Genetic diversity analysis of starch synthesis related genes (SSRGs) in rice varieties from Thailand, Laos and Yunnan province of China

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ABSTRACT

Genetic diversity analysis of 26 rice varieties; 7 from Thailand, 14 from Laos and 5 from Yunnan, China, was conducted using DNA markers specific to starch synthesis related genes (SSRGs). Screening of 31 markers from 17 SSRGs showed 16 polymorphic makers (51.62%). All polymorphic markers contain 2 alleles, which fragment size ranges from 101-700 bp. The dendrogram was constructed based on Jaccard's similarity coefficient using the marker data for all the rice genotypes following unweighted pair group method analysis. Examined rice varieties were divided into two major groups with a similarity coefficient of 0.68 correlated to their geographic distributions. The genetic variation of rice varieties between different countries was higher than genetic variation of rice varieties within country. Rice varieties from Laos seem to have close relationship with rice varieties from Yunnan, China. Our results showed that the SSRGs can be used as DNA makers to evaluate the genetic diversity in rice and the data generated from this study could be used in the parental selection in rice breeding program.

Keywords: rice; starch; starch synthesis related genes (SSRGs); genetic variation; DNA marker

INTRODUCTION

China and Thailand are major rice growing countries and rice exporters. The production of rice between the two countries can feed more than billion people each year (FAO, 2013). With the rapid increase of world population, the demand for rice is increasing (Sina & Artachinda, 2014) . Genetic diversity is an important source for germplasm utilization. Landrace rice cultivars contain unique characteristic, favoring to specific environment. Yunnan has the richest rice diversity in China, especially southwest area bordered to Myanmar and Laos (Zeng *et al*., 2007). Quality of rice is very crucial for the commercial aspect. The quality of the rice grain is a major characteristic that determines price and demand in the market. Starch is the major component of rice grain; the content and fine structure of its two constituents, amylose and amylopectin, determine rice eating and cooking quality. Biochemically, four classes of enzymes are involved in starch biosynthesis namely, ADP-Glucose pyrophosphorylase (AGPase) , starch synthase (SS) , starch branching enzymes (BEs) , and starch de-branching enzymes (DBEs) (Nakamura, 2002; James *et al.* , 2003; Hannah & James, 2008) . AGPase converts ADP-glucose to glucose-1-phosphate in rice kernel and provides substrate for starch synthase. Granule bound starch synthase-I enzyme (GBSS1) is a primary enzyme responsible for amylose production, while other SS, BEs and DBEs work together, but with distinct roles to synthesize amylopectin. Another important gene determining the quality of the starch is *Waxy* gene, *Wx*. It controls amylose content in rice grain endosperm (Tian *et al.*, 2010; Wanchana *et al.*, 2003). The 23-bp insertion in an allele of the *Wx* gene causes the lack of function that resulting in low amylose content of glutinous rice. When amplified with Wx M1 primer, PCR products of gDNA from non-glutinous and glutinous rice showed 252 and 275 bp

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bands, respectively (Tian *et al.,* 2013). Previously, starch synthesis related genes (SSRGs) were used as markers to assess rice genetic diversity (Liang *et al*., 2020; Young-Jun Mo *et al*., 2014). For example, SSRG markers were used to evaluate the genetic diversity of 187 Korean rice varieties. Ten SSRG markers showed polymorphisms and revealed the subspecies-specific allele distributions (Young-Jun Mo *et al*., 2014).

In this study, DNA markers specific to SSRGs, insertion and deletion DNA markers (InDel), were used to determine the genetic variation of 26 rice varieties: 7 rice varieties from Thailand, 14 rice varieties from Laos and 5 rice varieties from Yunnan, China. The genetic variation of InDel marker exists by the deletion or duplication of DNA fragment and it can be detected by the hybridization with specific DNA probe or the PCR technique using specific primer franking the deletion/duplication region. From 31 SSRGs markers screen, 16 markers showed polymorphism. The dendrogram from SSRG makers cloud divided the examined rice varieties into two groups: Group A with all varieties from Thailand and some varieties from China and Group B with varieties mainly from Laos and some varieties from China. The genetic variation of rice varieties between different countries was higher than within-country counterpart. Rice varieties from Laos seem to have close relationship with rice varieties from Yunnan, China. Our results showed that the SSRGs can be used as DNA makers to evaluate the genetic diversity in rice and the data generated from this study could be used in the parental selection in rice breeding program.

MATERIALS AND METHODS

Plant materials and DNA extraction

A total of 26 rice varieties from 3 countries: 7 varieties from Thailand (Mali Gomain 1, Mali Gomain 2, Mali NilSurin, Mali 105, RD6, RD8 and Chainat 1), 14 varieties from Laos (VTS-165-5, VTS-250-1, VTS-250-2, VTS-250-3, VTS-250-4, VTS-483-26, VTN-282, VTN-289-2, VTN-291, VTN-298-1, VTN-298-2, VTN-310, VTN-313-1 and VTN-315) and 5 varieties from Yunnan, China (Chujing, Duantun 502, Funingnuo, Linxian 31 and Xiguxiangnuo) were used. Genomic DNA was extracted from young leaves using a MATAB method (Agrawal *et al*., 1992) The quality of the extracted DNA was assessed by electrophoresis on 0.8% agarose gel run in 1X TAE buffer at 200 V for 20 min and DNA concentration was measured by Nano Drop spectrophotometer.

DNA markers screen for SSRGs

DNA amplification was performed using rice genomic DNA as template with specific primers for SSRGs. Previously developed 31 SSRG markers by Tian *et al*. (2010) comprising of 1 marker for *AGPlar* gene, 1 marker for *AGPiso* gene, 2 markers for *AGPsma* gene, 1 marker for *Wx* gene, 1 marker for *GBssII* gene, 1 marker for *SSI* gene, 3 markers for *SSII-1* gene, 3 markers for *SSII-2* gene, 1 marker for *SSII-3* gene, 1 marker for *SSIII-1* gene, 1 marker for *SSIII-2* gene, 1 marker for *SSIV-2* gene, 3 markers for *SBE1* gene, 2 markers for *SBE3* gene, 2 marker for *SBE4* gene, 1 marker for *ISA* gene and 6 markers for *PUL* gene, were used (Table 1). Each PCR amplification reaction (20 µl) contained: 20 ng of template DNA, 50 pmol of each primer, 0.25 mmol/L of each dNTP, 1xGCI buffer, and 1 U Taq DNA polymerase (Vivantis, Selangor, Malaysia). The program for the PCR amplification of DNA fragment of makers is one denaturing cycle (94°C, 4 min), 45 amplification cycles (94°C, 4 min; 58°C, 1 min, 72°C, 1 min) and a final extension at 72°C, 10 min. The DNA polymorphism was analyzed by gel electrophoresis.

Data analysis

Starch synthesis related genes (SSRGs) primers are codominant markers. The SSRG marker scores were used to create a data matrix to analyze genetic relationships using the NTSYS-pc program version 2.11a (Rohlf 1997). The dendrogram was constructed based on Jaccard's similarity coefficient using the marker data for all the rice genotypes following unweighted pair group method analysis (UPGMA). PIC score was calculated using PICcalc program(http://w3.georgikon.hu/pic/english/default.aspx). Principal component Analysis (PCA) and cluster analysis was performed using subroutine EIGEN, NTSYSpc 2.02i (Rohlf 1997).

Table 1 DNA polymorphism of 31 SSRG markers with 26 rice varieties from Thailand, Laos and China.

Markers	Polymorphism	Allele number	Size of bp	PIC
AGPlar M1	Mono	1	111	0
AGPiso M2	Mono	1	98	0
AGPsma M1	Poly	2	178 (A), 207(B)	0.47
AGPsma M2	Poly	2	150 (A), 200(B)	0.45
Wx M1	Poly	2	252 (A), 275(B)	0.26
GBSSII M1	Poly	2	256 (A), 280(B)	0.47
SSI M2	Mono	1	76	0
SSII M1	Poly	2	515(A), 596(B)	0.39
SSII-1 M3	Mono	1	150	0
SSII-1 M4	No clear band	-	-	-
SSII-2 M1	Poly	2	150(A), 167(B)	0.07
SSII-2 M2	No clear band	-	-	-
SSII-2 M3	Mono	1	250	0
SSII-3 M1	Mono	1	90	0
SSIII-1 M1	Poly	2	101(A), 113(B)	0.26
SSIII-2 M2	Mono	1	100	0
SSIV-2 M2	Mono	1	208	0
SBE 1 M1	Poly	2	290(A), 625(B)	0.45
SBE 1 M2	Mono	1	350	0
SBE 1 M3	Poly	2	300(A), 700(B)	0.43
SBE 3 M1	Poly	2	210(A), 238(B)	0.26
SBE 3 M2	No clear band	-	-	-
SBE 4 M1	Poly	2	233(A), 255(B)	0.47
SBE 4 M2	No clear band	-	-	-
ISA 1 M1	Poly	2	126(A), 136(B)	0.45
PUL M1	Poly	2	112(A), 128(B)	0.39
PUL M2	Mono	1	158	0
PUL M3	Poly	2	321(A), 373(B)	0.43
PUL M4	Mono	1	250	0
PUL M5	Poly	2	200(A), 300(B)	0.45
PUL M6	Poly	2	200(A), 250(B)	0.39

Results

From the screening of 26 rice varieties from Thailand, Laos and China with 31 SSRG markers, there were 11 monomorphic markers (35.48%), 16 polymorphic makers (51.62%) and 4 unamplified markers (12.90%) (Table 1 and Figure 1). All 16 polymorphic markers have 2 alleles, which the fragment size ranges from 101-700 bp (Table 1 and 2). Marker AGPsma M2 of *AGPsma* gene gave the private allele for Linxian 31 rice variety from China. The PIC score of polymorphic markers ranges from 0.07 (SSII-2 M1) to 0.47 (AGPsma M1, GBSSII M1 and SBE 4 M1). UPGMA was used to construct the dendrogram illustrating the genetic diversity (Figure 2). The resulted tree divided the examined rice varieties into two groups at the Jaccard's similarity coefficient = 0.68. Group A comprised of 14 varieties; one variety from Laos (VTS-250-3) was clearly delimited as a distant landrace from others. The other 13 varieties in Group A can be separated into four clusters using the Jaccard's similarity coefficient = 0.88; cluster I with 6 varieties of close geographic distributions from Thailand and Laos (Mali Gomen 1, Mali Nilsulin, Mali Gomen 2, Chainat1, RD8 and VTS-250-2), cluster II with two varieties from Thailand (Mali105 and RD6), cluster III with 5 varieties from Laos and China (VTS-165-5, VTS-250-1, Duantun 502, Linxian 31 and Xiguxiangnuo) and

cluster IV with 1 variety from Laos (VTS-250-3). Group B consisted of 12 rice varieties, which two rice varieties from Dehong in Yunnan, China (Chujing and Funingnuo) and 10 rice varieties from Laos. Rice varieties in this group can be separated into four clusters: cluster V with one variety from China (Chujing 27), cluster VI with 4 varieties from Laos (VTN-289-1, VTN-291, VTN-298-1, VTN-298-2 and VTN-310), cluster VII with 5 varieties from Laos (VTN-250-4, VTN-282, VTN-313-1, VTN-315 and VTN-483-2) and cluster VIII with one variety from China (Funingnuo). Phylogenetic analysis and principal component analysis were consistent with each other, which revealed the existence of two clear groups. All rice varieties from Thailand were grouped together in Group A and the majority of rice varieties from Laos were grouped together with 2 rice varieties from China in Group B (Figure 2 and Figure 3).

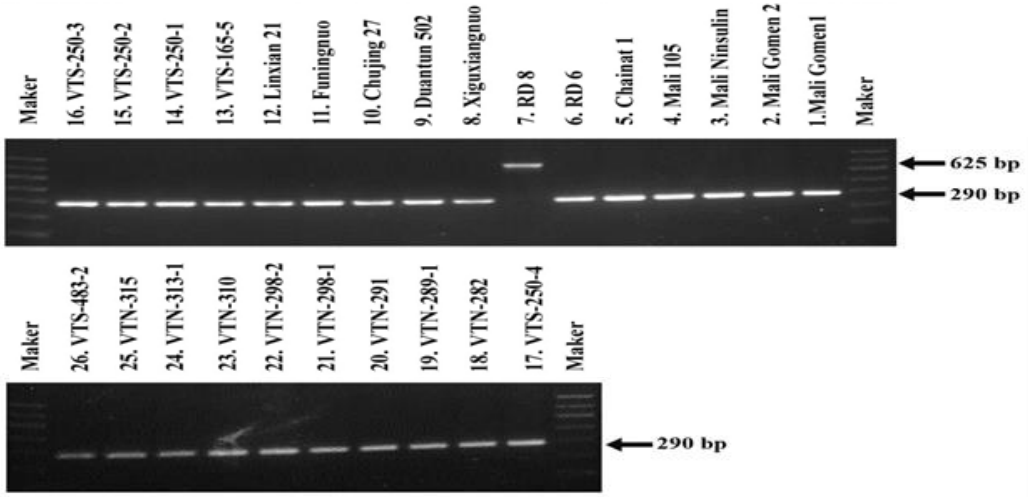

Figure 1 Ethidium bromide-stained agarose gel showing results of *SBE1* PCR product from 26 rice varieties genomic DNA amplified with SBE1 M1 primer.

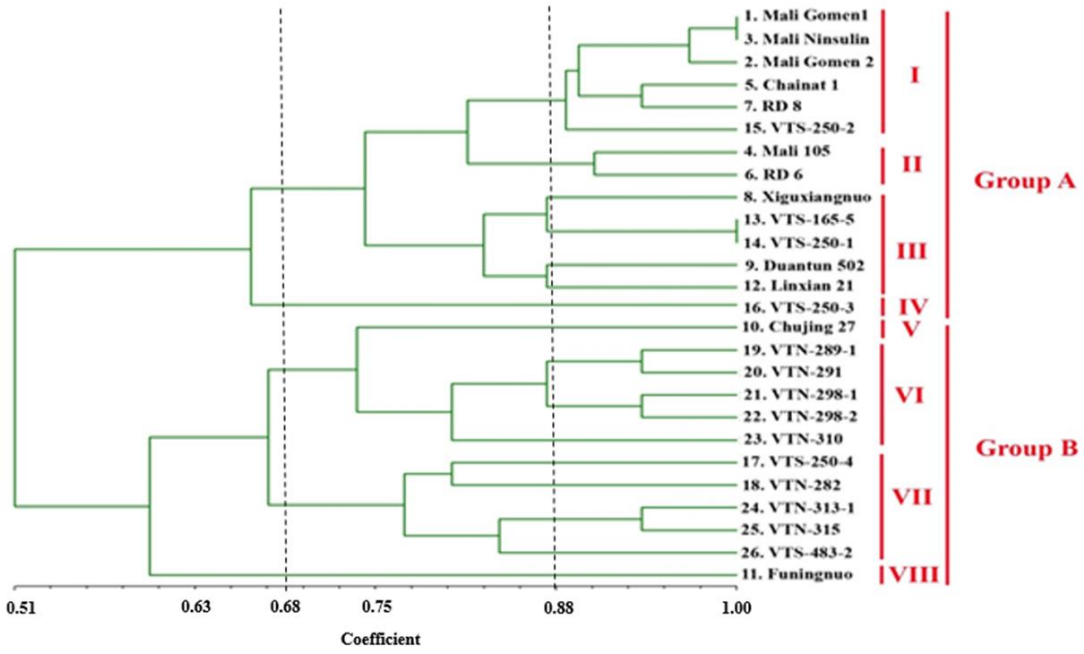

Figure 2 Dendrogram showing two major groups from (NTSYS-PC) of 26 rice genotypes using genetic diversity data from 16 polymorphic markers.

Table 2 DNA polymorphism of 16 polymorphic SSRG markers with 26 rice varieties from China, Laos and Thailand.

Rice variety	Polymorphic SSRG markers															
	AGPsma M1	AGPsma M2	Wx M1	GBSSII M1	SSII M1	SSII-2 M1	SSIII-1 M1	SBE 1 M1	SBE 1 M3	SBE 3 M1	SBE 4 M1	ISA 1 M1	PUL M1	PUL M3	PUL M5	PUL M6
THAILAND:																
Mali Gomain 1	B	B	A	A	A	A	A	A	A	A	A	A	A	A	A	AB
Mali Gomain 2	B	B	A	A	-	A	A	A	A	A	AB	A	A	A	-	AB
Mali NilSurin	B	B	A	A	A	A	A	A	A	A	AB	A	A	A	A	AB
Mali 105	B	B	A	A	A	A	A	A	A	A	A	A	A	A	A	A
RD6	A	B	B	A	A	A	A	A	A	A	A	A	A	A	A	A
RD8	-	B	B	A	A	A	AB	A	AB	A	A	A	A	A	A	A
Chainat 1	A	B	A	A	A	A	A	A	AB	A	A	A	A	A	A	AB
CHAINA:																
Chujing	-	B	B	A	A	A	AB	A	A	A	A	A	A	AB	AB	B
Duantun 502	-	B	-	A	A	A	A	A	A	A	A	A	A	A	A	-
Funingnuo	A	B	-	B	-	A	A	B	A	B	A	A	B	B	B	B
Linxian 31	A	A	B	B	A	A	A	A	A	B	A	A	B	B	B	B
Xiguxiangnuo	-	B	A	B	A	A	A	A	AB	A	A	A	B	A	A	-
LAOS:																
VTS-165-5	-	B	B	A	A	A	AB	A	AB	A	A	A	A	A	A	-
VTS-250-1	-	B	B	A	A	AB	AB	A	A	A	A	A	A	A	A	-
VTS-250-2	-	B	B	A	A	AB	AB	A	A	A	A	A	A	A	A	A
VTS-250-3	A	B	B	B	B	AB	AB	A	A	A	A	B	A	AB	AB	B
VTS-250-4	-	B	A	A	A	AB	A	A	A	A	B	A	A	A	A	AB
VTS-483-26	A	B	B	B	B	A	A	A	A	B	A	B	A	A	A	AB
VTN-282	A	B	B	B	B	A	A	A	A	A	B	B	A	AB	AB	B
VTN-289-2	A	B	B	B	B	A	A	A	A	A	A	B	A	AB	AB	-
VTN-291	A	B	-	B	B	A	A	A	A	B	A	B	A	AB	AB	-
VTN-298-1	A	B	-	B	B	A	A	A	A	B	A	B	A	B	B	-
VTN-298-2	A	B	B	B	B	A	AB	A	A	A	A	B	A	B	B	-
VTN-310	A	B	-	B	B	A	A	A	A	AB	A	B	A	A	A	AB
VTN-313-1	A	B	-	B	B	A	A	A	A	A	A	B	A	A	AB	-
VTN-315	A	B	-	A	A	B	B	B	A	A	B	A	A	A	A	AB

(A and B are different alleles, refers to the size of DNA band in Table 1) and '-' refers to no DNA band.

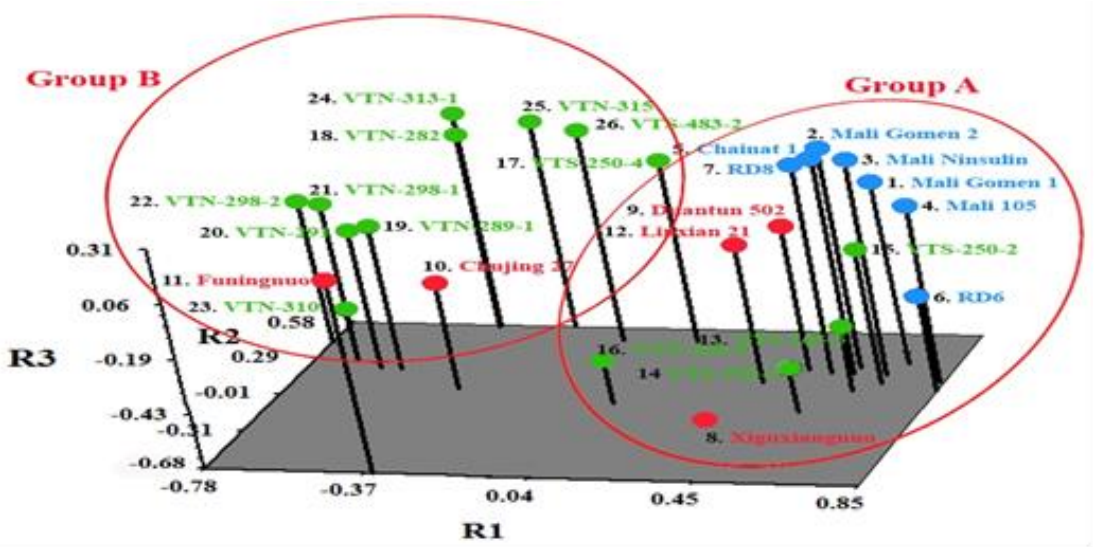

Figure 3 Principal Component Analysis of 26 rice varieties using genetic diversity data from 16 polymorphic markers of starch synthesis related genes. Group (A) is associated varieties of rice (Thailand and China) and Group (B) is associated varieties of rice from Laos (Blue color is rice varieties from Thailand, red color is rice varieties from China and green color is rice varieties from Laos).

DISCUSSION

A previous study from Yan *et al.*, (2016) employed 17 starch synthesis related genes (SSRB genes) to screen landrace rice varieties in Taihu basin, China and the results showed that the SSRB genes can be used to examine the genetic variation in rice germplasm. In this study, 31 starch synthesis related gene markers were used to examine with 26 rice varieties from Thailand, Laos and China. The results showed 16 polymorphic makers. These results indicated that there was genetic variation between 26 examined rice varieties. The dendrogram of 26 rice varieties base on 16 SSRGs DNA markers was correlated with the rice varieties geographic distribution. It should be noted that rice varieties from Thailand cluster together with rice varieties from China but separated from rice varieties from Laos. Our results were similar to the results from Angkhana *et al.*, (2016), which used SSR and InDel markers to show that rice varieties in southern of China and northern of Thailand were genetically related to each other. Fourteen rice varieties from Vientiane, Laos were separated by DNA markers into two groups, of which correlated to the northern and southern regions of Vientiane. From the results, rice varieties from Thailand were grouped together according to their pedigree including Mali Gomen 1, Mali Nilsulin and Mali Gomen 2 were placed in the same group, Chainat1 and RD8 were grouped together and Mali 105 and RD6 were grouped together. This result is consistent with the report from Moonsap *et al.* (2019), which used InDel markers to examine the genetic diversity of Thai elite rice varieties.

CONCLUSIONS

DNA markers specific to starch synthesis related genes (SSRGs) can be used as genetic marker and help to reveal the genetic variation and diversity of rice in Thailand, Laos and Yunnan province of China. The information generated from this study can be applied in the rice breeding and conservation program.

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