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A preliminary investigation of cultivated and wild species of rice for tocopherol contents

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Abstract

Rice (*Oryza sativa* L.), which provides calories to more than half of the world's population, has been considered as a source of vitamins, minerals, and proteins. The portion of nutrition quality of rice is determined by the content of tocopherols which exhibit an antioxidant effect. In this study, a total of 63 genotypes, including 35 wild accessions belonging to AA genome (*O. glaberrima, O. barthii, O. rufipogon and O. meridionalis*) and CC genome (*O. officinalis*), 9 Basmati and 19 non-basmati genotypes were analyzed for the total tocopherol content in brown rice. Wild rice accessions had considerably high total tocopherol content and it ranged from 9.7 mg/kg (*O. rufipogon*, IR105491) to 45.3 mg/kg (*O. rufipogon* CR100368). For the non-Basmati genotypes, it varied from 13.6 mg/kg (BPT5204) to 22.4 mg/kg (PR 128). Similarly, for Basmati cultivars, it ranged from 18.2 mg/kg (Basmati 370) to 25 mg/kg (Pusa Basmati 1509). The wild species that had high total tocopherol content could be used as donors to generate interspecific crosses which will ultimately lead to the development of nutritionally rich rice cultivars.

Keywords: Basmati, Non-basmati, Tocopherols, Wild rice , Nutrition

Introduction

Rice (*Oryza sativa* L.) is a staple food which provides up to 70% of daily calories to more than 3.5 billion population. The genus *Oryza* has 11 genome types, including six diploid species ($n = 12$) with AA, BB, CC, EE, FF and GG genomes and five polyploid species ($n = 24$) with BBCC, CCDD, HHJJ, HHKK and KKLL genomes. Rice contains phenolic acids, flavonoids, anthocyanins, proanthocyanidins, tocopherols, tocotrienols, γ-oryzanol, and phytic acid, all of which have antioxidant action (Goufo and Trindade, 2014). The quality of rice is also determined by the tocopherol content which exhibits an antioxidant effect in human body, and has long been regarded as one of the most potent natural antioxidants (Quereshi *et al.,* 2000). Tocopherols are categorized as, alpha tocopherol (α), beta tocopherol (β), gamma tocopherol(γ), and delta tocopherol(δ) (Goufo and Trindade, 2014). Vitamin E is a compound made up of tocotrienols and tocopherols. Tocopherols make about 20–53% of total vitamin E content, while tocotrienols make up 47–80%. The recommended dietary allowance (RDA) for vitamin E (α-tocopherol) for males and females is 7.5-10 mg (FSSAI 2017). Only α-tocopherol has the highest biological activity and has been found to meet human requirements. Contents of α-tocopherol in rice husk, rice bran, rice whole grain, rice endosperm, and rice germ are reported to be ranged from 0.06–2.13mg/kg, 7.34–107.7mg/kg, 2.40–49.14mg/kg, 0.17–5.43mg/k, and 60.61–457.9mg/kg, respectively. Overall, in rice varieties, the total tocopherol content ranged from 3 to 105.5 mg/kg in brown rice (Goufo and Trindade, 2014).

In relation to tocopherols and tocotrienols, hexane (Xu *et al*., 2001), methanol (Jeng *et al*., 2012), acetone (Gunaratne *et al*., 2013) and ethanol (Ghasemzadeh *et al*., 2015), are most often used for their extraction from rice grains. Tocopherols

and tocotrienols have also been extracted from rice using supercritical fluids (Imsanguan *et al*., 2008), ultrasonication (Moongngarm *et al*., 2012), soxhlation (Mohanlal *et al*., 2012) and vortexing (Gunaratne *et al*., 2013). The most extensively used technology for determining tocopherols is high-performance liquid chromatography (HPLC). After extraction, both normal phase-HPLC and reverse phase-HPLC have been used for the separation of tocopherols and tocotrienols (Goufo *et al.,* 2014). A spectrophotometric method for tocopherol analysis is simple to use in conventional laboratories for preliminary studies for breeding towards nutritional quality.

Different researchers have already reported on genetic diversity studies for vitamin E in various rice cultivars (Gunaratne *et al*., 2013; Kim *et al*., 2012; Lin and Lai, 2012; Kong and Lee, 2010). *OsγTMT* in rice is mainly responsible for the genetic diversity of the α-tocopherol content in rice (Wang *et al.,* 2015). In accordance with the consumer's preferences, grain and nutritional quality have become a primary goal for producers. In this perspective, it should provide all the essential minerals, vitamins and contain sufficient amount of proteins. Plant breeding is usually practiced to produce highly nutritious rice. So the first requirement of breeding program is to study variability in existing germplasm for target trait. Wild relatives of rice are also considered as a source of genetic variability for the enhancement of cultivated rice varieties or to develop new rice varieties. Hence, the present study was planned to study the variability for total tocopherol content in wild rice species, Basmati and non-Basmati cultivars. The present study is a continuation of our previous work (Kaur *et al.,* 2022), in which wild rice species, Basmati and non-Basmati cultivars were studied for protein content, fractionation of seed storage proteins and SDS gel electrophoresis. A total of 35 wild species which had high protein content (≥12%) along with Basmati and non-Basmati cultivars were selected for the analysis of total tocopherol content in brown rice as brown rice is nutritionally rich due to the presence of the bran layer, which is removed in case of polished rice.

Materials and Methods

The experimental material consisted of 63 genotypes, comprising 35 wild rice accessions from the AA (*O. glaberrima, O. barthii, O. rufipogon,* and *O. meridionalis*) and CC genome (*O. officinalis*), 9 Basmati and 19 non-Basmati genotypes. The seed of the wild rice accessions were procured from School of Agricultural Biotechnology, Punjab Agricultural University (PAU), Ludhiana. The Basmati and non-Basmati accessions were sown at experimental farm area of Rice section, Department of Plant Breeding and Genetics, PAU. Each entry was grown in a paired row with 10 plants per row with a uniform spacing of 20 cm between rows and 15 cm between plants. The crop was raised following the standard agronomic practices and harvested at maturity. The designations of genotypes are presented in table 1, table 2 and table 3.

The extraction of total tocopherols and their estimation was done by the method given by Kayden *et al.,* (1973). Seed of these 63 genotypes was dried to a moisture content of 13% and dehusked with hand dehusker to get brown rice and samples were ground to powder. For the extraction of tocopherols, about 50 mg of brown rice sample was homogenized with 4 ml of ethanol. The mixture was centrifuged at 4000 rpm for 30 min. The supernatant so obtained was used for the estimation of tocopherols. For estimation of tocopherols, 2.4 ml of the supernatant was pipetted in glass centrifuge tube and 2.4 ml of distilled water was added. The solution was vortexed properly. Then purified xylene (2.4 ml) was added in samples and again vortexed for 2 min and the glass tubes were then centrifuged at 5000 rpm for 5 min. About 1ml of xylene layer was taken into fresh glass tubes containing 0.8 ml of bathophenanthroline reagent (0.4% in absolute ethanol) and contents were mixed thoroughly. Then 0.8 ml ferric chloride reagent (60 mg $FeCl_3.6H_2O$ in 100 ml absolute ethanol) was added to tubes and contents were mixed thoroughly. To the above samples 0.9 ml *o*-phosphoric acid (0.5 ml of 85 % phosphoric acid in 100 ml of absolute ethanol) was added and mixed properly. The absorbance of the sample was read at 536 nm within 30 seconds. The sample should not be exposed to direct sunshine. The

Table 1. Variation in total tocopherols in wild species of rice (Mean ± SE, n = 3)

Sl. No.	Designation	Tocopherol content (mg/kg) (Mean ± SE)
1	*O. rufipogon* (CR100368)	45.3 ± 1.49
2	*O. rufipogon* (CR100372)	37.0 ± 1.43
3	*O. meridionalis* (IR105294)	33.9 ± 0.71
4	*O. rufipogon* (CR100334)	27.5 ± 0.89
5	*O. rufipogon* (IR80600)	25.6 ± 0.66
6	*O. glaberrima* (IR100983)	25.3 ± 0.39
7	*O. rufipogon* (CR100346A)	25.2 ± 1.03
8	*O. rufipogon* (CR100029)	24.9 ± 0.78
9	*O. rufipogon* (IR80610)	23.8 ± 0.76
10	*O. rufipogon* (CR100036)	23.1 ± 0.24
11	*O. barthii* (IR104103)	22.9 ± 0.53
12	*O. rufipogon* (IR80562)	22.5 ± 0.58
13	*O. rufipogon* (CR100334)	21.6 ± 1.06
14	*O. rufipogon* (IR104404D)	21.3 ± 0.64
15	*O. rufipogon* (CR 100201A)	21.2 ± 0.99
16	*O. meridionalis* (IR101146)	19.7 ± 0.72
17	*O. rufipogon* (CR100267)	19.2 ± 0.56
18	*O. rufipogon* (IR104433)	19.1 ± 0.60
19	*O. officinalis* (IR83809)	18.1 ± 1.02
20	*O. rufipogon* (CR 100201)	16.4 ± 1.23
21	*O. rufipogon* (CR 100309)	16.1 ± 0.47
22	*O. meridionalis* (IR105305)	15.7 ± 0.82
23	*O. meridionalis* (IR105300)	15.7 ± 0.34
24	*O. glaberrima* (IR102206)	15.5 ± 0.53
25	*O. meridionalis* (IR86538)	14.5 ± 0.31
26	*O. rufipogon* (CR100216)	14.2 ± 0.31
27	*O. barthii* (IR104076)	14.1 ± 0.35
28	*O. meridionalis* (IR86539)	13.6 ± 0.69
29	*O. meridionalis* (IR105290)	13.0 ± 0.38
30	*O. meridionalis* (IR93266)	12.7 ± 0.49
31	*O. glaberrima* (IR101800)	12.3 ± 0.27
32	*O. rufipogon* (CR100018A)	12.3 ± 0.59
33	*O. rufipogon* (IR105214B)	11.3 ± 0.72
34	*O. meridionalis* (IR104093)	10.7 ± 0.60
35	*O. rufipogon* (IR105491)	9.7 ± 0.56

SE=Standard error

Table 2. Variation in total tocopherols in Non-Basmati genotypes (Mean ± SE, n = 3)

Sl. No.	Designation	Tocopherol content (mg/kg) (Mean ± SE)
1	PR 128	22.4 ± 0.76
2	PR 127	21.4 ± 1.06
3	PR 122	21.0 ± 0.32
4	Pusa 44	20.5 ± 0.62
5	PR 126	20.2 ± 0.36
6	HKR 47	20.1 ± 0.26
7	PR 123	20.1 ± 0.30
8	PR 129	18.6 ± 0.64
9	PR 124	18.4 ± 0.60
10	PR 121	18.4 ± 0.36
11	RP5115-111-1	18.2 ± 0.72
12	PR 113	18.2 ± 0.44
13	PR 114	18.2 ± 0.46
14	IR82475-110-2	18.1 ± 0.41
15	R-RHZ-MI-81	17.8 ± 0.48
16	PAU 201	16.7 ± 0.69
17	IR64	14.9 ± 0.37
18	CR2826-1	14.5 ± 0.58
19	BPT5204	13.6 ± 0.54

SE=Standard error

Table 3. Variation in total tocopherols in Basmati genotypes (Mean ± SE, n = 3)

Sl. No.	Designation	Tocopherol content (mg/kg) (Mean ± SE)
1	Pusa Basmati 1509	25.0 ± 0.20
2	Punjab Basmati 4	24.8 ± 0.21
3	Pusa Basmati 1718	24.3 ± 0.18
4	Pusa Basmati 1637	24.3 ± 0.30
5	Pusa Basmati 1121	23.9 ± 0.14
6	Punjab Basmati 7	23.7 ± 0.27
7	Punjab Basmati 5	23.0 ± 0.18
8	CSR 30	18.7 ± 0.16
9	Basmati 370	18.2 ± 0.16

SE=Standard error

amount of tocopherols was estimated using a standard curve with tocopherol (2-10 g) as the reference.

The data presented in the tables represented the average of three observations, (±) standard error which was subjected to box plot analysis, one sample t-test and paired sample t-test using SPSS 20.0 at the 0.05 significance level.

Results and Discussion

The total tocopherol contents in brown rice of different accessions were significantly different among wild rice species, Basmati and non-Basmati cultivars (**Table 4**). The content of total tocopherols for wild, non-Basmati and Basmati genotypes is given in **Tables 1, 2 and 3**, respectively. For wild rice, it ranged from 9.7 mg/kg (*O. rufipogon*, IR105491) to 45.3 mg/kg (*O. rufipogon* CR100368). Seed morphology of wild rice accessions with highest and lowest tocopherol content is given in **Figure 1**. For the non-Basmati, it varied from 13.6 mg/kg (BPT5204) to 22.4 mg/kg (PR 128). Similarly, for Basmati cultivars, it ranged from 18.2 mg/kg (Basmati 370) to 25 mg/kg (Pusa Basmati 1509). Frequency distribution using box plots for total tocopherol content in wild, non-Basmati and Basmati genotypes is given in **Figure 2**. Paired t-test was applied to calculate significant differences between the different pairs *viz*: Wild-Basmati, Wild-Non-Basmati and Basmati–Non-Basmati. Significant differences were found to be present between means of these pairs (**Table 5**).

Table 4. Significant differences within wild rice species, Basmati and Non-Basmati genotypes for tocopherol content in present study

One-Sample Test			
Type	**t**	**df**	**p-value**
Wild	15.12	34	.000 (S)
Non-basmati	34.36	18	.000 (S)
Basmati	26.59	8	.000 (S)

Significant at p<0.05, S= significant

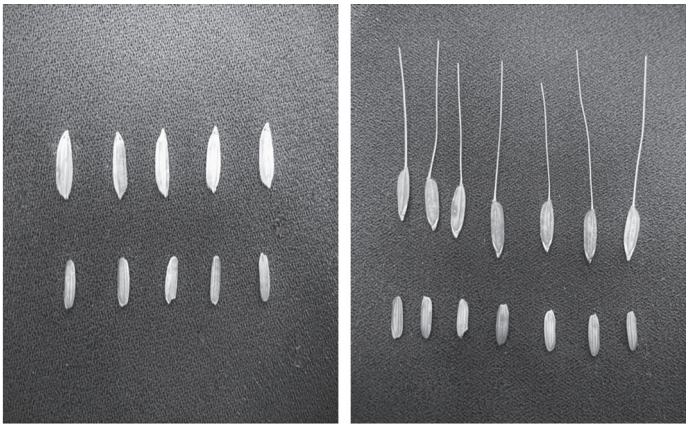

Figure 1: Seed morphology of wild rice accessions with highest and lowest tocopherol content

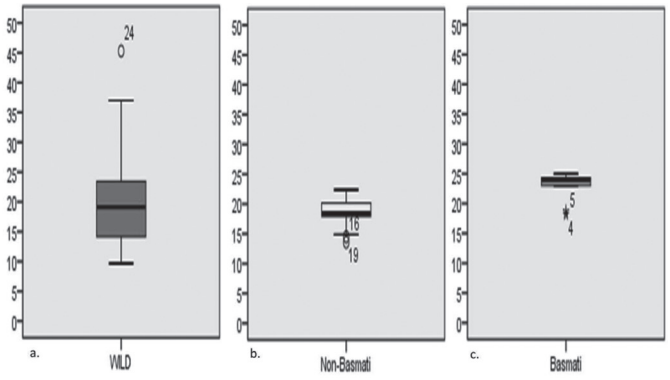

Figure 2: Frequency distribution using box plots for total tocopherol content (mg/kg) in wild, non-Basmati and Basmati genotypes

Total tocopherol content obtained in the wild rice accessions under study (9.7 to 45.3 mg/kg) was higher than (6.7 to 13.3 mg /kg, 10 to 32.5 mg/kg 6.24 – 12.52 mg/kg, 4.6 to 16.2 mg/kg, 9.3 to 19.9 mg/kg, 9.3 to 19.9 mg/kg, 6.52 to 21.51mg/kg and 9.74 to 30.64 mg/kg) previously reported by Aguilar-Garcia *et al.,* (2007), Heinemann *et al.,* (2008), Huang *et al.,* (2011), Fasahat *et al.,* (2012), Gunaratne *et al.,* (2013), Shammugasamy *et al.,* (2014) and Yu *et al.,* (2016), respectively. Brown rice from four varieties had total tocopherol content ranging from 31.3 to 48.7 mg/kg (Gopala Krishna *et al.,* 1984) and Zubair *et al.,* (2012) reported total tocopherol content ranged from 67.1 to 115.3 mg/kg which is higher than the observed range in the present study. The levels of total tocopherols observed in the present study for Basmati (25 to 18.2 mg/kg) and non-Basmati (13.6 to 22.4 mg/

kg) cultivars were in agreement with these previous studies. Furthermore, it is clear that differences in total tocopherol distribution may exist depending on the rice genotype and extraction method used. *O. rufipogon* accessions *viz*; CR100334 (27.5), CR100368 (45.3), CR100372 (37.0) and *O. meridionalis* accession, (IR105294) (33.9) had highest tocopherol contents.

With reference to our previous report (Kaur *et al.,* 2022), the results showed that the four accessions *viz*; *O. rufipogon* (CR100334), *O. rufipogon* (CR100368), *O. rufipogon* (CR100372) and *O. meridionalis* (IR105294) with high total tocopherol content also had high protein content (16.2%, 16.7%, 12.5% and 13.5%, respectively) in brown rice. Some high total tocopherol and high protein accessions along with their grain characteristics are given in **Table 6**. We cannot use these wild rice accessions directly for nutritional enhancement as they have short length, medium shape and low TGW as compare to Basmati and non-Basmati genotypes which are not preferred by consumers. But superior recombinants could be found by crossing these wild rice accessions with cultivated rice varieties which already had excellent grain quality and phenotypic acceptability.

In concusion, this study provides information on total tocopherol content of different rice wild accessions along with Basmati and non-Basmati cultivars**.** Wild rice accessions have high total tocopherol content and high protein content as compared to Basmati and non-Basmati accessions. Approximately 197g rice was available per person per day in India during 2021 (https://www.statista.com) and if rice contains 45.3

Table 5. Significant differences between the pairs of wild rice species, Basmati and Non-Basmati genotypes for tocopherol content

Paired Samples Test								
Pairs	Paired Differences					t	df	p-value
	Mean	SD	Mean SE	95% CI of the Difference				
				Lower	Upper			
Wild - Non-Basmati	6.63	5.08	1.17	4.18	9.08	5.68	18	.000 (S)
Wild - Basmati	6.96	6.20	2.07	2.19	11.73	3.36	8	.010 (S)
Non-Basmati - Basmati	-2.58	1.54	0.51	-3.76	-1.39	-5.02	8	.001 (S)

SD=Std. Deviation, SE=Std. Error, S= significant, NS= non-significant, Significant at p<0.05

Table 6. Wild rice accessions with high protein and high tocopherol content along with grain characteristics

S.No.	Wild accessions	Tocopherol	P (%)	GL	GB	L:B	TGW
1	*O. rufipogon* (CR100334)	27.5	16.2	5.5 (Short)	1.8	3.0 (Medium)	16.7
2	*O. rufipogon* (CR100368)	45.3	16.7	5.3 (Short)	2.1	2.6 (Medium)	15.0
3	*O. rufipogon* (CR100372)	37.0	12.5	5.3 (Short)	2.1	3.0 (Medium)	14.3
4	*O. meridionalis* (IR105294)	33.9	13.5	5.4 (Short)	1.7	3.2 (Medium)	10.8

Tocopherol (mg/kg), P=Protein%, GL=Grain Length (mm), GB=Grain Breadth (mm), L:B=Length/Breadth ratio, TGW= Thousand grain weight (gm)

mg/kg tocopherols (with reference to present study), thus tocopherol intake per person per day will be 8.92mg which is higher as compared to tocopherols provided by Basmati (4.92mg) and non-Basmati (4.41mg) genotypes. For instance, if general RDA of α-tocopherol for an average adult is 7.5-10 mg, the wild rice could assist to meet the required target in cultivated varieties in future rice breeding programs. So, this finding could provide rice breeders with new opportunities by allowing them to use wild rice species with high protein and tocopherol content as donors in interspecific crosses with elite rice cultivars to enhance their protein and tocopherol levels.

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