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INVESTIGATION

The Genetics and Genome-Wide Screening of Regrowth Loci, a Key Component of Perennialism in *Zea diploperennis*

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ABSTRACT Perennialism is common among the higher plants, yet little is known about its inheritance. Previous genetic studies of the perennialism in *Zea* have yielded contradictory results. In this study, we take a reductionist approach by specifically focusing on one trait: regrowth (the plant's ability to restart a new life cycle after senescence on the same body). 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. Segregation ratios of regrowth 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 technology indicated two major regrowth loci, *regrowth 1* (*reg1*) and *regrowth 2* (*reg2*), were on chromosomes 2 and 7, respectively. These findings lay the foundation for further exploration of the molecular mechanism of regrowth in *Z. diploperennis*. Importantly, our data indicate that there is no major barrier to transferring this trait into maize or other grass crops for perennial crop development with proper technology, which enhances sustainability of grain crop production in an environmentally friendly way.

KEYWORDS
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Perennialism is the phenomenon that a plant can live for more than two years; the ability of doing so is termed perenniality. Plants typically have a life cycle of growth, reproduction (sexual and/or vegetative) and senescence. Annuals and biennials have only one such cycle in their life, leaving behind seeds, bulbs, tubers, etc. to initiate another life cycle. Perennials maintain juvenile meristematic tissues capable of regrowth after senescence to start a new life cycle on the same body. How perennials do so remains as a mystery. Subterranean stems (such as rhizomes), polycarpy and tuberous roots are often cited as the means by which plants achieve perenniality. However, none of these traits is absolutely required by perennials. For instance, bamboos are essentially monocarpic perennial that regrow from rhizomes. Many perennial temperate grasses, such as switchgrass (Haferkamp and Copeland 1984), cordgrass (Boe *et al.* 2009) and eastern gamagrass (Jackson and Dewald 1994), regrow from the crowns instead of rhizomes. On the other hand, some annual/biennial plants, such as radish (*Raphanus sativus*), grow tuberous roots.

Although perennialism is common among higher plants, the study of its genetics and molecular biology is sporadic. So far, the only published research in molecular mechanism of plant perennialism was conducted in Arabidopsis. Melzer *et al.* (2008) successfully mutated this annual herb to show some perennial habits, such as increased woody fiber in the stem, by down-regulating two flowering genes coding for MADS-box proteins, SUPPRESSOR OF OVEREXPRESSION OF CONSTANT 1 and FRUITFUL. Unfortunately, this woody mutant was sterile, and no follow-up research was reported. Perennial-related genes and quantitative loci (QTL) have been reported in other species. Major QTL controlling rhizome development, regrowth and tiller number have been mapped on sorghum linkage groups C (chromosome 1) and D (chromosome 4) (Paterson *et al.* 1995; Hu *et al.* 2003), which are homeologous to regions of maize chromosomes 1, 4, 5 and 9, respectively (Wei *et al.* 2007). Hu at al. (2003) mapped two dominant, complementary QTL *Rhz2* (*Rhizomatousness 2*) and *Rhz3* that control rhizome production on rice (*Oryza sativa*) chromosomes 3 and

4 at the loci homeologous to the sorghum (*Sorghum bicolor*) QTL. Tuberous roots in a wild perennial mung bean (*Vigna radiate* ssp. *sublobata*) are conditioned by two dominant, complementary genes (Nguyen *et al.* 2012). However, after years of effort, these perennialism-related genes have yet to be cloned from any of the species despite that mapping data and complete genomic sequences of rice and sorghum are readily available. Therefore, no further research has been reported about these perennialism-related loci/genes. Recently, Ryder *et al.* (2018) reported a set of 98 expressed contigs in Johnsongrass (*Sorghum halepense*) that are likely associated with rhizome development.

In the genus *Zea* L., most species, including maize (*Z. mays* ssp. *mays*), are annual. However, two closely related species, tetraploid *Z. perennis* [Hitchc.] Reeves and Mangelsdorf and diploid *Z. diploperennis* Iltis, Doebley & R. Guzman, are perennial. Perenniality of these two teosintes is manifested as regrowth after seed production and senescence, which includes developing juvenile basal axillary buds and rhizomes, under favorable environment. A fertile F_1 hybrid between *Z. mays* and *Z. perennis* was made by R. A. Emerson in the 1920s (Emerson and Beadle 1930), and *Z. mays*' hybrids with *Z. diploperennis* were also obtained soon after the diploid perennial teosinte was discovered in the 1990s (Srinivasan and Brewbaker 1999). Evergreen stalks, bulbils (highly-condensed rhizomes), basal shoot development, stiff stalk and robust root system have all been cited as phenotypic features of perennialism in *Z. diploperennis* (Galinat 1981a; Westerbergh and Doebley 2004; Mary Eubanks, personal communication). For example, evergreen stalks, which was proposed as a component of perennialism in Z. *diploperennis* (Galinat 1981a), appears to be linked to *sugary 1* on the short arm of chromosome 4 (Galinat 1981b).

Conflicting conclusions have been reached in various studies on how perennialism is inherited in *Zea*. Shaver (1967), who worked with tetraploid *Z. perennis*, proposed that a triple homozygous recessive genotype is needed for the perenniality in *Zea*. In this model, *pe* (*perennialism*), interacting with *gt* (*grassy tillers*) and *id* (*indeterminate*), plays a key role in conferring totipotency to the basal axillary buds and rhizomes in the perennial teosintes (Shaver 1964, 1967). The nature of *pe* remains unknown and the *Z. perennis*-derived genotype from which *pe* was identified by Shaver (1967) was lost and never recovered despite decades of intensive efforts (D.L. Shaver, personal communication). *Pe*-d*, the *Z. diploperennis* allele of *pe* was mapped to the long arm of maize chromosome 4 (Mary Eubanks, personal communication). The *gt* gene (aka *gt1*), located on the short arm of maize chromosome 1, encodes a class I homeodomain leucine zipper that promotes lateral bud dormancy and suppresses elongation of lateral ear branches (Whipple *et al.* 2011). It appears that *gt1* depends on the activity of a major maize domestication gene, *teosinte branched 1* (*tb1*), and is inducible by shading (Whipple *et al.* 2011). The *id* gene (aka *id1*) alters maize's ability to flower (Singleton 1946). Both *tb1* and *id1* are located on the long arm of maize chromosome 1 and both encode transcription factors with zinc finger motifs (Whipple *et al.* 2011; Colasanti *et al.* 1998). Singleton (1946) believed that *id1* inhibits plantlet generation at the upper nodes of a maize stalk. Mangelsdorf *et al.* (1981) proposed that one or two dominant genes control annual growth habit in their *Z. diploperennis*-popcorn hybrid.

In contrast to the recessive inheritance model, Galinat (1981b) proposed that perennialism in *Z. diploperennis* is at least partially controlled by two dominant complementary genes. Also, Ting and Yu (1982) obtained three perennial F_1 hybrids by pollinating three Chinese field corn varieties with *Z. diploperennis*, which indicate that perennial factors are dominant. Unfortunately, there is no further report about these hybrids or their derivatives.

Westerbergh and Doebley (2004) regarded perennialism in *Z. diploperennis* as a quantitative trait and identified a total of 38 QTL for eight perennial-habit traits from a *Z. diploperennis* x *Z. mays* ssp. *parviglumis* (annual) mapping population. Intriguingly, they did not identify any QTL that shows a singularly large effect. Murray and Jessup (2013) indicated that non-senescence and rhizomatousness are essential traits in their perennial maize breeding practice.

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

MATERIALS AND METHODS

Plant materials and phenotyping

Zea diploperennis (PI 462368; Zd, hereafter in a cross combination) and *Z. mays* cv. Rhee Flint (PI 213764; RF, hereafter in a cross combination) were obtained from the USDA North Central Region Plant Introduction Station, Ames, IA. Maize B73 and Mo17 inbred lines were from the collection of Dr. Auger and are traceable back to the Maize Genetics Cooperation Stock Center, Urbana/Champaign, IL. Rhee Flint is small, fast-growing heirloom maize variety and usually has tillers, which affords serial plantings with an increased opportunity of a plant simultaneously flowering with *Z. diploperennis*. In our designations of F_1s and their derivatives, the female parental is shown first. All the plants used in this study were grown in the greenhouse during the winter and

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in the field during the summer in Brookings, SD. Controlled pollinations were done by covering tassels and ears with paper bags before and after the pollination was made. In the greenhouse, plants were maintained with a 16 h-light/8 h-dark cycle and 20/16° day/night temperature except that two-month old *Z. diploperennis* and its hybrid plants were treated with a 10 h light/14 h dark cycle for four weeks to induce the floral transition.

Plants were scored as regrowth if they produced shoots from the basal axillary buds after the original stalks finished flowering and senesced. Rhizome and tuber development were visually investigated on plants that were dug from the soil after senescence. Tiller number at tasseling (TNT) was investigated by counting numbers of tillers per plant when the tassel had fully emerged. Ear and kernel morphology was visually examined and photographed.

Table 1 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′

Pcr assay

DNA samples were isolated from young leaves using the CTAB procedure (Doyle and Doyle 1990) and used for PCR-based marker assay. Table 1 lists all the PCR primers used in this study. PCR assays were done using GoTaq Green Master Mix (Catalog# M7505, Promega, Madison, WI) at the following conditions: 95°, 35 cycles of 95° for 45 s, 55∼62° (primer dependent) for 1 min and 72° for 1 min, and 72° for 10 min. The annealing temperatures were determined using a 1°-touchdown PCR step starting from 65°.

SNP discovery and locus mapping

The GBS assay was conducted according to Elshire *et al.* (2011). The preparation and sequencing of the library were conducted by the University of Wisconsin Biotechnology Center (UWBRC). Generally, DNA samples were digested with *ApeK*I restriction enzyme (RE), and unique barcodes were annealed to each DNA fragments. A single-end 100 bp (1x100bp) sequencing run was carried out on an Illumina HiSeq 2500 platform. The raw data were pooled as a single fastq file and downloaded from UWBRC.

The TASSEL (Trait Analysis by Association, Evolution and Linkage) 3 pipeline was used under the guidance of TASSEL manual (Glaubitz *et al.* 2014) for the discovery of SNPs between *Z. diploperennis* and *Z. mays*. The barcoded sequence reads were collapsed into a set of unique sequence tags with counts. The tag count files were filtered for a minimum count threshold and merged into the master tag count file. The B73_RefGen_V4 reference genome sequence was downloaded from MaizeGDB and processed with Bowtie2 for alignment (Langmead and Salzberg 2012). Master tags were aligned to the B73 reference genome to generate a "Tags On Physical Map" (TOPM) file, which contains the genomic position of each tag with the best unique alignment. The occupancies of tags for each taxon were observed from barcodes information in the original FASTQ files. Tag counts were stored in a "Tags by Taxa" (TBT) file. The TOPM and TBT files were used to call SNPs at the tag locations on the genome. The SNPs were first subjected to a two-step filtration in TASSEL 3 with minimum tag counts of 5, genotype mismatch rate of 0.1, minimum taxon coverage of 0.01, minimum site coverage of 0.2 and minimum minor allele frequency of 0.01. Fastq files containing sequences of chromosomes 1 to 10 were merged by FASTX_Toolkit and indexed. All commands for SNP discovery were executed in Ubuntu 16.04 LTS platform. Steps and codes used in TASSEL pipeline including example command lines and brief descriptions are listed in Supplementary Table S1.

The SNPs resulted from the first filtration were filtered again by manually removing sites that had missing data in more than 20% of the B73-Zd F_2 plants. For those SNPs that have missing data in less than 20% of the B73-Zd F_2 plants, the missing data were imputed by treating them as heterozygote since both alleles can be embodied and considered to be moderate. The SNPs from the 2nd filtration were used for QTL mapping.

To understand the relationship between the mapped QTL with the genetic factors revealed by the genetic analysis, the SNPs from the 2nd filtration were further filtered (the 3rd filtration) with χ^2 ($P < 0.05$). This is based on our hypothesis that, if a SNP is associated with a regrowth locus, the teosinte allele of the SNP should be carried by all the regrowable F_2s but only by three sevenths non-regrowable F_2s. Therefore, for each regrowth-associated SNP, we expected, in the regrowable subpopulation, 33.3% plants to carry the homozygous Zd alleles (AA), 66.6% to have the Zd-B73 heterozygous allele combination (AB) and none to be with the B73 homozygous alleles (BB) and, in the non-regrowable subpopulation, 14% of the plants with AA, 28.6% with AB and 57.1% with BB. Altogether, a χ^2 contingency table were generated with expected $\chi^2_{0.05,\ 4} = 9.49$. Any SNPs with $\chi^2 < 9.49$ were kept fitting the 9:7 segregation model.

The 4th SNP filtration was performed by collapsing immediately neighboring SNPs, up to a 100-bp range, that share the same haplotypes into one cluster and using the first SNP to represent the cluster. In the locus analysis with the chi-square imputation, such a cluster of SNPs was treated as one locus. Removing the redundant SNPs makes the locus analysis more precise because repeated SNP sites would affect calculating LOD (logarithm of the odds) scores and influence interval estimation.

The SNPs after the 2nd and the 4th filtrations were used for candidate locus/QTL estimation, respectively. The locus analysis was executed by a standard QTL procedure in *R* using the *R*/qtl package (version 1.40-8) (Broman *et al.* 2003) to better observe the contribution of each SNP and its neighbors. Position simulation was drawn with a maximum distance of 1.0 cM and an error probability of $1x10^{-4}$. The conditional genotype probability (calc.genoprob), as well as simulated genotypes (sim.geno with n.draw = 32), were calculated. The "haldane" function was used to convert genetic distances into recombination fractions. Genome scan with a single locus model (scanone) was performed with a binary model using the expectation-maximization algorithm (Broman *et al.* 2003). A permutation test with 1000 replicates was performed in scanone to visualize the LOD thresholds. We determined a locus interval by selecting the first and last SNP sites with significant LOD value. Genes within the intervals were identified by searching the corresponding region on the Gramene website. The *R* codes used for candidate locus/QTL analyses in this study are listed in Supplementary Table S2.

Statistical analyses

For statistical analyses, all genotypes and phenotypes were transformed into numeric values. For phenotypes, the regrowth plants were scored as

"1" and the non-regrowth plants were scored as "2". For genotypes, the plants that were homozygous to the *Z. diploperennis* allele were scored as "1"; those that were homozygous to the B73 or Rhee Flint allele were scored as "2"; and those that were heterozygous were scored as "3". When conducting locus analysis, genotype "1" was transformed to "AA", "2" to "BB" and "3" to "AB".

A chi square goodness-of-fit test was used to find the best-fit model or linkage in the genetic analysis and reveal candidate loci on chromosomes. To determine if TNT has any correlation with regrowth, a One-Way ANOVA of TNT by regrowth was performed in JMP (JMP 11.2.0).

Data availability

All raw fastq data from this study are available at NCBI data deposition site (https://www.ncbi.nlm.nih.gov/bioproject/) with accession number PRJNA477673. Steps and codes in TASSEL pipeline including example command lines and brief descriptions are list in Supplementary Table S1. *R* codes used for candidate locus/QTL analyses in this study are listed in Supplementary Table S2. Additional phenotypic and genotypic data of the F_2 and F_3 populations are available in Supplementary Table S3 to S5 as well as Supplementary Figure S1 to S3. Supplemental material available at Figshare: https://doi.org/10.25387/g3.7766735.

RESULTS AND DISCUSSION

The production and growth of the hybrids

We made reciprocal crosses of *Z. diploperennis* with the following three maize lines: B73, Mo17 and Rhee Flint. The first F_1 was made with Rhee Flint in a greenhouse. Rhee Flint is small, fast-growing and usually has tillers, which affords serial plantings with an increased opportunity of a plant simultaneously flowering with *Z. diploperennis*. Because Rhee Flint is an open-pollinated variety, later F_1s were made with B73 and Mo17 to facilitate molecular analysis. All the F_1 plants are fertile and completed multiple cycles of growth, reproduction and senescence (Figure 1; Supplementary Figure S1). Regrowth (as opposed to accidental replanting from a seed) of F_1 plants was insured by inspection that new shoots were attached to the base of the F_1 and confirmed by the heterozygosity of polymorphic PCR markers (examples shown in Supplementary Figure S2). Regrowth of these F_1s originates from basal axillary buds after stem senescence in all the crosses (Figures 1D, 1E, 1F).

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

Because the F_1 plants and their perennial derivatives are not winter hardy, the regeneration cycles were alternated between the greenhouse and the field (Supplementary Figures S1 and S3). Interestingly, the ears and kernels of the F_1s of the six crosses all were more teosinte-like (*i.e.*, two rows of oppositely positioned spikelets with paired kernels encased by woody rachides and glumes) when grown in greenhouse but were more maize-like (*i.e.*, multiple rows of naked kernels with short soft glumes and rachides around a silica-filled soft core) when grown in the field (Figure 3). In the F_2 and higher generations, ear morphology segregated even under greenhouse condition (Figure 3). These observations suggest that environmental factors are important in the preferential expression of the teosinte or the maize alleles of the genes influencing ear morphogenesis in the hybrids. These observations also indicate that it should be possible to breed regrowable maize with maize-like ears and kernels.

The contrast between our observations and those of some previous reports is remarkable. While we focus on a single trait, regrowth after senescence, previous studies were interested in perennialism generally using varying criteria. Conclusions that perennialism in *Zea* is recessive might have resulted from the hypothesis that traits such as TNT or rhizome development are indispensable components of perennialism in *Zea*. Indeed, other studies have used rhizome development as an indicator of perennialism in *Zea* (Srinivasan and Brewbaker 1999; Shaver 1964, 1967; Mangelsdorf *et al.* 1981; Camara-Hernandez and Mangelsdorf 1981) and we have not observed rhizomes in any of our F_1s or the derived plants. When regrowth occurs, it is always from an axillary bud. Indeed, it is also our observation that the regrowth of *Z. diploperennis* is mainly from basal axillary buds, and only occasionally from rhizomes. The *Z. perennis* - 4X maize F_1s made by R. A. Emerson also were all "weakly perennial" under the environmental conditions with few or no rhizomes (Emerson and Beadle 1930).

Other possible explanations for contrasting results are that the perennial teosinte plants used in those studies were polymorphic for one or more regrowth genes, that the experimental environments were unfavorable for regrowth to happen, or that some plants needed more time to break up their dormancy. Shaver (1967) and Camara-Hernandez and Mangelsdorf (1981) observed that some of their F_1 plants eventually regrew from basal axillary buds after a period of dormancy. Indeed, some of our F_2 plants need about two months of dormancy before regrowth. This observation reinforces the view that even regrowth is a complex trait that is modified by genetics and environment.

TNT has been associated with perennialism in several studies (Camara-Hernandez and Mangelsdorf 1981; Doebley *et al.* 1997; Shaver 1964; Westerbergh and Doebley 2004), therefore we investigated the relationship of TNT with regrowth in the Zd-RF F_2s. One-way ANOVA of TNT by regrowth, however, revealed no significant difference of TNT ($F = 0.897$, $P = 0.353$) between the regrowth and the non-regrowth F_2s (Supplementary Table S3). Indeed, we observed regrowth from several single-stalked hybrid derivatives (Figure 4A) and non-regrowth of some multi-stalked plants (Figure 4B). These results suggest that TNT is not essential to regrowth in *Zea*.

The genetics of regrowth

All our F_1 plants regrew and underwent several life cycles alternating between the greenhouse and the field. This indicates that, with our materials and in our environment, regrowth is a dominant trait. Regrowable F_1 hybrids of maize with perennial teosintes were previously obtained by Emerson (Emerson and Beadle 1930), Shaver (1964), Galinat (1981b), Camara-Hernandez and Mangelsdorf (1981) and Ting and Yu (1982). Srinivasan and Brewbaker (1999) suggested cytoplasm may contribute to perennialism, but our reciprocal F_1s performed without difference from one another.

To analyze the genetics of regrowth further, 134 B73-Zd F_2s (derived from several F_1 plants where B73 was the female) and 159 Zd-RF F_2s (derived from a single F_1 plant where Zd was the female) were tested. Among the 134 B73-Zd F_2s, 81 regrew and 53 did not (Table 2). Similarly, among the 159 Zd-RF F_2s, 90 regrew after senescence and 69 did not (Table 3). One B73-Zd F_3 population (Supplementary Table S4) and three Zd-RF F_3 populations (Table 3), each of which was derived from a single regrowth F_2 plant, were also evaluated for their regrowth.

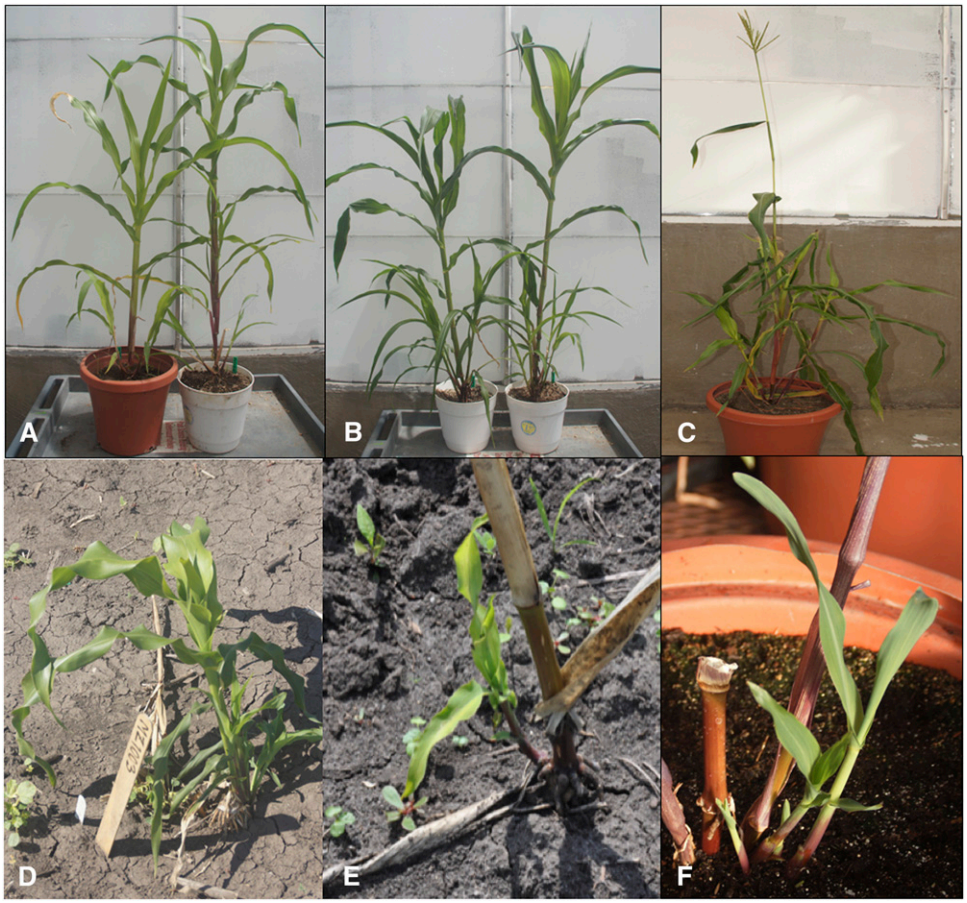

Figure 1 Photos of *Zea mays* and *Z. diploperennis* (Zd) F_1 plants. **A**: reciprocal Mo17-Zd (right) and Zd-Mo17 (left) F_1 plants; **B**: reciprocal B73-Zd (right) and Zd-B73 (left) F_1 plants; **C**: RF-Zd F_1 plant; **D**: regrowth of a Mo17-Zd F_1 plant; **E**: regrowth of a B73-Zd F_1 plant;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

A chi square (χ^2) goodness-of-fit test suggests that both of the F_2 populations and one Zd-RF F_3 population (Zd-RF F_3-5) we tested best fit a 9:7 regrowth to non-regrowth ratio (Table 4), and the B73-Zd F_3 population and the remaining two Zd-RF F_3 populations best fit a 3:1 ratio (Table 4). The simplest model that explains these results is that regrowth in the F_1s and their derivatives is mainly controlled by two dominant, complementary *regrowth* (*reg*) loci. The two dominant, complementary gene model parallels what has been found in other species, such as rice (Hu *et al.* 2003), Johnsongrass (Paterson *et al.* 1995; Hu *et al.* 2003; Washburn *et al.* 2013), basin wildrye (*Leymus cinereus*) (Yun *et al.* 2014) and wild mung bean (Nguyen *et al.* 2012), for perennialism-related traits.

The Zd-RF F_1 was also backcrossed to each parental line. All plants from the F_1-to-Zd backcross regrew, showing dominant effect of the Zd alleles. However, only one of the 20 plants from the F_1-to-RF backcross showed regrowth. Segregation of genomic fragments of *Z. diploperennis* in maize backcross derivatives is known to be highly distorted (Wang *et al.* 2012). Therefore, this observed 1:19 ratio may be due to distorted segregation of *Z. diploperennis* genomic fragment(s) carrying the allele(s) of one or both *reg* genes in the RF backcross derivative. Alternatively, other genetic models, such as one or three dominant complementary genes, two major genes with a few minor modifiers, or that regrowth is a complex trait controlled by many QTL, are not eliminated by this genetic analysis, but are less probable. Nevertheless, this could add difficulty to the effort of transferring the *Z. diploperennis* alleles to maize.

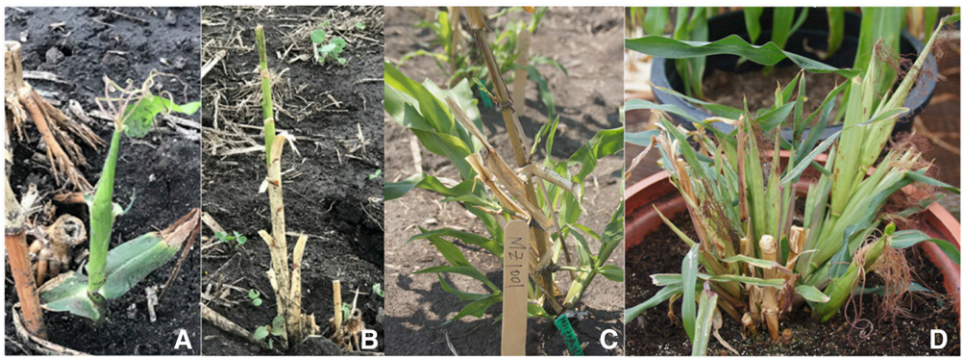

Figure 2 Photos of abnormal F_1 plants of crosses of *Zea diploperennis* with *Z. mays* inbred lines B73 (A & B) and Mo17 (C) or cv. Rhee Flint (D).

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

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

The F_2 and F_3 plants afforded an opportunity to investigate whether some factors previously implicated in perennialism may contribute to the regrowth trait. The rice rhizomatousness gene *Rhz2* has been mapped to rice chromosomes 3 (Hu *et al.* 2003) and sorghum chromosome 1 (Paterson *et al.* 1995; Hu *et al.* 2003; Washburn *et al.* 2013), which are both homeologous to parts of maize chromosome 1 (Wei *et al.* 2007). Additionally, *gt1* and *id1*, which have been implicated with perenniality in *Zea* (Shaver 1967), and *tb1*, which controls

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

Table 2 Phenotypes and genotypes of the *Zea mays* B73-*Z. diploperennis* F_2s*

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

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

Table 3 Segregation of regrowth among the *Zea mays* cv Rhee Flint-*Z. diploperennis* F_2s and F_3s*

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

*ZR2: F_2s; ZR3: F_3s; R: regrowth; NR: non-regrowth.

gt1 (Whipple *et al.* 2011), are all on chromosome 1 in *Zea* (Colasanti *et al.* 1998; Whipple *et al.* 2011). Therefore, we investigated the allele compositions of these three genes in the B73-Zd F_2s (Table 2), and 26 Zd-RF F_2 plants and the three Zd-RF F_3 populations (Supplementary Table S5), and assayed their association with regrowth. Of the 134 regrowth hybrid derivatives we examined, 5, 33 and 115 were homozygous for the maize *gt1*, *tb1* or *id1* alleles, respectively (Table 2 and Supplementary Table S5). Zd-RF F_3 family Zd-RF F_3-5 is homozygous for the *gt1* allele of *Z. diploperennis* (Supplementary Table S5) but segregates approximately 9:7 for regrowth and non-regrowth (Table 3). Therefore, our results are inconsistent with the model of Shaver (1967), and show that *gt1* and *id1* do not control regrowth in our F_1s and their derivatives. *Z. diploperennis*'s *gt1* allele may be helpful to regrowth because the majority of the plants that regrew had at least one copy, but it is not indispensable because some plants regrew without it.

Table 4 Results of the χ^2 goodness-of-fit tests of three simple genetic models

	Observed			No. dominant genes (the expected R to NR ratio) and P(χ^2)*		
Generations	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.

Interestingly, we observed no heterozygosity for *id1* and very low heterozygosity for *tb1* in all the hybrid derivatives that were examined, regardless of regrowth (Tables 2; Supplementary Table S5). Of 134 B73-Zd F_2 plants, only 16 had the *Z. diploperennis id1* allele (Table 2). Similar phenomena were observed in the derivatives of the Zd-RF cross (Supplementary Table S5). It seems that the maize chromosome fragment that carries *id1* was preferentially transmitted to the hybrid derivatives. Excess homozygosity of the maize *id1* allele indicates some sort of selection. It could be that a deficiency or other rearrangement adjacent to the teosinte *id1* allele causes it not to transmit efficiently, or it could be that the teosinte *id1* or tightly linked allele(s) cause the plant to grow poorly or be sterile in our experimental conditions.

Identifying regrowth loci with genotyping by-sequencing assay

A genome-wide mining of single nucleotide polymorphisms (SNPs) was conducted in a randomly selected sub-population of 94 (55 regrowth and 39 non-regrowth) B73-Zd F_2 plants with GBS technology (Table 2). We conducted the GBS assays to identify QTL for the regrowth trait. To prepare for these assays, a total of 2,204,834 (85.14%) Illumina sequencing tags that passed routine quality control filtrations were aligned with the B73_v4 reference genome. A total of 714,158 SNPs, covering all ten chromosomes with an average of 71,416 SNPs per chromosome, were then called from 83 (46 regrowth and 37 non-regrowth, labeled in bold in Table 2) of the 94 F_2 plants using TASSEL 3 pipeline (Supplementary Table S1). SNP-calling for the excluded 11 plants probably failed due to an error in barcode addition before sequencing. These SNPs were first subjected to a two-step filtration, which resulted in 37,925 and 10,432 SNPs among all ten chromosomes, respectively (Table 5).

To explore which chromosomal regions may control the regrowth phenotype, the 10,432 SNPs from the 2nd filtration step were pooled for QTL analysis using R/qtl (version 1.42-8) (Supplementary Table S2). The result is shown in Figure 5A. A total of 126 SNPs (104 real sites and 22 simulated sites) showed LOD scores higher than 3.00. A permutation test of 1,000 with the *p*-value of 0.05 resulted in a significant LOD score of 5.23 (Figure 5A). This significance threshold revealed two major QTL with one at 33,041,409 bp (the nucleotide position in the B73_v4 reference genome sequence) on chromosome 2 with a LOD score of 5.46 and one at 4,284,633 bp on chromosome 7 with a LOD score of 5.53.

To test if the two strongest QTL correspond to the two dominant and complementary loci suggested by the genetic analysis, we applied a χ^2 test with 9:7 allele segregation ratio model to the 10,431 SNPs to investigate if the observed and the expected genotypes are significantly different ($P \leq 0.05$). This step kept 946 SNPs (Table 5, 3rd filtration). Finally, to simplify the mapping effort, the 946 SNPs were filtered once more by collapsing immediately neighboring SNPs that share the same haplotypes into one cluster. This step resulted in 597 SNP cluster (Table 5, 4th filtration). Locus mapping with a threshold of $LOD_{95\%} = 4.17$ revealed two significant loci with one on chromosome 2 in the interval from 24,244,192 bp to 28,975,747 bp with the peak at 27,934,739 bp and another on chromosome 7 in the interval from 2,862,253 bp to 6,681,861 bp with the peak at 5,060,739 bp (Figure 5B). These two loci were mapped closely to the two major QTL indicated by the mapping without imputation. These results are consistent with the two-factor model, which warrants further investigation. To that end we are naming the factor underlying the chromosome 2 QTL *regrowth 1* (*reg1*) and the factor underlying the chromosome 7 QTL as *regrowth 2* (*reg2*).

Our LOD analysis located two minor peaks on chromosome 1 that are associated with regrowth (Figure 5B). We wanted to know if these two loci are related to *gt1* and *id1*. The SNPs at the peak of these loci are at 82,273,951 bp and 177,235,112 bp, far away from *id1* (around 243,201,405 bp) and *gt1* (around 23,625,801 bp), respectively (Figure 6). These observations further indicate that *id1* and *gt1* are not related to regrowth. Also, previous studies reported that *Z. diploperennis* carried perennialism-related *Pe*-d* (Mary Eubanks, personal communication) and an evergreen gene on chromosome 4 (Whipple *et al.* 2011). However, our data could not support these observations since no SNP on chromosome 4 is significantly associated with regrowth (Figures 5 and 6).

Table 5 Numbers of SNPs revealed in each chromosome of the B73-ZD F_2 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**

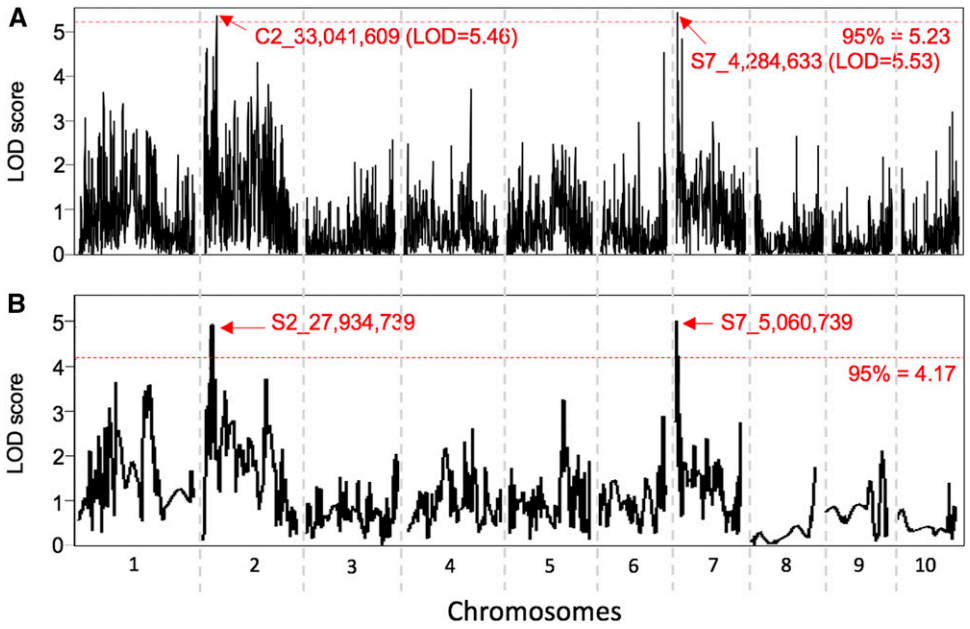

Figure 5 Graphics showing LOD scores of the QTL mapping the *Zea mays* B73-*Z. diploperennis* F_2 population without (A) or with (B) chi-square imputation. The 95% threshold lines (the parallel red dash lines) were calculated with 1,000 permutations. Significant QTL/loci are indicated by the location of the peak SNPs of the loci.

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

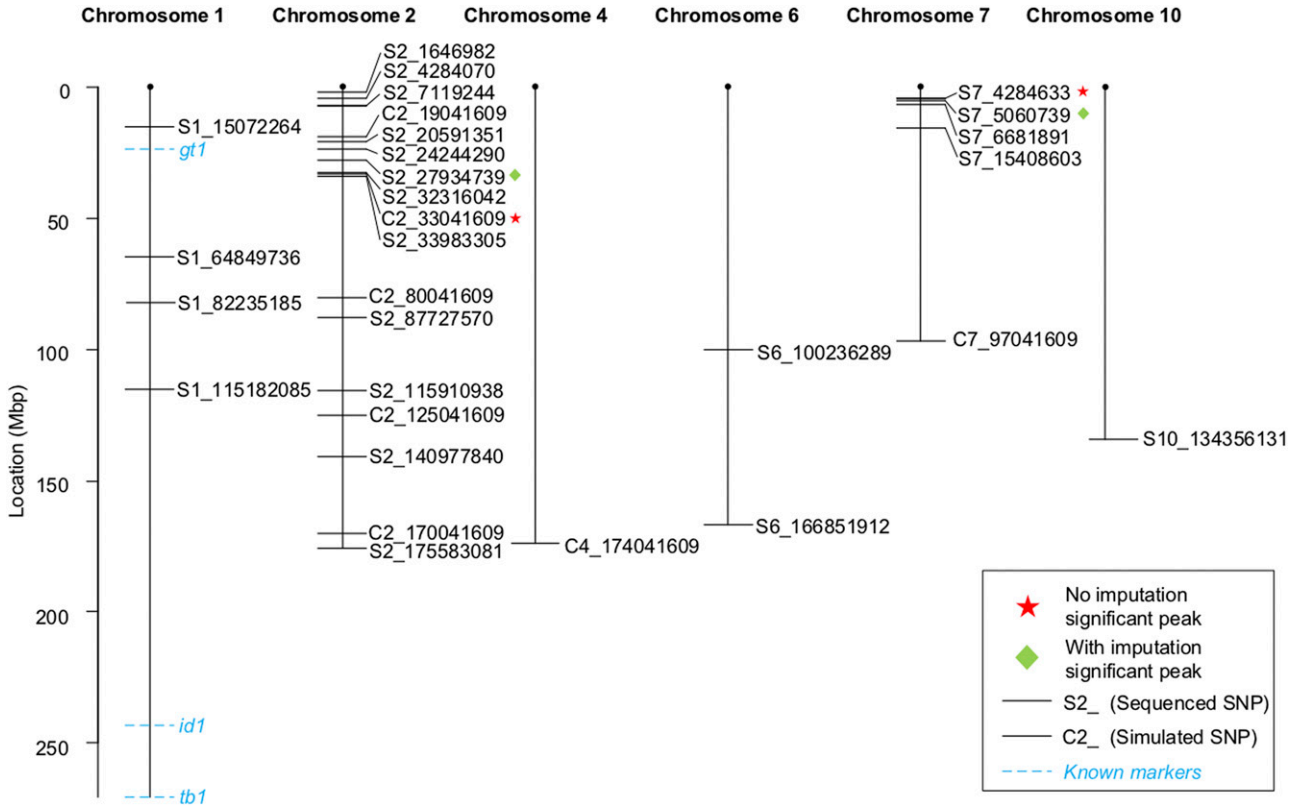

Figure 6 Genetic map of 30 representing SNPs and genes *gt1*, *id1*, and *tb1*. Each SNP represents a one-Mbp region except of SNP S2_27934739, which represent a SNP cluster.

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

LITERATURE CITED

Boe, A., V. Owens, J. Gonzalez-Hernandez, J. Stein, D. K. Lee *et al.*, 2009 Morphology and biomass production of prairie cordgrass on marginal lands. Glob. Change Biol. Bioenergy 1: 240–250. https://doi.org/10.1111/j.1757-1707.2009.01018.x

Broman, K. W., H. Wu, Ś. Sen, and G. A. Churchill, 2003 R/qtl: QTL mapping in experimental crosses. Bioinformatics 19: 889–890. https://doi.org/10.1093/bioinformatics/btg112

Camara-Hernandez, J., and P. C. Mangelsdorf, 1981 Perennial corn and annual teosinte phenotypes in crosses of *Zea diploperennis* and maize, pp. 3–37 in *Bussey Inst. Publication 10*, Harvard Univ., Cambridge, MA.

Colasanti, J., Z. Yuan, and V. Sundaresan, 1998 The *indeterminate* gene encodes a zinc finger protein and regulates a leaf-generated signal required for the transition to flowering in maize. Cell 93: 593–603. https://doi.org/10.1016/S0092-8674(00)81188-5

Doebley, J., A. Stec, and L. Hubbard, 1997 The evolution of apical dominance in maize. Nature 386: 485–488. https://doi.org/10.1038/386485a0

Doyle, J. J., and J. L. Doyle, 1990 Isolation of plant DNA from fresh tissue. Focus 12: 13–15.

Elshire, R. J., J. C. Glaubitz, Q. Sun, J. A. Poland, and K. Kawamoto, 2011 A robust, simple genotyping-by-sequencing (GBS) approach for high diversity species. PLoS One 6: e19379. https://doi.org/10.1371/journal.pone.0019379

Emerson, R. A., and G. W. Beadle, 1930 A Fertile Tetraploid Hybrid Between *Euchlaena perennis* and *Zea mays*. Am. Nat. 64: 190–192. https://doi.org/10.1086/280311

Galinat, W. C., 1981a Evergreen stalks as an indicator of perennialism. MNL 55: 107.

Galinat, W. C., 1981b The inheritance and linkage of perennialism derived from *diploperennis*. MNL 55: 107.

Glaubitz, J. C., T. M. Casstevens, F. Lu, J. Harriman, R. J. Elshire *et al.*, 2014 TASSEL-GBS: A high capacity genotyping by sequencing analysis pipeline. PLoS One 9: e90346. https://doi.org/10.1371/journal.pone.0090346

Haferkamp, M. R., and T. D. Copeland, 1984 Shoot growth and development of Alamo switchgrass as influenced by mowing and fertilization. J. Range Manage. 37: 406–412. https://doi.org/10.2307/3899625

Hu, F. Y., D. Y. Tao, E. Sacks, B. Y. Fu, P. Xu *et al.*, 2003 Convergent evolution of perenniality in rice and sorghum. Proc. Natl. Acad. Sci. USA 100: 4050–4054. https://doi.org/10.1073/pnas.0630531100

Jackson, L. L., and C. L. Dewald, 1994 Predicting evolutionary consequences of greater reproductive effort in *Tripsacum dactyloides*, a perennial grass. Ecology 75: 627–641. https://doi.org/10.2307/1941721

Langmead, B., and S. Salzberg, 2012 Fast gapped-read alignment with Bowtie 2. Nat. Methods 9: 357–359. https://doi.org/10.1038/nmeth.1923

Mangelsdorf, P. C., L. M. Roberts, and S. Rogers, 1981 The probable origin of annual teosintes, pp. 39–69 in *Bussey Inst. Publication 10*, Harvard Univ., Cambridge, MA.

Melzer, S., F. Lens, J. Gennen, S. Vanneste, A. Rohde *et al.*, 2008 Flowering-time genes modulate meristem determinacy and growth form in *Arabidopsis thaliana*. Nat. Genet. 40: 1489–1492. https://doi.org/10.1038/ng.253

Murray, S. C., and R. W. Jessup, 2013 Breeding and genetics of perennial maize: Progress, opportunities and challenges. In: *Perennial Crops for Food Security*, *Proceedings of the FAO Expert Workshop*, August 28–30, 2013, Rome, Italy, pp103–111.

Nguyen, T. D., R. J. Lawn, and L. M. Bielig, 2012 Expression and inheritance of perenniality and other qualitative traits in hybrids between mungbean cultivars and Australian wild accessions. Crop Pasture Sci. 63: 619–634. https://doi.org/10.1071/CP12263

Paterson, A. H., K. F. Schertz, Y. Lin, S. Liu, and Y. Chang, 1995 The weediness of wild plants: Molecular analysis of genes influencing dispersal and persistence of johnsongrass, *Sorghum halepense* (L.) Pers. Proc. Natl. Acad. Sci. USA 92: 6127–6131. https://doi.org/10.1073/pnas.92.13.6127

Ryder, N., K. M. Dorn, M. Huitsing, M. Adams, J. Ploegstra *et al.*, 2018 Transcriptome assembly and annotation of johnsongrass (*Sorghum halepense*) rhizomes identify candidate rhizome-specific genes. Plant Direct 2: e00065. https://doi.org/10.1002/pld3.65

Shaver, D. L., 1964 Perennialism in *Zea*. Genetics 50: 393–406.

Shaver, D. L., 1967 Perennial maize. J. Hered. 58: 271–273. https://doi.org/10.1093/oxfordjournals.jhered.a107611

Singleton, W. R., 1946 Inheritance of indeterminate growth in maize. J. Hered. 37: 61–64. https://doi.org/10.1093/oxfordjournals.jhered.a105582

Srinivasan, G., and J. L. Brewbaker, 1999 Genetic analysis of hybrids between maize and perennial teosinte. I. Morphological traits. Maydica 44: 353–369.

Ting, Y. C., and M. K. Yu, 1982 Further studies of F1 hybrids of maize x diploid perennial teosinte. MNL 56: 35–36.

Wang, G., Q. Q. He, Z. Xu, and R. T. Song, 2012 High segregation distortion in maize B73 x teosinte crosses. Genet. Mol. Res. 11: 693–706. https://doi.org/10.4238/2012.March.19.3

Washburn, J. D., S. C. Murray, B. L. Burson, R. R. Klein, and R. Jessup, 2013 Targeted mapping of quantitative trait locus regions for rhizomatousness in chromosome SBI-01 and analysis of overwintering in a *Sorghum bicolor* x *S. propinquum* population. Mol. Breed. 31: 153–162. https://doi.org/10.1007/s11032-012-9778-8

Wei, F., E. Coe, W. Nelson, A. K. Bharti, F. Engler *et al.*, 2007 Physical and genetic structure of the maize genome reflects its complex evolutionary history. PLoS Genet. 3: e123. https://doi.org/10.1371/journal.pgen.0030123

Westerbergh, A., and J. Doebley, 2004 Quantitative trait loci controlling phenotypes related to the perennial *vs.* annual habit in wild relatives of maize. Theor. Appl. Genet. 109: 1544–1553. https://doi.org/10.1007/s00122-004-1778-6

Whipple, C. J., T. H. Kebrom, A. L. Weber, F. Yang, and D. Hall, 2011 *grassy tillers1* promotes apical dominance in maize and responds to shade signals in the grasses. Proc. Natl. Acad. Sci. USA 108: E506–E512. https://doi.org/10.1073/pnas.1102819108

Yun, L., S. R. Larson, I. W. Mott, K. R. Jensen, and J. E. Staub, 2014 Genetic control of rhizomes and genomic localization of a major effect growth habit QTL in perennial wildrye. Mol. Genet. Genomics 289: 383–397. https://doi.org/10.1007/s00438-014-0817-5

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