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Fatty Acid Profiling among Samba Mahsuri Mutants (Rice) by Gas Chromatography

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ABSTRACT

Background: Rice is one of the most important crops worldwide, both as a staple food and as a model system for genomic research. Samba Mahsuri (BPT 5204) is a mega variety in India. A large population was developed and phenotyped leading to identifying mutants of varied agro-morphological, physiological and yield traits. The screening of the nutritional importance of mutants to identify the fatty acid composition and the total content of fatty acid will be the source for further studies. Rice contains bran, from which rice bran oil is obtained, contains both saturated and unsaturated fatty acids. Generally Monounsaturated fatty acids, Polyunsaturated fatty acids (MUFA and PUFA) are found in rice in higher concentrations than saturated fatty acids (SFA).

Methods: In this study around 22 promising mutants along with the wild type Samba Mahsuri were selected and screened for fatty acid composition by Gas Chromatography. Due to high resolution of effective capillary columns multiple components were separated from each sample. Among the 22 mutants Sb-87 has showed high saturated fatty acid content, high unsaturated fattyacid content, high polyunsaturated fatty acid content, high mono unsaturated fatty acid content, than compared to Samba Mahsuri (wild type).

Result: The mutant Ti-20 has shown lowest in saturated fatty acid content, unsaturated fatty acid content, mono unsaturated fatty acid content and polyunsaturated fatty acid content. After Sb-87, Ti-35, Ti-10, Ti-3, Ti-8 and Ti-113 has shown good results for fattyacid content, saturated fattyacid content, unsaturated fatty acid content, polyunsaturated fattyacid content, Linoleic acid and Linolenic acid content.

Key words: Gas chromatography, Monounsaturated fatty acids, Polyunsaturated fatty acids, Rice bran oil.

INTRODUCTION

Rice is the most important cereal crop of the world's population. Carbohydrates are the major source of energy in human diet. Rice is rich source of starch constitutes 75%, total proteins 4.3 to 20.2% (Chattopadhyay *et al.*, 2011; Santos *et al.,* 2013) and 2.4 to 3.9% total lipids (Juliano, 1977) and 0.5 to 2.0% of other compounds. Generally lipid content in rice is relatively low 1-2% in brown rice and 0.2-2% in polished (Zhang *et al.,* 2019). Rice grains contain lipids and free fatty acids (Prabhakar and Venkatesh, 1986; Proctor and Lam, 2001). Lipids are integral parts of cell membranes contributing structure, flexibility, permeability and involved in cell signaling as well as regulation of membrane bound enzymes. The fatty acids in rice grains are predominantly high quality unsaturated fattyacids Oleic acid, Linoleic acid, Linolenic acids which are nutritional with health benefits like prevention of arteriosclerosis and hypercholesterolemia (Liu *et al.,* 2013). The rice bran oil contains both saturated and unsaturated fatty acids. Generally monounsaturated fatty acids, polyunsaturated fattyacids (MUFA and PUFA) are found in rice in higher concentrations than saturated fatty acids (SFA). Among the fatty acids, C16 and C18 were present at higher concentrations. Notably, of the two essential fatty acids, linoleic and linolenic acids, were found in rice. Eventually, the proportion of unsaturated or polyunsaturated fatty acids (PUFA) may vary based on the rice genotype. Different types of fatty acids have different effects on the body, so understanding the fatty acid profile of a food can provide insight into its potential impact on health (Song *et al.,* 2015). Rice bran have the potential to be used in food sector as bioactive compound carrier, flavor enhancer, oil extraction, emulsifying agent, foaming agent and protein stabilizer (Arsheid Manzoor *et al.,* 2023). Fatty acids are a major component of most diets and can be synthesized endogenously in the human body (Calder and Burdge, 2004). Fatty acids are usually linked to other structures, frequently but not exclusively by ester linkages, to form more complex lipids like triglycerides, phospholipids and sphingolipids. Non-esterified fatty acids (NEFAs), often called 'free fatty acids'. The lipids have an important effect on starch physicochemical properties (Perez and Bertoft, 2010). Lysophosphatidylcholines (LPCs) are the main phospholipids in cereal starches as well as in rice kernels

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(Choudhury and Juliano, 1980) and LPC content is an important factor in the determination of starch quality (Blaszczak *et al.,* 2003; Hernández-Hernández *et al.,* 2011). Rice bran consumption in the form of rice branoil significantly improves blood lipid profiles (Liu *et al.,* 2024) with notable reductions in total cholesterol and LDL-Cholesterol for 1 the prevention of cardiovascular diseases (Park *et al.,* 2024); Rice bran is rich in bioactive compounds such as γ-oryzanol, phytosterols, vit. E provides antioxidant, anti-inflammatory, anticancer benefits (Sharif *et al.,* 2014).

Fatty acids get converted to into their more volatile methyl esters (Carrapiso and Garcia, 2000) required in gas chromatography analysis (Seppänen-Laakso *et al.,* 2002), in which the analysis identifies the fatty acids group. The best method for separating volatile chemicals is gas chromatography (McNair *et al.,* 2019). Analysis of both organic and inorganic materials are possible through gas chromatography. High resolution is provided by the effective capillary columns, allowing for the separation of multiple components in one sample. The general principles of sample preparation including the selection of solvents and modes of extraction of lipids (Shahidi *et al.,* 1998; Myher *et al.,* 1995) and the purification and separation of lipid classes by solid phase extraction and thin-layer chromatography (TLC) (Touchstone and Chromatogr, 1995; Folch *et al.,* 1957). Lipids in rice grains were obtained by ultra sound assisted extraction method reported by Tu *et al.* (2020). A convenient method which is simple and highly sensitive was designed to investigate the changes in free fatty acid (FFA) of rice during storage using a thin-layer chromatography and flame-ionization detection (TLC/FID) system. This seems to be an especially convenient method for small-scale storage tests or for experiments using many samples (Nishiba *et al.,* 2000).

Five different Japanese cultivars (Koshihikari, Haenuki, Akitakomachi, Hitomibore and Sasanishiki) were estimated for lipid composition and fatty acid profiling by combining thin layer chromatography and gas chromatography (Yoshida *et al.,* 2011). Some Cambodian rice varieties were analysed for fattyacid analysis and untargeted profiling of volatiles by using gas chromatography (Concepcion *et al.,* 2017). In India, fattyacid analysis was done on traditional Indian rice (*Oryza sativa* L.) landraces from Chhattisgarh (Sahu *et al.,* 2019). In 2022, three traditional rice varieties were screened for fattyacid profile in Srilanka by using gas chromatography and reported fattyacid profiles (Samaranayake *et al.,* 2022). Rice variety karupu kavuni was examined for the analysis of fatty acid methyl esters by using the GC-MS/FID. Indonesian black rice variety (in milk form) was analyzed for fattyacids profile by GC-FID (Romulo and Sadek, 2022). Chen *et al.* (2023) in 2024, measured fattyacid composition in a high yield rice cultivar Koshihikari by using gas chromatography mass spectrophotometer (GC-MS). Mostly Helium was used as carrier gas and temperatures maintained 200°C-250°C for injection and detector; the oven temperature was maintained 50°C-230°C and the used columns were varied length, diameter, thickness based on the model of gas chromatography machine. Fatty acid was identified by comparing the retention times with the Standard FAME.

Samba mahsuri is the most popular mega variety for its best cooking quality with intermediate amylose content. Hence, it was subjected to mutagenesis to develop mutants better than Samba Mahsuri. In this study around 22 promising mutants were selected based on yield and morphological data and same were screened for fattyacid profiling by gas chromatography.

MATERIALS AND METHODS

Samba Mahsuri (BPT-5204) is a mega variety of India released in 1984 highly popular in Southern and Eastern parts of India. It holds highest acreage in the states of Andhra Pradesh, Telangana, Tamil Nadu and Karnataka. It shows excellent cooking quality with good yield and desirable (low) Glycemic Index. However this variety is susceptible to biotic stresses like sheath blight, blast, bacterial leaf blight and insect pests such as yellow stem borer, brown plant hopper and gall midge. But it is favoured by consumers and farmers in India, because its good cooking and eating quality characteristics. Samba Mahsuri is an ideal genotype to develop a comprehensive mutant population due to its excellent combining ability. Mutant resources can be used to create variations and novel gene identification of specific traits and deciphering gene function. The mutants can be used as donors in rice improvement programs. The rice variety Samba Mahsuri mutants were developed by IIRR and CCMB jointly using Ethyl methane sulphonate as mutagen. The promising lines among samba Mahsuri mutants were cultivated and multiplied in IIRR*, Rajendra nagar field following standard package of practices. Hence in present study, Samba Mahsuri mutants (22) along with Samba Mahsuri, seed were collected during 2024 in two seasons (*Rabi* and *Kharif*) used for the estimation of fatty acid profile during 2024-2025*.

FAME synthesis protocol

The method of Folch *et al.* (1957) and Bligh and Dyer (1959) used to isolate total lipids. Weigh 0.250 gm of bran was taken in fresh centrifuge tubes in duplicates soaked for overnight with 1N NaOH and vortexed and methanol was added in the next day; then incubated for 1.5 hrs at 200 rpm at 55°C. The samples were cooled to room temperature. Then 0.58 ml of 24N H_2SO_4 was added through the walls and incubated for 1.5 hrs, at 55°C at 200 rpm. Then allowed to cool and vortexed for 5 min after adding 2 ml of hexane. The tubes were centrifuged for 8 min at 6000-7000 rpm and the supernatants were collected into GC vials. The samples were stored at -20°C till the analysis by gas chromatography.

The gas chromatography machine Agilent 8890 available in IIRR (Hyderabad, Rajendranagar) and analysis was done under standardized conditions. The machine

and the computer were switched on and the analysis in gas chromatography) was controlled using the software provided along with the instrument. The machine was provided with three cylinders were switched on and the gas flow was set upto flow continuously through (onto a liquid layer embedded on a solid support) the column (100 m) and an autoinjector. FAME analysis method was standarized and protocol is saved in the system connected to monitor the entire analysis. The run conditions were identified using standard fatty acid mixture (C8-C24 FAME Sigma Aldrich) at ICAR-IIRR. The injector and detector temperatures were maintained at 260°C constantly throughout the analysis. The column temperature was increased from 140°C to 240°C at the rate of 4°C/min with a total run time of each sample was 25 min. The spectrum obtained from the standard FAME sample was selected as reference to process (the spectrum of each rice sample). The area of each peak was converted into concentration using standard as reference.

RESULTS AND DISCUSSION

ANOVA indicates that variation in fatty acid content was highly significant for genotype (P-0) and fattyacid (P-0) and genotype -fatty acid type (P-0.05) among Samba Mahsuri mutants (Table 1).

The total fatty acid content present in Samba Mahsuri (wild type) is 11418.37 mg and the mutants ranged from 363.73 mg (Ti-20) to 13139.65 mg (Sb-87). The mutant Sb-87 has shown highest among mutants and it is higher than the wild type. The total saturated fattyacid concentration ranged from 177.23 mg (Ti-20) to 3239.52 mg (Sb-87) and Samba Mahsuri has shown 2790.92 mg only. The total unsaturated fatty acid content ranged from 186.50 mg (Ti-20) to 9900.14 mg (Sb-87) and it is higer than wild type (8627.45 mg). The total content of monounsaturated fatty acid ranged from 106.24 mg to 5166.60 mg (Sb -87) and for Samba Mahsuri it is 4589.15 mg only. The total concentration of polyunsaturated fattyacids ranged from 80.26 mg (Ti-20) to 4733.54 mg (Sb-87) and 4038.31 mg for Samba Mahsuri (Table 2).

The concentration of essential polyunsaturated fattyacids Linoleic acid ranged from 33.01 mg (Ti-20) to 2251.57 mg (Sb-87) and Linolenic acid ranged from 7.12 mg (Ti-20) to 115.19 mg (Sb-87). For Samba Mahsuri the concentration of linoleic acid is 1894.66 mg and linolenic acid is 124.49 mg (Table 3). One mutant among the 22 have shown higher values than wild type *i.e.* Sb-87. After Sb-87, Ti-35, Ti-10, Ti-3, Ti-8 and Ti-113 has shown more values among the mutants.The ratio of saturated fatty acid to unsaturated ranged from 0.29 (Ti-128) to 0.95 (Ti-20).

The unsaturated fatty acid content is higher than saturated fatty acid content among Samba Mahsuri mutants. While regarding polyunsaturated fattyacid content, the concentration of Linoleic acid is more than linolenic acid among the mutants. Generally, the composition of fatty acid content vary among rice genotypes. So the saturated fatty acid content,unsaturated fattyacid content, monounsaturated fattyacid content and polyunsaturated fattyacid content also varies among rice genotype (Samaranayake *et al.,* 2022). These variations may be due to application of fertilizer, increase in panicle density, genetic variations and external environmental conditions.

Rice is mainly consumed worldwide as polished rice. The polishing process is performed by rice industries in order to improve the physical characteristics and sensory properties of rice, as well as to increase its storage stability (Monks *et al.,* 2013). This processing technique removes the bran and germ of the rice caryopsis. The bran layers rich in proteins, fibers, vitamins and fat; while the germ is mainly rich in fat. Thus, although the polishing process provides benefits to the physical, sensorial and preservative properties of rice, the nutritional properties are diminished. Pigmented rice cultivars, present their bran layers rich in phenolic compound and thus, polishing cause a detrimental nutritional effect on pigmented rice (Paiva *et al.,* 2016). The increased thickness of aleurone layer cells lead to increase in grain oil content (Li *et al.,* 2022). Multiple gene engineering, knock out technology of enzymes which regulate fattyacid metabolism can be employed for boosting bran oil in rice (Liu *et al.,* 2024). Rice polishing is performed through a rice polisher which change the appearance and taste. The polishing is used to transform brown rice into white rice. Rice polish is derived from the outer layers of the rice caryopsis during milling and consists of pericap, seed coat, nucleus, aleurone layer, germ and part of sub-aleurone layer of starchy endosperm (Juliano, 1988). Rice contains medicinally important antioxidants and oryzanol content of rice polish has recognized it as a potential feed (Iqbal *et al.,* 2005; Moldenhauer *et al.,* 2003; Chatha *et al.,* 2006).

The best method for separating volatile chemicals is gas chromatography. The substances are often dissolved in volatile solvents and used to investigate gases, liquids

Table 1: ANOVA analysis of fatty acids in rice samples.

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Genotype	22	6772754	307852	3.259	2.84e-06 ***
Fatty acid	15	148198240	9879883	104.592	< 2e-16 ***
Genotype: Fatty acid	1	926	926	0.010	0.9212
Genotype: fa	278	23891022	85939	0.910	0.7836
Genotype: fa type	1	304554	304554	3.224	0.0737 .
Residuals	273	25787931	94461		

Table 2: Total saturated and unsaturated fatty acids content in mg/100 gm of rice bran.

Smmut	T.fatty acid	T.S aturated fa	T. Unsaturatedfa	T. Mufa	T. pufa	Sfatounsfa
BPT5204	11418.37±797.31	2790.92±360.54	8627.45±2376.41	4589.15±1245.05	4038.31±1131.36	0.32
Sb-6	5178.11±516.87	1335.98±239.39	3842.14±1652.00	2048.43±810.80	1793.71±841.20	0.35
Sb-8	5442.26±449.49	1409.96±205.18	4032.30±1418.40	2196.57±715.88	1835.73±702.52	0.35
Sb-87	13139.65±934.74	3239.52±450.75	9900.14±2699.45	5166.60±1377.43	4733.54±1322.02	0.33
Ti-10	10858.48±742.80	2668.20±321.03	8190.28±2230.09	4352.09±1151.74	3838.19±1078.35	0.33
Ti-113	9107.49±637.01	2331.08±328.78	6776.41±1767.04	3740.31±956.38	3036.10±810.66	0.34
Ti-125	5503.15±605.48	1323.96±272.24	4179.18±1940.53	2294.45±995.38	1884.73±945.15	0.32
Ti-128	5707.22±562.90	1274.39±225.91	4432.83±1850.50	2487.14±961.54	1945.69±888.96	0.29
Ti-13	6351.14±425.94	1590.12±193.09	4761.03±1254.72	2574.33±668.35	2186.70±586.37	0.33
Ti-132	7391.49±499.88	1890.65±240.21	5500.84±1434.85	3025.47±780.26	2475.37±654.59	0.34
Ti-14	4948.61±330.88	1199.57±139.91	3749.04±986.24	1988.33±511.36	1760.71±474.88	0.32
Ti-15	7886.50±552.01	1857.53±233.11	6028.97±1609.82	3403.62±886.70	2625.35±723.13	0.31
Ti-20	363.73±13.44	177.23±7.25	186.50±0.85	106.24±24.01	80.26±15.84	0.95
Ti-27	8571.80±573.73	2433.83±305.37	6137.98±1680.89	3281.98±871.47	2855.99±809.42	0.4
Ti-3	10483.40±725.81	2793.64±370.08	7689.76±2176.35	3792.63±1013.48	3897.13±1162.87	0.36
Ti-35	11244.14±828.42	2748.15±400.72	8495.99±2363.48	4304.63±1206.95	4191.36±1156.53	0.32
Ti-40	6034.52±421.14	1640.01±207.46	4394.51±1233.08	2433.01±660.50	1961.50±572.58	0.37
Ti-45	3648.21±284.62	734.64±125.36	2913.57±793.64	1562.68±443.76	1350.89±349.88	0.25
Ti-56	8175.82±605.33	2104.76±334.59	6071.05±1650.58	3163.55±840.85	2907.50±809.73	0.35
Ti-6	9232.12±664.78	2319.37±322.46	6912.75±1948.05	3739.90±1027.11	3172.85±920.94	0.34
Ti-60	5824.54±410.66	1559.36±219.27	4265.17±1151.82	2358.18±645.51	1906.99±506.30	0.37
Ti-8	10611.66±718.86	2638.20±331.92	7973.46±2139.01	4355.07±1207.41	3618.38±931.59	0.33
Ti-9	4043.88±390.46	1155.43±190.10	2888.46±1271.44	1578.58±599.59	1309.88±671.85	0.4

Table 3: Availability of polyunsaturated fatty acids.

Smut	rep1	rep2	Linoleic acid	rep1	rep2	Linolenic acid
BPT 5204	2487.914	1301.421	1894.66±838.97	160.0705	88.9023	124.49±50.32
Ti-9	36.50373	1212.284	624.39±831.40	61.09269	0	30.55±43.20
Ti-132	1369.021	965.939	1167.48±285.02	80.55537	59.85877	70.21±14.63
Ti-20	39.41715	26.61157	33.014±9.054	7.427553	6.803852	7.12±0.44
Ti-40	1247.142	620.7271	933.93±442.94	60.02152	33.6054	46.81±18.68
Ti-15	1506.991	1007.205	1257.09±353.40	65.39377	45.76051	55.58±13.88
Sb-87	2833.605	1669.551	2251.57±823.11	143.0835	87.3001	115.19±39.44
Ti-3	2426.596	1400.536	1913.56±725.53	8.477921	61.52047	35.00±37.51
Ti-6	2015.258	992.4014	1503.82±723.26	108.9005	56.28779	82.59±37.20
Ti-8	2015.908	1418.261	1717.08±422.60	97.05873	70.31241	83.69±18.91
Ti-10	2297.376	1370.479	1833.92±655.41	104.6911	65.63989	85.17±27.61
Ti-13	1223.764	850.1585	1036.96±264.17	65.79281	46.98244	56.39±13.30
Ti-14	991.6179	681.3939	836.50±219.36	50.93696	36.75812	43.85±10.03
Ti-27	1744.34	975.7719	1360.05±543.45	85.17196	50.70724	67.94±24.37
Ti-35	2416.966	1310.003	1863.48±782.74	114.6399	64.27031	89.46±35.62
Ti-45	806.1656	448.5472	627.35±252.87	48.45239	29.1367	38.79±13.66
Ti-56	1735.871	1027.725	1381.79±500.73	88.93373	54.97262	71.95±24.01
Ti-60	1041.101	767.7103	904.40±193.31	55.92054	42.2607	49.09±9.66
Ti-113	1595.023	1312.851	1453.93±199.52	70.08347	58.14159	64.11±8.44
Ti-125	1719.598	87.41632	903.50±1154.12	77.71996	0	38.86±54.96
Ti-128	1672.614	197.7217	935.16±1042.90	75.35569	0	37.68±53.28
Sb-6	1569.008	130.5071	849.75±1017.17	94.19693	0	47.10±66.61
Sb-8	1407.978	348.9264	878.45±748.86	78.82817	0	39.41±55.74

and solids. Analysis of pesticidal residue was donein chilli plant (Megawati *et al.,* 2022). The cholesterol present in different foods can be analyzed by using gas chromatography (Sharma *et al.,* 2022). The gas chromatography is used for analysis of secondary metabolites (Sirivella *et al.,* 2025). Advantages of gas chromatography: Rapid analysis, Reliable-simple operation, sensitive, quick analysis accurate results, needs small sample for analysis (in µl); The disadvantages are limitation to volatile and thermally stable samples,requires high temperatures and pressures, cost and maintenance of the machine is expensive and sample recovery (not possible in some cases), concentration of sample and presence of other substances may interfere in analysis.

The fatty acid content affects taste, cooking properties, nutritional properties (Guo *et al.,* 2024). Moreover changes in fatty acid composition and the ratio to unsaturated fatty acid to saturated fatty acid affect the storage stability and cooking characteristics. The lipid content improves the appearance of rice grain causes glossiness and enhances palatability and taste. The lipid content negatively affects starch digestion and glycemic index (GI). The polyunsaturated fatty acid content present in rice causes degradation of quality due to oxidative acid transformation by external environmental conditions (Guo *et al.,* 2024). Lipids play important role in growth and development, regulate seed longetivity, grain yield, grain quality, biotic and abiotic stress in rice (Zhou *et al.,* 2024).

CONCLUSION

In current study mutant lines promising for fattyacid content under saturated, unsaturated, monounsaturated, polyunsaturated were identified. Sb-87 mutant is the superior over the wild type and among the mutants. Sb-87, Ti-35, Ti-10, Ti-3, Ti-8 and Ti-113 were found promising for fattyacid content. The essential polyunsaturated fattyacid Linoleic acid content is higher than linolenic acid content. The linoleic acid content is higher in Sb-87 followed by Ti-3, Samba Mahsuri, Ti-35, Ti-10, Ti-8. The linolenic acid is higher in Samba Mahsuri followed by Sb-87, Ti-35, Ti-10, Ti-8. The mutant Ti-20 has shown lowest value for total fattyacid content,total unsaturated fattyacid content, total PUFA, total MUFA and linoleic acid and linolenic acid contents.

These findings will add value to rice regarding human nutrition and health, also showing the need to explore and identify rice varieties having healthy fatty acid profiles. Further study is required to know the interaction of fatty acids with starch, cooking and eating quality is required to provide a scientific basis for breeders to select and cultivate varieties with superior tastes with high nutritive values as well as good cooking and eating qualities.

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Disclaimers

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Conflict of interest

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