

**Food Science and Nutrition**

**REGULAR ARTICLE**

**Genotypic, grain morphological and locality variation in rice phytate content and phytase activity**

**H. H. Lee<sup>1</sup>, C. F. J. Bong<sup>1</sup>, S. P. Loh<sup>2,3</sup>, S. R. Sarbini<sup>1</sup> and P. H. Yiu<sup>1\*</sup>**

<sup>1</sup>*Faculty of Agriculture and Food Sciences, Universiti Putra Malaysia, 97000 Bintulu, Sarawak, Malaysia*

<sup>2</sup>*Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia*

<sup>3</sup>*Laboratory of Molecular Biomedicine, Institute of Bioscience, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia*

**Abstract**

Phytate complexes in whole grain rice are indigestible by human but can be broken down by endogenous phytase enzyme. The inherent phytate content and phytase activity could influence the nutritional quality of whole grain rice. This work aims to determine and identify their variability with genotypes, growing areas and grain morphology. It was found that the whole rice grain was largely high in phytate content (18.20 to 32.36 g/kg) but low in phytase activity (4.77 to 102.65 U/kg), with significant variation among cultivars. Phytate content was marginally different between growing locations but, with no significant difference among their genotypes and grain morphology. This variation could be due to locality factors such as cropping and fertilization practices in the cultivation site. Meanwhile, phytase activity appeared to be determined by genotype, grain width and grain length-to-width ratio. A relatively high phytase activity could be selected from the whole grain rice based on rounded grain and in the genotypic category of L. These types of rice cultivars could reduce the inherent phytate level and improve the nutritional quality of the whole grain rice.

*Key words:* Genotype, Grain morphology, Locality, Phytase activity, Phytate, Whole grain rice

**Introduction**

Whole grain diet is well recognized for counteracting the diet-related diseases and promoting good health (Shahidi, 2009). Whole rice grain supplies not only calories but contains more vitamins, minerals and fiber than its processed equivalents (Champagne et al., 2004; Jones and Engleson, 2010). A wide range of phytochemicals in the rice bran serves as good source of biomedical components for consumers. Nevertheless, they are often either beneficial or deleterious, depending on the dosage and their chemical compositions.

Phytic acid (myo-inositol-1,2,3,4,5,6-hexakis-dihydrogen-phosphate, IP6), is a strong chelating compound, naturally deposited in plant tissues as phytates in globoids (Raboy et al., 2000; Kumar et

al., 2010). The interactions of phytic acid with food components reduce the minerals including phosphorus, protein and starch availability for human body absorption (Oatway et al., 2001; Raboy et al., 2001). However, it provides therapeutic effects under gastro-intestinal conditions by reducing the iron and zinc mediated oxidative stress and blood sugar response, resulting in antioxidant, anti-carcinogenic and anti-diabetic properties (Graf and Empson, 1987; Norazalina et al., 2010).

Phytase is a class of phosphatase that catalyzes sequential dephosphorylation of phytate by releasing phosphates, lower inositol phosphates and chelated components (Bohn et al., 2008; Kumar et al., 2010). Phytases can be found in plant and microbes as the 4/6-phytases (EC 3.1.3.26) and 3-phytases (EC3.1.3.8), but it is absent in gastrointestinal tract of monogastric animals (Konietzny and Greiner, 2002; Bohn et al., 2008). Plant food offer good phytase source for monogastric animal in utilization of phytate complexes, as only minimal phytase activity is contributed by intestinal microflora (Pallauf and Rimbach, 1997).

Received 02 July 2014; Revised 02 August 2014; Accepted 12 August 2014; Published Online 16 September 2014

\*Corresponding Author

P. H. Yiu  
Faculty of Agriculture and Food Sciences, Universiti Putra  
Malaysia, 97000 Bintulu, Sarawak, Malaysia

Email: yiu-ph@upm.edu.my

The phytate level in whole rice grain would depend on food matrix, co-occurrence of other components and agronomic conditions, which are generally categorized as genotype and environmental effects (Kusmann et al., 2007). The influence from genotype and environment are widely reported in cereal plants but their effects often varied (Liu et al., 2005; Polycarpe Kayodè et al., 2006; Dai et al., 2007; Mahmood et al., 2010). Other co-occurrence factors such as inherent phytase activity of whole grain rice could also influence the phytic acid level in whole grain rice. These highlighted the important of current study to clarify the influential factors of phytate content and phytase activity in local rice.

In this study, the variability of phytate content and phytase activity with genotypes, growing locations and grain morphology among thirty Bario rice cultivars from Sarawak, Malaysia was investigated. The determinant factor for the variability was also identified to provide a baseline reference for rice breeders and food processing industries in their nutritional improvement programs.

## Materials and Methods

### Rice collection and samples preparation

Thirty Bario rice cultivars were collected in paddy form from seven locations (Figure 1) in Limbang, Miri and Bintulu division, Sarawak, Malaysia. The Bario rice cultivars were referred to the rice cultivars named after Bario or Adan cropped in the mentioned growing locations. The paddy samples were dehusked manually using mortar and pestle to obtain whole grain rice. Pulverization was done with an electrical blender and sieved through a 425 µm sieve. Moisture content was determined by using a moisture analyzer. Rice cultivars were grown and maintained for deoxyribose nucleic acid (DNA) extraction.

### Phytate content and phytase activity

Phytate content determination was conducted as described by Dost and Tokul (2006). The determination of phytate content using high performance liquid chromatography (HPLC) was based on the complexometric replacement of ferric ion by phytic acid. The HPLC system was equipped with CN3 analytical column (5 µm; 4 x 150 mm) manufactured by GL Science Inc. Chromatogram was monitored at 460 nm using a photodiode array detector. All cultivars were analyzed in triplicate with duplicate injections.

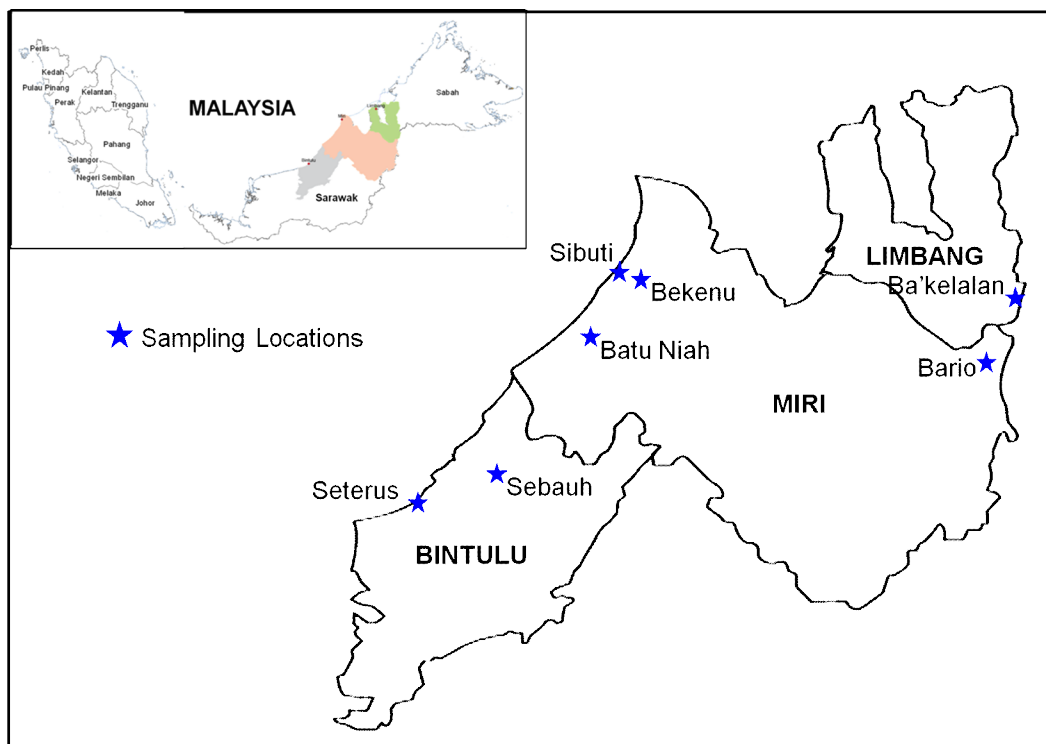


Figure 1. Sampling locations of the study.

Phytase activity was determined as described by Kim and Lei (2005), based on the inorganic phosphorus released during incubation of phytase enzyme for a specified period of time. One unit phytase activity was defined as the amount of enzyme required to liberate one micromole of inorganic phosphate per minute from sodium phytate at pH 5.5 and at 37°C.

### Genetic profiles, grain morphology and growing locations

Leaf samples were harvested from the 20 days old plants for DNA extraction and stored at -80°C. DNA was extracted according to the protocol of GF-1 Plant DNA Extraction Kit (Vivantis Technologies, USA). Polymorphism analysis was performed using the multiplex PCR technique, with four marker panels in triplex combination (Lee et al., 2010). The banding patterns were resolved in 10% polyacrylamide gel electrophoresis, running at 80 volts in a Bio-RAD Mini Protean system for 90 minutes.

Thousand kernels weight (TKW), grain length, grain width and lengths to width (L/W) ratios were characterized according to Juliano (1993). Grain size was deduced by measurement of grain length according Table 1. Grain shape was deduced from the ratio of grain length to width according to Table 2. Meteorological data was obtained from the meteorological department, drainage and irrigation department and soil survey department of Sarawak.

### Statistical analysis

The statistical differences among cultivars and variation with genotypes, grain morphology and growing locations in phytate and phytase activity were estimated from ANOVA test, followed by Duncan New Multiple's Range Test (DMRT) using SAS Version 9.0 (2002; SAS Institute Inc., Cary,

NC, USA). Correlation analyses was tested by the Pearson's Student Correlation at the significant level of P=0.05. The genetic relationship among the cultivars was inferred by a dendrogram generated using the NTSYSpc version 2.20r N software package (Rohlf, 2005).

Table 1. Grain length categories of whole grain rice.

Size	Length (mm)
Extra long	> 7.50
Long	6.61 – 7.50
Medium	5.51 – 6.60
Short	< 5.50

Table 2. Grain shape categories of whole grain rice.

Shape	Length/width ratio
Slender	> 3.0
Medium	2.1 – 3.0
Bold	1.1 – 2.0
Round	<1.0

## Results and Discussion

### Growing locations

Farmers often share their paddy seeds among relatives or villages. Same cultivar may be cropped in different locations under various cropping practices and named according to the seed characteristics. In this study, a total of nine uplands and twenty one lowland Bario rice cultivars were collected from seven growing locations under 3 meteorological stations (Table 3). Ba'kalalan and Bario cropping location gave a large variation in surface air temperature as compared to lowland cropping location. The total rainfall amount and number of rain days between upland and lowland location was similar, except Batu Niah, Bekenu and Sibuti.

Table 3. Growing environmental conditions of sampling locations.

Meteorological station	Sampling location	Total rainfall amount (mm)	No. of rain days	Surface air temperature (°C)	Sunshine hours
Mulu	Ba'kalalan	143.7 - 473.4	17 - 26	22.6 - 34.8	n.a.
	Bario				
Miri	Batu Niah	7.8 - 453.6	3 - 23	26.1 - 27.6	n.a.
	Bekenu				
	Sibuti				
Bintulu	Sebauh	133.2 - 505.8	10 - 26	26.4 - 27.5	5.0 - 7.3
	Seterus				

n.a. indicates data not available.

Table 4. Phytate content, phytase activity and grain morphology of 30 Bario rice cultivars from northern Sarawak, Malaysia.

Code name	Cultivar name	Sampling site/Location	Phytate content (mg/g)	Phytase activity (U/kg)	Max. length (mm)	Max. width (mm)	Length/width ratio	Thousand kernels weight (g)
SH01	Adan Halus	Bario Asal/Bario	22.67 ± 2.10 efghi	57.13 ± 1.01 cdefgh	5.96 ± 0.28cdef	1.71 ± 0.09 a	3.5	12.63 ± 0.64qr
SH02	Adan Sederhana	Bario Asal/Bario	20.92 ± 2.13 ijk	28.55 ± 1.99 jk	5.92 ± 0.27 defg	1.93 ± 0.08def	3.1	13.56 ± 0.03pq
SH03	Adan Merah	Ulong Palang/Bario	23.39 ± 1.31 efghi	17.12 ± 3.08 kl	6.47 ± 0.22cde	1.86 ± 0.23efg	3.5	16.74 ± 0.38 op
SH04	Adan Hitam	Bario Asal/Bario	27.74 ± 1.39bc	4.77 ± 1.63 l	6.10 ± 0.42 b	2.02 ± 0.10 a	3.0	14.22 ± 0.41pq
SH05	Adan Pulut	Bario Asal/Bario	28.49 ± 1.78b	51.97 ± 5.99 efghi	6.89 ± 0.30 a	2.18 ± 0.11 cde	3.1	19.84 ± 0.10 no
SH06	Adan Tuan	Ulong Palang/Bario	20.79 ± 1.98 ijk	102.65 ± 25.34 a	7.07 ± 0.31 a	2.18 ± 0.07bc	3.2	21.33 ± 0.07 n
SH07	Adan Halus	Pa' Ukat/Bario	26.67 ± 3.80bcd	57.89 ± 1.89 cdefgh	6.05 ± 0.28cde	1.77 ± 0.10 a	3.4	11.85 ± 0.20 l
SH08	Adan	Ba'kalalan	18.64 ± 2.53 jk	71.33 ± 1.73 bc	5.63 ± 0.26hijk	1.97 ± 0.10ghijk	2.9	12.45 ± 0.25qr
SH09	Adan Merah	Ba'kalalan	22.33 ± 2.17ghi	54.85 ± 10.21 defgh	5.90 ± 0.21efg	1.69 ± 0.12 a	3.5	10.08 ± 0.05 r
SL10	Bario Pendek	Rh. Henry/Sebauh	18.27 ± 1.95 k	56.19 ± 3.47 cdefgh	5.69 ± 0.27ghijk	2.10 ± 0.14 klmn	2.7	14.44 ± 0.05pq
SL11	Bario Banjal	Rh. Ruma/Seterus	28.26 ± 2.69bc	60.85 ± 7.75 cdef	5.57 ± 0.27jk	2.09 ± 0.10 mn	2.7	14.44 ± 0.09 pq
SL12	Adan Halus	Paya Selanyau/Bekenu	21.62 ± 4.07 hij	71.01 ± 2.22 bc	5.77 ± 0.21fghij	1.73 ± 0.07 b	3.3	10.92 ± 0.16 m
SL13	Adan Sederhana	Paya Selanyau/Bekenu	25.67 ± 1.74bcdef	37.72 ± 5.54 ij	5.49 ± 0.23k	2.10 ± 0.09 mn	2.6	13.33 ± 0.15jk
SL14	Bario	Kpg. Danau/Bekenu	28.05 ± 2.55bc	66.66 ± 8.84 bcd	5.69 ± 0.28ghijk	2.11 ± 0.07 lmn	2.7	14.30 ± 0.33 hi
SL15	Bario (A)	Rh. Unggeh/Sibuti	18.20 ± 2.64 k	48.73 ± 9.00 fghi	5.93 ± 0.40 defg	2.06 ± 0.13ghij	2.9	13.45 ± 0.36 jk
SL16	Bario (B)	Rh. Unggeh/Sibuti	21.66 ± 1.85hij	59.10 ± 18.83 cdefg	5.73 ± 0.23fghijk	1.98 ± 0.09ghi	2.9	13.22 ± 0.30 k
SL17	Bario Merah	Rh. Unggeh/Sibuti	23.38 ± 1.24 efghi	54.09 ± 18.83 cdefg	6.15 ± 0.29cd	1.93 ± 0.13bcd	3.2	14.19 ± 0.32 i
SL18	Bario Sederhana	Kpg. Hunai/Bekenu	22.61 ± 3.06 efghi	55.87 ± 5.25 cdefgh	5.85 ± 0.41efghi	2.22 ± 0.07 mn	2.6	16.32 ± 0.41d
SL19	Bario Brunei	Rh. Ramang/Batu Niah	22.39 ± 2.35fghi	79.04 ± 1.83 b	5.73 ± 0.33fghijk	2.09 ± 0.09 ijklmn	2.7	13.66 ± 0.03 j
SL20	Bario Pendek	Niah/Batu Niah	22.77 ± 1.45 efghi	59.81 ± 3.55 cdefg	5.87 ± 0.23efgh	2.06 ± 0.12hijkl	2.9	13.24 ± 0.29 k
SL21	Bario Pendek	Rh. Ramang/Batu Niah	23.27 ± 2.60 efghi	43.03 ± 5.95hij	5.75 ± 0.27 fghij	2.20 ± 0.09 mn	2.6	14.98 ± 0.16 g
SL22	Bario Pendek	Rh. Tinggang/Batu Niah	24.02 ± 2.14 dfghi	65.52 ± 4.51 bcde	5.77 ± 0.45fghij	2.11 ± 0.12 jklmn	2.7	15.77 ± 0.18 e
SL23	Bario Brunei	Rh. Gamang/Batu Niah	27.80 ± 1.82bc	65.33 ± 6.42 bcde	5.91 ± 0.36 efg	2.25 ± 0.13mn	2.6	16.98 ± 0.26 bc
SL24	Bario Merah	Rh. Daud/ Batu Niah	25.93 ± 1.71bcde	67.04 ± 6.05 bcd	6.55 ± 0.31b	2.21 ± 0.12fgh	3.0	16.67 ± 0.24 c
SL25	Bario Merah	Rh. Gamang/ Batu Niah	32.36 ± 2.31a	22.71 ± 0.93 k	5.61 ± 0.20jk	2.14 ± 0.08 mn	2.6	14.53 ± 0.20 h
SL26	Bario Pendek	Rh Daud/ Batu Niah	25.28 ± 1.45 bcdefg	39.92 ± 4.28 ij	5.56 ± 0.19 jk	2.13 ± 0.06 mn	2.6	14.13 ± 0.08 i
SL27	Bario Pendek	Rh. Gamang/ Batu Niah	24.29 ± 2.14 dfgh	56.23 ± 0.95 defgh	6.54 ± 0.74b	2.21 ± 0.17fgh	3.0	15.30 ± 0.03f
SL28	Bario Selepin	Rh. Gamang/ Batu Niah	25.16 ± 3.37 cdefg	40.64 ± 1.80 ghi	5.93 ± 0.38defg	2.15 ± 0.06ijklm	2.8	14.07 ± 0.17 i
SL29	Bario Tinggi	Rh. Daud/ Batu Niah	28.12 ± 3.44bc	60.56 ± 8.75 cdef	6.20 ± 0.55c	2.41 ± 0.13 n	2.6	17.63 ± 0.12 a
SL30	Bario Tinggi	Rh. Usek/ Batu Niah	25.44 ± 3.80 bcdefg	93.91 ± 8.66 a	5.87 ± 0.33efgh	2.41 ± 0.10 o	2.4	17.25 ± 0.16 b

Values reported were mean ± standard deviation of five replications. Means in the same column with different letter (a-r) are statistically different (Duncan New Multiple's Range Test, P ≤ 0.05).

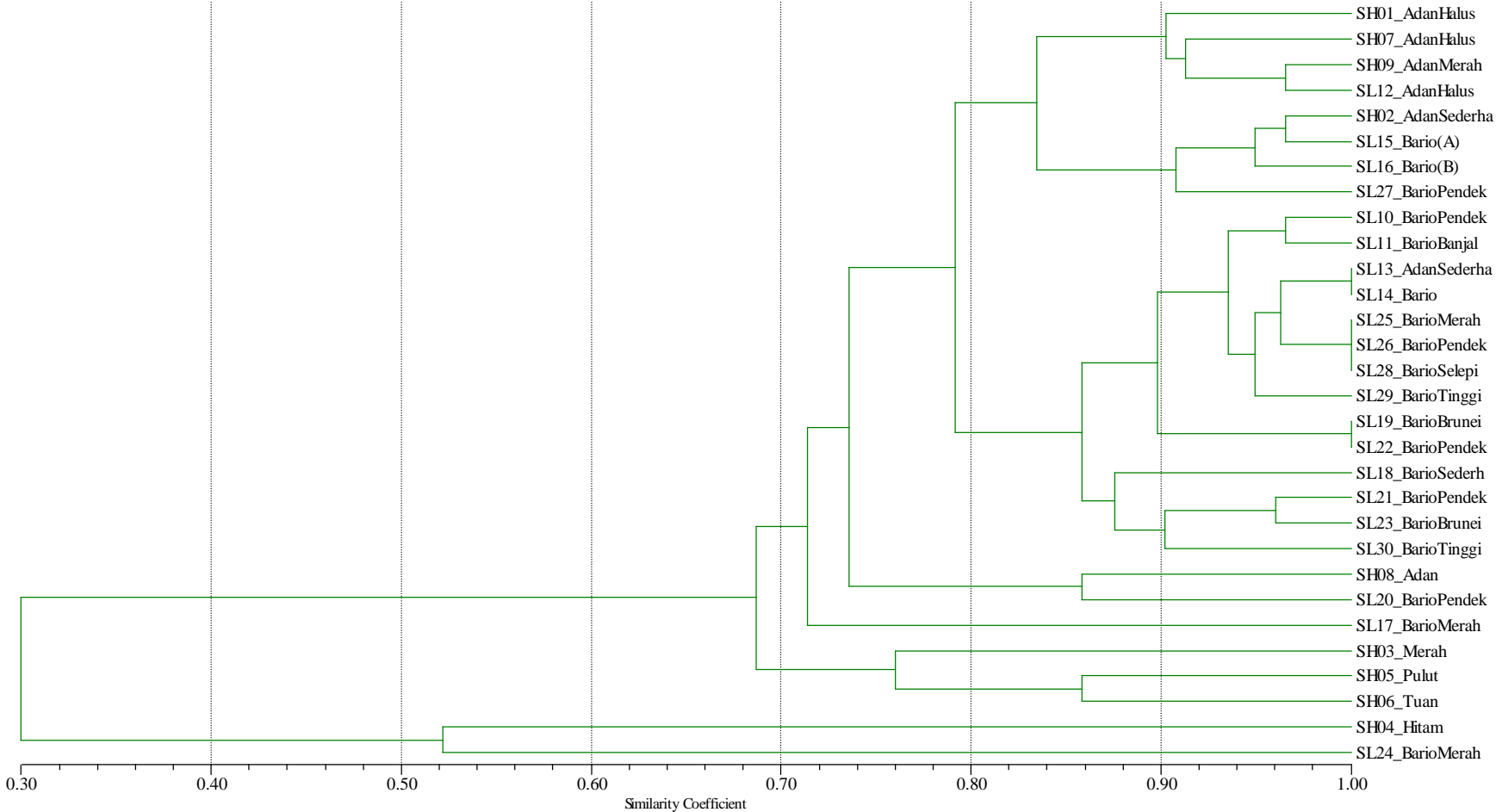


Figure 2. Genetic clustering of 30 Bario rice cultivars based on multiplex SSR marker panels (UPGMA, NTSYS-Pc).

### Grain morphology

Grain morphology is the inherited characters of a variety (Wan et al., 2008; Bai et al., 2010). Different morphology (Table 4) was shown in the rice cultivars although they are given a similar name by farmers. The rice cultivars were differentiated into four physical grain morphology groups, i.e. medium-medium grain, medium-slender grain, long-slender grain and short-medium grain. Medium grain length and shape was the dominance group among the cultivars. Wide range of thousand kernels weights was shown with the values ranging from 10.08 g to 21.33 g. Tuan and Pulut with genotypic similarity of 85.7% were found to be morphological distinct from the remaining rice cultivars.

### Genetic profiles

Genotypic characterization allowed further differentiation of the rice cultivars, being claimed as Bario rice. The rice cultivars are differentiated into fourteen genotypes, consisting of five clusters and nine individual genotypes at 90% similarity based on UPGMA clustering (Figure 2). Genotype A consisting of four rice cultivars related to Adan Halus, which represents the popular rice in Bario Highlands. Adan Sederhana, the second major rice cultivars in Bario Highlands was clustered as genotype B. The largest cluster (genotype C) was consisting of lowland Bario rice cultivars, which includes the Adan Sederhana (SL13) adopted from Bario highlands. Genotypic difference was found between genotype B and C, which could be due to the long term adaptation of Adan Sederhana in the lowland environment (Bajrasharya et al., 2006; Tu et al., 2007). Majority of the rice cultivars are closely related cultivars with similarity more than 70 %. The result showed that Adan Sederhana is the widely adopted highlands cultivar in lowland paddy field.

### Phytate content

Phytate content and phytase activity are the parameters which could influence the antinutritional or antioxidant properties of the whole rice grain. Low phytate content and high phytase activity is often preferable due to well-known anti-nutritional properties of phytic acid. Rice cultivars showed high phytate content (18.20 - 32.36 g/kg, mean of 24.21 g/kg) with significant variation at  $P \leq 0.05$ . The finding was comparable to Chinese rice (7.2 to 11.9 g/kg; mean of 9.6 g/kg), Chinese japonica rice (6.9 to 10.3 g/kg; mean of 8.7 g/kg) and Korean rice (8.6 to 17.6 g/kg; mean of

12.6 g/kg) (Lee et al., 1997; Liu et al., 2005; Liang et al., 2007). High phytate content in rice cultivars implied some advantages in antioxidant properties, yet it showed high anti-nutritional effect in the whole grain.

Phytate content of the rice cultivars revealed a marginal significant variation ( $P < 0.05$ ) among growing locations (Table 5), with no significant correlation with grain morphology and genotype. Accordingly, phytate content was dependent on external factors of growing locations. However, meteorological conditions did not contribute to the influence of growing locations on phytate content. Different cropping and fertilization practices could be the influential factors due to diverse elevation and coastal distance among growing locations. Previous studies reported that phosphorus and zinc fertilization during the grain filling stage greatly accelerates the precipitation and accumulation of phytate (Raboy and Dickinson, 1984; Medeiros Coelho et al., 2002; Kaya et al., 2009).

### Phytase activity

Phytase activity (4.77-102.65 U/kg, mean of 54.99 U/kg) varied significantly among cultivars at  $P < 0.05$ . The phytase activity level was less than 200 U/kg and therefore categorised under low phytase cereal group (Eeckhout and De paepe, 1994). Different phytate content and phytase activity in rice could influence the degradation of the phytate during food ingestion. The low phytase activity could limit the phytate hydrolysis which in turns influences the anti-nutritional effect from phytic acid (Pallauf and Rimbach, 1997).

Phytase activity varied significantly with grain morphology and genotypes but not growing locations. Positive correlation between phytase activity, grain width and length to width ratio (Table 6) suggested that rounded grain had higher phytase activity. Thicker bran layer in short grain rice could facilitate the hydrolysis of phytate (del Rosario et al., 1968; Li and Ding, 2010). Highest phytase activity was represented by genotype L (Table 7). The influence of genotypes on phytase activity may be explained by the gene dependent synthesis of the phytase enzyme (Dionisio et al., 2011). Nevertheless, phytase activity did not significantly varied between the growing locations. These showed that phytase activity is largely controlled by intrinsic factors of genotypes and grain morphology.

Table 5. Effect of growing locations on phytate content and phytase activity in Bario rice.

Location	Cultivars	No. of cultivars	Phytate content (mg/g)	Phytase activity (U/kg)
Ba'kalalan	SH08, SH09	2	20.48bc	63.09a
Bario	SH01 - SH07	7	24.38abc	45.72a
Batu Niah	SL18-SL30	13	25.34ab	57.61a
Bekenu	SL12-14	3	25.11ab	58.46a
Sebauh	SL15-17	3	18.27c	56.19a
Seterus	SL10	1	28.26a	60.85a
Sibuti	SL11	1	21.08bc	53.97a

Means in the same column with different letter (a-c) are statistically different (Duncan New Multiple's Range Test,  $P < 0.05$ )

Table 6. Phytate content and phytase activity of 14 genotype clusters from northern Sarawak Bario rice collection.

Genotype	Cultivars	No. of cultivars	Phytate content (mg/g)	Phytase activity (U/kg)
A	SH01, SH07, SH09, SL12	4	23.32a	60.22abc
B	SH02, SL15, SL16, SL27	4	21.27a	48.15bc
C	SL10, SL11, SL13, SL14, SL25, SL26, SL28, SL29	8	26.39a	48.16bc
D	SL21, SL23, SL30	3	25.51a	67.22ab
E	SL19, SL22	2	23.21a	72.28ab
F	SL18	1	22.61a	55.87bc
G	SH08	1	18.64a	71.33ab
H	SL20	1	22.77a	59.81abc
I	SL17	1	23.38a	54.09bc
J	SH03	1	23.39a	17.12cd
K	SH05	1	28.49a	51.97bc
L	SH06	1	20.79a	102.65a
M	SH04	1	27.74a	4.77d
N	SL24	1	25.93a	67.04ab

Means in the same column with different letter (a-d) are statistically different (Duncan New Multiple's Range Test,  $P < 0.05$ )

Table 7. Pearson correlation matrix between phytate content, phytase activity and grain morphology.

	Phytate content	Phytase activity	Length	Width	L/W Ratio	TKW
Phytate content	1	-0.256	0.263	0.042	0.144	0.194
Phytase activity		1	-0.164	0.509*	-0.538*	0.228
Length			1	0.129	0.470*	0.666*
Width				1	-0.805*	0.699*
L/W Ratio					1	-0.247
TKW						1

Values with asterisk (\*) are significant at  $P < 0.01$ .

## Conclusion

The whole rice grain was generally high in phytate content but low in phytase activity. The phytate content of the whole grain rice was locality dependent, while phytase activity was determined by their genotypes and grain morphology. Rice with rounded grain and in the genotype group of L (Tuan) showed a relatively high phytase activity. This enabled a dynamic phytase enzymatic activity

rice could be selected for reducing their inherent phytate level, and enhancing their nutritional quality of whole grain.

## Acknowledgement

We gratefully acknowledge the Ministry of Higher Education, Malaysia for their financial supports. Heartfelt thanks are also dedicated to the Department of Agriculture Sarawak, National

Paddy Board, and Department of Irrigation and Drainage for their assistance and supports during the course of the study.

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