CHAPTER 5

DISCUSSION AND CONCLUSION

5.0 DISCUSSION

5.1 Efficiency of Markers in Distinguishing Aromatic and Non-aromatic Varieties

Aroma is one of the most important traits of aromatic rice grain quality after grain appearance, amylose content, gelatinization temperature, and kernel elongation. The genetic control and environmental variation of this trait are reported to be quite complex. Recently, molecular markers that control the aroma are now available to facilitate direct selection in a rice breeding program. In this study, we have used markers from previous study (Bradbury et al., 2005a,b) for screening aroma in F_1 hybrids, namely EAP, ESP, INSP and IFAP primers in a multiplex PCR (as previously described in section 3.2.6). A primer combination of ESP and EAP could amplify an approximately 580 bp fragment corresponding to the positive control in all the samples. This band was used to indicate the success of the allele specific PCR amplification. This technique identifies homozygous aromatic, homozygous non-aromatic, and heterozygous non-aromatic individuals. During this screening process, homozygous aromatic, heterozygous non-aromatic, and homozygous non-aromatic appeared in the ratio 38: 35: 8, which also means that there are 38 aromatic and 43 (35+8) non-aromatic F_1 individuals. Mohamad et al. (2008) also observed a similar amplification pattern in their screening process and they identified 28 homozygous aromatic: 2 heterozygous non-aromatic: 45 homozygous non-aromatic, indicating 28 aromatic and 47 non-aromatic rice individuals. Meanwhile, another group of researchers, Bounphanousay et al. (2008), detected 36 homozygous aromatic: 3 heterozygous non-aromatic: 17 homozygous non-aromatic whilst Sarhadi et al. (2008) found 10 aromatic: 18 non-aromatic, also demonstrating the efficiency of these markers and 100 % accuracy to detect this aroma allele. The results also confirmed the previous findings of Bradbury et al. (2005a) who demonstrated that the aroma or fragrance of basmati or jasmine rice is associated with the presence of a gene encoding BADH2 on chromosome 8 of rice. This recessive gene (*fgr*) on chromosome 8 of rice which contains 8 bp deletions and 3 SNP's produced a non-functional BADH2 enzyme that most likely cause aroma in rice (Bradbury et al., 2005a).

5.2 Integration of Sensory Methods and Molecular Markers in Detection of Aroma in Rice

Aromatic rice varieties emit aroma from their leaves, grains and flowering organs at various stages of maturity (Wongpornchai et al., 2003). Paule and Powers (1989) mentioned that aroma in rice is a sensory character which was familiar to describe the odor of the aromatic rice. Several chemical constituents have been shown to be important for rice aroma. Of these aroma components, 2-acetyl-1-pyrolline (2AP), has been reported as an important aroma component of several aromatic rice varieties. There are many approaches used by researchers to determine the presence or absence of aroma in rice, such as chewing the rice grains (Reinke et al., 1991), smelling the leaves after heating with hot water (Nagaraju et al., 1975), evaluating aroma from leaves and grains with dilute KOH (Sood and Siddiq, 1978), analyzing the aroma using gas chromatography (Varaporn and Sarath, 1993), and molecular markers related to rice aroma (Bradbury et al., 2005b). In this study, we followed both sensory tests and molecular marker methods to detect the presence or absence of aroma in F_1 hybrids. The F_1 individuals which were classified having the aroma alleles also showed presence of aroma in sensory tests. This occurrence was less than 40 %. Although they had aroma alleles and showed presence of aroma in sensory tests, they varied from light aroma to strong aroma in leaf and grain. However, some of the F_1 individuals did not exhibit aroma in grain aromatic test although they had aroma alleles and produced leaf aroma. This phenomenon occurred in less than 10 % of the F_1. In another cases, around 30-40 % of F_1 that were classified as absent of aroma alleles, surprisingly produced aroma in both the leaf and grain aromatic tests. All of these were heterozygous at the BADH2 locus. There was also 30-40 % of F_1 that show aroma in leaf, but not grain and half of these were homozygous non-aromatic whilst the reminder were heterozygous at this locus. Less than 5 % F_1 do not have aroma alleles but produced grain aroma. Therefore, there are only less than 50 % F_1 that classified as aromatic or non-aromatic rice by using molecular method is well agreed with sensory methods in both leaf and grain. In Sarhadi et al. (2008) reports, they detected a coincidence among conventional methods, which is 1.7 % KOH sensory test, and molecular marker analysis in the classification between aromatic and non-aromatic rice, but sometimes molecular markers could not classify heterozygous and homozygous genotypes due to molecular nature. However, in a research by Bounphanousay et al. (2008), they used the chemical analysis (detect 2AP) with molecular marker analysis. They reported that the molecular markers are well agreed with the chemical analysis in most of the rice varieties, except some contrasting results such as in a local aromatic rice variety, Kai Noi Leuang. It was detected producing aroma but was identified as homozygous non-aromatic by molecular marker analysis. They claimed that different gene location might be responsible for the observed aroma in rice or would be the presence of another major aromatic compound. In Myint Yi et al. (2009) investigation, they mentioned that the variation in the sensory score may cause by minor genes or environmental factors or some rice varieties may carry the minor QTLs which would have an influence on rice aroma. As sensory quality has always been an important consideration in rice improvement (Paule and Powers, 1989), integration of sensory methods and molecular markers is reliable, fast and cost-effective ways essential for rice breeders to evaluate rice aroma in the breeding programs.

5.3 Genetic Basis and Factors Affecting Aroma in Rice

Aroma compound 2-acetyl-1-pyrolline (2AP) is a key component of rice aroma (Wongpornchai et al. 2003) and was determined synthesis by a non-functional BADH2 gene (Bradbury et al., 2005). More recently, Chen et al. (2008), Bradbury et al. (2008), and Huang et al. (2008) suggested there are two pathways of 2AP biosynthesis in rice: (a) BADH2-dependent 2AP synthesis, and (b) BADH2-independent 2AP synthesis. Our results matched these suggestions, for example, around 30-40 % of F_1 that classified as absent of aroma alleles, but produced aroma in both the leaf and grain aromatic tests. Moreover, the aroma smell emitted from those F_1 almost the same as the Pandan (*Pandanus amaryllifolius*) leaves smell which was linked to 2AP (Buttery et al., 1983b). Therefore, some aromatic rice varieties might be followed the second phenomenon described.

Different aroma score from light to strong which were found in $F_{1,}$ are homozygous aromatic, were reported in our experiments, as well as those heterozygous non-aromatic F_1 individuals produced aroma than expected. The aroma in rice might also be controlled by minor genes or minor QTLs (Myint Yi et al., 2009). Lorieux et al. (1996) reported that aroma is a quantitative trait. A major gene on chromosome 8 and two QTLs on chromosomes 4 and 12 regulated the formation of aroma in the rice cultivars Azucena. It is with the same line of Pinson (1994) where he mentioned aroma was controlled by genes in two different loci. However, more experiments need to be carried out to confirm the minor genes effect and their location.

Environmental factors might be another important role in determining the aroma in rice. In our observation, it was expected that F_1 individuals detected with aroma gene (BADH2) supposed to produce aroma in both leaf and grain, but we observed some of the individuals produced aroma only in leaf or only in grain during sensory tests. In most cases, leaf aroma was produced rather than grain aroma. This might be due to high temperature during their grain filling and ripening stage (Faruq et al., 2011). During the period of grain filling to ripening, the temperature of the planting field is around 30-34 ℃ at day time. High temperature may also affect the accumulation of 2-acetyl-1-pyrroline. The concentration may be very low when the grain exposed under high temperature for a long time. Further experiments can be carried out by controlling the temperature during the maturity period to confirm the accumulation of 2-acetyl-1-pyrroline in rice grains.

Aroma is mainly controlled by a major gene, but it is easily influenced by environmental conditions such as soil type, cultural practices, and temperature during the grain filling stage, storage conditions as well as storage time (Singh et al., 1997). During our observation in rice field, we found some F_1 hybrids emitted pleasant aroma from the flowering organs, especially in the morning from 8 a.m. to 10 a.m. However, pleasant aroma which we smelled in field during flowering is a result of a large number of compounds present in a specific proportion. In aromatic varieties, pleasant aroma is not only associated with cooked rice. Quite often these varieties emit aroma in the field when flowering (Mittal et al., 1995). It also means that aroma emit at different stages of rice plant growing. Whether volatile aromatic compounds released in field at the time of flowering differ from those released after cooking is an important question to be answered in further investigation.

5.4 Inheritance of Aroma in F_1 Hybrids

It is well known that aroma in rice is genetically controlled by cell nuclear genes and independent of cytoplasmic heredity. The inheritance of rice aroma probably involved modifier genes without maternal effect (Sun et al., 2008). 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. In order 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 produced hybrids with mild aroma or different aroma. In our experiments, when we crossed non-aromatic genotype (MR 219) with the aromatic genotype, we observed the decreasing in aroma score when compare with their male parent (aromatic genotype) and having the same aroma score as female parent (MR 219). A bad odour also emitted from the F_1 of MR 219/Gharib during the sensory tests. Moreover, in the molecular markers analysis, homozygous non-aromatic crossed with the homozygous aromatic produced F_1 of heterozygous non-aromatic. Whereas, the aromatic and aromatic rice crosses, the aroma score do not increase in F_1 during sensory tests, but maintain the same as their both parents. From the molecular markers analysis, homozygous aromatic F_1s were produced from homozygous aromatic crosses. However, to validate the Mendelian Model of inheritance, all the heterozygous individuals can be further tested by using segregation analysis of F_2 progenies.

Aroma in domesticated rice has apparently originated from a common ancestor and may have evolved in a genetically isolated population, or may be the outcome of a separate domestication event. This is an example of effective human selection for a recessive trait during domestication (Bounphanousay et al., 2008). In our research, similar amplification pattern in aroma (*fgr*) gene were observed in all of the 3 F_1 individuals from each cross, except for F_1 hybrid MRQ 50/Entry 11 (a: Lane 12), MR 219/Rato Basmati (b: Lane 5), MR 219/Sadri (b: Lane 7) and MRQ 50/Rato Basmati (b: Lane 9) (Figure 4.1). Most of them are from non-curve shaped F_1 which would be the unsuccessful crosses because the banding pattern followed the female parent. One of the F_1 hybrid from the curve shaped seed had different banding pattern between them may be the outcomes of homozygous aromatic and heterozygous non-aromatic cross.

Through comparison of both genotypic and phenotypic characteristics of aroma in F_1 rice individuals, it was observed that aroma from parents was successfully inherited in F_1 individuals derived from curve shaped seed compare to non-curved seeds.

5.5 Agronomic and Yield Performance of Selected F_1 Hybrids

In this research, agronomic traits or phenotypic evaluation were carried out for all F_1 seeds collected from 14 different crosses. There are only 11 of the crosses were selected, because 3 of the crosses do not produce any F_2 seeds. These three F_1 may be sterile in the reproductive organs and they are from aromatic into aromatic cross. According to Singh et al. (2000), most of the traditional aromatic rice varieties are low yielding. We also observed low yield in most F_1 hybrids (MRQ 50/Gharib). Many of them were found in aromatic with aromatic cross F_1, except for F_1 from MRQ 50/E13 which have high yield. However, most high yielding F_1s were found in crosses between the MR 219 (non-aromatic) with aromatic rice line. MR 219 is a Malaysia local variety. These results showed that the high yielding F_1s will be more advance in adaptation to tropical environment. Myint Yi et al. (2009), in their marker assisted backcross results clearly showed that all of their introgression line (IL) had agronomic characteristics similar to the place of origin Manawthukha and were well-adapted to the local environment (Myanmar).

The DMRT comparison and ANOVA analysis of the data presented suggests that the F_1s show considerable variations in growth and yield characters. F_1 of MRQ 50/E13 (9.30 g/plant), MR 219/Gharib (7.26 g/plant), MR 219/E 11 (6.28 g/plant), and MR 219/ Rato Basmati (5.84 g/plant) were selected as the four top yielders. The yield potential of these F_1s can be explained based on the higher number of fertile tillers (MR 219/Rato Basmati), fertile grain per panicle (Mr 219/E 11), increase panicle length (MRQ 50/E 13), and thousand grain weight (MR 219/Gharib). George et al. (2005) also explained the yield potential of the varieties checked based on their higher number of effective tillers, increased in panicle length, and higher number of grains per panicle. This showed that the mentioned agronomic traits are contributing to the yield.

Important agronomic traits such as plant height were also recorded. The plant height of the selected F_1s was ranged from 66.33-90.00 cm. According to IRRI index (IBPGR-IRRI, 1980), this range belongs to the short plant group. A short rice plant does not guarantee a high degree of lodging resistance (Ookawa and Ishihara, 1993). In the present study (Appendix 1.4), some of the F_1 plants have been lodged during grain filling period (hard dough stage) and some of them have been lodged after maturity. This may be due to the heaviness of the rice grains. However, in the findings of Sarhadi et al. (2008), short culm rice plants have been lodged after heading. Our results are in contrast, we do not found lodging during heading stage. The phenotypic acceptance score were found higher in two F_1s (MRQ 50/E13 and MR 219/E 7), because the overall performance is good and not lodged. Stem borer and rice buds were found in the experimental field. We also observed rice leaf blast and sheath blights diseases in the experimental plot (Appendix 1.5).

Comparison of agronomic characters along with their parents also been carried out. Grain size and shape with their grain cover color were also taken into consideration (Appendix 1.6). The grain cover color of F_2 seed from F_1 of MRQ 50/Rato Basmati was the same as their male parent, that is purple color. F_2 seeds of F_1 from MR 219/Rato Basmati were observed to have two different colors: 1) Purple (follow male parent), and 2) Yellowish (follow female parent). The awn was observed in F_2 seeds of F_1 from MRQ 50/Rambir Basmati, but the female parent does not have awn. It may inherit from the male parent. However, Myint Yi et al. (2009) reported the grain size and shape of the IL was similar to the original Manawthukha (female parent) except for the chalkiness. Thus, in our study, the agronomic traits and grain quality may have some variation between the F_2 seed with both their male and female parent results from the selection and segregation during crossing. Yet, hybridization and selection in segregating generations and mutation breeding for high yielding aromatic rice varieties have been practiced (Singh et al., 2000c).

5.6 Genetic Analysis

Yield of rice plant is a complex quantitative character controlled by many genes interacting with the environment. It is the product of many factors called yield components. Thus, the knowledge on relationship between yield and its contributing characters is needed for an efficient selection strategy for the plant breeders to evolve an economic variety. The information about phenotypic and genotypic interactions of various agronomic traits is the most importance to a plant breeder for the selection and breeding of different genotypes with increasing yield potential (Girish et al., 2006). In our investigation, phenotypic coefficients of variability were higher than genotypic coefficients of variability for all the traits in F_1 which is similar to the findings of Zahid et al., (2006), Buu and Truong, (1988) and Abdus et al. (2009). High heritability estimates were observed for DH, DM, GF, PH, PL, FGP and GYP, heritability was over 50% in all these characters. So, these estimates are helpful in making selection on the basis of phenotypic performance.

5.7 Correlation Analysis

Correlation analysis of the agronomic traits can be used as a tool for indirect selection in breeding program. Correlation studies help the plant breeder during selection and provide the understanding of yield components (Abdus et al., 2009). In our studies, the association of grain yield per plant with other traits was estimated by correlation. Only three of the agronomic traits were negatively correlated with grain yield per plant. They are days to maturity, grain filling period and plant height. However, Abdus et al. (2009) and Habib et al. (2005) reported that grain yield per plant was associated positively with days to maturity. Besides, positive correlation was found between plant height and grain yield per plant by research of Rasheed et al. (2002), Abdus et al. (2009), and Girish et al. (2006). Traits that positively correlated with grain yield per plant were observed in days to heading (DH), number of tiller (NT), number of fertile tiller (NFT), panicle length (PL), grain per panicle (GP), fertile grain per panicle (FGP) and thousand grain weight (TGW). However, Abdus et al. (2009) observed that number of tiller (NT) and grain yield per plant (GYP) was positive. On the other hand, Kibria et al. (2008) reported that grain yield was positively correlated with panicle length, number of fertile tillers, fertile grain per panicle and 1000-grain weight, respectively.

5.8 Conclusions

Several aromatic rice varieties such as MRQ 50 (Putri), PS 1297 and Mahsuri Mutant in Malaysia have been developed through mutation breeding. High cost and time consuming always related to mutation breeding and back crossing. Moreover, the selection for rice with aroma is not easy because of the large effects of the environment and the low sense heritability of aroma. However, with the integration of molecular markers and sensory tests, it is easy, inexpensive, only require small amount of tissue and fast. The aroma smell from the sensory tests also can be determined which meets the consumer needs. High aroma score was observed in F_1 of MRQ 50/E 13, MRQ 50/ Rambir Basmati and MRQ 50/Rato Basmati (leaf: 3; grain: 2). Agronomic traits and the selection strategy have been taken into consideration for selection of high yielding aromatic rice lines. Top yielder with aroma in both genotypic and phenotypic was found in F_1 of MRQ 50/E 13 (9.30 g/plant). The selected F_1 hybrids in this investigation may be used for future in aromatic rice improvement program.