VOLUME 9 NOMOR 2 DESEMBER 2022 ISSN 2548 – 611X

JURNAL
BIOTEKNOLOGI & BIOSAINS INDONESIA

Homepage Jurnal: http://ejurnal.bppt.go.id/index.php/JBBI

VOLATILE COMPOUND ANALYSIS OF AROMATIC RICE MUTANT LINES USING HS-SPME/GC-MS

Analisis Kandungan Senyawa Volatil Galur Mutan Padi Aromatik Menggunakan HS-SPME/GC-MS

Muh. Riadi*[1], Andi Muliarni Okasa[2], Rinaldi Sjahril[1], Meta Mahendradatta[3], Rafiuddin[1]

[1]Agrotechnology Study Program, Department of Agronomy, Faculty of Agriculture, University of Hasanuddin, Jl. Perintis Kemerdekaan KM 10, Makassar 90245, Indonesia.

[2] Plant Protection Study Program, Faculty of Agriculture, University of Ichsan Sidenreng Rappang, Jl. Jend. Sudirman, Sidrap 91611, Indonesia.

[3] Food Science and Technology Study Program, Department of Agricultural Technology, Faculty of Agriculture, University of Hasanuddin, Jl. Perintis Kemerdekaan KM 10, Makassar 90245, Indonesia.

*Email: riadimuh@yahoo.co.id

ABSTRACT

Volatile compound is one of the key factors for aromatic components of rice. This study aimed to identify the key aroma components and their relationship with plant productivity in the Pare Bau variety mutant lines and its wild type. Volatile extraction was carried out using the headspace solid-phase microextraction (HS-SPME) method and analysed by the Gas Chromatography-Mass Spectroscopy (GC-MS) instrument. The results of the identification of volatile compounds showed a total of 224 compounds in the mutant lines and wild type. However, only 14 compounds were suspected as key aroma compounds in Pare Bau rice, namely 2-Acetyl-1-pyrroline, Indole, 1-Octanol, 1-Octen-3-ol, 2,4-Nonadienal, (E,E)-, Octanal, 2-Nonenal, (E)-, 2-Octenal, (E)-, Decanal, Hexanal, Nonanal, Furan, 2-pentyl-, toluene and vanillin. The results on aroma compounds of mutant lines using principal component analysis showed that there were differences in the main characteristics of several strains and wild type. There was no relationship between increasing volatile concentration and plant productivity, vice versa.

Keywords: *2-AP, aromatic rice, GC-MS, HS-SPME, volatile compound*

ABSTRAK

Senyawa volatil merupakan salah satu faktor kunci sebagai komponen aromatik beras. Penelitian ini bertujuan untuk mengidentifikasi faktor kunci sebagai komponen aroma dan hubungannya dengan produktivitas pada galur mutan dan Pare Bau (genotipe tetua). Ekstraksi senyawa volatil dilakukan dengan metode headspace solid-phase microextraction (HS-SPME) dan dianalisis dengan instrumen Gas Chromatography-Mass Spectroscopy (GC-MS). Hasil identifikasi senyawa volatil menunjukkan sejumlah 224 senyawa yang terdapat dalam galur mutan dan genotipe tetuanya. Namun, hanya 14 senyawa yang diduga sebagai senyawa aroma utama pada beras Pare Bau, yaitu 2-Acetyl-1-pyrroline, Indole, 1-Octanol, 1-Octen-3-ol, 2,4-Nonadienal, (E,E)-, Oktanal, 2-Nonanal, (E)-, 2-Oktenal, (E)-, Dekanal, Heksanal, Nonanal, Furan, 2-pentil-, toluen dan vanilin. Hasil analisis senyawa aroma galur mutan dengan analisis komponen utama menunjukkan adanya perbedaan karakteristik utama dari beberapa galur dengan genotipe tetuanya. Tidak terdapat hubungan antara peningkatan konsentrasi senyawa volatil dengan produktivitas tanaman.

Kata Kunci: 2-AP, GC-MS, HS-SPME, padi aromatk, senyawa volatil

Received: *27 November 2022* ***Accepted:*** *20 December 2022* ***Published:*** *29 December 2022*

INTRODUCTION

Aromatic rice is a popular high-quality rice variety, each with a distinctive and unique aroma. Aromatic rice varieties have premium prices in local and international markets due to their quality (Verma and Srivastav 2017). The development of aromatic rice in Indonesia can be accomplished by adapting and improving existing local aromatic rice varieties.

Most of the aromatic rice cultivated is of high-quality texture and taste. Therefore, farmers need to focus on the production of higher-quality aromatic rice (Nagarajan et al. 2010). Indonesian aromatic rice has a soft rice texture, late maturing, which is cultivated in landrace area such as in the Toraja, one of which is the Pare Bau variety.

The chemical composition of aromatic rice is very complex, consisting of a wide variety of volatile and non-volatile compounds with various functions. About 300 volatile compounds have been identified in rice (Wakte et al. 2017). Volatile organic compounds are classified into several classes, i.e. aldehydes, alcohols, terpenes, and others (Dudareva et al. 2013). This compound can be correlated with the quality of rice such as aroma and taste. The aroma of rice is highly dependent on genetic factors (Routray and Rayaguru 2018).

There are several methods for the extraction of volatile compounds, including the Simultaneous Distillation Extraction (SDE) method (Verma et al. 2018b), head space solid-phase micro-extraction (HS-SPME) (Gao et al. 2021), and supercritical fluid extraction (SFE) (Verma et al. 2018a). Currently, HS-SPME has been widely used for the analysis of volatile compounds in foods and beverages such as bananas (Dou et al. 2020), sponge cake (Garvey et al. 2020), and coffee (Abdelwareth et al. 2021), due to its ease of use and sensitivity. Furthermore, the combination of gas chromatography-mass spectrometry (GC-MS) is a hyphenated analysis technique that is widely used to profile volatile compounds in rice because it can separate and identify different substances in test samples (Das et al. 2014, Lim et al. 2018).

Multivariate analysis is a method that simultaneously analyse all random variables measured in each unit of experiment or sampling (Yeater et al. 2015). Principal component analysis (PCA), one of the multivariate statistical approaches, divides the data into two proportions to describe comparisons and relationships between variables and genotypes based on percentage variability and correlation (Hameed et al. 2019). It is a technique for reducing the dimensions of a data set with several interrelated variables (Jollife and Cadima 2016). It is also useful in breeding programs to select genotypes that are superior in terms of agronomic properties during rice milling

The purpose of this study was to identify key aroma compounds and their relationship with plant productivity in the mutant lines M4 generation of Pare Bau, induced by heavy ion beam irradiation compared with its wild type. We predicted that there is a significant effect of heavy ion beam to volatile compound of aromatic rice.

MATERIALS AND METHODS

Location and time

Analysis of volatile compounds was conducted at the Laboratory Flavour and Sensory Evaluation, Indonesian Institute for Rice Research, Sukamandi, Subang Regency from September to October 2020.

Materials

Equipment used in this experiment were a set of head space solid-phase micro-extraction (HS-SPME), a set of gas chromatography-mass spectrometry (GC-MS) tools, a flour-making machine, and an analytical balance. The materials used were grain samples of 10 M4 mutant lines of aromatic Pare Bau rice and its wild type, which were harvested after planting from December 2018 to June 2019 in Buntu Limbong Village, Gandang Batu Sillanan District, Tanah Toraja Regency, and located at altitude of 898 m above sea level (03º13’765” S; 119º52’745” E). Mutant lines of aromatic rice derived from ‘Pare Bau’ which was generated by the treatment of heavy Ar-ion beam irradiation (Okasa et al. 2021a, Okasa et al. 2021b, Okasa et al. 2022). These mutants were selected for early heading and high yield characters component for M4 generations.

Methods

Rice from each of the mutant line and wild type were floured using a flour-making machine. Sample of 5 g rice flour was shifted into GC vials and wrapped in light excluding sachets of aluminium foil to minimize light-induced enzymatic reactions, odour contamination, and stored at 4ºC till further analysis.

Extraction of volatile compounds

The volatile compounds from rice lines were extracted using the HS-SPME. A sample of 5 g grains was weighed into a 20 mL vial and placed in a tightly closed septum. The samples were heated at 50 ºC for 30 min and then kept at room temperature for 10 min. Then SPME fibres were inserted into the septum for 30 minutes at 90 ºC.

GC-MS analysis

GC combined with MS was used to perform GC-MS analyses. The separation of the volatile compounds of rice lines was carried out using a DB-Wax capillary column (30 m × 250 µm × 0.25 µm). After extraction, the fibre was desorbed into the GC injector port for 5 minutes at 250 ºC in split-less mode. The oven temperature was initially held at 40 ºC, then increased to 120 ºC at 28 ºC/min. Then, increased to 200 ºC at 5 ºC/min, then increased to 230 ºC with level of 8 ºC/min. The mass scan range (m/z) was 29-550 amu.

Identification and quantification

Volatile compounds were identified by comparing the mass spectra of the compounds and the retention indices (RI) with the database of the National Institute of Standards and Technology (NIST Library, Gaithersburg, MD, USA), Wiley7n.1 (Hewlett-Packard, Palo Alto, CA) and literature study.

The GC-MS post-run software was used to determine the peak areas. The peak alignments were accomplished by comparing their retention time and mass fragments. Subsequently, for the statistical analysis, commonly existing peaks in all 12 samples were selected and imported to generate a data set.

Productivity

Harvesting was conducted when the panicles turned yellow and hardened. Harvest was done on the whole grain yields in non-destructive pots. Productivity (ton ha^{-1}) was weighed at the end of field experiment after being dried to reach a moisture content of 12%.

Statistical analysis

Data analysis was performed to identify significant differences between groups of samples, to identify compounds that contributed significantly to the observed differences. The obtained data sets were analyzed using principal component analysis (PCA) using standardized Peatherson data collection method, to avoid increasing the impact of variables with higher responses, and higher probability of variance.

RESULTS AND DISCUSSION

This study obtained 11 lines of mutant and wild type; each had different aroma content of volatile compounds. Based on the data on the results of volatile compounds between the lines and the wild type (Table 1), it was found that 14 volatile compounds were suspected as aroma markers of the samples, i.e. -Octen-3-ol,

Table 1. Volatile compounds in M4 generation mutant lines of aromatic rice

No	Compound	Type
1	2-Acetyl-1-pyrroline	Nitrogen
2	Indole	Nitrogen
3	1-Octanol	Alcohol
4	1-Octen-3-ol	Alcohol
5	2,4-Nonadienal, (E,E)-	Aldehyde
6	Decanal	Aldehyde
7	Octanal	Aldehyde
8	2-Octenal, (E)-	Aldehyde
9	Nonanal	Aldehyde
10	2-Nonenal, (E)-	Aldehyde
11	Hexanal	Aldehyde
12	Toluene	Phenol
13	Vanillin	Phenol
14	Furan, 2-pentyl-	Heterocyclic

2,4-Nonadienal, (E,E)-, Octanal, 2-Nonenal, (E)-, 2-Octenal, (E)-, Decanal, Hexanal, Nonanal, Furan, 2- pentyl-,toluene and vanillin (Mathure et al. 2014, Setyaningsih et al. 2019). The aroma in rice is obtained from volatile compounds which are the result of the synthesis of different biochemical pathways.

Based on the literature study, we grouped those 14 important volatile compounds that are assumed to be contributed to the aroma in Pare Bau rice lines from 224 volatile compounds detected (data not shown). The aroma component compounds were 2-Acetyl-1-pyrroline, Indole, 1-Octanol, 1-Octen-3-ol, 2,4-Nonadienal, (E, E)-, Octanal, 2-Nonenal, (E)-, 2-Octenal, (E)-, Decanal, Hexanal, Nonanal, Furan, 2-pentyl-, toluene and vanillin. The concentration values of each volatile compound are expected to affect the aroma of rice (Table 1).

Nitrogen compounds

Nitrogen group compounds (Figure 1), namely 2-AP compounds are compounds that are considered the main component of aroma in rice. Compound 2-AP is not formed in the cooking process or other postharvest processes, but this compound is formed during the planting period. The 2-AP compound is described by Americans as a popcorn-like aroma, and by Asians, it is described as a pandan-like aroma (Verma and Srivastav 2020). The content of 2-AP can be affected by the degree of grinding, packaging, and temperature, whereas a low degree of grinding can increase the amount of 2-AP, while high storage temperature will decrease the content of 2-AP (Rakhmi et al. 2013). This compound is an indicator in aromatic rice that can be used to distinguish between aromatic and non-aromatic rice.

Based on the results of the study, all lines had a 2-AP compound content higher than the wild type, except for the PB-A.7.1.30 line which didn't have a 2-AP compound. The presence or absence of 2AP compounds in rice is caused by the activity of the BADH2 enzyme. If the BADH2 enzyme is active, then this enzyme can convert GABAld into -aminobutyric acid (GABA). Conversely, if the BADH2 enzyme is inactive, GABAld will undergo acetylation (addition of an acetyl group) to form 2-acetyl-1-pyrroline (Bradbury et al. 2005). The BADH2 enzyme will be active when there is a dominant badh2 allele that codes for the enzyme. This is in accordance with the opinion of Chen et al. (2008) which states that the BADH2 amino acid encoded by the Badh2 gene will inhibit 2-AP biosynthesis by converting AB-ald (the presumed precursor of 2AP) to GABA, whereas the absence of BADH2 due to the allele malfunctioning badh2 results in the accumulation of AB-ald and thereby alters the pathway to 2-AP biosynthesis.

Another nitrogen group compound that

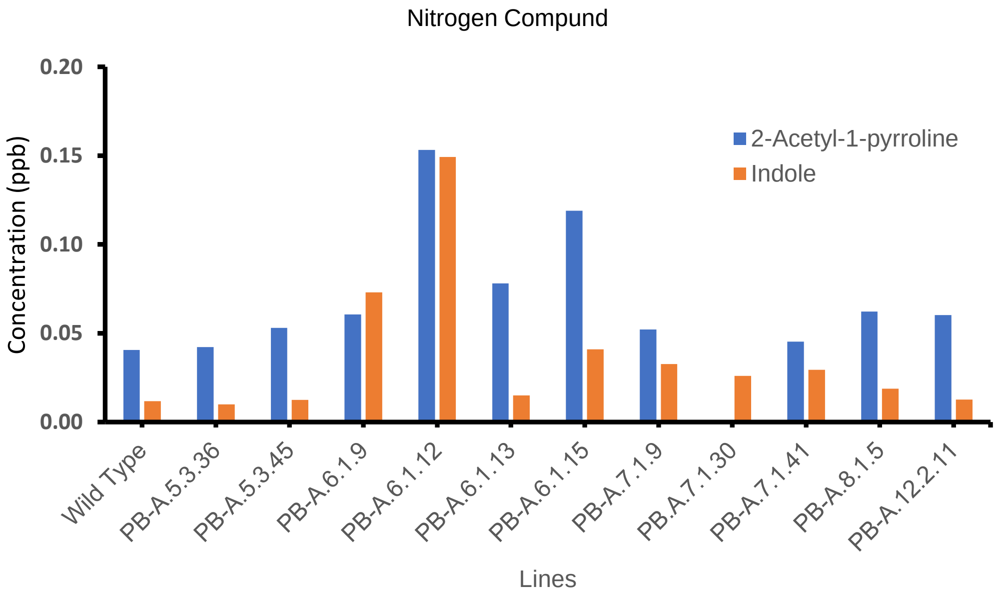

Figure 1. Concentration of nitrogen-containing compound of aromatic rice lines and wild type

also plays a role in the aroma of rice is indole compounds which are synthesized from the shikimic acid pathway. PB-A.6.1.12 line has highest concentration in 2-AP and Indole compound. In addition to causing the aroma of rice, this compound also functions as a biological agent (Erb et al. 2015). According to Kaiser (2006), indole compounds even at low concentrations can emit floral or fruity and herbaceous aromas. The two compounds were selected as the rice aroma index in the nitrogen compound group. The 2-AP compound is not the only standard for distinguishing between aromatic and non-aromatic rice. In addition to 2-AP, several alcohols, aldehydes, phenol-containing and heterocyclics compounds also play an important role in the aroma quality of rice.

Alcohol compounds

Alcohol group compounds (Figure 2) such as 1-Octanol which has a fruity aroma (Setyaningsih et al. 2019), and 1-Octen-3-ol which has a mushroom aroma (Wang et al. 2021) are volatile compounds that are thought to contribute to the aroma character of rice. The result showed that PB-A.6.1.12 had the highest concentration of 1-Octanol, while PB-A.6.1.9 had the highest concentration of 1-octen-3-ol. The 1-Octen-3-ol compound is a product of lipid oxidation, which is derived from the lipoxygenase activity of the linoleic acid precursor.

Aldehyde compounds

The aldehyde compounds (Figure 3) that are expected to play a role in the aroma of rice

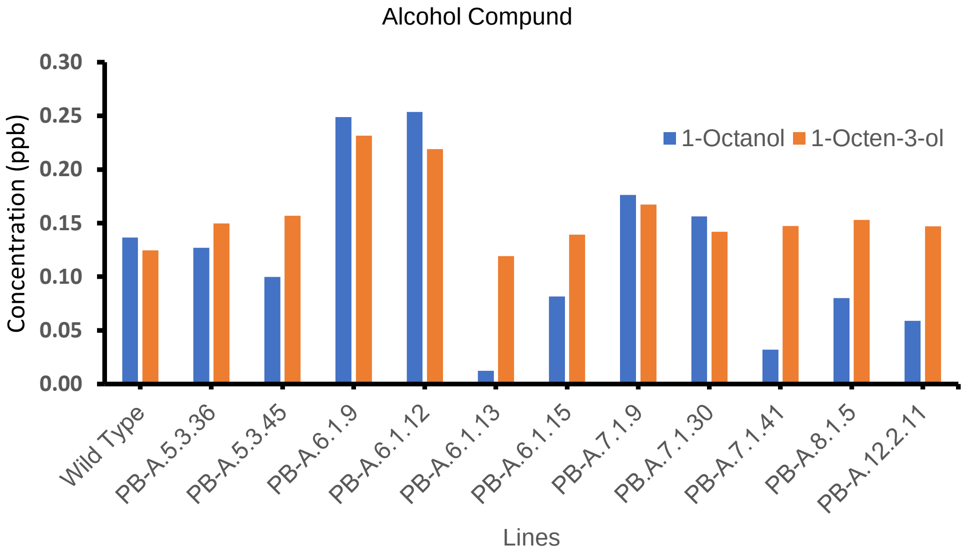

Figure 2. Concentration of alcohols compound of aromatic rice lines and wild type

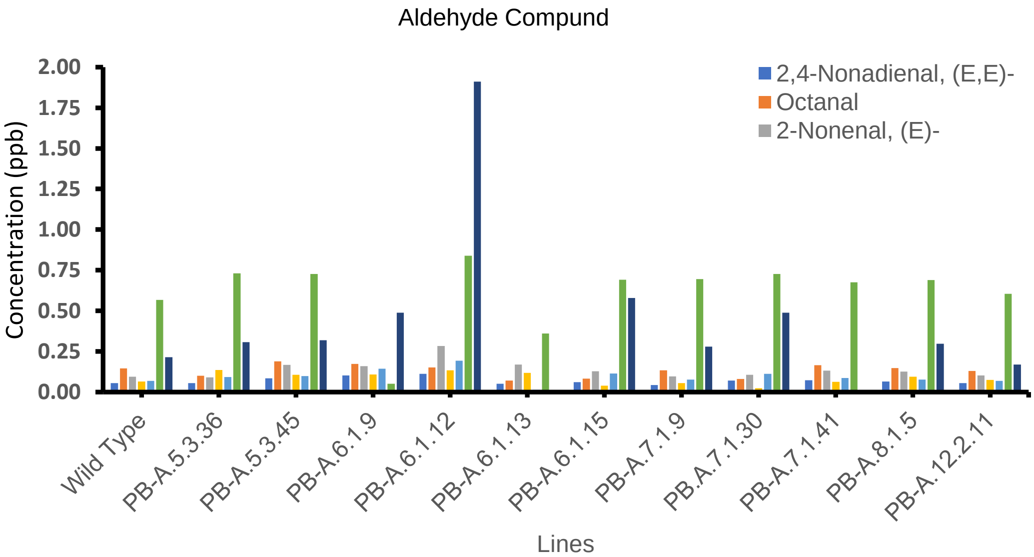

Figure 3. Concentration of aldehydes compound of aromatic rice lines and wild type

are 2,4-Nonadienal, (E,E)-, Octanal, 2-Nonenal (E)-, 2-Octenal, (E)-, Decanal, Hexanal, and Nonanal compounds. The compound 2,4-decadienal, (E,E)- has a fatty and waxy aroma (Chen et al. 2009). Meanwhile, decanal compounds have a citrus aroma (Mathure et al. 2014). Hexanal is a short-chain aldehyde compound that is produced from the breakdown of lipids. The presence of hexanal has been used to determine off-flavor in rice only high concentration (Yuan et al. 2022). During storage (E)-2-nonenal (rancid), octanal (fatty), and hexanal (green) will increase the off-flavors aroma which is not liked by consumers.

Phenol and heterocyclic compounds

Phenol group compounds (Figure 4) that are thought to contribute to the aroma of rice are Vanillin and Toluene. Vanillin compounds are also found in cooked black rice and contribute to the aroma (Choi et al. 2018). According to Ocan et al. (2020), one of the 3 important components of aroma in rice is Toluene. The heterocyclic compound (Figure 5) thought to play a role in the aroma of rice is Furan, 2-penthyl-. Furan, 2-penthyl- compound has a nutty aroma (Widjaja et al. 1996).

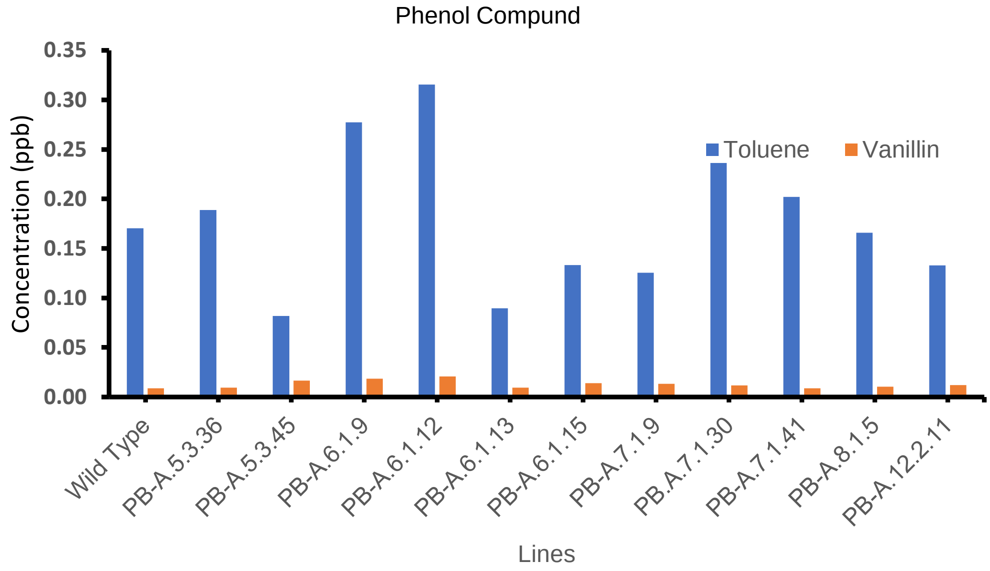

Figure 4. Concentration of phenol-containing compound of aromatic rice lines and wild type

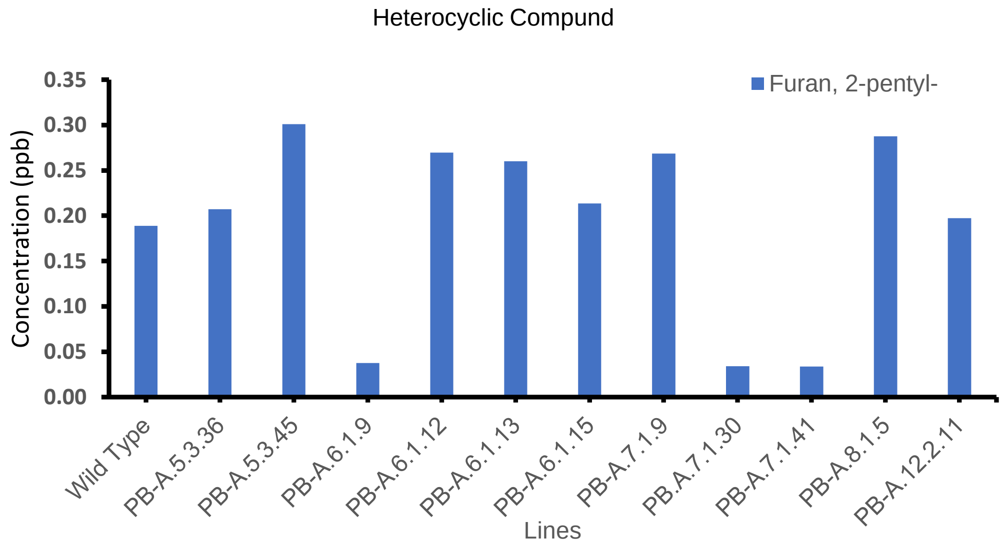

Figure 5. Concentration of aroma compound of aromatic rice lines and wild type

Principal component analysis

Principal component analysis was used to determine the volatile compounds which in this case were related to the dominant aroma in each line and the grouping of the lines based on the similarity of the volatile compound content. Both PCs accounted for the total diversity of 69.77%.

Figure 6 shows the distribution of rice samples based on the aroma component of the sample. Volatile components with large variations were represented as long vectors, while volatile components with small variations were represented as short vectors. In Figure 2, it can be seen that the longest line was the 2-AP compound. This indicates that there is a large diversity in the group of compounds.

The volatile compounds that have the shortest vectors were octanal and hexanal, so it can be assumed that these two compounds have small data variations. The highest hexanal and octanal concentration values were lines PB-A.6.1.12 and PB-A.5.3.45, while the lowest were lines PB-A.6.1.13 and PB-A.7.1.30, respectively.

Differences in compound composition between lines were thought to be influenced by genetic differences, given the irradiation treatment on these plants. Genetic differences will affect the formation of enzyme precursors and aroma-forming activity (Reineccius 2006).

In addition, information that can be obtained from the principal component analysis is the closeness between the lines where the lines that are located close together or in the same quadrant have similar characteristics on certain variables. Quadrant I showed that the lines PB-A.5.3.45 and PB-A.6.1.12 had a similar pandan aroma. This shows that the line has more specific characteristics than the 2-AP variable. The total content of these lines tends to be higher than the other lines. While the lines that had a similar aroma to the wild type were PB-A.7.1.30 and PB-A.7.1.41, the characteristic aroma of the three samples was not yet known.

A line that is located in the same direction as a variable can be said to have an above average value. On the other hand, a

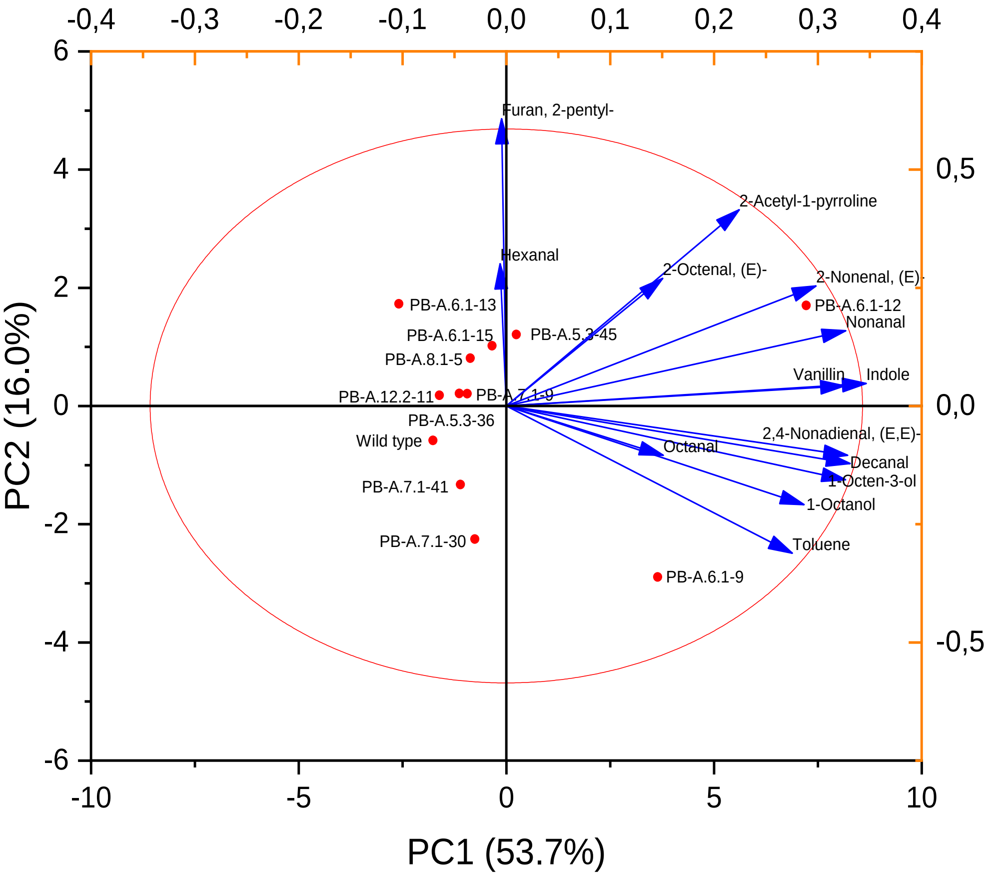

Figure 6. PCA biplot volatile aroma compound 11 lines irradiated by heavy ion beam and Pare Bau (wild type)

line that is located opposite the direction of a variable can be said to have a below-average value. Wild type has opposite direction vector with the volatile compound of 2-AP. So, it can be argued that the concentration of compound 2-AP in the wild type of these variables is below the average value.

Biplot can also explain the correlation between the volatile compound variables of aroma. The correlation between variables is indicated by the direction of the line on the biplot graph, the positive correlation is indicated by the direction of the line in the same direction. The closer or smaller the angle of the variable line, the stronger the correlation. It shows that several volatile compounds of the aroma have line graphs that are close together and coincide, i.e., 2 Acetyl pyrroline and 2-Octenal (E), indole and vanillin, 1-octanol and octanal, and hexanal and furan 2-penthyl. This shows that the pandan aroma is very close to the nutty aroma.

A correlation of the number of lines to the grouping of productivity and volatile compounds in the M4 generation mutant line of aromatic rice is presented in Figure 7. In this study, there was no relationship between increasing volatile concentration and plant productivity. The research of Kibria et al. (2008) explained that there was a significant negative correlation between rice production and the content of 2-AP compounds. This is because aromatic rice is generally very sensitive to abiotic stresses and has high disease resistance. This is thought to be caused by a lack of resistance genes, low GABA content, and malfunctioning of the BADH2 gene. This result is supported by research of Sansenya et al. (2017) where the gamma irradiation treatment increases the concentration of 2-AP compounds in the Thai aromatic local rice strain compared to wild type.

CONCLUSION

There are 14 components of volatile compounds that are thought to act as aroma characterizing compounds in the strain. The results of data on the aroma component of mutant lines using principal component analysis showed that there were differences in the main characteristics of several lines and wild types. In this study, there was no relationship between increasing volatile concentration and plant productivity.

ACKNOWLEDGMENT

Our gratitude to The Ministry of Research, Technology and Higher Education of Indonesia for the research grant through PMDSU Batch III scholarship and research scheme under grant number 1739/UN4.21/PL.01.10/2019. We also would

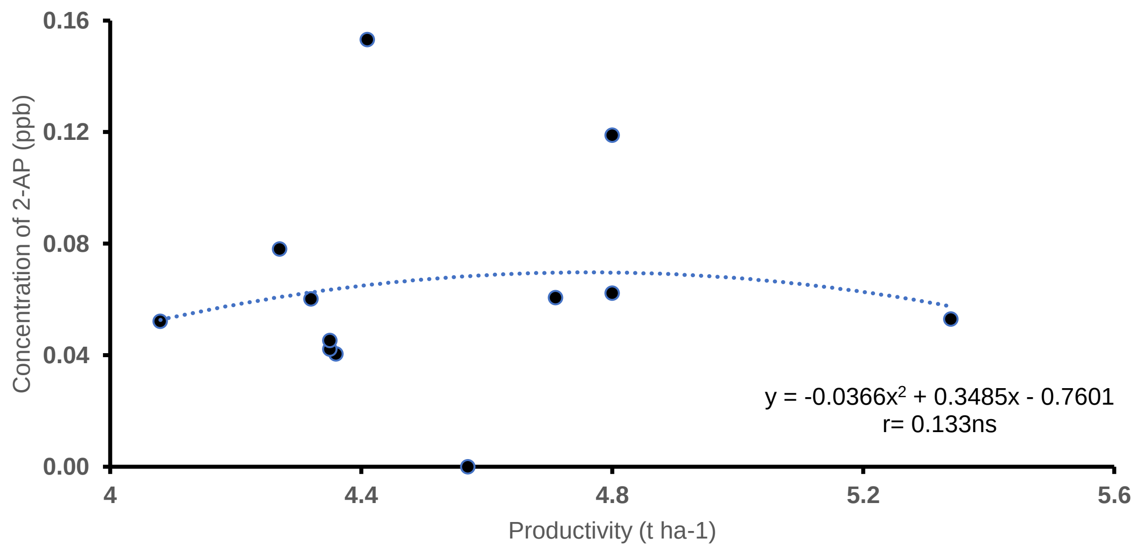

Figure 7. Correlation of productivity and 2-AP compounds in M4 generation mutant lines of aromatic rice irradiated by heavy ion beam

like to thank Dr. Tomoko Abe, from RIKEN Nishina Center for Accelerator-Based Science. Wako, Saitama, Japan, Dr. Tadashi Sato and Prof. Kinya Toriyama from Graduate School of Agricultural Science, Tohoku University. Sendai, Miyagi, Japan, for their consultation and visits.

REFERENCES

Abdelwareth A, Zayed A, Farag MA (2021) Chemometrics-based aroma profiling for revealing origin, roasting indices, and brewing method in coffee seeds and its commercial blends in the Middle East. Food Chem 349:129162. doi: 10.1016/j.foodchem.2021.129162

Bradbury LMT, Fitzgerald TL, Henry RJ, Jin Q, Waters DLE (2005) The gene for fragrance in rice. Plant Biotechnol J 3:363–370. doi: 10.1111/j.1467-7652.2005.00131.x

Chen G, Song H, Ma C (2009) Aroma-active compounds of Beijing roast duck. Flavour Fragr J 24:186–191. doi: 10.1002/ffj.1932

Chen S, Yang Y, Shi W, Ji Q, He F, Zhang Z, Cheng Z, Liu X, Xu M (2008) Badh2, encoding betaine aldehyde dehydrogenase, inhibits the biosynthesis of 2-acetyl-1-pyrroline, a major component in rice fragrance. Plant Cell 20:1850–1861. doi: 10.1105/tpc.108.058917

Choi S, Seo HS, Lee KR, Lee S, Lee J (2018) Effect of milling degrees on volatile profiles of raw and cooked black rice (*Oryza sativa* L. cv. Sintoheugmi). Appl Biol Chem 61:91–105. doi: 10.1007/s13765-017-0339-z

Das AJ, Khawas P, Miyaji T, Deka SC (2014) HPLC and GC-MS analyses of organic acids, carbohydrates, amino acids and volatile aromatic compounds in some varieties of rice beer from northeast India. J Inst Brew 120:244–252. doi: 10.1002/jib.134

Dou TX, Shi JF, Li Y, Bi FC, Gao HJ, Hu CH, Li CY, Yang QS, Deng GM, Sheng O, He WD, Yi GJ, Dong T (2020) Influence of harvest season on volatile aroma constituents of two banana cultivars by electronic nose and HS-SPME coupled with GC-MS. Sci Hortic (Amsterdam) 265:109214. doi: 10.1016/j.scienta.2020.109214

Dudareva N, Klempien A, Muhlemann JK, Kaplan I (2013) Biosynthesis, function and metabolic engineering of plant volatile organic compounds. New Phytol 198:16–32. doi: 10.1111/nph.12145

Erb M, Veyrat N, Robert CAM, Xu H, Frey M, Ton J, Turlings TCJ (2015) Indole is an essential herbivore-induced volatile priming signal in maize. Nat Commun 6:6273. doi: 10.1038/ncomms7273

Gao C, Li Y, Pan Q, Fan M, Wang L, Qian H (2021) Analysis of the key aroma volatile compounds in rice bran during storage and processing via HS-SPME GC/MS. J Cereal Sci 99:103178. doi: 10.1016/j.jcs.2021.103178

Garvey EC, O'Sullivan MG, Kerry JP, Kilcawley KN (2020) Optimisation of HS-SPME parameters for the analysis of volatile compounds in baked confectionery products. Food Anal Methods 13:1314–1327. doi: 10.1007/s12161-020-01740-4

Hameed K, Khan MS, Sadaqat HA, Awan FS (2019) Phenotypic characterization of super basmati ethyl methane sulfonate (EMS) induced mutants. Pak J Agric Sci 56:377–384. doi: 10.21162/PAKJAS/19.7671

Jolliffe IT, Cadima J (2016) Principal component analysis: A review and recent developments. Philos Trans A Math Phys Eng Sci 374:20150202. doi: 10.1098/rsta.2015.0202

Kaiser R (2006) Flowers and fungi use scents to mimic each other. Science 311:806–807. doi: 10.1126/science.1119499

Kibria K, Islam MM, Begum SN (2008) Screening of aromatic rice lines by phenotypic and molecular markers. Bangladesh J Bot 37:141–147. doi: 10.3329/bjb.v37i2.1720

Lim DK, Mo C, Lee DK, Long NP, Lim J, Kwon SW (2018) Non-destructive profiling of volatile organic compounds using HS-SPME/GC–MS and its application for the geographical discrimination of white rice. J Food Drug Anal 26:260–267. doi: 10.1016/j.jfda.2017.04.005

Mathure SV, Jawali N, Thengane RJ, Nadaf AB (2014) Comparative quantitative analysis of headspace volatiles and their association with BADH2 marker in non-basmati scented, basmati and non-scented rice (*Oryza sativa* L.) cultivars of India. Food Chem 142:383–391. doi: 10.1016/j.foodchem.2013.07.066

Nagarajan S, Jagadish SVK, Prasad ASH, Thomar AK, Anand A, Pal M, Agarwal PK (2010) Local climate affects growth, yield and grain quality of aromatic and non-aromatic rice in northwestern India. Agric Ecosyst Environ 138:274–281. doi: 10.1016/j.agee.2010.05.012

Ocan D, Rongrong Z, Odoch M, Nuwamanya E, Ibanda AP, Odong TL, Lamo J, Fitzgerald AM, Daygon VD, Rubaihayo PR (2020) Volatile organic compound based markers for the aroma trait of rice grain. J Agric Sci 12:92. doi: 10.5539/jas.v12n8p92

Okasa AM, Sjahril R, Riadi M, Mahendradatta M (2022) Multivariate analysis of agronomic traits in M4 generation of aromatic rice lines. Pak J Biol Sci 25:182–190. doi: 10.3923/pjbs.2022.182.190

Okasa AM, Sjahril R, Riadi M, Mahendradatta M, Sato T, Toriyama K, Ishii K, Hayashi Y, Abe T (2021a) Evaluation of Toraja (Indonesia) local aromatic rice mutant developed using heavy-ion beam irradiation. Biodiversitas 22:3474–3481. doi: 10.13057/biodiv/d220846

Okasa AM, Sjahril R, Riadi M, Mahendradatta M, Sato T, Toriyama K, Ishii K, Hayashi Y, Abe T (2021b) Correlation and path coefficient analysis of grain yield and its components in Toraja land-race aromatic rice mutants induced by heavy ion beam. Asian J Plant Sci 20:406–413. doi: 10.3923/ajps.2021.406.413

Rakhmi AT, Indrasari SD, Handoko DD (2013) Karakterisasi aroma dan rasa beberapa varietas beras lokal melalui quantitative descriptive analysis method. Informatika Pertanian 22:37–44

Reineccius G (2006) Choosing the correct analytical technique in aroma analysis. Pp. 81-97. In Voilley A, Etievant P (Eds). Flavour in Food. doi: 10.1533/9781845691400.1.81

Routray W, Rayaguru K (2018) 2-Acetyl-1-pyrroline: A key aroma component of aromatic rice and other food products. Food Rev Int 34:539–565. doi: 10.1080/87559129.2017.1347672

Sansenya S, Hua Y, Chumanee S, Phasai K, Sricheewin C (2017) Effect of gamma irradiation on 2-acetyl-1-pyrroline content, GABA content and volatile compounds of germinated rice (Thai upland rice). Plants 6:18. doi: 10.3390/plants6020018

Setyaningsih W, Majchrzak T, Dymerski T, Namieśnik J, Palma M (2019) Key-marker volatile compounds in aromatic rice (*Oryza sativa*) grains: An HS-SPME extraction method combined with GC×GC-TOFMS. Molecules 24:4180. doi: 10.3390/molecules24224180

Verma DK, Dhakane-Lad J, Mahato DK, Kapri M, Billoria S, Bhattacharjee P, Srivastav PP (2018a) Supercritical fluid extraction (SCFE) for rice aroma chemicals: Recent and advance extraction method. Pp. 179-198. In: Verma DK, Srivastav PP (Eds). Science and Technology of Aroma, Flavour and Fragrance in Rice. Apple Acad Press, New York. doi: 10.1201/b22468-14

Verma DK, Mahato DK, Srivastav PP (2018b) Simultaneous distillation extraction (SDE): A traditional method for extraction of aroma chemicals in rice. Pp. 99-108. In: Verma DK, Srivastav PP (Eds). Science and Technology of Aroma, Flavour and Fragrance in Rice. Apple Acad Press, New York. doi: 10.1201/b22468-14

Verma DK, Srivastav PP (2017) Proximate composition, mineral content and fatty acids analyses of aromatic and non-aromatic Indian rice. Rice Sci 24:21–31. doi: 10.1016/j.rsci.2016.05.005

Verma DK, Srivastav PP (2020) Extraction, identification and quantification methods of rice aroma compounds with emphasis on 2-acetyl-1-pyrroline (2-AP) and its relationship with rice quality: A comprehensive review. Food Rev Int 38:111–162. doi: 10.1080/87559129.2020.1720231

Wakte K, Zanan R, Hinge V, Khandagale K, Nadaf A, Henry R (2017) Thirty-three years of 2-acetyl-1-pyrroline, a principal basmati aroma compound in scented rice (*Oryza sativa* L.): A status review. J Sci Food Agric 97:384–395. doi: 10.1002/jsfa.7875

Wang SL, Lin SY, Du HT, Qin L, Lei LM, Chen D (2021) An insight by molecular sensory science approaches to contributions and variations of the key odorants in shiitake mushrooms. Foods 10:622. doi: 10.3390/foods10030622

Widjaja R, Craske JD, Wootton M (1996) Comparative studies on volatile components of non-fragrant and fragrant rices. J Sci Food Agric 70:151-161. doi: 10.1002/(SICI)1097-0010(199602)70:2<151::AID-JSFA478>3.0.CO;2-U. Corpus ID: 96183276

Yeater KM, Duke SE, Riedell WE (2015) Multivariate analysis: Greater insights into complex systems. Agron J 107:799–810. doi: 10.2134/agronj14.0017

Yuan H, Cao G, Hou X, Huang M, Du P, Tan T, Zhang Y, Zhou H, Liu X, Liu L, Jiangfang Y, Li Y, Liu Z, Fang C, Zhao L, Fernie AR, Luo J (2022) Development of a widely targeted volatilomics method for profiling volatilomes in plants. Mol Plant 15:189–202. doi: 10.1016/j.molp.2021.09.003