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Evaluation of kernel elongation ratio and aroma association in global popular aromatic rice cultivars in tropical environment

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Aroma and cooked kernel elongation are the most important quality traits of aromatic rice, which differentiate the highly valued aromatic rice from the other rice types. Previous studies on genetic analysis have shown that genes/ QTLs for these two traits are linked. In the present study, it tried to evaluate the expression of aroma, kernel elongation and their association in 55 fine rice genotypes in the tropical environment of Malaysia. Highest percentage of elongation ratio was observed in Genotype E2 followed by E11, Gharib, E6, E26, E34, E35, E36, E19, E20 and E27. Aroma was observed in 34 rice genotypes and 10 were identified as superior. They are E11, Sadri, Gharib, E7, Kasturi, Rambir Basmati, E21, E13, E24, and Rato Basmati. Positive correlation ($r = 0.59$, $p \leq 0.05$) was observed between aroma and kernel elongation in these selected 10 genotypes. Three of them had strong aroma (score 4) and there genotypes were E11, Sadri and Garib. We observed that the outstanding 10 genotypes for aroma and highest kernel elongation ratio are not the same except for two of the genotypes (Garib and E11). Aroma concentration was significantly different in highest kernel elongation ratio performance of 10 genotypes. Similar results were also observed in top 10 aroma performing genotypes and their kernel elongation ratio also varied among each other. In addition, out of 55 aromatic genotypes 17 did not have any aroma; comparatively low kernel elongation ratio was also observed in many of the genotypes. This investigation indicated that association of aroma and kernel elongation ratio can be highly influenced by tropical environment. However, since two genotypes (Garib and E11) perform their normal aromatic and Kernel elongation ratio and aromatic expression are 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.

Key words: Aromatic rice, kernel elongation ratio, aroma, kernel elongation ratio and aroma association, aromatic rice cultivars /varieties / advance lines tropical environment.

INTRODUCTION

Rice is the unique grain that is nearly entirely used as human food, unlike other cereals which are also used extensively used as feed (Swaminathan, 1999). Rice is the major food of most of the Asian countries and aromatic rice varieties are playing a vital role in trading (Huang

et al., 1991). So, attention should be focused toward improving aromatic rice in its cooking quality as well as its several biochemical and morphological characteristics. Development of aroma in rice is influenced by both genetic and environmental factors. Aroma in rice is detected through sensory test and genotypic analysis by using microsatellite markers. Tanchotikul and Hsieh (1991) observed that the biochemical basis of aroma was identified as 2-acetyl-1- pyroline (2AP). Bradbury et al. (2005a) successfully identified this aromatic gene. Our

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previous studies showed that the inheritance pattern of rice kernel elongation is controlled by a single gene and are influenced by some modifier genes (Faruq et al., 2004).

A group of researchers have developed marker for kernel elongation but the specific gene for kernel elongation could still not be found. Bao et al. (2001) used genetic models to analyze the cooked rice elongation traits of indica rice in two environments and found that the rice grain appearance quality was mainly controlled by genotype, major gene effects and environment interactions.

While describing these two traits, Dela Cruz et al. (1989) also reported environment factors, especially the temperature during ripening. The temperature of 25°C at day time and 21°C at night during the ripening stage has been found to have favorable effect in aroma as well as kernel elongation where the maximum elongation in matured grains was reported at this temperature. Sood et al. (1983) reported the involvement of both non-additive and additive types of gene affects with the former playing a predominant role.

Aroma and cooked kernel elongation ratio are the most important quality traits, which differentiate the highly valued aromatic rice from the other rice types. Previous studies on genetic analysis have shown that genes/ QTLs of these two traits are linked and present on chromosome number 8 (Neelu et al., 2005). The objective of the study was to evaluate aroma and kernel elongation and express their association in 54 different fine rice (*Oryza sativa L.*) genotypes in tropical environment

MATERIALS AND METHODS

Materials

A total of 55 rice genotypes including four local checks (MR 219, MRQ 50, Q70, and Q72) were used for this investigation. Twelve of them are global popular aromatic rice cultivar namely; Ratani Pagal, Katari Bhog, Khau Dau Mali, Gharib, Sadri, Chini Gura, Kasturi, Paheale, Tamahonami, Rambir Basmati, Rato Basmati, Rato basmati and 39 advance homozygous lines. Twelve global popular aromatic rice cultivars and 39 advance lines were received from International Rice Research Institute (IRRI) and 4 local checks were collected from Malaysia Agricultural Research and Development Institute (MARDI).

Methods

Experiment site and management practice

A multi dimensional experiment was laid out in small plots (1 x 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. The planting date was on 14th October 2009. Recommended rice production practices from Malaysian Agricultural Research Institute were followed. For the present investigation, samples were collected from this experimental field.

Sample preparation

30 g of sample from each genotype (moisture content 13%), were dehusked in a Satake Testing Rice Husker (THU-34A, Satake Co. Ltd. Tokyo, Japan). The brown rice obtained was polished in a single pass rice pearlier (BS08A Satake Co. Ltd. Tokyo, Japan). Only normal shaped rice kernels (unbroken) were used for kernel elongation ratio and aroma evaluation.

Preparing sample for kernel elongation

The average lengths of five milled rice were measured. Then, the individual milled kernels were put separately in a long labeled test tube. After that, they were soaked in tap water for 25 min. Then, each sample was put inside the water bath for 15 min while maintaining the temperature at 110°C. This method is a modification (time and temperature) of Azeez and Shafi's (1966) protocol. After that, the cooked rice was transferred to a Petri dish lined with filter paper.

Determination of kernel elongation ratio

The length of the cooked grain was measured and expressed as the ratio of cooked grain versus uncooked grain (Sidhu et al., 1975).

Leaf aromatic test (Sensory test)

During the flowering stage, 0.3g of leaf samples was taken from each genotype. Leaves were cut into tiny pieces and put into glass Petri-plates. 15 ml of 1.9% (1000 ml dH₂O+ 1.7 g KOH) 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 min 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 made up of five members each from Genetics and Molecular Biology Division, Institute of Biological Science, Faculty of Science University of Malaya were invited to score the aroma in each genotype. This is a modified protocol of Sood and Siddiq (1978), where amount of leaf and concentration of KOH had been changed.

Grain aromatic test (Sensory test)

Sixty grains of each genotype were soaked in 15 ml of 1.7% KOH solution at room temperature in a covered glass Petri-plate for about 1 h. 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. Again, four panels made up of five members each from Genetics and Molecular Biology Division, Institute of Biological Science, Faculty of Science University of Malaya were invited to score the aroma in each genotype. This protocol was followed based on the conception of Sood et al. (1983), where again the amount of grain and concentration of KOH had been changed.

Statistical analysis

Data were analysed with SAS Version 9.2 (Statistical Analysis System) program to get analysis of variance (ANOVA) and mean differences adjudged with Duncan's Multiple Range Test (DMRT). Correlation between aroma and kernel elongation was done to

Table 1. Description of 10 outstanding aroma performing rice genotypes.

Genotypes	Designation	Cross	Origin	Source
Entry 11	IR 78554-145-1-3-2	IR 72861-13-2-1-2/IR-68450-36-3-2-2-3	Irri	Irri
Sadri	Cultivar	unknown	Iran	Irri
Gharib	Cultivar	unknown	India/Pakistan	Irri
Entry 7	IR 7773493-2-3-2	NSIC RC 148/PSB RC 18//NSIC RC 148	Irri	Irri
Kasturi		unknown	India / Pakistan	Irri
Rambir Basmati		unknown	India	Irri
Entry 21	WAB515-B-10 A 1-4	unknown	Warda	Irri
Entry 13	IR77512-2-1-2-2	IR 68726-3-3-1-2/IR 71 730-51-2	Irri	Irri
Entry 24	WAS 197-B-4-1-22	IR 31851-96-2-3-2-1/ IR 66231-37-1-2	Senegal	Irri
Rato Basmati	Cultivar	unknown	India / Pakistan	Irri
MRQ50	Variety	unknown	Malaysia	Mardi
Q72	Variety	unknown	Malaysia	Mardi

Table 2. Description of 10 outstanding kernel elongation performing rice genotypes.

Genotypes	Designation	Cross	Origin	Source
E2	CT9882-16-4-2-3-2P-M	unknown	Ciat	Irri
Entry 11	IR 78554-145-1-3-2	IR 72861-13-2-1-2/IR-68450-36-3-2-3	Irri	Irri
Gharib	Cultivar	unknown	India/Pakistan	Irri
E6	IR 74	IR 19661-131-1-2/IR 15795-199-3-3	Irri	Irri
E26	WAS 197-B-5-2-16	IR 31851-96-2-3-2-1/ IR 66231-37-1-2	Senegal	Irri
E34	IR 50	IR 2153-14-1-6-2/IR 2061-214-3-82//IR 2071-6251-252	Irri	Irri
E35	IR 64	IR 5657-33-2-1/IR 2061-465-1-5-5	Irri	Irri
E36	IR 72	IR 19661-9-2-3/IR 15795-199-3-3//IR 9129-209-2-2-2-1	Irri	Irri
E19	WAS 197-B-4-1-22	IR 31851-96-2-3-2-1/ IR 66231-37-1-2	Senegal	Irri
E20	WAB337B-B-15-H1	ITA 135/WABC 165	Warda	Irri
E27	WAS 197-B-5-2-5	IR 31851-96-2-3-2-1/ IR 66231-37-1-2	Senegal	Irri
MRQ50	Variety	unknown	Malaysia	Mardi

observe the relationship between them.

RESULTS AND DISCUSSION

Aroma analysis in 55 rice genotypes

Details of the selected genotypes for aroma are described in Table 1 and kernel elongation ratio is also described in Table 2. Out of 55 rice genotypes (excluding the local checks MR219, MRQ50, Q70 and Q72), aroma was found in 35 genotypes. Of the 35 aromatic rice genotypes, 24 aromatic rice genotypes were of the advanced homozygous lines and 11 of them from the global popular aromatic rice cultivars. Three of these aromatic genotypes (E11 and Sadri and Gharib) performed strong aroma, seven (E7, E13, E21, E24, Kasturi, Rambir Basmati, and Rato Basmati) of them with moderate aroma (Figures 1 and 2) and 24 performed slight aroma

with scoring of 2. Susamma et al. (2005) mentioned that 'Caster' and 'Basmati' type rice are known for their aroma when cooked. Slight aroma was found in genotypes E6, Chini Gura and the rest. Das and Baqui (2000) reported that Chini Gura, Ratani Pagal and Katari Bhog are local aromatic rice in Bangladesh, while Parichat et al. (2005) reported that Khau Dau Mali is a Thai aromatic rice genotype. This result was similar to what was found during their sensory tests, but the results show them to be slightly aromatic. This is assumed because of high temperature during ripening stage (Day-night average $\geq 28^{\circ}\text{C}$). Local checks on MRQ50 and Q72 came with moderate aroma. This was similar to the result reported by Phang et al. (2004).

Comparisons of aroma through leaf aromatic test

We observed aroma in all of the global popular aromatic

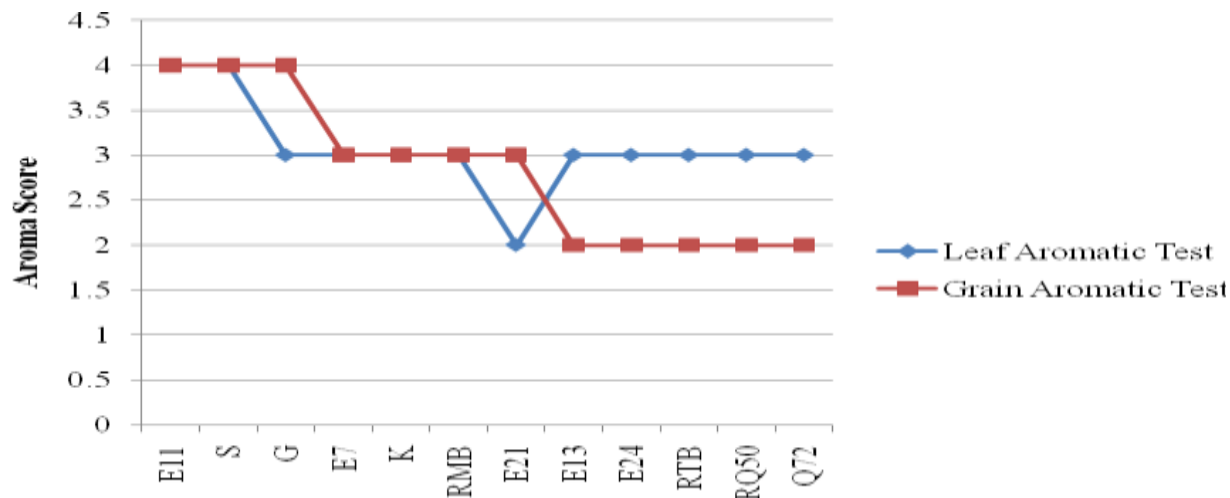


Figure 1. Comparison of aromatic performance of selected ten aromatic rice genotypes through two different methods with local check.

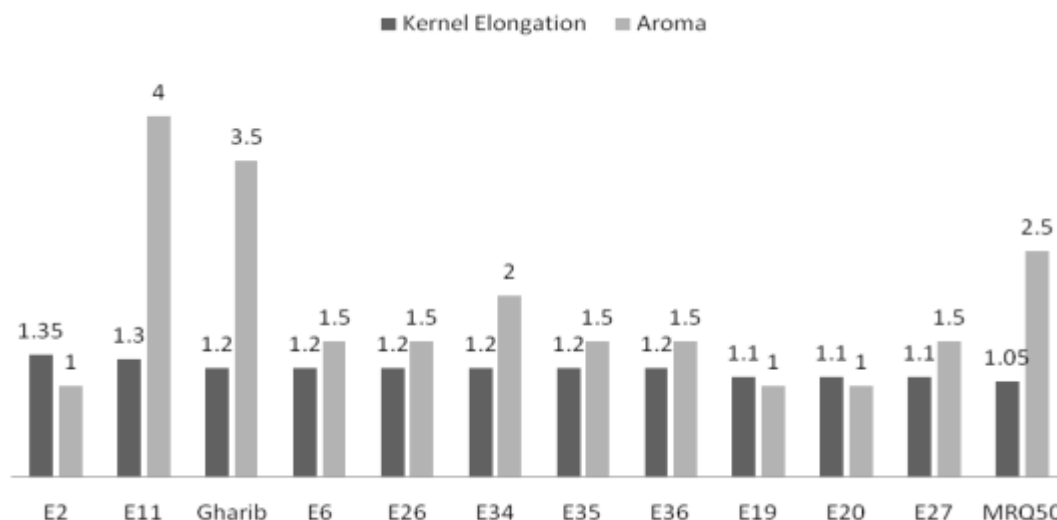


Figure 2. Top Kernel elongation ratio performing 10 genotypes showing different level of aroma score (Mean score of leaf and grain test have been used).

rice cultivars. Out of 35 aromatic rice genotypes, E11 (score 4) and Sadri (score 4) had strong aroma. Moderate aroma (score 3) was observed in 12 genotypes and they are Gharib, E7, Kasturi, Rambir Basmati, E21, E13, E24, Rato Basmati, Ratani Pagal, E22, E17 and Katari Bhog. Slight aroma was observed in the remaining 20 genotypes.

Comparisons of aroma through grain aromatic test

Only 18 rice genotypes performed aroma (excluding local check checks) from 50 genotypes in grain aromatic test. Eight genotypes (global popular aromatic rice cultivars)

performed aroma except Katari Bhog, Ratani Pagal and Chini Gura (all are from Bangladesh). This indicated severe environmental sensitivity especially high temperature of these three genotypes, because these three genotypes are grown in Rabi season (day night average 18 - 20°C) in Bangladesh. Aging time also may be another factor affecting the results. We conducted the test immediately after harvesting. Ten genotypes were found aromatic in 39 advance homozygous lines (E7, E9, E11, E13, E21, E24, E28, E32, E33, E34). However Chini Gura, Ratani Pagal and Katari Bhog also scored very low in leaf aroma test. Genotypes with strong aroma were found in 3 aromatic rice genotypes namely, E11, Sadri and Gharib. Moderate aroma was observed in the grains

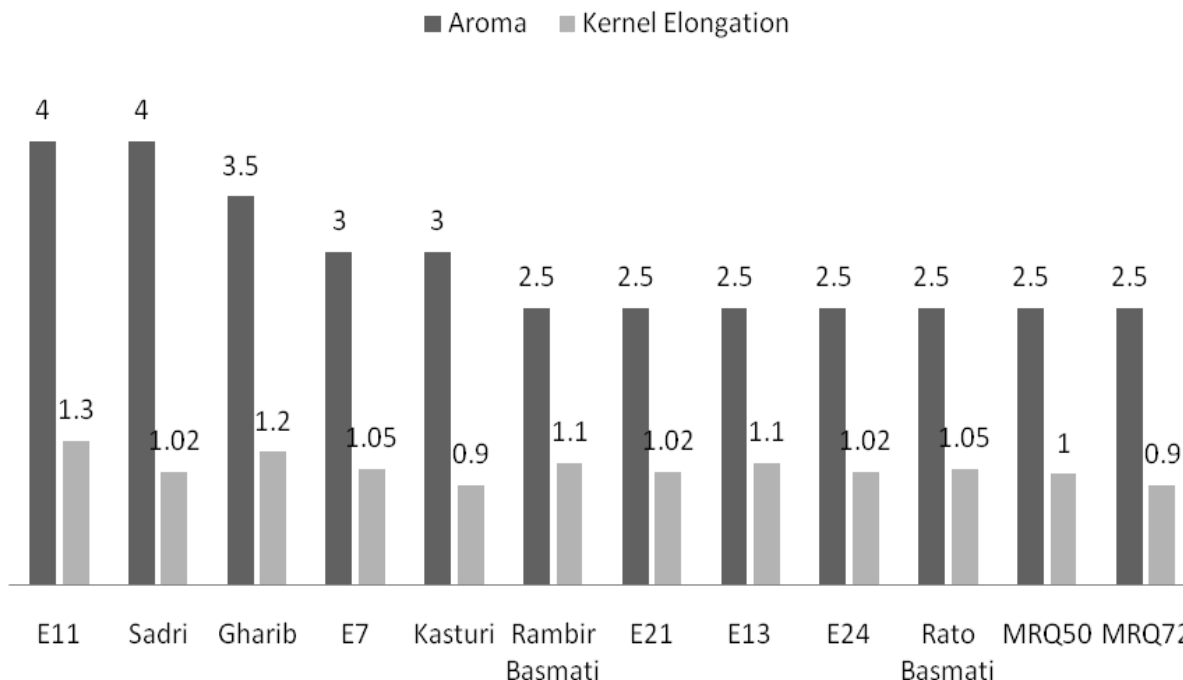


Figure 3. Top aroma performing 10 genotypes with Kernel elongation ratio (For aroma mean score of leaf and grain test have been used).

of genotypes E7, Kasturi, Rambir Basmati, and E21. Genotypes E13, Rato Basmati and others performed slight aroma.

Comparison of aromatic performance of ten selected aromatic rice genotypes through two different methods with local checks

From two sensory tests for aroma we observed variation in identifying aromatic genotypes. Though leaf test was observed as sensitive in identifying aromatic genotypes (35 genotypes) we cannot say that this test is more efficient for aroma. Our main concern is on grain and in our observation the grain test was more applicable (identified only 18 genotypes). However, during identification of the association of aroma and kernel elongation, the mean scores of these two tests were used.

Kernel elongation ratio analysis in 55 rice genotypes

Comparison of kernel elongation among the genotypes

Genotype E2 performed the highest percentage (1.35) of elongation ratio and it was significantly different (at $p \leq 5\%$ level) from other entries and local check Q70 performed significantly lower percentage (1.05) of elongation ratio.

Analysis of elongation ratio with 4 local check (MRQ50, MR219, Q70 and Q72)

Some rice show extreme elongation on cooking but most varieties have shown that the expansion is more breadth wise rather than lengthwise. Eleven genotypes (E2, E11, Gharib, E6, E26, E34, E35, E36, E19, E20, E27, Q70, Q72, MR219 and MRQ50) were identified as outstanding. Genotype E2 had the highest percentage of kernel elongation ratio, followed by E11 and Gharib. However, 4 local checks (MRQ50 MR219, Q70 and Q72) had lowest percentage of elongation ratio compared to 10 selected cultivars (Figure 3). Usually Basmati rice from India and Pakistan rice elongated 100% from its original length. The elongation of kernel is influenced by both genetic and environment factor especially temperature. Ambient temperature was reported to be at 25°C during day time and 21°C during night time. Compared to Basmati rice, observation shows that the Basmati rice cannot elongate properly while cooking. It may happen due to unsuitable temperature in Malaysia.

Aroma - kernel elongation ratio association analysis

In the present investigation, we selected 10 genotypes based on their aroma performance and another 10 genotypes based on their highest kernel elongation ratio. We found that only two genotypes (E11 and Garib) were

Table 3. Relation between aroma score from leaf aromatic test and grain aromatic test with kernel elongation ratio for selected genotypes and local check.

Genotypes	Aroma score		Kernel elongation ratio
	Leaf	Grain	
Entry 11	4	4	1.20
Sadri	4	4	1.02
Gharib	3	4	1.20
Entry 7	3	3	1.06
Kasturi	3	3	0.92
Rambir Basmati	3	3	1.06
Entry 21	2	3	1.03
Entry 13	3	2	1.06
Entry 24	3	2	1.02
Rato Basmati	3	2	0.90
MRQ50	3	2	1.00
Q72	3	2	0.97
Correlation coefficient between aroma and kernel elongation ratio of outstanding aromatic genotypes	0.31*	0.64**	0.59**

*P ≤ 0.05,

**P ≤ 0.01.

common in both selections. Genotype E11 and Garib performed excellent in aroma as well as in kernel elongation ratio. In the selected 10 rice genotypes, of which highest aroma was performed, their kernel elongation ratio varied significantly among each other. Similarly, in the selected 10 rice genotypes, of which the highest kernel elongation ratio was performed, aroma score differed significantly among the genotypes. In addition, within the total 55 aromatic genotypes 17 did not perform any aroma and comparatively low kernel elongation ratio were observed in many of the genotypes when compared to sub-tropical environment. Aroma score such as Rambir Basmati or Rato Basmati is always more than 4.0 in Indian sub-continent (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. Similar observations were observed in kernel elongation ratio. In subtropical environment kernel elongation ratio was always observed to be more than 2.0 in these two rice genotypes and in Malaysian tropical environment it was 1.1 for Rambir basmati and 1.05 for Rato basmati.

In the present investigation, we observed that the outstanding 10 genotypes for aroma and highest kernel elongation ratio are not the same except for two (Garib and E11). Aroma concentration was significantly different in highest kernel elongation ratio performing 10 genotypes. Similar results were also observed in top 10 aroma performing genotypes and their kernel elongation ratio varied significantly. In addition, of the 55 aromatic genotypes 17 did not have any aroma, comparatively low kernel elongation ratio in many of the genotypes was also observed when compared to sub-tropical environment.

This investigation indicated that association of aroma and kernel elongation ratio can be highly influenced by tropical environment and it severely affects the Bangladeshi indigenous aromatic rice cultivars. Interestingly two genotypes (Garib and E11) perform 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 of these two traits.

Correlation between aroma and kernel elongation ratio

Correlations between aroma and Kernel elongation ration were studied in 10 outstanding aromatic genotypes. Positive trend ($r = 0.31$) was observed between these two traits in leaf aromatic test, but highly positive correlation was observed in grain test ($r = 0.63$). The average value of aroma from the two tests also indicated strong positive correlation ($r = 0.59$) (Table 3).

Genotypic similarity in Aroma and Kernel Elongation Ratio

Only 2 genotypes (E11 and Garib) performed extremely well in aroma and kernel elongation ratio (Figure 4). Ahn et al. (2005) described the linkage between the two traits. Positive correlation between the two traits was observed among the top ten genotypes, but their strong association phenotypically was not expressed in the outstanding

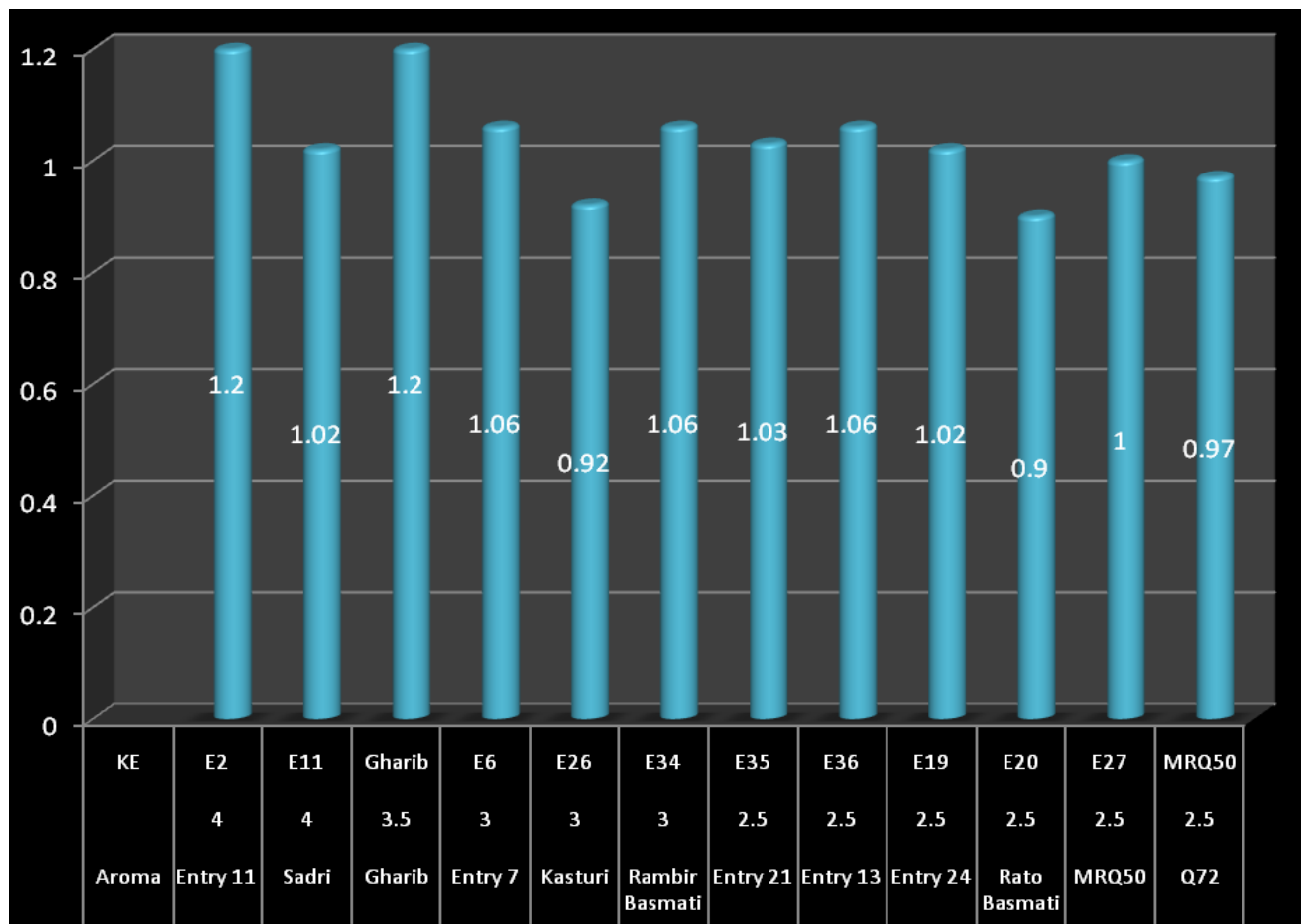


Figure 4. Genotypic similarity considering Aroma and Kernel elongation ratio. KE = Kernel elongation ratio, MRQ50 and Q72 are Malaysian local checks.

genotypes. So the association of these two traits seems to be complex phenotypically. It needs more integrated approach to get appropriate information of the association of aroma and kernel elongation ratio in aromatic rice.

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