APPENDIX

Pictures

Appendix 1.1

Curve shaped seed F_1 and non-curved shaped seed F_1

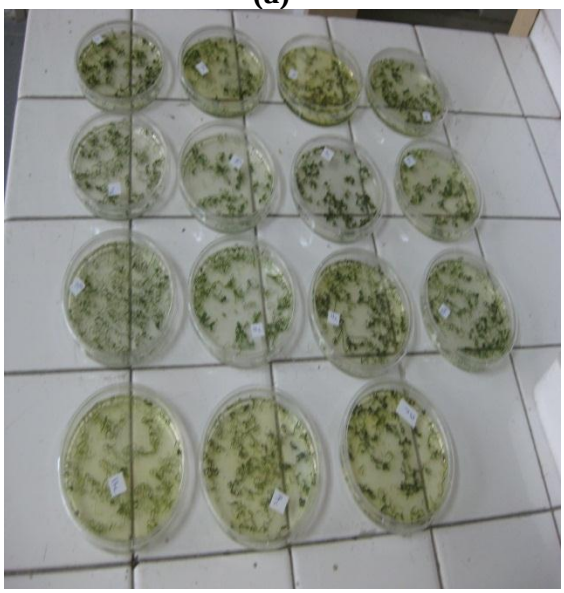
(a)  (b) 

(a) Curve shaped seed F_1. (b) Non-curve shaped seed F_1.

Appendix 1.2

Leaf Aromatic Test

(a) **(b)**

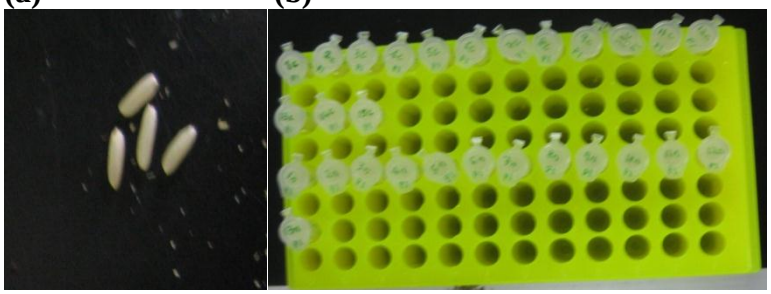
 

(a)Picture showing leaves soaked in 1.7% KOH. (b) Panels scoring for leaf aroma.

Appendix 1.3

Grain Aromatic Test

(a) **(b)**



(c)

(a) Picture showing milled rice.
(b) Picture showing centrifuge tube used for incubating rice.
(c) Panels smell and score for grain aroma.

Appendix 1.4

Lodging during grain filling period

Appendix 1.5

Pests and Diseases

(a) **(b)**

(a) Rice buds. (b) Rice leaf blast.

Appendix 1.6

F_2 seeds with parental grain

(a) **(b)** **(c)**

(a) F_2 seed from F_1 of MRQ 50/Rato Basmati.
(b) F_2 seeds of F_1 from MR 219/Rato Basmati.
(c) F_2 seeds of F_1 from MRQ 50/Rambir Basmati.

APPENDIX

Data

Appendix 2.1

Leaf Aroma Score from Panels

Leaf Aroma Score for Plant 1									
	F_1 Hybrids	Panel 1	Panel 2	Panel 3	Panel 4	Panel 5	Panel 6	Average	**Score**
	Curve shaped seed								
1	MR219/GHARIB	3	1	3	1	2	2	2.0	**2**
2	MR219/E7	1	2	1	2	2	1	1.5	**2**
3	MR219/RMB	1	2	2	1	1	1	1.3	**1**
4	MR219/E13	2	2	1	1	2	2	1.6	**2**
5	MR219/RTB	2	1	1	2	1	2	1.5	**2**
6	MR219/E11	1	2	3	2	1	3	2.0	**2**
7	MR219/SADRI	3	3	3	1	2	1	2.1	**2**
8	MRQ50/RMB	2	2	1	2	2	2	1.8	**2**
9	MRQ50/RTB	4	3	4	3	2	4	3.3	**3**
10	MRQ50/SADRI	3	4	3	2	3	3	3.0	**3**
11	MRQ50/GHARIB	2	4	4	2	2	4	3.0	**3**
12	MRQ50/E11	1	1	2	2	2	1	1.5	**2**
13	MRQ50/E13	4	3	4	3	3	4	3.5	**4**
14	MRQ50/E7	3	2	3	1	1	3	2.1	**2**
	Non-curve shaped seed								
1	MR219/GHARIB	2	3	3	1	1	2	2.0	**2**
2	MR219/E7	1	2	2	2	2	2	1.8	**2**
3	MR219/RMB	1	1	3	1	2	2	1.6	**2**
4	MR219/E13	2	3	1	2	2	2	2.0	**2**
5	MR219/RTB	3	2	1	1	1	2	1.6	**2**
6	MR219/E11	2	3	2	3	2	3	2.5	**3**
7	MR219/SADRI	2	3	1	2	3	3	2.3	**2**
8	MRQ50/RTB	3	4	4	3	2	4	3.3	**3**
9	MRQ50/SADRI	4	1	2	1	3	4	2.5	**3**
10	MRQ50/GHARIB	3	2	1	1	3	4	2.3	**2**
11	MRQ50/E11	4	3	2	1	2	4	2.6	**3**
12	MRQ50/E13	3	2	1	2	1	4	2.1	**2**
13	MRQ50/E7	1	2	1	1	1	3	1.5	**2**

Appendix 2.1: Continue.

Leaf Aroma Score for Plant 2									
	F_1 Hybrids	Panel 1	Panel 2	Panel 3	Panel 4	Panel 5	Panel 6	Average	**Score**
	Curve shaped seed								
1	MR219/GHARIB	3	3	3	2	2	1	2.3	**2**
2	MR219/E7	2	2	2	1	1	2	1.6	**2**
3	MR219/RMB	2	2	3	3	3	2	2.5	**3**
4	MR219/E13	1	3	2	2	2	1	1.8	**2**
5	MR219/RTB	1	2	1	1	1	2	1.3	**1**
6	MR219/E11	2	3	1	2	2	2	2.0	**2**
7	MR219/SADRI	2	2	2	1	2	2	1.8	**2**
8	MRQ50/RMB	3	3	3	3	3	3	3.0	**3**
9	MRQ50/RTB	4	1	1	1	4	2	2.1	**2**
10	MRQ50/SADRI	2	2	3	1	2	3	2.1	**2**
11	MRQ50/GHARIB	4	1	1	1	4	2	2.1	**2**
12	MRQ50/E11	2	2	4	1	3	4	2.6	**3**
13	MRQ50/E13	4	1	1	1	4	2	2.1	**2**
14	MRQ50/E7	3	4	4	2	3	4	3.3	**3**
	Non-curve shaped seed								
1	MR219/GHARIB	2	2	2	2	1	1	1.6	**2**
2	MR219/E7	1	2	1	1	2	1	1.3	**1**
3	MR219/RMB	2	1	1	1	1	2	1.3	**1**
4	MR219/E13	1	2	1	1	2	3	1.6	**2**
5	MR219/RTB	2	3	1	2	2	3	2.1	**2**
6	MR219/E11	2	3	1	2	1	3	2.0	**2**
7	MR219/SADRI	2	4	2	2	3	4	2.8	**3**
8	MRQ50/RTB	3	4	4	3	3	4	3.5	**4**
9	MRQ50/SADRI	3	3	3	2	1	4	2.6	**3**
10	MRQ50/GHARIB	2	3	4	3	2	4	3.0	**3**
11	MRQ50/E11	3	2	4	2	2	4	2.8	**3**
12	MRQ50/E13	2	4	4	3	1	3	2.8	**3**
13	MRQ50/E7	3	3	4	3	1	3	2.8	**3**

Appendix 2.1: Continue.

Leaf Aroma Score for Plant 3									
	F_1 Hybrids	Panel 1	Panel 2	Panel 3	Panel 4	Panel 5	Panel 6	Average	**Score**
	Curve shaped seed								
1	MR219/GHARIB	3	2	1	2	2	2	2.0	**2**
2	MR219/E7	3	2	1	1	2	2	1.8	**2**
3	MR219/RMB	2	3	3	2	3	3	2.6	**3**
4	MR219/E13	1	2	1	1	2	2	1.5	**2**
5	MR219/RTB	2	2	1	1	1	1	1.3	**1**
6	MR219/E11	1	3	1	1	1	2	1.5	**2**
7	MR219/SADRI	3	4	3	2	3	3	3.0	**3**
8	MRQ50/RMB	4	4	3	3	2	4	3.3	**3**
9	MRQ50/RTB	4	2	2	2	1	4	2.5	**3**
10	MRQ50/SADRI	2	2	1	1	1	4	1.8	**2**
11	MRQ50/GHARIB	4	1	1	1	3	2	2.0	**2**
12	MRQ50/E11	3	2	2	2	3	4	2.6	**3**
13	MRQ50/E13	3	3	1	2	2	4	2.5	**3**
14	MRQ50/E7	3	2	2	2	2	4	2.5	**3**
	Non-curve shaped seed								
1	MR219/GHARIB	4	1	1	1	4	2	2.1	**2**
2	MR219/E7	2	3	2	2	2	2	2.1	**2**
3	MR219/RMB	2	3	2	2	2	3	2.3	**2**
4	MR219/E13	1	2	1	2	1	2	1.5	**2**
5	MR219/RTB	1	3	1	2	1	2	1.6	**2**
6	MR219/E11	2	4	3	2	2	3	2.6	**3**
7	MR219/SADRI	2	4	1	1	3	2	2.1	**2**
8	MRQ50/RTB	3	3	3	3	2	4	3.0	**3**
9	MRQ50/SADRI	3	3	2	2	3	4	2.8	**3**
10	MRQ50/GHARIB	3	2	2	1	3	4	2.5	**3**
11	MRQ50/E11	3	3	1	2	2	3	2.3	**2**
12	MRQ50/E13	4	1	1	1	3	2	2.0	**2**
13	MRQ50/E7	3	3	2	1	2	2	2.1	**2**

Appendix 2.2

Grain Aroma Score from Panels

Grain Aroma Score for Plant 1							
	F_1 Hybrids	Panel 1	Panel 2	Panel 3	Panel 4	Average	**Score**
	Curve shaped seed						
1	MR219/GHARIB	1	1	1	1	1.0	**1**
2	MR219/E7	2	2	1	2	1.7	**2**
3	MR219/RMB	1	1	2	1	1.2	**1**
4	MR219/E13	1	1	1	1	1.0	**1**
5	MR219/RTB	1	1	2	1	1.2	**1**
6	MR219/E11	2	1	2	1	1.5	**2**
7	MR219/SADRI	2	1	1	2	1.5	**2**
8	MRQ50/RMB	2	2	1	3	2.0	**2**
9	MRQ50/RTB	3	2	1	4	2.5	**3**
10	MRQ50/SADRI	1	1	1	1	1.0	**1**
11	MRQ50/GHARIB	1	1	3	2	1.7	**2**
12	MRQ50/E11	1	1	1	1	1.0	**1**
13	MRQ50/E13	2	2	4	1	2.2	**2**
14	MRQ50/E7	2	1	2	2	1.7	**2**
	Non-curve shaped seed						
1	MR219/GHARIB	1	1	1	1	1.0	**1**
2	MR219/E7	1	1	1	1	1.0	**1**
3	MR219/RMB	1	2	1	1	1.2	**1**
4	MR219/E13	1	1	1	1	1.0	**1**
5	MR219/RTB	1	2	2	2	1.7	**2**
6	MR219/E11	1	1	1	1	1.0	**1**
7	MR219/SADRI	1	1	1	2	1.2	**1**
8	MRQ50/RTB	2	1	3	3	2.2	**2**
9	MRQ50/SADRI	1	1	3	1	1.5	**2**
10	MRQ50/GHARIB	1	1	1	1	1.0	**1**
11	MRQ50/E11	2	2	1	2	1.7	**2**
12	MRQ50/E13	2	2	1	2	1.7	**2**
13	MRQ50/E7	2	1	2	1	1.5	**2**

Appendix 2.2: Continue.

Grain Aroma Score for Plant 2							
	F_1 Hybrids	Panel 1	Panel 2	Panel 3	Panel 4	Average	**Score**
	Curve shaped seed						
1	MR219/GHARIB	1	1	1	1	1.0	**1**
2	MR219/E7	2	3	1	2	2.0	**2**
3	MR219/RMB	2	2	3	2	2.2	**2**
4	MR219/E13	1	1	2	1	1.2	**1**
5	MR219/RTB	2	1	1	2	1.5	**2**
6	MR219/E11	1	1	1	1	1.0	**1**
7	MR219/SADRI	2	1	1	2	1.5	**2**
8	MRQ50/RMB	2	3	2	4	2.7	**3**
9	MRQ50/RTB	2	1	2	4	2.2	**2**
10	MRQ50/SADRI	1	1	2	3	1.7	**2**
11	MRQ50/GHARIB	2	1	1	1	1.2	**1**
12	MRQ50/E11	1	1	1	1	1.0	**1**
13	MRQ50/E13	2	1	1	2	1.5	**2**
14	MRQ50/E7	1	1	2	2	1.5	**2**
	Non-curve shaped seed						
1	MR219/GHARIB	1	1	1	1	1.0	**1**
2	MR219/E7	1	1	2	3	1.7	**2**
3	MR219/RMB	1	2	1	2	1.5	**2**
4	MR219/E13	2	1	1	2	1.5	**2**
5	MR219/RTB	1	1	1	1	1.0	**1**
6	MR219/E11	1	1	1	1	1.0	**1**
7	MR219/SADRI	1	1	2	2	1.5	**2**
8	MRQ50/RTB	2	2	1	2	1.7	**2**
9	MRQ50/SADRI	1	1	1	3	1.5	**2**
10	MRQ50/GHARIB	1	1	3	2	1.7	**2**
11	MRQ50/E11	1	1	1	1	1.0	**1**
12	MRQ50/E13	2	2	1	3	2.0	**2**
13	MRQ50/E7	2	1	1	1	1.2	**1**

Appendix 2.2: Continue.

Grain Aroma Score for Plant 3							
	F_1 Hybrids	Panel 1	Panel 2	Panel 3	Panel 4	Average	**Score**
	Curve shaped seed						
1	MR219/GHARIB	1	1	1	1	1.0	**1**
2	MR219/E7	2	1	1	2	1.5	**2**
3	MR219/RMB	2	3	3	2	2.5	**3**
4	MR219/E13	2	1	2	2	1.7	**2**
5	MR219/RTB	1	1	3	2	1.7	**2**
6	MR219/E11	1	1	1	1	1.0	**1**
7	MR219/SADRI	1	1	1	1	1.0	**1**
8	MRQ50/RMB	2	1	1	2	1.5	**2**
9	MRQ50/RTB	2	1	1	2	1.5	**2**
10	MRQ50/SADRI	1	1	1	1	1.0	**1**
11	MRQ50/GHARIB	2	3	2	2	2.2	**2**
12	MRQ50/E11	1	1	1	1	1.0	**1**
13	MRQ50/E13	1	1	1	1	1.0	**1**
14	MRQ50/E7	1	1	2	2	1.5	**2**
	Non-curve shaped seed						
1	MR219/GHARIB	1	1	2	1	1.2	**1**
2	MR219/E7	1	1	1	1	1.0	**1**
3	MR219/RMB	1	2	3	2	2.0	**2**
4	MR219/E13	1	1	1	1	1.0	**1**
5	MR219/RTB	1	1	2	1	1.2	**1**
6	MR219/E11	1	1	1	1	1.0	**1**
7	MR219/SADRI	1	1	1	1	1.0	**1**
8	MRQ50/RTB	1	2	2	1	1.5	**2**
9	MRQ50/SADRI	1	1	1	2	1.2	**1**
10	MRQ50/GHARIB	1	1	1	1	1.0	**1**
11	MRQ50/E11	1	1	1	1	1.0	**1**
12	MRQ50/E13	1	2	1	2	1.5	**2**
13	MRQ50/E7	1	1	2	1	1.2	**1**

Australian Journal of Crop Science AJCS

AJCS 5(11):1318-1325(2011) ISSN:1835-2707

Analysis of aroma and yield components of aromatic rice in Malaysian tropical environment

Faruq Golam[*1], Y. Hui Yin[1], A. Masitah[1], N. Afnierna[1], Nazia Abdul Majid[1], Norzulaani Khalid[2], Mohamad Osman[3].

[1]Genetics and Molecular Biology, Institute of Biological Sciences, University of Malaya, 50603, KualaLumpur, Malaysia
[2]UM Biotechnology and Bio-product Research Cluster, University of Malaya, 50603, Kuala Lumpur, Malaysia
[3]Kulliyyah of Science, International Islamic University Malaysia, 25200, Kuantan, Pahang, Malaysia

***Corresponding author: faruq@um.edu.my**

Abstract

Low yield is a common phenomenon of aromatic rice and consequently rice breeders are trying to develop the agronomic characters to gain a better grain yield. In this study, a total of 53 rice genotypes including 12 globally popular aromatic rice cultivars and 39 advanced breeding lines were evaluated for yield and yield contributing characters in Malaysian tropical environment. Two local varieties MRQ 50 and MRQ 72 were used as check varieties. Correlation analysis revealed that the number of fertile tillers (r = 0.69), grain/panicle (r = 0.86) and fertile grain per panicle (r = 0.65) have the positive contribution to grain yield. Highest grain yield was observed in E36, followed by Khau Dau Mali, E26 and E13. E36 appeared with lowest plant height and it also produced highest number of fertile tillers. After evaluation of yield components four genotypes namely E36, Khau Dau Mali, E26 and E13 were selected as outstanding genotypes, which can be used as potential breeding materials for Malaysian tropical environment.

Keywords: Aromatic rice, Yield, Yield component, Tropical environment

Introduction

Rice is the major food of most Asian countries and aromatic rice varieties are playing a vital role in global rice trading. Major feature of these aromatic rice varieties is aroma which is being appreciated by many people and represents a high value added trait (Dela Cruz and Khush, 2000). So, rice needs attention toward improvement in its cooking qualities as well as several biochemical and morphological characteristics (Golam et al., 2004). The demand for aroma rice is increasing day by day. Unfortunately, the aromatic rice often has undesirable agronomic characters, such as low yield, susceptibility to pests and diseases, and strong shedding (Berner and Hoff, 1986). The agronomic value of a variety depends on many characteristics (Huang et al. 1991) and the most important characteristics are high yielding ability, resistance to diseases and pests, resistance to undesirable environmental factors and high quality of the products. But, the final aim is to increase the grain yield of rice (Swaminathan, 1999). Rice grain yield is determined by several agronomic characters such as heading days, days to maturity, grain filling period, number of fertile tiller, number of fertile grain per panicle, panicle length, 1000 grain weight and plant height (Halil & Necmi, 2005). The number of fertile tiller and number of grains per panicle cab be determined at vegetative and reproductive phase, respectively. The weight of 1000 grain which is important trait is normally determined during the ripening phase. Larger number of tillers can be expected at longer vegetative phase. But, the space available or optimum growth will limit the number of tillers which produce panicles. In determining the number of panicles the maximum tiller-number stage is the most important stage (Wang et al., 2007). Yield is a quantitative trait, greatly influenced by environmental fluctuations. Study on yield contributing characters assumes greater importance of fixing up characters that influence yield (Prasad et al., 2001; Kole and Hasib, 2008). A statistical analysis has been used to measure the mutual relationships between various characters and yield improvement. Genotypic evaluation of yield components can identify their relationship with grain yield in aromatic rice and the information of these relationships can be helpful to find superior aromatic rice genotypes (Tahir et al., 2002). In the present study, we tried to evaluate the extent of genetic variability of several diverse high yielding aromatic rice genotypes for yield contributing traits and to find out the correlation between yield and yield contributing traits.

Results and discussion

Genotypic variation in agronomic characters

A significant difference (5% level) was observed in all agronomic traits among the genotypes. Highest coefficient of variation observed in grain yield/plot followed by number of fertile tillers, fertile grains/panicle and grains/panicle. Number of tillers, grain filling period, 1000 grain weight, panicle length, plant height and heading days showed

moderate values for coefficient of variation. The lowest coefficient of variation was days to maturity (Table 3).

Grain yield per plot

The genotypes were significantly different for this trait at 1% level (Table 3). Coefficient of variation and coefficient of determination (R^2) for grain yield/plant were 3.62 % and 0.49 (Table 4). Higher coefficient of variation was recorded for grain yield/plot (Kole and Hasib 2008). Grain yield/plot ranged from 1.38 to 3.8 kg (Table 4). Minimum grain yield per plant was recorded in MRQ50, while maximum was recorded by E36.

Days to heading

A significant genetic variation was observed among genotypes in different replications (Table 3). Coefficient of variation and the coefficient of determination (R^2) for heading days were 7.26 % and 0.79, respectively (Table 4). Days to heading ranged from 75 to 94 (Table 4). The minimum days to 70 % heading were observed for genotype E15 while the maximum value was recorded in MRQ50. Weiya et al. (2008) also observed variation in heading days of several genotypes and they identified a regulatory gene responsible for this variation.

Days to maturity

For days to maturity variation was not significant among the tested genotypes (Table 3). Coefficient of variation and coefficient of determination (R^2) for days to maturity were 4.77 % and 0.84, respectively (Table 4). Days to maturity among rice genotypes ranged from 105 to 117 (Table 4). Minimum and maximum days to maturity was observed in E3 and MRQ50, respectively.

Grain filling period

Significant difference was observed for grain filling period in all genotypes. However, this variation was not significant in different replications (Table 3). Coefficient of variation and coefficient of determination (R^2) for grain filling period were 16.95 % and 0.37 (Table 4). Grain filling period ranged from 21 to 30 (Table 4). Minimum grain filling period was observed in MRQ70 while maximum were in 4 genotypes (E36, E11, E14, E15).

Plant height

Analysis of variance for plant height was significantly different among the genotypes at 1% level (Table 3). Coefficient of variation and coefficient of determination (R^2) for plant height were 8.65 % and 0.82, respectively (Table 4). Panicle length displayed moderate coefficient of variation values. Similar result was recorded by Kole and Hasib, 2008. Plant height ranged between 72 cm to 103 cm (Table 4). Minimum plant height were recorded in E36, while maximum height observed in E5.

Number of fertile tillers

Genetic differences were not significant among rice genotypes for tillers per plant (Table 3). Coefficient of variation and coefficient of determination (R^2) for number of fertile tillers were 34.32% and 0.49, respectively (Table 4). The number of tillers per plant ranged from 10 to 20 (Table 4). Minimum and maximum number of tillers observed in MRQ50 and in E36, respectively.

Panicle length

Significant variation was observed in length of panicle among the genotypes at 5% levels (Table 3). Coefficient of variation and coefficient of determination (R^2) for panicle length were 9.54 % and 0.68, respectively (Table 4). Panicle length displayed moderate values of variation coefficient. Kole and Hasib, (2008) also obtained the same results. The data for panicle length ranged 19.30 to 26.77 (Table 4). The minimum panicle length was recorded in E15 while maximum panicle length in E26. Ifftikhar et al. (2009) studied genetic variability for various traits and found that this trait is under the genetic control and could be used in the selection process of some desirable traits.

Fertile grain per panicle

Analysis of variance showed significant genetic variations among genotypes at 1% level in replications and non-significant among all varieties for fertile grains per panicle (Table 3). Coefficient of variation and coefficient of determination (R^2) for fertile grains per panicle were 26.26 % and 0.55, respectively (Table 4). Number of fertile grains/panicle ranged from 65 to 135 (Table 4). The least number of grains/panicle was observed by the genotype E3, while the maximum number for Khau Dau Mali.

1000 Grain weight

Significant variation was not observed among the tested genotypes in replications and among all varieties (Table 3). Coefficient of variation and coefficient of determination (R^2) for 1000 grain weight were 11.55 % and 0.78 (Table 4). The 1000 grain weight ranged from 15.33 g to 31.33 g (Table 4). Minimum 1000 grain weight was recorded in MRQ50, while maximum in E3.

Comparison of agronomic characters

The DMRT comparison of the data presented in Table 4, suggests that aroma rice genotypes show considerable variations in growth and yield characters. The E36, Khau Dau Mali, E26 and E13 were observed as the four top yielders, respectively. The yield potential of these genotypes can be explained based on the higher number of fertile tillers (E36), fertile grain per panicle (Khau Dau Mali and E13) and increased panicle length (E26). Khau Dau Mali is a popular and a well accepted Thai aromatic rice variety. Comparison of agronomic characters of 4 selected outstanding lines along with E11 (with good aroma and kernel elongation ratio), E2 (highest aroma) and two local checks MRQ50 and MRQ72 are presented in Fig 1.

Table 1. Description of 53 rice genotypes

Entry No.	Designation	Cross	Origin
1	E1 (88023-RE)	Unknown	CIAT
2	E2 (CT9882-16-4-2-3-2P-M)	Unknown	CIAT
3	E3 (H013-5-3-B4)	Unknown	ARGENTINA
4	E4 (H014-1-1-B2)	Unknown	ARGENTINA
5	E5 (IR 60080-46A)	IR 47686-08-4-3/CT 6516-21-4-4	IRRI
6	E6 (IR 74)	IR 19661-131-1-2/IR 15795-199-3-3	IRRI
7	E7 (IR 77734-93-2-3-2)	NSIC RC 148/PSB RC 18//NSIC RC 148	IRRI
8	E8 (IR 77736-54-3-1-2)	NSIC RC 148/PSB RC 64//NSIC RC 148	IRRI
9	E9 (IR 78006-55-2-3-3)	IR 67406-6-3-2-3/IR 72860-80-3-3-3	IRRI
10	E10 (IR 78537-32-1-2-10)	IR 65610-38-2-4-2-6-3/IR 60912-93-3-2-3-3	IRRI
11	E11 (IR 78554-145-1-3-2)	IR 72861-13-2-1-2/IR 68450-36-3-2-2-3	IRRI
12	E12 (IR 77298-14-1-2)	IR 64 (WH)/ADAY SEL//3*IR64	IRRI
13	E13 (IR 77512-2-1-2-2)	IR 68726-3-3-1-2/IR 71730-51-2	IRRI
14	E14 (IR 77629-72-2-1-3)	IR 71730-51-2/IR 71742-267-3-2	IRRI
15	E15 (M1-10-29 UL)	Unknown	MYANMAR
16	E16 (TOX 3226-5-2-2-2-2)	ITA 235/IR 9828-91-2-3//CT 19	IITA
17	E17 (TOX 3867-19-1-2-3-3)	Unknown	WARDA
18	E18 (WAB 272-B-B-5-H5)	3290/WASC165	WARDA
19	E19 (WAB 99-84)	ITA257/WABUKA	WARDA
20	E20 (WAB 337-B-B-15-H1)	ITA 135/WABC 165	WARDA
21	E21 (WAB 515-B-10 A 1-4)	Unknown	WARDA
22	E22 (WAS 169-B-B-4-2-7)	Jaya / Basmati 370	SENEGAL
23	E23 (WAS 169-B-B-4-2-9)	Jaya / Basmati 370	SENEGAL
24	E24 (WAS 197-B-4-1-22)	IR 31851-96-2-3-2-1 / IR 66231-37-1-2	SENEGAL
25	E25 (WAS 197-B-4-1-25)	IR 31851-96-2-3-2-1 / IR 66231-37-1-2	SENEGAL
26	E25 (WAS 197-B-5-2-16)	IR 31851-96-2-3-2-1 / IR 66231-37-1-2	SENEGAL
27	E27 (WAS 197-B-5-2-5)	IR 31851-96-2-3-2-1 / IR 66231-37-1-2	SENEGAL
28	E28 (WAS 197-B-6-3-12)	IR 31851-96-2-3-2-1 / IR 66231-37-1-2	SENEGAL
29	E29 (WAS 197-B-6-3-16)	IR 31851-96-2-3-2-1 / IR 66231-37-1-2	SENEGAL
30	E30 (WAS 197-B-6-3-2)	IR 31851-96-2-3-2-1 / IR 66231-37-1-2	SENEGAL
31	E31 (WAS 197-B-6-3-4)	IR 31851-96-2-3-2-1 / IR 66231-37-1-2	SENEGAL
32	E32 (WITA 7=TOX 3440-171-1-1-1-1)	TOX891-212-1-201-1-105/TOX3056-5-1	WARDA
33	E33 (BASMATI 370)	Unknown	PAKISTAN
34	E34 (IR 50)	IR 2153-14-1-6-2/IR 2061-214-3-8-2//IR 2071-625-1-252	IRRI
35	E35 (IR 64)	IR 5657-33-2-1/IR 2061-465-1-5-5	IRRI
36	E36 (IR 72)	IR 19661-9-2-3/IR 15795-199-3-3//IR 9129-209-2-2-2-1	IRRI
37	E37 (PSB RC2= IR 32809-26-3-3)	IR 4215-301-2-2-6/BG90-2//IR 19661-131-1-2	IRRI
38	E38 (PSB RC18=IR51672-62-2-1-1-2-3)	IR 24594-204-1-3-2-6-2/IR 28222-9-2-2-2-2	IRRI
39	E39 (PSB RC64=IR 59552-21-3-2-2)	IR 32809-26-3-3/IR 39292-142-3-2-3	IRRI
40	Ratani Pagal	Land race	Bangladesh
41	Katari Bog	Land race	Bangladesh
42	Khau Dau Mali	Land race	Thailand
43	Gharib	Land race	India/Pakistan
44	Sadri	Land race	Iran
45	Chini Gura	Land race	Bangladesh
46	Kasturi	Land race	India/Pakistan
47	Paheale	Land race	India/Pakistan
48	Tamahonami	Land race	Japan
49	Rambir Basmati	Land race	India
50	Rato Basmati	Land race	India
51	MRQ70	Variety	Malaysia
52	Q50	Variety	Malaysia
53	Q72	Advance line	Malaysia

Correlations analysis

Correlation analysis of characters can be used as tool for indirect selection. Correlation studies help the plant breeder during selection and provide the understanding of yield components. From the result in Table 5, out of eleven characters, only three variables such as number of fertile tillers, grains/panicle and fertile grain/panicle, show positive correlation with the most important character yield. Although negative trend was observed between heading date (HD), days to maturity (DM) and plant height (PH) but they were not correlated. Direct effect of days to flowering and positive indirect effect via days to maturity on grain yield was reported by Prasad et al. (2001), which is against with the present findings. Plant height has no significant correlation with yield. This is in contrast with the previous study of Bai et al. (1992) that presented the positive and significant correlation between plant height and yield. Usually, it is desired that a high yielding type of rice should be of short stature (Singh et al., 2000). However, number of tiller (NT), panicle length (PL) and thousand grain weight (TGW) suppose to show positive correlation with yield. However, no significant correlation observed between the above mentioned characters and yield in this study although their trend was positive. We suggest infertility of grains for lack of correlation. Zia-ul-qwamar et al. (2005) observed negative correlation between productive tillers per plant and fertility percentage. The indirect effects via total grain/panicle and 1000-grain weight were positive but low as compared to other traits. Positive correlation was observed between grain yield per plot (GYP) with grain/panicle (GP), fertile grain/panicle (FGP) and number of fertile tiller (NFT). Fertile tillers per plant exhibited a positive direct effect on grain yield/plot. Positive association of grain yield with productive tillers/plant was also studied by Meenakshi et al.(1999). Total grain per panicle also exhibited a positive direct effect and correlation coefficient with grain yield per plant. Kim et al. (1999) reported positive contribution of total grain towards grain yield, which supports the present finding. Correlation coefficients between different agronomic traits and grain yield have shown in Fig 2.

Association analysis of aroma-kernel elongation ratio

In the present investigation, genotype E11 and Garib performed excellent in aroma (score 3.5-4.0) as well as in kernel elongation ratio (1.2-135). In addition, within the total 53 aromatic genotypes, 17 did not produce any aroma. In addition, a significant number of genotypes showed relatively low kernel elongation ratio in this sub-tropical environment. Aroma score such as Rambir Basmati or Rato Basmati is always more than 4.0 in Indian subcontinent (in sub-tropical environment; day night average temperature 22-23°C) and in Malaysian tropical environment (day night average 28 - 30°C) and the score was 2.5 in both genotypes (Faruq et al., 2010). Similar observations were also observed in kernel elongation ratio. In subtropical environment kernel elongation ratio was always observed to be more than 2.0 in Rambir Basmati or Rato Basmati in Malaysian tropical environment. It was 1.1 and 1.05 for Rambir basmati and Rato basmati, respectively (Faruq et al., 2010). This investigation indicated that association of aroma and kernel elongation ratio can be highly influenced by tropical environment.

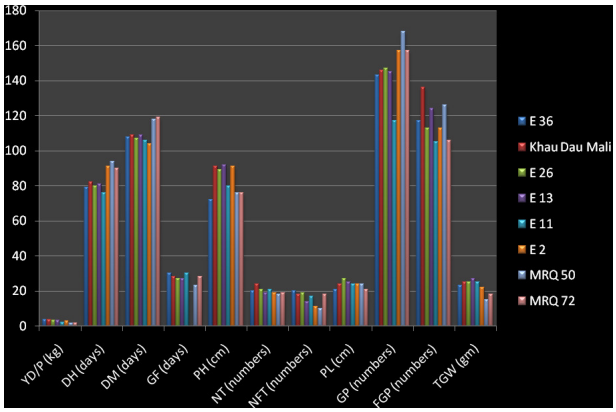


Fig 1. Comparison of agronomic characters of 4 selected outstanding lines along with E11 (with good aroma and kernel elongation ratio), E2 (highest aroma) and two local checks MRQ50 and MRQ72, Indicators: YD/P= Yield/plot, DH= Heading days, DM= Days to maturity, GF= Grain filling period, PH= Plant height, NT=Number of tillers, NFT=Number of fertile tillers, PL= Panicle length, GP= Grain/panicle, FGP= Fertile grain/panicle, TGW=1000 grain weight .

Interestingly, two genotypes (Garib and E11) produced their normal aromatic and Kernel elongation ratio and aromatic expression even in tropical Malaysian Environment. It can be concluded that this expression might be as a result of the influence of dominant nature of some associated genes in these two traits. However, both Garib and E11 were not placed in top 4 yielders, because of their low yield performance in Malaysian tropical environment.

Materials and methods

Plant Materials

A total of 53 rice genotypes including two local check varieties (MRQ50 and MRQ72) were used in this investigation. Twelve of them were globally popular aromatic rice cultivar such as Radhuni Pagal, Katari Bhog, Khau Dau Mali, Gharib, Sadri, Chini Gura, Kasturi, Paheale, Tamahonami, Rambir Basmati, Rato Basmati, Rato basmati. In addition, 39 advanced breeding lines plus 2 local check varieties (MRQ 50 and MRQ 72) were collected from International Rice Research Institute (IRRI) and Malaysia Agricultural Research and Development Institute (MARDI), respectively. A brief description of these 53 genotypes has been provided in Table 1.

Experimental design and growing condition

All of 53 genotypes raised in small plots (1 × 3 m) in three replications with Randomized Complete Block Design (RCBD) at the experimental field at Genetic and Molecular Biology, Institute of Biological Science, Faculty of Science University of Malaya situated at Latitude 3° 06' N and Longitude 101° 39' N with elevation of 60.8 m from sea level in October 2009. The climatic condition was hot and humid with frequent rain (Table 2). Recommended rice production practice of Malaysian Agricultural Research Institute was followed.

Table 2. Meteorological data recorded at the experimental site during the study period.

Months	Average temperature (^{0}C)	Rainfall (mm)	Relative Humidity (%)	Sunshine (h day^{-1})
October 09	27.7	64.5 (2.1 mm/day)	75.7	7.4
November 09	26.8	239.1 (8 mm/day)	82.0	6.7
December 09	25.2	242.3 (11 mm/day)	74.9	6.3
January 10	27.4	202.2 (6.5 mm/day)	78.0	6.9
February 10	27.5	192.3 (6.9 mm/day)	73.8	7.0

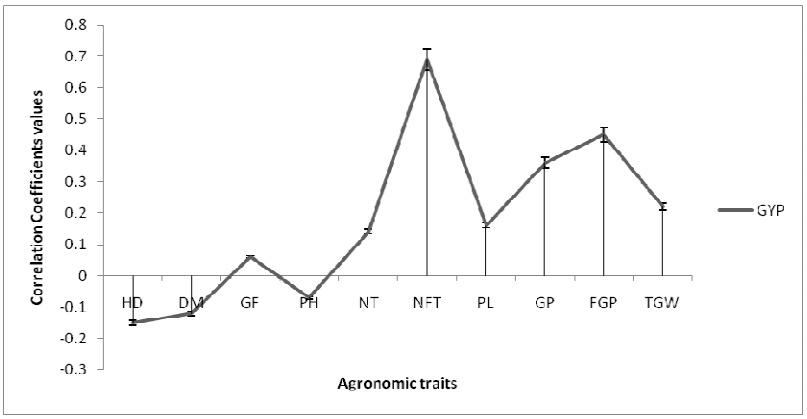


Fig 2. Correlation coefficients between different agronomic traits and grain yield. Indicators: HD= Heading days, DM= Days to maturity, GF= Grain filling period, PH= Plant height, NT= Number of tillers, NFT= Number of fertile tillers, PL= Panicle length, GP= Grain/panicle, FGP= Fertile grain/panicle, TGW= 1000 grain weight, GYP= Grain yield/plot

Data collection of agronomic traits

Data were collected at 70% flowering or heading stages (HD), days to maturity (DM), plant height (PH), number of total tillers (NT), number of fertile tillers (NFT), panicle length (PL), number of grain/panicle (GP), fertile grain/panicle (FGP), 1000 grain-weight and grain yield/plot (TGW). Days to flowering have been recorded as soon as 70% of the panicles appeared. Number of tillers were recorded when grain has set and the total number of fertile panicles emerged by each plant. The plant height was measured from ground level to the top the node (just below the panicle). Panicles were harvested at maturity and individually placed in an envelope. All panicles were taken out of the envelopes and air-dried at room temperature for one week. After that fertility of panicle, 1000 grain-weight and grain yield/plot were estimated.

Aromatic Test (Sensory Test)

Leaf Aromatic Test

To know the level of aromatic nature of each rice genotypes Leaf Aromatic Test (LAT) was conducted. An amount of 0.2 g of leaf samples was taken from each genotype. Leaves were cut into tiny pieces and put into glass petri-plates. 10ml of 1.7% potassium hydroxide (KOH) was added to each of the petri-plates containing the sample and was covered immediately. These petri-plates were left under room temperature for 10 minutes and then opened one by one for aroma test. The contents in each petri-plates was smelt and were scored on 1-4 scale with 1, 2, 3 and 4 corresponding to absence of aroma, slight aroma, moderate aroma, and strong aroma, respectively. Four panels of students and staffs from Division of Genetics and Molecular Biology, Institute of Biological Science, Faculty of Science University of Malaya were invited to score the aroma in each genotype.

Grain Aromatic Test

Fourty grains of each genotype were soaked in 10ml 1.7% KOH solution at room temperature in a covered glass Petri-plate for about 1 hour. The sample was scored on 1-4 scale with 1, 2, 3 and 4 corresponding to absence of aroma, slight aroma, moderate aroma, and strong aroma, respectively. The same four panels of students and staffs from Division of Genetics and Molecular Biology, Institute of Biological Science, Faculty of Science University of Malaya were invited to score the aroma in each genotype.

Measurement of elongation ratio, proportionate change and actual elongation

Ten measured (length and width) rice grain took into 20 ml glass test tube and soaked for 20 minutes in 5 ml of tap water. After soaking, the test tubes were put into the boil water for around 30 min. When the grains cooked properly test tubes were taken out from boiled water and water inside the test tubes removed. After that cooked grain were kept on a glass plate for few minutes to evaporate extra moisture and then measured the length and width of the cooked grain. Measurements were done through a digital slide calipers. Kernel elongation ratio means the proportionate change of rice grain after cooking. But different research group define it in different way. However, we followed the protocol described by Golam et al. (2004). Length and breadth of 10 kernels were measured before cooking and after cooking also length and width of these same 10 kernels were measured for calculation of actual elongation kernel ratio using the formula

Table 3. Variations of agronomic characters in 53 aromatic rice genotypes

		Mean square										
Source of variation	DF	HD	DM	GF	PH	NT	NFT	PL	GP	FGP	TGW	GYP
Replications	2	167**	172^{ns}	6.32^{ns}	62*	150^{ns}	46**	15*	839*	736*	3^{ns}	56.75**
Varieties	53	267^{ns}	275^{ns}	24.83*	502^{ns}	56^{ns}	42^{ns}	21^{ns}	2281^{ns}	1651^{ns}	48^{ns}	30.86^{ns}
Mean		83	110	26.99	87	20	14	24	123	100	23	3.38

*indicate significantly different at 5%, **significantly different at 1%, ns: not significant
(Indicators: HD= Heading days, DM= Days to maturity, GF= Grain filling period, PH= Plant height, NT= Number of tillers, NFT= Number of fertile tillers, PL= Panicle length, GP= Grain/panicle, FGP= Fertile grain/panicle, TGW= 1000 grain weight, GYP= Grain yield/plot)

Table 4. Mean comparison of four selected aromatic rice along with another 7 with the best Kernel elongation ratio and aroma performing including 2 local checks through Duncan Multiple Range Test (DMRT)

Genotypes	GYP (kg)	HD (days)	DM (days)	GF (days)	PH (cm)	NFT (number)	PL (cm)	FGP (number)	TGW (gm)
E36	3.80 a	79 f-o	108 e-o	30 a-d	72 o-q	20 a-c	21.37 g-n	117 a-g	22.67 e-i
Khau Dau Mali	3.56 ab	82 d-o	109 e-m	28 a-d	91 e-m	18 a-f	24.23 b-i	135 a	25.33 c-g
E26	3.40 a-c	80 e-o	107 g-o	27 a-d	89 f-n	19 a-e	26.77 b-d	113 a-g	24.67 c-h
E13	3.32 a-c	81 d-o	109 e-n	27 a-d	92 e-l	14 a-h	25.07 b-h	124 a-c	26.67 b-f
E15	3.24 a-d	75 j-o	105 j-p	30 a-d	85 h-o	18 a-f	19.30 k-n	98 a-g	22.00 e-i
E14	3.16 a-d	77 h-o	107 g-o	30 a-d	83 h-o	19 a-d	25.53 b-g	104 a-g	24.67 c-h
E11	2.92 a-e	76 i-o	106 i-p	30 a-d	80 j-q	17 a-g	23.73 d-l	105 a-g	24.67 c-h
E38	2.91 a-f	88 b-h	116 c-h	29 a-d	78 j-q	16 a-g	26.03 b-f	133 ab	22.00 e-i
E3	2.90 a-f	79 f-o	105 j-p	26 a-d	96 c-i	15 a-h	20.73 h-n	65 g-i	31.33 ab
E5	2.84 a-f	78 g-o	107 h-p	29 a-d	103 a-e	12 b-h	22.59 d-n	127 a-c	28.00 a-d
E37	2.82 a-f	88 b-h	114 e-j	26 a-d	73 o-q	14 a-h	22.27 d-n	119 a-e	22.00 e-i
MRQ 50	1.94 a-h	94 bc	117 c-g	23 b-d	76 m-q	10 c-h	24.83 b-h	126 a-c	15.33 kl
Q72	1.38 d-h	87 b-k	108 f-o	21 d	75 n-q	11 b-h	24.23 b-i	80 b-h	18.33 i-k
Range	1.38-3.8	75-94	105-117	21-30	72-103	10-20	19.30-26.77	65-135	15.33-31.33
R^2	0.49	0.79	0.84	0.37	0.82	0.49	0.68	0.55	0.78
CV	3.62	7.26	4.77	16.95	8.65	34.32	9.54	26.26	11.55
F-value	10.02	1.19	8.88	3.72	1.88	2.49	2.41	1.79	7.36

Mean followed by the same latter in a column are not significantly different from each other at 0.05 % probability level. Indicators: HD= Heading days, DM= Days to maturity, GF= Grain filling period, GYP= Grain yield/plot, PH= Plant height, NFT= Number of fertile tillers, PL= Panicle length, FGP= Fertile grain/panicle, TGW= 1000 grain weight

formula described by Sood et al., (1983).

$$PC = \frac{Lf/Bf - Lo/Bo}{Lo/Bo}$$

where, L_f, B_f are mean length and width of the 10 kernel after cooking; L_O, B_O: mean length and breadth of the 10kernel before cooking.

Statistical analysis

Data were analyzed using SAS 9.2 (2008) software and Microsoft Excel. Analysis of variance was used to test the significance of variance sources, while DMRT test (p=0.05) employed to compare the differences among treatment means. The correlation coefficient analysis was conducted to find the relationship of different attributes.

Conclusion

In this investigation several basmati type rice genotypes were studied. None of them performed better in term of aroma score, kernel elongation and yield related traits. It might be due high temperature during their grain filling and ripening stage. All of the 4 selected top yield genotypes such as E36 and E13 had been developed at IRRI, Philippines in the tropical environment. Also, E26 developed in Senegal and Khau Dau Mali is a cultivar originated from Thailand. However, their aroma performance and kernel elongation were moderate. Correlation analysis revealed that three agronomic traits such as number of fertile tillers (0.69), grain per panicle (0.86) and fertile grain per panicle (0.65) have the positive contribution to grain yield. Penicle length, 1000-grain weight, grain filling period suppose to have positive correlation with yield, but in this investigation superior

Table 5. Correlation coefficients among the agronomic traits using genotypes means

	HD	DM	GF	PH	NT	NFT	PL	GP	FGP	TGW	GYP
HD	1.00										
DM	0.90**	1.00									
GF	-0.25nc	0.19nc	1.00								
PH	-0.01nc	-0.05nc	-0.10nc	1.00							
NT	0.15nc	0.17nc	0.03nc	0.04nc	1.00						
NFT	-0.24nc	-0.16nc	0.17nc	-0.09nc	0.34*	1.00					
PL	0.31*	0.18nc	-0.29nc	0.30nc	0.18nc	0.06nc	1.00				
GP	0.32*	0.32nc	-0.02nc	-0.03nc	0.07nc	0.13nc	0.38*	1.00			
FGP	0.22nc	0.21nc	-0.03nc	0.03nc	0.12nc	0.18nc	0.37*	0.86**	1.00		
TGW	-0.22nc	-0.20nc	0.05nc	0.04nc	-0.22nc	-0.08nc	-0.12nc	-0.15nc	-0.04nc	1.00	
GYP	-0.15nc	-0.12nc	0.06nc	-0.07nc	0.14nc	0.69*	0.16nc	0.36*	0.45*	0.22nc	1.00

*=positive correlation, **=highly positive correlation, nc= no correlation

Indicators: HD= Heading days, DM= Days to maturity, GF= Grain filling period, PH= Plant height, NT= Number of tillers, NFT= Number of fertile tillers, PL= Panicle length, GP= Grain/panicle, FGP= Fertile grain/panicle, TGW= 1000 grain weight, GYP= Grain yield/plot

genotypes with these traits did not show reliable number of tillers, appeared with slack panicle and sterility. So they did not show any positive correlation with. In term of yield amount, E36, Khau Dau Mali, E26 and E13 were identified as outstanding genotypes. The gathered information can be useful for rice improvement research and the selected rice genotypes can be used as a potential breeding materials in the future rice research in Malaysia as well as other tropical countries.

Acknowledgements

Authors are thankful to the International Rice Research Institute (IRRI) and Malaysian Agricultural Research Development Institute for providing rice genotypes. The research was conducted with the financial support (UMRG grant No. RG 033 / 10 BIO) of the University of Malaya, 50603, Kuala Lumpur, Malaysia.

References

Bai NR, Devika R, Regina A, Joseph Ca (1992) Correlation of yield and yield components in medium duration rice cultivars. Environ Eco. 10(2):469-470.

Bemer DK, Hoff BJ (1986) Inheritance of scent in American long grain rice. Food Sci & Tech 26:876-878.

Dela Cruz N, Khush GS (2000) Rice grain quality evaluation procedures. In: Aromatic rices. Singh RK, Singh US, Khush GS (Eds). Oxford & IBH Publishing Co. Pvt. Ltd., New Delhi, India. pp 16-28

Faruq Golam, NorZulaani K, Jennifer AH, Subha B, Zulqarnain M, Nazia AM, Mohammad O (2010) Evaluation of kernel elongation ratio and aroma association in global popular aromatic rice cultivars in tropical environment. Afr J Agric Res. 5 (12):1515 – 1522.

Golam Faruq, Mohamad O, Hadjim K, Craig AM (2004) Inheritance of kernel elongation in rice. Int J Agri Biol. 1560 8530 /2004 /06 5: 813 815.

Halil S, Necmi B (2005) Selection for grain yield and its components in early generations in rice (*Oryza Sativa* L.). Trakya Univ J Sci. 6(1), 51-58

Huang J, David CC, Duff B (1991) Rice in Asia: Is it becoming an inferior good? Comment. American J Agric Econ. 73: 515- 521

Ifftikhar J, Khalil H, Abdul B, Sajid K, Islam Z (2009) Genetic variation for yield and yield components in rice. ARPN Journal of Agricultural and Biological Science 4 (6):60-64.

Kim L, Kimmel AR (2000) GSK3, a master switch regulating cell fate specification and tumorigenesis. Curr Opin Genet Dev. 10, 508–514.

Kole PC, Hasib KM (2008) Correlation and regression analysis in scented rice. Madras Agri J. 95(1-6): 178-182

Meenakshi T, Ratinam AAD, Backiyarani S (1999) Correlation and path analysis of yield and some physiological characters in rain fed rice. Oryza, 6: 154-156.

Prasad B, Patwari AK, Biswas PS (2001) Genetic variability and selection criteria in fine grain rice. Pak J of Biol Sci. 4 (10), 1188-1199

SAS 9.2 (2008) SAS Institute Inc. 100 SAS Campus Drive, Cary, NC 27513-2414, USA

Sood GB, Siddiq EA, Zaman UF (1983) Genetic analysis of kernel elongation in rice. Ind J Genet Plant Breed. 43: 40-43.

Singh RK, Singh US, Khush GS, Rashmi R (2000) Genetics and biotechnology of quality traits in aromatic rices. In Aromatic Rices. New Delhi: Oxford & IBH Publishing Co. Pvt. Ltd., p 47-71.

Swaminathan MS (1999). Rice in 2000 AD. In Abrol and Sulochana Gadgil (Eds.), Rice in a variable climate (pp. 217-238). APC Publications Pvt. Ltd., Post Box No. 2679, New Delhi-110005, India

Tahir M, Wandan D, Zada A (2002) Genetic variability of different plant yield characters in rice. Journal Title? 18(2)

Wang F, Cheng F, Zhang G (2007) Difference in grain yield and quality among tillers in rice genotypes differing in tillering capacity. Rice Science 14(2),135-140

Weiya Xue, Yongzhong Xing, Xiaoyu Weng, Yu Zhao, Weijiang Tang, Lei Wang, Hongju Zhou (2008). Natural variation in Ghd7 is an important regulator of heading date and yield potential in rice, Journal title??(40), 761 – 767

Zia-ul-qamar A, Cheema A, Ashraf M, Gulam Rasul Tahir GR (2005) Association analysis of some yield influencing traits in aromatic and non-aromatic. Pak J Bot 37(3): 613-627, 2005.

AROMA ANALYSIS IN FEW RICE GENOTYPES

*Yeap Hui Yin, Jennifer Ann Harikrishna, Golam Faruq**
Centre for Research in Biotechnology for Agriculture (CEBAR) and Genetics and Molecular Biology, Institute of Biological Science, Faculty of Science, University of Malaya.
**Author for Correspondence (e-mail: faruq@um.edu.my)*

Abstract

An investigation of aroma in different rice genotypes was carried out at University of Malaya. A total of 19 genotypes including three local checks (MR 219, MRQ 72 and MRQ 50) were used for aroma analysis. Five of the lines are globally popular aromatic rice cultivars namely, Sadri, Gharib, Kasturi, Rambir Basmati, Rato Basmati and these were studied together with 11 advanced homozygous lines from International Rice Research Institute (IRRI), Philippines. Sensory evaluation and molecular screening were carried out to determine aroma in rice. In sensory test, aroma was observed in 10 rice genotypes and they are E11, Sadri, Gharib, E7, Kasturi, Rambir Basmati, E13, Rato Basmati, MRQ 50 and MRQ 72. Of these three (E 11, Sadri and Gharib) have strong aroma (score 4). In allele specific amplification, E11 and Gharib which scored 4 for aroma were heterozygous for the fragrance gene and Sadri which also scored 4, was identified as homozygous for fragrance gene. Genotypes E13, Rato Basmati, E7, Rambir Basmati and two local checks MRQ 50 and Q72 which scored 3, were identified as homozygous for fragrance gene, but Kasturi with aroma score of 3 in sensory test was found as homozygous non-fragrant. Nine genotypes (including Popular Malaysian Cultivar MR219) showed scoring of 1 and were found as homozygous non-fragrant.

Introduction

The demand for aromatic rice has been increasing in recent years in local and international markets to such an extent that consumers are willing to pay a premium for aromatic rice. Because of this market opportunity, rice breeders have an interest in developing a simple and inexpensive method for distinguishing aromatic from non-aromatic rice. Buttery *et al.*, 1983; Lorieux *et al.*, 1996; Widjaja et al., 1996; Yoshihashi et al., 2002 have confirmed aroma in rice is associated mainly with the presence of 2-acetyle-1-pyrroline (2AP) which is most closely to the aroma of Basmati and Jasmine types of rice. Many other compounds are also found that cause aroma in aromatic rice cultivars (Widjaja et al., 1996). Methods for smelling leaf tissue, grains after heating in water, and reacting with solutions of 1.7% KOH are available (Sood and Siddiq 1978). Identification of 2AP using gas chromatography (Lorieux et al., 1996; Widjaja et al., 1996) and molecular marker, such as single nucleotide polymorphism (SNP) and simple sequence repeat (SSR) that are genetically linked to aroma (Jin et al., 2003;Cordeiro et al. 2002) have been developed for the selection of aromatic rice. The availability of rice genome sequences (Goff et al., 2002) provided an opportunity to discover the gene responsible by comparison of the sequences of fragrant and non-fragrant genotypes. More recently, an eight base pair-deletion and three SNPs in exon 7 of the gene encoding betaine aldehyde dehydrogenase 2 (BAD2) on chromosome 8 of rice were identified as the probable cause of aroma enzyme in aromatic rice (Bradbury et al., 2005b). In the present investigation it was tried to identify the status of aroma potentiality of 19 rice genotypes in Malaysian tropical environment.

Materials and Methods

Plant Materials
Five globally popular aromatic rice cultivars (Sadri, Gharib, Kasturi, Rambir Basmati and Rato Basmati) and 11 advanced homozygous lines were supplied by International Rice Research Institute, Philippines. The local checks (MR 219, MRQ 72 and MRQ 50) were provided by Malaysian Agricultural Research and Development Institute (MARDI).

Development of Plant materials
All of these 19 genotypes were raised at experimental field of Institute of Biological Science, Faculty of Science University of Malaya in a Complete Randomized Design (CRD) with three replications. The planting date was on 14th October 2009. Around 50 randomly chosen seeds from each genotype were planted in small pots with each contained 500g black soil and with labels. Water level was 1-2cm ensured all time during the whole crop season.

Aroma Evaluation (Sensory test)
0.2 g of leaf samples and 40 grains was taken from each genotype. A partial modified method of Sood and Siddiq (1978) was used for leaf aroma assessment and again their described method in 1983 was used for grain aromatic test. The contents in each petri-plates was smelt and were scored on 1-4 scale with 1,2,3 and 4 corresponding to absence of aroma, light aroma, moderate aroma, and strong aroma respectively. Four panels, undergraduates from Division of Genetics and Molecular Biology, Institute of Biological Science, Faculty of Science University of Malaya were invited to score the aroma in each genotype, respectively for leaf and grain.

DNA extraction, PCR and genotyping
Total genomic DNA from young leaves was extracted using CTAB method. Primers were designed based on Bradbury et al., 2005b and synthesised by Medigene. PCR was performed using 2.0 µl of 10X reaction buffer (with 20 mM Mg^{+}), 0.2 µl of 10 mM dNTPs mix, 0.25 µl of YEAtaq DNA Polymerase, 4.0 µl of DNA template, 0.4 µl of each primer (ESP, EAP, INSP and IFAP), in a total volume of 20 µl. Amplification was carried out using a BioRad, C1000 Thermal Cycler. Cycling conditions were 5 min at 94 ℃, followed by 35 cycles of 30 s at 94 ℃, 30 s at 53 ℃, 1 min at 72 ℃, concluding with a final extension of 7 min at 72 ℃ and a hold at 4 ℃ until recovery. PCR products were analysed by electrophoresis in 1.0% agarose gels and followed by staining in ethidium bromine. A 100 bp ladder (Vivantis) was used to estimate PCR fragment size.

Results

Table 1: Relation between aroma score from leaf aromatic test and grain aromatic test with genotypic analysis for selected genotypes and local check.

Genotypes	Aroma Score		Mean Aroma Score	Genotypic Analysis
	Leaf	Grain		
Entry 11	4	4	4	H
Gharib	3	4	4	H
Sadri	4	4	4	F
Entry 13	3	2	3	F
Rato Basmati	3	2	3	F
Entry 7	3	3	3	F
MRQ 50	3	2	3	F
Q 72	3	2	3	F
Kasturi	3	3	3	N
Rambir Basmati	3	3	3	F
MR 219	1	1	1	N
Entry 15	1	1	1	N
Entry 14	1	1	1	N
Entry 38	1	1	1	N
Entry 37	1	1	1	N
Entry 16	1	1	1	N
Entry 20	1	1	1	N
Entry 18	1	1	1	N
Entry 19	1	1	1	N

(1= Absence of aroma; 2= Slightly aroma; 3= Moderate aroma; 4= Strong aroma; H= Heterozygous; F= Homozygous Fragrant; N= Homozygous Non-Fragrant)

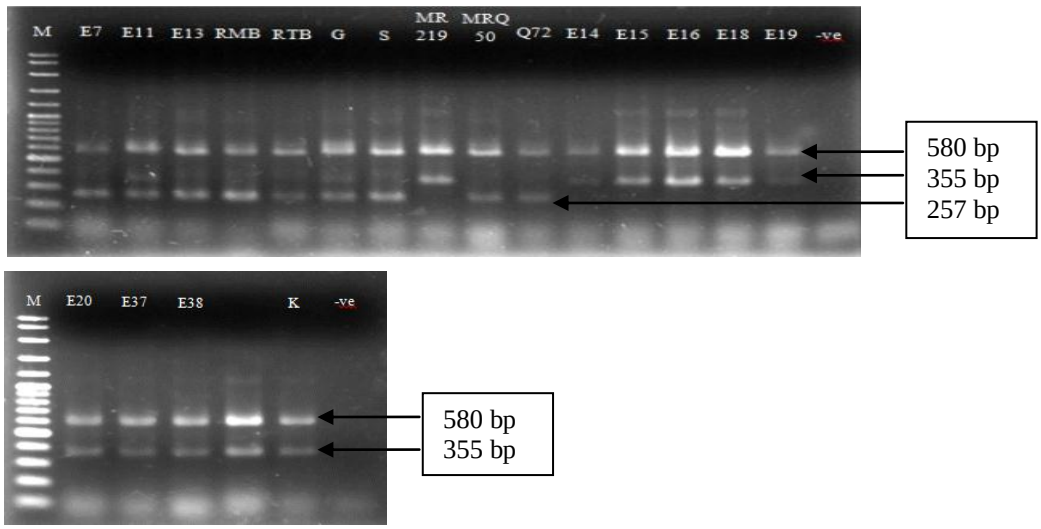


Figure 1: Two agarose gel showing individuals selected for fragrance analysed using single tube Allele Specific Amplification (ASA). The band of approximately 580 bp corresponds to the positive control amplified by both external primers (ESP and EAP). The 355 bp band corresponds to a PCR product amplified from the non-fragrant allele by Internal Non-fragrant Sence Primer (INSP) and External Antisence Primer (EAP). The 257 bp band corresponds to a PCR product amplified from the fragrant allele by the Internal Fragrant Antisence Primer (IFAP) and the External Sence Primer (ESP). A negative control (water) and Vivantis DNA ladder (100 bp) were used. Lane MR219, MRQ50 and Q72 are local checks.
(E: Entry; RMB: Rambir Basmati; RTB: Rato Basmati; G: Gharib; S: Sadri; K: Kasturi)

Discussion

Aroma was found in 8 genotypes out of 16 rice genotypes (excluding the local checks MR219, MRQ50, and Q72) through sensory test. Genotypes with the highest scoring of aroma were observed in E11, Sadri and Gharib. Moderate aroma was found in E7, E13, Kasturi, Rambir Basmati, and Rato Basmati (Table 1). According to Susamma et al. (2005), Kasturi and 'Basmati' type rice are known for their characteristic fragrance when cooked and present observations also with the line of their research findings. All 'Basmati' type genotypes were fall under scented category and Kasturi under lightly scented (Table 1). Local check MR219 did not perform aroma, whereas MRQ50 and Q72 were observed with moderate aroma.

These 19 genotypes including 3 local checks (MRQ50, MRQ72 and MR219) were chosen for further genotypic analysis for their aroma gene by using allele specific amplification (ASA) and according to Bradbury et al. (2005b) it is possible to discriminate between fragrant and non-fragrant rice varieties and identify homozygous fragrant, homozygous non-fragrant and heterozygous non-fragrant genotypes by using this method. This is due to fragrance in rice has an eight base pair deletion and three SNPs in exon 7 of the gene encoding betaine aldehyde dehydrogenase 2 (BAD2) on chromosome 8. Non-fragrant rice varieties possess what appears to be a fully functional copy of the gene encoding BAD2 while fragrant varieties possess a copy of the gene encoding BAD2 which contains the deletion and SNPs, resulting in a frame shift that generates premature stop codon that presumably disables the BAD2 enzyme. This polymorphism provides an opportunity for the construction of a perfect marker for fragrance in rice (Bradbury et al., 2005a). The PCR product of approximately 580 bp was presence in all fragrant and non-fragrant genotypes and serves as a positive control. The presence of a second PCR product of 355 bp identified homozygous non-fragrant individual. While, if the second PCR product is 257 bp, the individual is identified as homozygous fragrant. An individual gives all three PCR products, it can be discriminated as heterozygous. We observed that E11 and Gharib which scored 4 for aroma were heterozygous for the fragrance gene. Genotype Sadri which also scored 4, was identified as homozygous for fragrance gene. These three genotypes obtained the highest scoring for aroma in phenotypic analysis. Genotypes E13, Rato Basmati, E7, Rambir Basmati and two local checks MRQ 50 and Q72 which scored 3, were identified as homozygous for fragrance gene. Surprisingly Kasturi with aroma score of 3 in sensory test was found as homozygous non-fragrant (Figure 1). According to Bounphanousay et al., (2008), they observed the same incident in genotype named Kai Noi Leuang, a popular Lao local aromatic rice variety with

the highest 2-AP concentration did not possess a 257 bp fragment. Khush and De La Cruz (1998) mentioned that environmental factors are well known to affect the expression of aroma, such as temperature during the late reproductive stages of crop growth, soil properties, agricultural factors grain storage and processing. Further investigations are required to elucidate this question. Nine genotypes (including Popular Malaysian Cultivar MR219) showed scoring of 1 and were found as homozygous non-fragrant (Figure 1).

Acknowledgments

The authors are thankful to the International Rice Research Institute (IRRI) and Malaysian Agricultural Research Development Institute for the supply of rice genotypes. The research was conducted with the financial support (UMRG grant No. RG 033 / 10 BIO) of the University of Malaya, 50603 Kuala Lumpur, Malaysia.

References:

Bounphanousay, C.,Jaisil, P., Sanitchon, J., Fitzgerald, M. & Sackville Hamilton, N.R., 2008. Chemical and Molecular Characterization of Fragrance in Black Glutinous Rice from Lao PDR. Asian J. of Plant Sci., **7(1):** 1-7.

Bradbury, L.M.T., Fitzgerald, T.L., Henry, R.J., Jin, Q., Waters, D.L.E. (2005a). The gene for fragrance in rice. Plant Biotechnology Journal. **3:** 363-370.

Bradbury, L.M.T., Henry, R.J., Jin, Q., Reinke, R.F., Waters, D.L.E., 2005b. A perfect marker for fragrance genotyping in rice. Mol. Breed. **16**, 279–283.

Buttery, R.G., Juliano, B.O., and Ling, L.C. 1983. Identification of rice aroma compound 2-acetyl-1-pyrroline in leaves. *Chem. Ind. (London)* **23:** 478-479.

Cordeiro, G.M., Christopher, M.J., Henry, R.J. and Reinke, R.F., 2002. Identification of microsatellite markers for fragrance in rice by analysis of the rice genome sequence. *Mol. Breed.* **9:** 245-250

Goff, S.A. et al., 2002. A draft sequence of the rice genome (*Oryza sativa L. ssp japonica*). *Science*, **296:** 92-100

Jin, Q.S., Waters, D., Cordeiro, G.M., Henry, R.J. and Reinke, R.F., 2003. A single nucleotide polymorphism (SNP) marker linked to the fragrance gene in rice. *Plant Sci.* **165:** 359-364

Khush, G.S. and N. Dela Cruz, 1998. Developing Basmati Quality Rices with High Yield Potential. In: Rice Quality: A Pluridisciplinary Approach. Chataigner (Ed.). Proceeding of the International Symposium held in Nottingham, UK, 24-27 Nov., 1997, Montpellier, France, pp: 11-23.

Lorieux M, Petrov M, Huang N, Guiderdoni E, Ghesquiere A., 1996. Aroma in rice: genetic analysis of quantitative trait. *Theor Appl Genet*, **93:** 1145-1151

Sood, B. C. and E. A. Siddiq, 1978. A rapid technique for scent determination in rice. *Indian J. Genet. Plant Breed.* **38**: 268-271.

Sood, G.B., E.A. Siddiq and U.F. Zaman, 1983 Genetic analysis of kernel elongation in rice *Indian J. Genet. Plant Breed*., **43:** 40–3

Susamma P. George, Dijee Bastian, N.V. Radhakrishan and K.C. Aipe, 2005. Evaluation of aromatic rice varieties in Wayanad, Kerala. *Journal of Tropical Agriculture*, **43 (1-2):** 67-69.

Widjaja R., Craske J.D., Wootton M., 1996. Comparative studies on volatile components of non-fragrant and fragrant rices. *J. Sci Food Agric* **70:** 151-161

Yoshihashi, T., N.T.T. Huong and Kabaki, N. (2002). Quality evaluation of Khao Dawk Mali 105, an aromatic rice variety of Northeast Thailand. JIRCAS Working Report No. 30