Genetic Analysis of Superior Double Haploid Rice Lines Developed from Anther Culture

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Abstract

Rice is the most important food commodity in Indonesia. The need for rice continues to increase with the population increase. Plant breeding's objective to obtain high-yielding varieties is an attempt to increase production plateu. Biotechnology is one of the breeding aproaches using tissue culture and genetic engineering techniques. The availability and diversity of plant genetic resources are important factors to develop high yielding varieties with desirable traits. This study was aimed to investigate the agronomic characters of double haploid lines developed from anther culture, evaluate genetic diversity, phenotypic variations and broad sense heritability of double haploid lines developed from anther culture, and to obtain rice genotypes potential as superior lines. Analysis of 18 genotypes showed there was diversity of agronomic characters among the rice double haploid genotypes. Characters of dry weight-based grain (yield) per hectare and the number of empty grain per panicle had high level of genetic variability, while other characters had rather low to low level of genetic variability. Yield per hectare in KP44223 line was the highest (4537.8 kg / ha or 4.5 tons / ha) among the lines, even higher than that of conrol varieties, Ciherang and Celebes. The characters of plant height, flowering age, harvesting time, panicle length, number of empty grain/ panicle, 1000 grain weight and yield per hectarehad high broad sense heritability, suggested that those characters can be used as selection characters to improve crop performance

Keywords: anther culture, double haploid, rice

Introduction

Rice is the most important food commodity in Indonesia. In addition, rice is also a major source of carbohydrate material for Indonesian people. The need for rice continues to increase with population increase. (Prasetyo 2008), Plant breeding to obtain high-yielding varieties is one attempt to overcome rice production plateu. Utilizing biotechnology through tissue culture technique and genetic engineering could be one of the appraches in plant breeding (Somantri *et al*. 2003). The availability and diversity of rice genetic resources is an important factor in developing high yielding varieties with desirable traits. Development of new high yielding varieties of rice in Indonesia has long been conducted since the early 1970s. After the 1980's breeding toward new high yielding varieties (VUB) started to be intensified to obtain varieties that are responsive to fertilizer and high quality of rice, such as IR64, Membramo, Cisadane and Ciherang (Samaullah 2007). Conventional breeding to develop new rice varieties require a long time (7-10 years), especially the time for selection process to obtain pure lines. Utilization of modern technologies (biotechnology) such as anther culture is expected to shorten the selection process thus saving time, effort, and cost. Dewi and Purwoko (2001), states that the researchers have applied the anther culture technique to obtain rice lines that are resistant to pests and diseases, quality of rice as well as tolerant of environmental stress. Kim (1986), also reported that anther culture-based breeding approach have also been applied in Korea, Vietnam, China and India.

Materials and Methods

The study was conducted in January-May 2011 at IPB laboratory, Dramaga, Bogor. The materials used were 18 rice double haploid lines developed from anther culture (KP1-3-1-2, KP3-7-2-1, KP3-18-1-1, KP3-18 -1-2, KP3-18-1-3, KP3-19-1-1, KP3-19-1-2, KP3-19-1-3, KP3-19-1-4, KP4-19-1 -3, KP4-19-2-3, KP4-42-2-1, KP4-42-2-2, KP4-42-2-3, KP4-43-1-2-43-KP4 1-4 , KP4-43-2-3, KP4-43-2-4), and 2 control varieties (Ciherang and Celebes). The experiment was conducted at the Experimental station Babakan IPB, Darmaga, Bogor, using Randomized Block Design with four replicates. The treatments used were 18 double haploid rice genotypes and two control rice varieties. The 21 day old seedlings of each genotype were planted in field plots (0.8 x 5 m) with plant spacing of 20x20 cm having 2 seedlings/hole. Plants were fertilized with 200 kg/ha of Urea, 100 kg/ha of SP36 and 100 kg/ha of KCl applied three times at 10, 25 and 45 days after planting (DAP). Plants were maintained based on the paddy rice cultivation. The observations were performed on parameters of plant height (cm), total number of tillers (stems), number of productive tillers (stems), flowering date (days), harvesting date (days), panicle length (cm), number of filled grain per panicle (grains), number of empty grain per panicle (grains), total grain number per panicle (grains), weight of 1000 grains (g), and grain yield (kg/ha).

The data were analyzed using analysis of variance, Duncan multiple range test and analysis variance components (Singh and Chaudhary 1979). Variance component and heritability were determined based on Singh and Chaudhary (1979) as follow:

$$V_g = \frac{\mathrm{KTg} - \mathrm{KTe}}{\mathrm{r}}; \; V_P = V_g + Ve \; ; \; h^2{}_{bs} = \frac{Vg}{Vp} \; ; \quad KVG = \left(\frac{\sqrt{Vg}}{X}\right). \; 100\% \; ; KVP = \left(\frac{\sqrt{Vp}}{X}\right). 100\%$$

Which was:

Vg: various genotypes; Vp: various phenotypes; r: replication; X: average common genotype; KVG: coefficient of genetic diversity; KVP: coefficient of phenotypic diversity; KTg: sum square of genotype; KTE: sum square error. Broad sense heritability values according to Stanfield (1983) is high (12:50 $<h2<1.00$), moderate (12:20 $<h2<0.50$) and low ($h2<0.20$).

Results and Discussion

The results showed that the genotype effect was significant to all characters observed. This suggested that there was variability among genotypes for all the observed characteristics.

Plant Height

KP44323 and KP44324 lines were genotypes having highest average plant height (112.6 and 112.3 cm, respectively), while KP31812 and KP31813 lines had shortest average of the plant height (83.6 and 84.95 cm, respectively). Ciherang was classified as medium plant height (103 cm), while Celebes was classified as relatively short plant height (82.8 cm). From anther culture, the characters inheriteged from parent was the plant height, both on descent Ciherang and Celebes. This indiated that the plant height was dominant.

Total Productive Tillers and Tillers

Although not signicantly different among genotype, number of tillers and productive tillers per plant were varied among genotypes ranging from 14.1 to 19.55 and 11.55 to 15.25, respectively. KP31913 and KP44223 were the genotypes producing the highest and the lowest tillers. While KP1312 and KP44314 were the genotypes having the highest and the lowest productive tillers. The genotypes tested showed moderate number of tillers.

Flowering and Harvested

Flowering KP31812 of genotype lines and varieties Cilebes (control) i.e 63.25 consecutive days and 57 days compared to genotype lines KP41913, KP44312, KP44221, and KP44324 the consecutive 76 days and 75.75 days. This indicated that the lines have descended from varieties Ciherang age slower than lines flowering varieties descent Celebes. To determine the general harvesting age, the age of the rice crop can be classified into three groups calculated from the day after the scatterplot (HSS), namely age genjah (90-104 HSS), age was 105-120 HSS and age in at more than 120 HSS) (BB-padi 2009a). The average age of crop genotypes tested genjah belonging to moderate, with harvest age genjah of lines KP31811 and KP31813 was 91.75 day and most varieties genjah in Celebes (control) to 89.75 days old. While the maximum harvest age on genotype KP41913 and KP41923 a consecutive were 106 days and 105.75 days, respectively.

Panicle length

Panicle length characters in genotypes tested was quite short to medium. Longest panicle was found at KP44323 which was 24.96 cm. This was slightly shorter than that of varieties Ciherang at 25.82 cm, while the shortest panicle of KP44222 was 22.82 and 21.82 cm resulted from Celebes varieties (Table 3). DMRT test showed that all genotypes were not significantly different between the lines tested, except in the control varieties and Celebes Ciherang.

Number of Grain per Panicle

Line KP3721 had highest total amount of grain per panicle of 178.85 points, while the lowest was found in line KP31914 and KP31813 at 121.55 points and 121.88 points, respectively. Thus these genotypes were ssified as moderate (Table 4). All the genotypes tested were not significantly different with the second control test DMRT at 5%, except in line KP3721 and KP31914. Lines KP44312 and KP44324 had high in grain content / panicle at 100.35 points, respectively and 100.1 points higher than the control plants respectively 90.45 and 90.1 for the Celebes and Ciherang, while the lowest was found in line KP41923 had 56.45 points lower than the control varieties. KP41923 line had the most empty grain number/panicle at 106.3 points, while the lowest in line was KP31914 had 33.75 points. Although the line had a number of grain hollow KP31914/panicle, this line produced the lowest total amount of grain. That affected the yield per hectare. Calculation of the percentage (%) content of grain/panicle to total grain/panicle produced in line KP31914 was the highest at 72.23%, this value was higher than that of the varieties of Celebes (control) atf 70.06%. The lowest percentage was found at lines KP41923 and KP44314 which were 34.6 and 46.04%, respectively.

1000 Grain Weight

Weight of 1000 grains of rice indicated the size of grains of rice and beans level. The results showed that the highest weight of 1000 grains of rice was found at KP1312 line at 39 925 g. This value was higher than both the control varieties and Celebes Ciherang consecutive which were 36 025 and 30,575 g, respectively. The weight of 1000 grains of line KP3721 was the lowest at 26 325 g. This value was lower than the control Celebes varieties which was 30 575 g. KP3721 line had the lowest weight of 1000 grains allegedly due to low resistance to various diseases in the field, either by bacteria or viruses. Value of 1000 grain weight assessed with DMRT test at 5% level is shown in Table 3.

Grain Yield (kg /ha)

Grain yield per plot can be used to measure the amount of the plant production. Dry grain yield per plot was then converted to a hectare (kg/ha). The highest production was found at line KP44223 (no.entry 14) and KP44221 (no. entry 12) in a row having 4.5 and 4.3 t/ha, respectively, this significantly different with both the control varieties and Celebes Ciherang at 3:50 and 3:43 t/ha, respectively. The lowest dry grain was found at line KP41923 (no.entry 11) and KP31811 (no.entry 3) consecutive at 1:03 and 1.82 tonnes/ha, respectively. This differed markedly lower than the control based on test DMRT 5%. DMRT test at 5% for the grain yield/ha showed that all lines tested had distinct intangible, except in line KP44223 (highest) and line KP41923 (lowest) (Table 3).

Variety Component

Various components and heritability estimation is performed to determine the proportion of diversity caused by genetic factors and environmental factors. Heritability determines the success of selection for the appropriate environment, because it indicates the heritability of a trait is influenced by genetic or environmental factors. High heritability indicates the relative importance of genetic influences that can be used from the elders to their offspring as well as useful to determine the most appropriate selection method for improving a plant characters (Falconer and Mackay 1996). The value of plant genetic parameter estimation is shown in Table 3. Coefficient value of genetic diversity (KVG) ranged between 3.25 and 27.31 and phenotypic diversity coefficient (KVP) ranged between 4.51 and 36.83. Value KVG KVG 0.00-27.31% was the absolute value, that value was determined from the relative value of KVG. Absolute value of 27.31% KVG KVG was a relative value of 100%. According Moedjiono and Mejaya (1994), KVG relative value of the following criteria: low (0 <x <25%), somewhat lower (25%<x<50%), high (50%<x<75%) and high (75%x<100%). Based on the above criteria, it can be determined KVG absolute criteria in this trial, namely low (0.00 <x <6.83%), somewhat lower (6.83% <x <13.66%), high (13.66% <x <20:48%), and high (20:48% <x <27.31%). Murdaningsih *et al*. (1990) stated that the value was low and rather low KVG classified as narrow genetinya character variability, while quite high and high KVG classified as character mempynyai wide genetic variability.

Table 3. The value range of components and heritability estimates the value of agronomic characters and yield in rice

Agronomic character	mean	Vg	Vp	KVG (%)	KVP (%)	h^2bs (%)
Plant hight	93.79	87.61	103.04	9.98	10.82	85.03
Productive tillers	13.55	0.24	5.44	3.62	17.22	4.42
Total tiller	16.43	0.37	7.54	3.70	16.71	4.89
Flowering	69.59	30.54	31.56	7.94	8.07	96.79
Harvested	98.00	18.59	19.53	4.40	4.51	95.17
Panicle lenght	23.79	0.60	1.19	3.25	4.59	50.13
Number of grain/panicle	85.53	116.29	255.69	12.61	18.70	45.48
number of empty grain/ panicle	55.37	228.61	415.72	27.31	36.83	54.99
Total grain/panicle	140.90	165.78	372.94	9.14	13.71	44.45
1000 grain weight	34.43	9.46	14.01	8.93	10.87	67.53
Yield (ton/ha)	2.94	5.64	11.24	25.58	36.12	50.17

Remarks: Vg=varian genotype, Vp=varian phenotype, KVG=coefisien varian genotype, KVP=coefisien varian phenotype, h^2bs= boad sense heritability

Based on the criteria of absolute KVG, there were two characters having relatively high KVG ie grain yield / ha and the number of empty grain per panicle, five characters have a rather low KVG, namely plant height, flowering age, number of grain content per panicle, total grain number per panicle and 1000 grain weight, and the four characters have low KVG ie the number of productive tillers, total number of tillers, harvesting age, and length of panicle. Thus there are two characters having a broad genetic variability, and there are nine characters that have a narrow variability. Nugraha Lestari (2007) mentioned the opportunities for improvement of grain yield can be done with the selection of the character of the number of grain per panicle and grain yield dry. Broad sense heritability value of the characters observed was between 04:42 and 96.79% (Table 3). According to Stanfield (1983), broad sense heritability values can be grouped into three high heritability (12:50 <h2 <1.00), the heritability of moderate (0.2 <h 2 <0.50) and low heritability (h2 <0.20). Based on the above criteria that broad sense heritability values for characters: plant height, flowering age, age of harvest, panicle length, number of empty grain / panicle, 1000 grain weight and dry grain yield per hectare was high, whereas the character of grain number per panicle and the number of content total grains per panicle classified as being; then to the character of productive tillers and total number of tillers per hill had a low broad sense heritability values. Therefore, the improvment of grain yield can be done through the selection of plant height, flowering age, age of harvest, panicle length, number of empty grain / panicle, 1000 grain weight and grain yield per hectare dry.

Conclusions

Analysis of agronomic characters of 18 genotypes indicated that there was diversity in agronomic characters and character among the double haploid genotypes of rice anther culture results. Dry grain yield per hectare and the number of empty grain per panicle had a high level of genetic variability, agronomic characters and yield while others had a rather low level of genetic variability to low. Dry grain yield per hectare obtained from line KP44223 was the highest of 4.5 ton/ha than varieties Ciherang and Celebes. High broad sense heritability value generated on the characters were plant height, flowering age, age of harvest, panicle length, number of empty grain/panicle, 1000 grain weight and grain yield per hectare dry Therefore, the characters can be used as benchmarks for improving the character of the next generation of this crop.

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