Coincident light and clock regulation of *pseudoresponse regulator protein 37* (*PRR37*) controls photoperiodic flowering in sorghum

Rebecca L. Murphy[a,1], Robert R. Klein[b,1], Daryl T. Morishige[a], Jeff A. Brady[c], William L. Rooney[d], Frederick R. Miller[e], Diana V. Dugas[f], Patricia E. Klein[f], and John E. Mullet[a,2]

[a]Department of Biochemistry and Biophysics, Texas A&M University, College Station, TX 77843; [b]US Department of Agriculture-Agricultural Research Service, College Station, TX 77845; [c]Texas AgriLife Research and Extension Center, Stephenville, TX 76401; [d]Department of Soil and Crop Sciences, Texas A&M University, College Station, TX 77843; [e]MMR Genetics, Vega, TX 79092; and [f]Department of Horticultural Sciences and Institute for Plant Genomics and Biotechnology, Texas A&M University, College Station, TX 77843

Edited by Richard M. Amasino, University of Wisconsin, Madison, WI, and approved August 2, 2011 (received for review April 18, 2011)

Optimal flowering time is critical to the success of modern agriculture. Sorghum is a short-day tropical species that exhibits substantial photoperiod sensitivity and delayed flowering in long days. Genotypes with reduced photoperiod sensitivity enabled sorghum's utilization as a grain crop in temperate zones worldwide. In the present study, *Ma_1*, the major repressor of sorghum flowering in long days, was identified as the pseudoresponse regulator protein 37 (PRR37) through positional cloning and analysis of *SbPRR37* alleles that modulate flowering time in grain and energy sorghum. Several allelic variants of *SbPRR37* were identified in early flowering grain sorghum germplasm that contain unique loss-of-function mutations. We show that in long days *SbPRR37* activates expression of the floral inhibitor *CONSTANS* and represses expression of the floral activators *Early Heading Date 1*, *FLOWERING LOCUS T*, *Zea mays CENTRORADIALIS 8*, and floral induction. Expression of *SbPRR37* is light dependent and regulated by the circadian clock, with peaks of RNA abundance in the morning and evening in long days. In short days, the evening-phase expression of *SbPRR37* does not occur due to darkness, allowing sorghum to flower in this photoperiod. This study provides insight into an external coincidence mechanism of photoperiodic regulation of flowering time mediated by PRR37 in the short-day grass sorghum and identifies important alleles of *SbPRR37* that are critical for the utilization of this tropical grass in temperate zone grain and bioenergy production.

circadian rhythm | pseudo-response regulator

Sorghum [*Sorghum bicolor* (L.) Moench] is a C4 grass native to Africa that provides an indispensable food source for over 300 million people inhabiting food-insecure regions worldwide (1). Although primarily grown for its grain and forage, high-biomass sorghum is also an excellent drought-tolerant energy crop for sustainable production of lignocellulosic-based biofuels (2). Forage and energy sorghums are selected for delayed flowering to increase biomass yield through longer duration of vegetative growth, whereas grain sorghums are selected for early flowering to ensure sufficient time for grain maturation and to avoid drought and frost. Optimal production of each of these sorghum crops requires the precise regulation of flowering time, which varies depending on planting location and climate. Differences in photoperiod sensitivity confer a wide range of flowering times on diverse accessions of the sorghum germplasm collection (3). Due to its critical importance to crop yield and hybrid seed production, photoperiodic regulation of flowering has been an important trait characterized by sorghum improvement programs dating back to the early 1900s (4).

In *Arabidopsis*, flowering is induced in long days (LD) that expose plants to light in the evening during a phase of circadian clock oscillation required for induction of floral genes, consistent with the external coincidence model (5–7). Rhythmic expression of the core circadian clock components *CIRCADIAN CLOCK ASSOCIATED 1* (*CCA1*)/*LATE ELONGATED HYPOCOTYL* (*LHY*) and *TIMING OF CAB 1* (*TOC1*) during each day–night cycle regulates expression of the clock output gene *GIGANTEA* (*GI*), which in turn regulates the floral-inducing gene *CONSTANS* (*CO*). The stability and activity of these floral regulators are further altered through light-mediated posttranslational modifications and protein–protein interactions, allowing *FLOWERING LOCUS T* (*FT*), a known florigen, to accumulate to critical levels in LD but not in short days (SD) (7). In rice, a species induced to flower in SD, orthologs of *GI*, *CO* (*Hd1*), and *FT* (*Hd3a*) also regulate flowering time (8). In contrast to *Arabidopsis*, *Hd1* (*CO*) was found to repress flowering in LD in rice (9). In wheat and barley (LD-inductive grasses), an ortholog of *AtPRR7*, *PHOTOPERIOD 1* (*Ppd1*), plays an important role in regulating flowering time in response to photoperiod (10, 11). Other key regulators of flowering time have been identified in grass species with no known orthologs in *Arabidopsis*, including *EARLY HEADING DATE 1* and *2* (*Ehd1*, *Ehd2*) (12, 13), which activate *FT* expression, and *VRN2* (*Ghd7*), a repressor of *FT* in wheat and rice (14, 15). These and other reports indicate that the pathway regulating flowering time diversified as grass species adapted to different latitudes and environments.

Sorghum genotypes show a wide range of photoperiod sensitivity and critical floral-inductive day lengths (4, 16). Historic genetic studies uncovered four flowering-time (maturity) loci, which were designated *Ma_1*, *Ma_2*, *Ma_3*, and *Ma_4* (17–19). More recently, two additional maturity genes, *Ma_5* and *Ma_6* (20), which increase photoperiod sensitivity and lengthen the duration of vegetative growth in forage and high-biomass sorghum hybrids, were described. Dominant alleles at each maturity locus contribute to late flowering in LD. Of the four original maturity loci, *Ma_1* has the largest impact on flowering time in sorghum (4). Mutations in *Ma_1* were critical for the early domestication and dispersal of sorghum from its center of origin during the migration of people across Africa and Asia (19). During the first 40 years of the 20th century, growers and plant breeders in the United States and elsewhere selected recessive alleles of *Ma_1*

Author contributions: R.L.M., R.R.K., D.T.M., W.L.R., F.R.M., P.E.K., and J.E.M. designed research; R.L.M., R.R.K., D.T.M., J.A.B., D.V.D., and P.E.K. performed research; R.L.M., R.R.K., D.T.M., J.A.B., D.V.D., P.E.K., and J.E.M. analyzed data; and R.L.M., R.R.K., and J.E.M. wrote the paper.

The authors declare no conflict of interest.

This article is a PNAS Direct Submission.

Data deposition: The sequences reported in this paper have been deposited in the GenBank database (accession nos. JF801178–JF801190).

[1]R.L.M. and R.R.K. contributed equally to this work.

[2]To whom correspondence should be addressed. E-mail: jmullet@tamu.edu.

This article contains supporting information online at www.pnas.org/lookup/suppl/doi:10.1073/pnas.1106212108/-/DCSupplemental.

that resulted in the development of early flowering sorghum cultivars suitable for grain production in temperate regions worldwide (1, 4). More recently, the manipulation of flowering-time loci has been of fundamental importance to the production of high-biomass sorghum for biopower and lignocellulosic biofuels (2).

The overall importance of flowering time and the critical role that Ma_1 plays in this regulatory pathway led to this large-scale effort to understand the molecular basis of Ma_1 function and allelic variation. We report here the positional cloning of sorghum Ma_1 and describe how coordinated regulation of Ma_1 gene expression by light and the circadian clock provides a mechanism for regulating flowering time in response to photoperiod.

Results and Discussion

Map-Based Cloning of the Ma_1 Gene. The gene corresponding to Ma_1 was cloned using three mapping populations derived from genotypes that vary in flowering time due to differences in Ma_1 alleles (Fig. 1, *SI Methods*, and *SI Discussion*). Ma_1 was initially mapped using a population created by crossing two early flowering genotypes, ATx623 (ma_1, Ma_5) and R.07007 (Ma_1, ma_5), to generate photoperiod-sensitive, late-flowering F_1 hybrids that are useful for biomass production (Fig. 1*A*). Exposure of 65-d-old vegetative F_1 plants to SD resulted in flowering 36 d later, whereas F_1 hybrids kept in LD remained vegetative indefinitely (Fig. 1*B*). To create a population suitable for mapping Ma_1, F_1 plants were backcrossed to ATx623 to eliminate allelic effects of Ma_5, a flowering locus recessive in R.07007 (20). Genetic mapping using 1,821 BC_1F_1 plants identified a flowering-time locus within a 700-kb interval on chromosome SBI-06 (Fig. 1*D*) that mapped coincidently with the reported location of Ma_1 (21, 22). This region encodes 34 putative genes (Table S1), among which the most likely candidate gene was *PSEUDORESPONSE REGULATOR 37* (Sb06g014570, *SbPRR37*). The Ma_1 locus was fine-mapped using several historically important grain-producing cultivars that possess different Ma_1 alleles. 100M and SM100 are nearly isogenic lines that contain dominant and recessive Ma_1 alleles, respectively (19), and differ in flowering time by ~60 d when grown in LD (Fig. 1*C*). A mapping population was created by crossing 100M (Ma_1) to the elite inbred BTx406, which derives its ma_1 allele from the same source as

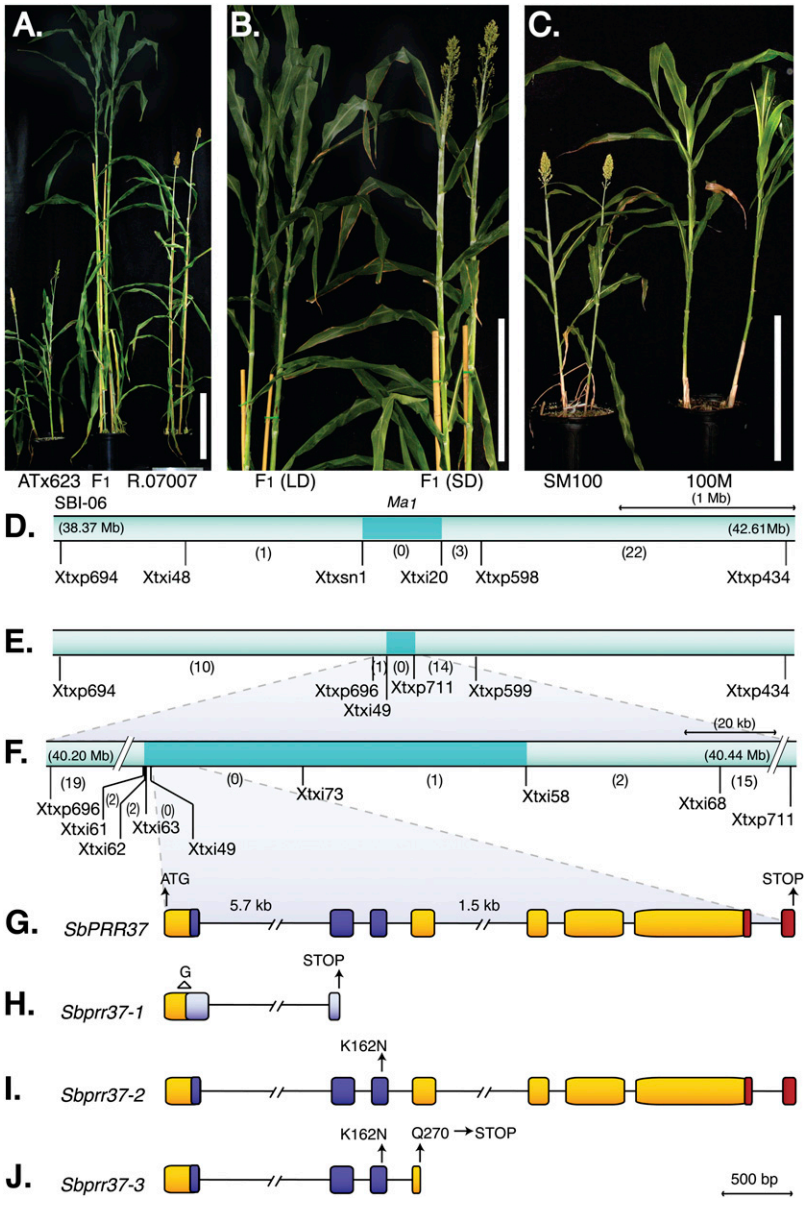

Fig. 1. Phenotypic and genetic analysis of Ma_1. (*A*) LD-entrained ATx623 and R.07007 flower by 54 and 68 d, respectively; ATx623 × R.07007 F_1 plants remain vegetative for >150 d. (*B*) Flowering is induced in LD-entrained ATx623 × R.07007 F_1 hybrids exposed to SD; continued exposure to LD represses flowering. (*C*) In LD, SM100 flowers in 54 d; 100M in 120 d. (Scale bar, 0.5 m.) (*D*) Ma_1 locus delimited to an ~700-kb region between *Xtxsn1* and *Xtxi20* in a BC_1F_1 population (n = 1,821) derived from ATx623 and R.07007 (*E*) Ma_1 mapped to an ~240-kb region delimited by *Xtxp696* and *Xtxp711* using a population of F_2 plants (n = 122) derived from 100M and BTx406. (*F*) The Ma_1 locus was refined between markers *Xtxi62* and *Xtxi58*. Recombination events are shown in parentheses, physical coordinates are at the end of each chromosome segment, and the Ma_1 locus is shaded in blue. (*G*) Functional *SbPRR37* allele in 100M and R.07007. (*H*) Recessive *Sbprr37-1* allele from SM100 and BTx406 with a single nucleotide deletion and frameshift upstream of the PRR domain. (*I*) *Sbprr37-2* allele from Blackhull Kafir with a missense mutation in the PRR domain at conserved Lys^{162} residue. (*J*) *Sbprr37-3* allele from ATx623 containing both the Lys^{162}Asn substitution and a nonsense mutation at Gln^{270} resulting in premature termination. Exons are shown as boxes, and introns as solid lines. Yellow boxes, protein coding sequence; blue boxes, pseudoreceiver domain; red boxes, CCT domain; light blue boxes, missense coding post frameshift.

SM100, but provides a level of polymorphism more suitable for mapping. BTx406 is also of historical importance as the genetic donor of the ma_1 allele used to convert tropical late-flowering sorghum to photoperiod-insensitive cultivars useful for grain sorghum breeding (23). Genetic analysis of this F_2 population refined the Ma_1 locus to a 240-kb region (Fig. 1*E*). Further Ma_1 fine mapping was accomplished using an F_2 population (n = 1,925) derived by crossing 100M to the photoperiod-insensitive cultivar Blackhull Kafir (ma_1), a founder genotype from an ancestral lineage different from 100M (17, 19). Analysis of F_2 plants from this population, in combination with derived F_3 families, allowed the Ma_1 locus to be reduced to an 86-kb interval delimited by markers *Xtxi62* and *Xtxi58* (Fig. 1*F*). The best candidate for Ma_1, *SbPRR37*, was the sole gene present among the stretches of repetitive DNA found in this region (Phytozome v5.0).

Sequence Analysis of *SbPRR37* Alleles in Historical Cultivars. To substantiate the identity of the Ma_1 gene as *SbPPR37* and to characterize alleles of this locus that modify photoperiod sensitivity, full-length cDNAs were sequenced for a select set of founder and elite sorghum cultivars. The structure of *SbPRR37* alleles was examined by aligning full-length cDNA sequences from photoperiod-sensitive (Ma_1) and -insensitive (ma_1) parental genotypes to genomic DNA sequences. The 3,165-nucleotide *SbPRR37* mRNA from R.07007 and 100M contained three untranslated and eight protein-coding exons (Fig. 1*G* and Fig. S1). This transcript encodes a 739-amino-acid, ∼93-kDa protein that contains a predicted N-terminal pseudoreceiver domain (residues 99–207) and a C-terminal CCT domain (residues 682–727), present in all known plant PRR proteins. Sorghum PRR37 was compared with other plant PRR proteins using the method described by Turner et al. (11) (Fig. S2). This analysis showed that sorghum PRR37 is most closely related to *Arabidopsis* PRR7, two maize PRR37-like proteins (encoded by GRMZM2G033962 and GRMZM2G005732), rice PRR37 (LOC_Os07g49460), and PRR proteins encoded by barley *Ppd-H1* and wheat *Ppd-D1a* (10, 11).

The coding sequences from photoperiod-insensitive ma_1 genotypes revealed mutations in the PRR37 protein that are predicted to disrupt function (Fig. 1 *H–J*) as well as other background nucleotide polymorphisms (Fig. S1 and Table S2). The nucleotide sequence of the coding region from the *Sbprr37-1* allele (genotypes BTx406 and SM100) was identical to *SbPRR37* except for a single nucleotide deletion upstream of the pseudoreceiver domain, resulting in the premature termination of the Sbprr37-1 protein (Fig. 1*H*). Allele *Sbprr37-2* (from cultivar Blackhull Kafir) differs from *SbPRR37* by three amino acid substitutions; two substitutions are present in regions of low conservation among PRR37 proteins (Fig. S1 and Table S2), but the third substitution occurs in the pseudoreceiver domain at Lys^{162}, a highly conserved amino acid in all pseudoreceiver and receiver-domain proteins (Fig. 1*I*). The substitution of an uncharged Asn for a positively charged Lys at this position could alter the functionality of the pseudoreceiver domain. Recessive allele *Sbprr37-3* from ATx623 harbored both the $Lys^{162}Asn$ substitution found in *Sbprr37-2* and an additional nucleotide substitution, resulting in an in-frame stop codon (Q^{270} → Stop) before the CCT motif (Fig. 1*J*). Quinby (4) proposed more than 50 y prior that a series of unique recessive ma_1 alleles have been preserved in sorghum germplasm from different temperate regions of the world, and the present results support this assertion. The *Sbprr37-1* functional mutation occurring in tropical Standard Milo was introduced into the United States from Columbia in the mid-1800s (1, 19). By comparison, the *Sbprr37-2* allele can be traced to Kafir cultivars from South Africa that were introduced in 1876 (4, 19). *Sbprr37-3* represents a second Kafir allele originally present in the progenitor cultivar Combine Kafir-60 (1). The full extent of the *Sbprr37* allelic series remains to be determined, but these results suggest that multiple independent mutation events in *SbPRR37* have occurred during sorghum's adaptation to temperate climates worldwide.

Photoperiod and the Circadian Clock Modulate *SbPRR37* and Floral Gene Expression. In wheat, misexpression of *Ppd-D1a* is correlated with reduced photoperiod sensitivity, indicating the importance of *PRR37* expression in photoperiodic regulation of flowering in this LD grass (10). Therefore, *SbPRR37* mRNA levels were quantified in photoperiod-sensitive 100M and F_1 plants under LD, SD, and circadian cycling conditions (Fig. 2). Analysis of cDNA revealed several PRR37 splice variants (Table S2 and Fig. S3*F*). The abundance of splice variants and full-length transcripts was regulated in a similar manner; therefore, overall *SbPRR37* transcript abundance was quantified by quantitative RT-PCR (qRT-PCR). In LD, 100M and F_1 plants show peaks of *SbPRR37* mRNA abundance in the morning and in the evening ∼3 and 15 h after lights were turned on, respectively (Fig. 2 *A* and *B*). The daily bimodal cycling pattern of *SbPRR37* mRNA abundance persisted in continuous light and temperature (LL) (Fig. 2 *A* and *B*, days 2/3, star), indicating that the circadian clock modulates *SbPRR37* expression under these free-running conditions. By

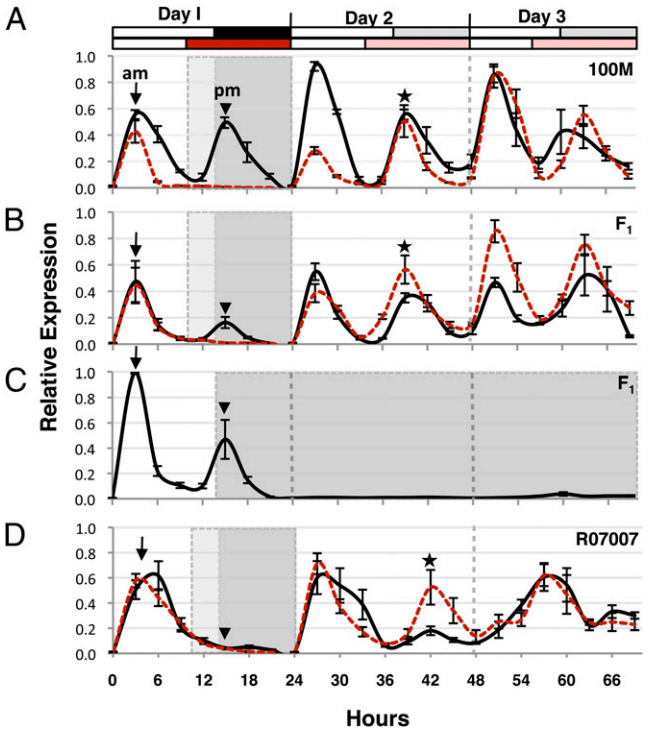

Fig. 2. Clock-regulated *SbPRR37* expression is light dependent. Plants were grown in 14-h light:10-h dark LD (solid line) or 10-h light:14-h dark SD (red dashed line) and then released into LL at time 24 h. Relative expression of *SbPRR37* was analyzed at 3-h intervals by quantitative RT-PCR. In 100M (*A*) and ATx623 × R.07007 F_1 plants (*B*), *SbPRR37* expression increased in the morning (arrow) and evening (arrowhead) of long days. (*C*) ATx623 × R.07007 F_1 plants grown in 14-h:10-h LD and then released into DD at time 24 h. (*D*) R.07007 plants grown in LD and then transferred to LL at time 24 h. The *Sbprr37-1* mutation in SM100 results in a nonfunctional protein whereas its expression profiles remain similar to 100M; therefore, these data are not shown. The ordinate represents normalized expression relative to a calibrator sample and is based on three biological replicates ± SEM (24). The black bar at the top of the figure indicates the dark period for LD-treated plants, and the gray bars indicate subjective dark during LL conditions. The red bar indicates darkness for SD-treated plants; pink indicates subjective dark during LL conditions. Open bars denote light periods. The light gray shading within the plot area indicates darkness for SD-treated plants only, and the dark gray shading indicates darkness for both LD- and SD-treated plants.

contrast, in SD, 100M and F_1 plants showed only the morning-phase peak of *SbPRR37* mRNA abundance (Fig. 2 *A* and *B*, day 1). However, when plants grown in SD were transferred to LL, both the morning and evening-phase peaks of *SbPRR37* mRNA abundance were observed (Fig. 2 *A* and *B*, days 2/3, star). This suggests that *SbPRR37* expression is light dependent and that the disappearance of the evening peak of *SbPRR37* expression in SD is caused by the lack of light during the evening. Expression of the core clock genes *TOC1* and *LHY* continues to cycle in LD, SD, and LL (Fig. S3 *A* and *B*), indicating that decreased *SbPRR37* expression in SD is likely due to lack of light during the evening rather than to disruption of clock function. The light dependence of *SbPRR37* expression was further analyzed by transferring F_1 plants grown in LD to continuous dark (DD) (Fig. 2*C*). In DD, neither peak of *SbPRR37* expression was observed, consistent with a requirement for light for *SbPRR37* expression.

The results described above indicate that *SbPRR37* expression is dependent on illumination of plants during times of the day when output from the circadian clock has the potential to activate *SbPRR37* expression. This mode of regulation is consistent with the external coincidence model of flowering-time regulation (6). In LL or LD, output from the circadian clock activates *SbPRR37* expression in the morning and evening, and the continuous production of PRR37 in LD is proposed to repress flowering. In SD, output from the clock increases *SbPRR37* expression during the morning but not in the evening because the evening phase of potential clock activation of *SbPRR37* expression occurs in darkness. In SD, lack of increased *SbPRR37* expression during the evening phase is proposed to reduce the level of the repressor PRR37, allowing floral initiation.

The important contribution of the evening peak of *SbPRR37* expression to floral repression in LD was supported by analysis of the genotype R.07007 (Fig. 2*D*). This genotype is photoperiod insensitive and flowers early in LD due to recessive ma_5 (20), despite possessing a functional *SbPRR37* allele. In LD, the morning-phase increase in *SbPRR37* expression was observed in R.07007 and 100M (Fig. 2*D*, arrow). However, the increase of *SbPRR37* expression in the evening that occurs in 100M was not observed in R.07007 (Fig. 2*D*, arrowhead). The evening peak is restored under LL conditions, although shifted three hours later than peaks of *SbPRR37* mRNA abundance observed in 100M or the F_1 (Fig. 2*D*, star). The molecular basis for altered *SbPRR37* expression during the evening and under LL conditions in R.07007 is not known. However, these results show that evening-phase expression of *SbPRR37* is correlated with repression of flowering in LD photoperiods in sorghum.

We next investigated the mechanism by which PRR37 represses flowering in LD in sorghum. Genes in the canonical *Arabidopsis* flowering pathway also contribute to the control of flowering time in rice and other grasses, and the most noted of these, *CO*, is a repressor of flowering in rice in LD (9). Therefore, to gain further understanding of how PRR37 modulates the floral induction pathway, we characterized the expression of the sorghum ortholog of *CO* over a 40-h time course in 100M and SM100 in LD and SD to determine if this gene was regulated by PRR37 as previously shown in barley (11). 100M grown in LD (Fig. 3*A*, solid line) showed two increases of *CO* mRNA abundance in leaves each day, similar to the daily bimodal *CO* expression pattern observed in maize in LD (25). The first peak of *CO* mRNA occurred in the evening ~15 h after lights on, and the second increase occurred during the last several hours of the

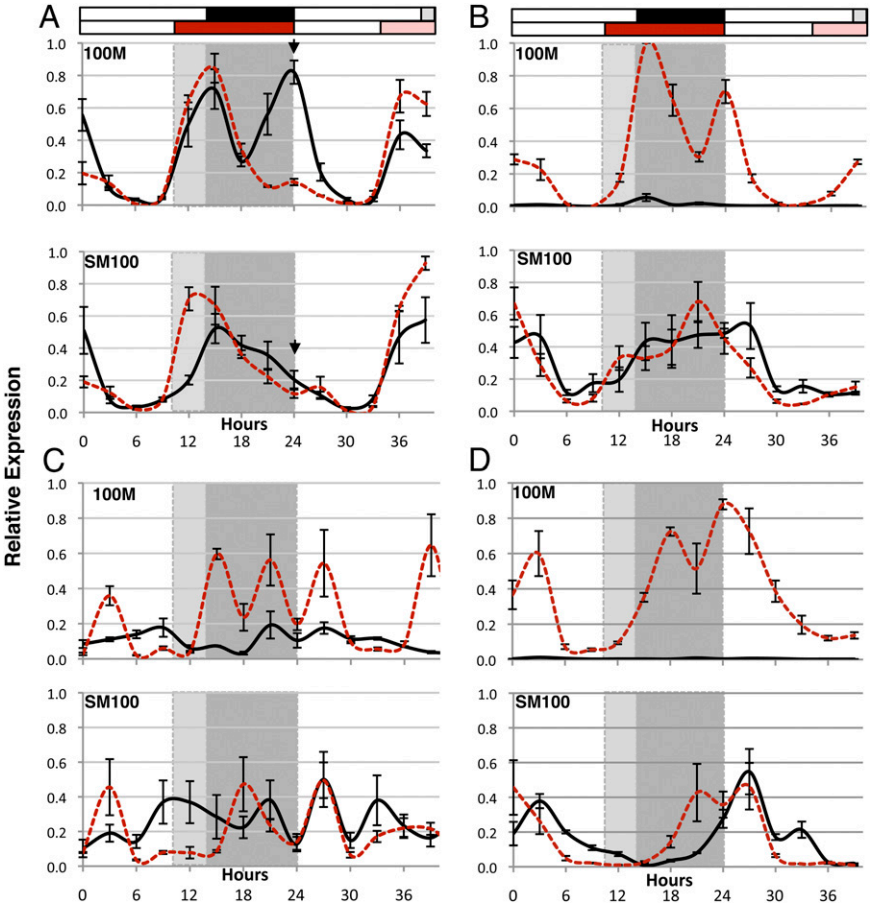

Fig. 3. SbPRR37 modulates expression of downstream flowering genes. Plants were treated under14-h light:10-h dark (LD, solid line) or 10-h light:14-h dark (SD, red dashed line) conditions. (*A*) Relative *CO* expression in 100M peaks at dawn (arrowhead) in plants treated in LD, but not in SD. This peak is absent in SM100 under either condition. (*B*) Relative *Ehd1* expression is repressed under LD in 100M, but is activated under both LD and SD in SM100. (*C*) Expression of *FT* is repressed in LD in 100M, but SM100 expression levels are equivalent in LD and SD. (*D*) Expression of *ZCN8* is elevated in SD-treated 100M plants but is repressed to near undetectable levels in LD. In SM100, expression is de-repressed in LD. The ordinate represents expression normalized to 18S ribosomal RNA expression and relative to a calibrator sample and is based on three biological replicates ± SEM (24). The black bar above the plot indicates the dark period for LD-treated plants; gray bars indicate subjective dark during LL conditions. Red bars indicate darkness for SD-treated plants; pink indicates subjective dark during LL conditions. Open bars denote light periods. Light-gray shading within the plot area indicates darkness for SD-treated plants only; dark-gray shading indicates darkness for both LD- and SD-treated plants.

night, peaking at dawn (24 h) (Fig. 3*A*, arrowhead). By contrast, in SD (Fig. 3*A*, red dashed line), the peak of *CO* expression at dawn was greatly reduced. In addition, the second peak of *CO* expression observed at dawn in 100M grown in LD is absent in SM100 (*Sbprr37-1*) (Fig. 3*A*, arrowhead). These results indicate that the reduction of *CO* mRNA abundance at dawn in SM100 plants grown in LD is due to prr37-1 and that PRR37 is required for differential expression of *CO* in response to photoperiod in sorghum. *CO* expression is regulated by the circadian clock through the action of GI in *Arabidopsis* and rice (26–28). Therefore, it is possible that PRR37 alters *CO* expression through an indirect effect on clock gene expression. Small differences in the patterns of *TOC1*, *LHY*, and *GI* expression in 100M and SM100 were noted but could not be directly connected to the altered expression of *CO* in SM100 compared with 100M (Fig. S3 *A–C*). We interpret these results to indicate that, although PRR37 may have an effect on clock gene expression, this protein also directly regulates *CO*. Regulation of *CO* expression by PRR7, an ortholog of SbPRR37, independent of the clock-*GI* pathway, was also proposed to occur in *Arabidopsis* (29).

In rice, *Ehd1* encodes a B-type response regulator transcription factor unique to grasses that has been shown to promote flowering (12). Because *Ehd1* has a role in floral activation via a pathway separate from *CO*, we identified the sorghum ortholog of *Ehd1* and found that 100M expression of this gene was strongly repressed in LD, whereas in SM100, LD and SD levels were similar (Fig. 3*B*). Moreover, when 100M plants grown in LD were transferred to SD, expression of *Ehd1* at 15 h after lights on increased ∼17-fold. In contrast, transfer of SM100 plants to SD increased *Ehd1* mRNA levels only 2.4-fold (Fig. S3*E*, *Upper*). These results are consistent with the de-repression of *Ehd1* in the *Sbprr37-1* background (SM100).

FT is part of a 6-member PEBP-domain gene family in *Arabidopsis* and a >20-member gene family in maize (30). Several members of the PEBP-domain gene family encode florigens that modulate flowering in rice and maize (30, 31). In rice, Hd3a and RFT1 act synergistically to promote the transition from vegetative to reproductive growth (31). No ortholog of *RFT1* was found in sorghum (31) (Phytozome v5.0). However, a collinear sorghum ortholog of rice *FT* (*OsHd3a*) was present in sorghum, and this gene was regulated by photoperiod and PRR37 (Fig. 3*C*). The sorghum ortholog of *OsHd3a* (*SbFT*) was expressed in 100M leaves at lower levels in LD compared with SD (Fig. 3*C*, *Upper*). In contrast, *SbFT* showed elevated expression in SM100 plants in LD and SD (Fig. 3*C*, *Lower*). When 100M plants were transferred from LD to SD for 1 wk, *SbFT* mRNA levels increased 7.1-fold during the evening phase (15 h after lights on). By contrast, transfer of SM100 plants from LD to SD for 1 wk increased *SbFT* levels in SM100 only 0.3-fold, indicating the absence of repression of *SbFT* expression in LD in the *Sbprr37-1* background (Fig. S3*E*, *Lower*).

Sorghum *ZCN8*, the collinear ortholog of the maize florigen *Zea mays CENTRORADIALIS 8* (30), was expressed at low levels in LD in 100M and at elevated levels in SD (Fig. 3*D*, *Upper*). Similar to *Ehd1* and *SbFT*, *SbZCN8* expression in SM100 plants was de-repressed regardless of photoperiod (Fig. 3*D*, *Lower*). In addition, expression analysis of *ZCN12*, a second florigen candidate gene in maize that responds to photoperiod (30), showed a pattern of expression in 100M and SM100 similar to *SbZCN8* (Fig. S3*D*). In summary, expression of sorghum orthologs of genes that are involved in floral induction in other grasses, including *Ehd1*, *FT*, *ZCN8*, and *ZCN12*, is regulated by photoperiod in 100M (*SbPRR37*), but not in SM100 (*Sbprr37-1*), a genotype that lacks a functional PRR37.

This study demonstrates that *SbPRR37* is a central repressor in a regulatory pathway that controls sorghum flowering in response to photoperiod. A working model for this regulatory network is shown in Fig. 4. In LD, light dependent, circadian-regulated increases in *SbPRR37* expression in the morning and evening are proposed to result in a sufficient level of PRR37 throughout the day to repress *FT*, other genes encoding florigens, and floral initiation. In SD, the evening peak of *SbPRR37* expression is reduced or eliminated, leading to floral induction consistent with the external coincidence model of flowering-time regulation (5, 6). By contrast, *Arabidopsis PRR7*, the ortholog of *SbPRR37*, shows only a single morning-phase peak of expression (32), indicating that evening-phase expression of this gene may be a special feature of grass species. The light-dependent induction of *SbPRR37* expression and the clock-mediated evening-phase peak of expression enable *SbPRR37* to regulate flowering time in response to photoperiod; PRR37 mRNA levels in the evening phase decrease as day length is reduced. The photoreceptor(s) that mediate light-induced *SbPRR37* expression are currently under investigation; however, phytochrome B is likely involved because recessive alleles of this gene cause early flowering in LD in sorghum (ma_3, ma_3^R) (33), barley (34), and rice (35). SbPRR37 is proposed to repress *FT*, *SbZCN8*, and *SbZCN12* and flowering in LD in part by inhibiting expression of *Ehd1*, an activator of *FT* and flowering in rice (12); by increasing expression of *CO*, a repressor of flowering in rice in LD (26); and possibly by other mechanisms that modulate *SbFT* and *SbZCN8* expression (Fig. 4).

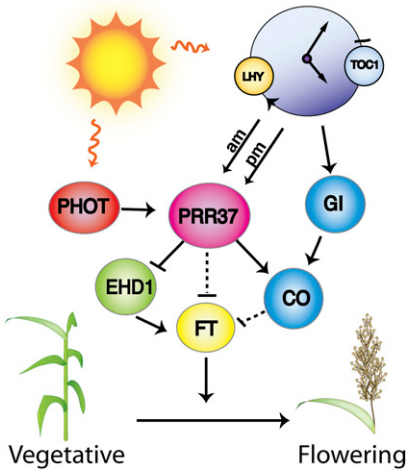

Fig. 4. Model of photoperiodic flowering-time regulation in sorghum. PRR37 is a central floral repressor that blocks transition from the vegetative phase to flowering in LD. PRR37 represses *FT*, *ZCN8*, and flowering by activating expression of *CO*, a repressor of *FT* in rice, and by inhibiting *Ehd1*, a grass-specific inducer of *FT*. *SbPRR37* expression is regulated by the circadian clock and light in a manner consistent with the external coincidence model. It is proposed that photoreceptors (PHOT) such as phytochromes mediate light activation of *SbPRR37* expression coincident with output from the circadian clock, resulting in increased *SbPRR37* expression in the morning and evening in LD. In SD, *SbPRR37* expression is not activated in the evening, leading to floral induction.

This study provides insight into the mechanism of photoperiodic regulation of flowering time in the SD grass sorghum. The importance of PRR37 in photoperiod regulation was first documented in LD barley (11) and wheat (10). In these grasses, PRR37 activates *FT* and flowering in LD whereas in sorghum PRR37 represses *FT*, *ZCN8*, and flowering in LD. The molecular basis of this difference in PRR37 activity in sorghum, a short-day plant, and the long-day grasses barley and wheat may relate to differences in CO activity in the formation of CCAAT-box–binding complexes involved in floral gene expression (26, 36). In addition to documenting how sorghum regulates flowering time in response to photoperiod, this study identified important alleles of *SbPRR37* that were critical for the domestication and utilization

PLANT BIOLOGY

of this tropical grass for grain production in temperate regions worldwide (1, 16). This information will allow plant breeders to more precisely control flowering time in grass species, thus increasing yield and sustainable production.

Methods

Genotyping and Phenotyping Mapping Populations. All sorghum accessions used in this study, as well as relevant descriptors, are listed in Table S3. For flowering-date determinations, plants were grown in different LD environments in the greenhouse or in the field in the summer seasons of 2003–2010. Days to midanthesis were evaluated in the greenhouse under 14-h light/10-h dark photoperiods (30–34/20–25 °C), and in field locations in College Station, Vega, and Plainview, all in Texas. For genotyping, plant DNA was extracted with either the FastDNA Spin Kit (MP Biomedicals) or by disruption of a leaf punch using a GenoGrinder (BT&C/OPS Diagnostics). Plants from all three mapping populations were subjected to PCR-based marker analysis as previously described (37).

***SbPRR37* Allele Sequencing.** To examine the *SbPRR37* gene for functional mutations that contribute to the temperate-zone adaptation of sorghum, either the full 10-kb *SbPRR37* genomic region or expressed cDNA was sequenced from six genotypes including historically prominent cultivars. PCR-amplified products from genomic DNA (Phusion High-Fidelity DNA Polymerase, New England BioLabs, Inc.) were isolated using the QIAquick PCR Purification and Gel Extraction Kits (QIAGEN). Sequencing of the purified PCR products was carried out in a reaction using the BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems). Capillary sequencing was performed on the Applied Biosystems 3130xl Genetic Analyzer. The results were assembled and analyzed using either Sequencher v4.8 (Gene Codes) or Phred/Phrap and Consed (http://www.phrap.org/phredphrapconsed.html).

Plant Materials for Gene Expression Studies. Sorghum genotypes 100M, SM100, ATx623, R.07007, and ATx623 × R.07007 F_1 plants were grown in a greenhouse in Metro-Mix 200 (Sunshine MVP; Sun Gro Horticulture Ltd.) under long-day conditions (14-h days) and were fertilized once after 2 wk using Peters Professional Allrounder fertilizer (The Scotts Company). After 32 d, the plants were transferred to a growth chamber for 1 wk under either long (14-h) days or short (10-h) days at a light intensity of ~300 $\mu mol \cdot s^{-1} \cdot m^{-2}$ at ~50% humidity with 30 °C day temperatures and 23 °C night temperatures. At day 39, 1 wk after SD or LD treatment, the three topmost leaves from three different plants (pooled) were harvested from each genotype every 3 h for one 24-h light–dark cycle and an additional 48-h constant light (constant 30 °C) or constant dark cycle (constant 23 °C), as indicated. Total RNA was extracted and used in downstream quantitative real time PCR (qRT-PCR) reactions. Relative expression was determined using the comparative cycle threshold (Ct) method, and relative transcript number was calculated using the standard curve method (24).

ACKNOWLEDGMENTS. This research was supported by the Office of Science (Biological and Environmental Research), US Department of Energy, Grant DE-FG02-06ER64306, the Perry Adkisson Chair (J.E.M.), and US Department of Agriculture-Agricultural Research Service (R.R.K.).

1. Smith CW, Frederiksen RA (2000) *Sorghum: Origin, History, Technology, and Production*, eds Smith CW, Frederikson RA (John Wiley & Sons, New York), pp 191–223.
2. Rooney WL, Blumenthal J, Bean B, Mullet JE (2007) Designing sorghum as a dedicated bioenergy feedstock. *Biofuel Bioprod Bior* 1:147–157.
3. Garner WW, Allard HA (1920) Effect of the relative length of day and night and other factors of the environment on growth and reproduction in plants. *J Agric Res* 18: 553–606.
4. Quinby JR (1974) *Sorghum Improvement and the Genetics of Growth* (Texas A&M University Press, College Station, TX).
5. Bünning E (1960) Circadian rhythms and the time measurement in photoperiodism. *Cold Spring Harbor Symp Quant Biol* 25:249–256.
6. Pittendrigh CS, Minis DH (1964) The entrainment of circadian oscillations by light and their role as photoperiodic clocks. *Am Nat* 198:261–293.
7. Hayama R, Coupland G (2004) The molecular basis of diversity in the photoperiodic flowering responses of Arabidopsis and rice. *Plant Physiol* 135:677–684.
8. Izawa T (2007) Adaptation of flowering-time by natural and artificial selection in *Arabidopsis* and rice. *J Exp Bot* 58:3091–3097.
9. Yano MK, et al. (2000) *Hd1*, a major photoperiod sensitivity quantitative trait locus in rice, is closely related to the Arabidopsis flowering time gene *CONSTANS*. *Plant Cell* 12:2473–2484.
10. Beales J, Turner A, Griffiths S, Snape JW, Laurie DA (2007) A *Pseudo-Response Regulator* is misexpressed in the photoperiod insensitive *Ppd-D1a* mutant of wheat (*Triticum aestivum* L.). *Theor Appl Genet* 115:721–733.
11. Turner A, Beales J, Faure S, Dunford RP, Laurie DA (2005) The pseudo-response regulator *Ppd-H1* provides adaptation to photoperiod in barley. *Science* 310:1031–1034.
12. Doi K, et al. (2004) *Ehd1*, a B-type response regulator in rice, confers short-day promotion of flowering and controls *FT-like* gene expression independently of *Hd1*. *Genes Dev* 18:926–936.
13. Matsubara K, et al. (2008) *Ehd2*, a rice ortholog of the maize *INDETERMINATE1* gene, promotes flowering by up-regulating *Ehd1*. *Plant Physiol* 148:1425–1435.
14. Xue W, et al. (2008) Natural variation in *Ghd7* is an important regulator of heading date and yield potential in rice. *Nat Genet* 40:761–767.
15. Yan L, et al. (2004) The wheat *VRN2* gene is a flowering repressor down-regulated by vernalization. *Science* 303:1640–1644.
16. Craufurd PQ, et al. (1999) Adaptation of sorghum: Characterisation of genotypic flowering responses to temperature and photoperiod. *Theor Appl Genet* 99:900–911.
17. Quinby JR, Karper RE (1945) Inheritance of three genes that influence time of floral initiation and maturity date in milo. *Agron J* 37:916–936.
18. Quinby JR (1966) Fourth maturity gene locus in sorghum. *Crop Sci* 6:516–518.
19. Quinby JR (1967) The maturity genes of sorghum. *Advances in Agronomy*, ed Norman AG (Academic Press, New York), Vol 19, pp 267–305.
20. Rooney WL, Aydin S (1999) Genetic control of a photoperiod-sensitive response in *Sorghum bicolor* (L.) Moench. *Crop Sci* 39:397–400.
21. Lin YR, Schertz KF, Paterson AH (1995) Comparative analysis of QTLs affecting plant height and maturity across the Poaceae, in reference to an interspecific sorghum population. *Genetics* 141:391–411.
22. Klein RR, et al. (2008) The effect of tropical sorghum conversion and inbred development on genome diversity as revealed by high-resolution genotyping. *Plant Genome* 48:S12–S26.
23. Stephens JC, Miller FR, Rosenow DT (1967) Conversion of alien sorghums to early combine genotypes. *Crop Sci* 7:396.
24. Bookout AL, Mangelsdorf DJ (2003) Quantitative real-time PCR protocol for analysis of nuclear receptor signaling pathways. *Nucl Recept Signal* 1:e012–e018.
25. Miller TA, Muslin EH, Dorweiler JE (2008) A maize *CONSTANS*-like gene, *conz1*, exhibits distinct diurnal expression patterns in varied photoperiods. *Planta* 227: 1377–1388.
26. Hayama R, Yokoi S, Tamaki S, Yano M, Shimamoto K (2003) Adaptation of photoperiodic control pathways produces short-day flowering in rice. *Nature* 422:719–722.
27. Park DH, et al. (1999) Control of circadian rhythms and photoperiodic flowering by the *Arabidopsis GIGANTEA* gene. *Science* 285:1579–1582.
28. Fowler S, et al. (1999) *GIGANTEA*: A circadian clock-controlled gene that regulates photoperiodic flowering in *Arabidopsis* and encodes a protein with several possible membrane-spanning domains. *EMBO J* 18:4679–4688.
29. Ito S, et al. (2009) A genetic study of the Arabidopsis circadian clock with reference to the *TIMING OF CAB EXPRESSION 1* (*TOC1*) gene. *Plant Cell Physiol* 50:290–303.
30. Meng X, Muszynski MG, Danilevskaya ON (2011) The *FT*-like *ZCN8* gene functions as a floral activator and is involved in photoperiod sensitivity in maize. *Plant Cell* 23: 942–960.
31. Komiya R, Ikegami A, Tamaki S, Yokoi S, Shimamoto K (2008) *Hd3a* and *RFT1* are essential for flowering in rice. *Development* 135:767–774.
32. Mizuno T, Nakamichi N (2005) *Pseudo*-response regulators (PRRs) or *true* oscillator components (TOCs). *Plant Cell Physiol* 46:677–685.
33. Childs KL, et al. (1997) The sorghum photoperiod sensitivity gene, Ma_3, encodes a phytochrome B. *Plant Physiol* 113:611–619.
34. Hanumappa M, Pratt LH, Cordonnier-Pratt M-M, Deitzer GF (1999) A photoperiod-insensitive barley line contains a light-labile phytochrome B. *Plant Physiol* 119: 1033–1040.
35. Takano M, et al. (2005) Distinct and cooperative functions of phytochromes A, B, and C in the control of deetiolation and flowering in rice. *Plant Cell* 17:3311–3325.
36. Wenkel S, et al. (2006) CONSTANS and the CCAAT box binding complex share a functionally important domain and interact to regulate flowering of *Arabidopsis*. *Plant Cell* 18:2971–2984.
37. Menz MA, et al. (2004) Genetic diversity of public inbreds of sorghum using mapped AFLP and SSR markers. *Crop Sci* 44:1236–1244.