CHAPTER 4
COMPARISON OF AGRONOMIC CHARACTERISTICS AND TOTAL PHENOLIC CONTENT OF NATIVE PURPLE GLUTINOUS RICE

Abstract

This research aims to study the variation of purple glutinous rice varieties in terms of developments, yield and total phenolic content of rice grain. In addition, the relationship between them was also determined. Hierarchical cluster analysis by average linkage method was used to sort varieties of rice on the basis of association of their grain yield and total phenolic content in grain. Twenty four varieties of native purple glutinous rice were grown under completely randomized block design with 3 replications. Phenological development namely heading and maturity date were recorded. Yield and yield components were collected at maturity. Sample of brown rice grains were analyzed to determine the amount of total phenolic content.

Analysis results showed that among 24 varieties of native purple glutinous rice, growing degree days required from planting to heading was 1,359 – 1,657 degree Celsius (82-100 days). Grain yield ranged from 1.4 – 4.7 t/ha. The amount of total phenolic content in grains ranged from 128 - 735 mg/ml GAE per gram of brown rice grain. It was found that grain yield was positively correlated with 1,000 - grain weight at significant level $p \leq 0.05$ and negatively correlated with number of sterile grain per panicle at significant level $p \leq 0.01$. Result indicated that early maturing varieties tended to take longer period of time for grain filling duration. Moreover, late maturing varieties produced longer panicle length than those of early maturing varieties. Total phenolic content were found positively correlated with panicle length at significant level $p \leq 0.01$. The cluster analysis result showed that purple glutinous rice varieties can be classified into four major clusters according to their grain yield and total phenolic content in grain. Analysis results suggested that native purple glutinous rice varieties used in this study shown potential as genetic sources for rice improvement in order to breed a variety with satisfied yield as well as high antioxidant in grain.

4.1 Introduction

Purple glutinous rice is commonly known as "Kao Kum" or black sticky rice in northern region of Thailand. Its name comes from the sticky shiny purple colored texture of the brown rice. The grain normally has a deep black color and turns deep purple when cooked. The dark purple color of brown rice is primarily due to its high in mixture of anthocyanins content which located in the aleurone layer (Hu *et al*., 2003). The mixture of anthocyanins are naturally occurring as compounds that belong to phenolic group. Phenolic compounds are considered as one of the main classes of secondary metabolites in plants (Van Sumere, 1989). They are involved in the growth and reproduction of plants as well as in the pigmentation of plants.

Black rice bran extract contains antioxidative and radical scavenging properties which include the properties to provide prevention of various disease associated with oxidative stress such as cancer (Nam and Kang, 1997; Nam and Kang, 1998; Chen *et al.*, 2006) and cardiovascular disease (Jerzy *et al.*, 2003). It was also reported that pigmented rice varieties have the potential to promote human health because they contain antioxidative compounds that have the ability of reactive cell-damaging free radicals (Acquaviva *et al*., 2003; Adom and Liu, 2002; Choi *et al*., 1996; Hu *et al*., 2003; Hyun and Chung, 2004; Ichikawa *et al*., 2001; Lee *et al.*, 2003; Oki *et al*., 2002; Parrado *et al*., 2003; Toyokuni *et al.*, 2002).

There are many purple glutinous rice varieties grown in north and north eastern Thailand of which are local varieties. Even though the purple glutinous rice is becoming popular among those who concern on healthy food product due to its sweet flavor and high in antioxidant content in grain. However, Thai farmers do not grow purple glutinous rice in large area but they normally grow it in small paddy. This is because grain yield per area of purple glutinous rice is quite low due to it contains characteristics of native rice and photo period sensitive. As such, fewer farmers would decide to grow purple glutinous rice each year. This potentially results in loss of genetic diversity of purple glutinous rice varieties. This research intended to collect various purple glutinous rice varieties grown under upland environment and study the variation of purple glutinous rice in terms of some agronomic characteristics and total phenolic content of its brown rice. In addition, the relationship between them also determined. Hierarchical cluster analysis by average linkage method was used to sort varieties of rice on the basis of association of their grain yield and total phenolic content in grain. Results from this study can be used as information for varietal selection as well as providing valuable data for rice breeders. Furthermore, it can provide understanding of growth and development for constructing the mechanistic model of purple glutinous rice which can be further use as a decision support tool for selecting variety and optimum growing conditions.

4.2 Materials and methods

Twenty four varieties of purple glutinous rice (Table 4.1) were selected for this study. Each variety is planted in 3 x 5 m experimental plots with 0.30 x 0.25 m. plant spacing at upland field experiment of Lamphun College of Agriculture and Technology, Mae Tha district, Lamphun province. Design of the experiment was randomized completed block design with 3 replications. After planting, phenological stages which include heading and physiological maturity stages is observed. Daily temperature were recorded using automatic weather recorder (HOBO Pro series) setting at hourly time interval. Growing degree days was calculated using daily temperature as following equation :

$$GDD = \frac{(T_{\max} + T_{\min})}{2} - T_{base}$$

Where

T_{max} is the daily maximum air temperature. T_{max} is set equal to 30°C when greater than upper threshold temperatures. The upper threshold temperature equal to 30°C for rice.

T_{min} is the daily minimum air temperature. T_{min} is set equal to T_{base} if less than T_{base}.

T_{base} is the temperature which is temperature below which plant growth is zero. The base temperature used for rice was 10°C.

The observed phenological data are used to construct the mathematical relationship with growing degree days. Agronomic characteristics included plant height, panicle length, grain yield and yield components i.e. number of panicle per hill, number of grain per panicle and 1,000-grain weight were recorded.

Table 4.1 Purple glutinous rice varieties used in this study.

No.	Variety name	Source
1	MHS 1	Mae Hong Son Province
2	Chiang Saen	Chiang Saen District, Chiang Rai Province
3	PGMHS 3	Mae Hong Son Rice Research Center
4	PGMHS 5	Mae Hong Son Rice Research Center
5	PGMHS 6	Mae Hong Son Rice Research Center
6	PGMHS 7	Mae Hong Son Rice Research Center
7	PGMHS 8	Mae Hong Son Rice Research Center
8	PGMHS 9	Mae Hong Son Rice Research Center
9	PGMHS 10	Mae Hong Son Rice Research Center
10	PGMHS 11	Mae Hong Son Rice Research Center
11	PGMHS 12	Mae Hong Son Rice Research Center
12	PGMHS 13	Mae Hong Son Rice Research Center
13	PGMHS 14	Mae Hong Son Rice Research Center
14	PGMHS 15	Mae Hong Son Rice Research Center
15	PGMHS 16	Mae Hong Son Rice Research Center
16	PGMHS 17	Mae Hong Son Rice Research Center
17	PGMHS 18	Mae Hong Son Rice Research Center
18	Samoeng No.1	Samoeng Rice Research Center, Chiang Mai
19	Samoeng No.2	Samoeng Rice Research Center, Chiang Mai
20	Samoeng No.3	Samoeng Rice Research Center, Chiang Mai
21	Samoeng No.4	Samoeng Rice Research Center, Chiang Mai
22	Samoeng No.7	Samoeng Rice Research Center, Chiang Mai
23	Samoeng No.8	Samoeng Rice Research Center, Chiang Mai
24	Nong Khao 2	Mae Hong Son Province

4.2.1 Determination of total phenolic content

Total phenolic content of brown rice extract was determined by spectrophotometric method using Folin-Ciocalteu's phenol reagent (Osawa and Namiki, 1981). The brown rice (0.5g) was extracted with 80% ethanol (100 ml) then shaked at room temperature for 30 min. The brown rice extract (0.5 ml) was placed in a test tube and added distilled water (5 ml). Folin-Ciocalteu's phenol reagent (5 ml) was added, and mixed thoroughly. After 3 min, 5 ml of 10% sodium carbonate solution was added then the mixture was left to stand for 1 h at room temperature. The absorbance of the mixture was measured by using a UV-Vis Spectrophotometer at 750 nm. The concentration of total phenolic content was determined using the gallic acid equation obtained from the standard gallic acid calibration curve and expressed as gallic acid equivalents (GAE), milligrams per gram of dry matter.

4.2.2 Statistical Analysis

Experimental data were analyzed with Statistix software version 9.0 (Analytical Software, Tallahassee FL). Differences of agronomic characteristics data including yield, yield components, total phenolic content and antioxidant activity among varieties was analyzed using descriptive statistics. Regression analysis was utilized for analysis of relationship between phenological stages and some agronomic characteristics. Pearson's correlation coefficients were computed to establish relationship between agronomic characteristics, yield, yield components and total phenolic content. Hierarchical cluster analysis by average linkage method was used to group purple glutinous rice varieties into clusters on the basis of grain yield and total phenolic content. SAS software version 9.0 (SAS Software Institute, Cary, NC) was used as analytical tool for performing the cluster analysis.

4.3 Results and Discussion

Field observation showed that growing degree days requirement from planting to heading ranges from 1,359 to 1,657 degree Celsius while requirement from planting to maturity ranges between 2,070 to 2,262 degree Celsius (Table 4.2). It was found that PGMHS 12 required least growing degree days and Samoeng No.7 required greatest growing degree days from planting to heading. Since rice varieties in this study are all photoperiod sensitive thus variation in phenological development depended on growing degree days (Summerfield *et al*., 1992; Kropff *et al*., 1993; Dingkuhn, 1995). The variation in degree days requirement from planting to heading indicated that photoperiod response occurred during photoperiod sensitive phase

(PSP) in which rice response to both photoperiod and temperature (Nakagawa and Horie, 1995). Analysis result showed that there was negative relationship between growing degree days required from planting to heading and panicle development (heading to maturity) (Figure 4.1). This analysis pointed out that rice variety which has shorter heading stage tended to have longer panicle development or grain filling period. Similarly rice variety that required shorter degree days for heading tended to have longer panicle length and greater grain weight (Figure 4.2 and 4.3). This analysis indicated that longer panicle and larger grain reflected larger sink which could accumulate photosyntate from source (leaf and stem) thus took longer period in panicle development. Yang *et al*. (2008) also pointed out that grain weight on area basis was positively associated with grain filling duration. In this study panicle length varied between 22.1 - 32.1 cm and 1,000 - grain weight varied between 26.44 - 42.33 g (Table 4.3).

Table 4.2 Growing degree days (^oC) required from planting to heading and maturity.

Variety name	Heading		Maturity		Panicle development	
	Day after planting	GDD (^oC)	Day after planting	GDD (^oC)	Day from heading to maturity	GDD (^oC)
PGMHS 12	82	1359	126	2070	44	727
Samoeng No.2	83	1375	126	2070	43	711
PGMHS 11	84	1391	126	2070	42	694
PGMHS 14	84	1391	126	2070	42	694
Samoeng No.4	84	1391	133	2177	49	802
PGMHS 5	85	1408	133	2177	48	786
Samoeng No.1	85	1408	133	2177	48	786
MHS 1	91	1507	126	2070	35	579
PGMHS 6	91	1507	126	2070	35	579
PGMHS 18	91	1507	126	2070	35	579
Samoeng No.3	91	1507	126	2070	35	579
Samoeng No.8	91	1507	133	2177	42	686
PGMHS 13	93	1540	126	2070	33	546

Table 4.2 (Continue)

Variety name	Heading		Maturity		Panicle development	
	Day after planting	GDD (°C)	Day after planting	GDD (°C)	Day from heading to maturity	GDD (°C)
PGMHS 17	93	1540	133	2177	40	653
PGMHS 7	94	1557	139	2262	45	722
PGMHS 15	94	1557	133	2177	39	637
Chiang Saen	96	1590	133	2177	37	604
PGMHS 8	96	1590	133	2177	37	604
PGMHS 9	96	1590	133	2177	37	604
Nong Khao 2	96	1590	139	2262	43	689
PGMHS 3	97	1607	133	2177	36	587
PGMHS 10	98	1624	133	2177	35	570
PGMHS 16	98	1624	133	2177	35	570
Samoeng No.7	100	1657	126	2070	26	429

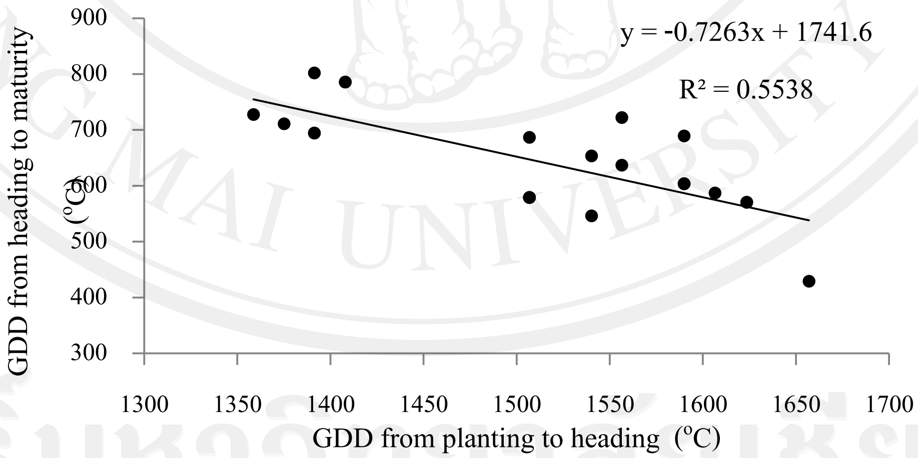

Figure 4.1 Relationship between GDD required from planting to heading and GDD required from to heading to maturity.

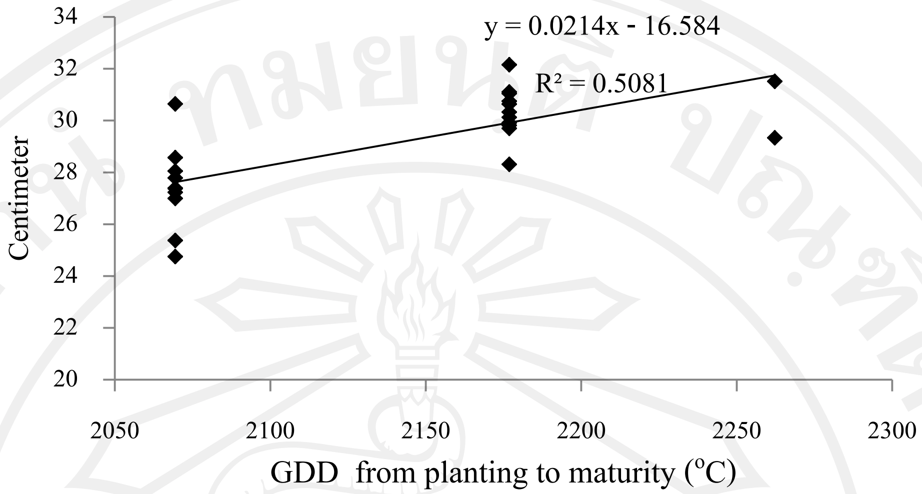

Figure 4.2 Relationship between GDD required from planting to maturity and panicle length.

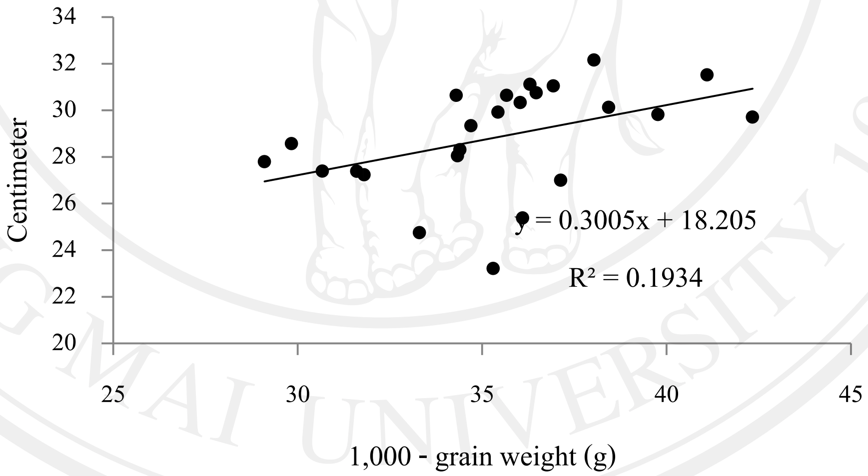

Figure 4.3 Relationship between 1,000 - grain weight and panicle length.

Analysis result demonstrated that rice variety PGMHS 15 produced the greatest grain yield which was 4.7 t/ha while rice variety PGMHS 18 gave the lowest grain yield which was 1.4 t/ha (Table 4.3). Correlation analysis result (Table 4.4) showed positive relationship between grain yield and grain weight ($p \leq 0.05$). In contrast, negative correlation was found among grain yield and number of sterile grain per panicle ($p \leq 0.01$). This finding indicated that grain weight was a major factor that contribute to grain yield among varieties in this study. Reducing sterility grain could also enhance yield. Number of panicle per hill and number of grain per panicle showed no relationship with grain yield even though they were yield components that could contribute to grain yield (Yoshida, 1981). This is probably because greater number of grain per panicle was found positively significant correlated to number of sterile grain ($p \leq 0.01$) (Table 4.4). Plant height varied between 106.2 – 144.3 cm in which Samoeng No.4 has the lowest plant height while PGMHS 14 was the tallest. Correlation analysis result found that plant height was positively correlated with number of grain per panicle ($p \leq 0.01$). This was probably due to greater source i.e. taller plant has more biomass thus could greater contribute to sink such as number of grain per panicle. Sheehy *et al.* (2001) also reported that there was positive relationship between leaf area index which was photosyntate source and number of grain per panicle.

Table 4.3 Agronomic characteristics of purple glutinous rice in this study.

Variety name	Plant height (cm)	Panicle length (cm)	Panicles per hill	Grains per panicle	1,000 – grain weight (g)	Grain yield (t/ha)
PGMHS 12	135.7 ± 6.5	27.8 ± 0.5	13 ± 0	215 ± 14	29.10 ± 4.16	3.44 ± 0.27
Samoeng No.2	125.7 ± 4.0	25.4 ± 0.9	12 ± 0	152 ± 6	36.10 ± 1.43	4.39 ± 0.31
PGMHS 11	134.7 ± 2.2	27.4 ± 0.9	15 ± 1	186 ± 13	31.60 ± 1.03	2.62 ± 0.32
PGMHS 14	144.3 ± 2.1	30.6 ± 0.6	14 ± 0	164 ± 20	35.67 ± 0.87	3.43 ± 0.43
Samoeng No.4	106.2 ± 2.7	28.3 ± 0.3	13 ± 0	116 ± 9	34.40 ± 1.60	2.21 ± 0.05
PGMHS 5	120.7 ± 0.5	29.7 ± 0.9	14 ± 0	136 ± 15	42.33 ± 0.66	3.35 ± 0.25
Samoeng No.1	119.3 ± 4.0	29.8 ± 0.7	15 ± 1	136 ± 15	39.77 ± 0.41	3.37 ± 0.55
MHS 1	132.2 ± 2.8	24.7 ± 1.0	14 ± 0	161 ± 10	33.30 ± 2.36	2.96 ± 0.31
PGMHS 6	136.9 ± 2.0	28.0 ± 0.3	13 ± 0	225 ± 11	34.33 ± 0.49	4.46 ± 0.39
PGMHS 18	130.2 ± 4.2	28.6 ± 1.0	15 ± 1	216 ± 22	29.83 ± 0.42	1.38 ± 0.28
Samoeng No.3	123.1 ± 4.4	27.4 ± 0.6	15 ± 1	163 ± 11	30.67 ± 0.69	1.66 ± 0.11
Samoeng No.8	119.5 ± 5.3	23.2 ± 0.9	15 ± 1	121 ± 5	35.30 ± 0.76	3.25 ± 0.07
PGMHS 13	142.1 ± 2.3	27.0 ± 0.3	12 ± 1	189 ± 4	37.13 ± 0.96	3.49 ± 0.35
PGMHS 17	129.7 ± 3.2	30.1 ± 0.3	16 ± 0	140 ± 7	38.43 ± 0.43	3.50 ± 0.10
PGMHS 7	135.4 ± 2.9	31.5 ± 0.1	12 ± 1	151 ± 4	41.10 ± 0.15	3.26 ± 0.54
PGMHS 15	126.9 ± 1.1	32.1 ± 0.4	15 ± 1	164 ± 8	38.03 ± 1.11	4.69 ± 0.48
Chiang Saen	121.2 ± 5.3	30.6 ± 1.4	14 ± 1	162 ± 13	34.30 ± 2.41	3.01 ± 0.31
PGMHS 8	126.8 ± 7.1	30.7 ± 1.5	14 ± 2	159 ± 15	36.47 ± 2.28	3.14 ± 0.13
PGMHS 9	111.5 ± 3.4	29.9 ± 0.9	14 ± 0	115 ± 7	35.43 ± 1.34	1.72 ± 0.19
Nong Khao 2	137.6 ± 6.0	29.3 ± 0.3	13 ± 1	203 ± 15	34.70 ± 2.61	3.07 ± 0.37
PGMHS 3	121.2 ± 1.2	31.0 ± 1.6	13 ± 0	138 ± 15	36.93 ± 3.27	4.40 ± 0.57
PGMHS 10	120.3 ± 2.8	30.3 ± 0.6	15 ± 1	152 ± 11	36.03 ± 1.02	3.46 ± 0.20
PGMHS 16	126.1 ± 3.6	31.1 ± 0.6	13 ± 1	179 ± 14	36.30 ± 0.92	2.99 ± 0.62
Samoeng No.7	124.3 ± 0.6	27.2 ± 0.4	15 ± 1	167 ± 15	31.80 ± 1.26	1.96 ± 0.33

Data present in above table reveal mean ± standard error (n=3)

Table 4.4 Pearson correlation between agronomic characteristics, grain yield and total phenolic content (n=24).

	Heading	Harvest	Panicle	GrainN	Sterile	GrainWt	Length	Height	Yield
Harvest									
Panicle									
GrainN		-0.4385*							
Sterile				0.5873**					
GrainWt		0.6164**		-0.5140*	-0.6687**				
Length		0.5340**				0.4390*			
Height				0.7219**					
Yield					-0.5777**	0.4908*			
Phenolic							0.6514**		

* = Significant at 5% level of *P*, ** = Significant at 1% level of *P*, Heading = GDD required to heading, Harvest = GDD required to harvest, Panicle = Panicle per hill, GrainN = Grain per panicle, Sterile = Sterile grain per panicle, GrainWt = 1,000 – grain weight, Length = Panicle length, Height = Plant height, Yield = Grain yield, Phenolic = Total phenolic content.

Figure 4.4 showed average amount of total phenolic content in brown rice grain among varieties studied. Analysis of grain total phenolic content showed that PGMHS 16 contained highest grain total phenolic content i.e. 736 mg/ml GAE per gram of brown rice grain while Samoeng No.8 contained the lowest grain total phenolic content i.e. 128 mg/ml GAE per gram of brown rice grain. Goffman and Bergman (2004) reported that variation of rice grain phenolic content depended upon grain color which varied among varieties. Research result from this study also demonstrated that total phenolic content has significant positive correlation with panicle length. This was probably due to greater number of grain on the longer panicle contained greater surface area of rice grain. Hu *et al*. (2003) reported that the anthocyanins content is located in the aleurone layer which covered outer layer of brown rice grain. Chung *et al*. (2003) reported positive correlation between phenolic content in rice and brown rice produced.

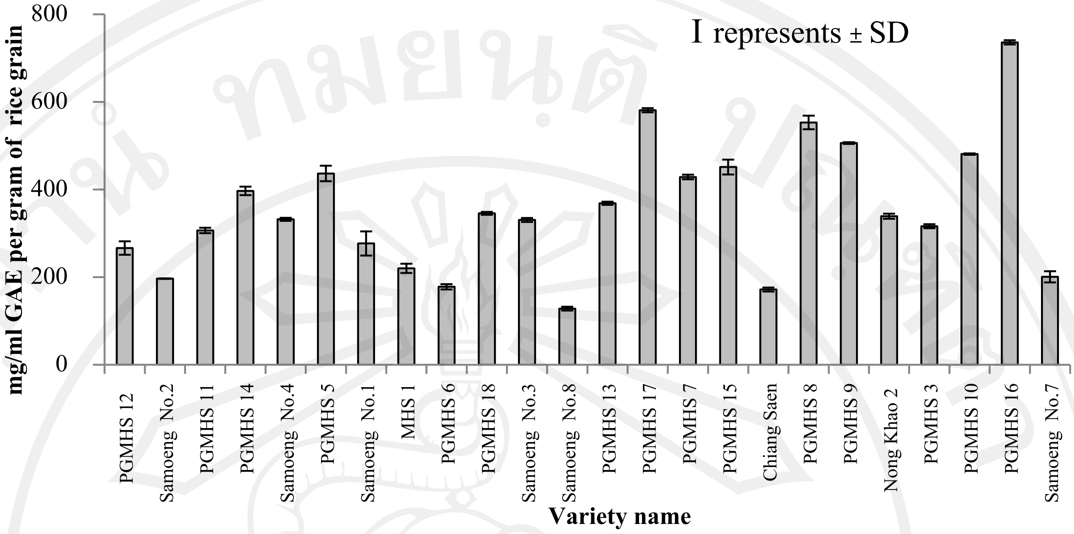

Figure 4.4 Average amount of total phenolic content in brown rice grain (n=3).

The hierarchical cluster analysis of total phenolic contents and grain yield from purple glutinous rice generated a dendogram showed that rice varieties can be grouped in four clusters (Figure 4.5). On the basis of similarities and differences in grain yield and total phenolic content, the four clusters were separated by a root-mean-square (RMS) distance of 1.52, 1.13 and 0.96.

In summary, group 1 contained highest yield but low in total phenolic content. Group 2 contained average total phenolic content but high yield. Group 3 contained average total phenolic content and average yield. The last group, only PGMHS 16 has the highest total phenolic content but average yield (Table 4.5)

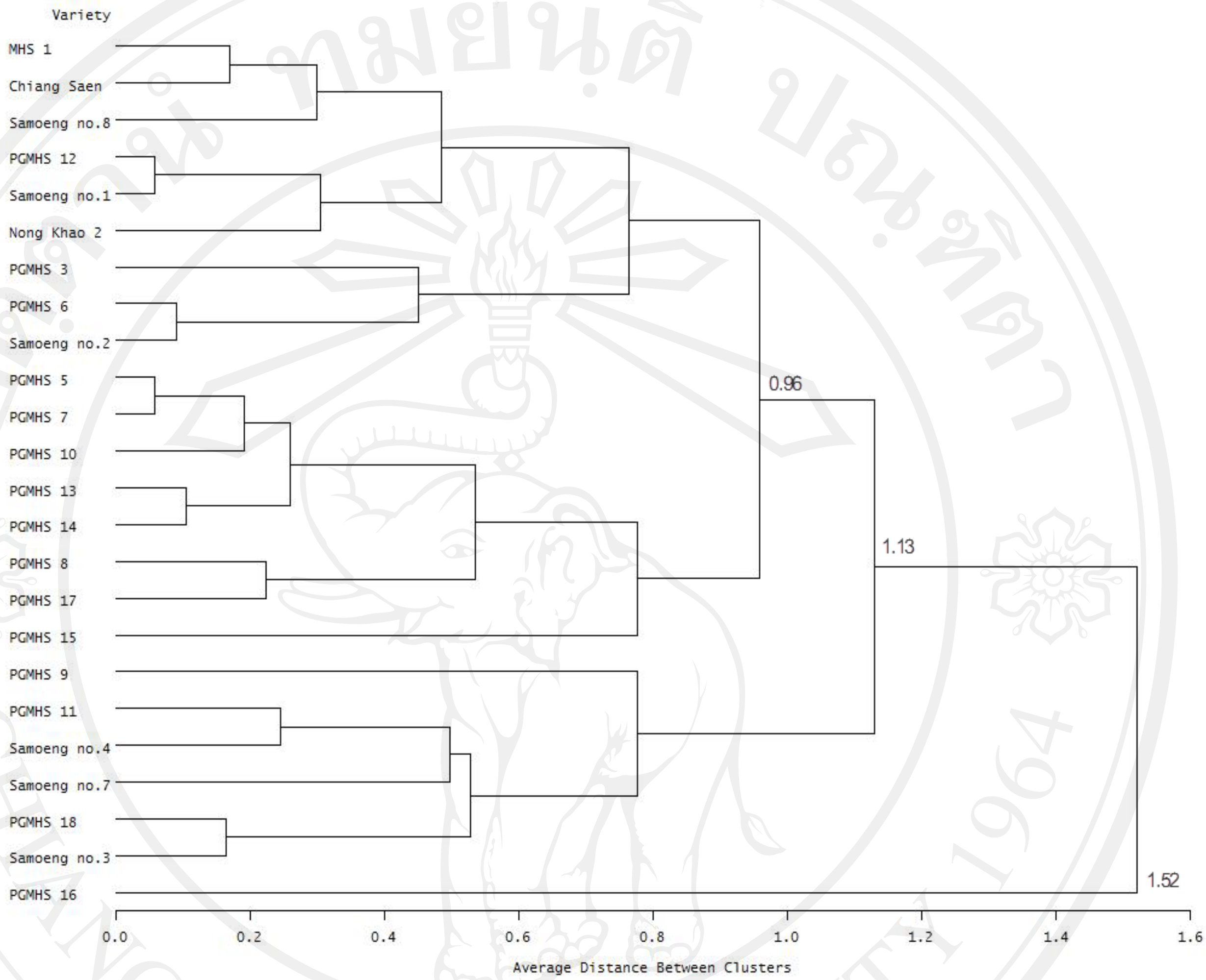

Figure 4.5 Dendogram generated by hierarchical cluster analysis of total phenolic contents and grain yield of purple glutinous rice.

Table 4.5 Rice variety group as summarized from dendogram generated by hierarchical cluster.

Group	Variety name	Total phenolic content (mg/ml) GAE per gram of rice grain	Yield (t/ha)
1	MHS 1	219.87	2.96
	Chiang Saen	171.70	3.01
	PGMHS 3	316.08	4.40
	PGMHS 6	177.84	4.46
	PGMHS 12	266.41	3.44
	Samoeng no.1	276.73	3.37
	Samoeng no.2	196.47	4.39
	Samoeng no.8	128.04	3.25
	Nong Khao 2	338.95	3.07
	Average	***236.95***	***3.59***
2	PGMHS 5	436.54	3.35
	PGMHS 7	428.43	3.26
	PGMHS 8	553.01	3.14
	PGMHS 10	480.78	3.46
	PGMHS 13	368.43	3.49
	PGMHS 14	396.73	3.43
	PGMHS 15	451.31	4.69
	PGMHS 17	581.05	3.50
	Average	***462.04***	***3.54***
3	PGMHS 9	506.08	1.72
	PGMHS 11	306.47	2.62
	PGMHS 18	345.42	1.38
	Samoeng no.3	330.46	1.66
	Samoeng no.4	331.83	2.21
	Samoeng no.7	200.65	1.96
	Average	***334.42***	***1.93***
4	PGMHS 16	***735.88***	***2.99***

4.4 Conclusion

This study demonstrated variation of phenological development, yield and yield components among purple glutinous rice varieties studied. These varieties can be classified into four groups on the basis of similarities and differences in grain yield and total phenolic content. Such results suggested that native purple glutinous rice varieties used in this study shown potential as genetic sources for rice improvement in order to breed a variety with satisfied yield as well as high antioxidant in grain.