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Development of High Yielding Rice Varieties Tolerant to Phosphorus Deficiency

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ABSTRACT

Improvement of nutrient-use efficiency (NUE) in rice is important for increasing productivity. Rice varieties with tolerance to phosphorus (P) deficiency increase the productivity in P-deficient fields. Therefore, the objective of this study was to develop high yielding, P-deficiency tolerant rice varieties to enhance the NUE. High yielding and popular rice varieties (Bg300 and Bg94-1) and known P-deficiency tolerant rice varieties (H_4 and At353) were used as female and male parents, respectively, in a crossing program. Single seed descent approach was used for rapid generation advancement. Bg300/At353 and Bg94-1/H_4 crosses were advanced from F_2 to F_6 in planthouse condition under minimum fertigation for restricted growth. In F_7 generation, 310 recombinant inbred lines (RIL) in both crosses were space-planted and 40 RILs were selected from each cross. Selected RILs were screened in a hydroponic system at 10 μM P level to select the best lines for P-deficiency tolerance. Multiple plant traits were used for selection and the most promising 20 rice lines were selected as the best performers under P-deficient hydroponic conditions. Marker assisted selection was carried out to confirm the presence of PSTOL1 gene. Selected rice lines were evaluated for agronomic traits and yield parameters. Ten promising rice lines were identified as high yielding lines with accepted agronomic traits.

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INTRODUCTION

Rice is the staple food that supplies primary caloric requirement of Sri Lankan people. Per capita annual rice availability of the country in 2018 was 138 kg/year (Central Bank Annual Report, 2018). Due to the limitations in land and water availability, development of high yielding short-age rice varieties has been identified as the best option to achieve this task (Khan et al., 2015) although the long-age rice varieties give higher yield in low fertile conditions (Kekulandara et al., 2019). High yielding rice varieties developed during and after the green revolution are high nutrient responsive and require more chemical fertilizers for growth to achieve expected yield (Zhang, 2007). All high yielding rice varieties remove large quantities of nutrients from soil and such quantities should be replaced externally as fertilizer to sustain yield levels. Almost all applied fertilizers are not utilized by the plants due to variation of plant performance under different soil and climatic conditions. Although higher rates of P accumulation in paddy soils has been noted in many occasions, P deficiency is common in acid soils and highly alkaline soils due to immobilizing behavior of P under those pH conditions (Fageria and Baligar, 1998). Under such context, overdosing of P fertilizer has become a burden to Sri Lankan economy. In the year 2020, 95,000 Mt of TSP has been imported annually expending foreign exchange around USD 38 Million (Media briefing on Fertilizer, 2021).

Screening and development of P-deficiency tolerant (PDT) genotypes are the primary requirement for improving use efficiency of P fertilizers (Rose et al., 2011). One option to address this is to develop high-yielding and nutrient-efficient rice varieties (Breseghello and Coelho, 2013).

Several phenotypes associated with P-absorption have been studied for precision screening (Kant et al., 2018). Among them, root dry weight and shoot dry weight are important characters for evaluating the selection index for low-P tolerance in rice (Aluvihare et al., 2015; *Ali et al*, 2018). Similarly, key morphological and physiological traits such as plant height, number of tillers, root length, relative shoot and root dry weights, shoot and root P concentration and root-attributes such as root diameter, root hair number and number of roots have also been used for screening and identifying tolerant genotypes under P deficiency conditions (Wissuwa and Ae, 2001; Ali et al., 2018). Increasing productivity of rice under P deficiency requires increasing both P uptake efficiency and P utilization efficiency because productivity is the result of dry matter produced per unit area utilizing the nutrients absorbed by roots. Thus; higher productivity indicates the efficient uptake and efficient utilization of nutrients. Therefore, breeding of rice for improving nutrient use efficiency is a major area of research at present and considered as an important strategy for increasing rice production and productivity (Ali et al., 2018).

Pup1 QTL in the rice chromosome 12 has been identified as the major QTL for P-deficiency (PD) tolerance. The PSTOL1 gene is considered as the major gene in Pup 1 QTL that contributes to PD tolerance trait in rice (Chin et al., 2011). Nevertheless, there is a great variability in P uptake efficiency in Sri Lankan rice varieties (Kekulandara *et al*, 2018). The authors identified that PD tolerant rice varieties are either low yielders, long-age varieties or missing some traits related to P-deficiency tolerance. In the normal procedure adopted for rice breeding, it takes more than five years to develop a rice variety tolerant to P deficiency. Therefore, rice breeders have identified Single Seed Descent method (SSD) as a rapid generation advancement method in rice breeding (Janwan et al., 2013). The objective of this study was to develop high yielding, PD tolerant rice lines by incorporating the major gene PSTOL1 together with PD tolerance-related traits to new superior rice lines through SSD method.

METHODOLOGY

Hybridization

New improved, high yielding and well adapted rice varieties Bg300 and Bg94-1, which were reported to performed well under low P condition (Kekulandara et al., 2015), were selected as the maternal parents of the breeding program. Rice varieties with early and high tillering, and high yield under low-P conditions, namely, At353 and H4 (Kekulandara et al., 2017; Aluwihare et al., 2016) were used as male parents in this study. Hybridization was carried out for Bg300/At353 and Bg94-1/H_4 crosses using hot water emasculation method (Tong and Yoshida, 2008) at around 9.00 am to 10.00 am at the plant house at the Rice Research and Development Institute (RRDI) at Batalagoda, Sri Lanka in the 2015/16 *Maha* season.

Line advancement through SSD approach

The F_1 plants were grown in the field and allowed to self-pollinate to get F_2 seeds. From F_2 generation, 400 plants were grown in trays with dimension of 100 cm (length) x 60 cm (width) x 14 cm (height), filled with sandy soil separating individual plants with a wire mesh and the lines were advanced until F_6 generation with SSD method by germinating one seed from each single plant for the next generation.

Minimum fertigation was done to avoid vigorous plant growth and to reduce the number of tillers as a single panicle was expected from each genotype, and only single seed from a plant is needed for the next generation, and to avoid delayed flowering as well. Plants were allowed to self-pollinate in each generation from F_2 to F_6 and seeds were collected separately from each individual plant. As described in SSD method, any kind of selection was not carried out and matured panicles were harvested at different times according to the maturity age of the segregating populations. The harvested seeds from each plant were stored separately and one seed from each harvested plant was used as one genotype for the next generation. Same procedure was carried out in each generation until F_6 generation and seeds were collected from each RIL separately.

Seed multiplication and Selection of RILs for PDT

The experiment was conducted at the greenhouse of RRDI. All the Recombinant Inbred Lines (RILs) of each cross were space planted as lines at 40 cm × 40 cm spacing for seed multiplication and required field evaluation for agronomic traits. Lines were selected based on visual observation of plant type, culm strength, panicle type, and the maturity age. Spreading type plants, plants having thin and weary culm, plants with open panicles and plants having extended maturity age over 135 days were rejected, and 40 plants from each cross were selected for further evaluation. However, all the multiplied seeds were collected and conserved to be used if there would not be PD tolerant lines in the 40 RILs selected based on the basic agronomic observations.

Evaluation for PD tolerance in hydroponic

Forty RILs each from the crosses Bg94-1/H_4 and Bg300/At353 were selected for PD tolerance evaluation. Each selected RILs and all four parents were grown in 7.5 L plastic buckets filled with Yoshida nutrient solution supplied with low-P (10 μM) while all the other nutrients were supplied at optimal level (Figure 1). The P level of 10 μM (low-P) was selected based on previous work (Kekulandara et al., 2016) and the experiment was arranged in a Completely Randomized Design with three replicates. Six plants were grown in each bucket at similar spacing and two RILs were grown in one bucket labelling them on the Styrofoam plate where plants were plugged in. The pH of the solution was maintained at 5.8 by adjusting it daily by adding HCl or NaOH and the solution was replaced twice a week. There is no single definite morphological marker available for PD tolerance screening in rice, several parameters including plant height, rooting depth (length of the longest root), number of tillers, seedling vigor and plant total dry weight were used in combination for the effective screening for low P response. The RILs were evaluated at six weeks after planting. Seedling vigour was scored for individual plants using a 1-3 scale; 3 was given for plants having good vigour, 2 for moderate, and 1 for the weakest plants considering the culm diameter and colour and width of leaves. The best performing RILs were selected using above parameters from both crosses for further generation advancement.

Statistical analysis was done using Proc GLM and mean separation was done using DMRT in SAS statistical software (SAS 2005) to test the differences in plant height, rooting depth and total dry weight among lines. Moreover, Kruskal-wallis test was performed to test the differences in number of tillers and seedling vigour among the lines.

Molecular evaluation for PSTOL1 gene

Rice varieties used as parents in hybridization and selected RILs were tested for the presence of PSTOL1 gene using K46-1 marker used in previous studies (Chin et al., 2011; Mukherjee et al., 2014). As the parents of Bg94-1/H_4 are PSTOL1 positive RILs were not subjected to molecular evaluation.

The DNA was extracted from the 4 parents and selected RILs of Bg300/At353 cross in PDT screening, using CTAB DNA extraction method (Doyle and Doyle, 1987). The PCR was carried out with a mixture consisted of 3 µl of diluted DNA template, 3.00 µl of 5X buffer, 0.9 µl of 25 mM $MgCl_2$, 0.15 µl of 2.5 mM dNTPs, 1 µl of 20 pmol/µl primers F: 5/TGAGATAGCCGTCAAGATGCT3/ and R: 5/ AAGGACCACCATTCCATAGC 3/ and 0.075 µl of 5 U/µl *Taq* DNA polymerase (Promega,USA) in a total volume of 15 µl. Amplification was performed in a Thermal Cycler (Bio Rad My cycler™, USA) with temperature profile of initial denaturing at 94 °C for 5 min followed by 35 cycles of 1 min at 94 °C, 1 min at 57 °C for 1 min for primer annealing and 2 min at 72 °C for extension and final extension cycle of 05 min at 72 °C. Five microliters of amplified PCR products were run in a horizontal electrophoresis system on 2% agarose gel prepared using 1X TBE included with 0.5 µg/ml Ethidium bromide at 5 V/cm in 1 X TBE buffer. Gel was visualized and images were captured by using a gel documentation system.

Evaluation for agronomic traits

The RILs which showed PD tolerance in evaluation and the PSTOL 1 gene were field planted and evaluated for agronomic traits. They were field planted as three row progenies containing 100 plants with 20 cm x 15 cm spacing were considered as plots. Three raw progenies were designed as Randomized complete block design with three replicates and were evaluated for plant characters and yield parameters.

Multiple traits reflecting final yield, plant height, number of productive tillers, panicle length, panicle weight and yield per plant were taken from 10 plants in the middle row of each progeny and average per plant value was considered as a replicate. Lines were compared for the tested variables using ANOVA (PROC GLM) procedure in SAS, except for number of productive tillers. Kruskal-wallis test was performed to test the differences in number of productive tillers among the lines. To study relationships between four variables and the yield, simple linear regression analysis was performed separately for the two crosses. Interpretations were made at P =0.05.

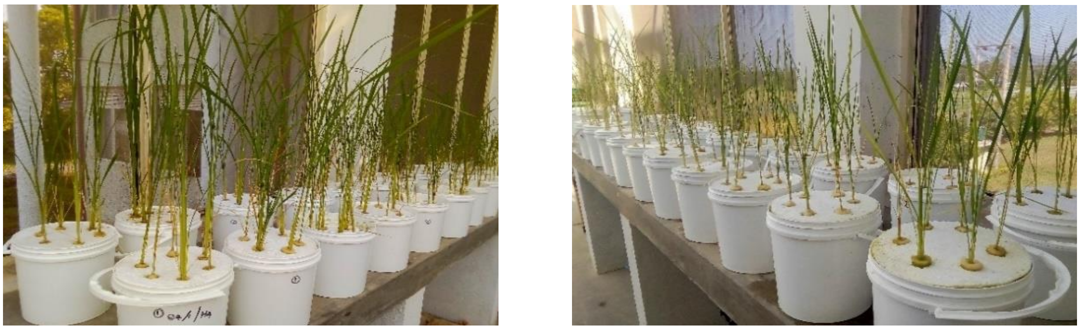

Figure 1: Evaluation of RILs for PD tolerance in hydroponic system

RESULTS AND DISCUSSION

Development of RILs through SSD approach

As dense planting is a characteristic feature of the SSD method, for developing recombinant inbred lines from F_2 to F_6 generation, plants were grown in a tray in a plant house with limited spacing. Although planned selection was not done in SSD method as in progeny selection in classical breeding procedure, there was a natural selection for removing weak plants from generation to generation as some genotypes did not produce panicles under the given conditions. Therefore, in some genotypes, there was a loss of number of plants from generation to generation and unfilled grains in panicles, weak growth and absence of panicles. At the F_6 generation, there were 160 plants from Bg94-1/H_4 cross and 150 plants from Bg300/At353 cross from initial 400 plants at F_2 generation of each cross

RIL selection for PD tolerance in hydroponic system (Bg 94-1/H_4 cross and Bg 300/At 353 cross)

The RILs were significantly different on parameters; plant height, rooting depth, total dry weight, number of tillers per plant and plant vigor ($P<0.01$). Therefore, all the parameters were considered when selecting the best performing RILs at low-P availability. The mean of three plant in each parameter is shown in Tables 1 and 2 in separate crosses.
The highest tiller number of four tillers per plant was recorded in Bg94-1 ($P<0.05$). Seven RILs had seedling vigour of three, similar to that of both the parents ($P>0.05$). The line 8 had the highest plant dry weight (2.3 g/plant) followed by the best parent Bg94-1, although it had lesser tiller number than Bg94-1 (Table 1).

Table 1: Mean values of parameters tested in selected RILs of Bg 94-1/H_4 cross at F_8 generation in hydroponic screening

Line/Variety	Plant height (cm)	Rooting depth (cm)	Number of tillers	Seedling vigor*	Dry weight/plant (g)
1	44	30	1	1	1.4
2	42	27	2	2	1.3
3	56	32	3	3	2.0
4	41	29	1	1	1.4
5	42	29	1	1	1.4
6	45	26	1	1	1.4
7	35	19	1	1	1.4
8	77	38	3	3	2.3
9	50	26	1	1	1.5
10	38	23	1	3	1.5
11	38	18	1	1	1.2
12	47	30	1	1	1.4
13	42	21	1	1	1.4
14	60	32	2	3	2.0
15	53	37	3	2	2.0
16	45	25	1	1	1.5
17	40	19	1	1	1.3

18	51	25	2	2	1.4
19	49	23	1	1	1.5
20	41	20	1	1	1.4
21	44	26	2	2	1.4
22	35	18	1	1	1.3
23	36	19	1	1	1.3
24	43	24	1	1	1.5
25	42	23	1	3	1.4
26	35	16	1	1	1.3
27	42	25	1	1	1.5
28	47	29	1	1	1.5
29	41	28	1	1	1.3
30	77	41	2	3	2.1
31	50	27	1	1	1.5
32	42	22	2	2	1.5
33	36	19	1	1	1.4
34	55	31	3	3	1.9
35	74	38	3	3	2.2
36	51	27	2	2	1.5
37	40	20	1	1	1.5
38	45	27	1	1	1.4
39	50	31	1	1	1.6
40	55	35	3	3	1.9
Bg 94-1	55	40	4	3	2.2
H4	77	39	1	3	1.9

*Seedling vigor; 1 – weak, 2- moderate, 3- good

All the RILs selected in Bg300/At353 cross were shorter than 63 cm (Table 2). The RIL numbers 58 and 72 had four tillers per plant and it was similar to the highest number of tiller bearing parent At353 (P>0.05). Both parents showed similar seedling vigor and there were 11 RILs with the same rank in seedling vigor (P>0.05). The highest mean plant dry weight of 2.1 g/plant was recorded in RIL No. 49 followed by 2.0 g/plant in RIL Nos. 40, 58, 61 and 69, which were higher than that of both the parents (P<0.05).

All the RILs selected in Bg300/At353 cross were shorter than 60 cm. The RIL numbers 58 and 72 showed four tillers per plant while the highest number of tiller-bearing parent At353 had the same number of tillers. Both parents showed similar seedling vigor and there were 11 RILs with the same rank in seedling vigor. The highest mean plant dry weight 2.1 g/plant was recorded in RIL No. 49 followed by 2.0 g/plant in RIL No. 40, 58, 61 and 69, which were higher than that of the parents Bg300 and At353 (Table 2).

The best performing RILs for each parameter are summarized in Table 3 and the number of parameters that promising RILs were included in, of each crosses Bg94-1/H_4 and Bg300/At353 are illustrated in Figures 2 and 3, respectively, As the varietal performances varied, two cut off levels were used for two crosses and RILs are selected if they are included in at least three parameters as the best performer in Bg300/At353 cross while RILs were selected if they were included in two parameters in Bg94-1/H_4 cross.

Table 2: Mean values of parameters tested in selected RILs of Bg300/At353 cross at F_8 generation in hydroponic screening

Line/Variety	Plant height (cm)	Rooting depth (cm)	Number of tillers/plant	Seedling vigor*	Total dry weight/plant (g)
41	54	32	3	3	2.0
42	47	27	1	2	1.3
43	38	22	2	2	1.3
44	56	35	3	3	1.9
45	45	25	1	2	1.6
46	39	22	2	2	1.2
47	56	36	3	3	1.8
48	50	27	2	1	1.4
49	55	36	3	3	2.1
50	62	41	2	2	1.5
51	36	23	2	2	1.5
52	56	35	3	3	1.8
53	36	22	2	1	1.4
54	38	22	1	2	1.3
55	42	24	2	2	1.2
56	56	36	3	3	1.7
57	32	22	2	2	1.4
58	55	35	4	3	2.0
59	60	37	2	1	1.6
60	46	25	2	2	1.3
61	55	33	3	3	2.0
62	47	25	2	1	1.5
63	40	22	2	1	1.3
64	56	36	2	2	1.6
65	56	34	3	3	1.8
66	40	26	2	2	1.3
67	51	35	1	2	1.6
68	50	33	2	2	1.6
69	54	33	3	3	2.0
70	51	32	2	2	1.7
71	56	33	1	1	1.5
72	60	43	4	3	2.0
73	56	34	2	2	1.6
74	53	31	2	1	1.7
75	60	39	2	2	1.5
76	45	23	1	1	1.4
77	46	24	1	2	1.3
78	40	21	2	1	1.3
79	47	31	2	1	1.6
80	42	22	2	2	1.3
At353	48	34	4	3	1.8
Bg300	55	41	2	3	1.9

*Seedling vigor; 1 - weak, 2 - moderate, 3 - good

Table 3: Promising RILs which are not significantly different for each parameters tested

Parameter	Plant height	Rooting depth	Number of tillers/plant	Seedling vigor	Dry weight/plant
Bg 94-1/ H_4	30, 08, 35, 14, 03, 34, 40, 15, 18, 36, 09	30, 35, 08, 15, 40, 14, 03, 34, 39, 01, 12	35, 08, 15, 40, 03, 34, 30, 14	35, 08, 40, 03, 34, 30, 14, 10. 25	08, 35, 30, 03, 14, 15, 40, 34, 39, 31
Bg300/At353	50, 75, 59, 72, 44, 65, 56, 73, 47, 64, 52	72, 50, 75, 59, 47, 49, 56, 64, 52, 44, 67	58, 72, 49, 61, 41, 47, 52, 65, 69	58, 41, 65, 72, 49, 44, 61, 47, 52, 69, 56	49, 58, 72, 69, 41, 61, 44, 47, 52, 65 56

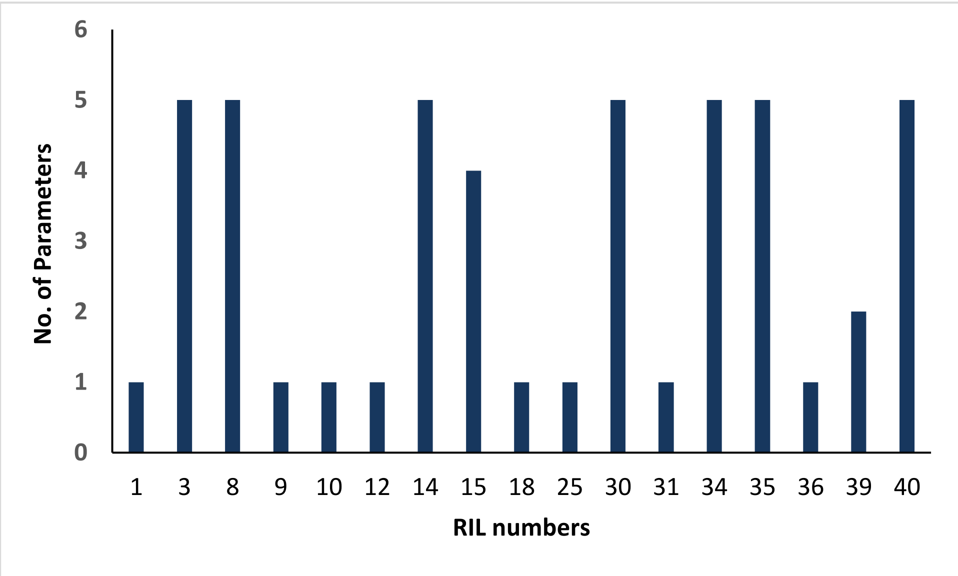

Figure 2: Number of parameters of promising RILs that were included in Bg94-1/H_4 cross
RILs included in 2 or more parameters align on or above the horizontal line at No. 2

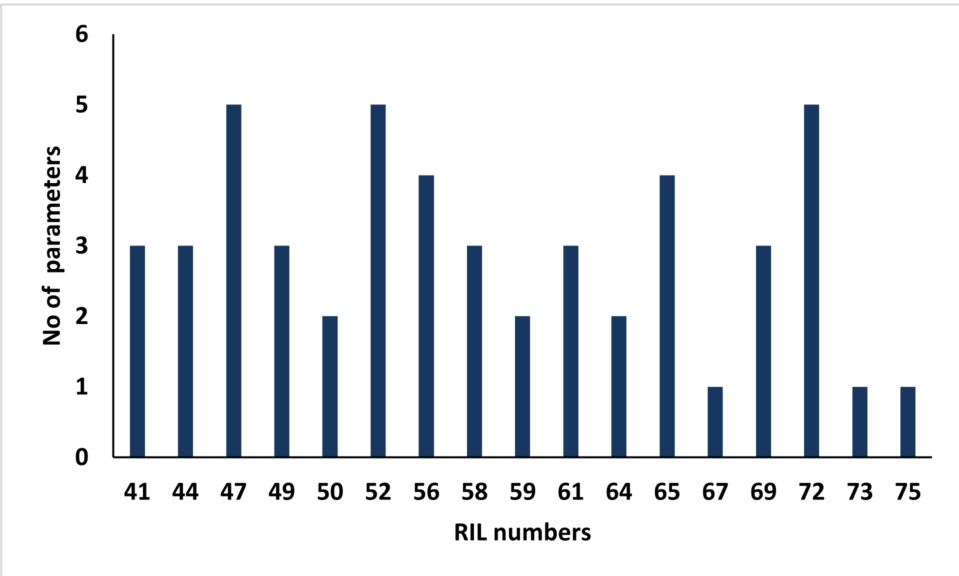

Figure 3: Number of parameters of promising RILs that were included in Bg300/At353 cross. RILs included in 3 or more parameters align on or above the horizontal line at No. 3

Out of 80 RILs used for PD tolerance evaluation, a total of 20 TILS were selected including 9 RILs from Bg94-1/H_4 cross, namely 3, 8, 14, 15, 30, 34, 35, 39 and 40 and 11 RILs from Bg300/At353 cross namely 41, 44, 47, 49, 52, 56, 58, 61, 65, 69 and 72 (Figure 2 and 3).

Molecular evaluation of selected 11 RILs of Bg300/At353 cross and 4 parental lines used in crosses

Varieties Bg300, H_4 and Bg94-1 had 523 bp PCR band suggesting the presence of PSTOL1 gene while only variety At353 was PSTOL1 negative. Therefore, molecular evaluation was not needed for selecting PSTOL1 positive genotypes from 9 RILs selected in the Bg94-1/H_4 cross and only 11 RILs selected from the Bg300/At353 cross were subjected to molecular marker assisted selection.

The RIL numbers 44, 52 and 56 did not show DNA band while others gave 523 bp band for K46-1 marker. It revealed that RIL No. 41, 47, 49, 58, 61, 65, 69 and 72 are PSTOL1 positive while 44, 52 and 56 are absent in PSTOL1 gene (Figure 4). However, PSTOL1 negative RIL No 52 included in the three of best performers under low-P condition while many of the PSTOL1 positive RILs did not show P tolerance attributes. This suggests the possibility of having another gene/QTL or mechanism either in Bg300 or in At353 that enhances PDT.

Evaluation of RILs for agronomic traits and yield parameters under optimum P availability

Field evaluated seventeen RILs were significantly different ($P<0.05$) for all the variables separately in each cross except the number of tillers in Bg94-1/H_4 cross. In Bg300/At353 cross, selected lines were significantly different in all the variables tested (Table 4).

Effect of measured parameters on yield

In Bg94-1/H_4 cross, there was no significant relationship ($P>0.05$) between the plant height or panicle length with yield. However, a positive relationship was observed between the yield and number of productive tillers and panicle weight (Figure 5). The Bg300/At353 cross showed positive correlation with all four parameters with yield. However, only the plant height showed a significant relationship with yield at $P<0.05$ (Figure 6).

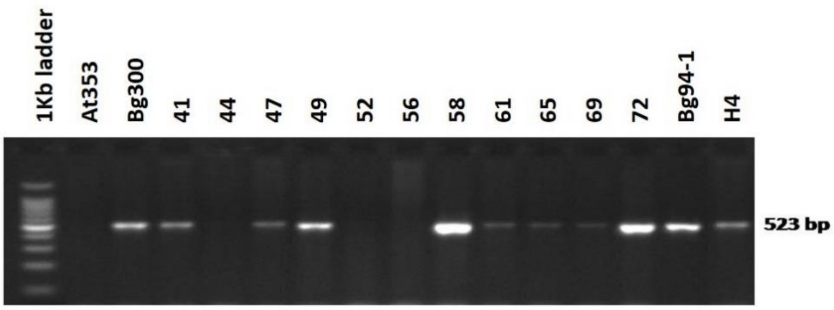

Figure 4: PCR amplicons of 523 bp for K46-1 dominant gene specific marker representing presence of PSTOL1 gene in all 4 parents and 11 RILs of Bg 300/At 353 cross. Note: line1 – 1 kb ladder, line 2 - At353, line 3 - Bg300, Line 4 – 14 RILs from Bg300/At353 cross, line15 - Bg94-1, line 16 - H_4

Table 4: Level of significance of variables studied in agronomic evaluation

	Plant height (cm)	**Number of tillers/plant**	**Panicle length (cm)**	**Panicle weight (g)**	**Yield/plant (g)**
RILs of Bg 94-1/ H_4	<0.01	0.12	<0.01	<0.02	<0.01
RILs of Bg 300/ At 353	<0.01	<0.01	<0.01	<0.01	<0.01

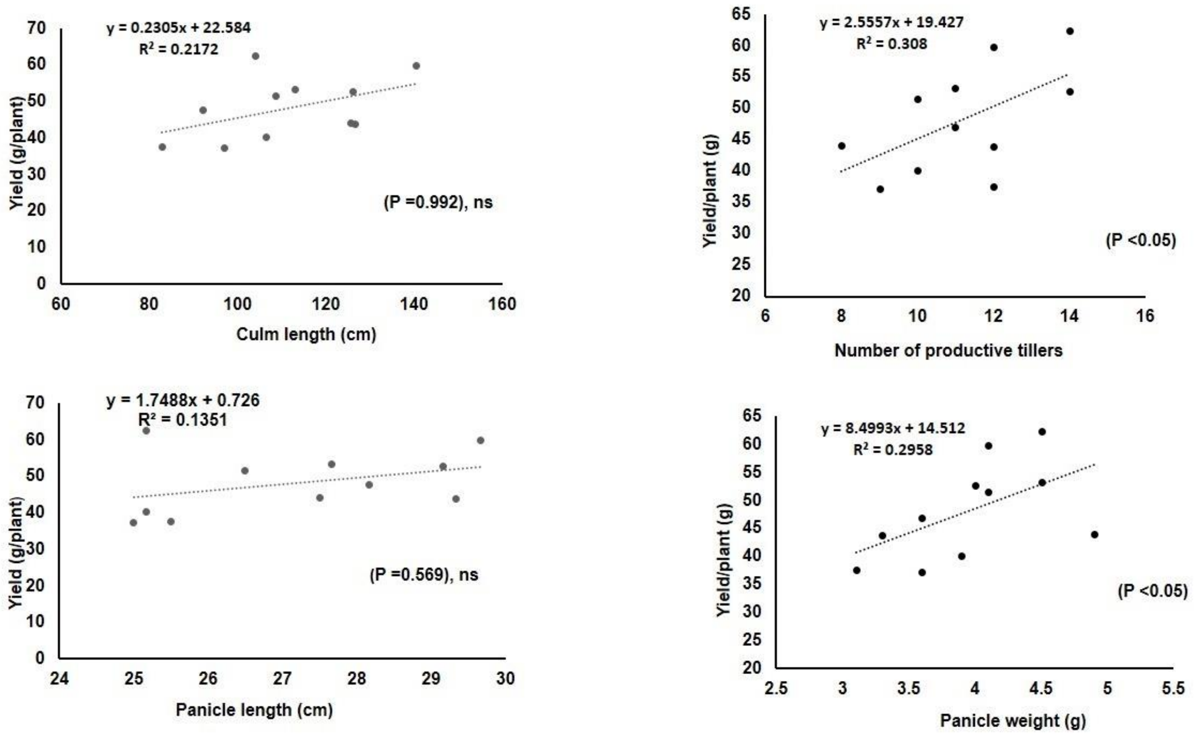

Figure 5: Relationships between the plant height, tiller number, panicle length and panicle weight of evaluated RILs of Bg94-1/H_4 cross (each point represents the mean values of respective parameter of each line).

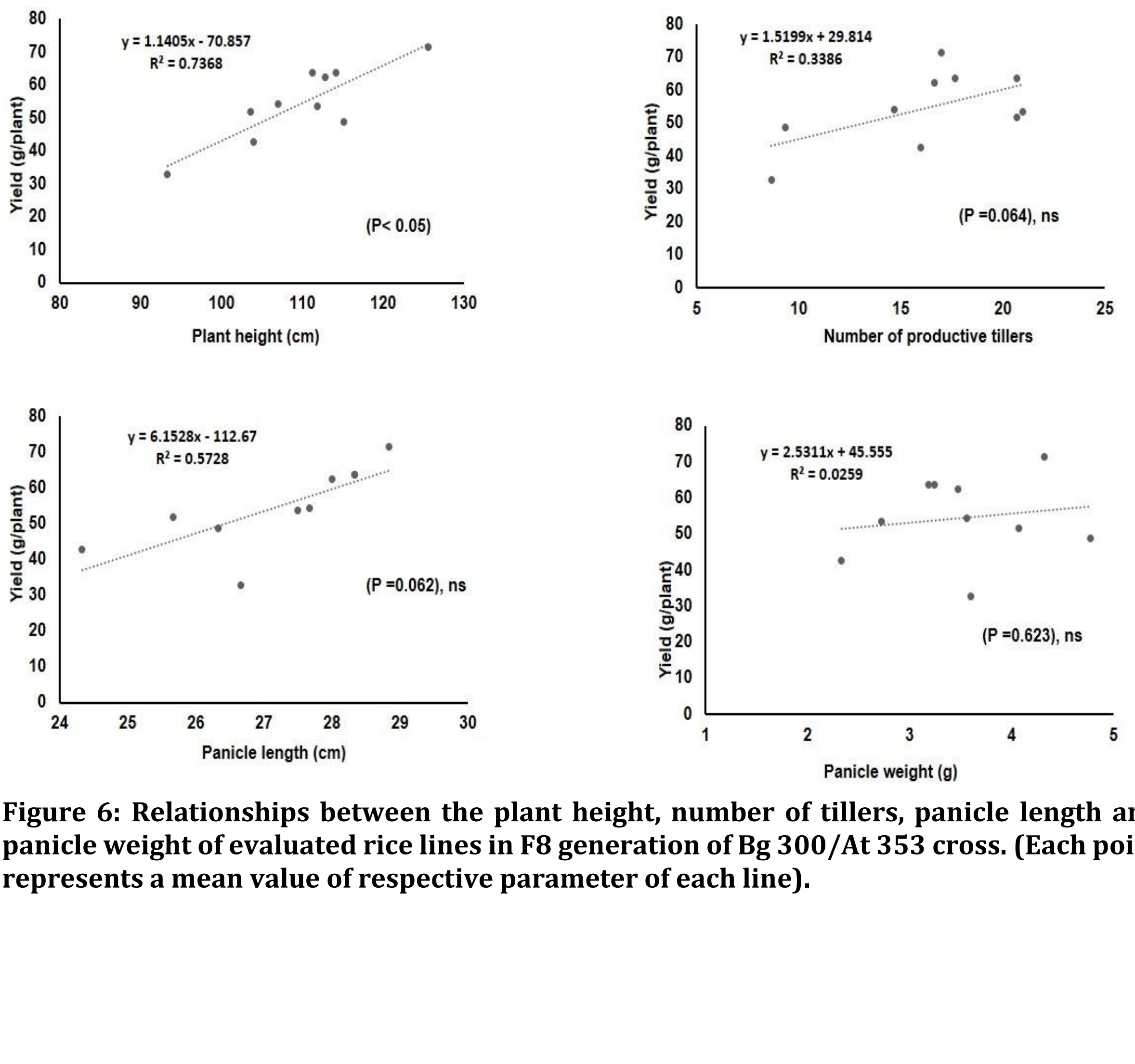

Figure 6: Relationships between the plant height, number of tillers, panicle length and panicle weight of evaluated rice lines in F8 generation of Bg 300/At 353 cross. (Each point represents a mean value of respective parameter of each line).

As the panicle weight and the number of productive tillers showed a significant relationship with yield In Bg94-1/H_4 cross, panicle weight and the number of productive tillers can be considered for evaluating the yield of each progeny of Bg94-1/H_4 cross.
In Bg300/At353 cross, only the plant height with yield shows a higher regression coefficient (Figure 6). Therefore, yield cannot be predicted using the panicle weight, panicle length or the number of productive tillers in this cross.

Evaluation of RILs for agronomic characters

Seventeen RILs were tested for agronomic traits including 9 from Bg94-1/H4 and selected 8 RILS from molecular selection of Bg300/At353 cross. Out of them fourteen RILs resulted higher yields than that of their parents. Line numbers 3, 15 and 49 gave lower grain yield than their respective parents while the highest mean yields were given by 47, followed by 58, 61, 35 and 41 (Tables 5 and 6).

The number of productive tillers was comparatively higher in lines derived from Bg300/At353 (Table 6). However, panicle weight was higher in lines obtained from Bg94/H_4 cross (Table 5). The highest tiller number of 21 was obtained from three lines; i.e. 58, 65 and 69 (Table 6).

The variety H_4 produced a higher yield than Bg94-1 (Table 5), although Bg94-1 was selected as the best variety for PD tolerance. This may be due to the age difference of the two varieties since maturity age of Bg94-1 is 3½ months while that in H_4 is 4½ months and it shows how plant behaviors change with P available condition than P deprived condition Bg300 gave a higher yield than At353 exhibiting high yielding capability of Bg300 which included in three month age group exceeding the yield of 3½ month age variety At353.

An ideal plant height is essential for the yield of rice. However, lodging resistance mainly depends on plant height. Therefore, four lines, namely, 8, 39, 40 and 47 were rejected due to their tall nature having more than 125 cm plant height although they gave higher yield. Finally, 10 lines; 14, 30, 34, 35, 41, 58, 61, 65, 69 and 72 were selected as PDT rice lines with PSTOL1 gene.

The single seed descent method has been applied for accelerating the classical breeding programs, increasing the favorable genotypes and reducing the breeding cost (Maruyama, 1987), Current results suggest the possibility of using SSD for developing nutrient use efficient rice varieties through proper screening method with the use of marker assisted selection.

Table 5: Means of the variables tested for each advanced line in F_8 generation of Bg94-1/H_4 cross in the field with DoA fertilizer recommendation

RIL No	Panicle length (cm)	Plant height (cm)	No. of tillers/plant	Panicle weight (g)	Yield/plant (g)
94-1	25.0 ± 0.5	97 ± 0.0	09 ± 1.0	3.6 ± 0.2	37.2±1.7
H_4	29.3 ± 0.7	127 ± 0.7	12 ± 0.7	3.3 ± 0.1	43.9±2.8
3	25.5 ± 0.8	83 ± 0.7	12 ± 0.9	3.1 ±0.2	37.6±1.1
8	27.5 ± 0.3	126 ± 0.1	08 ± 0.6	4.9±0.2	44.1±2.6
14	27.7 ± 0.9	113 ± 0.6	11 ±1.7	4.5±0.6	53.2±1.7
15	25.2 ± 0.6	107 ± 0.4	10 ± 0.3	3.9±0.2	40.2±1.9
30	28.2 ± 0.2	92 ± 0.7	11 ±1.3	3.6±0.3	47.0 ±2.9
34	26.5 ± 0.3	109 ± 0.5	10 ± 0.9	4.1±0.5	51.6 ±2.1
35	25.2 ± 0.7	104 ± 0.1	14 ± 2.0	4.5±0.2	62.4 ±2.8
39	29.1 ± 1.8	126 ± 0.7	14 ± 1.1	4.0±0.2	52.7±4.3
40	29.6 ± 0.9	144 ± 0.9	12 ± 2.5	4.1±0.5	59.9 ±1.2

Values are Mean ± Standard error of 3 replicates

Table 6: Means of variables tested for each advanced line in F_8 generation of Bg300/At353 cross in the field with DoA fertilizer recommendation

Line No	Panicle length (cm)	Plant height (cm)	No. of tillers/plant	Panicle weight (g)	Yield/plant (g)
At353	26.67 ± 1.2	93 ± 0.7	9 ± 1.7	3.6 ± 0.4	32.7 ± 1.8
Bg300	26.33 ± 0.4	105 ± 1.0	9 ± 0.9	4.8 ± 0.5	48.7 ± 3.3
41	28.00 ± 0.8	113 ± 0.6	17 ± 0.9	3.5 ± 0.1	62.3 ± 2.8
47	28.83 ± 1.2	126 ± 0.7	17 ± 1.0	4.3 ± 0.4	71.5 ± 2.8
49	24.33 ± 0.4	104 ± 0.2	16 ± 1.0	2.3 ± 0.1	42.6 ± 0.5
58	28.33 ± 0.2	114 ± 0.1	21 ± 4.3	3.2 ± 0.8	63.7 ± 2.0
61	28.33 ± 0.2	111 ± 0.2	18 ± 0.3	3.2 ± 0.1	63.7 ± 1.8
65	25.67 ± 0.9	104 ± 0.5	21 ± 5.2	4.1 ± 0.2	51.7 ± 1.3
69	27.50 ± 0.5	112 ± 0.6	21 ± 2.5	2.7 ± 0.2	53.6 ± 3.1
72	27.67 ± 0.6	107± 0.5	15 ± 1.8	3.6 ± 0.1	54.2± 2.5

Values are Mean ± Standard error of 3 replicates

CONCLUSIONS

Ten promising rice lines; 14, 30, 34, 35, 41, 58, 61, 65, 69 and 72 were identified as PD tolerant, high yielding rice lines. The SSD method can be effectively applied for specific target trait improvement in rice breeding.

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REFERENCES

Ali, J., Jewel, Z.A., Mahender, A., Anandan, Hernandez, J. & Li, Z. (2018). Molecular Genetics and Breeding for Nutrient Use Efficiency in Rice. *International Journal of Molecular Sciences* 19, 1762; doi: 10.3390/ijms19061762.

Aluwihare, Y.C., Ishan, M., Chamikara, M.D.M., Weebedde, C.K. and Sirisena, D.N., Samarasinghe, W.L.G. & Sooriyapathirana. S.D.S.S. (2016). Characterization and selection of phosphorus deficiency tolerant rice genotypes in Sri Lanka. *Rice Science,* 23(4): 184-195.

Annual Report 2018, Central Bank of Sri Lanka, Colombo, Sri Lanka

Breseghello, F. & Coelho, A. S. G. (2013). Traditional and modern plant breeding methods with examples in rice **(***Oryza sativa* L.**).** *Journal of Agricultural and Food Chemistry*, 61, 8277-8286.

Chin, J. H., Gamuyao, R., Dalid, C., Bustamam, M., Prasetiyono, J. Moeljopawiro, S., Wissuwa, M. & Heuer, S. (2011). Developing Rice with High Yield under Phosphorus Deficiency: Pup1 Sequence to Application. *Plant Physiology*. 156: 1202–1216.

Doyle, J.J. & Doyle, J.L. (1987). A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochemical Bulletin*. 19: 11- 15

Fageria, N. K., Wright, R. J. & Baligar, V. C. (1998). Cultivar evaluation for phosphorus use efficiency. *Plant and Soil.* 111, 105-109.

Janwan, M., Streewongchai, T. & Sripichitt, P. (2013) Rice breeding for high yield by advanced single seed descent method of selection. *Journal of Plant Sciences*. 8(1): 24-30.

Kant, J., Ishizaki, T., Pariasca-Tanaka, J., Rose, T., Wissuwa, M. & Watt, M. (2018).

Phosphorus Efficient Phenotype of Rice. In: Shah, F., Khan, Z.H., Iqbal, A. (Eds.). https://doi.org/10.5772/intechopen.

Kekulandara, D.S., Bandaranayake, P.C.G., Sirisena, D.N. Samarasinghe, W.L.G. & Suriyagoda, L.D.B. (2017). Temporal tillering behavior of Sri Lankan elite rice varieties in response to phosphorus availability. *Tropical Agriculture Research*. 28:133–143.

Kekulandara, D.S., Suriyagoda, L.D.B., Sirisena, D.N., Bandaranayake, P.C.G & Samarasinghe, W.L.G. (2016). Determination of deprived and optimum phosphorus concentrations for screening rice germplasm for P deficiency tolerance using hydroponics. *Proceeding of the Wayamba University International Conference* 2016: 299.

Kekulandara, D.S., Sirisena, D.N., Bandaranayake, P.C.G., Samarasinghe, G., Wissuwa, M. & Suriyagoda, L.D.B. (2019). Variation in grain yield, and nitrogen, phosphorus and potassium nutrition of irrigated rice cultivars grown at fertile and low-fertile soils. *Plant and Soil*. 434: 107–123.

Khan, M. H., Dar, Z. A. & Dar, S.A. (2015). *Agricultural Sciences*, Breeding Strategies for Improving Rice Yield-A Review 6, 467-478.

Maruyama, K., (1987). Using rapid generation advance with single seed descent in rice breeding. *Proceedings of the International Rice Research Conference*, September 21-25, 1987, Hangzhou, China, pp: 253-259.

Media briefing on fertilizer [online]. Available at https://agrimin.gov.lk/web/index.php/en/news-and-events/1492-2021-02-02.

Mukherjee, A., Sarkar, S., Chakraborty, A. S., Yelne, R., Kavishetty, V., Biswas, T., Mandal, N. & Bhattacharyya, S. (2014). Phosphate acquisition efficiency and phosphate starvation tolerance locus (PSTOL1) in rice. *Journal of Genetics* 93, 683–688.

Paddy Statistics 2020/21 Maha season (2021). Department of Census and Statistics. Sri Lanka.www.statistics.gov.lk/Agriculture/StaticalInformation/

Ratnayake, A.R.M.S.P. & Navaratna, A.N. (2014). Spectroscopic determination of metal impurities in commercial raw material fertilizer in Sri Lanka. *Ceylon Journal of Science (Physical Sciences)*. 18, 27 – 36.

Rose, T.J., Rose, M.T., Pariasca-Tanaka, J., Heuer, S. & Wissuwa, M. (2011). The frustration with utilization: why have improvements in internal phosphorus utilization efficiency in crops remained so elusive? *Frontiers in Plant Science*. 2, 73.

SAS (2005) Statistical analysis systems. Release 9. SAS Institute Inc, Cary.

Tong, L. & Yoshida, T. (2008). Can hot-water emasculation be applied to artificial hybridization of Indica-type Cambodian rice? *Plant Production Science* 11(1): 132-133.

Wissuwa, M. & Ae, N. (2001). Genotypic variation for tolerance to phosphorus deficiency in rice and the potential for its exploitation in rice improvement. *Plant Breeding.* 120: 43-48.

Zhang, Q. (2007). Strategies for developing green super rice*. Proceedings of National Academy of Science*, U. S. A. 104:16402-16409.