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Identification of QTLs for resistant starch and total alkaloid content in brown and polished rice

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ABSTRACT. An F_3 population consisting of 117 $F_{2:3}$ families derived from a cross between two varieties of rice, Gongmi No. 3 and Diantun 502, with a large difference in their resistant starch and total alkaloid content, was used for quantitative trait locus (QTL) mapping. Two QTLs of resistant starch for rice (*qRS7-1*, *qRS7-2*) were identified in a linkage group on chromosome 7, which could explain phenotypic variance from 7.6 to 17.3%, due to additive effects for resistant starch from Gongmi No. 3 or over-dominance effects for *qRS7-2* of the

marker interval (RM3404-RM478) on chromosome 7 from Gongmi No. 3, accounting for 13.8-17.3% of the phenotypic variance. Two QTLs of total alkaloids for brown rice (*qALb7-1*, *qALb7-2*) were identified in the same linkage group, which could explain phenotypic variance from 7.7 and 19.3%, respectively, due to dominance or over-dominance effects for total alkaloids on chromosome 7 from Diantun 502. To our knowledge, these are the first QTLs to be identified, which are related to resistant starch and total alkaloid content in rice. These results are beneficial for understanding the genetic basis of, as well as for developing markers linked with, resistant starch and total alkaloids of functional components for marker-assisted selection breeding in rice.

Key words: Resistant starch; Total alkaloids; Quantitative trait locus; Brown and polished rice

INTRODUCTION

Non-communicable diseases (NCD) have been established as a clear threat to human health, social development, and economic growth. It is estimated that NCD-associated costs will be over US$47 trillion in the next two decades, representing 75% of the global gross domestic product in 2010 (Bloom et al., 2011). Diabetes, affecting 366 million people worldwide, caused approximately US$465 billion in global healthcare expenditures in 2011, and it is estimated that 552 million people (129.7 million in China and 101.2 million in India) will have diabetes by 2030 (Kozak et al., 2012). Diabetes affected 11.6% (113.9 million) and prediabetes afflicted 50.1% (493.4 million) of Chinese adults in 2010 (Xu et al., 2013). In 2011, 1.5 billion people were diagnosed with hypertension globally, accounting for 21.4% of the world population. In China, 275 million people suffer from hypertension, which accounts for 18.3% of world's hypertensive population (Zeng et al., 2011). Major NCD risk factors include unhealthy diet, physical inactivity, tobacco use, and harmful alcohol use (WHO, 2011). Unhealthy diets typically include processed foods high in refined starch, sugar, salt, and unhealthy fats.

Rice is one of the most important cereals for human nutrition (Huang et al., 2012) and constitutes, on an average, about 21% of human nutrient intake and energy requirement. Resistant starch (RS) is starch that resists or escapes digestion in the small intestine, thus passing through to the large intestine where it is fermented. However, the lower activity of ADP-glucose pyrophosphorylase and starch-branching enzyme, and higher activity of starch synthase and starch de-branching enzyme observed in high-RS rice might be responsible for the formation of small, irregular starch granules with large spaces (Shu et al., 2014). Higher consumption of white rice is associated with a significantly increased risk of type II diabetes, especially in Asian populations (Hu et al., 2012), but rice with high RS could be the key for diabetes prevention in Asia, especially in China. According to Atkinson et al., (2008), the mean glycemic index values of rice are 92 for glutinous rice, 87 for standard milled rice, 55 for brown rice, 35 for high-RS rice, and 19 for rice bran. Foods high in RS are beneficial for preventing various diseases including diabetes, colon cancer, and chronic renal or hepatic diseases. Elevated RS in rice is important for public health since rice is a staple food for half of the world's population (Yang et al., 2012). Treatment with gamma irradiation has a potential

for increasing resistant starch content and reducing the digestibility of starch in common rice (Shu et al., 2013). Total alkaloids are derived from the products of amino acids, such as Phe, Tyr, Trp, Orn, and Lys (Bunsupa et al., 2012), and 12,000 alkaloids in plants play multiple roles in plant growth and human health. Kang et al. (2010) suggested that oryzadine (a new alkaloid of *Oryza sativa* cv. Heugjinjubyeo) protected cells against H_2O_2-induced cell damage via reactive oxygen species scavenging effect. Two alkaloids (4-carboethoxy-6-methoxy-2-quinolone and 4-carboethoxy-6-hydroxy-2-quinolone) were isolated from the ethyl acetate-soluble fraction of the *Oryza sativa* cv. Heugnambyeo bran, and were determined to exert significant inhibitory effects in macrophage cell line and murine splenocytes (Ryu and Chung, 2010).

Yunnan Province in China is not only the place where the origin of human evolution is closely related to the origin of rice evolution (Zeng et al., 2012a, 2013), but is also the geographic region from where most of the natural functional rice is sourced. Interestingly, Yunnan Province has the lowest mortality of cancers (0.541‰) in the world (Zeng et al., 2015). Functional rice is a type of rice providing superior nutrition; compared with common rice, it is not only rich in protein, essential amino acids, minerals and other nutrient components, but also contains resistant starch, dietary fiber, unsaturated fatty acids, flavonoids, sterols, alkaloids, and other physiologically active substances. Common rice has a low content of resistant starch (<1%) and high glycemic index. It is a matter of particular interest to obtain rice with higher levels of resistant starch and lower glycemic index. Kharabian-Masouleh et al. (2012) found that the gene encoding granule-bound starch synthase (Waxy gene) influenced amylose content and retrogradation in rice, but other genes contributing to retrogradation were glucose-6-phosphate translocator 1, soluble starch synthase I, branching enzyme I and starch synthase IIIa. Wild rice has been found to be effective in improving abnormal glucose metabolism and insulin resistance in rats (Han et al., 2013).

Quantitative trait locus (QTL) mapping has been applied for rice improvement; moreover, some QTLs that control agronomic traits have been successfully cloned. Yet, information about RS-related genes in rice is very limited; we found two reports about RS QTLs (Mou et al., 2008; Yang et al., 2012), and none about total alkaloid-related genes. Gong et al. (2013) performed a genetic analysis of the rice metabolome that provided over 2800 highly resolved metabolic QTLs for 900 metabolites. The *sbe3-rs* was fine-mapped to a 573.3-kb region between *InDel 2* and *InDel 6* on chromosome 2, which explained 60.4% of RS variation (Yang et al., 2012). In this report, we provide evidence of a relationship between rice simple sequence repeat (SSR) markers and high RS as well as total alkaloids, which will be useful for elucidating the mechanism of functional components and utilizing favorable genes from Gongmi No. 3 for functional rice breeding.

MATERIAL AND METHODS

Plant materials

Gongmi No. 3 is one of several cultivars sold under the brand known in China as Shitangmi of Yungong. Shitangmi means reducing postprandial sugar and tolerance starvation rice for major food of diabetes in Chinese. Gongmi No. 3 was identified from among *boro* accessions from 41 farmers of two villages in Xinping county in China, an early-maturing *indica* cultivar suitable for planting in winter (Zeng et al., 2013). Gongmi No. 3 contains 10-13% retrograded RS in dry cooked rice, 55 times that in an *indica* cultivar of aromatic soft

rice 'Diantun 502' (only 0.2% RS) (Zeng et al., 2013). When rice is cooked and then cooled, resistant starch tends to harden and become indigestible in the small intestine. This process of hardening of starch is called retrogradation. The resistant starch then passes through to the large intestine where it is fermented.

In 2007, Gongmi No. 3 was crossed with Diantun 502 in the experimental farm of Yunnan Academy of Agricultural Sciences (YAAS), and the resultant F_1 was grown in Xinping winter nursery. In 2009 and 2010, 117 F_2 individuals, 117 F_3 families, and the two parents were transplanted in a 20 x 10-cm grid pattern into flooded paddies at Yanhe town (altitude 1638 m) of Yuxi prefecture in Yunnan Province. Brown rice and polished rice for F_3 individuals (one random plant per F_3 families) and the two parents were harvested for detecting RS and total alkaloid content. DNA from fresh leaf tissue of 117 F_3 families (one plant per F_3 families) and the two parents was harvested for identifying QTLs of RS and total alkaloids via linkage mapping. There was exactly one plant per family for detecting components of one plant for QTLs.

Measurement of resistant starch and total alkaloids

Brown rice was de-hulled in a rice dehuller. Polished rice was processed in a rice polisher. Both were then ground in a rice mill (China), passed through a 100-mesh sieve and stored in a dryer until use. The total alkaloid content in rice was determined using a previously described acid dye colorimetry method (Li, 2007). In 10-mL centrifuge tubes, 0.5 g rice powder was added to 5 mL 95% ethanol, using diluted hydrochloric acid to adjust pH value to 3.0. The tubes were subjected to a vibrator (200 times/min) for 4 h in a water bath (50°C). After cooling to room temperature, they were centrifuged at 10,000 rpm for 6 min. Taking out 2 mL supernatant in 50-mL centrifugal tubes, ammonia water was used to adjust pH to neutral. After adding 2 mL bromocresol green phosphate buffer solution and 10 mL chloroform, and shaking for 1 min, the mixture was poured into a separating funnel and allowed to stand for 1 h. The absorbance value of 5 mL of the chloroform layer was determined at 416 nm wavelength, using chloroform as the blank. The alkaloid content in rice was calculated using the standard curve of standard solutions (1, 2, 3, 4 and 5 mg/100 g) based on berberine chloride from Guizhou Dida Science & Technology Limited Company (Guiyang, Guizhou Province, China). The standard curve had the linear relationship: $y = 0.1366x + 0.0255$, $R^2 = 0.9993$. All analyses were repeated three times for error control.

RS content in rice was measured according to Goñi method (Goñi et al., 1996), which has been widely used for RS determination in crops (Yang et al., 2012; Shu et al., 2013). According to this method, 0.1 g rice powder was taken in 10-mL centrifuge tubes each, 4 mL (3000 U/mL) reaction liquid of pancreatic alpha-amylase was added to each tube, and the tubes were placed in a vibrator (200 times/min) in a water bath at 37°C for 16 h. Then, 4 mL 95% ethanol was added and, after vortex mixing, the tubes were subjected to centrifugation at 5000 rpm for 10 min, and the supernatant discarded. After adding 2 mL ethanol (50%) to the sedimentation and then 6 mL ethanol (50%), vortex mixing and then centrifuging at 5000 rpm for 10 min, the supernatant was discarded. Next was addition of 2 mL 2 MKOH solution and oscillation in an ice bath for 20 min. Then, 6 mL 1.2 M sodium acetate buffer solution, pH 3.8, was added and centrifugation at 5000 rpm for 10 min was carried out and the supernatant discarded. In each tube, 4 mL sodium acetate buffer solution, pH 3.8, and 1 mL glucoamylase (3000 U/mL) solution was added to the sediment, vortex mixed, and put on a vibrator (200

time/min) in a water bath at 50°C for 50 min, followed by centrifugation at 3000 rpm for 2 min. By using glucose reagent box (GOD-PAP method; Jilin Huili Biotechnology Limited Company (Changchun, Jilin Province, China), the absorbance value of the supernatant was measured at 505 nm wavelength. The glucose content (GS) in the sample was also measured and then the RS content determined according to the formula (RS = GS x 0.9). All analyses were repeated three times for error control. Pearson's correlation analysis was performed using the SPSS 16.0 software package. Correlation analysis between RS and total alkaloids in brown and polished rice among 117 lines of F_3 populations from Gongmi No. 3 x Diantun 502 was carried out.

DNA extraction, SSR primers selection, and polymerase chain reaction (PCR)

Total genomic DNA was extracted from fresh leaves of each plant using the CTAB method (Rogers and Bendich, 1988), with minor modifications. The volume of PCR mixture was 20 mL, containing 20 µL PCR system (3 µL genomic DNA of 25 ng/µL, 3 µL 10X PCR buffer, 1 µL forward and reverse primer each, 0.5 µL dNTPs, 0.5 µL Taq polymerase of 2 U/µL, 11 µL ddH_2O). The PCR products were separated on 8% polyacrylamide gel by electrophoresis for 2 h in 180 V. Bands were detected using a silver-staining method (Varshney et al., 2007). A total of 500 SSR markers distributed on 12 chromosomes were selected from Gramene (http://www.gramene.org/markers/) to determine polymorphism between the parents Gongmi No. 3 and Diantun 502. In 117 F_3 families, using one random plant per family, high bulked segregant analysis (BSA) was performed for DNA mix of five plants for determining the highest RS contents in brown rice and polished rice, and low BSA was carried out for DNA mix of five plants for determining the lowest RS contents in brown rice and polished rice. The high-RS bulks was similar to Gongmi No. 3 but were different from those of low-RS bulks for Diantun 502; it was assumed that the SSR marker was probably linked to a high-RS gene based on BSA. Mark 1 was for the PCR products that had the same band size as of Gongmi No. 3, and mark 2 was for those that had the same band size as of Diantun 502, heterozygote of mark 3, others of mark '-'. Thirteen of 67 SSR markers with polymorphism in two parents are probably linked to a high-RS gene (see Figure 1), and these SSR markers were employed for PCR in 117 F_3 families. One-way ANOVA was used to analyze the correlation between molecular data and RS content. The SPSS for Windows statistical software, version 16.0 (SPSS Inc. Chicago, IL, USA) was used to analyze the phenotypic, molecular data, and one-way ANOVA.

Mapping and QTL analysis

The MAPMAKER/EXP3.0 (Lincoln et al., 1992) software was used for linkage group construction, and the Kosambi function was used to convert recombination values to genetic distances. Composite interval mapping approach in the WinQTL Cartographer V2.5 (Wang et al., 2012) software was used for QTL detection. RS and total alkaloid content in brown and polished rice, respectively, of 117 F_3 families were the phenotypic data for QTL analysis. Each data set was analyzed with 1000 permutations and a 5-cM walking speed. Logarithm of odds was set at 2.0 as all displayed traits' threshold value. The percentage variation explained (general contribution) by each QTL, additive and dominance effects were estimated. Nomenclature of QTL name followed McCouch et al. (1997).

RESULTS

Variation of resistant starch and total alkaloid content

RS in brown rice of Gongmi No. 3 (10.06%) was 28 times that of the other parent Diantun 502 (0.36%), and its RS in polished rice (8.00%) was 53 times than that of Diantun 502 (0.15%). Gongmi No. 3 polished rice had a very opaque endosperm due to increased chalkiness, while polished rice of Diantun 502 was transparent. Among the brown rice samples in F_3 population, 8 individuals were in the low-RS region of 0.6-1.0%, and 8 individuals were in the high-RS region of 6.0-8.0% (Figure 1A).

Remarkably, the lowest RS (0.59%) of F_3 population brown rice is higher than that of Diantun 502 (0.36%), while the highest RS (7.82%) is lower than that of Gongmi No. 3 (10.8%) (Table 1). With respect to polished rice in the F_3 population, 6 individuals were in the low-RS region of 0.5-1.0%, and 1 individual was in the high-RS region of 6.0-8.0% (Figure 1B). Correspondingly, the lowest RS (0.48%) of the F_3 population is higher than that of Diantun 502 (0.15%), whereas the highest RS (6.22%) is lower than that of Gongmi No. 3 (8.0%) (Table 1). This distribution indicated that the responsible QTLs were few for RS inheritance.

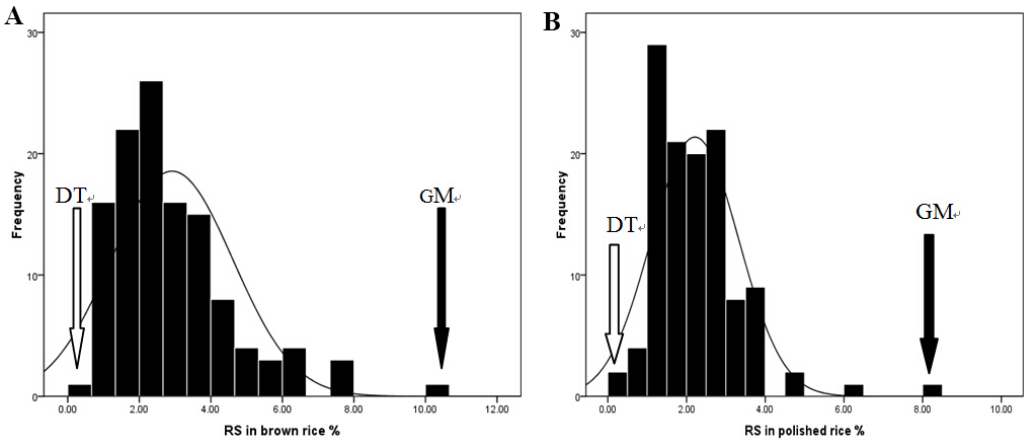

Figure 1. Resistant starch content in brown rice (**A**) and polished (**B**) of F_3 lines for Gongmi No. 3 (GM) x Diantun 502 (DT).

Table 1. Resistant starch (RS) and total alkaloid content in brown and polished rice for two parents and Gongmi No. 3 x Diantun 502 F_3 population.

Treatment		Parents		117 lines of F_3 population					
		GM	DT	Mean	CV %	Range	Skewness	Kurtosis	Variance
RS (%)	Brown	10.06	0.36	2.86	55.24	0.59-7.82	1.10	1.04	2.49
	Polished	8.00	0.15	2.17	45.16	0.48-6.22	0.93	1.59	0.95
Total alkaloids mg/100 g	Brown	43.73	52.84	29.13	20.83	15.22-49.75	0.88	1.58	36.85
	Polished	22.00	25.00	21.78	24.20	10.07-35.47	0.06	-0.16	27.81

GM = Gongmi No. 3, DT = Diantun 502.

Total alkaloids in brown rice of Diantun 502 (52.84 mg/100 g) was 1.2 times than that of Gongmi No. 3 (43.73 mg/100 g), and its total alkaloids in polished rice (25 mg/100 g) was 1.25 times than that of Gongmi No. 3 (20 mg/100 g) (Table 1). In brown rice samples in the F_3 population, four individuals were in the low-total alkaloid region of 15.0-20.0 mg/100 g, and eight individuals were in the high-total alkaloid region of 40.0-50.0 mg/100 g (Table 1). This distribution indicated that total alkaloid content is a quantitative trait controlled by a few main-effect genes.

Correlation analysis is a statistical method that can be used to establish relationships between metabolites belonging to a biological system. The correlations between RS and total alkaloids in 117 F_3 families are as follows (See Table 2): total alkaloids of brown and polished rice (0.447) > RS of brown and polished rice (0.411) > RS and total alkaloids in polished rice (-0.224) > RS in brown rice and total alkaloids in polished rice (-0.216) > RS in polished rice and total alkaloids in brown rice (-0.139) > RS and total alkaloids in brown rice (-0.015).

Table 2. Analysis of correlation among functional component contents in F_3 population.

Coefficient of correlation	RS content of brown rice	RS content of polished rice	Alkaloid content of brown rice
RS content of polished rice	0.411**		
Alkaloid content of brown rice	-0.003	-0.134	
Alkaloid content of polished rice	-0.204*	-0.225*	0.471**

$r_{0.05}$ = 0.182, $r_{0.01}$ = 0.237, N = 117.

QTL mapping and genetic effects on RS content in brown and polished rice

In total, 67 of 500 SSR markers were polymorphic between Gongmi No. 3 and Diantun 502. Using MAPMAKER/EXP.3.0, of the 67 markers were used for linkage map construction thorough QTL-mapping across the whole genome; only one linkage group consisting of 8 markers was constructed on chromosome 7. Based on RS data from 117 F_3 families, four QTLs on chromosome 7 were identified (Figure 2). One minor QTL (*qRS7-1*) in RM7110-RM3211 explained 7.6% of brown rice (*qRSb7-1*) of total variation in this population, and the alleles from Gongmi No. 3 increased RS by 17.3% for additive effect and dominant effect of -0.173; This minor QTL (*qRS7-1*) in RM7110-RM3211 explained 7.7% of polished rice (*qRSp7-1*) of total variation in this population, and the alleles from Gongmi No. 3 increased RS by 6.8% for additive effect and dominant effect of -0.056 (Table 3 and Figure 3). The *qRS7-1* (*qRSb7-1*, *qRSp7-1*) locus was flanked by RM7110 and RM3211 with a genetic distance of 10.8 cM approximately (Figure 2).

The other major QTL (*qRS7-2*) not only explained 13.8% of brown rice (*qRSb7-2*) of total variation in this population, and that the alleles from Gongmi No. 3 increased RS by 4.1% for additive effect and dominant effect of -0.399, but also explained 17.3% of polished rice (*qRSp7-2*) of total variation in this population, and the alleles from Gongmi No. 3 increased RS by 1.4% for additive effect and dominant effect of -0.173 (Table 3 and Figure 3). The *qRS7-2* (*qRSb7-2*, *qRSp7-2*) locus was flanked by RM3404 and RM478 with a genetic distance of 26.3 cM approximately; its effect was relatively great (Table 3).

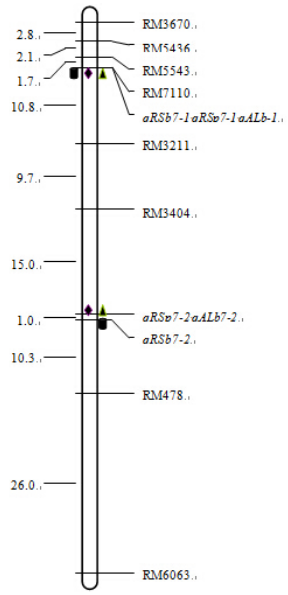

Figure 2. Mapping of QTLs for RS and total alkaloids on chromosome 7.

Table 3. QTLs and genetic effects on RS and alkaloid contents in rice.

QTL		Marker interval[1]	Distance[2] (cM)	LOD	Explained variance (%)	Effective value		Gene action
						Additive[3]	Dominance	
qRS7-1	*qRSb7-1*	RM7110-RM3211	0.01	2.03	7.6	0.173	-0.137	Partial dominance
	qRSp7-1		0.01	2.05	7.7	0.068	-0.056	Dominance
qALb7-1	*qALb7-1*		0.01	2.07	7.7	0.030	-0.025	Dominance
qRS7-2	*qRSb7-2*	RM3404-RM478	10.3	2.19	13.8	0.041	-0.399	Over dominance
	qRSp7-2		11.3	2.51	17.3	0.014	-0.173	Over dominance
qALb7-2	*qALb7-2*		11.3	2.69	19.3	0.005	-0.081	Over dominance

[1]Black marker indicates nearer marker from putative QTL. [2]Distance from nearer marker to putative QTL; [3]+ and - mean that positive alleles come from Gongmi No. 3 and Diantun 502, respectively.

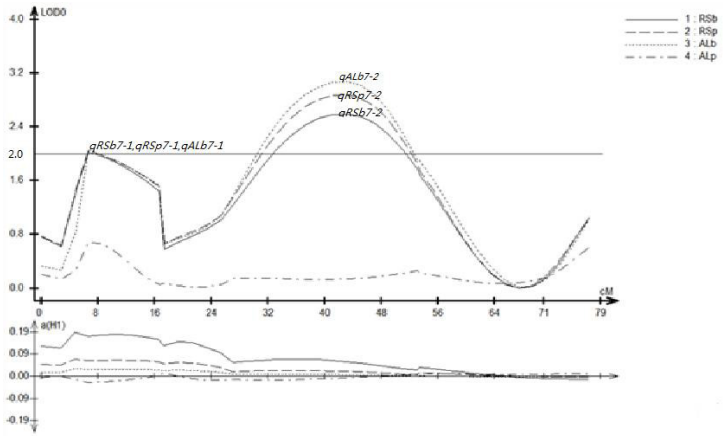

Figure 3. Graph for QTL mapping result.

In addition to the phenotype of resistant starch in rice being an endosperm trait, which is 3n, brown rice and polished rice for 117 F_4 plants from F_3 families (one plant per family) and the two parents were harvested for detecting the content of RS and total alkaloids. There is exactly one equivalent plant per family for detecting components to one plant for QTLs. Since this QTL mapping was accomplished in F_3 plants (using F_4 seed of one F_3 plant), the heterogeneity of the phenotype seed samples were not considered in the gene action interpretation.

QTL mapping and genetic effects on total alkaloids content in brown rice

Based on total alkaloids data from 117 F_3 families, two QTLs for total alkaloids in brown rice (named as *qALb7-1* and *qALb7-2*) on chromosome 7 were identified (Figure 3), according to QTL nomenclature (McCouch et al., 1997).

One minor QTL (*qALb7-1*) explained 7.7% of brown rice of total variation in this population. The allele from Diantun 502 increased total alkaloids by 3% and dominant effect of -0.025; this *qAL7b-1* locus was flanked by RM7110 and RM3211 with a genetic distance of 10.8 cM approximately (Table 3). The other major QTL (*qALb7-2*) explained 19.3% of brown rice of total variation in this population. This allele from Diantun 502 increased total alkaloids by 0.5% and dominant effect of -0.081. The *qALb7-2* locus was flanked by RM3404 and RM478 with a genetic distance of 26.3 cM approximately (Table 3).

DISCUSSION

Resistant starch and total alkaloids in rice

All modifiable factors causing hypertension and diabetes can probably be summed up in one's dietary habits, especially the loss of functional components from brown rice (whole grains) to white rice (wheat flour), especially good rice (wheat flour) (Zeng et al., 2011, 2012b). Total alkaloid content in polished rice was always negatively correlated with RS in brown and polished rice. Although RS in brown and polished rice of Gongmi No. 3 were 28 and 53 times than that of the other parent Diantun 502, respectively, among the 117 F_3 families, the highest RS content in brown and polished rice were 13.2 and 13.0 times than that of the lowest RS content, respectively. Similarly, although total alkaloids in brown and polished rice of Diantun 502 were 1.2 times that of the other parent Gongmi No. 3, respectively, among the117 F_3 families, the highest total alkaloid content in brown and polished rice were 3.3 and 3.5 times than that of the lowest total alkaloid content, respectively. The presence of amylose inhibits starch digestion and the level of crystalline structure of re-crystallized amylopectin affects the RS formation during retrogradation (Zhou et al., 2013). The retrograded starch content of uncooked and cooked polished rice for Gongmi No. 3 are 8.0-8.5 and 10-13%, respectively (Zeng et al., 2013). When retrograded rice was produced by repeated heating and cooling cycles, it contained significantly higher amounts of resistant starch (13.9 ± 0.98%) than is found in common rice (9.1 ± 1.02%) (Ha et al., 2012). In some products, retrogradation can provide a desirable quality such as in the manufacture of rice stick noodles, resistant starch type 3 croutons, and breadcrumbs (Charoenrein and Udomrati, 2013).

Gongmi No. 3 from *boro* groups is the natural variation of rice landrace in Yunnan Province, but using radiation at 0.5 kGy, an *indica* mutant RS4H produces up to 8.07% resistant

starch (Shu et al., 2013). Similarly, using young panicles for 0.015% of N-methylnitrosourea solution technology, a *japonica* mutant 'Jiangtangdao 1' produces up to 11.67% resistant starch (Yang et al., 2012). The differences between natural variation (Gongmi No. 3) and artificial mutagenesis (RS4H, Jiangtangdao 1) are due to the fact that whether *qRS7-1* or *qRS7-2* from Gongmi No. 3 is a quantitative trait controlled by multiple dominance genes, and *sbe3-rs* from Jiangtangdao 1 is a recessive gene for high RS mutated from SBE3 (Yang et al., 2012). Because of its effective control of postprandial blood glucose, diabetes prevention, anti-starvation, blood lipids control, weight control, and prevention of intestinal diseases, etc. (Zeng et al., 2013), Gongmi No. 3 with a high retrograded RS content has been commercially developed. The low productivity along with high market demand has resulted in Gongmi No. 3 being highly priced. Employing SSR marker-assisted breeding techniques should improve the productivity of Gongmi No. 3, and subsequently increase its availability to serve more diabetic patients.

QTLs affecting resistant starch and total alkaloids

Mapping QTLs and development of markers are essential for marker-assisted breeding, especially for RS and total alkaloid content, assessment of which is extremely difficult. One minor QTL was mapped between SSR markers RM7110 and RM3211 on chromosome 7 with a genetic distance of approximately 10.8 cM; it explained 7.6% of brown rice (*qRSb7-1*) and 7.7% of polished rice (*qRSp7-1*) as well as 7.7% of brown rice (*qALb7-1*) of total variation in this population for RS and total alkaloids. The other major QTL was mapped between SSR markers RM3404 and RM478 on chromosome 7 with a genetic distance of approximately 26.3 cM; it explained 19.3% of brown rice (*qRSb7-2*) and 17.3% of polished rice (*qRSp7-2*) as well as 19.3% of brown rice (*qALb7-2*) of total variation in this population for resistant starch and total alkaloids. To our knowledge, these are the first QTLs to be identified controlling resistant starch and total alkaloid content in brown and polished rice. Phenotypic variation of 20.36 and 40.13% from Gongmi No. 3 for the *qRS6-2* and *qRS6-3*, respectively, can be explained (Luo et al., 2014). However, a correlation analysis of resistant starch and total alkaloid content in brown and polished rice based on SSR markers has not been reported. One QTL cluster near the G1149 marker on chromosome 8 includes nine QTLs, which control 9 traits of the quality of cooked rice (Liu et al., 2011). QTLs affecting the secondary metabolites in rice are similar to those affecting the quality of cooked rice. Interestingly, one QTL cluster for three components (*qRSb7-1*, *qRSp7-1*, and *qALb7-1*) near the RM7110 and another QTL cluster for three components (*qRSb7-2*, *qRSp7-2*, and *qALb7-2*) near the RM478 marker on chromosome 7, which control resistant starch of brown rice, resistant starch of polished rice, and total alkaloids of brown rice (see Figures 2 and 3). It would be of greater value for our study to investigate the RS locations on chromosome 7, which would be different from Yang et al. (2012), who mapped RS locus to chromosome 2. This QTL cluster is probably similar to a truncated starch synthase II-3 (*SSII-3*) gene on chromosome 7 (Dian et al., 2005). Gelatinization temperature was controlled by the *ALK* gene, which encodes a putative soluble starch synthase II-3 (Gao et al., 2011). Two QTLs on chromosome 6 and one on chromosome 7 were detected for gel consistency, which accounts for 57% of phenotypic variation explained, meanwhile the position of QTLs for amylose content and gel consistency being close to each other may reflect linkage or pleiotropy (Lanceras et al., 2000). Two QTLs (*qPKV7.1*, *qHPV7.1*, *qCPV7.1*, and *qSBV7.1*) on chromosome 7 with contributions ranging from -31.8 to 53.2% were found

to have pleiotropy (Zhang et al., 2013). Shu et al. (2012), working with barley, found a large variation in RS content and identified a number of SNP markers that could be studied further for elucidation of the pathway and control of RS phenotype. There is significant negative correlation between total alkaloids and RS in polished rice as well as RS in brown rice in the 117 families of F_3 population from cross of Gongmi No. 3 and Diantun 502. Therefore, using the Gongmi No. 3, our future objectives are to map, verify, and elucidate the QTL gene for resistant starch associated with total alkaloids in rice.

Conflicts of interest

The authors declare no conflict of interest.

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