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Genetic Analysis of F_1 Hybrids Derived from Aromatic (Exotic) × Aromatic (Malaysian) Rice Crosses and Their Callus Induction Performance for Haploid Production

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Abstract

The aim of this study was basically to develop new aromatic rice lines through integrated breeding approaches. After screening and selection of superior parental materials, F_1s were raised from four different crosses (MRQ50×Gharib, MRQ50×RMB, MRQ50×RTB and MRQ50×E13) and used for haploid production through Anther culture. Phenotypic coefficient of variation % (PCV), genotypic coefficient of variation % (GCV), heritability % (h^2), genetic advance (GA) were estimated for yield and yield contributing traits such as for panicle length, grain per panicle, 1000 grain weight and yield per plant. In the cross MRQ50×RMB, the highest PCV was observed in grain per panicle (34.29) followed by yield per plant (31.73), 1000 grain weight (18.44), and panicle length (12.30). The same trends were occurred in cross MRQ50×RTB. The highest GCV was detected in grain per panicle (32.15) followed by yield per plant (30.90), 1000 grain weight (16.67) and panicle length (10.13). However, in the cross MRQ50×RTB, the highest GCV was obtained in yield per plant (37.89) followed by grain per panicle (30.54) and panicle length (9.33). Yield per plant was distinguished as the highest heritable trait in the cross MRQ50×RMB followed by grain per panicle (87.9), 1000 grain weight (81.7), and panicle length (67.8). On the contrary, in the cross MRQ50×RTB, this scenario was a bit different in which yield per plant was detected a highly heritable trait but the trends were different in grain per panicle, 1000 grain weight, and panicle length. Finally, the highest genetic advance was occurred in yield per plant followed by 1000 grain weight, grain per panicle, and panicle length. These trends were equal in both crosses. Study of callus induction ability of all hybrids studies showed that the best performance of callus induction was MRQ50×Gharib and N6 medium was found to be adequate for callus induction.

Keywords: Callus Induction, Haploid, Rice, Hybrid, Genetic Analysis

1. Introduction

Rice (*Oryza sativa* L.) is the most crucial crop in the developing countries in terms of production and consumption. It is also one of the major food resources and important cereal crop just after wheat and maize[1]. Aroma or fragrance is one of the most crucial traits in rice. Aromatic rice is a small group of rice which has a more advantage than that of non-aromatic rice. The fragrance itself somehow is a key factor in determining market price and value of rice[2]. The understanding of genetic variation which controls the inheritance of quantitative traits is crucial in genetic improvement program[3]. Recent trends have shifted to incorporate quality character in the

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centre approach apart from yield itself [4]. Study on genetic variability such as genetic coefficient of variation (GCV), heritability (H) and genetic advance are absolutely essential in which environmental conditions can have effect on them and also yield and its components[5]. Coefficient analysis is able to provide information on yield contributing traits. For establishing homozygous plants, doubled haploid system is a promising way. This type of system ensures the unique genetic property in which it allows the establishment of completely homozygous line from heterozygous parents in just a single generation. Pollen within the cultured anthers may be induced in order to form callus in which the haploid plants can be regenerated. Haploid plants upon chromosome doubling can be fully homozygous in the genotypes. This technique has been used successfully in producing homozygous breeding lines in *japonica* rice[6]. Callus induction has been studied by several researchers. Roy and Mandal[7] observed that callus induction was higher when N6 was added with different concentrations (0.5, 1.0, 2.0, and 3.0 mg L^{-1}) of NAA. Keeping the basic composition and altering the organic supplements and osmotic pressure might be helpful in increasing the rate of callus induction. Reinert et al.[8] proposed that 2 to 5% percent of sucrose is suitable for anther culture in rice. They concluded that maximum callus induction occurrence (7.5%) was obtained when 4% of sucrose was applied in the media. Shahjahan *et al.*[9] used the best microspore stage, mid-uninucleate stage, to obtain the highest callus induction frequencies in four *indica* rice varieties. In their research, the spikelet of the rice was seen to appear yellowish green color and the anthers were located at the middle of the spikelet. Yin *et al.*[10] used late-uninucleate stage as the best microspore stage for *japonica* cultivar callusing in which the spikelets were yellowish green in color and the length of stamen was one-third to half of the glume. In the present study, it was tried to analyze the genetic performances of F_1 hybrids developed from several aromatic rice crosses (Exotic aromatic land races × Malaysian aromatic rice variety) and their callus induction performance for haploid production with the aim to develop grain quality improvement of aromatic rice in the future.

2. Materials and Methods

2.1 Plant Materials and Field Experiments

The *indica* rice varieties which were proven to be well responsive to anther culture[11] were used in this study. Five different plant materials were used which consisted of four different crosses between F_1 of MRQ 50×Rambir Basmati, F_1 of MRQ 50×Rato Basmati, F_1 of MRQ 50×Ghambir, and F_1 of MRQ 50×E13. These materials were received from parts of ongoing research projects by the Institute of Biological Science, University of Malaya and the experiment was conducted on the net house of the Institute of Biological Science, University of Malaya. The number of seeds was 216 for MRQ 50×Rato Basmati, 240 for MRQ 50×Rambir Basmati, 4 for MRQ 50×Gharib, and 360 for MRQ 50×E 13. The parental genotype was local rice genotype of MRQ 50 as the female parent and 4 other varieties (Rato Basmati, Rambir Basmati, Ghambir, and E 13) as the male parents. The F_1 seeds of each cross were planted in small pot with 6 seeds placed in every hole which were irrigated every day. After 33 days of planting, all seedlings were transplanted to a 75×100 cm tank that were filled up with 2/3 loam soil. Just before transplanting, each tank was added with fertilizer comprising N: P: K: S (15: 15: 15: 2) as recommended by Hossain *et al.*[12].

2.2 Data Collection for Genetic Analysis

Three randomly selected plants from each cross were used in genetic analysis. The data was taken throughout the whole growing season and also after harvesting period. The traits were plant height (PH), total tiller (TT), panicle length (PL), grain per panicle (GP), fertile grain per plant (FGP), and thousand grain weight (TGW). PH was measured when the plant reached the matured state. After all the data was taken, the mean for each trait was calculated. Randomly three selected plants from each cross were chosen, and the mean was calculated and recorded respectively to the crosses.

2.3 Estimation of Genetic Parameter

Broad sense heritability (h^2) was estimated according to Falconer[13]. The resulting components of variances were then used to figure out the phenotypic coefficient of variance (PCV) and genotypic coefficient of variance (GCV) according to Singh and Chaudhury[14] and finally genetic advance (GA) was calculated according to Singh and Chaudhury[14].

2.4 Sample Collection

Panicles enclosed in leaf sheaths were harvested from primary tiller that were in booting stage. The panicles with the booting leaf sheath were correlated when the auricle

distance of the flag leaf to the penultimate leaf collar was around 5 to 9 cm. This technique was applied according to Gioi and Tuan[15]. The panicles were collected by cutting the stem, placed in a sealed plastic bag and labeled. Then, they were placed in an ice box and brought to the lab for pre-cold treatment.

2.5 Pre – cold Treatment and Preservation

Each harvested panicles were washed thoroughly in tap water and spread with 70% ethanol. They were covered with moist tissue paper, kept in sealed plastic bag, and labeled accordingly with important notes. Samples were then subjected to cold shocked at 8°C for eight days in refrigerator prior to anther plating. Following cold shock, on the day of culture, panicles were first rinsed with 70% ethanol for 20 seconds. The spikelets were removed one by one and surface sterilized by soaking with 30% commercial Clorox solution for 20 minutes and rinsed thoroughly with sterilized distilled water. Individual spikelets were then dried on filter paper and labeled accordingly to the genotype.

2.6 Anther Culture

Eighteen to hundred spikelets were cut at a time on sterile petri dishes under laminar air flow hood. They were planted aseptically onto sterile petri dish containing solidified callus induction medium (CIM) that were N6 and MS media which were supplemented with phytohormones, NAA, and 2, 4-D. One petri dish constituted one replication and at least two replications for each cross were applied in each callus induction media, MS and N6 media. The cultures were sealed with parafilm and kept in dark room at 28°C ± 2°C for 6 weeks[16]. The plates were examined periodically at weekly interval up to 5 weeks to observe the progress in respect of callus formation and callus induction frequency (percentage of anther performing calli) was recorded.

2.7 Media Preparation

To prepare 1 L of medium, 1 liter of schott bottle was first filled with distilled water until half of the bottle. After that, all of the components were mixed well on stirrer plate using magnetic stirrer. The pH of each medium was adjusted to 5.8. Adjustment was done with additional either 1M of NaOH or 1M of HCl. Each medium were then filled up with distilled water up until 1L. To solidify the medium, 2 grams of gelrite were added and sterilized by autoclaving at 15 psi and 121°C for 15 minutes. Then, medium was ready to be dispensed at the rate of 15 to 20 ml per petri dish.

2.8 Callus Induction from Cultured Anther

Callus development from cultured anther was observed during 4 and 8 weeks after anther placement in CIM; MS and N6 medium respectively for all four crosses that were studied. Callus induction efficiency was calculated as reported by Abbasi and Brar[17] by using the given formula:

$$\text{Callus induction (\%)} = \text{Number of anther forming callus / Total number of anther planted}$$

2.9 Statistical Analysis

The collected data were then being analyzed using SAS version 9.2 (Statistical analysis system) for analysis of variance (ANOVA) and mean differences with Duncan's Multiple Range Test (DMRT). As suggested by Falconer[13] and Singh and Chaudhury[14], genetic analysis of the agronomic traits for F_1 hybrids was performed for heritability (h^2), genotypic variance (σ^2_g), phenotypic variance (σ^2_{ph}), phenotypic coefficient of variance (PCV), genotypic coefficient of variance (GCV), and genetic advance respectively.

3. Results

3.1 Agronomic Traits of Aromatic Hybrids

The details of few important agronomic traits of aromatic hybrids were recorded for all four F_2 crosses (Table 1). According to Table 1, F_1 of MRQ 50×RTB genotypes had the highest plant height (105 cm) followed by F_1 of MRQ 50×Gharib (103 cm), F_1 of MRQ 50×RMB (86 cm), and F_1 of MRQ 50×E 13 (62 cm). In terms of tiller per plant and panicle length, F_1 of MRQ 50×RTB showed the highest rates, 13 and 20, while F_1 of MRQ 50×G demonstrated the lowest rates which were 5.3 and 17.2 respectively. For grain per plant (GP), F_1 of MRQ 50×G exhibited the higher number of grains, (104), while the lowermost rate was observed in F_1 s of MRQ 50×E 13 (46). Finally, F_1 of MRQ 50×E 13 had 43 fertile grains per penicle which was higher than other hybrids grains. Genetic parameters of yield and yield components of F_1 derived from MRQ50×Rato Basmati and MRQ50×Rambir Basmati cross are shown in Table 2. Phenotypic coefficient of variance (PCV) for all the traits including panicle length,

Table 1. Agronomic traits of aromatic hybrids for all four crosses

Genotypes/ Populations	Agronomic traits of aromatic hybrids						
	PH	TT	PL	GP	FGP	Stem color	Grain color
MRQ 50× RTB	105	13	20	76	32	Green	Green
MRQ 50× RMB	86	7	18.7	62	12	Yellowish green	Light green
MRQ 50× G	103	5.3	17.2	104	34	Light green and brownish	Green
MRQ 50× E 13	62	8.7	17.8	46	43	Green	Green

(RTB = Rato Basmati; RMB = Rambir Basmati; G = Gharib; E = Entry; PH = Plant height; TT = Total tiller; PL = Panicle length; GP = Grain per panicle; FGP = Fertile grain per plant; TGW = Thousand grain weight; GYP = Grain yield per plant)

Table 2. Genetic parameters of yield and yield components of F_1 derived from MRQ50× Rato Basmati and MRQ50× Rambir Basmati crosses

Characters	Phenotypic coefficient of variation % (PCV)		Genotypic coefficient of variation % (GCV)		Heritability % (h^2)		Genetic advance (GA)	
Cross	MRQ50× RMB	MRQ50× RTB	MRQ50× RMB	MRQ50× RTB	MRQ50× RMB	MRQ50× RTB	MRQ50× RMB	MRQ50× RTB
Panicle length	12.30	10.40(11.35)	10.13	9.33(9.73)=1(L)	67.8	65.3	1.10	1.02
Grain /panicle	34.29	32.89(33.59)	32.15	30.54(31.35)=3(H)	87.9	84.6	4.03	3.85
1000 Grain weight	18.44	17.74(18.09)	16.67	14.57(15.62)=2(M)	81.7	80.2	11.64	10.24
Yield/Plant	31.73	29.43(30.58)	30.90	38.79(34.85)=5(H)	94.8	92.5	20.85	18.53

(RTB = Rato Basmati; RMB = Rambir Basmati; G = Gharib; E = Entry; APC: Anther performing callus)

grain per panicle, 1000 grain weight, and yield per plant was higher than genotypic coefficient of variance (GCV). High PCV was observed in grain per panicle (33.59) and yield per plant (30.58), but low and moderate PCV rates were obtained in panicle length and 1000 grain weight respectively. Higher GCV value, 31.35 and 34.85, for grain per panicle and yield per pant was observed respectively. Highest heritability was recorded in yield per plant (94.8%) and the minimum was observed in panicle length (65.3%). The highest genetic advance was occurred in yield and the lowest was in panicle length. The number of anther planted and callus producing anther frequency are represented in Table 3. In this table, it is observed that F_1s of MRQ 50× G produced highest number of anther performing callus (APC), where F_1s of MRQ 50× E 13 appeared without any APC. In terms of callus induction percentage, F_1s of MRQ 50× G gave around 10% which was the highest rate compared to others.

3.2 Callus Induction Performance in Different Cultural Media (CIM, MS and N6)

MS and N6 media were used for callus induction. The frequency of callus formation varied between the two media and these variations also depending on the types of hybrids. Frequency and performance of callus induction are illustrated in Table 4 and Figure 1. N6 medium performed better results in terms of the number of APC and callus induction percentage compared to MS medium in hybrids of MRQ 50× RTB and MRQ 50× G, where the hybrids of MRQ 50× E 13 did not produce any APC (Table 4).

4. Discussion

4.1 Genetic Variability

Significant variations were observed in different yield contributing characters such as panicle length, grain per panicle, 1000 grain weight, and yield per plant among the genotypes (Table 2). The presence of variability is important for selection of the qualitative and quantitative traits which are crucial in conducting the improvement of rice breeding program. Thus, for a clear understanding of variation pattern, phenotypic variance was measured in which it was influenced by both genotypic and environmental variance. In attempt to conclude the range of variation in yield components that contribute to the

Table 3. Rate of callus formation from four different crosses of aromatic rice are comparing through Duncan Multiple Range Test (DMRT)

Rice hybrids	Total no. of anther planted	Total no. of anther performing callus (APC)	Frequency of APC	Callus induction (%)
MRQ 50× RTB	498 c	14 b	0.028 b	2.810 b
MRQ 50× RMB	660 b	2 c	0.003 c	0.300 c
MRQ 50× G	248 d	25 a	0.101 a	10.081 a
MRQ 50× E 13	762 a	0 d	0.000 d	0.000 d

(RTB = Rato Basmati; RMB = Rambir Basmati; G = Gharib; E = Entry; APC: Anther performing callus); The letters in different alphabate refres significant differences at 1% probability level

Table 4. Callus induction frequency on different callus induction media; MS and N6 for four different crosses of aromatic rice

Rice hybrids	Callus induction medium	No. of anthers cultured	No. of anther performing callus (APC)	Frequency of APC	Callus Induction (%)
MRQ 50× RTB	MS	276	1	0.0036	0.36
	N6	222	13	0.0586	5.86
MRQ 50× RMB	MS	330	1	0.0030	0.3
	N6	330	1	0.0030	0.3
MRQ 50× G	MS	124	6	0.0484	4.84
	N6	124	19	0.1532	15.32
MRQ 50× E 13	MS	408	0	0	0
	N6	354	0	0	0

(RTB = Rato Basmati; RMB = Rambir Basmati; G = Gharib; E = Entry; APC: Anther performing callus)

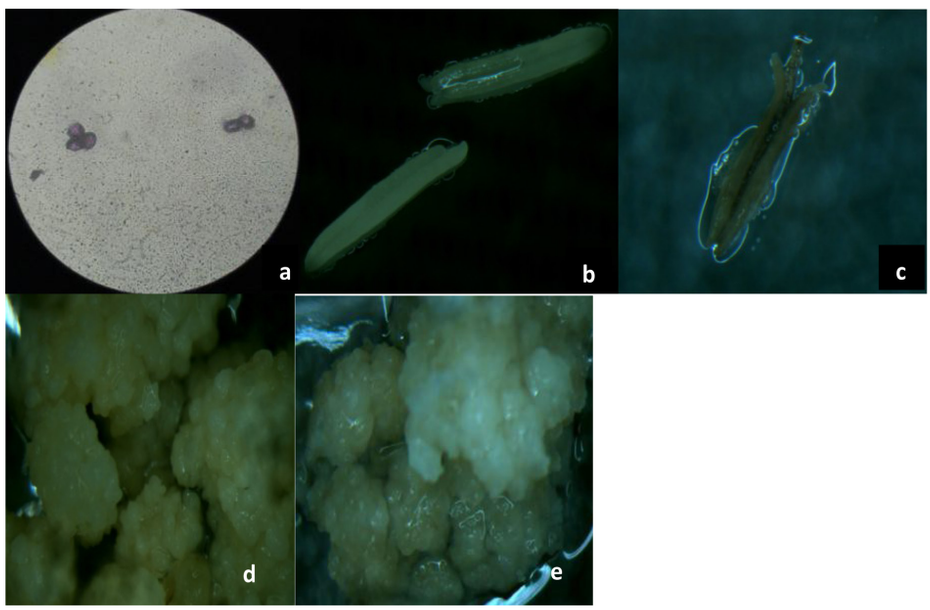

Figure 1. Callus induction from cultured anthers of aromatic rice hybrids
a - pollen of hybrid from MRQ50× RMB; b- cultured anther; c-brownish anther; d- brownish callus; e – whitish callus

variability in yield among the hybrids, important aspect need to be concerned in which the overall variability is linked with heritable and non- heritable components. Measurement of PCV and GCV in this study provided a useful indicator to compare the presence of variability in all the traits that had been studied. GCV indicates the variation among genotypes in particular traits based solely on genetic makeup while PCV shows the variation among genotypes in particular traits with association of environment with genetics[18]. In the present investigation, phenotypic coefficient of variance (PCV) was higher than genotypic coefficient of variance (GCV) for all traits that were studied. This magnitude indicated that there might be interactions among genotypes and environmental conditions that effect the phenotypic expression of the traits which is in the line with[18.] In some degrees, it may be due to other denoting environmental factors which affect the characters that were expressed by the hybrid plants. These results were similar to the results obtained by Akinwale et al.[18] and Iftekharuddeula et al.[18] All of the traits appeared with higher PCV than GCV. Thus, it can be concluded that panicle length, grain per panicle, 1000 grain weight, and yield per plant were obtained with the high influence of environment and did not depend solely on the genotype. In present study, PCV value was higher than GCV, thus this measurement were not suitable in making selection based on the phenotypic assessment for improving breeding scheme in this hybrid aromatic rice. The results were further revealed by looking at the value of each PCV and GCV. GCV provides a measure to compare the genetic variability in all qualitative characters studied (panicle length, grain per panicle, 1000 grain weight, and yield per plant). Higher GCV (more than 20%) observed in grain per panicle (32.15% for F_1s of MRQ50× RMB and 30.54% in F_1s of MRQ50× RTB) and in yield per plant (30.90% in F_1s of MRQ50× RMB and 38.79% in F_1s of MRQ50× RMB). Rangare et al.[20] also observed the same results of higher GCV value in grain per panicle and yield per pant which was similar to the result of this study. This indicates that high genetic variability in these two characters, moderate GCV in 1000 grain weight was obtained but low GCV (less than 10%) was observed in panicle length. However, Rangare et al.[20] observed low GCV in 1000 grain weight which was in contrast with the result obtained in this research. The higher GCV value clearly indicates high degree of genotypic variability in grain per panicle and yield per rice plant. In this study, high PCV was observed in grain per panicle and yield per plant, but low and moderate PCV rates were obtained in panicle length and 1000 grain weight respectively. Low PCV in panicle length was previously detected by Akinwale et al.[18] and Patil et al.[21]. Akinwale et al.[18] reveled that moderate to low PCV and GCV was due to the presence of both dominant and recessive alleles in the population. The low value of PCV and GCV shows a narrow range of variation which provides very low scope of selection. Genetic coefficient of variation (GCV) revealed the extension of genetic variability that present in the genotypes for various traits that were studied. However, it did not provide adequate information on determining the presence of variation that is heritable. As revealed by Singh[22], variation of heritable is useful especially for permanent genetic improvements.

4.2 Heritability

Variation of coefficient alone does not provide the full scope of heritable variation[23]. The present study indicated that among the yield components, all characters showed high heritability (more than 50%). Maximum value was recorded in yield per plant (94.8%) and the minimum was recorded in Panicle length (65.3%). Similar results for yield contributing traits for rice were reported in earlier studies[24]. Singh et al.[23] revealed that the estimation of heritability is crucial in genetic improvement of breeding programs. The purpose of heritability estimation in genetic analysis is to predict the reliability of the phenotypic value[25, 26, 27]. This provides an indication about the exploitable proportion of variation. GCV coupled with heritability provide information on the value of expected genetic advance through selection by phenotype[28]. High heritability of panicle length, grains per panicle, 1000 grain weight and yield per plant indicated that these traits have high response to selections. Shan and Mishra[29] have revealed high heritability for yield per plant, 1000 grain weight, and grain per panicle. Bhatti et al.[30] also reported high value of heritability for the number of spikelets per panicle, 1000 grain weight and the number of panicles per plant. Falconer[31] mentioned that heritability is the transmission portion of phenotypic variance. It is a good parameter in elucidating characters which were transmitted from the parent to the offspring. Ganapathy et al.[32] also explained that estimation in heritability may help plant breeders to make selection of elite genotype from divergent population. Therefore, characters especially yield per plant, grain per panicle, 1000 grain weight, and panicle length can be improved easily and selection will

be effective for upcoming breeding programs. However, estimation of heritability itself did not provide enough indication for selection the best individual as it is coupled with genetic advance.

4.3 Genetic Advance

Assessment of genetic advance elucidates the gene actions that contribute to the expression of various polygenic characters. In this study, all characters showed low genetic advance. According to Singh et al.[23], low genetic advance is an indication of non-additive gene action. So, selection for all characters therefore should be postponed to later generation to harness the non-additive gene action. Akinwale et al.[18] stated that when the heritability with genetic advance coupled, high heritability with low genetic advance in all characters was observed. This indicates that genotypes and interaction of genotypes with environmental conditions contribute a significant role in degree of phenotypes being expressed[18]. High heritability with low genetic advance was observed by Akinwale et al.[18] in panicle length, and 1000 grain weight. However, recent finding in yield per plant and grain per panicle for genetic advance was low and deviated from what they were observed. Singh et al.[23] also observed the same results as Akinwale et al.[18] where high heritability with high genetic advance was observed in yield per plant and grain per panicle. This may be due to difference in environmental conditions in the place where this study had been conducted. In present study, it was interpreted that among the four characters of yield contributing components, characters of yield per plant had the highest phenotypic and genotypic coefficient of variability followed by grain per panicle. Based on the two hybrids, F_1s of MRQ 50× RMB had the highest value of PCV and GCV compared to F_1s of MRQ 50× RTB. Yield per plant had the highest heritability followed by grain per panicle, 1000 grain weight and panicle length. The same pattern was observed for heritability where F_1s of MRQ 50× RMB has the highest heritability compared to F_1s of MRQ 50× RTB. In conclusion, yield per plant and grain per panicle were the most important traits which could be used for selection program among the yield contributing components. Selection based on these traits would be very effective in selection of aromatic hybrid rice for the best yield. Therefore, selection based on yield per plant and grains per panicle tend to increase grain yield for aromatic hybrid rice. Yet, selection could be postponed for these traits due to low genetic advance otherwise they could be improved by inter-mating of superior genotypes of segregating population from recombination breeding[23]. MRQ 50×RMB hybrids performed the best performance in terms of the highest PCV, GCV and heritability compared to MRQ 50×RTB. Thus, this hybrid was better in the performance of producing high yield of aromatic rice.

4.4 Callus Induction Ability

Data on the ability of rice genotypes to produce callus varied (Table 3). Previous studies by Li[33] proved that the earliest calli was formed on 10 to 15 days after inoculating anthers. In contrast, the earliest calli occurred after 5 weeks of culture which differed from the result obtained by the mentioned scientist. This was because visible bulk of the callus that was not standard. Common trend of variation in anther culture response was reported in *japonica* > *indica*/*japonica* > *indica*/*indica* >*indica*[34]. All hybrids used in the present study were genotypes of *indica*/*indica* so that culture efficiency was intermediate which was consistent with the study conducted by Shen et al.[34]. A clear difference in anther culture response was observed in the four aromatic rice hybrids in which the highest anther response was detected in F_1s of MRQ50×Ghambir followed by F_1s of MRQ50×Rato Basmati, F_1s of MRQ50× Rambir Basmati, and F_1s of MRQ50×E13. It can be seen that F_1s of MRQ50×Gharib was performed three times better than F_1s of MRQ50×RTB. Among the media evaluated, N6 medium was more efficient than MS medium. N6 medium was proved to be a better medium for callus induction especially in hybrid of MRQ50×Gharib. It was observed that callus was formed three times higher in the cultured anther than in MS medium from this hybrid. The same situation went to MRQ50× RTB hybrid where the cultured anther in N6 medium responded better than that of MS medium. Although all hybrids were cultured in the same composition of medium, there was a significant genotypic effect on callus induction frequency among the aromatic indica hybrids. It was clearly seen that MRQ50× Gharib hybrids had wide adaptation to the composition of the media since they had the highest callus induction frequency (15.32% in N6 and 4.84% in MS) in comparison with the other indica hybrids. The frequency of callus induction was depending on the genotype and also the types of media that was used to induce callus formation. MRQ50× Gharib performed the best callus induction both in MS and N6 medium but higher in N6. N6 medium

contained inorganic nitrogen in the form of nitrate and ammonium ion form. Media in which nitrogen in the ammonium form is considerably low were considered better for indica varieties[35]. The amount of nitrogen in N6 media was low compared with MS media. Callus induction frequency for MRQ50× Gharib was reasonably high by indica rice standard. However, manipulation of media components could have further improvements.

5. Conclusion

Several aromatic rice varieties already developed by Malaysian Agricultural Research and Development Institute (MARDI) such as MRQ 50 (Putri), PS 1297, and Mahsuri. All of these varieties were developed through mutation breeding and back crossing which were very costly and time consuming. Moreover, the selection for aromatic rice was not easy because of the large effect of environmental conditions. However, with integrated breeding approaches, where classical breeding and biotechnological concepts were used it is possible to improve the selection of aromatic rice traits with superior genotypes. Agronomic traits and the selection strategy have been taken into account for selection of high yielding aromatic rice hybrids and the performance of haploid production was obtained through anther culture technique. The overall results indicated that there was an adequate genetic variability in the materials studied. The PVC, GCV, broad sense heritability, and genetic advance revealed that the characters of yield per plant and grain per panicle were the most important yield components. These characters showed high variability and high heritability. Therefore, the results suggested that the yield per plant and grain per panicle were important yield contributing traits and selection on these traits would be most effective. However, these characters showed low genetic advance although their heritability was high. Thus, selection through simple selection methods could be postponed or these characters could be improved by inter-mating of superior genotype. So, it is concluded that these two traits may be considered as the selection criteria for the improvement of aromatic rice hybrids. Based on the types of genotypes studied, the present study exhibited that MRQ 50×RMB hybrids performed better than MRQ 50×RTB for all four traits and can therefore be recommended as aromatic rice varieties with promising high yield with good stability in Malaysia. Study of callus induction ability of these hybrids demonstrated that the best performance of callus induction was MRQ 50×Gharib followed by MRQ 50×Rato Basmati, MRQ 50×Rambir Basmati and the poor performance exhibited by MRQ 50×E13. It was concluded that variation did occur in plants development through anther culture and this finding was similar with previous studies by Raina[36]. Genotypic differences clearly influenced the anther culture ability of aromatic rice, and hybrid MRQ 50×Ghambir had good anther culture potential. The N6 medium was found to be adequate for callus induction in MRQ 50×Gharib. Further improvements may be possible by manipulating the components of the media.

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