

Genetic insights into fatty acid components of traditional Indian rice (Oryza sativa L.) landraces from Chhattisgarh

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Abstract

Knowledge about the contents and type of fatty acids (FAs) in rice bran is beneficial, particularly from a nutritional and health standpoint. An experiment was conducted to assess the genetic variability and diversity for FA components in 215 rice landraces during kharif 2015 and kharif 2016 by following the RCB design with two replications. Palmitic (C16:0), oleic (C18:1) and linoleic (C18:2) were the major fatty acids ranging from 12.59% to 20.25%, 37.60% to 49.17% and 31.55% to 44.67%, respectively. Analysis of variance revealed significant differences in all the FA components except for linolenic acid. Results showed that environmental factors play a significant impact on the expression of FA contents. Fatty acid components showed intermediate to low genotypic and phenotypic coefficient of variation, intermediate to high heritability and low to moderate genetic advance as percent of the mean. Oleic acid content was negatively correlated with palmitic acid, stearic acid, and linoleic acid contents. Principal component analysis and cluster analysis discriminated the 215 rice landraces into five main groups with a major contribution of oleic acid, linoleic acid, and palmitic acid contents. Landraces Kadamphool, Ratanchudi and Bathrash possessed the highest amount of oleic acid/linoleic acid ratio. Information generated through this study will be functionally useful in developing rice varieties having high-quality bran oil.

Keywords: Bran oil, fatty acids, genetic variation, landraces, rice

Introduction

Rice (Oryza sativa L.) is the major crop for the global food and nutritional security and socio-economic development, therefore, its production potentials have to be enhanced significantly to feed the burgeoning population along with good nutritional quality traits (FAO 2017). Carbohydrate is the major component of rice, however, it has a reasonable amount of protein and oil components mainly in the bran portion. Amount of vitamin-B complexes such as niacin, riboflavin, and thiamin has been also reported in rice bran. Attentiveness on health benefits and nutritive value and of rice is essential to increase the intake of rice in the daily diet of human beings (Fresco 2005).

Nowadays, rice bran and its oil have been getting special attention to use as a functional food due to their nutritional properties viz., phenolic compounds, beneficial fatty acids, vitamins, minerals and fibers (Wilson et al. 2002). Rice bran is the outer aleuron layer of the rice kernel which can be separated during milling/ whitening of brown rice and accounted approximately 10% of the weight of rice grain (Rondanelli et al. 2011). Bran portion has ample amount of antioxidants viz., tocopherols, tocotrienols, and ãoryzanol and phenolic compounds, which help to reduce LDL cholesterol, protect cardiovascular diseases and possess anticancer effects (Min et al. 2011).

Looking into the significance of bran oil, the characterization of rice landraces for FA composition may have great importance for future breeding programs. Despite abundant genetic resources available, breeders have utilized only limited rice germplasm for the improvement of fatty acid components, which cause their narrowed genetic base in improved varieties and advance breeding materials. Therefore, a study was conducted to characterize 215 rice landraces through genetic analysis based on fatty acid composition in the bran fraction. It is expected that the information obtained would help assure an effective breeding program for developing rice varieties

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having ample amount of desirable fatty acids.

Materials and methods

The experimental material comprises 213 traditional landraces and two improved rice varieties (Mahamaya and Rajeshwari as control) from Chhattisgarh state, India (Supplementary Table S1). All the genotypes were procured from R.H. Richharia Rice Biodiversity unit of the Indira Gandhi Krishi Vishwavidyalaya (IGKV), Raipur for research purpose. All 215 genotypes were grown at the rice research field of IGKV, Raipur for two years viz., kharif 2015 and kharif 2016 by following the randomized complete block design (RCBD) with two replications. Agronomic management practices were performed as per the standard evaluation system of IRRI, Philippines (IRRI 2002). Genotypes were harvested separately, cleaned properly, dried up to 12-14% moisture content and kept at room temperature for further utilization.

Extraction of bran, sample processing and estimation of fatty acid components

Hulling and milling process of each rice genotype was done by using a McGill No. 2 Huller & Miller (Rapsilver Supply Co. Inc., Brookshire, TX) at R.H. Richharia Rice Research Laboratory, Department of Genetics and Plant Breeding, IGKV, Raipur (C.G.), India. Bran of each sample of brown rice (decorticated rice) was collected into a small stripped polythene bag and labeled. Rice bran of each genotype was kept immediately at 4° C to avoid the harmful activities of lipase enzyme. Fatty acid estimation was done by the base-catalyzed trans-esterification method (Mondal et al. 2018) in Gas Chromatography (Shimadzu, Kyoto, Japan) at Nuclear Agriculture and Biotechnology Division (NA&BTD), Bhabha Atomic Research Centre (BARC), Mumbai, India. About 200 mg of rice bran of each genotype was taken into a 50 ml glass test tube in duplicates. One milliliter (1ml) of methanol (AR) and 1ml of 0.5M sodium methoxide (AR) were added in each tube. All the tubes were shaken thoroughly by vortex and kept at room temperature for 20 minutes. Then all the tubes were kept into a water bath at 50°C for 1 hour. After water bath treatment, tubes were kept for 10 min at room temperature for cooling. Thereafter, 2 ml of HPLC grade petroleum ether (boiling point = 60-80 ºC) and 2 ml of de-ionized water was poured in each tube and vortexed for 1 min. Then tubes were kept at room temperature for 30 min to allow the phase separation. The upper petroleum ether phase was

extracted from each tube by using 1ml micro-pipette and transferred them into 1.8 ml clear GC vials. Gas chromatography (GC2010, Shimadzu, Japan) technique was used to analyze the processed samples and the final concentration of fatty acid components were obtained from GC SOLUTION software (Shimadzu, Japan) after normalization of peak areas and the recorded data were converted into a percentage value.

Statistical analysis

Proportional contents of fatty acids were transformed by ArcSine transformation as per the method suggested by Sokal and Rohlf (1995) to normalize the dataset for further analysis. Descriptive statistics, box plots, and histogram were made by using XLSTAT v18.07 (Addinsoft, NY, USA). Analysis of variance (ANOVA), genetic parameters of variability and genotypic correlation coefficients were calculated by software WINDOSTAT v9.3. Principal component analysis (PCA) based on the covariance matrix and agglomerative hierarchical cluster (AHC) analysis by following the Euclidean distance and Ward's algorithm was performed in PAST v3.15 software (Hammer et al. 2001).

Results

Gas chromatography (GC) analysis of rice bran oil revealed five fatty acid components viz., oleic (C18:1), linoleic (C18:2), palmitic (C16:0), stearic (C18:0), and linolenic acid (C18:3). Analysis of variance (ANOVA) reported high significant differences for palmitic, stearic, oleic and linoleic acid during the year 2015, 2016 and over the year analysis, but they are nonsignificant for linolenic acid in all the conditions (Table 1). ANOVA over the year showed highly significant effects of the year for all the fatty acid components. Genotype x year interaction was significant for fatty acid components apart from for linolenic acid in rice landraces.

Genetic analysis of fatty acid contents

Since fatty acid components are very sensitive to environmental conditions therefore over the year (pooled data) analysis is more appropriate for describing the descriptive statistics, variability parameters, correlations and diversity parameters in the study. Pooled data may reduce the chances of error and combine the overall effect of environmental conditions. Palmitic acid ranged from 12.59% (Khajoor) to 20.25% (Lajini Super) with an average of 17.54%

Year	Source of variation	df	Mean sum of square					
			Palmitic acid	Stearic acid	Oleic acid	Linoleic acid	Linolenic acid	
2016	Replication	1	0.119	0.904	0.164	0.512	0.01	
	Treatment	214	$2.955**$	$1.321*$	$2.77***$	$2.261**$	0.626	
	Error	214	0.255	0.367	0.296	0.344	0.312	
	Total	429						
2017	Replication	1	1.653	1	0.67	0.738	0.119	
	Treatment	214	$3.827**$	$1.447**$	$3.261**$	$3.074**$	1.023	
	Error	214	0.414	0.415	0.444	0.596	0.189	
	Total	429						
Pooled	Replication	1	0.458	0.001	0.004	0.195	0.038	
	Year	1	425.96**	44.42**	40.89**	113.35**	75.51**	
	Variety	214	$3.93**$	$1.53**$	$4.29**$	$4.76***$	0.92	
	Year x Variety	214	$3.86**$	$1.24*$	$2.75***$	$4.58**$	0.73	
	Error B	430	0.34	0.39	0.37	0.47	0.25	
	Total	859	2.6	0.91	1.98	2.69	0.62	

Table 1. Year-wise Analysis of Variance (ANOVA) for fatty acid contents in rice landraces

*5% level of significance; **1% level of significance

(±1.29%). Stearic acid ranged from 1.01% (Memri Khedi) to 2.90% (Baiga Seeng) with an average 1.80% (±0.29%). A total of 25 genotypes had more than 46% oleic acid content and ranged from 37.60% (Antarved) to 49.17% (Kadamphool) with an average of 43.90% (±1.79%). Linoleic acid content ranged from 31.55% (Ratanchudi) to 44.67% (Antarved) with an average of 35.56% (\pm 1.83%) and a total of 16 genotypes had more than 38% linoleic acid content. While linolenic acid content ranged from 0.75% (Khajoor) to 2.0% (Dadbanko) with average 1.19% (±0.18%) (Table 2).

Coefficient of variation was highest for stearic acid (16.11%) and lowest for oleic acid (4.58%). According to box plot profiling, 75% landraces have equal to or less than 18.44% palmitic acid, 1.98% stearic acid, 44.87% oleic acid, 36.53% linoleic acid, 1.28% linolenic acid content while 25% landraces have equal to or less than 16.75% palmitic acid, 1.62% stearic acid, 42.94% oleic acid, 36.53% linoleic acid, 1.28% linolenic acid content (Fig. 1). Frequency distribution on the histogram for various fatty acids showed that none of the traits followed the exact normal distribution pattern (Fig. 2). Skewness values and histogram showed that palmitic acid and oleic acid were negatively skewed whereas stearic acid, linoleic acid, and linolenic acid contents were positively skewed. All the fatty acid components were showed positive kurtosis (Table 2) and followed the leptokurtic

Fig. 2. Histogram showing the frequency distribution of fatty acid components based on pooled data

Fatty acid contents	Palmitic acid	Stearic acid	Oleic acid	Linoleic acid	Linolenic acid
Minimum	12.59 % (Khajoor)	1.01% (Memri Khedi)	37.60% (Antarved)	31.55% Ratanchudi	0.75% (Khajoor)
Maximum	20.25 % (Lajini Super)	2.9% (Baiga Seeng)	49.17% (Kadamphool)	44.67% (Antarved)	2% (Dadbanko)
Mean	17.54	1.8	43.9	35.56	1.19
Standard deviation	1.29	0.29	1.79	1.83	0.18
Coefficient of variation	7.35	16.11	4.08	5.15	15.13
Skewness	-0.56	0.37	-0.21	1.43	0.83
Kurtosis	0.7	0.95	1.11	4.78	2.47
Phenotypic coefficient of variation	5.90	12.81	3.67	4.41	12.33
Genotypic coefficient of variation	5.41	9.84	3.375	4.00	9.34
Heritability(bs)	84.18	59.08	84.26	82.06	57.35
Genetic advance	2.53	1.19	2.65	2.73	0.90
Genetic advance as % of mean	10.24	15.59	6.38	7.47	14.57

Table 2. Genetic parameters of fatty acid components in rice landraces over the years

configurations. None of the traits showed mesokurtic and platykurtic configuration.

Genotypic coefficient of variations (GCV) was slightly lower than the corresponding phenotypic coefficient of variation (PCV) for oleic acid, palmitic acid, and linoleic acid content. Linolenic acid and stearic acid showed intermediate PCV with low GCV whereas Linoleic acid, oleic acid, and palmitic acid contents showed low GCV and PCV (Table 2). High heritability was expressed by oleic acid, palmitic acid, and linoleic acid content. The highest heritability exhibited for oleic acid content whereas moderate genetic advance as percent of the mean was observed for stearic acid, palmitic acid, and linolenic acid content.

Genetic correlations and diversity analysis for fatty acid components

The correlation coefficients in all possible combination of five fatty acids showed that Palmitic acid was positively correlated with linolenic acid $(r = 0.21)$; P<0.01), while it is negatively correlated with oleic acid content ($r = -0.39$; P<0.01) and linoleic acid content ($r = -0.29$; P<0.05). Stearic acid is significant negatively correlated with oleic acid ($r = -0.12$; P<0.05) and linolenic acid content ($r = -0.25$; P<0.05). Oleic acid is significantly negatively correlated with linoleic acid ($r = -0.75$; P<0.01). Linoleic acid content is significant negatively correlated with linolenic acid content ($r = -0.17$; P<0.05) (Fig. 3).

Fig. 3. Genotypic correlation plots among the fatty acid components (pooled)

The principal component analysis (PCA) revealed two major variables, which accounted for 98% of total variation from all the fatty acids in the tested landraces. The principal component (PC1) for the first set contributed about 67.14% with eigenvalue 5.57 and PC2 for the second set contributed about 31.08% with eigenvalue 2.58. Rest of the PCs had less than 1 eigenvalue and less than 5% variability (Supplementary Table S2). The analysis detected that linoleic acid and oleic acid were the main contributory traits for PC1 whereas palmitic acid and oleic acid are the main contributing traits for PC2. Thus, it was

postulated that about 98% of the total variation is represented by oleic acid, palmitic acid, and linoleic acid. Further, scattered diagram based on PCA has grouped the landraces into five groups across the PC1 and PC2 (Supplementary Fig. S1). The result of PCA was further confirmed by agglomerative hierarchical cluster analysis that divided the rice landraces into five groups in Supplementary Table S1 and Fig 4. This

Fig. 4. Scatter diagram of 215 rice landraces across the PC1 and PC2

demonstrated that the data obtained from this experiment were accurate, precise, and reliable. In AHC, cluster-V comprised of 82 genotypes (38.13%) which were the highest among all the clusters followed by Cluster-III which had 62 (28.83%) genotypes, out of 215 rice landraces. Cluster I, II and IV had 5, 38 and 28 rice genotypes, respectively (Supplementary Table S3). The Euclidean distance-based dendrogram showed that the genotypes Bansgathi, Gangachur, Nariyaljhoba, Anjaniya, and Antarved are highly diverse from the rest of the genotypes (Supplementary Fig. S1).

Discussion

Oleic acid (C18:1), linoleic acid(C18:2) and palmitic acid(C16:0) were identified as the major FAs and accounted for 77%-88% contribution of total FA components in the current study which are in accordance with the previous findings (Lugay and Juliano 1964; Yoshida et al. 2011; Yu et al. (2016). Higher oleic/linoleic (O/L) ratio determines the stability of bran oil and extends its shelf life (Holley and Hammons 1968) as well as high O/L ratio reduces the chances of cardiovascular diseases in the human

body (O'Byrne et al. 1997). The current study reported the highest oleic acid/linoleic acid ratio in landraces Kadamphool, Ratanchudi, Bathrash, Panwar, and Karigilash for the first time, which can either used as a donor to develop rice varieties for high-quality bran oil or released directly after selection. Results of analysis of variance illustrated the sufficient variation among the rice landraces for fatty acid components except for linolenic acid, indicating the huge possibility of their improvement and results are following the earlier findings in rice (Goffman 2003; Konate et al. 2016). Significant effect of year component and GxE interaction indicated that the environmental conditions and extraneous factors were not similar in two years and similar results were reported in rice (Goffman 2003), groundnut (Onemli 2012) and safflower (Gecgel et al. 2007).

Coefficients of variation studies indicated the negligible influence of extraneous factors and therefore, selection for such traits based on phenotype only could be gratifying. Nevertheless, the intermediate and low value of PCV and GCV indicated the presence narrow genetic base of FAs in rice landraces. Therefore, induced mutagenesis and hybridization could be the best method to broaden the genetic base for FAs in rice. High heritability with moderate GA for palmitic acid content has suggested the significant contribution of non-additive and additive both gene action in their inheritance. High heritability with low GA for oleic acid and linoleic acid content revealed the significant contribution of non-additive gene action. Hence, heterosis breeding may be worthwhile for the enhancement of these traits. The significant negative association of oleic acid with linoleic acid, palmitic acid and stearic acid indicated that the conversion of each fatty acid to another is dependent through various biochemical pathways. The higher negative correlation between oleic acid and linoleic acid exist due to a possible natural mutation in oleyl phosphatidylcholine desaturase gene which converts oleic acid to linoleic acid (Mondal et al. 2011) and similar results were also reported in earlier studies on rice (Ying et al. 2012) and groundnut (Onemli 2012).

PCA detected two main components of fatty acid variability in rice landraces where in palmitic, oleic and linoleic acid played a major role in total genetic variation. This result was in agreement with earlier reports (Yoshida et al. 2011; Yu et al. 2016) however, the materials used by them had different geographic origin. PCA elucidated the high level of genetic diversity in rice landraces, which were further confirmed by agglomerative hierarchical cluster analysis. A similar pattern of PCA and AHC was also reported by Lasalita-Zapico et al. (2010) and Khatun et al. (2015). The landrace viz., Antarved, Gangachur, Nariyal Jhoba of cluster-I posses less oleic acid components as compare to the landraces Kadamphool, Panwar, Rudra, Kalajeera, which belong to cluster IV which have higher oleic acid contents. Landraces of these two clusters were genetically diverse and thus could be useful in crossbreeding towards the development of high yielding genotypes with higher oleic acid content. It can, therefore, be concluded that the identified landraces viz., Kadamphool, Ratanchudi, Bathrash, Panwar and Karigilash with good monounsaturated fatty acids and high O/L ratio as well as landraces of the diverse group have the enormous value in breeding program. Further, the inverse relationship between oleic and linoleic acid suggested that future targeted mutagenesis in oleyl phosphatidylcholine desaturase or OsFAD2 which may be useful in generating higher oleic acid-containing genotypes in rice. The information, thus obtained, would be highly supportive in planning an effective rice-breeding program for developing rice varieties with a high content of desirable fatty acids for human welfare.

Authors' contribution

Conceptualization of research (SM, DS); Designing of the experiments (SM, VK, BKD); Contribution of experimental materials (DS, SM, VK, BKD); Execution of field/lab experiments and data collection (PKS, RS); Analysis of data and interpretation (PKS, SM, RS); Preparation of the manuscript (PKS, SM, RS).

Declaration

The authors declare no conflict of interest.

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Supplementary Fig. S1. Cluster diagram (dendrogram) for 215 rice landraces based on fatty acid components based on pooled data

Supplementary Table S1. List of rice genotypes used for the investigation

Note: All the collected landraces/ varieties are conserved in IGKV, Raipur (C.G.), India

Supplementary Table S2. Eigen value, variability % and eigen vectors based on principal component analysis

Supplementary Table S3. Grouping of rice landraces in different clusters

