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Basmati aroma: what it means and how it accumulates in fragrant rice?

Rice plant growth comprises vegetative, reproductive, grain filling and maturity phases. In these phases availability of primary and secondary metabolites including volatile organic compounds (VOC's) are greatly varied. Basmati rice aroma contains more than 100 volatile organic compounds (VOC's) including 13 hydrocarbons, 14 acids, 13 alcohols, 16 aldehydes, 14 ketones, 8 esters, 5 phenols etc. These VOC's were reported to be responsible for basmati type flavor (Hussain et al. 1987). But among this 2-Acetyl-1-pyrroline (2AP) has been identified as a principal aroma compound and detected in all aerial plant parts of scented rice (Yoshihashi et al. 2002, Maraval et al. 2010). In scented rice, aroma volatiles are synthesized in aerial plant parts and deposited in mature grains. It will be interesting to keep track of aroma volatiles across the developmental stages in scented rice. Therefore, the aroma volatiles contributing in aroma with special reference to 2AP were screened at 7 developmental stages (S1-Seedling, S2-Tillering, S3-Booting, S4-Flowering, S5-Milky grain stage, S6-Dough grain stage, S7-Mature grain stage) in scented rice cultivars Basmati-370(BA-370), Ambemohar-157(AM-157) along with non-scented rice cultivar IR-64 as a control following Head Space Solid Phase Micro Extraction Gas Chromatography Mass Spectrometry (HS-SPME-GC-MS) method. In addition, the expression levels of key genes and precursor levels involved in 2AP biosynthesis were studied.

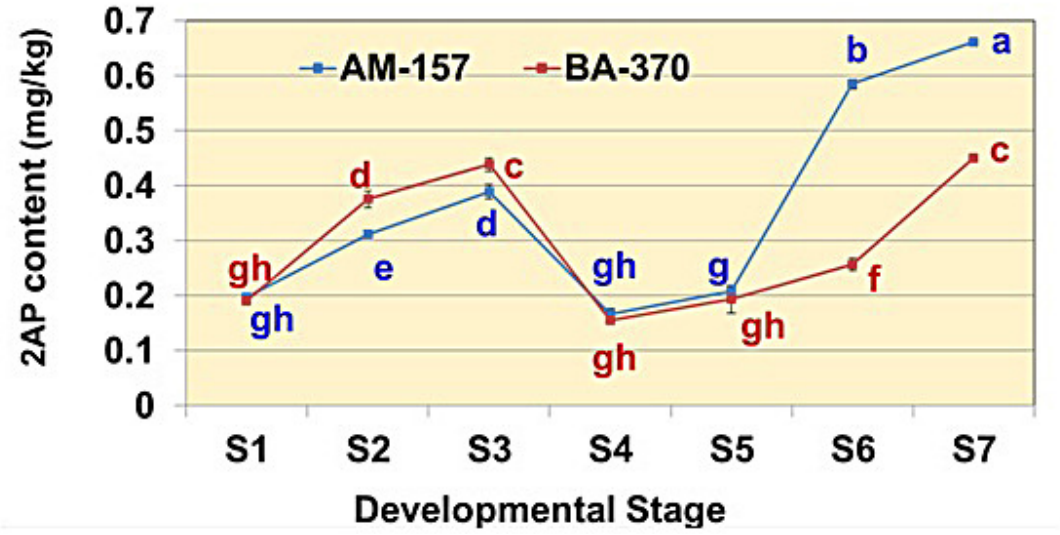

Fig. 1. 2AP content analysis at 7 developmental stages in 3 rice cultivars (S1: seedling, S2: tillering, S3: booting, S4: flowering, S5: miky grains, S6: dough grains, S7: mature grains, difference in lowercase letter indicates significant difference among the mean value presented at p = 0.05 level, a-most significant).

Qualitative analysis of aroma volatile indicated that volatilome of scented rice cultivars is more complex than non-scented rice cultivar. Among identified 88 volatile compounds in three rice cultivars, 2AP and other 13 compounds were detected specifically only in scented rice cultivars. N-heterocyclic class was the major distinguishing class between scented from non-scented rice. Maximum number of compounds were

synthesized at seedling stage and decreased gradually at reproductive and maturity. The seedling stage is an active phase of development where maximum number green leaf volatiles (GLVs) were synthesized which could be acting as defense molecules for protection of young plant parts.

Quantitative analyses of aroma volatiles identified 14 odor active compounds (OACs), which are contributing in the rice aroma. Among the 14 OACs, 9 OACs were accumulated at higher concentrations significantly in scented rice cultivars and contribute in the aroma. Five OAC (2AP, decanal, pentanal, phenylacetaldehyde and hexanal) were identified as major contributor towards the characteristic flavor of Ambemohar-157 rice. And six compounds ((E)-2-nonenal, nonanal, heptanal, 1-octanol, 1-octen-3-ol and 2-pentylfuran) were found associated with basmati flavor in Basamti-370 rice cultivar. 2AP content was highest in mature grains followed by booting stage (Fig. 1). Maximum 2AP accumulation in mature grains can be correlated with higher level of 2AP bound starch content in mature grains (Yoshihashi et al., 2005; Hinge et al. 2015). The present result revealed the mechanism of 2AP accumulation such that 2AP in mature grains might be transported from leaves and stem sheath and accumulation takes place in grains. And enhancement of 2AP content at or after booting stage could increase 2AP accumulation in mature grains. Therefore, agronomical and physiological measures could be taken at or after booting stage to enhance 2AP levels in mature grains. Based on 14 OACs, seven developmental stages could be clearly differentiated (Fig. 2A). Which showed that volatile accumulation pattern through developmental stages was specific for each developmental stage. The volatile compounds giving constant ratios across the developmental stages could be considered as biomarkers for defining specific rice cultivars (Fig. 2B). The ratio of octanal/1-octanol was served as common marker for AM-157 (3.41), BA-370 (2.66) and IR-64(4.41). Whereas Pentanal/phenylacetaldehyde (1.79) for AM-157, pentanal/heptanal for BA-370 (0.54) and pentanal/hexanal (0.47) for IR-64 were identified as cultivar specific biomarkers.

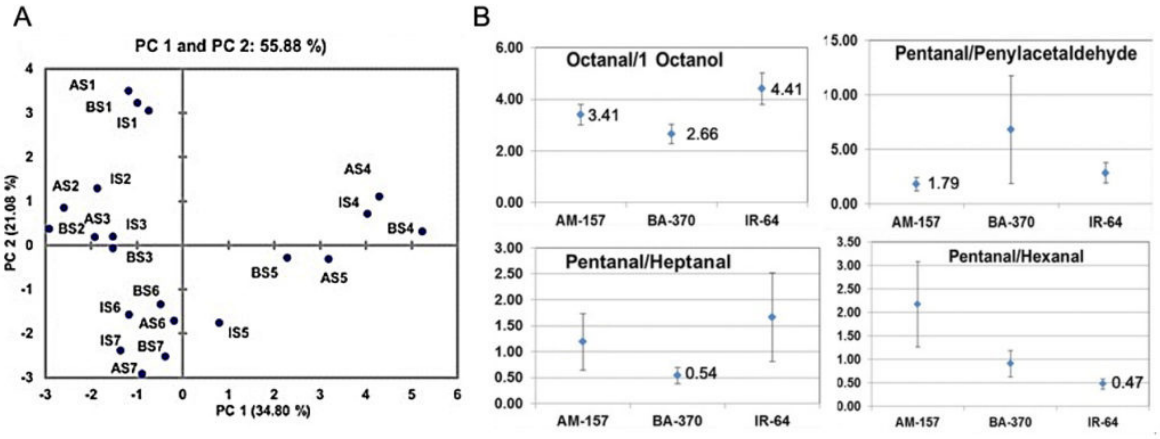

Fig. 2. PCA analysis of odor active compounds and developmental stages (b) of 3 rice cultivars A in AS1 to AS7; AM-157, B in BS1 to BS7; BA-370, I in IS1 to IS7;IR-64, S1; seedlings, S2; tillering, S3; booting, S4; flowering, S5; milky grains, S6; dough grains, S7; mature grains (a). OACs as cultivars specific biomarker identification, average values with standard error for ratio of OACs in respective rice cultivars under study (b).

Proline and methylglyoxal content was recorded higher in scented rice cultivars than non-scented rice cultivars IR-64 at all developmental stages and confirmed their contribution in accumulation of 2AP. Gene

expression analysis revealed that reduced expression of *betaine aldehyde dehydrogenase* 2 (*badh*2) and *glyceraldehyde-3-phosphate dehydrogenase* (*GAPDH*) and elevated level of *triose phosphate isomerase* (*TPI*) and *Δ1-Pyrolline-5-carboxylic acid synthetase* (*P5CS*) transcript enhances 2AP accumulation. The results of correlation analysis between 2AP, gene expression and metabolites revealed the negative association of *Badh*2 and *GAPDH* in 2AP accumulation and positive contribution of *TPI, P5CS*, methylglyoxal and proline in 2AP accumulation. Down regulation of *Badh2* and *GAPDH* and up expression of *TPI* and *P5CS* could be targeted for aroma enhancement in rice through genetic engineering. Specific aroma flavor like Basmati or Ambemohar could be enhanced through enhancement OACs associated with that aroma by using metabolic engineering approach.

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Publication

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