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Comparative analysis of eating quality and yield of selected non-waxy red-pericarp aromatic rice mutants

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Summary

Red-pericarp rice variety Kuanfu waxy aroma is highly valued for its grain quality in Taiwan, but it has undesirable traits of awned rough grain and taller plant height. The present study compared the palatability of cooked rice grains and yields of Kuanfu waxy aroma and its ten NaN_3-induced awnless non-waxy aromatic M_6-generation mutants developed through single-seed-descent selection plus a non-waxy aromatic rice variety TNG71 (reference variety with good eating quality). The palatability of cooked rice grains was assessed by using a rice taste meter. Results indicated that all the mutants exhibited awnless grain traits and reduced plant height. Significant differences in the palatability of cooked rice were also observed among the mutants with AM-425 (70.45) and AM-430 (73.75) having higher palatability scores than TNG71 (69.32). Mutant AM-425 also had higher aroma sensory score (1.33) than TNG71 (1.17). Two years yield trials indicated that AM-425 and AM-430 significantly out-yielded Kuanfu waxy aroma and can be recommended to rice growers.

Introduction

Rice (*Oryza sativa* L.) is a tremendously variable species and has worldwide distribution. It is estimated that about 120,000 distinct rice varieties exist in the world (Khush, 1997). Among these, aromatic rice varieties that release a unique and pleasant aroma detectable through sensory test, chemical assessment or molecular marker identification (Bradbury et al., 2005) constitute a small but important subgroup of rice varieties. Aromatic rice varieties are popular throughout Asia, and have also gained wider acceptance in Europe, Middle East, Australia and the United States of America (Sakthivel et al., 2009). However, most of the aromatic rice varieties have low yield compared to non-aromatic rice varieties, because the genes responsible for aroma also lead to a decreased ability to produce matured rice grains when under environmental stresses (Fitzgerald et al., 2010). Lack of high-yielding varieties is also associated with the low yield of aromatic rice that is mainly traditional and local landraces (Abdul Baset Mia et al., 2012). Therefore, various breeding efforts for increasing the yield of this type of rice varieties have been conducted in many countries. But in most cases, the outcomes are limited because the improvement of aromatic rice varieties is often challenged by the environment and genotype interactions for aromatic quality (Gay et al., 2010).

The rice variety Kuanfu waxy aroma, which releases a pleasant flavor during cooking, is a red-pericarp waxy landrace indigenous in Taiwan. It is primarily grown by the aboriginal peoples at Kuanfu Township of Hualien County in the eastern part of Taiwan. The produced Kuanfu waxy aroma grains are sold at a premium price in local market because of their aroma and red-pericarp characteristics. Moreover, the bran fraction of red-pericarp rice grains is endowed with many phytochemicals that are known to decrease oxidative stress *in vivo* and thus exert beneficial effects on human health (Jeng et al., 2012). Therefore, the produced Kuanfu waxy aroma grains not only can be directly used for human consumption, but also can be used to produce high-value nutraceuticals for food and cosmetic uses (Parrado et al., 2006, Revilla et al., 2009; Ryan, 2011). However, further expansion of Kuanfu waxy aroma is limited by three drawbacks. First, the harvested Kuanfu waxy aroma grains are mainly prepared and consumed in whole grain, as rice desserts or folk medicines, therefore, no rice brans milled off from Kuanfu waxy aroma are currently available for producing high value by-products. Secondly, the relative taller plant height (1.3-1.4 m) of Kuanfu waxy aroma makes it susceptible to lodging. Thirdly, the awned rough grains of Kuanfu waxy aroma (Fig. 1A) also cause problems during harvest and husking, even though this grain trait protects the developing rice grains from animal attack (Minaei et al., 2007; Hu et al., 2011). Thus, if an awnless non-waxy aromatic rice variety with red-pericarp is readily available for farming, not only the polished rice grains can be consumed as a staple food, the resulted bran parts enriched with phytochemicals may also be collected and used to produce high-value functional substances.

Mutation has been used with distinct effects to bring about desirable changes in traits usually controlled by single gene or polygenes. Previously, Agriculture Research Institute in Taiwan had used sodium azide to induce mutational changes in Kuanfu waxy aroma, and several awnless red-pericarp mutants with non-waxy endosperm and reduced plant height were identified. This study examined the grain eating qualities of these awnless non-waxy mutants derived from Kuanfu waxy aroma. The selected mutants were also grown in both spring and autumn crop seasons for grain yield comparisons.

Materials and methods

Plant materials and field planting

Ten M_6 generation mutants namely AM-419, AM-420, AM-422, AM-423, AM-424, AM-425, AM-426, AM-427, AM-430, AM-433, derived from wild type variety Kuanfu waxy aroma through NaN_3-induced mutation, with reduced plant height, awnless rough grain and non-waxy endosperm were selected in the autumn of 2010. These M_6 mutants with stabilized morphological traits were planted from spring 2011 to autumn 2012 for grain yield comparisons.

All the selected mutants and wild type Kuanfu waxy aroma were grown on the experimental farm of the Agricultural Research Institute in the spring and autumn of 2011 and 2012. A non-waxy aromatic rice variety TNG71 (a high eating quality aromatic variety with white-pericarp) was included to serve as a comparison. All the tested accessions were grown on the experimental farm of the Agricultural Research Institute in the spring and autumn of 2011 and 2012. Rice grains were sown in the nursery plots, where the seedlings grew up to the three-leaf stage before transplanting. The seedlings from the two spring crops were transplanted to the experimental plots on 5 February 2011 and 16 February 2012, respectively. The seedlings from the two autumn crops were transplanted to the experimental plots on

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5 August 2011 and 30 July 2012, respectively. The experiment was laid out in a completely randomized block design with three replicates. One seedling per hill was planted with a spacing of 30 cm × 15 cm in each experimental plot of 3 m x 6 m. Each plot received a basal application of fertilizer before transplanting (24 kg N ha^{-1}, 36 kg P_2O_5 ha^{-1} and 24 kg K_2O ha^{-1}) and three top-dressings of fertilizer on the 20th day (6 kg N ha^{-1}, 9 kg P_2O_5 ha^{-1} and 6 kg K_2O ha^{-1}), the 40th day (9 kg N ha^{-1}, 13.5 kg P_2O_5 ha^{-1} and 9 kg K_2O ha^{-1}) and the 60th day (9 kg N ha^{-1}, 13.5 kg P_2O_5 ha^{-1} and 9 kg K_2O ha^{-1}) after transplanting. Diseases and pests were controlled using the standard practices in the study areas.

Protein and amylose contents determinations

For grain protein and amylose contents analyses, the rice grains sampled from the spring crops of 2011 were used. Sampled rough rice grains (50 g) were polished followed in an abrasive polisher (Model Satake Pearler-TM05, Satake Corp., Horoshima-ken, Japan) with the degree of milling around 10%. Broken kernels were removed, and the polished rice samples were ground into fine powders in an RT-08 mill (Rong Tsong, Taichung, Taiwan) and stored at -20 °C until analysis. The total nitrogen content (with three replicates) was determined using the micro-Kjeldahl procedure (ZHANG et al., 2005). The grain protein content was calculated as the nitrogen content multiplied by the factor of 5.95. For amylose content (with three replicates) determination, milled rice grains were ground to pass through a 1.0 mm mesh screen by a Udy Cyclone mill (Udy Corp. Boulder, CO) to obtain rice flour. Fifty mg of rice flour was used to determine the amylose content following the iodine colorimetry at 608 nm detailed by Juliano (1971).

Aroma sensory and palatability tests

For aroma sensory tests, rice grains sampled from the spring crops of 2011 were used by using the method of LESTARI et al. (2011) with some modifications. Briefly, two g of polished rice grains were placed in a test tube of 15 mm × 150 mm previously filled with 10 ml of 1.7% KOH solution. The test tube was then covered with aluminum foil and heated in a 50 °C water bath for 10 minutes. After the samples were cool, the aluminum foil was removed, and samples were smelled and scored for aroma by six panelists. The scores were averaged and recorded with categories of non-aromatic (score 0), slightly aromatic (score < 1), moderately aromatic (score 1 ~ 2) and strongly aromatic (score 2 ~ 3).

Polished rice sample (30 g) were soaked in 40 ml of water for 30 min. The soaked rice samples were then cooked in an automatic electric cooker (SR-W180, Panasonic, Japan) for 25 min and held in the cooker covered for 10 min. Afterwards, the cooked rice was cooled down at room temperature for 90 min. The palatability scores (with three replicates), based on the appearance, hardiness and stickiness of cooked rice grains, were determined by using a Satake rice taste meter (Sta1A, Satake Corp., Hiroshima-ken, Japan) according to the manufacturer's instructions.

Grain yield and yield components determinations

Once the field-grown plants reached physiological maturity, 10 hills were marked in each experimental plot for the plant heights (from the plant base to the tip of the highest panicle) to be measured. Since there was only one plant per hill, the number of panicles per hill was counted to determine the number of panicles per plant. For grain yield determinations, the plants in the middle four rows of 2 m length in each plot were hand-harvested. The harvested panicles were oven-dried at 80 °C for 72 h to determine grain yields. A sub-sample of 20 panicles was taken at random and threshed to determine the number of spikelets per panicle, the percentages of fertility per panicle and the 1000-grain weights. The amount of developed and filled grains per panicle was recorded as the number of fully matured grains per panicle, while the sterile spikelets and grains with weight less than 10 mg were excluded from the calculation of fully mature grains. The percentage of fertility per panicle equaled the number of fully matured grains per panicle divided by the number of spikelets per panicle. The grain mass was weighed and the moisture content was measured. The grain yields were expressed at the 140 g kg^{-1} grain moisture content.

Statistical analysis

Variance analyses of the grain quality and agronomic traits data were performed with a Statistical Package for the Social Sciences (SPSS 10.0 for Windows: SPSS Inc., Chicago, IL, USA). Differences among means were evaluated using the Duncan's multiple range test. The correlation coefficients between palatability scores of tested grains vs. grain quality traits and between yield components vs. grain yield were also calculated using the same SPSS statistical package.

Results

Agronomic traits

The rough rice grains of Kuanfu waxy aroma had long awn (Fig. 1A), but some of NaN_3-induced mutants also produced awned grains (Fig. 1B). Nevertheless, all the selected mutants exhibited awnless grain trait (Tab. 1). Differentiation in other grain traits such as pericarp color (white-pericarp, red-pericarp and purple-pericarp grains) and grain shape (medium and short grains) also occurred among Kuanfu waxy aroma and selected mutants (Fig. 1B). Based on our breeding goals, only 10 non-waxy mutants that exhibited awnless (Tab. 1) and red-pericarp grain characteristics and wild type variety Kuanfu waxy aroma plus a non-waxy white-pericarp high-yielding aromatic variety TNG71 were selected and grown for agronomic traits comparisons.

As shown in Tab. 2 and 3, significant differences in plant height ($P < 0.05$) were observed among the tested mutants in both spring and autumn crop seasons. The plant heights of the selected mutants (ranged from 93 to 123 cm and from 83 to 112 cm for spring crop and autumn crop, respectively) were decreased considerably compared to Kuanfu waxy aroma in both crop seasons (129 and 141 cm for the spring and autumn crops, respectively). Significant differences in grain amylose and protein contents ($P < 0.05$) also existed among the selected non-waxy mutant (Tab. 1). The contents of grain amylose ranged from 11.69 (mutant AM-419) to 21.02% (mutant AM-422). Mutants AM-422, AM-424, AM-427 and AM-4337 showed higher grain amylose content than non-waxy aromatic variety TNG71 (18.70%). The protein contents of tested mutants ranged between 5.5 and 7.1% (Tab. 1). Mutant AM-430 had the lowest protein content of 5.5%, which was slightly lower than the protein content of TNG71 (5.8%).

Grain eating qualities

The aroma sensory test indicated that the milled grains of Kuanfu waxy aroma showed a moderate level of aromatic score (1.83) (Tab. 1). The aroma sensory scores of milled grains among the tested mutants ranged from 0.50 (AM-433) to 1.50 (AM-422) (Tab. 1). Mutants AM-425 and AM-422 had aroma sensory scores of 1.33 and 1.50, respectively, which were higher than the aroma sensory score of 1.17 recorded for non-waxy aromatic variety TNG71 (Tab. 1). The palatability scores of tested mutants ranged from 63.70 to 73.74 (Tab. 1). Two mutants AM-425 and AM-430 had the palatability scores of 70.45 and 73.75, respectively, which were higher than the

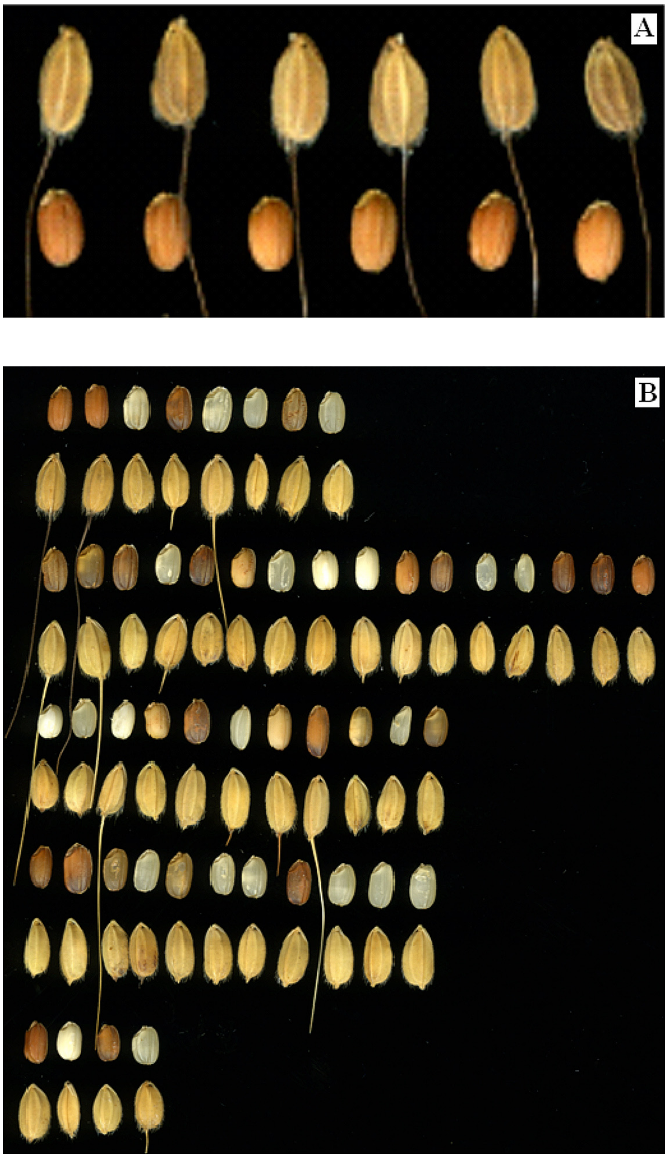

Fig. 1: The rough rice and brown rice grains of (A) wild type variety Kuanfu waxy aroma and (B) some of its NaN_3-induced mutants.

palatability score of 69.32 recorded for reference variety TNG71 (Tab. 1). The correlation analyses (data not shown) between the palatability of cooked rice grains and related grain quality traits across all the mutants were conducted, but the results indicated only the grain protein content and palatability was negatively correlated ($r = -0.9289$, $P < 0.01$).

Grain yield

The grain yields of selected aromatic mutants ranged from 6.26 to 8.09 ton ha^{-1} and from 4.72 to 6.63 ton ha^{-1} for spring and autumn crops, respectively (Tab. 3 and 4). Most of the mutants (except autumn-grown AM-420 and AM-426) produced higher grain yield than wild type Kuanfu waxy aroma. The variance analyses demonstrated that there were significant differences in yield components of panicle number per plant, grain number per panicle, and 1,000-grain weight ($P < 0.05$) among the mutants (Tab. 2 and 3). In both spring and autumn crop seasons, most of the mutants (except autumn-grown AM-423) produced more panicles per plant than Kuanfu waxy aroma (Tab. 2 and 3). Significant variations in the number of spikelets per panicle and the percentage of fertility per panicle ($P < 0.05$) were also obtained from the tested mutants (Tab. 2 and 3). In the spring crop season, mutants AM-423, AM-424, AM-425, AM-430 and AM-433 produced more spikelets per panicle than Kuanfu waxy aroma (Tab. 2). In the autumn crop season, mutants AM-423, AM-424, AM-425, AM-430 and AM-433 produced more spikelets per panicle than Kuanfu waxy aroma (Tab. 3). Some of mutants also exhibited higher percentages of fertility per panicle than Kuanfu waxy aroma in both seasons (Tab. 2 and 3). As for the 1000-grains weight, only mutant AM-422 showed heavier grains weight than Kuanfu waxy aroma in both crop seasons (Tab. 2 and 3). As for the crop season effect on grain yield, all the mutants grown in the spring crop season delivered higher grain yields ($P < 0.05$) than the same mutants grown in the autumn crop season (Tab. 2 and 3). To quantify the relationships among the examined yield components and grain yield, the correlation analysis was applied to the obtained data (Tab. 4). Correlation coefficients between the examined yields and yield ranged from 0.003 to 0.620. Overall, significant correlation coefficients were found for % fertility per panicle vs. grain yield ($r = 0.620$, $P < 0.01$) and panicles per plant vs. grain yield ($r = 0.340$, $P < 0.05$).

Discussion

The awn in cultivated rice is an undesirable trait that causing problems during harvesting and husking (MINAEI et al., 2007; HU et al., 2011). Therefore, de-awning is the first objective in our Kuanfu waxy aroma improvement program, and the results indicate that the NaN_3-induced mutation is an effective approach to remove undesirable awn characteristic from Kuanfu waxy aroma grain (Tab. 1). Another drawback of Kuanfu waxy aroma is its relative taller plant height (about 1.3 m). Nevertheless, all the selected mutants showed reduced plant height compared to Kuanfu waxy aroma in both crop seasons (Tab. 2 and 3).

Changing from waxy rice to non-waxy rice is a crucial criterion in determining whether the derived mutants are accepted and consumed as a daily staple by the general public in Taiwan. Therefore, selection of mutants with non-waxy endosperm is also one of our breeding objectives. As shown in Tab. 1, all the selected mutants derived from Kuanfu waxy aroma exhibited non-waxy type endosperm, each with different levels of grain amylose contents. However, breeding a rice variety with non-waxy endosperm does not guarantee that it will be accepted by the rice consumer. The consumers' choice of a given rice variety is largely based on its eating quality, which covers various grain characteristics such as grain protein and amylose contents and sensory properties (BHONSLE and SELLAPPAN, 2010; ZHU et al., 2010). The milled rice with amylose content below 20% is generally moist, soft and sticky once cooked, while the high amylose rice (25-30%) tends to absorb more water and have a fluffy texture after cooking. On the other hand, the milled rice with high protein content tends to reduce water absorption and starch swelling, and therefore is less sticky when cooked. As shown in Tab. 1, the high eating quality reference variety TNG71 had the grain amylose and protein contents of 18.7% and 5.83%, respectively. Several derived mutants including AM-426, AM-430 and AM-433 had the grain amylose and protein contents comparable to that of reference variety TNG71 (18.7%) (Tab. 1).

Aroma is a much valued quality factor for rice. Various volatile aroma compounds, with 2-acetyl 1-pyrroline being the principal aromatic compound (SAKTHIVEL et al., 2009), have been identified in cooked rice grains of varieties originated from diverse regions (JEZUSSEK et al. 2002). A 2AP quantification using gas chromatography-mass spectrometer is available but the assay requires large tissue samples, and is expensive and time consuming (HIEN et al., 2006). Thus, in this study, a simple and fast KOH sensory test (SARHADI et al., 2008; LESTARI et al., 2011) was used to evaluate the aroma sensory scores

Tab. 1: Awn, amylose contents, protein contents, aroma sensory scores, and palatability scores as well as their respective results from variance analyses of the tested aromatic mutants, their wild type variety Kuanfu waxy aroma and a non-waxy white-pericarp aromatice rice variety TNG71.

Mutant	Awn	Pericarp color	Amylose content %	Protein content %	Aroma sensory score	Palatability Score
AM-419	-	Reddish-brown	11.9d	7.11a	1.00de	65.32±de
AM-420	-	Reddish-brown	13.69cd	7.07a	1.17 cd	63.70e
AM-422	-	Reddish-brown	21.02a	6.72±bc	1.50ab	66.08cd
AM-423	-	Reddish-brown	18.85b	7.01a	0.67f	64.73e
AM-424	-	Reddish-brown	19.37a	7.14a	1.17cd	65.71de
AM-425	-	Reddish-brown	14.95c	6.33d	1.33bc	70.45±b
AM-426	-	Reddish-brown	18.14b	6.67c	1.17cd	67.78bc
AM-427	-	Reddish-brown	20.99a	6.97ab	1.00de	64.35e
AM-430	-	Reddish-brown	18.59ab	5.52f	0.83ef	73.75a
AM-433	-	Reddish-brown	19.30a	6.77bc	0.50f	66.01cd
TNG71	-	white	18.70b	5.83e	1.17cd	69.32b
Kuanfu waxy aroma	+	Reddish-brown	Waxy	Nd	1.83a	Nd
Analysis of variance						
Mut MS			16.41	0.81	0.69	22.49
Error MS			2.15	0.02	0.11	0.72
F value			7.63**	40.50**	6.27**	31.24**

**, Significant at P = 0.01.
Nd, not determined.
Values followed by different superscript letters in each column are statistically significantly different at P = 0.05.

Tab. 2: Various agronomic characteristics and their respective results from variance analyses of results of the tested mutants, wild type variety Kuanfu waxy aroma and non-waxy white-pericarp aromatic variety TNG71 grown in spring crop season.

Mutant	Plant height (cm)	Panicles plant^{-1}	% fertility panicle^{-1}	Spikelets panicle^{-1}	1000-grain weight (g)	Yield (ton ha^{-1})
AM-419	93.01g	14.63bc	88.91abc	87.06ef	27.86cde	6.95bcd
AM-420	93.17g	15.90ab	89.26abc	91.93de	28.34c	8.09ab
AM-422	97.17f	14.42bc	88.09abcd	78.16fg	30.21b	6.57cd
AM-423	102.17d	10.53def	85.37bcd	134.25ab	25.05f	6.92cd
AM-424	109.67c	10.07ef	89.79abc	128.44bc	27.04de	7.59bc
AM-425	120.11b	11.41de	92.32a	122.91c	28.16cd	7.99ab
AM-426	97.52ef	17.55a	90.04ab	69.04g	26.82e	6.36d
AM-427	94.17fg	16.53ab	87.72abcd	82.65ef	24.02gf	6.26d
AM-430	122.50b	11.26de	90.82ab	140.07ab	22.97±gh	7.31±bcd
AM-433	121.33b	12.65cd	87.35abcd	129.91bc	22.69h	7.15bcd
TNG71	103.17c	17.62a	82.01d	124.18c	23.07gh	8.97a
Kuanfu waxy aroma	140.83[a]	8.21f	83.63d	98.75d	28.77c	4.18e
Analysis of variance						
Mut MS	647.78	46.41	53.22	2917.16	49.84	6.57
Error MS	5.09	3.45	24.98	58.26	0.87	0.85
F value	127.27**	13.44**	2.13**	50.07**	57.12**	7.71**

**, Significant at P = 0.01.
Values followed by different superscript letters in each column are statistically significantly different at P = 0.05.

Tab. 3: Various agronomic characteristics and their respective results from variance analyses of variance analyses of the tested mutants, wild type variety Kuanfu waxy aroma and non-waxy white-pericarp aromatic variety TNG71 grown in autumn crop season.

Mutant	Plant height (cm)	Panicles plant^{-1}	% fertility panicle^{-1}	Spikelets panicle^{-1}	1000-grain weight (g)	Yield (ton ha^{-1})
AM-419	82.21g	17.32b	76.37bc	83.66efg	25.37e	6.17bc
AM-420	90.53f	12.31cd	70.32ef	88.7ef	26.85d	4.72e
AM-422	93.17f	13.82c	87.58a	74.66fgh	30.01b	5.96bcd
AM-423	97.21e	9.45e	73.88±cde	180.28a	23.88fg	6.63ab
AM-424	102.67d	10.37de	67.13fg	154.31bc	24.25fg	6.23bc
AM-425	107.17c	10.31de	70.55def	142.97bcd	27.13d	6.12bc
AM-426	83.67g	21.22a	71.11def	58.83h	24.94fg	4.85de
AM-427	84.67g	21.93a	75.56bcd	65.98gh	23.36g	5.45cde
AM-430	111.52b	9.70e	63.85g	159.69b	21.81h	5.72bcd
AM-433	105.17cd	11.45de	79.16b	125.83d	22.07h	5.53bcd
TNG71	98.11e	14.27c	76.42bc	137.27cd	23.89fg	7.64a
Kuanfu waxy aroma	129.02a	9.53e	75.69abcd	95.72e	28.58c	4.93de
Analysis of variance						
Mut MS	519.11	92.88	227.76	7760.24	43.64	3.63
Error MS	3.60	3.55	15.47	197.35	0.77	0.78
F value	144.35**	26.17**	14.72**	39.32**	56.98**	4.64**

**, Significant at P = 0.01.
Values followed by different superscript letters in each column are statistically significantly different at P = 0.05.

Tab. 4: The correlation coefficients, across all non-waxy aromatic mutants and TNG71, for the tested grain amylase contents, grain protein contents, aroma sensory scores and palatability scores.

Grain trait	1000-grain weight	% fertility panicle^{-1}	Spikelets panicle^{-1}	Yield
Panicles plant^{-1}	-0.203	0.085	-0.550**	0.340*
1000-grain weight		0.311	-0.444**	0.003
% fertility panicle^{-1}			-0.264	0.620**
Spikelets panicle^{-1}				0.144

*,**, Significant at 0.05 and 0.01 levels, respectively.

of milled rice grains of selected mutants by a panel (Tab. 1). The results indicate that significant differences in the sensory score ($P < 0.05$) existed among the tested aromatic mutants, with Kuanfu waxy aroma, mutant AM-422 (1.50) and AM-425 (1.33) showing high sensory score than reference variety TNG71 (1.17).

The palatability of cooked rice, which is comprehensively determined by aroma, appearance, taste and texture, is a very important factor affecting the consumer acceptance. It is frequently evaluated by trained tasting panel (YOON et al., 2009; BHONSLE and SELLAPPAN, 2010; LESTARI et al., 2011). However, several instrument-based methods for evaluating the palatability of cooked rice texture have also been developed with acceptable results (ARAI and ITANI, 2000; OKADOME, 2005; YOON et al., 2009). In Taiwan, the instrument-based technique has been standardized and used to determine the palatability of newly developed rice variety before its nomenclature and release (CHEN et al., 2009). In this study, the palatability of cooked rice samples were judged by a commercially-produced tasting meter that evaluated the palatability based on the hardiness, stickiness and appearance, and subsequently provided an overall score of cooked rice samples. Mutants AM-425 and AM-430 were found to have palatability scores of 70.45 and 73.75, respectively, which were higher than the palatability score of 69.32 recorded for non-waxy aromatic variety TNG71 (Tab. 1). The mutant AM-430 also had grain amylose and protein contents very close to TNG71.

The contents of amylose and protein are two major components influencing the palatability of cooked rice (ZHU et al., 2010). Therefore, the correlation analyses between the tested palatabilities of cooked rice grains and related grain quality traits across all mutants were conducted, but the statistical analyses indicated that only the grain protein content was negatively correlated to palatability (r = 0.929, significant at P = 0.01) (data were not shown). Similar finding was also reported by LESTARI et al. (2009). On the other hand, negative but insignificant correlation (r = -0.311) was found between grain amylose content and palatability (data not shown). This result is not unexpected because the existence of significant variation in grain amylose content (ranged from 11.69 to 21.02) among the tested mutants (Tab. 1). Moreover, selecting a proper water and grain ratio is important to obtain an optimum cooked rice texture for a given rice variety. The high amylose rice grains generally require more water for achieving optimum eating quality (SRISAWAS and JINDAL, 2007). But, in the present study, a fixed amount of water and grain ratio (30 g rice grains/40 ml water) was given to all the rice mutants that differing in grain amylose content. This result may affect the palatability scores of cooked rice to some extent.

As for the grain yield, aromatic rice varieties generally have fewer numbers of panicles and produce lower grain yield comparing to non-aromatic rice varieties. In the present study, all the selected aromatic mutants showed considerably higher grain yield than their wild type variety Kuanfu waxy aroma in both spring and autumn crop seasons. These results are mainly resulted from the improved % fertility per panicle (Tab. 2 and 3). Correlation analysis ($r = 0.620$, $P < 0.01$), across all the tested accessions in two crop seasons, confirms this finding (Tab. 4). However, some of the mutants (e.g., AM-420, AM-426 and AM-427) did show unstable yield responses when they were grown in autumn crop season (Tab. 2 and 3). The autumn-grown AM-420 exhibited significant declines in all the examined yield components comparing to spring-grown AM-420, and resulted in a substantial yield decline. JENG et al. (2006) reported that the low air temperature tended to decrease the number of spikelets per panicle and the % fertility per panicle. The low air temperature and insufficient interception of solar radiation during autumn crop season would further decrease the post-heading accumulated dry matter, and therefore cause a significant yield decline. However, the increased panicles per plant ($r = 0.340$, $P < 0.05$) is also a factor for improved grain yield in the tested mutants (Tab. 4). As shown in Tab. 1-3, the mutants AM-425 and AM-430, which scored relatively higher in aroma sensory and palatability tests, consistently produced significantly higher grain yield than Kuanfu waxy aroma in both spring and autumn crop seasons. However, the grain yield of mutant AM-430 was slightly lower than the reference variety non-waxy aromatic TNG71, even though AM-430 had greater palatability score (73.75) than TNG71 (69.32).

In conclusion, our results confirm the effectiveness of NaN_3-induced mutation on de-awning of the non-waxy red-pericarp mutants derived from awned rice variety Kuanfu waxy aroma. NaN_3-induced mutation also reduced the plant heights of selected mutants. Significant variations on aroma sensory and palatability scores existed among the tested mutants, with mutant AM-425 having aroma sensory and palatability scores slightly higher than reference variety TNG71. Large variations in the number of panicles per plant, number of spikelets per panicle, percentages of fertility per panicle, 1000-grain weights and grain yields were also observed among the tested mutants grown in the spring and the autumn seasons. The red-pericarp aromatic mutants AM-425 and AM-430 with waxy endosperm produced slightly lower grain yield than TNG71 in both spring and autumn crop seasons. These two mutants produced more yields than Kuanfu waxy aroma. Therefore, mutants AM-425 and AM-430 can be grown and the produced rice grains can be milled and used as a staple food for daily consumption. Additionally, the bran fraction milled off from these red-pericarp rice grains can be used to produce high-value nutraceutical supplements.

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