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Characterization of Doubled Haploid Derived from Anther Culture for New Type Upland Rice

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ABSTRACT

Anther culture is one of tissue culture methods which can be applied to plant breeding programs in order to accelerate the process of obtaining pure lines. The successful development of rice varieties is highly dependent on genetic diversity and desirable traits. To obtain the genetic variability of doubled haploid lines through anther culture techniques, anther F1 or F2 were used as explants sources. The objectives of the study were to select and characterize doubled haploid lines of upland rice having the characters of new plant type, and to study the genetic variability and agronomic characters of tested doubled haploid lines. A total of 58 doubled haploid lines, and four parental lines i.e. Fatmawati, SGJT-28, SGJT-36, and Way Rarem were used in this study. The experiment used completely randomized design with three replications. Results showed that the characters of the doubled haploid lines vary considerably. Selection of the character i.e. number of productive tillers, number of filled grain per panicle, and percentage of empty grain was more effective to be selected because they were well correlated to weight of grain per hill, possessed high heritability values, and have wide genetic variability. Based on productive tillers number, number of filled grains per panicle, fertility, weight of 1,000 grains, and weight of grains per hill, the lines of P3-26, P3-27, P3-28, P4-45, P5-50, P6-103, P6-105, P3-120, P3-134, P3-135, P3-150, P3-158, P3-248, P3-249, P6-271, P6-272, P6-274, P6-276, and P6-295 were potential for further selection for new type of upland rice.

Keywords: characters, doubled haploid, new plant type, upland rice, variability

INTRODUCTION

Superior rice varieties with high yields are expected to support self-sufficiency food program in Indonesia. IRRI have formulated prototypes of "new plant type" (NPT) of rice in 1989 (IRRI, 1990). The desired characters of the NPT rice are compact growth, number of productive tillers of 8-10, big panicles and good grain filling (200-250 grains), semi dwarf (80-100 cm in height), erect and thick leaves with dark green colour, medium earliness (100-130 days), deep rooting system, and high resistance to major pests and diseases (Khush, 1995).

There have been limited efforts to produce NPT of upland rice due to environmental constraints and biotic stresses. Modifications to NPT low land rice are required to produce NPT upland rice, including high number of grains (>150 grains per panicle), productive tillers (> 6), high proportion of grain filling (> 70%), plant height of less than 150 cm, earliness (less than 130 days), angle of flag leaves of 10º-15º, the second and third leaves are slightly bended to allow wider plant canopy, stem diameter of more than 70 mm, aluminum-tolerant, and resistant to blast diseases (Herawati, 2010).

Anther culture is a tissue culture technique which can be applied in plant breeding to accelerate the process of obtaining pure lines. The success of the NPT engineering depends on the genetic variability and the desired characters. High genetic variabilities in a single cross occurred on the second (F2) and third (F3) generation, and the F2 have all combinations of genes in the expected genotypes. Combinations of characters derived from the two parental lines occurred in the haploid plants, so if the chromosomes were doubled, or spontaneous doubling occurred during the culture, a double haploid or dihaploid (DH) pure lines will be obtained. Selection for the desired characters can be done earlier on the DH1 or DH2, hence will take lesser time compared to a conventional breeding.

Previous studies by Herawati *et al*. (2008) produced 348 dihaploid lines (DH0). These lines have been evaluated and 58 of the first generation of the upland dihaploids (DH1) have released. Seed multiplication and further evaluation of the second generation DH (DH2) were required in order to determine the agronomic character stabilities for further selection of desirable characters, particularly in terms of yield components and yields.

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The objectives of the study were to select and characterize doubled haploid lines of upland rice having the characters of NPT, and to study the genetic variability and agronomic characters of the tested doubled haploid lines for further selection.

MATERIALS AND METHODS

The experiment was conducted in greenhouses at Indonesian Center for Agricultural Biotechnology and Genetic Resources Research and Development, Bogor from December 2008 to June 2009.

Seeds used were derived from 26 lines of P3 (NPT Fatmawati x SGJT-36), 27 lines of their reciprocals, P6 (SGJT-36 x Fatmawati), one line of P1 (Fatmawati x Way Rarem), two lines of P2 (Fatmawati x SGJT-28), one line of P4 (Way Rarem x Fatmawati), and one line of P5 (SGJT-28 x Fatmawati) (Herawati *et al*., 2009). All lines were F-1 dihaploids from Fatmawati x upland rice crosses produced through anther culture. All seeds were planted in pots, each contains 10 kg of upland soil, and fertilized with 200 kg Urea ha^{-1} (5 g pot^{-1}), 100 kg SP-36 ha^{-1} (2.5 g pot^{-1}), and 100 kg KCl ha^{-1} (2.5 g pot^{-1}). Completely randomized design was used with three replications of three plants each replication.

Observations were conducted on plant height, number of productive tillers, number of grains per panicle, number of filled grains per panicle, percentage of empty grains, weight of 1,000 grains, grain weight per hill, time to flower and time to harvest. Scorings on agronomical characters used Standard Evaluation System (SES) for rice developed by IRRI (IRRI, 1996).

Multivariant analysis and correlations between variables using Pearson coefficient correlation were conducted using SAS version 9.1. Genetical variability was estimated from (σ^2_g) and standard deviation of $\sigma^2_{\sigma g}$. A character is considered to have a wide genetical variability if $\sigma^2_g > 2\,\sigma^2_{\sigma g}$ (Pinaria *et al.,* 1995). The genotypic variability coefficient (GVC) is considered narrow if 0 < GVC ≤ 10.94; moderatly narrow if 0 < GVC ≤ 21.88; moderately wide if 0 < GVC ≤ 32.83; wide if 0 < GVC ≤ 43.77; and very wide if 43.77 < GVC (Qosim *et al*., 2000). Heritability values (h^2_{bs}) were classified according methods developed by Stanfield (1983): high (0.50 < h^2_{bs} < 1.00); medium (0.20 < h^2_{bs} < 0.50); and low (h^2_{bs} < 0.20).

Lines were grouped based on number of productive tillers character, number of filled grains, percentage of empty grains, weight of 1,000 grain. Weight of grains per hill was used as a variable to identify high yielding lines with potentials for further selection, using NTSYS group analysis program version 2.02 (Numerical taxonomy and multivariate analysis system) as described by Rohlf (1998).

RESULTS AND DISCUSSION

Results of analysis of variance showed significant differences between all characters (Table 1), and that there were wide genetic variabilities in all characters.

Coefficient of genotypic and phenotypic variability of productive tillers, number of grains per panicle, number of filled grains per panicle, percentage of empty grains, and grain weight per hill were wide to very wide with medium to high heritability values, ranged from 0.63 for percentage of empty grains to 1.0 for time to flower and time to harvest (Table 1). It is important to determine correlation between agronomical characters, yields and yield components in order to do an efficient selection process, hence will enable selections for one or more characters to be conducted at the same time.

The number of productive tillers, number of filled grains, weights of 1,000 grain correlated positively,

Table 1. Analysis of variances and genetic variabilities of agronomical characters of DH upland rice lines from anther culture

Characters	MS	F value	GV	PV	2 x SDGV	GVC (%)	PVC (%)	h^2_{bs}
Time to flower (dap)	119.1	5.9**	39.7	39.7	25.1	6.4	6.4	1.00
Time to harvest (dap)	149.6	6.8**	49.8	49.8	31.5	5.4	5.4	1.00
Plant height (cm)	1685.8	41.5**	548.4	589.0	352.6	16.3	16.9	0.93
Productive tillers number	35.2	8.7**	10.4	14.4	8.1	31.0	36.5	0.72
Panicle length (cm)	42.1	22.3**	13.4	15.3	9.4	12.5	13.3	0.88
Number of filled grains per panicle	11867.9	8.6**	3498.1	4871.6	217.7	25.9	30.6	0.72
Number of total grains per panicle	31653.6	11.7**	9651.5	12350.6	450.7	27.6	31.2	0.78
Empty grains (%)	438.3	6.0**	121.9	194.4	92.3	31.8	40.2	0.63
1,000 grains weight (g)	58.4	47.6**	19.1	20.3	12.6	18.9	19.5	0.94
Grain weight per hill (g)	538.8	6.5**	151.8	235.2	84.9	33.1	41.2	0.65

Note: MS = Mean Square; GV = Genetical Variability; PV = Phenotypic Variability; SDGV = Standard Deviation of Genotypic Variability; GVC = Genotypic Variability Coefficient; PVC = Phenotypic Variability Coefficient; h^2_{bs} = heritability; dap = days after planting ** significant at α = 1%

whereas percentage of empty grains correlated negatively with grain weight per hill (Table 2). Effective selection for the number of productive tiller, number of filled grains and percentage of empty grains can be conducted since these characters correlated (positively) with grain weight per hill. In addition, these characters demonstrated wide genetic variability and high heritability.

Agronomical Characters of P3 Crosses of Low Land Dihaploids Population (Fatmawati x SGJT-36) and P6 (SGJT-36 x Fatmawati).

Agronomical characters of the DH population derived from F-1 reciprocals and their parents are presented in Table 3. There were significant variabilities in the all agronomical characters. Zhou (1996) and Roy and Mandal (2005) reported differences in the regeneration ability through anther culture between genotypes and between sub species. These differences were due to gene segregation during meiosis in the formation of microspores of F-1 (Dewi *et al.,* 2009).

Plant height of P3 population ranged from 104.7-184.0 cm, and 93.3-168 cm in the P6 population (Table 3). Therefore, the 26 lines or genotypes derived from P3 can be grouped into three medium height lines with (91-110 cm), two tall lines (111-130 cm), and 21 very tall lines (> 131 cm) according to plant height standards developed by IRRI (1996). Fatmawati has medium height, whereas SGJT-36 was very tall. Frequency of distribution range of DH1 population in both P3 and P6 tend to be very tall (SGJT-36).

The number of productive tillers in P3 ranged from 6.0-16.7, whereas P6 had 6.3-16.7 (Table 3). Therefore, the 26 lines or genotypes derived from P3 can be grouped into 14 low productivity lines with (5-9), 12 medium productivity lines (10-19), whereas those 27 genotypes derived from P6 had nine low productivity lines (5-9), 17 medium productivity lines (10-19) and one high productivity lines (>19) according to productivity standards developed by IRRI (1996). Both Fatmawati and SGJT-36 had low number of productive tillers (Table 3). Range of frequency distribution of DH1 population in both P3 and P6 tend to be low.

Time to flower and time to harvest of line P3 were 89-114 and 120-139 days after planting (dap), respectively, whereas for line P6 they were 93-115 and 124-150 dap. Line P6 had the longest time to harvest (150 dap), whereas line P3 was the earliest (120 dap, Table 3).

The lines or genotypes can be grouped into 15 medium earliness with (115-125 dap), and 38 late (125-150 dap), according to productivity standards developed by Lubis *et al*. (1993). Most DH lines tend to be late flowering like their parents, SGJT-36.

Panicle length of P3 and P6 were 24.7-37.9 cm and 22.0-35.6 cm, respectively. Generally long panicles had more grains than short panicles. However, grain density was also important, probably more than panicle length, in affecting productivity. Fatmawati had long panicles (31.3 cm) and had more grains (401.7), resulting in a higher grain density compared to the parental line of SGJT-36 which had panicles of 37.7 cm and number of grains of 395 (Table 3).

Line P3 had the most filled grains (363), whereas P6 had the least (86.4). There were quite a large variability in standard deviation of total grains and number of filled grain per panicle between lines (Table 3). The most important yield characters are number of filled grains, grain density, panicle length, followed by weight of 1,000 grains.

Four lines from P3 population had empty grains of less than 20%, 20 lines had 20-40%, and two lines had 40-50%. In P6 population, one line had less than 20%, 15 lines had 20-40%, and 11 lines had 40-70%.

Low fertility in DH population might have been due to low fertility of F1 explants used for the anther culture (Dewi *et al*., 2009). A character of high percentage of empty grain in the DH lines might have been derived from the parental line Fatmawati (41.9%).

Table 2. Coefficient of correlations between agronomical characters and grain yield per tiller of DH upland rice lines from anther culture

	PH	PTN	PL	FG	TGN	EG	FT	HT	1000W	GWH
PH	1	-0.29	0.12	0.45	0.24	-0.32	-0.05	-0.05	0.20	0.29
PTN		1	0.31	-0.09	-0.28	0.19	0.28	-0.25	-0.27	0.44
PL			1	0.42	-0.13	0.36	0.56	-0.71	-0.06	0.42
FG				1	0.57	-0.25	0.05	0.29	-0.21	0.57
TGN					1	0.16	0.05	0.29	-0.21	0.04
EG						1	0.33	-0.16	-0.23	-0.26
FT							1	0.1	-0.09	0.29
HT								1	0.11	-0.28
W1000									1	0.19
GWH										1

Note: PH = Plant Height; PTN = Productive Tillers Number; PL = Panicle Length; FG = Filled Grain per Panicle; TGN = Total Grains Number; EG = Percentage of Empty Grains ; FT = Time to Flower; HT = Time to Harvest; 1000W = 1,000 Grain Weight; GWH = Grain Weight per Hill

Lines derived from P6 had the most grain weight per hill (68.3 g), whereas lines derived drom P3 had 58.7 g. These values were higher than those from their parental lines, i.e. 55.1 g in SGJT-36 and 48.5 g in Fatmawati (Table 3). Therefore, there have been an increase in the number of total grains, number of filled grain per panicle, 1,000 grain weight, and grain fertility in the DH1 lines compared to their parents. All agronomical characters in the DH1 vary widely, some were similar to one of the parental line, some were intermediates, and some exceeded both parental lines. The wide genetic variabilities were beneficial for further selection processes.

Multivariant Analysis

Yield components of number of productive tillers, number of filled grains per panicle, empty grains, 1,000 grain weight, and grain weight per hill (Table 1, 2 and 3) can be used as variables to identify and to group potentially high yielding lines through group analysis (Figure 1). Group separation at similarity level of 47% resulted in 12 groups. Fatmawati, Way Rarem, SGJT-28, and SGJT-36 were classified into different groups to simplify identification of other groups (Table 4).

Groups III, IV, VII, and XII had high yield components in terms of number of productive tillers, number of filled grains, fertility, 1,000 grain weight, and grain weight per hill. Group II, V, and VI had medium yield components, whereas group I, VIII, IX, X, XI had low yield components (Table 4). Amongst the parental lines, Way Rarem, SGJT-28, and SGJT-36 had high yields, whereas Fatmawati had medium yields. Therefore, the high yielding groups have potentials for further selection to create NPT upland rice. The agronomical characters of the selected lines are presented in Table 5.

Modifications of NPT low land rice traits are required to engineer NPT upland rice, including high number of grains (> 150 grains per panicle), productive tiller (> 6), high proportion of grain filling (> 70%), plant height of less than 150 cm, early (less than 130 days), angle of flag leaves of 10º-15º, the second and third leaves are slightly bended to allow wider plant canopy, stem diameter of more than 70 mm, aluminum-tolerant and have resistance to blast diseases (Herawati, 2010).

Nineteen lines were selected as NPT upland rice (Table 5). The number of productive tillers in these lines were 7-23. Criteria of having more than six productive tillers for NPT upland rice is consistent with the high yields targeted for dry lands, and these criteria agreed with the reports by Peng *et al*. (1994) that low tillering lines with 3-4 productive tillers were suitable for direct planting in upland, whereas lines with 8-10 tillers were suitable for transplanting. Number of filled grains per panicle were quite large, i.e. 113.44-363.00 grains per panicle. Line P6-105 had the least filled grains, whereas P3-26 had the most (Table 5).

Percentage of empty grains was 19.04-43.13%, with line P3-135 being the lowest of 19.04% and line P6-105 being the highest of 43.13% (Table 5). Limited sink to source, or earlier senescence might have been the cause of the large percentage of empty grains. Li *et al*. (2008) reported that at the end of the filling period, or during the aging period, activities of Ribulose biphosphate carboxylase activase and Rubisco binding-protein alpha sub unit that regulate photosynthate accumulation during the filling period were reduced.

Weight of 1,000 grain ranged between 18.59-30.55 g. Line P5-50 had the least 1,000 grain weight of 19.59 g, whereas P3-150 had the heaviest of 30.55 g (Table 5). Grain weight of more than 30 g with long panicle (> 26 cm) and large number of grains are good sink size to increase grain yields of NPT upland rice. Increasing harvest index and grain yields of NPT rice should focus on number of grains per panicle (Yang *et al*., 2007). Grain weight per hill of the selected lines were ranged between 41.31-68.33 g. Line P3-248 had the least grain weight of 41.31 g, whereas P6-105 had the largest grain weight of 68.33 g (Table 5). These lines had high yields and had potentials for further selection.

Table 3. Agronomical characters of DH2 from P3 ((Fatmawati x SGJT-36) and P6 (SGJT-36 x Fatmawati))

Characters	X ± SD DH2*	Population DH2 range**		Mean***	
		P3	P6	Fatmawati	SGJT-36
Time to flower (dap)	96.5 ± 6.5	89.0 - 114.0	93.0 - 115.0	93.0	104.0
Time to harvest (dap)	129.0 ± 7.3	120.0 - 139.0	124.0 - 150.0	126.0	137.0
Plant height (cm)	152.8 ± 22.1	104.7 - 184.0	93.3 - 168.0	124.0	175.0
Productive tillers number	9.7 ± 3.4	6.0 - 16.7	6.3 - 16.7	12.0	7.0
Panicle length (cm)	29.1 ± 3.6	24.7 - 37.9	22.0 - 35.6	31.3	37.7
Number of filled grains per panicle	235.6 ± 61.3	124.9 - 363.0	86.4 - 304.8	243.0	339.3
Number of total grains per panicle	357.0 ± 104.7	223.0 - 525.4	190.9 - 660.1	401.7	395.0
Empty grains (%)	31.2 ± 12.6	18.3 - 49.0	19.0 - 64.3	41.9	14.9
1,000 grains weight (g)	22.3 ± 4.4	19.6 - 30.5	14.5 - 36.4	23.7	27.4
Grain weight per hill (g)	38.9 ± 13.6	15.1 - 58.7	11.7 - 68.3	48.5	55.1

Note: *X ± SD DH2 = MS ± standard deviation; ** DH2 P3 had 26 lines; DH2 P6 had 27 lines; ***Fatmawati and SGJT-36 of 9 plants each

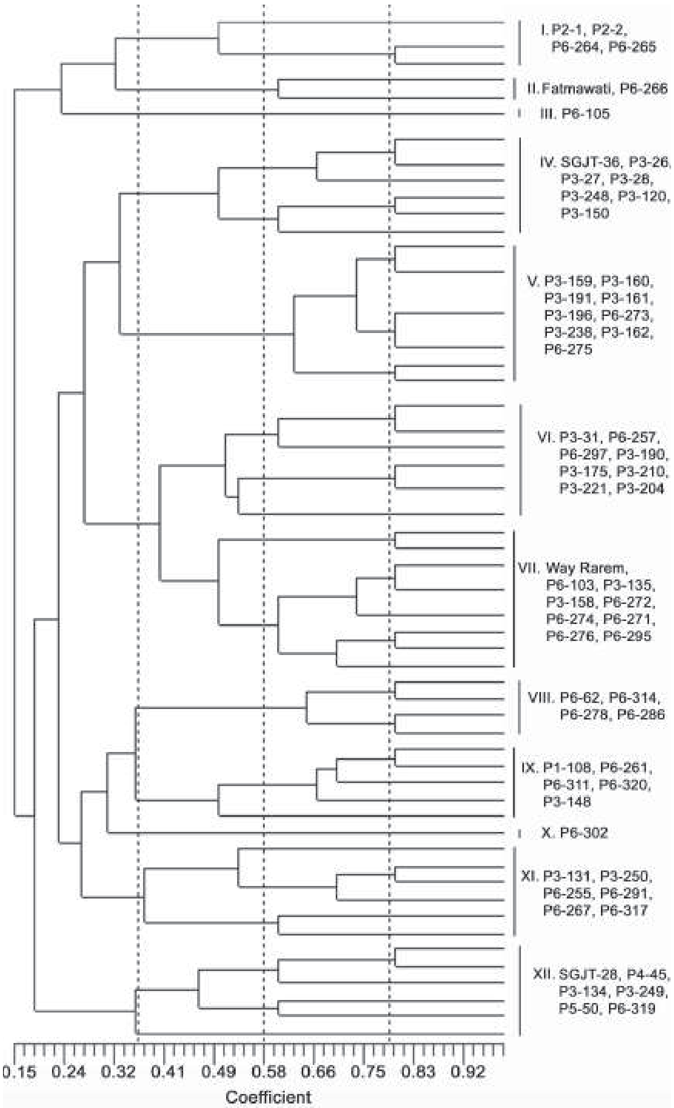

Figure 1. Dendogram population of double haploid lines of upland rice

Table 4. Average yield components for each group

Group	Number of productive tillers	Number of filled grains per panicle	Empty grains (%)	1,000 grains weight (g)	Grain weight per hill (g)	Criteria
I	15.03	225.14	50.01	15.84	29.98	Low
II	12.50	251.12	45.82	20.00	41.40	Medium
III	23.30	113.44	43.13	27.13	68.33	High
IV	7.24	323.59	24.99	27.74	51.72	High
V	8.49	272.92	26.11	21.49	36.24	Medium
VI	10.89	210.90	34.40	24.90	40.37	Medium
VII	10.43	272.04	26.78	22.87	48.81	High
VIII	8.73	155.12	29.69	26.81	26.37	Low
IX	9.28	201.45	42.41	23.12	24.91	Low
X	9.30	86.44	62.17	36.41	21.46	Low
XI	8.32	162.82	50.01	19.26	14.41	Low
XII	15.00	215.21	26.35	21.72	49.51	High

Table 5. Agronomical characters of some selected lines for NPT upland rice

Line	Number of productive tillers	Number of filled grains per panicle	Empty grains (%)	1,000 grains weight (g)	Grain weight per hill (g)
P3-26	6.70	363.00	30.96	28.80	53.45
P3-27	6.00	360.22	26.83	27.53	44.10
P3-28	7.00	349.00	31.10	27.28	52.44
P4-45	13.00	218.78	39.00	24.47	52.71
P5-50	19.00	227.56	26.82	18.59	55.50
P6-103	11.00	304.78	42.35	21.63	47.62
P6-105	23.30	113.44	43.13	27.13	68.33
P3-120	7.70	329.33	21.76	25.60	58.74
P3-134	13.30	207.89	30.71	26.00	52.30
P3-135	9.67	262.78	19.04	27.96	48.05
P3-150	9.00	283.56	20.90	30.55	57.30
P3-158	9.70	281.11	20.86	23.49	43.86
P3-248	7.30	240.00	27.82	27.02	41.31
P3-249	16.70	191.67	22.04	24.93	56.13
P6-271	11.30	280.00	22.71	20.56	53.92
P6-272	10.30	269.22	19.02	21.29	43.99
P6-274	10.30	259.00	27.32	21.56	46.44
P6-276	10.00	261.89	31.26	21.14	52.25
P6-295	11.30	236.56	31.43	26.99	56.62

CONCLUSION

DH population from F1 of crosses between NPT and upland rice P3 and P6 demonstrated wide variabilities and met the requirements for selection. The DH1 population had superior total grains, number of filled grain per panicle,1,000 grains weight, and fertility to both parental lines. Based on productive tillers, number of filled grains per panicle, fertility, 1,000 grains weight, and weight of grains per hill. The lines of P3-26, P3-27, P3-28, P4-45, P5-50, P6-103, P6-105, P3-120, P3-134, P3-135, P3-150, P3-158, P3-248, P3-249, P6-271, P6-272, P6-274, P6-276, and P6-295 showed potentials to be further selected for new types of upland rice.

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