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Review

The Genetics of Pandan-Like Fragrance, 2-Acetyl-1-Pyrroline, in Crops

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ABSTRACT

The main advantages of pandan (*Pandanus amaryllifolius* Roxb.) is the fresh leaves which mainly used for the pleasant fragrance in cuisine. 2-acytile-1-pyrroline (2AP) (also known as 1-(3,4-dihydro-2H-pyrrol-5yl) ethanone) is the principle volatile chemical responsible for the fragrance in pandan. 2AP was identified for the first time from the cooked rice. Many cultivars of certain crops also produce pandan-like fragrance/2AP including rice (*Oryza sativa* L.), sorghum (*Sorghum bicolor* (L.) Moench), mungbean (*Vigna radiata* (L.) Wilczek), soybean (*Glycine max* (L.) Merr.), coconut (*Cocos nucifera* L.), cucumber (*Cucumis sativus* L.), wax gourd (*Benincasa hispida*), and taro (*Colocasia esculenta* (L.) Schott). The fragrant crop varieties command higher price than non-fragrant cultivars. Breeding for fragrance is a main goal in breeding programs in these crops. Although genetics studies revealed that the presence of fragrance in crops is monogenic trait and that mutation(s) resulting in null or reduced function of betaine aldehyde dehydrogenase 2 (BADH2)/ amino aldehyde dehydrogenase (AADH) causes production of 2AP, the level of the fragrance is quantitative in nature. In this paper, we review and discuss the genetic controls of the fragrance in some crops.

INTRODUCTION

Pandan (*Pandanus amaryllifolius* Roxb.) is a fragrant crop widely known in South and Southeast Asia. The fresh leaves are mainly used for the pleasant fragrance in cuisine in Southeast Asia, especially Thailand. 2-acytile-1-pyrroline (2AP) (also known as 1-(3,4-dihydro-2*H*-pyrrol-5yl)ethanone) is the principle volatile chemical responsible for the fragrance in pandan (Buttery, Juliano, & Ling, 1983). 2AP was identified for the first time from the cooked rice (Buttery, Ling, Juliano, & Turnbaugh, 1983). Besides pandan, some varieties/cultivars of certain crops also produce pandan-like fragrance/2AP including rice (*Oryza sativa* L.), coconut (*Cocos nucifera* L.), sorghum (*Sorghum bicolor* (L.) Moench), mungbean (*Vigna radiata* (L.) Wilczek), soybean (*Glycine max* (L.) Merr.), cucumber (*Cucumis sativus* L.), wax gourd (*Benincasa hispida*), and taro (*Colocasia esculenta* (L.) Schott). The fragrant cultivars of these crops are highly-valued and have higher price than non-fragrant ones. The prices of Thai fragrant jasmine rice, India basmati rice, and aromatic Thai coconut are more than two-fold those of non-fragrant cultivars. Thus the fragrance is one of the most important grain quality traits in rice determining the market price. This trait is also related to local and national identity (Fitzgerald, Sackville Hamilton, Calingacion, Verhoeven, & Butardo, 2008). Aside from the said crops, some other plants also produce 2AP, i.e. bread flower (*Vallarisglabra* Ktze.) (Wongpornchai, Sriseadka, & Choonvisase, 2003) and *Bassia latifolia* Roxb.). Although 2AP is naturally produced from living organism, it can also be produced from Millard reaction of heated and processed foods. Since pandan-like fragrance is one important trait in food crops and determines the product price in the market, understanding the genetic basis of the fragrance in the crops is important to improve new fragrance cultivars.

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Genetic Basis of the Biosynthesis of 2AP in Crops

Rice (Oryza sativa (L.))

Rice is the third most important cereal crop of the world in term of cultivated areas. It is a staple food of about 400 million people of the world, mostly in Asia. Hundreds of thousands of rice varieties/cultivars are present, of which hundreds of landrace and improved varieties are fragrant ones. Among the fragrance rice cultivars, "Khao Dok Mali 105" (KDML105; also known as "jasmine rice" or "Hom Mali") from Thailand and "Basmati 370" and "Basmati 347" from India and Pakistan are well known for their fine and strong pandan-like fragrance.

Scientists have long been interested in the genetic basis giving rise to the presence of 2AP in crops, especially rice due to its socio-economical importance at both national and international levels. There was a long-standing debate of the genetic basis of the fragrance. Earliest report on genetics of the fragrance in crops came from rice. Jodon (1944) and Kadam & Patankar (1938) reported that a single dominant gene controls fragrance in rice. This gene was named as "*Fgr*". Berner & Hoff (1986), Ghose & Butany (1952), Pinson (1994), and Sood & Siddiq (1978) reported that a single recessive gene controls the fragrance. Two genes with different interaction are also reported to control the fragrance in rice (Chakravarty, 1948; Dhulappanavar, 1976; Nagaraju, Chaudhary, & Balakrishna-Rao, 1975; Reddy & Sathyanarayanaiah, 1980; Tripathi & Rao, 1979; Tsuzuki & Shimokawa, 1990). Richharia, Minsro, & Kulkarni (1965) reported that the fragrance is a polygenic trait. Pinson (1994) suggested that the different conclusions on the inheritance of the fragrance in rice is due to different fragrance rice cultivars were analyzed. Thus he used six different cultivars to investigate the genes controlling the fragrance and found that the fragrance in Jasmine 85, A-301, Della-X and PI45917 were each controlled by a single gene that are allelic. While those of Amber and Dragon Eyeball 100 were each controlled by two genes of which one is allelic to the former cultivars. Nonetheless, gene mapping for fragrance using molecular markers revealed a major locus, *fgr*, on chromosome 8 associates with the fragrance (Ahn, Bollich, & Tanksley, 1992).

Later, quantitative trait locus (QTL) mapping for fragrance and 2AP seed concentration showed that a major QTL for both traits were mapped to the same position between markers RG8 and RG1 on chromosome 8. A map-based cloning for 2AP in KDML105 identified three candidate genes for this trait. These genes were on chromosome 8, including *Methyl crotononyl CoA lyase* (*MCCase*), *hypothetical protein* and *betaine aldehyde dehydrogenase* (*BADH*) (Vanavichit et al., 2004; Wanchana, 2005). This *BADH* gene was named as *Os2AP* (Vanavichit et al., 2004). Sequence comparison revealed that *Os2AP* in KDML105 contains a continuous 8-bp deletion in exon 7 that lead to premature stop codon. In addition, gene expression analysis showed that expression of the *Os2AP* gene in KDML105 is lower than that of non-fragrant rice. Gene silencing of *Os2AP* in non-fragrance rice cultivar "Nipponbare" by RNA interference (RNAi) resulted in expression gene suppression and biosynthesis of 2AP in leaves (Wanchana et al., 2004). Thus, *0s2AP* is considered the major gene causing production of 2AP in rice. Fine mapping of the recessive gene for fragrance (*fgr*) in rice cultivar "Kyeema" identified 17 candidate genes on chromosome 8 for the fragrance (Bradbury, Fitzgerald, Henry, Jin, & Waters, 2005). Re-sequencing of these candidate genes in several fragrance and non-fragrance cultivars revealed that a gene encoding for *Betaine aldehyde dehydrogenase 2* (*BADH2*; Os08g0424500) showed significant sequence variation (Bradbury, Fitzgerald, Henry, Jin, & Waters, 2005). *BADH2* of Kyeema contains an 8-bp deletion in exon 7 that causes dysfunction of the gene.

The 8-bp deletion was also found in the other fragrant rice cultivars, including KDML 105. Thus *BADH2* is the gene for fragrance in rice (Bradbury, Fitzgerald, Henry, Jin, & Waters, 2005). The studies by Bradbury, Fitzgerald, Henry, Jin, & Waters (2005), Vanavichit et al. (2004), and Wanchana et al. (2004) demonstrated that the loss in function of *BADH2* is responsible for 2AP production in rice. The 8-bp deletion in exon 7 of rice was named as allele *badh2-7* (Kovach, Calingacion, Fitzgerald, & McCouch, 2009). Later, gene transformation and gene knockout studies (Chen et al. 2008; Niu et al. 2008; Shan, Zhang, Chen, Zhang, & Gao, 2015) confirmed that the loss infunction of *BADH2* is the major factor controlling 2AP biosynthesis in rice. Subsequently, other mutations (alleles) that cause the dysfunction of *BADH2* and 2AP production in rice have been identified (Kovach, Calingacion, Fitzgerald, & McCouch, 2009; Shi, Yang, Chen, & Xu, 2008).

Up to present, more than 10 fragrance alleles are known in rice of which *badh2-7* is the most common allele in fragrance rice (Fig. 1) (Kovach, Calingacion, Fitzgerald, & McCouch, 2009).

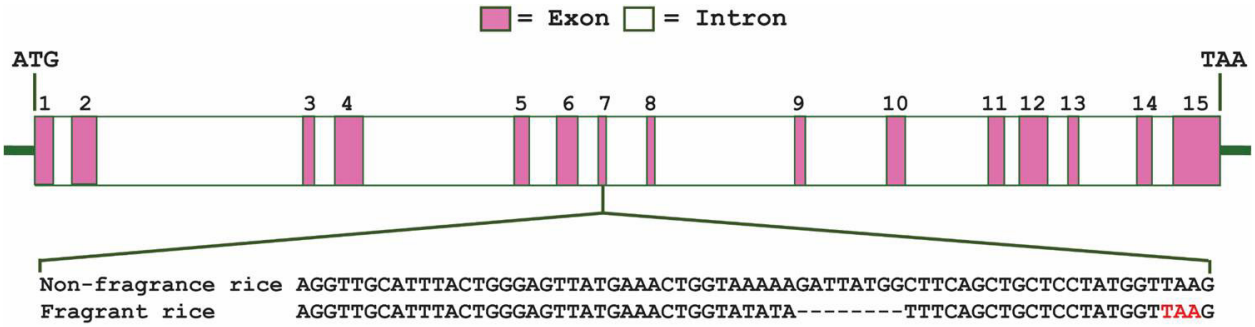

Fig. 1. Landscape of *betaine aldehyde dehydrogenase 2* (*BADH2*) gene in non-fragrant and fragrant rice cultivars. ATG and ATT represent initiation and termination codons, respectively. *BADH2* comprises 15 exons. Nucleotide sequence variations in the exon 7 between non-fragrant and fragrant rice cultivars is shown. The fragrant rice has a continuous 7-bp deletion and three single nucleotide polymorphisms (SNPs) in the exon 7. The 7-bp deletion causes frame-shift reading and introduces premature stop codon (shown in red) in this exon. The deletion leads to 2AP biosynthesis in the fragrant cultivar. The figure is re-drawn from Bradbury, Fitzgerald, Henry, Jin, & Waters (2005)

Apart from *BADH2* as the major gene causing 2AP production/fragrance in rice, minor loci on rice chromosomes 1, 3, 4 and 12 were also reported to associate with the fragrance (Amarawathi et al., 2008; Daygon et al., 2017; Lorieux, Petrov, Huang, Guiderdoni, & Ghesquière, 1996; Singh, Singh, Sharma, Singh, & Singh, 2007). *Betaine aldehyde dehydrogenase 1*(*BADH1*), a homologous gene of *BADH2*, is the candidate gene for the fragrance gene on chromosome 4 (Amarawathi et al., 2008; Singh, Singh, Sharma, Singh, & Singh, 2007), although the effect of this gene on the fragrance is much weaker (about 6 % variation of the fragrance score) than that of *BADH2*. Singh et al. (2010) sequenced *BADH1* in a diverse set of rice germplasm, including several fragrant varieties and found that two single nucleotide polymorphism (SNP) haplotypes, SH3 and SH4, of *BADH1* associates with fragrance in fragrant rice varieties. Both haplotypes have lysine$_{144}$ to asparagine$_{144}$ and lysine$_{345}$ to glutamine $_{345}$ substitutions in the BADH1. Due to similar biochemical function between *BADH1* and *BADH2*, *BADH1* may beimplicated in the 2AP production in rice in the same way as *BADH2*.

The locus for fragrance on chromosome 1 contributing to seed 2AP content was identified by genome-wide association analysis (GWAS). This locus was located between the positions 35.25 to 42.48 Mbp. A gene in this region that may involve in 2AP biosynthesis is *glycerol-phosphate acyltransferase* (*G3PAT*; *Os01g0855000*). The fragrance locus on chromosome 3 contributed moderately to seed 2AP content and fragrance score in rice, as compared to the locus containing *BADH2* gene (Amarawathi et al., 2008; Singh, Singh, Sharma, Singh, & Singh, 2007). Transcriptome profiling analysis coupled with QTL analysis for the fragrance on the same population used by Amarawathi et al. (2008) and Singh, Singh, Sharma, Singh, & Singh (2007) showed that one differentially expressed gene, *Os03g0327600*, was located in the QTL region for the fragrance on chromosome 3 (Pachauri et al., 2014). *Os03g0327600*is annotated as hydroxyproline-rich glycoprotein family, a stress responsive protein. Additional studies are necessary to confirm the involvement of *Os03g0327600* in 2AP synthesis.

Despite *BADH2* is the major locus responsible for the pandan-like fragrance in rice, some studies showed that the genome region containing *BADH2* alone contributed to only about 20 % of the variation infragrance score (Amarawathi et al., 2008). The low contribution suggests that environmental factors greatly affects expression of the *BADH2* gene and/or there may be other genes controlling the fragrance and 2AP. 2AP content in rice plant is associated with the expression of not only *BADH2* gene, but also *Δ1-pyrolline-5-carboxylic acid synthesis* (*P5CS*), *glyceraldehyde-3-phosphate dehydrogenase* (*GAPDH*) and *triose phosphate isomerase* (*TPI*) genes. Gene expression analyses revealed that 2AP content in rice was elevatedby reducing expression of *BADH* and *GAPDH* genes and increasing the expression of *TPI* and *P5CS* genes (Hinge, Patil, & Nadaf, 2016a; 2016b; Wu, Chou, Wu, Chen, & Huang, 2009). In addition, accumulation of 2AP in rice was found to be dependent of genotypes (cultivars), environmen-

tal conditions, production sites, time of planting and harvesting, and fertilizers (Mathure, Jawali, Thengane, & Nadaf, 2014; Yoshihashi, Huong, & Inatomi, 2002) by reductive the expression of *BADH* and *GAPDH* genes and increasing the expression of *TPI* and *P5CS* genes.

Soybean (*Glycine max* (L.) Merr.)

Soybean is the most important legume crop of the world in term of production area. The crop is grown in all the continents of the world in which its seed is used mainly as source of protein and oil. This legume crop has long been used as food since ancient time in East Asia, including China, Korea and Japan. Soybean can be classified into two major types based on its uses, viz. grain/field soybean and vegetable soybean. Vegetable soybean is a special type of soybean where seeds of young pods (generally at reproduction 6 (R6) stage) are cooked and consumed. Vegetable soybean has long been used by East Asian people, but only recently consumed by people in the other regions. China and Japan are the major producers of vegetable soybean. In Japan, fragrance vegetable soybeans are commercially marketed. There are two groups of the fragrance cultivars; "Kaori" (meaning fragrant/aromatic) and "Dadachamame" (also known as "Chamame"). The fragrance vegetable soybeans have a high market value, especially in Japan. 2AP is largely responsible for the fragrance in seeds of Dadachamame (Fushimi & Masuda, 2001) and Kaori (Juwattanasomran et al., 2011).

Genetic analysis revealed that a single recessive gene controls the fragrance in Dadachamame (Juwattanasomran et al., 2012; World Vegetable Center, 2003) and Kaori (Juwattanasomran et al., 2011). Gene mapping study showed that a major locus controlling seeds 2AP content (*q2AP*) and seed pandan-like fragrance (*qFgr*) in Kaori was on the chromosome 5 (linkage group A1) of soybean (Juwattanasomran et al., 2011). This locus accounted for about 17 % of the seed 2AP variation and 43 % of the fragrance score variation in the RIL mapping population. Both the positions of *q2AP* and *qFgr* coincided with the position of the marker BADH2-CDS6 that was developed from soybean *BADH2* sequence (*Glyma05g01770*). Sequence analysis of the *BADH1* on chromosome 6 (linkage group C) and *BADH2* genes demonstrated that there was no mutation in *BADH1* of Kaori but there were three synonymous and one non synonymous mutations in *BADH2* of Kaori (Juwattanasomran et al., 2011). The non synonymous mutation was at the position equivalent to nucleotide position 92 of the exon 10. This SNP is a G to A change in Kaori and results in amino acid change of glycine (G) to aspartic acid (D) at the position 334 of BADH2 protein. This amino acid change in Kaori occurs in a highly conserved motif EEGCRLGPIVS which is believed necessary for BADH function (Juwattanasomran et al., 2011).

Allelism test using DNA markers specific to the G/A SNP in the exon 10 of BADH2 gene suggested that Chamame did not possess the same fragrance allele or gene as in the Kaori (Juwattanasomran et al., 2012). However, sequencing the *BADH2* gene in Chamame revealed that this fragrance soybean has a continuous 2-bp deletion in the exon 10. The deletion causes a premature stop codon, and is thus resulted in a dysfunction of the BADH2 protein in Chamame (Juwattanasomran et al., 2012). The fragrance alleles in Kaori and Chamame were designed as *GmBADH2-1* and *GmBADH2-2*, respectively. The indel marker GmBADH2-EX10 specific to allele *GmBADH2-2*, was developed and used to map the QTL controlling the pandan-like fragrance in Chamame. A major QTL, *qFgr*, controlling the fragrance was mapped to chromosome 5. The position of *qFgr* was nearly the same as the marker GmBADH2-EX10. These results indicate that *BADH2* gene is responsible for the fragrance in Chamame vegetable soybean.

The major role of *BADH2* in 2AP production in vegetable soybean was demonstrated by gene silencing. Arikit et al. (2011) showed that when expression of *BADH2* gene in a non-fragrance soybean variety "Jack" was knocked down by RNA interference (RNAi) such soybean can produced 2AP. The authors also demonstrated that the expression of *BADH2* in fragrance soybean varieties was much lower than non-fragrance ones. These results clearly indicate recessive nature of *BADH2* fragrance allele in soybean. Although *BADH2* is the major locus responsible for the fragrance in soybean, its effect towards 2AP production and fragrance is not highly potent. In a mapping study using Kaori as the fragrant parent, this locus explained for only about 17 % and 43 % respectively of the 2AP variation and fragrance variation in the mapping population (Juwattansomran et al., 2011). Similarly, in another mapping study using Chamame the fragrant parent, this locus explained for 61 % of the total variation for fragrance in the mapping population

(Juwattanasomran et al., 2012). In addition, some plants having homologous fragrant alleles showed low 2AP or no fragrance (Juwattanasomran et al., 2011; 2012). These suggest that 2AP production or fragrance in soybean is a polygenic trait.

Sorghum (*Sorghum bicolor* (L.) Moench)

Sorghum is among the five most important cereal crops of the world. It is generally grown in Asia and Africa, especially in dry areas of the tropics and subtropics regions due to its tolerance to drought condition. Sorghum is a traditional food crop of Africa and Asia where several sorghum types have been described, including fragrant sorghums. The fragrant sorghum cultivars are only known in India and Taganyiga (Tanzania). Kottur (1919) found that a landrace cultivar "Ambemohor" from India has a fragrant, while sorghum cultivar "Kinungapembo" from Tanganyiga possessed fragrant seeds. However, it is not known what kind of fragrance in these sorghum varieties are. Nonetheless, Rao & Murty (1979) reported sorghum cultivar "Basmati" from Madhya Pradesh, India having grains with pandan-like fragrance as in Basmati rice. Seeds of an accession (IS19912) of Basmati sorghum cultivar possessed 2AP (Yundaeng, Somta, Tangphatsornruang, Wongpornchai, & Srinives, 2013).

The fragrance in sorghum variety "Kinungapembo" is controlled by a single recessive gene. The gene was named as "*sc*". The pandan-like fragrance in Basmati sorghum is governed by a single recessive gene (Murty, Nicodemus, & House, 1982; Yundaeng, Somta, Tangphatsornruang, Wongpornchai, & Srinives, 2013). A major quantitative trait locus (QTL) controlling 2AP content and pandan-like fragrance in basmati sorghum was located onto chromosome 7 of the sorghum (Yundaeng, Somta, Tangphatsornruang, Wongpornchai, & Srinives, 2013). This locus explained about 60 % of the total seed 2AP content variation in the F_2 mapping population. Sequencing of *BADH2* gene (*Sb07g020650*) on chromosome 7 in Basmati sorghum revealed that there is a continuous 1,444-bp deletion encompassing exon 12 to 15 in this gene (Yundaeng, Somta, Tangphatsornruang, Wongpornchai, & Srinives, 2013). The deletion causes a premature stop codon and thus resulting in truncated BADH2 protein in Basmati sorghum. A marker, SbBADH2-EX12-15, developed for this deletion showed association with the seed 2AP content. These results indicated that *BADH2* gene is responsible for the fragrance in sorghum. In the same study, the authors showed that there was no QTL for seed 2AP content detected on chromosome 6 of the sorghum where *BADH1* gene (Sb06g019210 and Sb06g019200) located on, indicating that the *BADH1* gene is not involved in the pandan-like fragrance in the Basmati sorghum. Despite *BADH2* is the major gene controlling 2AP biosynthesis in sorghum, its contribution in 2AP biosynthesis is only moderate (60 %). This suggests a considerable environmental effect in 2AP biosynthesis in sorghum.

Cucumber (*Cucumis sativus* L.)

Cucumber is one of the most important and widely cultivated vegetables which its fruits are consumed as a fresh or cooked vegetable or in pickled forms. Over 95 % of the cucumber production is in Asia in which China is the largest producer. India is believed to be center of origin, diversity and domestication of cucumber where cultivated (*C. sativus* var. *sativus*) and wild (*C. sativus* var. *hardwickii*) forms exist. Thailand is part of the center of diversity of cucumber, where the wild and primitive cucumber cultivars are found (de Wilde & Duyfjes, 2010). Fruit quality traits of cucumber include fruit color, spine color, stripes, fruit size and firmness. Recently, fragrant cucumber cultivars were reported from Thailand (Pramnoi, Somta, Chankaew, Juwattanasomran, & Srinives, 2013). Leaves and fruits of fragrant cucumbers show pandan-like fragrance. The fragrance in cucumber was also observed due to the presence of 2AP (Yundeang *et al*., 2015).

Inheritance study revealed that the pandan-like fragrance in cucumber is a monogenic trait with fragrance is recessive to non-fragrance (Pramnoi, Somta, Chankaew, Juwattanasomran, & Srinives, 2013). The authors named this gene as "*Fgr*". The study also revealed that there is no xenia effect for the fragrance. Unlike other flowering plants that generally have two or more homologous *BADH* genes, *in silico* analysis showed that cucumber has only one *BADH* gene *(Cucsa.197230*) (Yundeang *et al.* 2015). Phylogenetic analysis of the cucumber BADH protein with BADH proteins from other plants demonstrated that cucumber BADH was closely related to pea (*Pisum sativum* L.) BADH2. *BADH* is on the chromosome 1 of cucumber. Gene expression analysis of *BADH* in leaves of fragrant and non-fragrant cucumbers revealed that transcriptional expression of *BADH* was similar in both cucumber types. Sequence comparison of *BADH* showed that fragrant cucumber has a single base substitution

(A1855G) in exon 5 that causes an amino acid change, Y163C of BADH protein (Yundeang *et al*. 2015). Y163 is a highly conserved amino acid in both BADH1 and BADH2 proteins of plants. DNA marker specific to the A1855G SNP was developed. Gene mapping in two different populations having the same parents showed that the major locus for fragrance, *qFgr*, was co-localized with the *BADH.* This locus explained as high as 81 % of the fragrance score variation in one population, but explained only about 43 % in the other population. The two populations were grown in the same location but different years, suggesting that environments greatly affect the expression of the fragrance. In addition, the DNA marker specific to A1855G SNP showed no perfect association between *BADH* and the fragrance in cucumber. Although the inaccuracy of the sensory test for the fragrance may cause imperfect association between the marker and the fragrance. This suggests that besides *BADH* gene, other genes may affect the fragrance in cucumber (Yundeang *et al*. 2015).

Coconut (*Cocos nucifera* L.)

Coconut palm is an important tree crop of South and Southeast Asia. Coconut fruit is a portable source of food and water during time of human migration, trade, and colonization in the Pacific Rim and Old World tropics. In addition, coconut is used for fiber, construction material, charcoal and oil. Cultivated coconuts are broadly classified into two types; tall and dwarf. It is estimated that over 12 million ha of coconut are grown in about 90 countries of the tropical region. Center of diversity and domestication of coconut is Indian Ocean (India, Sri Lanka, Maldives and Madagascar), and Pacific Ocean (from Southeast Asia to Papua New Guinea) (Gunn, Baudouin, & Olsen, 2011). Thailand is included in the centers of diversity and domestication of coconut. The planting areas of coconut in Thailand is about 208,000 ha. The country is a major exporters of fresh coconut fruits. Ninety percent of the exported fresh coconut fruits is fragrant coconut, locally called as “Maphrao Nam Hom” (means fragrant-juice coconut). Liquid endosperm (juice) of the fragrant coconut provides a refreshing drink with a pleasant taste and fragrance. “Nam Hom” is a cultivar name. This cultivar is a mutant of the cultivar “Mu Si Khieo” (Chomcalow, 1999). The fragrant coconut is dwarf-green type native to Thailand. At present, fragrant coconut is the most popular economic tree crop of the country. The fragrance in Ma Prawo Nam Hom is pandan-like causing by the presence of 2AP (Luckanatinvong & Sornkeaw, 2011). Although several fragrance coconut cultivars are available, all of them are believed to be derived from the same cultivar. It is generally known that the presence of fragrance (2AP) in coconut is controlled by arecessive gene. In addition, pollen also exerts xenia effect on the 2AP synthesis in coconut (Pooprasert, Imsabai, Arikit, & Boonruangrod, 2015); liquid and solid endosperms from fruits pollinated by non-aromatic varieties had low to very low amounts of 2AP while fruits pollinated by the aromatic varieties were detected in high amount. This is in contrast to the 2AP synthesis in cucumber that shows no xenia effect (Pramnoi, Somta, Chankaew, Juwattanasomran, & Srinives, 2013). The 2AP in coconut fruit starts to present in liquid endosperm (juice) and solid endosperm (meat) at about 6 months after fruit setting, and increasing as the fruit becomes older (Krisanapook, Jaroonchon, & Imsabai, 2016).

The expression level of *BADH2* gene in the fruit shows negative trend against to the presence of the fragrance. This is the first study that suggested *BADH2* may be responsible for the fragrance in coconut. The open reading frame of coconut *BADH2* (*AADH*) gene is 1,512 bp in length from 15 exons encoding 503 amino acids (Saensuk et al., 2016; Vongvanrungruang et al., 2016). Comparison of coding sequence (CDS) of *BADH2* gene between fragrant and non-fragrant coconuts revealed only one SNP between the two coconut types (Saensuk et al., 2016; Vongvanrungruang et al., 2016). This SNP is in exon 14 and is a substitution of guanine (G) in non-fragrant coconut to cytosine (C) in fragrant coconut which resulted in conversion of alanine to proline at the position 442 of the BADH2 protein. This position is a substrate binding site of the BADH2. It is believed that the proline at this position destabilizes the structure leading to a non-functional BADH2 in fragrance coconut (Saensuk et al., 2016; Vongvanrungruang et al., 2016). These findings suggested that *BADH2* is the gene responsible for the fragrance because mutations that cause the functional change in BADH2 protein in several crops have shown to give rise to fragrance, although association between the mutation and the fragrance trait has not been shown in coconut.

Biosynthesis of 2AP in Plants

Based on molecular biology and biochemical

analyses in rice, two biosynthetic pathways for 2AP biosynthesis have been proposed. One pathway is involved with the ADH/BADH2, while the other is not. BADH is an enzyme belongs to the aldehyde dehydrogenase families 9 and 10 (Kirch, Schlingensiepen, Kotchoni, Sunkar, & Bartels, 2005). In the former pathway, Arikit, Yoshihashi, & Vanavichit (2007) noted that *Os2AP* is a member of amino aldehyde dehydrogenase (AADH). They reported that 2AP is synthesized in rice via polyamine pathway. In their proposal, ornithine is the prime precursor of nitrogen in 2AP via Δ^1-pyrroline andγ-aminobutyraldehyde (GABald) is the immediate precursor of both 1-pyrroline and γ-aminobutyric acid (GABA). AADH controls Δ^1-pyrrolineby converting GABald, to GABA, thus AADH controlled the biosynthetic switch of 2AP and GABA in rice. Functional study of purified BADH1 and BADH2 proteins encoded from rice genes annotated respectively as *BADH1* and *BADH2* using several substrates including betaine aldehyde (bet-ald), GABald, Δ^1-pyrroline, γ-guadino amino butyr aldehyde (GGBald), and N-acetyl-γ-amino butyr aldehyde/N-acetyl-pyrroline (NAGABald) demonstrated that both BADH1 and BADH2 proteins showed better affinity towards the GABald and GGBald. *BADH1* and *BADH2* in rice should be annotated as AADH (Bradbury, Gillies, Brushett, Waters, & Henry, 2008).

A very similar conclusion is reported by Chen et al. (2008). Based on their enzyme study, Bradbury, Gillies, Brushett, Waters, & Henry (2008) proposed that proline is the prime precursor of 2AP in plant as it is changed into GABald by putrescine. In non-fragrance plant functional BADH2 converts the majority of GABald to GABA, while in fragrant plant non-functional BADH2 is able to do so and the majority of GABald is spontaneously changed into Δ^1-pyrroline which is then acetylated to form 2AP. The pathways purposed by Arikit, Yoshihashi, & Vanavichit (2007) and Bradury et al. (2005) are the same, both highlighted AADH (BADH2) as the key player in 2AP production.

In the other pathway, Δ^1-pyrroline-5-carboxylic acid (P5C) is one of the main precursors for 2AP synthesis. Enzymes Δ^1-pyrroline-5-carboxylic acid synthetase (P5CS) that produce P5C, and methylglyoxal are found to be significantly higher in fragrant rice as compared to non-fragrant rice (Huang et al., 2008). Gene expression analysis showed that expression of *P5CS1* and *P5CS2* genes in fragrant rice was found to be significantly higher than non-fragrant rice. These findings were also found when fragrant and non-fragrant soybean cultivars were compared (Huang et al., 2009). Based on these findings, Huang et al. (2008) and Wu, Chou, Wu, Chen, & Huang (2009) purposed that P5C derived from proline and glutamic acid by P5CS and from ornithine reacts directly with methylglyoxal (MG) to form 2AP. On the other hand, P5C may also degrade to Δ^1-pyrroline and condense with MG to form 2AP. In this proposed pathway, P5CS is the key player. Proline synthesis in plant is mediated largely by P5CS and proline is proposed as the prime precursor of 2AP (Bradbury, Gillies, Brushett, Waters, & Henry, 2008). In addition to *P5CS in the involvement of 2AP synthesis*, gene expression analyses also revealed that reduced expression of *GAPDH* and increased expression of *TPI* genes elevated 2AP content in rice (Hinge, Patil, & Nadaf, 2016a; 2016b; Wu, Chou, Wu, Chen, & Huang, 2009) and soybean (Wu, Chou, Wu, Chen, & Huang, 2009). The positive correlation between *TPI* and *MG* and the negative correlation between *TPI* and *GAPDH* in rice suggest that TPI and GAPDH are major genes controlling MG in rice (Hinge, Patil, & Nadaf, 2016a). These findings prompted Hinge, Patil, & Nadaf (2016a) to modify the pathways proposed by Bradbury, Gillies, Brushett, Waters, & Henry (2008), Huang et al. (2008), and Wu, Chou, Wu, Chen, & Huang (2009) by including TPI and GAPDH into the pathways (Fig. 2).

However, in a recent study using a rice collection that includes diverse fragrant rice and recombinant inbred lines derived from fragrant rice parents showed four biochemical compounds, viz.6-methyl, 5-oxo-2,3,4,5-tetrahydropyridine (6M5OTP), Δ^1-pyrroline, 2-acetylpyrrole, and pyrrole highly correlated with 2AP contents in the fragrance rice (Daygon et al., 2017). 2AP and these four compounds are clearly discriminated between fragrant and non-fragrant rices. 2AP and 2-acetylpyrrolboth have the same sweet fragrance. 6M5OTP and Δ^1-pyrroline were identified for the first time in their study. 6M5OTP is a structural isomer of 2AP, and they both have similar scent perceivable. In the same study, applying GWAS technique demonstrated that *BADH2* is responsible for the production of these five compounds. Based on their findings and previous *in vitro* and *in vivo* biochemical studies on 2AP and related compounds, they proposed a pathway for biosynthesis of 2AP and 6M5OTP (Fig. 3). In this pathway, GABald is derived from putrescine and

changed into GABA if BADH2 functions normally. In case BADH2 does not function, GABald is changed into 6-amino-2,3-hexanedioneandΔ^1-pyrroline. The 6-amino-2,3-hexanedione is unstable and cyclises to 2AP and 6M5OTP (Fig. 3A). Δ^1-pyrroline can be oxidized to form pyrrole or theoretically can be changed into 2AP. The production of GABald and Δ^1-pyrroline occurs in peroxisome, while the production of 2AP and 6M5OTP takes place in cytosol (Daygon *et al*., 2017) (Fig. 3B).

Why Some Plants Synthesize 2AP?

BADH2/AADH enzymes have the main physiological role in detoxification and stress response, because natural substrates of BADH2/AADH are reactive metabolites that show considerable toxicity (Tylichová, Kopečný, Snégaroff, & Šebela, 2007). In plants, BADH2/AADH are necessary in the conversion of GABald, the precursor of 2AP, to GABA. Mutation(s) in *BADH2/AADH* gene that results in non-function BADH2/AADH can cause accumulation of GABald in a large proportion that is toxic to plants. To detoxify such toxic, GABald or its cyclic form Δ^1-pyrrolineis acetylated to form 2AP. 2AP biosynthesis is thus a necessary pathway in non-functional *BADH2/AADH* plants to detoxifythe toxic GABald. This is supported by association between mutations in *BADH2*/*AADH* and 2AP synthesis in rice (Bradbury, Fitzgerald, Henry, Jin, & Waters, 2005; Vanavichit et al., 2004; Wanchana 2005), soybean (Arikit et al. 2011; Juwattanasomran et al. 2011; Juwattanasomran et al. 2012), sorghum (Yundaeng, Somta, Tangphatsornruang, Wongpornchai, & Srinives, 2013), cucumber (Yundaeng, Somta, Tangphatsornruang, Chankaew, & Srinives, 2015) and coconut (Saensuk et al. 2016). Nonetheless, it is interesting to find out why *BADH2/AADH* in plant is prone to mutation.

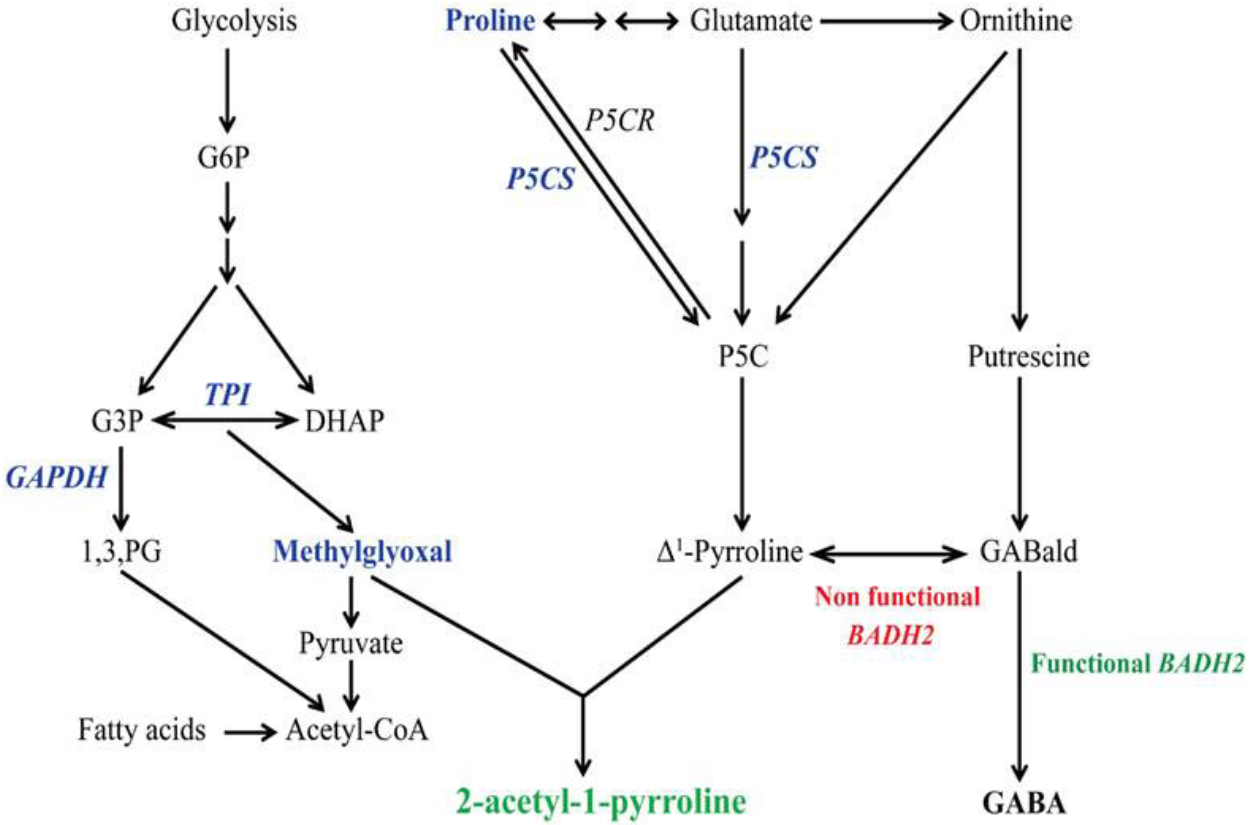

Fig. 2. Putative pathway of 2AP biosynthesis based on molecular biology and molecular genetics studies in rice. The pathway is believed to be conserved in soybean, sorghum, cucumber and coconut. The figure is redrawn from Hinge, Patil, & Nadaf, (2016a).

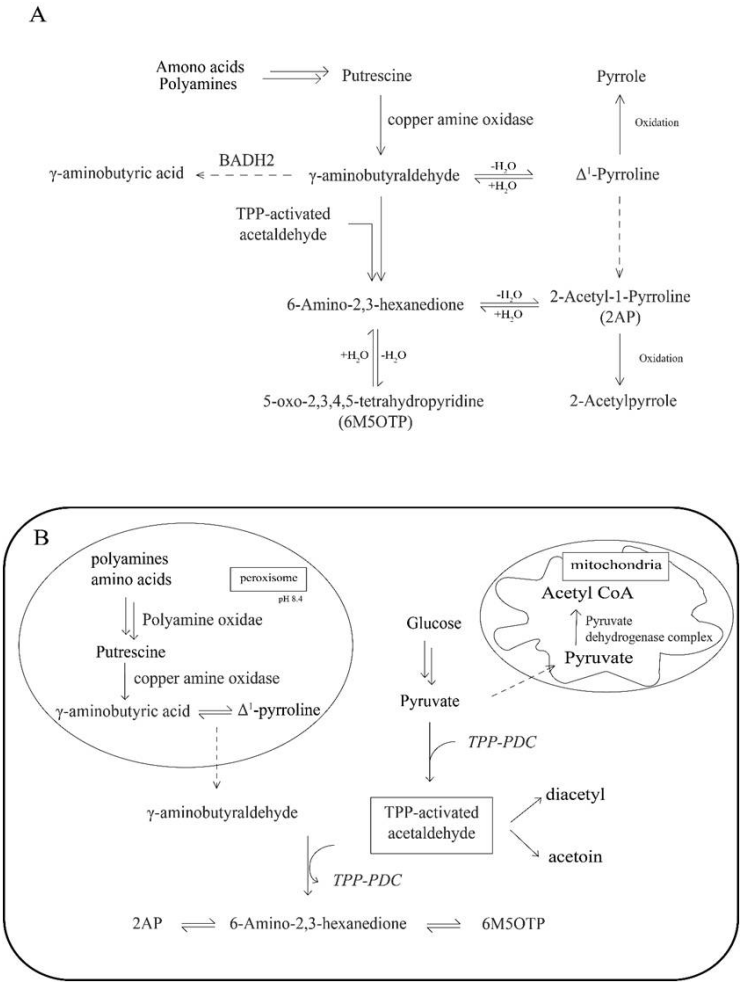

Fig. 3. Putative pathway of 2AP biosynthesis in rice and its related biochemical compounds as proposed by Daygon et al. (2017) based on the study in rice. In this pathway, TPP-activated acetaldehyde reacts with γ-aminobutyric acid to form diketone 6-amino2,3-hexadione which is unstable and cyclised into 2AP and 6-methyl, 5-oxo-2,3,4,5-tetrahydropyridine (6M5OTP). 6M5OTP is a structural isomer of 2AP and has a similar sweet fragrance. It is not known whether 6M5OTP and 2-acetylpyrrole present in other crop cultivars that produce 2AP.The figure is re-drawn from Daygon et al. (2017).

CONCLUSION AND SUGGESTION

Genetic study in rice, soybean, sorghum, cucumber and coconut demonstrated that 2AP biosynthesis is principally controlled by a single recessive gene. Mutation(s) that renders the non-function of *BADH2/AADH* gene is the major causeof the 2AP biosynthesis in these crops, indicating conserved pathway in the biosynthesis. By using *BADH2/AADH* as the candidate gene, genetic variation(s) that causes 2AP biosynthesis may be identified in some cultivars of other crops that possess pandan-like fragrance such as taro, wax gourd and mungbean. Although *BADH2/AADH* is the major cause of 2AP biosynthesis in crops, its effects on the level of 2AP synthesis is only low to moderate. Environmental factors have been found important in the biosynthesis. Besides *BADH2/AADH* gene expression of other genes including *P5CS*, *TPI* and *GAPDH* also affect 2AP biosynthesis in the crops. This indicates quantitative nature of 2AP production and explains why the fragrance level in a crop cultivar, for example KDML105 rice, is highly dependent upon planting location. In some cases, a more complex genetic background also involves in expression of the fragrance such as the xenia effect in coconut.

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