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AGRONOMIC PERFORMANCE OF BC_3F_5 RICE LINES INTROGRESSED WITH *PUP1* AND *ALT* LOCI

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SUMMARY

Phosphorus (P) is one of the important nutrients needed for rice growth. One of the reasons for the low availability of P in soil is due to its binding with aluminum. Combination of *Pup1* and *Alt* loci in one genotype is expected to produce good agronomic appearance and high productivity. This study aimed to evaluate agronomic performance of BC_3F_5 lines from three crosses containing *Pup1* and *Alt* loci. Field testing of the breeding material was conducted at Taman Bogo, Lampung while molecular marker analysis was carried out at Moleculer Biology Laboratory (ICABIOGRAD), Bogor, West Java, Indonesia. Plant material comprising 54 genotypes, consisted of 11 parental lines, 14 BC_3F_5 Dodokan-*Pup1+Alt* lines, 28 BC_3F_5 Situ Bagendit-*Pup1+Alt* lines, and 2 BC_3F_5 Batur-*Pup1+Alt* lines. The research field had various levels of Al toxicity with low availability of P, indicating that a large proportion of P was bound by Al. All tested lines in the field had *Pup1* locus, however, two tested lines Situ Bagendit-*Pup1+Alt* lines did not have *Alt* locus. *Pup1* locus had significant effect and improve the number of empty grains on Batur. *Alt* locus had significant effects on Dodokan for plant height, Situ Bagendit for plant height, number of total and productive tillers, and Batur for plant height, panicle length, total tillers and grain weight. Line number 13(A71)-9 could be one of the promising lines, because it has allele of donor parent and maximum weight of filled grains per clump.

Keywords: Agronomic characters, *Alt*, molecular markers, *Pup1*, rice

Key findings: Evaluation of agronomic performance of the genotypes consisting *Pup1* and *Alt* loci in field and molecular analysis showed *Pup1* locus had significant effect and improve the number of empty grains on Batur. *Alt* locus had significant effects on Dodokan for plant height, Situ Bagendit for plant height, number of total

and productive tillers, and Batur for plant height, panicle length, number of total tillers and grain weight. A promising line with both *Pup1*+*Alt* loci showed highest weight of filled grains per clump.

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INTRODUCTION

In tropical countries like Indonesia, the most common obstacle in cultivation of upland rice is phosphorus (P) deficiency and aluminum (Al) toxicity. The dry lands are generally infertile, dominated by acid soils which are characterized by low pH (<5.5), has relatively high aluminum and fixation of phosphorus (P), sensitive to erosion and poor in biotic elements (Prasetyo, 2001; Mulyani and Hidayat, 2009).

As a macro nutrient, P is the second most required element by plants after nitrogen (N). P element influences every stage of rice growth and development. Root development, total tillers, flowering, and maturation (especially at low temperatures) are influenced by P availability. Plants with proper amount of phosphorus will flower, ripe faster, and produce high grain yield (Ya-jie *et al.*, 2012; Atakora *et al*., 2015).

Aluminum toxicity is one of the problems those commonly encountered in upland rice cultivation. Under acidic soils, P elements cannot be absorbed maximally by plants due to binding with Al. Soil acidity can also cause ionization of Al elements into Al^{3+} which is toxic to plants. Aluminum toxicity affects many parts of the roots (Matsumoto *et al*., 2015). The roots will become short, brittle, thicken, and turn brownish until the tips become curled, and the fine root branches will diminish (Prasetiyono, 2010; Setiadi and Anira, 2015; Tasma, 2015). Another indicator of toxicity is decreasing dry weight of root and canopy (Prasetiyono, 2010).

The use of P deficiency and Al toxicity tolerant genotypes is considered as a practical and environmentally friendly solution for growing rice in the lands with these stresses. The selection stage is one of the important steps to obtain good performing genotypes. Marker-assisted breeding could be a promising method for developing new lines. Application of molecular markers can reduce the number of population and time needed for selection in the field (Prasetiyono, 2010; Tasliah *et al*., 2015). Out of several molecular marker techniques available, Simple Sequence Repeat (SSR) markers are relatively simple to use, inexpensive and can produce better polymorphism (Akagi *et al*., 1996; Wang *et al*., 2014).

Pup1 locus mapped by Wissuwa *et al*. (1998) is a QTL in rice that can be used for detection of P deficiency tolerance. The source of *Pup1* comes from Kasalath, an Indian landrace rice. Aluminum toxicity tolerance is controlled by many genes (Prasetiyono *et al*., 2003). *Alt* locus used in this study was mapped by Hidayatun (2014), which originated from Dupa, an Indonesian local rice. The combination of the *Pup1* and *Alt* loci in upland rice is expected to provide

positive correlation to vegetative and generative characters especially productivity. The existence of *Pup1* and *Alt* loci together in one genotypes expected to produce good agronomic appearance and high yielding plants cultivated in acid soils. According to Tasliah *et al*. (2011), the mechanism of P deficiency and Al toxicity are not related. Molecular analysis of P deficiency and Al toxicity tolerance was carried out separately in this study.

The development of rice lines since 2014 containing the *Pup1* and *Alt* loci using the marker-assisted backcrossing (MAB) method has been carried out by Indonesian Center for Agricultural Biotechnology and Genetic Resources Research and Development (ICABIOGRAD), Indonesia. *Alt* locus was introgressed into the Dodokan, Situ Bagendit, and Batur varieties with the earlier introgressed *Pup1* locus by the MAB method (Prasetiyono *et al*., 2016). These lines need to be tested in the field to determine the tolerance level to Al toxicity and P deficiency conditions. Therefore, this study aimed to examine 44 BC_3F_5 lines from three crosses containing the *Pup1* and *Alt* loci in the field.

MATERIALS AND METHODS

Field research was conducted at Taman Bogo, Lampung Province and molecular studies were conducted during November 2017 to May 2018 at Molecular Biology Laboratory, ICABIOGRAD, Bogor, West Java Province, Indonesia. Plant materials used were 54 genotypes, consisting of 11 parental lines, 14 BC_3F_5 Dodokan-*Pup1*+*Alt* lines, 28 BC_3F_5 Situ Bagendit-*Pup1*+*Alt* lines, and 2 BC_3F_5 Batur-*Pup1*+*Alt* lines. Molecular markers used for foreground selection were RM12031 and RM1361 for *Alt* locus (Mizan, 2018) and Kas46-2 for *Pup1* locus (Prasetiyono, 2010; Tasliah *et al*., 2011). The information of the used markers is described in Table 1.

Field testing

The research at Taman Bogo was conducted using a randomized complete block design with three replicates. The plants were grown in 3 m × 3 m plots, with spacing of 25 cm × 25 cm. Seeds (1-3 grains/hill) were direcly inserted into planting hole. Fertilization included urea 300 kg/ha, phosphate (SP-36) 100 kg/ha, and potassium (potassium chloride/KCl) 100 kg/ha. The plants were observed for agronomic characters consisting of plant height (PH [cm]), number of total tillers (TT), number of productive tillers (PT), days to flowering (FD [days]), panicle length (PL), number of filled grains per panicle (FG), number of empty grains per panicle (EG), total grains per panicle (TG),

Table 1. Markers used for foreground selection.

Marker Name		Base Chain	Locus
RM12031	*(forward)*	ATGCTTGCAGACAATCGATGC	*Alt*
	(reverse)	CTCTCCGCCTAAACAACTTGTGC	
RM1361	*(forward)*	TCCCTAGCTAGCTCTCCATCTCC	
	(reverse)	AGTACTACCGCTACATGTCTTCTTGG	
Kas46-2	*(forward)*	AGGAAGATGGTTGTCGTTGG	*Pup1*
	(reverse)	TTCACACCAAACAGTGTTGTC	

weight of 1,000 grains (WG), and weight of filled grains per clump (WF). Several soil samples were taken from different points in the field, bulked and analyzed.

Molecular studies

One leaf sample from was collected for each of 10 four week old plants and bulked for each genotype. DNA was isolated according to modified Dellaporta *et al*. (1983) method.

The PCR reaction was carried out at 20 µl of volume containing 1× PCR buffer (10 mM Tris-HCl [pH 8.3], 50 mM KCl, 1.5 mM $MgCl_2$, 0.01% gelatin), 100 µM dNTPs (dATP, dCTP, dGTP, dTTP), 0.5 µM primary (F and R), DNA, and 1 unit of Taq DNA polymerase. The PCR profile was: (1) 5 minutes at 94°C for initial denaturation, (2) 35 cycles consisting of 1 minute at 94°C denaturation, (3) 1 minute at 55°C for primer attachment, (4) 2 minutes at a temperature of 72°C for primer extension, and the final primer extension was (5) 7 minutes at 72°C. The amplified PCR product was visualized through electrophoresis using 2% agarose gel (Kas46-2) and 8% polyacrylamide gel (primer RM12031 and RM1361). DNA staining was done using ethidium bromide (Et-Br) 20 mg/l for ±5 minutes and gels were documented by using Chemidoc gel system.

Data analysis

The molecular data were analyzed by comparing the alleles from each BC_3F_5 line with check parents, *viz*. Dupa for RM12031 and RM1361 markers, and Kasalath for Kas46-2 marker.

Data analysis for the field experiment was carried out using analysis of variance for testing the genotypic effects. If the F-test is significant, the analysis was continued with the Dunnett's test. Comparisons between the check parents and introgressed lines were carried out by orthogonal contrast testing.

RESULT

Molecular observation

Amplification of Kas46-2 (Figure 1) indicated that all the progenies of three BC_3F_5 crosses (DP-A, SB-PA, and BP-A) have the same alleles as Kasalath. PCR amplification of RM1361 and RM12031 detected expected alleles of the parental genotypes (Table 2)(Figures 2 and 3). Two lines of Dodokan-*Pup1+Alt* and Situ Bagendit-*Pup1+Alt* have the same allele of RM1361 as recurrent parent (Dodokan-*Pup1* and Situ Bagendit-*Pup1+Alt*). Whereas for RM12031 marker, only one line of Situ Bagendit-*Pup1+Alt* progeny has the allele of the recurrent parent (Situ Bagendit-*Pup1*). In addition, heterozygous genotypes with RM1361 were observed in progenies of Batur-*Pup1+Alt* and RM12031 in progenies of Dodokan-*Pup1+Alt*, Situ Bagendit-*Pup1+Alt*, and Batur-*Pup1+Alt*.

Field testing

The soil texture in the Taman Bogo experimental field is dominated by sand particles (Table 3). The total P content was classified as high which may be resulted from previous season fertilization. The available P content was classified as very low, indicating a possible fixation of P by Al (Al-P).

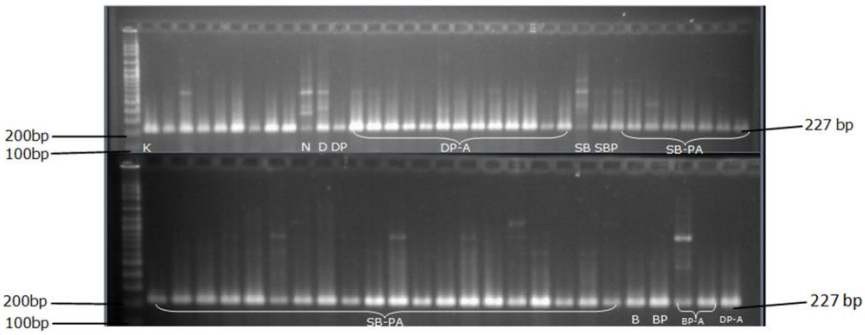

Figure 1. Amplification PCR of marker Kas46-2 on 2% agarose. K = Kasalath, N = Nipponbare, D = Dodokan, DP = Dodokan-*Pup1*, DP-A = BC_3F_5 Dodokan-*Pup1*+*Alt*, SB = Situ Bagendit, SB-P = Situ Bagendit-*Pup1*, SB-PA = BC_3F_5 Situ Bagendit-*Pup1*+*Alt*, B = Batur, BP = Batur-*Pup1*, BP-A = BC_3F_5 Batur-*Pup1*+*Alt*.

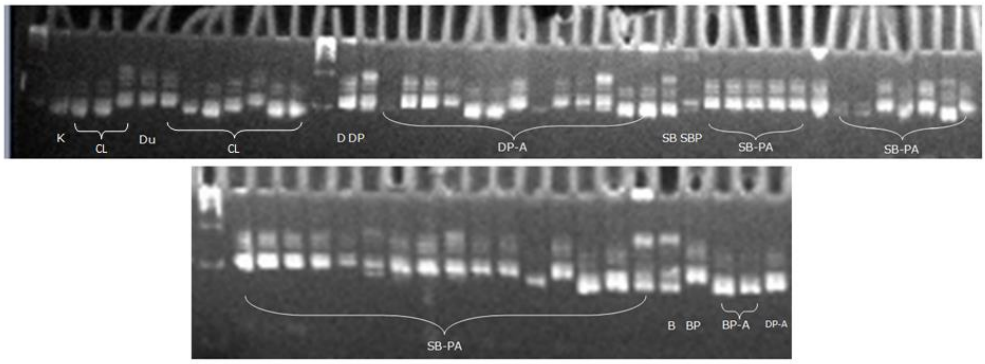

Figure 2. Amplification PCR of marker RM1361 on 8% polyacrylamide. K = Kasalath, CL = Comparison Lines, Du = Dupa, D = Dodokan, DP = Dodokan-*Pup1*, DP-A = BC_3F_5 Dodokan-*Pup1*+*Alt*, SB = Situ Bagendit, SB-P = Situ Bagendit-*Pup1*, SB-PA = BC_3F_5 Situ Bagendit-*Pup1*+*Alt*, B = Batur, BP = Batur-*Pup1*, BP-A = BC_3F_5 Batur-*Pup1*+*Alt*.

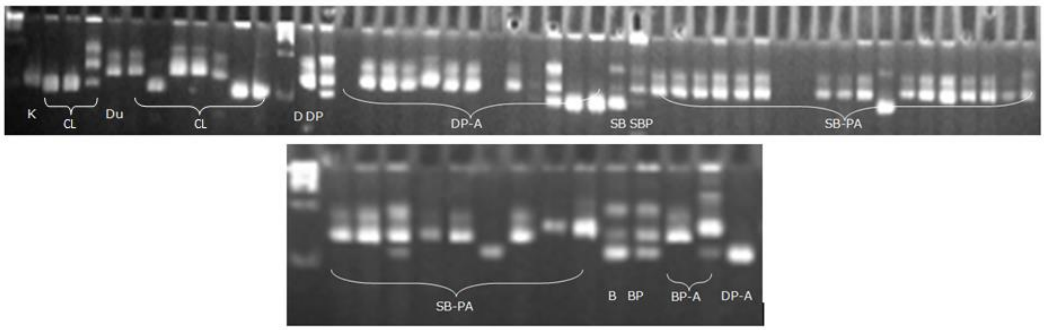

Figure 3. Amplification PCR of marker RM12031 on 8% polyacrylamide. K = Kasalath, CL = Comparison Lines, Du = Dupa, D = Dodokan, DP = Dodokan-*Pup1*, DP-A = BC_3F_5 Dodokan-*Pup1*+*Alt*, SB = Situ Bagendit, SB-P = Situ Bagendit-*Pup1*, SB-PA = BC_3F_5 Situ Bagendit-*Pup1*+*Alt*, B = Batur, BP = Batur-*Pup1*, BP-A = BC_3F_5 Batur-*Pup1*+*Alt*.

Table 2. Result of PCR amplification for confirmation of *Alt* locus.

No.	Genotypes	Markers		No.	Genotypes	Markers	
		RM1361	RM12031			RM1361	RM12031
1	Kasalath	Parent	Parent	28	21(B15)-2	D	D
2	Dupa	Parent	Parent	29	21(B15)-3	SB-P	D
3	Nipponbare	Parent	Parent	30	21(B15)-4	-	D
4	Dodokan	Parent	Parent	31	21(B15)-5	-	D
5	Dodokan-*Pup1*	Parent	Parent	32	21(B15)-6	D	D
6	11(A26)-2	D	D	33	21(B15)-8	D	D
7	11(A26)-3	D	H	34	22(B16)-1	D	D
8	12(A47)-2	D	D	35	22(B16)-8	SB-P	D
9	12(A47)-3	D	D	36	22(B16)-9	D	SB-P
10	12(A47)-4	D	D	37	24(B21)-10	D	D
11	12(A47)-5	D	D	38	25(B25)-1	D	D
12	12(A47)-7	DP	D	39	25(B25)-2	D	D
13	12(A47)-10	DP	D	40	25(B25)-7	D	D
14	13(A71)-4	D	D	41	27(B33)-3	D	D
15	13(A71)-9	D	D	42	22(B16)-5	D	D
16	13(A71)-10	D	D	43	27(B33)-8	D	D
17	14(A128)-2	D	D	44	27(B33)-9	D	D
18	14(A128)-7	D	H	45	27(B33)-10	D	H
19	12(A47)-6	D	D	46	28(B35)-1	D	D
20	Situ Bagendit	Parent	Parent	47	28(B35)-5	D	D
21	Situ B-*Pup1*	Parent	Parent	48	28(B35)-10	D	D
22	19(B5)-2	D	D	49	29(B45)-1	D	D
23	20(B8)-1	D	D	50	Batur	Parent	Parent
24	20(B8)-4	D	D	51	Batur-*Pup1*	Parent	Parent
25	20(B8)-8	D	D	52	71(C138)-1	H	H
26	20(B8)-10	D	D	53	71(C138)-3	H	H
27	21(B15)-1	D	D				

D = Dupa, DP = Dodokan-*Pup1*, SB-P = Situ Bagendit-*Pup1*, H = Heterozygous

The value of CEC (cation exchange capacity) is low, indicating that the field contains low base cations. Additionally, the base saturation is also low, indicating that the Al^{3+} cation is available in relatively high amount and absorbed on the surface of colloidal soil. When this experiment was conducted, the third replicate was suspected to have a higher Al content compared to the first and second replicates, indicated by a greater number of genotypes suffering from Al toxicity.

Based on Table 4, the *Pup1* locus had effect on total empty grains per panicle (EG) on Batur, but no significant effect for every characters on Dodokan and Situ Bagendit. Genotype had significant effects on almost all traits, except total number of tiller per plant (TT) (Tabel 5) and weight of 1,000 grains (WG) (Table 6).

The effect of *Alt* locus was significant on plant height (PH) when lines has *Alt* locus compared to parents which only has *Pup1* (Table 4). The effect of *Alt* locus on BC_3F_5 Situ Bagendit-*Pup1*+*Alt* progenies was also seen in total number of tillers (TT) and number of productive tillers (PT). Whereas in BC_3F_5 Batur-*Pup1*+*Alt* progenies, the *Alt* locus effects was also seen in panicle length (PL), total grains (TG) and weight of filled grains (WG).

Table 3. Soil characteristics at the experimental field (Taman Bogo, Lampung Province).

No.	SoilCharacteristics	Method	Unit	Value	Criteria
1	pH	H_2O	-	5.1	Acid
		KCl	-	4	Acid
2	Organic-C	Walkey & Black	%	1.07	Low
3	Total N	Kjeldahl	%	0.09	Very low
4	C/N ratio		-	12	Medium
5	Available P	Bray I	ppm	4.66	Very low
6	Ions:				
	Ca	NH_4OAc 1 N	ppm	448	Low
	Mg	NH_4OAc 1 N	ppm	31.2	Very low
	K	NH_4OAc 1 N	ppm	27.3	Very low
	Na	NH_4OAc 1 N	ppm	18.4	Very low
	Total			524.9	
	CEC		me/100gr	8.2	Low
7	Base saturation		%	32	Low
8	Al^{3+}	KCl 1 N	ppm	84.6	----
9	H^+	KCl 1 N	ppm	2.2	----
10	Texture				
	Sand	Hidrometer	%	49	----
	Silt	Hidrometer	%	13	----
	Clay	Hidrometer	%	38	----
11	Total P	HCl 25%	ppm	520	High
12	Al saturation		%	11.46	Low

*Based on Soil Testing at Soil Laboratory, Soil Research Center (Under Indonesian Center for Agricultural Land Resources Research and Development [ICALRRD], Ministry of Agriculture, Indonesia) in 2017.

Table 4. Comparison between parents and BC_3F_5 lines.

Varieties	PH	FD	TT	PT	PL	FG	EG	TG	WG	WF
Dodokan	**	ns	ns	ns	*	**	ns	ns	ns	ns
Dodokan vs Dodokan-*Pup1*	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Dodokan-*Pup1* vs BC_3F_5 Dodokan-*Pup1+Alt* lines	**	ns	ns	ns	ns	ns	ns	ns	ns	ns
Inter-BC_3F_5 Dodokan-*Pup1+Alt* lines	*	ns	ns	ns	*	**	ns	ns	ns	ns
Situ Bagendit	**	ns	ns	ns	*	ns	ns	ns	ns	ns
Situ Bagenditvs Situ Bagendit-*Pup1*	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Situ Bagendit-*Pup1* vs BC_3F_5 Situ Bagendit-*Pup1+Alt* lines	**	ns	*	*	ns	ns	ns	ns	ns	ns
Inter-BC_3F_5 Situ Bagendit-*Pup1+Alt*	ns	ns	ns	ns	*	ns	ns	ns	ns	ns
Batur	**	ns	ns	ns	*	ns	**	**	ns	ns
Batur vs Batur-*Pup1*	ns	ns	ns	ns	ns	ns	**	ns	ns	ns
Batur-*Pup1* vs BC_3F_5 Batur-*Pup1+Alt* lines	**	ns	ns	ns	*	ns	ns	*	ns	*
Inter-BC_3F_5 Batur-*Pup1+Alt*	ns	ns	ns	ns	ns	ns	ns	**	ns	ns

PH = plant height, FD = flowering days, TT = total tiller per plant, PT = productive tiller per plant, PL = panicle length, FG = filled grains per panicle, EG = empty grains per panicle, TG = total grains per panicle, WG = weight of 1,000 grains, WF = weight of filled grains per clump, ** significant at $P < 0.01$, * significant at $P < 0.05$, ns = not significant.

Based on plant height classification (IRRI, 2013), there were 32 lines tested classified as intermediate and 12 lines as short. Progenies of Dodokan-*Pup1+Alt* lines, 13(A71)-4, is the tallest lines compared to all tested lines. The lines which have *Pup1* and *Alt* loci was relatively higher than lines had *Pup1+Alt* loci or only *Pup1* locus (Table 5).

Table 5. Vegetative and generative means of parents and selected BC_3F_5 lines.

Genotypes	PH (cm)	FD (days)	TT	PT	PL (cm)
IR64	67	83	16	12	20.4
Dupa	117	97	9	6	19.8
Hawara Bunar	129	83	10	9	26.2
Inpago 7	95	86	13	10	25.2
Inpago 8	102	85	14	13	22.4
Dodokan	69	79	17	15	20.8
Dodokan-*Pup1*	74	77	15	14	20.7
12(A47)-7 [DP-A]	82	79	9	7	21.0
12(A47)-10 [DP-A]	88	79	10	9	22.2
13(A71)-4 [DP-A]	106	79	15	14	23.3
13(A71)-9 [DP-A]	83	78	11	9	22.4
13(A71)-10 [DP-A]	96	78	14	12	21.0
Situ Bagendit	60	81	19	17	17.4
Situ Bagendit-*Pup1*	63	84	21	19	18.2
20(B8)-10 [SB-PA]	91	82	14	13	24.3
21(B15)-4 [SB-PA]	88	83	13	12	19.8
21(B15)-5 [SB-PA]	92	79	16	14	21.8
24(B21)-10 [SB-PA]	90	87	17	15	20.6
27(B33)-9 [SB-PA]	87	83	12	11	20.2
28(B35)-5 [SB-PA]	88	84	16	14	21.3
Batur	88	84	10	7	23.8
Batur-*Pup1*	101	87	11	10	24.3
71(C138)-1 [B-PA]	75	86	13	12	19.4
71(C138)-3 [B-PA]	88	87	16	14	21.7
F test	**	**	ns	*	**
CV (%)	10.2	5.2	10.9^T	13.5^T	10.3
LSD (0.05)	78.4	18.3	17.3	16.4	4.6

PH = plant height, FD = days to flowering, TT = total tiller per plant, PT = number of productive tiller per plant, PL = panicle length, T = data transformed using log (x+0.5), ** significant at $P < 0.01$, * significant at $P < 0.05$, ns = not significant. The lines shown (Dodokan-*Pup1*+*Alt* and Situ Bagendit-*Pup1*+*Alt*) are lines that considered to have good character based on weight of filled grains per clumps (WF) character.

In this experiment, genotype had significant effects on days to flowering (Table 5). Each progenies from cross have no difference compared to wild type or recurrent parent. Progenies of BC_3F_5 Dodokan-*Pup1*+*Alt* lines flowered relatively earlier than other progenies.

The total number of tillers (TT) and productive tillers (PT) can also be indicators of P-deficiency tolerance. Genotypes did not differ significantly total number of tillers, but differed significantly for number of productive tillers (Table 5). The genotype 20(B8)-10 with the longest panicle (PL) (Table 5) did not have the maximum weight of filled grains per clump (WF) (Table 6).

Eventhough the plant formed many spikelets, only a portion of the grains were observed to be filled. Situ Bagendit-*Pup1*+*Alt* progenies have relatively high percentage of filled grain and small number of empty grains.

Genotypes did not significantly affect the weight of 1,000 grains (WG) (Table 6). A BC_3F_5 Dodokan-*Pup1*+*Alt* line, 13(A71)-9, had more weight of 1,000 grains and weight of filled grains per clump (WF) (Table 6). In this experiment, the genotype had no significant effects on weight of 1,000 grains (WG), indicating that P-

Table 6. Generative means of parents and selected BC_3F_5 lines.

Genotypes	FG	EG	TG	WG (g)	WF (g)
IR64	39	38	77	19.4	19.0
Dupa	93	26	119	23.6	11.3
Hawara Bunar	71	46	118	25.5	23.8
Inpago 7	62	52	113	19.8	10.3
Inpago 8	77	34	111	17.7	14.9
Dodokan	46	43	89	17.2	13.5
Dodokan-*Pup1*	25	47	72	20.3	8.8
12(A47)-7 [DP-A]	63	67	130	24.7	15.5
12(A47)-10 [DP-A]	63	62	125	20.6	15.2
13(A71)-4 [DP-A]	28	55	84	14.1	12.2
13(A71)-9 [DP-A]	81	44	125	27.6	18.7
13(A71)-10 [DP-A]	24	46	70	15.4	10.3
Situ Bagendit	35	31	66	18.3	9.7
Situ Bagendit-*Pup1*	39	35	74	16.6	18.0
20(B8)-10 [SB-PA]	27	31	58	20.0	10.6
21(B15)-4 [SB-PA]	29	30	59	33.9	11.8
21(B15)-5 [SB-PA]	59	28	86	19.5	14.4
24(B21)-10 [SB-PA]	40	45	85	20.2	10.1
27(B33)-9 [SB-PA]	26	26	52	21.2	10.4
28(B35)-5 [SB-PA]	27	42	79	18.0	10.55
Batur	57	93	149	25.3	9.4
Batur-*Pup1*	80	45	125	21.6	15.2
71(C138)-1 [BP-A]	27	31	58	18.7	6.5
71(C138)-3 [BP-A]	63	42	105	21.1	14.8
F test	**	**	**	ns	*
CV (%)	18.1^T	9.9^T	6.5^T	11.1^T	20.8^T
LSD (0.05)	406.2	227.2	528.2	0.3	19.5

FG = filled grains per panicle, EG = empty grains per panicle, TG = total grains per panicle, WG = weight of 1,000 grains (g), WF = weight of filled grains per clump (g), T = data transform log(x+0.5), ** significant at $P < 0.01$, * significant at $P < 0.05$, ns = not significant. The lines shown (Dodokan-*Pup1*+*Alt* and Situ Bagendit-*Pup1*+*Alt*) are lines that considered to have good character based on weight of filled grains per clumps (WF) character.

deficiency and Al-toxicity may alter photosynthesis, increase the percentage of empty grains, and reduce the value of weight of filled grain (WF). On the other hand, genotype had significant effects on weight of filled grains per clump (WF) (Table 6). Progenies of Dodokan and Batur had relatively more weight of filled grains than genotypes only had one locus or none. However, Situ Bagendit progenies did not have more weight of filled grains than Situ Bagendit-*Pup1*.

DISCUSSION

Kasalath is an Indian landrace rice and a type of *aus* (some classify in *indica* rice). It was chosen as donor parent which has *Pup1* locus and has good agronomic appearance in P-deficiency field (Prasetiyono, 2010). *Pup1* locus helps in multiplication and growth of roots for capturing P element. *Pup1* containing the Phosphorus-starvation tolerance (*PSTOL1*) gene, which can increase the number of productive tillers and canopy dry weight, but did

not increase yield (weight of filled grains) (Prasetiyono, 2010; Prasetiyono *et al.*, 2012; Gamuyao *et al*., 2012). Kas46-2 marker is a mandatory marker for rice exploration for identification of the *Pup1* locus (Gamuyao *et al*., 2012). In this experiment based on amplification on Kas46-2, all progenies from three crossing have *Pup1* locus. It was estimated effect of backcrossing in F_1, BC_1, and BC_2 population using recurrent parent (Dodokan-*Pup1*, Situ Bagendit-*Pup1* and Batur-*Pup1*), it already containing with *Pup1* locus. Beside that, Kas46-2 marker in advanced generation also helps to select genotypes had *Pup1* locus. It would remain *Pup1* locus in population.

Dupa is Indonesia local rice tolerant to Al-toxicity and used as the donor parent for *Alt* locus (Prasetiyono *et al*., 2003; Hidayatun, 2014). The use of national upland rice varieties as recurrent parents in backcross aims to return the genome of progenies lines from cross (other than *Alt* locus) as parent (Dodokan-*Pup1*, Situ Bagendit-*Pup1* and Batur-*Pup1*) into homozygous condition. Each generation of F_2 (F_2, BC_1F_2, BC_2F_2, BC_3F_2, etc), the progenies were selected based on similar allele as that of the donor parent to maintain the donor segment in advanced progenies (Prasetiyono *et al.*, 2008). Foreground marker selection was used to detect *Pup1* and *Alt* locus on BC_3F_5 lines by comparing the alleles with the donor parent. In this study, Batur progenies (BC_3F_5 Batur-*Pup1*+*Alt*) showed heterozygous on markers for *Alt* locus. The appearance of heterozygous lines could be because of non homologous crossing over (Tasliah *et al*., 2011).

Taman Bogo has sandy clay acid soils with high aluminium and iron content, and low organic substance (Table 3). The available P was very low than the total P because binding of P element with Al and also promoted by soil acidity (<5.5) (Subardja, 2007). In addition, several factors influence the available P such soil parent material, soil development, and the most dominant is land management (Nursyamsi and Setyorini, 2009). The plant ability to absorb P element also affect the value of available P (Nasution *et al.,* 2014).

Aluminum could be toxic for rice if the soil has >30% Al saturation, soil pH (H_2O) <5.0, and >1-2 mg Al/L (Dobermann and Fairhust, 2000). In this study, the experiment field had high Al concentration (Table 3), which causes the low availability of P due to its binding with Al. The high acidity level in the experimental field possibly causes the change of Al element into Al^{3+} which is toxic to plants. Aluminum toxicity can affect root ability to absorb water and mineral (Kochian *et al.*, 2005), and cause dwarf canopy (Miftahudin *et al.*, 2007; Anggraheni and Mulyaningsih, 2017).

The lack of *Pup1* and *Alt* effect on observed characters was possibly due to extreme soil enviroment for rice. The expression of *Pup1* locus in genotypes could not been observed in almost overall characters due to low concentration of P (Table 3). *Alt* locus effect was not clear in the present as aluminum concentration was too high. The effect was not clearly visible for the observed characteristics due to high exposure of Al, *viz.* (1) the roots become short and brittle, (2) the branches of roots diminish, and (3) become thick and brownish color (Prasetiyono, 2010; Tasma, 2015). Due to reduced branch roots, it will affect to ability of the roots to absorb water and mineral which causes a

decrease in root and canopy dry weight (Kochian *et al.,* 2005; Prasetiyono, 2010). The influence of *Pup1* and *Alt* loci together in one genotype still need further studies in controlled environment. According to Indrayani *et al.* (2016), tolerant lines in controlled environment might not be tolerant on acidic field (pH <5.0). It is because the field was influenced by many factors such as micro climate and soil fertility.

Plant height is the most easily observed trait for growth indicator, and it is closely related with photosynthesis. The dwarf plants usually use more photosynthate than the taller plants (Mulyaningsih *et al*., 2016). Avaliable-P deficiency and Al toxicity generally could affect plant height due to plant roots damage such as curling (Dobermann and Fairhust, 2000; Setiadi and Anira, 2015) The combination of *Pup1* and *Alt* loci in one genotype is expected to capture and use P optimally, and could minimize roots damaged due to Al.

According to Table 5, days to flowering (FD) the test progenies were not significantly different than the parent lines. It might because of backcrossing effect. Besides, the plant suffering from abiotic stress tend to flower early.

According to Prasetiyono (2010), the tolerant P-deficiency lines usually can be seen from the effect on number of tillers. Besides, avaliable-P content should be there during early to late vegetative stage to show the effect for the number of total tillers (Aluwihare *et al*., 2016). Formation of productive tiller was influenced by genotypes capability to absorb and use nutrients. In P-deficiency soil, the plants with *Pup1* locus could still capture and utilize P, and therefore the number of productive tillers was relatively less in comparison to the plants without *Pup1* locus.

Phosporus-deficiency can also affect the number and length of panicles (Dobermann and Fairhust, 2000). Genotypes with longer panicle may produce higher yield, but this not always the case because yield was also influenced by the percentage of filled grain. According to Mu *et al*. (2008), a decrease in the number and length of panicles can be due to reduced number and length of plant roots.

The weight of 1,000 grains is usually more influenced by the shape of hull of the seed of each genotype (Fujita *et al.,* 1984; Tahir *et al*., 2002). The percentage and total number of filled grains can be considered for identifying lines tolerant to P-deficiency. Limited P condition activates the *PSTOL1* gene in *Pup1* locus, resulting in more roots produced that can catch and utilize P to fill grains (Mu *et al*., 2008; Prasetiyono 2010; Hambali and Lubis, 2015). The condition of P-deficiency and Al-toxicity could reduce plant growth and cholorophyl concentration. Apart from very small concentration of available P element for plants, the high concentration of Al^{3+} was possibly to cause plant to use photosynthates for defense from P-deficiency and Al-toxicity than enlarging vegetative organs and filling grains. These would disrupt photosynthesis and development of plants, and could also disrupt filling and maturation of grains (Mu *et al*., 2008; Guo *et al*., 2012). Beside of photosynthate use competition between vegetative organs, competition for filling grains in panicles can also increase the number of empty grains (Sugiono and Saputro, 2016).

CONCLUSION

All tested lines contained the *Pup1* locus, two test lines did not have *Alt* locus (Situ Bagendit-*Pup1+Alt*). *Pup1* locus had significant effect on number of empty grains on Batur. *Alt* locus affected plant height on progenies of Dodokan, Situ Bagendit, and Batur, number of total and productive tillers on progenies of Situ Bagendit), panicle length, number of total grains, and weight of filled grains (on progenies of Batur). Line number 13(A71)-9 [DP-A] could be one of the promising lines, because it possessed both *Pup1+Alt* loci and had the highest weight of filled grains per clump.

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