

Research Application Summary

A review of available knowledge on aroma in rice

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Abstract

The 2-acetyl-1-pyrroline (2AP) is a compound responsible for the sweet popcorn aroma in aromatic rice and has attracted interest globally. Consequently, research efforts have been directed to improving aroma characteristics in rice through multiple platforms including inheritance and breeding. However, the progress in this direction has been slowed and needs further inquiries due to the complex nature of the genes, compounds and environments governing this trait. The complexity is further exacerbated by the simultaneous need to increase aroma alongside other important qualities and productivity traits in rice. This paper gives insights on the current knowledge of aroma in rice with particular emphasis on how the genetic, chemical and environmental interaction relates to the aroma strength and the prospects for breeding for aroma in rice.

Key words: 2AP, aroma, gene for aroma, rice

Résumé

La 2-acétyl-1-pyrroline (2AP) est un composé responsable de l'arôme du maïs sucré dans le riz aromatique qui suscite un intérêt au plan mondial. En conséquence, des recherches visant l'amélioration des caractéristiques aromatiques du riz ont utilisé plusieurs approches, notamment la sélection variétale et l'héritabilité. Cependant, des progrès peinent à se réaliser dans ce domaine, et des recherches supplémentaires sont nécessaires, en raison de la nature complexe de l'effet des gènes, des composés et de l'environnement régissant ce caractère. La complexité est encore exacerbée par le besoin simultané d'augmenter l'arôme ainsi que d'autres qualités et caractéristiques de productivité importantes du riz. Le présent article donne un aperçu des connaissances actuelles sur l'arôme du riz avec un accent particulier mis sur l'effet des influences génétique, chimique et environnementale et leurs interactions, et examine les perspectives pour la sélection de variétés de riz aromatique.

Mots clés: 2AP, arôme, gène pour arôme, riz

Background

Rice (*Oryza sativa*) which is presumed to originate in Asia (Hirst, 2017) is the second most essential food crop after wheat providing income to more than 1.5 billion people including 900 million of the world's most impoverished population (IRRI 2010). Being a critical source of energy and protein and mitigating conditions such as coronary heart disease, diabetes, dehydration and cancer in humans, rice is considered a health food. Rice varieties may be classified as aromatic or non-aromatic. The aromatic rice produces a distinct popcorn smell (Buttery *et al.*, 1982) and attract a preference and premium price (Calingaceon *et al.*, 2014) which is on the increase (Asante 2017).

Aromatic rice cultivars belong to three groups, namely: group I (the Indica from China), group V (the Indica from Thailand) and group VI (the Japonica from Indonesia) (Singh *et al.*, 2000). The Western Himalayan region of India with rich genetic diversity of rice. (Rana *et al.*, 2009) has been suggested the origin of the common alleles for the aroma (Pachauri *et al.*, 2010). Over 500 aromatic cultivars have been documented (Singh *et al.*, 2000) including the popular cultivars such as the Basmati type from India (Patil *et al.*, 2012) and the Jasmine type from Thailand (Wilkie *et al.*, 2004). Other popular aromatic rice include Khao Dawk Mali 105 from Thailand (Bradbury *et al.*, 2005). Likewise, the most popular nonaromatic rice include Doongara (Garland *et al.*, 2000), Nipponbare (Bradbury *et al.*, 2005), Gulfmont (Bradbury *et al.*, 2005), Japonica Lemont (Sun *et al.*, 2008), and IR-64 (Hinge *et al.*, 2015).

An African species of rice, *Oryza glaberrima*, domesticated in West Africa (Linares *et al.*, 2002) was crossed with *O. sativa* to form new rice (NERICA) varieties (AfricaRice 2010; Haneisha *et al.*, 2013). One of the upland Nerica rice varieties grown in Uganda, namely Nerica 1 was reported to have some aroma (Asante *et al.*, 2010; Tsuboi 2012). A landrace of Nerica, 'supa' cultivar has been reported to exhibit good aroma and taste (Massette *et al.*, 2013). In Tanzania, popular aromatic rice grown under lowland or irrigated conditions belonging to *O. sativa* indica include Super, Mbeya rice, Kyela and TXD 306 (Kilimo Trust, 2014). In Uganda, common aromatic rice found in the markets include Tilda aromatic, Dawaat Long Grain and Pearl Kenya Pishori (Massette *et al.*, 2013).

Volatiles in Rice

Of the more than 1,700 plant volatiles (Dudareva *et al.*, 2006), over 100 compounds have been reported to contribute to odour in rice including the hydrocarbons, alcohols, aldehydes, ketones and acids (Champagne *et al.*, 2008). The most common hydrocarbon is paraffin and is found in big quantities in both the aromatic and the nonaromatic rice. However, there is higher levels of alcohols (n-pentanol, n-heptanol n-pentanol, 1-octen-3-ol, menthol, estragol, n-butanol and n-hexanol) in aromatic rice in addition to aldehydes and ketones (n-pentanal, n-heptanal and n-nonanal) as compared to nonaromatic rice (Buttery *et al.*, 1983). Also major volatile compounds including 2-acetyl-1-pyrroline, indole and 2-phenylethanol were reported to constitute aroma in Basmati and Jasmine type rice (Buttery *et al.*, 1988). According to Wilkie *et al.* (2004), a rice variety with higher concentrations of volatiles known to contribute to aroma due to their low aroma threshold would have more desirable aroma qualities. The variety possessing significantly more 4-vinylguaicol and indole, which generally possess unpleasant odours at higher concentrations have lower aroma quality. The compounds produced under storage conditions include dimethyl trisulphide; nonan-2-one; methylcyclobutane; 2-furancarboxaldehyde; (E,E)deca-2,4-dienal; dodecan-2-one, octanoic, 4-vinyl guaiaicol, 4-vinylphenol and dodecanoic acid. The nonfragrant rice has approximately twice the amounts of 2-hexenal[E], nonanal, 2-pentylfuran,

and 2-octenal[E], and significantly more 2-nonenal[E] and hexanal compared to the fragrant rices (Wilkie *et al.*, 2004). A recent study on elite aromatic rice varieties from South East Asia and Australia by Daygon *et al.* (2017) reported a strong correlation between 2AP and 6-methyl, 5-oxo-2,3,4,5-tetrahydropyridine (6M5OTP), 2-acetylpyrrole, pyrrole and 1-pyrroline with aroma. Of all the compounds, the aromatic rice on average contains 4 times and in some specific cases 70 times more 2AP than the non-aromatic (Lin *et al.*, 1990). Owing to involvement of many compounds in determining the final aroma quality, Yang *et al.* (2008) suggested the use of GC-MS/GC-olfactometry (GC-MS/GC-O), and multivariate analysis in the selection of multiple flavor types using a reference standard with 13 odor-active compounds to characterize rice aroma without sensory test.

Determination and Quantification of Aroma

Aroma of rice has been variously determined through putting centrifuge tube containing milled grain samples and distilled water in a boiling water bath and smelling after cooling (IRRI 1971), chewing a few grains from individual plants and noting the aroma (Dhulappanavar *et al.*, 1976) and through elution of leaf or grain samples in 1.7% KOH and smelling (Sood and Siddiq, 1978). In these methods, panelist used a scale to score aroma with a nonaromatic (0), slightly aromatic (1), moderately aromatic (2) and strongly aromatic (3) (Sarahdi *et al.*, 2009; Chakraborty, *et al.*, 2016). Use of these methods were considered subjective (Lorieux *et al.*, 1996). A histochemical method of identifying 2AP by treating plant tissues with 2,4-dinitrophenyl hydrazine that react with 2AP and produce an orange-red colour of the compound 2-acetyl phenyl hydrazone has been reported (Nadaf *et al.*, 2006). In addition to qualitative methods, quantitative method of measuring concentration of 2AP has been used (Zackaria *et al.*, 2017). The chromatography techniques which include Gas liquid chromatography combined with mass spectrometry (GLC/MS) (Buttery *et al.*, 1983), Gas chromatography mass spectrometry combined with gas chromatography-olfactometry (GC-MS/GC-O) (Yang *et al.*, 2008), Gas chromatography-olfactometry (GC-O) (Limpawattana *et al.*, 2008), Headspace solid-phase micro-extraction (HS/SPME) (Goufo *et al.*, 2010), Headspace gas chromatography with a nitrogen-phosphorus detector (HSGC-NPD) (Arikrit *et al.*, 2011), headspace sorptive extraction combined with GC-MS (HSSE/GC-MS) (Grimm *et al.*, 2011), GC-MS analyses coupled with alternative moving window factor analysis (GC-MS/AMWFA) (Yang *et al.*, 2012), Stable isotope dilution assay (SIDA) combined with other techniques (SIDA/SPME/GC-PCI-MS-MS) (Poonlaphdecha *et al.*, 2012), and Mass-spectrometry-based electronic nose (MS-EN) for qualitative discrimination of flavor followed by quantitative headspace solid-phase microextraction coupled to gas chromatography-mass spectrometry combined with partial least-squares regression (HS/SPME/GC-MS/PLSR) (Kang *et al.*, 2014) and Gas chromatography mass spectrometry (GCMS) (Mo *et al.*, 2015) have been used.

In addition, Gas chromatography fitted with Ion diode (GC-FID) (Zackaria *et al.*, 2017), Stable isotope dilution assay technique involving SPME combined with GC-PCI-MS (SIDA/ SPME/ GC-PCI-MS), and Two-dimensional gas chromatography-time-of-flight mass spectrometry (GC × GCTOF-MS) (Daygon *et al.*, 2017) have also been employed in 2AP quantification. The use of molecular techniques involving allele specific markers such as badh2-E7 (Bradbury *et al.*, 2005; Roy *et al.*, 2012; Yeap *et al.*, 2013), FMBadh2-E4-5 (Shao *et al.*, 2011) and combinations of microsatellite markers (Priyadarshini *et al.*, 2018) have also been employed to identify aromatic lines. The molecular method has been described as a simple, inexpensive and convenient approach for screening of a wide range of rice varieties with 100% accuracy (Ashfaq *et al.*, 2015). A strong positive association of 80% among the sensory, chromatography and molecular markers was reported (Kumari *et al.*, 2012).

The sensory evaluation methods according to Sood and Siddiq (1978) have indicated the minimum concentration of 2AP consistently detectable by a 16 panel of judges was 7ppb although some members could detect up to 0.1ppb (Buttery *et al.*, 1983). In another study, a panel detected all the aromatic lines with 2AP concentration ranging from 0.03 to 0.04 ppm (Lorieux *et al.*, 1996). Mo *et al.* (2015) reported a detection limit of 0.01 ppm by a panel, whereas Hinge *et al.* (2016) detected 2AP at 0.001 ppm. According to Zackaria *et al.* (2017), the 2AP concentrations of five rice genotypes: Ranbir Basmati, Rati Basmati, E 7, E 13, and MRQ 50 ranged between 0.08-0.14 ppm and was given a sensory evaluation score of 4. The amount of 2AP in milled and brown rice obtained from various regions through IRRI ranged between 0.006-0.90 ppm and 0.0-0.2 ppm, respectively. The quantity of the 2AP, depends among others on the rice cultivar (Zackaria *et al.*, 2017), evaluation method (Chambers *et al.*, 2013), and state of the grain such as the degree of milling (Rodríguez-Arzuaga *et al.*, 2015).

Biosynthesis and Profile of 2AP in Rice

Accumulation of 2AP has been associated to metabolism of carbohydrates and proteins (Wongpia *et al.*, 2016). Three amino acids namely glutamate, proline and ornithine are converted to Pyrroline-5-Carboxylase (P5C) and Delta-1-pyrroline to 2AP, while another amino acid putrescine is converted to Delta-1-pyrroline through gama betaine aldehyde (GaBald) (Wongpia *et al.*, 2016). GaBald can be converted either to Delta-1-pyrroline in the absence of betaine aldehyde dehydrogenase (BADH) enzyme, leading to accumulation of 2AP or to Gama butyric acid (GABA) in the presence of BADH enzyme (Bradbury *et al.*, 2005). Also, the products of glycolysis, namely pyruvate, methylglyoxal (MG) and Acetyl CoA combines with Delta-1-pyrroline to form 2AP (Wongpia *et al.*, 2016). In addition, stronger aroma in brown rice grain of traditional aromatic cultivar was positively associated with the quantities of L-proline, total nitrogen content, total plant nitrogen (protein) and negatively associated with the soluble protein in brown rice (Yang *et al.*, 2012). Huang *et al.* (2008) suggested lack of involvement of BADH in 2AP synthesis arguing that the P5C acts directly with MG to synthesize the 2AP. According to Wongpia *et al.* (2016), BADH is 1.3 folds less in the aromatic lines. A recent study by Daygon *et al.* (2017) identified four other heterocyclic amines, namely, 6-methyl, 5-oxo-2,3,4,5-tetrahydropyridine (6M5OTP), 2-acetylpyrrole, pyrrole and 1-pyrroline with strong correlation to 2AP in rice varieties from Asia.

There are different views on the synthesis of 2AP, the first being that 2AP is possibly solely produced from different types of amino acids (proteins) independently from glycolysis (Fitzgerald *et al.*, 2009), the second is that the 2AP biosynthesis is from products of glycolysis, namely MG, pyruvate, Acetyl CoA, GADP and DHP (Fitzgerald *et al.*, 2009) and the third is a combination of the products of glycolysis mainly MG and the ultimate product from the protein, namely delta-1-pyrroline being combined to produce the 2AP (Wongpia *et al.*, 2016). The implication of possibilities of having the synthesis of 2AP being explained not only by the BADH enzyme pathway, gives the basis for the other 2AP independent pathways apparent credibility. The 2AP being a function of a particular gene, and with arguments that other chemical substances are involved in aroma of rice, contribution of other genes in determining aroma is a possibility (Daygon *et al.*, 2017).

The 2AP is constitutively produced as indicated by the emission of specific aroma in the fields at vegetative (Wilkie *et al.*, 2004), flowering and reproductive stages (Hinge *et al.*, 2016). Profile of 2AP was studied in Australia using SPME and the three leaf stage was reported the best earliest stage with highest 2AP accumulation (Wilkie *et al.*, 2004). On the other hand, Myint *et al.* (2012) in

a study to confirm functional 3-bp deletion in Myanmar rice evaluated aroma using sensory test on a one month and two months old leaves and by chewing of several seeds reported the two methods to consistently identify three rice varieties out of 52 as aromatic. During the reproductive stage, the highest concentration of 2AP in grains was reported at four to five weeks after heading (Itani *et al.*, 2004). Accumulation of 2AP was shown to increase from the early vegetative stages (0.2 ppm) through tillering (0.42 ppm) to the grain filling stage (0.662 ppm) of Ambemohar-157 rice cultivar (Hinge *et al.*, 2016). Under salinity, however, it was revealed that plant leaves accumulate higher 2AP (4.277 ppm) compared to the grains (0.998 ppm) (Poonlaphdecha *et al.*, 2012). Other studies have indicated the association of 2AP with soluble proteins, proline and P5C (Huang *et al.*, 2012) and substances such as BADH (Wongpia *et al.*, 2016). Similarly, Hinge *et al.* (2016) have reported 2AP to exist at all the growth stages of rice alongside 14 major compounds such as p-xylene and methyl 2-aminobenzoate in aromatic rice.

Inheritance of Aroma Trait

Genetic studies conducted on rice cultivars reported aroma to be under the control of four complementary dominant genes (Dhulappanavar, 1976), a single gene associated with inhibitory gene (Tsuzuki and Shimokawa, 1990), at least two genes (Pinson *et al.*, 1994), a dominant gene (Kuo *et al.*, 2005), two recessive genes (Hien *et al.*, 2006), a dominant suppression epistasis interaction between two genes (Kuat *et al.*, 2010) and a single recessive nuclear gene (Patil *et al.*, 2012). The different views on the nature of the gene conferring aroma has been suggested due to the materials used in the studies (Pinson *et al.*, 1994). For instance, the aromatic basmati group of rice has been reported to exhibit poor combining ability with other rice genotypes (Kaushik *et al.*, 2003). Comparison of aroma in Dragon eyeball known to harbor two aroma genes with basmati using sensory evaluation indicated scores of 0.509 ppm and 1.007 ppm, respectively suggesting non additive gene action (Pinson *et al.*, 1994). However, QTL mapping work on sorghum for aroma reported additive and dominant q2AP gene effect of -0.34 and -0.69, respectively. A recent study by Gonya *et al.* (2016) assessed heterosis from 16 crosses obtained from four aromatic lines and four nonaromatic lines and reported that heterosis for aroma (35.21%) was positive. The ratio of the variance for the general combining ability to the specific combining ability was 0.394. Another study was conducted to investigate the efficiency (time and cost) of developing a high-yielding fragrant rice in Malaysia through targeted introgression of *fgr* gene by marker-assisted backcrossing of rice. (Cheng *et al.*, 2017). Two sets of BC2F2 individuals derived from a cross of one donor parent MRQ74, with two recurrent parents; MR84 (“Cross-1”) and MR219 (“Cross-2”) carrying aromatic allele (*aa*) analysed for their agronomic performance of height, grain length and yield resulted into ten good fragrant backcross lines in rice. The study concluded that a high yielding fragrant rice could be obtained through targeted introgression of *fgr* gene through marker-assisted backcrossing in rice. The study also for the first time validated the utilisation of the single functional marker system (*fgr*-SNP) in introgressing the *fgr* gene into different rice varieties.

QTL and the Genes for the Aroma

The first QTL work using segregating F2 population, mapped aroma based on restriction fragment length polymorphism ‘RFLP’ marker reported the marker RG28 to be linked to the aroma gene in chromosome 8 at a distance of 4.5 cM (Ahn *et al.*, 1992). Subsequent a QTL study was conducted through bulk segregation analysis to construct map using RFLP and simple tag sequence (STSs) performed on the core map of the whole genome. Aroma gene was located on chromosome 8 at 6.4 cM from marker RG28 with maximum LOD score of 14.5 explaining 69% of the variance in the aroma

trait (Lorieux *et al.*, 1996). This study further reported minor QTLs on chromosome 4 and 12 with the LODs of 0.008 and 0.004, respectively. Later, SSR based mapping of 168 field grown F2 population segregating for aroma confined the aroma gene between markers RM515 and SSRJ07 at a distance of 386 and 591 bp, respectively (Bradbury *et al.*, 2005a). By cloning this region using significant bacteria artificial chromosome (BAC) clone AP004463 and re-sequencing the 17 genes in this BAC clone, a 8 bp deletions and three SNPs corresponding to badh2 gene were identified and based on this mutation, marker badh2-E7 was developed (Bradbury *et al.*, 2005b). Subsequent fine mapping study on 2891 segregating individuals for aroma reported the locus for aroma at an interval of 69 kb (Chen *et al.*, 2006). Based on this region, three candidate genes encoding eukaryotic-type carbonic anhydrase, 3-methylcrotonyl-CoA carboxylase beta chain, and betaine aldehyde dehydrogenase were identified.

A study that employed 23 markers on recombinant inbred lines (RILs) population derived from a cross between basmati rice and a non-aromatic rice identified candidate genes aro3-1(chr 3) and aro-8 (chr 8) at a physical interval of 390 kbp and 430 kbp, respectively (Singh *et al.*, 2007). Speculating another gene for aroma, fine mapping for the aroma gene was conducted using two populations identified the aroma gene to be at an interval of 142.85 kb on BAC clones AP005301 and AP005537 (Sun *et al.*, 2008). Later, sequence analysis revealed a new badh2 allele with identical 8-bp deletion and three SNPs in exon 7, but with a 7-bp deletion in exon 2 designated badh2-E2 (Shi *et al.*, 2008). Badh2 has also been reported as the major fragrant gene in Azucena (*O. sativa japonica*) rice and the gene has been found to contain miniature interspersed transposable element (MITE) in its promoter region (Bourgis *et al.*, 2008), and the presence of the MITE in 22 out of 81 rice varieties including KDML (indica, group I), Azucena (japonica, group VI) and basmati (group V) rices suggested a monophylogenetic origin of this badh2 mutation in Asian cultivated rices.

A sequence and BLAST analyses involving four nonaromatic and 24 aromatic rice identified a gene allelic to badh2-E7, but with a new deletion mutation of 803 bp between exons 4 and 5 (FMbadh2-E4-5) (Shao *et al.*, 2011). In the same badh2 gene, Myint *et al.* (2012) reported a 3-bp insertion in exon 13 as a major allele found in aromatic rice varieties from Myanmar (Myint *et al.*, 2012). In sorghum line IS19912, a continuous 1444 bp deletion encompassing exon 12 to 15 of SbBADH2 IS19912 was identified on chromosome 7 for aromatic line (Yundaeng *et al.*, 2013). Cheng *et al.* (2014) applied SSR markers on 90 F2 segregating population for aroma and reported four QTL for aroma on chromosome 8.

Pachauri *et al.* (2014) conducted fine mapping of three aroma QTLs in basmati rice using a biparental mapping population derived from a cross between Pusa 1121, a basmati rice variety, and Pusa 1342, a non-aromatic rice variety to identify candidate genes for rice grain aroma. By combining QTL mapping and transcriptome profiling approaches, ten genes: one on chromosome 3, eight on chromosome 4 and one on chromosome 8 were identified. In a recent study, Cheng *et al.* (2014), through comparative sequence analysis of two aromatic landrace in Vietnam using whole genome sequencing revealed that chromosomes 8 and 11 of both rice landraces contained higher number and islands of novel SNP polymorphism compared to other chromosomes. Hashemi *et al.* (2015) used 189 of F2 individuals from a cross of aromatic and a high yielding nonaromatic rice and detected two QTLs on chromosomes 4, frg4-1, and 8, frg8-1. The locus for frg8-1 was placed between RM223 (82.2 cM) and SCU015RM (83.82 cM). Profiling studies have since identified compounds associated with aroma and genomic wide association studies (GWAS) have mapped these compounds to chromosome 8 with a second QTL for levels of 2AP observed on chromosome 1 with three markers and candidate genes (Daygon *et al.*, 2017).

Environmental Factors Affecting 2AP Production in Rice

Many soil factors including nutrition and salinity have been reported to impact on 2AP accumulation (Asante *et al.*, 2017). Srivastava and Singh (2007) reported the effect of nitrogen (N) and sulphur (S) application in rice and reported that aroma was positively correlated with the grain N/S ratio. Kumar and Kureel (2017) reported increase in yield in aromatic rice due to application of nitrogen and phosphorus fertilizers compared to the control. Yang *et al.* (2012) investigated the role of nitrogen in aroma synthesis in traditional Chinese regional aromatic rice by sensory and GC-MS analyses coupled with alternative moving window factor analysis (AMWFA). Results showed that higher total soil nitrogen content corresponded to higher l-proline content and total nitrogen (protein) content of the brown rice, lower soluble protein content and stronger aroma of the brown rice. Another study investigated manganese (Mn)-induced regulations in aroma formation and enzymes involved in 2AP biosynthesis in two aromatic rice based on four levels of MnSO_4 of 100mg, 159mg, 200mg and 250mg per plot containing 15 kg of soil (Li *et al.*, 2016). Manganese was found to up-regulate proline, pyrroline-5-carboxylic acid (P5C) (precursors of 2-AP), soluble proteins and activities of proline dehydrogenase (ProDH), D1 pyrroline, pyrroline-5-carboxylic acid synthetase (P5CS), and ornithine aminotransferase (OAT) that led to enhanced 2AP production in rice grains. The study pointed out that the exact role of Mn in improving aroma is not understood and recommended other studies at molecular levels to understand the mechanisms of Mn in improving rice aroma formation. A similar study was conducted by Lei *et al.* (2017) to investigate the effect of exogenous application of mixed micro-nutrient such as copper, manganese, zinc and potassium on aroma in two aromatic rice and reported significant correlations among 2AP, Zn and Se ($P < 0.05$). The study suggested further investigation on the mechanism of these micronutrients in 2AP biosynthesis. In addition, the effect of salinity (NaCl salt solution with EC of $3800 \pm 400 \mu\text{S}\cdot\text{cm}^{-1}$) on 2AP, proline, and γ -aminobutyric acid (GABA) were investigated by Poonlaphdecha *et al.* (2012) who reported that increased salinity was positively associated with the levels of 2AP and proline, but not with GABA. The grains from the treatment plants contained significantly higher levels of 2AP ($733\text{-}998 \mu\text{g}\cdot\text{kg}^{-1}$) than those from the control ($592 \mu\text{g}\cdot\text{kg}^{-1}$). The highest 2AP synthesis occurred when the plants were subjected to the treatment during vegetative or reproductive phases. In Sri Lanka, however, Wijerantha *et al.* (2011) reported weak survival of aromatic rice under salinity conditions compared to the nonaromatic cultivars. In a glasshouse-scale experiment, however, difference in the ability of rice plants to produce mature seed under salinity condition of 22mM salt solution (from 11 weeks post-planting), with greater than 99% inhibition of mature seed production for fragrant rice plants exposed to 22mM salt solution (from 11 weeks post-planting) was observed (Fitzgerald *et al.*, 2010).

Besides the soil factors, the concentration of 2AP has been reported to increase with lower plant densities (Goufo *et al.*, 2010). The effect of solar radiation (shading) on the accumulation of 2AP and on the accumulation of plant stress response molecules, namely, proline and gamma-aminobutyric acid, which have also been implicated in pathways leading to 2-AP production in two elite Chinese fragrant rice varieties were investigated (Mo *et al.*, 2015) and the study revealed that 2AP and GABA content in grains were significantly increased for all shading treatments in both varieties. Goufo *et al.* (2010) investigated the effect of harvesting time on 2AP in Chinese rice varieties and reported the highest 2AP concentrations associated with lower planting densities, the earliest harvesting time of 10 days after heading, the shortest storage time of 3 months, and the lowest storage temperature of -4°C . The 2AP for grains kept for six months at 30°C reduced from $3.73\text{-}1.3 \mu\text{g}\cdot\text{g}^{-1}$ while those kept at -4°C contained up to 4 times more 2AP compared to those stored at 30°C . Similarly, Itani *et al.* (2004) showed that during grain development, the 2AP concentration in brown rice of Miyakaori reached a

peak at 4 to 5 weeks after heading (WAH) and then decreased rapidly reaching a final concentration of 20% and 40% of the peak at 7 to 8 WAH in early and later heading cultivars, respectively. The 2AP concentration was higher in brown rice ripened at a low temperature (day: 25 °C/night: 20 °C) than that ripened at a high temperature (day: 35 °C/night: 30 °C) in both short-grain cultivars.

Many storage and postharvest conditions such as temperature impact on the grain aroma. The effects of drying methods of rice at 30, 40, 50, and 70 °C and storage length of 10 months on 2AP were investigated (Worpongchai *et al.*, 2003). Drying rice grains at lower temperature of 30°C was found to maintain higher concentrations of the key aroma compound including 2AP and lower amounts of the off-flavour compounds such as n-hexanal and 2-pentylfuran, regardless of the storage time. At the same time 2AP concentrations decreased with storage whereas the quantities of n-hexanal and 2-pentylfuran contents were found to increase with storage duration. Accordingly, the average 2AP after one month of storage (4.02 ± 0.60 ppm) was slightly more than double that after four months (1.88 ± 0.27 ppm) and more than four times that after 10 months of storage (0.89 ± 0.12 ppm). Similarly, Yoshihashi *et al.* (2005) reported faster decrease of 2AP content at higher storage temperature than at a storage temperature of 5 °C. Champagne *et al.* (2008) also reported that the 2AP concentration was higher in brown rice ripened at a low temperature (day 25 °C; night 20 °C) than that those ripened at a high temperature (day 35 °C; night 30 °C) in two short-grain rice cultivars. Another study reported that rice samples stored at 30 °C had higher scores for pungent, oily, moldy/musty, sour (taste), bitter, sour (aroma), and muddy/earthy flavor, while those stored at -20 °C had higher scores for sweet (taste), fragrant, smooth (aroma), and sweet (aroma). The effect of degree of milling (DOM) measured by surface lipid content (SLC) was evaluated by panelist. The raw milled-rice samples varying in SLC level from 0.64% in brown rice to 0.25% typically were found not to tremendously affect sensory rating differences by the panelists, but milling beyond 0.25% SLC reduced the aroma level (Rodríguez-Arzuaga *et al.*, 2016).

Future Prospects in Breeding Rice for Aroma

For so long a time, the issues of quality could not largely be taken into consideration due to the need for self-sufficiency in rice (IRRI, 2010). Consequently, research previously focused on abiotic constraints (Kilimo Trust, 2014). Despite the increasing global population, the need for increased aroma and other grain quality attributes are gaining importance alongside the ongoing efforts towards increased rice productivity (Fitzgerald *et al.*, 2008). The aromatic rice improvement methods including pure breeding methods that produced aromatic cultivars such as Basmati 370 and KDML-105 (Singh *et al.*, 2000), hybridization and selection in segregating generations have led to basmati 385 and super basmati (Singh *et al.*, 2000) and super rice (Kilimo Trust, 2014) and mutation breeding resulted in development of aromatic mutants such as KLM-14 and KLM-24. Some of these varieties showed one or a few weaknesses including lower yield compared to the parents. Another attempt involved hybrid breeding in China which has led to high yielding hybrid (6.9 t/ha) with good grain quality, disease resistance, wide adaptability, moderate aroma and high acceptability (Singh *et al.*, 2000). This is a research direction for Africa and has been reported to increase yield by at least 20-30% above the parents (AfricaRice, 2010). The technique has also improved mean yield and quality performance in non-basmati aromatic rice (*Oryza sativa* L.) (Behera *et al.*, 2017). Molecular biology and biotechnology have opened new opportunities for genetic manipulation of the crop, especially use of marker assisted selection (MAS), metabolic profiling and sequence analyses (Ashfaq *et al.*, 2015). Through QTL and fine mapping work, the gene for aroma was identified (Bradbury *et al.*, 2005) and deeper understanding is further being explored using metabolomics and genomics (Daygon

et al., 2017). QTL studies have identified genes for important traits, thus selection for multiple traits may be possible through genomic selection (Asante *et al.*, 2017). Current challenge in aroma improvement, however, still remains causing a multiplicity of breeding objectives (Asante *et al.*, 2017) and subjectivity in aroma evaluation (Chambers *et al.*, 2013). In addition, the need for involving small effect QTLs/genes for aroma (Pachauri *et al.*, 2010; Daygon *et al.*, 2017) besides the need to determine the proportion of the resulting different compounds responsible for the specific flavor in rice has become apparent (Bryant and McClung, 2011). Prediction models for sensory descriptors based on the volatile components derived from gas chromatography-olfactometry (GCO) would be useful in selecting rice cultivars containing a satisfactory flavor to produce improved quality in rice breeding programs. The use of genetic and chemical based approaches and manipulation of environmental conditions can supplement efforts to accumulate and retain 2AP in rice.

Acknowledgements.

The financial support from Mak-Sida programme, National Crops Resources Institute (NaCCRI) and Gulu University is gratefully acknowledged. This paper is the contribution to the Sixth African Higher Education Week and RUFORUM Biennial Conference held 22-26 October 2018 in Nairobi, Kenya.

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