Rapidly Available Glucose (RAG) and *Waxy* Haplotype as Indicators for Glycemic Index in Some Lowland and Upland Thai Rice Varieties (*Oryza sativa* L.)

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Abstract

Glycemic index (GI) is generally used as an indicator for the response of blood sugar levels after food consumption. It was found that amylose content (AC) is a key factor that affects the rate of starch digestion and GI values. Previous studies indicated that amylose content in rice can be determined by *Waxy* haplotype at intron1 (In1G/T) and exon6 (Ex6 A/C). The rapidly available glucose (RAG) has also been reported to be used as an indicator of the GI, low RAG indicates a lower GI. However, *Waxy* haplotype and starch digestive properties in Thai rice have rarely been studied. The objective of this study was to rapidly evaluate available glucose and *Waxy* haplotype for GI indicators in Thai rice varieties. The results showed that four varieties of Thai rice contain high AC harboring G-A haplotype, nine varieties contain intermediate AC harboring G-C haplotype and thirteen varieties contain low AC harboring T-A or T-C haplotype. Furthermore, G-A exhibited significantly low RAG value (at $p<0.05$) compared to G-C haplotype, T-A haplotype and T-C haplotype. The study suggested that RAG, *Waxy* haplotype and AC can be used as an indicator of GI in Thai rice germplasm.

Keywords: Thai rice, rapidly available glucose, *Waxy* haplotype, amylose

Introduction

The prevalence of diabetes and chronic diseases in Thai people is increasing because over food consumption. Rice is the main staple containing 80% of starch (Wani et al., 2012). It has been reported that eating rice could increase the risk of type 2 Diabetes (Courage, 2010). Therefore, rice might play an important role in lowering the risk of getting this disease. Carbohydrate in rice composed of the two glucose polymers, amylose and amylopectin, respectively (Champagne, 1996). Amylose is controlled by *Waxy* gene, which codes for granule bound starch synthase (GBSS) (Chen et al., 2008). In rice, amylose content (AC) is a key factor that affects the rate of starch digestion and Glycemic index (GI) (Kharabian-Masouleh et al., 2012). Previous studies found that *Waxy* haplotype at intron1 (In1G/T) and exon6 (Ex6 A/C) can be used for determining of AC in rice (Chan et al., 2008; Larkin et al., 2003; Kharabian-Masouleh et al., 2012). At intron1, the Guanine (G) to Thymine (T) mutation at the 5'-leader intron splice site (Hirano et al., 1998) can be used to distinguish low AC (harboring T) from high AC (harboring G). However, *Waxy* haplotype at intron1 is insufficient to explain the genetic variations of AC in rice (Cheng et al., 2012). Larkin and Park (2003) reported that a change at exon 6 (A/C SNP) could be used to distinguish the intermediate AC (harboring C) from high AC (harboring A). AC in rice was successfully classified (Chen et al., 2008) which composed of 4 haplotypes; including (In1T- Ex6A), (In1G- Ex6C), (In1G- Ex6A) and (In1G- Ex6A). Fitzgerald et al., (2011) found that the amount of AC, *Waxy* haplotype and digestibility of rice are significantly correlated. Accordingly, this can be used for indicating of the quality of the rice, which helps to select appropriate varieties for diabetes. Moreover, Jenkins et al., (2002) found that food with low

GI could reduce the risk of diabetes and also coronary heart disease. The rice/flour with high AC tend to have low GI which has a benefit for people with diabetes since carbohydrates in rice are slowly digested and absorbed (Frei et al,. 2003). GI is generally used as an indicator for the responses of blood sugar levels after eating, which can be measured by rapidly available glucose (RAG). It has been reported that the RAG can be use as an indicator of the GI (99%) (Englyst et al., 1999). However, the study of *Waxy* haplotype and starch digestive property in Thai rice is still limited nevertheless there is a study of Charoensiri et al., (2008) which RAG values were determined in 49 varieties of Thai rice. Consequently, *Waxy* haplotype, RAG and SAG might be use as biomarkers for healthy carbohydrate investigation (Healthy food) and rice variety selection with low GI, for an alternative approach to prevent and reduce diabetes problems. The objective of this study was to rapidly evaluate available glucose and *Waxy* haplotype for GI indicators in Thai rice varieties.

Methodology

Rice sample

Glutinous and non-glutinous rice (*Oryza sativa* L.) including twenty-six Thai rice varieties obtained from Sooksatharana Company Ltd. and four Rice Research Centers including (Chiang rai, Pathumthani, Phitsanulok and Phatthalung)

Single Nucleotide Polymorphism of *Waxy* gene

Rice grains were germinated and genomic DNA extracted from leaves using the CTAB method (Doyle, 1991). The PCR amplification of the *Waxy* were performed using the G/T polymorphism at intron1 of the *Waxy* (In1 G/T SNP) as previously described by (Ayres et al., 1997), the A/C polymorphism at exon6 of the *Waxy* (Ex6 A/C SNP) previously described by (Fitzgerald et al., 2011) and PCR product quality analysis by gel electrophoresis on 2.0 % agarose gel. The sequences of primers showed in Table 1.

Table 1: List of Primers

Primer name	Sequence (5′→3′)	Tm(ºC)	Restriction enzyme	References
Waxy(In1)190F Waxy(In1)W2R	CTTTGTCTATCTCAAGACAC TTTCCAGCCCAACACCTTAC	47.8 55.3	*AccI*	(Ayres et al., 1997)
Waxy(Ex6)-F Waxy(Ex6)-R Waxy(Ex6)-R	CCCATACTTCAAAGGAACATA GGTTGGAAGCATCACGAGTT T CTTCAGG TAGCTCGCCAGT	48.5 51.8 53.8	-	(Fitzgerald et al., 2011).

Amylose content (AC) analysis

Rice grains were ground in an electric coffee grinder (PHILIPS HR 2001) 220-240V., 350W. Amylose content was determined using iodine spectrophotometer method previously described by Juliano (1985).

In vitro rapidly available glucose (RAG) and slowly available glucose (SAG)

Cooked rice, rice and distilled water were prepared in ratio of 1:3 using boiling water bath (Memmert) at 95±5°C for 30 minutes. After that, the cooked rice was ground for RAG and SAG analysis and determined by using method modified from Englyst et al., (1999).

Statistical analysis
All statistical analyses were performed using IBM SPSS statistics software program version 21.0 (Purchase Order: 10-58878) for analysis of variance (ANOVA) means were compared using the Duncan's multiple comparison tests. Correlation of RAG, SAG and their AC of Thai rice were analyzed by Pearson's correlation was used to measure the linear correlation between parameters at 95% confident interval, significance level $P \leq 0.05$.

Results

The results show four varieties harboring G-A include Chainat1, Leb Nok Lek, Phitsanulok2, and RD31. Nine varieties harboring G-C include Ba Nie, Basmati, FR13A, Khao Ta Kong, Leb Nok Yai, Nui Khuea, RD35, RD41, and Suphan Buri60, respectively.

Table 2: *Waxy* haplotype, AC, RAG and SAG of twenty-six Thai rice varieties

Varieties	*Waxy* (In1-Ex6)		g/100g cooked rice		AC%
			RAG	SAG	
Chainat1	G	A	15.72±1.50[bcdefg]	11.43±2.14[g]	23.46
Leb Nok Lek	G	A	10.42±0.62[a]	6.99±0.62[cdef]	32.46
Phitsanulok2	G[c]	A	13.03±1.55[abc]	3.61±2.26[abc]	26.15[c]
RD31	G	A	15.94±2.27[cdefg]	5.76±1.22[bcdef]	34.29
Ba Nie	G	C	20.57±2.39[ij]	8.25±2.38[efg]	18.89
Basmati	G	C	16.36±2.57[cdefg]	2.76±1.63[ab]	25.13
FR13A	G	C	12.18±1.95[ab]	6.26±1.11[bcdef]	32.04
Khao Ta Kong	G	C	15.10±2.05[bcde]	3.86±2.67[abcd]	30.83
Leb Nok Yai	G	C	16.59±1.53[cdefgh]	4.67±1.08[abcde]	29.74
Nui Khuea	G	C	16.44±1.25[cdefg]	1.51±0.85[a]	28.87
RD35	G	C	18.80±3.82[efghij]	8.94±3.20[fg]	33.29
RD41	G	C	19.57±0.99[ghij]	5.28±1.19[abcdef]	27.95
Suphan Buri 60	G	C	21.75±0.35[j]	5.53±1.03[bcdef]	29.07
Hang Pla Lai[a]	T	A	14.72±2.77[bcd]	4.12±3.52[abcd]	4.65
Hawm Thung[a]	T[c]	A	16.87±2.71[cdefghi]	6.73±2.08[cdef]	3.94[c]
Homnin Surin	T	A[b]	15.33±0.63[bcdef]	2.76±0.92[ab]	15.53
Kam Doi Saket[a]	T	A	18.77±1.87[efghij]	7.49±2.19[def]	5.45
Kam Phayao[a]	T	A	17.26±2.38[defghi]	6.38±0.69[bcdef]	5.95
Niaw Cham Mai Pai[a]	T	A	18.79±1.59[efghij]	3.37±1.37[abc]	5.16
Niaw Dam Chum Phae[a]	T	A	16.37±0.69[cdefg]	7.65±1.01[def]	6.16
Pathum Thani1	T	A	17.61±0.85[defghi]	5.26±0.11[abcdef]	17.51
Whan1[a]	T[c]	A	20.34±5.37[hij]	6.03±4.31[bcdef]	4.46[c]
Whan2[a]	T[c]	A	15.13±2.30[bcde]	4.01±2.46[abcd]	5.56[c]
Jow Khao Chiangmai	T	C	19.04±2.59[fghij]	5.95±3.98[bcdef]	18.47
Jow Lisaw	T	C	19.38±3.22[ghij]	4.57±1.43[abcde]	14.45
Jow Rai Khao	T	C	21.45±1.59[j]	3.34±2.49[abc]	14.47

[a] Glutinous rice
[b] Previous studies by Popluechai et al., 2012
[c] Previous studies by Popluechai et al., 2013

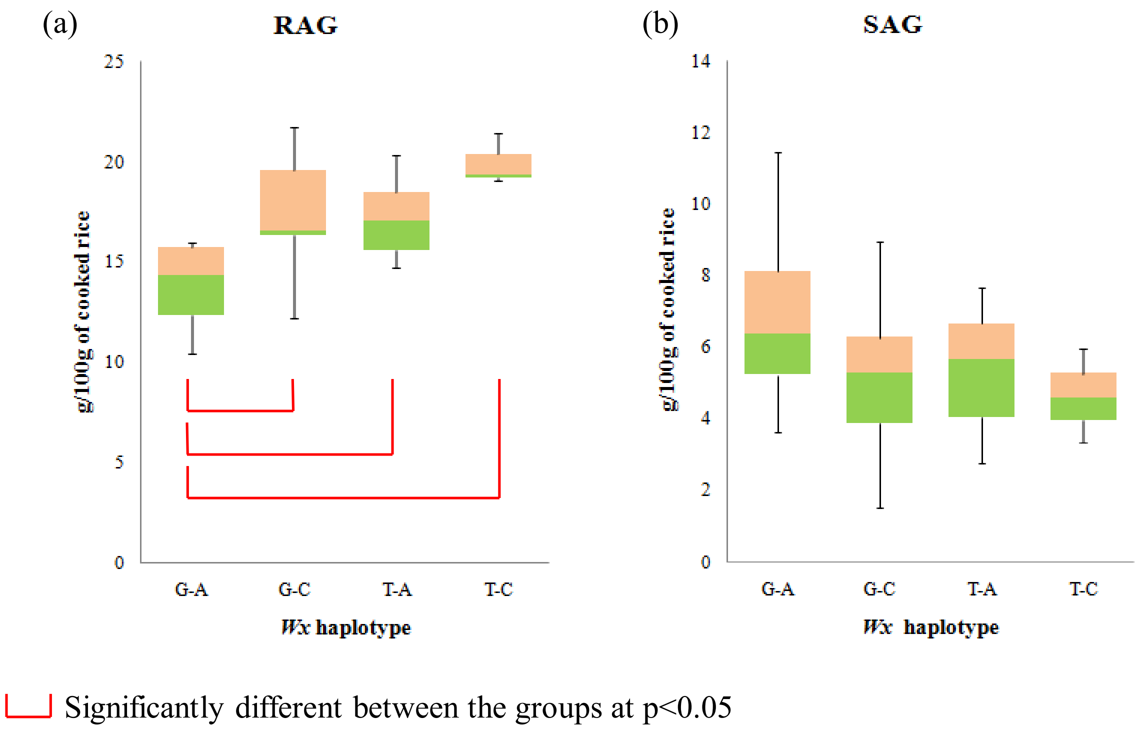

Figure 1: Digestible properties of twenty-six rice sample base on *Waxy* haplotype; (a) RAG, (b) SAG

Ten varieties harboring T-A include Hang Pla Lai, Hawm Thung, Homnin Surin, Kam Doi Saket, Kam Phayao, Niaw Dam Chum Phae, Niaw Cham Mai Pai, Pathum Thani1, Whan1, and Whan2, and three varieties harboring T-C include Jow Khao Chiangmai, Jow Lisaw, and Jow Rai Khao. The Amylose contents of twenty-six Thai rice varieties ranging from 3.94-34.29% (Table 2). RAG value ranging from 10.42-21.75 g/100g, and SAG value ranging from 1.51-11.43 g/100g (Figure 1). Twenty-six Thai rice varieties were classified using the Analysis of Variances (One-Way ANOVA). There were 4 haplotypes harboring G-A, G-C, T-A, and T-C, which each variety of Thai rice was classified according to the haplotype. It was found that AC (Sig=0.000) and RAG (Sig=0.018) were significantly different at $p<0.05$, while SAG was not significantly different at $p<0.05$. From Pearson's correlation coefficient of AC, RAG and SAG showed that there was no significant difference at $p<0.05$.

Discussion

From the results *Waxy* haplotype of twenty-six Thai rice varieties can be used to classify AC in rice (Chen et al., 2008) found harboring G-A haplotype exhibit high AC whereas harboring G-C haplotype exhibit intermediate AC which the results are consistent with Larkin and Park (2003) and Chen et al., (2008). However, in this study, AC of these two groups were not significantly different at $p<0.05$. Chen et al., (2008) have previously found a haplotype harboring T-A haplotype in non-glutinous rice samples and also found harboring T-C haplotype only in glutinous rice samples. In addition, the study found that harboring T-A haplotype exhibit low AC in non-glutinous rice samples while harboring T-C haplotype exhibit low AC in non-glutinous rice samples, which are consistent with Larkin and Park (2003) and Chen et al., (2008). Subsequently, a haplotype harboring T-C and T-A were found in glutinous rice samples (Chen et al., 2010) (Table 2).

Table 3: The correlation of AC, RAG and SAG of twenty-six Thai rice varieties

	AC	RAG	SAG
AC	1		
RAG	-0.264	1	
SAG	0.017	0.058	1

*Correlation is significant at the 0.05 level.

From the results revealed that AC in glutinous group were higher than previous report by Thai Rice Department. This might due to the long chains of amylopectin, which could form a complex compound with iodine (Chung et al., 2011). Furthermore, the study by Chen et al. (2008) found that variation of AC values might be affected by environment factors.
The amount of RAG and SAG were reported as glucose releases in gram per 100g of cooked rice from (Table 2). There was no difference of RAG values among twenty-six Thai rice varieties. From the data showed that Leb Nok Lek harboring G-A haplotype exhibited the lowest RAG value whereas Suphan Buri 60 harboring G-C haplotype exhibited the highest RAG value. However, there were significant differences (at $p<0.05$) between the groups of harboring G-A haplotype compared to harboring G-C haplotype (0.016), harboring T-A haplotype (0.027) and harboring T-C haplotype (0.003). Larkin and Park (2003) have described that rice variety harboring G-C haplotype (high AC) containing low RAG value can be used for GI prediction. We also found that a haplotype harboring G-A contain low RAG value can be grouped into high AC group according to Chen et al., (2008). For the SAG values, each group of *Waxy* haplotype were not significantly different at $p<0.05$. From the results obtained from Pearson's correlation coefficient found that, there were a negative correlation between AC and RAG ($r = -0.264$) and also a positive correlation between AC and SAG ($r = 0.017$), which consistent with Charoensiri et al., (2008), nevertheless, there was no significant difference at $p<0.05$ (Table 3).

Conclusion

Waxy haplotype and RAG can be used to predict starch digestible properties such as indicators of the GI in twenty-six Thai rice varieties. But there are other factors that related to starch digestion in rice, such as resistant starch and crude fiber which will be taken into consideration as well.

Acknowledgements

The authors are Thankful to Sooksatharana Company Ltd for funded. We would like to thank Rice Research Centers for providing seed samples.

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