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Metabolic diversity in the grains of Indian varieties of rice

Bratati De *, Gargi Nag

Phytochemistry and Pharmacognosy research Laboratory, Department of Botany, University of Calcutta, 35, Ballygunge Circular Road, Kolkata 700019, India

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ABSTRACT

The aim of the present work was to analyze metabolic diversity in 26 different indica varieties of rice grains. Seventy-six metabolites could be identified in the methanol extracts of each of the rice varieties analyzed by gas chromatography-mass spectrometry. These metabolites included 9 sugars/sugar alcohols, 17 amino acids/derivatives, 18 fatty acids, 5 free phenolic acids and 19 other organic acids, 3 phytosterols, 5 other constituents. Cluster analyses to extract information for similarity and differences in metabolites unveiled diversity in metabolite profile. Two hierarchical clusters were generated based on the metabolite contents of the rice varieties. The first cluster (cluster I) consisted of one variety only. The second cluster again segregated into four clusters (clusters II, III, IV and V). Very distinct differences were visible amongst the clusters with respect to their sugars/sugar alcohols, organic acid, amino acid and fatty acid, phenol, and sterol profiles. Metabolites determine nutritional quality, taste, aroma. This and future efforts on the metabolomic information would help biochemists and nutritionists to better understand the nutritional quality of such grains at varietal level and correlating metabolites and long term human health related issues.

1. Introduction

The nutritional quality of a food crop depends, in part, on the metabolite components present in it. Natural diversity of chemical composition of a food crop is also responsible for its taste, colour, fragrance and characters like resistance to disease and other stress factors. Qualitative and quantitative identification of the metabolome (the small molecules) using metabolomic technology would assist in identifying the biochemical markers responsible for such quality. The nutritional scientists also may precisely identify bioactive ingredients in foods and better understand their potentially beneficial (or harmful) consequences using metabolomic technology [1].

Rice (*Oryza sativa* L.) is the staple food source for a large proportion of the world's population and also has potential health benefits. In India rice gruel is used in disorganized digestion, in bowel complaints in diarrhoea and dysentery. Rice water is demulcent, nourishing drink in febrile diseases and inflammatory states of the intestine [2]. The tocotrienol rich fraction of rice bran is effective in lowering serum total and LDL-cholesterol levels. Tocotrienols have superior efficacy compared with α-tocopherol [3]. Red and black rice decrease atherosclerotic plaque formation in rabbits [4]. Rice grains have antioxidant properties [5].

Metabolomic methodology has been applied to the metabolic phenotyping of natural variants in brown rice from 68 varieties from the world rice core collection (WRC) and two other varieties. Ten metabolites were selected as metabolite representatives [6]. The correlative relationships between genetic and metabolic diversity among 18 accessions from the world rice collection based on their population structure were assessed. The variations in the metabolic fingerprint of the extracts of seed grains were analysed with one dimensional ^{1}H-nuclear magnetic resonance (NMR). The result indicated that there were no relationships between the genomic and metabolic

* Corresponding author.
E-mail address: bratatide@hotmail.com (B. De).

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diversity of solution metabolites [7]. Metabolomics technology has also been applied for determination of flavour profile [8], production of GM rice [9].

Analysis of the natural variations in rice using metabolomics techniques is thought to be not only useful to understand the biological traits of rice such as the yield and defence responses, but also helpful to improve rice quality, including its taste and nutritive value [10]. Variations in the chemical composition of grains are important to grain consumers and to both food/feed industries for the purposes of quality control [11]. Metabolomics is thought to be a technique that may be applicable to rice breeding by a combination of other omics approaches [12]. Detailed report regarding the metabolite profile of Indian rice varieties are lacking. The aim of the present work is to analyse metabolic diversity in 26 varieties of rice grains of India using gas chromatography-mass spectrometry (GC-MS) which is currently the most utilized global analysis method for data acquisition by metabolomics technology [13].

2. Materials and methods

2.1. Plant materials

Grains from 26 different indica rice varieties were obtained from the plants grown in the paddy field at Rice Research Station, Chinsurah, Directorate of Agriculture, Government of West Bengal, India. The varieties (code names used) were Raghusail (WA), Bhasamanik (WB), Biraj (WC), Radhunipagal (WD), Kumargone (WE), Bipasha (WF), Lalat (WG), Manassarovar (WH), Tilokkachari (WI), Mahananda (WJ), Swarna (WK), Sashi (WL), Sunil (WM), Jhingasail (WN), Rupsail (WO), Badshabhog (WP), Shalibahan (WQ), IE7-6141 Kunti (WR), Mandira (WS), SR-26 B (WT), Kalamkati 147 (RA), Meghal Patnai (RB), FR 13A (RC), Jaladhi I (RD), FR 43B (RE), NC 678 (RF). The size and colour of the grains are presented in Table 1.

2.2. Extraction of grains

Two grains were dehusked, powdered using mortar and pestle and extracted with 1 ml methanol (at 70 °C for 15 minutes) after addition of internal standards ribitol and norleucine (20 μl of 0.2 mg/ml solution). The supernatant was collected by centrifugation and distributed into 3 Eppendorf tubes (2×50 μl and 5 μl). This was repeated five times for each rice sample (biological replication).

2.3. Derivatisation

Before GC-MS analysis, the extracts were derivatised [14]. One dry residue of 50 μl was re-dissolved and derivatised for 120 min at 37 °C (in 20 μl of 30 mg/ml methoxyamine hydrochloride in pyridine) followed by a 45 min treatment with 40 μl of *N*-methyl-*N*-(*tert*-butyldimethylsilyl)trifluoroaceteamide + 1% *tert*-butyldimethylchlorosilane at 65 °C (for TBS analysis) and another 50 μl and the 5 μl dried residue were re-dissolved and derivatised for 120 min at 37 °C (in 20 μl of 30 mg/ml methoxyamine hydrochloride in pyridine) followed by a 120 min treatment with 40 μl *N*-methyl-*N*-(trimethylsilyl) trifluoroacetamide at 37 °C (for TMS analysis). 5 μl of a

Table 1
Morphological characters of rice grains.

Rice grains variety (code name)	Length (mm) ± sd	Breadth (mm) ± sd	Length: Breadth	Colour
Raghusail[a] (WA)	7.04 ± 0.1125	2.3 ± 0.2582	3.061	Sand dune
Bhasamanik[a] (WB)	5.705 ± 0.2598	2.2 ± 0.2297	2.593	Corn silk
CNM-539Biraj[c] (WC)	6.257 ± 0.3217	2.05 ± 0.1054	3.052	Gold sunset
Radhunipagal[a] (WD)	3.95 ± 0.3073	2 ± 0	1.795	Jonquil
Kumargone[a] (WE)	6.495 ± 0.3459	3.025 ± 0.0791	2.147	Ray of light
Bipasha[b] (WF)	6.115 ± 0.1248	2.795 ± 0.1921	2.188	Chick yellow
Lalat[b] (WG)	7.095 ± 0.1787	2.165 ± 0.1668	3.277	Sweet buttercup
Manassarovar[b] (WH)	5.87 ± 0.1735	2.455 ± 0.0956	2.391	Sunny
Tilokkachari[a] (WI)	6.645 ± 0.3104	3.1 ± 0.1291	2.144	Celestial sun
Mahananda[b] (WJ)	8.395 ± 0.4368	2.325 ± 0.2058	3.611	Sun rays
Swarna[b] (WK)	5.745 ± 0.1589	2.175 ± 0.1208	2.641	Cheers
Shashi[b] (WL)	7.07 ± 0.1719	2.125 ± 0.1318	3.327	Soft yellow
Sunil[b] (WM)	8.07 ± 0.2084	2.15 ± 0.1291	3.753	Indian corn
Jhingasail[a] (WN)	6.94 ± 0.1022	2.225 ± 0.0791	3.119	Cottage yellow
Rupsail[a] (WO)	6.245 ± 0.195	2 ± 0	3.123	Orange copper
Badshabhog[a] (WP)	4.625 ± 0.2946	2.025 ± 0.0791	2.284	Falling star
Shalibahan[b] (WQ)	5.865 ± 0.1226	2.9 ± 0.1291	2.022	Sparkler
IE7-6141Kunti[b] (WR)	7.415 ± 0.2739	2.115 ± 0.1248	3.506	Pale lemon
Mandira[b] (WS)	5.08 ± 0.1295	2 ± 0	2.540	Prairie grass
SR-26 B[a] (WT)	6.91 ± 0.1125	2.075 ± 0.121	3.330	Glorious
Kalamkati 147[a] (RA)	6.69 ± 0.3195	2.075 ± 0.121	3.224	Mocha treat
Meghal Patnai[a] (RB)	5.975 ± 0.03536	2.275 ± 0.0354	2.626	Wine
FR 13A[a] (RC)	5.715 ± 0.2897	2.325 ± 0.1687	2.458	Brunt umber
Jaladhi 1[a] (RD)	5.33 ± 0.25	2.075 ± 0.121	2.569	Tinder box
FR 43B[a] (RE)	5.25 ± 0.2357	2.325 ± 0.2058	2.258	Deep russet
NC 678[a] (RF)	6.075 ± 0.1687	2.375 ± 0.1768	2.558	Crimson velvet

[a] Indegenous variety.
[b] High yielding variety.
[c] Mutated variety.