

Proximate nutritional composition and antioxidant properties of *Oryza rufipogon*, a wild rice collected from Malaysia compared to cultivated rice, MR219

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Abstract

Wild rice (*Oryza rufipogon* L) is known as an important germplasm that has specific desirable attributes and a high genetic diversity. The physicochemical and antioxidant properties of *O. rufipogon* Griff. (acc. IRGC105491), a wild rice collected from Malaysia that was used in a breeding program and a QTL mapping study, were assessed to determine the rice's nutritional value compared with a commonly consumed rice cultivar MR219 (*O. sativa* subsp. *indica*). To identify and quantify the phenolic compounds in the rice extracts, Ultra Performance Liquid Chromatography (UPLC) coupled with a photodiode array detector (PDA) was applied. The results obtained were supported by a determination of the total phenolic compounds (TPC), 2,2-diphenyl-1-picrylhydrazyl (DPPH[•]) radical scavenging, tocopherols, tocotrienols, and the ferric reducing ability power (FRAP) of the respective extracts originating from the whole grain. The results indicated that *O. rufipogon*, irrespective of its poor appearance, contained high proximate nutritional composition and antioxidant properties. The grain of *O. rufipogon* consisted of 8.0% protein, 2.2% fat, and 25.0% amylose content all significantly higher than the ratios of MR219. Except for the percentage of inhibition DPPH[•] radical, which was statistically at par with MR219, the antioxidant activity of *O. rufipogon* was higher than that of MR219. The high antioxidant activity of *O. rufipogon* was supported by the presence of high tocochromanol content higher than MR219. *Oryza rufipogon* extracts were also the most effective in antioxidative reactions. This study demonstrated that wild rice can be considered a valuable source of bioactive components with high antioxidant properties in breeding programs.

Abbreviations: DPPH- 2,2-diphenyl-1-picrylhydrazyl; FRAP- Ferric reducing ability power; TP- Tocopherol; T3- Tocotrienol; TPC- Total phenolic content.

Introduction

Genus *Oryza* in rice consists of two cultivated species, *O. sativa* L. and *O. glaberrima* (Coffman and Herrera, 1980), as well as 22 wild species with a wide range of genetic diversity (Brar and Khush, 1997; Lu et al., 2002; Park et al., 2003; Ren et al., 2003; Sun et al., 2001). Wild rice varieties contain many favourable genes that have been removed from cultivated rice through forced selection. These favourable genes enable the wild rice species to resist undesirable environments as well as many diseases and pests (Shan et al., 2009). Common wild rice recognized as the main genetic source for both drought tolerance and pest resistance in rice breeding programs as it can be crossbred with cultivated rice to produce fertile plants (Punyalue et al., 2006; Zhou et al., 2006). In rice, the greater part of genetic variation in the genus *Oryza* remains intact in its wild relatives (Wang et al., 1992). In some studies, wild species of rice have been purposely used for the introgression of different traits, such as abiotic and biotic stress resistance (Albar et al., 2003; Jena and Khush, 1990; Khush, 1997; Khush et al., 1990; Nguyen et al., 2003), yield and its components (Brondani et al., 2002; Moncada et al., 2001; Sabu et al. 2006; Septiningsih et al., 2003a; Thomson et al., 2003; Wickneswari et al., 2012; Xiao et al., 1996; Xiao et al., 1998; Xiong et al., 1999), cytoplasmic male sterility (Dalmacio et al., 1995; Dalmacio et al., 1996; Hoan et al., 1997; Lin and Yuan, 1980), and

grain quality (Aluko et al., 2004; Li et al., 2004; Septiningsih et al., 2003b; Thomson et al., 2003) into cultivars. Alleles from wild species involving *O. rufipogon* demonstrated a beneficial effect in 35–58% of the QTLs in different studies (Moncada et al., 2001; Septiningsih et al., 2003a and b; Wickneswari et al., 2012; Xiao et al., 1998). *Oryza rufipogon*, which is assumed to be the wild ancestor of *O. sativa* is a perennial that has extensively spread in the tropics and subtropics of monsoon Asia (Vaughan, 1994). The two species are analogous (vegetatively they are similar) and hybridize easily. In most of the regions of the world, *O. rufipogon* is recognized as an aggressive weed of rice. It is classified as a weed because the seeds can go dormant, the grain coat is red, and the spikelet shatters (http://itp.lucidcentral.org/id/fnw/key/FNW_Grasses/Media/Html/fact_sheets/Oryza_rufipogon.htm). This wild rice species has the AA genome and reproduces by both seeds and horizontal stems (Khush, 1997; Xie et al., 2001). The nutritional value of wild rice is equal to or perhaps even higher than other cereals (Anderson, 1976). In contrast with milled white rice, wild rice contains all parts of a grain, including the embryo, endosperm, and bran from inside to outside. Thus, it was listed as a whole grain along with oats, barley, and wheat by the United States Food and Drug Administration in 2006. As wild rice has no direct

commercial value, most previous studies have focused on grain quality characteristics of white and red commercial varieties. Hence, no published information is available on the nutritional and antioxidant properties of *O. rufipogon* whole grain. The present study investigates the nutritional value of *O. rufipogon*, in comparison with a commercial white rice cultivar MR219 grown in more than 90% of rice fields in Malaysia.

Results

Physical characteristics and proximate nutritional composition

The agronomical characteristics of *O. rufipogon* IRGC105491 and MR219 are presented in Table 1. Based on the length and length to breadth ratio, *O. rufipogon* can be classified as a medium grain variety, while MR219 is a long and slender grain variety ($p < 0.01$) (see Table 2 and Fig 1). In terms of hull proportion, *O. rufipogon* has a value of less than 20 percent. No significant difference between *O. rufipogon* and MR219 was found in terms of moisture or fat and protein contents. *Oryza rufipogon* possessed significantly ($p < 0.01$) higher amylose content than MR219 (see Table 3). The total antioxidant activity of samples is presented in Table 4. The DPPH[•] scavenging activity results show no significant difference between the two samples. However, significant differences in TPC ($p < 0.01$) were observed between the samples. The total antioxidant capacity was measured using the FRAP assay; for *O. rufipogon*, the FRAP value ($253.35 \mu\text{molFeSO}_4 \text{ g}^{-1}$) was 3.6 times higher than that of MR219 ($69.7 \mu\text{molFeSO}_4 \text{ g}^{-1}$). Tocopherol and tocotrienol profiles of the whole grains are shown in Table 5. A typical run lasted for 30 min, and the five homologues of vitamin E were baseline separated and eluted as follows: α -tocopherol, α -tocotrienol, γ -tocopherol, γ -tocotrienol, and δ -tocotrienol. Results were compared with purified standards, and significant differences were observed for tocopherols, between both samples (Table 5). The content of tocopherols (including α -tocopherol and γ -tocopherol) varied between samples with the whole grain of *O. rufipogon* showing higher levels and the main tocopherols were α -T and γ -T. The main tocotrienols were only α -T3, γ -T3, and δ -T3 in both samples. The concentrations of α -tocopherol and α -tocotrienol were also affected by the pericarp colour of the wild rice (Table 5). The most abundant phenolic acids found in all samples were caffeic and ferulic acids (Fig 2). In addition, sinapic, vanillic, syringic and *p*-coumaric acids were minor constituents in both samples. However, vanillic acid could not be detected in *O. rufipogon*.

Discussion

Oryza rufipogon is a red-coloured, wild species of rice and a progenitor of *O. sativa* (Takeoka, 1962). Bhuiyan (2010) reported that the growth duration of *O. rufipogon* lasted about 105 days. *Oryza rufipogon* has similar ecological requirements as local and commercial cultivars and hence, tends to take advantage from most of the conditions provided by farmers in rice fields for its growth. In Malaysia, this species can be found only in the northern part of the Peninsular Malaysia (Abdullah et al., 1991) and is facing genetic erosion (Ngu et al., 2010). MR219, which was developed from a cross between MR137 and MR151, was released in 2001. Its important characteristics include a high resistance to bacterial leaf blight and brown plant hopper, a short maturation period and long grade grains. Based on the

rough rice hull colour, *O. rufipogon* is a “black hull” type and awned, whereas MR219 is “straw hull” type and awnless. Blackhull types are compact plants with a tall stature and dense tillers that mature late, producing awned seed with black lemma and palea. Meanwhile, *O. rufipogon*'s culm structure was open and spreading whereas that of MR219 was upright and compact. On average, blackhull red rice has been shown to produce 18% more straw biomass and 27% more tillers (Diarra et al., 1985). The black hull seed is common among the ancestors of cultivated rice. Maekawa (1984) reported that three complementary genes (symbolized as *Bh-a*, *Bh-b*, and *Bh-c*), control the black hull colour. Dehusked grains of *O. rufipogon* are red-pigmented while MR219 is light brown in colour (Fig 1). The pigments identified in wild rice seeds included anthocyanins, tannins, carotenoid, and chlorophylls (Withycombe, 1974). Wild rice grains, which are usually small and round in shape, are desirable under natural selection which is optimal for high fertility and dispersion by natural vectors (Fan et al., 2006). In an earlier study, the *spd6* allele in wild rice (*O. rufipogon*) was correlated with significantly smaller panicles containing fewer spikelets, low spikelet fertility, small grains, and shorter plants compared to the recurrent parent Teqing (Shan et al., 2009). Kasem et al. (2010) reported that the grain size of *O. rufipogon* accession from Australia (AusTRCF 309317) falls in the medium category as in the current study. Significant differences in the quantity of the fibrous hull ($p < 0.05$), covering the rice grain, were also pronounced between the samples. *Oryza rufipogon* showed a higher percentage of dehusked rice grain compared to MR219. The moisture content of both samples was approximately 12%, which shows similar agro-climatic situations and/or places where the paddy was stored. The moisture content of paddy rice samples to be analyzed should be 12 to 14% to minimize grain fissures (Patindol, 2000). Amylose content is one of the most important determinants of rice quality. Amylose is the final product of the granule-bound starch synthesis encoded by the waxy gene (*Wx*) (Sano, 1984). The amylose content in rice germplasm is classified as waxy (1–2%) or non-waxy (>2%). In addition, the non-waxy group is divided into four subgroups: very low (2–10%), low (10–20%), intermediate (20–25%), and high (>25%) amylose content (Juliano, 1979). Low-amylose cooked rice is prone to be moist, tender, and cohesive, whereas high amylose content is more likely to end up as dry, fluffy, and separated grains (Juliano, 1971). A significant difference was found between both samples ($p < 0.01$). MR219 is classified as intermediate amylose while *O. rufipogon* is a high amylose type (>25%). Red rice with high amylose levels could potentially be suitable as germplasm for rice cultivars to be used in niche applications in the canning industry (Moldenhauer et al., 2004) or some rice products, especially considering the nutritional benefits attributed to red-bran rice. High-amylose rice has been reported to display lower glycaemic values (Hu et al., 2004). In the current study, the protein content was appreciably high (>7%) for both samples. Although the protein content of rice is relatively low (8.5%) compared to other cereals, such as millet (13.4%), barley (12.8%), and wheat (12.3%), rice is considered to be one of the cereals with high quality protein, being rich in lysine (3.8%) (Shobha Rani et al., 2006). Coloured rices have been shown to be a valuable source of antioxidants and are considered to be strong origins of antioxidants for functional foods (Yawadio et al., 2007). Phenolic compounds are a good source of natural antioxidants that act by both decreasing the content of toxic compounds in foods and providing the human body with exogenous antioxidants. Increased consumption of phenolic

Table 1. Agronomical features of *O. rufipogon* and MR219.

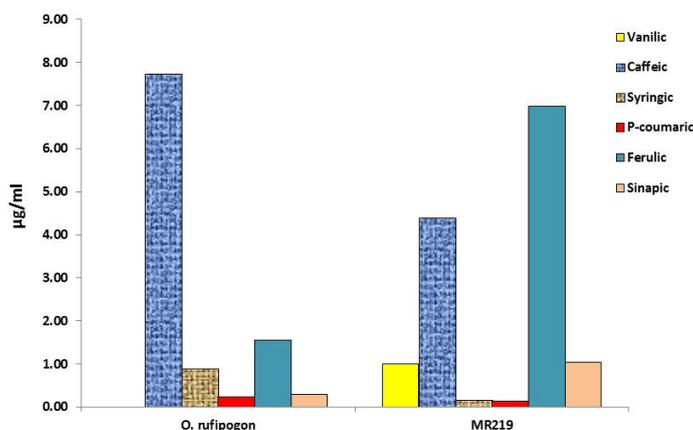
Popular name	Variety/origin	Crop maturation (days)	Parentage	Agronomical features	References
<i>Oryza rufipogon</i> (IRGC105491)	Wild, Peninsular Malaysia	105	Progenitor of <i>O. sativa</i>	Perennial tufted grass, 150 - 400 cm tall, with culms spongy below the lower parts	Ngu et al., 2010; Bhuiyan, 2010
MR219	Inbred MARDI, Malaysia	121	MR137 x MR151	Released in 2001 in Malaysia. Short, maturation period, highly resistant to bacterial leaf blight and brown plant hopper	MARDI, 2002

Table 2. Physical characteristics of *O. rufipogon* and MR219.

Species/cultivar	Rough rice (Lemma and Palea) colour description	Appearance	l/b*
<i>Oryza rufipogon</i>	black furrows on black background	Red, medium, medium	2.33 ± 0.03 ^a
MR219	straw furrows on straw background	White, long, slender	3.50 ± 0.08 ^b

Values are mean ± standard error, *Length/breadth ratio, ^aSignificant difference ($P < 0.05$) between *O. rufipogon* and MR219.

compounds has been associated with the reduced risk of certain cancers and cardiovascular diseases (Liu, 2004; Liu, 2007; Dykes and Rooney, 2007). Significant differences ($p < 0.01$) were observed for TPC between the samples. Compared with MR219, *O. rufipogon* contained more phenolic compounds. Recently, Shen et al. (2009) reported that the levels of phenolics in red rice ranged from 165.8 to 731.8 mg GAE 100 g⁻¹. The concept of the total antioxidant capacity, which represents the ability of different food antioxidants to scavenge free radicals, has been suggested as a tool for evaluating the health effects of antioxidant-rich foods. A significant difference ($p < 0.01$) in FRAP existed in both samples as well. The FRAP value ranged from 69.7 to 253.35 $\mu\text{molFeSO}_4 \text{ g}^{-1}$, with the lower value coming from MR219. Phenolic acids include two subclasses: hydroxycinnamic acids and hydroxybenzoic acids. Phenolic compounds commonly present in whole grains are phenolic acids and flavonoids (Al-Farsi and Lee, 2008). The common phenolic acids found in whole grains are ferulic acid, caffeic acid, syringic acid, vanillic acid, and *p*-coumaric acid (Sosulski et al., 1982). To understand the individual phenolic constituents of the wild rice sample, 6 phenolic standards were selected based on the most commonly found and studied phenolics in rice (Butsat and Siriamornpun, 2010; Laokuldilok et al., 2011). The concentration of caffeic acid found in *O. rufipogon* was high (7.74 $\mu\text{g ml}^{-1}$). In addition to ferulic acid and caffeic acid, sinapic acid was found in significant quantities; all three of these phenolic acids belong to hydroxycinnamic acids, indicating that hydroxycinnamic acids were the main phenolic acids in this sample. Meanwhile, the hydroxybenzoic acids (vanillic and syringic acids) did not occur at such high levels. Vitamin E, another antioxidant present in grains, protects polyunsaturated fatty acids in cell membranes against oxidative damage (Slavin et al., 1999). Significant differences ($p < 0.01$) were observed in the samples in terms of tocols. The content of tocopherols varied significantly. The highest level of tocopherols was found in the whole grain of *O. rufipogon* where the main tocopherols were α -tocopherol and γ -tocopherol. Meanwhile, γ -tocopherol was higher than α -tocopherol in MR219. A previous study reported a total tocopherol content of 31.3 to 48.7 $\mu\text{g g}^{-1}$ for brown rice from four varieties (Gopala Krishna et al., 1984). Khaton and Gopalakrishan (2004) reported the α -tocopherol contents of Basmati brown rice (12.1 $\mu\text{g g}^{-1}$) and Jaya brown rice (9.9 $\mu\text{g g}^{-1}$). Recently, Aguilar-Garcia et al. (2007) reported the levels of tocopherol in rice bran (<0.5–35.0 $\mu\text{g g}^{-1}$) and brown rice (<0.5–8.5 $\mu\text{g g}^{-1}$) for three varieties of Venezuelan rice. The main

**Fig 1.** Pericarp colour of *Oryza rufipogon* and MR219.**Fig 2.** Distribution of phenolic acids in whole grain of *O. rufipogon* and MR219.

tocotrienols found in both samples were α -T3, γ -T3, and δ -T3. The levels of α -tocotrienol in whole grain of *O. rufipogon* and MR219 were 0.63 and 0.06 mg 100 g⁻¹, respectively ($p < 0.01$). Despite being overshadowed by tocopherols (TP) over the past few decades, tocotrienol (T3) is now considered to be a more promising anti-cancer agent from the vitamin E family due to its effective reaction to a wide range of cancers. Given that most of the previous clinical studies on tocopherols have produced disappointing results, research has now been refocused on testing T3 as the next generation of vitamin E for cancer treatment and chemoprevention (Ling et al., 2011).

Table 3. Proximate composition of brown rice of *O. rufipogon* and MR219.

Components	<i>O. rufipogon</i>	MR219
Moisture Content (%)	12.15 ± 0.07 ^a	12.25 ± 0.07 ^a
Dehusked rice grains (%)	80.2 ± 0.01 ^a	79.5 ± 0.14 ^b
Protein (g per 100 g) (N x 5.95)	8.0 ± 0.14 ^a	8.35 ± 0.36 ^a
Fat content (%)	2.2 ± 0.02 ^a	2.3 ± 0.06 ^a
Amylose (%)	25.2 ± 0.04 ^a	21.1 ± 0.11 ^b

Values are mean ± standard error, ^a Significant difference ($P < 0.05$) between *O. rufipogon* and MR219, ns: not significant.

Table 4. Total phenolic content, % inhibition DPPH' radical and FRAP value of whole rice grain of *O. rufipogon* and MR219.

Species	TPC (mg GAE g ⁻¹)	% inhibition DPPH' radical	FRAP (μmol FeSO ₄ g ⁻¹)
<i>O. rufipogon</i>	1.78 ± 0.03 ^a	83.6 ± 0.50 ^a	253.35 ± 1.48 ^a
MR219	0.32 ± 0.0 ^b	85.5 ± 0.77 ^a	69.7 ± 0.14 ^b

Values are mean ± standard error, ^a Significant difference ($P < 0.05$) between *O. rufipogon* and MR219, ns: not significant, TPC=total phenolic content, FRAP= ferric reducing ability power.

Table 5. The contents of tocopherols (T) and tocotrienols (T3) in whole grains of *O. rufipogon* and MR219 (mg 100g⁻¹ d.m.)*.

Species/cultivar	T		T3			Total
	α	γ	α	γ	δ	
MR219	0.06 ± 0.0 ^a	0.34 ± 0.0 ^a	0.06 ± 0.0 ^a	0.62 ± 0.0 ^a	0.08 ± 0.0 ^a	1.17 ± 0.0 ^a
<i>O. rufipogon</i>	0.69 ± 0.02 ^b	0.30 ± 0.0 ^b	0.63 ± 0.01 ^b	1.25 ± 0.01 ^b	0.30 ± 0.01 ^b	3.16 ± 0.02 ^b

* Data expressed as means ± standard deviation, ^{ab} Significant difference ($P < 0.05$) between *O. rufipogon* and MR219, T=tocopherol, T3=tocotrienol.

Materials and methods

Materials

Paddy rice samples used for this study were collected from the 2008 harvest at the Rice Genebank of the Malaysian Agricultural Research and Development Institute at Seberang Perai, in northwestern Malaysia (Bhuiyan et al., 2011). Folin-Ciocalteu's (FC) phenol reagent was obtained from Merck (Darmstadt, Germany). Sodium carbonate, gallic acid, 1,1-diphenyl-2-picrylhydrazyl (DPPH') and 2,4,6-Tris (1-pyridyl)- 5-triazine (TPTZ) were purchased from Sigma (Steinheim, Germany), while ferrous sulphate was obtained from R&M Chemicals (Essex, UK). Samples of rough rice were hulled using a dehulling machine (Motion Smith Co., Singapore) to produce dehulled rice. The dehulled rice was passed through a 500μm sieve screen on a Sample Mill (Cyclotec 1093, Foss analytical, Sweden) to obtain rice powder.

Proximate analysis of grains

The moisture, protein, fat and amylose content were measured based on Fasahat et al. (2012). Hull colour was described according to the descriptors defined by the IBPGR-IRRI Rice Advisory Committee (1980). The length and width of the samples (50 grains per sample) were measured with the help of a digital micrometer (Steinmeyer, Germany). Size and shape were determined according to the scale from RTWG (1997). The percentage of de-husked rice grains was evaluated using the method described by Septiningsih et al. (2003b).

Extraction and determination of total antioxidants

The extraction and determination of total phenolic content (TPC), free-radical scavenging capacity (DPPH'), and ferric reducing ability power (FRAP) were carried out following the method reported by Fasahat et al. (2012).

Lipid extraction, HPLC analyses for tocopherol and tocotrienol, and UPLC analysis and quantification of individual phenolic compounds

The extraction and determination of tocopherols and tocotrienols as well as phenolic compounds were performed, using the method of Fasahat et al. (2012).

Statistical Analysis

All analyses were carried out in duplicate and in randomized order with mean values. T-tests, correlation analysis, and regression analysis were performed using SAS v.9 software (SAS Institute, Cary, NC, USA) with a significant level of 0.05.

Conclusion

This study showed that the dehulled rice sample of *O. rufipogon* has relatively high amylose, total phenolic content, ferric reducing ability power, and vitamin E content, making it beneficial for use in breeding programs to enhance nutritional and antioxidant properties of grains.

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