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Research article

Clustering of white, red and purple rice cultivars according to their total phenolic content, total flavonoid content and antioxidant capacity in their grains

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Abstract

Rice grains are a rich source of antioxidant compounds. The objectives of this study were to determine the total phenolic content (TPC), total flavonoid content (TFC) and antioxidant capacity (AC) in 174 rice germplasms, comprised of 152 white, 10 red and 12 purple pericarp cultivars, and to cluster the rice cultivars according to their TPC, TFC and AC levels using hierarchical cluster analysis (HCA). Among the tested germplasms, the TPC, TFC and AC levels of the red and purple pericarp cultivars were significantly higher than those of the white pericarp cultivars. A significant positive correlation between the TPC and TFC levels was found in all three color groups of rice cultivars. However, no significant correlation was found between the AC and TPC levels in the white pericarp rice. In contrast, a highly significant correlation (r = 0.918 and 0.806) was found between the AC and TPC levels in both the red and purple pericarp rice, while a significant correlation (r = -0.143 and 0.757) between the AC and TFC levels was found in the white and red pericarp rice. This suggested that the AC level in the red pericarp rice resulted from the phenolic and flavonoid compounds, while the major contribution to the AC level in the purple pericarp rice was from the phenolic compounds. The HCA revealed four groups with a distance value of 2.60. High TPC (5.134±0.459 mg GAE/g), TFC (10.034±0.668 mg RE/g) and AC (1844.769±294.773 µg AEAC/g) levels in the rice grains were found in six cultivars. Thus, these cultivars could be used as genetic resources for breeding to improve the antioxidant content in the future.

Introduction

Antioxidants are important compounds that inhibit the reactive oxygen species involved in oxidative stress (Liu, 2007). Oxidative stress is a serious health concern as it can lead to a number of chronic diseases, such as coronary heart disease, type-2 diabetes and cancer (Reddy et al., 1995; Tian et al., 2004; Butsat and Siriamornpun, 2010; Ravichanthiran et al., 2018). Antioxidant activities in plants are found among the phytochemicals, including phenolic acids, flavonoids, anthocyanidins, proanthocyanins, vitamins, amino acids and phytosterols (Newmark, 1996; Liu, 2007; Imam et al., 2012) and in a wide variety of fruits, leaves and whole grains (Adom and Liu, 2002; Areekul and Phomkaivon, 2015; Ouerghemmi et al., 2017). Thus, the consumption of fruits, legumes, vegetables and whole grains can supplement the diet with natural antioxidants (Liu, 2007; Min et al., 2012), besides other bioactive compounds.

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Rice (*Oryza sativa* L.) is culturally the main food in Asia (Shen et al., 2009). There are three major colors of the rice pericarp (white, red and purple) which represent a unique pigmentation feature of the rice bran as the bran layers covering the rice grain contain many antioxidant compounds, such as phenolic acids, flavonoids, tocopherols, tocotrienols, γ-oryzanol and γ-aminobutyric acid (Butsat and Siriamornpun, 2010; Kaur et al., 2017). Red and purple rice cultivars have been investigated as potential sources of phenolic compounds and have been shown to possess strong antioxidant activities compared to white rice cultivars (Goffman and Bergman, 2004; Shen et al., 2009) and the phenolic and flavonoid contents are correlated to the rice antioxidant activities (Shen et al., 2009; Sompong et al., 2011; Shao et al., 2018).

Phenolics are compounds that are comprised of one or more aromatic rings bearing a hydroxyl substituent. Phenolic compounds are also known to be antioxidants that can provide health benefits associated with reducing the risk of various chronic illnesses (Newmark, 1996; Imam et al., 2012). Flavonoids are phenolic compounds that generally consist of two aromatic rings linked by three carbons that become part of a heterocyclic ring (Hosoda et al., 2018). They are divided into several subclasses, which include the anthocyanins, flavanols, flavanones, flavonols, flavones and isoflavones.

The antioxidant capacity (AC) of food extracts can be determined by various antioxidant testing methods, such as ferric reducing antioxidant power, 2,2′-azino-bis-3-ethylbenzthiazoline-6-sulphonic acid (ABTS) and 2,2-diphenyl-1-picrylhydrazyl. Each of these arrays can independently account for more than one mechanism of the antioxidants (Benziea and Strain, 1996; Re et al., 1999; Molyneux, 2004). The ABTS radical method has excellent stability and is one of the fastest methods for the evaluation of the AC. This method provides reproducible results, maximum absorption and good solubility, and so it has been used for food products, pure compounds and plant extracts (Re et al., 1999).

Several studies have classified rice groups according to their antioxidant compounds using cluster analysis. One study investigated 481 rice accessions based on their grain size, 100-grain weight and the three antioxidative properties, the total phenolic content (TPC), total flavonoid content (TFC) and AC, based on the ABTS method (Shen et al., 2009). The study reported significant positive correlations among the three antioxidative properties, while five components that were extracted explained 83.7% of the total variance of the observed variables. In another study, Thai rice cultivars with purple, red and white pericarps were collected from 20 locations in the North of Thailand to determine their color parameters and antioxidant properties (Pramai and Jiamyangyuen, 2006). The results demonstrated that rice cultivars could be classified into four main clusters that differed by their antioxidant properties and colors.

Understanding the genetic variation and classification of rice collections is important for quality improvement and to increase the yield of rice (Chuchert et al., 2018). Therefore, the aims of this study were to determine the TPC, TFC and AC (based on ABTS assay) of Thai rice germplasms (174 cultivars), to assess the relationship of these parameters, and to classify the rice cultivars based on their antioxidant contents and AC using hierarchical cluster analysis (HCA).

Materials and Methods

Plant materials

The selected 174 indica rice cultivars were obtained from the Center of Excellence in Environment and Plant Physiology (Chulalongkorn University), Chum Phae Rice Research Center, Phatthalung Rice Research Center, and Maejo University, Thailand. They comprised 152 white, 10 red and 12 purple pericarp cultivars. The rice grains were dehulled and classified as A for white (Fig. 1A), B for red (Fig. 1B) and C for purple (Fig. 1C) pericarps.

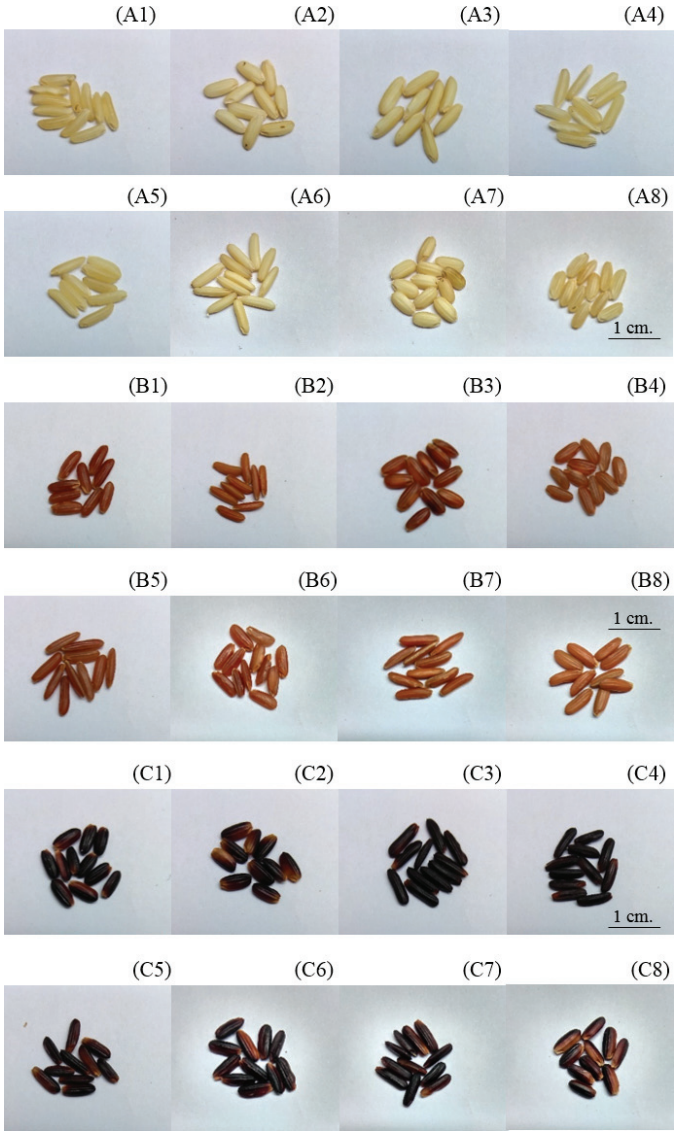

Fig. 1 Pericarp colors of three rice groups: (A) white pericarp group, A1–A8 = Leuang huan, Leuang dong, Plah sew, Ruang diaw, Khao dawk mali 105, Kaset daw, Ma fai, and Gwian hak, respectively; (B) red pericarp group, B1–B8 = Nok kum, Sang yod, Loi hah ruang, Lai mahk, Red rose rice, Rathu heenati, RD69, and Chaw pli khao, respectively; (C) purple pericarp group, C1–C8 = Gam feuang, Gam liaw, Mali dam, Riceberry, Khao hawm mea payah tawng dam, Gam nahng payah, Hawm nin, and Dam dahng, respectively

Sample extraction

The grain samples were powdered using a mixer mill (MM 400, Restsch; Germany). The extraction protocol was modified from Zhang et al. (2010). In brief, 0.5 g of rice was extracted with 10 mL of extraction solvent [1% volume per volume (v/v) hydrochloric acid in methanol] in a rotator shaker at room temperature for 24 hr. The mixture was then centrifuged at 4,000×*g* at 4°C for 15 min and the supernatant was harvested. The residue was extracted again in the same manner and the two supernatants were pooled and used for the measurements of the TPC, TFC and AC (based on the ABTS radical scavenging assay). All extracts were stored at 4°C until the analyses were performed. Three replicates of each rice cultivar were used in each of these assays.

Measurement of total phenolic content

The TPC was determined according to the Folin-Ciocalteu colorimetric method in the dark with minor modifications (Zhang et al., 2006). In brief, a 20µL sample of the extract was added to 100 µL working Folin-Ciocalteu reagent (10% Folin-Ciocalteu reagent solution) and 100 µL deionized water in a microplate, mixed and then incubated at room temperature for 1 min. Next, 80 µL of 7.5% weight per volume (w/v) sodium carbonate solution was added to the mixture and then incubated for 30 min in the dark at room temperature. The absorbance at 765 nm was measured using an ultraviolet visible absorbance microplate reader (SpectraMax® M3, Molecular Devices; USA). The TPC was expressed as milligrams of gallic acid equivalents per gram of rice (mg GAE/g rice).

Measurement of total flavonoid content

The TFC was estimated as described by Shen et al. (2009) and Herald et al. (2012) with slight modification. In brief, 200 µL of 50% (v/v) ethanol and 7.5 µL of 5% (w/v) sodium nitrite were combined as the reaction mixture in each well of a microplate, followed by 10 µL of the respective sample extract. The reaction mixture was incubated for 5 min before adding 15 µL of 10% (w/v) aluminum chloride to the mixture and incubating for 5 min. Then, 50 µL of 1 M sodium hydroxide was added and the mixture was incubated for 15 min before reading the absorbance of the final solution at 400 nm using the microplate reader (SpectraMax® M3, Molecular Devices, USA). The TFC was calculated as milligrams of rutin equivalent per gram of rice (mg RE/g rice).

Determination of the antioxidant capacity using 2,2'-azino-bis-3-ethylbenzthiazoline-6-sulphonic acid assay

The AC was determined using ABTS radical scavenging assay. First, a working solution of ABTS (ABTS·+ cation radical) was prepared overnight by the reaction of 7 mM of ABTS solution and 2.45 mM of potassium persulfate solution in the dark at room temperature (Re et al., 1999). Then, the ABTS·+ cation radical solution was diluted with deionized water to adjust the absorbance at 734 nm (A_{734}) to 0.700 ± 0.02. Next, 200 µL of diluted ABTS·+ cation radical solution was transferred to 96-well plates, followed by 20 µL of the sample extract being added to each well and incubated for 30 min in the dark. The A_{734} values were converted to a percentage inhibition using Eq. (1):

$$\text{Percentage inhibition} = \frac{\text{A734 of control} - \text{A734 of sample}}{\text{A734 of control}} \times 100 \quad (1).$$

The concentration and percentage inhibition of ascorbic acid was used to plot the standard curve. The AC of all the samples was measured in terms of micrograms of ascorbic acid equivalent antioxidant capacity per gram of rice (µg AEAC/g rice).

Statistical analyses and hierarchical cluster analysis

All the statistical analyses were performed using the IBM SPSS Statistics version 22 software (IBM; USA). One-way analysis of variance and Duncan's multiple range test were used to determine the significance differences between the groups of different pericarp colors, accepting significance at the $p < 0.05$ level. The data for the antioxidant compounds and AC were expressed as the mean ± SD and the range as boxplots.

Pearson correlation was used to estimate the correlation coefficients among the different parameters in the white, red and purple pericarp cultivars. The significance of differences in the correlation was determined at the $p < 0.05$ and 0.01 levels.

The Hierarchical cluster analysis (HCA) was performed on the 174 rice cultivars based on the three variables of TPC, TFC and AC. Distances between the samples were measured using agglomerative clustering, followed by cluster analysis using Ward's method (Milligan, 1980), as implemented in the JMP9 software (SAS Institute Inc., 2010). The results were used to construct a dendrogram showing the relationships among the 174 rice cultivars.

Results and Discussion

Measurement of total phenolic content

The TPC levels of the crude extracts from the 174 rice cultivars are summarized in Fig. 2A. The TPC levels of the white pericarp cultivars were in the range 0.86–1.93 mg GAE/g with a mean of 1.24 mg GAE/g. The red pericarp cultivars had the highest TPC levels (3.85–5.79 mg GAE/g) with a mean of 4.70 mg GAE/g, while the purple pericarp cultivars were slightly lower (3.01–4.74 mg GAE/g) with a mean of 4.07 mg GAE/g.

The top three TPC levels in each rice color group (Fig. 3A) were significantly different between each color group. The top three cultivars in the white rice group were Ai tai (1.93 mg GAE/g), Mahkyom (1.69 mg GAE/g) and E-lai (1.64 mg GAE/g), which had much lower TPC levels than the top three in the red rice cultivars of Lai mahk (5.79 mg GAE/g), Chaw pli khao (5.48 mg GAE/g) and Rathu heenati (5.29 mg GAE/g), and in the purple rice cultivars of Gam nahng payah (4.74 mg GAE/g), Gam feuang (4.60 mg GAE/g) and Dam dahng (4.40 mg GAE/g). The highest TPC cultivar (Lai mahk) in

the red pericarp group was about 3.0-fold higher than that in the white pericarp cultivar (Ai tai), while the highest TPC cultivar (Gam nahng payah) in the purple pericarp group was about 2.5-fold higher than that in the white pericarp cultivar (Ai tai). However, in a previous study with different rice cultivars the differences were greater, with the TPC in red grain cultivars being 4.6-fold higher than in the white grain cultivars (Xu et al., 2016).

Significant differences in the TPC were observed between the three groups of rice, which was in agreement with Goffman and Bergman (2004), who studied the phenolic content in the whole grain of 133 rice cultivars, where the red bran cultivars had the highest TPC levels. The current results showed that the TPC levels in these studied Thai rice grains mainly correlated with the pericarp color, with relatively minor variations between cultivars within each color group. Shen et al. (2009) also reported that the TPC was related to the color parameters of the rice grain. Jun et al. (2012) analyzed the phenolic acids of white, red and purple rice cultivars using high performance liquid chromatography. Their results indicated that protocatechuic acid, p-hydrobenzoic acid, gallic acid, vanillic acid, syringic acid, p-coumaric acid, ferulic acid and sinapic acid were the major phenolic compounds in the red and purple rice cultivars and were present at higher levels than in the white rice cultivars.

For Thai rice, Pramai and Jiamyangyuen (2006) analyzed the unpolished rice grains of 20 Thai white and colored pericarp cultivars and found that the highest TPC was in a purple cultivar (Niaw dam pleuk khow) while the lowest TPC was found in a white pericarp cultivar (Phitsanulok 2). In the current study, the purple cultivar Gam nahng payah (4.74 mg GAE/g) and the red cultivar Lai mahk (5.79 mg GAE/g) had the highest TPC level in each group. The lowest TPC in the white pericarp rice was in RD19 (0.86 mg GAE/g), while the Ai tai cultivar (1.93 mg GAE/g) was the white pericarp rice with the highest TPC.

Measurement of total flavonoid content

The TFC of crude rice extracts had levels in the ranges 2.03–5.07 mg RE/g, 6.49–11.13 mg RE/g and 6.57–10.25 mg RE/g for the white, red and purple pericarp rice groups, respectively, (Fig. 2B). A significant difference in the TFC levels was observed between the white pericarp cultivars and the two groups of pigmented pericarp cultivars, but no significant difference in the TFC was observed between the red and purple pericarp cultivars. These results suggested that the colored rice had higher TFC levels compared to white rice. Previous studies have indicated that the predominant flavonoids are proanthocyanins in the red pericarp cultivars and anthocyanins in purple pericarp cultivars (Thitipramote et al., 2016; Hosoda et al., 2018). Furthermore, proanthocyanins and anthocyanins are present in a range of colored plants, including orange, red, pink, mauve, purple and blue. These colors in plants depend on the number of hydroxyl groups on the B-ring and on glycosylation of the anthocyanidins (Tanaka et al., 2008).

The top three TFC cultivars in each of the white, red and purple rice groups are shown in Fig. 3B. For the white pericarp rice group, the top three TFC cultivars were Ai tai (5.07 mg RE/g), Plah khaeng (4.12 mg RE/g), and Tom meuang luang (4.05 mg RE/g), which had much lower TFC levels than the top three in the red and purple rice cultivars. The top three TFC cultivars in the red rice group were Chaw pli khao (11.13 mg RE/g), Rathu heenati (10.13 mg RE/g) and Lai mahk (9.85 mg RE/g), the same as the top three TPC cultivars. The top three cultivars in the purple rice group were Gam nahng payah (10.25 mg RE/g), Khao hawm mea payah tawng dam (9.85 mg RE/g) and Gam feuang (9.69 mg RE/g), with two of the cultivars (Gam nahng payah and Gam feuang) also having the highest TPC levels. The highest TFC in the red (Chaw pli khao) and purple (Gam nahng payah) pericarp cultivars were about 2.2- and 2.0-fold higher than that in the white pericarp cultivar (Ai tai), respectively.

Previously, in a study on the colors of the rice pericarp, Ghasemzadeh et al. (2018) suggested that quercetin, apigenin, catechin, luteolin and myrecitin were the most abundant flavonoid compounds in white and pigmented rice, but that red and purple rice had higher levels than white rice. The color of the rice pericarp might have been due to the anthocyanin content, a kind of flavonoid compound (Furukawa et al., 2007). Therefore, it should be noted here that the white rice cultivars had lower TFC levels than the red and purple cultivars (Shen et al., 2009).

Antioxidant capacity of different color groups of rice

The overall results for the AC, measured in terms of the ABTS radical scavenging assay, are expressed as equivalents of ascorbic acid in Fig. 2C. The AC of white rice was significantly lower than that of the two groups of colored rice. Among the white rice cultivars, the mean AC was 186.78 µg AEAC/g and the range was 78.32–349.33 µg AEAC/g, whereas among the red rice cultivars, the mean AC was 1,641.90 µg AEAC/g and the range was 1,363.67–2,301.92 µg AEAC/g. The purple rice had slightly lower AC levels than the red rice, in the range 727.24–1,880.62 µg AEAC/g with a mean of 1,232.55 µg AEAC/g.

The results showed that the top three AC cultivars in the white rice group were Gwian hak (349.33 µg AEAC/g), Nahng mon (306.72 µg AEAC/g) and E-khao yai (288.51 µg AEAC/g), whereas those in the red rice group, Lai mahk (2,301.92 µg AEAC/g), Chaw pli khao (2,043.73 µg AEAC/g) and Rathu heenati (1,737.65 µg AEAC/g), had markedly higher AC levels than the white rice. The top three AC cultivars in the purple rice group were Gam feuang (1,880.62 µg AEAC/g), Dam dahng (1,520.64 µg AEAC/g) and Gam nahng payah (1,520.00 µg AEAC/g), which were much higher than for the white group. Thus, the highest AC cultivars in the red (Lai mahk) and purple (Gam feuang) groups were about 6.6- and 5.4-fold higher, respectively, than the highest AC cultivar (Gwian hak) in the white group.

The difference in the AC between the colored pericarp rice and the white rice has been attributed to the presence of phenolic and flavonoid contents in the red and purple pericarp, which have strong antioxidant activities (Goffman and Bergman, 2004; Shen et al., 2009).

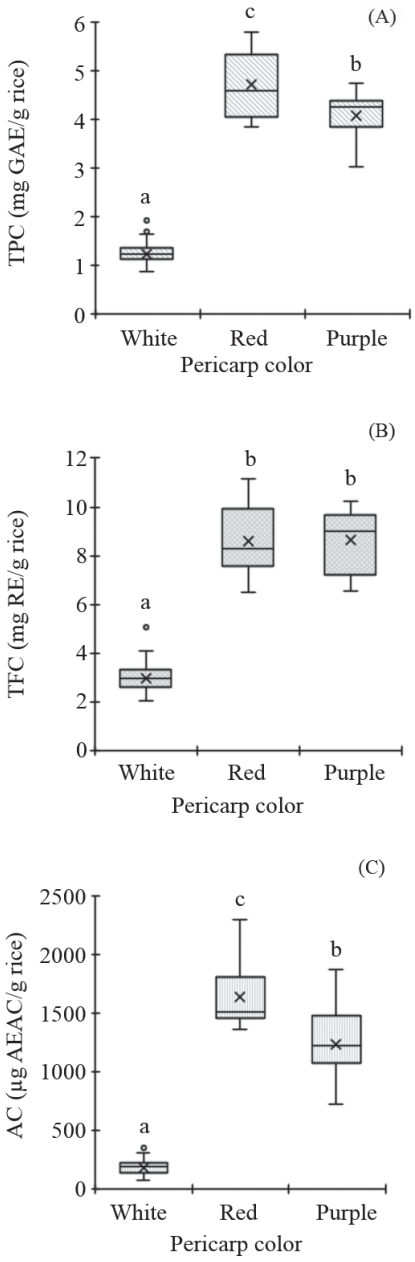

Fig. 2 Boxplots of: (A) total phenolic content (TPC); (B) total flavonoid content (TFC); (C) antioxidant capacity (AC) of white rice (152), red rice (10) and purple rice (12) cultivars, where boxplots represent median (horizontal line in the box), SD (bottom and top box lines), mean (X mark in the box) and range (upper and lower bars) and for each parameter, means with a different lowercase letter are significantly *($p < 0.05$),* different.

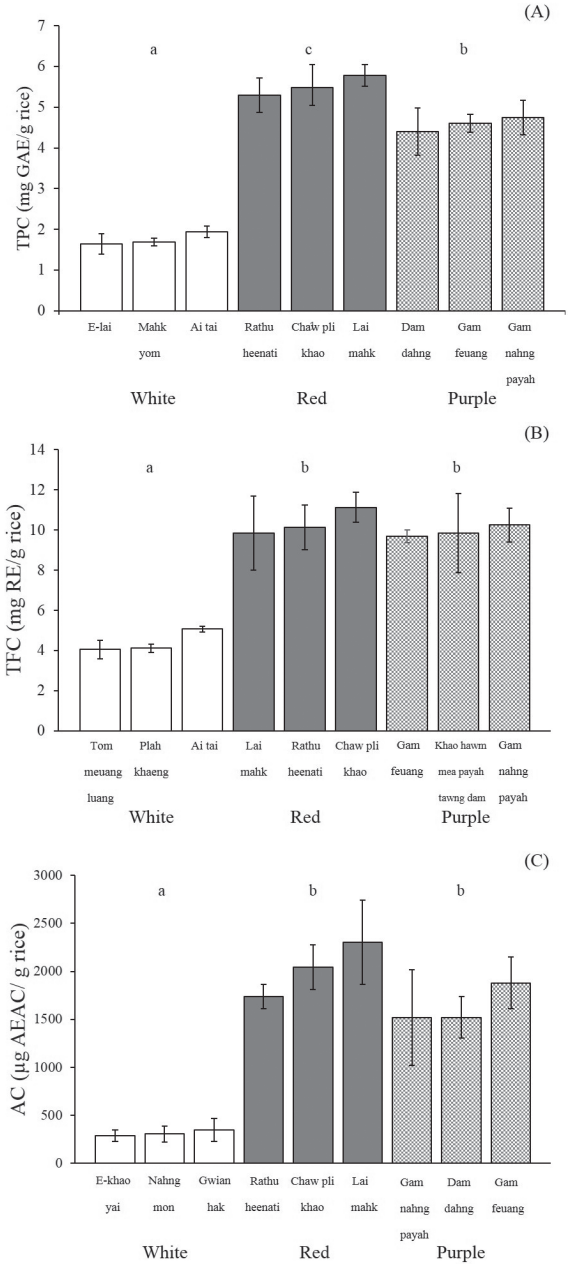

Fig. 3 The three cultivars with the highest levels of (A) total phenolic content (TPC); (B) total flavonoid content (TFC); (C) antioxidant capacity (AC) in each of white (□), red (■) and purple (▩) pericarp rice groups, for each parameter; different lowercase letters indicate significant ($p < 0.05$), difference among means (averaged across three best cultivars within a colour group) of different colour groups; GAE = gallic acid equivalents, RE = rutin equivalent and AEAC = ascorbic acid equivalent antioxidant capacity

The lowest AC (78.32 µg AEAC/g) was in the white pericarp cultivar Ma yom, while the highest activity (2,301.92 µg AEAC/g) was observed in the red pericarp cultivar Lai mahk, with this red cultivar having a smaller grain size than those in the purple group (Fig. 1). Therefore, for a given weight of rice, a smaller grain size would lead to a higher proportion of pericarp in the grain sample. Most of the antioxidant compounds are found in the pericarp layer, followed by the embryo, with a very low content in the endosperm (Shao et al., 2014). Shen et al. (2009) investigated the relationship between the grain size and antioxidant activities in Chinese rice cultivars and found that the grain size was negatively correlated with the antioxidant capacity in rice.

Correlation analysis

Pearson correlation coefficient values were used to assess the correlation between the TPC, TFC and AC of the Thai rice cultivars within the three groups of pericarp colors (Table 1). In the white group (Table 1), significant correlations were detected between the TPC and TFC ($r = 0.600$, $p < 0.01$), and between the TFC and AC ($r = -0.143$, $p < 0.05$). In the red group, significant ($p < 0.01$) correlations between all three parameters were detected, which was in agreement with Shen et al. (2009), who reviewed the correlation between the TPC, TFC and AC in white and red rice. In the purple group, significant positive correlations were detected between all three parameters, except for between TFC and AC which were not significantly correlated ($r = 0.486$). That these results are different from previous studies (Pramai and Jiamyangyuen, 2006; Shen et al., 2009) was likely because of the different rice cultivars used.

There were significant ($p < 0.01$) positive correlation coefficients between the TPC and TFC among all the rice groups. However, the correlation coefficient within the white rice cultivars ($r = 0.600$) was smaller than that within the purple rice cultivars ($r = 0.718$) or red rice cultivars ($r = 0.845$), with the latter being the highest.

Within the white rice cultivars, the TPC was not significantly correlated to the AC, while the TFC showed a low negative (but significant) correlation with the AC. However, the correlations of the TPC and AC were positive ($p < 0.01$) within the pigmented rice cultivars. This result indicated that the TPC was always the major contributor to the AC of the red and purple rice cultivars (Ghasemzadeh et al., 2018). The TPC and TFC levels were lower in the white rice cultivars, which might suggest that the major AC of white cultivars was contributed from other phytochemicals, such as the tocopherols and γ-oryzanols (Xu et al., 2001; Sanghamitra et al., 2017). However, the TPC had higher positive correlation coefficients with the AC parameters than the TFC, which indicated that the AC was most closely related to the TPC.

Rice cultivar grouping based on hierarchical cluster analysis

The dendrogram of the 174 rice cultivars based on the three antioxidant parameters (TPC, TFC and AC) using HCA is presented in Fig. 4. The distance values of HCA ranged from zero (lower difference) to 22 (greater difference). The results showed that the 174 rice cultivars were grouped into four clusters at a distance value of 2.60. Cluster I comprised 72 white rice cultivars and had the lowest levels in terms of antioxidant contents and AC. Cluster II, the largest cluster, comprised 80 white rice cultivars and had the same range of TPC and AC levels as found in cluster I. Cluster III comprised 16 cultivars, made up of six red rice cultivars and 10 purple rice cultivars. Finally, cluster IV had six cultivars, made up of four red and two purple cultivars; this cluster had the highest levels of antioxidant contents and AC. In the pigmented pericarp cultivars, Hawm nin had the lowest TFC and AC, while Hawm dam had the lowest TPC. The red rice Lai mahk and the purple rice Gam feuang in cluster IV had the highest antioxidant content and AC levels (Fig. 4 and Table 2).

A previous evaluation of 20 rice cultivars from the north of Thailand divided them into four clusters according to their colors and antioxidant properties, in terms of the total anthocyanin content, TPC, TFC, α-tocopherol, γ-oryzanol and AC (Pramai and Jiamyangyuen, 2006). Cluster I comprised one purple and four red rice cultivars, cluster II had eight purple, cluster III had four purple rice cultivars with the highest TPC, TFC and AC levels, and cluster IV had three white rice cultivars. Their cluster analysis suggested that white rice cultivars clearly showed different (lower) TPC, TFC and AC levels to the red and purple rice cultivars, whereas most red and purple rice cultivars had similar TPC, TFC and AC levels in clusters I and II, although some purple cultivars had higher levels of antioxidant content and AC than the other red and purple rice cultivars in cluster III. These results were in broad accordance with the HCA-based clustering results of the current study.

A significant ($p < 0.05$) difference in the TPC and TFC levels of the 174 rice cultivars was observed among the four groups (Table 3). Cluster IV had the highest AC value, whereas clusters I and II had the lowest AC levels.

High TPC, TFC and AC levels were found in six pigmented rice cultivars in cluster IV (Table 2). Previously, five Thai landraces (Gam feuang, Lai mahk, Chaw pli khao, Gam nahng payah and Hawn daeng) and one Sri Lankan rice cultivar (Rathu heenatic), located in cluster IV in the current study, were reported to exhibit a moderate level of resistance against the brown plant hopper, and so they are important genetic resources, but there was no report on their TPC, TFC and AC levels (Jairin et al., 2007). Rice breeders could provide cultivars that could lead to improved nutritional quality of rice in conventional and molecular breeding programs.

The current study indicated that HCA could clearly distinguish between rice cultivars in terms of high and low antioxidant content and AC. However, this clustering still needs to be supported by additional work based on a yield component and on morphological and physiological traits. The current study presented a useful tool to be used in the future for making recommendations and selecting rice cultivars with a high antioxidant content as well as providing a foundation for rice breeders to use as indices to directly or indirectly select rice breeding lines with a view to enhancing the value of the phytochemicals present in the rice.

Conflicts of Interest

The authors declare that there are no conflicts of interest.

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Table 1 The correlation coefficients between the TPC, TFC, and AC (in terms of the ABTS assay) among rice cultivars with white, red, and purple pericarps.

	TFC			AC		
Pericarp color	White	Red	Purple	White	Red	Purple
TPC	0.600**	0.845**	0.718**	-0.044	0.918**	0.806**
TFC				-0.143*	0.757**	0.486

* and ** indicate significant differences at the $p < 0.05$ and 0.01 levels, respectively.

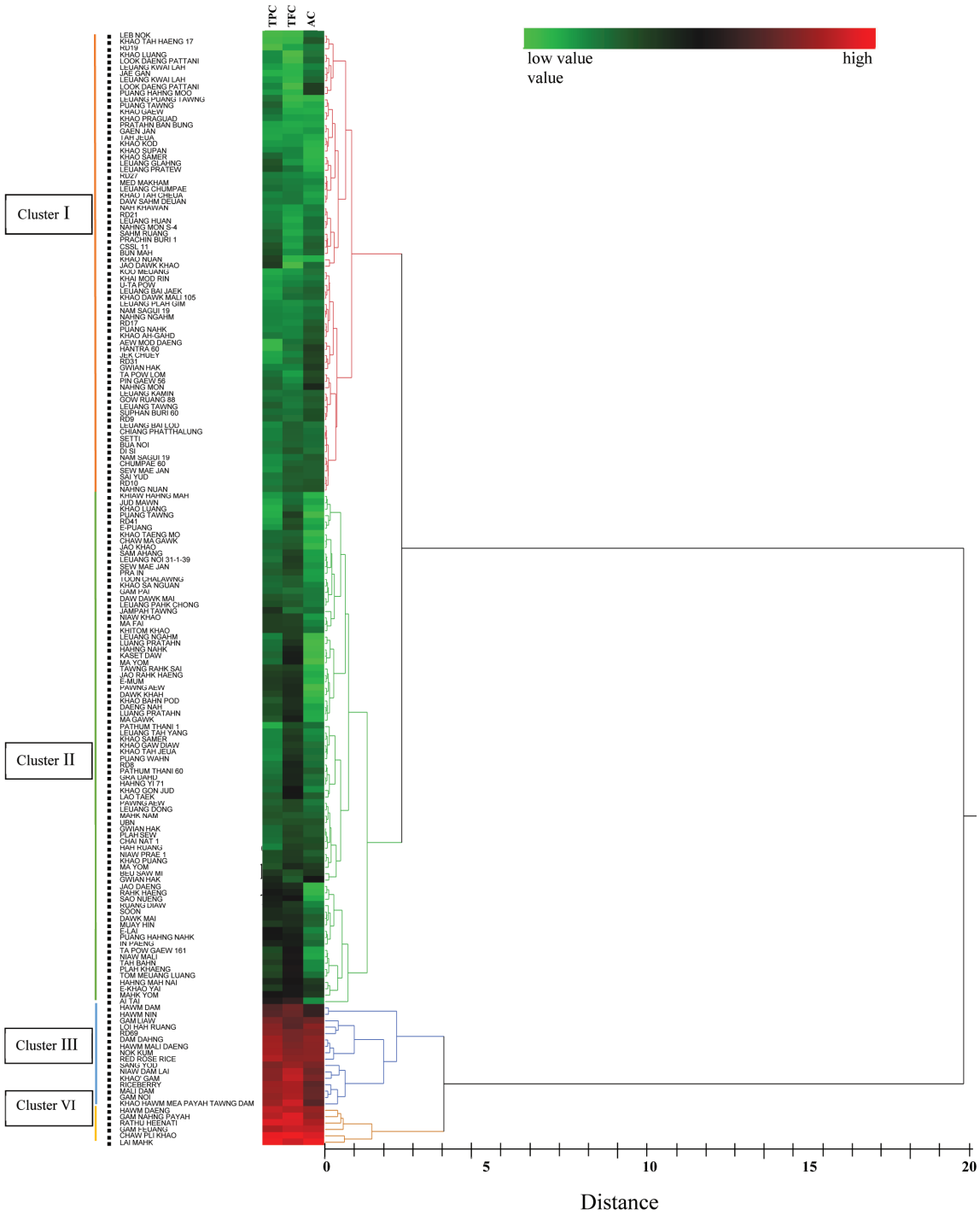

Fig. 4 Hierarchical cluster analysis dendrogram showing relationship between total phenolic content (TPC), total flavonoid content (TFC) and antioxidant capacity (AC) based on 2,2′-azino-bis-3-ethylbenzthiazoline-6-sulphonic acid assay in 174 Thai rice cultivars

Table 2 Lists of rice cultivars according to the HCA based on the TPC, TFC, and AC (ABTS assay).

Group number	Number of cultivars	Cultivars
Group I	72	Aew mod daeng, Bua noi, Bun mah, Chiang phatthalung, Chumpae 60, CSSL 11, Daw sahm deuan, Di si, Gaen jan, Gow ruang 88, Gwian hak, Hantra 60, jae gan, Jao dawk khao, Jek chuey, Khai mod rin, Khao ah-gahd, Khao dawk mali 105, Khao gaew, Khao kod, Khao luang, Khao nuan, Khao praguad, Khao samer, Khao supan, Khao tah cheua, Khao tah haeng 17, Koo meuang, Leb nok, Leuang bai jaek, Leuang bai lod, Leuang chumpae, Leuang glahng, Leuang huan, Leuang kamin, Leuang kwai lah, Leuang kwai lah, Leuang plah gim, Leuang pratew, Leuang puang tawng, Leuang tawng, Look daeng pattani, Look daeng pattani, Med makham, Nah khawan, Nahng mon, Nahng mon s-4, Nahng ngahm, Nahng nuan, Nam sagui 19, Nam sagui 19, Pin gaew 56, Prachin buri 1, Pratahn ban bung, Puang hahng moo, Puang nahk, Puang tawng, RD10, RD17, RD19, RD21, RD27, RD31, RD9, Sahm ruang, Sai yud, Setti, Sew mae jan, Suphan buri 60, Ta pow lom, Tah jeua, U-ta pow
Group II	80	Ai tai, Beu saw mi, Chai nat 1, Chaw ma gawk, Daeng nah, Daw dawk mai, Dawk khah, Dawk mai, E-khao yai, E-lai, E-mum, E-puang, Gam pai, Gra dahd, Gwian hak, Gwian hak, Hah ruang, Hahng mah nai, Hahng nahk, Hahng yi 71, In paeng, Jampah tawng, Jao daeng, Jao khao, Jao rahk haeng, Jud mawn, Kaset daw, Khao bahn pod, Khao gaw diaw, Khao gon jud, Khao luang, Khao puang, Khao sa nguan, Khao samer, Khao taeng mo, Khao tah jeua, Khiaw hahng mah, Khitom khao, Lao taek, Leuang dong, Leuang ngahm, Leuang noi 31-1-39, Leuang pahk chong, Leuang tah yang, Luang pratahn, Luang pratahn, Ma fai, Ma gawk, Ma yom, Ma yom, Mahk nam, Mahk yom, Muay hin, Niaw khao, Niaw mali, Niaw prae 1, Pathum thani 1, Pathum thani 60, Pawng aew, Pawng aew, Plah khaeng, Plah sew, Pra in, Puang hahng nahk, Puang tawng, Puang wahn, Rahk haeng, RD41, RD8, Ruang diaw, Sam ahang, Sao nueng, Sew mae jan, Soon, Ta pow gaew 161, Tah bahn, Tawng rahk sai, Tom meuang luang, Toon chalawng, UBN
Group III	16	Hawm mali daeng, Loi hah ruang, Nok kum, RD69, Red rose rice, Sang yod, Dam dahng, Gam liaw, Gam noi, Hawm dam, Hawm nin, Khao' gam, Khao hawm mea payah tawng dam, Mali dam, Niaw dam lai, Riceberry
Group IV	6	Chaw pli khao, Hawm daeng, Lai mahk, Rathu heenati, Gam feuang, Gam nahng payah

Table 3 Comparison of the TPC, TFC, and AC in the four different HCA groups (Mean (SD).

Character	Group I	Group II	Group III	Group IV
TPC	1.155 (0.112)[a]	1.325 (0.171)[b]	4.071 (0.452)[c]	5.134 (0.459)[d]
TFC	2.574 (0.251)[a]	3.349 (0.366)[b]	8.115 (1.085)[c]	10.034 (0.668)[d]
AC	174.615 (45.180)[a]	200.293 (55.697)[a]	1258.818 (254.150)[b]	1844.769 (294.773)[c]

Groups are from the HCA, see Fig. 4. Means in the same row with a different letter are significantly different ($p < 0.05$; DMRT).

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