CHAPTER I

INTRODUCTION

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1.1 Black rice

Black rice varieties are those rice with colored pericarp (other than white and red). Black rice has high anthocyanin content located in the pericarp layers, which gives it a dark purple color (Ryu et al.1998; Takashi et al.2001). In addition to traditional white or common rice, specialty rice that have unique properties like unique flavor, aroma (unique aromas), color (red, purple, black), nutrition (glossiness, stickiness and smooth texture), chemical composition, esthetic, waxy (very low amylose content) and superior processing quality which are increasing in demand and are widely grown in India, Pakistan and Thailand and are popular in Asia, the Middle East, Europe and the United States (Chaudhary et al. 2003, Choi et al. 2000, Yang et al. 2008a; 2010). The demand for various types of specialty rice has been increasing in recent years, which are sold for as much as50% more than traditional rice cultivars (Chaudhary et al. 2003).In addition to this, Black rice has been used in various traditional medicines and recently many researchers have reported that they have several health benefits in various studies and thus, black rice is being considered as the new superfood by US scientists.

1.2 Importance of black rice

In Asian countries, black rice is often consumed after mixing with white rice to enhance the flavor, color and nutritional value (Yang et al. 2008a) which includes high protein, total essential amino acids, vitamin B1 and minerals - Fe, Zn, Mn, and P (Suzuki et al. 2004) and intensely colored because of anthocyanin. One serving of black rice, even though contains some calories, but offers a high amount of flavanoid phytonutrients, important fiber, mineral content such as iron and copper and it is a good source of plant based protein which are hard to get to plant based eaters who

rely on grains and legumes for protein (Dr. Axe 2015). Black rice is antioxidant-rich, containing anthocyanin (Dr. Axe 2015; Wolf, 2015). Xu (2010) reported that a spoonful of black rice bran provides the same amount or more anthocyanins than a spoonful of blueberries. Anthocyanin antioxidants are very important which help the prevention of cardiovascular disease, protection against cancer, improving brain function, reducing inflammation and more.

1.3 Future potential of black rice

Black rice has great future potential; its bran is applicable in the food industries, as a natural food coloring, beverages and in pharmaceutical industries. Potential to export to the international markets as it is one of the super food.

1.4 Chemistry of fragrance

Suwansri et al. & Bhattacharjee et al. (2002) reported that flavor is the primary importance of specialty rice and the superior flavor increases consumer satisfaction and repeatedly purchase, aroma and taste are the most critical quality traits considered by the Indian consumers and flavor is one of the most important acceptance factors considered by the Asian consumers in the United States. Flavor is composed of taste and aroma while the aroma is conferred by volatile compounds emanating from cooked rice (Yang et al. 2008b). From cooked rice, a number of compounds have been identified, but only a few make up the characteristic aroma (Maga, 1984; Tsugita, 1986; Grosch & Schieberle, 1997; Yang et al. 2008b). Rice cultivars can be separated generally into aromatic and nonaromantic types, aromatic rice has a relatively diverse range of unique aromas (Yang et al. 2008b). A complex mixture of odor-active compounds comprises the aroma of both aromatic and nonaromatic rice approximately 300 volatile compounds have been identified from various cultivars of aromatic and nonaromatic rice (Widjaja et al. 1996) and several odor active

compounds in cooked aromatic rice has been determined using odor units (Buttery, 1988; Jezussek et al. 2002). In aromatic rice, 2-acetyl 1-pyrroline (2-AP) described as having a "popcorn-like" odor by American and "pandanlike" odor by Asian consumers (Paule et al. 1989) which is synthesized in aerial parts of aromatic rice during growth (Yoshihashi et al. 2002) is considered to be highly important and in some nonaromatic types, it is present at a very low negligible concentration. Yang et al. (2008b) reported that however, 2-AP is not only the compound responsible for the unique aromasbut their aromas are due to qualitative and quantitative variations in a diverse cross section of odor-active compounds. In cooked rice, lipid-derived odor-active compounds were formed during the degradation of oleic (octanal, heptanal, nonanal, (*E*)-2-nonenal, decanal, and 2-heptanone are formed from oleic acid), linoleic (hexanal, pentanol, pentanal, (*E*)-2-octenal, (*E*,*E*)-2,4-decadienal and 2-pentylfuran are formed from linoleic acid) and linolenic acid (Yang, 2007; Monsoor & Proctor; Zhou et al. 2002). The oxidation of lipid yields rancid odors and also induces various deteriorative reactions with proteins, amino acids and other components (Nawar, 1996). Examples of thermally derived flavor compounds formed in rice during cooking, which have seasoning-like and meaty-like aromas are 3-Hydroxy-4,5-dimethyl-2(5*H*)-furanone and bis (2-methyl-3-furyl) disulfide, respectively (Jezussek et al. 2002).

The volatile chemistry of rice grain suggests that both volatile as well as semi-volatile compounds either with a single predominant compound or a complex mixture of several compounds are associated with a unique flavor and strength of aroma in the diverse set of fragrant rice. Of the several volatile compounds identified from various fragrant and non-fragrant rice varieties, 2-acetyl 1-pyrroline (2AP) is regarded as the most important flavor compound in rice because of its popcorn-like aroma and low

odor threshold (Buttery et al. 1982). The popcorn-like smell, which gives both Basmati and Jasmine rice their distinctive fragrance, stemming primarily from its 2AP content, is considered desirable by many consumers (Bergman et al. 2000). 2AP was reported to be found in all parts of the rice plant, except for the roots (Sood & Siddiq, 1978) and is also found, at concentrations up to 100 times lower, in non-fragrant varieties (Grosch & Schieberle, 1997). In addition to its association with rice fragrance, 2AP has alsobeen associated with the flavor of a range of foods like popcorn (Schieberle, 1995), mung bean (Brahmachary & Ghosh, 2002), etc. A principal aroma compound 2AP and L-proline as a precursor of aroma have been identified in rice by many studies (Yoshihashi et al. 2002) but the biochemical pathways of 2AP synthesis is presently unknown (Niu et al. 2008; Fitzgerald et al. 2009). Vanavichit et al. (2005) elucidated the 2AP biosynthetic pathway and proposed that 2AP is synthesized via the polyamine pathway. 1-pyrroline (1P) is found to be the immediate precursor of2AP which is formed from 4-aminobutyraldehyde (AB-ald; the immediate precursor of 4-aminobutyricacid, GABA). A more compelling evidence for 2AP biosynthetic pathway was elucidated with the discovery of candidate gene for fragrance, recently. Chen et al. (2008) elucidated that the 4-aminobutyraldehyde (AB-ald) is maintained in an equimolar ratio withΔ1-pyrroline, an immediate 2AP precursor and the AB-ald levels appeared to be the important factor regulating the rate of 2AP biosynthesis (Fig. 1a). They suggested that in nonfragrant rice, the functional BADH2 enzyme, coded by the aroma gene *Fgr* inhibits 2AP biosynthesis while the non-functional badh2, coded by *fgr* result to the formation of 2AP in fragrant rice. It is also suggested that an effective substrate for BADH2 for the 2AP accumulation in rice is γ-aminobutyraldehyde (GABald) (Bradbury et al. 2008) (Fig. 1a). However, Huang et al. (2008) reported that increased

expression of Δ1-pyrroline-5-carboxylate synthetase in fragrant varieties compared with non-fragrant varieties, a concomitant elevated concentrations of its product and Δ1-pyrroline-5-carboxylate (the immediate precursor) of proline synthesized from glutamate, reacts with methylglyoxal forming 2AP, with no direct role proposed for BADH2 (Fig. 1b).

In addition to 2AP several other volatile as well as semi-volatile compounds either with a single predominant compound or a complex mixture of several compounds are associated with unique flavor and strength of aroma of diverse fragrant rice (Sakthivel et al. 2009). Alkanals, alk-2-enals, alka-2, 4-dienals, 2-pentylfuran and 2-phenylethanolare the other important compounds identified for the fragrance and many other compounds are also identified which contributed to the total aroma profile (Widjaja et al.1996). Similarly, some more new compounds which were present in high levels in Basmati 370 were also identified, 2-amino acetophenone and 3-hydroxy-4, 5-dimethyl-2(5H)-furanone (Jezussek et al. 2002). While the 2AP is associated with pleasant, popcorn-like aroma of fragrant rice, hexanal which developed from lipid oxidation has been related with off-odors (Bergman et al. 2000). In Basmati and Jasmine fragrant rice, a great difference exists 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 which may contribute to their respective flavors (Kirstin & Wootton, 2004). Champagne (2008) reported that each variety has a unique fragrance resulting from a number of volatile compounds which may vary from well characterized popcorn-like aroma/2APassociatedaroma although little is known about their relationships with aroma/flavor.

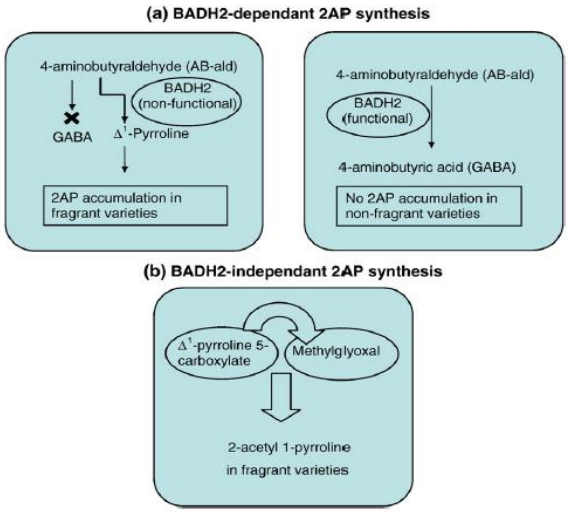

Fig.1: Pathway of 2AP biosynthesis (Sakthivel et al. 2009). (a) BADH2-dependant 2AP synthesis (Chen et al. 2008; Bradbury et al.2008), (b) BADH2-independant 2AP synthesis (Huang et al. 2008).

Table. 1: Molecular mapping of fragrance locus in rice (Sakthivel et al. 2009).

Gene(s)/QTL(s)	Marker type	Chr. location	Reference
1	RFLP	8	Ahn et al. (1992)
1	RFLP	8	Yano et al. (1992)
1	RAPD	-	Tragoonrung et al. (1996)
1 major gene and 2QTLs	RFLP, STS	8, 4 and 12	Lorieux et al. (1996)
1	SSR	8	Garland et al. (2000)
1	SSR	8	Cordeiro et al. (2002)
1	SNP	8	Jin et al. (2003)
1	SSR	8	Bradbury et al. (2005)
1	SSR, EST	8	Wanchana et al. (2005)
1	SSR	8	Chen et al. (2006)
1	SSR	8	Li et al. (2006)
3 QTLs	SSR	QTLs on 3, 4 and 8	Amarawathi et al. (2008)
1	SSR	8	Sun et al. (2008)

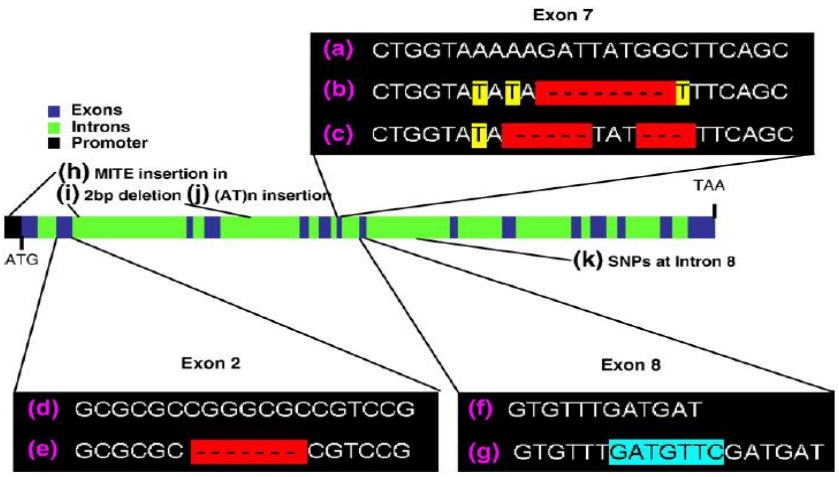

Fig. 2: The structure of *Badh2* gene showing various mutations as reported by Sakthivel et al. 2009. (a) exon 7 sequence of non-fragrant allele, (b) An 8-bp deletion (highlighted in red) and 3 SNPs (highlighted in yellow) in exon 7 of fragrant allele (Bradbury et al. 2005), (c) A discontinuous 8-bp deletion (highlighted in red) and a SNP (highlighted in yellow) of fragrant allele (Amarawathi et al. 2008), (d) exon 2 sequence of non-fragrant allele, (e) an 7-bp deletion (highlighted in red) in exon 2 of fragrant allele (Shi et al. 2008), (f) exon 8 sequence of non-fragrant allele, (g) an 7-bp insertion (highlighted in turquoise) in exon 8 of fragrant allele (Amarawathi et al. 2008), (h) a MITE insertion in promoter (Bourgis et al. 2008), (i) a 2-bp (TT) deletion in intron 2, (j) a (AT)n insertion in intron 4 (Chen et al. 2008) and (k) two SNPs in central section of intron 8 (Sun et al. 2008).

1.5 Gene responsible for fragrance/scent

A single recessive gene (*fgr)* on chromosome 8 is responsible for the fragrance. In rice (*Oryza sativa*), the presence of a dominant *Badh2* allele encoding betaine aldehyde dehydrogenase (BADH2) inhibits the synthesis of 2-acetyl-1-pyrroline (2AP), a potent flavor component in rice fragrance however, the two recessive alleles of BADH2 namely *badh2-E2* and *badh2-E7* induced 2AP formation. Sakthivel et al. (2009) reported that availability of high-density molecular marker maps and genome sequences in rice has allowed mapping, fine mapping and positional cloning of gene for fragrance, they also reported the studies of various workers, which put up many systematic attempts to map fragrance trait at molecular level (Table 1). The single recessive gene (*fgr)* has been mapped on chromosome 8 (Ahn et al. 1992; Wanchana et al. 2005; Chen et al.2006; 2008; Sun et al. 2008).

The *fgr* gene encodes badh2 (betaine aldehyde deydrogenase homologue 2) and reported an eight base-pairs (8-bp) deletion in exon 7 as the cause of fragrance in many of the fragrant rice, as the loss-of-function of the enzyme leading to development of fragrance corresponded well with the recessive nature of the trait, they suggested that *badh2* was indeed *fgr* (Bradbury et al. 2005). Sakthivel et al. (2009) reported that the 8-bp deletion and 3 single nucleotide polymorphisms (SNPs) in exon 7 of *badh2* have led to the introduction of premature stop codon to produce a truncated protein resulting in the abrogation of the function of the enzyme BADH2 which accumulate substrate 2AP in fragrant varieties, while the functional *Badh2* gene codes for a 503 amino acid mature protein which consumes the substrate in non-fragrant varieties. In addition to 8-bp deletion in exon 7, several variations including a 7-bp insertion in exon 8 (Amarawathi et al. 2008); a 7-bp deletion in exon 2 (Shi et al. 2008); absence of MITE (miniature interspersed transposable element) in promoter

(Bourgis et al. 2008); two new SNPs in the central section of intron 8 (Sun et al. 2008); a TT deletion in intron 2 and a repeated (AT)n insert in intron 4 (Chen et al. 2008) of badh2 were reported in various fragrant varieties. Although, the 8-bp deletion has been reported as the genetic cause for aroma, Sakthivel et al. (2006) found five indigenous aromatic rice genotypes without this deletion viz., Tarunbhog, Ganjeikalli, Bishnubhog, Bansphool A and Adamchini. Similarly, there are reports that some fragrant varieties also do not carry the deletion (Kuo et al. 2005; Navarro et al. 2007; Shi et al. 2008; Fitzgerald et al. 2008). Sakthivel et al. (2009) reported the existence of allelic/genic diversity for fragrance in the gene pool of aromatic rice.

1.6 Chemistry of purple pericarp color

In the aleurone layer of black rice (belonging to *Oryza sativa* L. *indica*) there is present of the pigments which have been reported to contain acetylated procyanidin, anthocyanins and other phenolic compounds which have significant free radical scavenging activity (Hu et al. 2003; Yawadio et al. 2007). Those colorings are due to the naturally occurring compounds belonging to the family flavonoids, there are more than 600 naturally occurring anthocyanins, among them, the most common are pelargonidin, cyanidin, delphinidin, peonidin, petunidin and malvidin (Anderson et al. 2006; Yawadio et al. 2007). Anthocyanins are found in plants in glycosylated forms linked with glucose, galactose, arabinose, rhamnose, xylose and fructose (Choia et al. 2007; Hosseinian & Beta, 2007; Mazza et al. 2004). Fig. 3: shows the basic structure of anthocyanins, that includes C-6 (A-ring), C-3 (C-ring), C-6 (B-ring), chromane ring which is an additional contribution to the aromaticity of the compound (Prior & Wu, 2006; Mazza & Miniati, 1993). Anthocyanins occur as glycosides of their respective aglycone anthocyanidin chromophores and the differences between individual anthocyanins are due to the number of hydroxyl groups, the nature and

number of sugars attached to the molecule, the position of this attachment and the nature and number of aliphatic or aromatic acids attached to sugars in the molecule (Han et al. 2006).

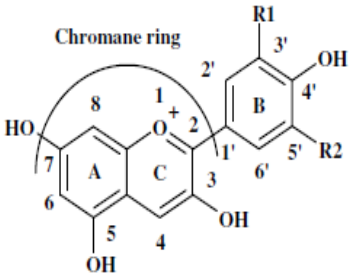

Fig 3: Basic structure of anthocyanin

Table 2: Chemical structures and molecular weights (MW) of six common anthocyanidins. (Wu & Prior, 2005; Prior & Wu, 2006; Mazza & Miniati, 1993)

Aglycone	R1	R2	colour	λ_{max} (nm)
Cyanidin (Cy)	OH	H	Red	535
Peonidin (Pn)	OCH3	H	Bluish-purple	532
Pelargonidin (Pg)	H	H	Orange-red	520
Malvidin (Mv)	OCH3	OCH3	Purple	542
Delphinidin (Dp)	OH	OH	Purple	546
Petunidin (Pt)	OCH3	OH	Purple	543

Anthocyanins are a major group of secondary metabolites believed to play an important role in plant function and human nutrition. Recently, due to the antioxidant activity of anthocyanins, they have been recognized as health promoting food ingredients (Nam et al. 2006; Philpott et al. 2006) and have anticancer (Hyun and Chung, 2004), hypoglycemic and anti-inflammatory effects (Tsuda et al. 2003). In mice, pigment-supplemented diets of black rice reduced oxidative stress (Xia et al. 2003) and they may have antiatherogenic activity (Xia et al. 2006). Park et al. (2008) reported that anthocyanins have antioxidant, free radical scavenging activities which may reduce the risks of cardiovascular diseases and cancer with anti-inflammatory, they also reported that cell invasion of human fibrosarcoma HT-1080 cell could be inhibited by delphinidin *in vitro.*

1.7 Gene responsible for the purple pericarp color

Many researchers have studied the biosynthetic pathway of anthocyanins with the isolation of the genes that encode the enzymes of plant secondary metabolism

(Fig. 4) and in anthocyanin biosynthesis, the activity of the genes involved is largely regulated by structural genes and their regulatory genes and specified at the transcriptional level, resulting in organ-specific accumulation of compound (Furukawa et al. 2007). Kinoshita (1995) summarized that in anthocyanin coloration, 26 genes are involved, 10 genes in the inhibition of anthocyanin coloration and 15 genes for the coloration by compounds other than anthocyanins, such as proanthocyanidins.

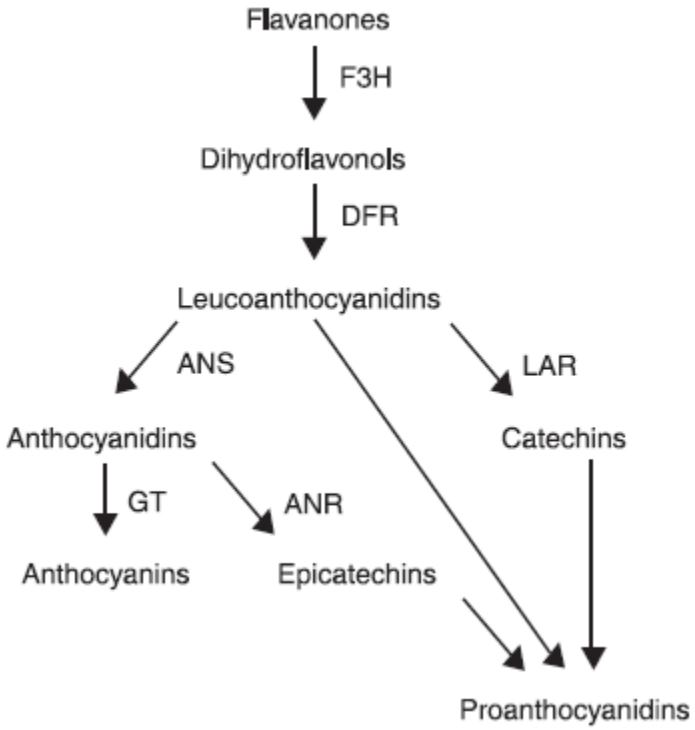

Fig. 4: Metabolic pathways for the synthesis of pro-anthocyanidins and anthocyanins (Furukawa et al 2007). The biosynthetic pathway for each compound and the enzymes are as follows: ANR, anthocyanidin reductase; ANS, anthocyanidin synthase; DFR, dihydroflavonol-4-reductase; F3H, flavanone-3-hydroxylase; GT, anthocyanidin glucosyl transferase; LAR, leucoanthocyanidin reductase (Xie and Dixon, 2005).

In maize, many structural genes are involved in the biosynthesis of anthocyanin pigments, they are *Activator 1* (*A1*), *A2*, *BZX1*, *BZ2*, *Chromogen 1* (*C1*), *C2*, *CHl* and *R* and two regulatory gene families, *R/B* and *C1*/Purple leaf (*Pl*) (Furukawa et al. 2007). Hu et al. (1996) reported the maize *R* and *B* genes of maize

comprise a very small gene family regulating anthocyanin biosynthesis by activating transcription of some of the structural genes in the pathway, this gene family can have as few as two members with a single *B* gene on chromosome 2 and a single *R* gene on chromosome 10**.** However, many researchers have described *R* complexes with multiple *R* genes and gene fragments (Robbins et al. 1991; Walker et al. 1995). The *R* and *B* genes encode homologous proteins related to the MYC type regulatory gene families which have a basic helix-loop-helix (bHLH) motif and nuclear localization signals and nuclear localization signals (Chandler et al. 1989; Shieh et al. 1993). *R* and *B* gene products can also activate transcription of chimeric genes containing structural gene promoters (isolated from the *Bzl* and *A1* genes) fused to reporter genes (Klein et al. 1989; Goff et al. 1990; 1992; Roth et al. 1991). The presence of a second transcriptional activator containing a MYB related regulatory protein, one of the largest plant transcription families encoded by the duplicate *C1* and *Pl* genes also activate the structural genes in the pathway (Paz-ares et al. 1987; Goff et al. 1992; Cone et al. 1993). Inspite of the action of two classes of regulatory proteins (*R* or *B* and *C1*or *Pl*) which activate the anthocyanin biosynthetic pathway, the diverse pigmentation patterns displayed by maize strains primarily reflect the allelic diversity of the *R* and *B* loci as indicated by genetic studies (Hu et al. 1996). Molecular studies have also shown that *R* and *B* alleles differ in their pattern of gene expression i. e. the presence or absence of the *R/B* protein determines whether a particular cell type will bepigmented (Ludwig et al. 1989; Radicella et al. 1992; Hu et al. 1996). Thus, additive effects of the particular allele at the *B* locus and the composition of the *R* locus or *R* complex represented the overall pattern of pigmentation (Ludwig & Wessler, 1990). Recent experiments suggest that anthocyanin regulation network have been conserved in all flowering plants (Hu et al. 1996). It has been reported that in

other plants also the maize *R* and *B* genes activate such as the grasses sorghum (Casas et al. 1993) and wheat (Bilang et al.1993) and the dicots tobacco, *Arabidopsis* and petunia (Lloyd et al. 1992; Quattrocchio et al.1993; Galway et al.1994). Furthermore, *R* homologues have been isolated from *Antirrhinum majus* (*delila*) and petunia (*jafl3*) and *R* homologues also appear to be members of small gene families (Goodrich et al.1992; Quattrocchio, 1994; Hu et al. 1996). Rice is another member of the grass family that, like maize, is the product of intensive human selection and also display diverse pigmentation patterns that may also reflect proliferation of *R* alleles but their genome architecture and evolutionary history differ dramatically from maize (Hu et al. 1996) in which the domesticated rice is a true diploid with no cytological or molecular evidence for an ancient polyploidization event (Oka, 1988) and rice has the smallest genome of all of the members of the grass family analyzed to date (Arumuganathan and Earle, 1991). As that of the maize, in rice also the genetic investigations have demonstrated that anthocyanin pigmentation involves at least the chromogen gene *C*, activator gene *A*, and tissue-specific regulator gene *Pl* for *C* and *A* (Nagao and Takahashi, 1963). Two loci, *Pb* (*Prp-b*) located on chromosome 4 (Cause et al. 1994) and *Pp* (*Prp-a*) on chromosome 1 (Yoshimura et al. 1997) have been identified by classical genetic analysis, which are required for the pericarp pigmentation with anthocyanins of purple rice. Considering the role played by polyploidization and transposable elements in shaping the *R/B* gene family of maize, Hu et al. (1996) determined whether *R/B* genes existed as a gene family in rice, probing the cDNA of the *Lc* gene that regulates anthocyanin biosynthesis in maize which identified two bHLH homologs, *Ra* and *Rb* in the leaf cDNA library of the purple leaf rice line (Purple 522), and mapped the *Ra* and *Rb* genes on chromosome 4 and chromosome 1, respectively. Further identified that the *Ra* locus contains two

genes, *Ra1* and *Ra2* (Hu et al. 2000). DNA sequence analysis of the PCR fragments of all the purple/black rice showed the 2 bp (GT) deletion, but that those of white rice showed the 2bp (GT) insertion, confirming that the rice *Ra* and *Pb* genes are the same gene (Wang & Shu, 2007; Rahman et al. 2013). Sakamoto et al. (2001) also probed the cDNA library prepared from purple leaves of T65-Plw using the maize *B-Peru* cDNA and identified the cDNA sequence of the *Plw* locus, which activates the anthocyanin biosynthesis pathway in most of the aerial tissues except the stem node in rice and demonstrated that the *Plw* locus composed of at least two genes, *OSB1* and *OSB2*, they further suggested that the *OSB1* gene was the *Ra* gene in nature.

1.8 Chromosomal location of the gene

The availability of a rice genome sequence (Goff et al. 2002) provided an opportunity to discover the gene by comparison of the sequences of fragrant and non-fragrant genotypes. Several molecular markers are available which are useful in seed purity testing, varietal fingerprinting and patenting, tagging, mapping and pyramiding major genes for a particular trait, pyramiding QTL's of importance traits, map based cloning, sequencing, etc.

1.9 BLAT: A new algorithm

The local alignment problem between two short sequences was solved by the Smith-Waterman algorithm in 1980 (Smith & Waterman, 1981). The FASTA (Pearson & Lipman 1988) and the BLAST family of alignment programs, including NCBI BLAST (Altschul et al. 1990; 1997), MegaBLAST (Zhang et al. 2000) and WU-BLAST (Altschul et al. 1990; Gish and States 1993) provide flexible and fast alignments involving large sequence databases and are available free on many web sites. The cDNA alignment by Sim4 (Florea et al. 1998) is very fine. The SAM program (Karplus et al. 1998) and PSI-BLAST (Altschul et al. 1997) find sure remote

homologs even though the process is slow. Many algorithms of Gotoh robustly deal with gaps (Gotoh, 1990; 2000) and SSAHA (Ning et al. 2001) maps sequence reads to the genome with significant efficiency.

Kent (2002) developed a new algorithm called BLAT, which is a "BLAST-like alignment tool," BLAT is very similar to BLAST but BLAT differs from BLAST in some significant ways. The BLAT program rapidly scans for relatively short matches (hits) and extends these into high-scoring pairs (HSPs) (Kent, 2002). BLAT builds an index of the database and then scans linearly through the query sequence, it can trigger extensions on any number of perfect or near-perfect hits, BLAT stitches them together into a larger alignment and it has special code to handle introns in RNA/DNA alignments and is available in several forms. Building an index of the whole genome is a relatively slow procedure but this has been solved using BLAT server, which builds the index and keeps it in memory and thus, a BLAT client can then query the index through the server. The client/server version is available via a web interface at http://genome.ucsc.edu and is suitable interactive applications. A more suitable stand-alone BLAT is also available for batch runs on one or more CPUs and both the client/server and the stand-alone can do comparisons at the nucleotide, protein or translated nucleotide level. Throughout the world, several researchers used BLAT to perform thousands of interactive sequence searches per day. BLAT produced multiple alignments for an mRNA/DNA Alignments which scored only the highest scoring alignment. The BLAT can also be used in translated mode to align proteins or mRNA from one species against genomic DNA of another species.

1.10 Gene expression

Cells in all organisms regulate gene expression by turnover of gene transcripts (messenger RNA): The amount of an expressed gene in a cell can be measured by the number of copies of an mRNA transcript of that gene present in a sample. Amplification of the gene transcript is necessary, to robustly detect and quantify gene expression from small amounts of RNA.

The polymerase chain reaction (PCR) is a sensitive technique by which a single DNA molecule can serve as a template for amplification (Azevedo et al. 2003). A variant of this method is real-time quantitative PCR (qRT-PCR), which allows quantification of a specific region of DNA. The template used during RNA quantification is complementary DNA (cDNA), which is product of reverse transcription of ribonucleic acid (RNA). In the conventional PCR, electrophoresis is used to assess the product of amplification, while in qRT-PCR, fluorescent molecules are used for the chemical reaction, which allows the quantification of the amplicon. The SYBR Green provided the simplest method for the detection and quantification of the PCR products in real-time reactions with high sensitivity (Nygard et al. 2007).This technique has been successfully used to evaluate the levels of mRNA in a given cell type. Reference or housekeeping genes are expressed constitutively by different cell types and are used to normalize the data (Karge et al. 1998). The β-actin gene encodes a structural protein of cytoskeleton and is perhaps the most widely used gene for normalization in the experiments of gene expression (Pohjanvirta et al. 2006).

1.11 Black scented rice of Manipur

The current study is on the black scented rice of Manipur State, which lies in the North- eastern part of India bordering Myanmar. Manipur has a large varieties of

indigenous rice germplasms which range their adaptation from low lying lake areas to rain fed uplands of Manipur hills. There is also a diverse set of locally adapted aromatic rice from black colored aromatic rice to white colored aromatic rice. These rice varieties are locally called *Chakhao*. The literal meaning of *Chakhao* in Manipuri language is delicious rice (*Chak* means rice and *ahoaba* means delicious). The black scented rice of Manipur are poor yielders (about 2,50 2,500 kg/ha as paddy) and are usually grown during Kharif season The black scented rice, has their importance as aromatic and its dark purple color and they are very glutinous in nature. They are very important in the community feast as well as ceremonial purposes as delicacy. These are one of the high rated dish serve as desserts, flakes, bread, cakes, beverages and a special snack "Utong Chak" prepared within bamboo sticks. They are sold in the local markets at a premium rate of about Rs. 80-120 per kg of rice. In the recent years, the black scented rice of Manipur is served in standard hotels as a top rated variety of rice.

1.12 Significance of the study

The black scented rice of Manipur is very poorly studied. Recently, there has been only a few studies on the black scented rice of Manipur regarding the germplasms collection, conservation, genetic diversity (Singh and Baghel, 2003; Singh and Sharma 1998; Roy et al. 2014) and Das et al. 2014, reviewed on its potential values. The present study is the first and foremost study, so far, no such work considering the phytochemical profiling and studies on the genes responsible for the pericarp color and fragrance/scent of the *Chakhao* has not been reported before. The present study mainly focuses on the phytochemical studies using Spectrophotometry, HPLC and GCMS of the black scented rice cultivars *Chakhao Poireiton* and *Chakhao Amubi*. The study also aimed at identification of the genes

responsible for the pericarp color and the fragrance/scent from the cv. *Chakhao Poireiton*, further cloning and characterization of these valuable genes. The isolated gene sequences were analyzed using bioinformatics tools and software and the gene expression profiling was studied. Despite of being their great values in nutrition, medicinally and economically, the farmers of Manipur neglect the cultivation of the black scented rice due its low yield. Thus, the current study was taken to have a better understanding of the neutraceutical values of *Chakhao*. As well as the present study would let understand the genic molecular level, which would make easier the exploration of black scented rice and furthermore, the inclusion of the black scented rice in the crop improvement program.

1.13 Objectives of the study

Considering the above information and background, the programme of the study was designed with the following objectives.

I. Determination of bioactive compounds causing the scent and the pericarp color using High Performance Liquid Chromatography (HPLC) and Gas chromatography-mass spectrometry (GC-MS).

II. Molecular mapping of the genes responsible for the scent and pericarp color of the black scented rice.

III. Sequencing and analysis of the sequences using bioinformatics tools and softwares.