8. Thailand

Rice Mutation Breeding for Various Grain Qualities in Thailand

S. Taprab, W. Sukviwat, D. Chettanachit, S. Wongpiyachon and W. Rattanakarn

Bureau of Rice Research and Development, Rice Department,
Ministry of Agriculture and Cooperatives, Thailand

8.1 Introduction

Rice breeding through mutagenesis in Thailand had been continuously done for long period of time. Recommended varieties of mutated glutinous rice (RD6) and RD 15 an earlier matured mutant of KDML 105 were released. They are photoperiod sensitive varieties that can be grown once a year in Thailand. RD10 was later a glutinous mutated variety released. It was a photoperiod insensitive variety that can be grown more than one crop per year. Those released mutants were purposed for higher yield per unit area. Rice breeders are consequently carrying on mutation breeding for better grain quality. We reviewed previous mutation breeding activities in Thailand prior to FNCA Mutation breeding project. The results showed some success in improving grain qualities of various purposes. Seeds of recommended varieties were widely used for mutation induction through gamma ray and fast neutron irradiations. We have joined FNCA Sub-project on improvement of composition or quality in rice since 2007. Using nuclear technique to induce mutation in rice for various grain qualities was our breeding program proposal. We have just started by mutation induction by gamma ray irradiation in 2008. We have not joined the Ion-beam mutation breeding project.

Collaborated with FNCA Mutation Breeding Project, five years plan of rice mutation breeding for improvement of grain quality in Thailand started in 2007. Main objective is to develop mutants with various grain qualities such as amylose content, protein content and low phytic acid. Two groups of varieties used for mutation induction are low amylose content varieties (KDML105 and RD15) and high amylose content varieties (SPR1 and CNT1) including one international check variety, IR64. Gamma ray with 200 and 300Gy, and fast neutron with 20 and 30Gy are used for irradiation. KDML105 and RD15 are supreme grain quality aromatic rice in Thailand contain about 15% amylose. They can be grown only one crop a year due to their photoperiod sensitivity. Mutants with various level of amylose content retained excellent physical grain quality with good cooking quality (aroma) and photoperiod insensitivity are intended to be obtained. KTH17, SPR1 and CNT1 are high yielding varieties and high amylose content of about 27%. They have very good physical grain quality. Irradiated grains of those varieties were screened for low amylose content and low phytic acid. IR64 was used for international check variety of the FNCA mutation breeding project.

8.2 Materials and Methods

Rice seeds of varieties: KDML105, RD15, KTH17, SPR1, CNT1 and IR64

Mutation induction: -200 and 300Gy of Gamma ray irradiation,
-20 and 30Gy of fast neutron irradiation

Grain analyses: -IRRI's standard protocol for amylose content analyses,
-SDS-PAGE technique (Laemmli, 1970) for protein and its component analyses,
-Screening technique developed by Victor Raboy (2005) for low phytate analyses

Mutant selection: -Pedigree selection method

Seeds had been irradiated and M_1 obtained in Wet season 2007. M_2 plants of KDML105 and RD15 had been grown in Dry season 2008 where their wild types could not flower. During this period, M_2 plants could be selected for photoperiod-insensitive mutants. Then, M_3 seed of 200 mutant lines had been separately harvested as single hill per line. Photoperiod sensitivity is an obvious trait and easy to be selected in dry season whereas long day period presented. Those 200 photoperiod-insensitive mutants were continuously screened for important agronomic traits and to be analysed for amylose content. They were again tested for photoperiodism, tillering ability and maturity in Dry season 2009. A half of single hill harvested M_4 seeds had been grown in Wet season 2009 and another half of seed had been analyzed for amylose content. In wet season, flowering time of the mutants were earlier than their wild types but not much differ in plant height. Expected higher amylose content with aroma would be identified from those mutants. While, lower amylose mutants would be used for breeding germplasm and for special purposes. Selected M_5 mutants had been grown in Dry season 2010 and M_6 and M_7 seeds had been grown in Wet season 2010 and 2011. In 2008, we developed simple technique for amylose content determination on breeding lines and distributed to FNCA members.

While amylose content was being analyzed, we developed a technique for protein analysis. Protein extraction technique reported by Iida et al. (1993) and Tanaka et al. (2004) had been modified and tested for indica rice protein analysis. Extracted proteins such as globulin, albumin, prolamine and glutelin from KDML105 and Koshihikari had been identified using SDS-PAGE technique (Laemmli, 1970).

In case of low phytic acid mutants, 2 mutant lines are going to be tested for bioavailability in artificial intestine digestion. New irradiated populations were screened for low phytic acid mutants.

8.3 Results

Wet Season 2010 and Dry Season 2011

8.3.1 Protein component analyses

Using SDS-PAGE technique adapted from Laemmli (1970) for protein and its component analyses to determine total protein, albumin, globulin, glutelin and prolamine of KDML105 and Koshihikari. The result showed clear bans of albumin, globulin and glutelin but not of prolamine (Figure 1). We could distinguish KDML105 from Koshihikari by their pattern of protein components on SDS-PAGE algarose gel (Figure 2).

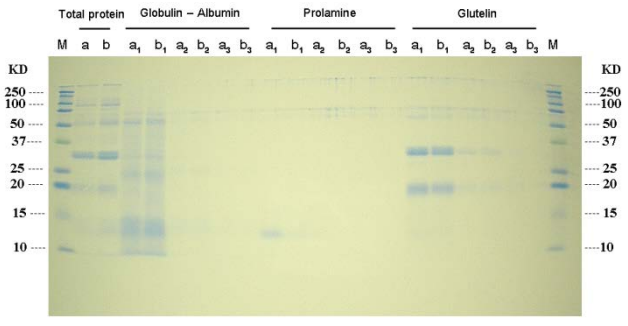

Figure 1. SDS-PAGE analyses on total protein, globulin, albumin, prolamine and glutelin of rice variety, KDML105.

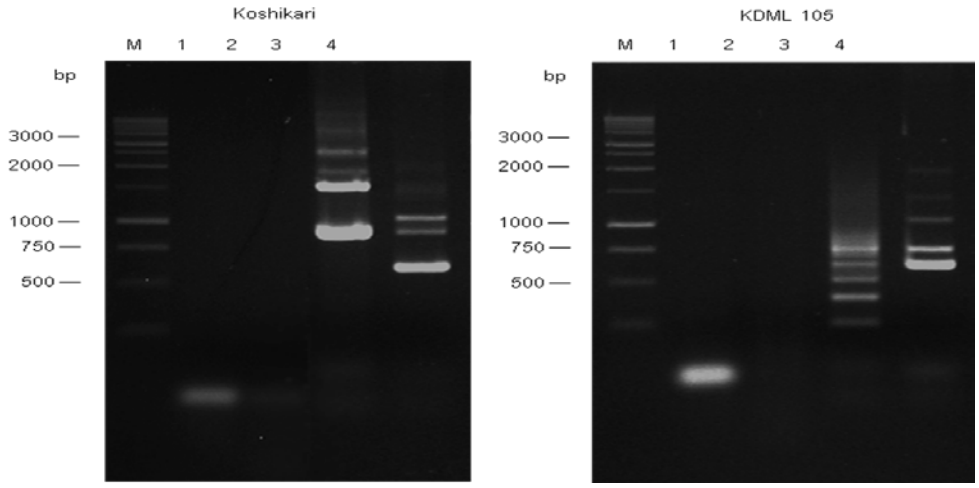

Figure 2. Agarose gel electrophoresis of Koshihikari and KDML 105, lane M, DNA marker (GeneRuler™ 1 kb DNA Ladder, Fermentas), lane 1, the PCR product amplified from genomic DNA with the primer set F2 and F8, lane 2, the nested RT-PCR product amplified with the primer set F2 and F8, lane 3, the PCR product amplified from genomic DNA with the primer set FC and F5, lane 4, the nested RT-PCR product amplified with the primer set FC and F5.

Molecular cloning of Glu-B from KDML105 was done by Nested reverse transcriptase-mediated PCR. The cDNA was made from total RNA of 20 days seedling using RetroTools® Two Step Kit (BIOTOOLS B&M LABS., S.A.) and random hexamers ($pd(N)_6$)as primers for 1st – Step Reverse Transcription (RT). The second round F2(5'-GTTTTGGAACGTTAATGCCCATAG -3') -F8(5'-ATGAGCACCAAAAGATCCAC-3') primers were used to amplified the read through LCC1 and the PCR product amplified from LCC1 genomic DNA with the primer set FC (5'-CTCCTAGATATCAACAACAGAC -3') and F5(5'- AGTTGTTGCTCTATATGTCTTCGACT-3'). Then the PCR product was process to insert in pGEM®-T Easy(Promega, USA). Nucleotides sequences analysis of Glu-B product were done by ABI 3130 automatic sequencer (Applied Biosystems) using BigDye® Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems). Results show the nucleotide sequences with 600 bps are homologous to *Oryza sativa* glutelin precursor (GluB4) gene exon 1 through 4 and complete cds.

8.3.2 Mutant lines selection

Since 2008, mutant lines with photoperiod insensitiveness had been achieved. In the year 2010, two hundreds M_5 mutant lines of both KDML105 and RD15 derived from 2008 had been analyzed for amylose content and protein components. Low amylose mutant lines had been achieved from KTH17 which is originally high amylose content local variety. Various amylose content mutant lines from original low amylose content varieties KDML105 and RD15 had been obtained. Some of those mutants contained lower and higher amylose content than did of their wild types. 293 mutant lines of RD15 showed wide variation of amylose contents from 10.58 – 28.08 % (Figure 3). 241 mutant lines showed less variation of amylose contents than did of RD15, from 11.96-16.02 % (Figure 4). 191 mutant lines of KTH17 showed wide variation of amylose contents from 13.30 – 29.98 % (Figure 5). Protein contents of those mutant lines are being analyzed.

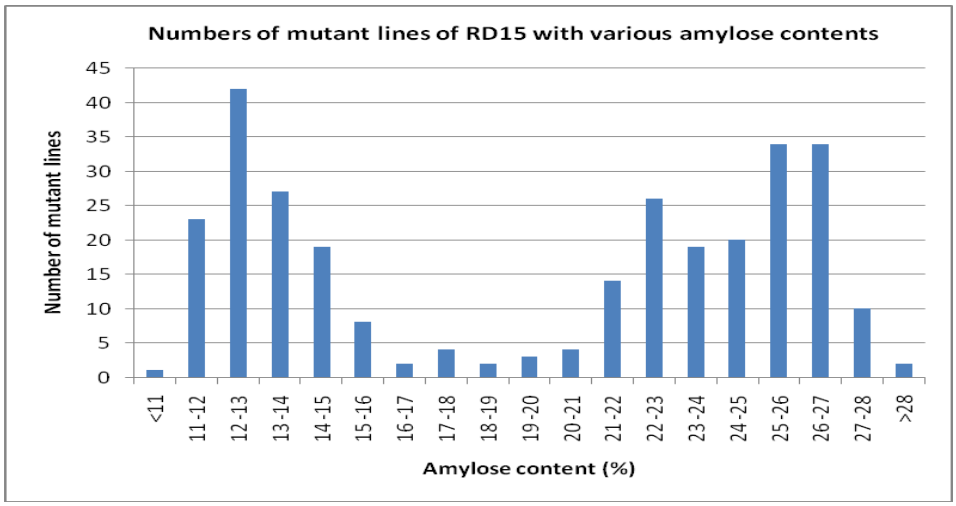

Figure 3. Number of mutant lines of RD15 with various amylose contents.

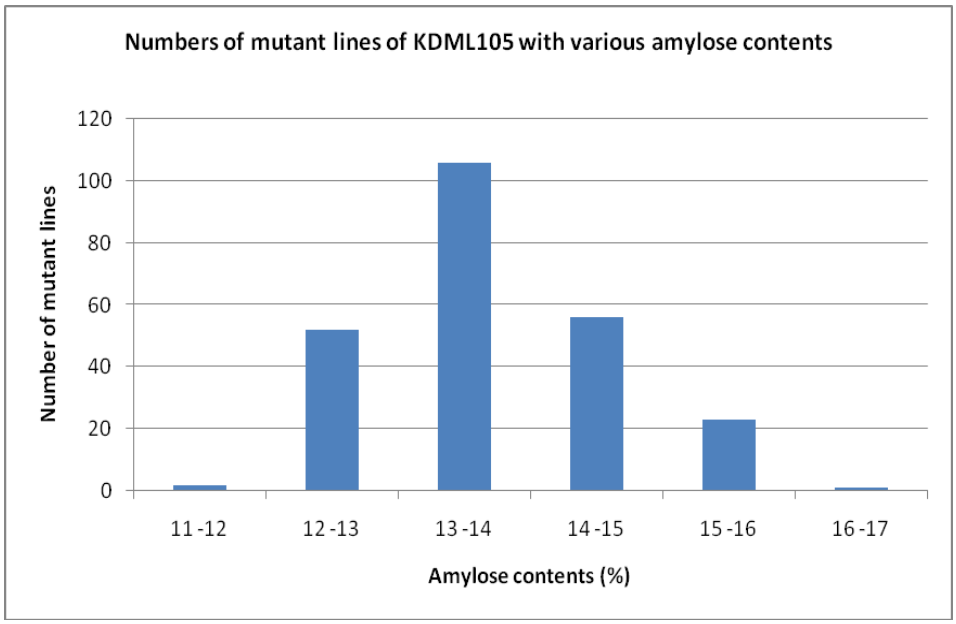

Figure 4. Numbers of mutant lines of KDML105 with various amylose contents.

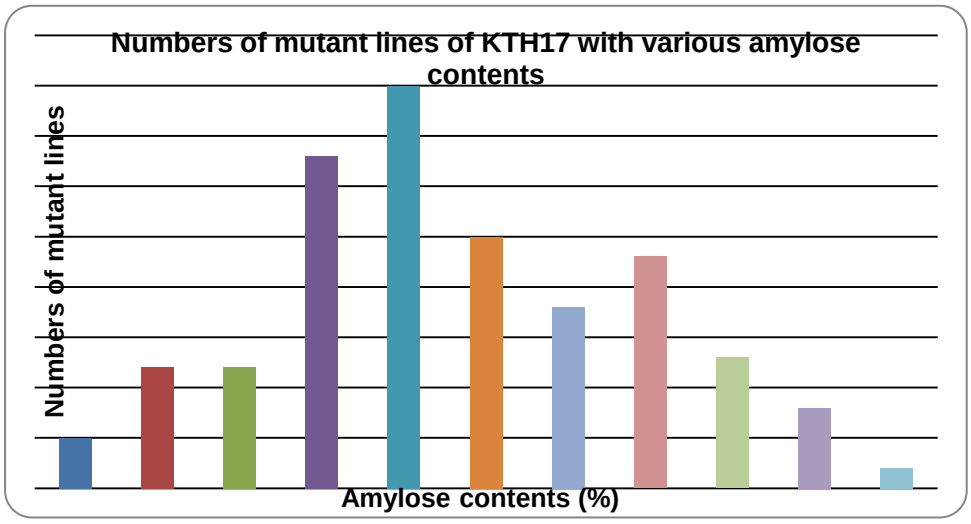

Figure 5. Numbers of mutant lines of KTH17 with various amylose contents.

When photoperiodism and dosages of gamma ray were considered, it was found that 20Kr (200Gy) and 30Kr (300Gy) induced different frequency of mutation on amylose content. Photoperiod sensitive mutants of RD15, KDML105 and KTH17 mostly contained lower amylose than wild types did. From RD15, photoperiod sensitive mutants derived from 20Kr irradiation contained lower amylose while 30Kr mutants contained slightly higher amylose (Figure 6). Highest frequency of 20Kr mutants of RD15 was 45 lines with 12% amylose content while 30Kr mutants was 43 lines with 13% amylose content. From KDML105, photoperiod sensitive mutants derived from 20Kr and 30Kr were similar distribution to RD15 mutants. But 20Kr created wider range of amylose with 1 line contained 18% amylose while 30 Kr had highest amylose of 15%. Both 20Kr and 30Kr had highest frequency at 13% amylose content with 45 and 61 lines, respectively (Figure 7). Similarly, high amylose variety KTH17 had been also induced lower amylose mutants. KTH17 wild type contained 27-28% amylose while its mutants derived from both 20 Kr and 30 Kr contained lower amylose. Their highest frequencies are 24 and 16 lines at 23% amylose content (Figure 8). In contrast, photoperiod insensitive mutants showed different distribution from photoperiod sensitive mutants. From RD15, lower dose of 20Kr induced higher amylose content than higher dose of 30Kr. It produced very higher amylose content up to 28% mutants compared to its 15% amylose wild type. It had highest frequency at 25% amylose with 32 mutant lines. The dose of 30Kr induced lower amylose to the lowest of 10% amylose content. Its highest frequency was 40 mutant lines at 12% amylose content (Figure 9).

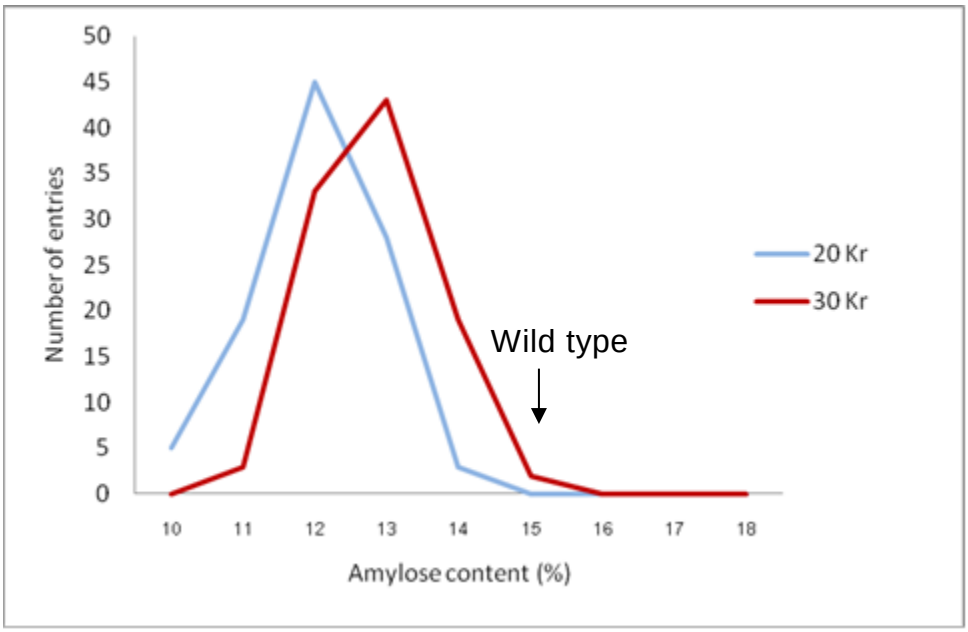

Figure 6. Amylose content (%) of RD15 photoperiod sensitive mutants irradiated with Gamma Ray 20Kr and 30Kr

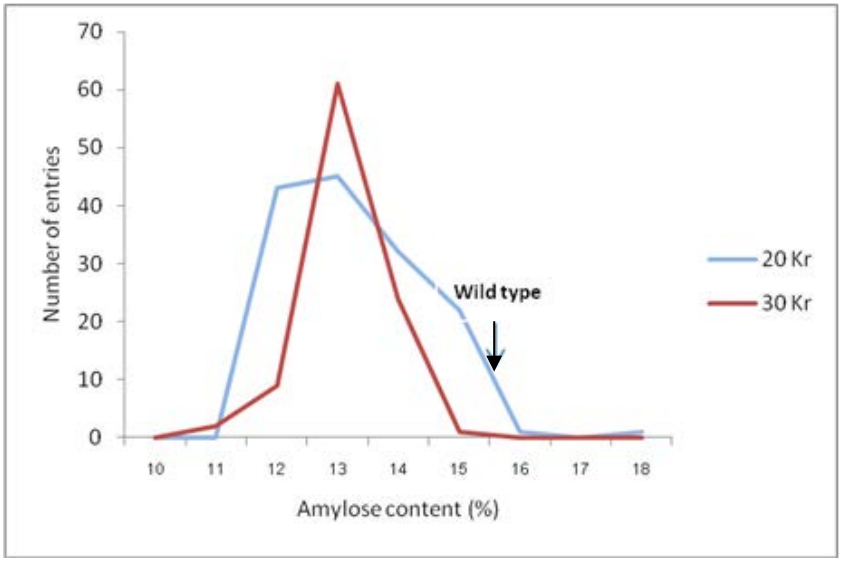

Figure 7. Amylose content (%) of KDML105 photoperiod sensitive mutants irradiated with Gamma Ray 20Kr and 30Kr

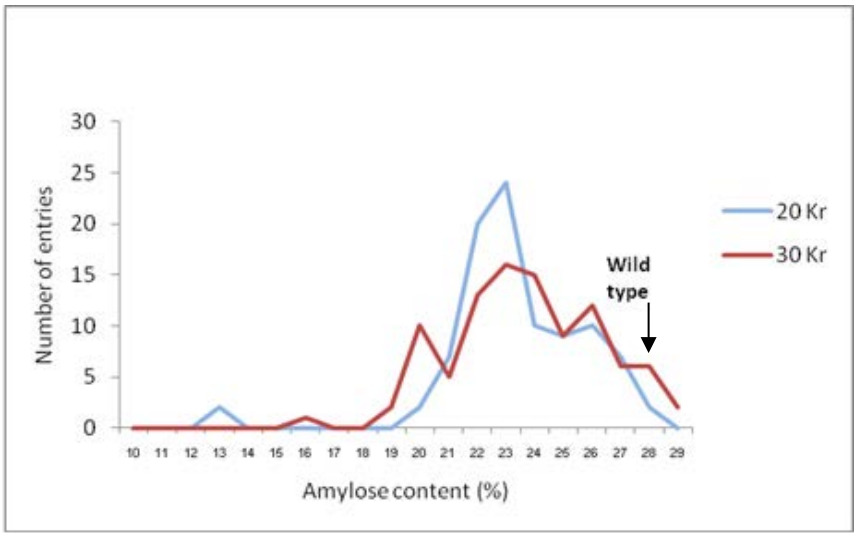

Figure 8. Amylose content (%) of KTH17 photoperiod sensitive mutants irradiated with Gamma Ray 20Kr and 30Kr

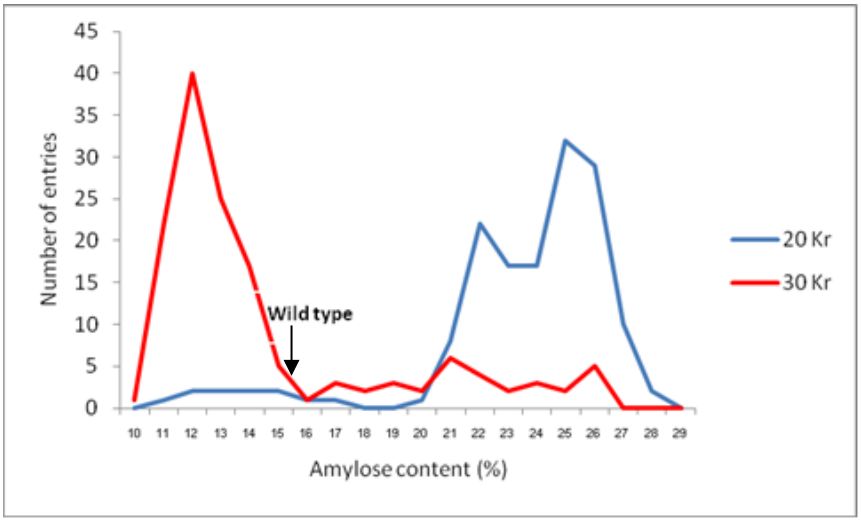

Figure 9. Amylose content (%) of RD15 photoperiod insensitive mutants irradiated with Gamma Ray 20Kr and 30Kr

8.3.3 Grain analyses of mutants

Twenty five M_7-mutant lines of KDML105 and RD15 had been randomly selected to be analyzed on grain physical properties and chemical quality. Grain size had been measured on brown rice in length, width and thickness. Grain shape was determined by Length/Width ratio (L/W). The ratio of L/W that was greater than 3.0 indicated slender grain shape. Visually observation on chalkiness, pericarp color and hull color had been also practiced. Means and standard deviations of mutants and wild types were shown in Table 1.

Table1. Grain physical properties of 25 M_7 mutants of KDML105 and 25 M_7 mutants of RD15 compare to its wild type.

Designation	Size of brown rice (mm)			L/W ratio	Chalkiness	Pericarp color	Hull color
	Length	Width	Thick				
M7-KDML105 mutants	7.16 ± 0.08	2.05 ± 0.02	1.68 ± 0.03	3.34 ± 0.05	dull	White	Straw
M7-RD15 mutants	7.46 ± 0.09	2.14 ± 0.02	1.75 ± 0.03	3.48 ± 0.04	dull	White	Straw
KDML105 (wild type)	7.40	2.12	1.76	3.49	dull	White	Straw
RD15 (wild type)	7.49	2.11	1.74	3.55	dull	White	Straw

It had been found that all mutants shown dull endosperm as well as their wild type. Chalkiness could not be identified in these samples. We have an experience that aromatic rice especially KDML105 and RD15 shown dull endosperm when grown in acid soil. Since our experimental field was acid sulfate soil, dull endosperm occurred. All mutants had white color pericarp and straw color hull as well as their wild types. Mutants of KDML105 had shorter grain length (7.16mm) than their wild type (7.40mm) and shown slightly bold shape (3.34 L/W) compared to their wild type (3.49 L/W). Mutants of RD15 shown almost same grain length but they were wider and thicker that gave smaller L/W ratio. This indicated that mutants of RD15 had bigger grain size than their wild type (Table 1).

Grains from 25 mutants of KDML105 and 25 mutants of RD15 that had been divided to be analyzed on physical properties were synchronously analyzed on chemical quality. Amylose content, Gel consistency, Alkali spreading value, Elongation ratio were important chemical quality that had been analyzed. Total protein (%) was analyzed by Kjaldal Method. Aroma or odor of milled rice had been tested by human sensory test. Aroma expressed in 4 levels of sensory test are 0 = odorless (not aroma), 1 = mild aroma, 2 = aroma and 3 = strong aroma. 2-Acetyl-1-pyrroline (2 AP) (Fig 10) was also analyzed through gas chromatography. **2-Acetyl-1 pyrroline** is a pyrroline that is 1-pyrroline in which the hydrogen at position 2 is replaced by an acetyl group. It is an aroma and flavor compound present in jasmine rice and basmati rice. It is responsible for the 'popcorn' aroma in a large variety of cereal and food products. It is one of the key odourants of the crust of bread and considered to be responsible for the cracker-like odour properties. In bread, it is primarily generated during baking but amounts are influenced by ingredient composition and fermentation conditions.

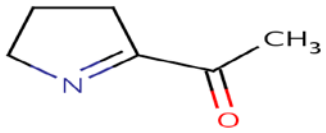

Figure 10. Structural formula of 2-Acetyl-1-Pyrroline

It found that M_7 mutants of KDML105 had low amylase content similar to their wild type. They contained amylose within range of 15.03 to 16.06% of amylose or 15.50≠0.2% in average, while their wild type contained 15.42% amylose. In contrast, M_7 mutants of RD15 contained higher amount of amylase than did of their wild type. The mutants had wider range of amylose content. Their amylase content varied from 15.40 to 27.79% while their wild type contained 15.55% of amylase. Out of these 25 mutants, there were three mutants that contained 15.40, 15.87 and 15.93% of amylose. Mutants of RD15 had harder gel than mutants of KDML105 and their wild type. Mutants of KDML105 had softer gel than their wild type. Alkli spreading value of all mutants was almost not differed from their wild type as well as elongation ratio. Total protein content of mutants averagely higher than their wild type in both KDML105 and RD15. But it was still in normal level of those varieties. Unfortunately, we could not find low protein content mutants in these samples. Mutants of KDML105 contained 9.1% while those of RD15 contained 9.8% protein. Aroma tested by smelling shown different levels of aroma scented between KDML105 and RD15 mutants. Mutants of KDML105 had strong aroma as well as their wild type with score of 3. Mutants of RD15 showed aroma in score 2 of sensory test level as well as their wild type. When 2-acetyl-1-pyrroline had been detected it was found that all mutants of KDML105 contained 2 AP in higher amount than their wild type in both milled rice and brown rice. Brown rice and milled rice of KDML105 mutants contained 2.00 and 2.32ppm of 2 AP, respectively. Their wild type KDML105 contained 2.06 and 2.24ppm of 2 AP in its brown rice and milled rice. We could detect 2 AP in only 3 mutants of RD15 that contained low amylase content. The mutants of RD15 those contained high amylose were not aroma with 2 AP detection, but sensory test detected aroma from those mutants. It would be an expression of other volatile compound. One mutant with 15.87% amylase content showed 2.18 and 2.16ppm 2 AP in its brown rice and milled rice which was almost similar to its wild type (2.99 and 2.06ppm 2 AP) (Table 2). It was very rare opportunity to find aromatic rice with high amylose content in our breeding material. This will be new chance for us to produce various products from our aromatic mutants for world market.

Table 2. Chemical quality of M7 mutants of KDML105 and RD15 compare to their wild type

Designation	amylose (%)	Gel[1] (mm)	Alkali	E.R.[2]	Protein (%)	Aroma[3]	2 AP[4] (ppm) Milled Rice	2 AP[4] (ppm) Brown Rice
M7-KDML105 mutants	15.5±0.2	72.9±3.9	6.9±0.28	1.6±0.03	9.1±0.39	2.7±0.48	2.32±0.35	2.00±0.28
M7-RD15 mutants	25.6±3.8	54.0±9.9	7.0±0.0	1.7±0.03	9.8±0.62	2.0±.00	0.1±0.43	0.1±0.44
KDML105 (wild type)	15.42	69	7.0	1.66	8.77	3	2.24	2.06
RD15 (wild type)	15.55	71	7.0	1.66	9.24	2	2.06	2.99
	Gel[1]	= Gel consistency			Aroma[3]	= aroma by sensory test		
	E.R.[2]	= Elongation Ratio			2 AP[4]	= 2 Acetyl-1-pyrroline detection		

In other purpose, low phytate mutant lines achieved from SPR1 are being evaluated on their grain yield and other agronomic characters. Forty eight mutant lines of low phytic SPR1 are going to be examined their protein content.

8.4 Conclusion

Gamma ray irradiation can be applied for mutation induction in rice. Dosages of 20Kr (200Gy) and 30Kr (300Gy) showed advantage on mutation induction for grain quality improvement. Thai rice varieties namely, SPR1, KTH17, KDML105 and RD15 had been irradiated by 20Kr and 30Kr of gamma ray to induce mutation for various grain qualities such as phytic acid, amylose content and protein and its compositions. Amylose content had been analyzed using standard method for rice developed by IRRI. Globulin, albumin, prolamine, and glutelin were detected using SDS-page analyses. Low phytate mutants had been screened using technique developed by Raboy (2004). Low amylose mutant lines had been achieved from KTH17 which is originally high amylose content local variety. Various amylose content mutant lines from original low amylose content varieties KDML105 and RD15 had been obtained. Some of those mutants contained lower and higher amylose content than did of their wild types. Photoperiod sensitive mutants from RD15, KDML105 and KTH17 had lower amylose content than did of their wild types. Both 20Kr and 30Kr induced similar distribution of mutants in case of photoperiod sensitive. Among photoperiod insensitive mutants derived from RD15, 20Kr induced higher amylose while 30Kr induced lower amylose than its wild type. M_7 mutants shown bigger grain size but shorter length than did of their wild types. Aromatic rice with low amylose content obtained from KDML105 mutants. Newly found aromatic rice with high amylose content derived from RD15 mutants could not be detected on its 2-acetyl-1 pyrroline compound. Those mutants might contain other volatile aromatic compound.