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Alternate Phenotype-Genotype Selection Method for Developing Photoperiod Insensitive, Good Cooking Quality and Potential High Yielding Rice Lines

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ABSTRACT

The Khao Dawk Mali 105 (KDML105) rice variety is photosensitive but considered to be good quality rice in Thailand. Crossing between KDML105 and a new plant type variety Qiqnizhan (Chinese origin), was performed to select photoperiod-insensitive, good cooking quality and high yielding lines. Switching between phenotype and genotype selections was made based on target traits. Selection for photoperiod insensitivity was made in the F_2 population grown under long day conditions with a DNA marker linked to the *fgr* and *Wx* genes. The F_3 population confirmed the recombinants with desirable ideotypes having photoperiod insensitivity and good cooking quality. Moreover, six lines were selected in the F_4 generation with more fragrant grain, lower amylose content and higher yield than those of their parents and standard varieties. The informative genetic markers linked to target traits were utilized which accelerated the breeding program.

Keywords: fragrance, low amylose, new plant type, marker assisted selection, pedigree selection

INTRODUCTION

Rice (*Oryza sativa* L.) is the most important staple food crop of half the global population (Food and Agriculture Organization, 2004). In many rice-producing areas of the world, cooking quality is always considered a major criterion in evaluating rice grain and it is mainly determined by the starch composition (Juliano, 1985). The amylose content (AC) is one of the most important parameters determining cooking and eating quality (Juliano, 1971). Waxy rice has almost zero amylose and is used for special foods such as desserts and snacks (Luh, 1999). Amylose rich varieties (greater than 25%) are common in Indica rice, and are dry and fluffy on cooking, but often become hard after cooling while intermediate amylose (20–25%) rice is soft, but not sticky, and varieties with low amylose content (10–19%) are soft and sticky, and include nearly all-temperate Japonica cultivars (Juliano, 1971). Low amylose content, fragrant rice is widely preferred by most consumers in Thailand and is considered of good cooking quality (Kumer and Khush, 1986). In fact, AC is predominantly a genetically determined trait which is controlled by the waxy locus (*Wx*) on chromosome-6 (Wang *et al*., 1995; Bao *et al*., 2008). A polymorphic microsatellite DNA marker having a dinucleotide cytosine-thymine repeat (CT_n) in the *Wx* gene was identified by Bligh *et al*. (1995).

Khao Dawk Mali 105 (KDML105) is the most popular aromatic rice variety grown in Thailand and is preferred for its good cooking

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quality being soft, tender and fluffy when cooked (Rice Department, 2012). A number of volatile compounds have been reported to be responsible for aroma in rice (Yajima *et al*., 1979; Buttery *et al*., 1988; Zeng *et al*., 2009). The volatile aromatic compounds of KDML105 have been identified and among them, 2-acetyl-1-pyrroline (2-AP) is considered to be the most important aroma producing compound in KDML105 (Tanchotikul and Hsieh, 1991). Until now, genetic analysis has revealed that a recessive gene (*fgr*) on chromosome-8 is responsible for rice fragrance (Jin *et al*., 2003). Bradbury *et al*. (2005) reported that the *badh2* gene is the *fgr* gene since it has an 8-bp deletion in its exon-7 compared to the functional *badh2* gene which encodes putative betaine aldehyde dehydrogenase 2 (BADH2). Molecular markers for fragrance genotyping were developed to identify this region (Rattanapol *et al*., 2011).

Rice grains with fragrance are appealing to consumers, and the retail price of fragrant rice is higher than that of conventional rice (Shi *et al*., 2008). The demand for KDML105 for export is increasing annually because of its very good cooking quality and aroma (Rice Department, 2012). However, such high demand has not yet been met. The major constraint in the low production of the photoperiod-sensitive rice is due to its prolonged growing period resulting in only one crop per year and in addition, it is prone to lodging and susceptibility to many diseases and insects (Rice Research Institute, 1984). The International Rice Research Institute began developing the new plant type (NPT) rice through ideotype breeding approaches in 1989 (Khush, 1995). The goal was to develop an NPT to increase the yield potential through simulation modeling on the basis of morphological traits due to their ease of measurement (Peng *et al*., 1994; Peng *et al*., 2005).

Pedigree breeding has been a popular selection method in various rice improvement programs (Hargrove *et al*., 1988). It involves continuous phenotypic selection of lines from a large number of segregating populations following hybridization or crossing until homozygosity for the desired trait is attained. Since pedigree selection is largely based on phenotypic selection, it is labor intensive, time consuming and requires large nursery or field space for screening (Stuber *et al*., 1999). Marker-assisted selection (MAS) may greatly increase the success of selection of desirable lines as it directly target the genotype without the influence of environment and thus speeds up the conventional selection procedures (Collard *et al*., 2005). This allows breeders to focus attention on fewer high-priority lines in subsequent generations (Sreewongchai *et al*., 2010; Matthayatthaworn *et al*., 2011). Hence, practicing both phenotypic and genotypic selection may increase efficiency by complementing each other.

The objective of the current study was to select new rice lines with aromatic, low amylose content and high yield using MAS and the pedigree method through crossing KDML105 with the Qiqnizhan rice variety.

MATERIALS AND METHODS

Plant materials

KDML105 (Thai rice with good cooking qualities) and Qiqnizhan (a new plant type variety of China with the potential to provide higher yields) were used as the female and male parent, respectively, to produce a cross. The commonly grown Thai rice varieties, Pathumthani 1 (PTT1), Supanburi 1 (SPR1) and Chainat 1 (CNT1) were used as standards. The F_1 hybrids and F_2, F_3, F_4 and F_5 populations were generated under the pedigree selection method.

Selection for photoperiod insensitivity character

In total, 1200 F_2 plants from the KDML105/Qiqnizhan cross were grown individually under long day conditions (March–

June) in a nursery. Flowering plants were selected and kept for marker-assisted selection.

Marker-assisted selection for fragrance and amylose content

The selected F_2 plants for photoperiod insensitivity were used for genotyping. The functional marker tagged for the *fgr* gene with the primer pair Naro1F (5'-AGGTTGCATTTACTGGGAG-3') and Naro1R (5'-TGGCTACTAGAATGATGCT-3') was used for identifying fragrant and nonfragrant rice (Rattanapol *et al*., 2011). Markers for microsatellite CT_n alleles of the *Wx* gene, amplified by the primer pair OSR19F (5'-CTCTCTCACCATTCCTTCAG-3') and OSR19R (5'-GATCTGAATAAGAGGGGAAAC-3'), were used to identify plants carrying the favorite *wx* gene (Akagi *et al*., 1996). PCR amplifications were performed with a Phire® Plant Direct PCR Kit (Finnzymes; Keilarata, Espoo, Finland), designed to amplify DNA directly from rice leaf samples. No DNA extraction was required prior to the PCR. The PCR reactions were performed using a 10.0 µL volume containing five pieces of 0.5 mm leaf sample used as DNA source together with 5.0 µL 2× plant PCR buffer and 3.8µL sterile distilled H_2O, 1.0 µM of forward and reverse primers along with 0.2 µL Phire® Hot Start DNA polymerase (Finnzymes; Keilarata, Espoo, Finland). The PCR reaction method consisted of DNA denaturation at 98 °C for 5 min followed by 40 amplification cycles of 98 °C for 5 s, 57 °C for 5 s and 72 °C for 30 sec. Finally, extension was carried out for 1 min at 72 °C. After PCR amplification, 5.0 µL of loading dye buffer was added. Polymorphism of each PCR product was detected using silver nitrate ($AgNO_3$) staining after electrophoresis on 6% polyacrylamide gels following Benbouza *et al*. (2006).

Plant type selection

The seeds of selected F_3 plants were grown under field conditions with the plant and row spacing both 20 cm. Parents and standard varieties were also grown in the same field with the same spacing. Plants showing NPT with a high number of seeds per panicle and a high number of panicles per plant were selected and used for evaluation again in the F_4 generation. Data for agronomic traits and yield components were collected from individual plants to validate the pedigree method of selection.

Fragrance and amylose content measurement in F_5 seeds

AC was determined by the colorimetric method with iodine-potassium iodide, as described by Juliano (1971). In addition, fragrance was determined using sensory evaluation based on a simple laboratory technique developed by International Rice Research Institute (1971). The samples were scored as fragrant and nonfragrant. A strongly scented variety (KDML 105) was used as a check variety for comparison.

RESULTS

Selection for photoperiod-insensitivity character

Rice is a short-day plant as flowering of photoperiod-sensitive varieties is promoted by a short photoperiod. Several major genes controlling this trait have been identified in rice showing a dominant gene effect (Ohshima *et al*., 1993). Thus, photoperiod-insensitive lines could be selected in the F_2 population of the cross between photoperiod-sensitive and photoperiod-insensitive varieties. The F_2 population, when growing under the long day conditions, had a good flowering response due to the presence of the photoperiod-insensitive gene. In contrast, photoperiod-sensitive plants did not flower. In total, 220 photoperiod-insensitive plants having the desired plant type were selected from the F_2 population (Figure 1 and Table 1).

Marker-assisted selection in the F_2 and F_3 generations

The selected F_2 plants were genotyped using the functional marker of the *fgr* gene (Naro1) (Rattanapol *et al*., 2011). KDML105 has the 8 bp deletion on the *fgr* gene which is identified from fragrance in rice. Out of 220 F_2 plants, 22 and 96 plants showed homozygosity for 8 bp deletions (*fgr/fgr*) and heterozygosity (*Fgr/fgr*), respectively (Figure 1 and Table 1). Since only 22 plants were selected for fragrance and the heterozygous plants were also used for the next marker screening, the number of selected lines in the next screening could be increased. This selection technique is known as stepwise screening (Sreewongchai *et al*., 2010).

After using the OSR19 marker for screening the *Wx* gene, KDML105 was found to have the *wx* locus for low AC. Among 22 plants carrying the homozygous *fgr* gene, 4 plants were homozygous for low AC. From the 96 heterozygous plants for the *fgr* gene, 16 plants were homozygous for low AC. Therefore, it can be concluded that 4 plants were homozygous for both *fgr* and *wx* genes in this population. Besides those 16 plants which were heterozygous for the

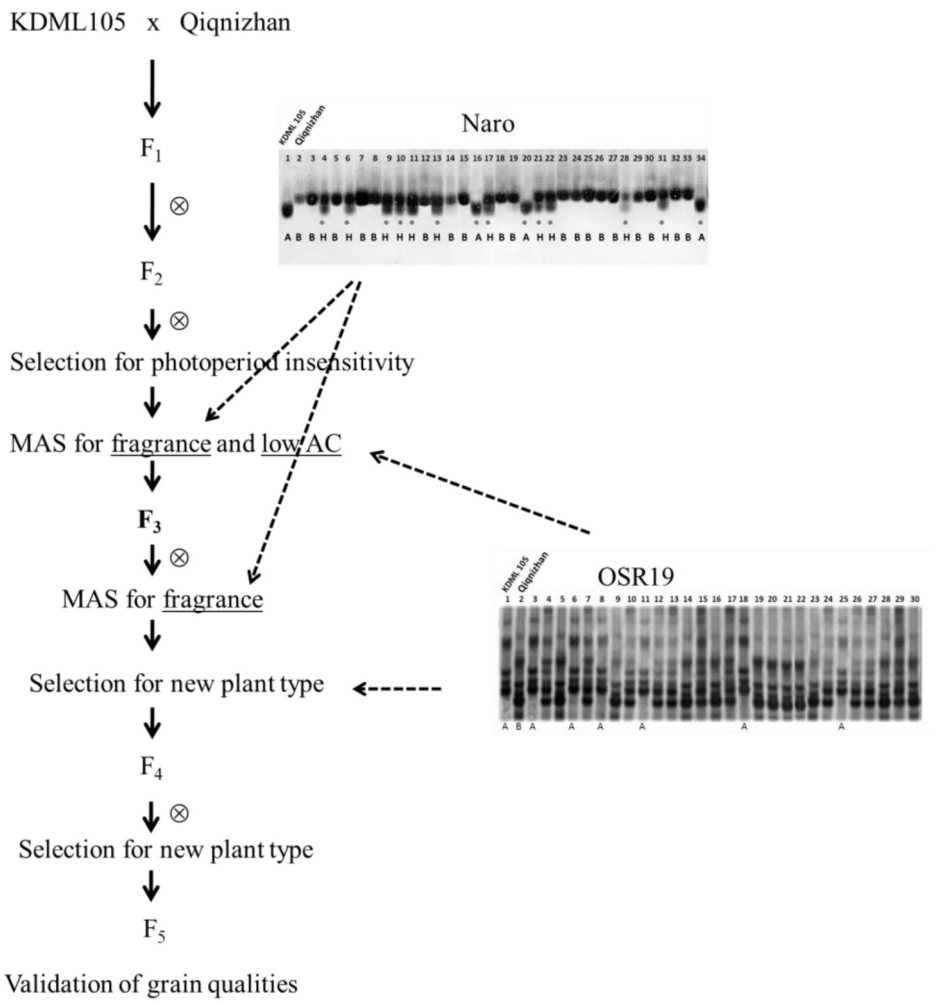

Figure 1 Flow chart of phenotype-genotype-phenotype selection in rice breeding for photoperiod insensitivity, good cooking quality and high yield rice lines in cross between KDML105 and Qiqnizhan varieties. (AC = Amylose content.)

Table 1 Procedure of phenotype and genotype selection and number of selected plants in each procedure of progenies from crosses between KDML 105 and Qiqnizhan.

Generation	Trait of selection	Type of selection	Selection method	Number of plants evaluated	Number of selected plants
F_2	Photoperiod insensitive	Phenotype	Grow under long day conditions (day length >12 $hr.d^{-1}$)	1,012	220
	Aromatic grain	Genotype	Naro DNA marker	220	22 with *fgr/fgr* 96 with *Fgr/fgr*
	Amylose content	Genotype	OSR19 DNA marker	22 (*fgr/fgr*) 96 (*Fgr/fgr*)	4 with *fgr/fgr* and *wx/wx* 16 with *Fgr/fgr* and *wx/wx*
F_3	Aromatic grain	Genotype	Naro DNA marker at seedling stage	150 from *Fgr/fgr* and *wx/wx*	38 with *fgr/fgr* and *wx/wx*
	Grain yield	Phenotype	Grain yield per plant evaluation	75 (38 selected plants with *fgr/fgr* and *wx/wx* + progenies of 4 selected plant in F_2 generation)	11
F_4	Grain yield	Phenotype	Grain yield per plant evaluation	230	6 with higher than check variety (CNT1)

fgr gene and heterozygous for the *wx* genes, a total of 20 selected plants were harvested for the next screening (Figure 1 and Table 1).

In the F_3 generation, plants carrying the heterozygous *fgr* gene and the homozygous *wx* gene were screened using the Naro1 marker at the seedling stage. From a total of 150 F_3 plants, 38 plants were tagged for the homozygous *fgr* gene. In addition, the lines fixed in both target genes were included. A total of 75 selected F_3 individual plants were selected for ideotypes. These plants were identified as *fgr* and *wx* genes. These could be traced back to nine plants of the F_2 population (Figure 1 and Table 1).

Plant type selection in F_3 and F_4 generation

In the F_3 generation, the 75 selected F_3 plants were grown under field conditions following the pedigree method of selection. The parents and check varieties (PTT1, SPR1 and CNT1) were included in the selection. Those lines which showed good plant type were observed and evaluated by visual screening compared with that of the NPT variety (male parent). The yield potential of each plant was compared with the check varieties. In total, 20 plants were selected and kept for the next procedure. These could be traced back to six plants of the F_2 population the so-called six families.

In the F_4 generation, 10 plants derived from each of the 20 selected F_3plants were grown under field conditions. Therefore, 200 plants were selected for plant type and yield potential. The yields of individual plants were compared with those of the parents and standard varieties. The result showed that the line ATN0701-857-1-2, was the highest yielder (66.05 g per plant). The six selected lines were higher yielders than those of the parents and standard varieties. Moreover, individual plant comparisons showed that the plant type of the best line in each selected plant was the same as that of the male parent, especially for the number of grains per panicle (Table 2).

Validation for fragrance and amylose content in F_5 seed

The evaluation of fragrance and AC in F_5 seeds of the six selected families including parents and standard varieties was made. The results showed that of the six selected lines, KDML105 and PTT1 were fragrant. The AC of the six selected lines ranged between 14.16 and 17.58%. The AC levels for the female parent, male parent, PTT1, SPR1 and CNT1 were 17.9, 21.3, 19.61, 24.96 and 25.16%, respectively (Table 2). These results showed the efficiency and accuracy of MAS.

DISCUSSION

A cross between KDML105 and Qiqnizhan followed by pedigree selection could create rice lines among progeny with superior yields compared with their parents. This may have been due to heterosis resulting from different sources or the varied genetic base of the parents (Khush, 1995). Conventional breeding is an efficient method for the selection of favorite plants but it is time consuming.

Since DNA markers linked to target traits have the potential to accelerate the breeding program, selection could be applied at any stage of rice growing. The favorite genotypes could be selected with co-dominance markers (Zhou *et al*., 2003; Collard *et al*., 2005, Ulukan, 2011). In addition, stepwise screening could minimize the population and increase the number of selected lines in a screening (Sreewongchai *et al*., 2010, Min *et al*., 2012).

The current study selected for four different traits through the pedigree method of selection. Traits controlled by complete or major genetic factors could be selected under field conditions using morphological or phenotypic markers. On the other hand, informative genetic markers linked to target traits could accelerate the breeding program by employing MAS. Moreover, selection was performed by switching between

Table 2 Agronomic and grain quality traits of the six selected lines of F_4 compared to parents and check varieties.

Line/variety	Plant height (cm)	Day to 50% flowering	Main panicle length (cm)	Number of seeds per panicle	Effective tillers per plant	Seed setting (%)	Grain weight (g per 1,000)	Grain yield (g per plant)	Fragrance	AC (%)
ATN0701-11-1-3	142.0	80	29.5	313.0	7.0	75.1	21.91	55.16	+	14.8
ATN0701- 42-1-1	166.0	75	27.0	209.0	7.0	82.3	23.84	51.48	+	14.6
ATN0701-214-3-2	133.0	83	18.0	200.0	7.0	70.0	25.00	63.63	+	16.0
ATN0701-214-10-1	133.5	83	28.5	248.0	7.0	86.7	21.40	54.88	+	15.2
ATN0701-314-1-1	150.0	95	28.0	229.0	7.0	83.8	23.39	64.73	+	17.3
ATN0701-857-1-2	180.0	85	32.5	255.0	6.0	85.1	22.26	66.05	+	17.6
KDML 105*	173.0	85*	33.0	95.0	4.0	75.0	19.52	14.18	+	17.9
Qiqnizhan	120.0	75	30.0	253.7	4.3	53.2	22.77	32.38	-	21.3
CNT1	136.6	75	29.0	261.3	9.3	70.4	22.24	46.29	-	25.2
PTT1	135.4	78	31.4	262.4	5.4	57.2	22.51	21.37	+	19.6
SPR1	139.0	75	25.3	249.7	3.5	66.0	22.66	19.69	-	25.0

+ = Fragrance present; - = Nonfragrant.

AC = Amylose content

* = KDML 105 is photoperiod sensitivity variety.

phenotype and genotype the so-called alternate phenotype-genotype selection method and MAS could be used to reduce the field trial size by excluding unfavorite genotypes before planting the rice population in the field. This method might prove to be one of the successful ways to accelerate breeding programs in rice and other crops.

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