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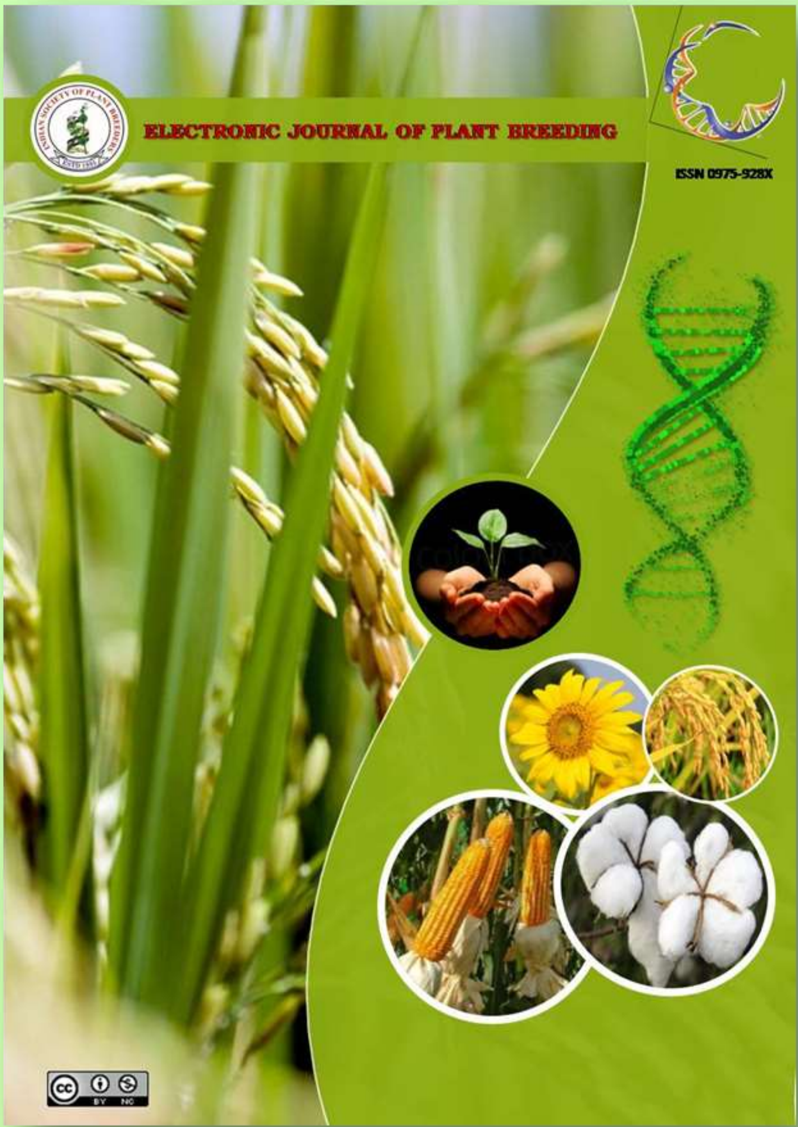

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Research Article

Comparative profiling of volatile compounds in the grains of rice varieties differing in their aroma

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Abstract
Rice (*Oryza sativa* L.) is a staple food for more than half of the world's population. Aromatic rice is highly preferred by most of the rice consumers and the aroma in Pusa Basmati 1 is said to be contributed by the accumulation of 2-acetyl-1-pyrroline (2AP). Our preliminary sensory tests identified that some of the rice genotypes including a traditional genotype of North-Eastern hill regions namely Chakhao Amubiexhibit entirely different aroma. Identifying alternate sources for aroma in rice and understanding its genetic basis will accelerate the development of rice varieties with desirable aroma. This study was aimed at developing a reliable extraction and profiling method for analyzing aromatic compounds in rice. KOH mediated digestion of grain flour followed by trapping of volatile compounds using sample collection tube containing Sorbent: Tenax GR and GC-MS separation of volatiles identified key differences between non-aromatic Improved White Ponni and aromatic Pusa Basmati 1. More than 50 volatile organic compounds (VOCs) were detected. A major aromatic compound 2-acetyl-1-pyrroline (2AP, (C_6H_9NO)) was detected only in the grains of Pusa basmati 1 but not in Improved White Ponni. Apart from 2AP, several other aromatic hydrocarbons and aldehydes such as hexanal, benzaldehyde, and2-pyrroline were detected in Pusa Basmati 1.Outcomes of this study are identification of several aromatic compounds in rice and also paved way for identifying novel aromatic compounds in rice.

Keywords
Rice, Aroma, Pusa Basmati 1, Gas Chromatography-Mass Spectrometry (GC-MS)

Introduction

Consumption of rice and rice-based meals are steadily increasing among western countries. This warrants a continuous increase in the rice production. Cultivation and adoption of these improved varieties is mainly driven by market demand based on the consumer's preference. The appearance of the grain (size and shape) determines the demand in the market and cooking quality traits determines popularization of the variety. If the variety fails to attract the consumer's preference, cultivation and popularization of newly developed rice variety are not guaranteed. This emphasizes the identification of traits that will attract the consumers/farmers and formulating breeding programs to achieve the goal.

Nearly 40% of people throughout the world especially USA, Canada, Europe, the Middle-East and South-East Asian countries prefer consuming aromatic rice irrespective of grain types. In India, preferences for rice grains are highly varying across states ranging from short slender to long bold. Among, sensory properties gel consistency and aroma are two commonly preferred characters among the farmers and consumers. Aromatic or fragrant rice occupies a top rank in pricing both locally and globally because of its superior quality and taste(Ahuja *et al.*, 1995). Aromatic rice varieties are of great importance in the market and acquire higher prices in comparison with non-aromatic rice cultivars (Calingacion *et al.*, 2014). The genetic structure of aromatic and quality rice has been studied in the past (Nagaraju *et al.*, 2002). The fragrance of aromatic rice is due to the presence of 2-acetyl-1-pyrroline, which is responsible for a distinct popcorn-like flavor(Widjaja *et al.*, 1996; Yoshihashi *et al.*, 2002).Apart from 2-acetyl-1-pyrroline, there are more than 100 volatile organic compounds have been reported. Some of them are hexanal, nonanal, octanal, nonenal, 2,4-nonadienal, heptanal,(E)-2-octenal, 4-vinylphenol, 4-vinylguaicol,1-octen-3-ol, decanal (Mathure *et al.*, 2014; Mathure *et al.*, 2011).

Several extractions and quantitative detection methods have been developed over a past few years, some of these are traditional extraction methods include purge and trap(Buttery *et al.*, 1988), solvent extraction (Buttery *et al.*, 1986), steam distillation (Widjaja *et al.*, 1996). Previous studies have identified a simple, rapid and reliable sensory evaluation method. This method utilizes 1.7% of Potassium hydroxide(KOH)for the testing aroma from a leaf or individual grains by treating the samples with Potassium hydroxide and later evaluation with olfactory sensors(Hien *et al.*, 2006).Potassium hydroxide acts as a nucleophile, it attacks the polar bonds in both organic and inorganic materials. The molten KOH is also used as aromatic reagents. Considering this background knowledge an attempt was made to establish an efficient protocol as well as to understand the metabolite profiles of aromatic and non-aromatic rice cultivars.

Materials and Methods

The present study was undertaken with the aim of understanding the variation in the volatile profiles of aromatic (Pusa Basmati 1) and non-aromatic rice (Improved White Ponni) genotypes. Freshly harvested rice seeds of Pusa Basmati 1 and Improved White Ponni(IWP) werede-husked and used. Ten grams of de-husked seeds were ground separately using clean and odour-free mixture grinder. Fine rice flour was transferred to a sterile conical flask and 100 ml of 1.7% Potassium hydroxide was added as described earlier (Hien *et al.*, 2006). The conical flask was tightly closed using a rubber cap containing a provision for a collector tube and sealed using the para film wrap to avoid leakage of volatiles. Stainless steel ATD pre-packed sample collection tube containing Sorbent: Tenax GR was inserted into the flask through the cap for collecting volatiles. The conical flask containing sample was retained at room temperature for overnight to facilitate the collection of volatiles into collector tube.

Gas Chromatography-Mass Spectrometry was carried out using a Clarus SQ 8C GC/Mass Spectrometer (Perkin Elmer, USA). DB-5 ms capillary standard non-polar column with 30Mts dimension and Helium (He)as carrier gas were used. Sample collection tubes were removed from the flask and directly fed into the GC-MS. The analysis system is equipped with data acquisition and evaluation was carried out using a Perkin Elemer Turbo Mass software Ver6.1.0.Data analysis was carried out using Xcalibur (Thermo Fisher) software. Obtained mass spectra was compared against NIST2014 (Version 2.2) library.

Results and Discussion

Aromatic rice ranks first in respect of consumer's preference because of its pleasant aroma and cooking quality, hence it acquires a premium price in the global markets. Several attempts were made to understand the molecular and biochemical basis of aroma in rice, which resulted in the identification of a recessive gene (*fgr*)and presence of the principle compound 2-acetyl-1-pyrroline(Bradbury *et al.*, 2005). Extraction and quantification of 2-acetyl-1-pyrroline as volatile aromatic compound using purge and trap (Buttery *et al.*, 1988), solvent extraction (Buttery *et al.*, 1986), steam distillation (Widjaja *et al.*, 1996;Yajima *et al.*, 1978) has been reported. Recently numerous researchers has reported HS-Solid Phase Micro Extraction (SPME)/GC-FID (Stashenko and Martínez, 2007). Regardless of 2-acetyl-1-pyrroline more than fifty volatile organic compounds (VOCs) were identified in Pusa Basmati 1 in comparison with Improved White Ponni (non-aromatic). The identified volatile compounds can be summarized into aromatic compounds (2-acetyl-1-pyrroline, 2pyrroline), aldehyde (Benzaldehyde), alkane hydrocarbons (Tetradecane, Dodecane) and other volatile organic compounds (Fig.1; Table1). Identification and quantification of 2-acetyl-1-pyrroline as the principle aromatic compound were investigated previously by several researchers in rice(Buttery *et al.*, 1988; Hinge *et al.*, 2016), sorghum(Attar *et al.*, 2017),vegetable soybean (Fushimi and Masuda, 2001; Wu *et al.*, 2009)and *P.amaryllifolius* (Bhattacharjee *et al.*, 2005; Laohakunjit and Noomhorm, 2004; Thimmaraju *et al.*, 2005).

In this study, we have identified few aromatic volatile compounds in Pusa Basmati1 such as, 2-pentylfuran imparting floral, fruity, nutty and caramel-like aroma to rice (Hinge *et al.*, 2016) and also found in various other food substances like alcoholic beverages, coffee, potatoes, tomatoes, and soybean oil. 2-pentylfuran is also used as a flavoring agent in many food products. Similarly, Benzaldehyde gives nutty and sweet flavour. Other volatile compounds like hexanal have proven to emit a green grassy like odour but it contributes to the off-flavour in rice (Buttery *et al.*, 1983; Buttery *et al.*, 1988; Yang *et al.*, 2007;Mathure *et al.*, 2014;Nadaf *et al.*, 2016). Hexanal is formed from the degradation of lipid products(Hinge *et al.*, 2016) and it was found to be present in both Pusa Basmati 1 and Improved White Ponni. Dimethyl trisulfide emits sulfury, cabbage-like odour. Volatile profiling of Improved White Ponni resulted inthe identification of VOCs such as 2-Heptanone, 2-Decenal, Decanal, and 4,5dimethyl-Nonane. These results clearly indicated the presence of 2AP (C_6H_9NO) a potent aromatic compound along with other volatiles present only in aromatic Pusa Basmati 1 and not in non-aromatic Improved White Ponni. This study also developed a simple protocol for extracting volatiles from rice grains. Established protocol for profiling volatile compounds in rice grains may serve as a basis for further exploration of rice germplasm to broaden the genetic base in rice for aroma.

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Table 1. List of volatile compounds identified in Pusa Basmati 1 and Improved White Ponni

Volatile compound (Pusa Basmati 1)	Retention Time (RT)	Volatile Compound (Improved White Ponni)	Retention Time (RT)
2-Nitro-1-propanol	1.64	Methylene chloride	1.884
N-ethylethanamine	1.74	Furan, 2-methyl	2.094
Glutaraldehyde	1.76	cis-3-Methylcyclohexanol	2.589
2,3-Dihydro-2-methyl-5-ethylfuran	1.85	2-(4-methylcyclohex-3-en-1-yl)propanal	3.039
1,2-Butadiene	1.91	1,3,5-Cycloheptatriene	3.309
Trichloromethane	1.97	1-Heptene, 5-methoxy-4-methyl-	3.539
1,5-Hexadiyne	2.17	Hexanal	3.689
Thiopropanalsulfoxide	2.4	Cyclotrisiloxane	3.815
2-Pyrroline	2.83	Hexane, 3,3-dimethyl-	3.955
2H-Pyran, 5,6-dihydro-2-methyl-	3.12	1H-Pyrrole, 2-methyl-	4.255
2-t-Butyl-4-methyl-5-oxo-[1,3]dioxolane-4-carboxylic acid	3.19	Phenyl-α-D-glucoside	4.615
4,4-Dimethyl-2-cyclopenten-1-one	3.38	m-Xylene	4.735
Hexane	3.45	Ethanone, 1-(3-methylenecyclopentyl)-	4.95
Hexanal	3.59	2-Heptanone	5.025
Cyclotrisiloxane, hexamethyl-	3.64	Oxime-, methoxy-phenyl	5.235
Heptane, 2,4-dimethyl-	3.74	2-Dimethylaminobicyclo[2.2.1]hept-2-ene	5.595
2,4-Dimethyl-1-heptene	4.03	3,5-dimethyl- Phenol,	5.76
Benzenepropanal	4.38	Oxirane, 2-methyl-2-pentyl	6.17
1,3,6-Cyclooctatriene	4.52	2,2-dichloro-2-fluoro-1-phenyl	6.406
2-methyl- Octanoic acid,	4.69	Dimethyl trisulfide	6.566
Benzenepropanoyl bromide	4.87	Tetrakis(trimethylsilyl) silicate	6.656
Benzaldehyde	6.1	2-pentyl-furan,	6.966
Dimethyl trisulfide	6.24	Benzene	7.106
2-acetyl-1-pyrroline	6.31	Hydroxylamine, O-decyl-	7.356
2-pentyl-furan	6.65	Benzene, 1,4-dichloro-	7.671
cis-7-Decen-1-al	7.66	2-Decenal	8.001
2-hydroxy- Benzaldehyde	8.05	2(3H)-Furanone, 5-ethenyldihydro-5-methyl-	8.251
4,7-dimethyl-Undecane	8.29	Bicyclo[3.2.0]hept-2-ene	8.506
2,4-dimethyl-1-Decene	8.81	Undecane, 4,7-dimethyl-	8.591
Dodecane	9.36	Dodecane, 4,6-dimethyl-	8.726
Nonanal	9.49	2-Oxazolamine	9.032
Heptan-2-yl formate	9.61	3-Decene, 2,2-dimethyl	9.092
N,N-dimethyl-formamide	9.82	Prop-1-en-2-yl undecyl ester	9.457
N,N-Dimethylacetamide	10	Nonane, 4,5-dimethyl-	9.632
Acetic acid, 2-propylpentyl ester	10.39	Nonanal	9.767
1-Methylene-1H-indene	11.18	Phenylethyl Alcohol	10.042
Acetic acid, octyl ester	11.63	Cyclopentasiloxane	10.322
Phenylethyl Alcohol	11.83	1-Adamantanol	10.637
Benzothiazole	12.02	8-Methylenecyclooctene-3,4-diol	10.937
2,6,11-trimethyl-Dodecane	12.78	1,7,7-Trimethyl-2-vinylbicyclo[2.2.1]hept-2-ene	11.177
Megastigma-4,6(E),8(E)-triene	13.15	1H-Indene	11.483
Cyclohexasiloxane	13.22	Dodecane	11.673
Pentadecane, 2,6,10-trimethyl-	13.48	Decanal	11.813
Dodecane, 2,6,11-trimethyl-	13.55	Benzothiazole	12.298
Tridecane, 3-methyl-	13.75	Pentadecane	12.983
Tetradecane, 2,6,10-trimethyl-	13.85	Carbonic acid, dodecyl vinyl ester	13.413
Tert-Hexadecanethiol	14.03	Methoxyacetic acid, 3-tridecyl ester	14.649
1-Hexadecanol, 2-methyl-	14.37	Naphthalene	15.649
α-ylangene	14.43	α-Cedrene	15.809
Cedrene	14.71		
Di-epi-α-cedrene	15.13		
5-Hydroxy-6-methoxy-8-[(4-amino-1-methylbutyl)amino]quinolinetrihydrobromide	15.77		

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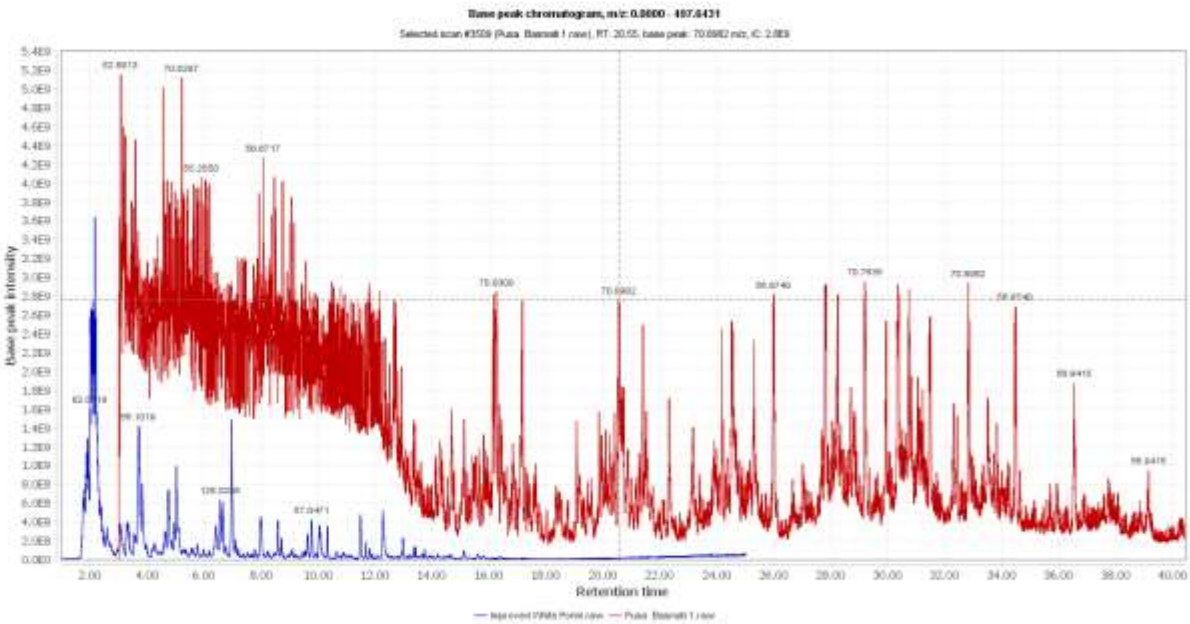

Fig. 1. Chromatogram of Pusa Basmati 1 (aromatic rice) and Improved White Ponni (non-aromatic rice) with overlay of selected ions

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