CHAPTER II

REVIEW OF LITERATURE

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2.1 Studies on the antioxidant activity, total phenol content, total anthocyanin content and the anthocyanin compositions of the colored rice

Xia et al. (2006) performed the HPLC and LC-MS analysis on the extract black rice and identified 2 major anthocyanins cyanidin-3-glucoside and peonidin-3-glucoside. They investigated the influence of the anthocyanin-rich extract from black rice on vulnerability of advanced plaques in E-deficient mice. Apo E deficient mice (n =30; 30 wk old) were randomly divided into 3 groups: a control group (fed the AIN-93G diet), the simvastin group (simva; fed the AIN-93G diet containing simva statin or the anthocyanin-rich extract group (antho; fed the AIN-93G diet supplemented with anthocyanin-rich extract from black rice). After 20 wk of intervention, the plaque area that developed in the brachiocephalic artery of mice in the antho group was smaller than that of the control mice. Both the antho and simva groups had lower frequencies of the large necrotic core and thin fibrous cap in plaques than the control group and there was an increased of Collagen I and matrix metalloproteinase-1 contents were reduced in the brachiocephalic lesion of both the antho and simva groups compared with the control group. They found out that in the antho and simva groups, mRNA levels of tissue factor and inducible nitric oxide synthase in aortae were decreased. The lipid profile was improved in the supplementation of anthocyanin-rich extract by decreasing serum triglyceride, total cholesterol and non-HDL cholesterol. Their results suggested that chronic diet intake of anthocyanin-rich extract from black rice may enhance plaque stabilization in old apoE-deficient mice.

Yawadio et al. (2007) the profile of anthocyanins and ferulic acid from black rice and pigmented brown rice, respectively from Osaka, Japan was determined using

an HPLC system. From the black and pigmented brown rice (*Oryza sativa* L. *japonica*), two anthocyanins (cyanidin-3-O-b-glucoside and peonidin-3-O-b-glucoside) and other phenolic (ferulic acid) were isolated, respectively and their complete structures were determined by spectroscopic analyses. The HPLC profile of anthocyanins extracted from black rice showed cyanidin-3-O-glucoside as the first peak (85%) and peonidin 3-O-D-glucoside as the second (15%), while that of pigmented brown rice showed ferulic acid as the first peak (85.7%) and tocols as the second (14.3%). Several tocols were isolated and identified from the unsaponifiable fractions of both the rice having some difference on their structures and amounts. The aldose reductase inhibitory activity of isolated compounds was determined and they were in the following decreasing order: cyanidin-3-glucoside >quercetin>ferulic acid > peonidin-3-glucoside >tocopherol. They concluded that all the isolated compounds showed significant inhibitory activity against aldose reductase suggesting that both the pigmented rice might contribute significantly in combating diabetic complications as health-promoting food ingredients in food processing.

Kim et al. (2008) identified and quantified anthocyanin pigments from different varieties of black, red and wild rice. The acidified methanol extraction of anthocyanin was done and identification of anthocyanin, aglycone and sugar moieties was conducted by HPLC, Ultraviolet-Visible absorption spectrophotometer and paper chromatography. By HPLC analysis, Black and wild rice gave three different types of pigments while red rice variety did not show any anthocyanins. Out of three pigments detected, cyanidin-3-glucoside (C3G) was the most abundant and another was tentatively identified as cyanidin-fructoside on the basis of spectroscopic properties.

Park et al. (2008) analyzed the anthocyanins in the Korean black rice (*Heugjinjubyeo*), quantitatively and qualitatively with HPLC and UV-Vis

spectrophotometer and found out to contain cyanidin 3-O glucoside (95%) and peonidin 3-O glucoside (5%). Investigation of the antioxidant activities of the anthocyanin extract using various *in vitro* methods was conducted. The 100g/ml concentration of the anthocyanin extracted exhibited 88.83% inhibition on the peroxidation of linoleic acid, 55.20% DPPH free radical scavenging activity, 54.96% superoxide anion radical scavenging activity and 72.67% hydrogen peroxide scavenging activity and also showed high ferrous ion reducing capability. Thus, from their results, they suggested that the anthocyanin extracted from black rice may be utilized as a possible antioxidant agent against ROS.

Lee (2010) determined the anthocyanin content for 10 Korean black rice (*O. sativa*) varieties, Jeonbuk 2, Heugjinjubyeo, Heugnambyeo, Sanghaehyanghyeolna, Josaengheugchal, Dragon Eyeball 100, Heughyang, Jeonbuk 1, Heuggwang and Suwon 512. The primary constituents including protein and oil were evaluated. Anthocyanins, cyanidin-3-O-glucoside and peonidin-3-O-glucoside, were isolated and elucidated using reverse phase C18 chromatography, nuclear magnetic resonance (NMR) spectroscopy and high performance liquid chromatography (HPLC) with diode array detection and electrospray ionization/mass spectrometry (DAD-ESI/MS). Anthocyanin showed significant differences and cyanidin-3-O-glucoside (52.1±6.3-1,601.0±8.5 μg/g) exhibited a markedly higher content than peonidin-3-O-glucoside (82.6±1.2 μg/g). Among varieties, Heugjinjubyeo showed the highest anthocyanin (1: 1,601.0±8.5, 2: 82.6± 1.2 μg/g), whereas Heughyang was the lowest (1: 52.1±6.3 μg/g). Protein and oil exhibited the minor differences and their contents ranged from 10.7±0.0 to14.1±0.1% and from 2.1±0.0 to 2.9±0.0%. Overall results suggested that anthocyanin can be a key factor in functional property of black rice and could be an important source concerning nutritional value.

Muntana and Prasong (2010) in their study, different Thai rice white color, red color and black rice cultivars were taken. Their total phenol content using Folin-Ciocalteau method and antioxidant activities using thiocyanate method and DPPH free radical-scavenging assay were investigated. Their results indicated that the total phenol content of white, red and black rice bran extract were in the range 0.8931-0.984, 1.0103- 1.0494 and 1.0810-1.2239 mg gallic acid equivalent (GAE mg^{-1}), respectively. The percentage inhibition were in the range 10.15-2068, 30-64-38.80 and 25.52-26.28 for white, red and black rice, respectively. The antioxidant activity of all the rice bran extracts indicated high antioxidant efficiency in which colored rice showed the highest that is red rice the highest followed by black rice and white rice the lowest. They suggested that the Thai rice are potential antioxidant sources.

Saenkod et al. (2013), eight rice varieties were taken for their study, two Chinese black rice - Heimi and Jing Nian, three Chinese white rice- JiNong Da, T-You 597, Xiang Wan Nuo 1 Hao, two Thai red rice – Niaow Deang and Brown Dok Kam and one Thai black rice -Nang Dum. The Heimi variety of Chinese rice showed the highest total phenol content and total flavanoid content (634.83 and 158.47 mg/kg, respectively) followed by Jing Nian variety, also from China, Dok Kam and Niaow Deang varieties both from Thailand, respectively. Highest antioxidant activity was observed in Heimi followed by Jing Nian, Dok Kam and Niaow Deang varieties. The color of Heimi and Jing Nian is black and that for Dok Kam and Niaow Deang is red, so total phenol content and total flavanoid content were correlated with the color of rice.

Moko et al. (2014) analyzed two non colored (Superwin and Cigeulis) and one colored rice red (rice variety) from Minahasa Regency, North Sulawesi, Indonesia and determined the phytochemicals and antioxidant properties as natural antioxidant

sources. They determined the antioxidant properties by means of radical, 1,1-diphenyl-2-picrylhydrazyn (DPPH) assay, total phenol content (TPC), total anthocyanin content, and thiobarbituric acid (TBA) assay. In the result, red variety had the highest DPPH scavenging radical activity (88.29 ± 5.62%), with the lowest IC50 value (26.26 ± 0.95 μg/ml) and highest total anthocyanin content (68.61 ± 1.98 mg/g). Thus, they showed that the colored varieties had better antioxidant properties than non colored varieties and concluded that colored varieties could be used as a natural antioxidant source.

2.2 Studies on the volatile oil compositions of the fragrant/scented rice

Yang et al. (2008a) studied the volatile profiles of cooked black rice and characterized the odor-active compounds. By using gas chromatography-mass spectrometry with a dynamic headspace system with Tenax trapping, thirty-five volatile compounds were identified from a Korean black rice cultivar. 80.1% of total relative concentration of volatiles was accounted by aldehydes and aromatics compounds. They reported a high concentration of 2-acetyl-1-pyrroline (2-AP) but exceeded only by hexanal, nonanal and 2-pentylfuran. 2-AP, guaiacol, indole and *p*-xylene largely influenced the difference between the aroma in cooked black and white rice. Their results showed that 2-AP and guaiacol were major contributors to the unique character of black rice based on odor thresholds, relative concentrations and olfactometry.

Yang et al. (2008b) studied the aroma chemistry of six distinctly different rice flavor types (basmati, jasmine, two Korean japonica cultivars, black rice, and a nonaromatic rice) was analyzed using a dynamic headspace system with Tenax trap, GC-MS, GC-olfactometry (GC-O), and multivariate analysis. They characterized 36 odorants from cooked samples out which twenty-five odorants had an intermediate or

greater intensity and were considered to be major odor-active compounds. Their odor thresholds in air were determined using GC-O, 2-Acetyl-1-pyrroline (2-AP) had the lowest odor threshold followed by 11 aldehydes, guaiacol and 1-octen-3-ol. The importance of each major odor-active compound was assessed, based on the odor thresholds and odor activity values (OAVs). Although the relative proportion varied among samples but the OAVs for 2-AP, hexanal, (*E*)-2-nonenal, octanal, heptanal, and nonanal comprised >97% of the relative proportion of OAVs from each rice flavor type. Thirteen odor-active compounds comprisesd the primary compounds explaining the differencs in aroma, they are 2-AP, hexanal, (*E*)-2-nonenal, octanal, heptanal, nonanal, 1-octen-3-ol, (*E*)-2-octenal, (*E*,*E*)-2,4-nonadienal, 2-heptanone, (*E*,*E*)-2,4-decadienal, decanal, and guaiacol amongthe six flavor types. Multivariate analysis on their study demonstrated that the individual rice flavor types could be seperated and characterized using these compounds.

Yang et al. (2008c) reported volatile compounds emanating from three cultivars- Ilpumbyeo (traditional white rice), Heugjinjubyeo (black pigmented), and Jeogjinjubyeo (red pigmented) of cooked rice milled to different degrees (0, 8, and 30% by weight) were compared to track their site of origin and the effect of pigmentation on synthesis. In dehulled, unmilled Ilpumbyeo, Heugjinjubyeo and Jeogjinjubyeo, 29, 38, and 27 volatile compounds were identified, respectively. Their results showed significant quantitative and qualitative differences among the cultivars in their volatile profiles. In Heugjinjubyeo, 2-Acetyl-1-pyrroline, guaiacol, 1-nonanol, 3-octen-2-one, 1,2-dimethoxybenzene, pyridine, and pyrrole were found and in Jeogjinjubyeo, phenylacetaldehyde only. They observed that removal of the bran, partial endosperm, and pigment qualitatively and quantitatively affected the volatile compounds formed, with certain volatiles higher in unmilled rice (0% milling),

indicating the pericarp and aleurone layer (bran) as their primary site of origin. Volatiles emanating from the 8 and 30% milled samples indicated the outer and middle endosperm (8%) and core endosperm (30%) as the primary sites of origin. Therefore, their results showed that differences in chemical composition with location within the grain appear to be responsible for the quantitative and qualitative variation in volatile synthesis.

Yang et al. (2010) took ten cultivars for their study, including six premium-quality, (*Oryza sativa* L. *japonica*cv. Hwaseongbyeo, Ilpumbyeo, Gopumbyeo, Taebongbyeo, Chucheongbyeo, and Samkwangbyeo), two waxy cultivars (*O. sativa* L. *japonica* cv. Hwaseonchalbyeo, and *O. sativa* L. *indica* cv. Hangangchalbyeo), and two black-pigmented (*O. sativa* L. *japonica* cv. Heugjinjubyeo, and Heugkwangbyeo) were grown at the National Institute of Crop Science, Suwon, South Korea in 2006. After harvest and milling, the rice was sealed in glass containers and held at −20 ◦C. The stored rice samples (100 g) were cooked in distilled water (150 mL) for30 min at 100◦C in a specially constructed 1L glass beaker with entry and exit ports, the extraction were analysed using GC-MS and the odorants were identified based on their mass spectra using NIST 02 and Wiley 7 libraries. Using authentic standards, the identification was confirmed using Kovats retention indices (RI) and odor descriptors. Twenty-one (21) and twenty three (23) odorants were detected using GC-O for cooked samples of premium-quality, waxy and black pigmented rice cultivars, respectively. They obtained hexanal as the main odorant in premium-quality and waxy cultivars, waxy cultivars had 16 times higher hexanal odor activity values than premium-quality cultivars which indicated premium-quality rice had a less overall aroma. 2-Acetyl-1-pyrroline was the main contributor to overall aroma in black-pigmented rice, followed by guaiacol. Six odor-active compounds (2-acetyl-1-

pyrroline, guaiacol, hexanal, (*E*)-2-nonenal, octanal and heptanal) contributed the most in discriminating the three types of specialty rice.

2.3 Studies on the *Pb/Ra* gene for the purple pericarp color

Zhuang et al. (1994) tagged the gene *Prp-b (Pb*) for purple pericarp at 19.0cM to RG329.

In china, according to the crosses made by Zhang et al. (1995) involving several parents with varying amounts of pigments (varying intensity of purple color) showed that the intensity of color in the plants varies, depending on the number of genes present in the F1 plants, the alleles for high pigment content being dominant to those for low pigment content, indicating additive-dominance. These authors report that two pairs of genes were found to control the pigment content in the pericarp, with high pigment content (colored pericarp) being dominant to low pigment content (light-colored pericarp). The deep purple pericarp was expressed as dominant over purple, with the light purple pericarp being dominant to nonpigmented (white), indicating that two pairs of dominant genes control black pericarp pigmentation.

Hu et al. (1996) focused on the role played by polyploidization and transposable elements in shaping the *R/B* gene family of maize, they were interested in determining if *R/B* genes existed as a gene family in rice. As a first step, they took advantage of the cloned maize *R* (*LC)* gene to isolate its homologue from rice and described the identification of two *R* genes in *Oryza sativa,* designated as *Ra* and *Rb,* that are mapped to chromosome 4 and 1, respectively. The active *Ra* gene showed similarity along its entire length with the maize *R (LC)* gene and the Antirrhinum *delila* gene and is able to induce anthocyanin expression in maize. Their data indicated that the common ancester of maize and rice may have had only a single *R*

gene and that the small *R* gene families of grasses have arisen recently and independently.

Wang & Shu (2007) reported the rice *Pb* gene controlling purple pericarp character is known to be on chromosome 4 and the purple color is dominant over white color. Using two F2 segregating populations, i.e. Pei'ai64S (white) × Yunanheixiannuo (purple) and Pei'ai 64S × Chuanheinuo (purple), they fine mapped the *Pb* gene using two F2 segregating populations. In the first-pass mapping, the *Pb*gene was located in the region downstream the SSR marker RM3820. In the fine mapping, the candidate region was saturated with the markers developed specifically for their study. The *Pb* gene was mapped within the 25-kb region delimited by the upstream marker RID3 and the downstream marker RID4. The delimited region contained two annotated genes, *Ra* and *bhlh16* (TIGR Rice Genome, R.5). The *Ra* gene is a homologue of the *Myc* transcription factor *Lc* controlling anthocyanin biosynthesis in maize and the *bhlh16* is a homologue of the *TT8* gene, which is also an Myc transcription factor gene controlling the pericarp pigmentation in *Arabidopsis thaliana*. Sequence analysis showed that the *Ra* gene of Yunanheixiannuo and Chuanheinuo had a 2-bp (GT) deletion in the exon 7 compared with those of the white rice varieties Pei'ai 64S, 9311 and Nipponbare. A CAPS marker, CAPSRa, was developed according to the GT deletion for analysis of the two F2 segregating populations and 106 rice lines. The results showed that all F2 plants with white pericarp and all non-purple rice lines (63 white and 22 red) contained no GT deletion, but all 20 purple rice lines contained the GT deletion. Their results suggested that the *Ra* gene may be the *Pb* gene and the purple pericarp characteristic of rice is caused by the GT deletion within exon 7 of the *Ra* gene.

Kim et al. (2011) assessed expression of anthocyanin in black rice cultivars using a newly designed 135 K *Oryza sativa* microarray. A total of 12,673 genes exhibited greater than 2.0-fold up- or down-regulation in comparisons between three rice cultivars and three seed developmental stages. The 137 transcription factor genes found to be associated with production of anthocyanin pigment were classified into 10 groups. In addition, 17 unknown and hypothetical genes were identified from comparisons between the rice cultivars. Finally, 15 out of the 17 candidate genes were verified by RT-PCR analysis. Among the genes, nine were up-regulated and six exhibited down-regulation. These genes likely play either a regulatory role in anthocyanin biosynthesis or are related to anthocyanin metabolism during flavonoid biosynthesis.

Rahman et al. (2013) used the purple pericarp rice*Oryza sativa* L. japonica var. Kewha and *O. sativa* L. japonica var. Heugnambyeo, while the white pericarp rice *O. sativa*L. japonica var. Hwayongbyeo, *O. sativa* L. japonica var. Ishikari, *O. sativa* L. japonica var. Ilpoombyeo, and *O. sativa* L. indica var. Kumgangbyeo were used as wild-type controls. They conducted the genetic studies to analyze the inheritance pattern of the pericarp color of rice. They visually assessed the pericarp color of matured seeds from the F1 and F2 populations for individuals with purple, brown or white seed pericarps. Evaluation of the inheritance pattern of purple pericarps, segregation analysis of the purple pericarps was carried out using F1 and a large population of F2 progeny from crosses among Oryza sativa L. japonica var. ‘Heugnambyeo’ with purple pericarps as a pollen receptor, and O. sativa L. japonica var. ‘Hwayongbyeo’, O. sativa L. japonica var. ‘Ishikari’, and O. sativa L. japonica var. ‘Ilpoombyeo’ with white pericarps as pollen donors. A total of 20 fertilized seeds for each cross were obtained and the resultant F1 seeds were grown in the field to

produce F1 plants. The F1 plants were then allowed to self-fertilize to produce F2 seeds, which were collected from a single F1 plant and grown in the field under natural conditions. The phenotypic data of the F2 segregations were documented and F3 seeds from a single panicle were harvested separately from each F2 plant at the mature stage. Segregation analysis of pericarp color was also conducted using F1 and a large population of F2 and F3 progeny from the cross between *O. sativa* L. *japonica* var. Kewha with purple pericarps as a pollen receptor (P1) and *O. sativa* L. *indica* var. Kumgangbyeo with white pericarps as apollen donor (P2). The genotype of the parents was determined using the seed pericarp phenotype of the F1, F2 and F3 populations. Genomic DNA was extracted from leaf tissues using the CTAB buffer method and the determination of allelic differences in *Pb* genes among the progeny of black pericarp rice and white pericarp rice crosses was performed based on PCR-based polymorphism of the *Ra* gene, which is a homologue of the *Pb* gene. Briefly, polymerase chain reaction (PCR) using primers for the transcriptional activator *Ra* gene (accession number U39860) was performed (forward primer 5'-GGGAGAAGCTCAACGAGATG and reverse primer 5'-GGGTGGCAGATTCATCACTT). The PCR amplified fragments were then sequenced to define the *Pb* gene. For genotype analysis, the PCR products were digested with BamH1 restriction enzyme and run on 1.2% agarose gels. The purple pericarp color in rice was controlled by two dominant complementary genes, *Pb* and *Pp*. Crossing black rice Heugnambyeo variants with three varieties of white pericarp rice gave a segregation ratio of 9 purple: 3 brown:4 white. The *Pp* genes were segregated by homozygous *PpPp* alleles for the dark purple pericarps, heterozygous *Pppp* alleles for the medium and mixed purple pericarps and homozygous *pppp* alleles for either brown or white pericarps with a 1 *PpPp*: 2 *Pppp*: 1 *pppp* segregation ratio,

indicating that the *Pp* allele in rice is incompletely dominant to the recessive *pp* allele. Among the purple seeds, the amount ofcyanidin-3-O-glucoside was higher in the dark purple seeds (*Pb_PpPp*) than in the medium purple seeds (*Pb_Pppp*). Moreover, no cyanidin-3-glucoside was detected in brown (*Pb_pppp*) or white pericarp seeds (*pbpbpppp*). These findings indicated that the level of cyanidin-3-glucoside was determined by the copy number of the *Pp* allele. Further genotype investigation of the F3 progeny demonstrated that the dominant *Pb* allele was present in either purple or brown pericarp. A 2-bp (GT) deletion from the DNA sequences of the dominant and functional *Pb* was found in the same DNA sequences of the recessive and non-functional *pb* allele. These findings suggested that the presence of at least a dominant *Pb* allele was an essential factor for color development in rice pericarps. In conclusion, the *Pp* allele in rice is incompletely dominant to the recessive *pp* allele; thus, the number of dominant *Pp* alleles determines the concentration F. They have performed the molecular cloning of the *Rc* and *Rd* loci from the rice genome. They demonstrated the introduction of the *Rc* gene into *rcrd* rice altered the coloration of seed from white to brown in transgenic rice. In addition, the introduction of *Rd* into *Rcrd* rice changed the seed color from brown to red. They have elucidated that *Rc* encodes a transacting regulatory factor with a bHLH motif and that *Rd* encodes a DFR protein Genetic segregation analysis suggested that the *Rd* and *A* loci are identical, and both encode dihydroflavonol-4-reductase (DFR). The introduction of the DFR gene into *Rcrd* mutant resulted in red-colored rice, which was brown in the original mutant, demonstrating that the *Rd* locus encodes the DFR protein. Accumulation of proanthocyanidins was observed in the transformants by the introduction of the *Rd* gene into the rice *Rcrd* line. Protein blot analysis showed that the DFR gene was translated in seeds with alternative translation initiation. A search for the *Rc* gene,

which encodes a transacting regulatory factor, was conducted using available DNA markers and the Rice Genome Automated Annotation System program. They identified three candidate genes and cloned from a rice *RcRd* line and subsequently introduced into a rice *rcrd* line. Brown-colored seeds were obtained from transgenic plants by the introduction of a gene containing the basic helix–loop–helix (bHLH) motif, demonstrating that the *Rc* gene encodes a bHLH protein.

2.4 Studies on the *fgr* gene

Ahn et al. (1992) reported that the single recessive fragrance gene (*fgr*) was linked to the RFLP clone RG28 on chromosome 8, at agenetic distance of 4.5 cM.

Lorieux et al. (1996) confirmed the close linkage between RG28 and *fgr* (5.8 cM) and also identified two quantitative trait loci for fragrance, one on chromosome 4 and the other on chromosome 12.

Garland et al. (2000) DNA was extracted from the leaf tissue for 50 F2 individuals derived from crossed of the parent cultivars, (Pelde/Della//Kulu) (tall, jasmine-style fragrant, long grain, Australian cultivar) and Doongara (Bluebelle/Calrose//Jojutla) (semi-dwarf, non-fragrant, long-grain, Australian cultivar). The genomic DNA clone RG28 was linked to the major fragrance gene of rice (*fgr*) and produced a PCR-based marker for fragrance. They identified a small mono-nucleotide repeat that was polymorphic between a pair of fragrant and non-fragrant cultivars and developed into a co-dominant PCR-based marker.

Jin et al. (2003) the whole rice genome sequence was used to assist in the identification of a single nucleotide polymorphism (SNP) marker linked to the fragrance gene (*fgr*) in rice. Genes flanked by restriction fragment length polymorphism and microsatellite markers known to be linked to the fragrance gene were identified by DNA sequence alignment of EST sequences against BAC clones

covering this region of chromosome eight. Re-sequencing and comparison of parts of these genes derived from a fragrant and a non-fragrant cultivar revealed only one SNP (a C/T transition) in more than 6 kbp of sequence from 14 genes. Ten of eleven fragrant genotypes and six of 14 non-fragrant genotypes tested carried the C allele. This approach indicated a generally low level of SNP polymorphismin cultivated rice suggesting that association of SNP with phenotypes should be an efficient path to gene discovery in cultivated rice.

Chen et al. (2006) four initial crosses were made between indica and japonica rice varieties with contrasting fragrant traits. These crosses were Wuxiangxian/ Zhongai91A (indica, fragrant/ japonica, non-fragrant), Wuxiangjing/ Nanjing11 (japonica, fragrant/ indica, non-fragrant), Suyunuo/ Zhongxian3037 (japonica, fragrant/indica, non-fragrant), and Suyunuo/Guichao2 (japonica, fragrant/ indica, non-fragrant). A total of eight segregant populations (four BC1 and four F2) consisting of 2291 individuals were prepared from the above four crosses by either backcrossing to the fragrant parents or self pollinating. In addition, several recombinants screened from the Suyunuo/ Zhongxian3037, F2 population were used to further develop F3 populations (600 individuals in total) for the fine-mapping purpose. From more than 100 volatile flavor components, 2-acetyl-1-pyrroline (2AP) stands out as the key component of aroma in both basmati- andjasmine-style fragrant rice. It has been reported that a single recessive gene (*fgr*) on chromosome 8 is responsible for the production of 2AP. They made an initial mapping efforts with SSR markers confirming the previous reported mapping result, and placed the *fgr* locus between RM8264 and RM3459 with the physical distance of 800 kb. Then, they saturated the *fgr* region with high-density markers developed by exploiting sequence diversities between indica and japonica rice subspecies and after mapping with segregant

populations consisting of totally 2891 individuals, the *fgr* locus was restricted to an interval of 69 kb flanked by the left marker L02 and the right marker L06. And they furthermore confirmed the *fgr* locus by simultaneous investigation of both genotypes and 2AP levels for the key recombinants and their offspring. Sequence analysis of the *fgr* region revealed three candidate genes encoding respective eukaryotic-type carbonic anhydrase, 3-methylcrotonyl-CoA carboxylase beta chain, and betaine aldehyde dehydrogenase.

Chen et al. (2008) took a local Chinese fragrant rice cv, Suyunuo (*Oryza sativajaponica*), anda nonfragrant rice cv. Nanjing11 (*Oryza sativaindica*) were used toconstruct BAC libraries. Multiple Badh2 transcript lengths were detected, and the complete, full-length Badh2 transcript was much less abundant than partial Badh2 transcripts. The full-length BADH2 protein (503residues) appeared exclusively in non fragrant transgenic lines and rice varieties. Their results indicated that the full-length BADH2 protein encoded by *Badh2* renders rice nonfragrant by inhibiting 2AP biosynthesis. The BADH2 enzyme was predicted to contain three domains: NAD binding, substrate binding, and oligomerization domains. BADH2 was distributed throughout the cytoplasm, where it is predicted to catalyze the oxidization of betaine aldehyde, 4-aminobutyraldehyde (ABald) and 3-aminopropionaldehyde. The presence of null *badh2* alleles resulted in AB-ald accumulation and enhanced 2AP biosynthesis. Therefore, these data supported the hypothesis that BADH2 inhibits 2AP biosynthesis by exhausting AB-ald whish is presumed as 2AP precursor.

Shi et al. (2008) collected a total of 24 Chinese fragrant rice varieties, they were including Suyunuo (japonica), Wuxiangjing (japonica), Pangxiegu (japonica), Wuxiang9915 (japonica), Xiangjing111 (japonica), Zhenxiangjing5 (japonica), Guanglingxiangnuo (japonica), Xiangxuenuo (indica), Wuxiangjing9 (japonica),

Xiangjing02-5855 (japonica), Xiangjing49 (japonica), Guanglingxiangjing (japonica), Xiangjing111/ C9083 (japonica), XiangjingT37 (japonica), Gehuxiangjing (japonica), Wuxiangjing14 (japonica), Wuxiang99-8 (japonica), (japonica), Suxiangjing1 (japonica), Wuxiang075 (japonica), Basmati385 (indica), Basmati370 (indica), Ganxiangnuo (indica) and Meiguomolixiang (indica). Ten non-fragrant rice varieties were employed as controls—3037 (indica), Ballila (japonica), Wuyunjing7 (japonica), 02428 (japonica), Laolaiqing (japonica), Miyang46 (indica), Liaojing5 (japonica), Zhenzhuai (indica), Baxizaodao (japonica) and 'Dullar'(indica). All the rice varieties were grown in the experimental field in Yangzhou University, Jiangsu Province, People Republic of China, in 2005. They reported the discovery of a new *badh2* allele and the development of functional markers for the *badh2* locus. They designed a total of ten primer pairs based on the full-length *Badh2/badh2* sequences. These primers amplified the overlapping *Badh2/badh2* fragments for all fragrant and non-fragrant varieties and the PCR products were cloned into the vector pGEMT and sequenced. The full length *Badh2/badh2* sequences were assembled based on identical overlapping regions. Sequence alignment of the *Badh2/badh2* alleles was conducted among the 24 fragrant and ten non-fragrant rice varieties and the sequence divergences were used to develop markers by designing flanking primers and then amplifying all fragrant and non-fragrant rice varieties. They prepared a F2 population from the cross between Xiangjing02-5855 (*badh2-E2*) and Xiangxuenuo (*badh2-E7*). And each F2 individual was checked for its genotype at the *badh2* locus using the functional markers developed in the study. Of the 24 fragrant rice varieties, 12 were found to have the known *badh2* allele (*badh2-E7*), which has an8-bp deletion and three single nucleotide polymorphisms (SNPs) in exon 7; the others had a novel null *badh2* allele (*badh2-E2*), which has a sequence identical to that of the *Badh2* allele in

exon 7, but with a 7-bp deletion in exon 2. Both null *badh2* alleles are responsible for rice fragrance. Based on sequence divergence amongst the functional *Badh2* and two null *badh2* alleles, they developed functional markers which can be easily used to distinguish nonfragrant from fragrant rice and to differentiate between two kinds of fragrant rice. Hence, they suggested the functional markers would find their usefulness in breeding for fragrant rice varieties via marker-assisted selection.

Srivong et al. (2008) four cultivars of Thai rice (*Oryza sativa* L. *indica*) seeds; of which two are fragrant rice i.e. Khao Dawk Mali 105 (KDML105) and Kor Khor 6 (RD6) while Niew-San-Pah-Tawng (NST) and Sew-Mae-Jan (SMJ) are non fragrance were collected from Khon Kaen, Thailand. They aimed to characterize the partial *Badh2* gene in other aromatic and non-aromatic of Thai rice cultivars and determine thebetaine aldehyde dehydrogenase activity. The primers for their study were designed on the basis of sequence differences of the *Badh1* and *Badh2* gene that flank 8-bp deletion and 3 SNPs in exon 7 inorder to generate specific primers for *indica* rice *Badh2* gene. Sequence analysis of exon 7 of *Badh2* gene of the aromatic rice cultivars, KDML105 and RD6, resulted sequence polymorphisms containing a total of 3 SNPs and 8-bp deletion, whereas, non-aromatic rice; SMJ and NST did not. Deduced amino acid of the partial *Badh2* genes of Thai rice predicted the mutation at exon 7 of aromatic rice, which would lead to an early stop codon, result in production of truncated BADH2 and possibly loss of its function. They also determined BADH activity from partially purified leaf extracts and indicated that BADH activities of nonaromatic rice were higher than aromatic rice. From their kinetic analysis, they indirectly suggested the loss of BADH2 function in aromatic rice, which plays important role in aroma synthesis in rice.

Parthepa (2008) collected a total of 64 black rice samples from several regions of Thailand and Loas and evaluated for the fragrance gene (*fgr*). A PCR assay was used to evaluate and predicted the genotypes of each individuals within a seed lot. A total number of 45, 5 and 15 seed lots were genotyped for DD (allele D, 8 bp deletions), ND (heterozygote) and NN (allele N, non-deletion), respectively. Their data showed from the 65 samples 23% of the samples were heterozygous and non-fragrant homozygous, respectively. They suggested that heterozygous individuals, black rice plants that carry both the fragrant allele and non-fragrant allele of the fragrance gene and non-fragrant seeds should be avoided because they are non-fragrant and will give rise to a mixture of fragrant and non-fragrant seed lots.

Parthepa (2009) carried out the study to determine the presence of the recessive allele (*badh2*) of the fragrance gene of the weedy rice population *(Oryza sativa* f. spontanea) in an important rice growing area of Northeastern Thailand. 215 weedy rice plants were examined using PCR assay with specific primer, three genotypes, *BADH2/BADH2*, *BADH2/badh2* and *badh2/badh2* were detected. Frequencies of the *badh2* allele showed a high value of 0.547.