CHAPTER 2

LITERATURE REVIEW

2.0 LITERATURE REVIEW

2.1 Origin of Aromatic Rice

Aromatic rice has been introduced into the global market. Most of the trade in aromatic rice is from India, Pakistan and Thailand. Aromatic rice from India and Pakistan consists of Basmati types, while Thailand is the supplier of Jasmine rice. Other important aromatic varieties in the world market are Khao Dawk Mali 105, Siamati (Thailand), Bahra (Afganistan), Sadri (Iran), Della, Texami and Kasmati (USA) (Singh et al., 2000d).

Singh et al. (2000d) also mentioned that aromatic rice varieties which are popular in the world market are long grained, but a majority of the Indian indigenous aromatic rice varieties are small and medium-grained. A large number of land races of these varieties are found in the Himalayan Tarai region of the state of Uttar Pradesh and Bihar of India. This indicates that this region is probably the origin of aromatic rice (Singh et al., 2000d).

Khush (2000) had grouped the aromatic cultivars into three groups: Group I (*indica*), Group VI (*japonica*) and Group V (which includes world famous high quality Basmati rice of India and Pakistan). Most of the aromatic rice cultivars come from Group V, in which the grains are long and medium in size such as Basmati, Kataribhog, and Sadri, as well as cultivars with very small grains such as Nama Tha Lay. Only a few aromatic cultivars belong to Group I (from Thailand, Vietnam, Cambodia, and China) and Group VI (from Indonesia, Philippines and China). The evolutionary pathway of the two cultivated species of rice was shown by Khush (2000) in Figure 2.1. Figure 2.2 shows the centre of diversity and dispersal routes of aromatic rice of Group V.

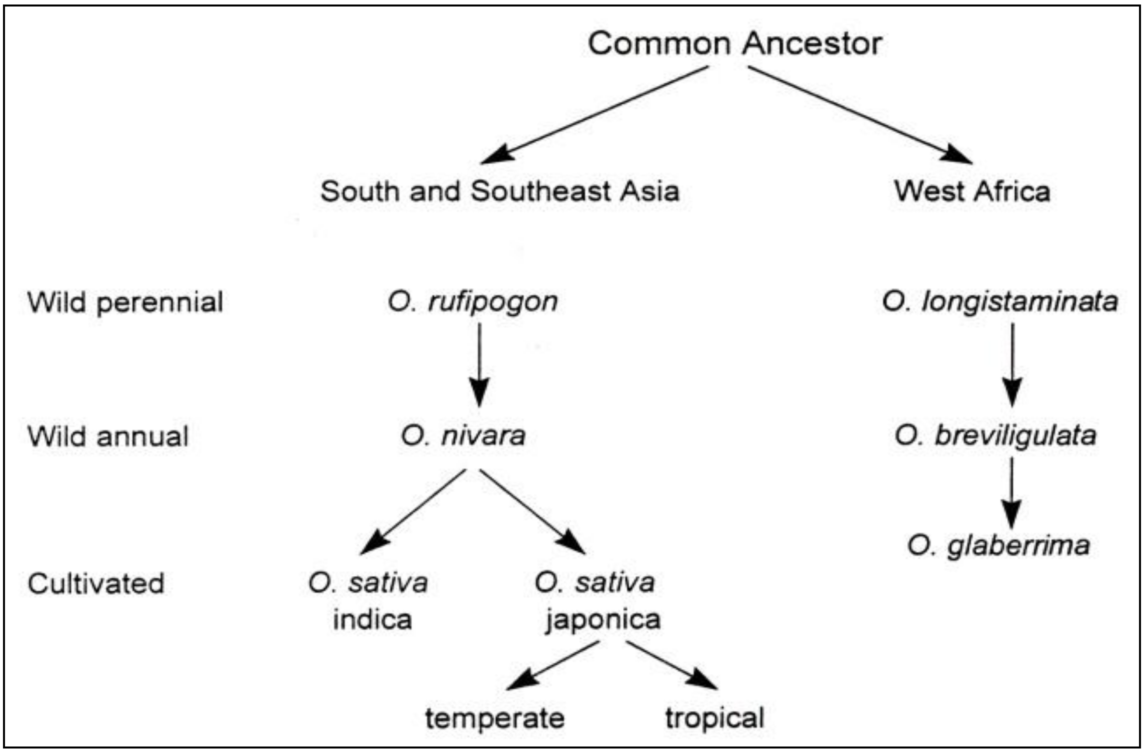

Figure 2.1: Evolutionary pathway of the two cultivated species of rice (Khush, 2000)

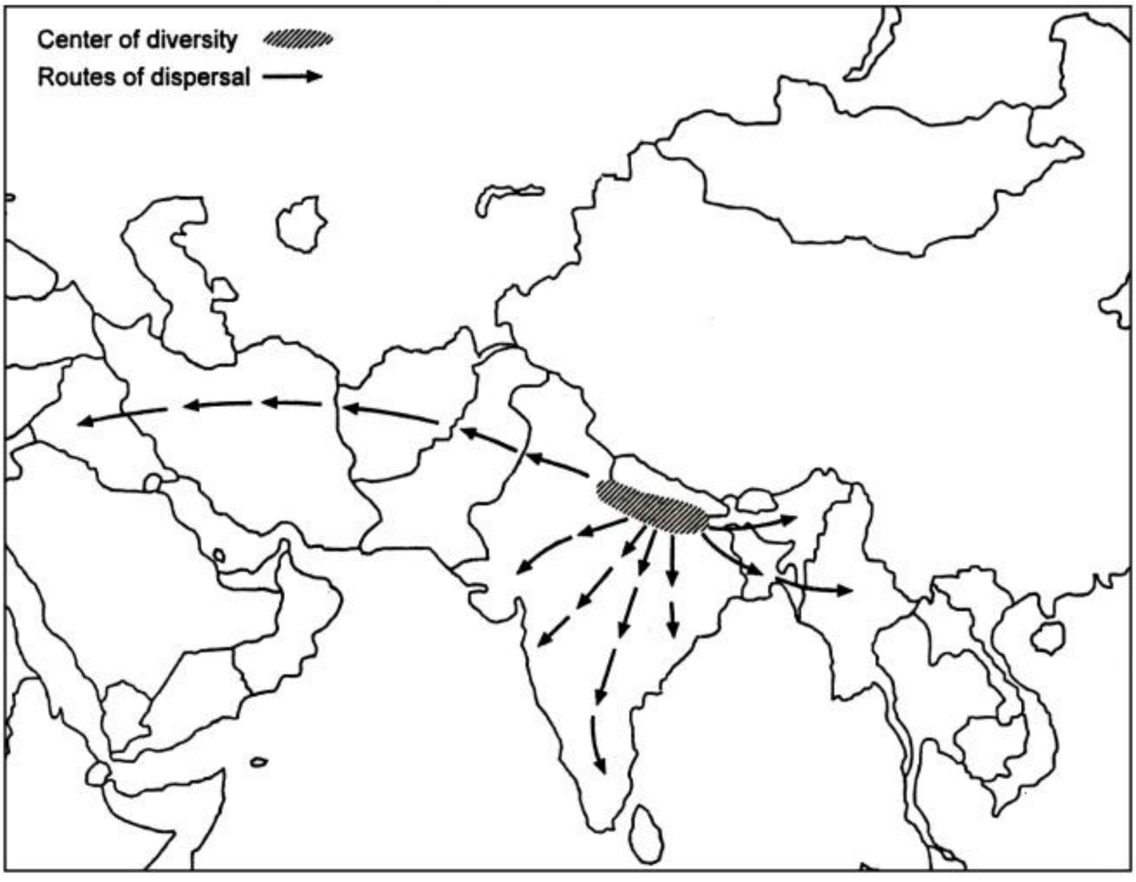

Figure 2.2: Centre of diversity and dispersal routes of aromatic rice of Group V (Khush, 2000)

2.2 Grain Quality in Aromatic Rice

Rice grain quality affects the nutritional and commercial value of grains. Genotype and environment are the two main factors that influence rice quality. Grain quality, along with crop yield and resistance to pests and diseases, is an important criterion in most rice breeding programs especially in rice variety selection and development (Dela & Khush, 2000). Besides, physiochemical characteristics of rice grains also are important indicators of grain quality. It is mainly determined by the combinations of many physical as well as chemical characters. However, it is very difficult to define grain quality because it involves objective and subjective criteria. According to Zhang and Yu (2000), cooking and eating quality are the most important components of rice quality. Based on Kaosa-ard and Juliano (1991), country and culture result in different preferences for rice quality. Thus, defining quality is often difficult since it is defined by the end user and their preferences are highly variable (Dela & Khush, 2000). For example, Middle East consumers prefer long grain, well milled rice with strong aroma while the European community generally prefers long grain rice with no scent because the presence of any scent signals spoilage and contamination (Efferson, 1985). In West Africa, grain quality is based on the type of food people prepare for eating. Long grain and aromatic rice are used with sauces, short and medium grain rice are used in porridge mixed with sugar, salt and milk, and broken rice is used in Senegal, Gambia and Mali as fried rice. Long grain aromatic rice has the greatest demand and is the most expensive rice in local markets. Although preferences vary from one group of consumers to another, rice grains with a pleasant fragrance or aroma and soft texture usually achieve higher price in national and international markets (Nguyen & Bui, 2008).

Several researchers (Dela & Khush, 2000; Juliano & Duff, 1991) concluded that grain quality is second after yield as the major rice breeding objective as well as other crop improvement. In the future, grain quality will be even more important as very poor consumers, who depend largely on rice for their daily food, demand higher quality rice (Juliano & Villarreal, 1993). The primary constituents of cooking and eating quality of rice grains are: fragrance or aroma of cooked rice, grain appearance, cooked grain elongation, amylose content (AC), gelatinization temperature (GT), and gel consistency (GC). Thus, grain quality in rice plays an important role in consumer acceptability.

2.2.1 Aroma

Aroma is the most important feature of aromatic rice. Several chemical constituents are important to the aroma of cooked rice (Grosch & Schieberle, 1997). Researchers have observed around 40 to 200 volatile compound in different types of rice aroma. Most of the researchers concluded that 2-acetyl-1pyrroline is the vital compound for aroma in aromatic rice. The flavor of a range of foods, including popcorn (Schieberle, 1995), corn tortillas (Buttery & Ling, 1995), baguettes (Zehentbauer & Grosch, 1998), ham (Carrapiso et al., 2002), cheese (Zehentbauer & Reineccius, 2002), green tea (Kumazawa & Masuda, 2002) and wine (Herderich et al., 1995), has been associated with the presence of 2-acetyl-1-pyrroline. The 2-acetyl-1-pyrroline had lower odor threshold than other volatile compounds in aromatic rice varieties (Buttery & Ling, 1982). Odor quality evaluation of 2-acetyl-l-pyrroline was described as popcorn-like, and the evaluation order of the amount of popcorn-like odor in 10 different rice varieties ranked them in general order of the concentration of this compound (Tsugita, 1985-86). 2-acetyl-1-pyrroline is found in all parts of the rice plant, except for roots (Lorieux et al., 1996), and is also found, at concentrations up to 100-times lower, in non-aromatic varieties (Grosch & Schieberle, 1997). The desirability of aroma has resulted in strong human preference and selection for this trait. Non-aromatic rice varieties contain very low levels of 2-acetyl-1-pyrolline, while the levels in aroma genotypes are much higher

(Widjaja et al., 1996). It was found to be the key element for the roasty, popcorn-like aroma of freshly baked wheat bread (Schieberle & Grosch, 1985) and cooked pandan leaves (Buttery et al., 1983). Buttery et al. (1988) was the first scientist to do a systematic study on the contribution of the different volatiles on a long rice variety from California. Other studies conducted elsewhere revealed that the field location (Fushimi et al., 1995), the temperature during ripening and drying (Itani & Fushimi, 1996), and the storage or aging affected the level of 2-acetyl-1-pyrroline in scented rice (Laksanalamai & Ilangantileke, 1994; Widjaja et al., 1996). Weber et al. (2000) concluded that the pleasant odor of raw or cooked non-aromatic or aromatic rice was controlled by a blend of various volatiles. Most of the volatiles found in aromatic and non-aromatic rice were similar, but their proportion differed. Lorieux et al. (1996) reviewed the genetics of aromatic fragrance and concluded that a single recessive gene was responsible for the production of aromatic rice plants. This single recessive fragrance gene (*fgr*) was linked to the RFLP clone RG28 on chromosome 8, at genetic distance of 4.5 cM (Ahn at al., 1992), controlling the presence of 2-acetyl-1-pyrroline, the main compound of rice aroma (Lorieux et al., 1996).

2.2.2 Grain Size, Shape and Appearance

Dela Cruz and Khush (2000) mentioned that preference for grain size and shape varies from one group of consumers to the other. Some ethnic groups prefer short bold grains, some have a preference for medium long grains, and long slender grains are highly prized by others. In general, long grains are preferred in the Indian subcontinent, but in Southeast Asia the demand is for medium to medium long rice. In temperate areas short grain varieties are prevalent. There is a strong demand for long grain rice on the international market.

Grain appearance depends upon the size and shape of the kernel, translucency and chalkiness of the grain. The physical dimensions of rice kernels are of vital interest to those engaged in the many facets of the rice industry. Rice varieties may be objectively classified into grain-type categories based upon two physical parameters: length and shape (Dela & Khush, 2000).

Length or size of rice grain is a measure of the rice kernel in its greatest dimension. They are categorized in four categories: (1) very long (more than 7.50 mm), (2) long (6.61- 7.50 mm), (3) medium (5.51- 6.60 mm), and (4) short (less than or equal to 5.50 mm). According to grain shape, rice is primarily classified into long, medium, and short categories, by which the ratios of grain length to grain width are more than 3.0, between 2.1 and 2.9, and smaller than 2.0, respectively (Dela & Khush, 2000).

Dela and Khush (2000) also mentioned that grain appearance is largely determined by the amount of chalkiness. The chalkiness of rice grain is based on endosperm opacity, which is classified as waxy and non-waxy (Shoba et al., 2006). Waxy rices have only traces of amylose content and are opaque. Non-waxy rices have varying amylose level (2.1-32 %) and are dull, hazy or translucent. While, Dela and Khush (2000) classified the endosperm chalkiness based on the percentage area with chalkiness, but they concluded that the greater the chalkiness, the lower the market acceptability.

2.2.3 Kernel Elongation

Sood and Siddiq (1979) said that linear elongation of kernel on cooking is one of the major characteristics of fine or scented rice. Genetic evaluation of kernel appearance and its cooking quality form important objectives in rice grain quality improvement programs. Grain size and shape largely determine the market acceptability of rice, while cooking quality is influenced by the properties of starch. Some varieties expand more in size than others upon cooking. Lengthwise expansion without increase in girth is considered a highly desirable trait in high quality rice (Khush et al., 1979; Sood et al., 1983). Grain elongation of pre-soaked milled rice seems greater with intermediate-amylose, low-gelatinization temperature (Juliano & Perez, 1984). Meanwhile, Villarial et al., (1976); Perez & Juliono, (1981), (1982) and Faruq et al. (2003) mentioned that ageing is a process that can develop rice-cooking quality. Aged rice is popular in many South and South East Asian countries. It helps to increase kernel elongation rate during cooking time. It is very simple to practice, through ageing or storing rough rice for 3-4 months after harvest also affects grain quality. Higher head rice (whole grain) yields are obtained from aged rice.

2.2.4 Amylose Content

Sanjiva et al. (1952) was the first to identify that there was a possible relation between amylose and rice grain quality. Amylose content, also called apparent amylose or amylose-to-amylopectin ratio, is the most important element influencing the cooking quality of rice (Bao et al., 2001). The amylose content of rice is known to play a crucial role in determining its cooked texture and processing functionality. Rice varieties are grouped on the basis of their amylose content into waxy (0-2 %), very low (3-9 %), low (10-19 %), intermediate (20-25 %) and high (>25 %) (Kumar & Khush, 1986). Zhang et al. (2003) reported that amylose content varied among grains from the same panicle. It varied also between outer and inner layers of the same grain. Grains harvested from the upper position, the first branches or early flowers tend to have higher amylose content. The trends appeared to be the same for both *Japonica* and *Indica* rice types. Amylose content was found not to be affected by timing of harvest or length of grain storage (Juliano, 1971; Muraue, 1997). Seguchi et al. (2003) concluded that amylose plays an important role in the maintenance of the structures of starch granules. The principal elements that compose starch granules are amylose, amylopectin, lipids, and proteins. When low amylose rice is boiled, the product is viscous and elastic. The swollen trait could be due to the characteristics of amylopectin.

2.2.5 Gelatinization Temperature

Amylose contents determine the texture of cooked rice. However, gelatinization temperature determines the cooking time mentioned by Heda and Reddy (1986). Gelatinization temperature (GT) is the range of temperature wherein at least 90% of the starch granules swell irreversibly in hot water with loss of crystallinity and birefringence or double refraction (Dela & Khush, 2000). The GT ranges from 55 to 79 ℃. Juliano (1972) found 3 classes which were: low GT (55 to 69 ℃), intermediate GT (70 to 74 ℃) and high GT with (more than 74 ℃). Ghosh and Govindaswamy (1972) declared that the cooking quality of rice is greatly influenced by the GT added to the quality and quantity of starch. In a study conducted by Tomar and Nanda (1985), they declared that the GT played an important role concerning water uptake, volume expansion and kernel elongation. High GT rice has a final soft texture when overcooked; it elongates less and can be undercooked if the standard cooking procedure is applied. Heda and Reddy (1984); McKenzie and Rutger (1983) and Maningat and Juliano (1978) mentioned that the gelatinization temperature of a plant is usually determined from a bulk sample of its seeds those are in the following generation. Such as bulk F3 and F4 endosperm populations represent F2 and F3 respectively. The alkali digestion test allows the scoring of individual rice endosperm, so observation of the F3 endosperms from a single F2 plant provides a progeny test allowing classification of the genotype of the F2 plants. Jennings et al. (1979) reported that the inheritance of gelatinization temperature is not entirely clear, but it appears to be fairly simple, involving one or two major genes. Gelatinization temperature is reasonably high in heritability, although it may vary as much as 10℃ within a variety in exceptional cases, depending on environment. High air temperature after flowering raises the gelatinization temperature (which lowers grain quality) and low air temperature reduces it (Faruq et al., 2004).

2.3 Composition of Aroma in Aromatic Rice

The volatiles produced by rice are normally determined by gas chromatography mass spectrometry (GC-MS) (also see section 2.5). The volatile compounds from cooked rice were analyzed by a few researchers such as Bullard and Holguin (1977), Yajima et al. (1978) and Buttery et al. (1983b). Tsuzuki et al. (1980) evaluated the effect of milling on cooked rice aroma. A total of 40 compounds were identified. They concluded that the outer surface layers of rice play an important role in the formation of cooked rice odour. Maga (1984) and Tsugita (1985-1986) indicated that more than 100 aroma components have been identified in cooked non-aromatic rice. The total analysis of raw and cooked rice volatiles suggested a number of aromatic compounds were involved in the aroma of rice. However, from an odour point of view, there was no individual compounds identified have been found to have the characteristic aroma of raw (Bullard & Holguin, 1977) and cooked rice. Therefore, Bullard & Holguin (1977) concluded that aroma in rice is formed by a blend of various volatiles.

Tsuzuki et al. (1981) and Buttery et al. (1983b) analyzed volatiles of cooked aromatic rice. Over 114 compounds were reported. Traditional rice had higher amounts of 4-vinylphenol, 1-hexanol and 1-hexanal but lower amounts of indole as compared to aromatic rice. Aromatic rice had an unidentified compound and *a* -pyrrolidone, which was not present in traditional rice. However, none of the individual compounds identified had the characteristic odour of cooked aromatic rice. Buttery et al. (1988) determined that the major contributors to the rice odour in California long grain rice (non-aromatic rice) were 2-acetyl-1-pyrroline, (*E*, *E*)-2,4-decandienal, nonanal, hexanal,

(*E*)-2-nonenal, octanal, decanal, 4-vinyl-guaiacol and 4-vinylphenol. Buttery et al. (1983a) analyzed 2-acetyl-1-pyrroline in the atmospheric steam volatile concentrate of different varieties of rice. The detection of this compound was only in cooked rice and not in raw rice; therefore, Tsugita (1985-86) suspected that this compound was generated during the cooking process. Yajima et al. (1979) analyzed cooked Kaorimai (aromatic rice) and found 105 compounds. Suvamalatha et al. (1994) developed callus tissue from Basmati rice and compared the volatile compounds from regularly cooked Basmati rice and found the compounds to be very similar. However, they did not detect 2-acetyl-1-pyrroline in the callus or in the cooked rice. Weber et al. (2000) said that perhaps their detection method was not sensitive enough.

The flavor chemistry of rice grain reveals the existence of numerous volatiles in aroma although the relationships among them and with aroma are not established except for the major aromatic compound, 2-acetyl-1-pyrroline (Yoshihashi et al., 2002). Even though many studies have identified 2-acetyl-1-pyrroline as a principal aroma compound (Cordeiro et al., 2002; Bradbury et al., 2005a), the biochemical pathways of its synthesis leading to aroma remained unknown (Niu et al., 2008). Moreover, some contradictions were reported about the origin of pyrroline, a precursor for 2-acetyl-1-pyrroline (Sakthivel et al., 2009). Vanavichit et al. (2005) attempted to elucidate the 2-acetyl-1-pyrroline biosynthetic pathway and proposed that 2-acetyl-1-pyrroline is synthesized via the polyamine pathway. The immediate precursor of 2-acetyl-1-pyrroline was found to be 1-pyrroline (1P) which is formed from 4-aminobutyraldehyde (AB-ald; the immediate precursor of 4-aminobutyric acid (GABA)).

Orientals normally describe the aroma of the aromatic rice as being pandan-like. Paule and Powers (1989) reported 2-acetyl-l-pyrroline in Basmati 370, an aromatic rice from Pakistan, and positively correlated the 2-acetyl-l-pyrroline concentration with the characteristic aroma of aromatic rice. Lin et al. (1990), Ahmed et al. (1996), and Tanchotikul and Hsieh (1991) confirmed the reports of Buttery et al. (1983a) and Paule and Powers (1989) that 2-acetyl-1-pyrroline was the characteristic odour of aromatic rice varieties. It is a common practice in Asia to include Pandan (*Pandanus amaryllifolius*) leaves in cooking non-aromatic rice to give it an aroma. Buttery et al. (1983b) analyzed Pandan leaves and found that the major volatile component was 2-acetyl-1-pyrroline. They found that the concentration of 2-acetyl-1-pyrroline in Pandan leaves was 10 times greater than aromatic rice and 100 times greater than non-aromatic rice. The concentration of 2-acetyl-1-pyrroline was lower in aged aromatic rice. Kim (1999) reported that hydrocarbon compounds were not significantly different between aromatic and non-aromatic rice varieties but aromatic rice had higher levels of alcohol (mainly n-pentanol, l-octen-3-ol, menthol and estragol), aldehydes and ketones (e.g. n-pentanal, n-heptanal and n-nonanal), acids and other compounds. Aromatic rice had 15 times more 2-acetyl-1-pyrroline than non-aromatic rice.

Biosynthetic pathways for the rice aroma compound, 2-acetyl-1-pyrroline were recently proposed (Fig. 2.3). According to Chen et al. (2008), 4-aminobutyraldehyde (AB-ald) is known to be maintained in an equimolar ratio with an immediate 2-acetyl-1-pyrroline precursor, Δ1-pyrroline, and the AB-ald levels appear to be an important factor regulating the rate of 2-acetyl-1-pyrroline biosynthesis. They suggested that the functional betaine aldehyde dehydrogenase 2 (BADH2) enzyme (coded by the aroma gene *fgr*) inhibits 2-acetyl-1-pyrroline biosynthesis in non-fragrant rice by converting AB-ald, a presumed 2-acetyl-1-pyrroline precursor, to GABA while the non-functional badh2 (coded by *fgr*) result in AB-ald accumulation leading to the formation of 2-acetyl-1-pyrroline in fragrant rice. Bradbury et al. (2008) also suggested that γ-aminobutyraldehyde (GABald) is an effective substrate for BADH2 and that accumulation and spontaneous cyclisation of GABald to form Δ1-pyrroline due to a non-functional BADH2 enzyme as the likely cause of 2-acetyl-1-pyrroline accumulation in rice. However, in another study, increased expression of Δ1- pyrroline-5-carboxylate synthetase in aromatic varieties compared with non-aromatic varieties, as well as concomitant elevated concentrations of its product, led to the conclusion that Δ1-pyrroline-5-carboxylate, usually the immediate precursor of proline synthesized from glutamate, reacts directly with methylglyoxal to form 2-acetyl-1-pyrroline (Huang et al., 2008), with no direct role proposed for BADH2.

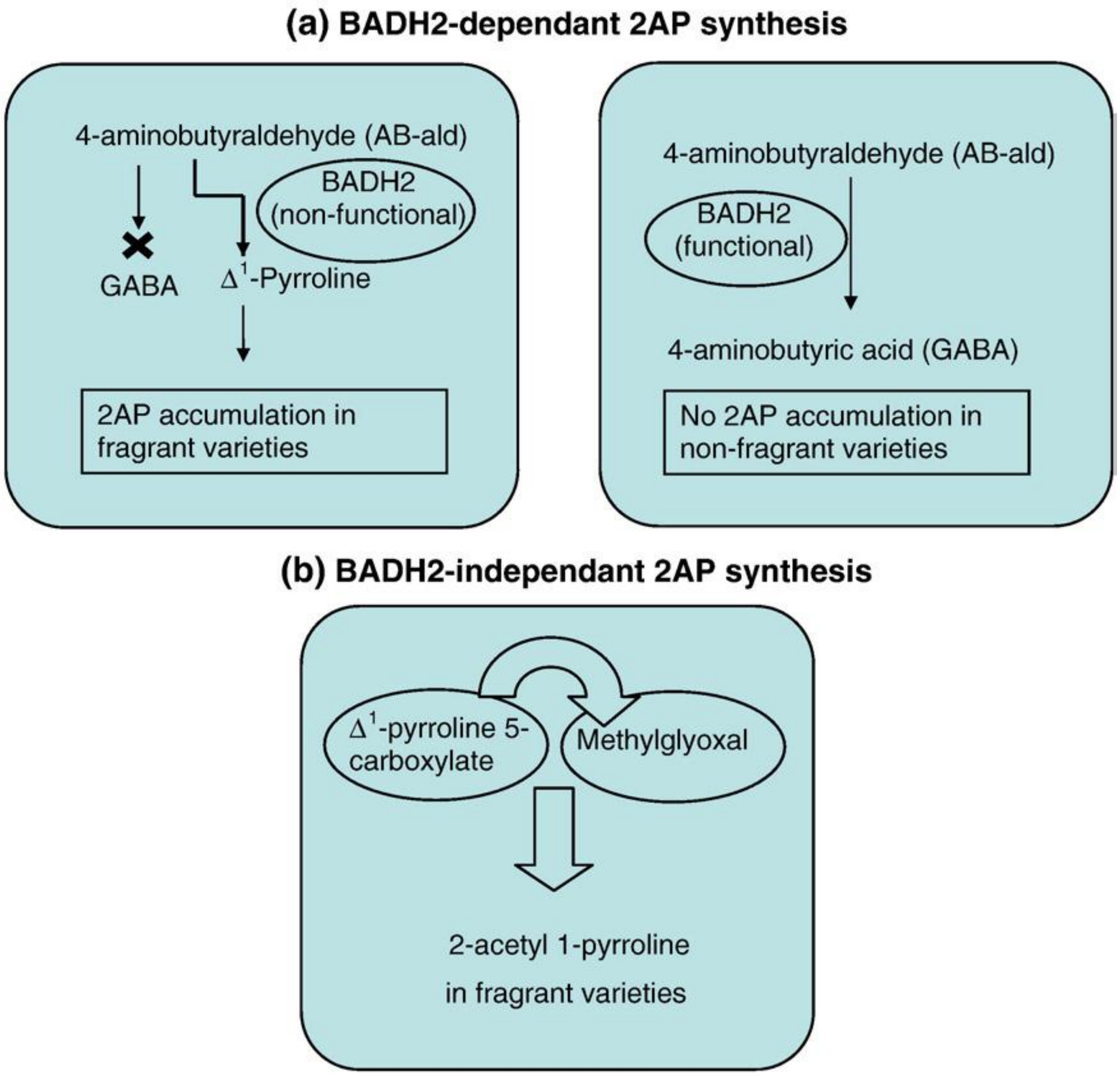

Figure 2.3: Pathway of 2-acetyl-1-pyrroline (2AP) biosynthesis in rice. (a) BADH2-dependant 2AP synthesis (Chen et al. (2008); Bradbury et al. (2008)), (b) BADH2-independant 2AP synthesis (Huang et al., 2008).

Although Basmati and Jasmine rice varieties are considered as world class premium aromatic rice, significant differences exist in concentrations of various flavor/off-flavor compounds such as methyl salicylate, deca-2,4-dienal, hexanal, hept-2-enal; 2-butenal and 2-pentylfuran (Kirstin & Wootton, 2004) which may contribute to their respective flavors. Thus, these studies showed that each variety has a unique aroma resulting from a number of volatile compounds which may vary from well characterized popcorn-like aroma or 2AP associated aroma to the unknown aroma (Champagne, 2008).

2.4 Genetic and Molecular Basis of Aroma in Aromatic Rice

Plant breeders in the USA developed a Jasmine type of aromatic rice variety named Jasmine 85 (Bollich, 1989) and the variety Della (Jodon & Sonnier, 1973). When Thai taste panels evaluated Jasmine 85, it was criticised for its dull off-white colour and less pronounced aroma (Rister et al., 1992). However, Lin et al. (1990) determined that the aromatic variety, Della, contained a higher level of 2-acetyl-l-pyrroline than the imported Thai Jasmine varieties. This suggested that the aroma in rice is complex and influenced by several compounds. Bemer and Hoff (1986) concluded that a single recessive gene controlled the aromatic nature of Della. This gene was located on chromosome 8 as determined by RFLP technology (Ahn et al., 1992). Several others also (Ghose & Butany, 1952; Sood & Siddiq, 1978; Reddy & Reddy, 1987) concluded that the aroma in rice was controlled by a single recessive gene. In contrast, Kadam and Patankar (1983) results suggested a single dominant gene controlling rice aroma. However, Dhulappanavar and Mensinkai (1969) observed a different scenario and indicated that two dominant aroma genes that interacted in a duplicate or complimentary manner to produce rice aroma. Later on, Dhulappanavar (1976) suggested there were four complementary recessive aroma genes. But, Reddy and Sathyanarayanaiah (1980) concluded that there were three complementary recessive aroma genes. Pinson (1994) suggested that one of the reasons for the confusion over the aroma gene was that different rice cultivars were analyzed. He looked at six cultivars to evaluate the type of aroma genes involved. He found that Jasmine 85, A-301, Della-X and PI45917 each contained a single gene for aroma and that they were allelic, but in Amber and Dragon Eyeball 100 each contained two aroma genes, a novel gene plus one allelic to the gene in A-301, Della-Z, Jasmine 85 and PI 457917. Buttery et al. (1986) suggested that the difference between aromatic and non-aromatic rice was not the presence or absence of 2-acetyl-l-pyrroline but due to a difference in the quantity of the chemical in the grain.

2.5 Detection of Aroma in Aromatic Rice

Different techniques to detect aroma were developed by several scientists around the world. The technique of chewing a half of a single seed was developed by Bemer and Hoff (1986). Chewing a few seeds was developed by Dhulappanavar (1976). Tasting individual grains has been the preferred method for the quality selection of aromatic rice varieties within an Australian breeding program (Reinke et al., 1991) and is still the principal means of identifying aroma in many breeding programs worldwide. Sood and Siddiq (1978) developed an assay for determining the aroma from plant material by adding KOH or I_2-KI to the plant sample, which released the aroma. Identification of 2AP using gas chromatography was introduced by Lorieux et al. (1996) and Widjaja et al. (1996) but requires large samples of tissue and is time consuming. The objective evaluation of fragrance is however difficult. More recently molecular markers, such as single nucleotide polymorphisms (SNPs) and simple sequence repeats (SSRs), which are genetically linked to aroma in rice have the advantage of being inexpensive, simple and rapid (Kibria et al., 2008).

2.5.1 Sensory Test

The method of heating of leaf tissues in water and noting the aroma was developed by Nagaraju et al. (1975). Evaluating the aroma from both leaf and grain with 1.7% KOH solution developed was a method developed by Sood and Siddiq (1978). Pinson (1994) said that it can cause damage to the nasal passages if carried out for a large amount of samples. The methods of smelling or chewing individual seeds (Ghose & Butany, 1952) for evaluation of aroma are subjective and not always reliable as ability to distinguish between aromatic and non-aromatic samples diminishes with each successive analysis. An analyst's ability declines as the senses become saturated or physical damage occurs from abrasions to the tongue often result from chewing the hard grain. A panel of analysts is required as the ability to detect aroma or taste varies between analysts. Sensory methods therefore have their limitations when processing large numbers of samples (Reinke et al., 1991). However, it has been practiced by many researchers (Bounphanousay et al., 2008; Sarhadi et al., 2009; Myint Yi et al., 2009; Varzirzanjani et al., 2011) until now and become a standard protocol for sensory test in Grain Quality Laboratory lab at International Rice Research Institute (IRRI).

2.5.2 Gas Chromatography-Mass Spetrometry (GC-MS)

Bryant and McClung (2011) determined the volatile profiles of nine rice cultivars before and after storage using solid phase microextraction (SPME) fibers in conjunction with gas chromatography mass spectrometer (GC-MS). This method determines the presence or absence of compounds rather than the quantity (gas chromatography). Ninety-three volatile compounds were identified, 64 of which had not been previously reported in rice. Wide differences were found in volatile compounds of aromatic and non-aromatic rice cultivars. A number of compounds were identified that were unique to aromatic rice cultivars other than 2-acetyl-1-pyrroline. This study also showed that storage time and temperature had little effect on the volatile profiles. Varaporn and Sarath (1993) detected the compound from the volatile oil of pandan leaf similar to 2-acetyl-1-pyrroline in molecular structure, but was not detected in non-aromatic rice and occurred in low concentrations in the aged Khao Dawk Mali-105 rice when using a steam distillation extraction method followed by gas chromatography. Identification of 2-acetyl-l-pyrroline using gas chromatography is possible but requires large samples of tissue and is time consuming (Lorieux et al., 1996; Widjaja et al., 1996). Therefore, it seemed appropriate to develop a method for quantification of 2-acetyl-1-pyrroline in aromatic rice varieties using smaller samples without steam distillation for extraction which was time saving and smaller sample needed.

2.5.3 Molecular Markers Related to Rice Aroma

Genetic control of aroma production has fascinated rice researchers resulting in numerous studies. Two or three recessive or dominant genes were reported to determine the aroma mentioned by Tripathi and Rao (1979); Reddy and Reddy (1987). However, most researchers believed that rice aroma was controlled by one single recessive gene (Huang et al., 1994; Sood & Siddiq, 1978; Jin et al., 2003). A recessive gene, on chromosome 8 of rice, largely controlling the level of 2-acetyl-1-pyrroline, has been identified in genetic studies. Genetic markers for this gene have been developed to allow selection for this trait in rice breeding. Cordeiro et al. (2002) defined the chromosomal location of the gene by mapping in segregating populations using simple sequence repeat (SSR) or microsatellite and single nucleotide polymorphism (SNP) markers (Jin et al., 2003). The availability of a rice genome sequence provided an opportunity to discover the gene responsible by comparison of the sequences of aromatic and non-aromatic genotypes (Goff et al., 2002). This will allow the future research to target the re-sequencing of genes and genomic sequences in an aromatic genotype to the most likely parts of chromosome 8.

In 2005, Bradbury et al. described a perfect marker to distinguish between aromatic and non-aromatic rice varieties. This method uses allele specific amplification of a gene encoding betaine aldehyde dehydrogenase 2 (BADH2) on chromosome 8, to differentiate between aromatic and non-aromatic varieties. . Non-aromatic rice varieties possess a full functional copy of the gene encoding BADH2 while aromatic rice varieties possess a copy of gene encoding BADH2 which contains an eight base pair deletion and three SNPs in exon 7, resulting in a frame shift that generates a premature stop codon that presumably disables the BADH2 enzyme.

The application of marker-assisted selection for this gene would have advantages over more traditional methods. It is objective and requires small amounts of tissue, thereby allowing a more accurate analysis of greater sample numbers in less time. Plants from breeding programs for aromatic lines could in the early stages of variety development be assessed for this trait using only a single seedling leaf. This offers the additional advantage of early screening of breeding populations compared to other methods, which require seeds to be produced for aroma analysis (Zhang, 2007; Liu et al., 2006; Toojinda et al., 2005; Zhou et al., 2003).

2.6 Heritability of Aroma in Aromatic Rice

Aroma development in rice grain is influenced by both genetic and environmental factors. Most of the rice varieties have been developed traditionally by selection, hybridization and back crossing with locally adapted high-yielding lines. The conventional methods of plant selection for aroma are not easy because of the large effects of the environment and the low narrow sense heritability of aroma (Kibria et al., 2008).

From many investigations in the past, aroma in rice is known to be genetically controlled. From the research of Ghose and Butany (1952), Sood and Siddiq (1978), and Berner and Hoff (1986), it was reported that aroma was monogenic recessive inherited while Kadam and Patankar (1938) reported it to be monogenic dominant. In the investigation on non-aromatic to aromatic F_2 segregation patterns, the ratio of 15:1 (Pinson, 1994), 37:27 (Reddy & Sathyanarayaniah, 1980), and 175:81 (Dhulappanavr, 1976) were reported. Hence, Sun et al. (2008) said that different flavors or aromas occur in different aromatic genotypes arising from diverse origins and there is no consensus as yet on the nature of rice aroma. However, Singh et al. (2000) said that aroma is a highly heritable trait as some of the released high yielding aromatic rice varieties show strong aroma.

In research by Faruq et al. (2011), it was reported that aroma in rice is also affected by high temperature during grain filling and ripening stages. The aroma of the global popular aromatic cultivars such as Rato Basmati and Rambir Basmati, which have distinct aroma in their cultivated country, was found to be only moderate when grown in a tropical environment. This further confirms the findings in Juliano (1972) and Mann (1987) that Basmati rice cultivars require relatively cooler temperature (25 ℃/ 21 ℃- day/ night temperature during crop maturity) for better retention of aroma. Table 2.1 shows the factors affecting aroma formation/ retention in aromatic rice cultivars as perceived by farmers (Singh et al. 1997).

Selection of parents for crossing to produce aromatic rice varieties is also very important. Islam (1983) and Khush et al. (1988) found that rice hybrids derived from one or both aromatic parents did possess aroma of different intensities. To maintain the aroma from the parental rice lines, Khush et al. (1988) had suggested using the cross between two aromatic varieties as aromatic varieties crossed with non-aromatic varieties produce hybrids with mild aroma or different aroma.

Table 2.1: Factors affecting aroma formation/ retention in aromatic rices as perceived by farmers.

Factors favouring aroma	Factors adversely affecting aroma
Cool weather during flowering and grain development	Hot weather during flowering and grain development
Farm yard manure	Nitrogenous fertilizers particularly urea
Fertile soil	Poor soil
Direct sowing	Transplanting
Lighter soil and upland conditions	Heavy soil
Low soil moisture during grain filling	Delayed harvesting after maturity
Manual dehulling	Mechanical dehulling

(Singh et al., 1997)

2.7 Importance of High Yielding Aromatic Rice Variety (HYV)

Most diseases and pests attacking normal rice cultivars also attack aromatic rice. Bemer and Hoff (1986) reported that aromatic cultivars were often associated with undesirable agronomic traits, such as low yield, susceptibility to pests and diseases, and strong shedding. Singh et al. (2000c) also reported that the diseases and pests are likely to be more serious on long duration and tall aromatic rice cultivars as compared to short and medium duration and dwarf and semi-dwarf non-aromatic rice varieties. Thus, breeders wish to develop aromatic rice varieties with high yield and good resistance to pests and diseases (Bemer & Hoff, 1986).

Most of the traditional aromatic rice varieties are low yielding. These low yielding aromatic rice varieties have been the major casualty of green revolution where the main emphasis was on yield rather than quality. A large number of aromatic rice cultivars have been lost and many are at the verge of extinction. Recently, rapid progress was made in standardizing the methodology for transformation in rice and also a large number of genes determining different traits have been cloned. These developments in field of biotechnology are expected to give momentum to the improvement of aromatic rice varieties (Singh et al., 2000b).

The earlier attempts to improve aromatic rice varieties were mainly through pure-line breeding. Later on, hybridization and selection in segregating generations and mutation breeding for high yielding aromatic rice varieties have been practiced (Singh et al., 2000b). He also mentioned that pedigree selection, backcross and convergent improvement methods are among the most commonly used breeding methods.

In this regard, the first high yielding aromatic rice variety was developed in India Subcontinent in 1925 (Azeez & Shafi, 1966) and was followed by others. Most of them are from direct crosses and subsequent generations handled by pedigree method of selection. PAU 29-295, a superfine aromatic rice variety was developed from a cross between Basmati 370 and Hamsa, grown in Andhra Pradesh (India) (Saini & Gagneja, 1973). Haryana Basmati-1, a semi-dwarf aromatic rice variety for Haryana (India) was developed by Panwar et al. (1991) from a cross between Sona and Basmati 370. The process involved growing of large F_2 population after selecting out the dwarfs in nursery stage in all crosses involving Basmati 370. Saini and Kumar (1978) said that this technique is not only reduced competition between tall and dwarfs but also permitted dwarfs to express better. In F_2 and onward generations, plants with improved plant type and long slender grains were selected and tested for aroma by the chewing test.

2.8 Conclusion

Aromatic rice is gaining importance in global rice trading. Increasing numbers of consumers are willing to pay a premium price for aromatic rice which is generally associated with good cooking and eating quality. Aroma is the most important feature in aromatic rice, followed by grain appearance, amylose content, gelatinization temperature, and kernel elongation. In order to detect rice aroma and its traits, the use of sensory tests, gas chromatography-mass spectrometry and molecular markers have been practiced. Thus, in the research on marker assisted breeding for aroma, we can use molecular markers to check the presence or absence of aroma gene/trait in the earlier stage (before flowering) of the breeding program followed by sensory methods (leaf aromatic test) to confirm the aroma or flavor which matches the requirements of the end users.

However, aromatic rice varieties have some problems in agronomic characters, such as low yield, susceptibility to diseases and pests and strong lodging. Thus, breeders should aim to solve these problems in order to develop high yielding aromatic rice varieties. There are many approaches for aromatic rice improvement. One of the techniques is hybridization between high yielding land races with aromatic rice.

Research on fine rice in Malaysia, including inheritance of kernel elongation (Faruq *et al*., 2004), inheritance of gelatinization temperature (Faruq *et al*., 2003) and grain quality evaluation (Mohamad *et al*., 2006) indicated that there is a scope to improve grain quality through an integrated conventional and molecular breeding approach. Therefore, Malaysian rice breeders should develop a few good quality fine rice varieties to meet the market demand and decrease Malaysia's expenses in import of fine rice.