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1 **Mapping and Analysis of QTL for Early Maturity Trait in Tetraploid Potato (*Solanum tuberosum* L.)**

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16 **Abstract**

17 Maturity is one of the important traits of potato. In order to get the genetic segment of potato early maturity trait,
18 a tetraploid potato maturity segregation population of Zhongshu 19 × Zhongshu 3 was used for genetic analysis
19 through the combination of high throughput simplified genome sequencing (2b-RAD) and bulked segregation
20 analysis (BSA). A genetic segment related to the early maturity trait at the 3.7~4.2 Mb locus on the short arm of
21 chromosome 5 was obtained and eight markers were developed based on this segment, while five of them were
22 closely linked to the early maturity trait loci. Moreover, 42 SSR markers were developed based on the reference
23 sequence of DM. Finally, a genetic map of chromosome 5 contained 50 markers was constructed using the
24 Tetraploidmap software. The total map length was 172 cM with an average genetic distance of 3.44 cM.
25 Combining with phenotypic data of the segregation population, we mapped the early maturity trait QTL with the
26 contribution of 33.55% on the short arm of chromosome 5, located at 84cM between the flanking markers
27 SSR5-85-1 and SCAR5-8 with the physical interval of 471kb. Gene annotation showed that there exist 34 genes
28 in this region, 12 of them are unknown function. Among the other 22 annotated genes, E3 ubiquitin ligase gene
29 *PUB14* may be related to maturity and regulate tuber formation. Our fine mapping of the early maturity QTL
30 made a solid foundation for cloning of the early maturity controlled gene or genes.

31 **Key words**：potato ·genetic segment · early maturity trait · marker development · QTL mapping

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Author contribution statement

GL and LJ conceived the project, provided idea and designed the whole experiments; XL and JZ conceived the project and performed the molecular marker development and analysis; JX contributed to the maturity phenotyping of the mapping population; SD performed the parents crossing of the mapping population; CB and JH performed the field management of potato materials. All authors read and approved the final manuscript.

Key message

Early maturity site was mapped using a tetraploid potato segregation population derived from cv. Zhongshu 19 and Zhongshu 3. One major QTL with 33.55% contribution to early maturity was fine mapped in physical interval of 471kb on chromosome 5.

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62 **Introduction**

63 Potato (*Solanum tuberosum* L.) is the third largest food crop worldwide and also serves as an important
64 industrial raw material. Maturity is one of the important features for identifying the characteristics of potato
65 varieties. It is also an important agronomic trait and a major breeding target. Different maturity varieties can
66 meet the production and consumption demand in different regions and seasons. The breeding of different
67 maturity varieties is greatly significant in the development of the potato industry. Previous studies showed that
68 potato maturity traits were controlled by minor polygenes and were recessive, and the potato maturity
69 quantitative trait loci (QTLs) were distributed on 12 chromosomes of potato. However, most of the studies have
70 mapped the major QTL for maturity on chromosome 5 and linked to the QTL for resistance to late blight (Van
71 Eck et al. 1995; Collins et al. 1999; Oberhagemann et al. 1999; Ewing et al. 2000; Draffehn et al. 2013). In 2007,
72 Sliwka et al. (2007) used a diploid potato population to map a QTL associated with growth stages and closely
73 linked to molecular marker BA47f2t7 (P1) on chromosome 5 (Sliwka et al. 2007). Later, scientist found that the
74 major QTLs that control maturity on chromosome 5 were closely related to the QTLs controlling late blight
75 resistance but were independent of each other and closely linked to molecular marker GP21 (Danan et al. 2011).
76 Using tetraploid materials, a major QTL was also obtained to be associated with maturity on chromosome 5,
77 which showed a contribution rate of 54.7% (Bradshaw et al. 2004). Later, by increasing the number of progeny
78 populations, some minor QTLs with contribution rates between 5.4% and 16.5% were identified (Bradshaw et al.
79 2008). In recent years, a large number of single nucleotide polymorphisms (SNPs) have been developed, and a
80 high-density genetic linkage map containing 3,839 SNPs has been constructed by analyzing dose information of
81 SNPs in tetraploid potato. One QTL associated with maturity closely linked to molecular marker c2_476095 has
82 also been mapped on chromosome 5, with a contributing rate of 55% (Hackett et al. 2014). In 2013,
83 Kloosterman et al. (2013) through fine mapping cloned the *StCDF1.2* gene related to potato maturity near
84 molecular marker GP21 on the short-arm of chromosome 5. This gene belongs to the DNA-binding One Zinc
85 Finger transcription factor family, which involves in tuber formation as an intermediate regulatory factor in the
86 potato tuber induction pathway.

87 Tetraploid potato (2n = 4x = 48) is highly heterozygous, with high frequency of genetic recombination and
88 severe recession after self-fertilization (Manrique-Carpintero et al. 2018). The molecular marker is difficult to
89 develop. The diploid potato chromosome number is small, the genetic background is relatively simple, and it is
90 easy to carry out genetic analysis and operation. Therefore, the development of potato markers and genetic map
91 construction is mainly at the diploid level, and is done mainly with the use of the traditional QTL mapping
92 analysis method, whereas fewer studies are conducted on tetraploid materials. Although most of the studies have
93 mapped QTLs on chromosome 5, there are no more genes related to potato physiology maturity are cloned
94 except for *StCDF1.2* gene.

95 With the development of high-throughput sequencing technology, marker development and genetic segment
96 mining in complex genomic species becomes simple. The high-throughput simplified genome sequencing
97 2b-RAD technique uses DNA type II B restriction endonucleases (*BsaXI* and *AlfI*) to cleave DNA from the
98 upstream and downstream sites of the target site on genomic DNA to obtain DNA fragments of consistent length
99 (tags). The 2b-RAD technique avoids the process of fragment size selection in other genome-wide sequencing

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100 technologies, making tags more uniform in the genome, allowing for the development of the complex genomic
101 marker, mining of chromosomal segments, and construction of linkage map and genetic variation map in natural
102 populations (Wang et al. 2012). At present, this technique has been applied to construct high-density genetic
103 linkage maps of animals, plants, and marine organisms. The high-density genetic linkage map of Chlamys Farrer
104 includes 3,806 molecular markers, the average distance between markers is 0.41 cM with genome coverage of
105 99.5%, and is it mapped to growth-and-sex-related QTLs (Jiao et al. 2013, 2014).

106 In this study, we intend to use the tetraploid potato maturity segregation population, combine
107 high-throughput simplified genome sequencing 2b-RAD with the BSA method, to identify the genetic segment
108 related to the early maturity trait and develop molecular markers based on the genetic segment. Linkage Map
109 was constructed by using software TetraploidMap for Windows (Hackett et al. 2007), which was designed
110 for calculating linkage maps from the maker phenotypes of the parents and segregating progeny of a cross
111 in an autotetraploid species. Combined with 4 years foliage maturity phenotypic data, QTL analysis was
112 performed using this software, which will provide a foundation for cloning and functional verification of the
113 maturity gene or genes.

114 **Materials and methods**

115 **Plant materials**

116 The mapping population, consisted of 221 individuals, was from a cross between two tetraploid varieties：
117 'Zhongshu19'× 'Zhongshu 3'. The female parent 'Zhongshu 19' with a pedigree of (CIP92.187×CIP93.154)
118 ×(Bierma ×Colmo) is a late maturing variety with a growth period of 110 days after emergence, and the male
119 parent 'Zhongshu 3' with a pedigree of (Jingfeng 1×BF77A) is an early maturing variety with a growth period
120 of 75 days. They were bred by the Institute of Vegetables and Flowers, Chinese Academy of Agricultural
121 Sciences, Beijing.

122 **Field tests**

123 During four consecutive years (2014-2017), the field experiment was carried out in Zhangbei (ZB) County of
124 Hebei Province (41˚15' N, 114˚07' E, 1500 m a.s.l.) and Liaocheng(LC) City of Shandong Province (36˚45' N,
125 115˚97' E, 38 m a.s.l.) in China. The mean daily temperature ranged from 10.05 ˚C to 21.66 ˚C and 12.59 ˚C to
126 24.37 ˚C corresponding to the average minimum and maximum temperatures. The average sunshine duration is
127 8.54 h and 6.45 h respectively. Four tubers of each progeny were sown in early May and mid-March and
128 harvested in late September and late June in ZB and LC respectively in each year.

129 **Phenotypic identification**

130 Growth period was defined the time from emergence (seedling emergence) to physiological maturation (50% of
131 plant leaves appear yellow) in this study. Individual emergence was investigated every five days from 20 days
132 after sowing. Physiological maturation time of the individuals was investigated every five days from 60 days
133 after emergence (DAE), continued to investigate every five days, and finally calculated the growth period of
134 each individual.

135 The maturity of the F1 population was divided into five groups according to the growth period, and scored from
136 one to five scales. 1: very early mature type with a growth period less than 70 DAE; 2: early mature type with a

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137 growth period 71-80 DAE; 3: middle mature type with a growth period 81-100 DAE; 4: late maturity type with
138 a growth period 101 -110 DAE; 5: very late mature type with a growth period more than 110 DAE.

139 **DNA extraction and progeny mixed pool construction**

140 Two-hundred twenty-one samples were collected from young leaves of Zhongshu 3, Zhongshu 19, and F1 plants.
141 The genomic DNA was extracted by the CTAB method, and DNA quality was detected with 1% agarose gel and
142 BioDrop for marker development and validation.
143 We used a punch to take one young leaf from the same part of each F1 generation early and late maturing
144 material with a growth period of less than 70 DAE and greater than 110 DAE, respectively, mixed them in equal
145 amount, extracted their DNA, and constructed an early and late maturing mixed DNA pool for simplified
146 genome sequencing. The DNA extraction method and quality testing method are the same as mentioned above.

147 **Simplified genome sequencing analysis and marker development**

148 A high-throughput simplified genomic 2b-RAD sequencing technique was used to construct a tag sequencing
149 library of parental DNA and extreme progeny DNA pool. The single-terminal sequencing was performed on the
150 Hiseq 2500 v2 platform. After removing from original reads of the sequences that contain no *BsaXI* recognition
151 sites, low quality sequences and sequences with more than 10 consecutive identical bases, the individual high
152 quality reads were mapped to the potato DM reference sequence using SOAP software. The number and depth
153 of specific tags that can be used for typing were obtained, and genome-wide SNP screening and typing was
154 conducted. According to the classification results, we constructed a chromosome tag density distribution map of
155 four samples with Matlab software, further, constructed specific tag density distribution and absolute value
156 distribution of the specific tag density between the two mixed pools, respectively, and then screened out
157 chromosome segments with a large difference in tag densities.
158 Based on the tag information obtained in the different segments, the synthetic primers were designed
159 according to the genomic sequences on the potato genome sequence website
160 (http://solanaceae.plantbiology.msu.edu/cgi-bin/gbrowse/potato/). The quality and specificity of the primers
161 were detected by using the genomic DNAs of the late maturing parent Zhongshu 19 and early maturing parent
162 Zhongshu 3 as the templates. The amplified products were detected by agarose gel electrophoresis with a
163 concentration of 1.2%. The PCR reaction system consisted of: 5.6 μL ddH$_2$O, 1.0 μL buffer (10 × PCR), 0.8 μL
164 dNTPs (10 mmol L^{-1}), 0.2 μL forward primer (10 μmol L^{-1}), 0.2 μL reverse primer (10 μmol L^{-1}), 0.2 μL Taq
165 enzyme (2.5 U μL^{-1}), and 2 μL DNA (25 ng μL^{-1}). PCR was performed as follows: 94°C for 3 min, followed by
166 35 cycles of 94°C for 30 s, 59°C for 30 s, and 72°C for 50 s, and finally, 72°C for 10 min.
167 The PCR products were digested by *BsaXI* restriction endonuclease if the primers could amplify clear and
168 non-different bands in the parents (Zhongshu 3 and Zhongshu 19). The enzyme digestion system was 8 μL PCR
169 product, 1.5 μL CutSmart® buffer, 0.2 μL *BsaXI* endonuclease (0.5 μL/U), and 5.3 μL ddH$_2$O. The primers
170 whose PCR products can be digested only in one parent and not digested in the other parent were used to
171 develop CAPs markers. The primers that amplified a band in one parent but not in the other parent, or amplified
172 different size bands in the two parents, were used to develop SCAR markers. Finally, the progeny early and late
173 maturing materials were used for validation.

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174 **Genetic map construction and QTL mapping**
175 Polymorphism screening of the molecular markers developed in this study and the previously published 152
176 SSR markers on chromosome 5, was conducted using the genomic DNAs of Zhongshu 3, Zhongshu 19, and 16
177 F1 generation materials as the templates (Feingold et al. 2005; Ghislain et al. 2009; Zhou, 2014), and the
178 selected polymorphic markers were verified using the population of 221 progenies. According to the
179 requirement of tetraploid mapping software TetraploidMap (Hackett et al. 2007), we selected the following
180 types of makers for map construction: (1) simplex dominant markers (segregating 1:1) with a *p* value greater
181 than 0.001 from a Chi-square test for goodness of fit; (2) duplex dominant makers (segregating 5:1)with a *p*
182 value greater than 0.01; (3) double-simplex dominant makers (segregating 3:1) with a *p* value generally greater
183 than 0.01 and which were known to be linked to at least one simplex marker; While dominant makers present in
184 both parents are extremely uninformative about recombination unless the segregation ratio is 3:1.(Hackett at al.
185 2007). So makers with the segregation ratio is 11:1 (simplex × duplex)， were in fact omitted from the linkage
186 map. Because of all makers we chose all from chromosome 5, so most of makers were selected to construct
187 linkage map for chromosome 5, except for a few makers that may belong different linkage group since add them
188 the software would show an error in subsequent QTL analysis. After the makers are selected, the TetraploidMap
189 ordered them for proper sequence and then we assigned 50 makers to four homologous chromosomes
190 considering with the relationship between makers is couple or repulsive (Simonsen and McIntyre. 2004). The
191 primer information for makers in this research are listed in Table 1. And genotypes information for 221
192 progenies are listed in Table S1. Consequently, combination with the mean value of 4 years progeny phenotype
193 data, details see Table S2. the maturity QTL was mapped by using TetraploidMap.
194 **Data availability**
195 The authors state that all data necessary for confirming the conclusions presented in the manuscript are
196 represented fully within the manuscript and supplemental information at
197 Figshare: https://figshare.com/s/876e1539f3f42b8ec8ef . TetraploidMap for Windows is freely available at:
198 http://www.bioss.ac.uk/ (user-friendly software).

199 **Results**
200 **Distribution of the segregation population maturity**
201 In ZB, the materials were planted on 20th May and emerged in mid-June. Part of the genotypes reached
202 physiological maturity in late August, while others were still keep vigorous in late September. In LC, the
203 corresponding date above were 10th March, mid-April and late June respectively. According to the field
204 investigation in the four consecutive years, the statistical analysis of the population for maturity trait was
205 consistent with polygenic genetic characteristic of quantitative traits. The offspring showed a normal distribution
206 over the entire scoring range (Fig. 1). Detail phenotypic data among four years was shown in Table S1. Genomic
207 DNA was extracted from 35 very early and early maturing genotypes and 33 very late maturing genotypes, and
208 the corresponding early maturing DNA pool and late maturing DNA pool were constructed.

209 **Screening and marker development of early maturity traits**
210 Simplified genome sequencing on four samples of parents Zhongshu 3 and Zhongshu 19, the early maturing
211 pool, and the late maturing pool were carried out using the 2b-RAD technique, and a total of 52,714,218 reads

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212 were obtained. The average number of reads of each sample was 13,178,554 with an average sequencing depth
213 of 42× (Table 2). High-quality reads containing *BsaXI* digestion sites in the four sequencing libraries were all
214 higher than 90%, and finally 125,556 unique tags in average of each sample were obtained.
215 SNP marker typing was performed according to the obtained sequencing tag sequences and the tag
216 distribution and density profiles on 12 potato chromosomes were got, and the results showed that the tags were
217 evenly distributed on all 12 potato chromosomes, and there was no large-scale tag information missing (Fig. 2).
218 Furthermore, according to the specific tag density of early maturing and late maturing pools, the density
219 difference map of the specific tag between early maturing and late maturing pools was obtained, and the results
220 showed that the segments with a large difference in tag density between early and late maturing pools were
221 mainly concentrated on chromosomes 4 and 5, where in the segment with the most significant difference in tag
222 density was found at 3.68–6.19 Mb on chromosome 5, followed by 18.6–20.9 Mb and 27.4–30.3 Mb on
223 chromosome 4 (Fig. 3). Moreover, the specific tag density of the early maturing pool among the differential
224 segments of chromosome 5 was relatively high, suggesting that this segment may be related to the early
225 maturity trait. But all the specific tag densities of the late maturing pool were relatively high among the
226 differential segments of chromosome 4, which might be related to the late maturity trait (Fig. 3).
227 Based on the above specific tag positions of chromosomal differential segments, combined with potato
228 reference genomic sequences, a total of 92 primers were designed with 52 pairs of primers in the 3.68–6.19 Mb
229 region of chromosome 5 and 40 pairs of primers in the differential segments of chromosome 4. Twenty
230 polymorphic markers including 4 CAPS and 4 SCAR markers located on chromosome 5 were obtained from 92
231 designed primers. Further test on the F1 individual plants showed that only five markers of SCAR5-5, SCAR5-8,
232 CAPS5-3-2, CAPS5-21-2 and CAPS5-24 were closely linked to the early maturity trait. Polymorphic markers
233 on other chromosomes were not linked with maturity trait (data not shown), suggesting that only the genetic
234 segment on chromosome 5 was associated with the early maturity trait. The marker SCAR5-8 on chromosome 5
235 was used to validate 70 early and late maturing Chinese cultivars, and the results showed that the coincidence
236 rate of marker detection results and trait identification results was as high as 81.4% (Li et al., 2017), indicating
237 that this marker could be applied in potato molecular marker-assisted breeding.

238 **QTL mapping for early maturity trait**

239 In order to verify the accuracy of genetic segments for the early maturity trait that were mined, we constructed a
240 genetic linkage map of chromosome 5 and performed QTL mapping for the early maturity trait. First, early and
241 late maturing parents and their 16 progenies were used to test the polymorphism of the 152 pairs of SSR primers
242 on chromosome 5, and a total of 32 polymorphic SSR markers were obtained. Then the 32 polymorphic SSR
243 markers and the previous eight molecular markers (4 CAPS, 4 SCAR markers) developed on chromosome 5
244 were further used to test the F1 generation. In this study, we focused on early maturity, and the genetic linkage
245 map for early type parent 'zhongshu 3' was constructed (Fig 4). The map including a total of 50 makers, of
246 which 33 were simplex, 4 were duplex, and 13 were double simplex makers (TetraploidMap generate
247 automatically combined with the segregation ratio) (Table 3). The total length of the map coverage was 172 cM,
248 and the average distance between markers was 3.44 cM. The 50 markers of this map were four CAPS markers,
249 four SCAR markers, and 42 SSR markers, respectively. 42 SSR markers were consisted of 25 single markers

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250 and 17 polymorphic SSR markers. The 25 single markers were come from the division of 11 polymorphic SSR
251 markers, and they were SSR5-22-1, SSR5-22-2, SSR5-36-1, SSR5-36-2, SSR5-38-1, SSR5-38-2, SSR5-40-1,
252 SSR5-40-2, SSR5-40-3, SSR5-55-1, SSR5-55-2, SSR5-85-1, SSR5-85-2, SSR5-85-3, SSR5-85-4, SSR5-100-1,
253 SSR5-100-2, SSR5-103-3, SSR5- 103-4, PM0333-2, PM0333-3, STI049-2, STI049-3, STG0021-1, and
254 STG0021-2. Actually, among the 32 polymorphic SSR markers only 28 markers were used to construct the
255 genetic linkage map.

256 QTLs for foliage maturity type were identified using TetraploidMap. Initially, the significance of individual
257 markers for each year trait was tested by analysis of variance (ANOVA) and the analysis procedure
258 Kruskall-Wallis in TetraploidMap. This two tests were used to compare the differences of the mean value of
259 different makers genotype（Simonsen and McIntyre, 2004）. A P-value of less than 0.01 was used as a threshold
260 criterion for QTL detection. Results from above two tests suggested the existence of QTLs for foliage mature
261 type on chromosome 5. There are 22 makers on chromosome 5 may associated with maturity. The maker cluster
262 including maker CAPS 5-21-2, CAPS5-24, CAPS5-3-2，SCAR5-5，SCAR5-8 were closely linked to the QTL
263 locus, since not only their P value are 0.000 but also their smallest SED with 0.0937 (Table 3).

264 Furthermore, in combination with phenotypic identification data of F1 segregation population, three
265 significant QTLs for early-maturity trait loci were identified with LOD scores above the threshold value of 2.97
266 (The blue dotted line) located on chromosome 5 (Figure 5). The most remarkable QTL was mapped at 84 cM of
267 the 3rd homologous chromosome of chromosome 5 between marker SSR5-85-1 and maker cluster including
268 CAPS5-21-2，CAPS5-24，CAPS5-3-2，SCAR5-5，SCAR5-8, but more closely linked to the 5 markers, which
269 was in line with the position of the maturity genetic segment mined in this study. The maximum LOD value of
270 this QTL was 19.491 explaining 35.55% of the phenotypic variation. And there were other two locus may also
271 associated with maturity type, one QTL linked to marker STM5148 with the LOD value 12.50, another one
272 mapped at 113cM in correspondence of marker PM0263 with the LOD value nearly 11.30.

273 In conjunction with the marker locus and reference genome of the DM sequence, the QTL of early maturity
274 trait was mapped to the 471 Kb physical interval between the flanking markers of SSR5-85-1 and SCAR5-8,
275 which contained six molecular markers (Fig. 6). The order of all makers in this QTL region is consistent with
276 the physical order of the makers in the potato DM genome sequence. (PGSC. Tuberosum group Phureja DM1-3
277 Pseudomolecules (v4.03)). Gene prediction and annotation showed that there are 34 genes in this 471 Kb
278 physical range based on the comparison with the reference genome of DM sequence,
279 (http://solanaceae.plantbiology.msu.edu/pgsc_download.shtml), of which 22 genes have been annotated and 12
280 genes functions are unknown (Table 4). The 22 annotated genes have different physiological functions and
281 participate in different physiological regulation pathways. For example, E3 ubiquitin ligase PUB14 is involved
282 in the photoperiodic regulatory pathway. The auxin export carrier is involved in plant hormone transport. Heat
283 shock protein binding, Quinolinate phosphoribosyl transferase, phosphatidylinositol kinase fyv1, resistance
284 protein BS2, and resistance protein PSH-RGH6 are involved in plant defensive reactions. The WD repeat
285 protein is involved in protein transport and nucleic acid processing modification. The Myb transcription factor is
286 involved in the plant secondary metabolic regulatory pathway.

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287 **Discussion**

288 **Genetic segments mining for early maturity trait based on 2b-RAD simplified genome sequencing**

289 At present, many researchers have used traditional molecular marker technologies to carry out potato QTL
290 mapping. Most of the studies mapped maturity major genetic loci on chromosome 5 and found that other
291 chromosomes may also have maturity micro-genetic sites. In this study, for the first time, we used the
292 high-throughput simplified 2b-RAD sequencing technique to identify and mark genetic segments for potato
293 complex quantitative traits, found a segment with large difference in specific tags on chromosome 4 and 5, and
294 further tag validation showed that the genetic segment on chromosome 5 is associated with the early maturity
295 trait, which is consistent with previous studies (Collins et al. 1999; Oberhagemann et al. 1999; Ewing et al. 2000;
296 Bradshaw et al. 2004, 2008; Danan et al. 2011; Hackett et al. 2014).

297 Tetraploid potato has a complex genetics, and molecular marker development is difficult. Previously
298 developed markers were mainly based on diploid (Bakker et al. 2004; Asano et al. 2012; Zhu et al. 2015;
299 Hara-Skrzypiec et al. 2018), but markers developed directly at the tetraploid level were rarely reported. This
300 study based on the maturity-related genetic segments mined on chromosome 5, a total of 52 pairs of specific
301 primers were designed and synthesized, and eight molecular markers were developed with a development
302 efficiency of 15.4%. The eight markers were further verified by early and late maturing progenies in the
303 tetraploid segregation population, five markers were found to be closely linked to the early maturity trait.
304 Therefore, the method can be used for the development of markers of complex genomes such as potato.
305 Combined with published potato reference genomes, this method can greatly shorten the marker development
306 cycle and map the markers. Which will also provide a reference for marker development of other polyploid
307 complex genomic organisms.

308 In this study, we found that not only was there a maturity related genetic segment on chromosome 5, but there
309 were also some segments with large difference in specific tag density on chromosome 4. Although there are a
310 few reports that mention the existence of a maturity-related genetic segment on the chromosome 4, the tag
311 validation results in this study showed that the genetic segment on chromosome 4 was not related to maturity.
312 This may be due to several reasons: First of all, the tetraploid potato genome is highly heterozygous, more
313 complicated than diploid potato in heredity, and in the high-throughput sequencing process, may be due to
314 sequencing depth not being enough or *BsaXI* recognition sequence preferences; some information was not
315 measured. Secondly, due to the filtering out some of the low quality reads resulted in partial loss of genetic
316 information, and eventually the differential tag interval appears. Thirdly, when performing specific tag filtering,
317 removing the tags with fewer occurrences may also cause a difference in tag density.

318 **Genetic linkage map construction and QTL mapping**

319 In this study, a 50-marker genetic linkage map was constructed using tetraploid potato segregation population,
320 and an early maturity trait QTL was mapped at the position of 84 cM near the marker SCAR5-8 (or SCAR5-5,
321 CAPS5-3- 2, CAPS5-24, CAPS5-21-2) on the short arm of chromosome 5. Which indicates that the QTL for the
322 early-maturity trait obtained by genetic map mapping is consistent with the maturity genetic segment results
323 obtained by high-throughput simplified genome sequencing, and also proved the feasibility and accuracy of
324 high-throughput simplified genome sequencing 2b-RAD method in mining potato genetic segments for

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325 important traits. The five molecular markers (SCAR5-8, SCAR5-5, CAPS5-3-2, CAPS5-24, CAPS5-21-25) that
326 are closely linked to the early maturity trait loci are from the same genetic segment and are relatively close to
327 each other, and no significant separations between the markers in the genetic map. Furthermore, the five
328 molecular markers were clustered on the 3rd homologous chromosome and belonged to the same linkage group.
329 The other three molecular markers, CAPS5-16, SCAR5-18, SCAR5-25 probably linked to the late maturity trait
330 loci, were clustered on the 1st homologous chromosome, and belonged to another linkage group (Fig. 4).
331 Previous studies on the maturity QTL mapping showed that the genetic interval of maturity QTL was
332 relatively large. The major QTL for maturity was mapped at 0–6 cM on chromosome 5 in diploid materials, and
333 the genetic distance between two flanking markers was 6 cM (Visker et al. 2003). In tetraploid materials, the
334 major QTL for maturity was also mapped at 14–22 cM on chromosome 5, and the genetic distance between two
335 flanking markers was 8 cM (Hackett et al. 2014). In this study, based on simplified genome genetic segment
336 mining and marker development, the QTL for the early maturity trait was mapped in a physical interval of 471
337 kb, which greatly approached the early maturity trait loci and the average distance between two makers was 3.44
338 cM. Bioinformatics analysis revealed that there were a total of 22 annotated genes in the 471kb region, and the
339 E3 ubiquitin ligase PUB14 gene was most likely related to potato maturity. In Arabidopsis, this gene regulates
340 flowering of *Arabidopsis thaliana* (Ma Da et al. 2014). Among the photoperiod, it regulated flowering pathways
341 that currently have been discovered, a variety of components are E3 ubiquitin ligase target proteins and can be
342 mediated by E3 ubiquitin ligase to achieve its ubiquitination degradation, thus affecting the photoperiod signals
343 for flowering regulation and may affect photoreceptor stability, circadian clock function, and flowering regulator
344 CO stability (Chen et al. 2011; Imaizumi et al. 2003; Takase et al. 2011; Ma Da, et al. 2014). Photoperiod has a
345 great influence on potato plant growth, tuber formation, and development. Under long daylight, stems and
346 leaves grow vigorously, a large number of stolons appear, but the tuber formation is delayed and the yield is
347 decreased. Under short daylight, plant growth is normal, and the tuber production is faster. Assimilation
348 products are transported to tubers faster, thus tuber yield is high. Early maturing varieties are sensitive to
349 daylight, while late maturing varieties have to form tubers under short daylight conditions. Therefore, the signal
350 transduction pathway of potato flowering and tuber formation may be similar to that of *Arabidopsis thaliana*
351 flowing regulation. Thus, E3 ubiquitin ligase PUB14 may act as an intermediate mediator during potato
352 flowering or tuber formation, regulating flowering and tuber formation. In addition, the auxin export vector gene
353 may also be involved in potato maturity. Auxin participates in the regulation and control of many physiological
354 and biochemical processes, such as root occurrence, photoreaction, apical dominance, flowers development,
355 leaves and fruits shedding, and distribution of assimilation products. In the process of auxin transport, this
356 protein, as a specific auxin output vector, can induce IAA passively flowing to the cell wall, then enter into next
357 cell, thus forming polar transport. In the late growth and development of potato, this gene may be involved in
358 assimilation product distribution as well as leaf and fruit shedding, thus plants show early maturation. However,
359 whether these two genes are related to maturity needs further analysis and validation. In this study, we also
360 examined the segregation of the maturity-related gene *StCDF1* (Kloosterman et al. 2013) in the population of
361 this study, and the results showed that the late maturing parent Zhongshu 19 contained the gene *StCDF1.1* (late
362 maturity related gene) and *StCDF1.2* (early maturity related gene), the early maturing parent Zhongshu 3 only
363 contained *StCDF1.1*, both the parents do not contained *StCDF1.3* (early maturity related gene), and also there

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364 was no polymorphism in our segregation population. Moreover, we also test the *StCDF1.2* gene in 83 Chinese
365 varieties, and the result showed that only 4 out of 36 early maturity varieties and 5 out of 47 late maturity
366 varieties contained the early maturity related gene *StCDF1.2* (data not shown). Moreover, the alignment of
367 *StCDF1* with the reference genome showed that the *StCDF1* was located in 4.537-4.542Mb, but the maturity
368 locus in our study is located in 3.7-4.2Mb. Pedigree investigation showed that the *StCDF1.2* gene was cloned
369 from CE3130 that should originated from phureja (Kloosterman et al., 2013), the early maturity genes of
370 Zhongshu 3 maybe come from cv. Katahdin. All the above indicated that there should exist other different
371 maturity-related genes in our maturity segregation population of this study from the gene *StCDF1*.

372 The tetraploid mapping software TetraploidMap (Hackett et al. 2007) used in this study only can identify
373 three marker types. For a single dominant marker, the parental genotype is AOOO × OOOO, and the segregation
374 ratio of progeny is 1:1. For double dominant markers, the parental genotypes are AAOO × OOOO and AOOO ×
375 AOOO, and the segregation ratios are 5:1 and 3:1. There are still two types of markers the software does not
376 recognize, i.e. markers of parental genotypes AOOO × AAOO and AAOO × AAOO with the segregation ratios
377 of 11:1 and 35:1. Therefore, among the 32 polymorphic markers selected from 152 SSR markers, four markers
378 belonged to the 11:1 marker type, and the software did not recognize them. Therefore, only 28 polymorphic SSR
379 markers could be used in the study to construct the map. Because the tetraploid mapping software limits the
380 recognition of marker types, some markers are not available, and made the number of markers less than normal
381 in the genetic map. Nevertheless, we have mapped the QTL for the early maturity trait in the physical region of
382 471 kb and analyzed the genes within this interval. Which provides a solid foundation for the cloning of the
383 major gene or genes that control the early maturity trait.
384

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388 **Compliance with ethical standards**

389 **Conflict of interest** Authors declare that they do not have conflict of interest.
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Table Captions

Table 1 Information of the 50 primers used in the genetic map

Table 2 Basic analysis of sequencing data

Table 3 Makers associated with physiological maturity KWSig: the significant of Kruskal-Wallis test. AVSig: the significant of the analysis of variance. Mean (0):the mean when the maker is absent. Mean (1): the mean when the maker is present. SED: the stand error of difference between the means.

Table 4 Gene annotation and location in the physical interval

Table S1 Phenotypic data 2014-2017

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Figure Captions

Fig. 1 Maturity frequency distribution of the mapping population individuals. X-axis indicates maturity type of the mapping population, Y-axis indicates the ratio between individuals of different maturity types and the whole mapping population. The entire mapping population consists of 221 individuals.

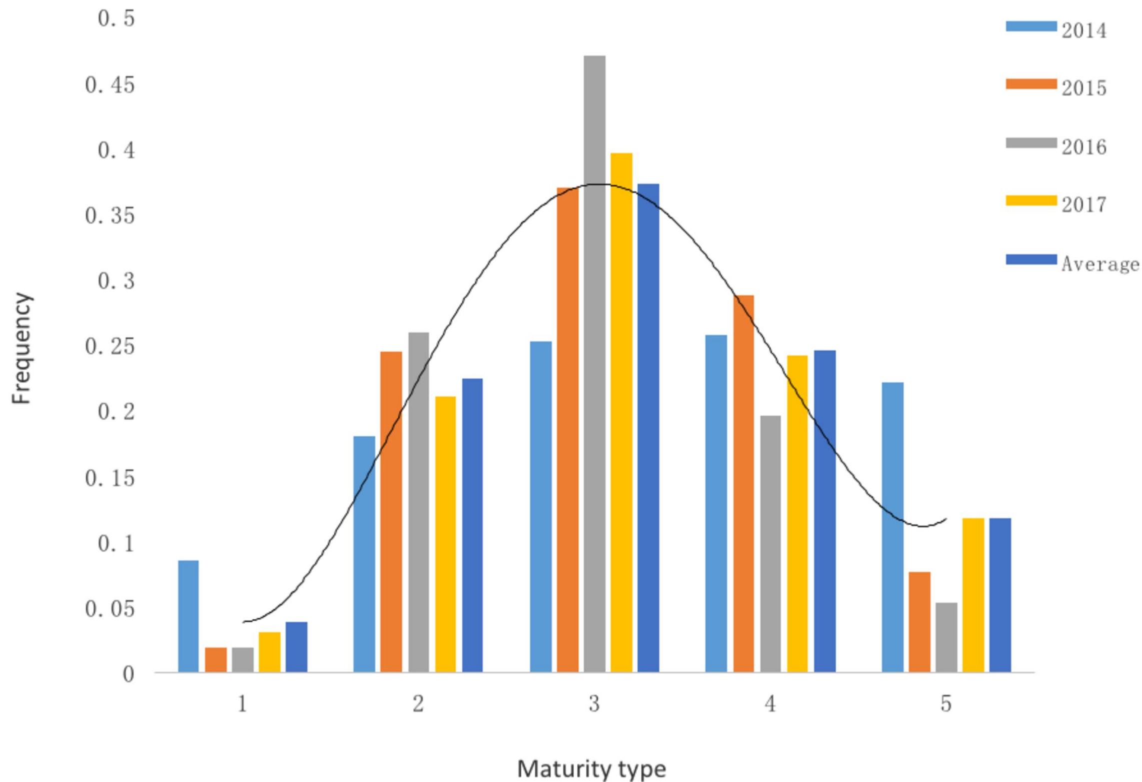
Fig. 2 Distribution of tags on the chromosome and tag density map. 0-80 M: chromosome physical distance (unit: M). Chr1-Chr12: 12 potato chromosomes, respectively. Right histogram: different colors indicate different tag numbers, and numbers indicate specific tag numbers. In each chromosome, the upper long histogram shows the tag density distribution on the chromosome, and the long histogram below shows the tag distribution on the chromosome.

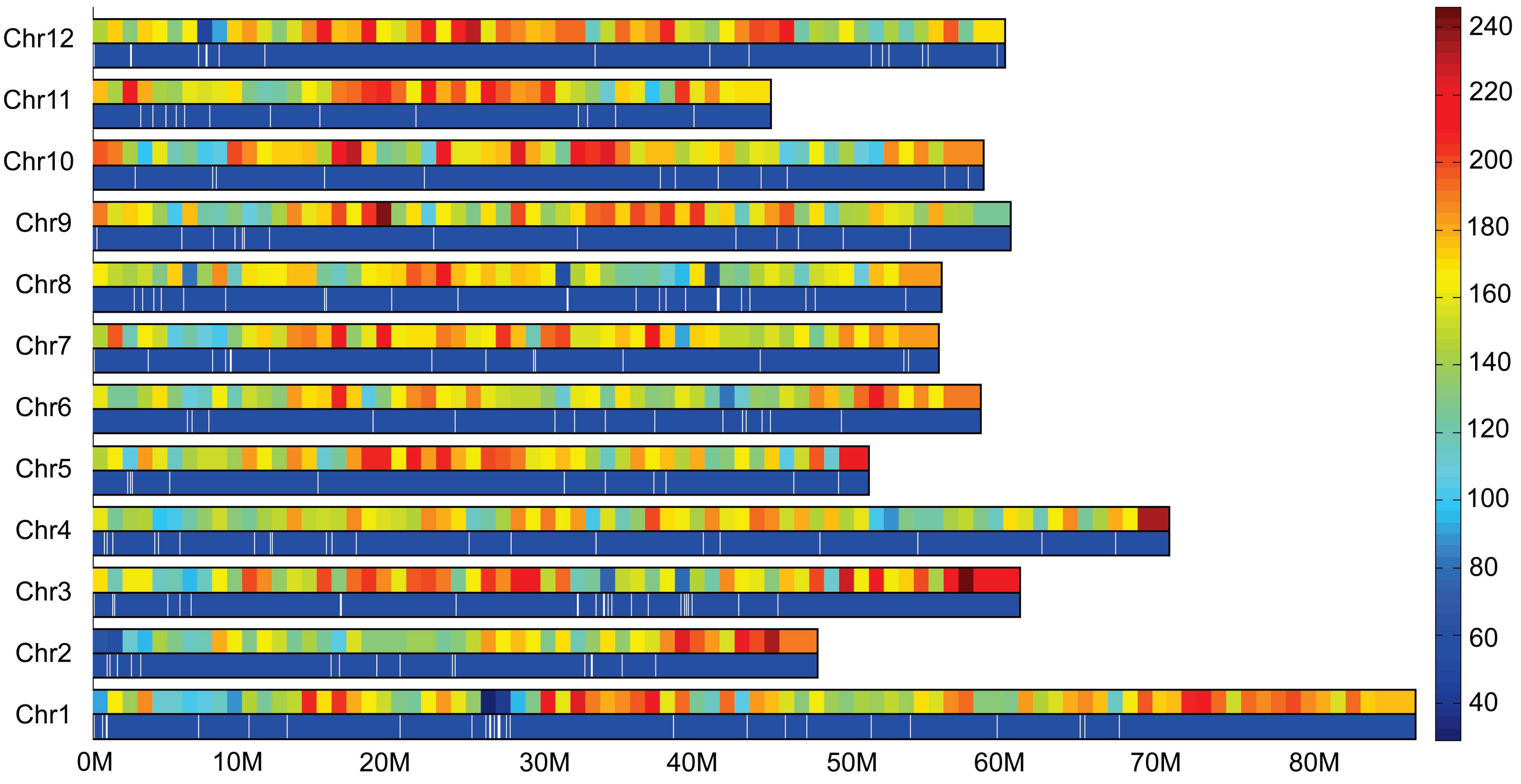
Fig. 3 Differential tag density map of early and late maturing mixed pool. 0-80 M: chromosome physical distance (unit: M). Chr1-Chr12: 12 potato chromosomes, respectively. In each chromosome, the upper long histogram shows the specific tag density of the early maturing pool, the middle long histogram shows the specific tag density of the late maturing pool, and the long histogram below shows the absolute value of differential tag density between the two pools. Right histogram, different colors indicate different tag numbers, and numbers indicate the specific tag numbers.

Fig. 4 Genetic map of chromosome 5 in potato. The left side of the map is 50 molecular markers. The numbers on the right indicate the corresponding genetic distances (unit: cM). Overall: chromosome 5. C1-C4: represent four homologous chromosomes of chromosome 5, respectively.

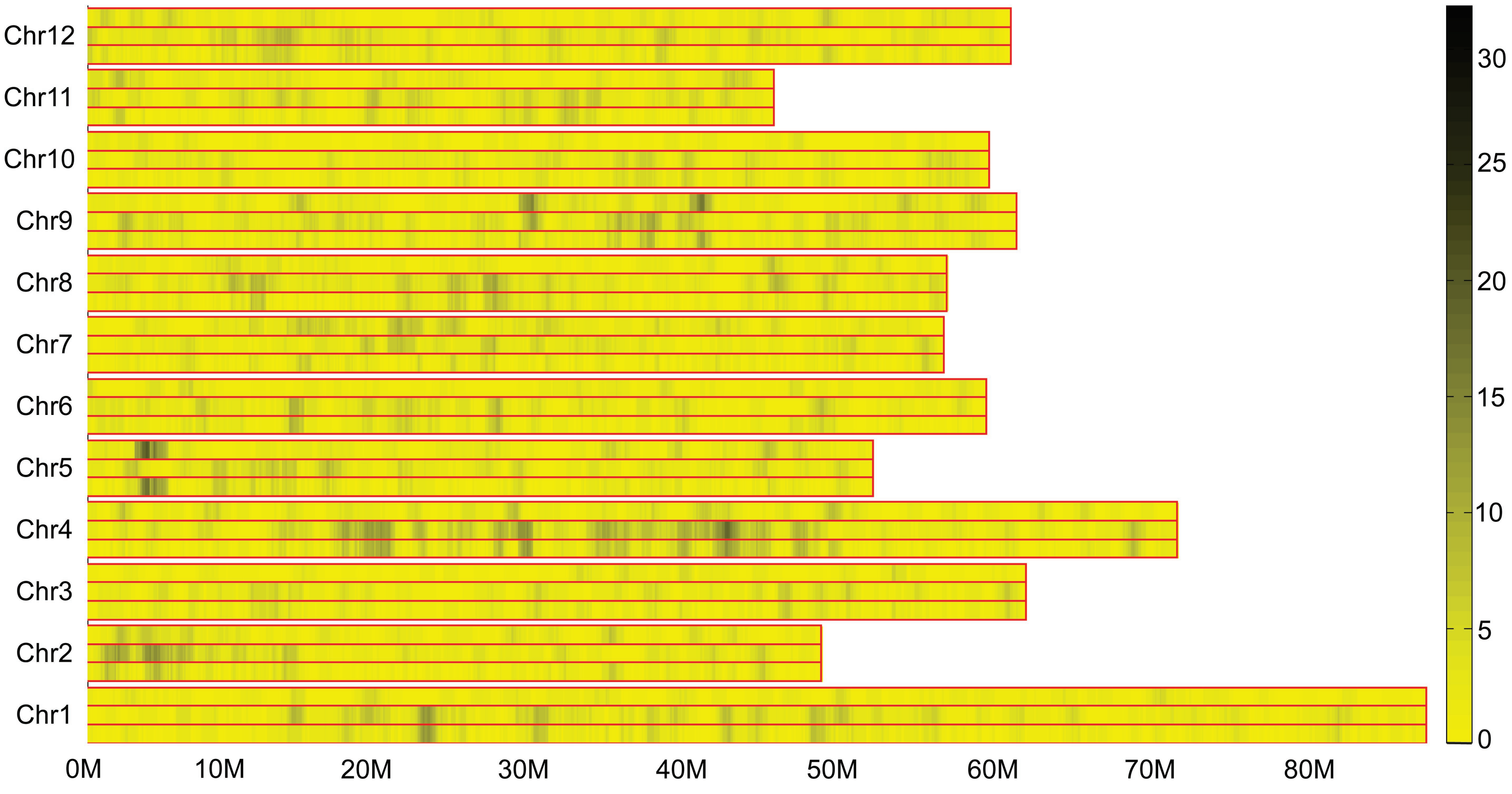
Fig. 5 QTL mapping for Maturity. Line shows LOD values over the chromosome 5. The horizontal line indicates the confidence threshold (LOD value =2.97).

Fig. 6 Physical interval of the early maturity QTL on chromosome 5 . The left side is genetic linkage map of 3rd homologous chromosome 5, and the right one is corresponding physical map.


0.5
0.45
0.4
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
Frequency
1
2
3
4
5
Maturity type
2014
2015
2016
2017
Average



Chr12
Chr11
Chr10
Chr9
Chr8
Chr7
Chr6
Chr5
Chr4
Chr3
Chr2
Chr1
0M
10M
20M
30M
40M
50M
60M
70M
80M
240
220
200
180
160
140
120
100
80
60
40


Chr12
Chr11
Chr10
Chr9
Chr8
Chr7
Chr6
Chr5
Chr4
Chr3
Chr2
Chr1
0M
10M
20M
30M
40M
50M
60M
70M
80M
0
5
10
15
20
25
30

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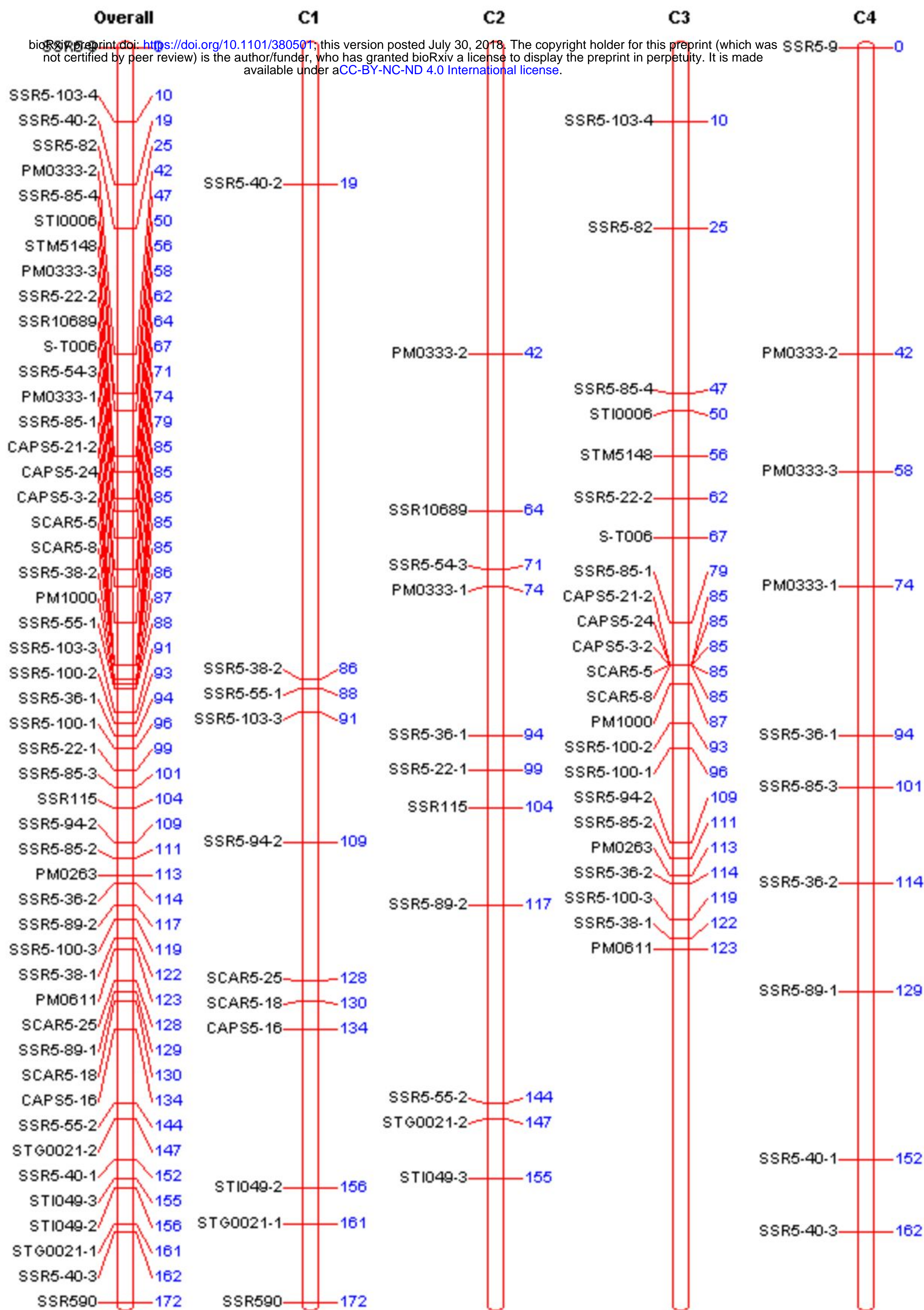
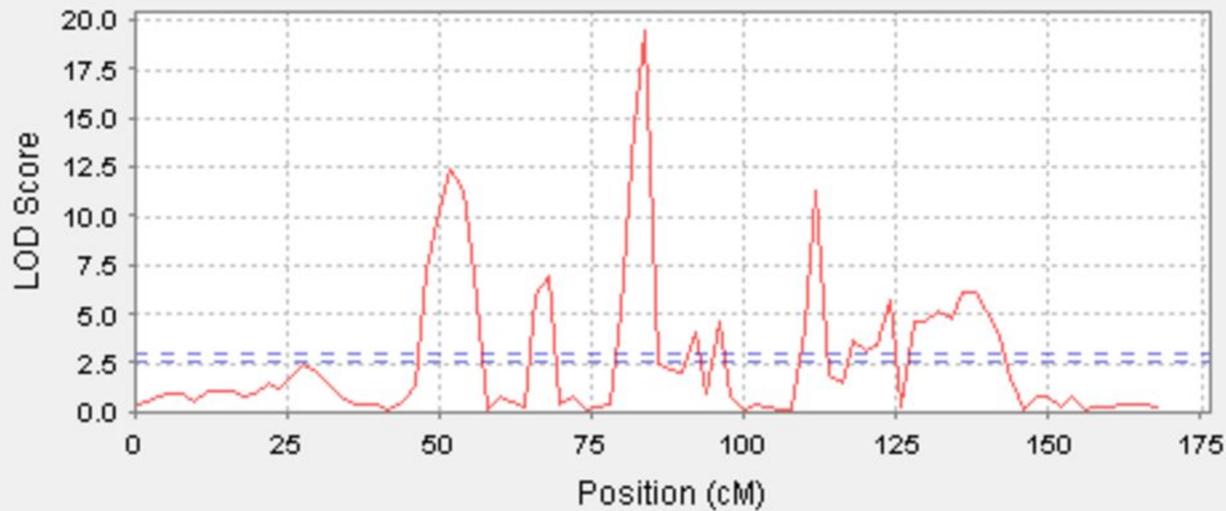
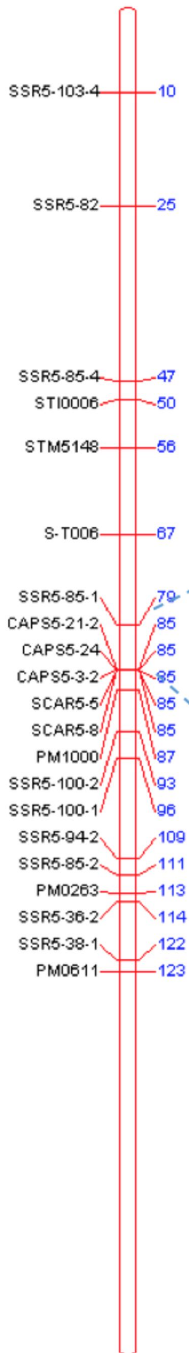




20.0
17.5
15.0
12.5
10.0
7.5
5.0
2.5
0.0
LOD Score
0
25
50
75
100
125
150
175
Position (cM)


C3
SSR5-103-4 10
SSR5-82 25
SSR5-85-4 47
STI0006 50
STM5148 56
S-T006 67
SSR5-85-1 79
CAPS5-21-2 85
CAPS5-24 85
CAPS5-3-2 85
SCAR5-5 85
SCAR5-8 85
PM1000 87
SSR5-100-2 93
SSR5-100-1 96
SSR5-94-2 109
SSR5-85-2 111
PM0263 113
SSR5-36-2 114
SSR5-38-1 122
PM0611 123

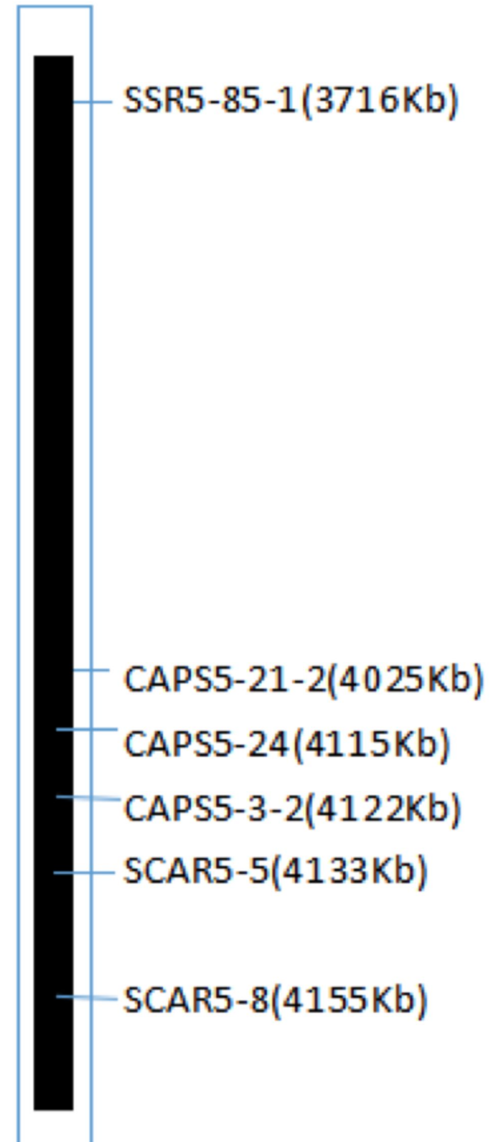

SSR5-85-1(3716Kb)
CAPS5-21-2(4025Kb)
CAPS5-24(4115Kb)
CAPS5-3-2(4122Kb)
SCAR5-5(4133Kb)
SCAR5-8(4155Kb)

Table4. Gene annota

Start position	Terminal position	Gene
3794799	3797561	PGSC0003DMG400030514
3800502	3803746	PGSC0003DMG400030561
3804570	3804947	PGSC0003DMG400042076
3808243	3812259	PGSC0003DMG400030560
3812966	3817781	PGSC0003DMG400030559
3819129	3825291	PGSC0003DMG400030513
3826477	3835460	PGSC0003DMG400030558
3846875	3847435	PGSC0003DMG400035916
3855942	3861025	PGSC0003DMG40003051
3872716	3876866	PGSC0003DMG400030557
3897559	3898635	PGSC0003DMG400030556
3903471	3909624	PGSC0003DMG400030510
3927363	3930287	PGSC0003DMG400030509
3943033	3943669	PGSC0003DMG400030508
3945081	3955925	PGSC0003DMG400030507
3960120	3964565	PGSC0003DMG400030555
3966729	3968667	PGSC0003DMG400030554
3969058	3970107	PGSC0003DMG400030506
3975677	3980357	PGSC0003DMG400030553
3991348	3993998	PGSC0003DMG400030552
4009033	4012803	PGSC0003DMG400030551
4016212	4017445	PGSC0003DMG400030505
4025454	4033006	PGSC0003DMG400030550
4033954	4042260	PGSC0003DMG400030549
4046634	4053740	PGSC0003DMG400030548
4055474	4059978	PGSC0003DMG400030547
4062234	4063922	PGSC0003DMG400030504
4068242	4070295	PGSC0003DMG400030503
4083569	4086820	PGSC0003DMG400030546
4088794	4097482	PGSC0003DMG400030502
4097590	4098620	PGSC0003DMG400030582
4107529	4113266	PGSC0003DMG400030544
4111951	4118400	PGSC0003DMG400030501
4169120	4173221	PGSC0003DMG400030543

tion and location in the physical interval

Gene function
Histone chaperone ASF1A
HB06p
Gene of unknown function
ATP synthase subunit beta
Fruit protein PKIWI502
Sarcoplasmic reticulum histidine-rich calcium-binding protein
ALG2-interacting protein X
Conserved gene of unknown function
1Repressor of RNA polymerase III transcription MAF1
Membrane associated ring finger 1,8
Gene of unknown function
Conserved gene of unknown function
Ribose-5-phosphate isomerase
By genscan and genefinder
Abc transporter
Acetylglucosaminyl transferase
Pentatricopeptide repeat protein
FLA20
ATP binding
Chloroplast-targeted copper chaperone
Spore coat protein
Conserved gene of unknown function
Conserved gene of unknown function
Conserved gene of unknown function
Myb-like transcription factor 6
E3 ubiquitin ligase PUB14
Conserved gene of unknown function
Conserved gene of unknown function
Conserved gene of unknown function
Fyve finger-containing phosphoinositide kinase, fyv1
Gene of unknown function
WD-repeat protein
Quinolinate phosphoribosyl transferase
Gene of unknown function