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The boosting of anthocyanin accumulation in black rice cv. Riceberry by spraying $MgSO_4$ at two different altitudes under field conditions

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Abstract The three $MgSO_4$ treatments were sprayed to the Riceberry variety at booting stage in high altitude (Chiang Rai; CR) and low altitude (Nakhon Pathon; NKP). The results of $MgSO_4$ foliar spraying at different concentrations did not affect by agronomic traits and grain yield in each location. However, most agronomic traits and grain yield except grain weight in CR were significantly higher than those in NKP. The grain yield in CR ranged from 6.23–6.95 t/ha, while that of NKP ranged from 3.89–4.43 t/ha. When consider the anthocyanin content, spraying $MgSO_4$ at 100 mM in CR showed the highest anthocyanin content (192 mg/100 g) and increased approximately 50% over the control (128 mg/100g). On the other hand, the anthocyanin content after spraying $MgSO_4$ at 100 mM (76 mg/100 g) and 50 mM (70 mg/100 g) in NKP was not significant, but it was higher than that of 0 mM $MgSO_4$ (48 mg/100 g). In addition, the anthocyanin contents from CR were significantly higher than from NKP for any amount of $MgSO_4$ treatment. The phenolic compound and antioxidant capacity determined by the DPPH assay were positively correlated with anthocyanin content. However, foliar application of $MgSO_4$ had no clear impact on color intensity or pericarp thickness. Thus, growing Riceberry variety at high altitude and boosting anthocyanin synthesis with 100 mM Mg can produce high anthocyanin contents and grain yields.

Keywords: Anthocyanin, Altitude, Black rice, Grain yield, Magnesium

Introduction

Black rice is landrace varieties mostly grown in China, Korea, Japan, Thailand, the Philippines and India (Kong *et al.,* 2008). Recently, Asian black rice has gained more attention in the international market (Yamuangmorn and Prom-u-Thai, 2021), and it is becoming popular among rice conscious consumers and dietitians due to its high nutritive and

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medicinal value (Kushwaha *et al.,* 2016a). Black rice refers to unpolished rice in which the husk is removed and the black pericarp, seed coat and nucellus remain intact (Kim *et al*., 2008). The pericarp color of rice grains is an important agronomic trait affected by domestication and color pigment (Wang *et al.,* 2020). However, rice pericarp color is diverse, ranging from white, brown, and red to black (Maeda *et al*., 2014). The black pericarp of rice grain has been reported as anthocyanin, that is one of the key determinants of rice nutritional quality. Anthocyanin is a flavonoid pigment with various colors from pink to blue hues (Castaneda-Ovando *et al.,* 2009; Tisarum *et al.,* 2018). Cyanidin-3-glucoside (C3G) is a major anthocyanin in black rice, but minor anthocyanins have been reported to be either malvidin-3-glucoside (Yoon *et al*., 1995) or peonidin-3-glucoside (Pt3G) (Hou *et al*., 2013). Moreover, anthocyanins are an enriched source of antioxidants that have the ability to inhibit the formation or to reduce the concentrations of reactive, cell-damaging free radicals and neuronal and cardiovascular illnesses (Castaneda-Ovando *et al*., 2009).

The synthesis of anthocyanin in plants is controlled by an interaction between genetic and environmental factors (Yamuangmorn and Prom-u-Thai, 2021). The intensity of grain color is dependent on many environmental factors during cultivation, such as sunlight (quality and duration), altitude level, day and night temperature, moisture content, rainfall, water conditions, and nutrient availability in the soil (Jaksomsak *et al*., 2020; Rerkasem *et al*., 2015). In addition, the accumulation of anthocyanins depends on plant hormones, osmotic stress and acidic conditions (Shaked-Sachray *et al*., 2002; Kovinich *et al*., 2014). The influenced differently of the environment such as altitude and temperature may be affected the biologically active of black rice varieties (Somsana *et al*., 2013). Temperature is one of the most important factors affecting anthocyanin biosynthesis, and low temperature regulates anthocyanin accumulation (Shaked-Sachray *et al*., 2002). In addition, the difference in altitude affected the anthocyanin content in black rice varieties (Rerkasem *et al.,* 2015). The anthocyanin content of black rice increased significantly with the increase in altitude of its cultivation site (Kushwaha, 2016b).

Micronutrient such as Mg, Ca, Fe, Mn, Zn and Cu stabilize anthocyanins by forming a complex with anthocyanins without increasing enzyme activities in the anthocyanin biosynthesis pathway (Takeda, 2006; Yoshida *et al.,* 2006). Bennett *et al.* (2020) reported that calcium dramatically increased the total grain anthocyanin concentration by threefold compared to the control, while selenium effectively increased anthocyanin in the leaf. In addition, ZnO increased anthocyanin in rice grain pericarp and were accompanied by an increase in enzyme antioxidant activity (Samart and Chutipaijit, 2019). Moreover, an increase in anthocyanin content using $MgSO_4$ treatment has been established in aster flowers (Shaked-Sachray *et al*., 2002), red grapevine cell suspensions

(Sinilal *et al*., 2011), and purple wheat grains (Bustos *et al*., 2012). Tisarum *et al.* (2018) reported that the total anthocyanin concentration in the pericarp of the Jao Hom Nin rice variety treated with 100 mM $MgSO_4$ at low temperature was increased 3.8 times over that of the control, while leaf chlorosis was observed together with low spikelet fertility and reduced grain yield under growth chamber conditions.

In Thailand, new elite black rice variety has been bred and cultivated as premium rice. Riceberry rice is a popular back rice in Thailand that derived from cross-breeding between the Jao Hom Nin variety, which is well known to have high antioxidant properties, and Khao Dawk Mali 105, which is well known as a fragrant rice (Vanavichit, 2021). In addition, Riceberry possesses longer and more translucent grains that retain the cooking qualities of softness, good taste, and good smell (Vanavichit, 2021). Riceberry has been established in many areas around central, northern and northeastern Thailand, where the climate is favorable for the growth of high-quality rice. However, the anthocyanin content ,color intensity in pericarp and grain yield varied according to the planting area and the growing season. Therefore, the aim of this study was to focus on the impact of Mg foliar spray under field conditions at different altitudes on anthocyanin content, antioxidant capacity, agronomic traits and grain yield in Riceberry.

Materials and methods

Growth condition

This experiment was conducted in the wet season of 2020 (June to October) in two locations, Tana Grain Polish, Ltd., Phan district, Chiang Rai province (CR) (19°35'09.4" N, 99°44'42.7" E, 413 m above sea level; soil series Phan) and Kasetsart University, Kamphaeng Saen Campus, Nakhon Pathom province (NKP) (14°01′ N, 99°58′ E, 10 m above sea level; soil series Kamphaeng Saen), which are located in northern and central parts of Thailand, respectively. Weather parameters, including the air temperature, relative humidity, and the amount of rain in the field, were measured every 3 h by a data logger (WatchDog 2000 Series Micro Stations, Spectrum Technologies, Inc., USA). The soil sampling for soil analysis was performed according to methods described by the Rice Department (Rice Department, 2021). Six samples from each site were collected at a depth between 0-40 cm before sowing. In addition, the water properties (phosphate, potassium, calcium, magnesium, sodium, EC and pH) were analyzed by collected six samples of water from farm ditches. The soil and water properties were analyzed by the Department of Soil Science, Faculty of Agriculture at Kamphaeng Sean , Kasetsart University, Nakhon Pathom province. Seeds of the Riceberry variety were sown in a field nursery. After 30 d, the rice seedlings were transplanted into experimental plots. Additionally, fertilizer was applied at 15 d after planting at a rate of 75 kg/ha of N , 37.5 kg/ha of P_2O_5 and 37.5 kg/ha of K_2O. The second split of fertilizer was applied at the booting stage (65 d after planting) at a rate of 37.5 kg/ha of N. Other management practices were performed in accordance with conventional high-yield cultivation approaches.

Mg treatment

The plot size for each treatment was 2.5 m x 2.5 m (6.25 m^2), with a spacing of 25 cm x 25 cm. The experiment was conducted as a randomized complete block design with three replications. The three $MgSO_4$ (Agricultural grade; Kemaus, Australia) treatments were set as follows: foliar spraying with 0 mM (water), 50 mM (6.92 g $MgSO_4$/l of water) and 100 mM (13.84 g $MgSO_4$/l of water). $MgSO_4$ was mixed with surfactant (0.5 cc/1 of water). Foliar spray at 20 liter per plot was applied twice with 2 days intervals using a hand-held sprayer (Tisarum *et al.,* 2018) at the booting stage (60 d after transplanting). At the harvesting stage, paddy grains in each plot were separated and dried to 14% moisture at room temperature. The paddy grains were dehulled for brown rice using a mini-polisher. Then, the anthocyanin content, antioxidant activity and phenolic compounds in the pericarp of rice grain were analyzed.

Agronomic trait collection

Agronomic traits were collected, including days to 100% flowering, number of tillers per plant, number of panicles per plant, plant height, 1000 grain weight and grain yield. The days to flowering were recorded when 100% of the individual plants in each plot flowered. The number of tillers per plant, number of panicles per plant and plant height were measured at maturity. The grain yield in each plot was determined per harvested area of 6.25 m^2. The grain yield moisture was adjusted to 14% and then extrapolated to kg per ha. After threshing, the grains were weighed to obtain a 1000 grain weight.

Pericarp thickness and pigment color analysis

The pigment intensity of unpolished rice was measured with a colorimeter meter (model CR-300, Minolta, Japan). L*, a* and b* values were calculated to determine the color of the rice pericarp of each treatment, where ‘L*’ indicates the degree of lightness or darkness (L* = 0 indicates perfect black and L* = 100 indicates most perfect white; hue chart); ‘a*’ indicates the degree of redness (+) and greenness (−); and ‘b*’ indicates the degree of yellowness (+) and blueness (Lamberts *et al.,* 2006).

The pericarp thickness was measured in each treatment. The unpolished rice grains were soaked in water for 24 h and dissected into longitudinal sections using a razor blade. The dissected grain was placed on agar in a Petri dish and observed under a light stereoscope (Leica model EZ4, Switzerland). The pericarp thickness was measured at five points on the pericarp using the program GIMP 2.10.12.

Determination of the anthocyanin content and phenolic compounds

The anthocyanin content in pericarp grains was measured according to the procedures described by Rahman *et al.* (2015). One hundred grams of unpolished rice grains was extracted in 100 ml of methanol containing 1% HCl. The extracted solution was filtered twice, and then the supernatant was pipetted to 2 ml and diluted with 1% HCl in methanol to 100 ml. The absorbance was measured at 535 nm using a Spectrophotometer (SpectraMax M2 Multilabel Microplate Reader). The total phenolic content was evaluated using Folin-Ciocalteu reagent (Kähkönen *et al.,* 1999). The total phenolic content was calculated from the calibration curve of gallic acid equivalents (GAE) according to the formula $Y = 0.0094x + 0.0028$, $R^2 = 0.998$. The total phenolic contents were expressed as mg GAE per 100 g dry weight (DW).

Determination of antioxidant capacity

The inhibitory effect of rice grain water extracts on DPPH radical scavenging was determined following the method of Boskou *et al*. (2006). Five hundred milliliters of the rice extraction solution was mixed with 200 µl of 0.5 mM DPPH methanol solution and 500 µl of 0.1 M sodium acetate buffer (pH 5.5). After shaking, the mixture was incubated in the dark at room temperature for 30 min, and then the absorbance was measured at 517 nm. Tert-butyl hydroxytoluene (BHT) was used as a positive control, while water and methanol were used as controls for calculation. ABTS radical scavenging was investigated using the method of Hsu *et al.* (2011). The $ABTS^+$ solution was generated by mixing 7 mM $ABTS^+$ and 2.45 mM potassium persulfate in water, which was placed in the dark at room temperature for 16 h to give the complete oxidation of ABTS. Before use, the $ABTS^+$ solution was diluted with water to obtain an absorbance of 0.700 ± 0.050 at 734 nm. Two hundred microliters of $ABTS^+$ solution was added to 20 µl of rice extracted solution and mixed thoroughly. The reactive mixture was incubated at room temperature for 6 min, and the absorbance was immediately recorded at 734 nm. BHT was used as a positive control, and water was used as a control.

Statistical analysis

All the data were analyzed using R program version 3.6.1 (R Core Team, 2014) to test the significance of the results in terms of agronomic traits and phytochemical and antioxidant capacity. The means were separated using Duncan's test at alpha levels of 0.01 and 0.05. If there was a significant difference among the experiments for a given parameter, then the values from all the experiments for that parameter were used to obtain the mean and standard error. The combined analysis was analyzed to compare the results between two locations. In addition, paired sample *t* tests (degrees of freedom, df =5) were performed at an alpha level of 0.05 to compare each soil and water property between both locations. Relationships between grain yield, color intensity, anthocyanin content, antioxidant activity and phenolic content attributes were calculated using Microsoft Excel software (n=18).

Results

Weather and soil properties

The mean daytime temperatures (7.00–16.00 h) from June–October 2020 in CR and NKP were 27.7 ℃ and 29.6 ℃, respectively, while the mean nighttime temperatures (19.00–4.00 h) in CR and NKP were 24.7 ℃ and 26.5 ℃, respectively. Thus, the mean air temperature in CR was lower than that in NKP during both the day and night by approximately 3.0 ℃ (Figure 1A and B). The mean maximum and minimum values from the same period in CR were 30.7 ℃ and 23.2 ℃ (7 ℃ interval), respectively, while those in NKP were 32.7 ℃ and 25.2 ℃ (7 ℃ interval), respectively. Therefore, both maximum and minimum temperatures in CR were lower than those in NKP by approximately 2.0 ℃ (Figure 1A and B). The daytime relative humidity levels in CR were different from those in NKP (81.6% RH in CR and 72.8% RH in NKP). However, the nighttime relative humidity in CR was slightly different from that in NKP (91.9% RH in CR and 87.3% RH in NKP). In addition, the relative humidity in CR was more stable than that in NKP (Figure 1C and D). The rainfall amount during four months in this experiment in CR was lower than that in NKP (384.9 mm in CR and 945.3 mm in NKP). However, the frequency of rain in CR was more uniform than that in NKP (Figure 1E and F).

The soil of the experiment in CR had clayey texture, while the soil of the other experiment in NKP had loamy texture. Chemical properties of these two soils were different. The soil pH values in CR (6.56) and NKP (5.80) were identified as slightly acidic and moderately acidic, respectively. The electrical conductivity, total nitrogen and exchange magnesium between CR and NKP were similar. However, the organic matter and available phosphorus in NKP were higher than those in CR, while the exchanged potassium and calcium in CR were higher than those in NKP. However, the soil nutrients in the two locations were adequate for rice cultivation (Table 1).

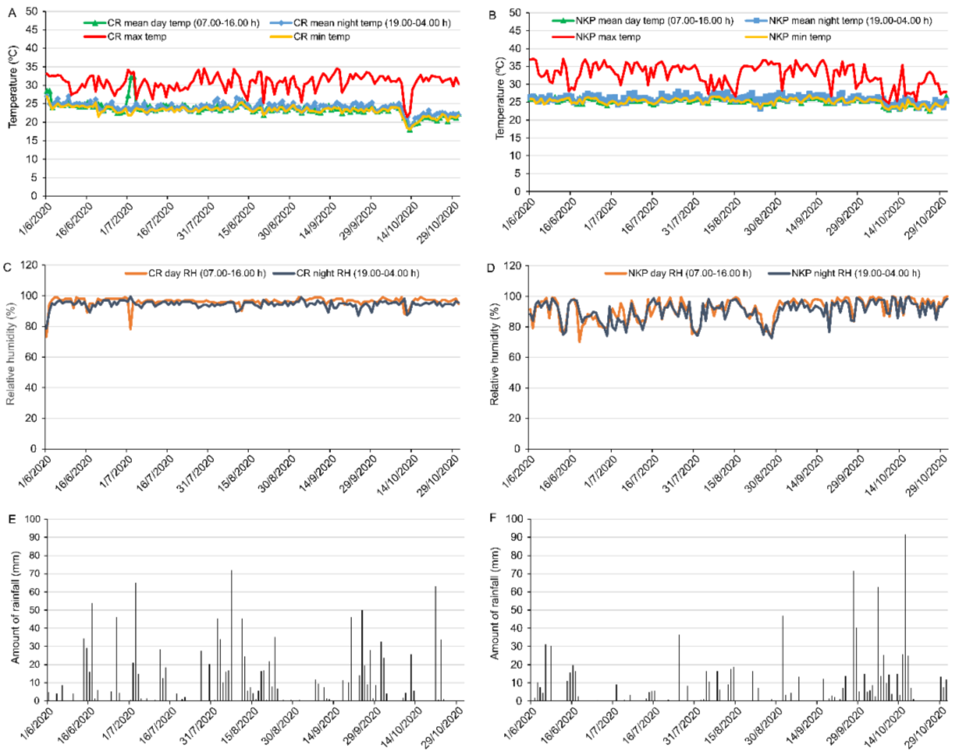

Figure 1. Weather data including air temperature, mean relative humidity and amount of rainfall of the experiment areas in Chiang Rai (A, C, E) and Nakhon Pathom (B, D, F) from June to October 2020

Agronomic traits and grain yield

The agronomic traits and grain yield among the three $MgSO_4$ concentrations at both sites are shown in Table 2 and Figure 2. The agronomic traits including days to flowering, plant height, number of tillers per plant and number of panicles per plant were not significant among $MgSO_4$ treatments in each location. However, the grain weight and grain yield were slightly different among $MgSO_4$ treatments in each location. The grain yield in the control (0 mM $MgSO_4$) at each location was slightly higher than that of the grain yield treated with $MgSO_4$. Therefore, foliar spraying of $MgSO_4$ did not affect by agronomic traits or grain yield. Interestingly, most agronomic traits and grain yield except grain weight in CR were significantly higher than those in NKP. In CR Province, the grain yield ranged from 6.23–6.95 t/ha, while that of NKP ranged from 3.89–4.43 t/ha. Thus, the rice in CR yielded more than two times that of NKP.

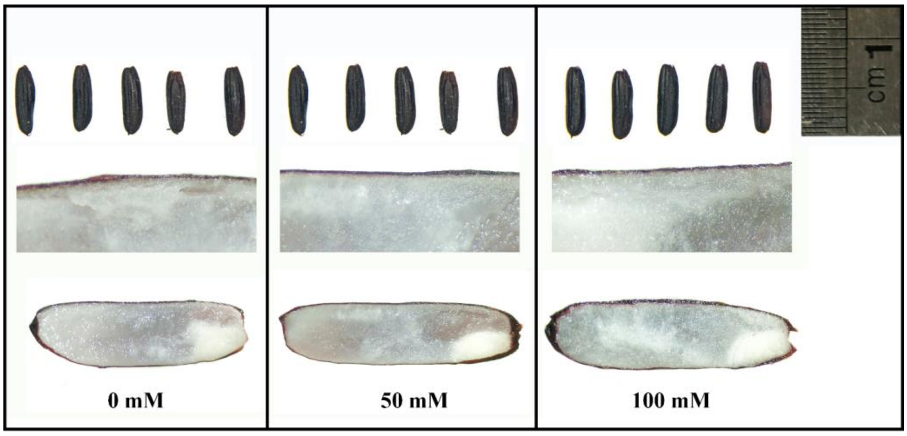

Figure 2. Grain color and cross section of grains of Riceberry compared with different $MgSO_4$ treatments

Table 1. Soil and water properties derived from Chiang Rai and Nakhon Pathom were tested in 2020

Soil properties	Chiang Rai	Nakhon Pathom	Soil properties for rice (Rice Department, 2020)
pH	6.56 ±0.56a[1/]	5.80 ±0.61b	5.50–6.50
Electrical conductivity (dS/m)	0.31 ±0.05a	0.48 ±0.03a	1.00–2.00
Organic matter (g/kg)	12.3 ±2.3b	38.2 ±3.5a	n/a
Total Nitrogen (mg/kg)	700 ±5.1a	1000 ±7.2a	n/a
Available phosphorus (mg/kg)	13.65 ±1.25b	29.45 ±0.95a	5.00–10.00
Exchange potassium (mg/kg)	110.29 ±2.35a	29.30 ±1.42b	60.00–80.00
Exchange calcium (mg/kg)	1981.58 ±10.25a	1120.20 ±13.58b	n/a
Exchange magnesium (mg/kg)	66.16 ±5.23a	69.73 ±4.55a	n/a
Soil type	Clay	Clay loam	n/a
Water properties	**Chiang Rai**	**Nakhon Pathom**	**Irrigation water quality for rice (Kahimba *et al.*, 2016)**
pH	8.01 ±0.78a	7.44 ±0.65a	6.5–8.4
Electrical conductivity (dS/m)	0.21 ±0.05a	0.17 ±0.08a	< 3.0
Nitrate (mg/l)	9.45 ±0.45a	9.86 ±0.61a	n/a
Phosphate (mg/l)	0.05 ±0.01a	0.02 ±0.01a	n/a
Potassium (mg/l)	13.56 ±0.78a	2.16 ±0.68b	n/a
Calcium (mg/l)	18.79 ±0.85a	18.24 ±0.74a	0–20
Magnesium (mg/l)	9.11 ±0.65a	7.46 ±0.42a	0–5
Sodium (mg/l)	44.38 ±0.88a	3.80 ±0.42b	0–40

[1/]Different small lowercase letters in the same row in two locations indicate a significant difference at the 0.05 level based on a *t* test. Values presented as mean ±SD

Table 2. Agronomic traits and grain yield of Riceberry compared with different $MgSO_4$ treatments under field conditions in Chiang Rai and Nakhon Pathom

$MgSO_4$ concentration (mM)	DF (days)[3]		PH (cm)		NTP		NPP		TGW (g)		GY (t/ha)	
	CR	NKP	CR	NKP	CR	NKP	CR	NKP	CR	NKP	CR	NKP
0	113B[2]	120A	110A	106B	27A	13B	27A	13B	21.7A	21.4a[1]A	6.96aA	4.43aB
50	113B	120A	108A	107A	25A	12B	25A	12B	21.2A	21.5aA	6.23abA	3.98bB
100	115B	120A	112A	105B	25A	13B	25A	13B	21.0A	20.0bA	6.84aA	3.89bB
Mean	114	120	110	106	26	13	16	13	21.3	20.9	6.68	4.10
F-test (P<0.05)	ns	ns	ns	ns	ns	ns	ns	ns	ns	*	*	*
CV%	1.23	2.12	4.25	3.12	8.54	11.35	7.56	6.53	2.98	3.61	10.25	7.39

1/ Different lowercase letters in the same column indicate significant differences at the 0.05 level using Duncan's test.
2/ Different capital letters of each trait in the same row indicate significant differences at the 0.05 level using Duncan's test.
3/ DF=Days to flower, PH=Plant height, NTP=Number of tillers per plant, NPP=Number of panicles per plant, TGW=1000 grain weight, GY=Grain yield

Pericarp thickness and color intensity

The color intensity identified by the L^* value on the rice pericarp did not differ among $MgSO_4$ treatments or between the two locations. Riceberries from three $MgSO_4$ treatments in both locations were identified as dark purple color, as shown in Table 3 and Figure 3. When considering the pericarp thickness, the results showed that the Riceberry pericarp among the three $MgSO_4$ treatments and between the two locations were not significant (Table 3 and Figure 3). Therefore, foliar spraying of $MgSO_4$ at different concentrations did not increase color intensity, and the pericarp thickness was not changed.

Table 3. Pericarp thickness, pigmentation intensity and pericarp color of the Riceberry compared with different $MgSO_4$ treatments in Chiang Rai and Nakhon Pathom

$MgSO_4$ concentration (mM)	Pericarp thickness (µm)		Pigmentation intensity						Pericarp color
			L^*		a^*		b^*		
	CR	NKP	CR	NKP	CR	NKP	CR	NKP	
0	39	37	7.36	7.16	6.53	6.63	5.14	5.23	Dark purple
50	39	35	6.78	6.97	6.15	6.33	4.83	4.70	Dark purple
100	39	37	7.65	7.17	6.58	6.53	5.40	5.20	Dark purple
Mean	39	36	7.26	7.10	6.42	6.50	5.12	5.04	
F-test (P<0.01)	ns	ns	ns	ns	ns	ns	ns	ns	
CV%	7.87	8.69	18.93	14.85	15.71	14.41	15.92	16.53	

Anthocyanin content and antioxidant capacity

The total anthocyanin content was extracted with methanol from brown rice grains among the three different $MgSO_4$ concentrations and between locations was significant. In CR province, the spraying $MgSO_4$ at 100 mM showed the highest of anthocyanin content (192 mg/100 g) and 0 mM was the lowest. On the other hand, the anthocyanin content after spraying $MgSO_4$ at 100 mM (76 mg per 100 g) and 50 mM (70 mg per 100 g) in NKP was not significant, but it was higher than that of 0 mM $MgSO_4$ (48 mg per 100 g). When considering between locations, it was found that the anthocyanin contents from CR was significantly higher than from NKP for any amount of $MgSO_4$ treatment (Figure 3A).

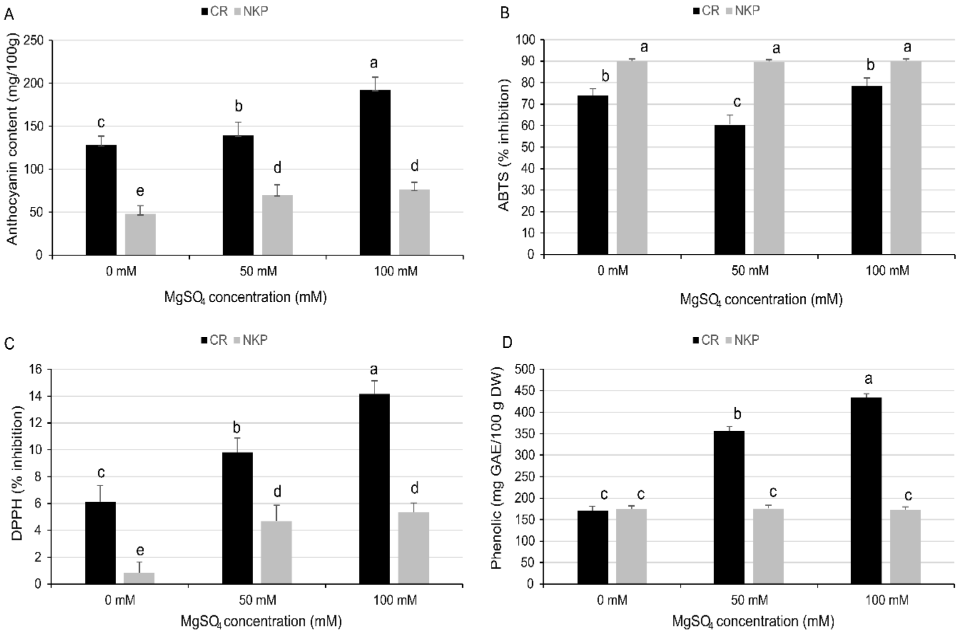

Figure 3. The anthocyanin content (A) ABTs (B) DPPH (C) and phenolic content in rice grain compared with different $MgSO_4$ treatments in the Chiang Rai and Nakhon Pathom, where different lowercase letters above each columns indicate significantly different ($p < 0.05$). Error bars indicate +SD.

The antioxidant capacity was determined by ABTs and DPPH assays, as shown in Figure 3B and C. The ABTs sprayed with $MgSO_4$ in CR showed higher at 0 and 100 mM (73.69% and 78.38%, respectively) than those at 50 mM (60.48 %), while ABTs sprayed with $MgSO_4$ in NKP was not significantly differed when compared among all concentrations. In addition, the ABTs in NKP at all concentrations of $MgSO_4$ were higher than those of CR. On the other hand, the DPPH results showed the same trend of anthocyanin content. DPPH was significant among the three $MgSO_4$ concentrations and between the two locations. DPPH was highest in 100 mM $MgSO_4$ (14.51% in CR and 5.36% in NKP), while the concentrations at 0 and 50 mM $MgSO_4$ were not significant in either location. However, the DPPH in CR was higher than that in NKP in every $MgSO_4$ treatment.

The phenolic compound content in NKP was not significantly different among the concentrations of $MgSO_4$ (Figure 3D), while the phenolic compound content at rates of 50 and 100 mM $MgSO_4$ (356.77 and 434.38 mg GA/100 g extract) in CR was significantly higher than that at 0 mM. Moreover, the phenolic compound content when spraying $MgSO_4$ at rates of 50 and 100 mM in CR was higher than that in NKP, but it was not significant at 0 mM.

The regression analysis

The regression between anthocyanin content and grain yield was positively significant (R^2 = 0.86) (Figure 4A). In addition, a positive correlation was found between anthocyanin content and DPPH radical scavenging (R^2 = 0.96) and between phenolic content (R^2 = 0.83) (Figure 4B and D). Surprisingly, the correlation between anthocyanin content and ABT radical scavenging showed a negative significance (R^2 = -0.71) (Figure 4C). In addition, anthocyanin content and color intensity were not significantly correlated (data not shown).

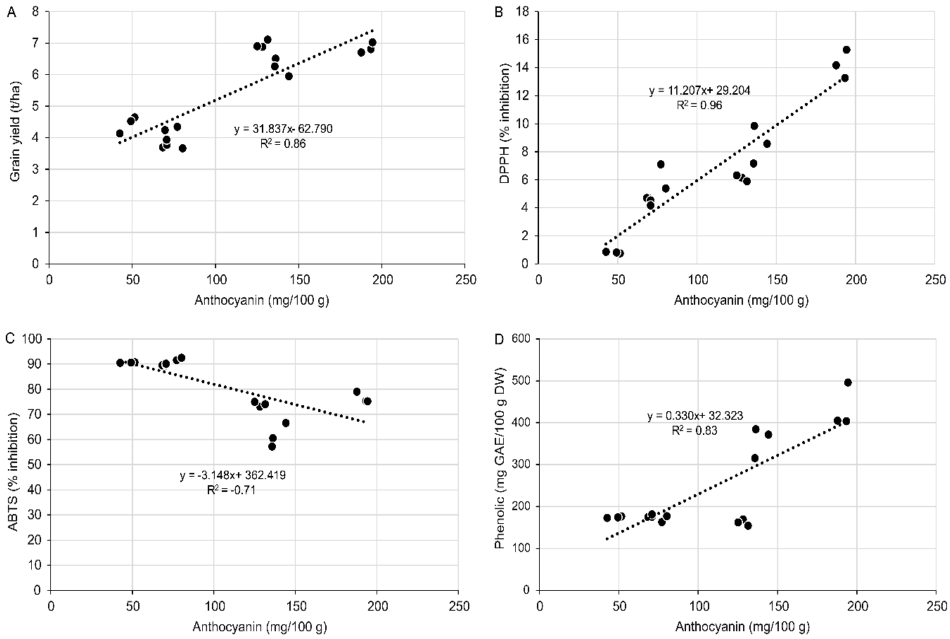

Figure 4. The relationship between anthocyanin with grain yield (A) DPPH (B) ABTS (C) and phenolic content (D)

Discussion

In terms of agronomic traits and grain yield, the different $MgSO_4$ concentrations were not affected in either location. These results were the same as those of Tisarum *et al*. (2018), who reported that agronomic traits and grain weight per panicle were not significant among different $MgSO_4$ concentrations at the same temperature (16 ℃ growth chamber). However, the agronomic traits and grain yield of rice that grew in CR showed higher performance than those of NKP. When considering the temperature, it was found that the mean, maximum and minimum temperatures in CR were lower than that of NKP by approximately 2 ℃. The mean temperature in CR (26 ℃) was slightly inappropriate for rice cultivation. Kushwaha (2016a) suggested that the optimum temperature for black rice should be at a mean of 24 ℃ during the rice crop standing period, while the mean temperature in NKP (28 ℃) was over the optimum by 4 ℃. However, the growth performance at temperatures lower than 16 ℃ decreased compared with 32/26 ℃ (day/night) (Tisarum *et al*., 2018). In addition, the altitude of CR (413 m above sea level) was higher than that of NKP (10 m above sea level), which may influence the difference in air temperature, humidity and amount of rainfall between the two locations. These results may suggest that the difference in grain yield between the two altitudes might be explained by the physiological responses of rice grown at different elevations, such as average day/night temperature, photoperiod, growing degree days and irradiance level (Rerkasem *et al.*, 2015; Kushwaha, 2016b). In addition, Riceberry is suitable for growing at temperatures approximately 26 ℃, and the northern region of Thailand is appropriate for the cultivation of this type of rice.

Anthocyanin variability in rice grains may depend on many factors, such as variety, accumulation form, and location that could be affected by environmental factors during cultivation (Yamuangmorn and Prom-u-Thai, 2021). In black rice, anthocyanin was started to synthesis from flowering stage (Jiamyangyuen *et al*., 2017) and the high level of anthocyanins accumulation occurs during grain filling stage (Reddy *et al*., 1995; Shao *et al*., 2014). Thus, if $MgSO_4$ was applied lately at grain filling stage, it may not help to increase anthocyanin content in rice pericarp. Tisarum *et al.* (2018) suggests that 10 mM $MgSO_4$ exogenous foliar spray did not promote Mg concentration in plant tissues. Therefore, the concentration in this study was applied at 50mM and 100mM. Interestingly, the accumulation of anthocyanin content in the rice pericarp sprayed with 100 mM $MgSO_4$ showed the highest content in CR, while $MgSO_4$ at 50 mM and 100 mM in NKP was not significant, but it was higher than that of $MgSO_4$ at 0 mM. Tisarum *et al*. (2018) reported that anthocyanin had the most enrichment in the pericarp of the Jao Hom Nin variety when spraying with $MgSO_4$ at 100 mM, especially in plants grown under 16 ℃ air temperature for 28 d. In contrast, Kim *et al*. (2007) and Mackon *et al*. (2021) reported that growing rice at 22–27 ℃ resulted in higher expression levels of anthocyanin biosynthetic genes during seed maturation related to the accumulation of cyanidin, cyanidin-3-glucoside, and peonidin-3-glucoside in black rice grains, whereas gene expression was reduced at lower temperatures (21–24 ℃). In addition, high temperatures >35 ℃, especially during grain filling, have an inhibitory effect on anthocyanin accumulation (Mackon *et al.*, 2021). Therefore, the amount of anthocyanin content in the rice pericarp in two locations was not the same even though the same cultivar was used in the experiment. It may suggest that the different of anthocyanin content between two locations might be affected by soil properties and environment condition. However, spraying $MgSO_4$ can boost the anthocyanin content in Riceberry.

Improving Mg uptake in rice can form a metalloid copigment complex for stabilizing anthocyanin, thus increasing the concentration in the rice pericarp (Tisarum *et al*., 2018). Sinilal *et al*. (2011) reported that Mg^{2+} acts as a coenzyme regulator in anthocyanin biosynthesis that could sustain a high level of anthocyanin in the rice pericarp. Therefore, in this study, the increasing of anthocyanin content may cause by spraying $MgSO_4$, which stimulated the efficiency of anthocyanin synthesis in rice grain. In addition, the anthocyanin content in all treatments in CR (high altitude) was higher than that in NKP (low altitude). Kushwaha (2016b) reported that growing black rice at high altitudes may impact the anthocyanin content in grain. Moreover, the relationship between anthocyanin content and grain yield was positive, while Jaksomsak *et al.* (2021) reported that grain yield was not correlated with anthocyanin content because anthocyanin deposition in the pericarp of black rice reduced the photosynthetic rate that it can decreased grain weight (Rahman *et al.*, 2015). However, in this study, growing at high altitudes and treating with $MgSO_4$ may help to enhance the accumulation of anthocyanin content (Somsana *et al*., 2013; Kushwaha, 2016a) together with grain yield but the color intensity between two different altitudes in this study was not different.

The antioxidant capacity and total phenolic compound content of black rice increased with increasing concentrations of anthocyanin (Rerkasem *et al.*, 2015; Settapramote *et al*., 2018; Yamuangmorn *et al*., 2018). This supports the results of our study, especially in the results of DPPH radical scavenging and phenolic content. However, ABT radical scavenging was not related to the trend of anthocyanin content. Thus, DPPH can be used to identify antioxidant capacity in black rice grain better than ABTs. Sompong *et al*. (2011) demonstrated that anthocyanins were some main compounds of total phenolic content in black rice. In addition, Zhang *et al*. (2010) reported that the total antioxidant activity of black rice bran was correlated with the contents of total anthocyanins and total phenolics.

In conclusion, grain yield and anthocyanin content in black rice cv. Riceberry is controlled by an interaction between variety and environmental factors. In addition, $MgSO_4$ spraying increased anthocyanin accumulation in Riceberry grain pericarp in both locations. However, Riceberry that grew at high altitudes (CR) had higher anthocyanin content, antioxidant and phenolic than those of low altitudes (NKP). Furthermore, CR was suitable for growth performance, giving higher grain yield than those of NKP. Therefore, growing of Riceberry in the northern part of Thailand and boosting anthocyanin synthesis with 100 mM Mg can produce high grain yield and high anthocyanin content.

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