CHAPTER VI

SUMMARY AND CONCLUSION

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Colored rice are becoming popular due to their nutraceuticals properties and economical values. Colored rice are of red and purple. There is an availability of diverse array of purple colored rice in Manipur and all the purple colored rice are usually called black scented rice (*Chakhao*), which are scented and very glutinuous in nature. In the present study, two black scented rice cultivars, (*Oryza sativa* cv. *Chakhao Poireiton* and *Oryza sativa* cv. *Chakhao Amubi* were taken to study the phytochemical profile and *Chakhao Poireiton* was taken to study the genes responsible for the purple pericarp color and scent. The major anthocyanin compositions of the two black scented rice cultivars (*Chakhao Poireiton* and *Chakhao Amubi*) were studied using HPLC. Four main anthocyanins, i.e. delphinidin 3-galactoside, delphinidin 3-arabinoside, cyanidin 3-galactoside and cyanidin 3-glucoside were identified in *Chakhao Poireiton* while three main anthocyanins, delphinidin 3-galactoside, delphinidin 3-arabinoside and cyanidin 3-galactoside were identified in *Chakhao Amubi*. In both the cultivars, delphinidin 3-galactoside is the most predominant anthocyanin. Both the cultivars, *Chakhao Poireiton* and *Chakhao Amubi* showed high anthocyanins, phenolic content and strong antioxidant activity of which *Chakhao Poireiton* is higher from *Chakhao Amubi*.

Black rice (*Oryza sativa* L.), an aromatic specialty rice popular in Asia, has a unique flavor, only a few volatile chemistry has been reported. The volatile profiles of the black scented rice, C*hakhao Poireiton* and *Chakhao amubi* were studied using Gas chromatrography mass spectrophotometry (GC-MS). Twenty-six volatile compounds were identified by gas chromatography-mass spectrometry from *Chakhao Poireiton* and from *Chakhao Amubi*, eleven volatile compounds were identified. Earlier studies on volatile oils profiling of aromatic rice reported that the 2-acetyl-1-

pyrroline is the major compound for the fragrance but the present study reported hexadecanoic acid, pentatriscontene and octadecenal are the major compounds giving fragrance which concluded that a complex cross section of several volatile compounds, causes the aroma in the black scented rice. The aroma increases during cooking, this may be due to the interaction of the complex mixture of the compounds with the lipids and the proteins present in the black scented rice.

From, among the two cultivars, *Chakhao Poireiton* was found to be more superior in all aspects of the phytochemical analysis conducted during the study, thus, this cultivar was taken for molecular studies for the characterization of the genes responsible for the purple pericarp color and the scent.

Using a modified CTAB method, the sample genomic DNA was extracted from young leaf sample (*Oryza sativa cv. Chakhao Poireiton*). The isolated genomic DNA was pure, free from the contaminants protein and polyphenolics/ polysaccharides compound and with a concentration of 115.7 ng/µl at 260 nm. Gene specific primers (*Pb* forward primer: 5'-GGGAGAAGCTCAACGAGATG and reverse primer: 5'-GGGTGGCAGATTCATCACTT) for the pericarp color (*Pb*) gene and (*fgr* forward primer: 5'-GCAAGTGACGGAGTACGCCT-3' reverse primer: 5'-GCTAACTTCCGCTCACGCAA-3') for the fragrance (*fgr*) gene were used for PCR reactions. The desired PCR products were visualized and purified by eluting from the gel and were cloned into pGEM-T Easy Vector System.

The cloning and sequencing of the *Pb* gene and *fgr* gene were carried out using the gene-specific primers, 1122 bp *Pb* gene sequence and 396 bp *fgr* gene sequence were obtained with the Genebank accession no. KP830117 and KP830118, respectively. The BLAT Alignment Tool mapped the 1122 bp *Pb* and 396 bp *fgr* gene fragments on chromosome 4 and 8, respectively. Geneticists use maps to describe the

location of a particular gene on a chromosome. This map uses the molecular location based on the sequence of DNA and describes the gene's precise position (molecular location) on a chromosome and indicates the size of the gene. In addition to this, the data would allow researchers to determine exactly the distance between genes on the same chromosome.

Sequence analysis of the 1122 bp of *Pb* revealed to be identical with four candidate genes encoding respective anthocyanin regulatory *Lc* protein (*Ra*) gene, anthocyanin regulatory *Lc* protein (*Ra*) gene mRNA for R-type basic helix-loop-helix protein and transcriptional anctivator (*Ra*) mRNA which concluded that the gene fragment identified is involved in the anthocyanin biosynthesis. Multiple sequence analysis of all the *Pb* gene fragment obtained from the cv. *Chakhao Poireiton* showed 2 bp (GT) deletion on the exon 7 which are also present in other black/purple rice but in the white rice there is present of the 2bp (GT) insertion, which concluded that like other black/purple rice the rich anthocyanin in the black scented rice of Manipur is also due to a mutation.

Similarly, the 396 bp of *fgr* gene fragment was found to be identical with two candidate genes encoding respective 3-methylcrotonyl-CoA carboxylase beta chain and betaine aldehyde dehydrogenase which suggests that identified *fgr* gene fragment characterized might be responsible for the fragrance/scent. The major gene for fragrance (*badh2*) on chromosome 8 and an 8-bp deletion in the exon 7 of this gene resulted in truncation of betaine aldehyde dehydrogenease enzyme whose loss-of-function lead to the accumulation of a major aromatic compound, 2-acetyl 1-pyrroline (2AP) in other fragrant rice. However, *fgr* gene fragment from *Chakhao Poireiton* is an exception to this mutation. Here, in *Chakhao Poireiton* there is a complex mixture of volatile compounds imparting the completely different aroma and the sequence of

the *fgr* region is also completely different from the previously reported mutation (the 8 bp deletion which causes the accumulation of 2-AP for aroma). From these results, it can be concluded that there is an availability of a wide diverse set of rice fragrance. The absent of 2-AP and the exception from the previously reported mutation in *Chakhao Poireiton* concludes that neither 2AP nor 8 bp deletion which were reported earlier could be the cause of fragrance, thus, indicating the involvement of different gene(s)/ loci or other mutation altogether. From the results, it could also be concluded that the presence of modifiers and the genetic background of the variety/cultivar may play a role in producing different or modified fragrance/scent different from the typical popcorn-like aroma produced by 2AP.

Gene expression study of the *Pb* and *fgr* genes showed the level of mRNA transcribed for the *Pb* gene was found to be higher from that of the actin, housekeeping gene while that of the *fgr* gene was similar with that of actin gene. Hence, the *Pb* gene likely play either a regulatory role in anthocyanin biosynthesis or are related to anthocyanin metabolism during flavonoid biosynthesis. Similarly, the *fgr* gene might involved in emanating scent by the black scented rice cultivar *Chakhao Poireiton*.

Now a days, people are seeing forward more to the supplementation of natural antioxidant in the diet, thus, the consumption of pigmented rice will be a great thinking for the improvement of human health. Therefore, the black scented rice extracts could be a potential source of antioxidative phytochemicals and useful ingredient for nutraceuticals or functional food products. The supplementation of the black scented rice (*Chakhao*) in the diet would have a great impact on human health.

The gene specific primers used for *Pb* and *fgr* genes amplification could be used as molecular markers for discriminating the cultivars from mixing up with others

at the early stage of their growth which would help in the pure germplasm conservation. The present study also suggests that the full gene sequence of *Pb* and *fgr* genes from the *Chakhao Poireiton* could be isolated and could be included in the crop improvement programme. Furthermore, the study provides guidance for effective control programs and assist in the exploration of the black scented rice which would pave the way for both cloning and marker-assisted selection of the *Pb* and *fgr* genes. A better understanding of these cultivars would encourage the agricultural scientists to include these cultivars in the crop improvement programme for higher production. Therefore, crop improvement using important germplasms with the application of modern techniques would fulfill the increasing demand and provide nutraceutical properties for the developing country like India.

Limitations and future prospective of this study

The present study provides a precise knowledge of the importance of black scented rice (*Chakhao*) and the understanding of the gene at the molecular level. However, further research on the genes is suggested so as to make applicable of these valuables genes in the crop improvement programme. With the help of molecular breeding and the advancement in transgenic crops, efforts can be taken up for developing high yielding varieties without losing the grain quality characters, cooking quality, aroma and its applications in pharmaceutical sciences. The improved varieties can be exported to Southeast and East Asian countries where there is a huge demand of black scented rice and further, drawing the attraction of the global attention regarding the wonderful quality of black scented rice.

CHAPTER VII

BIBLIOGRAPHY