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1 **Association mapping of starch physicochemical properties with starch synthesis**
2 **related gene markers in nonwaxy rice (*Oryza sativa* L.)**

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16

17 **Abstract**

18

19 Starch physicochemical properties strongly influence eating and cooking quality of
20 rice. The CAPS, dCAPS and InDel markers for 13 starch synthesis related genes
21 (SSRGs) were developed, and together with markers developed before, there are 35
22 markers tagged for 23 SSRGs, with each gene tagged with at least one marker. These
23 and 108 other markers were used for association mapping for 20 starch
24 physicochemical property parameters. A total of 64 main-effect loci or QTLs were
25 detected. In addition, 56 and 62 loci were identified under the *Wx* and *SSIIa*
26 background, respectively. *Wx* was a major main-effect QTL for AAC, pasting
27 viscosity, gel texture, and retrogradation property ($P < 0.0001$). *SSIIa* was a major
28 main-effect QTL for pasting temperature, thermal and retrogradation properties ($P <$
29 0.0001), but it was a minor main-effect QTL for some pasting viscosity parameters,
30 such as BD, CS, Stab and SBratio. Four other SSRGs, *SSIIa*, *BE1*, *SSIIc* and *GBSSII*
31 were detected for AAC under *Wx* background. *Wx* was detected for Tc and ΔHg under
32 the *SSIIa* background. *PUL* was detected for HD as main-effect QTL and under *SSIIa*
33 background. *AGPL2* and *ISAI* were detected respectively for ΔHg and retrogradation
34 as main-effect QTL as well as under both *Wx* and *SSIIa* backgrounds. This study
35 suggested that retrogradation properties were mainly controlled by *Wx*, *SSIIa* and
36 *ISAI* with the relative effects in the order of $SSIIa > Wx > ISAI$. These results have
37 direct applications to quality breeding programs.

38 **Keywords:** Rice; eating quality; amylose; gelatinization temperature; RVA pasting
39 viscosity; association mapping; QTL

40

41 **Abbreviations used:**

42 AAC, apparent amylose content; ADH, gel adhesiveness; ASV, alkali spreading value;
43 BD, breakdown viscosity; BE, starch branching enzyme; CAPS, cleaved amplified
44 polymorphic sequences; dCAPS, derived CAPS; COH, gel cohesiveness; CPV, cold
45 paste viscosity; CS, consistency; DBE, debranching enzyme; DSC, differential

46 scanning calorimetry; GBSS, granule-bound starch synthase; GT, gelatinization
47 temperature; HD, gel hardness; HPV, hot paste viscosity; ISA, isoamylase; PT, pasting
48 temperature; PV, peak viscosity; PUL, pullulanase; QTL: quantitative trait locus; R%,
49 retrogradation percentage; RVA, rapid visco analyser; SB, setback viscosity; SBratio,
50 setback ratio. Stab: stability; SS, starch synthase; SSRG, starch synthesis related genes;
51 T_o , onset temperature; T_p , peak temperature; T_c , conclusion temperature; ΔH_g ,
52 enthalpy of gelatinization; ΔH_r , enthalpy of retrogradation; $\Delta T_{1/2}$, width at half peak
53 height.

54 **Introduction**

55

56 Rice serves as a staple food for about half of the world's people. New varieties with
57 high yield, high quality and high resistance to biotic and abiotic stresses are bred and
58 released continuously in order to meet the ever-increasing demand for more food as a
59 consequence of human population growth coupled with the decrease in arable land.
60 Improvement of rice quality is among the most important goals in current breeding
61 programs, especially its eating and cooking quality as most rice is consumed as
62 cooked rice. Starch is the major component of rice grain, the content and fine
63 structure of its two constituents, amylose and amylopectin, determine rice eating and
64 cooking quality. Biochemically, four classes of enzymes are involved in starch
65 biosynthesis, i.e. ADP-Glucose pyrophosphorylase (*AGPase*), starch synthase (*SS*),
66 starch branching enzymes (*BEs*), and starch de-branching enzymes (*DBEs*)
67 (Nakamura, 2002; James et al. 2003; Hannah et al. 2008). *AGPase* converts
68 ADP-glucose to glucose-1-phosphate in rice kernel, provides substrate for starch
69 synthase. Granule bound starch synthase-I enzyme (*GBSSI*) is a primary enzyme
70 responsible for amylose production in rice endosperm while other *SS* (soluble starch
71 synthase), *BEs* and *DBEs* work together but with distinct roles to synthesize
72 amylopectin. Many of these enzymes have multiple isoforms. The *AGPase* consists of
73 four large (*AGPL1-4*) and two small (*AGPS1*, *AGPS2*) subunits (Ohdan et al. 2005;
74 Lee et al. 2007). There are a total of 10 isoforms for starch synthase enzymes: *GBSS*
75 (*I*, *II*), *SSI*, *SSII* (*SSIIa*, *SSIIb*, *SSIIc*), *SSIII* (*SSIIIa* and *SSIIIb*), and *SSIV* (*SSIVa* and
76 *SSIVb*) (Hirose and Terao 2004; Tetlow et al. 2004; Ohdan et al. 2005; Zhang et al.
77 2011). Rice *BE* has three isoforms: *BEI*, *BEII* (*BEIIa*, *BEIIb*) (Nakamura 2002; Ohdan
78 et al. 2005). Two types of *DBE*: isoamylase and pullulanase are both found involved
79 in amylopectin biosynthesis in rice endosperm (Nakamura et al. 1996; Kubo et al.
80 1999; Fujita et al. 2003; Wong et al. 2003; Ohdan et al. 2005).

81

82 Genetic studies indicated that the starch physicochemical properties, such as AAC,
83 gelatinization temperature, gel consistency, RVA pasting viscosity, gel texture, DSC

84 thermal property, and retrogradation, might be controlled by one or a few genes with
85 major effects (He et al. 1999; Bao et al. 2000; Bao et al. 2004a; Wan et al. 2004; Fan
86 et al. 2005). Linkage mapping of the quantitative trait locus (QTL) for AAC and
87 pasting viscosity (Bao et al. 1999; He et al. 1999; Bao et al. 2000, 2003; Larkin et al.
88 2003; Septiningsih et al. 2003; Aluko et al. 2004; Wan et al. 2004; Fan et al. 2005;
89 Wang et al. 2007; Lapitan et al. 2009; Traore et al. 2011) shows that they are largely
90 controlled by the *Wx* locus on chromosome 6. Since *GBSSI* is responsible for amylose
91 synthesis, *GBSSI* alleles correlated with variation in AAC of rice grain is not
92 surprising. Linkage mapping studies have identified only one major QTL, i.e. the
93 alkali degeneration (*alk*) locus on chromosome 6, as having major responsibility for
94 different gelatinization temperatures in diverse rice germplasm (He et al. 1999; Aluko
95 et al. 2004; Bao et al. 2004b; Fan et al. 2005; Tian et al. 2005; Wang et al. 2007;
96 Lapitan et al. 2009). Map-based cloning of the *alk* locus reveals that it encodes *SSIIa*,
97 which is the major gene responsible for GT (Gao et al. 2003). QTL mapping shows
98 that the amylopectin chain length distribution is also controlled by the *SSIIa* locus
99 (Umemoto et al. 2002). The function of *SSIIa* is to elongate the short A and B1 chains
100 with degree of polymerization (DP) < 10 to form long B1 chains of amylopectin
101 (Nakamura et al. 2005). Although the functions of several genes in determining the
102 rice eating and cooking qualities have been gradually clarified, such as *Wx* and *SSIIa*,
103 many of others still remain unknown even though their functions in starch
104 biosynthesis have been revealed.

105

106 In addition to *Wx* and *SSIIa*, the contribution of other genes to rice eating and cooking
107 quality is derived from analysis of allele variations and their association with the
108 quality parameters. Association analysis is a popular method to test the relationship
109 between specific sequence polymorphisms in candidate genes and phenotypic
110 variations (Thornsberry et al. 2001; Gupta et al. 2005). Molecular markers specific to
111 a simple sequence repeat polymorphism with respect to (CT)_n repeats and to a single
112 nucleotide polymorphism of *Wx* gene have been successfully designed to distinguish
113 rice varieties with low amylose content from varieties with intermediate or high

114 amylose content (Ayres et al. 1997). Likewise, a marker specific to a SNP of *SSIIa*
115 can differentiate varieties with low gelatinization temperature from those with
116 intermediate or high gelatinization temperature (Bao et al. 2006b). By sequencing
117 starch biosynthesis related genes, more and more allele variations have been revealed
118 either in coding regions or un-translated regions of genes. For association analysis,
119 previous studies (when SNP data were not available) focused more on the allele
120 variations occurred in un-translated regions (Bao et al. 2006a), but recent studies have
121 focused on the SNP of coding regions (Tian et al. 2009; Kharabian-Masouleh et al.
122 2012; Teng et al. 2013). Since starch biosynthesis is a complex network of many
123 isoforms, both types of allele variations should be incorporated into a systematic
124 association analysis. Furthermore, recently others genes have also been reported to
125 affect the production of amylose or amylopectin. For instance, a SNP of
126 *glucose-6-phosphate translocator 1* gene has been reported as highly associated with
127 amylose content and retrogradation properties (Kharabian-Masouleh et al. 2012).

128

129 Previous studies often focused on three parameters affecting eating and cooking
130 quality, apparent amylose content (AAC), gelatinization temperature (GT) and gel
131 consistency. However, starch physicochemical properties consist of many parameters
132 such as pasting, textural, thermal and retrogradation properties; and few genetic
133 studies of other physicochemical properties have been conducted. Previously, we have
134 established an association mapping panel consisting of 416 rice accessions (Jin et al.
135 2010) and genotyped the markers tagged for *Wx*, *SSI*, *BE1*, *BEIIb* (Bao et al. 2006a),
136 *SSIIa* (Bao et a. 2006b), and *AGPase* (Bao et al. 2012). In this study, we aim to
137 develop more markers for tagging other starch synthesis related genes (SSRGs), and
138 to investigate the associations between SSRG markers and starch physicochemical
139 properties. The results from this study will enhance our understanding of the genetic
140 control of starch physicochemical properties, and provide markers for carrying out
141 molecular breeding to improve rice grain quality.

142

143 **Materials and Methods**

144

145 **Rice materials and physicochemical properties**

146 Of the 416 rice accessions developed for association mapping (Jin et al. 2010), 379
147 accessions are nonwaxy rice with measured AAC, RVA pasting viscosity parameters
148 and gel texture properties (Bao et al. 2006c), and 205 accessions with measured
149 thermal and retrogradation properties (Bao et al. 2007). In brief, RVA pasting profile
150 was determined using a Rapid Visco Analyser (RVA, Model 3-D, Newport Scientific,
151 Warriewood, Australia) with the parameters including peak viscosity (PV), hot paste
152 viscosity (HPV), cool paste viscosity (CPV) and their derivative parameters
153 breakdown (BD, = PV-HPV), setback (SB, = CPV-PV), consistency (CS, =CPV-HPV),
154 stability (Stab, =HPV/PV), and setback ratio (SBratio, =CPV/HPV), and pasting
155 temperature (PT). The viscosities were measured in Rapid Visco Units (RVU). Gel
156 texture properties including hardness (HD, g), adhesiveness (ADH, g.s) and
157 cohesiveness (COH) were measured on a TA-XT2i Texture Analyzer (Texture
158 Technologies Corp., Scarsdale, NY) equipped with the Texture Expert software
159 program (Version 5.16). Thermal properties were analyzed using a DSC 2920 thermal
160 analyser (TA Instruments, Newcastle, DE, USA) and the parameters included onset
161 (T_o), peak (T_p), and conclusion (T_c) temperature, width at half peak height ($\Delta T_{1/2}$) and
162 enthalpy (ΔH_g) of gelatinization. The retrogradation properties were measured with
163 the same sample after measurement of the thermal properties, stored, and rescanned
164 with DSC. The enthalpy (ΔH_r) of the retrograded starch was used to calculate the
165 percentage of retrogradation (R%) as $(\Delta H_r) / (\Delta H_g) \times 100$.

166

167 **DNA isolation**

168 Fresh leaf tissue was harvested at the flowering stage from plants grown in the field.

169 DNA was extracted following a CTAB procedure (Doyle 1991).

170

171 **Development of CAPS, dCAPS, and InDel markers and genotyping**

172 Development of the cleaved amplified polymorphic sequences (CAPS) and derived

173 CAPS (dCAPS) follow the methods of Konieczny and Ausubel (1993) and Neff et al.
174 (1998).

175

176 The primers for PCR were synthesized by the Shanghai Shenggong BioTech Co. Ltd.
177 (Table 1). The PCR was carried out in a total volume of 20 μ L containing 10 mM
178 Tris-HCl (pH 9.0), 50 mM KCl, 0.1% Triton X 100, 2 mM MgCl₂, 0.1 mM dNTPs,
179 200 nM primers, 1 unit of *Taq* polymerase, and 30 ng of genomic DNA. All
180 amplifications were performed on a PTC-100 thermal cycler (MJ Research, Inc.)
181 under the following conditions: 5 min at 95°C; 35 cycles of 1 min at 95 °C, 45 s at 55
182 °C and 1 min at 72 °C; and a final extension step at 72 °C for 10 min.

183

184 Amplified PCR products were digested using suitable restriction endonucleases in a
185 total volume of 20 μ L according to the manufacturer's instructions (Table 1). The
186 digests were resolved by electrophoresis in 1.5–2.0% agarose gel and visualized using
187 a VersaDoc imaging system (Bio-Rad) after staining with ethidium bromide.

188

189 **Statistical analysis**

190 The polymorphism information content (PIC) values were determined using
191 PowerMarker version 3.25 (Liu and Muse 2005). Nei's genetic distance (Nei et al.
192 1983) was calculated and used for unweighted pair-group method with arithmetic
193 means (UPGMA) analysis as implemented in PowerMarker, with the UPGMA tree
194 viewed using MEGA 4.0 (Tamura et al. 2007).

195

196 Analysis of variance (ANOVA) and principal component analysis were performed
197 using the SAS System for Windows version 8 (SAS Institute Inc., Cary, NC, USA).

198 Duncan's multiple range test was conducted for comparison of means at $P < 0.05$.

199 Cluster analysis of the starch properties parameters were performed in SPSS Statistics

200 20 (Windows) using Ward's method based on squared Euclidean distance.

201

202 **Association mapping**

203 The population structure (Q) was determined using the STRUCTURE program
204 (Pritchard et al. 2000), with 100 simple sequence repeat (SSR) markers (Jin et al.
205 2010), and the kinship coefficients (K) between accessions were estimated using the
206 SPAGeDi program (v. 1.2g) (Hardy and Vekemans, 2002) with the same set of SSR
207 markers (Shao et al. 2011). Association analysis between marker alleles and different
208 starch physicochemical properties was performed with TASSEL Version 2.1 software,
209 taking the gross level population structure (Q) and kinship (K) into account (Yu et al.
210 2006; Bradbury et al. 2007). The *P*-value determining whether a marker is associated
211 with a trait was set at $P < 0.01$.

212

213 **Results**

214

215 **Marker development for starch biosynthesizing genes and their genotypes**

216 In addition to available SNP, InDel, and SSR markers tagged for *Wx*, *SSI*, *BEI*, *SSIIa*,
217 and all *AGP* genes (two small subunits and four large subunits) by Bao et al. (2002,
218 2006a, 2006b, 2012), we further developed CAPS, dCAPS and InDel markers for
219 tagging other starch biosynthesis related genes, i.e. *GBSSII*, *SSIIc*, *SSIIb*, *SSIIIa*,
220 *SSIIIb*, *SSIVa*, *SSIVb*, *BEIIa*, *BEIIb*, *ISA1-3* and *PUL* (Table 1) in this study. Most of
221 SNPs tagged were derived from the study of Tian et al. (2009) who reported many
222 functional SNPs for starch genes. All 13 new gene-specific markers (Table 1)
223 produced two or more alleles each, with the polymorphic information content (PIC)
224 ranging from 0.005 (InDel marker *SSIVb*) to 0.500 (marker *ISA2*). A deletion of 23bp
225 in *SSIVb* was reported by Tian et al. (2009) in rice Suyunuo; we developed the
226 primers to genotype this InDel in our rice accessions, and also found only Suyunuo
227 had this deletion. Due to its low PIC, we adapted another CAPS marker from Tian et
228 al. (2010) and Yan et al. (2011) to genotype this gene and found this marker had the
229 PIC value of 0.381 among all rice accessions.

230

231 In total, there are 35 markers tagged for 23 starch synthesis related genes (SSRGs),
232 with each gene tagged with at least one marker. The UPGMA tree made with these 35
233 SSRG markers showed that all the 416 rice could be assigned into two large groups,
234 representing *indica* and *japonica* subspecies, respectively (Supplementary Fig. 1),
235 with rice BP459, BP464, BP465, BP470, BP474, BP476, BP487 and BP532 loosely
236 affiliated with the *japonica* group. These same accessions were also assigned to the
237 *japonica* group based on 100 SSRs, but with a much smaller membership probability
238 (Jin et al. 2010). The waxy rice could also be classified into *indica* and *japonica*
239 groups using the SSRGs markers (Xu et al. 2013). These results clearly indicated that
240 SSRGs have diverged between the two subspecies during domestication and/or
241 improvement.

242

243 The association mapping panel has been genotyped with 100 SSRs before with a few
244 additional SSRs and other gene markers, such as *Gnla*, *fgr*, *Ra*, and *Rc* (Shao et al.
245 2011). In total, there are 143 markers that can be used for association mapping. Fig. 1
246 shows the position of each marker in the chromosome of rice genome in physical
247 distance.

248

249 **Phenotypes of starch properties of nonwaxy rice**

250 The starch physicochemical properties of each nonwaxy rice used in the present
251 analysis were based on Bao et al. (2006c, 2007). It is not surprising that this set of
252 association panel harbors wide genetic diversity in all starch physicochemical
253 properties. AAC had significant correlation with pasting viscosity and gel texture
254 traits except for PV, but had no relationship with thermal properties such as To, Tp, Tc
255 and ΔH_g . Tp had no correlation with HPV, SB and ADH, but had significant
256 correlation with PV ($P < 0.05$), CPV ($P < 0.05$), CS ($P < 0.01$), Stab ($P < 0.01$), and
257 SBratio ($P < 0.01$) (Supplementary Table 1). From the correlation analysis, all the traits
258 could be divided into two groups, AAC related traits (pasting viscosity and gel texture)
259 and gelatinization temperature (GT) related traits (thermal property). Retrogradation

260 trait could be related with both groups. As a result, after normalization of all the trait
261 value to between 0 and 1, a cluster analysis based on Ward's method showed that all
262 the traits could be classified into three groups (Fig. 2). Group 1 includes To, Tp, Tc
263 and PT, Group 2 includes BD, ΔHg , COH, $\Delta T_{1/2}$, and ADH, while Group 3 includes
264 HPV, CPV, PV, AAC, Stab, SB, CS, SBratio, ΔHr , R% and HD. Thus, Group 1
265 represents the GT-related traits, Group 3 represents AAC-related traits, while Group 2
266 is a mixture of AAC and GT related traits (Fig. 2).

267

268 Since the AAC and GT were the most important factors affecting other traits, the
269 AAC-GT combination for each rice accession could be clearly visualized in the plot
270 for both AAC and GT (Fig. 3). For the low AAC rice accessions ($AAC < 20\%$), the GT
271 could be divided into high GT and low GT classes. The high GT rice had the peak
272 temperature (Tp) ranging from 77.6 to 79.8 °C, while the low GT rice ranging from
273 65.8 to 71.0 °C. For the high AAC rice accessions, the GT could also be divided into
274 two groups. One is intermediate GT group with GT ranging from 72.8 to 76.6 °C, and
275 the other is the low GT group with GT ranging from 63.2 to 67.7 °C. It should be
276 noted that the two low GT groups with contrasting AAC differed in the range of
277 temperature, with the high-AAC rice having much lower GT than the low-AAC rice
278 (Fig. 3).

279 All the rice accessions have been divided into seven groups or subpopulations (Jin et
280 al. 2010). The difference in each starch property parameter among these seven
281 subpopulations is listed in Table 2. For apparent amylose content and pasting viscosity
282 parameters, POP3 and POP6 had higher AAC as well as higher PV, HPV, and CPV
283 than other subpopulations. POP7 had the lowest AAC and SB, but had the highest PV
284 and BD among the seven subpopulations. For gel texture parameters, POP3 had the
285 highest HD and the lowest ADH and COH, and by contrast, POP5 and POP7 had the
286 lowest HD but the highest ADH and COH. For pasting temperature (PT) and thermal
287 property parameters, POP1 and POP2 had similarly lower PT than the other five

288 subpopulations. Similar results were also found for the thermal property parameters,
289 i.e. POP 1 and POP2 had lower T_o , T_p , T_c and ΔH_g than the others. POP5 had the
290 highest mean $\Delta T_{1/2}$ and POP3 had the lowest mean $\Delta T_{1/2}$. For retrogradation property
291 parameters, POP3 had the highest mean ΔH_r and R%, whereas POP5 and POP7 had
292 the lowest ΔH_r and R% (Table 2).

293

294 **QTLs for starch physicochemical properties**

295 Association mapping of starch physicochemical property parameters were performed
296 using the 35 SSRG markers and 108 other markers (mostly SSRs) based on the Q+K
297 model. The identified QTLs are hereafter called main-effect QTLs. To uncover more
298 QTLs concealed by the *Wx* and *SSIa* loci for the starch physicochemical traits,
299 further association mapping were conducted with *Wx* (G/A) and *SSIa* (GC/TT) SNPs
300 as covariate based on the same Q+K model. The QTLs identified are called QTLs in
301 the *Wx* or *SSIa* background. The results are summarized in Table 3 and Fig. 4.

302 **AAC**

303 A total of five main-effect QTLs were detected for AAC. *Wx* gene locus was detected
304 as a major QTL ($P=1.45 \times 10^{-95}$). *SSI* locus, RM122, RM346 and RM222 were also
305 detected. Using *Wx* SNP (A/G) as a covariate, six QTLs were detected including four
306 SBRGs, *GBSSII*, *BE1*, *SSIIc*, and *SSIa*. Using *SSIa* SNP (GC/TT) as a covariate, the
307 same five main-effect QTLs were detected.

308

309 **Pasting viscosity**

310 As expected, the *Wx* locus was detected as a main-effect QTL for all the pasting
311 viscosity parameters. *SS1* was detected for SB and CS; *SSIa* was detected for BD, CS,

312 Stability and SBratio. In addition, RM346 was detected for CPV, SB, CS, and
313 SBratio.

314 Under the *Wx* locus background, no main-effect QTL was detected for PV, HPV and
315 CPV. *SSIIa* was still detected with much smaller *P* value for BD ($P=3.72\times 10^{-8}$),
316 SBratio ($P=0.0062$) and Stability ($P=8.24\times 10^{-8}$). No other SSRGs were detected for
317 pasting viscosities. More SSRs were detected for BD (RM237, RM276, RM340,
318 RM48 and RM507) and Stability (RM209, RM237, RM276, and RM507).

319 Under the *SSIIa* locus background, the QTLs identified for each trait were similar to
320 those of main-effect QTLs; the only difference was that one less QTL (*SSI*) for CS
321 and one more QTL (*GS3*) for SBratio were detected.

322

323 Gel texture

324 *Wx* was the common main-effect QTL for HD, ADH and COH, while *SSI* was
325 detected as the main-effect QTL for HD and COH. PUL was detected for HD.

326 Under the *Wx* locus background, *GBSSII*, RM252 and RM3 were detected for HD,
327 and only RM252 was detected for COH, while no QTL was detected for ADH.

328 Under the *SSIIa* locus background, the QTLs identified for each trait were the same as
329 those of main-effect QTLs.

330 Thermal property and pasting temperature (PT)

331 For the thermal property and PT, *SSIIa* was a common main-effect QTL. Other
332 SSRGs, *SSIIIb* (PT), *AGPL2* (ΔHg), and *Wx* ($\Delta T_{1/2}$) were also detected. In addition,
333 some SSRs were detected for PT (RM276, RM253 and RM346), T_o (RM346), T_c
334 (RM484), ΔHg (RM1 and RM48) and $\Delta T_{1/2}$ (RM161 and RM346).

335 Under the *Wx* locus background, all the QTLs identified for each trait were the same
336 as those of main-effect QTLs except that one more QTL for Tc (RM484) and ΔHg
337 (RM152), and one less (RM346) for $\Delta T_{1/2}$.

338 Under the *SSIIa* locus background, it was interesting to find that *Wx* locus was
339 detected for Tc ($P=0.0018$) and ΔHg ($P=0.0016$). However, *Wx* was not detected for
340 $\Delta T_{1/2}$, and instead, other SSRGs, such as *SSIIb* and *AGPL3* were detected for $\Delta T_{1/2}$.
341 *AGPL2* was also detected for ΔHg . RM346 was still detected for PT, To and Tp, and
342 RM1 and RM161 were still detected for ΔHg and $\Delta T_{1/2}$, respectively. More additional
343 SSRs were identified for PT, To, Tp, Tc and $\Delta T_{1/2}$.

344 Retrogradation property

345 For the retrogradation property, five common SSRGs, *Wx*, *SSIIa*, *ISAI*, *AGPL3* and
346 *BEIIa*, were identified as main-effect QTLs for both ΔHr and R%. RM346 and
347 RM161 for ΔHr and RM346 for R% were also detected.

348 Under the *Wx* locus background, *ISAI* and *SSIIa* were still identified as QTLs for both
349 ΔHr and R%. By contrast, *BEIIa* was only significant for R%. Another SSRG
350 (*AGPL2*) and RM87 were detected for both traits.

351 Under the *SSIIa* locus background, the QTLs identified were similar to those of
352 main-effect QTLs: *Wx*, *ISAI*, *AGPL3* and *RM346* were significant for both traits, but
353 *BEIIa* was only detected for R%.

354

355 **Discussion**

356 Association mapping has become a robust technology for quickly identifying the
357 genotype-phenotype relationships among diverse germplasms. The success of
358 association mapping depends on the diversity of the germplasms being investigated,
359 the marker coverage of the target genome, and the appropriate methodologies used.

360 New advances in association mapping has been made in plants recently, with some
361 great achievements coming from rice, such as mapping genes for cooking quality
362 (Tian et al. 2009) and agronomic traits (Huang et al. 2010; Zhao et al. 2011).

363

364 Due to the diverse origins of the rice accessions used in different studies, a wide range
365 of variations has always been found in different populations, for example, from the
366 African and USA germplasm (Asante et al. 2013), Italian germplasm (Caffagni et al.
367 2013) and Korean germplasm (Lu et al. 2012a, 2012b; Zhao et al. 2013). The present
368 study covers more than 20 starch physicochemical property traits that have been
369 measured for 379 rice accessions. Compared with other studies on eating and cooking
370 quality of rice, our research ranks the highest in both number of traits and accessions
371 analyzed. In addition to the wide genetic diversity revealed for each trait, we found
372 that all the traits could be classified into three groups: AAC-related traits, GT-related
373 traits, and the mixture (Fig. 2). In another linkage mapping study, Wang et al. (2007)
374 also found that eating and cooking traits of rice could be divided into two groups: the
375 first class consists of AC, GC, and most of the paste viscosity parameters that form a
376 major determinant of eating quality, the second class includes alkali spreading value,
377 pasting temperature and pasting time, which characterize the cooking process.
378 Furthermore, with plotting AAC and GT (measured as the Tp), we have another
379 important finding that both AAC and GT could be divided into two classes, and in
380 combination, there are four classes among nonwaxy rice (Fig. 3). For the low AAC
381 rice accessions (AAC<20%), the GT could be divided into high GT, and low GT class.
382 For the high AAC rice accessions, the GT could also be divided into two groups. The
383 GT of low-AAC- low-GT group are much higher than the high-AAC-low-GT group
384 (Fig. 3). The GT of waxy rice is similar to that of low-AAC rice that have high and
385 low GT classes (Xu et al. 2013). Juliano and Villareal (1993), Juliano (1998) have
386 long before indicated that high-AAC rice usually has intermediate or low-GT;
387 low-AAC rice or waxy rice usually has high or low-GT among rice accessions. By
388 contrast, it is difficult to find the combinations of high-AAC and high-GT rice, and
389 low-AAC and intermediate-GT rice (Juliano and Villareal 1993; Juliano, 1998). No
390 other genetic analyses have indicated this fact. The tight link between AAC and GT
391 suggests that there might be interaction between AAC and GT. However, in depth
392 screening of rice germplasm may find other rare combinations of AAC and GT. For
393 example, Juliano et al. (2009) found the combination of high-AAC and high-GT rice.

394 These diverse materials provide precious resources for further genetic studies and
395 molecular analysis of the related genes.

396

397 Many studies have focused on the genetic basis of the starch physicochemical
398 properties in relation to the cooking and eating quality of rice. AAC is mainly
399 controlled by the *Wx* region on chromosome 6 (He et al. 1999; Tan et al. 1999; Bao et
400 al. 2000; Lanceras et al. 2000; Septiningsih et al. 2003; Aluko et al. 2004). GT and
401 thermal properties are genetically determined by a major QTL, i.e. the alkali
402 degeneration (*alk*) locus on chromosome 6, also known as *SSIIa* (He et al. 1999;
403 Aluko et al. 2004; Bao et al. 2004c; Fan et al. 2005; Tian et al. 2005; Wang et al.
404 2007). Genetic analysis with QTL mapping approach showed that the RVA
405 parameters are mainly controlled by the *Wx* gene (Bao et al. 1999, 2000, 2003; Larkin
406 and Park 2003; Wang et al. 2007; Traore et al. 2011). Gel texture parameters were
407 also mainly controlled by the *Wx* locus (Bao et al. 2000; 2004a). The aforementioned
408 results mostly came from linkage analyses. Association mapping for these traits have
409 been conducted recently (Chen et al. 2008a, 2008b; Tian et al. 2009; Lu et al. 2012a,
410 2012b; Zhao et al. 2013). Similar results of main-effect QTLs have been reported, i.e.
411 *Wx* and *SSIIa* controlled AAC-related traits and GT-related traits, respectively (Chen
412 et al. 2008a, 2008b; Tian et al. 2009; Caffagni et al. 2013). However, due to different
413 germplasms used, some of the studies have not identified the *Wx* for AAC (Lu et al.
414 2012a, 2012b; Zhao et al. 2013). Lu et al. (2012a, 2012b) detected *AGPS1*, *AGPL4*
415 and *SSIIb* for AAC and pasting viscosity in their rice materials. Kharabian-Masouleh
416 et al. (2012) reported that a SNP of *glucose-6-phosphate translocator* gene was highly
417 associated with amylose content and retrogradation property. This study also found
418 some new loci for starch physicochemical properties, such as *SSIIIb* for PT, *PUL* for
419 HD, *AGPL2* for Δ Hg; *BEIIa* and *AGPL3* for retrogradation properties (Table 3). The
420 retrogradation property traits were comprehensively studied for the first time, and we
421 identified five common SSRGs, *Wx*, *SSIIa*, *ISAI*, *AGPL3* and *BEIIa*, as main-effect
422 QTLs for both Δ Hr and R%. Either under the *Wx* locus or *SSIIa* background, *ISAI*

423 could also be identified for both ΔH_r and R%. Thus, it can be concluded that
424 retrogradation properties are mainly controlled by *Wx*, *SSIIa* and *ISA1*. Their relative
425 effects are in the order of *SSIIa* > *Wx* > *ISA1*. However, the effects of other SSRGs
426 such as *BEIIa*, *AGPL2* and *AGPL3* could not be neglected.

427

428 Genetic linkage mapping studies showed that *Wx* not only controls AAC, gel
429 consistency and pasting viscosity, but also affects GT (Wang et al. 2007; Lapitan et al.
430 2009), and the QTL cluster at *SSIIa* locus also contains individual QTL for gel
431 consistency and some paste viscosity parameters (Wang et al. 2007). Tian et al. (2009)
432 reported by association mapping that *Wx* not only affects AAC and gel consistency as
433 a major gene but also regulates GT as a minor one; *SSIIa* plays an essential role not
434 only in controlling GT, but also AC and gel consistency (Tian et al. 2009). In this
435 study, we analyzed the effects of *Wx* and *SSIIa* background QTLs using the two
436 functional SNPs for *Wx* (G/A) and *SSIIa* (GC/TT) as an additional covariate on the
437 physicochemical property traits by association mapping. We found that more QTLs
438 that were concealed by the *Wx* and *SSIIa* could be discovered. In the *SSIIa*
439 background, the AAC-related traits were found to be controlled by similar QTLs as
440 the main-effect QTLs, but it is possible to detect more other QTLs for the GT-related
441 traits. Specifically, the Tc and ΔH_g were found to be controlled by *Wx*. RM447 on
442 chromosome 8 and RM17 on chromosome 12 were found to control PT, To, Tp and
443 Tc. *SSIIb* and *AGPL3* were identified for $\Delta T_{1/2}$. Similarly, under the background of
444 *Wx*, the GT-related traits were found to be controlled by similar main-effect QTLs,
445 while it is possible to detect more QTLs for the AAC-related traits. Specifically, AAC
446 was found to be controlled by more SSRGs, such as *SSIIa*, *BEI*, *SSIIc* and *GBSSII*.
447 The Stab was found to be controlled by more SSRs. Zhao et al. (2013) also detected a
448 locus near *SSIIa* (RM276) for AAC. All these findings confirm the importance of *Wx*
449 and *SSIIa* in determining the eating and cooking quality of rice, and suggest that there
450 might be epistatic interaction between *Wx* and *SSIIa*. Tian et al. (2009) introduced an
451 antisense and sense *Wx* RNA to a high AAC rice and waxy rice respectively, and they
452 found that GT changed simultaneously with significant decrease or increase in AAC.
453 Tian et al. (2009) and Gao et al. (2011) introduced *SSIIa* gene to low-GT rice by
454 transgenic engineering and found that it also affected the AAC, gel consistency and

455 pasting properties, suggesting that *SSIIa* is a modifier gene for AAC, gel consistency,
456 and pasting properties in rice. These genetic transformation studies provide
457 supporting evidence for the hypothesized interaction between *Wx* and *SSIIa*. However,
458 it is also possible that the complex effects of *Wx* and *SSIIa* are derived from their
459 close physical position in the chromosome 6, as suggested by strong linkage
460 disequilibrium for these starch related traits. Thus, the exact roles played by *Wx* and
461 *SSIIa* remain to be untangled.

462

463 Biochemically, starch biosynthesis in the cereal endosperm involves complex
464 interactions among multiple isoforms of starch synthase, branching and debranching
465 enzymes, leading to a fine amylopectin structure (Jeon et al. 2010; Tetlow et al. 2011).
466 Multi-enzyme complexes (protein-protein interactions) have been indentified in wheat
467 and maize endosperms during the period of grain filling (Tetlow et al. 2004, 2008;
468 Hennen-Bierwagen et al. 2008, 2009). The multi-enzyme complex components in the
469 normal maize endosperm include SSI, SSIIa, BEI, BEIIa, BEIIb, and SP (starch
470 phosphorylase) (Liu et al. 2009). Based on the facts that *Wx* and *SSIIa* exert joint
471 control over both AAC and GT, and the complex AAC and GT combinations exist in
472 rice germplasm, Bao (2012) proposed a multi-enzyme complex model to explain the
473 interaction between GBSSI (*Wx* protein) and SSIIa, and how they contribute to
474 diverse combination of AAC and GT in rice germplasm. The model hypothesizes that
475 BEIIb, SSI and SSIIa are the major multi-enzyme complex components in the
476 intermediate-GT (SSIIa active) rice, the high activity of GBSSI in the stroma
477 negatively regulates the function of the multi-enzyme complex, resulting in the
478 synthesis of high-AAC intermediate-GT starch. In low-AAC high-GT rice, the lower
479 GBSSI activity and hence the higher activity of the multi-enzyme complex, allows the
480 synthesis of more chains with DP>12, thus producing high-GT starch. Low-GT rice
481 can have high, intermediate, low or zero (waxy rice) AAC. Due to the loss of function
482 of SSIIa, the components that comprise the multi-enzyme complex are unknown and
483 thus need to be further studied. However, the multi-enzyme complex in SSIIa-active
484 rice grains should be clarified first before a more realistic hypothesis can be put
485 forward.

486

487 Undoubtedly, our results have direct applications to rice quality breeding programs.
488 The well-known markers of *Wx* and *SSIIa* genes have been used in rice breeding in

489 which they greatly facilitate the precise picking of the desirable alleles from the good
490 quality parent using marker-assisted selection (Wang et al. 2007; Jin et al. 2010). This
491 study also points to a new direction in rice grain quality research, that is, to identify
492 the protein-protein interactions among the related enzymes during grain filling. The
493 knowledge gained from these new researches will enhance our understanding of
494 starch biosynthesis, and ultimately contribute to the improvement of rice cooking and
495 eating quality.

496

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503

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746. **Legends for Figures**

747

748 **Fig. 1** The position of markers in physical distance on 12 chromosomes of rice
749 genome

750

751 **Fig. 2** Dendrogram generated with the starch properties parameters using Ward's
752 method based on squared Euclidean distance

753

754 **Fig. 3** Plot of AAC-GT combination for nonwaxy rice accessions

755

756 **Fig. 4** Plots of main-effect QTLs (**A**), QTLs identified in the *Wx* background (**B**)
757 and *SSIa* background (**C**) for 20 starch physicochemical properties. The SSRGs with
758 the largest $-\log(P)$ values were highlighted with red (*Wx*), blue (*SSIa*), green (*ISA1*)
759 and pink (*SSIb*) colors

760

Fig 1

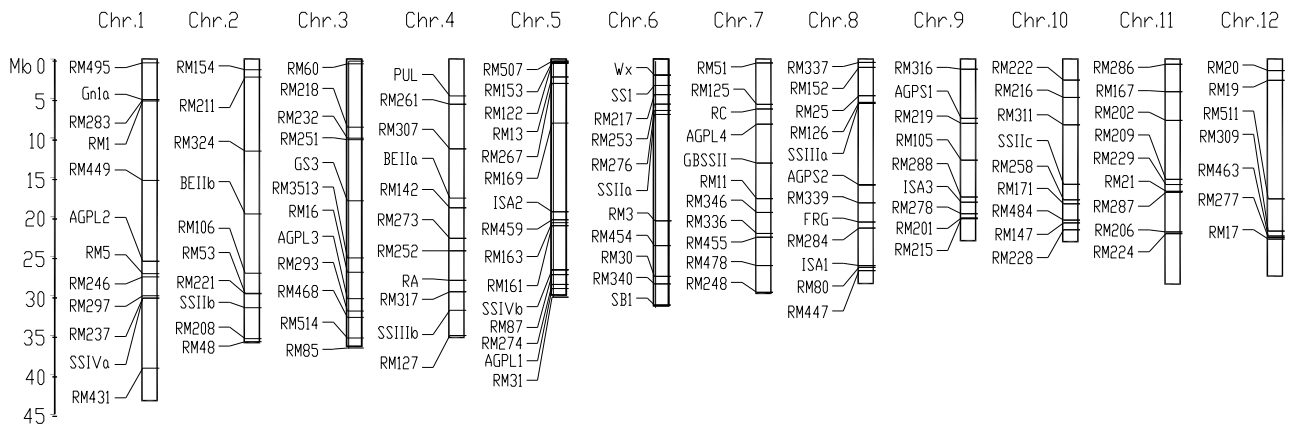


Fig 2

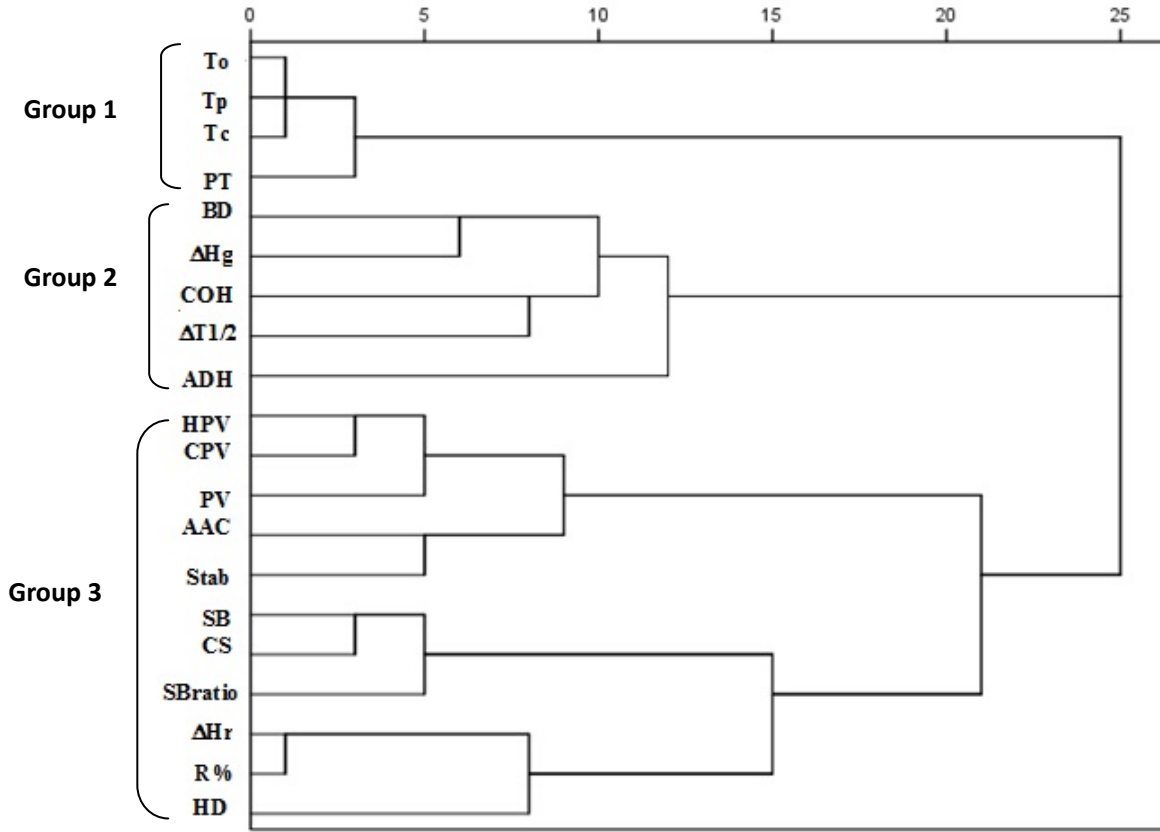


Fig 3

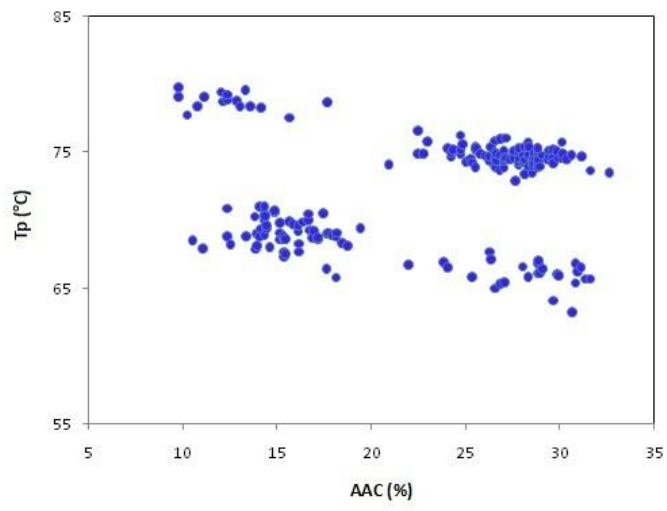
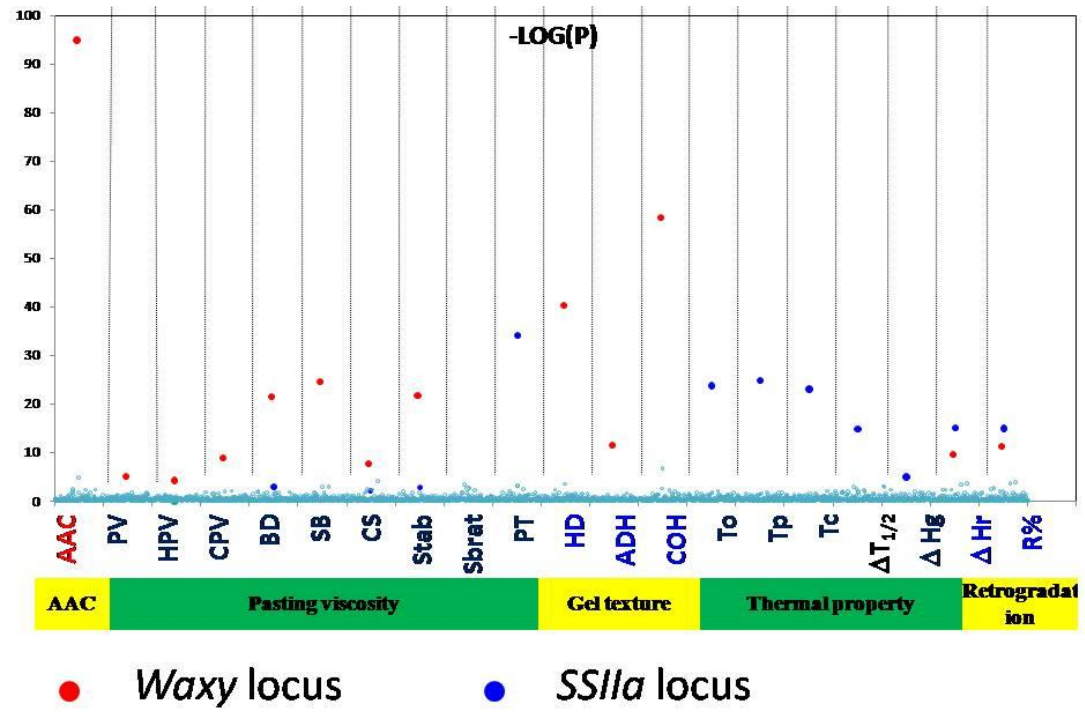
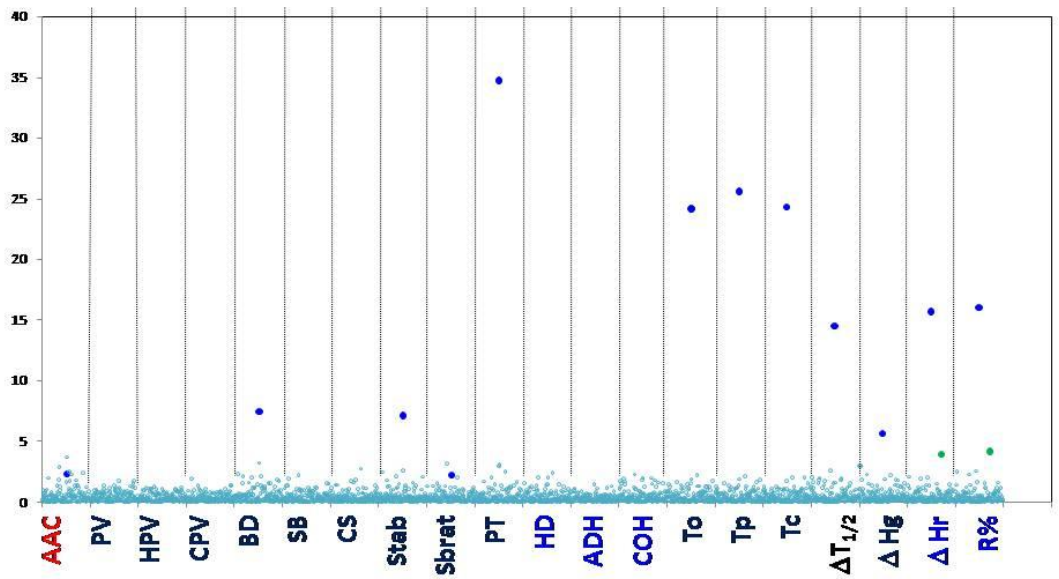


Fig 4.

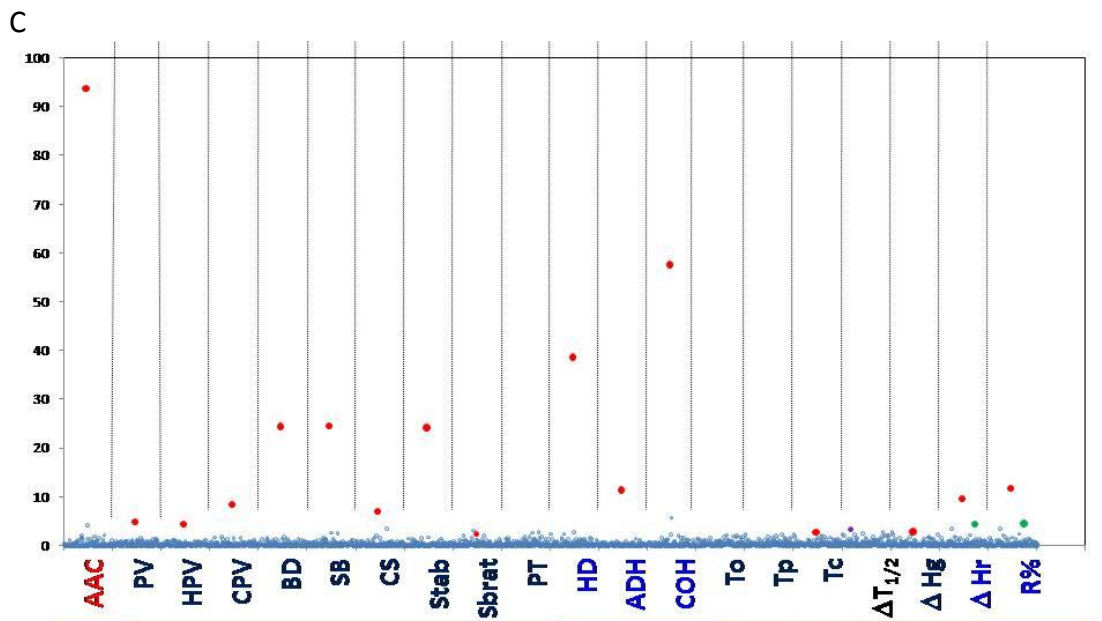
A



B



● *SSIIa* locus ● *ISA1* locus



● *Waxy* locus ● *ISA1* locus ● *SSIIb* locus

Table 1 Summary of CAPS, dCAPS and InDel markers for starch synthesizing genes used in this study

| Genes | Chromosome | Forward primer | Reverse primer | R.E. ¹ | Allele size (bp) | PIC ¹ |
|-------------------------|------------|---------------------------------------|----------------------------------|-------------------|---------------------------|------------------|
| <i>GBSSII</i> | 7 | TGACCTGAAAATCATATTATTAC | CACTTTCGTTTGGTGCATCTG | SpeI | 180 (A), 158 (T) | 0.473 |
| <i>SSIIc (SSII-1)</i> | 10 | CGGTGGCAAGGAGAGCCGGGGT | GCGGCACGGATCTGGAGAAG | MboII | 210 (G); 181 (T) | 0.109 |
| <i>SSIIb (SSII-2)</i> | 2 | TTCGCAAAGCATGAGACAATAAG | GAGGCCCAAGTCATTCAACAA | - | InDel (155; 172) | 0.280 |
| <i>SSIIIb (SSIII-1)</i> | 4 | AGCAGAATGAATCTGACAATCTAG | CGTGATTTCCACCATAAGAGCAA | XbaI | 182 (AG), 164+20(AGAG) | 0.302 |
| <i>SSIIIa (SSIII-2)</i> | 8 | TGTTAAAATTTCCCCCAAGTAC | GCATAATGTTCAACTGTAGATAAAGAA G | MobII | 203(C); 166+37(T) | 0.471 |
| <i>SSIVa (SSIV-1)</i> | 1 | GTCGCTTCTAGGAGGGCAACGT | GATAACTGCTAAGATATTGAGAG | Acl | 198 (C); 176 (T) | 0.260 |
| <i>SSIVb (SSIV-2)</i> | 5 | CCAAGTGGGGATCATCAACCTC | CCCGCAAAAATGAAGCTAAGC | - | InDel (252; 230) | 0.005 |
| | 5 | CTTCTGATTGATGGTTGGTTGC ² | GGAAGAATAATCTCTACTAGGTGGC | SphI | 728 (G); 523+205 (A) | 0.381 |
| <i>BEIIb (SBE3)</i> | 2 | GCATCCTCAACCTAAAAGACCA | GAATCAACCATCCAGCAAAGG | Scal | 301 (G); 140+160 (A) | 0.462 |
| <i>BEIIa (SBE4)</i> | 4 | CTGGGTGCTCCTGTTTGTCT | CGTGCTTATTCGCTGTATTCT | MnlI | 192 (C); 163 (G) | 0.358 |
| <i>ISA1</i> | 8 | CGCACTGGATTTCAAGATGAGC | TCCATAGATGCTTTCGGCTGT | AluI | 213 (C); 191 (T) | 0.305 |
| <i>ISA2</i> | 5 | CAGGCGTGTAGCAAGATCACTCAT | TGACCCGGTCTTTCCATGAC | Nla III | 181 (A); 157(G) | 0.500 |
| <i>ISA3</i> | 9 | TGACTGATTGGATGCTGCTAAAC | GCCGCTCTTGTGGAAATG | HinfI | 246 (A); 174+72 (G) | 0.336 |
| <i>PUL</i> | 4 | AGAGAAGGAGAAAGAAGTGGAGAC ² | GTCCAAACTGAATCACTCAATCG | - | InDel (128; 115) | 0.334 |

1. R.E: restriction enzyme; PIC: polymorphism information content.

2. The CAPS (*SSIV-2*) and InDel (*PUL*) markers were adapted from [Tian et al. \(2010\)](#) and Yan et al. (2011).

Table 2 Comparison of the mean values of the phenotypic traits among seven subpopulations

| Subpop | AAC | PV | HPV | CPV | BD | SB | CS | Stab | SBratio | PT | HD | ADH | COH | To | Tp | Tc | □ΔT1/2 | □ΔHg | □ΔHr | R% |
|--------|--------|----------|----------|----------|---------|--------|----------|---------|---------|--------|---------|----------|-------|---------|---------|---------|--------|--------|--------|---------|
| POP1 | 24.5 b | 241.1 b | 180.8 ab | 319.6 ab | 60.3 c | 78.5 a | 138.8 b | 0.7 ab | 1.8 bcd | 73.7 b | 29.2 cd | -30.0 bc | 0.6 b | 63.0 c | 69.2 c | 75.9 c | 7.2 b | 7.0 d | 1.1 de | 14.8 de |
| POP2 | 21.2 c | 224.1 c | 158.3 d | 309.5 b | 65.8 bc | 85.3 a | 151.2 a | 0.7 cd | 2.0 a | 73.9 b | 25.0 d | -6.2 ab | 0.6 b | 67.0 b | 72.2 ab | 78.7 ab | 6.4 cd | 7.8 c | 1.8 cd | 21.7 cd |
| POP3 | 28.4 a | 257.4 a | 189.8 a | 336.9 a | 67.7 bc | 79.4 a | 147.1 ab | 0.7 abc | 1.8 bcd | 76.2 a | 41.6 a | -36.1 d | 0.5 c | 69.4 c | 74.1 a | 80.1 a | 5.9 d | 9.1 a | 3.6 a | 38.9 a |
| POP4 | 24.9 b | 245.3 b | 176.4 bc | 323.9 ab | 68.9 bc | 78.6 a | 147.6 ab | 0.7 bcd | 1.8 b | 75.9 a | 32.2 c | -36.3 d | 0.6 b | 68.5 ab | 73.8 a | 80.0 a | 6.4 cd | 8.4 b | 2.5 bc | 28.7 bc |
| POP5 | 18.0 d | 235.5 bc | 163.9 d | 280.5 c | 71.6 b | 45.0 b | 116.6 c | 0.7 d | 1.7 d | 76.3 a | 17.1 e | -21.6 a | 0.7 a | 64.1 c | 70.7 bc | 77.9 b | 8.0 a | 7.8 c | 0.9 e | 10.5 e |
| POP6 | 27.6 a | 244.4 b | 184.3 ab | 329.7 a | 60.1 c | 85.3 a | 145.4 ab | 0.8 a | 1.8 bc | 76.3 a | 37.0 b | -33.3 cd | 0.5 c | 68.5 ab | 73.6 a | 79.8 a | 6.3 cd | 8.5 ab | 2.9 ab | 34.0 ab |
| POP7 | 17.7 d | 258.4 a | 166.3 cd | 288.8 c | 92.1 a | 30.3 c | 122.4 c | 0.6 e | 1.8 cd | 75.1 a | 18.6 e | -21.4 a | 0.7 a | 66.6 b | 72.3 ab | 78.6 ab | 6.7 bc | 8.2 bc | 1.0 e | 11.5 e |

Different letters in the same column were significant at $P < 0.05$.

Table 3 The marker loci associated with starch physicochemical property traits detected with Q+K model and with *Wx* or *SSIIa* as additional covariate.

| Trait | Main effect loci | | | <i>Wx</i> covariate | | | <i>SSIIa</i> covariate | | | |
|-------------------|------------------|--------------------|------------------------|------------------------|---------------|-----------------------|------------------------|------------|------------------------|------------------------|
| | Locus | chro. ¹ | p_Marker | Locus | Chro. | p_Marker | Locus | Chro. | p_Marker | |
| AAC | AAC | RM122 | 5 | 3.8×10^{-3} | RM507 | 5 | 1.3×10^{-3} | RM122 | 5 | 5.1×10^{-3} |
| | | <i>Wx</i> | 6 | 1.45×10^{-95} | <i>SSIIa</i> | 6 | 5.3×10^{-3} | <i>Wx</i> | 6 | 2.14×10^{-94} |
| | | <i>SSI</i> | 6 | 1.57×10^{-5} | RM276 | 6 | 2.1×10^{-4} | <i>SSI</i> | 6 | 4.67×10^{-5} |
| | | RM346 | 7 | 4.6×10^{-3} | <i>BE1</i> | 6 | 3.2×10^{-3} | RM346 | 7 | 9.0×10^{-3} |
| | | RM222 | 10 | 5.2×10^{-3} | <i>GBSSII</i> | 7 | 5.5×10^{-3} | RM222 | 10 | 5.6×10^{-3} |
| | | | | | <i>SSIIc</i> | 10 | 4.1×10^{-3} | | | |
| Pasting viscosity | | | | | | | | | | |
| PV | <i>Wx</i> | 6 | 1.18×10^{-5} | | | | <i>Wx</i> | 6 | 1.52×10^{-5} | |
| HPV | <i>Wx</i> | 6 | 6.46×10^{-5} | | | | <i>Wx</i> | 6 | 4.92×10^{-5} | |
| CPV | <i>Wx</i> | 6 | 1.52×10^{-9} | | | | <i>Wx</i> | 6 | 4.88×10^{-9} | |
| | | RM346 | 7 | 7.9×10^{-3} | | | | | | |
| BD | <i>Wx</i> | 6 | 3.92×10^{-22} | RM237 | 1 | 4.9×10^{-3} | <i>Wx</i> | 6 | 3.95×10^{-25} | |
| | <i>SSIIa</i> | 6 | 1.2×10^{-3} | RM48 | 2 | 7.5×10^{-3} | | | | |
| | | | | RM507 | 5 | 9.3×10^{-3} | | | | |
| | | | | RM276 | 6 | 6.3×10^{-4} | | | | |
| | | | | <i>SSIIa</i> | 6 | 3.72×10^{-8} | | | | |
| | | | | RM340 | 6 | 8.2×10^{-3} | | | | |
| SB | <i>Wx</i> | 6 | 2.87×10^{-25} | RM317 | 4 | 6.2×10^{-3} | <i>Wx</i> | 6 | 2.83×10^{-25} | |
| | <i>SSI</i> | 6 | 1.2×10^{-3} | | | | <i>SSI</i> | 6 | 1.6×10^{-3} | |
| | RM346 | 7 | 1.5×10^{-3} | | | | RM346 | 7 | 2.6×10^{-3} | |

| | | | | | | | | | | |
|-------------|------------|---------------|------------------------|------------------------|---------------|----------------------|------------------------|-----------|------------------------|------------------------|
| Gel texture | CS | RM161 | 5 | 4.5×10^{-3} | RM346 | 7 | 1.8×10^{-3} | RM161 | 5 | 5.9×10^{-3} |
| | | <i>Wx</i> | 6 | 2.29×10^{-8} | | | | <i>Wx</i> | 6 | 1.26×10^{-7} |
| | | <i>SSI</i> | 6 | 9.9×10^{-3} | | | | RM346 | 7 | 2.65×10^{-4} |
| | | <i>SSIIa</i> | 6 | 5.3×10^{-3} | | | | | | |
| | | RM346 | 7 | 8.77×10^{-5} | | | | | | |
| | PT | <i>SSIIIb</i> | 4 | 9.1×10^{-3} | <i>SSIIIb</i> | 4 | 9.9×10^{-3} | RM346 | 7 | 1.9×10^{-3} |
| | | RM253 | 6 | 9.39×10^{-4} | RM253 | 6 | 1.1×10^{-3} | RM447 | 8 | 1.4×10^{-3} |
| | | RM276 | 6 | 8.59×10^{-4} | RM276 | 6 | 8.02×10^{-4} | RM17 | 12 | 3.2×10^{-3} |
| | | <i>SSIIa</i> | 6 | 8.28×10^{-35} | <i>SSIIa</i> | 6 | 2.06×10^{-35} | | | |
| | | RM346 | 7 | 4.3×10^{-3} | RM346 | 7 | 3.1×10^{-3} | | | |
| | Stability | <i>Wx</i> | 6 | 2.45×10^{-22} | RM237 | 1 | 3.5×10^{-3} | <i>Wx</i> | 6 | 6.67×10^{-25} |
| | | <i>SSIIa</i> | 6 | 1.3×10^{-3} | RM507 | 5 | 7.5×10^{-3} | | | |
| | | | | | RM276 | 6 | 2.4×10^{-3} | | | |
| | | | | | <i>SSIIa</i> | 6 | 8.24×10^{-8} | | | |
| | | | | | RM209 | 11 | 8.4×10^{-3} | | | |
| | SBratio | RM161 | 5 | 3.74×10^{-4} | RM161 | 5 | 7.37×10^{-4} | GS3 | 3 | 6.6×10^{-3} |
| | | <i>Wx</i> | 6 | 3.0×10^{-3} | <i>SSIIa</i> | 6 | 6.2×10^{-3} | RM161 | 5 | 5.77×10^{-4} |
| | | <i>SSIIa</i> | 6 | 2.5×10^{-3} | RM346 | 7 | 1.0×10^{-2} | <i>Wx</i> | 6 | 4.4×10^{-3} |
| | | RM346 | 7 | 7.5×10^{-3} | | | | RM346 | 7 | 7.7×10^{-3} |
| HD | <i>PUL</i> | 4 | 4.6×10^{-3} | RM252 | 4 | 9.8×10^{-3} | <i>PUL</i> | 4 | 3.0×10^{-3} | |
| | <i>Wx</i> | 6 | 5.68×10^{-14} | RM3 | 6 | 9.3×10^{-3} | <i>Wx</i> | 6 | 2.48×10^{-39} | |
| | <i>SSI</i> | 6 | 3.32×10^{-4} | <i>GBSSII</i> | 7 | 4.2×10^{-3} | <i>SSI</i> | 6 | 1.4×10^{-3} | |
| ADH | <i>Wx</i> | 6 | 3.58×10^{-12} | | | | <i>Wx</i> | 6 | 4.9×10^{-12} | |
| COH | <i>Wx</i> | 6 | 4.96×10^{-59} | RM252 | 4 | 5.6×10^{-3} | <i>Wx</i> | 6 | 2.83×10^{-58} | |
| | <i>SSI</i> | 6 | 2.9×10^{-3} | | | | <i>SSI</i> | 6 | 4.7×10^{-3} | |

| | | | | | | | | | |
|------------------|--------------|----|------------------------|--------------|----|------------------------|--------------|----------------------|-----------------------|
| | RM346 | 7 | 6.4×10^{-3} | | | | RM346 | 7 | 7.6×10^{-3} |
| Thermal property | | | | | | | | | |
| T _o | <i>SSIIa</i> | 6 | 2.0×10^{-24} | <i>SSIIa</i> | 6 | 7.37×10^{-25} | RM346 | 7 | 5.5×10^{-3} |
| | RM346 | 7 | 9.1×10^{-3} | RM346 | 7 | 6.4×10^{-3} | RM447 | 8 | 8.7×10^{-3} |
| | | | | | | | RM215 | 9 | 6.5×10^{-3} |
| | | | | | | | RM17 | 12 | 4.4×10^{-3} |
| T _p | <i>SSIIa</i> | 6 | 1.91×10^{-25} | <i>SSIIa</i> | 6 | 2.67×10^{-26} | RM346 | 7 | 7.9×10^{-3} |
| | | | | RM346 | 7 | 7.3×10^{-3} | RM447 | 8 | 4.1×10^{-3} |
| | | | | | | | RM17 | 12 | 4.7×10^{-3} |
| T _c | <i>SSIIa</i> | 6 | 9.87×10^{-24} | <i>SSIIa</i> | 6 | 5.64×10^{-25} | <i>Wx</i> | 6 | 1.8×10^{-3} |
| | RM484 | 10 | 8.5×10^{-3} | RM346 | 7 | 9.9×10^{-3} | RM125 | 7 | 7.6×10^{-3} |
| | | | | RM484 | 10 | 9.1×10^{-3} | RM17 | 12 | 3.4×10^{-3} |
| ΔH_g | RM1 | 1 | 9.90×10^{-4} | RM1 | 1 | 1.0×10^{-3} | RM1 | 1 | 1.5×10^{-3} |
| | <i>AGPL2</i> | 1 | 1.4×10^{-3} | <i>AGPL2</i> | 1 | 1.1×10^{-3} | <i>AGPL2</i> | 1 | 4.6×10^{-3} |
| | RM48 | 2 | 5.1×10^{-3} | RM48 | 2 | 5.5×10^{-3} | <i>Wx</i> | 6 | 1.6×10^{-3} |
| | <i>SSIIa</i> | 6 | 1.24×10^{-5} | <i>SSIIa</i> | 6 | 2.38×10^{-6} | RM224 | 11 | 3.9×10^{-3} |
| | | | | RM152 | 8 | 6.2×10^{-3} | | | |
| $\Delta T_{1/2}$ | Gn1a | 1 | 8.1×10^{-3} | Gn1a | 1 | 9.3×10^{-3} | Gn1a | 1 | 8.1×10^{-3} |
| | RM161 | 5 | 1.1×10^{-3} | RM161 | 5 | 2.5×10^{-3} | <i>SSIIb</i> | 2 | 5.13×10^{-4} |
| | <i>Wx</i> | 6 | 4.5×10^{-3} | <i>SSIIa</i> | 6 | 3.47×10^{-15} | <i>AGPL3</i> | 3 | 3.3×10^{-3} |
| | <i>SSIIa</i> | 6 | 1.46×10^{-15} | | | | RM161 | 5 | 2.3×10^{-3} |
| | RM346 | 7 | 5.5×10^{-3} | | | | RM278 | 9 | 9.1×10^{-3} |
| | | | | | | RM215 | 9 | 1.3×10^{-3} | |
| | | | | | | RM286 | 11 | 6.6×10^{-3} | |
| | | | | | | RM202 | 11 | 6.0×10^{-3} | |
| | | | | | | RM224 | 11 | 5.3×10^{-3} | |

| Retrogradation | | | | | | | | | |
|----------------|--------------|---|------------------------|--------------|---|------------------------|--------------|---|------------------------|
| ΔHr | <i>AGPL3</i> | 3 | 5.0×10^{-3} | <i>AGPL2</i> | 1 | 8.8×10^{-3} | <i>AGPL3</i> | 3 | 2.69×10^{-4} |
| | <i>BEIIa</i> | 4 | 5.2×10^{-3} | RM87 | 5 | 1.02×10^{-2} | <i>Wx</i> | 6 | 2.30×10^{-10} |
| | RM161 | 5 | 5.4×10^{-3} | <i>SSIIa</i> | 6 | 2.22×10^{-16} | RM346 | 7 | 4.9×10^{-3} |
| | <i>Wx</i> | 6 | 3.18×10^{-10} | <i>ISA1</i> | 8 | 1.18×10^{-4} | <i>ISA1</i> | 8 | 4.67×10^{-5} |
| | <i>SSIIa</i> | 6 | 1.06×10^{-15} | | | | | | |
| | RM346 | 7 | 4.33×10^{-4} | | | | | | |
| | <i>ISA1</i> | 8 | 2.03×10^{-4} | | | | | | |
| R% | <i>AGPL3</i> | 3 | 4.2×10^{-3} | <i>AGPL2</i> | 1 | 3.4×10^{-3} | <i>AGPL3</i> | 3 | 2.31×10^{-4} |
| | <i>BEIIa</i> | 4 | 2.5×10^{-3} | <i>BEIIa</i> | 4 | 5.5×10^{-3} | <i>BEIIa</i> | 4 | 5.1×10^{-3} |
| | <i>Wx</i> | 6 | 7.28×10^{-12} | RM87 | 5 | 2.9×10^{-3} | <i>Wx</i> | 6 | 2.25×10^{-12} |
| | <i>SSIIa</i> | 6 | 1.32×10^{-15} | <i>SSIIa</i> | 6 | 1.08×10^{-16} | RM346 | 7 | 3.2×10^{-3} |
| | RM346 | 7 | 2.25×10^{-4} | <i>ISA1</i> | 8 | 7.18×10^{-5} | <i>ISA1</i> | 8 | 3.59×10^{-5} |
| | <i>ISA1</i> | 8 | 1.62×10^{-4} | | | | | | |
| | | | | | | | | | |

Chro.: chromosome.