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Variation of grain nutritional quality among Thai purple rice genotypes grown at two different altitudes

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ABSTRACT: Genotypic variation and nutritional quality of rice has been established, but environmental effects on the genotype are unknown. This study determines how nutritional quality, such as pericarp colour and antioxidant capacity, of purple rice can vary when grown under different environments. Nine purple rice genotypes and Khao Dok Mali 105 (KDML105, a non-pigmented rice) were grown at 2 different altitudes (330 m and 800 m above mean sea level, designated lowland and highland, respectively) at Chiang Mai, Thailand. Grain yield, Zn, anthocyanin concentration, and anti-oxidative capacity of the rice genotypes varied significantly in direction and magnitude. Grain Zn was higher in the lowland, but with differences between altitudes ranging from 16% to 50% among the purple rice genotypes, while non-pigmented KDML105 was among the lowest in grain Zn concentration at both altitudes. Some genotypes produce rice with more intense pigmentation and higher concentration of monomeric anthocyanin in the highland, some did so in the lowland, while no altitude effects were seen in others. Antioxidant capacity (Trolox equivalent) of the rice increased with increasing concentration of anthocyanin ($R^2 = 0.72$, $p < 0.01$), and varied in a multiple regression with anthocyanin and Zn concentration ($R^2 = 0.75$, $p < 0.01$). The effect of altitude on variation of grain nutritional quality among purple rice genotypes between the two growing conditions should be taken into consideration in efforts to enhance valuable nutrients in agronomic and breeding programmes.

KEYWORDS: pigmented rice, coloured rice, anthocyanin, iron, zinc, elevation

INTRODUCTION

Purple or black rice with pigmented pericarp is well established in Asia's traditional pharmacopoeias[1,2]. Traditional medicine in China uses pigmented rice to prevent anaemia and to improve blood circulation, kidney function, and eyesight[3]. Black rice porridge is given to aid recovery of invalids; one Chinese genotype is known as "healing of broken bones"[4]. Claims of medicinal properties of purple rice in Thailand, where it is known as "Khao Kam", include stopping bleeding after childbirth, reducing fever to curing skin disease and diarrhoea[5]. In Korea black rice is known as a health food[6].

Pigmented layers of plant cells and tissues have been reported to contain suites of compounds with anti-oxidative properties which can protect against oxidative damages implicated in a range of diseases, including cancer and cardiovascular ailments[7]. The anti-oxidative activities have been found to be associated mainly with phenolic compounds[8–11]. In purple rice, the main phenolic has been identified as anthocyanin[8], specifically cyanidin-3-glucoside and peonidin-3-glucoside[12], malvidin, pelargonidin-3,5-diglucoside, cyanidin-3-glucoside and cyanidin-3,5-diglucoside[9]; cyanidin-3-glucoside, pelargonidin-3-glucoside[10].

A wide variation of the antioxidant compounds has been reported among different Thai purple rice genotypes. Yodmanee et al[13] reported the variation of anthocyanin, polyphenol and iron among 8 coloured rice genotypes (red and purple pericarp) varied from 9–245 mg cyanidin-3-glucoside/100 g DW, from 58–329 mg gallic acid equivalent/100 g DW, from 0.9–1.6 mg/100 g DW, respectively. Genotypic variation in the concentration of anti-oxidative compounds has thus been clearly established. Among the very few studies that examined

Table 1 Local Thai purple rice genotypes used in the experiment and a non-pigmented KDML105.

Variety (Symbol)	Original ecotype†	
	Altitude	Water regime
Hom Luem Pua (HLP)	Highland	Upland
Kham Doi Saket (KDK)	Lowland	Wetland
Kham Nongbualumpu 1 (KNL1)	Lowland	Wetland
Kham Nongbualumpu 2 (KNL2)	Lowland	Wetland
Kham Nongbualumpu 3 (KNL3)	Lowland	Wetland
Kham Nongbualumpu 4 (KNL4)	Lowland	Wetland
Kham Petchaboon 1 (KPB1)	Highland	Upland
Kham Petchaboon 2 (KPB1)	Highland	Upland
Bieisu (BES)	Highland	Upland
Khao Dok Mali 105 (KDML105)	Lowland	Wetland

† Described by the local condition from which the genotype originated: lowland, grown at elevation < 500 m; highland, grown at elevation > 500 m; upland, grown in aerobic soil; wetland, grown in waterlogged soil submerged under a few centimetres of water.

the effect of the environment, a possibility that biologically active property of purple rice genotypes may be influenced differently by environment was suggested by a field experiment of 7 back glutinous upland rice grown in different altitudes and a small pot study of two Thai purple rice varieties which were affected differently by altitudes and nitrogen fertilizer in their anthocyanin content[14,15]. However, the genotype by environmental interaction effects on characteristics and nutritional qualities such as pericarp colour and antioxidant capacity have as yet to be clearly established. This study evaluated the effect of altitude on grain yield, pericarp colour, zinc, and monomeric anthocyanin content and antioxidant capacity in 9 Thai purple rice genotypes.

MATERIALS AND METHODS

Plant culture

Nine purple rice genotypes (Table 1) and Khao Dok Mali 105 (KDML105), a non-pigmented rice used as standard check, were grown in the field in the wet season (June–November) of 2012. The experiment was carried out in 2 altitudes in Chiang Mai, northern Thailand designated lowland and highland (Chiang Mai University: 18.8026° N, 98.9516° E, 330 m above msl, and Tung Luang village, Mae Win subdistrict, Mae Wang district: 18.6125° N, 98.7750° E, 800 m above msl). Soil fertility characteristics at the altitudes determined on 4 replicated samples to 30 cm depth were pH (1:1, soil:water), available phosphorus (Bray II), and DTPA extracted Zn. Rice plants at both altitudes were grown as upland rice, i.e., on aerobic soil. Briefly, the seed was soaked in water overnight and incubated moist until germinated and raised as seedlings for 30 days. Single seedlings were transplanted into hills at 25 × 25 cm spacing in prepared field of each site. Nitrogen fertilizer was applied at the rate of 75 kg N/ha, half at maximum tillering and half at flowering at both sites. Rice seed was harvested at maturity. Grain yield was adjusted to 14% moisture content.

Sample preparation

The rice seed was de-husked to produce unpolished rice with a laboratory husker (Model P-1 from Ngek Seng Huat Co. Ltd., Thailand). The metal parts of de-husker were carefully cleaned to avoid zinc contamination between samples. Optical quality was measured with a chroma meter (model CR-300, Minolta, Osaka, Japan), with the $L^*a^*b^*$ values, with the L^* value indicating brightness. The difference in optical quality between the 2 altitudes was calculated as in the following equation[16]:

$$\Delta E^*_{ab} = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2}$$

where ΔE^*_{ab} is the magnitude of difference of colour between 2 samples, ΔL^*, Δa^*, and Δb^* are the differences of L^*, a^*, and b^* values between 2 altitudes, respectively. The high value of ΔE^*_{ab} indicated the extreme difference of the pigmented colour according to the equipment capacity.

Chemical analysis

Samples were oven dried at 75 °C for 72 h before Zn was determined using a Hitachi Z-8230 atomic absorption spectrophotometer[17]. Monomeric anthocyanin was determined by the pH-differential method[18,19]. Briefly, 2.5 g freeze dried samples were extracted in double deionized water (DDI) water at 50 °C for 30 min. The extracted solution was filtered with filter paper before preparing two dilutions. Volume of one fraction was adjusted with KCl buffer, pH 1.0, and the other fraction with sodium acetate buffer, pH 4.5. Each dilution was allowed to equilibrate for 15 min. Absorbance of the first dilution was measured at 520 nm and the second dilution at 700 nm, against a blank cell filled with distilled water. Absorbance readings were made after 15 min and completed within 45 min. The absorbance of the diluted sample (A) was calculated as $A = (A_{520} - A_{700})_{\text{pH 1.0}} - (A_{520} - A_{700})_{\text{pH 4.5}}$.

The monomeric anthocyanin pigment concentration (mg/l) in the original sample was calculated as $(AMfB)/(\varepsilon L)$, which was converted to mg of total anthocyanin content per 100 g sample; here $M =$ 449.2 g/mol is the molecular weight of cyanidin-3-glucoside, f is the dilution factor, $B = 1000$ mg/g is a conversion factor, $\varepsilon = 26\,900$ l mol^{-1} cm^{-1} is the molar absorptivity of the pigment, and $L =$ 1 cm is the path length. The trolox equivalent antioxidant capacity (TEAC) was performed using the DPPH Free Radical Scavenging Method[20]. The DPPH reagent (0.395 g) was dissolved in 1000 ml of methanol for preparing the DPPH reagent solution. About 0.1 g of ground sample was extracted with methanol and filtered with 0.5 μm nylon membrane before measuring for the DPPH free radical scavenging test. Then 0.5 ml of DPPH solution and 1.6 ml methanol were added into the sample solution and transferred to a spectrophotometer cuvette. The reaction solution was carried out in a dark room at 25 °C for 20 min. Then the absorbance of the reaction mixture was monitored at 517 nm using a UV-Vis spectrophotometer. Radical scavenging activity was calculated as $(A_c - A_s)/A_c$, where A_s and A_c are the absorbances of the sample and control, respectively.

The DPPH scavenging activity percentage of the absorbance of DPPH was calculated by plotting against each quantity of the extraction to produce a regression line. Trolox (0.4 mM) in methanol was used as a standard to convert the inhibition capability of the samples to the trolox equivalent antioxidant activity. The ratio of the slopes of the regression lines of the extract solution and the trolox solution was defined as the trolox equivalent antioxidant capacity. Then it was converted to μmol trolox equivalent/g rice.

The Fe reducing antioxidant power (FRAP) was performed according to the method described by Benzie and Strain[21]. Briefly, freshly prepared FRAP reagent consisted of 0.1 M acetate buffer (pH 4.0), 0.5% (w/v) 1, 10 phenanthroline in 10% methanol, and 0.3 mM $FeCl_3$ in a ratio of 1:1:1 (v/v/v). The 2 ml of rice extract was mixed with 0.5 ml of the phenanthroline and FRAP reagent. After 20 min of incubation at 37 °C, absorption was measured at 510 nm using a spectrophotometer. Aqueous or methanolic solutions of known Fe(II) concentration were used for calibration in the FRAP assay. FRAP values, expressed as μmol of Fe(II) equivalent per 100 g rice, were obtained by comparing the absorption change in the test mixture with doses obtained from Fe(II) standard curve.

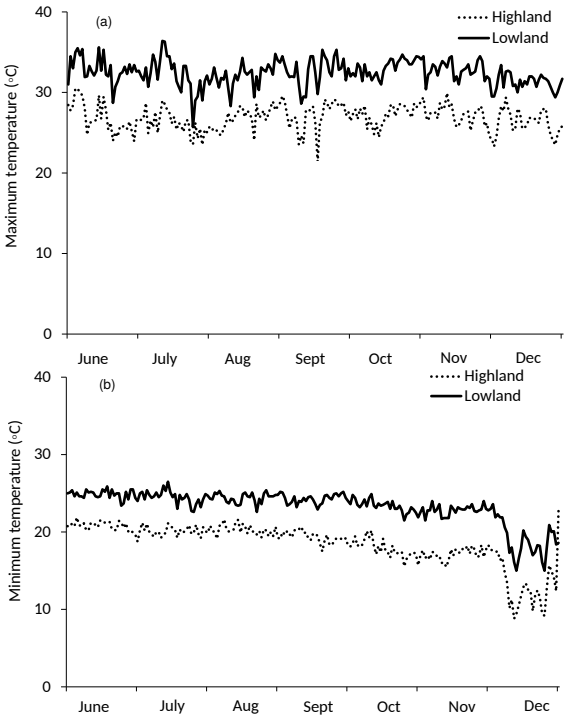

Fig. 1 Meteorological data during planting (June–Dec. 2012) at lowland and highland altitudes: (a) maximum temperature, (b) minimum temperature.

Data analysis

The data were subjected to combined ANOVA and the means that were significantly different were separated at $p < 0.05$ by the least significant difference test. Certain sets of data were also subjected to correlation and regression analysis.

RESULTS

During the growing season the highland site was cooler (18–27 °C for highland, compared with 23–32 °C for lowland) and more humid (69–100% for highland and 55–91% for lowland) than the lowland site with similar average rain fall at both altitudes (Fig. 1). More than half of the genotypes grown at the highland reached flowering before lowland site, except in KNL1, KPB2, and BES which had the reverse result, while KDK and KDML105 had similar flowering date in the two altitudes (Table 2). However, when counted the day from seeds planting until flowering, almost all genotypes grown at lowland had fewer days than highland site with the difference of day ranged from 1–36 days between the 2 altitudes (Table 2). All genotypes at both altitudes matured after 43 days after flowering, except the genotype KNL1 which took 35 days from flowering

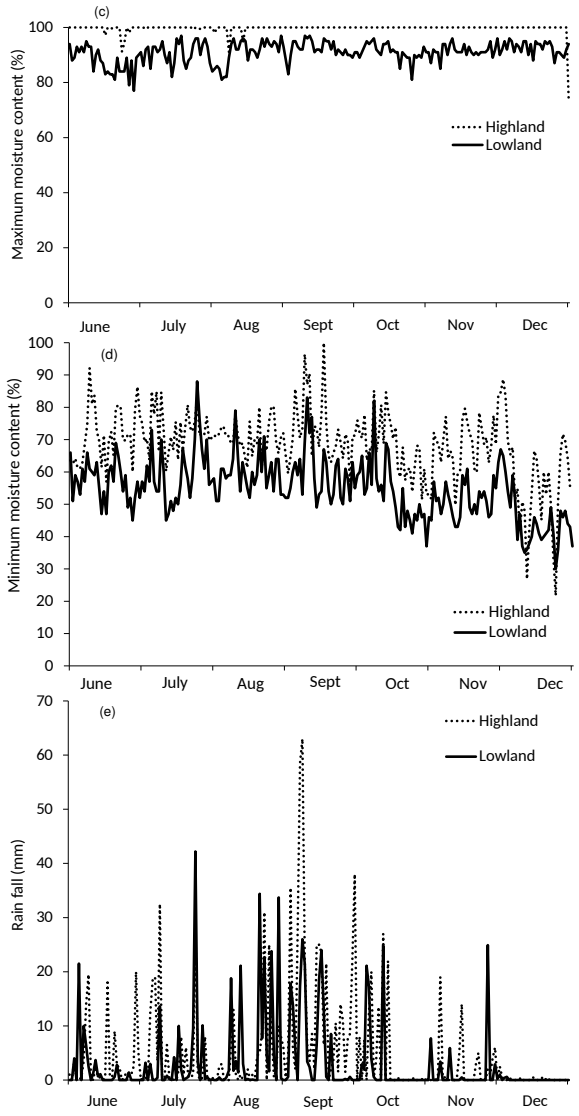

Fig. 1 (Cont.) Meteorological data during planting (June–Dec. 2012) at lowland and highland altitudes: (c) maximum moisture content, (d) minimum moisture content, (e) rain fall.

to maturity in the lowland but only 30 days in the highland (Table 2).

The soil was acidic sandy loam at both sites, with soil pH of 4.1 and 0.4 mg DTPA extractable Zn/kg at the highland altitude and soil pH of 5.5 and 1.5 mg DTPA extractable Zn/kg at the lowland altitude (Table 3).

Altitude effects

Altitude had different effects on grain yield of different genotypes ($p < 0.05$) (Fig. 2). The genotypes with higher grain yield at the highland than lowland altitude were HLP, KNL2, KNL3, KPB1, and KPB2, the reverse was found with KDK, KNL1 and the lowland variety KDML105, while altitude had no effect on the yield in KNL4 and BES. Effects of altitude on grain pigmentation differed among the rice genotypes in both magnitudes and direction (Table 4). The 2 genotypes with the largest effect of altitude on pigmentation intensity, KNL1 and KNL3, differed in the altitude at which the pigmentation was more intense. The lower brightness measure of L value which indicated more intense pigmentation was found in the highland in KNL1 but in the lowland in KNL3. Incidentally, there was a significant altitude effect on the optical quality of the non-pigmented KDML105 which showed a slightly brighter hue in the lowland than in the highland. There was a strong interaction between the effect of genotype and environment ($p < 0.01$) on grain Zn concentration, monomeric anthocyanin concentration, and anti-oxidative capacities determined by DPPH and FRAP methods in rice of the 10 genotypes (9 Thai purple rice genotypes and non-

Table 2 Flowering date, days from planting to flowering, and days from flowering to maturity of 10 rice genotypes at 2 different locations.

Genotype	Flowering date†		DPF		DFM	
	L	H	L	H	L	H
HLP	26/09	19/09	82	90	43	42
KDK	22/10	22/10	108	123	40	46
KNL1	17/10	25/10	103	126	35	30
KNL2	24/09	10/09	80	81	45	47
KNL3	26/09	12/09	82	83	43	45
KNL4	26/09	15/09	82	86	43	42
KPB1	26/09	10/09	82	81	43	47
KPB2	26/09	27/10	82	118	43	43
BES	26/09	27/10	82	118	43	38
KDML105	26/10	27/10	92	118	43	41

† Date of 2012.
DPF: days from planting to flowering; DFM: days from flowering to maturity; L: lowland; H: highland.

Table 3 Soil fertility characteristics at 2 experimental altitudes (lowland and highland).

Soil fertility characteristics†	Lowland	Highland
Texture	Sandy loam	Sandy loam
pH (1 : 1, Soil : Water)	5.5	4.1
P (mg/kg) (Bray II)	59.0	52.1
Zn (mg/kg) (DPTA extraction)	1.5	0.4

† Values are means of 4 replications.

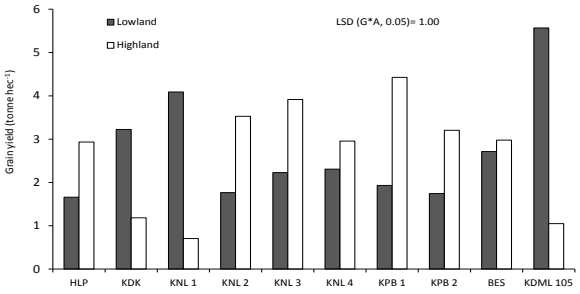

Fig. 2 Grain yield (14% moisture content) of 9 Thai purple rice genotypes and non-pigmented KDML105 grown at 2 altitudes (lowland and highland).

Table 4 Variation in pigmentation intensity between locations (ΔE^*_{ab}) and L^* value for brightness.

Genotype	L^* value†		ΔE^*_{ab}
	lowland (300 m)	highland (800 m)	
HLP	24.52	23.06	3.7 ± 0.3‡
KDK	21.59	21.41	3.0 ± 0.6
KNL1	23.92[a]	38.45[b]	17.5 ± 0.7
KNL2	24.61	23.92	3.0 ± 0.3
KNL3	31.08[b]	22.85[a]	15.5 ± 2.0
KNL4	23.28	24.23	3.8 ± 0.4
KPB1	26.52[b]	22.36[a]	4.8 ± 1.2
KPB2	23.88	22.46	4.0 ± 2.4
BES	24.50	26.56	5.8 ± 2.0
KDML105	62.66	59.69	5.2 ± 1.0

† For the same variety, the means marked with different letters were significantly different between 2 altitudes ($p < 0.05$).

‡ Values are mean ± standard error of mean with 3 replications.

pigmented KDML105) grown at 2 different altitudes (Table 5). Grain Zn concentration was affected by both genotype and altitude ($p < 0.01$) (Fig. 3). Purple rice grown in the lowland had higher Zn concentration than that grown at the highland, but with the difference between altitudes ranging from 16–50% among the genotypes. For the non-pigmented KDML105, which was among the lowest in grain Zn, there was no altitude effect on its grain Zn concentration. The highest grain Zn concentration (33–35 mg Zn/kg) was found in the genotypes HLP, KNL2 and KNL3 grown in the lowland, but in the highland the grain Zn in these genotypes was one third lower.

Monomeric anthocyanin concentration of the purple rice genotypes varied with the altitude where they were grown ($p < 0.01$) (Fig. 4). The genotypes

Table 5 Mean square of the combined ANOVA for grain Zn concentration.

Source†	df	Zn (mg/kg)	Monomeric anthocyanin (mg/100 g)	Anti-oxidative capacity	
				DPPH (g/100 g)	FRAP (mol/100 g)
G	9	50.36**	1920.8**	28.45**	2.113**
A	1	758.99**	8.8^{NS}	12.74**	2.743**
G×*A*	9	24.32**	150.0**	10.91**	1.535**
Pooled error	36	1.22	18.3	0.62	0.060

† *G*: genotype; *A*: altitude.

**Significance $p < 0.01$, ^{NS} Not significant.

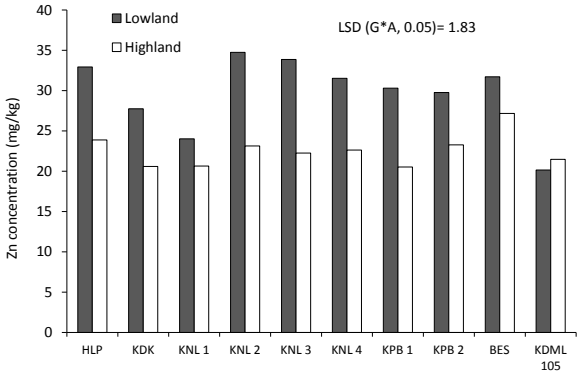

Fig. 3 Zinc concentrations in unpolished rice of 9 Thai purple rice genotypes and non-pigmented KDML105 grown at 2 altitudes (lowland and highland).

with the higher anthocyanin concentration in the lowland were HLP and KDK, while those with higher anthocyanin in the highland were KNL3, KNL4, KPB1, and BES. The remaining genotypes and non-pigmented KDML105 with negligible anthocyanin, showed no effect of altitude on their anthocyanin concentration. The antioxidant capacity determined by DPPH and FRAP methods, that were only weakly correlated ($R^2 = 0.13$, $p < 0.05$), showed different variation by genotype and altitude. The antioxidant capacity determined as TEAC by the DPPH method was high in all purple rice genotypes compared with the non-pigmented KDML105, but the capacity varied with genotype and altitude (Fig. 5). The TEAC of rice grain grown at the highland was about 4 times higher than at the lowland in KNL1 and KDML105, while the other genotypes showed much milder altitude effect. FRAP was also affected by the genotype and altitude (Fig. 6). The effect of altitude on FRAP was more marked, with the activity in the genotypes HLP, KDK, KNL1, KNL2, BES, and KDML105 grown in the lowland 2–4 folds higher than when grown in the highland, while the geno-

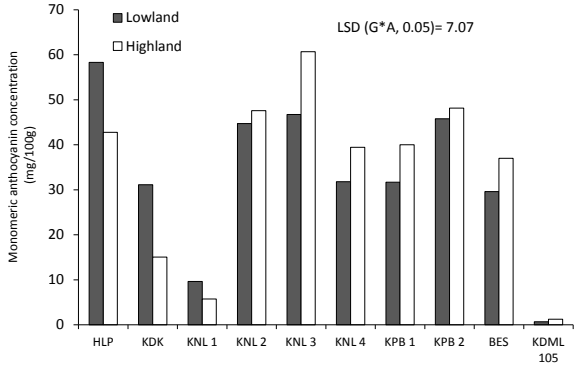

Fig. 4 Monomeric anthocyanin concentrations in unpolished rice of 9 Thai purple rice genotypes and non-pigmented KDML105 grown at 2 altitudes (lowland and highland).

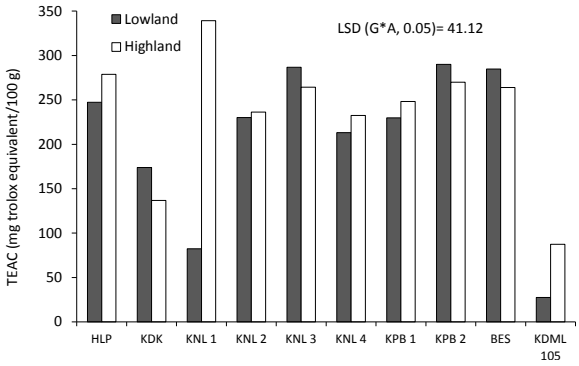

Fig. 5 Trolox equivalent antioxidant capacities (TEAC) in unpolished rice of 9 Thai purple rice genotypes and non-pigmented KDML105 grown at 2 altitudes (lowland and highland).

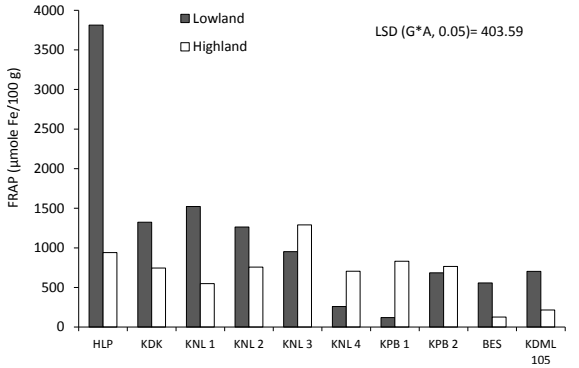

Fig. 6 Ferric reducing antioxidant power (FRAP) of unpolished rice 9 Thai purple rice genotypes and non-pigmented KDML105 grown at 2 altitudes (lowland and highland).

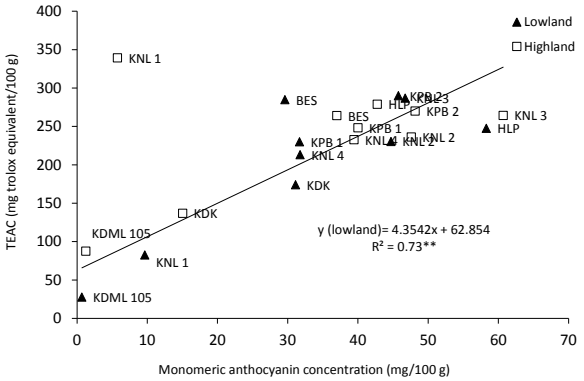

Fig. 7 Relationship between monomeric anthocyanin concentration and trolox equivalent antioxidant capacity (TEAC) of 9 Thai purple rice genotypes and non-pigmented KDML105 grown at 2 altitudes (lowland and highland) ($n = 10$).

types KNL4 and KPB1 grown in the highland had 3–7 folds of their FRAP value in lowland, with no altitude effect in the remaining genotypes.

Relationship between quality characteristics and between yield and quality

There was a close association between anthocyanin content and TEAC of the rice genotypes when grown at the lowland altitude ($R^2 = 0.73$, $p < 0.05$), but not when the rice genotypes were grown at the highland altitude (Fig. 7), while the association was much weaker between FRAP and the anthocyanin ($R^2 = 0.17$, $p < 0.05$). The TEAC also varied with Zn and anthocyanin concentration in a multiple regression ($R^2 = 0.75$, $p < 0.05$),

$$\mathrm{DPPH} = 31.399 + 2.656\,[\mathrm{Zn}] + 3.133\,[\mathrm{Antho}], \quad (1)$$

and so did FRAP but with much weaker association ($R^2 = 0.15$, $p < 0.05$),

$$\mathrm{FRAP} = -163.798 + 22.164\,[\mathrm{Zn}] + 14.700\,[\mathrm{Antho}]. \quad (2)$$

The association between grain Zn, anthocyanin and antioxidant capacity and grain yield varied significantly between the two altitudes (Fig. 8). The concentration of grain Zn in the highland did not vary with grain yield, but in the lowland grain Zn concentration declined when grain yield increased ($R^2 = 0.84$, $p < 0.05$) (Fig. 8a). On the other hand, altitude had different effects on the relationship between grain yield and anthocyanin concentration (Fig. 8b). With increasing grain yield the concentration of anthocyanin increased at the highland altitude ($R^2 = 0.82$, $p < 0.05$) but declined at the

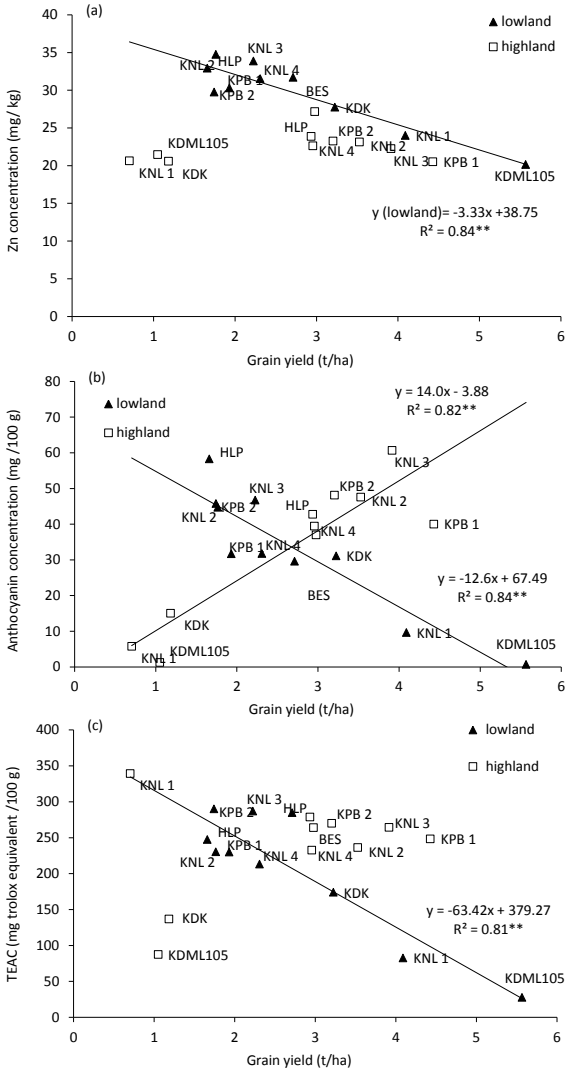

Fig. 8 The relationship between grain yield (14% moisture content) and (a) grain Zn concentration, (b) anthocyanin concentration, and (c) antioxidant capacity, of 9 Thai purple rice genotypes and non-pigmented KDML105 grown at 2 altitudes (lowland and highland) ($n = 10$).

lowland altitude ($R^2 = 0.84$, $p < 0.05$). No correlation was observed between TEAC and grain yield in the highland, but in the lowland the correlation was negative ($R^2 = 0.81$, $p < 0.05$, Fig. 8c).

DISCUSSION

This study established that there may be a strong interaction between genotype and altitude in their effects on the nutritional quality characteristics and grain yield of the purple rice. There was also different association between the quality characteristics and yield and quality in the highland and lowland. A previous investigation reported that tannin concentration, phenolic content and antioxidant efficiency among 133 coloured rice genotypes tested were independent of seasonal changes when grown in different years at the same altitude[22]. In contrast, the variation in altitude effect on the monomeric anthocyanin (a water soluble form of anthocyanin and considered as the primary bioactive compound in purple rice[3,23,24]) of different purple rice genotypes found here suggested a much larger environmental effect than the previous study. It remains to be elucidated whether the effect is the result of differences in the soil or micro-climate due to 500 m difference in elevation.

The effects of genotype and soil condition on variation in grain Zn concentration and anthocyanin, but not on pigmentation or antioxidant capacity of rice have been previously reported[15,25,26]. Genotype by environment interaction contributed to the total variation in grain anthocyanin content of 7 black glutinous upland rice varieties grown at different altitudes[15]. In pear, low temperature was found to induce red coloration, while anthocyanin levels increased with increasing irradiance level with genotype dependent[27]. It has also been reported that antioxidant capacity of strawberry varied among different genotypes and growing condition[28]. Stability of traits over different locations is desirable and an important issue in breeding programmes, especially when highly promising genotypes for the traits is identified. However, stability associated with low performing genotypes, as found here, is of little value.

Anthocyanin has been well established as a major inhibitor of malignant growth in human cells[29] and demonstrated excellent anti-inflammatory and antioxidant property[30,31]. The significant correlation between the TEAC and anthocyanin concentration both at the highland ($R^2 = 0.73$, $p < 0.05$) and lowland ($R^2 = 0.72$, $p < 0.05$) altitudes in this study is in agreement with these previous reports regarding the role of anthocyanin in antioxidant capacity of purple rice grain. The significant variation of the TEAC (1) and FRAP (2) in a multiple regression with anthocyanin in combination with Zn suggested an active role of Zn in antioxidant capacity. The additional effect of Zn on the antioxidant capacity in purple rice could be associated with its role in scavenging enzymes such as superoxide dismutase (SOD) in the form of CuZn-SOD. The enzyme, located in the mitochondria[32] and glyoxysomes[33], acts as a free-radical scavenging enzyme in plant cell[34], detoxifies superoxide radicals occurring from

photosynthetic activities and physiological stress responses, so mitigating their adverse effects on enzyme activity, integrity of polysaccharides, cell membrane and DNA and prevent cell death[35]. The role of Zn in antioxidant capacity of purple rice needs to be further investigated.

The antioxidant capacity determined with TEAC and FRAP agreed in that both reflected the interaction effects of genotype by altitude. The weak correlation between results from the two methods is in accordance with a previous report[36], which discussed that antioxidant activity values determined by the FRAP method were not distinctive as those found by TEAC. The TEAC method is now more commonly used for evaluating antioxidant activity of plant material due to the simplicity of the assay and the fact that it can be used in aqueous and lipid phases[37].

The negative correlation between grain yield and Zn concentration in the lowland altitude could be explained by the dilution of Zn in the tissue which might be related to the lower Zn concentration in the soil compared with those in the highland altitude which has higher Zn concentration in the soil and the concentration of grain Zn did not vary with grain yield. Similar findings on variation of grain Zn concentration when grown in different Zn concentration in the soil have been reported[25,38]. The reason for such increases in the anthocyanin by increasing of grain yield in the highland altitude is unknown. It might be related to the physiological function on enhancing grain yield as previously reported that increasing of phenolic and antioxidant activity also increased grain yield of black sorghum hybrids[39]. On the other hand, a negative correlation between grain yield and the anthocyanin was found in the lowland. Such different association between grain quality and yield between the 2 altitudes might be explained by the physiological responses of rice grown at different elevation such as average day/night temperature, photoperiod, and irradiance level which would require further studies to bring better understanding. Further research should also pay attention to physiological responses among rice genotypes from different original on-farm ecotype of altitude (highland and lowland) and water regime (wetland and upland varieties). This experiment was carried out by growing all the genotypes at both altitudes in the same aerobic condition, which is the predominant mode of rice production in the highlands. How the grain quality characteristics of different genotypes are affected by water regimes of their growing condition, i.e., aerobic versus waterlogged soil, is another avenue of exploration that should bring benefits to rice producers and consumers.

This study has established how nutritional quality, particularly anthocyanin content and the antioxidative capacity, as well as the intensity of pigmentation of purple rice genotypes may vary with the environment in which they are grown. Stability of these nutritional characteristics and environmental effects need to be considered when dealing with special quality of purple rice, while the controlling factors remain to be described.

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