



Evolution and expression of tandem duplicated maize flavonol synthase genes

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Flavonoids are specialized compounds widely distributed and with diverse functions throughout the plant kingdom and with several benefits for human health. In particular, flavonols, synthesized by flavonol synthase (FLS), protect plants against UV-B radiation and are essential for male fertility in maize and other plants. We have recently characterized a UV-B inducible *ZmFLS1*, corresponding to the first to be described in monocot plants. Interestingly, the new assembly of the B73 maize genome revealed the presence of a second putative *FLS* gene (*ZmFLS2*), with very high identity with *ZmFLS1*. *ZmFLS*s expression was analyzed in different maize tissues, and by combining electrophoretic mobility shift assays and transient expression experiments, we show that both genes are direct targets of anthocyanin (C1/PL1 + R/B) and 3-deoxy flavonoid (P1) transcriptional regulators. *ZmFLS* expression analyses show higher levels of both transcripts in high altitude landraces than inbred lines, and both genes are regulated by UV-B radiation in all lines analyzed. Moreover, the high sequence conservation of the *ZmFLS* promoters between maize lines suggests that the differences observed in *ZmFLS* expression are due to allelic variations in the transcription factors that regulate their activities. Finally, we generated pFLS1::FLS1-RFP transgenic plants and analyzed *ZmFLS1* expression in different maize tissues; we found that this enzyme is localized in the ER and the perinuclear region.

Keywords: UV-B, duplication, grasses, natural variation, maize

INTRODUCTION

Flavonoids are widely distributed plant metabolites with diverse biological functions. There is considerable evidence showing a role for flavonoids in contributing to the human health associated to their antioxidant, anti-proliferative and anti-inflammatory properties, and consequently, their indication in prevention against cancer and cardiovascular disease (Knekt et al., 2000; Hirvonen et al., 2001; Mak et al., 2006; Vargo et al., 2006; Geleijnse and Hollman, 2008; Kaur et al., 2008; Kang et al., 2009). Flavonols, the most abundant and widespread subgroup of flavonoids, play important functions in plant physiology, growth, and development, including the modulation of basipetal auxin transport in *Arabidopsis* (Brown et al., 2001; Peer et al., 2004; Kuhn et al., 2011; Lewis et al., 2011), attraction of and defense against insects (Gronquist et al., 2001), pollen fertility (Mo et al., 1992; Taylor and Jorgensen, 1992; Ylstra et al., 1994; Taylor and Hepler, 1997), and UV-B protection (Solovchenko and Schmitz-Eiberger, 2003; Jaakola et al., 2004; Stracke et al., 2007, 2010a; Kusano et al., 2011). Moreover, flavonols have recently been shown to participate in the ethylene-signaling pathway (Lewis et al., 2011).

The biosynthesis of flavonols from dihydroflavonols is catalyzed by the enzyme flavonol synthase (FLS), a soluble 2-oxoglutarate-dependent dioxygenase (2-ODD). *FLS* cDNAs were cloned from a

large number of dicot plants, and they were functionally expressed in bacteria, yeast, and plants (Martens et al., 2010). However, the regulation of flavonol biosynthesis has only been well studied in *Arabidopsis thaliana*. In this species, both the regulators and the biosynthetic genes are mainly regulated at the level of transcription (Quattrocchio et al., 2006; Jenkins, 2008). One subgroup of R2R3-type MYB proteins, called PFG family for Production of Flavonol Glucosides, is constituted of PFG1/MYB12, PFG2/MYB11, and PFG3/MYB111, which exhibit differential spatial expression patterns and regulate flavonol accumulation in a tissue- and developmental-specific manner (Stracke et al., 2007). However, a PFG1-3-independent flavonol accumulation occurs in pollen and siliques/seeds (Stracke et al., 2010b). Moreover, it has been demonstrated that the bZIP transcription factor long HYpocotyl5 (HY5), an important participant in the UV-B-induced signal transduction cascade mediated by UVR8, regulates the expression of *PFG1/MYB12* under UV-B radiation (Stracke et al., 2010a). Thus, *AtFLS1* and other genes encoding enzymes involved in flavonol biosynthesis are targets of these regulators (Mehrtens et al., 2005; Stracke et al., 2007, 2010a).

We recently characterized an FLS enzyme from maize, *ZmFLS1*, which converts dihydroflavonols to the corresponding flavonols, partially complementing the flavonol deficiency of the *Arabidopsis*

fls1 mutant. In addition, we showed that the *ZmFLS1* transcript level is increased by UV-B radiation, induction that is at least in part mediated by the activation of the regulators P1 and C1/PL1 + R/B (Falcone Ferreyra et al., 2010). The most recent release (version 5b.60) of the maize genome (inbred B73) allowed us to identify a second *ZmFLS* gene with very high identity to *ZmFLS1*. Thus, the aim of this work is to investigate these two maize *FLS* genes, and comparatively evaluate this paralogous pair in evolutionarily closely related grasses. Here, we show the *ZmFLS*s expression pattern in different tissues of the B73 maize line, and demonstrate that both genes are regulated by the P1 and C1/PL1 + R/B regulators. We further analyze *ZmFLS* expression in different maize inbreds and landraces from high altitudes, and we find that *ZmFLS*s are induced by UV-B exposure in all lines analyzed. Higher transcript levels of both *ZmFLS* genes were found in high altitude lines compared to inbred lines in the absence of UV-B, indicating that higher flavonol levels may be a constitutive mechanism of protection against high irradiance in these landraces. Moreover, the high sequence conservation of the *ZmFLS* promoters between maize lines suggests that different expression levels are probably a result from allelic variations in the *trans*-acting regulatory machinery.

RESULTS

IDENTIFICATION AND ANALYSES OF FLAVONOL SYNTHASES IN MAIZE AND OTHER GRASSES

We recently described *ZmFLS1* (GRMZM2G152801), encoding the first monocot flavonol synthase enzyme (Falcone Ferreyra et al., 2010). Interestingly, the new assembly of the B73 genome (release 5b.60) revealed the presence of a second putative *FLS* gene (GRMZM2G069298, *ZmFLS2*) with very high identity to *ZmFLS1*. Both genes are located in chromosome 5 separated by ~50 kb (Figure 1A) and have identical structure, with two exons separated by an intron (Figure 1B). *ZmFLS1* and *ZmFLS2* share 96% identity, with higher identity in the coding regions (99%), and major differences between the respective 3'UTR regions. The predicted amino acid sequences exhibit 99% identity, with only two differences at positions 49 and 184, corresponding to isoleucine (I) and glycine (G) to methionine (M) and serine (S) for *ZmFLS1* and *ZmFLS2*, respectively. Their putative upstream regulatory regions (arbitrarily defined here as 1.5 kb upstream of the start codon) exhibit 61% identity (Figure A1 in Appendix) while 95% identity is observed for the region from -350 to the start codon. In comparison with *FLS* genes from other grasses with completed sequenced genomes, like sorghum (*Sorghum bicolor*), rice (*Oryza sativa*), and *Brachypodium distachyon*, a high percentage of identity is observed at the nucleotide level (70–89%) with the highest homology found with one of the *FLS* genes in sorghum (*SbFLS1*). Based on the available genome sequences, *Brachypodium* and rice contain only one *FLS* gene (Bradi3g57910 and Os02g52840, respectively), while two *FLS* genes are present in *S. bicolor* (BTx623 line; Figure 1B). But unlike what is found in maize, the two sorghum *FLS* genes are located in different chromosomes [4 and 3 for *SbFLS1* (Sb04g034240) and *SbFLS2* (Sb03g002040), respectively] and *SbFLS1* is more closely related to *ZmFLS*s (87–89% identity) than to *SbFLS2* (72% identity), which is consistent with synteny analyses (see below). The length and

the structure of the *FLS* genes are conserved between *ZmFLS*s, *SbFLS1*, and *BdFLS1*, each having two exons and one intron (Figure 1B), while *OsFLS1* has three exons and two introns, a structure similar to *AtFLS1* (Stracke et al., 2009). Although the annotation of *SbFLS2* suggests the presence of a second intron, this region includes a region that, in all *FLS*s from other grasses, encodes a part of the FLS protein. Thus, the deduced protein sequence of *SbFLS2* lacks 27 amino acids. In addition, the second intron of *OsFLS1*, *SbFLS2*, and the only one for *ZmFLS*s and *SbFLS1* are placed at the same position in all these genes (Figure 1B).

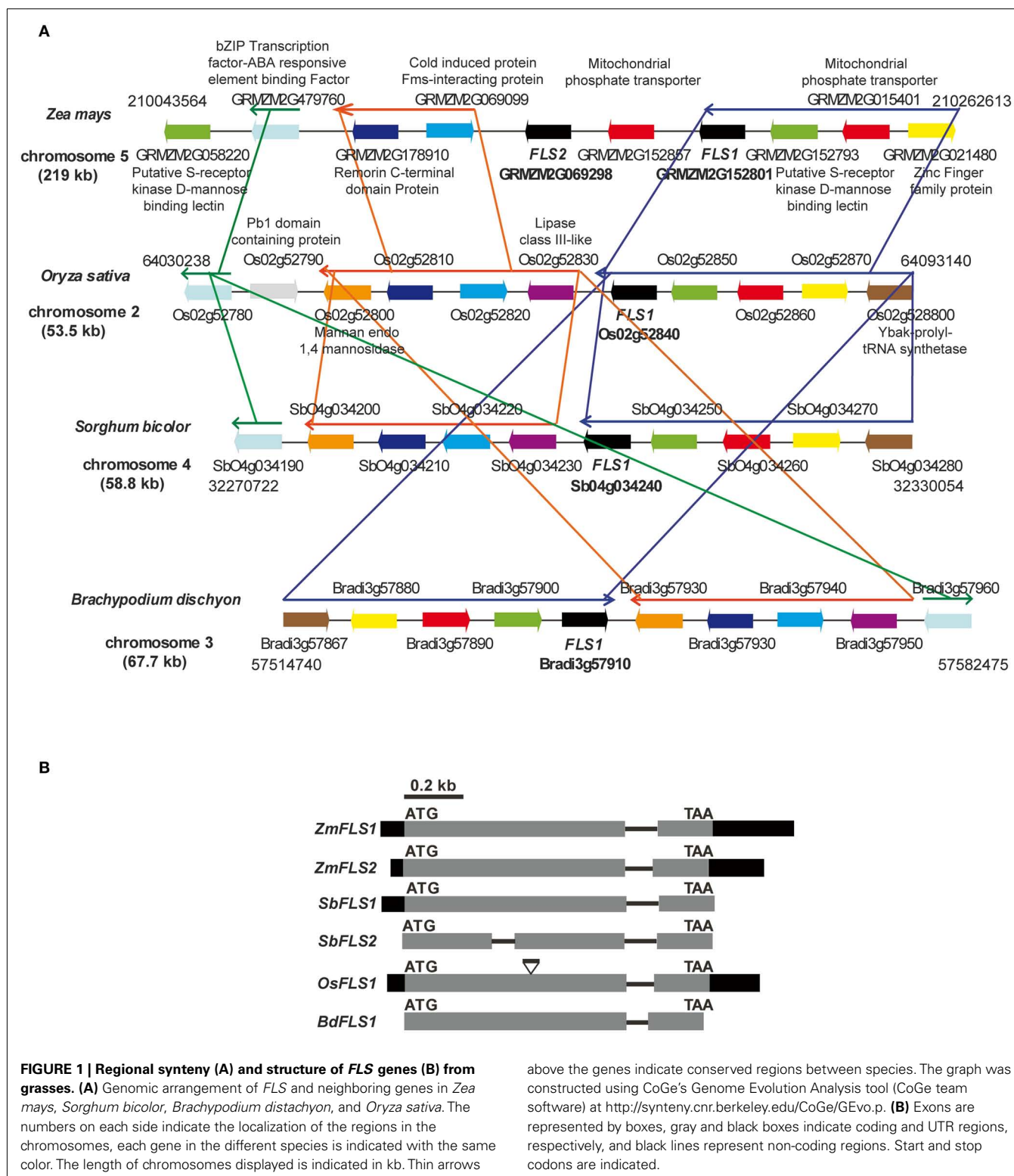
To verify the presence of the two tandemly arranged *ZmFLS* genes in the B73 genome, we designed primers to amplify fragments downstream of the 3'UTR of each gene, using the same forward primer that hybridizes in the 3'UTR of both genes, and specific reverse primers for each gene hybridizing elsewhere proximal in the chromosome. In addition, we amplified the intron of both *ZmFLS* genes using primers that hybridize in exons 1 and 2 of each gene (Figures A2B,C in Appendix). Moreover, to verify that both *ZmFLS* genes are localized in chromosome 5, we were able to amplify both genes using the same BAC clone (c0247D19) as a template (Figure A2D in Appendix). After sequencing the PCR products, we confirmed that the two different *ZmFLS* genes are present in chromosome 5 of the B73 maize inbred.

In order to analyze the orthologous *FLS* genes in detail, we explored the organization of the corresponding genes in the chromosomes. The analysis shows that *FLS* neighboring genes are conserved in maize (*ZmFLS1*, *ZmFLS2*), rice (*OsFLS1*), *Brachypodium* (*BdFLS1*), and sorghum (*SbFLS1*, with the exception of *SbFLS2* gene); however, some re-arrangements among blocks of linked genes are observed as well as inversions, duplication, and deletions of certain genes, particularly in maize (Figure 1A). Interestingly, synteny is spread over a region of 220 kb in maize while in other grasses this region is only 53–68 kb, as it was previously described for different regions of maize chromosomes (Li and Gill, 2002; Ilic et al., 2003; Bruggmann et al., 2006; Goette and Messing, 2009; Wei et al., 2009).

A phylogenetic tree generated with the amino acid sequences of plant FLS enzymes with demonstrated and predicted functionality showed a marked separation between enzymes from dicotyledonous and those from grasses monocotyledonous plants (Figure 2).

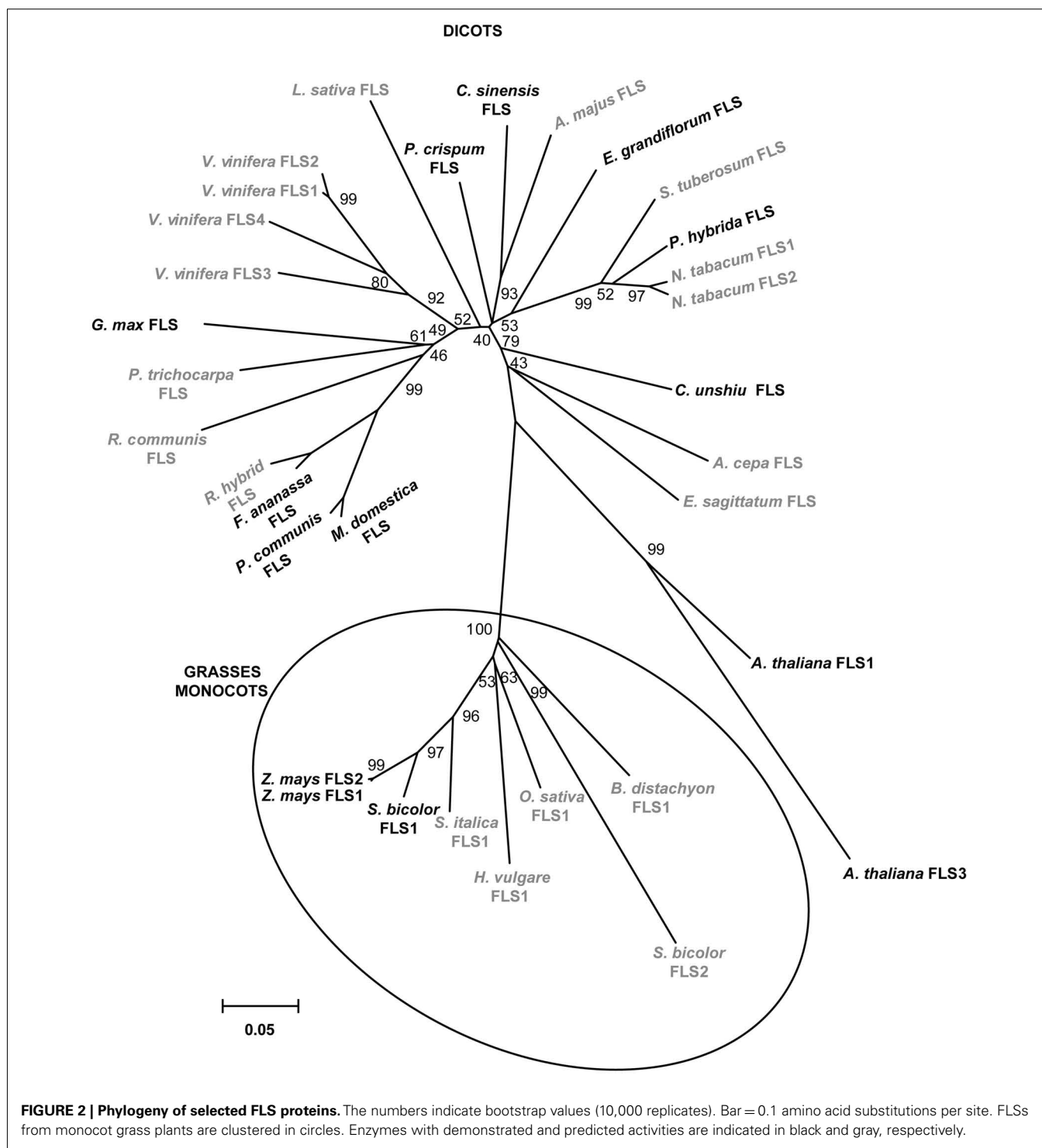
EXPRESSION ANALYSIS AND REGULATION OF *ZmFLS*s BY P1 AND C1 + R

To analyze the expression of *ZmFLS1* and *ZmFLS2*, we conducted quantitative RT-PCR (RT-quantitative PCR, qPCR) on RNA extracted from 7-day-old seedlings and juvenile leaves, roots, anthers (before anthesis), silks, 14 and 25 days after pollination (DAP) pericarps lacking (*PI-ww*) or accumulating (*PI-rr*) the phlobaphene pigments controlled by the maize *PI* gene (Grotewold et al., 1994), Black Mexican Sweet (BMS) maize cells, ectopically expressing the C1 + R anthocyanin regulators (BMS^{C1+R}, Grotewold et al., 1998), and untransformed control cells (BMS). Transcripts for *ZmFLS1* and *ZmFLS2* were detected in all the tissues analyzed, with the highest levels found in young seedlings



(Figure 3). It is worth mentioning that the expression patterns described are consistent with the microarray database from a genome-wide atlas of transcription in different tissues and developmental stage of maize (Sekhon et al., 2011).

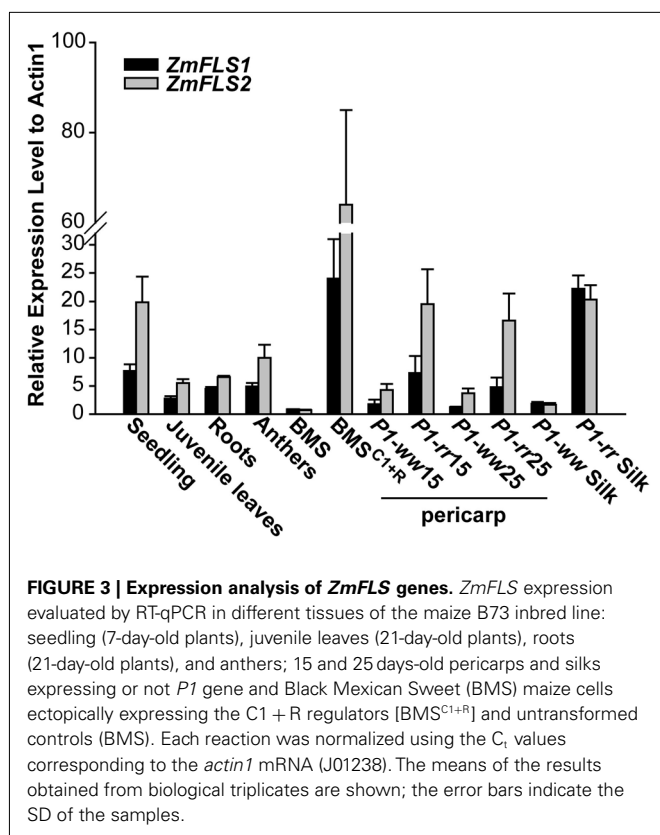
For both genes, transcripts were present at significantly higher levels in *P1-rr*, compared to *P1-ww* pericarps and silks, indicating that both genes are regulated by *P1*. Similarly, both *ZmFLS1* and *ZmFLS2* showed higher expression in BMS^{C1+R} than in BMS cells,



suggesting that both genes are also under the control of the C1 and R anthocyanin regulators (Figure 3).

Previously, we showed by transient expression experiments in BMS cells that *ZmFLS1* is positively regulated by P1 and C1 + R (Falcone Ferreyra et al., 2010). To investigate whether *ZmFLS2* is similarly regulated, we carried out transient co-transfection experiments of maize protoplast cells with the regulators driven

by the 35S promoter (p35S::P1 or p35S::C1 + p35S::R) and *ZmFLS2* promoter-luciferase reporter fusion (pZmFLS2::Luc; 1.5 kb upstream of the start codon). In addition, given that the proximal promoter regions (from -350 to the start codon) of *ZmFLS* genes are highly similar, we investigated to what extent the respective regions of *ZmFLS1* and *ZmFLS2* were sufficient for regulation by P1 and C1 + R.



Both *ZmFLS1* and *ZmFLS2* are robustly activated by C1 + R, with a significantly higher induction of *ZmFLS2*, compared to *ZmFLS1* ($P < 0.05$, **Figure 4A**). The analysis of candidate P1/C1 binding sites present in the *ZmFLS* promoters show additional candidate motifs in the distal region of the *ZmFLS2* promoter (**Figure 4A**). To investigate whether these binding sites could contribute to a major activation of *ZmFLS2* by C1 + R, we tested the proximal promoter regions up to -350 bp from the translation start codon; and, in this case, both genes were similarly activated, in accordance to the high identity of both *ZmFLS* proximal promoters (95%). For both genes, the 5' deletion of the promoter caused a significant reduction in their activation, indicating that the additional binding sites present in the distal promoter regions are important for C1 + R regulation. Additionally, when the most proximal binding site in both promoters was mutated (**Figure 4A**), regulation of both promoter::luciferase constructs by C1 + R remained the same, suggesting that this binding site is not the main contributor to C1 + R regulation. Thus, for *ZmFLS* genes, it is possible that the binding of C1 + R to DNA involves only one *cis*-regulatory element in the promoter (at -340 and -343 bp in *ZmFLS1* and *ZmFLS2*, respectively) or alternatively, another element could be involved in the binding and regulation, such as an E-box that is present in both promoters (at -296 and -293 bp, respectively), as it was suggested for the *Bz1* promoter (Roth et al., 1991).

Both *ZmFLS* genes were activated by P1, with higher levels of activation for *ZmFLS2* than for *ZmFLS1*. When the proximal

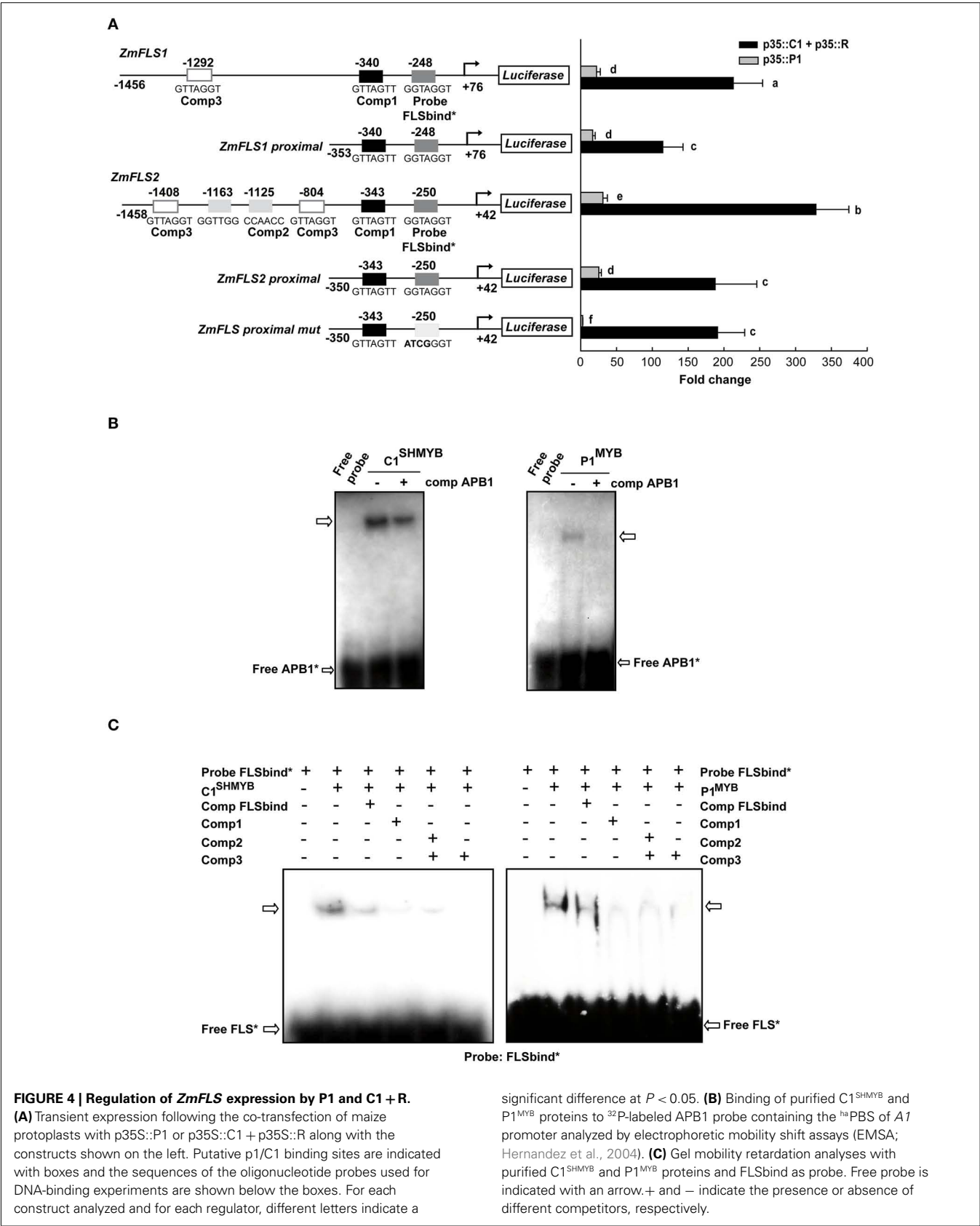
promoter regions were analyzed, *ZmFLS1* showed similar activation as when the full promoter was used, while a decreased activation was observed for *ZmFLS2*, suggesting that the additional P1-binding sites, absent in *ZmFLS1*, can contribute to *ZmFLS2* regulation by P1. Finally, when the most proximal binding site was mutated, the *ZmFLS* activation by P1 was almost completely lost, indicating an important and major contribution of this site in P1 regulation of *ZmFLS* genes (**Figure 4A**).

To verify which P1/C1 binding sites identified in the *ZmFLS* promoters are recognized by these transcription factors, we conducted electrophoretic mobility shift assays (EMSA). For these experiments, we expressed and affinity-purified from *E. coli* as N-terminal histidine-tagged fusions the P1 MYB domain (P1^{MYB}), as well as a version of the MYB domain of C1, C1^{SHMYB}, that binds DNA with higher affinity than C1^{MYB} and with comparable affinity to P1^{MYB} (Hernandez et al., 2004; N₆His-*ZmP1*^{MYB} and N₆His-*ZmC1*^{SHMYB}), obtaining 90–95% pure proteins (**Figure A3** in Appendix). C1^{SHMYB} and P1^{MYB} proteins bind to the APB1 probe, the positive control in our experiments that contains the ^hapBS present in the *A1* promoter (**Figure 4B**), which was previously demonstrated to bind both proteins with high affinity (Hernandez et al., 2004). C1^{SHMYB} and P1^{MYB} proteins can also bind effectively FLSbind (**Figure 4C**), the labeled probe that contains the most proximal binding site present in both *ZmFLS* promoters at -248 and -250 bp for *ZmFLS1* and *ZmFLS2*, respectively (**Figure 4A**). Oligonucleotides containing other sites (comp 1–3) competed with the binding to FLSbind (**Figure 4C**), as FLSbind does itself. These results indicate that C1 and P1 can bind to all P1/C1 binding sites identified in the *ZmFLS1* and *ZmFLS2* promoters.

IN VIVO LOCALIZATION OF *ZmFLS1*

To confirm the *in vivo* expression pattern of *ZmFLS1*, and to investigate its subcellular localization, we generated transgenic maize plants expressing *ZmFLS1* fused to the red fluorescent protein (RFP) under its own promoter (p*ZmFLS1::ZmFLS1-RFP*). The 3'UTR region and introns can contain regulatory functions (Bailey-Serres and Dawe, 1996; Patel et al., 2004; Rose et al., 2008; Parra et al., 2011); thus, the construct included the endogenous 3'UTR. It is noteworthy that we only generated transgenic plants expressing *ZmFLS1* fused to RFP, since at the time these transgenic plants were constructed, the B73 maize genome was being sequenced and only the sequence of *ZmFLS1* was available in GenBank.

The FLS1-RFP fusion protein showed strong localization to the ER, as indicated by the “patchwork” pattern of fluorescence within leaf and root cells (**Figures 5A,C**), and was most easily detected in the perinuclear region in all cells in which fluorescence was detected (illustrated by **Figure 5B**). In developing inflorescence primordia, although expression was detected in all cells, higher levels of FLS1-RFP fluorescence could be observed in cells subtending spikelet pair (spm) and spikelet meristems (sp), as well as the L1 layer of suppressed bracts (sb; **Figures 5F–H**). In mature anthers, FLS1-RFP could be found in the degraded tapetum tissue surrounding pollen grains (**Figures 5D–E**), in accordance with the flavonol accumulation described in the later stages of maize anther development by Deboo et al. (1995). How the tissue and



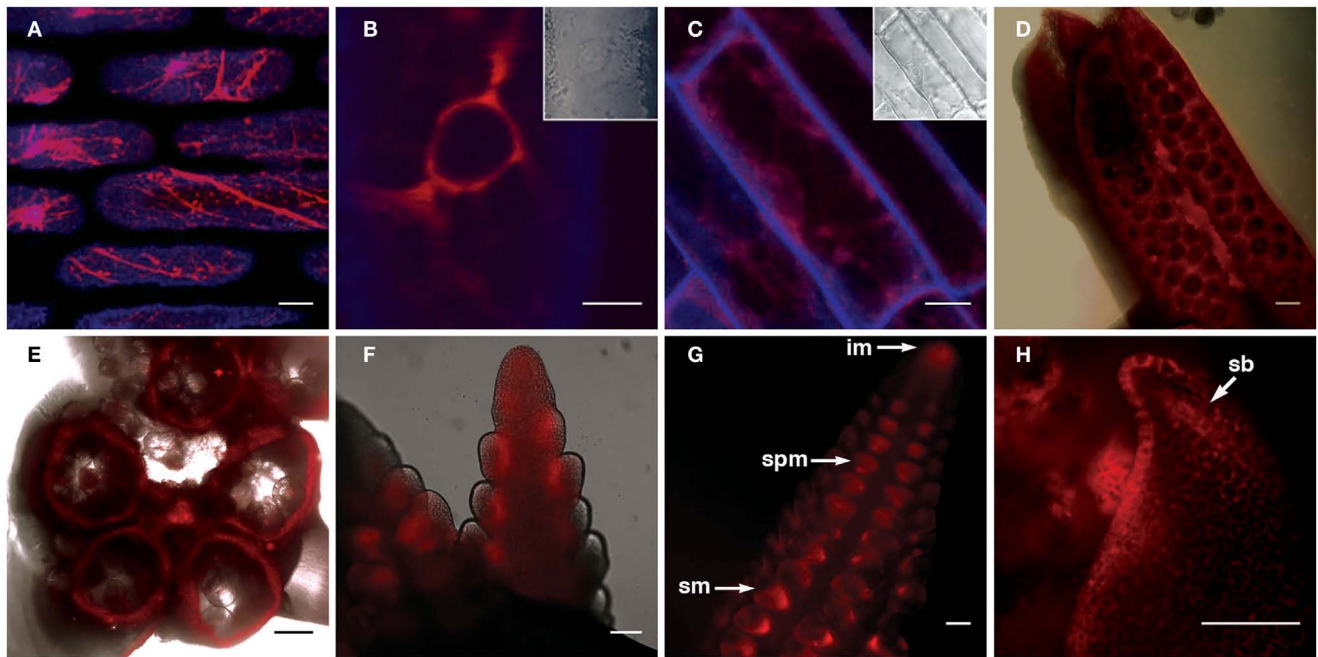


FIGURE 5 | Transgenic expression of pZmFLS1::ZmFLS1-RFP. Confocal laser-scanning micrographs showing localization of ZmFLS1-RFP in maize leaf epidermal cells (A,B); root cells (C); anthers (D,E), tassel branch primordia (F), and ear primordia (G,H). FLS1-RFP was most easily detected in the ER (A) and perinuclear region (B) of cells. Red represents FLS1-RFP fluorescence,

while blue represents cell wall autofluorescence. Brightfield images indicate the position of the nucleus and cell boundaries [(B–C) insets and (D–E) overlay]. Scale bars represent 5, 10, 20, 100 μm [(A–H), respectively]. White arrows indicate the inflorescence meristem (im), spikelet pair meristem (spm), spikelet meristem (sm), and suppressed bract (sb).

subcellular localization of the ZmFLS1 fusion protein relates to the function of the enzyme, remains to be determined.

EXPRESSION OF ZmFLSs IN DIFFERENT MAIZE LINES

In order to investigate the evolution of *ZmFLS* genes, we amplified by PCR *ZmFLS1* and *ZmFLS2* genes in different maize lines, covering from position +739 (exon 1) to the 3'UTR using specific reverse primers for each gene. A high conservation at nucleotide level was observed between maize lines for both genes, showing minor differences mainly in the intron and in the 3'UTR (Figure A4 in Appendix). The phylogenetic tree generated from these sequences showed that *ZmFLS1* and *ZmFLS2* genes group in different clusters in all lines analyzed (Figure A5 in Appendix) indicating that paralogous genes arose by duplication before maize lines divergence.

Previously, we showed that the combined expression of *ZmFLS1* and *ZmFLS2* is induced by UV-B radiation in the B73 inbred, as well as in the W23 inbred line with (*W23^{B^{PL}}*) and without (*W23^{b^{pl}}*) the dominant alleles for the *B* and *PL1* anthocyanin regulators. We examined *ZmFLS1* and *ZmFLS2* expression levels in different maize inbred lines (A619, Mo17, W22, B73, and W23 with or without the dominant alleles for the *B* and *PL1* regulators) and in five maize landraces from high altitudes (Arrocillo Amarillo, Cacahuacintle, Conico, and Confite Puneño) by RT-qPCR using specific primers for each *FLS* gene covering the regions previously sequenced. Under control conditions without UV-B radiation, higher transcript levels of *ZmFLS1* and *ZmFLS2* were observed in 28-day-old seedlings from high altitude lines, compared to

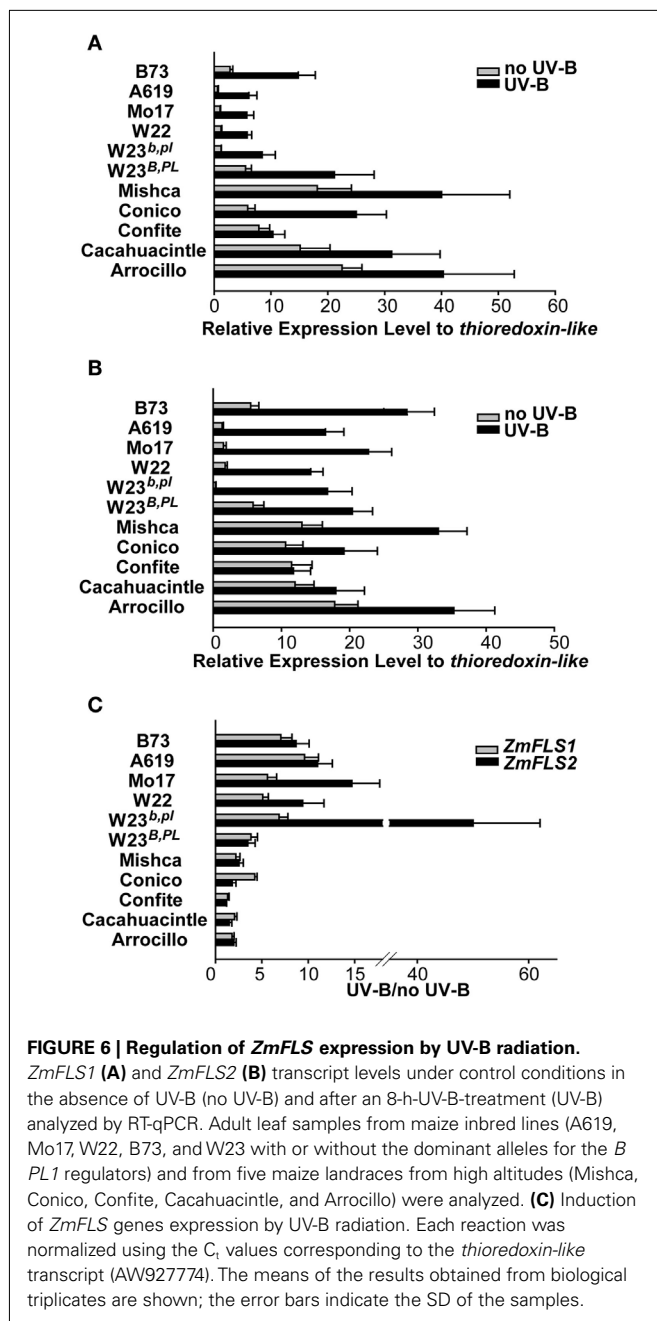
levels in the low-altitudes inbreds (Figures 6A,B). When the plants were irradiated with UV-B, *ZmFLS* transcripts increased in all lines, showing greater induction in the low-altitudes inbreds (Figure 6C). However, it is important to emphasize that despite the greater increase in *ZmFLS* expression by UV-B in low-altitudes inbreds, transcript levels, particularly for *ZmFLS1* after the UV-B treatment, are in general lower than those in high altitude plants.

CONSERVATION OF ZmFLS PROMOTERS BETWEEN MAIZE LINES

To study if the differences observed in *ZmFLS1* and *ZmFLS2* expression levels between the maize inbred lines and the landraces from high altitudes (Figure 6) are a consequence of allelic variations in their promoters, we amplified the proximal promoters of *ZmFLS* genes from these maize lines. A high similarity of sequence was found in both promoters in all lines, with the conservation of the C1/P1-binding sites present in the B73 line, showing only short insertions or deletions at the 3' promoter end, downstream of the putative TATA box (Figure A6 in Appendix). Consequently, the increased expression of *ZmFLS* genes in the maize landraces from high altitudes could be explained by differences in the activities and/or expression levels of C1 + R and P1 regulators in these landraces.

DISCUSSION

The recently completed maize genome sequence (release 5b.60) shows that two *FLS* genes are present in the B73 line, resulting from complete genome duplication during evolution (Schnable et al., 2009). Consequently, this study was aimed at characterizing



both *ZmFLS* genes and their patterns of expression and regulation, in order to analyze if duplication/divergence resulted in them being expressed under different conditions with functional specificity, or if there is functional redundancy between these genes. The analysis of both genes showed that there is an extremely high degree of identity between them, both at the nucleotide and amino acid levels, and also in their promoters. In addition, we found that there is a very high level of conservation in the sequences and genomic synteny with *FLS* genes from other grasses like rice, *Brachypodium*, and sorghum (*SbFLS1*, Figure 1). Although two *FLS* genes are also present in *S. bicolor* (*SbFLS1* and *SbFLS2*), unlike in *O. sativa* and *B. distachyon* which have only one *FLS* gene, *SbFLS1* is more closely

related to the maize counterparts than to *SbFLS2*, and the annotated amino acid sequence of *SbFLS2* lacks 27 amino acids. It is possible that *SbFLS2* is a pseudo-gene that has not yet been lost, perhaps a result of a whole-genome duplication of the common ancestor of the grasses (Salse et al., 2008; Paterson et al., 2009). This hypothesis is consistent with the fact that no matching transcript could be found in the sorghum EST collections, and no expression of *SbFLS2* could be observed under standard growth conditions, or upon exposure to salt stress or UV-B radiation in contrast to *SbFLS1* (unpublished data). Moreover, according to the evolution of grass genomes from a common ancestor (50–70 mya), maize, and sorghum diverged from each other ~12 mya, and maize underwent a complete genome duplication (~5 mya) followed by re-arrangements leading to the current chromosomes (Salse et al., 2008; Buell, 2009; Paterson et al., 2009). Based on this, we can hypothesize that *ZmFLS* genes resulted from gene duplication ~5 mya.

It is important to mention that, although we had previously reported an analysis of *ZmFLS1* expression by RT-qPCR (Falcone Ferreyra et al., 2010); because there was no evidence of a second closely linked gene with very high identity to *ZmFLS1*, the primers used recognized both transcripts. The two *ZmFLS* genes studied here are expressed in all maize tissues analyzed, showing higher levels of expression in tissues where the P1 and C1 + R regulators are present, indicating that both genes are regulated by these transcription factors (Figure 3). To validate this hypothesis, we combined *in vitro* DNA-binding experiments with *in vivo* transient co-expression studies in maize protoplasts, showing that *ZmFLS2* is more activated by the two regulators than *ZmFLS1*, probably due to the presence of additional P1/C1 binding sites in the distal region of its promoter (Figure 4).

Differences in regulatory elements present in *ZmFLS*-3'UTRs genes may contribute to their differential expression regulation. In humans, *C. elegans* and yeasts, regulatory elements present in the 5' and 3'UTRs affect mRNA stability and translation rate; however, information of this type of regulation in plants is scarce (Zubiaga et al., 1995; Yang et al., 2003; Wilusz and Wilusz, 2004; Shalgi et al., 2005; Merritt et al., 2008). The role of mRNA sequence determinants in posttranscriptional regulation of the *adh1* gene was investigated in maize protoplasts under hypoxia, demonstrating that the 5' and 3'UTRs are required for the regulation of the expression of this gene under this stress condition (Bailey-Serres and Dawe, 1996). Moreover, the 5' and 3'UTRs of the amaranth *RbcS* gene function as translational enhancers in different tissues (Patel et al., 2004). RNA binding proteins that complex with 5' or 3'UTR are involved in the stability and translation of chloroplast mRNA (Bruick and Mayfield, 1999). However, additional studies are needed to investigate the contribution of the *ZmFLS* UTRs to gene regulation, for example by transient experiments with constructs containing the 3'UTR of each *ZmFLS* gene. Furthermore, it is well-known that mRNA levels do not necessarily correlate with protein levels in plant cells (Kawaguchi et al., 2004; Branco-Price et al., 2008; Mustroph et al., 2009). It has been reported that mRNA translation state is perturbed by stressful conditions like hypoxia, dehydration, light availability, cadmium intoxication, elevated temperature and high salinity in *Arabidopsis* (Kawaguchi

et al., 2004; Mustroph et al., 2009; Matsuura et al., 2010; Sormani et al., 2011; Juntawong and Bailey-Serres, 2012). Therefore, we cannot rule out that *ZmFLS* mRNAs could be differentially translated.

We also show that both *ZmFLS* genes are regulated by UV-B radiation in both high altitude landraces and low-altitudes inbreds. However, under control conditions in the absence of UV-B radiation, higher transcript levels for both genes are present in high altitude plants compared with levels in the low-altitudes inbreds (Figure 6).

Maize lines growing at high altitudes have developed mechanisms to prevent damage caused by high UV-B exposure, such as the accumulation of C-glycosyl flavones in leaves, maysin, and its biosynthetic precursor rhamnosylisorientin, flavones commonly found in silks (Snook et al., 1993; Casati and Walbot, 2005). Previously, we also showed that *B*, *PL*, and *P1* are up-regulated by UV-B in W23 and B73 lines, while in high altitude landraces these regulators are also expressed in leaves at high levels (Casati and Walbot, 2005). Thus, given the protective role of flavonols to UV-B radiation (Stafford, 1990; Ryan et al., 2001, 2002; Solovchenko and Schmitz-Eiberger, 2003; Jaakola et al., 2004; Stracke et al., 2010a; Kusano et al., 2011; Pollastri and Tattini, 2011), it is possible that the high transcript levels of both *ZmFLS* genes may also contribute to the adaptation to this stress condition with higher UV-B fluxes.

The analysis of the *ZmFLS* proximal promoters in high altitude landraces and low-altitude inbreds showed a high degree of conservation in the distribution of *cis*-regulatory elements, perhaps suggesting allelic variations in the *trans*-regulating machinery, either provided by differences in the activities and/or in the expression levels of the transcription factors that regulate the expression of both *ZmFLS* genes. Previous reports have shown that flavonols are essential for pollen germination and conditional male fertility in maize and petunia (Mo et al., 1992; Taylor and Jorgensen, 1992; Ylstra et al., 1994; Taylor and Hepler, 1997), but not in *Arabidopsis* (Burbulis et al., 1996; Ylstra et al., 1996). However, maize plants lacking the *P1* and *R/B + C1/PL1* anthocyanin regulators are fertile (Coe and Neuffer, 1988; Dooner et al., 1991; Neuffer et al., 1997), suggesting that additional regulators, not yet identified, are involved in the regulation of *ZmFLS* genes in anthers.

The FLS1-RFP fusion protein was detected in all tissues analyzed in agreement with transcript pattern established by RT-qPCR (Figures 3 and 5). At the subcellular level, this enzyme was localized at the ER and the perinuclear region, but not in the nucleus, contrary to what was recently reported for FLS1 in *Arabidopsis* (Kuhn et al., 2011), and previously for other flavonoid enzymes (Saslowky et al., 2005). Interestingly, FLS1-RFP was observed in tapetum tissue of mature anthers, suggesting a role in late pollen development. Future experiments of localization will be necessary to investigate the specific participation of FLS1 and FLS2 proteins in maize anther development and pollen viability by analyzing maize *fls* mutant plants and complementation. While we cannot guarantee the functionality of the fusion protein, additional experiments such as the complementation of *A. thaliana* mutant plants in the *fls1* gene with the construct pFLS1::FLS1-RFP would allow to demonstrate its functionality in planta. Initial

supporting evidence demonstrated that 35S:FLS1-GFP was able to complement *A. thaliana fls1* mutant plants, restoring the levels of anthocyanins and flavonols (Falcone Ferreyra et al., 2010). Finally, based on the high identity between *ZmFLS1* and *ZmFLS2*, we can speculate that *ZmFLS2* would be able to complement the *A. thaliana fls1* mutants similarly as *ZmFLS1*. These proteins show only two differences in their amino acid sequences at positions not participating in 2-oxoglutarate and substrate binding, or the coordination of the ferrous iron necessary for the enzyme activity.

In conclusion, although it is expected that paralogous genes may be expressed in different tissues, at different levels and also regulated by distinct factors, our results indicate that it is not the case for duplicated *ZmFLS* genes. Moreover, while pseudogenization process eliminates unnecessary genes, it is notably that none of the *ZmFLS* genes turned non-functional or were removed from the genome. It is possible that *ZmFLS* genes are still diverging to specific functions. However, we cannot rule out that unidentified regulators can be involved in the differential regulation of *ZmFLS* genes to meet the physiological needs of the plants under specific conditions not investigated in our study.

MATERIALS AND METHODS

PLANT MATERIAL, GROWTH CONDITIONS, AND UV TREATMENTS

The *Zea mays* highland lines Confite Puneño, Mischa, Conico, Arrocillo Amarillo, and Cacahuacintle were obtained from the Germplasm Resources Information Network (GRIN, <http://www.ars-grin.gov/cgi-bin/npgs/acc/>).

The two near isogenic maize (*Z. mays*) lines that differ in flavonoid content from the genotype W23 (W23^{B^{PL}} and W23^{b^{PL}}) correspond to those previously described (Casati and Walbot, 2003). B73 seeds were obtained from the Instituto Nacional de Tecnología Agropecuaria (INTA, Pergamino, Buenos Aires, Argentina) while A619, W22, Mo17 seeds were maintained as laboratory stocks by self-pollination. The generation and analysis of the BMS cells expressing p35S::C1 and p35S::R were previously described (Grotewold et al., 1998).

Maize plants were grown in greenhouse conditions with supplemental visible lighting to 1000 $\mu\text{E m}^{-2} \text{s}^{-1}$ with 15 h of light and 9 h of dark without UV-B for 28 days. UV-B treatments were performed by illuminating plants with UV-B lamps for 8 h using fixtures mounted 30 cm above the plants (TL 20 W/12; Phillips) at a UV-B intensity of 2 W m^{-2} and a UV-A intensity of 0.65 W m^{-2} . The bulbs were covered with cellulose acetate filters (100 mm extra clear cellulose acetate plastic, Tap Plastics, Mountain View, CA, USA); the cellulose acetate sheeting does not remove any UV-B radiation from the spectrum but excludes wavelengths lower than 280 nm. No UV-B-treated plants (control) were exposed for the same period of time under the same lamps covered with polyester filters (100 mm clear polyester plastic; Tap Plastics, 0.04 W m^{-2} , UV-A, 0.4 W m^{-2}), which absorbs both UV-B and wavelengths lower than 280 nm. Lamp outputs were recorded using a UV-B/UV-A radiometer (UV203 A β B radiometer; Macam Photometrics) to ensure that bulbs and filters provided the designated UV light dosage in all treatments. Adult leaf samples (leaf 9 or 10) were collected immediately after irradiation and stored at -80°C . The UV-B treatment experiments were repeated at least three times.

For gene expression analyses in tissues of the B73 maize line, plants were grown in greenhouse conditions as described above and samples were collected from anthers, roots (21-day-old plants), seedlings (7-day-old plants), and juvenile leaves (21-day-old plants). Pericarps and silks were obtained from maize A619^{P-rr} and A619^{P-ww} plants grown in the field under natural sunlight conditions.

GENE EXPRESSION ANALYZES BY RT-qPCR

Tissues from three independent biological replicates were frozen in liquid nitrogen and stored at -80°C . Total RNA was extracted using the RNeasy Plant Mini kit with optional DNase treatment (Qiagen) or alternatively with Trizol Protocol (Invitrogen) followed by DNase treatment (Promega). cDNAs were synthesized from 4 μg of total RNA using Superscript Reverse Transcription Enzyme II (Invitrogen) with oligo-dT as a primer. The resulting cDNAs were used as templates for qPCR amplification in a iCycler iQ detection system with the Optical System Software version 3.0a (Bio-Rad), using the intercalation dye SYBR Green I (Invitrogen) as a fluorescent reporter and Platinum Taq Polymerase (Invitrogen). Primers were designed to generate unique 150–250 bp-fragments using the PRIMER3 software (Rozen and Skaletsky, 2000). Three biological replicates were used for each sample plus negative control (reaction without reverse transcriptase). To normalize the UV treatment data, primers for *thioredoxin-like* transcript (AW927774) were used, while for tissue dependent expression studies, primers for *Actin1* (J01238) were used for maize species (Table A1 in Appendix). Amplification conditions were as follows: 2 min denaturation at 94°C ; 40–45 cycles at 94°C for 10 s, 57°C for 15 s, and 72°C for 20 s, followed by 5 min at 72°C . Melting curves for each PCR product were determined by measuring the decrease of fluorescence with increasing temperature (from 65 to 95°C). To confirm the size of the PCR products and to check that they corresponded to a unique and expected PCR product, the final PCR products were separated on a 2% (w/v) agarose gel and also sequenced. Primers used for *ZmFLS1* and *ZmFLS2* are listed in Table A1 in Appendix (*ZmFLS*-RT-forward, *ZmFLS1*-RT-reverse, and *ZmFLS2*-RT-reverse, respectively).

AMPLIFICATION OF ZmFLS GENES AND PROMOTERS, CLONING, AND MUTAGENESIS

To verify the presence of two *ZmFLS* genes in B73, primers were designed to amplify fragments downstream of the 3'UTR of each gene using the same forward primer (*ZmFLS*-3'UTR-forward, *ZmFLS1*-crom5-reverse, and *ZmFLS2*-crom5-reverse, Table A1 in Appendix). PCR reactions were made with Platinum Pfx Polymerase (Invitrogen) under the following conditions: 1 \times Pfx buffer, 1 \times enhancer, 2 mM MgSO_4 , 0.5 mM of each dNTP, 0.5 μM of each primer, 0.3 U Platinum Pfx Polymerase, and sterile water added to obtain a volume of 20 μl . Cycling conditions were as follows: 5 min denaturation at 95°C , 35 cycles at 20 s denaturation at 95°C , 30 s annealing at 50°C , 90 s amplification at 68°C .

The BAC clone (c0247D19) was obtained from Arizona Genomics Institute (AGI, Tucson, USA). *ZmFLS* genes were amplified from the BAC clone by PCR using the same forward primer (*ZmFLS*-cfs-forward) and specific primers for each

gene (*ZmFLS1*-RT-reverse and *ZmFLS2*-RT-reverse, Table A1 in Appendix) that hybridize in the 3'UTR. PCR reactions were performed with Platinum Pfx Polymerase (Invitrogen) as described above but with annealing at 60°C . The PCR products were purified from the gels and sequenced.

To amplify the introns of the *ZmFLS* genes from B73, primers were designed to hybridize in the exon 1 and 2 of genes (Table A1 in Appendix). PCR reaction were performed with GoTaq (Promega) and Pfu Polymerases (Invitrogen; 10:1) under the following conditions: 1 \times buffer, 2 mM MgCl_2 , 0.5 μM of each primer, 0.5 mM of each dNTP and 0.5 U of enzyme, in 25 μl of final volume under the following cycling condition: 5 min denaturation at 94°C ; 35 cycles at 94°C for 20 s, 57°C for 25 s, and 72°C for 40 s, followed by 7 min at 72°C .

To amplify *ZmFLS1* and *ZmFLS2* genes in different maize lines, PCR reaction were performed as described above for B73 using primers that hybridize in the exon 1 and 3'UTR of each gene (*ZmFLS*-intron2-forward, *ZmFLS1*-RT-reverse, and *ZmFLS2*-RT-reverse, respectively, Table A1 in Appendix).

To clone the promoter of *ZmFLS2* from the B73 genotype, primers were designed to amplify a 1.5-kb fragment upstream of the start codon, as predicted from www.maizesequence.com release 5b.60. Restriction sites *NotI* and *KpnI* were included in the forward and reverse primers, respectively (*NotI*-*ZmFLS2*-prom-forward and *KpnI*-*ZmFLS2*-prom-reverse, Table A1 in Appendix). Genomic DNA was isolated from leaf tissue using a DNA isolation kit (Qiagen). PCR reactions were performed with Platinum Taq Polymerase (Invitrogen) under the following condition: 1 \times buffer, 0.3% DMSO, 2 mM MgCl_2 , 0.5 μM of each primer, 0.5 mM of each dNTP, 100 ng genomic DNA, and 0.3 U Platinum Taq Polymerase in a volume of 25 μl . Cycling conditions were as follows: 30 s denaturation at 95°C , 30 s annealing at 68°C , 2 min amplification at 72°C , with a 1°C decrement of annealing temperature in each cycle until it reached 58°C , followed by 25 cycles of 30 s denaturation at 95°C , 30 s annealing at 58°C , 2 min amplification at 72°C . The PCR products were purified from the gels, digested with the corresponding restriction enzymes and purified. The *pZmFLS1::Luc* construct (pMSZ011; Falcone Ferreyra et al., 2010) was restricted with *NotI* and *KpnI* and the *ZmFLS1* promoter was replaced by the *ZmFLS2* promoter, resulting in the *pZmFLS2::Luc* construct. Proximal promoters of *ZmFLS1* and *ZmFLS2* from B73 were amplified by PCR using the *pZmFLS1::Luc* and *pZmFLS2::Luc* constructs as templates with Platinum Taq Polymerase and Pfu Polymerases (Invitrogen; 10:1) under the following conditions: 1 \times buffer, 2 mM MgCl_2 , 0.5 μM of each primer, 0.5 mM of dNTPs and 0.5 U of enzyme, in 25 μl of final volume under the following cycling condition: 5 min denaturation at 94°C ; 35 cycles at 94°C for 20 s, 59°C for 30 s, and 72°C for 50 s, followed by 7 min at 72°C . The PCR products were purified from the gels, digested with the corresponding restriction enzymes and purified, and the *ZmFLS* promoters (1.5 kb) in pMSZ011 were replaced by the *ZmFLS* proximal promoters. For *ZmFLS* proximal promoter analysis from different maize lines, DNA was extracted from leaves using the CTAB Method, and PCR reactions were performed with Platinum Taq Polymerase under the following conditions: 1 \times buffer, 2 mM MgCl_2 , 0.4% DMSO, 0.5 μM of each primer, 0.5 mM of each dNTP and 0.5 U of enzyme,

in 25 μ l final volume. Amplification conditions were as follows: 5 min denaturation at 94°C; 35 cycles at 94°C for 25 s, 59°C for 35 s, and 72°C for 50 s, followed by 5 min at 72°C. PCR products were purified, cloned into pGEM®-T-Easy vector (Promega) and sequenced.

Mutagenesis of the P1/C1 binding site at –250 bp in *ZmFLS* promoters was made by PCR using the same forward primer described above for amplification of proximal promoters but using a reverse oligonucleotide with the mutated binding site (*ZmFLS*prom-mut-reverse; **Table A1** in Appendix). PCR reactions were performed with GoTaq (Promega) and *Pfu* Polymerases (Invitrogen; 10:1) under the following conditions: 1× buffer, 2 mM MgCl₂, 0.5 μ M of each primer, 0.5 mM of each dNTP, and 0.5 U of enzyme, in 25 μ l final volume under the following cycling condition: 5 min denaturation at 94°C; 35 cycles at 94°C for 20 s, 50°C for 35 s, and 72°C for 50 s, followed by 7 min at 72°C. The PCR product (mega primer) was purified from the gel and then used as a forward primer (0.16 μ M) in a new PCR with the reverse primer (1 μ M) described above for proximal promoters (**Table A1** in Appendix). The PCR products were purified from the gels, digested with the corresponding restriction enzymes and purified, and the *ZmFLS1* proximal promoter in pMSZ011 was replaced by the *ZmFLS* mutated proximal promoter. Finally, mutations in the P1/C1 binding site were confirmed by sequencing.

CLONING, EXPRESSION AND PURIFICATION OF RECOMBINANT PROTEINS

The MYB domain of P1 (P1^{MYB}) cloned in pET19b (Invitrogen) was used for its expression in *E. coli* (Williams and Grotewold, 1997). The plasmid for the expression of the MYB domain of C1^{SH} was obtained by subcloning from pTYB2 vector (Hernandez et al., 2004) to pET19b. Thus, the C1^{SH} MYB domain was cut with *NdeI* and *XhoI* restriction enzymes and inserted in pET19b previously digested with the same enzymes. BL21(DE3)pLys cells were transformed with the clones described; P1^{MYB} expression was achieved by induction of the cell culture (250 ml) with 1 mM IPTG at 37°C for 3 h, while C1^{SHMYB} expression was done at 30°C with 0.5 mM IPTG for 6 h. Protein purification was essentially done as described by Williams and Grotewold (1997) with the following modifications. After binding the proteins to a Ni-NTA resin (Invitrogen) by rocking at 4°C for 1 h, the resin was loaded onto a column, washed three times with 15 volumes of binding buffer (50 mM sodium phosphate, pH 8.0, 500 mM NaCl, 20 mM imidazole, 5% glycerol and 1 mM phenylmethylsulfonyl fluoride), followed by three washes with seven volumes of washing buffer (50 mM sodium phosphate, pH 8.0, 500 mM NaCl, 5% glycerol, 50 mM imidazole). Elution was carried out by seven sequential additions of 1.5 ml of elution buffer (50 mM sodium phosphate, pH 8.0, 500 mM NaCl, 5% glycerol, and 200 mM imidazole). Finally, recombinant proteins were desalted in desalting buffer (10 mM Tris-HCl pH 7.5, 50 mM NaCl, 1 mM EDTA, 5% glycerol, and 1 mM DTT) by four cycles of concentration and dilution using Amicon Ultra-15 3K (Millipore) and stored at –80°C. Protein levels were estimated both by comparison with dilution series of bovine serum albumin on a Coomassie Blue-stained SDS-PAGE and also using the Bradford reagent (Bio-Rad, Bradford, 1976). The yield of 90–95%

pure recombinant proteins obtained in these conditions was 4 and 6 mg/L of culture for P1^{MYB} and C1^{SHMYB}, respectively.

ELECTROPHORETIC MOBILITY SHIFT ASSAYS

End labeling of synthetic oligonucleotide probes (APB10, FLSbind-forward, comp1-forward, comp2-forward, and comp3-forward, **Table A1** in Appendix) was carried out using T4 polynucleotide kinase (Invitrogen) in the presence of a 2-M excess of [γ -³²P]ATP (>8,000 Ci/mmol). The labeled oligonucleotides were then annealed to equal amounts of complementary oligonucleotides (APB01, FLSbind-reverse, comp1-reverse, comp2-reverse, and comp3-reverse) by heating to 95°C and slowly cooling down to room temperature to generate APB1, FLSbind, comp1, comp2, and comp3 probes, respectively. A fraction of the double-stranded labeled oligonucleotides was precipitated on glass filters for quantification by scintillation of the radiation incorporated. The probe used as positive control (APB1) contains the high affinity P1-binding sites from the *a1* gene promoter. Protein-DNA incubations were performed essentially as described previously (Heine et al., 2004) with the following modifications. Approximately 2 μ g of purified proteins were incubated in a buffer containing 10 mM Tris, pH 7.5, 50 mM NaCl, 1 mM EDTA, 5% glycerol, 2 μ g of salmon sperm DNA, 1 mM DTT with 1 ng of end-labeled, double-stranded DNA probes, for 1 h at 4°C in the presence or absence of competitors as indicated in the Figures. Protein-DNA complexes were resolved on 8% polyacrylamide gels (30:0.8 acrylamide:bis-acrylamide) in 0.25× Tris-borate/EDTA (22.5 mM Tris-Borate and 0.5 mM EDTA) at 100 V for 90 min at 4°C. The gels were then dried onto Whatman paper and subjected to autoradiography at –80°C overnight.

TRANSIENT EXPRESSION EXPERIMENTS IN MAIZE PROTOPLASTS

The p35S::C1 + p35S::R, p35S::P1, p35S::Renilla, and p35S::BAR and p35S::GFP plasmids have all been previously described (Grotewold et al., 1994; Sainz et al., 1997; Hernandez et al., 2004, 2007).

Protoplasts from 11- to 13-day-old etiolated maize seedlings were obtained from kernels of B73xMo17 plant. After chopping second or third leaves into small pieces, leaf stripes were digested in 3% cellulase RS, 0.6% macerozyme R10 (both from Yakult Honsha Co., Japan), 0.6 M mannitol, 10 mM MES (pH 5.7), 5 mM CaCl₂, and 0.1% (w/v) BSA for 15 min under vacuum followed by 2:30 h gentle shaking (40 rpm) at 25°C in the dark. After releasing the protoplasts at 80g, the protoplasts were filtered through a 35- μ m nylon mesh and collected by centrifugation at 150g for 1 min. The protoplasts were washed in ES buffer (0.6 M mannitol, 5 mM MES, pH 5.7, 10 mM KCl) and counted with a hemocytometer. Electroporation was carried out on ~105 protoplasts with 40 μ g of total DNA per transformation, using 100 V/cm, 10 ms, and one pulse with a BTX Electro-Square-Porator T820. After electroporation, protoplasts were incubated for 18–22 h in the dark at RT before performing the luciferase reaction. Transformation efficiency was estimated following GFP expression by fluorescence microscopy. Transient expression assays for Luciferase and Renilla were performed essentially as previously described (Sheen, 1991; Feller et al., 2006; Hernandez et al., 2007).

GENERATION OF MAIZE TRANSGENIC PLANTS EXPRESSING pZmFLS1::ZmFLS1-mRFP

Construct was generated using the MultiSite Gateway® Pro 3.0 system (Invitrogen).

Two sets of primers (P1/P2, P3/P4; **Table A1** in Appendix) were designed for the amplification of two genomic fragments. The first set of primers amplified a fragment (P1–P2) that extends 2.483 kb upstream of the start codon to the tag insertion site within the coding sequence of *ZmFLS1* (just before the stop codon). The second set of primers amplified a fragment (P3–P4) from the tag insertion site to 2.1 kb downstream of the gene to include 3' UTR and regulatory sequences. P1 and P4 contained, in addition to gene-specific sequences, sequences partially overlapping the attB1 and attB2 Gateway forward and reverse primers, respectively (used for a second PCR). P2 and P3 contained sequences partially overlapping the RFP primers. A second PCR reaction, designated triple-template PCR (TT-PCR), utilized two primers containing the complete attB1 and attB2 Gateway sequences and partially overlapping the P1 and P4 primers. Thus, the RFP fluorescent tag was introduced into the *ZmFLS1* gene (C-terminal) and resulted in an internally tagged full-length gene sequence flanked by attB1 and attB2 sites ready for Gateway recombination cloning. The construct was recombined into the maize pTF101.1 binary vector, tagging *ZmFLS1* with *mRFP1* at the C-terminus. *Agrobacterium*-mediated transformation of maize HiII was performed by the Iowa State University Plant Transformation Facility. T0s were crossed to the inbred line B73 and T1s imaged live using a Zeiss 510 or 710 confocal laser-scanning microscope. Construct sequences and image metadata are available at <http://maize.jcvi.org/cellgenomics/index.shtml>.

PHYLOGENETIC ANALYSIS

The trees were constructed using MEGA 4.0 Software with the Neighbor-Joining method based on ClustalW multiple alignments (Tamura et al., 2007). For analysis of FLS proteins from monocot and dicot plants the following sequences were analyzed: *Pyrus communis* FLS (ABB70118), *Fragaria x ananassa* FLS (ABH07784.1), *Rosa hybrid* FLS (BAC66468), *Ricinus communis* FLS (XP_002513774), *Populus*

trichocarpa (XP_002301003.1), *Glycine max* FLS (AB246668.1), *Vitis vinifera* FLS1 (XP_002285838.1), *V. vinifera* FLS2 (BAE75809.1), *V. vinifera* FLS3 (XP_002284410.1), *V. vinifera* FLS4 (XP_002285839.1), *Lactuca sativa* FLS (BAG12186.1), *Petroselinum crispum* FLS (AAP57395.1), *Camellia sinensis* FLS (ACL98052), *Antirrhinum majus* FLS (ABB53382.1), *Eustoma grandiflorum* FLS (BAD34463), *Solanum tuberosum* FLS (ACN81826.1), *Petunia x hybrida* FLS (Q07512.1), *Nicotiana tabacum* FLS1 (ABE28017.1), *N. tabacum* FLS2 (BAF96939.1), *Citrus unshiu* FLS (BAA36554.1), *Allium cepa* FLS (AAO63023.1), *Epimedium sagittatum* FLS (ABY63659.1), *Hordeum vulgare* FLS (BAJ98444.1), *Setaria italica* FLS (Si017742m), *S. bicolor* FLS1 (Sb04g034240), *S. bicolor* FLS2 (Sb03g002040), *B. distachyon* FLS1 (Bd5g57910), *O. sativa* FLS1 (Os02g52840).

STATISTICAL ANALYSIS

Data presented were analyzed using one-way analysis of variance (ANOVA). Minimum significant differences were calculated by the Bonferroni, Holm–Sidak, Dunnett, and Duncan tests ($P < 0.05$) using the Statgraphics Plus 5.0 Software.

ACCESSION NUMBERS

Sequence data from this article can be found in the maize genome sequence (version 3b.60 at maizesequence.org), sorghum genome sequence (release Sb1.4 at gramene.org), TIGR Rice Genome Annotation Project (release 7 at rice.plantbiology.msu.edu), *Brachypodium* genome sequence (GBrowse v1.0 at brachypodium.org), and GenBank databases under the following accession numbers: *ZmFLS1* (GRMZM2G152801); *ZmFLS2* (GRMZM2G069298); *SbFLS1* (Sb04g034240); *SbFLS2* (Sb03g002040); *OsFLS1* (Os02g52840); *BdFLS1* (Bradi3g57910); *Z. mays* thioredoxin-like, AW927774; *Z. mays actin1*, J01238.

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APPENDIX

Table A1 | Primers used for cloning, sequencing, RT-qPCR generation of maize transgenic plants, and EMSA.

| Name | Sequence |
|---|--|
| <i>ZmFLS</i> -3'UTR-forward | 5'TGTAAGGGGCACTAATACATG3' |
| <i>ZmFLS</i> -cds-forward | 5'ATGGGGGGGCGAGACGCACCTGAG3' |
| <i>ZmFLS1</i> -crom5-reverse | 5'GAAAAGGCTCACTCGGTCTA3' |
| <i>ZmFLS2</i> -crom5-reverse | 5'GCCCTGCAGTTCAGTAACTT3' |
| <i>ZmFLS</i> -intron2-forward | 5'GTCAATGGTACGAGGCCAAG3' |
| <i>ZmFLS</i> -intron2-reverse | 5'CATGGGGAGCTTGTTGATCT3' |
| <i>NotI</i> - <i>ZmFLS2</i> prom-forward | 5'CACCGCGGCCGAGCCATGAAAAATGACAGT3' |
| <i>KpnI</i> - <i>ZmFLS2</i> prom-reverse | 5'CTATGGTACCCGCGAGCAGGAAGCAGGACC'3 |
| <i>NotI</i> - <i>ZmFLS</i> prom-int-forward | 5'CACCGCGGCCGCGCTATTACTCGTTAGTTGG'3 |
| <i>ZmFLS</i> prom-mut-reverse | 5'GGCCGTACGACGCACCCGATAGAAACTCGTTTA'3 |
| <i>KpnI</i> - <i>ZmFLS1</i> prom-reverse | 5'CTATGGTACCCGCGAGCAGGAAGCAGGGCC'3 |
| <i>ZmFLS</i> -RT-forward | 5'GACGGTGAACAAGGAGAAGAC'3 |
| <i>ZmFLS1</i> -RT-reverse | 5'GGCCATGCATGCGACTGGAAT'3 |
| <i>ZmFLS2</i> -RT-reverse | 5'ACAGAAGCGGGTGCACTGC'3 |
| <i>ZmActine1</i> -forward | 5'CTTCGAATGCCCAGCAAT3' |
| <i>ZmActine1</i> -reverse | 5'CGGAGAATAGCATGAGGAAG3' |
| <i>ZmThioredoxine</i> -like-forward | 5'GGACCAGAAGATTGCAGAAG3' |
| <i>ZmThioredoxine</i> -like-reverse | 5'ACGGATGTCCCATGAAGA3' |
| APB10 | 5'GATCCGGGTCAGTGTACCTACCAACCTTAAACAC3' |
| APB01 | 5'GATCGTGTTTAAGGTTGGTAGGTACACTGACCCG3' |
| FLSbind-forward | 5'GAGTTTCTGGTAGGTGCGTCGTACGCCAG3' |
| FLSbind-reverse | 5'CTGGCCGTACGACGCACCTACCAGAAACTC3' |
| Comp1-forward | 5'CTATTACTCGTTAGTTGGATTTTAGTTT3' |
| Comp1-reverse | 5'AAACTAAAATCCAATAACGAGTAATAG3' |
| Comp2-forward | 5'CCAAGTCAAAATCCAACCATTATCTCTTGA3' |
| Comp2-reverse | 5'TCAAGAGATAATGGTTGGATTTTGACTTGG3' |
| Comp3-forward | 5'TAGATGTGATTCGTTAGGTGTGTTTTATAA3' |
| Comp3-reverse | 5'TTATAAAAACACACCTAACGAATCACATCTA3' |
| P1 | 5'ggggacaagttgtacaaaaagcaggcttaGCGACGAATCCATTCTTGA3' |
| P2 | 5'ggggacaacttgtatagaaaagttgggtgCATGGGAGCTTGTGATCTTGC3' |
| P3 | 5' ggggacaacttgtataataaagttgcaTAATTATGTAGCTCGGGTTC3' |
| P4 | 5'ggggaccacttgtacaagaagctgggtaTATTGCTGGCGTGAATAACC3' |

A

| | | |
|---------------|---|-----------|
| GRMZM2G152801 | -----AGATAGGCCACTAGACACGTTTGGTGCATATTAGACCAA | -1417 |
| GRMZM2G069298 | AGCCATGAAAAATGACAGTAAATTAGATCCCTTCACCAACACCTGAGGGGTAGGTAA | -1398 |
| | * * * * * | **** * |
| GRMZM2G152801 | AGAGTTCAATTACATGCTCTCTCTAAGAGAAACAATATTATATAATTAGACCTTCGGT | -1357 |
| GRMZM2G069298 | AAACATCATTGAACA-----ATTAAAAAGGAAAAATCTCAACTTACTCAAATGCTTTAT | -1343 |
| | * * * * * | * * * * * |
| GRMZM2G152801 | TCA---AGCATGCAAGCGAGCCTCCTATATGTTAGATCATGATCGAACCTAGATATAGA | -1300 |
| GRMZM2G069298 | TAATTGGACAAAAAGATGAGCTGCTCACTAGTGCTACAAGATCCAAC-----AACTATA | -1288 |
| | * * * * * | * * * * |
| GRMZM2G152801 | TGTAGTTCGTTAGGTGTGTTTTTTTATAAGTCTACATAAGTTATGGCAGAAGTGTTTAAG | -1240 |
| GRMZM2G069298 | TGTGATGCGCTAGAAGCTCTCTAATCGCACCCAAATGATGAAGTGTAGATGGAATGAG | -1228 |
| | *** * * * * | * * * * * |
| GRMZM2G152801 | TGTTAAATGTGATATATGATTGAATCAGTAT-CTAACGGACCAAGAGCCTCACTTATAGG | -1181 |
| GRMZM2G069298 | TGAAATGTGTTTCTCTAGTCTCTAATAGGTGTTCTCAAGTGTTAGGAG--TTAAGGGAAC | -1170 |
| | ** * * * | * * * * * |
| GRMZM2G152801 | GTGTTTGGTTTGAGGAATAAGCTAGTCCACCATCT--TCTCACTTCTCACTTTTTTTTGT | -1124 |
| GRMZM2G069298 | TAGCCAAGGTTGGACAAAGGGTCTATTTATAGCCCAAGTCAAATCCAACCATTAATCTCT | -1110 |
| | * * * * * | * * * * * |
| GRMZM2G152801 | TTGATTGTGGAATGGAATGGGTTGATCCATCACCACCTTATTTCTTATAGTTAATAATA | -1064 |
| GRMZM2G069298 | TGAAGGGATAAAATGGAGGCATCAAAC-ATGTCTATGCACTATCAGTTTTTGCACCAG | -1051 |
| | * * * * * | * * * * |
| GRMZM2G152801 | ACATGAGGAATGAGGTCATCCC-ACCAAATTTGA---GGAATAAACTCATGATGAACCA | -1008 |
| GRMZM2G069298 | ACACATCCAGTGCTTTCACAATGACTATATTCTCACTGGTATAAAGTGTGTTAATTG | -991 |
| | *** * * * * | * * * * * |
| GRMZM2G152801 | CATCATATTAGATGGAGTGATTACACAAACCAACACCCC-TTAGGCAATCTCTAGCAGG | -949 |
| GRMZM2G069298 | TGCCATAT--ATCTGGTG---CATCCACCAGATATGCTACTAGACTGTTTGGTGCATA | -938 |
| | ***** * * * | * * * * * |
| GRMZM2G152801 | CCGTGTAAAGATCGTGCAAAGTAC-TGTTTTGTAACGTAGATTACACTATTTCTAGAGT | -890 |
| GRMZM2G069298 | TTAGACCAAAGA--GTTCAATTTTACATGCTCTCTCTAAGAGAAACAATATT-----AT | -886 |
| | ***** * * * | * * * * * |
| GRMZM2G152801 | GATGTTTGAATAATGAGCGAGATTGCATTTAAAGCCACATTGCTGTGTAGAGATGGCTAA | -830 |
| GRMZM2G069298 | GTAATTAGGCATTAGGTTTAAGCGTGCAAGTGA-GCCTCCTATCTGTAGATCA---TGA | -830 |
| | * * * * * | * * * * * |
| GRMZM2G152801 | ACGGGCGCGCCGCGCCGCGCCGCGCGTTTGTAGTCTGGCCCGCCAAGCACGGTTAGAAA | -770 |
| GRMZM2G069298 | TCGTACCCTAGATATAGATGTGATTCGT--TAGGTGTGTTTTTATAAATCTGCATAAGAT | -772 |
| | ** * * | * * * * |
| GRMZM2G152801 | ATCGGGTCGGACCGTCTAAGCACGCGAGCTCAATTTTCATGTCTGAGTTCGGCTCGCAGCG | -710 |
| GRMZM2G069298 | GGTGG-TGGAAGGGTTAAGTGT-TAAATGTAACAT-ATGATTGAAT-CAATATCTAACG | -716 |
| | * * * * * | * * * * * |
| GRMZM2G152801 | TGCCGAAAAGCGGGCTATACGGGCTGTAAACCACGTTTGTAGTGTAAAAAGCAGGCTTAAC | -650 |
| GRMZM2G069298 | GAACAAGAGCCTCACTTAAAGGGTGTGG-----TTTGAGGAATAAGCTAGCTCATT | -663 |
| | * * * * | * * * * * |
| GRMZM2G152801 | GGTCTTAGAGATAAACGGGTCGTGCGGGGCTAGCGCTA---GCCCACCAT-GCCTAGTT | -595 |
| GRMZM2G069298 | ATCTTCTCACTTCTCAGTTTTTTGTTTGTGTTTGTGGAATGGAATCCATCACCACCTTATT | -603 |
| | * * * * | * * * * * |
| GRMZM2G152801 | TCCTGTCTGAGCCCCGACCGTATAGAACCTGGTCGTGCGGACTCGGGCCGATCCAAC | -535 |
| GRMZM2G069298 | CCCTATAGTAATT-AGTTAGTATTAACATGAGAAATGAGGTCATCTACCAAATTTGAAG | -544 |
| | *** * * | * * * * * |
| GRMZM2G152801 | AACGGGCT--TCGTAC-CGGCCTCGCGGACTCGTGCTTATT--GGCCATCTATAATTGC | -480 |
| GRMZM2G069298 | AATAAACTCATGATACACCACATCATATTAGATGGAGTGATTCCACACCAACACCCAT | -484 |
| | ** * * * | * * * * * |

FIGURE A1 | (Continued).

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GRMZM2G152801      GGT-TGCTCTC-TCTAATCGCACATGCACAGTACCGAGTGGTATGGTACGCTGGCTGGCA -422
GRMZM2G069298      TACACGCTTGCATTTAAAACCGCATTGCCGTTGCTCTCTCTAAGCGCACAGTG-CTGGCA -425
                    *** * * *** * *** * * * * * * * * * * * * * * * * * * * * * * *

GRMZM2G152801      TTTCCGATGTACTGTAGAGCTGAAGTGCGGCCCTTTTAAAGTGCCGTTGCTGTTTGTGCT -362
GRMZM2G069298      TATCCGATGCACTGTAGAGCTGAAGTGCG-TCTCTTTCAAGTGCCCTTGTCTTTGTGCT -336
                    * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * *

GRMZM2G152801      ACAAACCGGTGCGCTATTACTCGTTAGTTGGATTTTAGTTTTCTAAGAAGTTGAAGTAG -302
GRMZM2G069298      ACAAACCGGTGCGCTATTACTCGTTAGTTGGATTTTAGTTTTCTAAGAAGTTGAAGTAG -306
                    * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * *

GRMZM2G152801      GGTCAAGGCACAGTTGAA-GCTGGTGACATGTCGCAGCGAACATAAAAGAGTTTCTGGTA -243
GRMZM2G069298      GGTCAAGGCACAGTTGAAAGCTGGTGACATGTCGCAGCGAACATAAACGAGTTTCTGGTA -246
                    * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * *

GRMZM2G152801      GGTGCGTCGTACGCCAGAAACACACAACGCGGCCCTATCTTCGCGCTCGCTCTCGGGTC -183
GRMZM2G069298      GGTGCGTCGTACGCCAGAAACAC--AACAACGCGCTATCTTCGCGCTCGC----- -197
                    * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * *

GRMZM2G152801      TGTTCCGTGACCAGCATGGCAAGGCTGCCACGTTCCAGCCACCCC-----TGGGCACCA -128
GRMZM2G069298      -----ATAGCAAGGCTGCCACGTTCCGAGCCACCCCGGCCCGGGCACCACCA -152
                    ** * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * *

GRMZM2G152801      AGTAACCGGCCACACAATCCGCACCGCGTCTGTGCCCTACGCCCATACCCAAACGCACG -68
GRMZM2G069298      AGCAACCGGCCACACAATTTCGCACCGGCTGTGTGCC-----ATACCCAAACGCACG -99
                    ** * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * *

GRMZM2G152801      TTTCCAGCGCAGCGGCCGGTCTACCACACCACACGCCACTCACTCCTGTCCCGTGCCCCA -8
GRMZM2G069298      TTTCCAGCACAGCGGCCGGTCTACCACACCACACGCCACTCACTCCTGTCCCGTGCCCCA -99
                    * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * *

GRMZM2G152801      CAACACACAACACTATTAATTCAGTGCTCTTCCCTTCGCTGCGTCTGCGTGCAGCGTGC +52
GRMZM2G069298      CAACACA--AACTATTAATTCAGTGCTCTTCCCT--GCCTGCGTCTGCGTGCAGCGTGC +17
                    * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * *

GRMZM2G152801      TCGCGAGGCCCTGCTTCTCTGCTGCG +76
GRMZM2G069298      TCGCGAGGTCCTGCTTCTCTGCTGCG +42
                    * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * *

```

FIGURE A1 | Alignment of *ZmFLS* promoters of B73 maize.

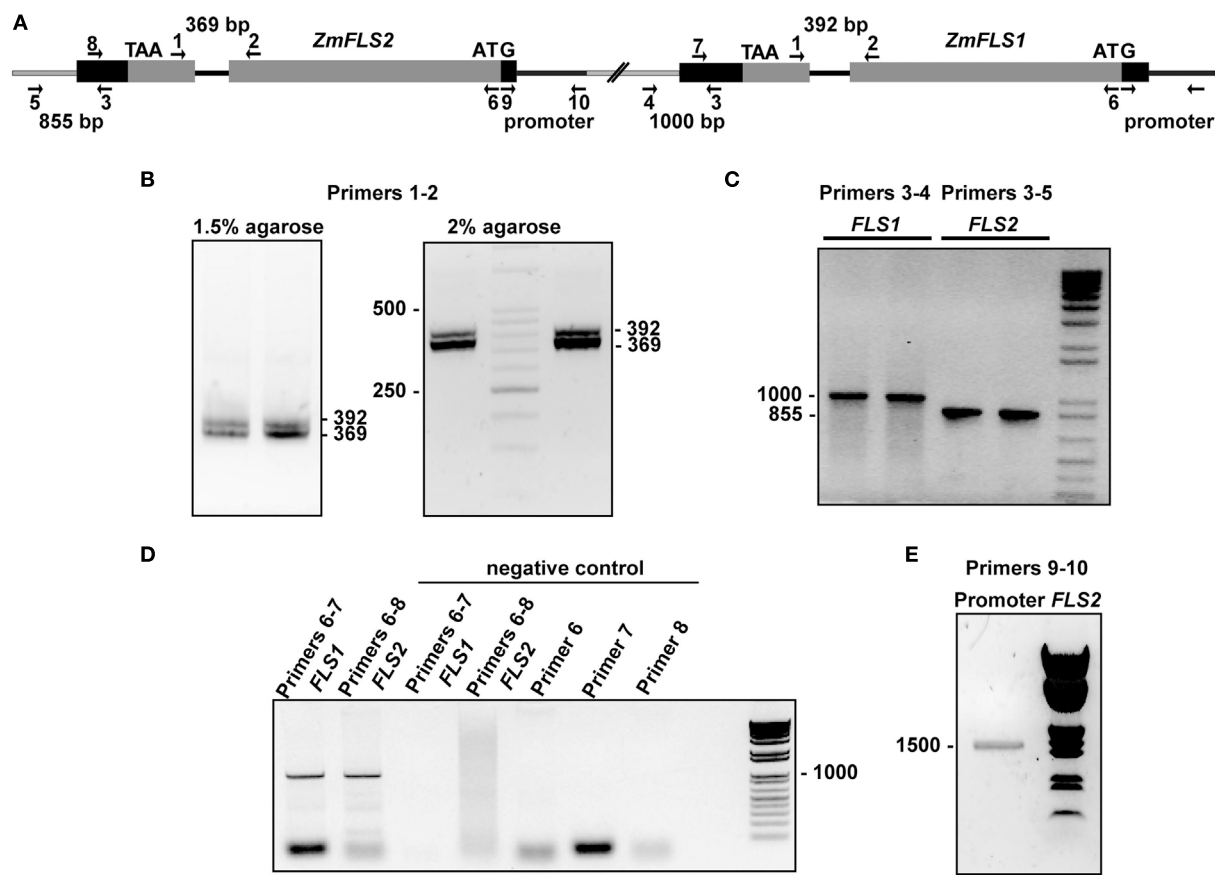


FIGURE A2 | Two *ZmFLS* genes are present in the B73 maize line. (A) Organization of *ZmFLS* genes in the chromosome 5. Primers used for amplification are indicated by arrows, and the predicted PCR product size is shown with numbers above them. Amplification of *ZmFLS* introns (B), 3'UTR regions downstream of *ZmFLS* genes (C), *ZmFLS1* and *ZmFLS2* genes from the BAC clone (D), and *ZmFLS2* promoter (1.5 kb upstream the start codon) (E).

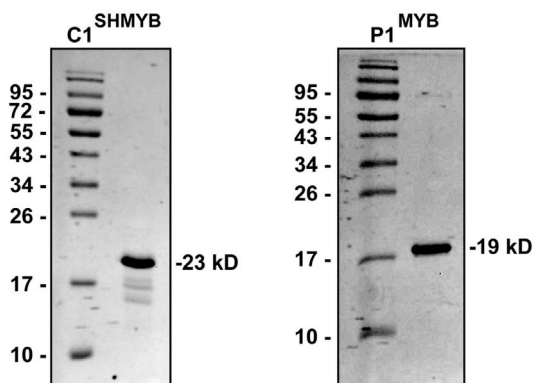


FIGURE A3 | SDS-PAGE analysis (15%) of the purified MYB domains of P1 and C1^{SH}. The numbers on the sides of the gels indicate the molecular mass of standard proteins in kD.

| | | |
|--------------|--|-----|
| A | | |
| Mishca | CCAAGTACGTGCCCCGACGCACTCATCGTCCATATCGGGGATCAGATGAGGCAAGCGACC | 60 |
| W22-1 | CCAAGTACGTGCCCCGACGCACTCATCGTCCATATCGGCGATCAGATCGAGGCAAGCGACC | 60 |
| Cachuacintle | CCAAGTACGTGCCCCGACGCACTCATCGTCCATATCGGCGATCAGATCGAGGCAAGCGACC | 60 |
| W23b,pl | CCAAGTACGTGCCCCGACGCACTCATCGTCCATATCGGCGATCAGATCGAGGCAAGCGACC | 60 |
| Mo17 | CCAAGTACGTGCCCCGACGCACTCATCGTCCATATCGGCGATCAGATCGAGGCAAGCGACC | 60 |
| Arrocillo | CCAAGTACGTGCCCCGACGCACTCATCGTCCATATCGGCGATCAGATCGAGGCAAGCGACC | 60 |
| A619 | CCAAGTACGTGCCCCGACGCACTCATCGTCCATATCGGCGATCAGATCGAGGCAAGCGACC | 60 |
| B73 | CCAAGTACGTGCCCCGACGCACTCATCGTCCATATCGGCGATCAGATCGAGGCAAGCGACC | 60 |
| Confite | CCAAGTACGTGCCCCGACGCACTCATCGTCCATATCGGCGATCAGATCGAGGCAAGCGACC | 60 |
| Conico | CCAAGTACGTGCCCCGACGCACTCATCGTCCATATCGGGGATCAGATGAGGCAAGCGACC | 60 |
| ***** | | |
| Mishca | TACGTAGCATCTTTTTTTTTTTCATTCTATTGTGTTGTGATGATCTTGCTCCC-TCAAGTA | 119 |
| W22 | TACGTAGCATCTTTTTTTTTT-CATTCTATTGTGTTGTGATGATCTTGCTCCC-TCAAGTA | 118 |
| Cachuacintle | TACGTAGCATCTTTTTTTTTT-CATTCTATTGTGTTGTGATGATCTTGCTCCC-TCAAGTA | 118 |
| W23b,pl | TACGTAGCATCTTTTTTTTTT-CATTCTATTGTGTTGTGATGATCTTGCTCCC-TCAAGTA | 118 |
| Mo17 | TACGTAGCATCTTTTTTTTTTTCATTCTATTGTGTTGTGATGATCTTGCTCCC-TCAAGTA | 119 |
| Arrocillo | TACGTAGCATCTTTTTTTTTTTCATTCTATTGTGTTGTGATGATCTTGCTCCC-TCAAGTA | 115 |
| A619 | TACGTAGCATCTTTTTTTTTTTCATTCTATTGTGTTGTGATGATCTTGCTCCC-TCAAGTA | 119 |
| B73 | TACGTAGCATCTTTTTTTTTTTCATTCTATTGTGTTGTGATGATCTTGCTCCC-TCAAGTA | 119 |
| Confite | TACGTAGCATCTTTTTTTTTT-CATTCTATTGTGTTGTGATGATCTTGCTCCC-TCAAGTA | 118 |
| Conico | TACGTAGCTTTCTTTTTTTTTT-CATTCTATTGTGTTGTGATGATCTTGCTCCC-TCAAGTA | 118 |
| ***** | | |
| Mishca | TTTCTTTGATGTCGGACAACACTACTAAGTGGTACGAACCGATCGAGCAGATTTTCAGCAAC | 179 |
| W22 | TTTCTTTGATGTCGGACAACACTACTAAGTGGTACGAACCGATCGAGCAGATTTTCAGCAAC | 178 |
| Cachuacintle | TTTCTTTGATGTCGGACAACACTACTAAGTGGTACGAACCGATCGAGCAGATTTTCAGCAAC | 178 |
| W23b,pl | TTTCTTTGATGTCGGACAACACTACTAAGTGGTACGAACCGATCGAGCAGATTTTCAGCAAC | 178 |
| Mo17 | TTTCTTTGATGTCGGACAACACTACTAAGTGGTACGAACCGATCGAGCAGATTTTCAGCAAC | 179 |
| Arrocillo | TTTCTTTGATGTC-----CCGATCAGTAGATTTTCAGCAAC | 152 |
| A619 | TTTCTTTGATGTCGGACAACACTACTAAGTGGTACGAACCGATCGAGCAGATTTTCAGCAAC | 179 |
| B73 | TTTCTTTGATGTCGGACAACACTACTAAGTGGTACGAACCGATCGAGCAGATTTTCAGCAAC | 179 |
| Confite | TTTCTTTGATGTCGGACAACACTACTAAGTGGTACGAACCGATCGAGCAGATTTTCAGCAAC | 178 |
| Conico | TTTCTTTGATGTCGGACAACACTACTAAGTGGTACGAACCGATCGAGCAGATTTTCAGCAAC | 178 |
| ***** | | |
| Mishca | GGGGCATAAAGGCGGTGCTGCACCGTACGACGGTGAACAAGGAGAAGACGCGGATGTCA | 239 |
| W22 | GGGGCATAAAGGCGGTGCTGCACCGTACGACGGTGAACAAGGAGAAGACGCGGATGTCA | 238 |
| Cachuacintle | GGGGCATAAAGGCGGTGCTGCACCGTACGACGGTGAACAAGGAGAAGACGCGGATGTCA | 238 |
| W23b,pl | GGGGCATAAAGGCGGTGCTGCACCGTACGACGGTGAACAAGGAGAAGACGCGGATGTCA | 238 |
| Mo17 | GGGGCATAAAGGCGGTGCTGCACCGTACGACGGTGAACAAGGAGAAGACGCGGATGTCA | 239 |
| Arrocillo | GGGGCATAAAGGCGGTGCTGCACCGTACGACGGTGAACAAGGAGAAGACGCGGATGTCA | 212 |
| A619 | GGGGCATAAAGGCGGTGCTGCACCGTACGACGGTGAACAAGGAGAAGACGCGGATGTCA | 239 |
| B73 | GGGGCATAAAGGCGGTGCTGCACCGTACGACGGTGAACAAGGAGAAGACGCGGATGTCA | 239 |
| Confite | GGGGCATAAAGGCGGTGCTGCACCGTACGACGGTGAACAAGGAGAAGACGCGGATGTCA | 238 |
| Conico | GGGGCATAAAGGCGGTGCTGCACCGTACGACGGTGAACAAGGAGAAGACGCGGATGTCA | 238 |
| ***** | | |
| Mishca | TGGCCGATGTTCTGTGGAGCCGCCGGGGAGCTCGTCGTCGGGCCGACCCCAAGCTGGTC | 299 |
| W22 | TGGCCGATGTTCTGTGGAGCCGCCGGGGAGCTCGTCGTCGGGCCGACCCCAAGCTGGTC | 297 |
| Cachuacintle | TGGCCGATGTTCTGTGGAGCCGCCGGGGAGCTCGTCGTCGGGCCGACCCCAAGCTGGTC | 298 |
| W23b,pl | TGGCCGATGTTCTGTGGAGCCGCCGGGGAGCTCGTCGTCGGGCCGACCCCAAGCTGGTC | 298 |
| Mo17 | TGGCCGATGTTCTGTGGAGCCGCCGGGGAGCTCGTCGTCGGGCCGACCCCAAGCTGGTC | 299 |
| Arrocillo | TGGCCGATGTTCTGTGGAGCCGCCGGGGAGCTCGTCGTCGGGCCGACCCCAAGCTGGTC | 272 |
| A619 | TGGCCGATGTTCTGTGGAGCCGCCGGGGAGCTCGTCGTCGGGCCGACCCCAAGCTGGTC | 299 |
| B73 | TGGCCGATGTTCTGTGGAGCCGCCGGGGAGCTCGTCGTCGGGCCGACCCCAAGCTGGTC | 299 |
| Confite | TGGCCGATGTTCTGTGGAGCCGCCGGGGAGCTCGTCGTCGGGCCGACCCCAAGCTGGTC | 298 |
| Conico | TGGCCGATGTTCTGTGGAGCCGCCGGGGAGCTCGTCGTCGGGCCGACCCCAAGCTGGTC | 298 |
| ***** | | |

FIGURE A4 | (Continued).

| | | |
|--------------|--|-----|
| Mishca | ACGGAGGAGAGCCCGGCCAAGTACAAGGCCAAGAAGTACAAGGACTACCAGCACTGCAAG | 359 |
| W22 | ACGGAGGAGAGCCCGGCCAAGTACAAGGCCAAGAAGTACAAGGACTACCAGCACTGCAAG | 357 |
| Cachuacintle | ACGG---AGAGCCCGGCCAAGTACAAGGCCAAGAAGTACAAGGACTACCAGCACTGCAAG | 355 |
| W23b,pl | ACGGAGGAGAGCCCGGCCAAGTACAAGGCCAAGAAGTACAAGGACTACCAGCACTGCAAG | 358 |
| Mo17 | ACGGAGGAGAGCCCGGCCAAGTACAAGGCCAAGAAGTACAAGGACTACCAGCACTGCAAG | 359 |
| Arrocillo | ACGGAGGAGAGCCCGGCCAAGTACAAGGCCAAGAAGTACAAGGACTACCAGCACTGCAAG | 332 |
| A619 | ACGGAGGAGAGCCCGGCCAAGTACAAGGCCAAGAAGTACAAGGACTACCAGCACTGCAAG | 359 |
| B73 | ACGGAGGAGAGCCCGGCCAAGTACAAGGCCAAGAAGTACAAGGACTACCAGCACTGCAAG | 359 |
| Confite | ACGGAGGAGAGCCCGGCCAAGTACAAGGCCAAGAAGTACAAGGACTACCAGCACTGCAAG | 358 |
| Conico | ACGGAGGAGAGCCCGGCCAAGTACAAGGCCAAGAAGTACAAGGACTACCAGCACTGCAAG | 358 |
| | ***** | |
| Mishca | ATCAACAAGCTCCCCATGTAAATTATGTAGCTCGGGTTCTACTGTCGTGTGCACCCGCTTC | 419 |
| W22 | ATCAACAAGCTCCCCATGTAAATTATGTAGCTCGGGTTCTACTGTCGTGTGCACCCGCTTC | 417 |
| Cachuacintle | ATCAACAAGCTCCCCATGTAAATTATGTAGCTCGGGTTCTACTGTCGTGTGCACCCGCTTC | 415 |
| W23b,pl | ATCAACAAGCTCCCCATGTAAATTATGTAGCTCGGGTTCTACTGTCGTGTGCACCCGCTTC | 418 |
| Mo17 | ATCAACAAGCTCCCCATGTAAATTATGTAGCTCGGGTTCTACTGTCGTGTGCACCCGCTTC | 419 |
| Arrocillo | ATCAACAAGCTCCCCATGTAAATTATGTAGCTCGGGTTCTACTGTCGTGTGCACCCGCTTC | 392 |
| A619 | ATCAACAAGCTCCCCATGTAAATTATGTAGCTCGGGTTCTACTGTCGTGTGCACCCGCTTC | 419 |
| B73 | ATCAACAAGCTCCCCATGTAAATTATGTAGCTCGGGTTCTACTGTCGTGTGCACCCGCTTC | 419 |
| Confite | ATCAACAAGCTCCCCATGTAAATTATGTAGCTCGGGTTCTACTGTCGTGTGCACCCGCTTC | 418 |
| Conico | ATCAACAAGCTCCCCATGTAAATTATGTAGCTCGGGTTCTACTGTCGTGTGCACCCGCTTC | 418 |
| | ***** | |
| Mishca | TGTAATTTTCAGTCTACCATATTTAAGTCACATAACCGTTATTCCAGTCGCATGCATGGCC | 479 |
| W22 | TGTAATTTTCAGTCTACCATATTTAAGTCACATAACCGTTATTCCAGTCGGGGGATGGGG | 477 |
| Cachuacintle | TGTAATTTTCAGTCTACCATATTTAAGTCACATAACCGTTATTCCAGTCGGGGGCATGGCC | 475 |
| W23b,pl | TGTAATTTTCAGTCTACCATATTTAAGTCACATAACCGTTATTCCAGTCGGGGGCATGGCC | 478 |
| Mo17 | TGTAATTTTCAGTCTACCATATTTAAGTCACATAACCGTTATTCCAGTCGAGGGCATGGCC | 479 |
| Arrocillo | TGTAATTTTCATTTCTACCATATTTAATTCACATAACCGTTATTCCAGTCGCATGGATGGCC | 452 |
| A619 | TGTAATTTTCAGTCTACCATATTTAAGTCACATAACCGTTATTCCAGTCGCATGGATGGCC | 479 |
| B73 | TGTAATTTTCAGTCTACCATATTTAAGTCACATAACCGTTATTCCAGTCGCATGGATGGCC | 479 |
| Confite | TGTAATT-CAGTCTACCATATTTAAACATATGACCATAAATCCAGTCGCATGCATGGCC | 477 |
| Conico | TGTAATT-CAGTCTACCATATTTCTGTCTTTCACCGTAATTCCAGTCGCATGCTTGGCC | 477 |
| | ***** | |

FIGURE A4 | (Continued).

B

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B73          CCAAGTACGTGCCGACGCACTCATCGTCCATATCGGCGATCAGATCGAGG-CAAGCGAC 59
Arrocillo    CCAAGTACGTGCCGACGCACTCATCGTCCATATCGGCGATCAGATCGAGG-CAAGCGAC 59
A619         CCAAGTACGTGCCGACGCACTCATCGTCCATATCGGCGATCAGATCGAGG-CAAGCGAC 59
Cacahuacintle CCAAGTACGTGCCGACGCACTCATCGTCCATATCGGCGATCAGATCGAGG-CAAGCGAC 59
Conico       CCAAGTACGTGCCGACGCACTCATCGTCCATATCGGCGATCAGATCGAGG-CAAGCGAC 59
Confite      CCAAGTACGTGCCGACGCACTCATCGTCCATATCGGCGATCAGATCGAGG-CAAGCGAC 59
W23b,pl     CCAAGTACGTGCCGACGCACTCATCGTCCATATCGGCGATCAGATCGAGG-CAAGCGAC 59
Mishca       CCAAGTACGTGCCGACGCACTCATCGTCCATATCGGCGATCAGATCGAGG-CAAGCGAC 59
Mo17         CCAAGTACGTGCCGACGCACTCATCGTCCATATCGGCGATCAGATCGAGG-CAAGCGAC 60
W22          CCAAGTACGTGCCGACGCACTCATCGTCCATATCGGCGATCAGATCGAGG-CAAGCGAC 59
*****

B73          CTACG-----TAGCATCTTTTTTTTT-CTTCTATTGTGTTGTGATGATCTTGCTCCCT 113
Arrocillo    CTACG-----TAGCATCTTTTTTTTT-CTTCTATTGTGTTGGGATGATCTTCTCCCT 113
A619         CTACG-----TAGCATCTTTTTTTTT-CTTCTATTGTGTTGTGATGATCTTGCTCCCT 113
Cacahuacintle CTACGCTACGTAGCATCTTTTTTTTT-CTTTTATTGTGTTGTGATGATCTTGCTCCCT 118
Conico       CTACG-----TAGCATCTTTTTTTTT-CTTCTATTGTGTTGTGATGATCTTGCTCCCT 113
Confite      CTACG-----TAGCATCTTTTTTTTT-CTTCTATTGTGTTGTGATGATCTTGCTCCCT 113
W23b,pl     CTACG-----TAGCATCTTTTTTTTT-CATTCTATTGTGTTGTGATGATCTTGCTCCCT 112
Mishca       CTACG-----TAGCATCTTTTTTTTT-CATTCTATTGTGTTGTGATGATCTTGCTCCCT 112
Mo17         CTACG-----TAGCATCTTTTTTTTTTATTCTATTGTGTTGTGATGATCTTGCTCCCT 114
W22          CTACG-----TAGCATCTTTTTTTTT-CTTCTATTGTGTTGTGATGATCTTGCTCCCT 113
*****

B73          CAAGTATTTCTTTGATGTC-----CCGATCGAGTAGATTTTC 150
Arrocillo    CAAGTATTTATGTGATGTC-----CCGATCAAGTAGATTTTC 150
A619         CAAGTATTTCTTTGATGTC-----CCGATCGAGTAGATTTTC 150
Cacahuacintle CAAGTATTTCTTTGATGTC-----CCGATCGAGTAGATTTTC 155
Conico       CAAGTATTTCTTTGATGTC-----CCGATCGAGTAGATTTTC 150
Confite      CAAGTATTTCTTTGATGTC-----CCGATCGAGTAGATTTTC 150
W23b,pl     CAAGTATTTCTTTGATGTCGGACAACCTAACTGGTACGAA CCGATCGAGCAGATTTTC 172
Mishca       CAAGTATTTCTTTGATGTCGGACAACCTAACTGGTACGAA CCGATCGAGCAGATTTTC 172
Mo17         CAAGTATTTCTTTGATGTCGGACAACCTAACTGGTACGAA CCGATCGAGCAGATTTTC 174
W22          CAAGTAATTATTTGATGTTA-----CGATCGAGTAGATTTTC 150
*****

B73          AGCAACGGGGCATACAAGGCGGTGCTGCACCGTACGACGGTGAACAAGGAGAAGACGCGG 210
Arrocillo    AGCAACGGGGCATACAAGGCGGTGCTGCACCGTACGACGGTGAACAAGGAGAAGACGCGG 210
A619         AGCAACGGGGCATACAAGGCGGTGCTGCACCGTACGACGGTGAACAAGGAGAAGACGCGG 210
Cacahuacintle AGCAACGGGGCATACAAGGCGGTGCTGCACCGTACGACGGTGAACAAGGAGAAGACGCGG 215
Conico       AGCAACGGGGCATACAAGGCGGTGCTGCACCGTACGACGGTGAACAAGGAGAAGACGCGG 210
Confite      AGCAACGGGGCATACAAGGCGGTGCTGCACCGTACGACGGTGAACAAGGAGAAGACGCGG 210
W23b,pl     AGCAACGGGGCATACAAGGCGGTGCTGCACCGTACGACGGTGAACAAGGAGAAGACGCGG 232
Mishca       AGCAACGGGGCATACAAGGCGGTGCTGCACCGTACGACGGTGAACAAGGAGAAGACGCGG 232
Mo17         AGCAACGGGGCATACAAGGCGGTGCTGCACCGTACGACGGTGAACAAGGAGAAGACGCGG 234
W22          AGCAACGGGGCATACAAGGCGGTGCTGCACCGTACGACGGTGAACAAGGAGAAGACGCGG 210
*****

B73          ATGTCATGGCCGATGTTCTGTTGAGCCGCGGGGGAGCTCGTCGTCGGGCCGCACCCCAAG 270
Arrocillo    ATGTCATGGCCGATGTTCTGTTGAGCCGCGGGGGAGCTCGTCGTCGGGCCGCACCCCAAG 270
A619         ATGTCATGGCCGATGTTCTGTTGAGCCGCGGGGGAGCTCGTCGTCGGGCCGCACCCCAAG 270
Cacahuacintle ATGTCATGGCCGATGTTCTGTTGAGCCGCGGGGGAGCTCGTCGTCGGGCCGCACCCCAAG 275
Conico       ATGTCATGGCCGATGTTCTGTTGAGCCGCGGGGGAGCTCGTCGTCGGGCCGCACCCCAAG 270
Confite      ATGTCATGGCCGATGTTCTGTTGAGCCGCGGGGGAGCTCGTCGTCGGGCCGCACCCCAAG 270
W23b,pl     ATGTCATGGCCGATGTTCTGTTGAGCCGCGGGGGAGCTCGTCGTCGGGCCGCACCCCAAG 292
Mishca       ATGTCATGGCCGATGTTCTGTTGAGCCGCGGGGGAGCTCGTCGTCGGGCCGCACCCCAAG 292
Mo17         ATGTCATGGCCGATGTTCTGTTGAGCCGCGGGGGAGCTCGTCGTCGGGCCGCACCCCAAG 294
W22          ATGTCATGGCCGATGTTCTGTTGAGCCGCGGGGGAGCTCGTCGTCGGGCCGCACCCCAAG 270
*****

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FIGURE A4 | (Continued).

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B73          CTGGTCACGGAGGAGAGCCCGGCCAAGTACAAGGCCAAGAAGTACAAGGACTACCAGCAC 330
Arrocillo    CTGGTCACGGAGGAGAGCCCGGCCAAGTACAAGGCCAAGAAGTACAAGGACTACCAGCAC 330
A619         CTGGTCACGGAGGAGAGCCCGGCCAAGTACAAGGCCAAGAAGTACAAGGACTACCAGCAC 330
Cacahuacintle CTGGTCACGGAGGAGAGCCCGGCCAAGTACAAGGCCAAGAAGTACAAGGACTACCAGCAC 335
Conico       CTGGTCACGGAGGAGAGCCCGGCCAAGTACAAGGCCAAGAAGTACAAGGACTACCAGCAC 330
Confite      CTGGTCACGGAGGAGAGCCCGGCCAAGTACAAGGCCAAGAAGTACAAGGACTACCAGCAC 330
W23b,pl     CTGGTCACGGAGGAGAGCCCGGCCAAGTACAAGGCCAAGAAGTACAAGGACTACCAGCAC 352
Mishca       CTGGTCACGGAGGAGAGCCCGGCCAAGTACAAGGCCAAGAAGTACAAGGACTACCAGCAC 352
Mo17        CTGGTCACGGAGGAGAGCCCGGCCAAGTACAAGGCCAAGAAGTACAAGGACTACCAGCAC 354
W22         CTGGTCACGGAGGAGAGCCCGGCCAAGTACAAGGCCAAGAAGTACAAGGACTACCAGCAC 330
*****

B73          TGCAAGATCAACAAGCTCCCATGTAAATTATGTAGCTCGGGTTCTACTGTCTGTG----- 384
Arrocillo    TGCAAGATCAACAAGCTCCCATGTAAATTATGTA TGTCTGGTTTCTACTGT CGGTG TGCAGG 390
A619         TGCAAGATCAACAAGCTCCCATGTAAATTATGTAGCTCGGGTTCTACTGTCTGTG----- 384
Cacahuacintle TGCAAGATCAACAAGCTCCCATGTAAATTATGTAGCTCGGGTTCTACTGTCTGTG----- 389
Conico       TGCAAGATCAACAAGCTCCCATGTAAATTATGTAGCTCGGGTTCTACTGTCTGTG----- 384
Confite      TGCAAGATCAACAAGCTCCCATGTAAATTATGTAGCTCGGGTTCT GCTG C CATG----- 384
W23b,pl     TGCAAGATCAACAAGCTCCCATGTAAATTATGTACCTCGGGTTCTACTG----- 401
Mishca       TGCAAGATCAACAAGCTCCCATGTAAATTATGTAGCTCGGGTTCTACTG----- 401
Mo17        TGCAAGATCAACAAGCTCCCATGTAAATTATGTAGCTCGGGTTCTACTGTCTGTG----- 408
W22         TGCAAGATCAACAAGCTCCCATGTAAATTATGTAGCTCGGGTTCTAT TGT----- 380
*****

B73          ---CAGTGTGCACCCGCTTCTGT 404
Arrocillo    CTT CAGTGTGCACCCGCTTCTGT 414
A619         ---CAGTGTGC C CCGGCTTCTGT 404
Cacahuacintle ---CAGTGTGCACCC C CTTCTGT 409
Conico       ---CAGTGTGC C C C CTTCTGT 404
Confite      ---CA TGTGCACCCGCTTCTGT 404
W23b,pl     ---CAGTGTGCACCC C CTTCTG C 421
Mishca       ---CAGTGTGCACCC C CTT TTT 421
Mo17        ---CAGTGTGCACCCGCTTCTGT 431
W22         ---AGTGTGCACCCGCTTCTGT 399
*****

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FIGURE A4 | Sequence comparison of partial *ZmFLS1* (A) and *ZmFLS2* (B) genes in different maize lines. The regions of *ZmFLS1* and *ZmFLS2* genes range from +740 bp (exon 1) to +1218 and +1143 bp, respectively (3' UTR). The introns in *ZmFLS* genes are

highlighted in gray, different nucleotides and insertions or deletions are highlighted in yellow. Stop codons are indicated in bold-underlined letters. Primers used for RT-qPCR are in bold-underlined letters on B73 sequences.

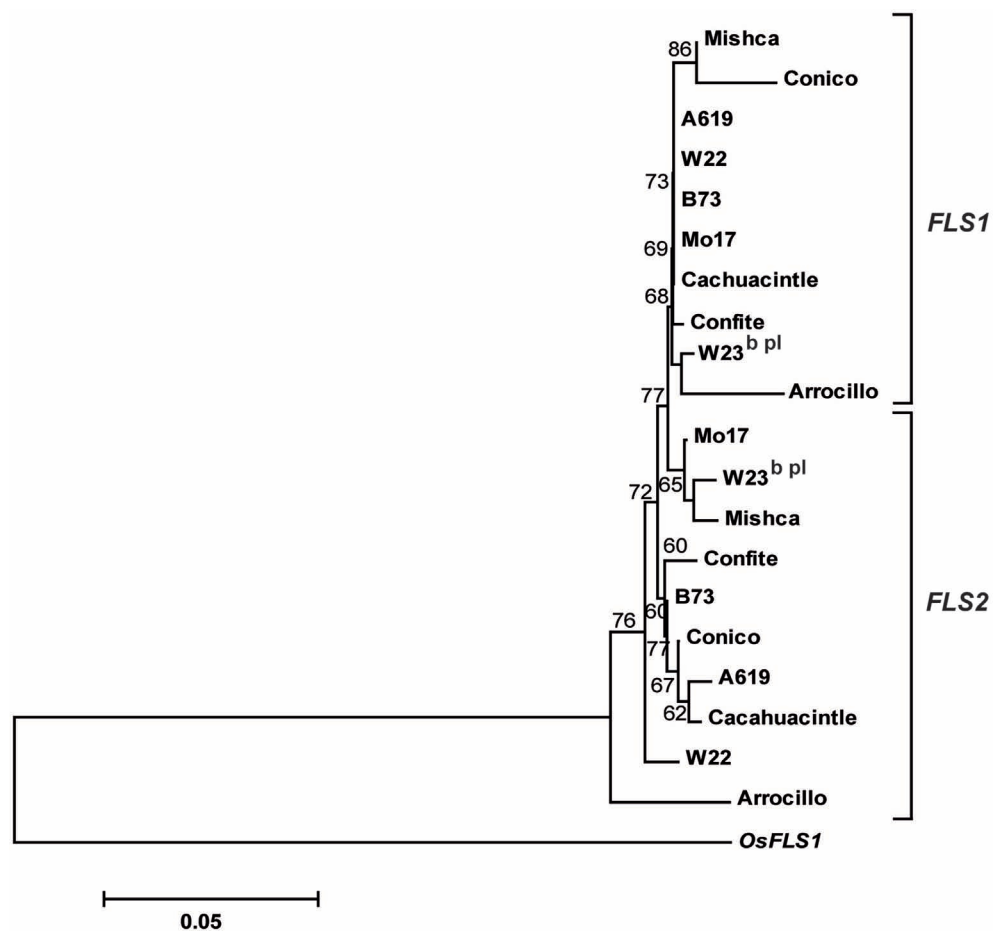


FIGURE A5 | Phylogenetic tree based on nucleotide sequences of *ZmFLS1* and *ZmFLS2* genes in maize inbred lines and landraces from high altitudes. The numbers indicate bootstrap values (10,000 replicates). Bar = 0.1 amino acid substitutions per site. *OsFLS1* was defined as the out-group.

A

B73 GCTATTACTC**CTTAGTT**GGATTTTAGTTTCTAAGAAGTTGAAGTAGGGTCAGGGCA**CAG** 60
W23b, pl GCTATTACTC**CTTAGTT**GGATTTTAGTTTCTAAGAAGTTGAAGTAGGGTCAGGGCA**CAG** 60
Mo17 GCTATTACTC**CTTAGTT**GGATTTTAGTTTCTAAGAAGTTGAAGTAGGGTCAGGGCA**CAG** 60
Confite **CGT**ATTACTC**CTTAGTT**GGATTTTAGTTTCTAAGAAGTTGAAGTAGGGTCAGGGCA**CAG** 60
Conico GCTATTACTC**CTTAGTT**GGGTTTAGTTTCTAAGAAGTTGAAGTAGGGTCAGGGCA**CAG** 60
Mishca GCTATTACTC**CTTAGTT**GGGTTTAG**ATT**TTAAGAAGTTGA**TA**AGGGTCAGGGCA**CAG** 60

B73 **TTG**AAGCTGGTGACATGTCGCAGCGAACATAAAAGAGTTTCT**CTAGG**GCGTCGTACGG 120
W23b, pl **TTG**AAGCTGGTGACATGTCGCAGCGAACATAAAAGAGTTTCT**CTAGG**GCGTCGTACGG 120
Mo17 **TTG**AAGCTGGTGACATGTCGCAGCGAACATAAAAGAGTTTCT**CTAGG**GCGTCGTACGG 120
Confite **TTG**AAGCTGGTGACATGTCGC**G**CGAACATAAA**C**GAGTTTCT**CTAGG**GCGTCGTACGG 120
Conico **TTG**AAGCTGGTGACATGTCGCAGCGAACATAAAAGAGTTTCT**CTAGG**GCGTCGTACGG 120
Mishca **TTG**AAGCTGGTGACATGTCGCAGCGAACAA**AAAA**AGAGTT**TT****CTAGG**GCGTCGTACGG 120

B73 CCAGAAACACACAACGGCGGCTATCTTCGCGCTCGCTCTCGGGTCTGTTCCGTGACCAG 180
W23b, pl CCAGAAACACACAACGGCGGCTATCTTCGCGCTCGCTCTCGGGTCTGTTCCGTGACCAG 180
Mo17 CCAGAAACACACAACGGCGGCTATCTTCGCGCTCGCTCTCGGGTCTGTTCCGTGACCAG 180
Confite CCAGAAACACACAACGGCGGCTATCTTCGCGCTCGCTCTCGGGTCTGTTCCGTGACCAG 180
Conico CCAGAAACACACAACGGCGGCTATCTTCGCGCTCGCTCTCGGGTCTGTTCCGTGACCAG 180
Mishca CCAGAAACACACAACGGCGGCTTTTTCGCGCTCGCTCTCGGGTCTGTTCCGTGACCAG 180

B73 CATGGCAAGGCTGCCACGTTCCAGCCACCCCTGGGC-----ACCAAGTAACCGGCCA 234
W23b, pl CATGGCAAGGCTGCCACGTTCCAGCCACCCCTGGGC-----ACCAAGTAACCGGCCA 234
Mo17 CATGGCAAGGCTGCCACGTTCCAGCCACCCCTGGGC-----ACCAAGTAACCGGCCA 234
Confite CATGGCAAGGCTGCCACGTTCCAGCCACCCCTGGGC**CTGGG**ACCAAG**C**AACCGGCCA 240
Conico CATGGCAAGGCTGCCACGTTCCAGCCACCCCTGGGC-----ACCAAGTAACCGGCCA 234
Mishca CATGGCAAGGCTGCCACGTTCCAGCCACCCCTGGGC-----ACCAAG**C**AACCGGCCA 234

B73 CACAATCCGCACCGCGTCTGTGCCCTACGCCATACCCAAACGCACGTTTCCAGCGCAGC 294
W23b, pl CACAATCCGCACCGCGTCTGTGCCCTACGCCATACCCAAACGCACGTTTCCAGCGCAGC 294
Mo17 CACAATCCGCACCGCGTCTGTGCCCTACGCC**T**ATACCCAAACGCACGTTTCCAGCGCAGC 294
Confite CACAATCCGCACCGCGTCTGTGCCCTACGCCATACCCAAACGCACGTTTCCAGCGCAGC 300
Conico CACAAT**T**CGCACCGCGTCTGTGCCCTACGCCATACCCAAACGCACGTTTCCAGCGCAGC 294
Mishca CACAAT**T**CGCACCGCGTCTGTGCCCTACGCCATACCCAAACG**A**ACGTTTCCAG**G**CGCAGC 294

B73 GGCCGGTCTACCACACCACAGCCACTCACTCCTGTCCCGTGCCCCACAA-----CA 346
W23b, pl GGCCGGTCTACCACACCACAGCCACTCACTCCTGTCCCGTGCCCCACAA-----CA 346
Mo17 GGCCGGTCTACCACACCACAGCCACTCACTCCTGTCCCGTGCCCCACAA-----CA 346
Confite GGCCGGTCTACCACACCACAGCCACTCACTCCTGTCCCGTGCCCCACAA-----CA 352
Conico GGCCGGTCTACCACACCACAGCCACTCGCT**T**TGTCCCGTGCCCCACAAAGGCCACAA**A** 354
Mishca GGCCGGTCTACCACACCAC**C**CCACTC**CATT**CTGTCCCGTGCCCC**G**CTGGCCACAA**A** 354

B73 CACAACAC**TATTAAT**TCAGTGCTCTTCCCTTCGTCTGCGTCTGCGT-----GCAGCGTG 400
W23b, pl CACAACAC**TATTAAT**TCAGTGCTCTTCCCTTCGTCTGCGTCTGCGT-----GCAGCGTG 400
Mo17 CACAACAC**TATTAAT**TCAGTGCTCTTCCCTTCGTCTGCGTCTGCGT-----GCAGCGTG 400
Confite CA--ACA**TATTAAT**TCAGTGCTCTTCCCT**G**CGTCTGCGTCTGCGTCTGCGT**G**CGTGCA**G**CGTG 410
Conico CACAACAC**TATTAAT**TCAGTGCTCTTCCCT**G**CGTCTGCGT-----**T**CGTG 399
Mishca CACAACAC**TATTAAT**TCAGTGCTCTTCCCT**G**CGTCTGCGT-----GCAT**T**CGTG 402

B73 CTCGCGAGGCCCTGCTTCTGCTGCG 426
W23b, pl CTCGCGAGGCCCTGCTTCTGCTGCG 426
Mo17 CTCGCGAGGCCCTGCTTCTGCTGCG 426
Confite CTCGCGAGG**T**CTGCTTCTGCTG**GGG** 436
Conico CTCGCGAGG**T**CTGCTTCTGCTG**GGG** 425
Mishca CTCGCGAGGCCCTGCTTCTGCTGCG 428

FIGURE A6 | (Continued).

B

| | | |
|---------------|---|-----|
| Arrocillo | GCTATTACTCCTTAGTTGGATTTTAGTTTCTAAGAAGTTGAAGTAGGGTCAGGGCA | 60 |
| Cacahuacintle | GCTATTACTCCTTAGTTGGATTTTAGCTTTCTAAGAAGTTGAAGTAGGGTCAGGGCA | 60 |
| Mo17 | GCTATTACTCCTTAGTTGGATTTTAGTTTCTAAGAAGTTGAAGTAGGGTCAGGGCA | 60 |
| W23b,pl | GCTATTACTCCTTAGTTGGATTTTAGTTTCTAAGAAGTTGAAGTAGGGTCAGGGCA | 60 |
| Mishca | GCTATTACTCCTTAGTTGGATTTTAGTTTCTAAGAAGTTGAAGTAGGGTCAGGGCA | 60 |
| B73 | GCTATTACTCCTTAGTTGGATTTTAGTTTCTAAGAAGTTGAAGTAGGGTCAGGGCA | 60 |
| ***** | | |
| Arrocillo | TTGAAAGCTGGTGACACGTACAGCGAACATAAATGAGTTTCTCTAGGTCACGTGCTACG | 120 |
| Cacahuacintle | TTGAAAGCTGGTGACACGTACAGCGAACATAAATGAGTTTCTCTAGGTCACGTGCTACG | 120 |
| Mo17 | TTGAAAGCTGGTGACACGTACAGCGAACATAAATGAGTTTCTCTAGGTCACGTGCTACG | 120 |
| W23b,pl | TTGAAAGCTGGTGACACGTACAGCGAACATAAATGAGTTTCTCTAGGTCACGTGCTACG | 120 |
| Mishca | TTGAAAGCTGGTGACACGTACAGCGAACATAAATGAGTTTCTCTAGGTCACGTGCTACG | 120 |
| B73 | TTGAAAGCTGGTGACACGTACAGCGAACATAAATGAGTTTCTCTAGGTCACGTGCTACG | 120 |
| ***** | | |
| Arrocillo | GCCAGAAACACAACACGGCCTATCTTCGCGCTCGCATAGCAAGGCTACACGTTCCGAG | 180 |
| Cacahuacintle | GCCAGAAACACAACACGGCCTATCTTCGCGCTCGCATAGCAAGGCTACACGTTCCGAG | 180 |
| Mo17 | GCCAGAAACACAACACGGCCTATCTTCGCGCTCGCATAGCAAGGCTACACGTTCCGAG | 180 |
| W23b,pl | GCCAGAAACACAACACGGCCTATCTTCGCGCTCGCATAGCAAGGCTACACGTTCCGAG | 180 |
| Mishca | GCCAGAAACACAACACGGCCTATCTTCGCGCTCGCATAGCAAGGCTACACGTTCCGAG | 180 |
| B73 | GCCAGAAACACAACACGGCCTATCTTCGCGCTCGCATAGCAAGGCTACACGTTCCGAG | 180 |
| ***** | | |
| Arrocillo | CCACCCCGG-----GCACCAAGCAACCGGCCACACAATTTCGACCCGGGTCTGTGCCCA | 234 |
| Cacahuacintle | CCACCCCGG-----GCACCAAGCAACCGGCCACACAATTTCGACCCGGGTCTGTGCCCA | 234 |
| Mo17 | CCACCCCGG-----GCACCAAGCAACCGGCCACACAATTTCGACCCGGGTCTGTGCCCA | 234 |
| W23b,pl | CCACCCCGG-----GCACCAAGCAACCGGCCACACAATTTCGACCCGGGTCTGTGCCCA | 234 |
| Mishca | CCACCCCGG-----GCACCAAGCAACCGGCCACACAATTTCGACCCGGGTCTGTGCCCA | 234 |
| B73 | CCACCCCGGCCCCGGGCACCAAGCAACCGGCCACACAATTTCGACCCGGGTCTGTGCCCA | 240 |
| ***** | | |
| Arrocillo | TACCCAAACGCACGTTTCCAGCACAGCGGCCGGTCTACCACACCACAGCCACTCACTCC | 294 |
| Cacahuacintle | TACCCAAACGCACGTTTCCAGCACAGCGGCCGGTCTACCACACCACAGCCACTCACTCC | 294 |
| Mo17 | TACCCAAACGCACGTTTCCAGCACAGCGGCCGGTCTACCACACCACAGCCACTCACTCC | 294 |
| W23b,pl | TACCCAAACGCACGTTTCCAGCACAGCGGCCGGTCTACCACACCACAGCCACTCACTCC | 294 |
| Mishca | TACCCAAACGCACGTTTCCAGCACAGCGGCCGGTCTACCACACCACAGCCACTCACTCC | 294 |
| B73 | TACCCAAACGCACGTTTCCAGCACAGCGGCCGGTCTACCACACCACAGCCACTCACTCC | 300 |
| ***** | | |
| Arrocillo | TGTCCCGTGCCCCACAACACAACACATATTAATTCAGTGCTCTTCC | 353 |
| Cacahuacintle | TGTCCCGTGCCCCACAACACAACACATATTAATTCAGTGCTCTTCCCTGCCTGCGTCTGCG | 354 |
| Mo17 | TGTCCCGTGCCCCACAACACAACACATATTAATTCAGTGCTCTTCCCTGCCTGCGTCTGCG | 354 |
| W23b,pl | TGTCCCGTGCCCCACAACACAACACATATTAATTCAGTGCTCTTCCCTGCCTGCGTCTGCG | 354 |
| Mishca | TGTCCCGTGCCCCACAACACAACACATATTAATTCAGTGCTCTTCCCTGCCTGCGTCTGCG | 354 |
| B73 | TGTCCCGTGCCCCACAACACAACACATATTAATTCAGTGCTCTTCCCTGCCTGCGTCTGCG | 360 |
| ***** | | |
| Arrocillo | TGCAGCGTGCTCGCGAGGTCCTGCTTCCTGCTGCG | 389 |
| Cacahuacintle | TGCAGCGTGCTCGCGAGGTCCTGCTTCCTGCTGCG | 389 |
| Mo17 | TGCAGCGTGCTCGCGAGGTCCTGCTTCCTGCTGCG | 389 |
| W23b,pl | TGCAGCGTGCTCGCGAGGTCCTGCTTCCTGCTGCG | 389 |
| Mishca | TGCAGCGTGCTCGCGAGGTCCTGCTTCCTGCTGCG | 389 |
| B73 | TGCAGCGTGCTCGCGAGGTCCTGCTTCCTGCTGCG | 395 |
| ***** | | |

FIGURE A6 | Alignment of *ZmFLS1* (A) and *ZmFLS2* (B) proximal promoters of maize inbred lines and landraces from high altitudes.
C1/P1-binding sites are highlighted in different colors. Insertions or deletions

in promoters are highlighted in gray, while differences in nucleotides from B73 are highlighted in yellow. The putative TATA boxes are indicated in bold-underlined letters.