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Evaluation of uniformity, variability, and stability of agronomic traits of doubledd haploid rice lines resulting from anther culture

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Abstract. *Sasmita P. 2010. Evaluation of uniformity, variability, and stability of agronomic traits of doubled haploid rice lines resulting from anther culture. Nusantara Bioscience 2: 67-72.* The formation of doubled haploid lines in anther culture aims to accelerate the acquisition of pure lines. Selection of the desired traits can be done directly to the progeny of anther culture results at early generations. This experiment aims to determine agronomic traits , uniformity, and stability of the doubled haploid lines, and obtain the putative doubled haploid lines as the material for further evaluation to obtain expected lines. The first experiments used completely randomized design which was repeated five times. The treatments were 111 doubledd haploid lines of first generation of anther culture results (DH1). The second experiment used split plot design with the main plot treatments were doubled haploid lines resulting from anther culture and the sub plot treatment were the second generation of doubled haploid lines (DH2) until the fifth generation (DH5). The results show that each plant within the same line have uniform agronomic traits, while the plants between different lines have different agronomic traits. The results of further evaluation on three out of 111 doubled haploid lines derived from the second to fifth generations show no difference between generations for each trait of the same lines. The results also show that the agronomic traits of the doubled haploid line were stable from generation to generation.

Key words: doubled haploid lines, uniform, stable, promising lines.

Abstrak. *Sasmita P. 2010. Evaluasi keseragaman, keragaman, dan kestabilan karakter agronomi galur-galur padi haploid ganda hasil kultur antera. Nusantara Bioscience 2: 67-72.* Pembentukan galur haploid ganda dalam kultur antera bertujuan untuk mempercepat perolehan galur murni. Seleksi karakter yang diinginkan dapat dilakukan langsung terhadap progeni hasil kultur antera pada generasi awal. Percobaan ini bertujuan untuk mengetahui karakteristik agronomi, keseragaman, dan kestabilan galur haploid ganda, serta mendapatkan putatif galur-galur haploid ganda sebagai bahan evaluasi lebih lanjut untuk mendapatkan galur harapan. Percobaan pertama menggunakan rancangan acak lengkap diulang lima kali. Perlakuannya adalah 111 galur haploid ganda hasil kultur antera generasi pertama (DH1). Percobaan kedua menggunakan rancangan petak terpisah dengan perlakuan petak utama adalah galur haploid ganda hasil kultur antera dan perlakuan anak petaknya generasi galur haploid ganda kedua (DH2) hingga kelima (DH5). Hasil percobaan menunjukkan bahwa setiap tanaman dalam galur yang sama memiliki karakter agronomi seragam, sedangkan tanaman antar galur berbeda memiliki karakter agronomi beragam. Hasil evaluasi lebih lanjut terhadap tiga dari 111 galur haploid ganda yang berasal dari generasi kedua hingga kelima menunjukkan tidak terdapat perbedaan karakter antar generasi untuk setiap galur yang sama. Hasil penelitian tersebut menunjukkan pula bahwa karakteristik agronomi galur haploid ganda stabil dari generasi ke generasi.

Kata kunci: galur haploid ganda, seragam, stabil, galur harapan.

INTRODUCTION

Anther culture is one of tissue culture techniques that can be applied to plant breeding programs in order to accelerate the process of obtaining a pure line. The technique is done *in vitro* technically through two stages, i.e. callus induction stage of pollen contained in the anther, and stage of plant regeneration from the callus. Stages of plant regeneration produces haploid plants, it is obtained through embryogenesis induction from repeated division of monoploid spores of F1 or F2 plants resulting from the crossing among parents those has the desired trait. When the chromosomes are doubledd or a spontaneous doubling occurs during culture process, it will obtain homozygous doubledd haploid plants. The traits controlled either by dominant genes and recessive genes can be expressed in the early generation of plants.

The results of previous studies show that the doubledd haploid plants can be obtained directly, together with other plants that have other ploidi on rice anther culture techniques (Chu 1982; Dodds and Robert 1987; Goddard et al. 1996). According to Chen (1983) these plants originated from pollen cells, because only pollen cells that initiate to develop callus and develop into plants regeneration on rice anther culture. The result of genetic analysis shows that 90% of fertile progeny resulted from anther culture were doubledd haploid (dihaploid) plants (Chu 1982). Trait of doubledd haploid plants of the same line was uniform and remains stable from generation to generation, so selection can be done directly on the early generation plants (Zhang 1989).

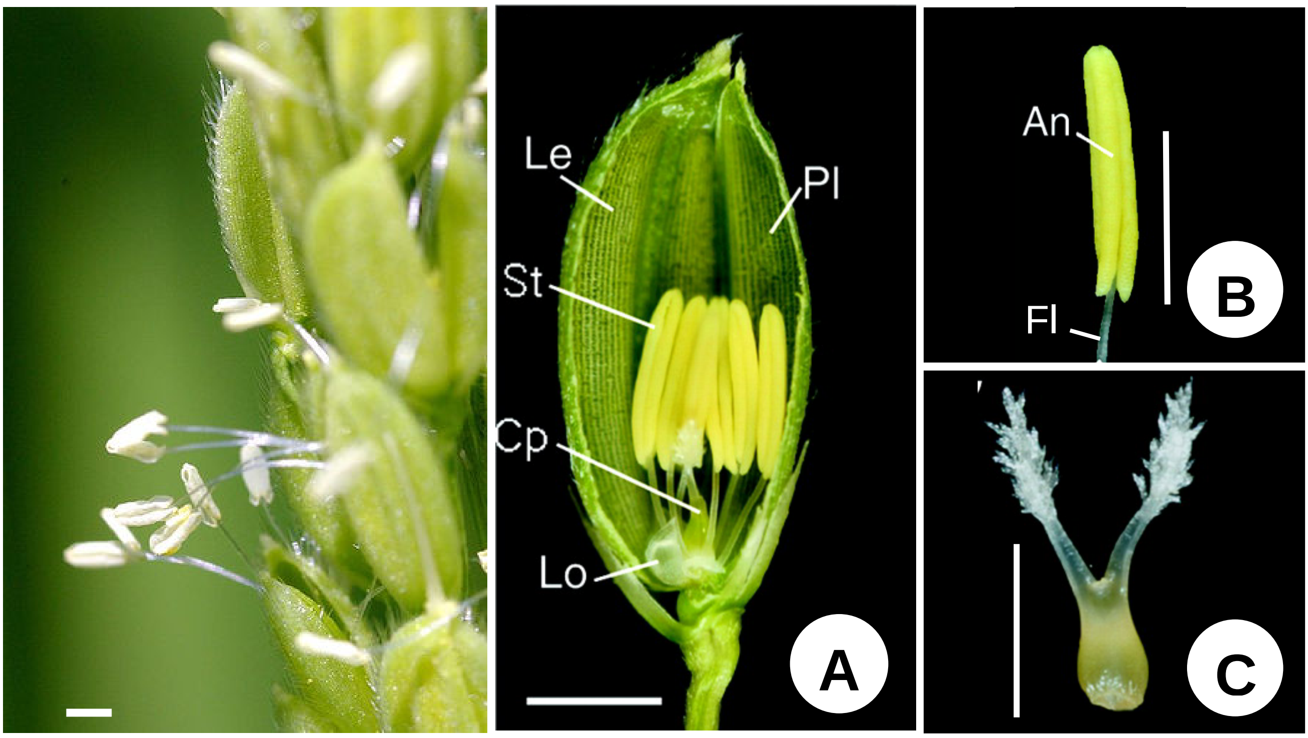

Figure 1. Flowers of rice plants. A. Close-up view of the inside grain. Cp, carpel; Le, lemma; Lo, lodicle; Pl, palea; St, stamen. B. Close-up view of the stamens. An, anther; Fl, filament. C. Close-up view of the pistils. Bars = 2 mm. (photos from several sources)

The formation of spontaneous doubledd haploid plants on rice anther culture is very beneficial, because it does not need to doubled the haploid plants as material selection. This method has been developed as an alternative in rice breeding to obtain pure lines as selection materials in order to accelerate the development of new superior varieties (Chahal and Gosal 2002).

To obtain genetic variability of doubledd haploid plants through anther culture techniques, we use explants (anthers) from plants that have high heterozygosity, F1 or F2 plants (Fehr 1987). Those anthers can be collected from part of rice flower (inside young panicle) at booting stage. Plant genetic variability caused by segregation of genes randomly during meiosis in microspore formation process of used F1 or F2 plants. The traits controlled by dominant genes and recessive genes can be expressed in early generation of doubled haploid plants, so the selection of the desired traits can be done in early generations. According to Zhang (1989) and Chung (1992), selection of main agronomic traits such as yield and grain quality and also tolerance to biotic or abiotic stress which were controlled by minor genes can be done at the generation of DH1 and DH2. Therefore the use of anther culture in breeding programs beside to improve the efficiency of selection, also to reduce the cost, the time and the labor (Chung 1992; Goddard et al. 1996; Niizeki 1997). Application of anther culture in rice breeding program has been reported to create a variety of superior varieties such as in China and Korea (Hu 1985; Li 1992; Chung 1992). Parts of the rice flower are showed in Figure 1.

Sasmita et al. (2002) report that results of anther culture of F1 upland rice obtained genetic material as many as 111 doubledd haploid lines. The plants were resulted from regeneration of various callus at regeneration stage in anther culture. The plants originating from one callus or same pollen and expressing uniform phenotype were grouped into one line and they were estimated to be doubledd haploid lines (homozigos). These lines could potentially be used as a population of selection material to get a new superior rice line (promising lines). To prove that these lines are pure lines, it is necessary to evaluate the uniformity of agronomic traits of each line and its stability between generations. This experiment aims to obtain information of uniformity and stability of agronomic and morphological traits of doubledd haploid lines resulted from anther culture as identifier of pure lines (homozygous).

MTERIALS AND METHODS

The experiment was conducted in September 2004 through January 2005 at Greenhouse of Research Institute for Agriculture Biotechnology and Genetic Resources, Bogor, West Java. This study consists of two experiments: first, the evaluation of the uniformity of agronomic traits in the same line and its variability among the lines, and second, the evaluation of the stability of agronomic traits of doubledd haploid lines from generation to generation. The genetic material used in the first experiment were 111 genotypes (lines) of first generation of doubledd haploid

(DH1) upland rice lines resulted from anther culture, while the material used in the second experiment were three doubledd haploid lines of the second generation to fifth generation (DH2, DH5), namely GI-8, IG-19 and IW-56 lines.

The first experiment used a completely randomized design with five replications. The treatments consist of 111 doubled haploid lines (DH1) resulted from anther. One experimental unit was one pot containing two hills of plants for each genotype. The planting and maintenance was done based on upland rice cultivation. Seeds of each genotype were planted in one pot (as a plot) that contains the media of soil and manure. Each pot was planted by two seeds from the same line on the two planting points. Fertilizer was given at a dose of 200 kg Urea, 100 kg SP36, and 100 kg KCl per hectare. Half dose of Urea, the whole dose of SP36 and KCl were given as a basic fertilizer mixed with the planting medium, while the remaining half dose of urea was given to the plants at 45 days after seed planting. Weeding is done twice, i.e. 30 and 40 days after seed planting. Pest control was done based on integrated pest management.

Observations was done on 13 agronomic traits at vegetative phase and reproductive phase. At the vegetative phase observation was done on plant height and the number of tiller per hill, while at the reproductive phase was done on flowering time (days), harvesting time (days), plant height at harvest time which was measured from the root neck to the panicle neck (cm), and the total number of tillers and productive tillers at harvest time (tiller/hill). Observations was also done on the yield and yield components, namely, panicle length which was measured from the panicle neck to the tip of the panicle (cm), number of grain/panicle (grains), filled and empty grain number, per panicle, 100 grains weight (g), and the grain production weight per hill (g).

The second experiment used split plot design with four replications. The main plot treatment were doubledd haploid lines, namely GI-8, IG-19 and IW-56, while the subplot treatment were generation of those doubledd haploid lines, i.e. second (DH2), third (DH3), fourth (DH4) and fifth generation (DH5) of those lines used. Each line grows on plot with 2.4 m x 1.5 m size and plant spacing of 30 cm x 20 cm. The planting and maintenance was done based on upland rice cultivation. Three seeds per hole were planted for each line. One experimental unit consists of five rows of plants. The sample plants were considered to be 10 plants, i.e. plants which were located on the middle row.

Fertilizer was given at a dose of 200 kg Urea, 100 kg SP36, and 100 kg KCl per hectare. Half dose of Urea, the whole dose of SP36 and KCl were given as a basic fertilizer at 15 days after seed planting, while the remaining half dose of urea was given to the plants at 45 days after seed planting. Crop arrangement was done at 14 days after planting by leaving two seeds per planting hole. Weeding was done twice: first, at 14 days after planting, and the second at 45 days after planting. The observation were done on 13 agronomic traits as it was done on the first experiment.

For the first experiment, traits homogeneity of each line was determined based on the Z value or the value of data standardization of each trait of individual plants from all five replications (10 plants). The uniform traits (homogeneous) were the traits that have frequency of observation data below the Z 97.5% curve which was bounded by $-1.96 \leq Z \leq +1.96$ with the data deviation < 20%. Furthermore, the variability among lines were analyzed by analysis of variance. For the second experiment, data was analyzed using analysis of variance, if the generation treatment has no significant effect on a trait, then the trait was considered to be stable treatment has no significant effect on a trait, then the trait was considered to be stable.

RESULTS AND DISCUSSION

Uniformity of the traits

Uniformity of the traits in the same line as well as the high variability among different lines is an important characteristic of the selection material population. The traits uniformity in the same line is one of pure line identifier (homozygous), while the population with a high variability among lines is the expected population that providing a great opportunity to get a genotype with the desired trait.

Based on the standardization results onto the 'Z' value from the observation data of each trait for each line, the overall data deviations were < 10%, except for the observation data of plant height and number of tiller on 45 days after planting. The data average deviation from normal distribution of Z values for each agronomic trait of the entire doubled haploid lines tested were presented in Table 1. These data indicate that the average of agronomic trait in the same line following the normal distribution of Z97.5% curve which was limited by the Z value = -1.96 and Z = +1.96, meaning that every trait in the same line shows uniformity.

Table 1. Data deviations of agronomic traits in the same line and variability among the lines.

Agronomic traits	The same lines	Among lines	
	Deviation of Z 97.5 (%)	F value	CV (%)
Plant height at 45 days after planting	10.8	97.8**	33.0
Number of tiller at 45 days after planting	10.6	39.6**	52.7
Flowering time	6.1	29.7 **	13.3
Harvesting tine	6.5	46.3**	11.3
Plant height at harvest time	7.9	51.0 **	22.0
The number of total tiller	7.2	9.8 *	25.4
The number of productive tiller	8.1	27.6 **	40.7
Panicle length	6.8	23.0**	24.5
The number of grains per panicle	5.2	46.3 **	19.9
The number of filled grain per panicle	5.9	44.6 **	25.1
Grain sterility	5.5	36.7**	65.3
The weight of 100 grains	7.2	158.7**	44.1
Grain weight per hill	8.1	36.8 **	57.9

Note: CV = coefficient of genetic variance, * = significant at 5% level, ** = significant at 1% level.

According to Baihaki (2000), the amount of variation in populations of pure lines can be presented as a scale with a normal distribution curve. Theoretically, in a plant population of pure line, there is no genetic variation; variation that occurs is mostly caused by environmental factors. The results of this evaluation show that the first generation rice lines from anther culture are indeed doubled haploid lines or pure line.

Evaluated Doubledd haploid lines in this study were result of callus induction from pollen and were not derived from anther somatic cells. Results of previous studies have proved that only the initiated (induced) pollen that can develop in anther culture process, whereas the somatic cells were not induced but act as a source of metabolites necessary for the development of callus (Chu 1982; Chen 1983). Plants derived from callus or pollen which were used in this study demonstrate the phenotypic expression which were uniform and can be grouped into one line. The results of this study indicate that lines of the early generations result from anther culture are pure lines (homozygous). These results also supports the previous reports indicating that each individual plant in a population of same doubledd haploid lines (from the same callus) have a uniform agromorphologic traits (Suhartini and Somantri 2000; Goddard 2002).

Variability of agronomic traits among the lines

Traits variability of plant populations determines the success of plant breeders in getting a new genotype with expected combination of superior traits. The greater variability of population selection is available, the greater the probability of obtaining a genotype with the expected trait. Genetic variability of agronomic traits of first generation doubled haploid lines (DH1) which result from are anther are presented in Table 1.

Results of variance analysis showed that the agronomic traits among the doubled haploid lines were significant different (Table 1). Agronomic traits which have the genetic variability and relatively high coefficients of genetic variance were the plants height at 45 days after planting (33.0%), number of tiller (52.7%), productive tillers (40.7%), sterility (65.3%), weight of 100 grains (44.1%), and grain weight per hill (57.9%). The variance value indicates a great probability to have the expected trait from the evaluated population. These results support the results of research by Dewi (2002) which show that there was great agromorphologic variability in the population of doubled haploid rice lines (DH1) obtained from anther culture. The agronomic traits appearance of doubled haploid lines obtained from anther culture were presented in Table 2.

Table 2 shows that the agronomic traits of the plant height at harvest time range from short to medium category (72.2 to 119.6 cm), while their productive tiller were in categories of little to many (5.3 to 28.4 tillers). The flowering time of the evaluated lines ranges from 58.4-82.0 days after planting and harvesting time at 96.8 to 131.7 days after planting. Based on the classification by Siregar (1981), harvesting time of the evaluated lines are classified into the category of very early maturing (harvest time < 110 days after planting), early maturing (110 ≤ harvest time <125 days after planting), medium (115 ≤ harvest time < 125 days after planting), as well as the long maturity (harvest > 125 days after planting).

Table 2. Appearances of agronomic traits of doubled haploid lines obtained from anther culture.

Traits	Value		Average
	Minimun	Maximun	
Plant height at 45 days after planting (cm)	34.6	66.8	50.4
Number of tiller at 45 days after planting	4.0	16.2	11.3
Flowering time (days after planting)	58.4	82.0	70.1
Harvesting tine (days after planting)	96.8	131.7	109.8
Plant height at harvest time (cm)	72.2	92.6	119.6
The number of total tiller	31.4	19.8	7.1
The number of productive tiller	5.3	28.4	16.1
Panicle length (cm)	18.8	31.2	24.4
The number of grains per panicle	99.2	196.1	139.9
The number of filled grain	78.2	132.4	110.7
Weight of 100 grains (g)	2.22	4.43	2.67
Sterility (%)	8.8	35.0	15.6
Grain weight/hill (g)	10.6	44.2	23.2

Stability of agronomic traits among generations

The stability trait of a plant genotype resulted from breeding is a requirement that must be fulfilled before being released as a new variety. Stability analysis basically aims to measure the variation of a genotype in different environments. In this study, the stability is intended to determine variations of a genotype trait on several generations of plants. The experiment was done on the same environment, with the aim that if there are variations, they are only caused by genetic variation. The results of the variability analysis to the effect of genotype, generation, and interaction between genotype and generation to agronomic traits of doubled haploid lines obtained from anther culture are presented in Table 3.

Table 3. Result of variance analysis of the effect of genotype (line), generation, and interaction of genotype and the generation to agronomic traits of doubled haploid lines obtained from anther culture.

Agronomic traits	F value		
	Line (L)	Generation (G)	LxG
Plant height at 45 days after planting	8656.1**	$<1^{ns}$	2.0^{ns}
Number of tiller at 45 days after planting	1463.5**	2.2^{ns}	1.4^{ns}
Flowering time	374.9 **	1.2^{ns}	1.9^{ns}
Harvesting tine	1365.4**	1.7^{ns}	$<1^{ns}$
Plant height at harvest time	1574.9 **	$<1^{ns}$	$<1^{ns}$
The number of total tiller	404.5**	1.9^{ns}	$<1^{ns}$
The number of productive tiller	1223.7**	1.6^{ns}	1.1^{ns}
Panicle length	386.9**	2.2^{ns}	$<1^{ns}$
The number of grains per panicle	157.8**	$<1^{ns}$	$<1^{ns}$
The number of filled grain per panicle	96.0**	$<1^{ns}$	$<1^{ns}$
Grain sterility	7.7^{ns}	$<1^{ns}$	$<1^{ns}$
The weight of 100 grains	2120.0**	$<1^{ns}$	$<1^{ns}$
Grain weight per hill	20.8**	1.7^{ns}	$<1^{ns}$

Note: **= significantly different at level 1%, ns = not different significantly

Results of variance analysis show that genotype (line) significantly affect to observed agronomic traits. The evaluated agronomic traits of the three lines are GI-8, IG-19, and IW-56 are presented in Table 4. The results show that the IW-56 line is the shortest line and has the highest number of tiller. At the time of harvest, the plant height was 72.3 cm with the number of productive tiller are 19.2, while the two other lines, namely GI-8 and IG-19, have plant height are 84.2 cm and 86.5 cm, and the number of tiller are 8.6 and 9.9 tillers.

Harvesting time, yield components and yield of the three lines were significantly different in general from each other. The longest harvesting time was shown by the GI-8 (123.7 days) while the two other lines were shorter. The highest yield (grain weight per hill and yield per plot) were achieved by the IW-56 line. It was estimated that the number of tiller per hill which were more gives contribution on the yield of this line. Grain weight per hill and yield per plot of the line were 57.20 g and 2.80 kg (Table 4). The two other lines show yield that were not significantly different from each other and were lower than the IW-56. In general, agronomic traits of the three doubled haploid lines, i.e. GI-8, IG-19 and IW-56, different from one another, but there were no significant differences in agronomic traits among generations (DH2-DH5) for the same line. It means that the agronomic traits are stable from generation to generation. Performance of different generation dobled haploid lines (DH2-DH5) in the same genotype (line) at vegetative stage were presented on Figure 2.

Table 4. Agronomic traits of doubled haploid rice genotype resulting from anther culture.

Traits	Genotypes		
	GI -8	IG-19	IW-56
Plant height at 45 days after planting	67.0 a	69.1 a	39.2 b
Number of tiller at 45 days after planting	9.1 b	9.0 b	16.1 a
Flowering time	85.9 a	63.4 c	68.9 b
Harvesting tine	123.7 a	103.8 b	107.6 b
Plant height at harvest (cm)	84.1 b	86.5 a	72.3 c
The number of total tiller	11.9 c	14.6 b	24.2 a
The number of productive tiller	8.6 c	9.9 b	19.2 a
Panicle length (cm)	22.1 c	28.3 a	27.1 b
The number of grains per panicle	145.2 b	138.4 c	154.9 a
The number of filled grain per panicle	133.1 b	124.1 c	140.4 a
Grain sterility (%)	13.8 a	14.5 a	13.5 a
The weight of 100 grains (g)	3.22 b	4.16 a	2.57 c
Grain weight per hill (g)	47.27 b	47.31 b	57.20 a
Yield weight per plot (kg)	1.97 b	2.04 b	2.80 a

Note: number in the same row followed by same letters indicates not different significantly based on 5% of DMRT.

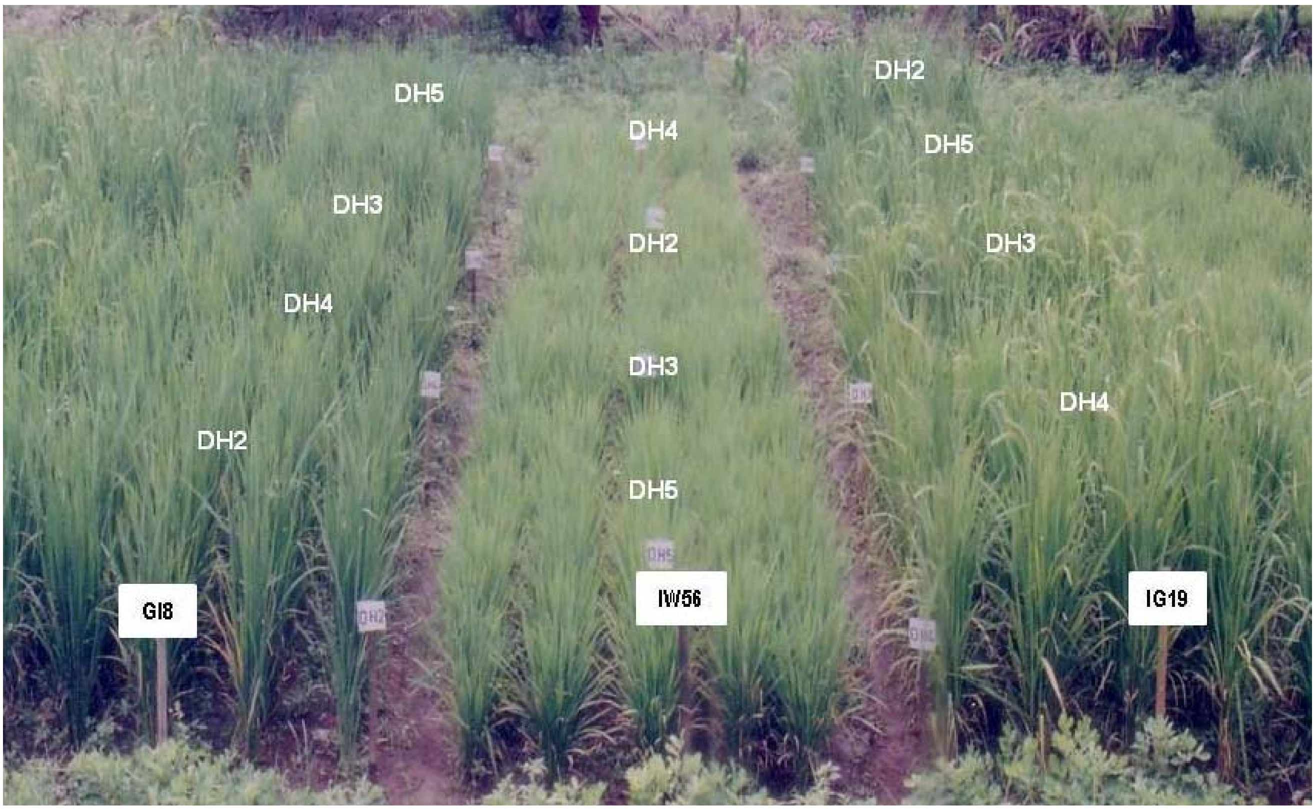

Figure 2. Performance of different generation doubledd haploid lines (DH2-DH5) in the same genotype (GI8, IW56, IG19) at vegetative stage

These results are consistent with Zhang's research result (1989) which indicates that all the traits of doubledd haploid rice lines originated from cultured one pollen trough antera culture which were planted from second generation to next generations showed no different significantly. It means that the appearance of all traits in this study are stable from 2nd generation to 5th generation. The previous research also indicate that more than 90% of diploid plants progeny which originated from pollen trough antera culture were homozygous and stable from generation to generation (Chu 1982; Niizeki 1997; Zhang 1989).

Chen (1983) shows that only pollen cells initiating to develop callus and then into plant regenerants in *in vitro* rice anther culture. In his research, he also indicates that anther wall tissue (tapetum cells) were not induced during the culture but plays an important role as a source of metabolites required for division and further proliferation of pollen. The use of metabolites from the anther wall was indicated by the formation of suspensor cells (multilayer) that connects the anther wall with microspores at the time of developing into globular embryos. This factor also supports the anther culture success rate higher than the culture of pollen and ovule.

Developed lines in breeding plants will eventually be planted by farmers in various different environments, while the phenotype appearance is determined by genetic factors, environmental, and the interaction of genetic and environmental factors. Therefore, the doubled haploid lines resulted from anther culture that have been identified as pure line as shown in this study, need to be further evaluated through multi-location test to determine theier traits stability among environments. The use of stable varieties or lines are useful for the development of a variety in a region, and also in extending the use of seeds as it can be planted in the next several generations.

CONCLUSION

Agronomic traits of doubled haploid rice resulted from anther culture which in the same line (genotype) were uniform, whereas the same agronomic traits among different lines were varied. Resulted doubled haploid lines from anther culture that have been evaluated are really pure lines, so they can directly be used as material selection to obtain lines with the expected superior traits. Agronomic traits of doubled haploid lines were stable from generation to generation, allowing these lines can be used as seed in a long time.

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