RESEARCH ARTICLE
doi: 10.2306/scienceasia1513-1874.2019.45.509

ScienceAsia 45 (2019): **509–514**

Analysis of nutritional composition, antioxidant activity and callus induction of *Oryza sativa* cultivars Khumthan and Norprae

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Received 2 Apr 2019
Accepted 10 Oct 2019

ABSTRACT: The purpose of this study was to investigate the nutritional composition, antioxidant activity and callus induction of *Oryza sativa* cultivars Khumthan and Norprae, which are the local rice cultivars in Chiang Saen district, Chiang Rai province, Thailand. Nutritional information was examined by AOAC method. It was found that fat, proteins, carbohydrates, crude fibre and ash composition of both rice cultivars were varied in the range of 2.56–2.91, 6.53–9.09, 71.87–76.36, 1.60–2.03, and 1.14–2.10%, respectively. Furthermore, twelve kinds of minerals including N, P, K, Ca, Mg, Mn, S, B, Na, Fe, Zn, and Cu were also detected in both cultivars. For antioxidant analysis, *O. sativa* cultivar Norprae had antioxidant activity of 19.39 μmol TE/g, while cultivar Khumthan had the lower value of antioxidant activity (2.4 μmol TE/g). Callus induction of both cultivars was examined. The seeds were surface sterilized with 15% Clorox for 10 min. The sterilized seed embryos were transferred to MS (Murashige and Skoog, 1962) medium supplemented with 0, 0.5, 1.0, 2.0 mg/l 2,4-D (2,4-dichlorophenoxyacetic acid) or kinetin. The result revealed that MS medium supplemented with 0.5 mg/l 2,4-D provided the highest callus induction of cultivars Khumthan and Norprae with 1.344 and 1.385 g fresh weight, respectively.

KEYWORDS: *Oryza sativa*, antioxidant, 2,4-dichlorophenoxyacetic acid, nutrition, mineral

INTRODUCTION

Rice (*Oryza sativa*) is the most important natural resource for worldwide consumption. There are a lot of *O. sativa* cultivars distributed among various countries and each country has valuable local rice. It is a major source of nutrients, especially carbohydrates. Furthermore, it has antioxidant capacity and other medicinal properties[1]. There are several rice varieties worldwide with different characteristics and chemical compositions. For example, there are differences in the nutrient content (e.g., protein, amylose and fat acidity) between Vietnamese and Japanese rice cultivars[2]. The nutrient and chemical composition of local black rice species in Indonesia has great potential as functional food due to its nutraceutical properties and ability to reduce non-communicable diseases[3]. Some varieties of African (NERICA) rice were analyzed, they contained moisture, ash, fibre, fat, proteins, and carbohydrates in the range of 8.2–9.8, 0.02–0.09, 0.3–0.5, 2.4–5.7, 5.9–13.0, and 74.4–82.8%, respectively. In addition, they contained iron (30–69.3 mg/kg), zinc (2.4–10.4 mg/kg), copper (0.5–4.6 mg/kg), calcium (43.4–146 mg/kg), and magnesium (85.5–368 mg/kg)[4]. The mineral contents vary among rice varieties. The previous research revealed that brown rice provided an essential source of vitamins and minerals[5]. Antioxidant activity was also discovered in the rice extract. For example, in two Iranian rice varieties (Fajr and Tarem), DPPH free radical-scavenging activity was highest (93.91%) at 50 mg/l concentration[6].

Regeneration by callus induction of the rice plant tissue culture was previously examined. Plant growth regulators used for callus induction were cytokinin and auxin, which played a role of callus development and differentiation. The callus induction of some selected basmati rice cultivars of Pakistan (*O. sativa* cv. Basmati 370, basmati 385, Super basmati and Shaheen basmati) were studied. The experiment showed that N6 media containing 5.0 g/l agar and 2.0–2.5 mg/l 2,4-D provided the highest callus induction of 53.0–85.07%[7]. The effect of synthetic auxin 2,4-D was optimized for callus induction of indica rice (*O. sativa*) cultivar ADT 43. Embryogenesis in callus cultures was achieved using LS medium supplemented with 2.5 mg/l 2,4-D and 1.0 mg/l thiamine-HCL[8]. *O. sativa* culti-

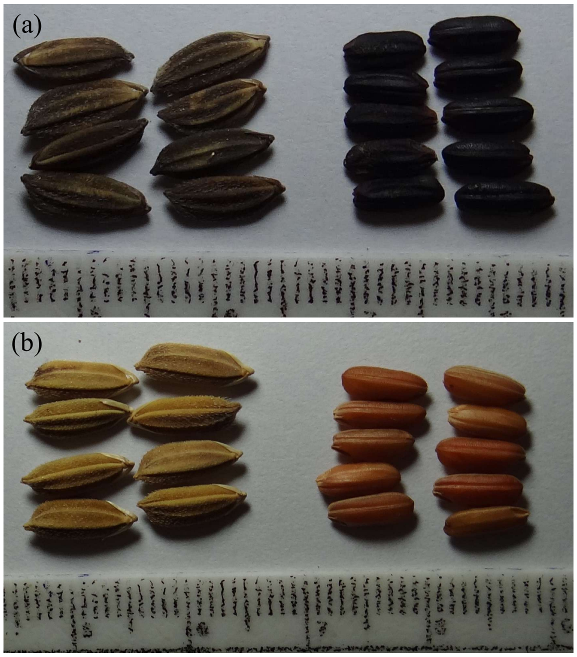

Fig. 1 Grains of *O. sativa* cultivars Khumthan (a) and Norprae (b).

vars Khumthan and Norprae are found in Chiang Sean district, Chiang Rai province, Thailand. Both cultivars have black and red grains, respectively (Fig. 1). The nutritional composition, antioxidant activity, as well as conservation method, were not investigated before. Hence this study aimed to study *O. sativa* cultivars Khumthan and Norprae in the aspects of nutritional information, antioxidant activity and callus induction for conservation and further development for valuable metabolites.

MATERIALS AND METHODS

Nutritional and mineral information of *O. sativa* cultivars Khumthan and Norprae

O. sativa cultivars Khumthan and Norprae were harvested from Chiang Sean district, Chiang Rai province. All of samples were dried, dehulled and weighted (100 g). Then, the proximate analysis of the dried seed was performed by the AOAC method for the composition of fat, protein, carbohydrate, crude fibre, and ash[9]. The total amounts of nitrogen (N), phosphorus (P), boron (B), and sulfur (S) were measured by Kjedahl, Vanadomolybdate, Azomethine-H and $BaCl_2$ method, respectively. Atomic Absorption Spectroscopy was used to quantify calcium (C), magnesium (Mg), iron (Fe), manganese (Mn), copper (Cu), and zinc (Zn). Potassium (K) and sodium (Na) were quantified by Atomic Emission Spectroscopy. All treatments were performed in triplicate.

Antioxidant activity of *O. sativa* cultivars Khumthan and Norprae

The seeds of *O. sativa* cultivars Khumthan and Norprae were dried, dehulled, weighted (100 g), ground and extracted by methanol (Merck, HPLC grade, Germany). Then the sample solution was filtered and evaporated by a rotary vacuum-evaporator. The crude extract was investigated for DPPH (2,2-diphenyl-1-picrylhydrazyl) radical-scavenging activity by the method slightly modified from Brand-Williams et al and Miliauskasa et al[10,11]. Three ml of DPPH solution were added and mixed with 77 (38 or 19 in additional assays) ml methanol extract solution in microcuvettes (ratio of extract to DPPH was approximately 3:1, 1.5:1, 0.75:1). The absorbance (515 nm) was recorded by a UV/visible spectrophotometer after being kept in the dark for 15 min at room temperature. The experiment was performed in triplicate. Trolox was used as a standard antioxidant. The results were expressed in µmol TE/g.

Callus induction of *O. sativa* cultivars Khumthan and Norprae

O. sativa cultivars Khumthan and Norprae seeds were surface sterilized with 15% Clorox for 10 min followed by three times washing with sterile distilled water. The sterilized seed embryos were cut and transferred to MS[12] medium supplemented with 0, 0.5, 1.0, 2.0 mg/l 2,4-D or kinetin for 15 replicates. Sucrose at 30 g/l was added to the medium and the pH was adjusted to 5.8 with 1 M KOH. Agar at 7 g/l was melted and added for solidification. The cultures were placed at 25 ± 2 °C under 16 h/d photoperiod for 4 weeks. Callus induction (%) and callus fresh weight (g) were recorded.

Statistical analysis

The statistical significance of nutritional, mineral information and antioxidant activity were determined by analysis of an independent sample t-test. For callus induction, statistical significance was determined by ANOVA with an adjustment for multiple comparisons with Turkey's test. Values are expressed in triplicate by means ± standard deviation. Values with different superscripts are significantly different ($p \leqslant 0.05$).

Table 1 Nutrient compositions of *O. sativa* cultivars Khumthan and Norprae seeds.

Nutrient composition	Khumthan	Norprae
Fat	2.91 ± 0.04^{a}	2.56 ± 0.06^{b}
Protein	9.09 ± 0.03^{a}	6.53 ± 0.07^{b}
Carbohydrate	71.87 ± 0.09^{b}	76.36 ± 0.12^{a}
Crude fibre	2.03 ± 0.04^{a}	1.60 ± 0.05^{b}
Ash	2.10 ± 0.05^{a}	1.14 ± 0.01^{b}

Values are means ± standard deviation of triplicate determinations. Values in the same row with different superscripts are significantly different ($p \leqslant 0.05$).

Table 2 Mineral compositions of *O. sativa* cultivars Khumthan and Norprae seeds.

Mineral composition	Khumthan	Norprae
N (%)	1.42 ± 0.05^{a}	1.29 ± 0.01^{b}
P (%)	0.41 ± 0.02^{a}	0.36 ± 0.02^{b}
K (%)	0.38 ± 0.03^{a}	0.32 ± 0.04^{a}
Ca (%)	0.27 ± 0.01^{a}	0.20 ± 0.03^{b}
Mg (%)	0.09 ± 0.005^{a}	0.07 ± 0.004^{b}
Mn (mg/kg DW)	29.75 ± 0.50^{a}	28.75 ± 0.15^{a}
S (%)	0.09 ± 0.008^{a}	0.08 ± 0.002^{a}
B (mg/kg DW)	2.77 ± 0.04^{b}	3.26 ± 0.06^{a}
Na (mg/kg DW)	55.52 ± 0.12^{b}	59.04 ± 1.20^{a}
Fe (mg/kg DW)	10.66 ± 0.08^{b}	16.95 ± 0.15^{a}
Zn (mg/kg DW)	47.74 ± 0.14^{a}	38.29 ± 0.23^{b}
Cu (mg/kg DW)	2.62 ± 0.03^{b}	2.92 ± 0.06^{a}

DW = dry weight.

RESULTS AND DISCUSSION

Nutritional and mineral information of *O. sativa* cultivars Khumthan and Norprae

The nutrient compositions of *O. sativa* cultivars Khumthan and Norprae rice are described in Table 1. The result revealed that *O. sativa* cultivar Khumthan had higher amounts of fat, protein, crude fibre, and ash (2.91, 9.09, 2.03, and 2.10%) than *O. sativa* cultivar Norprae. On the other hand, *O. sativa* cultivar Norprae had a higher content of carbohydrates (76.36%) than *O. sativa* cultivar Khumthan. Other rice varieties, such as red and white rice cultivars procured from the local market of Uttarakhand in India, contained 70.16 and 78.34%, respectively [13]. *O. sativa* cultivars Khumthan (black coloured rice) contained high protein content (9.09%) as well. Similarly with the previous experiment of CIC Black rice, the local rice variety from Sri Lanka, which was composed of 10.45% proteins [14] while *O. sativa* cultivar Norprae (red coloured rice) had 6.53%. There were many coloured rice cultivars that contained the amount of protein similar to *O. sativa* cultivars Khumthan. For example, crude protein content was in the range of 5.43–13.83% in brown rice cultivars of Assam, India [15]. *O. sativa* cultivars Khumthan and Norprae was found to have a higher level of protein than some rice varieties grown in Ebonyi state of Nigeria such as Sipi (1.58%), E4077 (4.00%), and Awilo (4.82%) [16]. Furthermore, *O. sativa* cultivar Khumthan could produce more ash content than two varieties of pigmented rice from Thailand (Thai black rice Khao Nim and Thai Jasmine red rice) and one variety of wild rice (*Zizania aquatica*) from Canada. All of the rice provided ash content in the range between 1.40 and 1.61% [17].

The results of mineral analysis of *O. sativa* cultivars Khumthan and Norprae are shown in Table 2. Mineral compositions (N, P, Ca, Mg, and Zn) of cultivar Khumthan were higher than cultivar Norprae. While, other mineral compositions, including Na, Fe, and Cu, of cultivar Khumthan were less than those of cultivar Norprae. However, for some minerals (K, Mn, and S), there was no notable different in quantity. Similarly with previous research, the study presented that black rice had higher contents of Zn, Ca, and Mg than red rice [18]. The levels of mineral compositions of *O. sativa* cultivars Khumthan and Norprae were higher than other rice varieties. For example, the content level of Zn (3.829–4.774 mg%) was higher than some pigmented hill rice cultivars of Assam from India (3.42–4.28 mg%) [19]. *O. sativa* cultivars Khumthan and Norprae also had higher mineral levels in comparison to other rice varieties. The contents of Zn, Mn, and Cu of Irri-6, Irri-9, Sarshar, and DR-83 Pakistani rice varieties were ranged from 1.44–2.97 mg%, 1.57–2.33 mg%, and 0.58–0.92 mg% [20]. In addition, new rice varieties in Ghana were found to contain P, Ca, and K levels of 26.3–51.0 mg%, 5.55–9.53 mg%, and 48.2–76.2 mg%, respectively [21]. From the mineral analysis of *O. sativa* varieties in Nigeria [22], the result revealed that the mineral contents were in the range of 113.07–118.75 mg% for K, 27.96–31.31 mg% for Ca, 24.36–29.81 mg% for Mn, 113.91–121.34 mg% for P, 1.43–2.75 mg% for Zn, 1.18–2.00 mg% for Mn, and 0.14–0.32 mg% for Cu. The nutritional composition could be affected differently between varieties, due to the nature of the soil, environmental conditions, and fertilizers used [23]. The nutritional and medicinal values of some rice varieties varied among different areas and colours of rice. The coloured rice varieties were comparatively identified as more nutritious [24]. The

Table 3 Antioxidant activity of *O. sativa* cultivars Khumthan and Norprae seeds.

O. sativa cultivar	DPPH (µmol TE/g)
Khumthan	2.40 ± 0.06[b]
Norprae	19.39 ± 0.07[a]

study showed that *O. sativa* cultivars Khumthan and Norprae could be functional foods in the future as they contain both essential nutrients and minerals.

Antioxidant activity of *O. sativa* cultivars Khumthan and Norprae seed extract

Antioxidant activity of *O. sativa* cultivars Khumthan and Norprae were quantified and the result is presented in Table 3. It was found that *O. sativa* cultivar Norprae had higher antioxidant activity than *O. sativa* cultivar Khumthan. Antioxidative properties of different rice varieties of pigmented rice in northern Thailand (Chiang Mai black rice, Mali red rice and Suphanburi-1 brown rice) were previously reported. The results revealed that they highly produced phytochemicals, anthocyanin, and free radical scavenging compounds[25]. Furthermore, these pigmented rice varieties had a higher antioxidant efficiency than the white rice. The contents of phenolic compounds, total flavonoid and antioxidants were also detected in a higher quantity than those of non-pigmented rice[26, 27]. Bioactive compounds and antioxidative activities of six coloured rice varieties were measured. The result revealed that the extract contained phenolic compounds, flavonoids, proanthocyanidins, and anthocyanins. Proanthocyanidins could only be detected in red rice, whereas anthocyanins could be detected in black rice. The antioxidant activity of red rice was higher compared to black rice[28]. In addition, antioxidant properties of pigmented rice were related to total soluble phenolic compounds found in each rice variety[29]. Hence it could be concluded that the pigmented rice (especially *O. sativa* cultivars Norprae) were composed of bioactive compounds that attributed to the antioxidant activity. Hence they became valuable natural sources of nutritional foods with antioxidant activity and potentials for other medicinal properties that could lead to development for useful functional products in the future.

Callus induction of *O. sativa* cultivars Khumthan and Norprae

The callus induction of *O. sativa* cultivars Khumthan and Norprae is described in Tables 4 and 5. The

Table 4 Callus induction of *O. sativa* cultivar Khumthan.

Medium	Induction (%)	Fresh weight (g)
MS	0	0[c]
MS+0.5 mg/l 2,4-D	100	1.344 ± 0.091[a]
MS+1.0 mg/l 2,4-D	100	0.121 ± 0.017[b]
MS+ 2.0 mg/l 2,4-D	100	0.113 ± 0.010[b]
MS+0.5 mg/l Kinetin	0	0[c]
MS+1.0 mg/l Kinetin	0	0[c]
MS+2.0 mg/l Kinetin	0	0[c]

Table 5 Callus induction of *O. sativa* cultivar Norprae.

Medium	Induction (%)	Fresh weight (g)
MS	0	0[c]
MS+0.5 mg/l 2,4-D	93.33	1.385 ± 0.058[a]
MS+1.0 mg/l 2,4-D	60.00	0.452 ± 0.040[b]
MS+ 2.0 mg/l 2,4-D	54.00	0.384 ± 0.018[b]
MS+0.5 mg/l Kinetin	0	0[c]
MS+1.0 mg/l Kinetin	0	0[c]
MS+2.0 mg/l Kinetin	0	0[c]

results showed that MS medium supplemented with 2,4-D could induce callus formation and MS medium supplemented with 0.5 mg/l 2,4-D was most efficient for callus induction (1.344 and 1.385 g fresh weight, respectively). MS medium without plant growth regulator and MS medium supplemented with kinetin could not induce callus formation. Callus induction of rice was induced by 2,4-D. From the callus induction of Malaysian rice (*O. sativa* cv. Panderas), callus induction (90%) could be obtained using the medium supplemented with 2,4-D and NAA[30]. Furthermore, 2,4-D also in-

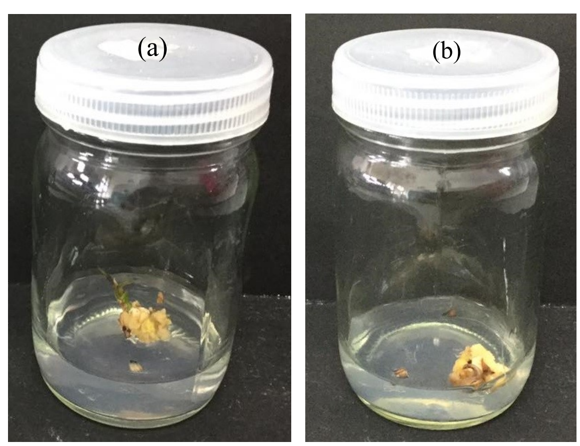

Fig. 2 Callus proliferation of *O. sativa* cultivars Khumthan (a) and Norprae (b).

duced callus formation of *O. sativa* variety PAU 201. It was found that embryogenic calli could be derived from MS medium containing 560 mg/l proline and 1.5–3.5 mg/l 2,4-D and 0.5–1.5 mg/l kinetin[31]. In addition, callus induction of Thai rice (*O. sativa* cv. Khao Daw Mali 105) was investigated. The result presented that modified MS medium supplemented with 2 mg/l 2,4-D, 10 mM proline and 3% sucrose exhibited callus proliferation[32] (Fig. 2).

Multiple callus induction of *O. sativa* cultivar Norprae was performed by MS medium supplemented with 0.5 mg/l 2,4-D treatment. The callus was extracted and quantified for antioxidant activity. The result revealed that the callus exhibited DPPH radical-scavenging activity of 182.18 µmol TE/g, more than that produced from the seeds. The rice callus (*O. sativa*) with supplemented exogenous elicitors could enhance enzymatic antioxidant activity[33]. The callus extraction for valuable compounds has also been investigated in many plant species. In vitro callus cultures of *Hildegardia populifolia* presented the highest antioxidant activity of 3077.98 µmol TE/g extract via internode derived callus[34]. Antioxidant activity estimation from callus culture was also determined in several medicinal plants, e.g., *Gynura procumbens*[35] and *Arachis hypogaea*[36]. Rice callus could be further studied to investigate rapid production of beneficial compounds across elicitor supplementation culture. Furthermore, it has the potential use in rice breeding and genetic improvement under various techniques.

CONCLUSION

O. sativa cultivars Khumthan (black) and Norprae (red) were found to be composed of nutritional information (fat, proteins, carbohydrates, crude fibre and ash) and minerals (N, P, K, Ca, Mg, Mn, S, B, Na, Fe, Zn, and Cu). The antioxidant activity was detected in the range of 2.4–19.39 µmol TE/g. The highest callus induction was obtained in MS medium containing 0.5 mg/l 2,4-D (1.344 and 1.385 g fresh weight for cultivars Khumthan and Norprae, respectively).

Acknowledgements: I gratefully acknowledge the support from the Research and Development Institute, Chiang Rai Rajabhat University, Chiang Rai, Thailand and the Institute of Biodiversity and Environment for Local and Asean Development, Chiang Rai Rajabhat University.

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