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Variation on Grain Quality in Vietnamese Rice Cultivars Collected from Central Vietnam

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Characterization of grain quality of rice germplasm is important for broadening the genetic base for breeding program. In this study, we characterize the grain size and apparent amylose content of ninety eight rice cultivars which collected from Central of Vietnam. The endosperm types of ninety eight Vietnamese rice cultivars were classified as waxy, opaque, white core and translucency, and GBSS level were divided into four groups, including absent, low, intermediate and high. In non–waxy group, the wide variation of apparent amylose content was observed in thirty four rice cultivars. The continuous variation on the apparent amylose content was observed in three respective groups of GBSS. The grain length showed the positive correlation with apparent amylose content while grain width showed negative correlation. The very low amylose class was not found in two regions including Central highland and South coastal central whereas the intermediate amylose class was not detected in the North central region of Vietnam.

Keywords: amylose, GBSS, genetic resources, grain quality, rice

INTRODUCTION

Rice (*Oryza sativa* L.) is one of the most important crops in the world, providing staple food for nearly one half of the global population. Particularly in Asia, rice provides 50–60% of dietary energy supply. Quality of rice is determined by key components such as appearance, milling quality, cooking and eating quality, and nutritional quality. The grain length, grain width, grain shape and endosperm type are important quality traits which greatly effects commercial value of grain. Previous studies reported that the grain shape is quantitatively inherited (Kuo and Hsien, 1982; Chen *et al*., 1998). To obtain different combination of allele conferring particular grain shapes and size has implications for yield improvement (Melissa *et al*., 2008). A great diversity of grain size and shape found in rice resources (Koutroubas *et al*., 2003; Zeng *et al*., 2003) suggested that the local rice germplasm may play an important role in improving rice quality.

Comprise up 90 percent of milled rice, endosperm starch plays a critical role in eating and cooking quality of rice. The physicochemical properties of rice starch are affected by the ratio of amylose to amylopectin (Larkin *et al*., 2003) and the structure of starch molecule (Nakamura *et al*., 2006). Amylose is considered the most important predictor of sensory quality in rice, which is essentially linear polymer of α–1, 4 linked glycosidic chain.

Starch biosynthesis is catalysed by at least four classes of enzymes: Adenosine disphosphate glucose pyrophosphorylase (AGPase), starch synthase (SS), starch branching enzyme (BE), and starch debranching enzyme (DBE) (Smith *et al*., 1997; Myers *et al*., 2000; Nakamura, 2002, Satoh *et al*., 2007; Hannah and James, 2008). The waxy gene, which encodes the granule–bound starch synthase (GBSS), is one key gene influencing starch synthesis in the rice endosperm (Larkin and Park, 2003). There are several alleles encoded by the *Wx* locus involved in amylose content, (Chen *et al*., 2008, Mikami *et al*., 2008). The allele Wx^a has been recognized as being distributed in *Indica* while the allele Wx^b is distributed in *Japonica* subspecies (Sano, 1984; Isshiki *et al*., 1998). Mikami *et al*. (2008) reported that an SNP in exon 6, which is identified as Wx^{in} allele, separating high and intermediate amylose varieties. An SNP in exon 4, which associates with opaque phenotype, is defined as the Wx^{op} allele. Furthermore, mutants with low amylose content with dull endosperm, called "dull" mutant, were characterized (Yano *et al*., 1988; Satoh *et al*., 2003; Satoh *et al*., 2004). The *waxy* mutants carry a deletion in the *Wx* gene that is fatal to activity of GBSS, and consequently they contain no amylose (Mikami *et al*., 1999). The spontaneous mutation of amylose were found in local rice cultivars from Asian and African countries (Nakagahara and Nagamine, 1986; Satoh *et al*., 1990; Jahan *et al*., 2002, Hoai *et al*., 2008).

Central and Northern mountainous areas in Vietnam are considered to be the regions of richness in rice genetic resources (Chang, 1976; Okuno *et al*., 1996). Therefore, in this study we investigated the variation on grain quality in rice cultivars from Central Vietnam for promoting utilization and improvement of high quality rice varieties.

MATERIALS AND METHODS

Plant materials

Ninety eight rice cultivars collected from three regions of Central Vietnam were used in this study. Three

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rice cultivars including IR36 (*Indica*), TC65 (*Japonica*) and EM21 (waxy mutant) were used as controls.

Variation in morphological character and measurement of grain size

Based on the rice descriptors of IRRI, variation on endosperm type was characterized. The endosperm type was classified as 4 scales including waxy, opaque, white core and translucency. Three characters including grain length, grain width and the ratio grain length per grain width have been conducted.

Determine the apparent amylose content

Amylose content was estimated by using the DU 7500 Spectrophotometer (Beckman) according to Satoh (1990e).

GBSS extraction and SDS–PAGE analysis

GBSS was isolated from the polished rice flour according to Echt and Schwartz (1981). 20 mg rice flour was homogenized in 1.5 ml of GBSS–extraction buffer (0.055M Tris–HCl, 2.5% SDS (w/v), 5% 2–mercaptoetanol adjusted at pH 6.8). The suspension was sonicated and centrifuged at 1,200 rpm for 5 minutes. After washing three times with GBSS–extraction buffer and twice in the distilled water, the pellet was dissolved in 2–mercaptoetanol adjusted at pH 6.8. The sample was centrifuged at 1,200 rpm for 10 minutes and the resulting supernatant was loaded on a 10% polyacrylamide gel. SDS–PAGE was performed according to Laemmli (1970) using 10% polyacrylamide gel. 60 kD band corresponding to GBSS could be seen by Coomassie Brilliant Blue staining and was measured by a densitometer. The GBSS level was classified as high, intermediate and low and absent based on staining intensity of 60kD.

Western blotting

700 μl SDS–PAGE sample buffers was added to each 20 mg sample and then vortex for 3 hours at room temperature, centrifuged at 20°C, 6,000 to 7,000 rpm in 10 minutes. SDS–PAGE was performed by using 10% SDS polyacrylamide gel. The PVDF membrane was used for blotting the band of proteins and the membrane was incubated with the primary antibody (GBSS–anti wheat waxy protein). Immunoreactive proteins were detected by incubating the secondary antibody. Goat Anti–rabbit IgG H+L. Equal amount of the ECL substrate was mixed and the membrane was incubated for 1 min in this mixture before using. The X–ray film was developed, washed and fixed.

RESULTS

Morphological variation of grain size

Length of grain ranged from 7.26 mm to 11.56 mm among 98 rice cultivars. They were divided into two

Table 1. Vietnamese rice examined for grain type (grain length, grain width, endosperm type) , apparent amylose content and GBSS level

Cultivar name	Collection site	Grain length (mm)	Grain width (mm)	L/W ratio	Endosperm Type	Apparent amylose content (%)	GBSS level
Nep cam dang 1	NC	9.98	3.76	2.65	Waxy	3.42	absent
Vang me	NC	9.82	3.56	2.76	Opaque	11.4	low
Lua song	NC	8	3.7	2.16	Waxy	5.7	absent
Nep hat cau dang 1	NC	7.54	3.86	1.95	Waxy	1.9	absent
Nep vang som	NC	8.56	3.7	2.31	Waxy	2.66	absent
Nep ech dang 1	NC	7.98	4.72	1.69	Waxy	1.14	absent
Cam vo vang	NC	9.66	3.02	3.20	Waxy	0.76	absent
Nep truong	NC	8.4	4.58	1.83	Waxy	3.8	absent
Cham song	NC	8.1	3.64	2.23	Translucency	14.82	low
Nep tron	NC	8.92	3.66	2.44	Waxy	2.66	absent
Nep cai rau	NC	7.48	3.64	2.05	Waxy	5.32	absent
Nep cai can	NC	7.96	4.56	1.75	Waxy	3.04	absent
Lua dam xoong	NC	8.28	3.32	2.49	White core	15.58	low
But veng	NC	8.44	3.22	2.62	Waxy	3.8	absent
But kia	NC	9.32	3.24	2.88	White core	13.68	low
Nep cam den	NC	9.78	3.56	2.75	Waxy	5.32	absent
Nep cam	NC	9.92	3.88	2.56	Opaque	14.82	low
Te mun	NC	9.76	3.14	3.11	Waxy	3.42	absent
Nep thuong	NC	8.34	3.8	2.19	Waxy	1.52	absent
Te nuong	NC	8.42	3.52	2.39	White core	13.3	low
Lo cang	NC	9.04	3.28	2.76	Waxy	1.52	absent
Lo xo	NC	10.46	3.62	2.89	Waxy	4.94	absent
Lo cam	NC	8	3.28	2.44	White core	29.64	high
Nep cu dat	NC	7.26	3.32	2.19	Waxy	5.32	absent
Cu man	NC	7.56	3.66	2.07	Waxy	0.38	absent
Sang chu	NC	8.38	4.64	1.81	Waxy	3.04	absent

Table 1. Continued

Cultivar name	Collection site	Grain length (mm)	Grain width (mm)	L/W ratio	Endosperm Type	Apparent amylose content (%)	GBSS level
Nep bo giua	NC	9.6	3.56	2.70	Waxy	3.04	absent
Boc bo	NC	9.44	3.52	2.68	Waxy	2.66	absent
Nep man	NC	9.54	3.64	2.62	Waxy	4.94	absent
Luot cay	NC	8.22	3.5	2.35	Waxy	5.32	absent
Khau cho lon	NC	9.7	3.74	2.59	Waxy	4.94	absent
Khau cam panh	NC	10.58	3.56	2.97	Waxy	2.66	absent
Khau ma giang	NC	8.96	3.44	2.60	Waxy	0.38	absent
Khau non	NC	10.18	3.3	3.08	Waxy	4.94	absent
Khau hin	NC	7.98	3.76	2.12	Waxy	1.14	absent
Khau vai nieu	NC	9.58	3.64	2.63	Waxy	0.76	absent
Khau moong mo	NC	9.26	3.66	2.53	Waxy	0.76	absent
Khau chao den	NC	9.26	3.42	2.71	White core	29.26	high
Khau cham cam	NC	8.02	3.12	2.57	White core	26.22	high
Khau luong cuoi	NC	10.26	3.12	3.29	Waxy	1.52	absent
Chao luu	NC	8.04	3.6	2.23	White core	15.58	low
Khau luong con	NC	7.6	3.98	1.91	Waxy	1.52	absent
Khau san	NC	8.84	3.74	2.36	Waxy	1.52	absent
Khau vai do	NC	10	3.9	2.56	Waxy	0.76	absent
Khau do don	NC	9.02	3.58	2.52	Waxy	3.42	absent
Khau danh dan	NC	9.46	3.5	2.70	Waxy	3.42	absent
Khau cam	NC	8.76	3.84	2.28	Waxy	1.52	absent
Chao meo	NC	9.16	2.98	3.07	Opaque	10.64	low
Khau pan	NC	9.32	3.7	2.52	Waxy	1.14	absent
Ngoo vai	NC	10.12	3.06	3.31	Waxy	3.04	absent
Ngoo nac	NC	10.16	3.64	2.79	Waxy	1.14	absent
Khau chao vai	NC	9.36	2.8	3.34	White core	14.82	low
Khau do sang	NC	8.28	3.8	2.18	Waxy	1.52	absent
Khau pa pan	NC	9.76	3.72	2.62	Waxy	3.42	absent
Nep lau	NC	10.08	3.36	3.00	Waxy	5.32	absent
Nep rang chau	NC	9.4	3.98	2.36	Waxy	4.94	absent
Nep man	NC	8.36	3.56	2.35	Waxy	4.56	absent
Nep thai	NC	10.12	3.04	3.33	Waxy	5.32	absent
Nep chac rat	NC	8.48	3.66	2.32	Waxy	3.42	absent
Khau lac	NC	9.22	3.46	2.66	Waxy	3.42	absent
Khau cu	NC	8.7	3.92	2.22	Waxy	1.14	absent
Khau pe lanh	NC	9.6	3.02	3.18	Waxy	1.14	absent
Cham nanh	NC	8.86	3.2	2.77	White core	11.78	low
Cham noi	NC	9.42	3.2	2.94	White core	13.3	low
Khau tao	NC	10.14	3.54	2.86	Waxy	4.94	absent
Lep muoi	NC	8.76	4.04	2.17	Waxy	1.9	absent
Knon	NC	10.38	2.94	3.53	Waxy	5.7	absent
Cu hon	NC	8.42	3.44	2.45	Waxy	5.7	absent
Nhan pung	NC	9.78	4.06	2.41	Waxy	3.42	absent
Lua na	SCC	8.26	3.12	2.65	White core	25.46	high
Ca do	SCC	9.66	3.52	2.74	Translucency	20.9	high
Nep tro	SCC	9.24	3.34	2.77	Waxy	3.8	absent
Lua nhe	SCC	8.7	3.04	2.86	Translucency	25.46	high
A hach	SCC	10.02	2.72	3.68	Translucency	27.74	high
Ca ang	SCC	9.82	2.72	3.61	Translucency	27.74	high
A vay	SCC	9.36	2.62	3.57	White core	17.48	intermediate
Nep den	SCC	8.52	2.96	2.88	Waxy	3.8	absent
Nep bo	SCC	9.62	2.32	4.15	Waxy	5.32	absent
Djang kloih	CH	9.76	3.6	2.71	White core	22.42	high
Me loll	CH	9.82	3.54	2.77	White core	19.38	intermediate
M bat	CH	9.86	2.66	3.71	Opaque	13.68	low
Coi tung rling dạng 1	CH	9.9	3.12	3.17	Translucency	22.42	high
Koi djang wir	CH	11.56	2.54	4.55	Translucency	28.12	high
M bet dang	CH	11.34	2.6	4.36	Waxy	3.42	absent

Table 1. Continued

Cultivar name	Collection site	Grain length (mm)	Grain width (mm)	L/W ratio	Endosperm Type	Apparent amylose content (%)	GBSS level
Koi me loh	CH	9.2	3.1	2.97	White core	24.7	intermediate
M bet krop	CH	10.4	3.66	2.84	Waxy	5.7	absent
Koi N ken	CH	10.04	3.12	3.22	White core	22.8	high
Koi soai	CH	9.98	2.86	3.49	Translucency	15.2	low
Koi koe	CH	9.46	3.2	2.96	Translucency	15.96	low
Ba keen	CH	10.64	2.74	3.88	Translucency	15.2	low
Ba ke	CH	10.4	2.74	3.80	Translucency	14.82	low
Ba lo	CH	9.62	3.62	2.66	White core	17.48	intermediate
Ba N kon	CH	9.42	3.26	2.89	White core	21.66	high
Ba M bet dia	CH	8.92	3.2	2.79	Waxy	5.7	absent
Ba M bet ler	CH	9.98	3.26	3.06	Waxy	5.32	absent
Nep heo	CH	11.54	2.64	4.37	Waxy	2.66	absent
Nep heo	CH	10.96	2.78	3.94	Waxy	3.42	absent
Lua rau	CH	9.88	2.96	3.34	Translucency	25.46	high
IR 36		8.84	2.42	3.65	Translucency	23.56	high
Nipponbare		7.6	3.12	2.44	Translucency	20.14	low

CH: Central Highland; NC: North Central; SCC: South Coastal Central

Table 2. Variation on the length–to–width ratio in Central Vietnamese rice cultivars

Scale	L/W ratio	Number of cultivars	Frequency (%)
1	Bold (1.1–2.0)	6	6
2	Medium (2.1–3.0)	66	67
3	Slender (>3.0)	26	27
	Total	98	100

Table 3. Distribution of endosperm type in Central Vietnam rice cultivars

Scale	Endosperm types	Number of cultivars	Frequency (%)
1	Waxy	64	65
2	Opaque	4	4
3	White core	18	18
4	Translucency	12	12
	Total	98	100

groups: long grain (from 6.61 to 7.5 mm) and very long grain (>7.5 mm). Most of cultivars (98%) belonged to the very long grain group.

Variation of length–to–width ratio (L/W) was shown in Table 1 and Table 2. The length–to–width ratio (L/W) varied from 1.69 to 4.55 among 98 rice cultivars. Based on the ratio of grain length to grain width, 98 rice cultivars were grouped as bold (scale 1: 1.6–2.0), medium (scale 2: 2.1–3.0) and slender (scale 3: >3.0) with frequency 6%, 67% and 27%, respectively.

Classification of non–waxy and waxy by endosperm type, iodine staining intensity and GBSS level

Endosperm type variation of 98 studied rice cultivars was shown in Table 1, Table 3 and Fig. 1A. The

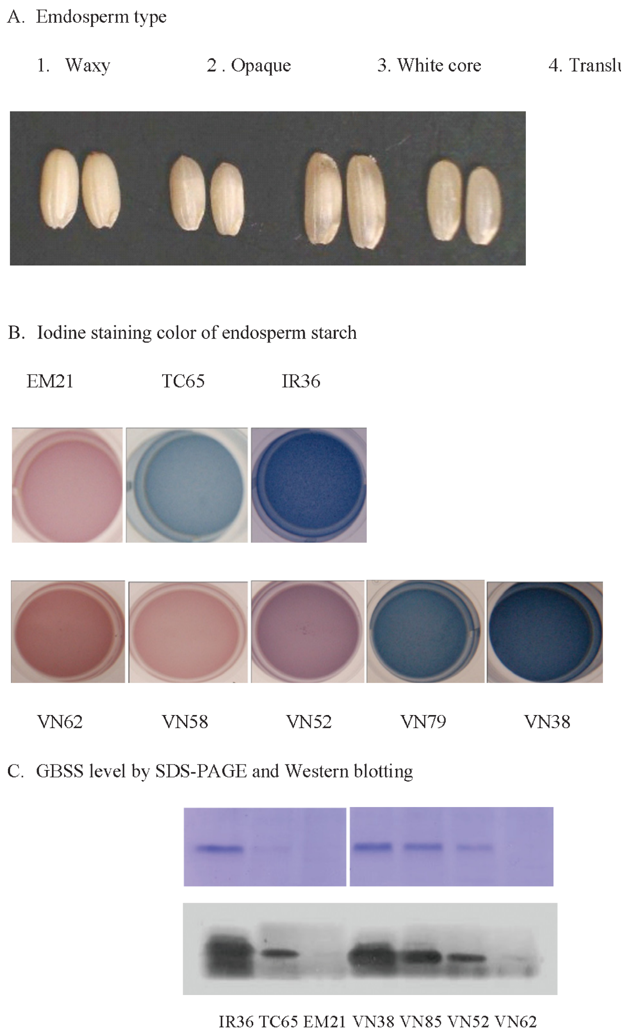

Fig. 1. Variation in endosperm type, iodine staining colour of endsperm starch and GBSS level among central Vietnam rice cultivars.

endosperm types were classified as four scales including waxy, opaque, white core and translucency. The waxy endosperm type was found popular with frequency 65% of total cultivars. The opaque, white core and translucency were exhibited in 4%, 18% and 12% of total cultivars, respectively.

Ninety eight rice cultivars have I2–KI staining color varied from reddish, reddish brown, purple, purple to blue and dark blue (Fig. 1B). The waxy endosperm type had reddish color while other endosperm type showed the purple, purple to blue and dark blue ones.

The variation in GBSS level was observed among 98 samples (Fig. 1C). TC65 and IR36 were distinguished from each other by the staining intensity of 60 kDa. High intensity of GBSS (Wx^a) was found in IR36 while low intensity of GBSS (Wx^b) was found in TC65. GBSS was absent in EM21 (Wx). GBSS level of 98 rice cultivars were classified into four groups, including absent (EM21 type), low (TC65 type), intermediate (intermediate between TC65 and IR36) and high (IR36 type) with frequency of 65.3%, 16.3%, 4.1% and 14.3%, respectively. All the waxy endosperm type of rice cultivars belonged to absent GBSS group.

Table 4. Variation in apparent amylose content among Central Vietnam rice cultivars

Apparent amylose content (%)	Amylose class	No of cultivars	Frequency (%)
0~6	Waxy	64	65.3
6~12	Very low	3	3.1
12~20	Low	16	16.3
20~25	Intermediate	6	6.1
>25	High	9	9.2
Total		98	100

Variation in apparent amylose content in non–waxy rice

In non–waxy group, the wide variation of apparent amylose content was observed in 34 rice cultivars (Table 1). The apparent amylose content in non– waxy rice varied from 10% to 29% and they were grouped into very low (10–12), low (12–20) high (20–25 and very high (≧25) with frequency 3.1%, 16.3%, 6.1% and 9.2% of total cultivars, respectively (Table 4).

Relationship between apparent amylose content and GBSS level

As mentioned above, waxy rice cultivars showed the absence of GBSS level. In non–waxy rice group, the correlation between the GBSS level and the apparent amylose content were observed in Table 5. The continuous

Table 5. Distribution of Central Vietnam rice cultivars in GBSS level and apparent amylose content

GBSS types	Apparent amylose content (%)					Total of cultivars	Frequency (%)
	0~6	6~12	12~20	20~25	>25		
Absent (waxy)	64	0	0	0	0	64	65.3
Low	0	3	13	0	0	16	16.3
Intermediate	0	3	0	1	0	4	4.1
High	0	0	0	5	9	14	14.3
Total	64	6	13	6	9	98	100.0

Table 6. The correlation of grain size and apparent amylose content

	Grain length	Grain width	L/W Ratio	Amylose content
Grain length	1			
Grain width	–0.449	1		
L/W Ratio	0.792	–0.878	1	
Amylose content	0.129	–0.432	0.332	1

Significant at 5%.

Table 7. Regional distribution of Vietnamese local rice cultivars on apparent amylose content

Amylose content (%)	Regions			Total	Frequency (%)
	CH	NC	SCC		
Waxy (0~6)	6	55	3	64	65.3
Very low (6~12)	0	3	0	3	3.1
Low (12~20)	7	8	1	16	16.3
Intermediate (20~25)	5	0	1	8	8.1
High (>25)	2	3	4	9	9.2
Total	20	69	9	98	100

CH: Central Highland; NC: North Central; SCC: South Coastal Central variation on the apparent amylose content was observed in three respective groups of GBSS. The low GBSS level cultivars had apparent amylose content varied from 10% to 16%, while intermediate and high GBSS cultivars showed the apparent amylose content from 17% to 24% and 20% to 29%, respectively.

Relationship between the grain length, grain width and grain shape and apparent amylose content

The correlation of grain size and apparent amylose content was showed in Table 6. Grain length showed the positive correlation with apparent amylose content and grain shape while negative with grain width. Grain width showed negative correlation with apparent amylose content.

Geographical distribution of rice cultivars on apparent amylose content

Table 7 shows the regional distribution of rice cultivars on apparent amylose content. Each of three regions showed four per five amylose classes. The very low amylose class was not found in two regions including Central highland and South coastal central whereas the intermediate amylose class was not detected in the North Central. Waxy cultivars were found in all regions. However, almost waxy rice cultivars were distributed in North Central region (55 cultivars).

DISSCUSSION

The shape, uniformity and translucence of rice grain are important traits which define the market value. The value of each trait, for example grain length, varies according to local cuisine and culture (Melissa *et al.*, 2008). Some studies on the variation of quality traits from rice germplasm have been reported. Koutroubas *et al.* (2003) showed that there was considerable genetic variation on grain length, grain width, grain length–to–width ratio and amylose content among 318 rice lines. Studying the variability in grain quality from 100 upland genotype *India* rice, Rathi *et al.* (2010) revealed the highly significant difference among the genotypes for grain size and other starch properties. In this study, almost of rice belonged to long grain length group while the medium is popular for grain shape (the length–to–width ratio) (Tables 1 and 2). The correlation between grain size and apparent amylose content in this research (Table 6) is in consistent with the finding of other research works (Chakraborty *et al.*, 2009). The information on the relationship between rice grain shape and quality trait may be useful for developing selection indices in rice breeding program.

The waxy gene, which encoded the GBSS enzyme, is responsible for amylose synthesis in the rice endosperm. Some studies reported that the G–T single nucleotide polymorphism (SNP) at the 5' splice junction of the first intron is contributed to variation in the expression of the *Waxy* gene. Moreover, CT repeat and the SNP in exon 4, exon 6 and exon 10 are strong associated with different amylose class (Frances *et al.*, 1998; Chen *et al.*, 2008, Mikami *et al.*, 2008; Larkin and Park, 2003; Bao *et al.*, 2008). In this study, the strong correlation between apparent amylose content and GBSS level was detected (Table 1). This continuous variation on the apparent amylose content may be caused by the intragenic variation of GBSS in addition to the amylopectin structure. Thus, rice cultivars from Central of Vietnam exhibited the high diversity both in GBSS level and apparent amylose content.

Some previous studies reported that the colorimetric method for estimating apparent amylose content is affected by the amylopectin structure (Nishi *et al.*, 2001) and some rice amylopectin have long chain showing a high affinity for binding iodine (Takeda and Hizukuri, 1987). As mentioned above, sixty four waxy rice cultivars showed the variation in iodine staining intensity while they were observed absence of GBSS (Table 1 and Fig. 1B). This result suggested that the variation in iodine staining intensity among waxy rice was caused by the amylopectin structure.

The relation between amylose content and geographical distribution as well as GBSS level has been shown in several studies (Nakagahara and Nagamine, 1986; Jahan *et al.*, 2002, Aung *et al.*, 2003; Hoai *et al.*, 2008; Liu *et al.*, 2008). The geographical distribution of amylose content in 872 Asian rice cultivars from five Asian countries have been investigated by Nakagahara and Nagamine (1986). The clear difference in the pattern of geographical distribution on amylose content of rice cultivars from five countries was observed. Aung *et al.* (2002) reported that the variation in GBSS level and apparent amylose content in mountainous region was wider than these from other regions. When studying geographical distribution of GBSS level in 185 local rice cultivars from mountainous areas in Northern Vietnam, Hoai *et al.* (2008) reported the wide variation on apparent amylose content in Northwest and Northeast regions. The geographical distribution on apparent amylose content in this study was proved that Central region of Vietnam has rice germplasm with high variation on starch properties. Moreover, a huge number of waxy rice cultivars existed in this area supported that the Southeast Asian (encompassing portions of Lao, Myanmar, Thailand, Cambodia and Vietnam) are considered as the "glutinous rice zone" (Olsen and Purugganan, 2002).

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