Quantitative Genetic Assessment of the Phosphorus Deficiency Tolerance in Selected F_2 Progenies of Rice (*Oryza sativa* L.) Genotypes in Sri Lanka

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ABSTRACT: *Phosphorus deficiency (PD) is a limiting factor in rice farming and hence PD tolerant varieties are essential. The PD tolerant quantitative trait loci (QTL) have been identified using the rice landraces Kasalath and Nipponbare. However, PD tolerant Sri Lankan rice landraces or varieties have not been used for QTL mapping. It is important to study the genetics of the PD tolerance associated traits of the locally grown rice genotypes before mapping QTL. Thus, aim of this study was to unravel the genetics of PD tolerant associated traits in segregating progenies generated by crossing PD tolerant and sensitive rice genotypes. Four F_2 progenies (H-4 × Bg 352, Suduheenati × Bg 352, Mas × Bg 352 and Suduheenati × Bg 357) had been created by crossing PD sensitive genotype Bg 352 with PD tolerant genotypes H-4, Suduheenati and Mas and PD sensitive genotype Bg 357 with PD tolerant genotype Suduheenati, respectively. The F_2 seeds from each cross along with the parents were grown in a greenhouse using a P deficient soil. The growth and quantitative colour parameters were measured at flowering stage. The normality, broad sense heritability and heterosis were calculated for each trait. Goodness of fit tests were conducted for colour metrics considering the common epistatic dihybrid ratios. The growth parameters were normally distributed with higher heritability indicating a potential basis for QTL mapping. The colour metrics were not normally distributed and fitting into the epistatic ratios 9:3:4, 9:6:1, 10:3:3 or 12:3:1 implying that they should be controlled by major genes. All the studied traits showed higher heritability estimates. According to this information, it would be logical to map QTL for growth parameters and colour variation occurred due to PD.*

Keywords: *Phosphorus deficiency tolerance, phosphorus efficient rice genotypes, rice landraces*

INTRODUCTION

Rice is arguably the most important plant species on earth. Majority of the human population mostly depend on rice for their daily energy needs (Elert, 2014). Rice is a progenitor species of many of the cereal and grass species and therefore considered as a model organism in plant molecular biology (Shimamoto and Kyozuka, 2002). Rice production is affected by many limiting factors out of which nutrient deficiencies are critical in many parts of the

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world (Dobermann and Fairhurst, 2000). Phosphorus (P) is one of the key plant nutrientsand a major determinant of the productivity of rice (MacDonald *et al*., 2011). Although artificial P application is common, it can lead to many repercussions. P fertilizer is very expensive (Childers *et al*., 2011), more than two billion hectares of farming lands in tropics lack available P for plant growth (Fairhurst *et al*., 1999) because the applied or naturally available P usually gets fixed with Fe^{3+} and Al^{3+} ions (Sample *et al*., 1980). Application of P fertilizer can also cause trivial consequences to the environment (Bennett *et al*., 2001).

The cost effective solutions to combat the problems associated with P limitations for rice productionare extremely important. Rather than working on the application of effective P fertilizer mixtures to rice fields, the development of P efficient or P deficiency (PD) tolerant rice varieties is much more promising as they would require minimal or no application of P fertilizer (Wissuwa and Ae, 2001; Cordell *et al*., 2009). The PD tolerant rice varieties could be produced quickly using Marker Assisted Breeding (MAB) (Alpuerto *et al*., 2009). The genomic resources important for MAB of PD tolerance (PDT) in rice are currently emerging. A major QTL named *Pup1* on rice Chromosome 12 (Heuer *et al*., 2009), a candidate gene named Phosphorus Starvation Tolerance 1 (*PSTOL 1*) (Gamuyao *et al*., 2012) and their allelic / haplotype variants have been recently identified. However, in specific rice growing countries and geographical locations, locally selected and appreciably tolerant landraces to PD could be identified (Wissuwa *et al*., 1998; Aluwihare *et al*., 2016). However, no specific attention has given yet to characterise the QTL or associated genes / genomic regions present in many landraces which could be potentially used in MAB of rice and to identify the genes which confer additional and hither to unknown molecular genetic mechanisms.

Although the identification of QTL associated with the PDT in interesting rice landraces is extremely important, the effectiveness of the attempts to elucidate these secrets is completely depending on the nature of the genetic inheritance and distribution of the associated traits. It is a prerequisite to identify the nature of the distribution, the heritability estimates and awareness about the quantitative or qualitative genetic nature of the traits for efficient QTL mapping studies (Darvasi *et al*., 1993; Doerge and Churchill, 1996). Accurately measured and highly correlated traits to PDT such as plant height (PlH), number of tillers (NT), shoot dry weight (SDW), flag leaf width (FLW) and variation in unusual colour development in leaves can be used as indicative traits of PD tolerance or sensitivity (Sarkar *et al*., 2011; Parentoni *et al*., 2012; Chen *et al*., 2013; Wang *et al*., 2014).

Aluwihare *et al*., (2016) has identified tolerant, moderately tolerant and sensitive rice genotypes for PD which contained landraces and improved varieties in Sri Lanka. The objective of the present study was to identify the inheritance and the distribution patterns of PDT associated traits in F_2 segregating populations created by crossing PD tolerant and sensitive rice genotypes reported in Aluwihare *et al*., (2016) to lay a foundation for future QTL mapping studies to facilitate MAB.

MATERIALS AND METHODS

Plant Material

A total of thirty rice genotypes including 18 varieties developed by Rice Research and Development Institute (RRDI), Bathalagoda, Sri Lanka and 12 landraces were screened under PD soil conditions. The screened rice genotypes were classified into three categories based on the degree of PDT (reported in Aluwihare *et al*., 2016). Four F_2 progenies (H-4 ×

Bg 352, *Suduheenati* × Bg 352, *Mas* × Bg 352 and *Suduheenati* × Bg 357) were generated by crossing PD sensitive genotype Bg 352 with PD tolerant genotypes H-4, *Suduheenati* and *Mas* (also known as *Mas*) and PD sensitive genotype Bg 357 with PD tolerant genotype *Suduheenati*, respectively using clipping method in *Maha* Season, 2012. The F_1 seeds were collected and carefully planted at RRDI. The F_1 plants were examined and any off types due to selfing were discarded. The F_2 seeds from F_1 plants were collected and 75 seeds (i.e. individuals) from each progeny were randomly selected andplanted in a greenhouse (mean temperature of 30 °C and relative humidity of 60%) at University of Peradeniya, Sri Lanka. As the growth medium, ultisol soil was used that was collected from a non-fertilized field at RRDI. This field has been maintained without addition of any fertilizer in last 40 years. This soil was characterised for the presence of very low concentrations of P (1 mg of P in 1 Kg of soil) and other nutrients (Kumaragamage and Indraratne, 2011; Sirisena and Wanninayake, 2014). The standard fertilizer dressings (without P) and other management practices were applied based on the recommendations provided by Department of Agriculture (DOA), Sri Lanka.

Data Collection

Growth Parameters

Plant height (PlH) and number of tillers (NT) were measured at the flowering stage (soon after the expansion of first panicle). The SDW was measured from the aerial parts of plants which were cut and collected at the same flowering stage. Harvested shoots were rinsed with distilled water and dried at 60 °C to acquire a constant dry weight. At the same stage, the flag leaf width (FLW) was also measured at the middle region.

Leaf Colour Measurements

The quantitative colour metrics L*, a*, b*, Chroma (C*) and hue angle (h*) were recorded in four replicates using a colorimeter (CR-10, Konika Minolta, Tokyo, Japan) to capture the colour variation of F_2 plants caused by PD. The metric L* represents the blackness or whiteness where negative values indicate blackness and positive values indicate whiteness. The metric a* represents the redness or greenness where negative values indicate greenness and positive values indicate redness. The metric b* represents the blueness or yellowness where negative values indicate blueness and positive values indicate yellowness. The colour metrics C* and h* were calculated using a* and b* as shown in the following formula. The C* represents the overall colour of an object and h* represents the sharpness or the dullness of that overall colour.

$$(C^* = a^{*2} + b^{*2})^{\frac{1}{2}} \qquad h^* = \tan^{-1}\left(\frac{b^*}{a^*}\right)$$

Data Analysis

Correlation and Normality

The Pearson's Correlation Coefficients (PCC) were calculated among all tested parameters using the statistical package Minitab 16 (Minitab Inc., USA). The normality of the trait distributions was tested using Kolmogorov-Smirnov (KS) Coefficient and the respective skewedness and kurtosis were calculated in Minitab 16. The frequency distribution of each parameter was plotted and the positions of the parental trait values were also marked.

Heritability and Heterosis

The Broad Sense Heritability (BSH) was calculated according to the following formula for each trait using the mean phenotypic variance of the parents (V_{P1} and V_{P2}) as the environmental variance and the variance observed in the F_2 progeny as the total phenotypic variance (V_{F2}). The genotypic variance was estimated as the difference between the total phenotypic variance and the environmental variance.

$$BSH = \frac{V_{F_2} - \frac{(V_{P_1} + V_{P_2})}{2}}{V_{F_2}}$$

The three different forms of Heteosis (H) were considered namely Mid Parental H (MPH), Better Parental H or Heterobeltiosis (BPH) and Worse Parental H (WPH) using the following formula (Falconer and Mackay, 1996; Mukamuhirwa *et al*., 2015).

$$MPH = \frac{F_2 \text{ mean} - \frac{(P_1 \text{ mean} + P_2 \text{ mean})}{2}}{\frac{(P_1 \text{ mean} + P_2 \text{ mean})}{2}}$$

$$BPH = \frac{(F_2 \text{ mean} - P_1 \text{ mean})}{P_1 \text{ mean}}$$

$$WPH = \frac{(F_2 \text{ mean} - P_2 \text{ mean})}{P_2 \text{ mean}}$$

Where P_1 is the better parent and P_2 is the worse parent for a given trait.

Goodness of Fit Analysis for Dihybrid Epistatic Ratios

Colour metrics L*, a*, b*, C* and h* were subjected to goodness of fit analyses based on the chi square (χ^2) tests. The epistatic dihybrid ratios, 9:3:4, 9:6:1, 10:3:3 and 12:3:1 were considered for analyses of the goodness of fit. The total sum of the deviation (i.e. calculated $\chi 2$ value) of the observed ratio from the expected ratio was calculated as given in the following formula.

$$\text{Calculated } \chi 2 \text{ value} = \sum \frac{(\text{observed trait value} - \text{expected trait value})^2}{\text{expected trait value}}$$

The calculated χ^2 value was compared with the critical reference χ^2 values at the probability levels of $P<0.05$ (expected χ^2 value of 5.99), $P<0.01$(expected χ^2 value of 9.21), $P<0.001$ (expected χ^2 value of 13.82).

RESULTS AND DISCUSSION

Correlation Among the Trait Data

The growth parameter PlH was significantly and negatively correlated with NT in the F_2 progenies of H-4 × Bg 352, *Suduheenati* × Bg 352 and *Mas* × Bg 352 (PCC of -24%,

P<0.05). PlH and NT were not correlated in the progeny *Suduheenati* × Bg 357. In all the progenies, PlH and NT were significantly correlated with SDW (P<0.05). However, none of the colour parameters; L*, a*, b*, C* and h* was correlated with the growth parameters; PlH, NT or SDW. In *Mas* × Bg 352 progeny, PlH was significantly correlated with FLW but it was not observed in other progenies. In H-4 × Bg 352 progeny, NT and SDW were significantly correlated (PCC of 34%, P<0.05) and in *Suduheenati* × Bg 357 progeny, only SDW was significantly correlated to FLW (PCC of 27%, P<0.05). However, strong correlations cannot be seen among PlH, NT, SDW and FLW. The colour metrics L* and a* were significantly and negatively correlated in the progenies of H-4 × Bg 352 and *Mas*× Bg 352 (P<0.05). Only in *Mas* × Bg 352 progeny L* was negatively correlated with b* (PCC of -52%, P<0.001). The L* and C* were significantly correlated in the progenies of H-4 × Bg 352 and *Mas* × Bg 352. Although a* and b* are used for the calculation of C*, only a* was strongly correlated with C* (98%, P<0.001). Except in H-4 × Bg 352 progeny, L* was very highly significantly correlated with h* (P<0.01) and in all the progenies a* and b* were very highly correlated with h* (P<0.001). The PCC values and associated significant levels for all the traits measured in four F_2 progenies are given in Tables 1 and 2.

Distribution of Trait Data

Growth Parameters

The distribution of PlH was not normal and the KS values were significant (P<0.01) in all the progenies (Figures 1A, 1B and 1D) except in the progeny *Mas* × Bg 352 and *Suduheenati* × Bg 357 (Figure 1C, Table 3). The distribution of NT was normal in all four progenies as indicated by non-significant KS values (Figures 2). The SDW was normally distributed in the progeny H-4 × Bg 352 but not normally distributed in other three progenies (Figure 3). The FLW was normally distributed in all four progenies (Figure 4). The KS values and the skewedness and kurtosis parameters of the each trait in four progenies are shown in Table 3.

Colour Parameters

The colour metric L* was normally distributed in the progenies H-4 × Bg 352 and *Mas* × Bg 352 but not normally distributed in other two progenies. Except the colour metric b* in *Mas* × Bg 352 progeny, in all the other progenies colour metrics were not normally distributed (P<0.01). In all the F_2 progenies transgressive segregants were observed for all the colour metrics (Figures 5, 6, 7, 8 and 9). Moreover, the colour metrics exhibited larger values for skewedness and kurtosis along with the significant KS values indicating discontinuous distribution patterns which are not inherent to polygenic traits (Table 3).

Heritability of the Traits

The growth parameter PlH had very high BSH ranging from 87% to 98% in all four progenies and NT also had higher BSH in all four progenies ranging from 45% to 72%. The FLW also had the higher BSH in the range of 58% to 99% and SDW had the BSH in the range of 62% to 87%. It was obvious that all four growth parameters had higher BSH values (45% to 99%). The colour metrics L*, a*, b*, C* and h* were all having very high BSH values ranging from 65% to 99%. The mean BSH was 94% for all the progenies and all the colour metrics (Table 3).

Heterosis

Negative values were calculated for MPH and BPH for PlH whereas positive values were calculated for WPH for the same parameters. The MPH and BPH values were mostly negative and highly variable for all the parameters in four progenies. Depending on the genetic combinations negative and positive heterosis could be seen for the tested parameters in all four F_2 progenies (Table 3).

Table 1. Pearson's Correlation Coefficients (PCC) for traits measured under PD in F_2 progenies

Progeny		NT	SDW	FLW	L*	a*	b*	C*	h*
H-4 × Bg 352	**PlH**	-0.25 *	0.33 **	0.04	-0.04	0.12	0.06	-0.12	0.02
	NT		0.49 ***	0.33 **	-0.10	0.15	0.03	-0.15	0.02
	SDW			0.36 **	-0.20	0.10	0.003	-0.09	0.02
	FLW				-0.19	0.07	0.11	-0.09	-0.08
	L*					-0.47 ***	-0.13	0.50 ***	0.04
	a*						-0.04	-0.98 ***	0.37 ***
	b*							-0.14	-0.91 ***
	C*								-0.25 *
***Suduheenati* × Bg 352**	**PlH**	-0.24 *	0.66 ***	0.05	-0.06	-0.05	0.04	0.05	-0.11
	NT		0.26 *	0.05	0.06	0.01	0.06	-0.01	-0.05
	SDW			0.05	0.04	0.05	-0.06	-0.05	-0.03
	FLW				0.04	-0.02	-0.05	0.02	0.04
	L*					-0.09	0.03	0.09	-0.03
	a*						-0.13	-0.99 ***	0.44 ***
	b*							0.11	-0.71 ***
	C*								-0.42 ***

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

PlH: Plant Height, NT: Number of Tillers, SDW: Shoot Dry Weight, FLW: Flag Leaf Width. L*: indicates colour range from black (-) to white (+), a*: indicates the colour range from green (-) to red (+), b*: indicates colour range from blue (-) to yellow (+), Chroma $(C^* = a^{*2} + b^{*2})^{\frac{1}{2}}$ and Hue angle: $h^* = \tan^{-1}\left(\frac{b^*}{a^*}\right)$.

Table 2. Pearson's Correlation Coefficients (PCC) for traits measured under PD in F_2 progenies

Progeny		NT	SDW	FLW	L*	a*	b*	C*	h*
	PlH	-0.22 *	0.50 ***	0.35 **	-0.09	-0.14	-0.13	0.14	0.06
	NT		0.39 ***	-0.05	-0.04	0.11	0.08	-0.12	-0.02
	SDW			0.16	0.05	-0.13	-0.05	0.12	-0.02
	FLW				-0.04	-0.03	-0.05	0.02	0.01
***Mas* × Bg 352**	**L***					-0.27 *	-0.52 ***	0.35 **	0.32 **
	a*						0.11	-0.98 ***	0.30 **
	b*							-0.27 *	-0.84 ***
	C*								-0.14
	PlH	0.06	0.65 ***	0.05	0.11	0.08	-0.06	-0.07	0.08
	NT		0.29 **	-0.06	0.14	-0.23	0.20	0.17	-0.27
	SDW			0.27 *	0.09	0.11	0.05	-0.12	-0.06
	FLW				0.18	0.03	-0.07	-0.01	0.08
***Suduheenati*× Bg 357**	**L***					-0.23	0.06	0.22	-0.07
	a*						-0.07	-0.96 ***	0.45 ***
	b*							-0.17	-0.86 ***
	C*								-0.24 *

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

PlH: Plant Height, NT: Number of Tillers, SDW: Shoot Dry Weight, FLW: Flag Leaf Width. L*: indicates colour range from black (-) to white (+), a*: indicates the colour range from green (-) to red (+), b*: indicates colour range from blue (-) to yellow (+), Chroma $(C^* = a^{*2} + b^{*2})^{\frac{1}{2}}$ and Hue angle: $h^* = \tan^{-1}\left(\frac{b^*}{a^*}\right)$.

Table 3. The normality, heritability and heterosis estimates of the traits measured under P deficient conditions for four F_2 progenies

Cross	Trait	Mean	Normality as indicated by the KS coefficient[#]	Skewedness	Kurtosis	Broad Sense Heritability (%)	Mid Parental Heterosis (%)	Better Parental Heterosis (%)	Worse Parental Heterosis (%)
H-4 × Bg 352	PLH (cm)	74.85	0.13**	-1.13	1.17	97.93	-2.73	-20.02	19.58
	NT	1.23	0.04	0.49	0.16	45.73	31.35	11.64	59.49
	SDW (g)	4.23	0.05	0.14	-0.75	81.99	-9.88	-43.30	119.51
	FLW (cm)	0.97	0.05	-0.55	0.18	58.31	-2.60	-20.49	24.35
	L*	58.76	0.07	0.25	-0.62	98.50	11.77	11.42	12.13
	a*	-56.53	0.12*	3.60	22.67	65.12	17.16	12.14	22.66
	b*	-8.73	0.13**	-1.56	5.25	99.49	-37.41	-34.60	-40.00
	C*	57.33	0.13**	-3.25	20.42	73.30	4.57	-0.96	10.76
	h*	0.15	0.13**	1.22	2.86	94.09	-44.44	-43.87	-44.99
Suduheenati × Bg 352	PLH (cm)	76.87	0.11*	-0.37	-0.26	92.85	-1.92	-25.53	21.69
	NT	0.73	0.06	0.86	0.06	69.07	-3.76	-21.25	23.74
	SDW (g)	3.26	0.20**	2.47	8.06	86.98	-38.65	-60.08	32.43
	FLW (cm)	1.07	0.04	0.12	-0.40	74.16	4.20	-12.03	27.76
	L*	58.39	0.33**	-7.20	56.56	97.82	9.76	8.80	10.73
	a*	-58.45	0.331**	-5.46	42.17	99.45	13.24	12.33	14.16
	b*	-8.11	0.154**	-1.47	3.58	95.76	-62.50	-61.79	-63.18
	C*	59.03	0.34**	5.69	44.53	99.73	10.86	7.84	14.06
	h*	0.15	0.207**	1.89	7.54	99.02	-42.27	-41.65	-42.89
Mas × Bg 352	PLH (cm)	69.51	0.08	-0.88	1.25	93.34	2.74	-7.44	15.46
	NT	1.83	0.03	0.47	0.51	72.11	-43.80	-57.60	-1.66
	SDW (g)	4.85	0.13**	1.19	1.35	61.75	-22.06	-51.42	96.99
	FLW (cm)	1.04	0.04	0.33	0.10	81.91	-7.96	-14.75	2.14
	L*	55.77	0.08	0.15	2.83	83.71	6.46	5.75	7.17
	a*	-50.37	0.25**	2.69	7.62	97.76	0.25	-1.64	2.13
	b*	-10.30	0.09	-1.05	2.65	98.55	-32.54	-30.73	-34.27
	C*	51.80	0.24**	-2.41	6.52	99.89	0.88	0.09	1.69
	h*	0.19	0.13**	1.08	4.43	97.37	-25.02	-23.66	-26.34
Suduheenati × Bg 357	PLH (cm)	69.97	0.04	0.08	-0.23	87.26	-12.12	-37.91	13.68
	NT	0.74	0.06	0.61	1.21	48.55	-3.55	-21.09	24.01
	SDW (g)	2.42	0.20**	2.72	9.24	64.88	10.75	0.98	22.62
	FLW (cm)	1.03	0.06	1.25	2.79	98.57	-33.05	-51.68	8.97
	L*	59.71	0.19**	-3.95	22.81	92.08	-2.20	-12.76	11.26
	a*	-56.41	0.30**	2.71	6.98	98.56	1.24	-5.03	8.41
	b*	-6.75	0.25**	-6.25	47.56	97.86	-35.53	-1.63	-4.26
	C*	57.19	0.29**	-2.33	6.32	98.76	-0.12	-4.34	4.48
	h*	0.12	0.23**	3.35	15.48	98.57	-33.04	8.96	-44.17

[#]Significant levels for Kolmogorov–Smirnov (KS) Coefficient: * P <0.05, ** P <0.01 (significant KS indicates the deviation from the normality)

PlH: Plant Height, NT: Number of Tillers, SDW: Shoot Dry Weight, FLW: Flag Leaf Width,

L*: indicates colour range from black (-) to white (+), a*: indicates the colour range from green (-) to red (+), b*: indicates colour range from blue (-) to yellow (+), Chroma: $(C^* = a^{*2} + b^{*2})^{\frac{1}{2}}$, Hue angle: $h^* = \tan^{-1}\left(\frac{b^*}{a^*}\right)$

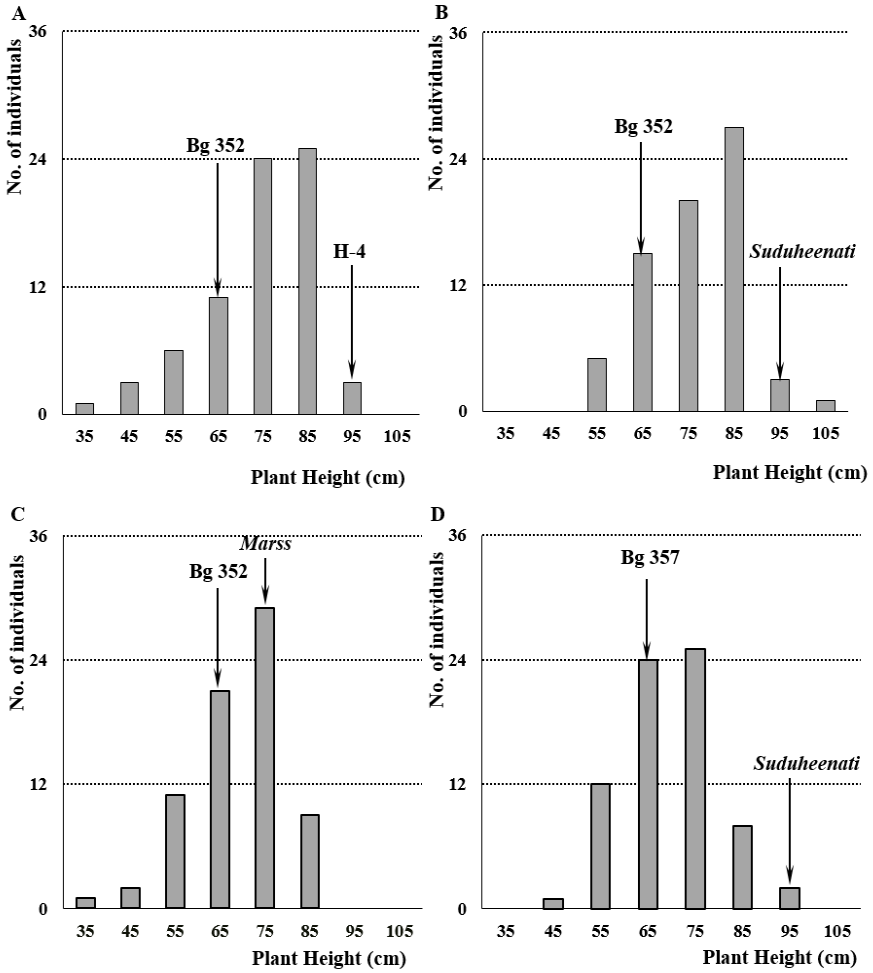

Figure 1. Frequency distributions of the F_2 progenies for plant height (PlH). A: H-4 × Bg 352; B: *Suduheenati* × Bg 352; C: *Mas* × Bg 352; D: *Suduheenati* × Bg 357. The positions of the parental trait values are shown with the arrows. The class midpoints of each phenotypic trait are shown in the X axis. Any individual located at the left of the parent with lower trait value, or located at right with the parent with higher trait value are the transgressive segregants.

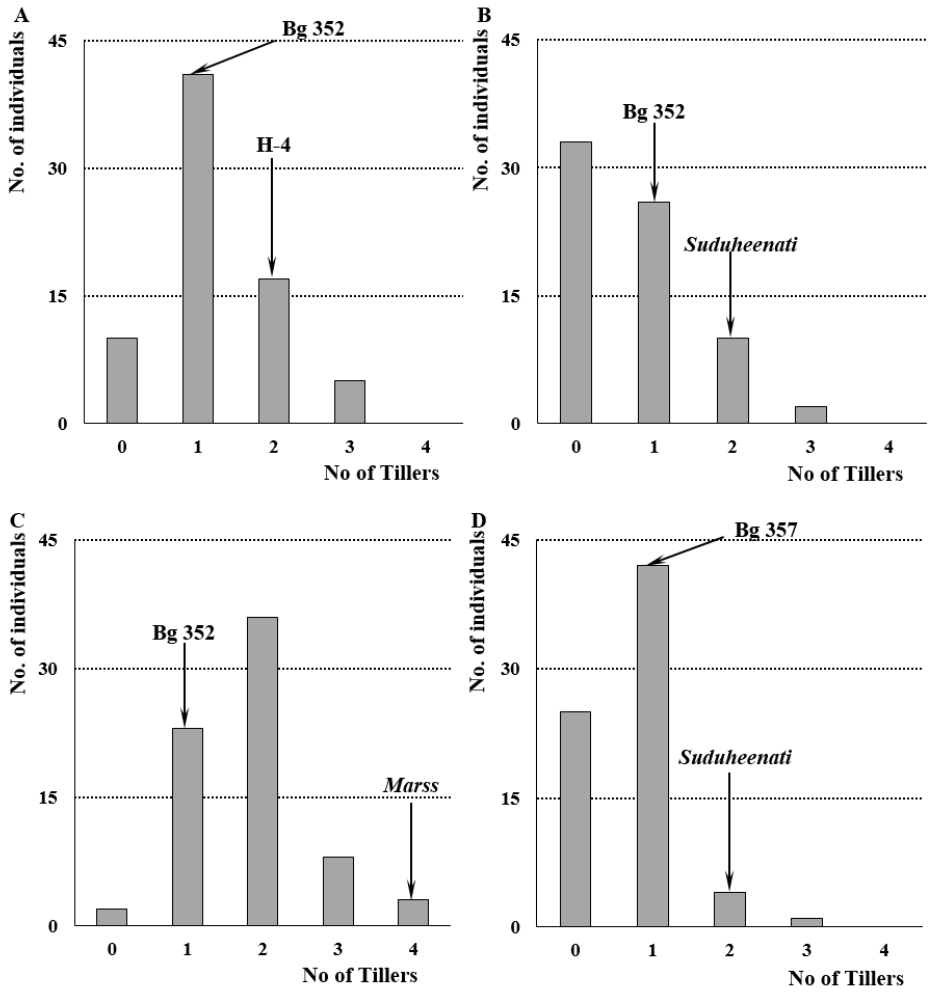

Figure 2. Frequency distributions of the F_2 progenies for number of tillers (NT).A: H-4 × Bg 352; B: *Suduheenati* × Bg 352; C: *Mas* × Bg 352; D: *Suduheenati* × Bg 357. The positions of the parental trait values are shown with the arrows. The class midpoints of each phenotypic trait are shown in the X axis. Any individual located at the left of the parent with lower trait value, or located at right with the parent with higher trait value are the transgressive segregants.

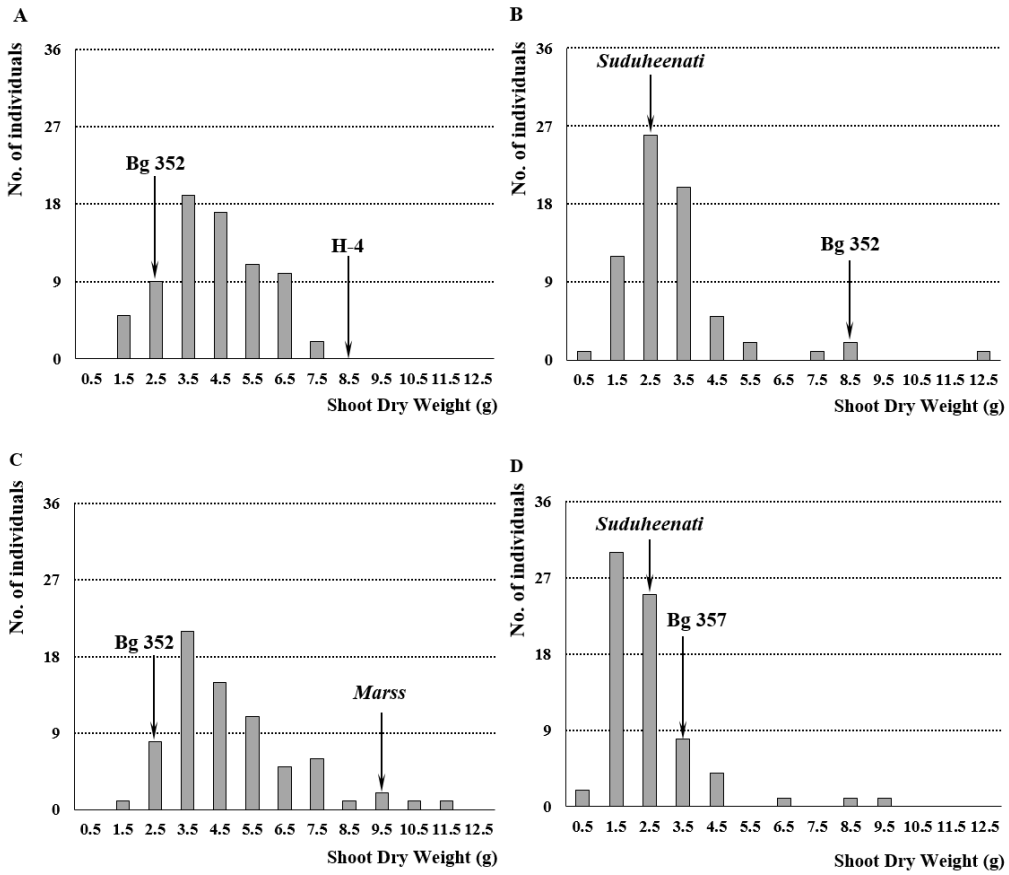

Figure 3. Frequency distributions of the F_2 progenies for shoot dry weight (SDW). A: H-4 × Bg 352; B: *Suduheenati* × Bg 352; C: *Mas* × Bg 352; D: *Suduheenati* × Bg 357. The positions of the parental trait values are shown with the arrows. The class midpoints of each phenotypic trait are shown in the X axis. Any individual located at the left of the parent with lower trait value, or located at right with the parent with higher trait value are the transgressive segregants.

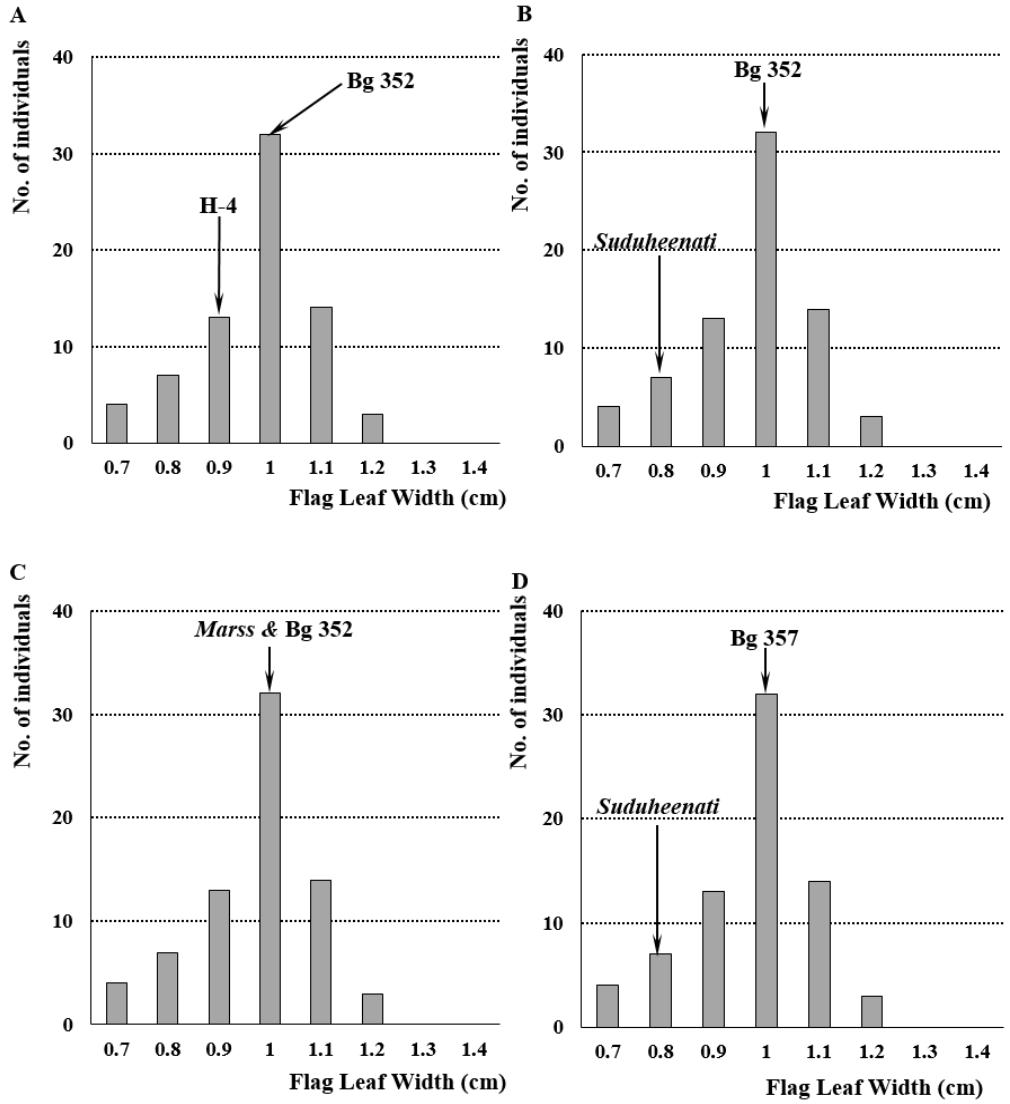

Figure 4. **Frequency distributions of the F_2 progenies for the flag leaf width (FLW). A: H-4 × Bg 352; B: *Suduheenati* × Bg 352; C: *Mas* × Bg 352; D: *Suduheenati* × Bg 357The positions of the parental trait values are shown with the arrows. The class midpoints of each phenotypic trait are shown in the X axis. Any individual located at the left of the parent with lower trait value, or located at right with the parent with higher trait value are the transgressive segregants. When two parents are having the trait values within the same class, they are indicated together with one arrow which is pointing in to the relevant class.**

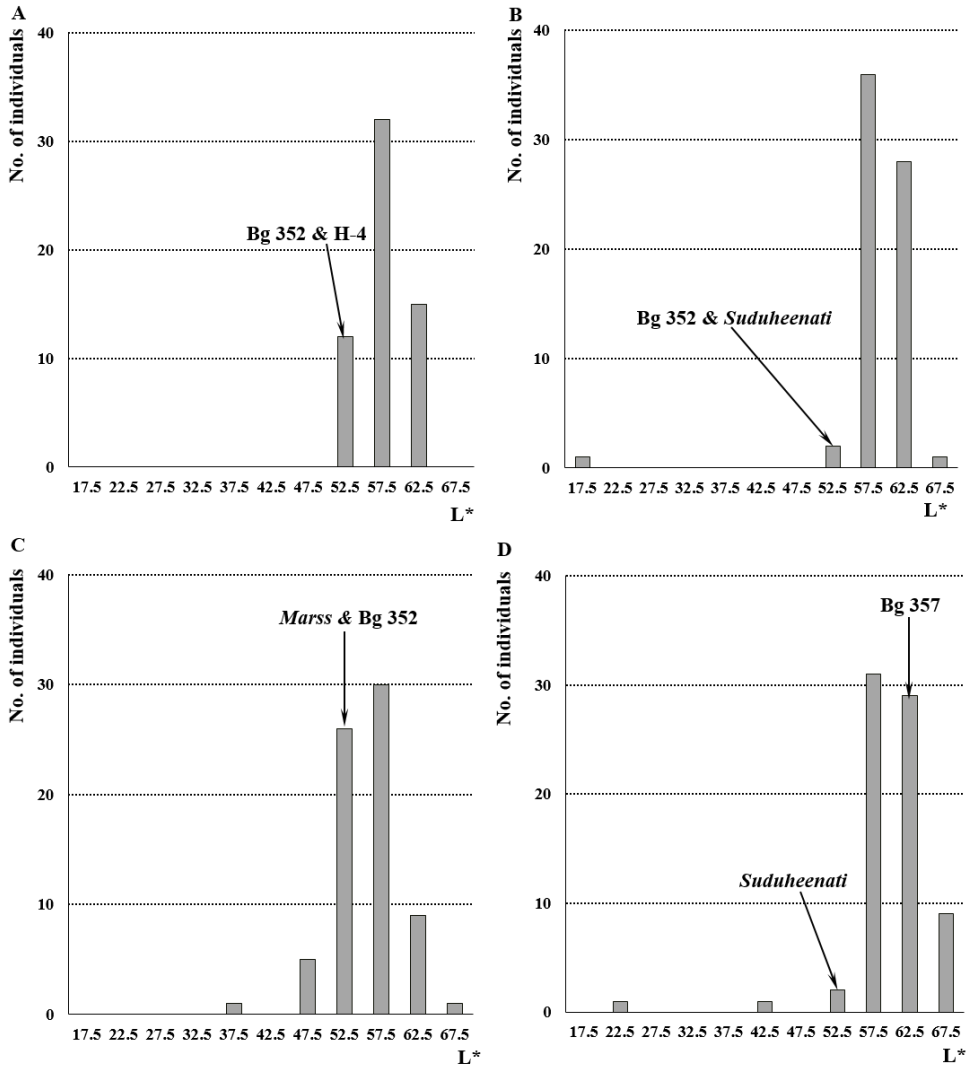

Figure 5. **Frequency distribution of the F_2 progenies for L*[indicates colour range from black (-) to white (+)]. A: H-4 × Bg 352; B: *Suduheenati* × Bg 352; C: *Mas* × Bg 352; D: *Suduheenati* × Bg 357. The positions of the parental trait values are shown with the arrows. The class midpoints of each phenotypic trait are shown in the X axis. Any individual located at the left of the parent with lower trait value, or located at right with the parent with higher trait value are the transgressive segregants. When two parents are having the trait values within the same class, they are indicated together with one arrow which is pointing in to the relevant class.**

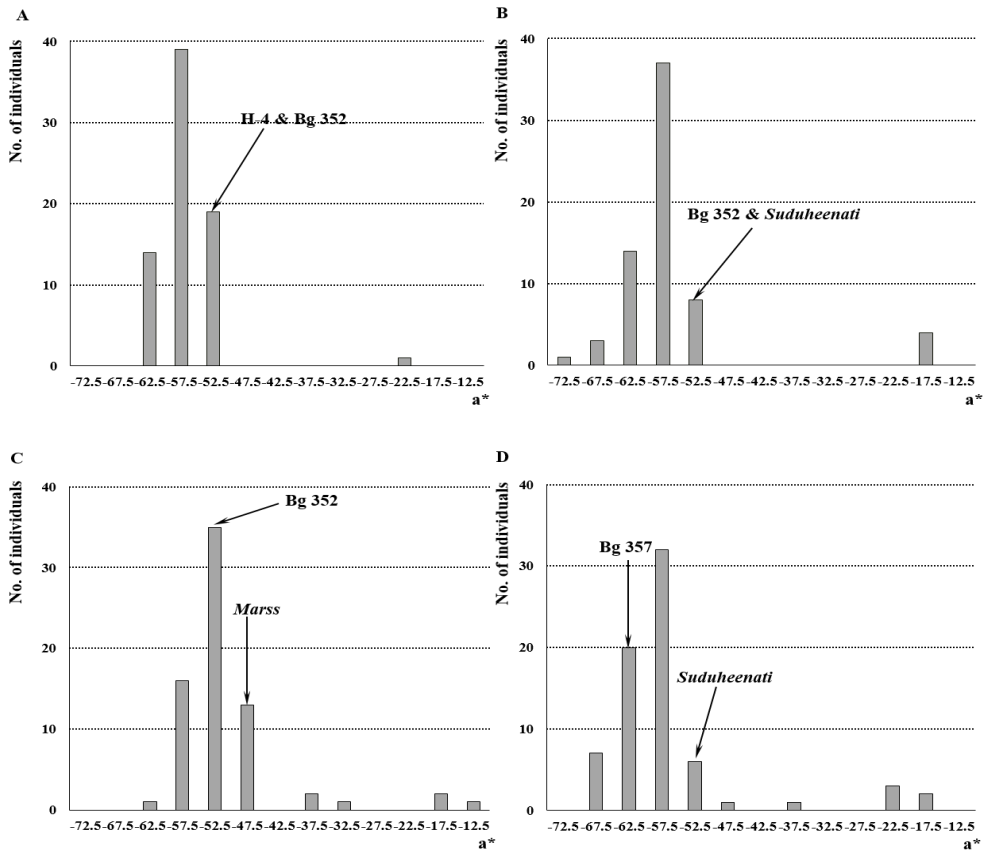

Figure 6. Frequency distribution of the F_2 progenies for a*[indicates the colour range from green (-) to red (+)]. A: H-4 × Bg 352; B: *Suduheenati* × Bg 352; C: *Mas* × Bg 352; D: *Suduheenati* × Bg 357. The positions of the parental trait values are shown with the arrows. The class midpoints of each phenotypic trait are shown in the X axis. Any individual located at the left of the parent with lower trait value, or located at right with the parent with higher trait value are the transgressive segregants. When two parents are having the trait values within the same class, they are indicated together with one arrow which is pointing in to the relevant class.

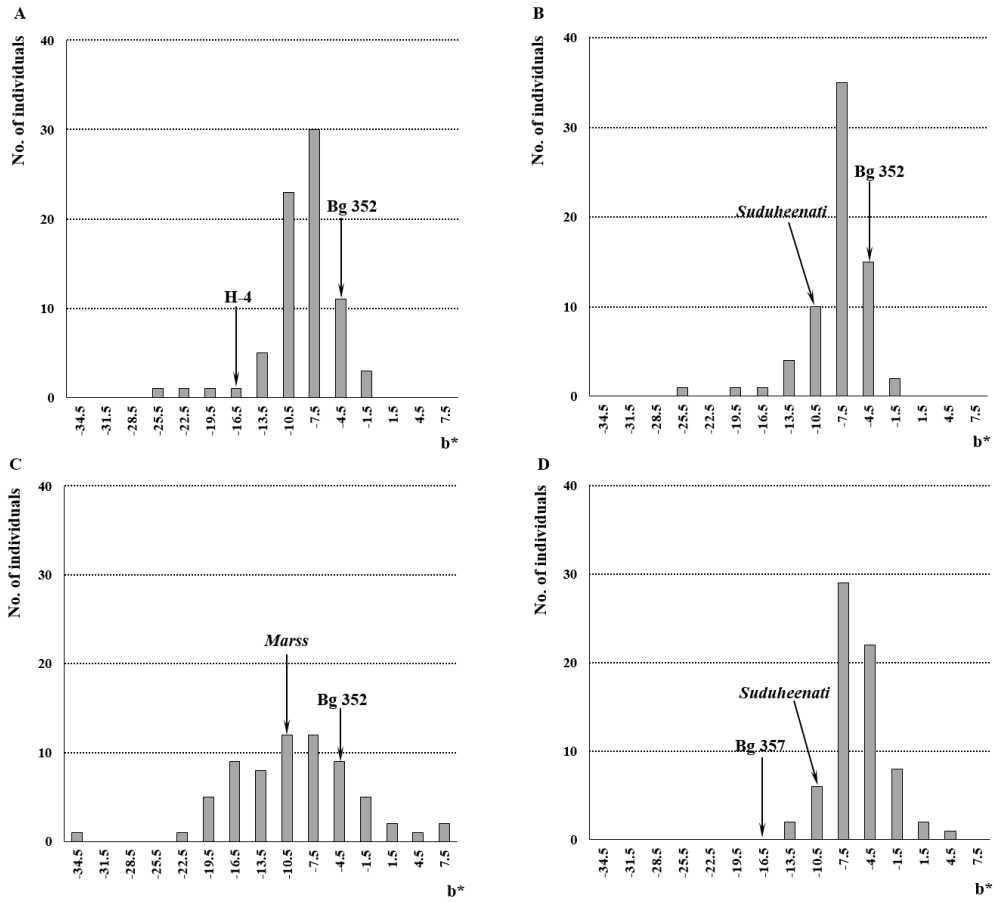

Figure 7. Frequency distribution of the F_2 progenies for b*[indicates colour range from blue (-) to yellow (+)]. A: H-4 × Bg 352; B: *Suduheenati* × Bg 352; C: *Mas* × Bg 352; D: *Suduheenati* × Bg 357. The positions of the parental trait values are shown with the arrows. The class midpoints of each phenotypic trait are shown in the X axis. Any individual located at the left of the parent with lower trait value, or located at right with the parent with higher trait value are the transgressive segregants.

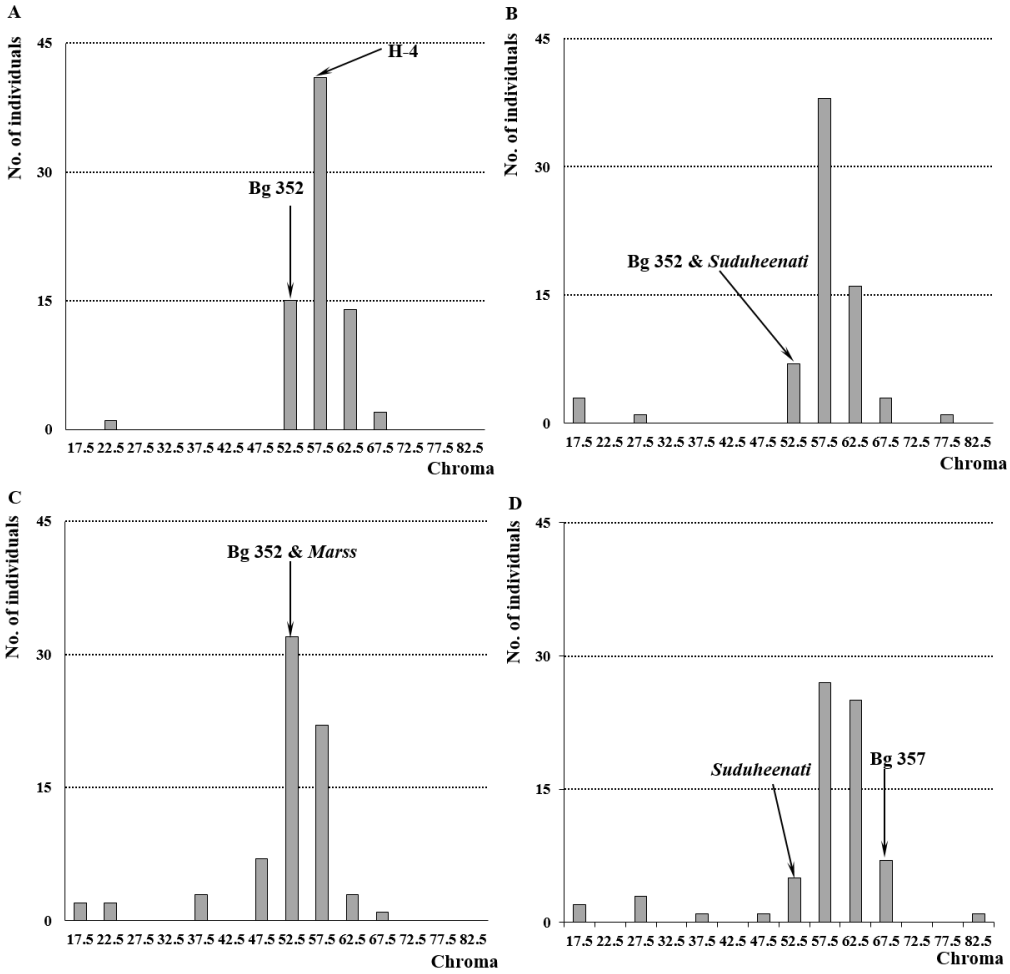

Figure 8. Frequency distribution of the F_2 progenies for chroma [$(C^* = a^{*2} + b^{*2})^{\frac{1}{2}}$].A: H-4 × Bg 352; B: *Suduheenati* × Bg 352; C: *Mas* × Bg 352; D: *Suduheenati* × Bg 357. The positions of the parental trait values are shown with the arrows. The class midpoints of each phenotypic trait are shown in the X axis. Any individual located at the left of the parent with lower trait value, or located at right with the parent with higher trait value are the transgressive segregants. When two parents are having the trait values within the same class, they are indicated together with one arrow which is pointing in to the relevant class.

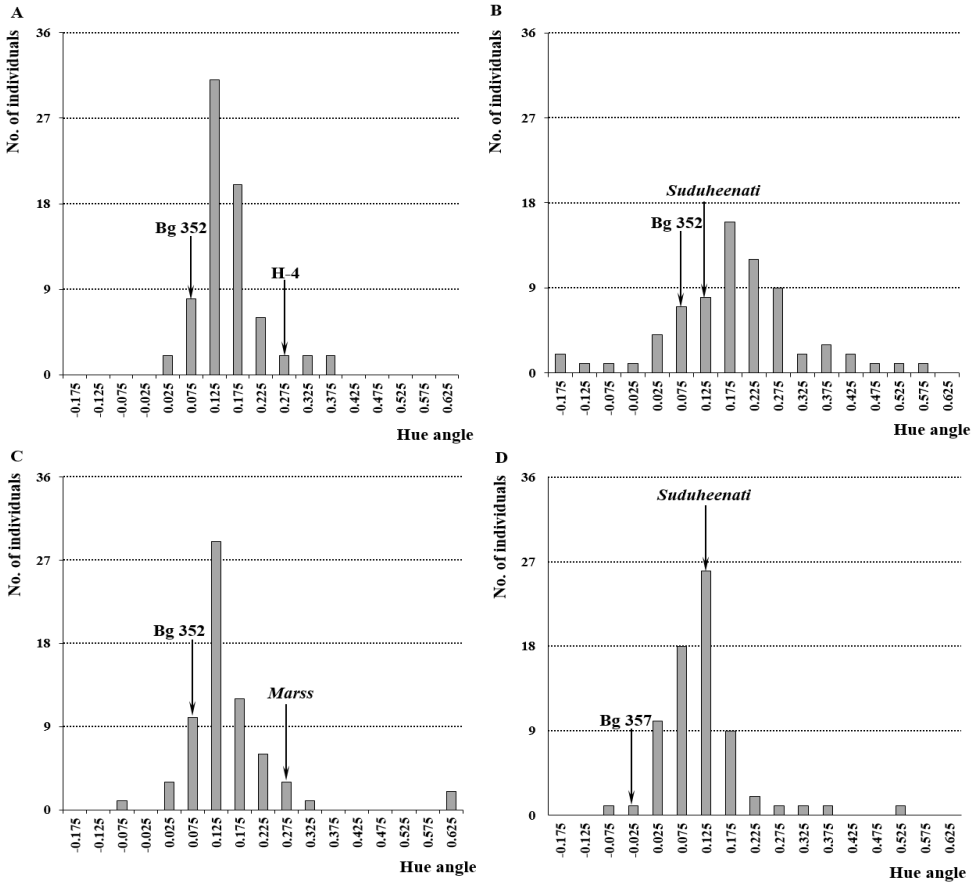

Figure 9. Frequency distribution of the F_2 progenies for hue angle [$h^\circ = \tan^{-1}\left(\frac{b^*}{a^*}\right)$].A: H-4 × Bg 352; B: *Suduheenati* × Bg 352; C: *Mas* × Bg 352; D: *Suduheenati* × Bg 357. The positions of the parental trait values are shown with the arrows. The class midpoints of each phenotypic trait are shown in the X axis. Any individual located at the left of the parent with lower trait value, or located at right with the parent with higher trait value are the transgressive segregants.

Goodness of Fit Analysis of the Colour Data

As the colour metric data were mostly distributed in non-normal passion (Table 1 and 2; Figures 5, 6, 7, 8 and 9), a goodness of fit analyses were conducted considering the dihybrid ratios representing the deviations to Mendelian postulates. By examining the general layout of the data distributions of colour metrics L*, a*, b*, C* and h*, the frequency values in class intervals were categorized into three groups for each trait. The observed ratio of individuals among the groups was tested against non-Mendelian dihybrid ratios of 9:3:4, 9:6:1, 10:3:3 and 12:3:1. The classical chi (χ^2) square test for the goodness of fit was performed to check whether the observed ratio is following the expected ratio (Table 4).

In H-4 × Bg 352 progeny, the colour metrics L*, C* and h* were fitting into the epistatic ratios of 9:3:4 and 10:3:3 (P<0.001). In *Suduheenati* × Bg 352 progeny, L* was fitting into 9:6:1 ratio where as a*, b* and C* were fitting into both 9:3:4 and 10:3:3 ratios (P<0.001). In *Mas* × Bg 352 progeny, C* was fitting into 9:3:4 and h* was fitting into both 9:3:4 and 10:3:3 ratios (P<0.001). In *Suduheenati* × Bg 357 progeny, L* was fitting into 9:6:1 ratio, b* was fitting in to 10:3:3 ratio, h* was fitting in to 9:3:4 and 10:3:3 ratios and C* was fitting into 10:3:3 and 12:3:1 ratios (P<0.001), and the same C* was also fitting into 9:3:4 ratio at P<0.01. The colour metric a* was not fitting into any of the dihybrid ratios tested in all four F_2 progenies (P<0.05). Mostly the colour metrics were following 9:3:4 or 10:3:3 ratios indicating a putative goodness of fit for an epistatic interaction between two genes / loci which could be further modified by other factors in producing unusual leaf colouration under PD (Table 4).

Table 4. Goodness of fit analyses for colour metric data measured under P deficient conditions in four F_2 progenies

Cross	Colour metric	Calculated Chi Square Values of Goodness of Fit for potential expected dihybrid ratios in F_2progenies			
		9:3:4	**9:6:1**	**10:3:3**	**12:3:1**
H-4 × Bg 352	L*	1.956	21.074***	2.124	23.530***
	a*	64.815***	75.007***	71.643***	129.375***
	b*	21.427***	20.795***	22.470***	46.386***
	Chroma	0.668	33.399***	1.251	32.516***
	Hue angle	5.538	25.557***	1.834	13.374**
Suduheenati × Bg 352	L*	32.372***	0.980	29.161***	25.491***
	a*	3.714	16.610***	2.947	20.424***
	b*	1.654	40.745***	4.083	44.024***
	Chroma	0.745	65.634***	4.754	61.009***
	Hue angle	12.236**	51.617***	17.053***	68.343***
Mas × Bg 352	L*	31.63***	10.37**	31.259***	42.741***
	a*	14.54***	75.517***	14.807***	59.273***
	b*	15.686***	57.417***	21.342***	76.522***
	Chroma	5.359	110.765***	15.349***	111.704***
	Hue angle	1.802	19.793***	0.207	16.443***
Suduheenati × Bg 357	L*	22.713***	5.307	21.209***	28.570***
	a*	16.673***	17.840***	17.275***	38.519***
	b*	21.870***	22.171***	13.454**	2.784
	Chroma	8.988*	14.32***	3.497	2.963
	Hue angle	0.968	33.588***	0.346	26.878***

* P<0.05 (Expected χ^2 value: 5.99), ** P<0.01(Expected χ^2 value: 9.21), *** P<0.001 (Expected χ^2 value: 13.82)
L*: indicates colour range from black (-) to white (+), a*: indicates the colour range from green (-) to red (+), b*: indicates colour range from blue (-) to yellow (+), Chroma: $(C^* = a^{*2} + b^{*2})^{\frac{1}{2}}$, Hue angle:$h^* = \tan^{-1}\left(\frac{b^*}{a^*}\right)$

Phosphorus is a key element in plant growth and reproduction. When it is limited stunted growth, reduced tillering and dark colouration of leaves could be seen in rice plants (Wissuwa and Ae, 2001; Mghase *et al*., 2011; Chen *et al*., 2013). The traits such as PlH, NT, SDW, FLW and leaf colour are segregating in significant quantities in F_2 populations and these variations could be efficiently used in QTL mapping. The parameters indicating the PDT such as shoot P concentration (SPC) and shoot P uptake (SPU) have also been studied and subjected to QTL mapping (Wissuwa and Ae, 2001). In the present study, the parameters PlH, NT, SDW, FLW and leaf colour were selected because they are easy to record, cost effective and highly correlated with the overall degree of PDT (Chaubey *et al*., 1994; Dingkuhn *et al*., 2006; Fageria and Knupp, 2013). The parental genotypes were selected to establish segregating progenies by primarily considering their degree of PDT and having the final aim of uncovering important genomic regions that could be present in the rice landraces which have not yet been manipulated by the geneticists. The utilization of the landraces or indigenous cultivars as parents to find the rare 'jewels of the genomes' (i.e. agriculturally important QTL) is a common practice in breeding many species such as rice (Wissuwa *et al*., 1998), wheat (Schmidt *et al*., 2005), barely (Qi *et al*., 1998), apple (Gharghani *et al*., 2009), cherry (Olmstead *et al*., 2008) and tomato (Carelli *et al*., 2006).

As the tolerant parental landraces / genotypes, H-4, *Suduheenati* and *Mas* were selected based on the PD screening data reported in Aluwihare *et al*., (2016). The variety *Mas* is a parent of H-4. Therefore the inheritance of the alleles / genotypes could be validated across the F_2 progenies parented by H-4 and *Mas* considering their pedigree relationships and the identity by descent. In establishing segregating populations it is important to select sensitive parental varieties to bring the counter path alleles which should be opposite in their effects to the alleles provided by the tolerant parents. Based on the P screening data reported in Aluwihare *et al*., (2016), the newly improved rice varieties Bg 352 and Bg 357 which are also the very highly grown mega rice varieties in Sri Lanka (www.agridept.gov.lk) were used as sensitive parents. The four F_2 progenies produced were H-4 × Bg 352, *Suduheenati* × Bg 352, *Mas* × Bg 352 and *Suduheenati* × Bg 357. The variety Bg 352 was used as the common sensitive parent in three crosses having the aim of comparing information across the progenies upon gene mapping. The landrace *Suduheenati* was also used in two progenies having the objective of comparing the mapping information across the populations sharing the common parents. The selection of common parents or parents with pedigree relationships in establishing segregating populations for QTL mapping is common in many species. It has been reported in potato (Bink *et al*., 2002), maize (Bardol *et al*., 2013), apple (Guan *et al*., 2015) and cherry (Zhang *et al*., 2010). As rice is predominantly a self-pollinating species it is very difficult to cross and obtain viable F_1 seeds. It is customary to obtain fewer F_1 seeds compared to the number of crossing attempts done artificially. Also the resulting F_1 seeds are often sterile and weak in germination and seedling development (Maruyama *et al*., 1991). Therefore, the F_1 seeds collected from the crosses were not given the PD treatment but were subjected to space planting under standard growing conditions to obtain as many F_2 seeds as possible. The parental genotypes were grown side by side as controls to thin out any off types of the F_1 plants. This has been the practice for producing large number of F_1 plants in rice breeding and genetic studies over the years (Zhao *et al*., 2005), although recently microsatellite marker based genotyping is proposed to confirm the hybridity in F_1 rice populations. However, most of the times visual confirmation of hybridity is in parallel with the confirmation using SSR based DNA fingerprinting (Zhao *et al*., 2005). In the present study F_1 plants were phenotypically confirmed to possess a combination of parental phenotypic traits by the expert rice breeders working at RRDI, Sri Lanka. The F_2 seeds collected from all progenies along with the parents were grown in a greenhouse using the

ultisol soil which has not been fertilized for last 40 years. A greenhouse experiment was preferred because precise screening of the F_2 progenies for PDT is not possible in the field since unintended P ions could come to the field through irrigation or overflowing water. Hydroponic systems were not preferred as many studies on P screening in rice reported that water based growth media would not mimic the P limited field conditions and such experimental setups would yield PD screening data of limited value (Chin *et al*., 2011). It would have been better to conduct another trial with the same rice F_2 genotypes grown under standard P levels for the comparison purposes. However, each of the seed in F_2 represents a unique genotype and there is no easy way to produce a clonal replicate to be tested under opposite conditions.

The PlH was negatively correlated with NT in three progenies indicating when plants are taller, the NT tends to go down. This is expected as the resource sequestration is based on the genetic basis available for either taller plants or more tillers (Sugiyama, 1995). However, PlH and NT were always significantly correlated with SDW as it includes all areal tissues in its estimation. In the progenies H-4 × Bg 352 and *Suduheenati* × Bg 357, it was reported that SDW was significantly correlated with FLW but it was not seen in other two progenies (Table 1 and Table 2).

The colour variation due to the P starvation in F_2 progenies were measured using standard L*, a* and b* colour metrics. The delicate colour variations that could be felt but cannot be accurately ranked using human eye could be efficiently measured using these colour metrics (Sooriyapathirana *et al*., 2010). Although visual difference of the colours could be seen among the F_2 rice plants, they cannot easily be recorded using a categorical scale such as Munsell colour chart (www.munsell.com). In such situations the quantitative colour metric assessments were employed by QTL mapping studies. However, the colour metrics L*, a*, b*, C* and h* were not correlated with any of the growth parameters recorded under PD in the present study. This strongly emphasises the fact that growth retardation due to PD is independent of the development of colour variation in leaves of the plants. In PD sensitive genotypes, it is noted that leaves get darker in colour as opposed to the brighter and healthy green colour in tolerant genotypes.

From the normality testing, it is evident that the tested growth parameters are mostly distributed in normal manner and some of the deviations observed could either be attributed to the sample size or to the presence of QTL with major effects in specific rice landraces. However, slight deviation from the normality could be used with caution to map QTL Therefore, F_2 progenies assessed in the present study suggest that PD tolerance linked QTLs could be detected for growth parameters PlH, NT, SDW and FLW. The distribution pattern of the colorimetric data and associated KS values along with the larger skewedness and kurtosis parameters indicate that these trait distributions are significantly deviated from the normality and showing discontinuous distributions that are commonly reported for qualitative traits. Discontinuous trait distributions are mainly occurring because of the presence of major genes underling the trait or presence of QTL with very high effects such as 80% or more. Because of this reason, a simple goodness of fit analysis was carried out using chi square test to see whether the colour metric data following the commonly known epistatic dihybrid ratios. The altered dihybrid ratios from the Mendel's postulates were selected because none of the traits showed apparently classical Mendelian ratios of 3:1, 9:3:3:1, 1:1 or 1:2:1. The goodness of fit analyses revealed that the colour metrics are either following 9:3:4, 9:6:1, 10:3:3 or 12:3:1. The 9:7 ratio or so called complementary gene action was not selected because there was no apparent breakdown for just two classes within the colour metric distribution. It is logical to think the presence of two or few genes / QTLs

which are epistatically interacting to decide the awkward colouration of rice leaves due to PD based on the results of goodness of fit analyses. When P is adequately supplied no such colour variance is observed in rice plants (Mghase *et al*., 2011).

The BSH estimates of the traits are important for efficient gene mapping studies. Under PD, all the growth parameters and colour metrics have extremely high BSH indicating the strong genetic basis for the degree of PD tolerance. The extremely high BSH values indicate the presence of either major genes or QTL with major effects for the colour metrics and growth parameters. The growth parameters generally have normal distributions indicating the presence of QTL with major effects and colour metrics which have discontinuous distributions indicating the possibility of having major genes rather than having the QTL with major effects.

The estimates of the MPH, BPH and WPH of the growth parameters and colour metrics in all F_2 progenies have indicated that no strong heterosis is present with regard to the P starvation. It is premature to conclude but hints that PD tolerance could not be achieved through heterosis and the more promising approach would be to pyramid favourable alleles (enhancing marker haplotypes) in breeding and producing RILs at the end to include all of them. In the present study the heterosis was calculated as MPH, BPH or heterobeltisosis and WPH using the data from F_2 progenies. Usually heterosis is calculated by comparing the F_1 means with the parental means. However, Falconer and Mackay (1996) developed formula to use F_2 data for heterosis estimations. When F_2 progenies are sufficiently large two homozygous classes within the F_2 progeny are cancelled out only leaving the mean effect of heterozygotes. This approach to calculate heterosis was also used by Mukamuhirwa *et al*., (2015).

CONCLUSIONS

The assessment of the phosphorus deficiency tolerance in rice using F_2 progenies of crosses created between tolerant and sensitive parents indicated that the phosphorus deficiency tolerance associated traits have complex quantitative and qualitative modes of inheritance with very high broad sense heritability. The genetic architecture of the phosphorus deficiency associated traits in the present study provides a strong platform to find the genomic resources for marker assisted breeding in rice and to undertake further molecular genetic studies to characterise the underlying genes.

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