RESEARCH ARTICLE

SABRAO Journal
of Breeding and Genetics
45 (3) 523-532, 2013

STABILITY AND GENOTYPE BY ENVIRONMENT INTERACTIONS FOR GRAIN ANTHOCYANIN CONTENT OF THAI BLACK GLUTINOUS UPLAND RICE (O*ryza sativa*)

P. SOMSANA[1], P. WATTANA[2], B. SURIHARN[2] and J. SANITCHON[1*]

[1]Department of Plant Science and Agricultural Resources, Faculty of Agriculture, Khon Kaen University, Khon Kaen, 40002, Thailand
[2]Department of Biology, Faculty of Science, Khon Kaen University, Khon Kaen, 40002, Thailand
* Corresponding author's email: jirawat@kku.ac.th

SUMMARY

Grain anthocyanin content is an important trait for breeding and production of black indigenous upland rice as functional food, and the interaction between genotype and environment for this trait has not been clearly investigated. The objectives of this study were to evaluate the effect of genotype, environment and interaction between genotype and environment on grain anthocyanin content in rice grains and to investigate the stability of these black upland rice varieties. Seven genotypes of Thai black indigenous upland rice were laid out in a randomized complete block design with 4 replications in 8 upland field environments during 2010-2011 in the North and the Northeast of Thailand. The results indicated that environment (E), genotype (G), and genotype by environment interaction (G×E) significantly affected anthocyanin content in rice grains. Genotype by environment interaction explained 42.79% of total variation, whereas G and E captured 14.94% and 22.46% respectively. Stability analysis using methods of Eberhart and Russell and GGE-biplot indicated that ULR238 and ULR046 were desirable in terms of the highest ability and stability for anthocyanin content in rice grains and ULR017 was desirable in terms of the highest ability and stability for grain yield. The test site in Khon Kaen province was the best representative of the overall environments and the best environment to discriminate rice genotypes. This is the first detailed investigation in the interaction between genotype and environment effects on grain anthocyanin content in black glutinous upland rice.

Keywords: Breeding, field environments, functional food, grain yield, production

Manuscript received: April 26, 2013; Decision on manuscript: August 8, 2013; Manuscript accepted: August 17, 2013
Society for the Advancement of Breeding Research in Asia and Oceana (SABRAO) 2013

Communicating Editor: Bertrand Collard

INTRODUCTION

Rice is a widely consumed food source for over half of the world's population. There are many different kinds of rice including lowland, deepwater and upland rice, for different growing ecologies. The pericarp colors vary from white to various colors, such as brown, red and black due to different types of pigments. Among them, black rice contains a high level of anthocyanin in aleurone layer (Sutharut and Sudarat, 2012). In Thailand, farmers grow local varieties of black glutinous rice, which are still diverse in genetic and geographical areas. Currently, more attention has been focused on upland rice due to their adaptability to a wide range of unfavorable environments and their resistance to diseases, insects and their high tolerance to drought.

These varieties vary in grain anthocyanin content.

Anthocyanins are present in fruits and vegetables as natural colorants, and are important for human health as a free radical scavenging and antioxidant activity (Chen *et al*., 2006; Stoner, 2009) and can protect metabolic syndrome (Guo *et al*., 2007). In recent years, the diverse protective effects of anthocyanin has been investigated and reported including antioxidant, anti-allergic, anti-inflammatory, anti-viral, anti-proliferative, anti-mutagenic, anti-microbial, anti-carcinogenic, protection from cardiovascular damage, microcirculation improvement, peripheral capillary fragility prevention, diabetes prevention and vision improvement (Dilip and Tetsuya, 2007). In addition to human health benefit, anthocyanin affected growth and development of leaf blight disease in rice caused by *Xanthomonas oryzae* (Padmavati *et al*., 1997; Gandikota *et al*., 2001).

Anthocyanins content in many fruits such as in blueberry, blackberry and black seed coat soybean were greatly affected by genotypes and environments, and high interaction between genotype and environment was also observed (Connor *et al*., 2002; Connor, 2005; Ha *et al*., 2009). In rice, many types of anthocyanins were founded, such as cyanidin 3-O-glucoside, peonidin 3-O-glucoside, malvidin 3-O-glucoside, pelagonidin 3-O-glucoside and delphinidin 3-O-glucoside. Approximately 95% of anthocyanins was cyanidin 3-O-glucoside (Park *et al*., 2008). The degradation rate of anthocyanins increased with increasing temperature and pH values (Hou *et al*., 2011). However, the information on the interaction between genotype and environment on grain anthocyanin content in black indigenous upland rice has not been available. The objectives of this study were to evaluate the effect of genotype, environment and interaction between genotype and environment on anthocyanin content in rice grains and to investigate grain yield and anthocyanin stability of black glutinous upland rice varieties. This information would aid to understand the suitable conditions for production of black glutinous rice with higher grain anthocyanin content and be helpful for plant breeding programs aiming to improve anthocyanin in black glutinous rice.

MATERIALS AND METHODS

Plant material and experimental design

Seven promising landraces (ULR012, ULR017, ULR038, ULR046, ULR238, ULR239 and ULR291) of Thai black indigenous upland rice (*Oryza sativa*) were used in this study (Table 1). These landraces were selected from previous yield trials of glutinous upland rice landraces collected from most parts of the country and stored in germplasm facility at the Preservation and Utilization of Indigenous Rice Project, Faculty of Agriculture, Khon Kaen University.

The study was conducted at 5 experimental sites, consisting of one location in the North (560 m above sea level (m asl)) and 4 locations in the Northeast (180-900 m asl) of Thailand during the year 2010 and 2011. The experiment in the North was conducted in Mae Hong Son (19°32′30″N, 98°13′07″E; 560 m asl) (MHS), and the experimental sites in the Northeast included Khon Kaen University (16°28′30″N, 102°48′36″E; 180 m asl) (KKU), Ban Had district, Khon Kaen (16°12′49″N, 102°48′48″E; 230 m asl) (BNH), Phu Rua district, Loei (17°17′59″N, 101°24′36″E; 600 m asl) (PHR) and Dan Sai district, Loei (17°22′59″N, 101°16′06″E; 900 m asl) (DS). Because of the problem of accessibility to the remote test sites, the experiment at PHR and DS was undertaken for 1 year only, whereas the experiment at KKU, BNH and MHS was conducted for 2 years. Therefore, there were 8 environments totally, and the 7 landraces were evaluated in a randomized complete block design with 4 replications at 8 environments under rainfed conditions (Tables 2 and 3)

Crop management and data collection

Conventional tillage was practiced for soil preparation. The seeds were sown by drilling in 2 x 4 m^2 plots consisting of 8 rows with a spacing of 25 cm between rows and 25 cm between hills within rows. There were 16 hills in a row. The seeding rate was about 160 seeds m^{-2}. The first split of Fertilizers was applied to the plots at the rates of 23 kg N ha^{-1}, 23 kg P_2O_5 ha^{-1} and 23 kg K_2O ha^{-1} at 30 days after planting (DAP) and the second split of fertilizers was

applied to the plots at the rates of 38 kg N ha^{-1}, 23 kg P_2O_5 ha^{-1} and 23 kg K_2O ha^{-1} at 60 DAP or before stem elongation. At harvest, the rows at the border of each plot were discarded and the area of 1.5 m x 3.5 m was harvested. The yield was determined in t/ha. Soil and meteorological data of each environment were collected and summarized in Table 2.

The data were recorded for tiller number, plant height, leaf length, leaf width, ligule length, days to flowering, panicle number, panicle length, dry weight, harvested index, seeds per panicle, filled grains, unfilled grains, grain length, grain width, grain thickness, 100 grain weight and grain yield. The detailed explanations of data collection for rice were described previously (IRRI, 1996)

Anthocyanin Extraction

The rice grain sample of 5 grams for each plot was hulled, and the hulled sample was ground into powder using mortar and pestle. The ground rice power of 0.2 g for each plot was used for extraction of anthocyanin in ethanol containing 1.0% (v/v) trifluoroacetic acid (TFA) for 24 hours at room temperature. The extracts were passed through a 0.45-µm filter before injection into a reverse-phase column of high performance liquid chromatography (HPLC) machine (Shimadzu LC-20AD) (Ryu *et al*., 2003). C18 interstill 25x with guard column were initially compared with direct sample injection. Flow rate was 0.6 ml/min; injection volume 20 µl. The mobile phase was (A) 0.5% formic acid in water and (B) 100% methanol.

Statistical Analysis

Data collected from 8 environments were analyzed individually according to a randomized complete block design (Gomez and Gomez, 1984).

$$Y_{ij} = \mu + G_i + B_j + e_{ij}$$

where Y_{ij}= observed phenotype of *i*th genotype in *j*th block, µ=overall mean, $G_{i=}$ effect of *i*th genotype, B_j= effect of *j*th block, e_{ij}= effect of experiment error

Error variances for each traits were test for variance homogeneity using Bartlett's test (Snedecor and Cochran, 1983), and data the traits with homogenous variances were combined.

$$Y_{ijk} = \mu + G_i + E_j + B_{j/k} + (GxE)_{ij} + e_{ijk}$$

where Y_{ijk}= observed phenotype of *i*th genotype in *k*th block under *j*th environment, µ=overall mean, $G_{i=}$ effect of *i*th genotype, E_j= effect of *j*th environment, $B_{j/k}$= effect of *k*th block under *j*th environment, $(GxE)_{ij}$= effect of interaction between *i*th genotype and *j*th environment, e_{ijk}= effect of experiment error.

When the main effects (genotype and location) were significant, least significant difference (LSD) at 0.05 probability level was used to separate means.

Stability of grain anthocyanin content and grain yield were determined by method of regression analysis (Eberhart and Russell, 1966) using computer program statistix8 analytical software (Statistix8, 2003) and by method of GGE-biplot (Yan *et al*., 2000). The concept of GGE-biplot was developed by Gabriel (1971) to graphically display a rank 2 matrix that is a matrix resulting from multiplying a matrix with 2 columns by a matrix with 2 rows. GGE-biplot was constructed by 2 principle components (PC1 and PC2), derived from subjecting the environment-centered data to singular value decomposition. An ideal cultivar should have a large PC1 score (high yielding ability) and a small PC2 score (high stability) (Yan *et al*., 2000).

RESULTS

Analysis of variance

Significant differences ($P < 0.01$) among environments were observed for most characters that were studies except for grain length (Table 4). Genotypes were also significantly different ($P < 0.01$) for most characters except for total dry weight and grain yield. Genotype by environment interactions were also significant ($P < 0.01$) for most characters except total dry

weight, harvest index, grain length and grain width.

For grain anthocyanin content and grain yield which were the most important traits in this study, environment and genotype by environment interactions played an important role for both characters, whereas genotype was important for variation in anthocyanin only. Environment explained 22.46% of total variation for grain anthocyanin content, whereas genotype and genotype by environment interaction captured 14.94% and 42.79% respectively. In contrast to grain anthocyanin content, environment accounted for the largest portion of total variation in grain yield (46.25%) followed by genotype by environment interaction (16.25%) and genotype (2.67%), respectively.

Means of genotypes and environments for grain yield and grain anthocyanin content

Grain anthocyanin contents ranging from 0.94 mg/g for the variety ULR239 in DS11 to 5.98 mg/g for the variety ULR239 in KKU10 were observed across environments (Table 5).

Grain yields ranging from 0.97 t/ha for the variety ULR038 at in MHS10 to 3.95 t/ha for ULR239 in BNH10 were observed across environments (Table 6).

Stability for grain anthocyanin content and grain yield

Means for grain anthocyanin content of 7 glutinous rice genotypes averaged across 8 environments ranged from 2.45 mg/g in ULR038 to 4.03 mg/g in ULR238, and means for grain yield averaged across 8 environments ranged from 2.37 t/ha in ULR012 to 2.66 t/ha in ULR017 (Table 7).

Regression coefficients (b_i values) ranging from -0.10 to 2.29 were observed for grain anthocyanin content. Significant deviations from 0.0 were indicated in ULR107 (0.89**), ULR239 (2.29**), whereas deviation from 1.0 was detected in ULR239 (2.29**) only. The range of regression coefficients indicated differential responses of genotypes to environmental changes.

Table 1. List of Thai black indigenous upland rice genotypes and character of seeds.

Genotype	Source	Morphology of seeds
ULR012	Chiang Mai province	Short, length/width similar, brown/red
ULR017	Phitsanulok province	Long, less thickness, brown
ULR038	Phitsanulok province	Short, spherical, brown/red
ULR046	Phitsanulok province	Short, light brown
ULR238	Phetchabun province	Long, dark brown/black
ULR239	Phetchabun province	Short, less thickness dark brown/black
ULR291	Phatthalung province	Long, less thickness, brown

Table 2. Soil data of the 8 environments.

Environment	Soil type	Soil pH	O.M (%)	Total N (%)	Available P_2O_5(ppm)	Exchangeable K_2O (ppm)	Altitude (m.asl)
KKU10	sandy	7.15	0.435	0.019	51.43	64.32	180
BNH10	sandy	5.26	0.203	0.015	5.14	20.31	230
MHS10	sandy loam	6.67	1.556	0.082	17.48	167.31	560
KKU11	sandy	7.38	0.422	0.018	51.12	63.53	180
BNH11	sandy	5.43	0.266	0.013	7.38	51.20	230
MHS11	sandy loam	6.27	2.295	0.104	22.73	123.31	560
PHR11	sandy clay loam	5.18	1.164	0.027	10.13	144.12	600
DS11	sandy clay loam	4.66	1.215	0.072	4.35	143.14	900

Table 3. Meteorological data of the 8 environments.

Environment	Crop Rain-fall (mm)	Max-temp. (°C)	Min-temp. (°C)	Sowing Date	Harvest Date
KKU10	725	32.81	24.47	07.07.10	26.11.10
BNH10	772	32.86	24.20	05.06.10	21.10.10
MHS10	1402	31.04	24.34	22.06.10	28.10.10
KKU11	1072	32.18	23.97	14.06.11	27.10.11
BNH11	846	31.89	23.92	15.06.11	26.10.11
MHS11	1459	30.34	21.52	28.05.11	20.10.11
PHR11	823	29.6	19.07	13.06.11	30.10.11
DS11	1291	30.66	22.83	02.07.11	30.10.11

Table 4. Grain anthocyanin content of black indigenous rice genotypes on 8 environments.

Genotype	Grain anthocyanin content (mg/g seed)							
	KKU10	BNH10	MHS10	KKU11	BNH11	MHS11	PHR11	DS11
ULR012	3.84	3.14	3.64	2.89	2.69	2.91	2.21	3.92
ULR017	4.75	3.32	3.76	2.98	2.72	2.88	2.83	1.87
ULR038	3.03	1.49	1.41	2.92	1.83	2.51	5.17	1.24
ULR046	4.38	4.06	4.57	3.00	3.09	3.95	5.34	3.56
ULR238	5.32	5.75	4.55	2.92	5.52	1.75	3.50	2.93
ULR239	5.98	3.69	3.06	2.03	3.61	2.18	4.97	0.94
ULR291	3.09	4.54	3.60	2.49	3.89	2.65	2.86	2.44
Mean	4.34	3.71	3.51	2.75	3.34	2.69	3.84	2.41
F-test	**	**	**	ns	**	*	**	**
$LSD_{(0.05)}$	0.94	0.66	0.82	0.92	1.12	1.04	0.84	0.85
C.V. (%)	14.49	12.02	15.70	22.62	22.50	26.07	14.71	23.81

ns = not significant; * = significant at 0.05 probability level; ** = significant at 0.01 probability level.

Table 5. Grain yield of black indigenous rice genotypes on 8 environments.

Genotype	Grain yield (t/ha)							
	KKU10	BNH10	MHS10	KKU11	BNH11	MHS11	PHR11	DS11
ULR012	2.81	3.28	1.27	2.32	3.61	1.19	1.61	2.89
ULR017	2.81	3.72	1.89	2.18	3.31	2.36	2.01	2.97
ULR038	3.01	3.69	0.97	2.58	3.78	1.26	2.19	2.66
ULR046	2.74	3.44	1.85	2.65	3.26	1.46	1.37	2.91
ULR238	3.54	2.67	1.65	3.09	2.43	1.28	1.81	2.98
ULR239	3.07	3.95	1.79	3.02	2.87	1.48	1.66	2.97
ULR291	2.30	3.65	1.92	2.27	3.12	2.20	1.79	2.85
Mean	2.90	3.49	1.62	2.59	3.20	1.61	1.78	2.89
F-test	*	ns	**	**	*	**	ns	ns
$LSD_{(0.05)}$	0.61	0.87	0.40	0.51	0.74	0.28	0.78	0.53
C.V. (%)	14.25	16.87	16.73	13.18	15.49	11.66	29.39	12.30

ns = not significant; * = significant at 0.05 probability level; ** = significant at 0.01 probability level.

Table 6. Sum of square percentage of 19 traits of 7 black indigenous upland rice genotypes analyzed over environments.

Traits	Sum of squares percentage (%)				C.V. (%)
	Environment	Genotype	G x E	Error	
Anthocyanin content	22.46 **	14.94 **	42.79 **	18.81	18.39
Yield	46.25 **	2.67 ^{ns}	16.25 **	34.83	16.16
Tiller number/hill	51.24 **	5.05 **	16.67 **	27.04	15.37
Plant height	58.57 **	10.47 **	11.60 **	19.36	6.05
Leaf length	26.47 **	8.87 **	31.67 **	32.98	6.69
Leaf width	32.78 **	21.16 **	13.40 **	32.66	7.27
Ligule length	17.11 **	46.10 **	17.57 **	19.22	8.42
Days to flowering	59.00 **	28.53 **	9.26 **	3.21	1.18
Panicle no./hill	24.29 **	10.13 **	17.70 **	47.88	11.83
Panicle length	20.87 **	48.84 **	13.51 **	16.78	4.06
Dry weight	21.02 **	1.05 ^{ns}	16.47 ^{ns}	61.46	28.78
Harvested index	29.77 **	11.96 **	15.56 ^{ns}	42.71	15.13
Seeds/panicle	34.29 **	18.98 **	14.48 **	32.25	15.35
Filled grains/panicle	50.12 **	12.13 **	12.24 **	25.51	15.44
Unfilled grains/panicle	16.25 **	14.01 **	35.69 **	34.05	33.44
Grain length	0.55 ^{ns}	88.70 **	2.44 ^{ns}	8.31	2.94
Grain width	4.16 **	85.26 **	2.35 ^{ns}	8.23	4.00
Grain thickness	6.18 **	62.55 **	11.27 **	20.00	2.88
100 grain weight	33.45 **	42.36 **	12.98 **	11.21	4.48

ns = not significant; ** = significant at 0.01 probability level.

Table 7. Estimates of stability and adaptability parameters of grain anthocyanin content and grain yield.

Genotype	Grain anthocyanin content (mg/ g seed)				Grain yield (t/ha)			
	Mean	b_i [(1)]	s^2_{di} [(2)]	R^2_i [(3)]	Mean	b_i	s^2_{di}	R^2_i
ULR012	3.16	-0.10 ns[a]	0.64*	0.01	2.38	1.21**[a], ns[b]	0.22	0.95
ULR017	3.14	0.89*[a], ns[b]	0.62	0.53	2.66	0.79**[a], ns[b]	0.32	0.80
ULR038	2.45	0.86 ns[a]	1.25	0.21	2.52	1.29**[a], ns[b]	0.37	0.89
ULR046	4.00	0.75 ns[a]	0.63	0.45	2.46	1.04**[a], ns[b]	0.20	0.94
ULR238	4.03	1.40 ns[a]	1.18	0.44	2.43	0.80*[a], ns[b]	0.55	0.58
ULR239	3.31	2.29**[a], **[b]	0.42**	0.94	2.60	1.10**[a], ns[b]	0.29	0.91
ULR291	3.13	0.50 ns[a]	0.72	0.22	2.51	0.75**[a], ns[b]	0.34	0.76
Mean	3.31±0.40				2.51±0.003			

[(1)] = Regression coefficient; [(2)] = Deviation from regression; [(3)] = Coefficient of determination
* = significant at 5% level; ** = significant at 1% level
a = different from 0.0; b = different from 1.0

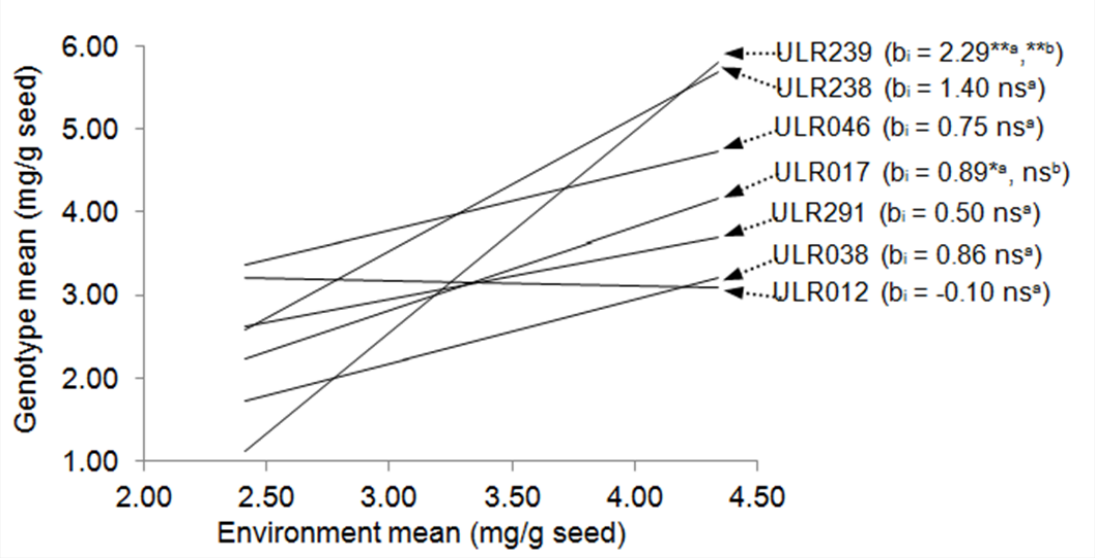

Figure 1. Regression lines of 7 black upland rice genotypes on grain anthocyanin content.

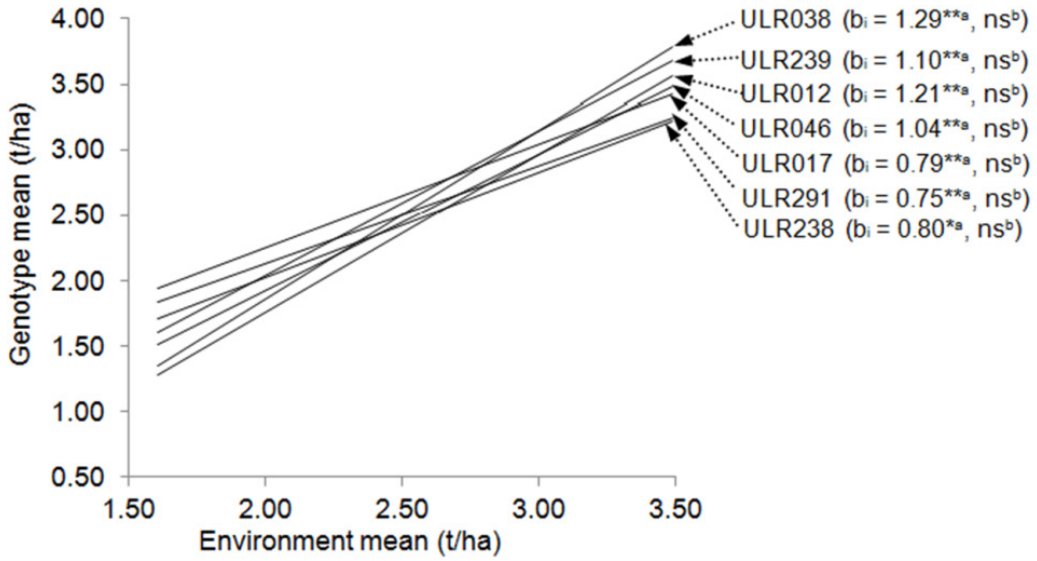

Figure 2. Regression lines of 7 black upland rice genotypes on grain yield.

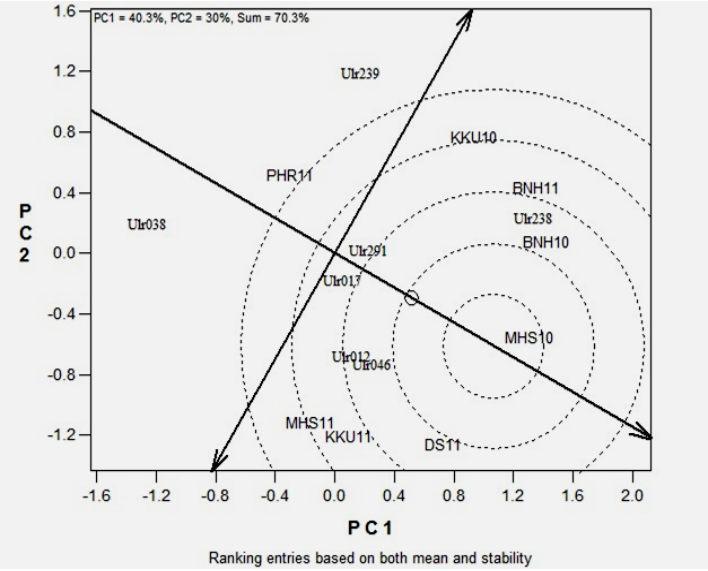

Figure 3. GGE-biplot based on genotype focused scaling for comparison the genotypes with the ideal genotype on grain anthocyanin content

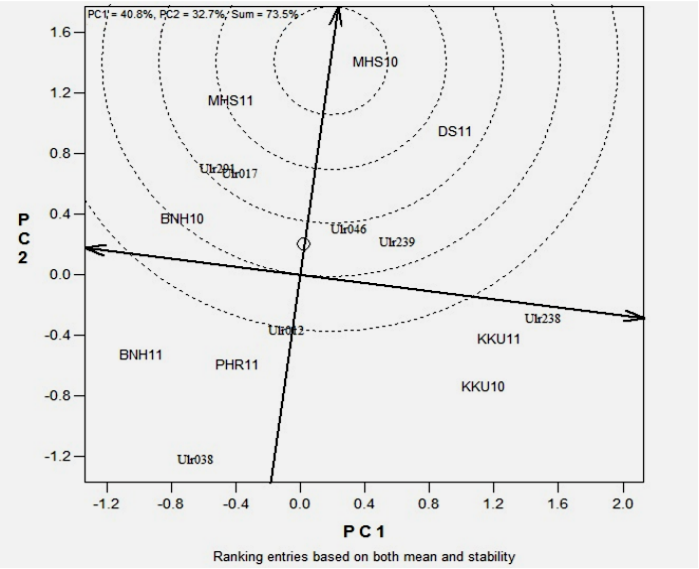

Figure 4. GGE-biplot based on genotype focused scaling for comparison the genotypes with the ideal genotype on grain yield.

DISCUSSION

Means of genotypes and environments for grain yield and grain anthocyanin content

Genotypes were significantly different for grain anthocyanin content in most environments except for KKU11. On average, DS11 environment had the lowest mean (2.41 mg/g), whereas KKU10 had the highest mean (4.34 mg/g).

Grain yield was highly unstable. This performance varied across environments. However, genotypes in 3 of 8 environments were not statistically different for grain yield, and the significant differences among glutinous rice varieties were observed in KKU10, MHS10, KKU11, BNH11 and MHS11 only. BNH10 had the highest environmental mean (3.49 t/ha), whereas MHS10 and MHS11 had the lowest environmental means (1.62 and 1.61 t/ha, respectively).

Stability for grain anthocyanin content and grain yield

According to Eberhart and Russell (1966) analysis, effects of genotype by environment were further partitioned into linear (b_i) and non-linear (s^2_{di}) components to estimate the stability of genotypes for grain anthocyanin content and grain yield of black glutinous rice.

Regression coefficients (b_i) approximating 1.0 coupled with standard deviation (s^2_{di}) of zero indicates average stability of genotypes. Regression coefficients greater than 1.0 show high sensitivity to environmental changes for greater specificity of adaptability to favorable environments, while regression coefficients below 1.0 indicate greater resistance to environmental change for greater specificity of adaptability to unfavorable environments (Wachira *et al*., 2002).

ULR238 and ULR046 were stable genotypes with high grain anthocyanin content (Figure 1). In addition, ULR239 was the most sensitive genotype to environmental change with a high specific adaptability (b_i = 2.29) for environment of KKU10 (Table 7). ULR017 had rather stable anthocyanin as indicated by its regression coefficient (b_i = 0.89**), but its grain anthocyanin content was rather low.

Stability analysis for grain yield and grain anthocyanin content using GGE-biplot method and the method suggested by Eberhart and Russell (1966) provided similar results, and ULR238 and ULR046 were the most stable genotypes for grain anthocyanin content (Figure 3), whereas ULR017 and ULR291 were the most stable genotypes for grain yield (Figures 2 and 4).

This is the first detailed investigation in the interaction between genetic and environmental effects on grain anthocyanin content of black upland rice. G x E interaction contributed to large portion of variation in grain anthocyanin content in black glutinous rice. Favorable environment for high grain anthocyanin content of each genotype was different. The highest grain anthocyanin content genotype recorded for each environments were ULR239 and ULR238 at KKU10, ULR238 at BNH10, ULR046 ULR238 and ULR017 at MHS10, ULR238 at BNH11, ULR046 and ULR012 at MHS11, ULR046 ULR038 and ULR239 at PHR11 and ULR012 and ULR046 at DS11. Although anthocyanin content in KKU10 as significant difference, not significant difference was presented in KKU11.

CONCLUSIONS

The effects of genotype, environment and genotype by environment interactions and stability of 7 black indigenous upland rice varieties for grain yield and grain anthocyanin content were investigated across 8 environments. Genotype by environment interaction contributed to the largest portion of total variation in grain anthocyanin content in rice grains, whereas environment contributed to the largest portion of total variation in grain yield. The results of stability analysis obtained from method of Eberhart and Russell (1966) corresponded to those from GGE-biplot. ULR238 and ULR046 were identified to have high stability for grain anthocyanin content, whereas ULR017 has high stability for grain yield. The experimental site in Khon Kaen

province was the best environment for grain yield and grain anthocyanin content.

ACKNOWLEDGEMENTS

Financial support for this research was provided by the Food and Functional Food Cluster under the National Research University Program of Khon Kaen University.

REFERENCES

Chen PN, Kuo WH, Chiang CL, Chiou HL, Hsieh YS, Chu SC (2006). Black rice inhibit cancer cells invasion via repressions of MMPs and u-PA expression. *Chem Biol. Interact*. 163: 218–229.

Connor AM (2005). Genotype and environmental variation in anthocyanins and their relationship to antioxidant activity in blackberry and hybridberry cultivars. *J. Am. Soc. Hortic. Sci.* 130: 680–687.

Connor AM, Luby JJ, Tong CS (2002). Genotype and environment variation in antioxidation activity, total phenolic content, and anthocyanin content among blueberry cultivars. *J. Am. Soc. Hortic. Sci*. 127: 89–97.

Dilip G, Testuya K (2007). Anthocyanins and anthocyanin-rich extracts: role in diabetes and eye function. *Asia Pac. J. Clin. Nutr*. 16: 200–208.

Eberhart SA, Russell WA (1966). Stability parameter for comparing varieties. *Crop Sci*. 6: 36–40.

Gabriel KR (1971). The biplot graphic display of matrices with application to principal component analysis. *Biometrika* 58: 453-467.

Gandikota M, Kochko A, Chen L, Ithal N, Fauquet C, Reddy AR (2001). Development of transgenic rice plants expressing maize anthocyanin genes and increased blast resistance. *Mol. Breed*. 7: 73–83.

Gomez KA, Gomez AA (1984). Statistical Procedures for Agricultural Research. 2nd ed. John Wiley and Sons, New York.

Guo H, Ling W, Wang Q, Liu C, Hu Y, Xia M, Feng X, Xia X (2007). Effect of anthocyanin-rich extract from black rice (*Oryza sativa* L. *indica*) on hyperilipidemia and insulin resistance in fructose-fed rats. *Plant Foods Hum. Nutr*. 62: 1–6.

Ha TH, Lee JH, Shin SO, Shin SH, Han SI, Kim HT, Ko JM, Lee MH, Park KY (2009). Change in anthocyanin and isoflavone concentration in black seed-coated soybean at different planting locations. *J. Crop Sci. and Biotechnol*. 12: 79–86.

Hou Z, Qin P, Zhang Y, Cui S, Ren G (2011). Identification of anthocyanins isolate from black rice (Oryza sativa L.) and their degradation kinetics. Food Research International 50: 691-697. IRRI (1996). Standard Evaluation System for Rice. IRRI. Philippines.

Lee JH (2010). Identification and Quantification of anthocyanin from the grains of black rice (*Oryza sativa* L.) varieties. *Food Sci. Biotechnol*. 19: 391–397.

Padmavati M, Sakthivel N, Thara KV, Reddy AR (1997). Differential sensitivity of rice pathogens to growth inhibition by flavonoids. *Phytochemistry* 46: 499–502.

Park, Young S, Sun-Joong K, Hyo-Ihl C (2008). Isolation of anthocyanin from black rice (Heugjinjubyeo) and screening of its antioxidant activities. *Kor. J. Microbiol. Biotechnol*. 36: 55–60.

Ryu SN, Park SZ, Kang SS, Hang SJ (2003). Determination of C3G content in blackish purple rice using HPLC and UV-Vis spectrophotometer. *Korean Journal. Crop Sci*. 48: 369–371.

Snedecor GW, Cochran WG (1983). Statistical Methods (6th Ed.) Oxford and IBH, New Delhi.

Statistix8 (2003). Statistix8: analytical software user's manual. Tallahassee, Florida.

Stoner GD (2009). The preclinical and clinical development of berries. *Cancer Prev. Res*. 2: 187–194.

Sutharut J, Sudarat J (2012). Total anthocyanin content and antioxidant activity of germinated colored rice. *International Food Research Journal* 19: 215–221.

Wachira F, Wilson NG, Omolo J, Mamati G (2002). Genotype x environment interactions for tea yields. *Euphytica* 127: 289–296.