The genetics and genome-wide screening of regrowth loci, a key component of perennialism in *Zea diploperennis*

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Classification:

Major: Biological Science

Minor: Plant Biology

Short Title: Regrowth loci in *Zea diploperennis*

Key Words: Perennial maize, teosinte, *Zea mays*, corn, genetics, perenniality, GBS

This manuscript was previously deposited as a preprint at http://dx.doi.org/10.1101/388256.

Abstract

Perennialism is common among the higher plants, yet little is known about its inheritance. To address this, six hybrids were made by reciprocally crossing perennial *Zea diploperennis* Iltis, Doebley & R. Guzman with inbred lines B73 and Mo17 and Rhee Flint, a heirloom variety, of *Z. mays* L. ssp. *mays*. All the F_1 plants demonstrated several cycles of growth, flowering, senescence and regrowth into normal flowering plants, indicating a dominant effect of the Z. *diploperennis* alleles. The regrowability (i.e. the plants' ability to regrow after senescence) was stably transmitted to progeny of the hybrids, so we focused on this trait. Segregation ratios in the F_2 generations are consistent with the trait controlled by two dominant, complementary loci, but do not exclude the influence of other modifiers or environment. Genome-wide screening with genotyping-by-sequencing (GBS) indicated two major regrowth loci, *regrowth 1* and *regrowth 2*, were on chromosomes 2 and 7, respectively. These findings lay the foundation for further exploration of the molecular mechanism of regrowth in *Z. diploperennis*.

Significance Statement: This study contributes to the general understanding of inheritance of perennialism in the higher plants. Previous genetic studies of the perennialism in *Zea* have yielded contradictory results. We take a reductionist approach by specifically focusing on one trait, the plant's ability to restart a new life cycle after senescence on the same body. While traits, such as rhizome formation, tillering and dormancy may be important to converting *Z. mays* to becoming truly perennial, understanding the conditions for regrowth after senescence will be substantial first step. Importantly, our data indicate that there is no major barrier to transferring

47 this trait into maize or other grass crops for perennial crop development with proper technology,
48 which enhances sustainability of grain crop production in an environmentally friendly way.

49 **Introduction**

50 Perennialism is the phenomenon that a plant can live for more than two years; the ability
51 of doing so is termed perenniality. Plants typically have a life cycle of growth, reproduction
52 (sexual and/or vegetative) and senescence. Annuals and biennials have only one such cycle in
53 their life, leaving behind seeds, bulbs, tubers, etc. to initiate another life cycle. Perennials
54 maintain juvenile meristematic tissues capable of regrowth after senescence to start a new life
55 cycle on the same body. How perennials do so remains as a mystery. Subterranean stems (such
56 as rhizomes), polycarpy and tuberous roots are often cited as the means by which plants achieve
57 perenniality. However, none of these traits is absolutely required by perennials. For instance,
58 bamboos are essentially monocarpic perennial that regrow from rhizomes. Many perennial
59 temperate grasses, such as switchgrass[1], cordgrass[2] and eastern gamagrass[3], regrow from the
60 crowns instead of rhizomes. On the other hand, some annual/biennial plants, such as radish
61 (*Raphanus sativus*), grow tuberous roots.

62 Although perennialism is common among higher plants, the study of its genetics and
63 molecular biology is sporadic. So far, the only published research in molecular mechanism of
64 plant perennialism was conducted in Arabidopsis. Melzer et al. successfully mutated this annual
65 herb to show some perennial habits, such as increased woody fiber in the stem, by down-
66 regulating two flowering genes coding for MADS-box proteins, SUPPRESSOR OF
67 OVEREXPRESSION OF CONSTANT 1 and FRUITFUL[4]. Unfortunately, this woody mutant
68 was sterile, and no follow-up research was reported. Perennial-related genes and quantitative loci
69 (QTL) have been reported in other species. Major QTL controlling rhizome development,

70 regrowth and tiller number have been mapped on sorghum linkage groups C (chromosome 1)
71 and D (chromosome 4)[5,6], which are homoeologous to regions of maize chromosomes 1, 4, 5 and
72 9, respectively[7]. Hu at al. mapped two dominant, complementary QTL *Rhz2* (*Rhizomatousness 2*)
73 and *Rhz3* that control rhizome production on rice chromosomes 3 and 4 at the loci homoeologous
74 to the sorghum QTL[6]. Tuberous roots in a wild perennial mungbean (*Vigna radiate* ssp.
75 *sublobata*) are conditioned by two dominant, complementary genes[8]. However, after years of
76 effort these perennialism-related genes have yet to be cloned from any of the species despite that
77 mapping data and complete genomic sequences of rice and sorghum are readily available.
78 Therefore, no further research has been reported about these perennialism-related loci/genes.
79 Recently, Ryder and the associates reported a set of 98 expressed contigs in Johnsongrass (*S.*
80 *halepense*) that are likely associated with rhizome development [9].
81 In the genus *Zea* L., most species, including maize (*Z. mays* ssp. *mays*), are annual.
82 However, two closely related species, tetraploid *Z. perennis* [Hitchc.] Reeves and Mangelsdorf
83 and diploid *Z. diploperennis* Iltis, Doebley & R. Guzman, are perennial. Perenniality of these
84 two teosintes is manifested as regrowth after seed production and senescence, which includes
85 developing juvenile basal axillary buds and rhizomes, under favorable environment. A fertile F_1
86 hybrid between *Z. mays* and *Z. perennis* was made by Emerson[10] in the 1920s, and *Z. mays*'
87 hybrids with *Z. diploperennis* were also obtained soon after the diploid perennial teosinte was
88 discovered in the 1990s[11]. Evergreen stalks, bulbils (highly-condensed rhizomes), basal shoot
89 development, stiff stalk and robust root system have all been cited as phenotypic features of
90 perennialism in *Z. diploperennis*[12-14]. For example, evergreen stalks, which was proposed as a
91 component of perennialism in Z. *diploperennis*[12], appears to be linked to *sugary 1* on the short
92 arm of chromosome 4[15].

93 Conflicting conclusions have been reached in various studies on how perennialism is
94 inherited in *Zea*. Shaver, who worked with tetraploid *Z. perennis*, proposed that a triple
95 homozygous recessive genotype is needed for the perenniality in *Zea*[16]. In this model, *pe*
96 (*perennialism*), interacting with *gt* (*grassy tillers*) and *id* (*indeterminate*), plays a key role in
97 conferring totipotency to the basal axillary buds and rhizomes in the perennial teosintes[16, 17]. The
98 nature of *pe* remains unknown and the *Z. perennis*-derived genotype from which *pe* was
99 identified by Shaver[16] was lost and never recovered despite decades of intensive efforts (Shaver,
100 personal communication). Mangelsdorf and Dunn mapped *Pe*-d,* the maize allele of the *pe*
101 homologue in *Z. diploperennis,* to the long arm of maize chromosome 4[18]. The *gt* gene (aka *gt1*),
102 located on the short arm of maize chromosome 1, encodes a class I homeodomain leucine zipper
103 that promotes lateral bud dormancy and suppresses elongation of lateral ear branches[15]. It appears
104 that *gt1* depends on the activity of a major maize domestication gene, *teosinte branched 1* (*tb1*),
105 and is inducible by shading[19]. The *id* gene (aka *id1*) alters maize's ability to flower[20]. Both *tb1*
106 and *id1* are located on the long arm of maize chromosome 1 and both encode transcription
107 factors with zinc finger motifs[19, 21]. Singleton believed that *id1* inhibits plantlet generation at the
108 upper nodes of a maize stalk[20]. Mangelsdorf and the associates proposed that one or two
109 dominant genes control annual growth habit in their *Z. diploperennis*-popcorn hybrid[22].
110 In contrast to the recessive inheritance model, Galinat proposed that perennialism in *Z.*
111 *diploperennis* is at least partially controlled by two dominant complementary genes[15]. Also, Ting
112 and Yu obtained three perennial F_1 hybrids by pollinating three Chinese field corn varieties with
113 *Z. diploperennis*[23], which indicate that perennial factors are dominant. Unfortunately, there is no
114 further report about these hybrids or their derivatives.
115 Westerbergh and Doebley regarded perennialism in *Z. diploperennis* as a quantitative

116 trait and identified a total of 38 QTL for eight perennial-habit traits from a *Z. diploperennis* x *Z.*
117 *mays* ssp. *parviglumis* (annual) mapping population[12]. Intriguingly, they did not identify any
118 QTL that shows a singularly large effect. Murray and Jessup indicated that non-senescence and
119 rhizomatousness are essential traits in their perennial maize breeding practice[24].

120 Perennialism appears to be a complex trait, strongly influenced by genetic and
121 environmental factors. A perennial plant in one environment usually cannot survive in another
122 due to the lack of the required adaptability. For example, *Z. diploperennis*, which is perennial in
123 the highlands of Mexico, cannot survive the harsh winter in the American Midwest. The various
124 criteria for what constitutes perennialism in *Zea* may have contributed to contradictory
125 observations. Traits such as rhizome formation, evergreen stalks, and dormancy are important
126 adaptive features that support the viability of various perennial plants in various environments. In
127 this study, we take a reductionist approach and specifically focus on a plant's regrowability (i.e.
128 the ability to maintain some juvenile meristematic tissues after each life cycle that can initiate a
129 new life cycle). Although this trait by itself is insufficient for functional perenniality, it appears
130 to be an essential component of perenniality in *Zea* L. Here we report the results of our genetic
131 analysis and genome-wide screening of the regrowth trait with genotyping-by-sequencing (GBS)
132 technology.

133 **Results and Discussion**

134 ***The production and growth of the hybrids***

135 We made reciprocal crosses of *Z. diploperennis* (Zd, hereafter in a cross combination)
136 with the following three maize lines: B73, Mo17 and Rhee Flint (RF, hereafter in a cross
137 combination). B73 and Mo17 are inbred lines and Rhee Flint is an heirloom maize variety. The
138 first F_1 was made with Rhee Flint in a greenhouse. Rhee flint is small, fast-growing and usually

139 has tillers, which affords serial plantings with an increased opportunity of a plant simultaneously
140 flowering with *Z. diploperennis*. Because Rhee Flint is an open-pollinated variety, later F_1s were
141 made with B73 and Mo17 to facilitate molecular analysis. All the F_1 plants are fertile and
142 completed multiple cycles of growth, reproduction and senescence (Fig. 1; Supplementary Fig.
143 S1). Regrowth (as opposed to accidental replanting from seed) of F_1 plants was insured by
144 inspection that new shoots were attached to the base of the F_1 and confirmed by the
145 heterozygosity of polymorphic PCR markers (examples shown in Supplementary Fig. S2).
146 Regrowth of these F_1s originates from basal axillary buds after stem senescence in all the crosses
147 (Figs. 1D, 1E, 1F).

148 Some of the basal regrowth immediately developed into a female (Fig. 2A) or a male
149 (Fig. 2B) inflorescence, or a forest of them (Fig. 2D). These F_1 plants with abnormal regrowth
150 most often can later undergo normal regrowth in an alternative environment, such as being
151 moved from the greenhouse to the field, etc., which suggests a strong environmental influence.
152 No such abnormal growth has been seen in advanced generations. Sometimes, plantlets also can
153 develop at the upper nodes of some hybrid plants when B13 and Mo17 were used as the parent
154 (Fig. 2C). The plantlets developed at the upper nodes, however, can only survive if transplanted
155 into soil. This indicates that the senescent stalks do not function to provide the necessary
156 nutrients to the plantlets.

157 Because the F_1 plants and their perennial derivatives are not winter hardy, the
158 regeneration cycles were alternated between the greenhouse and the field (Supplementary Figs.
159 S1 & S3). Interestingly, the ears and kernels of the F_1s of the six crosses all were more teosinte-
160 like (i.e. two rows of oppositely positioned spikelets with paired kernels encased by woody
161 rachides and glumes) when grown in greenhouse but were more maize-like (i.e. multiple rows of

162 naked kernels with short soft glumes and rachides around a silica-filled soft core) when grown in
163 the field (Fig. 3). In the F_2 and higher generations, ear morphology segregated even under
164 greenhouse condition (Fig. 3). These observations suggest that environmental factors are
165 important in the preferential expression of the teosinte or the maize alleles of the genes
166 influencing ear morphogenesis in the hybrids. These observations also indicate that it should be
167 possible to breed regrowable maize with maize-like ears and kernels.

168 The contrast between our observations and those of some previous reports is remarkable.
169 While we focus on a single trait, regrowth after senescence, previous studies were interested in
170 perennialism generally using varying criteria. Conclusions that perennialism in *Zea* is recessive
171 might have resulted from the hypothesis that traits such as tiller number at tasseling (TNT) or
172 rhizome development are indispensable components of perennialism in *Zea*. Indeed, other
173 studies have used rhizome development as an indicator of perennialism in *Zea*[11, 16, 17, 22, 25] and we
174 have not observed rhizomes in any of our F_1s or the derived plants. When regrowth occurs, it is
175 always from an axillary bud. Indeed, it is also our observation that the regrowth of *Z.*
176 *diploperennis* is mainly from basal axillary buds, and only occasionally from rhizomes. The *Z.*
177 *perennis* - 4X maize F_1s made by R. A. Emerson also were all "weekly perennial" under the
178 environmental conditions with few or no rhizomes[10].

179 Other possible explanations for contrasting results is that the perennial teosinte plants
180 used in those studies were polymorphic for one or more regrowth genes, that the experimental
181 environments were unfavorable for regrowth to happen, or that some plants needed more time to
182 break up their dormancy. Shaver[16] and Camara-Hernandez and Mangelsdorf[25] observed that some
183 of their F_1 plants eventually regrew from basal axillary buds after a period of dormancy. Indeed,
184 some of our F_2 plants need about two months of dormancy before regrowth. This observation

185 reinforces the view that even regrowth is a complex trait that is modified by genetics and
186 environment.

187 TNT has been associated with perennialism in several studies[14, 17, 26, 27], so we investigated
188 the relationship of TNT with regrowth in the Zd-RF F_2s. One-way ANOVA of TNT by regrowth
189 (Supplementary Table S1), however, revealed no significant difference of TNT ($F = 0.897$, $p =$
190 0.353) between the regrowth and the non-regrowth F_2s. Indeed, we observed regrowth from
191 several single-stalked hybrid derivatives (Fig. 4A) and non-regrowth of some multi-stalked
192 plants (Fig. 4B). These results suggest that TNT is not essential to regrowth in *Zea*.

193 ***The genetics of regrowth***

194 All our F_1 plants regrew and underwent several life cycles alternating between the
195 greenhouse and the field. This indicates that, with our materials and in our environment,
196 regrowth is a dominant trait. Although this contrasts with some reports, regrowable F_1 hybrids of
197 maize with perennial teosintes were previously obtained by Emerson[9], Shaver[16], Galinat[15], Ting
198 and Yu[24] and Camara-Hernandez and Mangelsdorf[25]. Brewbaker suggested cytoplasm may
199 contribute to perennialism[28], but our reciprocal F_1s performed similarly.

200 To analyze the genetics of regrowth further, 134 B73-Zd F_2s (derived from several F_1
201 plants where B73 was the female) and 159 Zd-RF F_2s (derived from a single F_1 plant where Zd
202 was the female) were tested. Among the 134 B73-Zd F_2s, 81 regrew and 53 did not (Table 1).
203 Similarly, among the 159 Zd-RF F_2s, 90 regrew after senescence and 69 did not (Table 2). One
204 B73-Zd F_3 population (Supplementary Table S1) and three Zd-RF F_3 populations (Table 2), each
205 of which was derived from a single regrowth F_2 plant, were also evaluated for their regrowth.

206 A chi square (χ^2) goodness-of-fit test suggests that both of the F_2 populations and one Zd-
207 RF F_3 population (Zd-RF F_3-5) we tested best fit a 9:7 regrowth to non-regrowth ratio (Table 3),

208 and the B73-Zd F_3 population and the remaining two Zd-RF F_3 populations best fit a 3:1 ratio
209 (Table 3). The simplest model that explains these results is that regrowth in the F_1s and their
210 derivatives is mainly controlled by two dominant, complementary *regrowth* (*reg*) loci. A two
211 dominant, complementary gene model parallels what has been found in other species, such as
212 rice (*Oryza sativa*)[6], Johsongrass (*Sorghum halepense*)[5, 6, 29], basin wildrye (*Leymus cinereus*)[30] and
213 wild mungbean (*Vigna radiate* ssp. *sublobata*)[8], for perennialism-related traits.

214 The Zd-RF F_1 was also backcrossed to each parental line. All plants from the F_1-to-Zd
215 backcross regrew, showing dominant effect of the Zd alleles. However, only one of the 20 plants
216 from the F_1-to-RF backcross showed regrowth. Therefore, alternative models, such as one or
217 three dominant complementary genes, two major genes with a few minor modifiers, or that
218 regrowth is a complex trait controlled by many QTL, are not eliminated by this genetic analysis,
219 but are less probable.

220 The number of regrowth plants observed in any generation might be understated, because
221 some plants initially recorded as non-regrowth eventually regrew after about two months of
222 dormancy. Therefore, some plants recorded as non-regrowth and discarded to open up
223 greenhouse space may have possessed the ability to regrow. Furthermore, transplanting from the
224 field to the greenhouse and *vise versa* is very stressful so that some regrowable plants may have
225 been killed this way.

226 The F_2 and F_3 plants afforded an opportunity to investigate whether some factors
227 previously implicated in perennialism may contribute to the regrowth trait. The rice
228 rhizomatousness gene *Rhz2* has been mapped to rice chromosomes 3[6] and sorghum chromosome
229 1[5, 6, 29], which are both homoeologous to parts of maize chromosome 1[7]. Additionally, *gt1* and *id1*,
230 which have been implicated with perenniality in *Zea*[8],and *tb1*, which controls *gt1*[19], are all on

231 chromosome 1 in *Zea*[19,21]. Therefore, we investigated the allele compositions of these three genes
232 in the B73-Zd F_2s (Table 1), and 26 Zd-RF F_2 plants and the three Zd-RF F_3 populations
233 (Supplementary Table S2), and assayed their association with regrowth. Of the 134 regrowth
234 hybrid derivatives we examined, 5, 33 and 115 were homozygous for the maize *gt1*, *tb1* or *id1*
235 alleles, respectively (Table 1 and Supplementary Table S2). Zd-RF F_3 family Zd-RF F_3-5 is
236 homozygous for the *gt1* allele of *Z. diploperennis* (Supplementary Table S2) but segregates
237 approximately 9:7 for regrowth and non-regrowth (Table 2). Therefore, our results are
238 inconsistent with the model of Shaver[16], and show that *gt1* and *id1* do not control regrowth in our
239 F_1s and their derivatives. *Z. diploperennis*'s *gt1* allele may be helpful to regrowth because the
240 majority of the plants that regrew had at least one copy, but it is not indispensable because some
241 plants regrew without it.

242 Interestingly, we observed no heterozygosity for *id1* and very low heterozygosity for *tb1*
243 in all the hybrid derivatives that were examined, regardless of regrowth (Tables 1;
244 Supplementary Table S2). Of 134 B73-Zd F_2 plants, only 16 had the *Z. diploperennis id1* allele
245 (Table 1). Similar phenomena were observed in the derivatives of the Zd-RF cross
246 (Supplementary Table S2). It seems that the maize chromosome fragment that carries *id1* was
247 preferentially transmitted to the hybrid derivatives. Excess homozygosity of the maize *id1* allele
248 indicates some sort of selection. It could be that a deficiency or other rearrangement adjacent to
249 the teosinte *id1* allele causes it not to transmit efficiently, or it could be that the teosinte *id1* or
250 tightly linked allele causes the plant not to grow well or flower in our experimental conditions.

251 ***Identifying regrowth loci with genotyping-by-sequencing assay***

252 A genome-wide mining of single nucleotide polymorphisms (SNPs) was conducted in a
253 randomly selected sub-population of 94 (55 regrowth and 39 non-regrowth) B73-Zd F_2 plants

254 with GBS technology (Table 1). We conducted the GBS assays to identify QTL for the regrowth
255 trait. To prepare for these assays, a total of 2,204,834 (85.14%) Illumina sequencing tags that
256 passed routine quality control filtrations were aligned with the B73_v4 reference genome. A total
257 of 714,158 SNPs, covering all ten chromosomes with an average of 71,416 SNPs per
258 chromosome, were then called from 83 (46 regrowth and 37 non-regrowth, labeled in bold in
259 Table 1) of the 94 F_2 plants using TASSEL 3 pipeline[31, 32]. SNP-calling for the excluded 11 plants
260 probably failed due to an error in barcode addition before sequencing. These SNPs were first
261 subjected to a two-step filtration in TASSEL 3 (MergeDuplicateSNPsPlugin and
262 GBSHapMapFiltersPlugin) to remove SNPs with poor qualities (see the Materials & Methods
263 section and Supplementary Table S3 for the details), which resulted in 37,925 SNPs (Table 4, 1st
264 filtration). The 37,925 SNPs were then manually filtered with high missing data rate > 20%,
265 which resulted in 10,432 remaining SNPs among all ten chromosomes (Table 4, 2nd filtration).
266 To explore which chromosomal regions may control the regrowth phenotype, the 10,432
267 SNPs from the 2nd filtration step were pooled for QTL analysis using R/qtl (version 1.42-8)
268 (Supplementary Table S4). The result is shown in Figure 5A. A total of 126 SNPs (104 real sites
269 and 22 simulated sites) showed LOD scores higher than 3.00. A permutation test of 1,000 with
270 the *p*-value of 0.05 resulted in a significant LOD score of 5.23 (Fig. 5A). This significance
271 threshold revealed two major QTL with one at 33,041,409 bp (the nucleotide position in the
272 B73_v4 reference genome sequence) on chromosome 2 with a LOD score of 5.46 and one at
273 4,284,633 bp on chromosome 7 with a LOD score of 5.53, respectively.
274 To test if the two strongest QTL correspond to two dominant and complementary loci
275 suggested by the genetic analysis, we applied a χ^2 test with 9:7 allele segregation ratio model to
276 the 10,431 SNPs to investigate if the observed and the expected genotypes are significantly

277 different ($p \leq 0.05$). This is based on our hypothesis that, if a SNP is associated with a *reg* locus,
278 the teosinte allele of the SNP should be carried by all the regrowable F_2s but only by three
279 sevenths non-regrowable F_2s. This step kept 946 SNPs (Table 4, 3rd filtration). Finally, to
280 simplify the mapping effort, the 946 SNPs were filtered once more by collapsing immediately
281 neighboring SNPs that share the same haplotypes into one cluster. This step resulted in 597 SNP
282 cluster with each being represented by the first SNP in the cluster (Table 4, 4th filtration). Locus
283 mapping was then conducted with a threshold of $LOD_{95\%} = 4.17$. This analysis revealed two
284 significant loci with one on chromosome 2 in the interval from 24,244,192 bp to 28,975,747 bp
285 with the peak at 27,934,739 bp and another on chromosome 7 in the interval from 2,862,253 bp
286 to 6,681,861 bp with the peak at 5,060,739 bp (Fig. 5B). These two loci were mapped closely to
287 the two major QTL indicated by the mapping without imputation. These results are consistent
288 with the two-factor model, which warrants further investigation. To that end we are naming the
289 factor underlying the chromosome 2 QTL *regrowth 1* (*reg1*) and the factor underlying the
290 chromosome 7 QTL as *regrowth 2* (*reg2*).

291 Our LOD analysis located two minor peaks on chromosome 1 that are associated with
292 regrowth (Fig. 5B). We wanted to know if these two loci are related to *gt1* and *id1*, respectively.
293 The SNPs at the peak of these loci are at 82,273,951 bp and 177,235,112 bp, far away from *id1*
294 (around 243,201,405 bp) and *gt1* (around 23,625,801 bp) (Fig. 6). These observations further
295 indicate that *id1* and *gt1* are not related to regrowth. Also, previous studies reported that *Z.*
296 *diploperennis* carried perennialism-related *Pe*-d* and an evergreen gene on chromosome 4[15, 19].
297 However, our data could not support these observations since no SNP on chromosome 4 is
298 significantly associated with regrowth (Figs. 5 and 6).

299 In summary, the results presented here indicate that regrowth in Zea is inherited

300 dominantly in our experimental conditions. Both the genetic and GBA analyses support a model
301 where the regrowth trait is mainly controlled by two major regrowth loci, *reg1* and *reg2* on
302 chromosomes 2 and 7, respectively. Even so, the data do not eliminate more complex models.
303 Identification and functional study of the candidate genes for *reg1* and *reg2* and their possible
304 modifiers will initiate an understanding about the molecular mechanism of perenniality in *Zea* L.
305 We recognize that adaptability is very important for a plant to realize perennialism in a certain
306 environment. However, this issue can be addressed separately after we understand molecularly
307 how *Z. diploperennis* is regrowable.

308 **Materials and Methods**

309 Plant materials and phenotyping

310 *Zea diploperennis* (PI 462368) and *Z. mays* cv. Rhee Flint (PI 213764) were obtained
311 from the USDA North Central Region Plant Introduction Station, Ames, IA. B73 and Mo17
312 inbreds were from the collection of D. Auger and are traceable back to the Maize Genetics
313 Cooperation Stock Center, Urbana/Champaign, IL. In our designations of F_1s and their
314 derivatives, the female parental is shown first. All the plants used in this study were grown in the
315 greenhouse during the winter and in the field during the summer in Brookings, SD. Controlled
316 pollinations were done by covering tassels and ears with paper bags before and after the
317 pollination was made. In the greenhouse, plants were maintained with a 16 h-light/8 h-dark cycle
318 and 20/16 °C day/night temperature except that two-month old *Z. diploperennis* and its hybrid
319 plants were treated with a 10 h light/14 h dark cycle for four weeks to induce the floral transition.

320 Plants were scored as regrowth if they produced shoots from the basal axillary buds after
321 the original stalks finished flowering and senesced. Rhizome and tuber development were
322 visually investigated on plants that were dug from the soil after senescence. TNT was

323 investigated by counting numbers of tillers per plant when the tassel had fully emerged. Ear and
324 kernel morphology was visually examined and photographed.

325 PCR assay

326 DNA samples were isolated from young leaves using the CTAB procedure[33] and used for
327 PCR-based marker assay. Table 5 lists all the PCR primers used in this study. PCR assays were
328 done using GoTaq Green Master Mix (Catalog# M7505, Promega, Madison, WI) at the
329 following conditions: 95°C, 35 cycles of 95°C for 45 sec, 55~62°C (primer dependent, see Table
330 6 for detail) for 1 min and 72°C for 1 min, and 72°C for 10 min. The annealing temperatures
331 were determined using a 1°C-touchdown PCR step starting from 65°C.

332 SNP discovery

333 The GBS assay was conducted according to Elshire and the associates[31]. The preparation
334 and sequencing of the library were conducted by the University of Wisconsin Biotechnology
335 Center (UWBRC). Generally, DNA samples were digested with *ApeK*I restriction enzyme (RE),
336 and unique barcodes were annealed to each DNA fragments. A single-end 100 bp (1x100bp)
337 sequencing run was carried out on an Illumina HiSeq 2500 platform. The raw data were pooled
338 as a single fastq file and downloaded from UWBRC.

339 The TASSEL (Trait Analysis by Association, Evolution and Linkage) 3 pipeline was
340 used under the guidance of TASSEL manual[32] for the discovery of SNPs between *Z.*
341 *diploperennis* and *Z. mays* (Supplementary Table S3). The barcoded sequence reads were
342 collapsed into a set of unique sequence tags with counts. The tag count files were filtered for a
343 minimum count threshold and merged into the master tag count file. The B73_RefGen_V4
344 reference genome sequence was downloaded from MaizeGDB and processed with Bowtie2 for
345 alignment[34]. Master tags were aligned to the B73 reference genome to generate a "Tags On

346 Physical Map" (TOPM) file, which contains the genomic position of each tag with the best
347 unique alignment. The occupancies of tags for each taxon were observed from barcodes
348 information in the original FASTQ files. Tag counts were stored in a "Tags by Taxa" (TBT) file.
349 The TOPM and TBT files were used to call SNPs at the tag locations on the genome. The SNPs
350 were filtered by minimum tag counts of 5, genotype mismatch rate of 0.1, minimum taxon
351 coverage of 0.01, minimum site coverage of 0.2 and minimum minor allele frequency of 0.01.
352 Fastq files containing sequences of chromosomes 1 to 10 were merged by FASTX_Toolkit and
353 indexed. All commands for SNP discovery were executed in Ubuntu 16.04 LTS platform.
354 SNPs resulted from TASSEL filters plugin with a minimum minor allele frequency of
355 0.01 were filtered again by removing sites that had missing data in more than 20% of the B73-Zd
356 F_2 plants. For those SNPs that have missing data in less than 20% of the B73-Zd F_2 plants, the
357 missing data were imputed by treating them as heterozygote since both two alleles can be
358 embodied and considered to be moderate. The SNPs from the 2nd filtration identification were
359 used for QTL mapping. To understand the relationship between the mapped QTL with the
360 genetic factors revealed by the genetic analysis, the SNPs from the 2nd filtration were further
361 filtered (the 3rd filtration) with χ^2 ($p < 0.05$). For each regrowth-associated SNP, we expected, in
362 the regrowable subpopulation, 33.3% plants to carry the homozygous Zd alleles (AA) and 66.6%
363 to have the Zd-B73 heterozygous allele combination (AB) and none with the B73 homozygous
364 alleles (BB) and, in the non-regrowable subpopulation, 14% of the plants with AA, 28.6% with
365 AB and 57.1% with BB. Altogether, a χ^2 contingency table were generated with expected
366 $\chi^2_{0.05,4} = 9.49$. Any SNPs with $\chi^2 < 9.49$ were kept fitting the 9:7 segregation model. The 4th SNP
367 filtration was performed to keep the first SNP and remove the rest in a cluster in which all the
368 SNPs in the range of 100 bp share the same haplotypes. In the locus analysis with the chi-square

369 imputation, such a cluster of SNPs was treated as one locus. Removing the redundant SNPs
370 makes the locus analysis more precise because repeated SNP sites would affect the LOD value
371 and influence the interval estimation.

372 The SNPs after the 2nd and the 4th filtrations were used for candidate locus/QTL
373 estimation, respectively. The locus analysis was executed by a standard QTL procedure in *R*
374 using the *R*/qtl package (version 1.40-8)[35] to better observe the contribution of each SNP and its
375 neighbors. The *R* codes used for the analyses are listed in Supplementary Table S4. Position
376 simulation was drawn with a maximum distance of 1.0 cM and an error probability of $1x10^{-4}$. The
377 conditional genotype probability (calc.genoprob), as well as simulated genotypes (sim.geno with
378 n.draw=32), were calculated. The "haldane" function was used to convert genetic distances into
379 recombination fractions. Genome scan with a single locus model (scanone) was performed with a
380 binary model using the expectation-maximization algorithm[35]. A permutation test with 1000
381 replicates was performed in scanone to visualize the LOD thresholds. We determined a locus
382 interval by selecting the first and last SNP sites with significant LOD value. Genes within the
383 intervals were identified by searching the corresponding region on the Gramene website.

384 Statistical analyses

385 For statistical analyses, all genotypes and phenotypes were transformed into numeric
386 values. For phenotypes, the regrowth plants were scored as "1" and the non-regrowth plants were
387 scored as "2". For genotypes, the plants that were homozygous to the *Z. diploperennis* allele
388 were scored as "1"; those that were homozygous to the B73 or Rhee Flint allele were scored as
389 "2"; and those that were heterozygous were scored as "3". When conducting locus analysis,
390 genotype "1" was transformed to "AA", "2" to "BB" and "3" to "AB".

391 A chi square goodness-of-fit test was used to find the best-fit model or linkage in the

392 genetic analysis and reveal candidate loci on chromosomes. To determine if TNT has any
393 correlation with regrowth, a One-Way ANOVA of TNT by regrowth was performed in JMP
394 (JMP® 11.2.0).

395 Sequencing Data availability

396 All raw fastq data from this study are available at NCBI data deposition site
397 (https://www.ncbi.nlm.nih.gov/bioproject/) with accession number PRJNA477673.

398 **Acknowledgement** This research was partially supported by funds from USDA-NIFA via
399 South Dakota Experiment Station and Department of Biology and Microbiology, South Dakota
400 State University. We greatly appreciate Dr. Frank M. You of Agriculture and Agri-Food Canada
401 for his help in statistics.

402 **Author Contributions** Y.Y. designed and supervised this project and all the experiments,
403 and drafted the manuscript; Y.Q., A.M, T.R., B.P., A.G., Y.Z., Y.Y. & D.A. performed the
404 experiments and collected data; Y.Q., A.M., T.R., D.A. & Y.Y. analyzed the data; all authors
405 discussed the results and communicated on and approved the final manuscript.

406 **Competing financial interests** The authors declare no competing financial interests.

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485 **Table legends**:

486 Table 1. The regrowth (R) and the non-regrowth (NR) phenotypes and the marker genotypes of
487 *Zea diploperennis* (Zd), *Z. mays* B73 and their 134 F_2 plants*.

488 Table 2. Segregation of regrowth among the *Zea diploperennis-Z. mays* cv Rhee Flint F_2s and F_3s.

489 Table 3. Results of the χ^2 goodness-of-fit tests of three genetic models.

490 Table 4. The actions taken and the numbers of SNPs revealed in each filtration step of SNP
491 analysis of *Zea mays* B13 - *Z. diploperennis* F_2 plants.

492 Table 5. PCR primers used in this study.

493 **Figure legends**:

494 Figure 1. Photos of *Zea mays* and *Z. diploperennis* (Zd) F_1 plants. **A**: reciprocal Mo17-Zd (right)
495 and Zd-Mo17 (left) F_1 plants; **B**: reciprocal B73-Zd (right) and Zd-B73 (left) F_1 plants; **C**: RF-Zd
496 F_1 plant; **D**: regrowth of a Mo17-Zd F_1 plant; **E**: regrowth of a B73-Zd F_1 plant; and **F**: regrowth
497 of a RF-Zd F_1 plant. B73, Mo17 and RF represent, respectively, inbred lines B73 and Mo17 and
498 cultivar Rhee Flint of *Z. mays*.

499 Figure 2. Photos of abnormal F_1 plants of crosses of *Zea diploperennis* with *Z. mays* inbred lines
500 B73 (A & B) and Mo17 (C) or cv. Rhee Flint (D).

501 Figure 3. Photos of the ears produced from a *Zea mays* cv Rhee Flint x *Z. diploperennis* F_1 plant
502 in different seasons (the upper panel) and from F_2 in summer 2014 in greenhouse (the lower

503 panel).

504 Figure 4. Photos of *Zea mays* Mo17-*Z. diploperennis* F_2 plants, showing regrowth from the basal
505 node of a single-stalked plant (A) or non-regrowth from a multi-stalked plant (B).

506 Figure 5. Graphics showing LOD scores of the QTL mapping the B73-Zd F2 population without
507 (A) or with (B) chi-square imputation. The 95% threshold lines (the parallel red dash lines) were
508 calculated with 1,000 permutation. Significant QTL/loci are indicated by the location of the peak
509 SNPs of the loci.

510 Figure 6. Genetic map of 30 representing SNPs and genes *gt1*, *id1*, and *tb1*. Each SNP represents
511 a one- Mbp region except of SNP S2_27934739, which represent a SNP cluster.

512 **Supplementary documents**:

513 Supplementary Table S1. Segregation of regrowability among the B73-Zd F_3s.

514 Supplementary Table S2. Phenotypes and the *gt1*, *id1* and *tb1* haplotypes of 26 F_2 plants and
515 three F_3 populations of the *Zea mays* cv. Rhee Flint x *Z. diploperennis* cross.

516 Supplementary Table S3. Steps and codes in TASSEL pipeline including example command
517 lines and brief descriptions. Parameters are highlighted and described when first be used.

518 Supplementary Table S4. R codes used for candidate locus/QTL analyses.

519 Supplementary Figure S1. A photo showing the growth of *Zea diploperennis* and its F_1 with Z.
520 mays B73 or Mo17 in the field in the Summer 2017.

521 Supplementary Figure S2. An agarose gel image showing that two molecular markers confirmed

522 the heterozygosity of a *Z. diploperennis-Z. mays* cv. Rhee Flint F_1 plant over three life-cycles.

523 Supplementary Figure S3. A photo showing the regrowth of the B73 - *Z. diploperennis* F_4s in the

524 summer, 2017.

Table 1. Phenotypes and genotypes of the *Zea mays* B73-*Z. diploperennis* F2s

Plant*	PT	*gt1*	*tb1*	*id1*	Plant*	PT	*gt1*	*tb1*	*id1*	Plant*	PT	*gt1*	*tb1*	*id1*
Zea diploperennis	1	1	1	1	**BZ2-006-9**	1	1	1	2	BZ2-009-6	1	1	1	2
B73	2	2	2	2	**BZ2-006-10**	1	1	3	2	BZ2-009-7	2	1	-	2
BZ2-001-1	1	1	3	2	BZ2-006-11	2	3	2	2	**BZ2-009-8**	2	3	2	2
BZ2-001-2	1	3	3	1	**BZ2-006-12**	2	3	3	2	**BZ2-009-9**	1	3	1	2
BZ2-001-3	1	1	3	2	BZ2-006-13	2	3	2	1	**BZ2-009-10**	2	1	3	2
BZ2-001-4	1	1	3	2	**BZ2-006-14**	1	3	2	2	BZ2-009-11	1	1	3	2
BZ2-001-5	1	1	1	2	**BZ2-007-1**	2	3	2	2	**BZ2-009-12**	1	3	3	2
BZ2-002-1	2	1	3	2	BZ2-007-2	2	3	2	2	**BZ2-010-1**	1	3	2	2
BZ2-002-2	2	1	2	2	**BZ2-007-3**	1	1	2	2	**BZ2-010-2**	2	-	3	2
BZ2-002-3	1	3	3	2	BZ2-007-4	1	3	2	2	**BZ2-010-3**	1	3	1	2
BZ2-002-4	1	3	2	1	**BZ2-007-5**	1	3	3	2	BZ2-010-4	1	3	2	1
BZ2-002-5	2	3	3	2	**BZ2-007-6**	2	3	3	2	**BZ2-010-5**	2	3	1	2
BZ2-002-6	2	3	2	2	**BZ2-007-7**	1	1	3	2	**BZ2-010-6**	1	3	2	2
BZ2-002-7	2	3	2	2	BZ2-007-8	1	3	1	2	**BZ2-010-7**	2	3	1	2
BZ2-002-8	1	1	-	2	BZ2-007-9	1	3	1	2	**BZ2-010-8**	1	3	2	2
BZ2-002-9	1	3	2	2	**BZ2-007-10**	2	1	3	2	**BZ2-010-9**	1	1	3	2
BZ2-002-10	1	3	3	2	BZ2-007-11	2	3	1	2	**BZ2-010-10**	1	3	2	2
BZ2-002-11	2	1	2	1	**BZ2-007-12**	1	1	2	2	**BZ2-010-11**	1	2	3	2
BZ2-002-12	1	1	2	2	**BZ2-007-13**	1	3	1	2	**BZ2-010-12**	2	2	3	2
BZ2-002-13	1	3	3	2	**BZ2-007-14**	2	1	3	2	**BZ2-010-13**	1	3	3	2
BZ2-002-14	2	1	1	2	BZ2-007-15	2	3	1	2	BZ2-010-14	1	1	2	2
BZ2-002-15	1	1	3	1	**BZ2-007-16**	1	1	3	2	**BZ2-010-15**	2	3	3	2
BZ2-002-16	1	3	3	1	**BZ2-007-17**	2	1	-	1	**BZ2-010-16**	2	1	3	2
BZ2-002-17	2	3	2	2	**BZ2-007-18**	1	1	1	2	BZ2-010-17	1	1	3	2
BZ2-002-18	1	1	2	2	**BZ2-007-19**	1	3	2	2	**BZ2-010-18**	2	3	3	2
BZ2-002-19	1	1	2	2	**BZ2-007-20**	1	3	2	2	**BZ2-010-19**	1	2	2	1
BZ2-002-20	1	1	2	2	**BZ2-007-21**	2	3	2	2	**BZ2-010-20**	2	1	2	1
BZ2-002-21	1	1	2	1	BZ2-008-1	1	1	1	2	BZ2-010-21	2	3	3	2
BZ2-002-22	1	3	2	2	**BZ2-008-2**	1	3	1	2	BZ2-011-1	2	1	-	1
BZ2-002-23	1	3	2	1	BZ2-008-3	1	3	1	2	**BZ2-011-2**	1	3	2	2
BZ2-002-24	1	3	2	2	BZ2-008-4	2	3	2	2	BZ2-011-3	1	2	3	2
BZ2-002-25	2	1	2	2	**BZ2-008-5**	1	3	3	2	**BZ2-011-4**	1	3	1	2
BZ2-004-1	1	3	1	2	**BZ2-008-6**	2	3	2	2	BZ2-011-5	1	1	-	1
BZ2-004-2	1	1	3	2	**BZ2-008-7**	2	3	3	2	**BZ2-011-6**	2	3	2	2
BZ2-004-3	2	3	2	2	**BZ2-008-8**	1	3	2	2	**BZ2-011-7**	2	1	2	2
BZ2-004-4	1	1	3	2	**BZ2-008-9**	2	3	2	2	**BZ2-011-8**	2	2	1	1
BZ2-004-5	1	3	2	2	**BZ2-008-10**	1	1	1	2	**BZ2-011-9**	1	3	2	2
BZ2-004-6	1	3	1	2	**BZ2-008-11**	2	3	2	2	BZ2-011-10	1	3	1	2
BZ2-006-1	2	3	1	2	**BZ2-008-12**	1	3	3	2	BZ2-011-11	1	1	2	2
BZ2-006-2	2	1	1	2	**BZ2-008-13**	1	1	2	2	**BZ2-011-12**	1	1	3	2
BZ2-006-3	1	3	3	2	**BZ2-008-14**	2	1	3	2	**BZ2-011-13**	1	3	1	2
BZ2-006-4	1	3	2	2	**BZ2-009-1**	2	3	3	2	**BZ2-011-14**	2	3	1	2
BZ2-006-5	1	3	3	2	BZ2-009-2	1	1	3	1	BZ2-011-15	2	3	3	2
BZ2-006-6	2	3	3	2	**BZ2-009-3**	2	3	2	2	**BZ2-011-16**	2	1	2	2
BZ2-006-7	1	3	1	2	**BZ2-009-4**	1	1	3	2					
BZ2-006-8	1	3	1	2	**BZ2-009-5**	2	3	3	2					

* Bold: used for SNP calling in GBS; 1: homozygous Zd allele; 2:homozygous B73 allele; 3: heterozygous; -: missing data

Table 2. Segregation of regrowth among the *Zea mays* cv Rhee Flint-*Z. diploperennis* F2s and F3s.

Plant	PT	Plant	PT	Plant	PT	Plant	PT	Plant	PT	Plant	PT	Plant	PT
ZR2-001-1	1	ZR2-001-33	2	ZR2-001-69	0	ZR2-001-102	1	ZR2-001-132	2	ZR2-001-162	2	ZR3-005-7	1
ZR2-001-2	2	ZR2-001-34	1	ZR2-001-71	1	ZR2-001-103	1	ZR2-001-133	1	ZR2-001-163	1	ZR3-005-8	1
ZR2-001-3	1	ZR2-001-35	2	ZR2-001-72	0	ZR2-001-104	1	ZR2-001-134	1	ZR2-001-164	1	ZR3-005-9	1
ZR2-001-4	2	ZR2-001-36	2	ZR2-001-73	1	ZR2-001-105	1	ZR2-001-135	2	ZR2-001-165	2	ZR3-005-10	1
ZR2-001-5	1	ZR2-001-37	2	ZR2-001-74	1	ZR2-001-106	1	ZR2-001-136	2	ZR2-001-166	1	ZR3-005-11	2
ZR2-001-6	1	ZR2-001-38	2	ZR2-001-75	1	ZR2-001-107	2	ZR2-001-137	1	ZR2-001-167	2	ZR3-005-12	2
ZR2-001-7	2	ZR2-001-39	1	ZR2-001-77	0	ZR2-001-108	2	ZR2-001-138	1	ZR2-001-168	2	ZR3-005-13	1
ZR2-001-9	1	ZR2-001-40	2	ZR2-001-78	0	ZR2-001-109	2	ZR2-001-139	1	ZR2-001-169	2	ZR3-005-14	2
ZR2-001-10	2	ZR2-001-42	2	ZR2-001-79	1	ZR2-001-110	1	ZR2-001-140	2	ZR2-001-171	1	ZR3-005-15	2
ZR2-001-11	1	ZR2-001-43	1	ZR2-001-80	1	ZR2-001-111	2	ZR2-001-141	1	ZR3-003-1	1	ZR3-005-16	2
ZR2-001-12	1	ZR2-001-44	1	ZR2-001-81	1	ZR2-001-112	1	ZR2-001-142	1	ZR3-003-2	1	ZR3-009-1	1
ZR2-001-13	2	ZR2-001-45	1	ZR2-001-82	0	ZR2-001-113	2	ZR2-001-143	1	ZR3-003-3	1	ZR3-009-2	1
ZR2-001-14	2	ZR2-001-47	2	ZR2-001-83	0	ZR2-001-114	2	ZR2-001-144	2	ZR3-003-4	2	ZR3-009-3	1
ZR2-001-15	1	ZR2-001-48	2	ZR2-001-84	1	ZR2-001-115	2	ZR2-001-145	1	ZR3-003-6	1	ZR3-009-4	1
ZR2-001-16	2	ZR2-001-49	1	ZR2-001-85	1	ZR2-001-116	1	ZR2-001-146	1	ZR3-003-7	1	ZR3-009-5	1
ZR2-001-17	1	ZR2-001-51	2	ZR2-001-86	1	ZR2-001-117	1	ZR2-001-147	1	ZR3-003-8	1	ZR3-009-6	2
ZR2-001-18	2	ZR2-001-53	2	ZR2-001-87	0	ZR2-001-118	2	ZR2-001-148	1	ZR3-003-9	1	ZR3-009-7	1
ZR2-001-19	2	ZR2-001-54	1	ZR2-001-88	0	ZR2-001-119	1	ZR2-001-149	1	ZR3-003-10	1	ZR3-009-8	1
ZR2-001-20	1	ZR2-001-55	1	ZR2-001-89	0	ZR2-001-120	1	ZR2-001-150	1	ZR3-003-11	2	ZR3-009-9	1
ZR2-001-21	2	ZR2-001-56	1	ZR2-001-90	1	ZR2-001-121	2	ZR2-001-151	1	ZR3-003-12	1	ZR3-009-10	1
ZR2-001-22	2	ZR2-001-57	1	ZR2-001-91	1	ZR2-001-122	1	ZR2-001-152	2	ZR3-003-13	1	ZR3-009-11	1
ZR2-001-23	1	ZR2-001-58	2	ZR2-001-92	1	ZR2-001-123	2	ZR2-001-153	1	ZR3-003-14	1	ZR3-009-12	1
ZR2-001-24	1	ZR2-001-59	1	ZR2-001-93	1	ZR2-001-124	1	ZR2-001-154	1	ZR3-003-15	1	ZR3-009-13	1
ZR2-001-25	2	ZR2-001-60	1	ZR2-001-94	0	ZR2-001-125	1	ZR2-001-155	2	ZR3-003-16	2	ZR3-009-14	2
ZR2-001-26	1	ZR2-001-62	1	ZR2-001-95	0	ZR2-001-126	2	ZR2-001-156	1	ZR3-005-1	1	ZR3-009-15	2
ZR2-001-27	2	ZR2-001-63	1	ZR2-001-97	1	ZR2-001-127	2	ZR2-001-157	2	ZR3-005-2	1	ZR3-009-16	1
ZR2-001-28	2	ZR2-001-64	1	ZR2-001-98	1	ZR2-001-128	2	ZR2-001-158	2	ZR3-005-3	2		
ZR2-001-30	1	ZR2-001-65	1	ZR2-001-99	1	ZR2-001-129	1	ZR2-001-159	2	ZR3-005-4	2	PT: phenotype	
ZR2-001-31	1	ZR2-001-67	2	ZR2-001-100	1	ZR2-001-130	2	ZR2-001-160	1	ZR3-005-5	1	1: regrowth	
ZR20-01-32	1	ZR2-001-68	2	ZR2-001-101	1	ZR2-001-131	2	ZR2-001-161	1	ZR3-005-6	1	2: non-regrowth	

Table 3. Results of the $\chi 2$ goodness-of-fit tests of three simple genetic models

Generations	Observed			No. dominant genes (the expected R to NR ratio) and P($\chi 2$)*		
	Total	**R**	**NR**	**1 (3:1)**	**2 (9:7)**	**3 (27:37)**
B73-Zd F_2	134	81	53	0.0001	**0.2964**	0.0001
B73-Zd F3	72	52	20	**0.5862**	0.0063	0.0001
Zd-RF F_2	160	92	68	0.0001	**0.7499**	0.0001
Zd-RF F_3-3	15	12	3	**0.6547**	0.0639	0.3000
Zd-RF F_3-5	16	9	7	0.0833	**1.0000**	0.2547
Zd-RF F_3-9	16	13	3	**0.5637**	0.0438	0.0016

*: the best fit models are in bold.

Table 4. Numbers of SNPs revealed in each chromosome of the B73-ZD F2 population after each filtering step

Chr	Raw SNP number	1st filter	2nd filter	3rd filter	4th filter
1	109,543	5,751	1,628	82	51
2	85,283	4,966	1,476	120	77
3	81,625	4,708	1,200	120	75
4	75,832	3,376	942	112	82
5	77,314	4,409	1,197	198	111
6	58,195	2,938	761	87	49
7	62,280	3,108	877	144	98
8	57,748	3,210	877	20	16
9	57,231	2,982	741	29	16
10	49,107	2,477	732	34	22
Total	**714,158**	**37,925**	**10,431**	**946**	**597**

Table 5. PCR primers used in this study

Primers	Sequences
tb1MF	5′-AGTAGGCCATAGTACGTAC-3′
tb1MR	5′-CTCTTTACCGAGCCCCTACA-3′
tb1ZF	5′-ACTCAACGGCAGCAGCTACCTA-3′
tb1ZR	5′-CGTGTGTGTGATCGAATGGT-3′
tga1cF:	5′-AATAAAATAGAGGAACGTCA-3′
tga1cR:	5′-TGCTGCAAAGGATTACTGAT-3′
mmc0381F	5′-GTGGCCCTGTTGATGAG-3′
mmc0381R	5′-CGACGAGTACCAGGCAT-3′
gt1-ZF:	5′-TCGCCTACATGACCGAGTAC-3′
gt1-ZR:	5′-ATACTCTCAGCTGCTACGCG-3′
gt1-MF:	5′-GAGACCGAGCTGCTGAAGAT-3′
gt1-MR:	5′-TGTAGCTGTTGTAGGCGTACT-3′

Supplementary Table S1. Segeagation of regrowability among the B73-Zd F3s

Plant	PT	Plant	PT	Plant	PT	Plant	PT
BZ3-010-1-1	1	BZ3-010-1-20	2	BZ3-010-1-46	2	BZ3-010-1-69	1
BZ3-010-1-2	1	BZ3-010-1-22	1	BZ3-010-1-47	1	BZ3-010-1-70	1
BZ3-010-1-3	1	BZ3-010-1-23	1	BZ3-010-1-48	1	BZ3-010-1-71	1
BZ3-010-1-4	1	BZ3-010-1-24	1	BZ3-010-1-50	1	BZ3-010-1-72	1
BZ3-010-1-5	1	BZ3-010-1-25	1	BZ3-010-1-51	2	BZ3-010-1-73	1
BZ3-010-1-6	1	BZ3-010-1-26	1	BZ3-010-1-52	1	BZ3-010-1-74	1
BZ3-010-1-7	1	BZ3-010-1-27	1	BZ3-010-1-53	1	BZ3-010-1-75	1
BZ3-010-1-8	2	BZ3-010-1-30	1	BZ3-010-1-54	1	BZ3-010-1-76	2
BZ3-010-1-9	2	BZ3-010-1-31	1	BZ3-010-1-56	1	BZ3-010-1-77	2
BZ3-010-1-11	2	BZ3-010-1-32	1	BZ3-010-1-57	1	BZ3-010-1-78	1
BZ3-010-1-12	1	BZ3-010-1-33	2	BZ3-010-1-68	2	BZ3-010-1-79	1
BZ3-010-1-13	2	BZ3-010-1-34	1	BZ3-010-1-59	1	BZ3-010-1-80	1
BZ3-010-1-14	2	BZ3-010-1-35	1	BZ3-010-1-61	2	BZ3-010-1-81	2
BZ3-010-1-15	1	BZ3-010-1-38	2	BZ3-010-1-62	1	BZ3-010-1-82	2
BZ3-010-1-16	1	BZ3-010-1-39	1	BZ3-010-1-63	1	BZ3-010-1-83	2
BZ3-010-1-17	1	BZ3-010-1-40	1	BZ3-010-1-64	2	BZ3-010-1-84	1
BZ3-010-1-18	1	BZ3-010-1-41	2	BZ3-010-1-67	1	BZ3-010-1-85	1
BZ3-010-1-19	1	BZ3-010-1-42	2	BZ3-010-1-68	1	BZ3-010-1-87	1

1: regrowth; 2: non-regrowth

Suplementary Table S2. Phenotypes and the *gt1* , *id1* and *tb1* haplotypes of 26 F2 plants and three F3 populations of the *Zea mays* cv Rhee Flint x *Z. diploperennis* cross*.

Line	PT	*tb1*	*id1*	*gt1*	Line	PT	*tb1*	*id1*	*gt1*	Line	PT	*tb1*	*id1*	*gt1*
F_2-1	1	3	1	3	F_3-3-2	1	1	2	1	F_3-5-13	1	1	2	1
F_2-2	2	2	2	2	F_3-3-3	1	1	2	1	F_3-5-14	2	1	2	1
F_2-3	1	1	2	3	F_3-3-4	2	1	2	3	F_3-5-15	2	1	2	1
F_2-4	2	2	2	2	F_3-3-6	1	1	2	3	F_3-5-16	2	1	2	1
F_2-5	1	1	2	1	F_3-3-7	1	1	2	1	F_3-9-1	1	1	2	1
F_2-6	1	3	1	1	F_3-3-8	1	1	2	3	F_3-9-2	1	1	2	2
F_2-7	2	2	2	2	F_3-3-9	1	1	2	3	F_3-9-3	1	1	2	2
F_2-9	1	1	2	3	F_3-3-10	1	1	2	3	F_3-9-4	1	1	2	3
F_2-10	2	3	1	3	F_3-3-11	2	1	2	3	F_3-9-5	1	1	2	1
F_2-11	1	2	2	3	F_3-3-12	1	1	2	1	F_3-9-6	2	1	2	2
F_2-12	1	3	1	1	F_3-3-13	1	1	2	3	F_3-9-7	1	1	2	3
F_2-13	2	3	1	3	F_3-3-14	1	1	2	1	F_3-9-8	1	1	2	1
F_2-14	2	2	2	3	F_3-3-15	1	1	2	1	F_3-9-9	1	1	2	3
F_2-15	1	3	1	1	F_3-3-16	2	1	2	2	F_3-9-10	1	1	2	3
F_2-16	2	2	2	1	F_3-5-1	1	1	2	1	F_3-9-11	1	1	2	1
F_2-17	1	3	1	3	F_3-5-2	1	1	2	1	F_3-9-12	1	1	2	3
F_2-18	2	3	1	2	F_3-5-3	2	1	2	1	F_3-9-13	1	1	2	1
F_2-19	2	3	1	2	F_3-5-4	2	1	2	1	F_3-9-14	2	1	2	3
F_2-20	1	3	1	3	F_3-5-5	1	1	2	1	F_3-9-15	2	1	2	3
F_2-21	2	2	2	3	F_3-5-6	1	1	2	1	F_3-9-16	1	1	2	1
F_2-22	2	2	2	3	F_3-5-7	1	1	2	1	F_3-12-1	1	1	2	1
F_2-23	1	2	2	3	F_3-5-8	1	1	2	1	F_3-12-2	1	1	2	1
F_2-24	1	1	2	3	F_3-5-9	1	1	2	1	F_3-12-3	1	1	2	1
F_2-25	1	2	1	1	F_3-5-10	1	1	2	1	F_3-12-4	1	3	1	1
F_2-26	1	3	2	1	F_3-5-11	2	1	2	1	F_3-12-5	1	3	1	1
F_3-3-1	1	1	2	3	F_3-5-12	2	1	2	1					

* For phenotype (PT), "1" is for regrowth and "2" is for non-regrowth; for genotypes, "1" is homozygous for the *Zea diploperennis* allele, "2" is homozygous for the *Z. mays* 's allele and "3" is for heterozygous.

Supplementary Table S3. Steps and codes in TASSEL pipeline including example command lines and brief descriptions. Parameters are highlighted and described when first be used.

Step 1	**FastqToTagCountPlugin**		
	Description	Derives a tagCount list for each FASTQ file. Keeps only good reads having a barcode and a cut site and no N's in the useful part of the sequence. Trims off the barcodes and truncates sequences that (1) have a second cut site, or (2) read into the common adapter.	
	Code	*/home/tassel/run_pipeline.pl -fork1 -Xms8G -Xmx16G -FastqToTagCountPlugin -i fastq -k key.txt -e ApeKI -o tagcounts -endPlugin -runfork1*	
	Parameters	-Xms8G, -Xmx16G	Set minimum and maximum usage of RAM.
Step 2	**MergeMultipleTagCountPlugin**		
	Description	Merges each tagCount file into a single "master" tagCount list.	
	Code	*/home/tassel/run_pipeline.pl -Xms8G -Xmx16G -fork1 -MergeMultipleTagCountPlugin -i tagcounts -o mergedtagcounts/BCA965ANXX_4.cnt* ***-c 5*** *-endPlugin -runfork1*	
	Parameters	-c 5	Minimum number of times a tag must be present to be output, typically between 5 and 20. The lower value is, the more sequencing errors will be included analysis. Default: 1
	Note:	Sequencing errors can be tolerated and will be removed by following steps.	
Step 3	**TagCountToFastqPlugin**		
	Description:	Converts a master tagCount file containing all the tags of interest from binary (.cnt) format into a FASTQ format file (.fq) that can then be used as input to bowtie2.	
	Code	*/home/tassel/run_pipeline.pl -Xms8G -Xmx16G -fork1 -TagCountToFastqPlugin -i mergedtagcounts/BCA965ANXX_4.cnt -o BCA965ANXX_4.fq* ***-c 5*** *-endPlugin -runfork1*	
Step 4	**Bowtie2 Index**		
	Description	Creates a series of support files needed to operate bowtie2.	
	Code	*bowtie2-build chr1.fa,chr2.fa,chr3.fa,chr4.fa,chr5.fa,chr6.fa,chr7.fa,chr8.fa,chr9.fa,chr10.fa /home/gbs/b73ref/b73_ref.fa*	
	Notes	Chromosome 1 to 10 from B73_Ref_V4 genome are used to create index files.	
Step 5	**Alignment with bowtie2**		
	Description	Aligns the master set of GBS tags to the reference genome.	
	Code	*bowtie2* ***-p 8 -N 0 -L 20 -i S,1,0.50 -D 20 -R 3*** *-x /home/b73ref/b73_ref.fa -U /home/gbs/analysis/BCA965ANXX_4.fq -S /home/gbs/analysis/BCA965ANXX_4.sam*	
	Parameters	-p 8	The number of processors to be used.
		-N 0	The number of mismatches to allowed in a seed alignment during multiseed alignment. Can be set to 0 or 1. Higher value makes alignment slower but increases sensitivity. Default: 0.
		-L 20	The length of the seed substrings to align during multiseed alignment. Smaller values make alignment slower but more sensitive. Default: 20.
		-I S, 1, 0.50	Sets a function governing the interval between seed substrings to use during multiseed alignment. Decide the seed frame length and interval which can influence the accuracy of alignment.

		-D 20	The times that consecutive seed extension attempts can "fail" before Bowtie 2 moves on. Default: 15.
		-R 3	the maximum number of times Bowtie 2 will "re-seed" reads with repetitive seeds. Default: 2.
	Note	Arguments and values used here are identical to –very-sensitive-local.	
Step 6	**SAMConverterPlugin**		
	Description	Converts a SAM format alignment (.sam) file produced by bowtie2 into a binary tagsOnPhysicalMap (.topm) file that can be used by the TagsToSNPByAlignmentPlugin for calling SNPs.	
	Code	*/home/tassel/run_pipeline.pl -Xms8G -Xmx16G -fork1 -SAMConverterPlugin -i BCA965ANXX_4.sam -o topm/BCA965ANXX_4.topm -endPlugin -runfork1*	
Step 7	**FastqToTBTPlugin**		
	Description	Generates a TagsByTaxa file to parse FASTQ files containing raw GBS sequence data for good reads	
	Code	*/home/tassel/run_pipeline.pl -Xms8G -Xmx16G -fork1 -FastqToTBTPlugin -i fastq -k key.txt -e ApeKI -o tbt -y -t mergedtagcounts/BCA965ANXX_4.cnt -endPlugin -runfork1*	
	Parameters	-y	Output in TBTByte format (counts from 0-127) instead of TBTBit (0 or 1).
Step 8	**MergeTagsByTaxaFilesPlugin**		
	Description	Merges all .tbt.bin and/or (preferably) .tbt.byte files present in the input directory.	
	Code	*/home/tassel/run_pipeline.pl -Xms8G -Xmx16G -fork1 -MergeTagsByTaxaFilesPlugin -i tbt -o mergedtbt/BCA965ANXX_4.tbt.byte -endPlugin -runfork1*	
Step 9	**TagsToSNPByAlignmentPlugin**		
	Description	Aligns tags from the same physical location against one another, calls SNPs from each alignment, and then outputs the SNP genotypes to a HapMap format file (one file per chromosome).	
	Code	*/home/tassel/run_pipeline.pl -Xms8G -Xmx16G -fork1 -TagsToSNPByAlignmentPlugin -i mergedtbt/BCA965ANXX_4.tbt.byte -y -m topm/BCA965ANXX_4.topm -o hapmap/raw/BCA965ANXX_4_chr+.hmp.txt* ***-mnF "-0.1"*** *-ref /home/b73ref/b73_chr.fastq* ***-sC 1 -eC 10*** *-endPlugin -runfork1*	
	Parameters	-mnF “-0.1”	Minimum value of F. Samples that are not inbreed line should invoke this to be negative value.
		-sC 1	Start chromosome.
		-eC 10	End chromosome.
	Note	This command is only available in TASSEL 3. The DiscoverySNPCaller in TASSEL 5 replaces this command and remains the same SNP calling functions but generate a db file rather than hapmap file. Except listed parameters, all others were used as default.	
Step 10	**MergeDuplicateSNPsPlugin**		
	Description	Finds duplicate SNPs in the input HapMap file, and merges them if they have the same pair of alleles	
	Code	*/home/tassel/run_pipeline.pl -Xms8G -Xmx16G -fork1 -MergeDuplicateSNPsPlugin -hmp hapmap/raw/BCA965ANXX_4_chr+.hmp.txt -o hapmap/mergedSNPs/BCA965ANXX_4_chr+.hmp.txt* ***-misMat 0.1 -callHets*** *-sC 1 -eC 10 -endPlugin -runfork1*	
	Parameters	-misMat 0.1	Threshold genotypic mismatch rate above which the duplicate SNPs won't be merged. Default: 0.05. For lines not fully inbred, value of 0.1 is recommended.
		-callHets	When two genotypes at a replicate SNP disagree for a taxon, call it a heterozygote. If the germplasm is not fully

			inbred and contains residual heterozygosity, then -callHets should be on.
	Note	The -callHets parameter is to guarantee heterouzygotes can be correctly output, rather than omitted as errors.	
Step 11	**GBSHapMapFiltersPlugin**		
	Description:	Filtering SNPs with specific requirements.	
	Code:	*/home/tassel4/run_pipeline.pl -Xms10G -Xmx16G -fork1 -GBSHapMapFiltersPlugin -hmp hapmap/mergedSNPs/BCA965ANXX_4_chr+.hmp.txt -o hapmap/filt/BCA965ANXX_4_chr+.hmp.txt* ***-mnTCov 0.01 -mnSCov 0.2 -mnMAF 0.01 -hLD -mnR2 0.2 -mnBonP 0.005*** *-sC 1 -eC 10 -endPlugin -runfork1*	
	Parameters:	-mnTCov 0.01	Minimum taxon coverage. The minimum SNP call rate for a taxon to be included in the output
		-mnSCov 0.2	Minimum site coverage. The minimum taxon call rate for a SNP to be included in the output
		-mnMAF 0.01	Minimum minor allele frequency
		-hLD True	filtered for those in statistically significant LD with at least one neighboring SNP

Supplementary Table S4. R codes used for candidate locus/QTL analyses

```
library(qtl)
all <- read.cross("csv", file="SNP.csv", genotypes = c("AA","AB", "BB"),
na.strings = "NA", alleles = c("A", "B"))
all <- calc.genoprob(all, step=1.0, off.end = 0.0, error.prob = 1.0e-
4,map.function = "haldane",stepwidth = "fixed")
all <- sim.geno(all, n.draws=32, step=1.0, off.end = 0.0, error.prob = 1.0e-
4,map.function = "haldane",stepwidth = "fixed")
all.scan1 <- scanone(all, pheno.col=2, model="binary", method = "em")
all.scan1.perm <- scanone(all, pheno.col = 2, model = "binary", method="em",
n.perm = 1000)
plot(all.scan1,main="LOD plot of regrowth",ylim = c(0,6))
threshold <- summary(all.scan1.perm, alpha=c(0.1, 0.05, 0.01))
abline(h=threshold[1], lty="dashed", lwd=1, col="blue")
abline(h=threshold[2], lty="dashed", lwd=1, col="yellow")
abline(h=threshold[3], lty="dashed", lwd=1, col="red")
summary(all.scan1, perm=all.scan1.perm, lodcolumn=1, alpha=0.1)
mkname1 <- find.marker(all, chr=2, pos=24.244290)
mkname2 <- find.marker(all, chr=7, pos=5.060739)
effectplot(all,pheno.col=2,mname1= mkname1), ylim=c(0,1))
effectplot(all,pheno.col=2,mname1= mkname2), ylim=c(0,1))
write.csv(all.scan1, "all.scan1.csv",row.names = TRUE)
```

Figures

Figure 1:

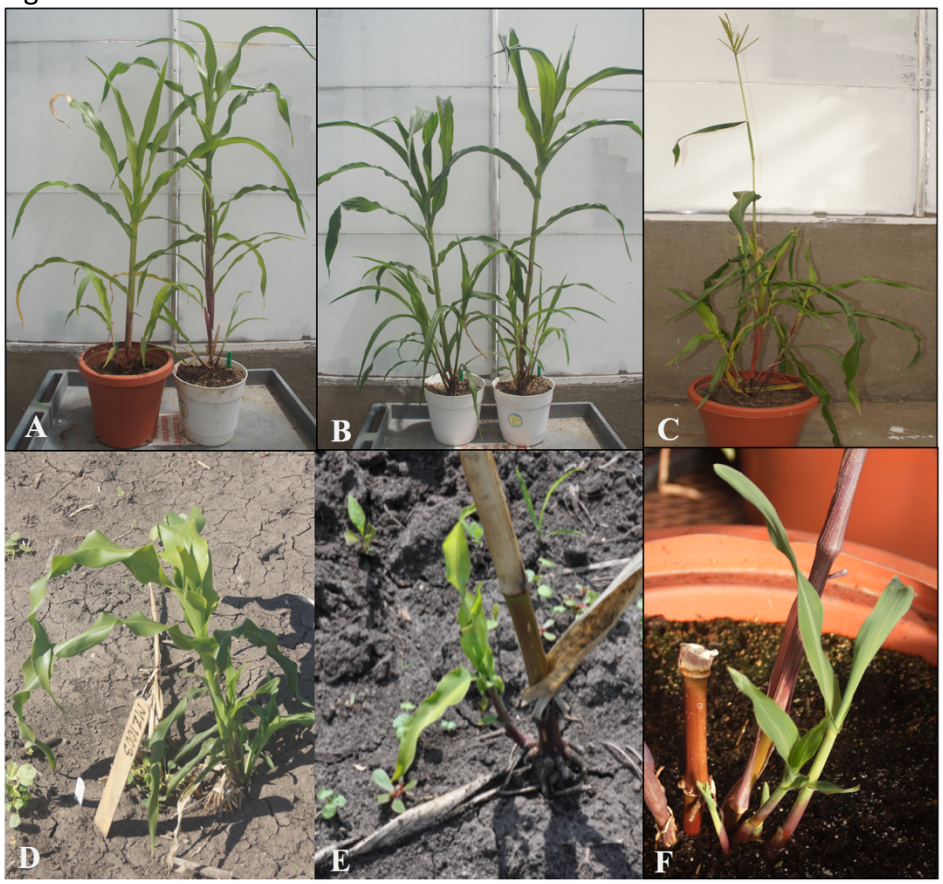

Figure 2:

Figure 3:

Figure 4:

Figure 5:

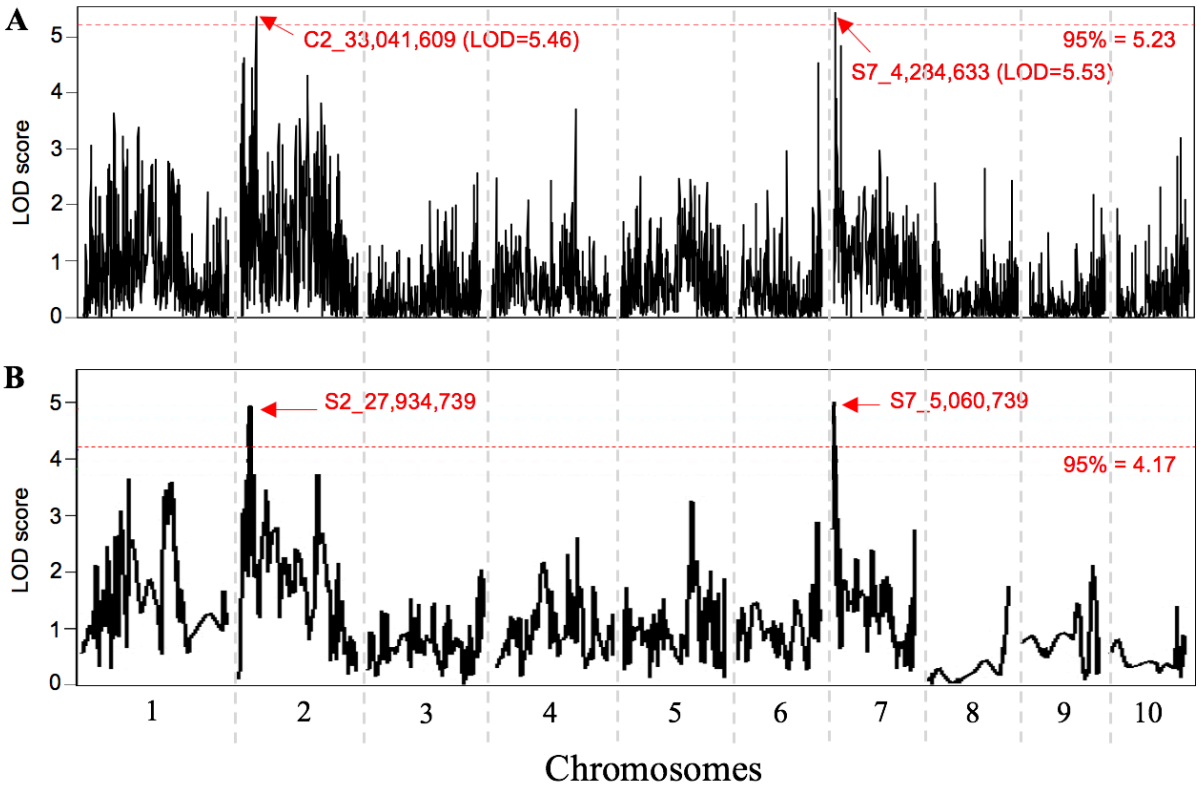

Figure 6:

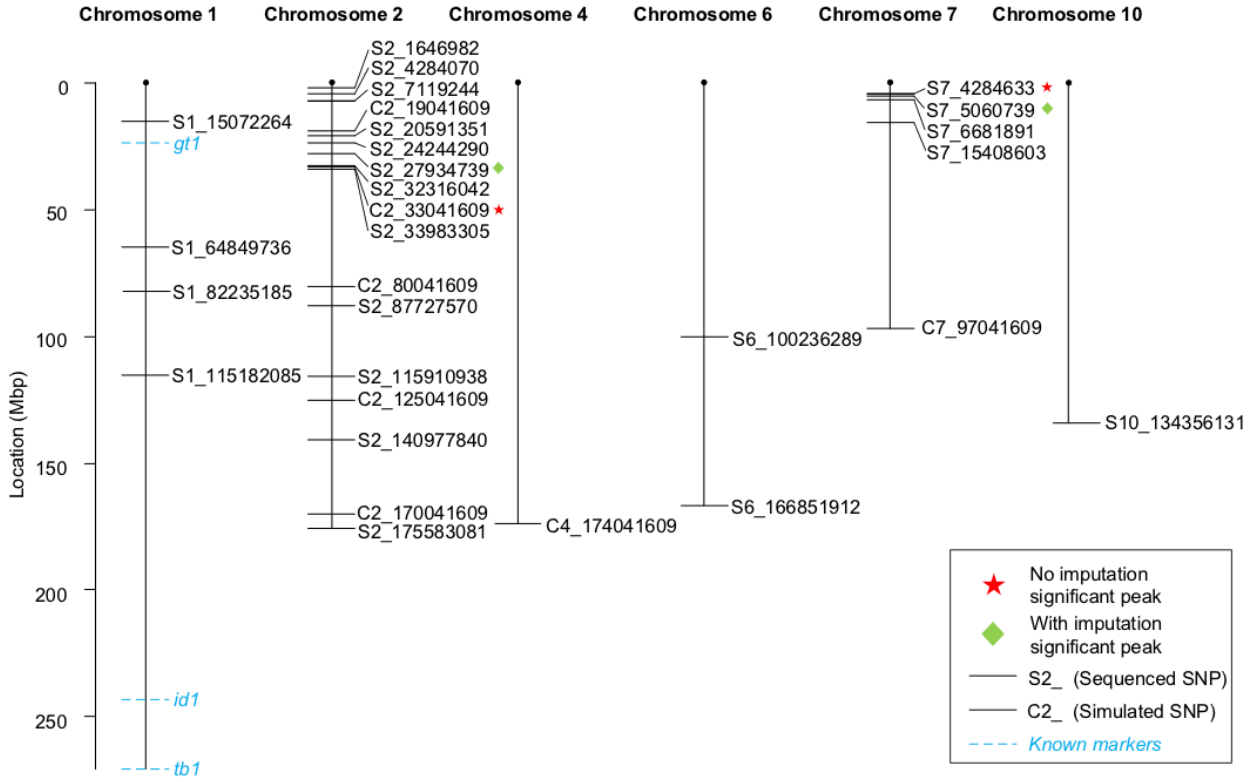

Figure S1:

Figure S2:

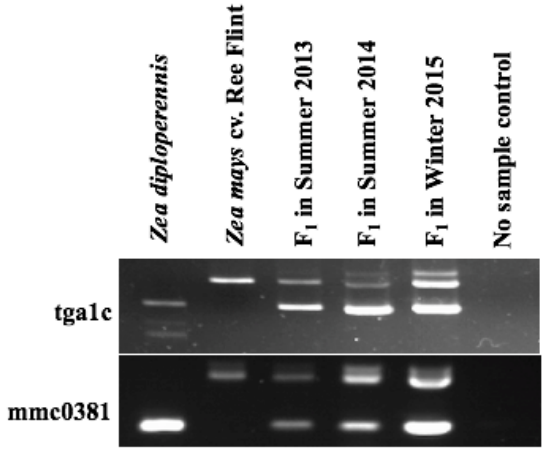

Figure S3: