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Identifying rice grains with premium nutritional quality among on-farm germplasm in the highlands of Northern Thailand

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RESEARCH ARTICLE

Abstract

Local rice varieties with premium nutritional quality grains are beneficial to consumers, and their genetic traits may be deployed in breeding programmes for many purposes. This study explores grain quality characteristics with health implications of rice germplasm maintained and used by farmers in the highlands of Northern Thailand. Concentrations of iron (Fe), zinc (Zn), phenols and anthocyanin and anti-oxidative capacity were determined in the caryopsis without husk of 77 samples of rice seed collected from farmers. Entries with the highest grain quality characteristics identified were grown together with four standard check varieties at two different elevations in a farmer's field at Mae Wang District of Chiang Mai province (800 m above mean sea level), as well as in pots at Chiang Mai University (CMU) (330 m above mean sea level). The grain quality characteristics were determined at maturity, separately for 10 individual plants grown at CMU. A wide variation in all the grain quality characteristics was found among samples grown in the farmer's field. There were approximately twofold differences in the lowest and highest Fe and Zn concentrations, especially high variation in contents of phenols and anthocyanin and anti-oxidative capacity found in grain with purple pericarp. The top entries identified from the farmer's seed had significantly higher anthocyanin concentration and anti-oxidative capacity than the check varieties when grown together at Mae Wang and CMU, in spite of the strong locality-specific effects on these characteristics. Further variation was found in the grain quality characteristics within each of the selected farmer's seed samples. Quality improvement could thus be made by either eliminating the poorest performing lines or development of single-seed descent lines from the top-performing plants. The desirable genetic traits can also be used in breeding programme for improvement of grain yield as well as cooking and nutritional quality.

Keywords: local rice, iron, zinc, phenols, anthocyanin, anti-oxidative capacity

1. Introduction

Local rice varieties are a valuable resource for traits that confer grain quality characteristics with health benefit implications (Panomjan *et al.*, 2016; Prom-u-thai and Rerkasem, 2001; Xiongsiyee *et al.*, 2018), as well as for adaptation to environmental factors of both biotic and abiotic stresses that are lost among the modern improved varieties, resulting in susceptibleness to a wide spread environmental stresses (Kumar *et al.*, 2004; Laenoi *et al.*, 2015; Naruebal, 2009; Oupkaew *et al.*, 2011; Wissuwa and Ae, 2001). The quality characteristics of rice with health implications, which are a concern among rice consumers, include contents of the minerals Fe and Zn, the bioactive compounds anthocyanin and phenols and anti-oxidant capacity. Rice consumers are especially prone to health problems associated with these minerals and compounds because their presence in rice is much lower in

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concentration than in other staple crops such as wheat and barley (Demirbas, 2005; White and Broadley, 2005). Inadequate consumption of Fe and Zn results in impaired growth, development and immune system, especially among the children in rice consumption regions (Black, 1998; Keen and Gershwin, 1990; Lozoff and Georgieff, 2006). On the other hand, bioactive compounds with medicinal and industrial potential, such as anthocyanin and antioxidants, have been reported to prevent free radicals that stimulate cancer and cell injury (Kehrer, 1993) and inhibit activity of some digestive enzymes that can increase the impact of diabetes (Boue *et al.*, 2016).

Improving the minerals and bioactive compounds in rice grain by bio-fortification, for example, breeding strategy and agronomic managements, is suggested as a promising way to alleviate the problems among population whose staple food is rice (Cakmak, 2010; Kutman *et al.*, 2010). To do this, the source of genetic material with wide variation of nutrition qualities is required to achieve the desired goal in both grain nutrition quality and its production, especially in breeding programme. Interestingly, local rice varieties with desirable quality traits are being exploited in the emerging market for rice with special quality (e.g. Boonsit *et al.*, 2010; Panomjan *et al.*, 2016) as well as to directly benefit those who consume the rice they grow and they also provide a source of genetic traits in breeding programmes. On the other hand, commercialisation of rice production, especially in the countries such as Thailand, Vietnam, China and India where a great amount of the production is consumed and exported annually, has led to the replacement of local rice varieties by only a few mega-varieties; however, local rice varieties are still grown by farmers in a few areas, including the highlands of Laos (Xiongsiyee *et al.*, 2018) and Northern Thailand (Jamjod *et al.*, 2017), which are part of the centre of diversity for *Oryza sativa* (Harlan, 1992). Thus, identifying nutritional quality traits among the local rice varieties is not only beneficial to health-conscious consumers, but would also allow the further in-depth physiological study and breeding programme. In particular, programmes assisting with modern molecular techniques would allow to rapidly improve new rice varieties for the farmer's desirable traits, such as photoperiod insensitivity with high yield, which is generally lacking in the local varieties.

This study attempted to explore grain nutritional quality characteristics of rice germplasm maintained and used by farmers in three provinces—Chiang Mai, Chiang Rai and Mae Hong Son—where a substantial proportion of the local rice germplasm in Thailand may still be found. In the previous study, 77 samples of rice seed were collected and analysed for Fe, Zn, phenols, anthocyanin and anti-oxidative capacity (Jamjod *et al.*, 2017). The highest quality traits were selected for two samples each (Kam Wiangsa; KWS was selected for both Fe and phenol qualities) for the current study. The nutritional quality of the selected lines was also determined between the growing locations. The results of this study would be very useful information, especially in breeding programmes for rice of high nutritional values as well as its utilisation for health benefit purposes.

2. Materials and methods

Farmers' seed samples

In the previous study, 77 samples of rice seed were collected from farmers in Chiang Mai, Chiang Rai and Mae Hong Son (Figure 1) (Jamjod *et al.*, 2017). Information recorded for each sample included the Global Positioning System (GPS) position of the farm and the soil-water system under which the rice was grown (upland = grown on aerobic soil on slope; wetland = grown on levelled field, soil submerged under a few cm of water). A100 g subsample was taken from each collected sample and dehusked in a sample mill machine (Ngek Seng Huat model P-1, Thailand) to produce brown rice (endosperm with intact embryo and pericarp). Pericarp pigmentation was determined visually as white (not pigmented), purple and red, and the amylose content of the brown rice was determined by iodine–amylose colour development (Juliano, 1971) using a spectrophotometer (Hitachi model U-1900, Japan) at 620 nm. Grain nutritional quality characteristics of the brown rice were determined: Fe and Zn by atomic absorption spectrophotometry (Hitachi Model Z-8230, Japan) after dry-ashing at 535 °C for 8 h (Allan, 1961); phenols by Folin Ciocalteu's method (Folin and Denis, 1912), anthocyanin by the pH-differential method (Escribano-Bailón *et al.*, 2004; Wrolstad *et al.*, 2001) and trolox equivalent anti-oxidative capacity (TEAC) by the DPPH free radical scavenging method (Yue and Xu, 2008). The highest concentration of Fe, Zn, phenols and anthocyanin and anti-oxidative capacity were selected for two samples each (Kam Wiangsa; KWS was selected for both Fe and phenol qualities) for the current study.

Comparing selected lines with top quality at Mae Wang

The nine selected lines were grown together with four standard check varieties at Mae Wang District of Chiang Mai province, Thailand (N18.4254°, E98.3412°; 800 m above mean sea level) from July to December 2014, with soil-water condition in accordance with their management on-farm (Table 1). The upland rice lines were grown on well-drained soil with flood irrigation when necessary; the wetland lines were grown on waterlogged soil (5–10 cm of water maintained above the soil surface from transplanting to maturity). Each of the selected lines and check varieties was grown as single plants, with 0.25 m × 0.25 m spacing in 2×4 m^2 plots, arranged in duplicated blocks. The crop was fertilised with

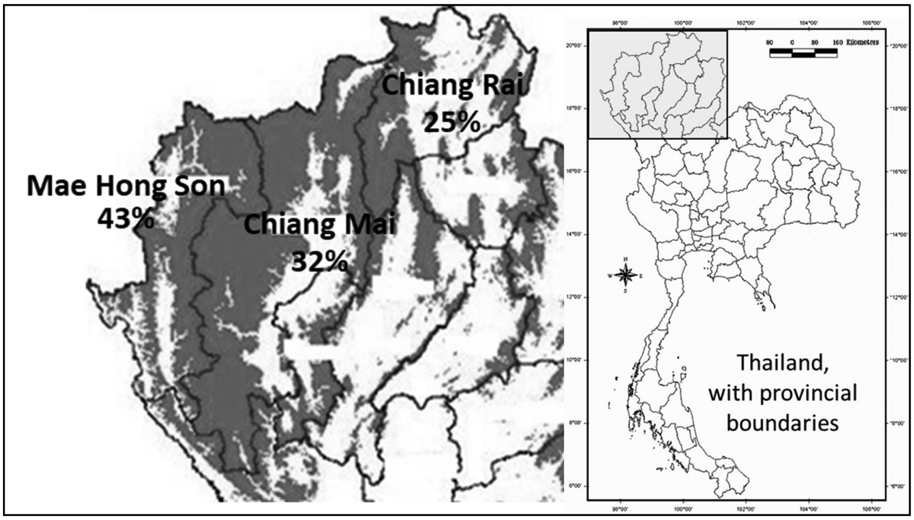

Figure 1. Local rice varieties sampling area in the highlands of Northern Thailand. The number under the province name is the percentage of samples evaluated. The symbol ■ in the map indicates mountainous area 500 m above mean sea level and lower lying areas between the mountains.

Table 1. Selected local rice varieties with the highest iron, zinc, phenol and anthocyanin concentrations and anti-oxidative capacity grown together with four check varieties for comparison at Mae Wang, a highland site in Northern Thailand.

Top quality for	Variety	Location (°)	Soil-water management
Iron	KWS (Kam Wiang Sa)	N19.4052, E98.1242	Wetland
	BK (Bian Koo)	N19.8921, E100.2967	Upland
Zinc	BTN (Bue Tor Na)	N17.8770, E97.9248	Upland
	BB (Bue Bang)	N17.5353, E97.5224	Upland
Phenols	KWS (Kam Wiang Sa)	N19.4052, E98.1242	Wetland
	KS (Kao Saeng)	N19.4052, E98.1242	Upland
Anthocyanin	PES037 (Pee-Ee-Su 1)	N18.3748, E98.3748	Upland
	PES057 (Pee-Ee-Su 2)	N18.3748, E98.3748	Upland
Anti-oxidative capacity	DMO (Daew Ma O)	N19.7332, E99.5280	Upland
	KAK (Kam Akha)	N19.7332, E99.5280	Upland
Check varieties for			
Iron, Zinc	KDML105 (non-glutinous, mega-variety)		Wetland
	RD6 (glutinous, mega-variety)		Wetland
Phenols, anthocyanin,	KDK (purple rice)		Upland
anti-oxidative capacity	KHCMU (purple rice)		Upland

60 kg nitrogen (N) ha^{-1}, 30 kg phosphorus (P) ha^{-1} and 30 kg potassium (K) ha^{-1}, which was split two times between tillering and flowering. Days to flowering were determined from the germination date to 50% flowering of each plot. Plant height and the number of panicles per hill were determined on 10 representative plants of each line and grain yield from 1 m^2 internal area of each plot, at maturity. Nutritional quality characteristics were determined in the same way as for farmers' seed described above.

Variation within seed samples

Variation was evaluated in the seed of 10 individual plants of each selected seed sample and the check varieties grown at Chiang Mai University (CMU), Thailand (N19.4052°, E98.1242°; 330 m above mean sea level) from July to December 2014. The plants were grown in pots (Ø30 cm, 30 cm deep) containing 15 kg of sandy loam soil of the Sansai series, at five plants per pot, with soil-water management in accordance with their management on-farm, that is, aerobic well-drained soil for the upland ecotype and waterlogged soil for the wetland ecotype. The plants were fertilised on a surface area basis in the same way as described above. At maturity, plants were harvested individually and threshed by hand. Quality characteristics of the grain were determined as described above, separately for each plant.

Data analysis

The data were evaluated using the analysis of variance (ANOVA) and mean that were significantly different and were separated by the least significant difference test (LSD) at $P < 0.05$.

3. Results

A considerable variation was found among the selected lines that were grown in farmers' fields in the highlands of Northern Thailand (Table 2). Among individual entries, there were approximately twofold differences in the lowest and highest Fe and Zn concentrations, but much larger variation in the contents of phenols and anthocyanin and anti-oxidative capacity. The highest total phenolic content was five times the lowest one, while anthocyanin ranged from 0 to 166.4 mg 100 g^{-1}, and anti-oxidative capacity ranged from 1.9 to 1811.5 mg Trolox100 g^{-1}. Thus, entries with exceptionally high levels of nutritional quality characteristics measured were identified. Two entries with the highest anthocyanin concentration, PES037 and PES057, were from the same village (N18.3748°, E98.3748°), and similarly were the two with the highest anti-oxidative capacity, Daew Ma O (DMO) and Kam AKha (KAK) (N19.7332°, E99.5280°).

The entries of local rice varieties selected for grain quality were all grown on-farm as upland rice, except KWS which was a wetland ecotype, and had very different appearance from the mega-variety checks, KDML105 and RD6 (Figure 2 and Table 3). They all had medium to bold grain type, in contrast to the slender grain KDML105 and RD6, and all except three had glutinous grain with pigmented pericarp, although the hull colour ranged from purple, straw colour

Table 2. Grain quality characteristics of farmers' rice seed samples from the highlands of Northern Thailand by plant and grain type.

Plant and grain type	%	Iron (mg kg^{-1})	Zinc (mg kg^{-1})	Phenol (mg gallic acid 100 g^{-1})	Anthocyanin (mg 100 g^{-1})	Anti-oxidative capacity (mg Trolox 100 g^{-1})
Overall mean		11.2 ± 1.8	23.2 ± 3.3	247.1 ± 72.4	44.2 ± 41.8	422.0 ± 423
Range		7.7–14.3	13.9–28.5	72.0–346.0	0.0–166.4	1.9–1811.5
Ecotype						
Upland	92	11.1 ± 1.7	23.1 ± 3.4	241.9 ± 72.9	45.5 ± 43.1	420.3 ± 435.0
Wetland	8	12.5 ± 1.4	24.4 ± 2.2	308.5 ± 19.3	39.9 ± 34.3	440.1 ± 284.2
Difference[a]		$NS_{0.05}$	$NS_{0.05}$	$P < 0.05$(49.8)	$NS_{0.05}$	$NS_{0.05}$
Grain type, by % amylose[b]						
Non-glutinous	49	11.7 ± 1.6	24.2 ± 2.9	212.8 ± 56.8	10.6 ± 20.6	169.0 ± 167.3
Glutinous	51	10.8 ± 1.8	22.2 ± 3.4	280.4 ± 71.0	59.3 ± 40.1	648.3 ± 454.9
Difference[a]		$NS_{0.05}$	$NS_{0.05}$	$P < 0.001$(29.2)	$P < 0.001$(22.8)	$P < 0.001$(164.8)
Grain type, by pericarp pigmentation[c]						
Purple	43	11.1 ± 1.7	22.0 ± 3.3	299.0 ± 61.2	64.4 ± 36.0	695.3 ± 399.5
White	35	11.4 ± 1.7	25.0 ± 3.1	187.1 ± 34.9	0.0 ± 0.0	115.0 ± 93.2
Mixed	22	11.3 ± 1.8	22.6 ± 2.5	246.8 ± 59.7	3.5 ± 11.5	311.7 ± 434.1
Difference[a]		$NS_{0.05}$	$NS_{0.05}$	$P < 0.001$(37.9)	$P < 0.001$(48.4)	$P < 0.001$(249.6)
Check variety (with origin in brackets: LL = lowland variety; HL = highland variety)						
KDML105 (LL)		7.2	22.4	nd	nd	nd
RD6 (LL)		6.8	21.4	nd	nd	nd
KDK (LL)		11.7	22.7	301.0	28.6	421.0
KHCMU (HL)		17.9	29.1	300.9	20.7	259.9

Grain quality characteristics were analysed for three replicates of each farmers' rice seed samples. Values of grain quality characteristics are presented as mean ± standard deviation among farmers' rice seed samples.
[a]Significant difference by type, by analysis of variance, with LSD at $P < 0.05$ in brackets.
[b]Grain type: >10% amylose = non-glutinous; <10% amylose = glutinous.
[c]White = not pigmented; mixed = not pigmented, purple and red.
nd, not determination.

Figure 2. Paddy and brown rice grain of selected lines for high grain Fe, KWS (a) and BK (b); Zn, BTN (c) and BB (d); anthocyanin, PES037 (e) and PES057 (f); phenol, KWS and KS (h); anti-oxidative capacity, DMO (i) and KAK (j); with check varieties KDML105 (k), RD6 (l), KDK (m) and KHCMU (n).

Table 3. Selected local rice varieties with the highest iron, zinc, phenol and anthocyanin concentration and anti-oxidative capacity.

ID[a]	Variety name	Ecotype	Grain dimension[b]		Grain starch	Hull colour	Pericarp colour
			Length (mm)	Shape	Type[c]		
56-037	PES037	Upland	5.5	Bold	Waxy	Straw + purple tint	Purple
56-057	PES057	Upland	5.8	Bold	Waxy	Straw + purple tint	Purple
56-078	BB	Upland	6.4	Medium	Non-waxy	Straw	White
56-092	BTN	Upland	6.4	Medium	Non-waxy	Straw	White
56-151	DMO	Upland	7.3	Medium	Waxy	Straw + purple tint	Purple
56-153	KAK	Upland	7.4	Medium	Waxy	Straw	Purple
56-242	KWS	Wetland	7.2	Medium	Waxy	Purple	Purple
56-260	BK	Upland	7.2	Medium	Non-waxy	Straw	Purple
56-279	KS	Upland	7.5	Medium	Waxy	Straw + purple tint	Purple
Check variety							
KDML105		Wetland	7.5	Slender	Non-waxy	Straw	White
RD6		Wetland	7.2	Slender	Waxy	Straw	White
KDK		Wetland	6.2	Medium	Waxy	Straw + purple tint	Purple
KHCMU		Upland	5.9	Medium	Waxy	Straw + purple tint	Purple

[a]Several of the selections had entries with lower quality characteristic with the same variety name, while KWS was identified as having the highest Fe and phenols.
[b]Brown rice: length—extra-long (>7.50 mm), long (6.61–7.50 mm), medium (5.51–6.50 mm) and short (<5.50 mm); shape by length-to-width ratio: slender (>3), medium (2–3) and bold (1–2) (Juliano, 1993).
[c]Waxy or glutinous (≤10% amylose); non-waxy or non-glutinous (>10% amylose).

with purple tint, to straw colour with no pigmentation. The non-glutinous Bue Bang (BB) and Bue Tor Na (BTN) both had straw coloured hull and white pericarp.

The Fe concentration averaged higher, but Zn was in the same range as the mega-variety checks KDML105 and RD6 (Table 2). Anthocyanin concentration also averaged significantly higher than the purple rice checks, Kam Doisaket (KDK) and Kam Hom Chiang Mai University (KHCMU), although phenols and anti-oxidative capacity did not. Neither the plant ecotype (upland vs. wetland) nor the grain starch (glutinous vs. non-glutinous) and pigmentation type were differentiated by their Fe and Zn contents. Entries of the wetland and upland ecotype were not significantly different in anthocyanin content and anti-oxidative capacity, although higher phenol levels were found among the wetland entries. Glutinous rice entries were significantly higher than non-glutinous entries in anthocyanin and phenolic contents as well as anti-oxidative capacity. Purple rice had significantly higher anthocyanin and phenolic content, and anti-oxidative capacity than entries that were without pigmentation or mixtures with non-pigmented, red and purple pericarps. The selected materials also exhibited different agronomic characteristics from the lowland checks when grown together at a highland site in Mae Wang (Table 4). The selected lines were generally earlier and shorter in stature compared with the lowland check varieties. Three of the nine selected lines, namely Kam Wiang Sa (KWS), Bian koo (BK) and Kao Saeng (KS), yielded in the range as the lowland checks, while the rest yielded significantly less as was the highland check KHCMU. Regression analysis showed that there was no association between the number of panicles and grain yield ($R^2 = -0.32$, NS at $P > 0.05$).

Nutritional and bioactive qualities of the selected entries were found to vary when grown at three different locations (Table 5). All quality characteristics of individual entries were lower when grown at Mae Wang and CMU than in farmers' fields, except that the Zn and phenolic contents were uniform among the three locations. KWS and BK had the highest Fe content in rice grain among on-farm samples, with Fe contents of 14.3 and 14.2 mg kg^{-1}, respectively, but these were only half those at Mae Wang and CMU, in the same range as the check varieties, KDML105 and RD6. Two selected lines with high grain Zn, BTN and BB, had grain Zn at 28.5 and 28.1 mg kg^{-1}, and were slightly higher in grain Zn only when grown at Mae Wang. The highest levels of phenols among on-farm samples were found in KWS and KS at 346.0 and 336.7 mg gallic acid equivalent (gae) 100 g^{-1}, respectively. KWS produced uniformly high phenolic content among the three locations, while KS produced slightly higher phenolic content at Mae Wang and substantially higher at CMU. Anthocyanin was highest in rice from the farmer's field, at 166.4 mg 100 g^{-1} in PES037 and 115.1 mg 100 g^{-1} in PES057, but only a fraction of these when grown at Mae Wang and CMU. Similarly, anti-oxidative capacities were higher in the rice grown in the farmer's fields, with entries DMO and KAK having 1811.5 and 1806.9 mg Trolox100 g^{-1},

Table 4. Agronomic parameters of selected rice varieties from the highlands compared with check varieties grown at Mae Wang, a highland site in Northern Thailand.

Variety	Days to flowering	Plant height (cm)	Panicles/hill	Grain yield (t ha^{-1})
PES037	83 ± 5[a]	94 ± 9	6 ± 2	1.83 ± 0.10
PES057	81 ± 6	94 ± 11	5 ± 2	2.30 ± 0.05
BB	92 ± 3	96 ± 12	7 ± 3	2.13 ± 0.07
BTN	98 ± 2	75 ± 13	7 ± 2	1.85 ± 0.06
DMO	89 ± 5	73 ± 8	5 ± 2	2.10 ± 0.04
KAK	98 ± 2	102 ± 15	3 ± 1	2.11 ± 0.05
KWS	106 ± 4	130 ± 10	5 ± 2	2.79 ± 0.05
BK	99 ± 1	113 ± 10	3 ± 1	2.72 ± 0.07
KS	100 ± 2	111 ± 8	3 ± 2	2.45 ± 0.04
Check variety				
KDML105	114 ± 4	152 ± 10	5 ± 1	2.57 ± 0.03
RD6	115 ± 1	139 ± 8	4 ± 1	2.65 ± 0.04
KDK	118 ± 1	125 ± 10	3 ± 1	2.63 ± 0.03
KHCMU	85 ± 2	94 ± 6	3 ± 1	2.08 ± 0.04

[a]Standard deviation.

Table 5. Variation in grain quality of farmers' selected rice seed samples grown at three locations in Northern Thailand.

Selected for	Variety name	Highland		Lowland
		Farmer's fields	Mae Wang	CMU
Iron (mg Fe kg^{-1})	KWS	14.3 ± 1.0[a]	8.6 ± 0.3	6.8 ± 1.4
	BK	14.2 ± 0.0	7.2 ± 0.1	7.5 ± 1.1
Zinc (mg Zn kg^{-1})	BTN	28.5 ± 0.1	36.4 ± 2.1	27.1 ± 4.8
	BB	28.1 ± 0.6	34.8 ± 0.5	30.5 ± 4.8
Phenols(mg gae 100 g^{-1})	KWS	346.0 ± 2.3	334.7 ± 18.4	323.0 ± 46.2
	KS	336.7 ± 3.7	363.4 ± 0.5	439.0 ± 112.1
Anthocyanin (mg 100 g^{-1})	PES037	166.4 ± 12.6	40.3 ± 1.8	22.5 ± 7.6
	PES057	115.1 ± 7.0	70.9 ± 1.0	18.5 ± 5.7
Anti-oxidative capacity	DMO	1811.5 ± 16.5	580.0 ± 14.1	524.0 ± 173.3
(mg Trolox100 g$^{-1)}$	KAK	1806.9 ± 3.9	449.2 ± 5.6	541.0 ± 213.4
Check variety				
Iron (mg Fe kg^{-1})	KDML105		6.8 ± 0.8	6.8 ± 0.1
	RD6		6.3 ± 0.7	6.3 ± 1.0
Zinc (mg Zn kg^{-1})	KDML105		22.0 ± 0.8	22.0 ± 0.8
	RD6		23.4 ± 0.0	23.4 ± 0.0
Phenols(mg gae 100 g^{-1})	KDK		337.8 ± 0.0	283.5 ± 2.2
	KHCMU		336.8 ± 4.9	302.3 ± 0.6
Anthocyanin (mg 100 g^{-1})	KDK		25.6 ± 2.2	25.6 ± 2.2
	KHCMU		29.8 ± 0.1	29.8 ± 0.1
Anti-oxidative capacity	KDK		303.1 ± 3.3	242.1 ± 29.3
(mg Trolox100 g$^{-1)}$	KHCMU		386.5 ± 28.8	269.7 ± 3.0

KWS, Kam Wiang Sa; BK, Bian Koo; BTN, Bue Tor Na; BB, Bue Bang; KWS, Kam Wiang Sa; KS, Kao Saeng; PES037, Pee-Ee-Su 1; PES057, Pee-Ee-Su 2; DMO, Daew Ma O; KAK, Kam Akha; KDML105, Khaodawk Mali 105; RD6, Rice Department 6; KDK, Kam Doisaket; KHCMU, Kam Hom Chiang Mai University; CMU, Chiang Mai University.
[a]Standard deviation.

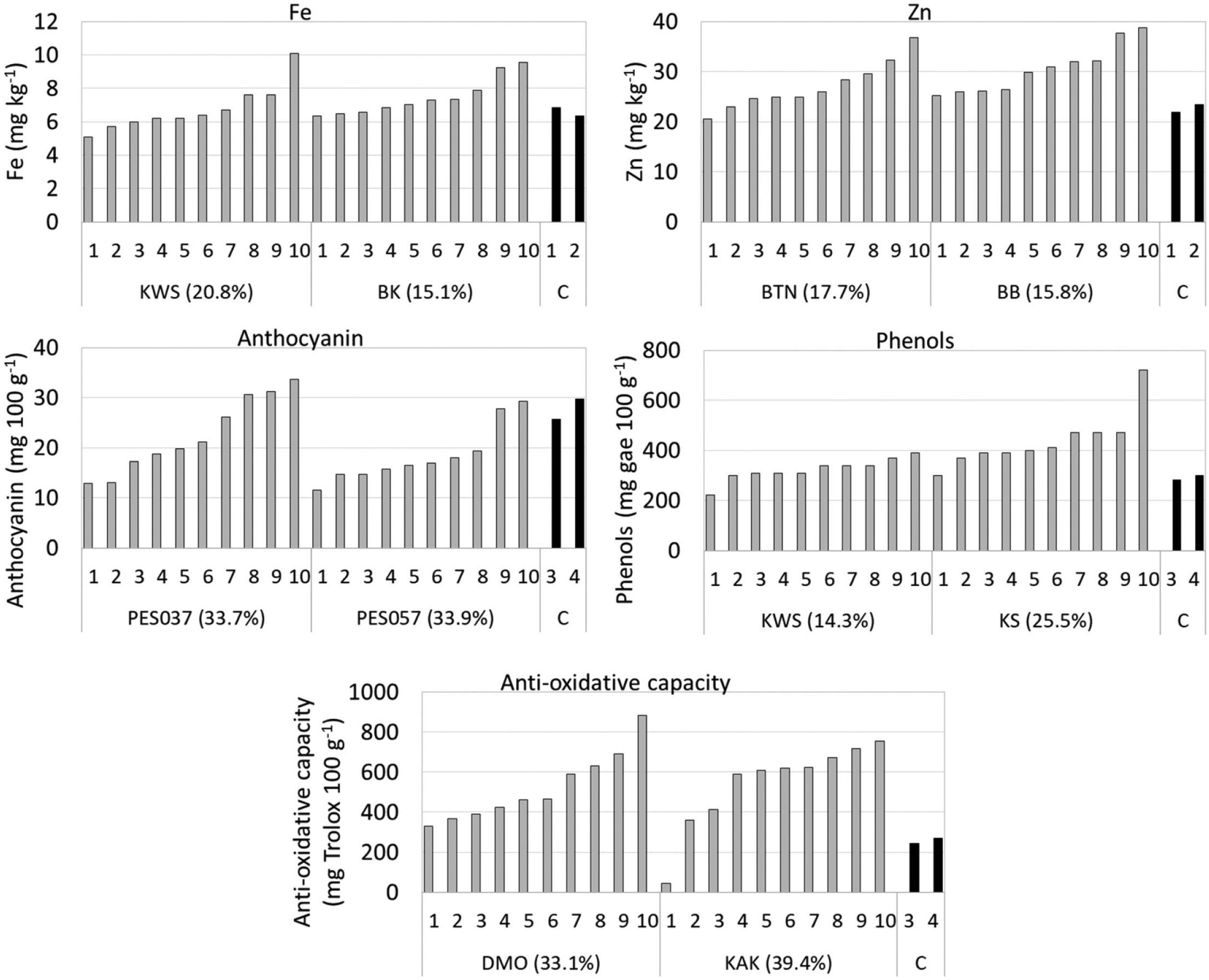

Figure 3. Variation in grain quality among 10 single seed decent lines from selected farmers' rice seed from Northern Thailand (in brackets after variety name is coefficient of variation for the sample; C = check, 1 = KDML105; 2 = RD6; 3 = KDK; 4 = KHCMU).

respectively, and were lower by 1/3 when the entries were grown at Mae Wang and CMU. Substantial variation in nutritional and bioactive qualities was found among plants within the selected lines grown together at CMU (Figure 3). Individual plants with exceptional quality were identified, which exceeded the level in the higher check by up to 48% for Fe, 65% for Zn, 138% for phenols and 239% for anti-oxidative capacity. Anthocyanin concentration was also variable among individual plants of the same selected entry, but plants with higher concentration than the check varieties KDK and KHCMU were rare.

4. Discussion

In spite of the replacement of local rice varieties by modern high-yielding varieties that have been reported across the world's rice growing regions (Cheng *et al.*, 2007; Dalrymple, 1986; Rerkasem and Rerkasem, 2002), the highlands of Northern Thailand is one of the few places where local rice varieties are still to be found (Jamjod *et al.*, 2017). This present study has shown that considerable variation in grain quality characteristics with health benefits existed in local rice varieties which are grown on-farm in Northern Thailand at the time this study was conducted. The rice with exceptional grain quality therefore provides the farm families with other health benefits in addition to dietary energy. Environmental constraints and limited access to modern varieties contribute to the use of locally adapted rice varieties grown with minimal inputs. Farmers continue grow these local rice varieties with seed kept from the previous season or through exchanging their seeds with other farmers (Oupkaew *et al.*, 2011; Sirabanchongkran *et al.*, 2004). The local rice varieties with top quality characteristics in this study contained twice the Fe and Zn of mega-varieties, KDML105 and RD6, while anthocyanin and anti-oxidative capacity were more than double the standard check varieties, KDK and KHCMU. This highlights the direct benefits of rice with high nutritional and bioactive quality to the farm families that grow it. It has been previously suggested that high seed Zn would be beneficial to germination and early growth in upland rice in Zn-deficient soils exacerbated by the ash from the slash and burn practice (Jaksomsak *et al.*, 2015). Ranking of the local varieties based on grain quality characteristics in this study has also shown that quality characteristics with health implication varied widely among local varieties distributed across the highlands of Northern Thailand. KDML105 and RD6, the check varieties for Fe and Zn, are among Thailand's mega rice varieties that are generally low in these nutrients (Saenchai *et al.*, 2012). The marginally higher Fe and Zn in BB and BTN, the only white rice varieties which are grown for home consumption among the selected samples, would therefore directly benefit those farm families that produced the rice for their own use. However, as a source of high Fe and Zn traits, genotypes with much higher concentration of the nutrients have been identified in previous studies on local rice germplasm in Thailand (Jaksomsak *et al.*, 2015; Pintasen *et al.*, 2007). The potential for development of rice varieties with special quality for the highlands is suggested by the identification of local rice varieties with exceptional levels of anthocyanin, phenols and anti-oxidative capacity.

Variation in the rice grain quality by location (Table 5) indicated a degree of instability of individual characteristics. Iron and Zn concentrations in the rice grain can be influenced by climatic and soil factors (Gregorio *et al.*, 2000; Prasad *et al.*, 2014). Zinc is primarily dependent on the native soil Zn, in which the grain Zn of a single rice genotype was reported to range from 8 to 47 mg Zn kg^{-1} (Wissuwa *et al.*, 2008). On the other hand, the grain Zn in some local rice varieties was shown to be lowered by the dilution effect of higher yield brought about by the application of nitrogen fertiliser (Jaksomsak *et al.*, 2017). In the case of bioactive compounds and anti-oxidative capacity, strong genotype by environment (GxE) effects have been demonstrated in few studies in which the performance of the rice genotypes was evaluated under different environmental conditions (e.g. Banterng and Joralee, 2015; Somsana *et al.*, 2013). Genotypes may respond differently to the different conditions. For example, increasing N supply depressed anthocyanin content in one purple rice genotype but increased it in another (Kathuai *et al.*, 2013). Some pigmented rice genotypes grown in a farmer's field in the highland (elevation 800 m) had higher anthocyanin concentration and anti-oxidative capacity than when it was grown in the lowland (elevation 330 m) at CMU; however, for other genotypes, the reverse was true (Rerkasem *et al.*, 2015). Among the bioactive components, phenol concentration was the most stable across locations, while the anthocyanin and anti-oxidative capacity of rice grown in the on-farm trial at Mae Wang were only one quarter of those produced on the original location, thus clearly indicating the location specificity of these quality attributes of the pigmented rice. The rice samples with the highest anti-oxidative capacity, of the varieties DMO and KAK, were grown at an exceptionally high elevation of 1031 m (for the highlands of Thailand), in Wawi, Chiang Rai. The samples with the highest anthocyanin concentration, however, were grown at a village close to the on-farm trial at Mae Wang (0.05° to the south, 0.03° to the west), but at 200 m higher elevation. Confirmation and reproducibility of the exceptional quality features of the rice grown, even without definitive identification of the responsible factors, would enable some of these highland villages to benefit from the legal protection provided by the geographical indication (GI), within Thailand (Department of Intellectual Property [DIP], 2003) and internationally (World Intellectual Property Oganization [WIPO], 2019), as has been the case of special-quality rice from the south, Sangyod Muang Phattalung (DIP, 2006), and north-east, Hom Mali Thung Kula Rong-hai (DIP, 2007), of Thailand. While some selected rice varieties were comparable in productivity to the check varieties, most others were much lower yielding. However, a well-developed market for premium-priced special-quality rice has enabled farmers in Thailand (Rerkasem, 2017) and also in Cambodia (International Finance Corporation [IFC], 2015; Vannak, 2017; Vent *et al.*, 2015) to compensate for their inability to take advantage of modern rice varieties with higher yield by growing local varieties with higher quality and price.

When grown at CMU in the lowland (330 m), the farmers' rice varieties maintained their higher phenol concentration and anti-oxidative capacity than the check varieties, although not in the anthocyanin concentration. Genetic diversity within the farmers' seed lots is a common feature of local rice varieties in Thailand, and has been demonstrated with molecular markers (Pusadee *et al.*, 2009, 2014) and in functional traits (Jaksomsak *et al.*, 2015; Laenoi *et al.*, 2018; Pintasen *et al.*, 2007). The variation in the grain quality characteristics of the 10 single seed descent lines from each sample, that is, the rice grown from individual seeds found here (Figure 3), demonstrates the range of bioactive components in the on-farm germplasm that were averaged out in the chemical analyses involving samples each containing 50–100 rice kernels. Intensity of staining, Perls' Prussian blue specific for Fe and dithizone specific for Zn have been suggested as a means for rapidly detecting variation in the concentration of these nutrients in individual rice grains (Jaksomsak *et al.*, 2015; Pintasen *et al.*, 2007). Visual ranking of the intensity of the purple pigmentation of the pericarp correlated significantly with anthocyanin concentration (Kathuai *et al*., 2013), but unfortunately no similar quick and easy method for preliminary screening is available for phenols and anti-oxidative capacity. The close association between anthocyanin concentration and anti-oxidative capacity previously reported (e.g. Nam *et al*., 2006; Rerkasem *et al*., 2015; Xiongsiyee *et al*., 2018) is also found here with the single seed descent lines (R^2 = 0.61, P < 0.001).

Enhancing the nutritional quality of staple food crops is a powerful tool to increase the daily intake of nutritional compounds to combat malnutrition and afford better health. Breeding rice varieties with high grain nutritional quality is a sustainable solution that benefits consumers as well as farmers (Graham *et al*., 1999; Welch and Graham, 2004). However, improvement in grain quality of pigmented rice needs to be considered separately from that of the ordinary white rice normally consumed as staple food. Pigmented rice has long been part of the Asian rice culture (Appa *et al*., 2006; Sukhonthara *et al*., 2009). Pigmented rice has been reported to have much lower genetic diversity and higher homozygosity (Pusadee *et al*., 2019; Vilayheuang *et al*., 2016) compared with the local germplasm of ordinary white rice (Huang *et al*., 2010; Pusadee *et al.*, 2009, 2014; Roy *et al.*, 2016; Wunna *et al*., 2016), possibly due to the more conservative management of a small area of the crop (<5% of the total rice area) for special usages in making ceremonial preparation and desserts. In spite of this, there is clear evidence of variation in functional traits in the range of anthocyanin, phenols and anti-oxidative capacity found in this study among the 10 single-seed decent lines of each of the selected farmer's rice seed stock. The top-performing lines for the bioactive compounds are being further investigated for potential development of single genotypes. Alternatively, quality could be raised by elimination of the lower performing lines. It should, however, be pointed out that grain quality improvement in pigmented rice is unlikely to directly benefit the majority of rice consumers as it is not regularly used as the staple food. The economic potential of pigmented rice lies in the emerging market for premium-priced special-quality rice as health food and supplements (e.g. see Boonsit *et al*., 2010; Panomjan *et al*., 2016; Pusadee *et al*., 2019; Saeton, 2010).

5. Conclusions

In conclusion, a wide range of variation in grain nutritional and bioactive quality was found among the local rice varieties grown in different locations in the highlands of Northern Thailand. Strong location effects were observed on the selected genotypes in their anthocyanin content and anti-oxidative capacity, but not their phenol content. Variation was also found in the grain nutritional and bioactive quality among single-seed descent lines of each of the selected farmer's seed -lots. With respect to bioactive compounds, potential for development of pigmented rice with improved grain quality may be found in further investigation of the top-performing lines or elimination of the inferior lines.

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Conflict of interest

The authors have declared no conflict of interest in this article.

Compliance with ethical standards

This article followed all ethical standards for a research without direct contact with human or animal subjects.

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