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Full Length Article

Micronutrients, Antioxidant Activity, and Tocochromanol Contents of Selected Pigmented Upland Rice Genotypes

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Abstract

Pigmented rice genotypes have high nutritive value compared to white rice genotypes. These genotypes contain high levels of micronutrients and antioxidant compounds, such as polyphenols, tocochromanols and oryzanol. In this experiment, 42 pigmented rice genotypes obtained from the International Rice Research Institute (IRRI) were evaluated to determine micronutrient content, antioxidant activity and vitamin E content. The results revealed that the micronutrient content, antioxidant activity and tocochromanol content of all genotypes varied. Iron (Fe) was the most abundant micronutrient followed by Zn, Mn and Cu. The antioxidant DPPH scavenging effect among all genotypes ranged from 31.85 to 98.45%. The tocotrienol content was higher than tocopherol in grains of selected pigmented rice genotypes. Among rest of genotypes, IR3257-13-56, IR5533-14-1-1 and Khao gam (niaw) contained high micronutrient content and antioxidant properties compared to others. These genotypes could also be potentially used further for breeding program to improve the rice plant with high micronutrient content and antioxidant properties.

Keywords: Pigmented rice; Nutritional quality; Micronutrient; Tocochromanols
Abbreviation: DPPH =1; 1-Diphenyl-2-picrylhydrazyl; α=Alpha; β=Beta; γ=Gama; δ=Delta

Introduction

Rice (*Oryza sativa* L) is a staple food and an important cereal crop throughout Asia and also cultivated in many countries including America, Africa and Europe under several agro-climatic conditions (Chaudary and Tran, 2001). It can grow in upland and lowland areas which allow rice to be widely planted around the world.

Pigmented rice is categorized based on the red, black or purple colour on their bran layer. The colour pigment anthocyanin gives the pigment to bran layer and genetic factor causes the different bran layer among the rice genotypes (Maekawa and Kita, 1984). Rice bran is also known to have higher amount of phytonutrient including phenolic compounds (Chen and Bergman, 2005). The content of anthocyanin and other phenolic compounds in red and black rice are higher compared to white rice (Ryu *et al*., 1998; Zhang *et al*., 2006).

The public is currently more concerned with consuming healthy and nutritious food. According to World Health Organization, every third person in developing countries is affected by vitamin and micronutrient deficiency. The lack of these micronutrients in food can cause several diseases and health problems. Approximately 40% of unmarried and 50% of pregnant women suffer from anemia and 1500 million children have Zn deficiency (Zeng *et al*., 2004). The micronutrient content of pigmented rice, i.e. Fe and Zn are higher than commercially available rice cultivar. Additionally, the micronutrient contents varied among rice genotypes because these controlled by several genes. The genes controlling Fe content in rice are identified at chromosome 7, 8 and 9 (Meng *et al*., 2005).

Plants are natural source of antioxidant compounds, such as caratenoids, flavanoids, cinnamic acid, benzoic acid, folic acid, ascorbic acid, tocopherols and tocotrienols (Shahnawaz *et al*., 2010). Pigmented rice is healthier alternative food because its antioxidant compounds, such as tocochromanols, oryzanol and phenolic compounds, are high (Ling *et al*., 2001; Walter and Marchesan, 2011). These antioxidant compounds can reduce chronic diseases, such as heart attacks, diabetic and cancer. Although the white rice cultivars are generally consumed, pigmented genotypes have been traditionally consumed in some Asian countries (Fasahat, 2012).

Tocochromanols, or vitamin E, are lipid soluble molecules with an important function in human health (Falk

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and Munn-Bosch, 2010). The term vitamin E was introduced by Evans (1922) and it is associated with antioxidant activities (Epstein *et al.*, 1966). Two isomers of tocochromanols have been identified: tocopherol (α, β, γ and δ) and tocotrienol (α, β, γ and δ). The position and number of methyl group on the chromal ring differ in these isomers. The content and composition of both tocochromanol isomers are different in each tissue. Red and purple rice brans are rich natural source of phytochemicals and nutraceuticals for functional food development.

The variation in the phytochemical concentration and composition among studied pigmented rice bran genotypes may lead to production of rice plants with increased nutritional value via a breeding program. However, research on the nutritional quality of pigmented rice, such as the micronutrient content and antioxidant activities is still scanty. There is also need to identify the nutritional status of pigmented upland rice genotypes. This experiment has therefore been undertaken to determine the micronutrient, antioxidant and tocochromanols content in selected pigmented upland rice genotypes.

Materials and Methods

Site and Design of the Experiment

Forty-two pigmented rice genotypes were grown in greenhouse at Faculty of Agriculture, Universiti Putra Malaysia from August 2011 to January 2012. The climatic conditions of the study area are hot, humid and tropical with high rainfall. The experiment was conducted in randomized complete block design (RCBD) with three replications. The seeds for each genotype were collected. All the biochemical analysis was carried out at the Institute of Tropical Agriculture (ITA) central laboratory, Universiti Putra Malaysia (UPM) and Malaysia Palm Oil Board (MPOB), Bangi, Malaysia.

Experimental Method

The seeds of 42 pigmented upland rice genotypes were used to determine the micronutrient and antioxidant DPPH activities. The list of genotypes is presented in Table 1. Based on the total antioxidant activities, the content of tocochromanols was determined in 6 genotypes (Table 2).

Determination of Micronutrient Contents

The micronutrient contents (Fe, Mn, Zn and Cu) were determined using the wet digestion method of Jones Jr. *et al.* (1991). Approximately 0.25 g of rice flour was placed in digestion tube with 5 mL of concentrated sulfuric acid. The digestion block was heated to 150°C for 2 h and the temperature was increased to 320°C for the next 1 h. Approximately 1 mL of hydrogen peroxide (H_2O_2) was slowly added to the plant material inside the tube until solution became colorless. The digestion tube was removed from the digestion block after 30 min and allowed to cool. Then solution was diluted using distilled water and filtered into a 100 mL volumetric flask. The solution was then diluted to 100 mL with distilled water. The solution was transferred into a plastic vial to further analyze the mineral contents. All samples were digested in triplicate.

Determination of Antioxidant DPPH Activities

Rice flour was extracted using the method of Perez-Jimenez *et al.* (2008). The total antioxidant activities were determined using the method of Butsat and Siriamornpun (2010). An approximately 0.1 mM freshly prepared DPPH solution was mixed with the concentrated extract. The absorbance was measured at 517 nm relative to control using 2 mL of 1 mM DPPH. All samples were run in triplicate. The equation used to determine the percentage of the scavenging effect was.

$$\% \text{ scavenging}=[1-(A_{517} \text{ of sample}/A_{517} \text{ of control})] \times 100.$$

The A_{517} is the absorbance of the sample at 517 nm.

The A_{517} is the absorbance of the control (no sample) at 517 nm.

Tocochromanols Content

Vitamin E was extracted and its content (tocochromanols and tocotrienols) was estimated according to the method of Fasahat *et al.* (2012). Approximately 3 g of rice flour sample were weighed and placed in a capped centrifuge tube. Subsequently, 30 mL of chloroform and methanol (2/1 ratio, v/v) were added, and the mixture was shaken vigorously at room temperature. The supernatant was collected in a new tube and remaining residue was extracted again four times. The collected supernatants were combined in the same tube. The solvent was filtered using a 45 µm Whatman syringe filter. The combined filtrate was then evaporated at 70°C using a rotary evaporator (Rotavapor R-210, Buchi, Switzerland). The dry residue was re-dissolved in 1 mL hexane and filtered into the tube.

The contents of tocopherol and tocotrienol isomers of oil obtained from the extraction process were determined by HPLC analysis. The HPLC system consisted of 250 × 4.6 mm column packed with 5 µm of silica (Phenomenex, Torrance, USA) using a mobile phase of iso-propanol (Merck, Darmstadt, Germany) at a flow rate of 1 mL min^{-1}. The peaks were detected by a fluorescence detector (Perkin Elmer, Massachusetts, USA) using an excitation wavelength of 295 nm and emission wavelength of 330 nm. The content of tocochromanols was identified using the standard retention time for tocopherols and tocotrienols (Isomer kits of chromodex, Santa Ana, USA).

Statistical Analysis

An analysis of variance (ANOVA) was performed to

Table1: List of 42 selected pigmented upland rice genotypes

Germplasm no	Seed coat color	Genotype name	IRGC accession	Country Origin	Status
v1	Red	Black banni	10181	India	Landrace/Traditional cultivar
v2	Red	258	14887	Liberia	Breeding and inbred line
v3	Purple	Khao gam (niaw)	15005	Thailand	Landrace/Traditional cultivar
v4	Red	Bi-e-gaw	15053	Thailand	Landrace/Traditional cultivar
v5	Red	C	15165	Ivory Coast	Released/Improved/advanced cultivar
v6	Red	Choke tang	24085	Vietnam	-
v7	Red	Chokoto 14	25988	Brazil	-
v8	Red	Jahau	27654	Thailand	Landrace/Traditional cultivar
v9	Red	Ja la shau	27655	Thailand	Landrace/Traditional cultivar
v10	Red	Jaloy	27656	Thailand	Landrace/Traditional cultivar
v11	Red	Ja no naq	27666	Thailand	Landrace/Traditional cultivar
v12	Purple	Ja nu ne ne	27671	Thailand	Landrace/Traditional cultivar
v13	Red	Bibili al	31369	Sri Lanka	Landrace/Traditional cultivar
v14	Purple	Ngacheik	33453	Myanmar	Breeding and inbred line
v15	Red	IR 9669-22-2-6	40315	Philippines	Breeding and inbred line
v16	Red	IR 9669-23-12-7	40316	Philippines	Breeding and inbred line
v17	Red	IR 9669-PP 823-1	40317	Philippines	Breeding and inbred line
v18	Red	IR 9669-PP 830-1	40319	Philippines	Breeding and inbred line
v19	Red	IR 9669-PP 836-1	40320	Philippines	Breeding and inbred line
v20	Red	IR 5533-13-1-1	40425	Philippines	Breeding and inbred line
v21	Red	IR 5533-14-1-1	40426	Philippines	Breeding and inbred line
v22	Red	IR 5533-15-1-1	40427	Philippines	Breeding and inbred line
v23	Red	IR 5533-50-1-10	40428	Philippines	Breeding and inbred line
v24	Red	IR 5533-55-1-11	40429	Philippines	Breeding and inbred line
v25	Red	IR 5533-56-1-12	40430	Philippines	Breeding and inbred line
v26	Red	IR 5533-PP 854-1	40432	Philippines	Breeding and inbred line
v27	Red	IR 5533-PP 856-1	40434	Philippines	Breeding and inbred line
v28	Red	IR 9559-3-1-1	40437	Philippines	Breeding and inbred line
v29	Red	IR 9559-4-1-1	40440	Philippines	Breeding and inbred line
v30	Red	IR 9559-5-3-2	40441	Philippines	Breeding and inbred line
v31	Red	IR 9559-PP 871-1	40446	Philippines	Breeding and inbred line
v32	Red	IR 3257-13-56	40497	Philippines	Breeding and inbred line
v33	Red	Chirikata 2	66264	India	-
v34	Red	Ippa	67833	Bhutan	Landrace/Traditional cultivar
v35	Purple	Beu e-soo	73363	Thailand	-
v36	Purple	Daeng se leuad	73403	Thailand	-
v37	Red	Chirikata 1	74580	India	-
v38	Red	Biawboodpae	76318	Thailand	-
v39	Red	Blaunoc	90567	Vietnam	Landrace/Traditional cultivar
v40	Red	Blechucau	90579	Vietnam	Landrace/Traditional cultivar
v41	Red	Ble la	90584	Vietnam	Landrace/Traditional cultivar
v42	Red	Bleliasu	90587	Vietnam	Landrace/Traditional cultivar

Table 2: Rice genotypes used for tocochromanols content

Genotypes	Seed coat color
IR 3257-13-56	red
Khao gam (niaw)	purple
Biawboodpae	red
Chirikata 2	red
IR 5533-55-1-11	red
IR 5533-14-1-1	red

measure the significant differences in the micronutrient content, antioxidant DPPH scavenging activity and tocochromanol content among the genotypes using the SAS software version 9.2.

Results

Micronutrient Contents

The Cu content among all rice genotypes varied from 77.07 to 113.6 mg kg^{-1}, with a mean of 96.65 mg kg^{-1} (Table 3). Highest Cu content were recorded in Ja Loy genotype, followed by Bi-E-Gaw and lowest for Beu E-Soo genotype. The average Zn content of the 42 pigmented rice genotypes was 157.85 mg kg^{-1} and highest Zn content for Biaw Bood Pae and minimum for Blau Noc type. The Mn content ranged from 78.4 to 202.53 mg kg^{-1}, with average of 130.07 mg kg^{-1}. The Mn content were of the Biaw Bood Pae genotype, followed by Mn content of the Bibili al type, while the minimum Mn content were of genotype IR 5533-14-1-1. Similarly, the average Fe content of genotypes ranged from 161 to 523.80 mg kg^{-1}, with mean of 212 mg kg^{-1}. Highest Fe content was recorded in Khao Gam (Niaw) genotype and minimum for Choke Tang type. The abundance of micronutrients ranked in following decreasing order: Fe > Zn > Mn> Cu and the contents of all nutrients except Mn and Zn varied significantly in all rice genotypes ($p \leq 0.05$ and $p \leq 0.01$).

Table 3: Micro-nutrient content of 42 pigmented upland rice genotypes

Accession name	Fe	Mn	Zn	Cu
	mg/kg			
Black Banni	234.80±1.39	88.00±26.82	176.13±8.51	102.80±27.20
258	291.80±10.97	124.13±17.43	176.00±7.94	100.40±24.80
Khao Gam(Niaw)	523.80±5.66	106.13±4.90	159.60±8.24	104.00±26.69
Bi-E-Gaw	218.80±18.01	134.60±8.96	186.27±7.74	104.40±18.31
C	245.60±17.32	104.50±8.02	167.33±2.27	100.00±20.08
Choke Tang	161.00±9.12	98.20±3.35	164.13±19.48	93.73±25.95
Chokoto 14	197.40±16.51	125.07±21.03	159.47±4.14	98.93±26.26
Ja Hau	160.40±15.01	104.67±24.07	173.60±11.35	96.00±23.71
Ja La Shau	163.86±33.76	93.80±5.66	149.73±19.87	97.60±21.41
Ja Loy	226.00±18.24	130.60±12.12	169.87±6.24	113.60±14.83
Ja No Naq	178.80±30.95	123.23±8.08	169.20±5.56	99.60±20.93
Ja Nu Ne Ne	167.80±7.51	166.60±13.51	170.80±5.6	104.13±19.18
Bibili Al	179.00±19.51	200.00±10.85	178.27±7.53	97.47±14.06
Ngacheik	179.80±28.75	146.40±4.16	152.27±9.61	98.93±18.57
IR 9669-22-2-6	235.20±21.48	107.40±12.12	171.33±4.85	102.40±17.33
IR 9669-23-12-7	201.20±18.24	115.20±7.39	134.80±8.65	99.73±15.09
IR 9669-PP 823-1	363.80±23.90	116.80±2.31	143.73±10.10	101.20±15.56
IR 9669-PP 830-1	174.00±18.71	106.80±6.24	144.40±12.98	94.27±14.48
IR 9669-PP 836-1	194.40±19.17	132.20±2.89	160.00±8.83	101.20±17.44
IR 5533-13-1-1	164.60±12.36	116.40±16.21	164.27±11.81	100.40±17.80
IR 5533-14-1-1	181.40±19.98	78.40±29.41	153.73±16.68	110.53±12.75
IR 5533-15-1-1	194.80±17.32	192.27±84.61	163.20±10.36	97.87±15.14
IR 5533-50-1-10	185.60±11.55	171.73±61.36	150.80±1.51	97.73±18.34
IR 5533-55-1-11	185.8±10.51	82.93±41.75	150.27±10.33	109.07±15.85
IR 5533-56-1-12	218.40±16.17	112.80±28.46	164.13±17.41	107.07±9.85
IR 5533-PP 854-1	189.00±7.04	198.53±82.42	159.07±7.79	102.27±15.40
IR 5533-PP 856-1	177.20±14.78	103.20±7.62	168.80±6.04	107.33±11.09
IR 9559-3-1-1	195.80±9.12	110.40±17.33	172.00±9.99	86.93±16.80
IR 9559-4-1-1	199.80±9.12	151.73±56.94	138.67±19.47	96.53±10.96
IR 9559-5-3-2	228.20±7.51	97.60±8.08	154.80±7.61	88.53±15.28
IR 9559-PP 871-1	197.20±12.47	145.33±44.34	149.73±6.81	85.20±20.48
IR 3257-13-56	199.00±8.89	160.53±48.54	147.07±23.87	90.00±19.06
Chirikata 2	203.00±11.43	110.00±13.31	134.27±22.19	92.40±14.42
Ippa	195.80±9.12	93.40±4.73	141.20±13.54	81.33±17.55
Beu E-Soo	197.40±4.04	169.07±68.13	134.40±26.82	77.07±21.80
Daeng Se Leuad	254.20±13.74	106.00±2.77	135.20±17.33	84.00±13.19
Chirikata 1	231.60±10.62	104.40±12.24	141.87±27.20	83.07±15.42
Biaw Bood Pae	193.60±20.55	202.53±92.36	224.67±71.18	87.60±14.41
Blau Noc	225.40±5.20	182.67±73.00	133.20±28.85	86.67±16.46
Ble Chu Cau	207.00±4.50	182.93±74.20	154.40±22.39	93.33±11.61
Ble La	194.80±5.54	156.80±37.03	142.80±14.62	91.07±17.09
Ble Lia Su	187.00±11.20	108.80±3.60	144.13±16.69	92.93±13.28
Mean	212	130.07	157.85	96.65
Std deviation	26.93	30.78	63.19	65.09
CV (%)	27.86	19.5	48.58	30.7

Antioxidant DPPH Scavenging Activity

The total antioxidant DPPH activities ranged from 31.85 to 98.45% (Fig. 1). The DPPH free radical scavenging activity significantly differed ($p \leq 0.01$) among all rice genotypes evaluated in the present study. The DPPH free radical scavenging effect in 9 rice genotypes exceeded 95%: IR 5533-14-1-1, IR 9669-PP 830-1, Ble La, IR 9669-22-2-6, IR 5533-PP 854-1, IR 5533-15-1-1, IR 5533-PP 856-1, IR 9669-PP 836-1, and IR 9669-23-12-7. In addition, the DPPH free radical scavenging effect of 3 genotypes was less than 40%: KhaoGam (Niaw), Ja Loy, and IR 3257-13-56. The average value of the total antioxidant activity of all genotypes was 75.92%.

Tocochromanols Contents

The tocochromanol contents ranged from 18.62 to 28.48 ug g^{-1} for all rice genotypes (Fig. 2). The tocopherol content varied from 6.36 to 11.67 µg g^{-1} in all evaluated rice genotypes. The average values of the total tocopherol and tocotrienols contents were 8.64 and 14.49 µg g^{-1}, respectively. The most abundant tocopherol was α-tocopherol, followed by γ-tocopherol and δ-tocopherol. The content of α-tocopherol was highest in the Khao Gam (Niaw) genotype, followed by IR 5533-55-1-11 (Fig. 3). The concentration of γ-tocopherol was highest in IR 3257-13-56 (5.07 µg g^{-1}), but the concentration of δ-tocopherol was highest in IR 5533-14-1-1 and IR 5533-55-1-11 at 0.32 µg g^{-1}.

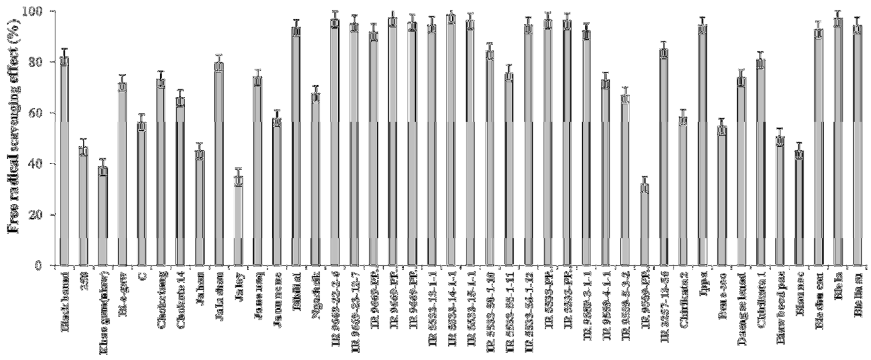

Fig. 1: Free radical scavenging effect of 42 pigmented upland rice genotypes

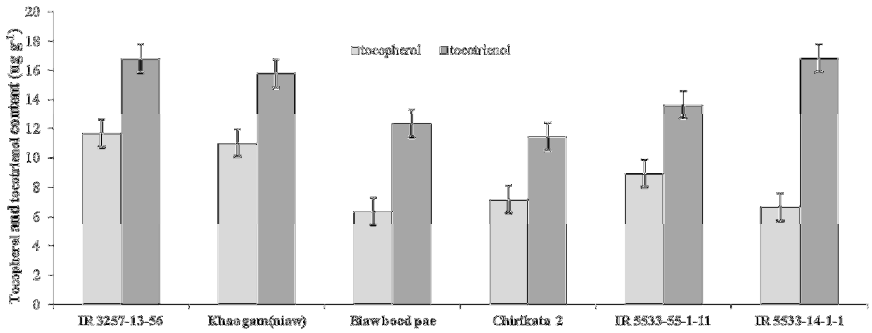

Fig. 2: Tocopherol and tocotrienol content of six selected pigmented upland rice genotypes

The tocotrienol contents ranged from 11.43 to 16.85 µg g^{-1} in remaining evaluated rice genotypes. The most abundant tocotrienol was γ-tocotrienol (10.64 µg g^{-1}), followed by α-tocotrienol (2.56 µg g^{-1}) and δ-tocotrienol (1.29 µg g^{-1}). The content of γ-tocotrienol was highest in genotype IR 5533-14-1-1 (13.63 µg g^{-1}). The contents of α-tocotrienol and δ-tocotrienol were highest in Khao Gam (Niaw) (4.80 µg g^{-1}) and IR 3257-13-56 (1.85 µg g^{-1}), respectively. The tocopherol and tocotrienol contents strongly correlated and the total tocotrienol content was higher than the total tocopherol content in all evaluated rice genotypes.

Discussion

Of all the micronutrients, Fe was the most abundant, followed by Zn, Mn and Cu. Our results differed previously reports in which Zn was most abundant in basmati rice genotypes, followed by Mn, Cu and Fe (Sood *et al.*, 2006). In addition, Fe was most abundant at 49.87 mg kg^{-1} in a core collection of Yunnan rice (Zeng *et al.*, 2004). This value is lower than found in present study due to genotypic characterization and types of soil, varied in nutrient elements. Moreover, the contents of Zn and Fe are two or three times higher in pigmented rice genotypes compared to commercial white rice varieties (Ahuja *et al*., 2007). These pigmented rice genotypes can potentially be used as an alternative food to overcome nutrient deficiency problems. In addition, nutrient deficiency is related to chronic diseases, such as heart conditions, chronic bronchitis and asthma. Nutrients also interact with each other in the body's metabolic functions and can play significant role in maintenance of human health (Marler and Wallin, 2006).

The antioxidant capacity and phenolic compounds in pigmented rice are usually higher than of non-pigmented rice (Norzaleha *et al*., 2012). This difference is due to the abundance of anthocyanin and phenolic compounds in pigmented rice (Zhang *et al*., 2006). The antioxidant activities of red and black rice bran extracts were higher than of white bran extract (Muntana and Prasong, 2010). A study of the antioxidant DPPH scavenging effect in red and black Thailand rice genotypes reported values of 12.99 and 76.38%, respectively (Sompong *et al*., 2011). This range is slightly narrower than reported in our present study due to the differences in the extraction method and evaluated genotypes.

The total tocochromanol content varied in our study. In addition, the tocopherol and tocotrienol contents varied among southern US rice cultivars (Bergman and Xu, 2003). The tocopherol and tocotrienol contents strongly correlated

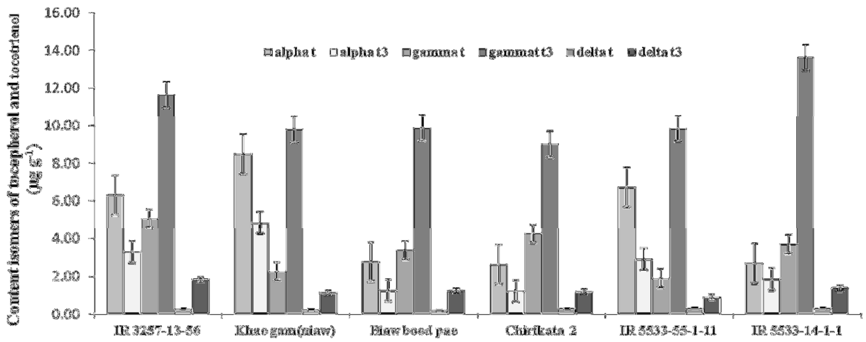

Fig. 3: Isomer of tocopherol and tocotrienol content of six selected pigmented upland rice genotypes

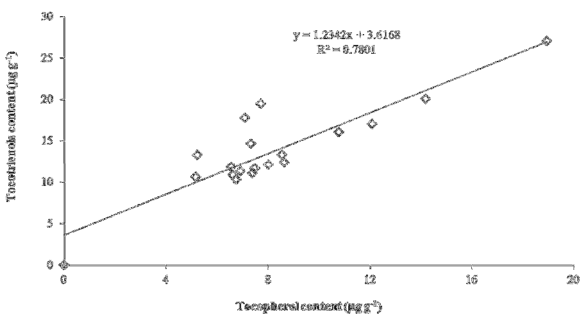

Fig. 4: The correlation between tocopherol and tocotrienols content of six selected pigmented upland rice genotypes

with each other (Fig. 4). The total tocotrienol content was higher than total tocopherol content in all evaluated rice genotypes. Tocotrienol is abundant in cereal grains, such as rice and barley bran (Wang *et al*., 1993). Tocotrienols also have powerful neuro-protective, anti-cancer and cholesterol-lowering properties that significantly affect human health (Colombo, 2010). The tocotrienol and tocopherol contents of red and white pericarp rice variants had been reported 0.68 and 0.40 mg100 g^{-1}, respectively (Fasahat *et al*., 2012). Of the isomers of tocotrienols present, the most abundant isomer was γ-tocotrienol. Schroeder *et al*. (2006) reported that the antioxidant activities of γ-tocotrienols have been shown to be more effective than of α-tocopherol and α-tocopherol was more abundant than the other detected isomers of tocopherol.

Conclusion

All rice accessions showed variation in grain micronutrients and order of increase was Fe > Zn > Mn > Cu. Khao Gam (Niaw) and Ja Loy accessions had highest Fe and Cu content respectively. The total antioxidant activities using the percentage free radical scavenging effect of the stable 1, 1-Diphenyl-2-picrylhydrazyl (DPPH) ranged from 31.85 to 98.45%. Additionally, it also contained high tocochromanols content in seed part. Tocotrienol content was higher than tocopherol among 6 accessions evaluated in the experiment. Among the remaining genotypes, IR3257-13-56, IR5533-14-1-1 and Khao gam (niaw) contained high micronutrient content and antioxidant properties compared to others. Thus, the improvement of nutritional quality in rice plant can be done by using pigmented upland rice genotypes through breeding program.

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