APPEAR AMYLOSE CONTENT AND SINGLE NUCLEOTIDE POLYMOPHISMS (SNPs) OF *Wx* GENES IN SOME THAI RICE (*Oryza sativa* L. var indica)

Siam Popluechai[1]*, Nittaya Laosat[1], Rungarun Sasanatayart[2], Kongkiat Kespechara[3]

[1]School of Science, Mae Fah Luang University, Chiang Rai 57100, Thailand
[2]School of Agro-industry, Mae Fah Luang University, Chiang Rai 57100, Thailand
[3]88/9 Moo 2, Muang, Phuket 83000, Thailand
*e-mail: s.popluechai@sci.mfu.ac.th

Abstract

Appearance amylose content (AAC) is key factor for determinant of eating and cooking quality in rice (*Oryza sativa* L.). AAC is largely controlled by the *Wx* gene encoding a granule-bound starch synthase (GBSS). In this study, single nucleotide polymorphisms (SNPs) of *Wx* genes and AAC of eleven Thai rice varieties including Homnin Surin, Khao yeepun, Maligomen, Pakaaumpuel, Sinlek, Srinil, Nuikhuea, Malinin Surin, Riceberry, Homnin, and Homcholasit were investigated. It was found that all varieties contain AAC range from 18 to 22.92 %. SNPs analysis show that all varieties harboring haplotype In1T-Ex6A. The results indicate that all varieties can be classified into low amylose varieties. These data can be use for further determination of eating and cooking quality of those eleven Thai rice varieties.

Keywords: Appear amylase content, SNPs, *Wx* genes, Thai rice

Introduction

Rice (*Oryza sativa* L.) is a staple food for half of world's population. Rice eating and cooking quality are controlled by the properties of its endosperm starch. Starch is composed of amylose and amylopectin. Appearance amylose content (AAC) is determined rice cooking and eating (Juliano, 1985). It has been reported that amylose synthesis in endosperm requires granule-bound starch synthase (GBSS) activity (Smith et al, 1997). GBSS encodes by *Waxy (Wx)* gene (Sano, 1984).

It has been reported that single nucleotide change of G to T at position 144 of *Wx* gene (+1 position of the intron 1, hereafter refer to In1SNP) strongly associate with amylose content (Ayres et al, 1997). The In1SNP can be used to discriminate low AAC (harboring T at In1SNP) from intermediate and high AAC-type rice (harboring G at In1SNP). Larkin and Park (2003) reported that substation of A to C at position 2389 (exon 6, hereafter refer to Ex6SNP) of *Wx* gene can be used to distinguish the intermediate AAC-type rice accessions (harboring C at Ex6SNP) from high AAC-types rice (harboring A at Ex6SNP). Chen et al (2008a) proposed four haplotypes based on a combination of SNP in In1SNP and Ex6SNP. The haplotypes are In1G-Ex6C, In1G-Ex6A, In1T-Ex6A, and In1T-Ex6C. The haplotypes In1G-Ex6C, In1G-Ex6A, In1T-Ex6A associate with high, intermediate, and low AAC-type rice. The In1T-Ex6C found in glutinous rice however it requires further confirmation with larger diverse germplasm.

Thailand used to be world largest rice exporter. However, improvement varieties are needed because global warming issues effect rice production. Eleven Thai rice varieties including Homnin Surin, Khao yeepun, Maligomen, Pakaaumpuel, Sinlek, Srinil, Nuikhuea, Malinin Surin, Riceberry, Homnin, and Homcholasit were developed and released to the market. Although basic information of these varieties is available however, genetic information of starch biosynthesis gene is needed for further varieties improvement. In this study, appear amylose content and SNP of *Wx* of these varieties were investigated.

Material and methods

Appear amylose content analysis

Appear amylose content (AAC) of rice grains were determined using iodine spectrophotometric method described by Juiano (1985).

Single Nucleotide Polymorphism of Wx gene

Genomic DNA of each accession was extracted using modified CTAB method (Rajendrakumar et al, 2006) from rice grain. The G/T polymorphism in intron 1 of *Wx* gene was analyzed by using cleaved amplified polymorphic sequences (CAPS) method as previously described in Prathepha and Baimai (2004) and Ayres et al (1997). The A/C polymorphism in exon 6 of *Wx* gene was analyzed by allele-specific primers PCR method as previously described by Fitzgerald *et al* (2011).

Table 1: Primers use in this study

Primer name	Sequence (5'==> 3')	Tm (C)	PCR product (bp)
Waxy-F and Waxy-R (In1SNP)	ACCATTCCTTCAGTTCTTTGTCT	61	250 (Prathepha and Baimai, 2003)
	TAGCATGTATGAGACTACTTGTAA	55	
Allele-specfic primers (Ex6 SNP)	CCC ATA CTT CAA AGGAAC ATA	58	292 for A 292 and 202 for T (Fitzgerald *et al*, 2011)
	GGT TGG AAG CAT CAC GAG TT	64	
	TCT TCA GGT AGC TCG CCA GT	64	

Results and discussion

Appear amylose content of eleven Thai rice varieties

Appear amylose content (AAC) of eleven Thai rice varieties range from 18 to 22.92 % (Table 2). AAC value of eleven Thai rice varieties is not identical to previous reported by Thai Rice Department and Rice Science Center. The variation of AAC value might be affected from environment as previously reported by Chen *et al* (2008a).

Single Nucleotide Polymorphism of Wx gene of eleven Thai rice varieties

The G/T polymorphism in intron 1 of *Wx* gene was investigated by PCR amplification using Waxy-F and Waxy-R primers (Table 1). Then, the 250 bp of PCR products were digested with *Acc*I. Result showed that all of PCR products amplified from those eleven varieties did not digest with *Acc*I indicating that those eleven varieties harboring thymine (T) at putative leader intron1 (position 144) of *Wx* gene (Table 2).

The A/C polymorphism in exon 6 of *Wx* gene was analyzed by allele-specific primers (Table 1) PCR method. Result showed that two PCR products of 200 and 292 bp were obtained from genomic DNA of those verities indicating adenine (A) at exon6 (position 2389) of *Wx* gene (Table 2). It has been reported that appear amylose content and SNP of *Wx* gene indicated eating and cooking quality of rice (Chen *et al*, 2008b). The authors demonstrated that *Wx* gene haplotype associated with pasting properties. AAC and SNP of *Wx* gene has also been used to predicted in vitro glycemic index (GI, Fitgerald *et al* 2011).

Table 2: Appear amylose content and single nucleotide polymorphism of *Wx* of eleven Thai rice varieties

Varieties	AAC-Type	AAC content (%)	SNP-In1 (G/T)	SNP-Ex6 (A/C)
Homnin Surin	Low	19.39	T	A
Khao yeepun	Low	21.24	T	A
Maligomen	Low	19.65	T	A
Pakaaumpuel	Low	20.75	T	A
Sinlek	Low	21.09	T	A
Srinil	Low	19.23	T	A
Nuikhuea	Low	20.11	T	A
Malinin Surin	Low	21.06	T	A
Riceberry	Low	20.92	T	A
Homnin	Low	22.92	T	A
Homcholasit L	Low	18	T	A

Conclusion

This study presents appear amylose content in related to SNP of *Wx* of eleven Thai rice varieties. The data can be used to predict eating and cooking quality of those varieties.

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