culling levels), Bg 300, Bg 357 and At 306, were grouped together (Fig. 1-A) though At 354 was separately clustered and these genotypes also got independently grouped by PCA based cluster analysis (Supplemental Fig. 1). Under  $P_{30}$  conditions, the tested genotypes grouped or clustered in mixtures

without grouping according to the PDT as shown by Fig. 1-B and Supplemental Fig. 2, respectively.

#### Correlation among tested parameters for PDT

Plant height was significantly and positively correlated with SPU (46% to 56%,  $P < 0.05$ ). Under  $P_0$  soil conditions in Maha season, plant height was positively correlated with SDW (71%), but this correlation was not detected under  $P_{30}$  conditions. Similarly, number of tillers per plant was also positively correlated with SDW only in Maha season in  $P_0$  soil (40%) and SPU was correlated with number of tillers per plant in both Yala and Maha seasons in  $P_0$  soil (36% and 63%, respectively). Under  $P_0$  conditions, SPC and SDW were significantly and negatively correlated under greenhouse conditions (-35%) and non-significant negative correlation (-35%) was observed under field conditions. In both seasons, under  $P_0$  soil conditions in greenhouse and field, SDW and SPU were significantly and positively correlated (80%,  $P < 0.001$ ).

## DISCUSSION

The PDT rice genotypes would be immensely important for increasing the productivity with less input (Cordell et al, 2009). In the present study, the landraces which prefer upland conditions were selected because generally they were identified as having relatively robust performance under P deficient soil conditions in other countries (Hedley et al, 1994;



Kirk et al, 1998; George et al, 2001; Fageria and Santos, 2002; Fageria et al, 2003; Chin et al, 2010). The inclusion of landraces, old improved and new improved varieties are useful as it has been always questioned that the new improved varieties do not possess genes/alleles for tolerant traits, because of the intensive selection in breeding for other important agronomical traits (Wissuwa and Ae, 2001; Lafitte et al, 2004). Through screening, tolerant old improved and new improved varieties were found out and they can be used in future studies to discover diverse physiological and molecular mechanisms conferring PDT. Thus, in the present study, a nice comparison was made among landraces, old improved and new improved rice varieties for PDT in Sri Lanka.

It is very important to choose a proper screening procedure for PDT of rice genotypes. Hydroponic systems with precise controlling of P ions along with the other conditions in the solution could be employed for screening (Chaubey et al, 1994; Ni et al, 1998). However, practically the maintenance of accurate hydroponic system is not an easy task. Maintenance of uniform pH by adjusting the buffer condition for the constant osmosis and regular even mixing of solution are always tedious in practise. Chin et al (2011) also pointed out that soil based screening is the most preferred method for PDT screening. The ideal system would be a natural rice growing soil which does not contain P (Chin et al, 2011). They also argued that the absences of  $Al^{3+}$ ,  $Fe^{3+}$  and  $Ca^{2+}$  ions and clay particles in the hydroponic system making it really different from natural soils. Thus, the PDT results obtained from a hydroponic system cannot be used to understand the natural rice growing conditions. However, in the normal soils these binding ions and particles are present in naturally available quantities, which could fix P, making it unavailable or limitedly available for the plants. This expected condition could not be achieved by employing a hydroponic system for PDT screening studies. In almost all the past studies, rice scientists have used soil systems for PDT screening (Fageria et al, 1988a, b; Hedley et al, 1994; Fageria and Baligar, 1997; Wissuwa and Ae, 2001) and it is hard to find a study which used hydroponic systems. In the present study, we successfully employed the PDT screening system proposed by Chin et al (2011) by using the Ultisol soil which has not been fertilized in the last 40 years by the RRDI, Sri Lanka. The P concentration of this Ultisol soil was measured and confirmed accurate for the absence of P

(Kumaragamage and Indraratne, 2011; Sirisena and Wanninayake, 2014).

In Sri Lanka, rice farming is mainly undertaken as two major cropping seasons known as *Yala* and *Maha*. The present study was also conducted as two trials of *Yala* and *Maha* to find out the tolerance of the rice genotypes. Similar multi-season trials for PDT were reported by Wissuwa and Ae (2001) and Wissuwa (2005).

In the present study, as the PDT indicators, plant height, number of tillers per plant, SDW, SPC, SPU and PUE were used and measured at three growth stages (early vegetative stage, late vegetative stage and flowering stage). These indicators were used in the previous studies on PDT (Fageria et al, 1988a, b; Wissuwa and Ae, 2001; Fageria and Knupp, 2013). The results implied that plant height and number of tillers per plant varied significantly ( $P < 0.05$ ) among genotypes and between the two P levels (Tables 2 to 4). Plant height is an important morphological trait for PDT screening as it correlates with dry weight and yield (Fageria and Knupp, 2013). Availability of P significantly affects plant height (Cancellier et al, 2012). According to the present study, at the early vegetative stage, no significant difference in plant height was obtained among the two P levels (Table 2). Hedley et al (1994) indicated that seed P supplies adequate P for early plant growth regardless of the external P conditions. This may be the reason for insignificant difference in plant height between the plants at the two P levels at the early vegetative stage. Moreover, Hedley et al (1994) reported that seeds supply required P for early root development, and seed P content is correlated with SDW and root dry weight up to six weeks of growth in P deficient soils but this phenomenon was not observed in the P sufficient soil. The occurrence of reduced number of tillers per plant was also observed under limited P conditions (Dobermann and Fairhurst, 2000). Number of tillers per plant was higher under  $P_0$  conditions at the initial growth stage, and later at the flowering stage it was lower, indicating the importance of P for continuous tillering. In *Maha* season, tillering was generally low possibly because of the reduced amount of sunlight received by the plants than that in *Yala* season. However, all the genotypes responded to application of P by increasing number of tillers in both seasons.

The highest SDW was shown by H4 under all conditions and one of the parents of H4, Marss also

showed higher SDW, implying the potential involvement of enhancing alleles for PDT in H4 pedigree. SDW is one of the best parameters indicating PDT (Fageria et al, 1988a, b; Wissuwa and Ae, 2001). SPC indicates the concentration of P in shoot tissues and lower SPC indicates higher PUE, hence higher PDT. However, SPU (the combined product of SDW and SPC) better represents PDT (Wissuwa and Ae, 2001). Under  $P_0$  conditions in *Yala* season, Bg 358 showed the highest SPU and in *Maha* season Marss showed the highest SPU. PUE is also an important parameter implying PDT. PUE was going up when P was limitedly available in soil (Table 4). Under  $P_{30}$  conditions, PUE was going down, indicating the importance of applying the minimum required amount of P to the particular rice genotype of choice to get the maximum benefit and to avoid over application. PUE is given by the ratio of SDW and SPU (Rose et al, 2011; Rose and Wissuwa, 2012). However, SPU is given by the product of SDW and SPC. This simplified to an equation that PUE is equal to the inverse of SPC. In general, when SPC is low, the PUE is high. This means that plants keep less P in the above ground part while mobilizing most of P for its metabolic activities or to the below ground parts. P availability for the rice plants and parameters such as SPU and SDW were found to be correlated with the yield and other harvesting indices (Fageria et al, 1988a; Wissuwa and Ae, 2001; Fageria and Knupp, 2013). Therefore, the assessments of SDW, SPU, SPC and PUE at the vegetative stage would clearly provide an indirect idea of the final performance (i.e. rice yield) of the rice genotypes. This permits the possibility of conducting destructive sampling for P assessment on or before the flowering stage.

It was ambiguous to group the rice genotypes for PDT when single traits that measured at different seasons were considered (Supplemental Table 2). The PCA was employed to remove the collinearity among the PDT parameters. In addition, PCA provides a sort of an index to identify the overall degree of PDT for each genotype. Therefore, a cluster procedure and independent culling levels of SDW and PUE were applied to the rice genotypes generated in *Maha* season under greenhouse conditions. As the independent culling level parameters, SDW and PUE were considered because SDW is the best parameter for PDT and PUE is directly proportional to the PDT. SPU and SPC were not considered as independent culling level parameters because they were related to

SDW and PUE. Plant height and number of tillers per plant were also not used as parameters of culling, because the variability of plant height is determined mainly by genetic factors than the soil P effect (Huang et al, 1996; Yan et al, 1998) and number of tillers per plant is always enhanced by the higher P in soil and the presence of favorable alleles for tillering (Chaubey et al, 1994; Xing and Zhang, 2010). In animal breeding independent culling levels are prominently used for selection (Xu and Muir, 1992). The independent culling levels of SDW and PUE revealed the grouping of tolerant, moderately tolerant and sensitive genotypes for PDT separately (Fig. 1-A). This grouping was consistent with the clusters obtained through PCA (Supplemental Fig. 1). When  $P_{30}$  condition is considered (P is adequately available), the genotypes grouped in mixtures regardless of their PDT, indicating the PDT mechanisms are coming into function when P is limited. In the cluster diagram developed for  $P_0$  soils (Supplemental Fig. 1), H4 and H10 are in a single cluster and all the others are in another cluster, except Bg 403, indicating the presence of potentially different mechanisms for PDT. When SDW was plotted against PUE under  $P_{30}$ , some of the sensitive genotypes were also grouped with the tolerant genotypes (Fig. 1-B), indicating that PDT is active when P is scarce. However, Rathel, H4, Marss, At 362 and Murungakayan exhibited higher SDW while absorbing less P even when P is sufficiently available in the soil. It seems that PCA based on plant height, number of tillers per plant, SDW and SPC followed by the cluster analyses using the first four PCs provide a strong basis for classification of rice genotypes for the degree of PDT. As a cross validation, the regression between SDW and PUE can be considered to identify the standout tolerant and sensitive genotypes for genetic and breeding studies.

Fig. 1-A provides the basis of clustering according to the PDT. We propose this scheme of classification (listed clearly in Table 5) for the selection of varieties or landraces for the organic rice production and for the selection of genotypes for future genetic and breeding studies. The scoring system can also be suggested for the screening of any rice genotype for PDT in the future.

## CONCLUSIONS

It could be concluded that H4, Marss, Suduheenati, H10, Rathel, Kaluheenati, Murungakayan, Kokuwellai,

**Table 5. Proposed scoring scheme for phosphorus deficiency tolerance (PDT) screening of rice genotypes.**

Type	Variety
Tolerant	H4 <sup>a</sup> , Marss, Suduheenati, H10, Rathel, Kaluheenati, Murungakayan, Kokuwellai, H7, Sudubalawee, Bg 94-1, Bg 403, At 362
Moderately tolerant	Pokkali, Bg 358, Bg 450, Sudurusamba, Bg 379-2, Hondarawala, Bg 352, Bg 250, Bw 364, At 353, Suwandel, Ld 356, Rathuheenati
Sensitive	Bg 357 <sup>b</sup> , At 306, At 354, Bg 300

<sup>a</sup> Highest tolerant genotype; <sup>b</sup> Highest sensitive genotype.

Rice genotypes listed in the order of decreasing PDT.

The classification was obtained by conducting regression analysis between shoot dry weight and phosphorus utilization efficiency under P<sub>0</sub> greenhouse condition in *Maha* season (Fig. 1) and cluster analysis based on the four principle components calculated using plant height, number of tillers per plant, shoot dry weight and shoot phosphorus concentration (Supplemental Fig. 1).

H7, Sudubalawee, Bg 94-1, Bg 403 and At 362 are phosphorous deficiency tolerant rice genotypes and Bg 357, At 306, At 354 and Bg 300 are phosphorous deficiency sensitive ones. Pokkali, Bg 358, Bg 450, Sudurusamba, Bg 379-2, Hondarawala, Bg 352, Bg 250, Bw 364, At 353, Suwandel, Ld 356 and Rathuheenati can be considered as moderately tolerant genotypes. H4, Suduheenati, H10 and Rathel can be recommended as tolerant parents and Bg 357, At 306, At 354 and Bg 300 can be recommended as sensitive parents for future breeding programs and genetic studies on PDT to detect potentially novel genetic mechanisms.

## ACKNOWLEDGEMENT

This study was supported by the National Research Council (NRC), Sri Lanka (Grant No. 11-087).

## SUPPLEMENTAL DATA

The following materials are available in the online version of this article at <http://www.sciencedirect.com/science/journal/16726308>; <http://www.ricescience.org>.

Supplemental Table 1. Climatic parameters of the locations and seasons.

Supplemental Table 2. Rice genotypes with highest and lowest recorded trait values for the tested trait.

Supplemental Table 3. Eigen values and the percentage variance explained by the principal components.

Supplemental Fig. 1. Dendrogram constructed based on the principal components computed using the studied traits of PDT resulted under greenhouse conditions, P<sub>0</sub> soil in *Maha* season, 2012.

Supplemental Fig. 2. Dendrogram constructed based on

the principal components computed using studied traits of PDT under greenhouse conditions, P<sub>30</sub> soil in *Maha* season, 2012.

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(Managing Editor: LI Guan)