

# Comparative Analyses for Aroma and Agronomic Traits of Native Rice Cultivars from Central Asia

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## Abstract

Aromatic rice has become popular owing to its aroma. Growing demand for aromatic rice has spurred interest in the development of domestic cultivars that offer similar combinations of grain attributes such as texture, cooking characteristics, aroma, and taste. In this study, the most important agronomic attributes and aroma of 26 cultivars from Afghanistan, Iran, and Uzbekistan, and controls from Japan, Thailand, and India were characterized. Also F<sub>2</sub> populations derived from the cross between (Jasmine 85 aromatic x Nipponbare non-aromatic) and (Jasmine 85 x Basmati 370 aromatic) were obtained. Tasting individual grains, cooking test, 1.7% KOH sensory test, and molecular marker analysis have been applied to distinguish between aromatic and non-aromatic rice. Diversity for some traits of agronomic importance, such as plant height was detected among countries, e.g. Afghan cultivars classified as tall, and Iranian and Uzbek intermediate and short, respectively. Differentiations of panicle, grain, leaf, basal internode, and culm dimension among rice cultivars, indicating the source of rice diversity in Central Asia. According to the results, 6 of 10, 2 of 7, and 0 of 6 of Afghan, Iranian, and Uzbek rice cultivars were scored as aromatic, respectively. Therefore, Afghan cultivars are a good source of aromatic rice germplasm for Central Asia. The expression between aromatic and non-aromatic, and aromatic and aromatic combinations has been evaluated. The observed segregation ratio of these crosses in the F<sub>2</sub> populations was tested by  $\chi^2$  analysis against the expected ratio for a single gene. A segregation ratio of 3:1 between non-aromatic and aromatic combination has been detected, while segregation has not been detected between the aromatic and aromatic combinations. Also, parallel results were obtained from the tested aromatic rice cultivars. Thus, our results suggest that a single recessive gene controls aroma in all aromatic rice cultivars.

Key words: 1.7% KOH test, Aroma, Agronomic trait, Genetic resources, PCR. Rice (*Oryza sativa* L.)

## Introduction

The demand for aromatic rice has been increasing in recent years in both traditional and non-traditional rice-growing countries. Rice breeders have an interest in developing a simple and inexpensive method for distinguishing aromatic from non-aromatic rice. The genetic diversity of modern rice cultivars has been reduced due to intensive breeding efforts (Cuevas-Perez et al. 1992; Dilday 1990). Genetic uniformity could become a

problem for the selection of germplasm to develop improved varieties. A more diverse germplasm would enhance the selection efficiency of desirable varieties in the rice breeding programs. Culm strength in rice depends on the physical properties of culm, i.e. stoutness and stiffness of culm, in addition to morphological traits, environmental factors, and meteorological conditions (Hien 2001). Aroma in rice is associated mainly with the presence of 2-acetyl-1-pyrroline. This compound is most closely associated with the aroma of Basmati and Jasmine types of rice (Buttery et al. 1983; Hien et al. 2006a; Lorieux et al. 1996; Widjaja et al. 1996; Yoshihashi et al. 2002). 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

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are available (Sood and Siddiq 1978). The identification of 2-acetylcysteine-1-pyrroline, using gas chromatography mass spectrometry selected ion monitoring (GC-MS-SIM) is also available (Hien et al. 2006b; Lorieux et al. 1996; Widjaja et al. 1996; Yoshihashi et al. 2004).

Molecular markers, such as single nucleotide polymorphism (SNP) and simple sequence repeats (SSR) that are genetically linked to aroma have been developed for the selection of aromatic rice (Cordeiro et al. 2002; Jin et al. 2003). The availability of rice genome sequences provided an opportunity to discover the gene responsible by comparing the sequences of aromatic and non-aromatic genotypes (Goff et al. 2002; IRGSP 2005).

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). The objectives of this research were to classify aromatic and non-aromatic rice, and to study agronomic traits of Afghan, Iranian, and Uzbek native rice cultivars, comparatively.

## Materials and methods

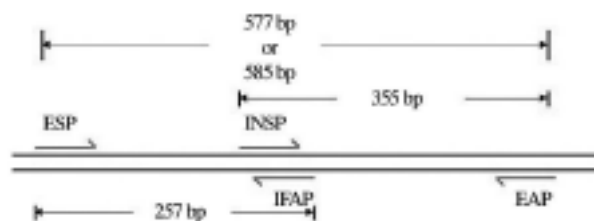
Ten Afghan native rice cultivars (Permela, Sherkati, Luke Qasan, Monda Mashruqi, Pashadi Konar, Sarda Bala, Sela Doshi, Sela Takhar, Germa Bala, and Lawangi), seven Iranian cultivars (Fajr, Poya, Shiroudi, Nemat, Kodus, Doroudzan, and Qaserdashti), and six Uzbek cultivars (Jaihon, Sanam, Shortamby, Gulnar, Nokos-2, and Nokos-70) were used as materials in this study. Basmati 370 (India) and Jasmine 85 (Thailand) were used as controls for aroma and Nipponbare (Japan) for non-aroma. A population of 200 F<sub>2</sub> individuals derived from the cross between Jasmine 85 aromatic and Basmati 370 aromatic, in addition to a population of 200 F<sub>2</sub> individuals derived from the cross between Jasmine 85 aromatic and Nipponbare non-aromatic were also used as material. All cultivars and progenies were grown in Honmachi experimental paddy field at Tokyo University of Agriculture and Technology (Japan).

The experiment for varietal comparison was performed in a completely randomized block design with three replications. Each replication consisted of 26 plots with the area of 2 m<sup>2</sup>. The seedlings at 21 days after sowing were randomly transplanted with a 20 x 20 cm spacing. Fertilizer with the rate of 70N:40P:70K kg ha<sup>-1</sup> was applied after the last paddling and 50 days after the transplanting. Morphological and agronomical characterization was conducted on 25 plants per plot, 75 plants per cultivars (total of 1,950 plants of 26 cultivars), and on all F<sub>2</sub> populations. Statistical analysis was applied using analysis of variance (ANOVA) and Duncan's multiple-comparison test.

In the 1.7% KOH method, 100 mg of young rice leaves at the heading stage were weighed, cut into small pieces, and placed into Petri dishes. Then 10 ml of 1.7% KOH solution was added onto the leaves, covered by a Petri dish cap, and left for one hour at room temperature. A panel of six persons smelled the samples one by one and their evaluation was recorded. The average grade was scored as (+) for aroma and (-) for non-aroma.

Polished kernels of each cultivar were used for tasting the kernel and the cooking test. Fifty kernels of each cultivar were chewed by a panel of analysts having the ability to distinguish between aromatic and non-aromatic rice. For the cooking test, 500 g of grain of each cultivar were weighed and then cooked. The cooked rice was tasted by a panel of six persons and the average grade was scored as (+) for aromatic and (-) for non-aromatic rice.

Genomic DNA from young leaves was extracted by the rapid DNA extraction (CTAB) (Doyle et al. 1987). PCR (ASTEC, Gene Amp PC system 320 Japan) was performed using 0.1  $\mu$ l Platinum<sup>®</sup> Taq DNA Polymerase (Fermentas Inc<sup>®</sup>), 1  $\mu$ l of genomic DNA 10 ng/ $\mu$ l, 2.5  $\mu$ l of 10X buffer (Fermentas Inc<sup>®</sup>), 2  $\mu$ l of 50 mM MgCl<sub>2</sub> (Fermentas Inc<sup>®</sup>), 2  $\mu$ l of dNTPs [5mM], 2.5  $\mu$ l of each primer external sense primer (ESP), internal fragrant antisense primer (IFAP), internal non-fragrant sense primer (INSP), and external antisense primer (EAP) (Fig. 1). PCR amplification was performed in a 32  $\mu$ l of reaction buffer. The



**Fig. 1.** Relative positions of PCR primers used for determination of aromatic and non-aromatic rice. External primers (EAP and ESP) generate a fragment of approximately 580 bp as a positive control for each sample. Internal (INSP) and corresponding external (EAP) primers produce a 355 bp fragment from a non-aroma allele. Internal (IFAP) and corresponding external (ESP) primers produce a 257 bp fragment from an aroma allele (Bradbury et al. 2005a).

PCR conditions were initial denaturation at 94 °C for 2 min followed by 30 cycles at 94 °C for 30 s, at 58 °C for 30 s, at 72 °C for 30 s, and final extension at 72 °C for 5 min. PCR products were separated by electrophoresis on a 1.0% agarose gel in 1x TAE buffer. MassRuller™ was used to estimate fragment size. The gel was stained with ethidium bromide (5  $\mu$ l EtBr in 100 ml of 1x TAE) for 20 min, and then photographed.

## Results and Discussion

### Identification of SS STS markers

In this research, agronomic traits of Afghan, Iranian, and Uzbek aromatic and non-aromatic native rice cultivars and control cultivars from the different countries were studied in the field. The results showed that there was a wide range of agronomical variations among cultivars. Important agronomical characters such as plant height, panicle length, panicle weight, grain index, leaf index, basal internode length, and culm dimension were analyzed. A short rice plant does not guarantee a high degree of lodging resistance (Ookawa and Ishihara 1993). In the present study, Luke Qasan and Jaihon with short culm have been lodged after heading. The height of Permela, Sherkati, Sarda Bala, Sela Doshi, Sela Takhar, Germa Bala, Lawangi

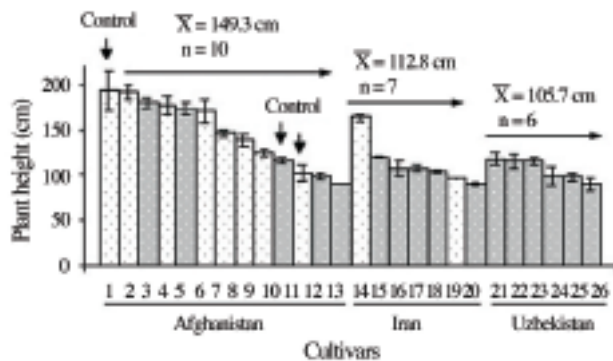


Fig. 2. Distribution of plant height, among aromatic and non-aromatic rice cultivars (white columns and black columns are aromatic and non-aromatic rice cultivars, respectively). 1: Basmati 370, 2: Germa Bala, 3: Sherkati, 4: Lawangi, 5: Permel, 6: Sarda Bala, 7: Sela Takhar, 8: Sela Doshi, 9: Pashadi Konar, 10: Nipponbare, 11: Jasmine 85, 12: Monda Mashruqi, 13: Luke Qasan, 14: Qaserdashti, 15: Poya, 16: Doroudzan, 17: Nemat, 18: Kodus, 19: Fajr, 20: Shiroudi, 21: Gulnar, 22: Sanam, 23: Shortamby, 24: Nokos-70, 25: Jaihon, and 26 Nokos-2 . Critical linkage has not been found between aroma and plant height.

(Afghanistan), Qaserdashti (Iran), and Basmati 370 (control) ranged from 138.7±7.2 to 194.2±21.4 cm. According to the IRR1 index (IBPGR-IRRI 1980), this range belongs to the tall plant group. The plant height of Pashadi Konar (Afghanistan), Poya (Iran), Gulnar, Sanam and Shortamby (Uzbekistan), and Nipponbare (control) ranged from 110.8±2.6 to 125.2±3.1 cm, and belong to the intermediate plant group. The plant height of Luke Qasan (Afghanistan), Doroudzan, Nemat, Kodus, Fajr, and Shiroudi (Iran), Nokos-2, Nokos-70, and Jaihon (Uzbekistan) and Jasmine 85 (control) ranged from 89.3±0.4 to 107±8.6 cm, and belong to the short plant group. Afghan rice cultivars were taller than Iranian and Uzbek and shorter than Basmati 370 (Fig. 2). Premium Basmati rices have extremely slender grains, substantial kernel elongation after cooking, fluffy appearance, high amylose content, and low gel consistency (Sing et al. 2000). Based on the results, most Afghan and Iranian rice cultivars have slender grains and desirable flavor. These cultivars are useful for development of aromatic rice in breeding programs. In this study, we detected that Pashadi Konar from Afghanistan with the average of 33±0 cm of panicle length had the longest panicle among cultivars, while Iranian rice cultivars with the total average of 28.2±3.7 cm displayed longer panicle than Afghan, the control, and Uzbek, respectively. In terms of panicle weight, Shortamby from Uzbekistan with an average of 7.1±0.5 g exhibited the heaviest panicle among cultivars. Among countries, Uzbek rice cultivars with a total average of 4.7±1.3 g panicle weight were found to be heavier than Iranian, Afghan, and the control, respectively. Iranian rice cultivars with the 4.4 indices had larger grain index than Afghan, control, and Uzbek rice cultivars, respectively. The basal internode was measured among Afghan, Iranian, Uzbek, and control rice cultivars. Afghan rice cultivars exhibited longer basal internode than the others. Culm strength in Afghanistan cultivars was weaker than the others (Hien et al. 2006b). In the present study, Afghan was also evaluated more susceptible to lodging than Iranian and Uzbek rice cultivars. The results show huge diversity of agronomic traits among rice cultivars in Central Asia (Table 1).

Analysis of variance (ANOVA) was applied to clarify the significant difference among rice cultivars. The results indicated that there is a significant difference in panicle length, panicle weight, basal internode, and culm dimension among rice cultivars at the 5% level (Table 1).

Duncan's multiple-comparison test was applied to compare the significant difference of agronomic traits at the 5% level among rice cultivars. A significant difference was detected between Pashadi Konar with longer panicle length, and Jaihon, Nokos-70, Nokos-2, Sanam, Shortamby, Gulnar, Luke Qasan, Nipponbare, Jasmine 85, Basmati 370, Monda Mashruqi, Doroudzan, and Qaserdashti, while there was no significant difference with the others. In the case of panicle weight, there was no significant difference between Shortamby and Sherkati, Nokos-70, Doroudzan, Jasmine 85, and Pashadi Konar, while there was a significant difference with the others. There was no significant difference between Sherkati with the longer basal internode and Luke Qasan, Gulnar, Sarda Bala, and Qaserdashti, while there was a significant difference with the others. Sherkati with larger culm dimension did not show a significant difference with Nokos-70, Jaihon, Germa Bala, Shortamby, Pashadi Konar, Permel, Sela Takhar, Jasmine 85, Basmati 370, Lawangi, and Sela Doshi, while it exhibited a significant difference with the others (Table 1).

Plant height was analyzed in the F<sub>2</sub> generation obtained from the cross between Jasmine 85 (P<sub>1</sub>) and Basmati 370 (P<sub>2</sub>). The height of P<sub>1</sub> plants ranged between 100-120 cm, P<sub>2</sub> plants 180-200 cm, and the F<sub>1</sub> generation 140-160 cm. The plant height in five (2.5%) of the F<sub>2</sub> populations ranged between 80-100 cm, 26 (13%) were 100-120 cm, 72 (36%) were 120-140 cm, 48 (24%) were 140-160 cm, 39 (19.5%) were 160-180 cm, and 10 (5%) were 180-200 cm (Fig. 3). The plant height exhibited a continuous segregation in the F<sub>2</sub> population. The distribution of plant height at different ranges could be useful for the improvement of short and intermediate aromatic rice in breeding programs.

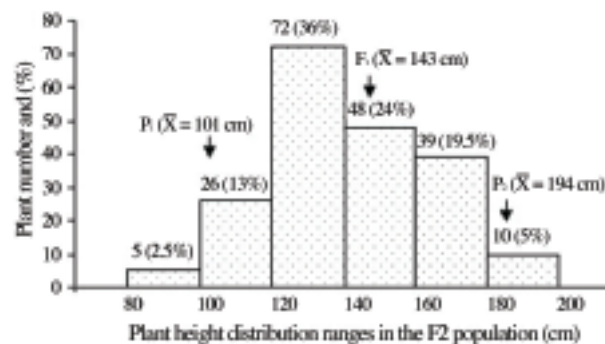


Fig. 3. Frequency distribution of plant height in the F<sub>2</sub> (200) individuals derived from the cross between Jasmine 85 and Basmati 370. The average of plant height in the P<sub>1</sub>, P<sub>2</sub>, and the F<sub>1</sub> inserted at the top of the figure. The plant height displayed a continuous segregation in the F<sub>2</sub> population.

### Chemical and molecular characterization of aroma in rice

Tasting individual kernels is one of the original methods for

**Table 1.** Comparison of agronomic traits among Afghan, Iranian, and Uzbek native rice cultivars using ANOVA and Duncan's multiple-comparison test.

Cultivars	Panicle length (cm)	Panicle Weight (g)	Basal internode (cm)	Culm dimension (cm)	Grain index	Leaf index
Jaihon	15.6 ± 1.0a <sup>1)</sup>	3.9 ± 0.4abcd	4.5 ± 2.1abcd	0.73 ± 0.0d	2.4	12.5
Nokos-70	17.6 ± 1.0a	5.3 ± 1.3cde	1.3 ± 0.3a	0.76 ± 0.0d	2.3	12.3
Nokos-2	18.0 ± 2.0ab	3.8 ± 1.0abcd	3.0 ± 1.1abc	0.60 ± 0.0abc	2.3	12.0
Sanam	18.6 ± 0.0ab	3.8 ± 0.4abcd	1.9 ± 0.1ab	0.60 ± 0.0abc	3.0	18.7
Shortamby	18.6 ± 0.0ab	7.1 ± 0.5e	1.7 ± 0.3a	0.73 ± 0.0d	2.3	11.7
Gulnar	19.0 ± 2.0ab	4.7 ± 0.7bcd	5.9 ± 0.0de	0.60 ± 0.0abc	2.0	17.0
Luke Qasan	19.3 ± 0.5ab	1.5 ± 0.3a	6.6 ± 0.6e	0.40 ± 0.1a	2.0	27.2
Nipponbare	23.4 ± 0.2bc	3.8 ± 0.7abcd	2.0 ± 0.0ab	0.46 ± 0.0abc	2.1	21.0
Jasmine 85	23.6 ± 0.5bc	4.9 ± 0.6cde	2.6 ± 0.2abc	0.66 ± 0.0cd	3.9	14.5
Basmati 370	25.7 ± 0.9cd	2.4 ± 0.6ab	2.6 ± 0.5abc	0.66 ± 0.0cd	4.2	47.1
Monda Mashruqi	25.9 ± 0.3cde	2.0 ± 0.5a	2.1 ± 0.2ab	0.53 ± 0.1abc	3.2	15.7
Doroudzan	26.3 ± 1.3cde	5.1 ± 0.4cde	3.4 ± 1.7abc	0.60 ± 0.1abc	4.1	35.7
Qaserdashti	26.3 ± 1.3cde	3.1 ± 0.8abc	4.9 ± 1.2bcde	0.60 ± 0.1abc	3.0	44.5
Germa Bala	27.3 ± 2.5cdef	3.1 ± 0.4abc	3.5 ± 1.3abcd	0.73 ± 0.0d	3.8	35.3
Shiroudi	27.5 ± 1.9cdef	4.5 ± 0.3bcd	3.0 ± 1.3abc	0.53 ± 0.0abc	4.8	35.0
Fajr	27.6 ± 1.6cdef	3.3 ± 0.3abcd	3.0 ± 0.9abc	0.53 ± 0.0abc	5.0	33.8
Kodus	27.8 ± 2.4cdef	2.3 ± 0.4ab	1.4 ± 0.1a	0.43 ± 0.0ab	4.7	34.4
Sela Takhar	28.0 ± 4.3cdef	3.6 ± 0.3abcd	2.5 ± 0.8abc	0.66 ± 0.0cd	4.6	29.5
Lawangi	28.6 ± 5.7cdef	3.0 ± 0.5abc	4.3 ± 0.5abcd	0.66 ± 0.0cd	4.2	27.7
Sarda Bala	29.3 ± 5.5cdef	3.9 ± 0.9abcd	5.3 ± 2.5cde	0.60 ± 0.3abc	4.1	20.3
Sela Doshi	29.3 ± 1.5cdef	4.4 ± 0.6bcd	4.0 ± 2.7abcd	0.63 ± 0.0bcd	3.7	32.8
Sherkati	29.6 ± 2.2cdef	5.6 ± 2.4de	6.6 ± 0.5e	0.76 ± 0.0d	4.5	25.5
Poya	30.0 ± 2.0cdef	3.3 ± 0.1abcd	2.3 ± 0.0abc	0.43 ± 0.0ab	4.5	48.2
Permel	32.3 ± 0.2def	3.1 ± 0.9abc	3.4 ± 0.0abcd	0.70 ± 0.1d	3.9	30.0
Nemat	32.5 ± 1.3ef	4.3 ± 1.0bcd	2.5 ± 0.4abc	0.40 ± 0.1a	4.7	42.8
Pashadi Konar	33.0 ± 0.9f	4.9 ± 0.6cde	1.8 ± 0.5ab	0.70 ± 0.0d	4.4	32.9
ANOVA	*	*	*	*		

a) Values with different letters are significantly different at the 5% level by Duncan's multiple-comparison test.

\*: Significant difference at 5% level.

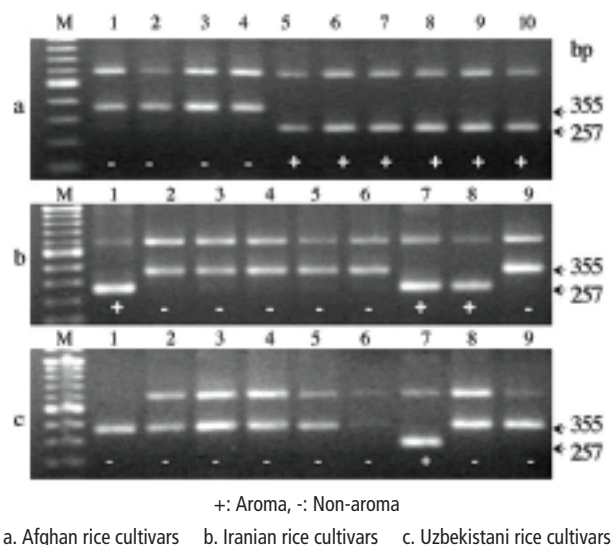
Note: Statistical analysis did not apply in the grain and leaf indices.

the quality selection of aromatic rice (Reinke et al. 1991). The 1.7% KOH test (Sood and Siddiq 1978), gas chromatography (Lorieux et al. 1996; Widjaja et al. 1996), and specific PCR amplification (Bradbury et al. 2005a) are also available for classification of aromatic and non-aromatic rice.

In this study, aromatic and non-aromatic rice cultivars from Afghanistan, Iran, and Uzbekistan with control were characterized by conventional methods (tasting kernel and cooking test), 1.7% KOH sensory test, and molecular marker analysis. The results of the 1.7% KOH test were similar to those of the PCR analysis as described later.

The results of conventional methods (tasting kernel and cooking test) varied among cultivars. It was recorded (+) for aromatic and (-) for non-aromatic rice. Cultivars that classified into aromatic or non-aromatic rice by the molecular method and 1.7% KOH test well agreed with the conventional methods. Six of ten Afghan native rice cultivars were scored (+) such as Pashadi Konar, Sarda Bala, Sela Doshi, Sela Takhar, Germa Bala, and Lawangi, while it was scored (-) in the others, two of seven Iranian such as Fajr and Qaserdashti were also scored (+), while all Uzbek cultivars were scored (-). Based on these results, a predominance of aromatic rice in Afghanistan (Table 2) was observed.

For molecular marker analysis we used allele specific DNA amplification by PCR (Bradbury et al. 2005a). This technique



**Fig. 4.** Estimation of aromatic and non-aromatic rice cultivars by PCR amplification and 1.7% KOH test (+: Aroma, -: Non-aroma). M: Marker, A: 1: Permel, 2: Sherkati, 3: Luke Qasan, 4: Monda Mashruqi, 5: Pashadi Konar, 6: Sarda Bala, 7: Sela Doshi, 8: Sela Takhar, 9: Germa Bala, and 10: Lawangi. B: 1: Fajr, 2: Poya, 3: Shiroudi, 4: Nemat, 5: Kodus, 6: Doroudzan, 7: Qaserdashti, 8: Basmati 370, and 9: Nipponbare. C: 1: Jaihon, 2: Sanam, 3: Shortamby, 4: Gulnar, 5: Nokos-2, 6: Nokos-70, 7: Jasmine 85, 8: Nipponbare, and 9: Koshihikari.

## Agronomic Traits of Native Rice from Central Asia

**Table 2.** Identification and characterization of aromatic and non-aromatic rice cultivars by four methods.

Cultivars	Origin	Tasting kernel	Cooking test	1.7% KOH	Molecular marker
Permel	Afghanistan	-	-	-	-
Sherkati	Afghanistan	-	-	-	-
Luke Qasan	Afghanistan	-	-	-	-
Monda Mashruqi	Afghanistan	-	-	-	-
Pashadi Konar	Afghanistan	+	+	+	+
Sarda Bala	Afghanistan	+	+	+	+
Sela Doshi	Afghanistan	+	+	+	+
Pashadi Konar	Afghanistan	+	+	+	+
Germa Bala	Afghanistan	+	+	+	+
Lawangi	Afghanistan	+	+	+	+
Fajr	Iran	+	+	+	+
Poya	Iran	-	-	-	-
Shiroudi	Iran	-	-	-	-
Nemat	Iran	-	-	-	-
Kadus	Iran	-	-	-	-
Doroudzan	Iran	-	-	-	-
Qaserdashti	Iran	+	+	+	+
Basmati 370	India	+	+	+	+
Jaihon	Uzbekistan	-	-	-	-
Sanam	Uzbekistan	-	-	-	-
Shortamby	Uzbekistan	-	-	-	-
Gulnar	Uzbekistan	-	-	-	-
Nokos-2	Uzbekistan	-	-	-	-
Nokos-70	Uzbekistan	-	-	-	-
Jasmine 85 (control)	Thailand	+	+	+	+
Nipponbare (control)	Japan	-	-	-	-
Koshihikari	Japan	-	-	-	-

+: Aroma, -: Non-aroma

can be used to discriminate alleles that differ by SNP's insertions or deletions with a single PCR. This technique identifies homozygous aromatic, homozygous non-aromatic, and heterozygous non-aromatic individuals. External primers produce a fragment of approximately 580 bp as a positive control for each sample. Internal and corresponding external primers generate a 355 bp fragment from a non-aromatic allele and a 257 bp fragment from an aromatic allele (Bradbury et al. 2005a).

Here we report the results of our study on aromatic and non-aromatic rice cultivars in some Asian countries. The results showed that non-aromatic rice cultivars produced a 355 bp fragment, and aromatic rice cultivars produced a 257 bp fragment. Nipponbare was used as a control for non-aromatic and Jasmine 85 for aromatic rice. According to the results, six of 10 Afghan cultivars and two of seven Iranian were aromatic, followed by all Uzbek as non-aromatic (Fig. 4).

Based on the previous study of our group, a conflict has been included between conventional methods (tasting and chewing kernels) and gas chromatography among rice cultivars (Hien et al. 2007). In the present study, a coincidence was detected among conventional methods, 1.7% KOH test, and molecular marker analysis in the classification between aromatic and non-aromatic rice (Table 2).

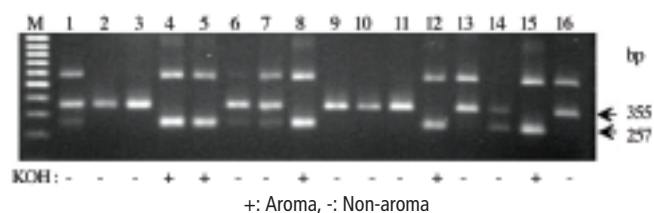
The segregation of non-aromatic and aromatic rice in the F<sub>2</sub> generation derived from the cross between Jasmine 85 (aromat-

**Table 3.** Genetic analysis of aroma nature by using 1.7% KOH test and PCR analysis in the F<sub>1</sub> and F<sub>2</sub> generations derived from the crosses between Jasmine 85 (aromatic) and Nipponbare (non-aromatic), and Jasmine 85 and Basmati 370 (aromatic).

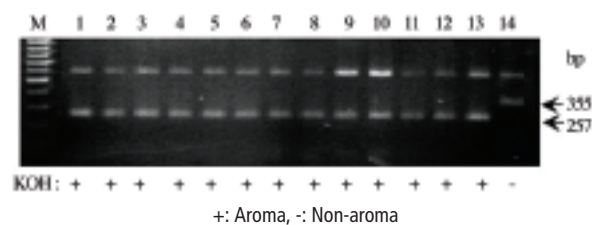
Genotype	Non-aroma	Aroma	Segregation ratio	$\chi^2$ value	Probability (%)
Nipponbare (♀)	15	0	1:0		
Jasmine 85 (♂)	0	15	0:1		
F <sub>1</sub>	2	0	1:0		
F <sub>2</sub>	152	48	3:1	0.1	70-80
Basmati 370 (♀)	0	15	0:1		
Jasmine 85 (♂)	0	15	0:1		
F <sub>1</sub>	0	2	0:1		
F <sub>2</sub>	0	200	0:1	ns*	ns*

\*: Non-significant (♀): Female (♂): Male

ic) and Nipponbare (non-aromatic) was analyzed by using 1.7% KOH solution and PCR amplification. The results showed a strong coincidence between the 1.7% KOH test and molecular marker method. Lanes 1, 6, 7, and 14 in the F<sub>2</sub> individuals obtained from the cross between Jasmine 85 and Nipponbare displayed heterozygous bands, which can classify non-aromatic. Some times molecular markers cannot classify heterozygous and homozygous genotypes due to molecular nature. Segregation ratio was detected in the F<sub>2</sub> generation as 3 (non-aroma): 1 (aroma). Segregation has not been detected in the F<sub>2</sub> generation derived from the cross between Jasmine 85 (aromatic) and Basmati 370 (aromatic) (Figs. 5 and 6, Table 3).



**Fig. 5.** Segregation of aromatic and non-aromatic rice in the F<sub>2</sub> individuals derived from the cross between Jasmine 85 and Nipponbare by PCR amplification and 1.7% KOH test (+: Aroma, -: Non-aroma). M: Marker, Lane 1-16: F<sub>2</sub> individuals. Based on the results phenotype ratio is fit to 3 (non-aroma): 1 (aroma).



**Fig. 6.** Segregation pattern in the F<sub>2</sub> generation derived from the cross between Jasmine 85 (aromatic) and Basmati 370 (aromatic) by PCR amplification and 1.7% KOH test (+: Aroma, -: Non-aroma). M: Marker, Lane 1-13: F<sub>2</sub> individuals and Lane 14: Nipponbare as a control. Segregation has not been detected.

## Conclusion

The diversity of agronomic traits in rice within a country and among countries has been evaluated in this study, and it may help breeders in the improvement of aromatic rice. Our results suggest that Central Asia, especially Afghanistan, is a good source of genetic diversity for rice breeding.

Until now, aromatic rice cultivars such as Basmati and Jasmine have been introduced from India and Thailand. New aromatic rice cultivars, especially Germa Bala, Sarda Bala, Sela Takhar, Sela Doshi, Pashadi Konar, and Lawangi from Afghanistan, and Fajr and Qaserdashti from Iran can be also used as aromatic germplasm resources. The demand for the above-mentioned rice cultivars, which have an extremely slender grain and strong aroma, is very high in Central Asia. Using molecular marker and chemical methods in this research, which could be a background and framework for the breeding program for further research in Central Asia. Up to now, the aroma trait has not been studied in Central Asia except through the farmers' experience; therefore this study is original and expects to be applicable and useful in breeding programs in Central Asia in the near future.

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