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Genetic Regulation of Development in *Sorghum bicolor*[1]

X. Greatly Attenuated Photoperiod Sensitivity in a Phytochrome-Deficient Sorghum Possessing a Biological Clock but Lacking a Red Light-High Irradiance Response

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The role of a light-stable, 123-kD phytochrome in the biological clock, in photoperiodic flowering and shoot growth in extended photoperiods, and in the red light-high irradiance response was studied in *Sorghum bicolor* using a phytochrome-deficient mutant, 58M (ma_3^R ma_3^R), and a near-isogenic wild-type cultivar, 100M (Ma_3 Ma_3). Since chlorophyll *a/b*-binding protein mRNA and ribulose bisphosphate carboxylase small subunit mRNA cycled in a circadian fashion in both 58M and 100M grown in constant light, the 123-kD phytochrome absent from 58M does not appear necessary for expression or entrainment of a functional biological clock. Although 58M previously appeared photoperiod insensitive in 12-h photoperiods, extending the photoperiod up to 24 h delayed floral initiation for up to 2 weeks but did not much affect shoot elongation. Thus, although 58M flowers early in intermediate photoperiods, a residual photoperiod sensitivity remains that presumably is not due to the missing 123-kD phytochrome. Since rapid shoot elongation persists in 58M under extended photoperiods despite delayed floral initiation, long photoperiods uncouple those processes. The observed absence of a red light-high irradiance response in 58M, in contrast to the presence of the response in 100M, strengthens the suggestion that the 123-kD phytochrome missing from 58M is a phyB.

Phytochromes are a class of photoreceptors that mediate a myriad of plant photomorphogenetic responses. At present, five phytochromes have been well characterized, phyA, phyB, phyC, phyD, and phyE (Sharrock and Quail, 1989; Clack et al., 1994). Our understanding of the physiological roles of phyA and phyB has been expanded because of phytochrome-overexpressing transgenic plants and phytochrome-deficient mutants (Koornneef and Kendrick, 1994). Of the phyB-deficient plants, *phyB Arabidopsis thaliana* has been conclusively demonstrated to result from a mutation in the *PHYB* gene (Reed et al., 1993). *A. thaliana* with *phyB* has greater hypocotyl and petiole elongation, lower Chl and anthocyanin levels, increased apical dominance, and earlier flowering compared to *PHYB A. thaliana.* This mutant also shows severely attenuated responses to FR end-of-day treatments and low R:FR, and it does not exhibit an R-HIR inhibition of hypocotyl elongation. The *lh* mutant of *Cucumis sativus* (López-Juez et al., 1992) and the *ein* mutant of *Brassica rapa* (Devlin et al., 1992) have been shown to lack phytochromes that react to antibodies that are antigenic to phyB. These mutants share many of the same phenotypes of *PHYB A. thaliana.*

Similar to the known and suspected phyB mutants is the ma_3^R mutation in *Sorghum bicolor* (L.) Moench. Sorghum plants homozygous for ma_3^R are taller and have longer and narrower leaf blades, fewer tillers, less Chl, less anthocyanin, and higher concentrations of GAs than Ma_3-containing sorghum (Pao and Morgan, 1986a; Beall et al., 1991; Childs et al., 1991; Foster et al., 1994). The ma_3^R mutation also results in the absence of a light-stable 123-kD phytochrome that is present in Ma_3 sorghum. A distinctly different light-labile 126-kD phytochrome is present in both ma_3^R and Ma_3 sorghum (Childs et al., 1992). Because of the similarities with the other known phyB mutants and because the 123-kD sorghum phytochrome is light stable, we believe that Ma_3 codes for phyB and that the other aberrant characteristics of ma_3^R sorghum may then be explained by the absence of the 123-kD phytochrome (Childs et al., 1992).

Of particular interest to us is the extreme photoperiod insensitivity of ma_3^R sorghum, which is the behavior that first led to the recognition of this mutant (Quinby and Karper, 1961). Sorghum is an SDP, but under photoperiods that delay non-ma_3^R-containing genotypes, floral initiation and anthesis occur very early in ma_3^R sorghum (Quinby, 1973; Pao and Morgan, 1986a). Why the absence of 123-kD phytochrome hastens flowering is uncertain. The roles that phytochromes play in photoperiodism are several and incompletely understood (Vince-Prue, 1994). In addition to the commonly known phytochrome control of the night-break response in SDPs, phytochrome is also believed to set the phase of the biological clock that is involved in time measuring in photoperiodism. Phytochrome has been shown to phase shift the rhythmic sensitivity to night-

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Abbreviations: FR, far-red light; HIR, high-irradiance response; Lhcb, chlorophyll *a*/*b*-binding protein; R, red light; RbcS, ribulose-1,5-bisphosphate carboxylase.

break in the SD *Pharbitis nil* (Lumsden and Furuya, 1986; Lumsden, 1991). Phytochrome has not been directly demonstrated to affect photoperiod time-keeping in LDPs, but in *Hordeum vulgare* FR can phase shift the rhythmic response to FR promotion of flowering (Deitzer et al., 1982). The entrainment of the circadian oscillator is clearly regulated by phytochrome in circadian leaf movements in *Samanea saman* (Simon et al., 1976), the circadian release of CO_2 by leaves of *Bryophyllum fedtschenkoi* (Harris and Wilkins, 1976), and circadian *Lhcb* gene expression in *Phaseolus vulgaris* (Tavladoraki et al., 1989).

One hypothesis to account for the photoperiod insensitivity of ma_3^R sorghum is that the 123-kD phytochrome of sorghum normally entrains the circadian clock that regulates photoperiodism. In ma_3^R sorghum the lack of 123-kD phytochrome would then render the oscillator dysfunctional, and the rhythmic sensitivity to night-break inhibition would never come into phase. Thus, at no time during a long night would a night-break cause inhibition of flowering in ma_3^R sorghum, and a short night would also not act to inhibit flowering. One way to test this hypothesis is to examine a convenient, circadian phenomenon and observe the presence or absence of a rhythm in ma_3^R sorghum. Here we report the circadian cycling of Lhcb and RbcS mRNA expression in Ma_3 and ma_3^R sorghum. We then reexamine photoperiod effects on growth and flowering. Additional evidence is also presented to support the assertion that 123-kD sorghum phytochrome is a phyB.

MATERIALS AND METHODS

Plant Material

Two near-isogenic cultivars of *Sorghum bicolor* (L.) Moench, differing only in the allele that they contain at the third maturity gene locus, were used in this study: 100M (Ma_3Ma_3) and 58M ($ma_3^Rma_3^R$).

Lhcb and RbcS mRNA Analysis

Seedlings of 58M and 100M were grown as described previously (Beall et al., 1991), but the growth chamber used here contained metal halide lamps (400 W, Philips) that produced a fluence rate of 875 μmol m^{-2} s^{-1} (400–700 nm). Plants were grown under a 12-h light/12-h dark photoperiod for 15 d. For the continuous dark experiment, plants were transferred on d 15 to a growth chamber with high output fluorescent and incandescent lamps (300 μmol m^{-2} s^{-1}, 400–700 nm). Harvesting of the fully expanded leaf blades began at lights-on of d 16 and continued every 3 h thereafter for 54 h. At lights-off of d 16, the lights remained off for the duration of the experiment. Tissue was immediately frozen in liquid N_2 and stored at −80°C. Harvests during the dark period were carried out with the aid of a dim green safelight. For the continuous light experiment, the plants remained in the growth chamber with the metal halide lights during the harvest, and harvesting began 1 h before lights-on of d 15 and continued at lights-on and every 3 h thereafter for 72 h. At lights-on, the lights remained on for the duration of the experiment. The tissue was stored as in the continuous dark experiment.

The extraction of RNA was based on the procedure of Lu et al. (1990). Three to 5 g of frozen tissue were ground with a mortar and pestle and liquid N_2. RNA was extracted with 15 mL of homogenization buffer (7.5 M guanidine-HCl, 25 mM Tes-NaOH buffer, 10 mM EDTA, 100 mM 2-mercaptoethanol, pH 7.5). This mixture was centrifuged and the supernatant decanted and strained through Miracloth (Calbiochem). A 7/13 volume of 10 M LiCl was added to the supernatant. This solution was mixed, placed on ice overnight, and then centrifuged at 10,000*g* for 15 min at 4°C. The precipitated RNA was resuspended in 4 mL of homogenization buffer, phenol/chloroform extracted, reprecipitated, and resuspended in a solution of 10 mM Tris-HCl, 1 mM EDTA, 0.5% SDS, pH 7.4. Twenty-microgram aliquots from each RNA sample were used for electrophoresis. A 1% (w/v) agarose gel with 0.66 M formaldehyde and 10× Mops buffer (0.2 M Mops, 50 mM sodium acetate, 10 mM EDTA, pH 7.0) was used for electrophoresis at 50 V for 4 h. The gel was stained with ethidium bromide and examined with a UV transilluminator to check for equal RNA loading and RNA integrity. For the Lhcb hybridization, RNA was transferred to a nylon membrane (GeneScreen; DuPont) by capillary action. For the RbcS RNA hybridization, RNA samples were electrophoresed as above to check for integrity, but for subsequent analyses 20-μg aliquots of RNA were applied directly to a nylon membrane using a dot blot apparatus (Minifold I; Schleicher & Schuell). In both experiments, the RNA was fixed to the membrane by baking at 80°C for 2 h.

Lhcb and RbcS mRNAs were detected by hybridization with either a partial sorghum Lhcb clone (pSbcab3) or a partial sorghum RbcS clone (pSbrbcs16) isolated from a cDNA library constructed from sorghum BTx623 shoots and identified by sequence homology to *Zea mays* Lhcb and RbcS by J.-L. Lu and J.E. Mullet (unpublished data). Probes were labeled with [^{32}P]dCTP using a random-primer labeling kit (GIBCO-BRL). Hybridization at 65°C with dextran sulfate but without formamide followed the procedure provided with the GeneScreen membrane. After hybridization, membranes were briefly washed twice with 2× SSC (0.3 M NaCl, 0.03 M sodium citrate), twice for 30 min with 2× SSC plus 1.0% SDS at 65°C, and twice for 30 min with 0.1× SSC. Radioactivity was detected and quantitated using a Betascope 603 blot analyzer (Betagen, Waltham, MA).

Photoperiod Effects on Growth and Floral Initiation

Seed of 58M and 100M were sown and cared for as above. The plants were grown in a growth cabinet with a mixture of high-output fluorescent and incandescent lamps giving a fluence rate of 300 μmol m^{-2} s^{-1} (400–700 nm). The controls on the growth cabinet were set to give a temperature regime that followed a cycle of 12 h at 30°C/12 h at 20°C. Regardless of the photoperiod, the 30 to 20°C shift of the thermoperiod consistently corresponded to the lights-off transition. Floral initiation was examined by sacrificing four plants and observing the apices. Lane's (1963) classification of apex differentiation was used to score apices. Initiation was determined to have taken place

when at least one of the four examined plants had reached stage 2 as described by Lane.

HIR Inhibition of Mesocotyl Growth

Seed of 58M or 100M were shallowly sown along one edge of a square, 10-cm pot. Germination was allowed to take place for 40 h in a dark room with 100% humidity. Using a dim green safelight, we either placed the barely emergent seedlings in front of a light source or returned them to darkness. After 40 h of treatment, mesocotyl lengths of treated and control seedlings were measured. R was obtained by using a bank of 36 light-emitting diodes (model AND180BRP; Kensington Electronics, Irvine, CA) with a maximum wavelength of 660 nm and a half-peak height width of 22 nm. FR was created by filtering the light from a 100-W incandescent bulb through a CBS Far Red 750 filter (Carolina Biological Supply, Burlington, NC). Fluence rates were determined from 600 to 700 nm (R source) or from 700 to 800 nm (FR source) with a Li-Cor 1800 spectroradiometer (Li-Cor Inc., Lincoln, NE).

RESULTS

Circadian Cycling of Lhcb and RbcS mRNAs

Circadian modulation of Lhcb mRNA abundance was examined in 58M and 100M sorghum. During a normal 12-h light/12-h dark photoperiod (first 24 h of Fig. 1), diurnal expression of Lhcb mRNA was observed. Just after lights-on, there was little Lhcb mRNA present in the leaves. The amount of Lhcb mRNA increased and peaked by the middle of the day. Before lights-off, the level of Lhcb mRNA had already begun to decline dramatically. During the first subjective night there was little Lhcb mRNA accumulation, and while the plants were maintained in continuous darkness there was very little Lhcb mRNA present in the leaves (Fig. 1). When sorghum was grown in continuous light, Lhcb mRNA levels cycled in a circadian manner

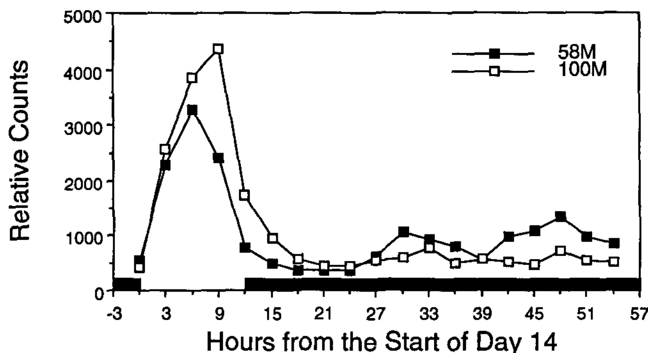

Figure 1. Expression of Lhcb mRNA in sorghum treated with continuous darkness. Sorghum cv 58M (*ma*$_3^R$) and cv 100M (*Ma*$_3$) were grown in 12-h photoperiods for 15 d. At dusk of d 15, the lights were turned off and remained off until the end of the experiment. The solid bar on the *x* axis indicates lights off. Harvesting of fully expanded leaf blades began at dawn of d 15. Total RNA was extracted, electrophoresed, blotted onto a nylon membrane, and hybridized with a ^{32}P-labeled sorghum Lhcb probe.

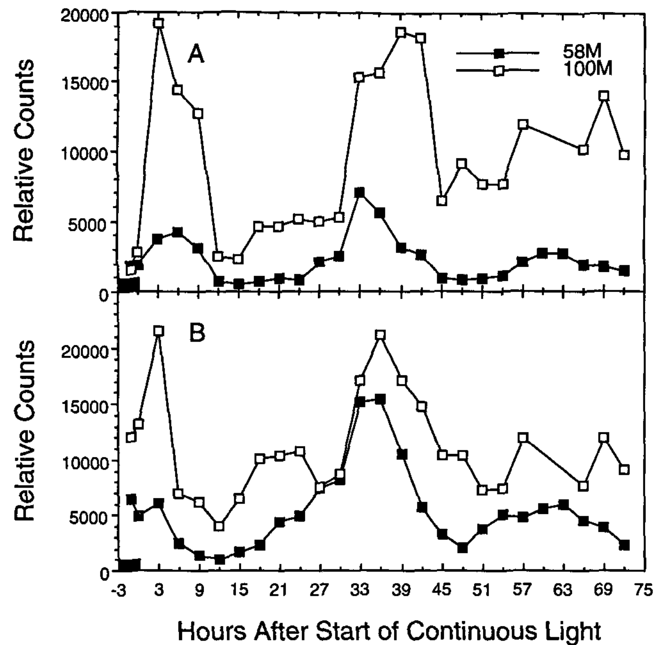

Figure 2. Expression of Lhcb (A) and RbcS (B) mRNAs in sorghum treated with continuous white light. Sorghum cv 58M (*ma*$_3^R$) and cv 100M (*Ma*$_3$) were grown in 12-h photoperiods for 15 d. On the morning of d 16 (h = 0), the lights were turned on and remained on for 72 h. The solid bar on the *x* axis indicates when lights were off. Fully expanded leaf tissue was harvested at the times indicated. For Lhcb analysis, total RNA was extracted, electrophoresed, blotted onto a nylon membrane, and hybridized with a ^{32}P-labeled sorghum Lhcb probe. For RbcS analysis, total RNA was extracted, directly blotted onto a nylon membrane, and hybridized with a ^{32}P-labeled RbcS probe.

in both 58M and 100M (Fig. 2A). Circadian cycling of RbcS mRNA abundance was also found to occur in 58M and 100M that were kept in continuous white light (Fig. 2B).

Photoperiod Effects on Growth and Flowering

The length of the photoperiod was also found to determine the degree of photoperiod insensitivity expressed by 58M (Table I). Even the 4-h difference between the 12- and 16-h photoperiods resulted in an 8-d delay in initiation. Daylengths greater than 18 h postponed floral initiation by nearly 2 weeks.

After 30 d of growth, both 58M and 100M had reduced internode elongation in the longer photoperiods (Table II). Regardless of the daylength, the internodes from 30-d-old 58M plants were about 15 times longer than those from 100M plants.

HIR Inhibition of Mesocotyl Elongation

An R fluence-response curve for mesocotyl inhibition was obtained for 58M and 100M (Fig. 3). An increase in R fluence rate over 3 orders of magnitude resulted in progressively greater inhibition of mesocotyl elongation in 100M. The same range of fluence rates resulted in uniform inhibition of mesocotyl elongation in 58M. 58M and 100M

Table I. *Effect of photoperiod on floral initiation in 58M sorghum*

Plants were grown under various photoperiods in a growth cabinet. Beginning on d 15, individual plants were sampled for panicle differentiation. Apices were scored as initiated if they had reached stage 2 as defined by Lane (1963). The experiment did not proceed after d 30. L, Light; D, dark.

Photoperiod	d to Initiation
12 h L/12 h D	16
16 h L/8 h D	24
18 h L/6 h D	28
23 h L/1 h D	>30
24 h L/0 h D	28

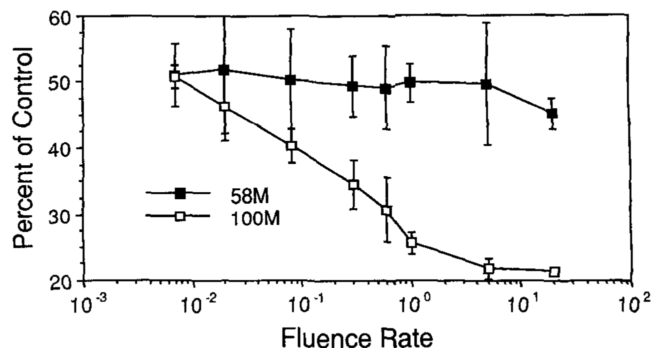

Figure 3. R-induced inhibition of mesocotyl elongation of sorghum cv 58M (ma_3^R) and cv 100M (Ma_3). Seedlings were grown in the dark for 40 h and then treated with R for 40 h or returned to darkness for 40 h. R was obtained by using 36 light-emitting diodes with a maximum wavelength of 660 nm and a half-peak height width of 22 nm. Fluence rates are presented in units of μmol m^{-2} s^{-1}. Data are averages of three repetitions of 20 seedlings each expressed as percent of control ± SD.

sorghum each showed approximately 50% mesocotyl inhibition when treated with the lowest intensity of R (7×10^{-3} μmol m^{-2} s^{-1}) for 40 h. In contrast to the responses to R, when 40-h-old dark-grown 58M and 100M were treated with 19.4 μmol m^{-2} s^{-1} FR for 40 h, mesocotyl lengths were, respectively, 40.6 ± 7.7 and 40.6 ± 1.1% of the dark-grown controls.

DISCUSSION

Photoperiod sensitivity in *S. bicolor* is controlled by at least four known maturity genes, which are refered to as Ma_1, Ma_2, Ma_3, and Ma_4 (Quinby, 1973). Of particular interest to this laboratory is the ma_3^R mutant allele of the Ma_3 locus. The ma_3^R allele was initially isolated because it caused field-grown plants to be photoperiod insensitive (Quinby and Karper, 1961). Photoperiodism is a phenomenon that relies on a biological clock. Proper time-keeping by a biological oscillator is possible only through the daily entrainment that prevents the clock from drifting out of phase. Phytochrome has been shown to be able to entrain the clock (Lumsden, 1991; Vince-Prue, 1994). After it became clear that ma_3^R sorghum plants lack a 123-kD phytochrome and that the absence of 123-kD phytochrome leads to photoperiod insensitivity, questions arose as to whether photoperiod insensitivity in ma_3^R sorghum is due to an inoperative biological clock and whether 123-kD phytochrome is responsible for entraining that clock. The aim of the experiments presented here was to test for the existence of a functioning biological clock in ma_3^R sorghum and to gain a better understanding of the role of 123-kD phytochrome in the control of photoperiodism in sorghum.

The phenomenon chosen to conveniently test for the existence of a biological clock in ma_3^R sorghum was the accumulation of Lhcb and RbcS mRNAs. It has been shown in *P. vulgaris* (Tavladoraki et al., 1989), *Triticum aestivum* (Nagy et al., 1988), *A. thaliana* (Pilgrim and McClung, 1993), and *Nicotiana tabacum* (Paulsen and Bogorad, 1988) that Lhcb mRNA levels cycle in a circadian fashion. RbcS mRNA also cycles in a circadian manner in *A. thaliana* (Pilgrim and McClung, 1993). When sorghum plants were exposed to constant darkness, Lhcb mRNA levels remained low, and cycling could not be easily determined (Fig. 1). The first 24 h of the data in Figure 1 show that Lhcb mRNA levels fluctuate similarly during a normal day/night cycle in both ma_3^R and Ma_3 sorghum. In continuous light, circadian cycling of both sorghum Lhcb and RbcS mRNAs was readily observed (Fig. 2). It is not uncommon for a circadian phenomenon to be evident only in either constant light or constant dark (Paulsen and Bogorad, 1988). Because both ma_3^R and Ma_3 sorghum demonstrated rhythmicity of Lhcb and RbcS mRNA expression, it may be concluded that both genotypes possess a functioning biological clock. Thus, 123-kD phytochrome is not necessary for the daily phase setting of the clock that drives cyclic Lhcb and RbcS mRNA expression. This is the first time to our knowledge that the viability of a biological oscillator has been examined in a phytochrome-deficient mutant.

Work by Nagy et al. (1993) and Casal et al. (1994) can be interpreted to indicate that phyA is the phytochrome that entrains the circadian oscillator. A very-low-fluence response can phase shift the circadian cycling of Lhcb mRNA in *T. aestivum* (Nagy et al., 1993). The very-low-fluence response is very likely a result of phyA action (Casal et al., 1994). The results presented here do not contradict these previous findings.

The presence of a functional biological clock in ma_3^R sorghum led us to reexamine the photoperiod sensitivity of

Table II. *Total length of internodes of 58M and 100M sorghum grown under different photoperiods*

Plants were grown for 30 d under 12-, 23-, or 24-h photoperiods. Ten plants of each genotype were harvested, and the total lengths of internodes from the root bases to the apices were measured. Data are presented as average lengths in mm ± SD. Observation of apical meristems indicated that no 58M plants other than those grown under 12 h light (L)/12 h dark (D) initiated earlier than d 28 (Table I).

Photoperiod	Genotype	
	58M	100M
12 h L/12 h D	341.7 ± 37.7	19.8 ± 1.3
23 h L/1 h D	214.0 ± 19.5	16.4 ± 1.8
24 h L/0 h D	239.5 ± 53.6	15.3 ± 2.1

ma_3^R sorghum. Constant light caused floral initiation to be relatively late (Table I). Compared to the 12-h daylength, photoperiods of 16 to 24 h resulted in notable delays in time to floral initiation. The ma_3^R mutation has always been characterized as causing photoperiod insensitivity (Quinby, 1973; Pao and Morgan, 1986a). Major et al. (1990) found no difference in floral initiation dates in ma_3^R sorghum grown with 10- to 15-h photoperiods. Pao and Morgan (1986a) observed ma_3^R sorghum to flower at the same time under 10- and 12-h d, but a 14-h d caused some postponement in floral initiation. When Sorrells and Myers (1982) grew ma_3^R sorghum in the field under photoperiods that varied from 14.5 to 16 h of daylight, ma_3^R genotypes were retarded in floral initiation by as much as 20 d. The study by Sorrells and Myers (1982) involved conditions under which temperature and other environmental factors were not held constant. These nonphotoperiodic factors may have been responsible for some delay in panicle initiation. However, in view of the results presented here, photoperiod probably accounted for most of the observed differences in floral initiation dates (Table I). Presumably, if Major et al. (1990) and Pao and Morgan (1986a) had used longer photoperiods, substantial postponement of floral initiation would have been apparent. The description of ma_3^R sorghum as photoperiod insensitive is true only when compared to ma_3 or Ma_3 genotypes, which can initiate panicles more than 80 d later than ma_3^R plants when grown under 14-h d (Pao and Morgan, 1986a). When comparing the responses of ma_3^R plants grown under a wide range of photoperiods, the photoperiod sensitivity becomes apparent (Table I). Although Pao and Morgan (1986a) and Sorrells and Myers (1982) had shown this before, the importance of these observations were not clearly recognized until now. Thus, ma_3^R causes relative photoperiod insensitivity, demonstrated as early floral initiation when grown under any photoperiod, but ma_3^R does not remove the requirement for a 10- to 12-h dark period to express the earliest possible floral initiation (Pao and Morgan, 1986a; Table I).

Several phytochrome actions have been implicated in the control of photoperiodism in SDPs (Takimoto and Saji, 1984; Vince-Prue and Takimoto, 1987; Vince-Prue, 1994). The Pfr form of one phytochrome is stimulatory toward flowering throughout an inductive night. The Pfr form of another pool of phytochrome is inhibitory to flowering in a cyclic fashion during an inductive night. A biological clock drives the rhythmic sensitivity to flowering inhibition and is itself entrained by a phytochrome. The data presented here suggest that 123-kD sorghum phytochrome does not entrain the biological clock. The most basic explanation is that the Pfr form of 123-kD phytochrome acts to either cyclically inhibit flowering or continually stimulate flowering during an inductive night. The precise role of 123-kD phytochrome in photoperiodism cannot be determined from these experiments.

Another explanation for the results presented here is that there are two or more biological clocks in sorghum and that Lhcb and RbcS mRNA expression and photoperiodism are regulated by different clocks, which are entrained by different phytochromes. Two clocks have been shown to exist in *P. vulgaris* (Hennessey and Field, 1992). In this scenario 123-kD phytochrome could be the phytochrome to entrain the photoperiodism-related clock, and proper operation of the Lhcb- and RbcS-related clock would not be a useful indicator of the photoperiodism-related clock. Although the existence of two clocks in sorghum is possible, the conclusion that 123-kD phytochrome does not entrain a photoperiodism-related biological clock is still tenable, since time measurement, indicated by photoperiod sensitivity, was demonstrated in ma_3^R sorghum (Table I).

Although sorghum is an SDP, *phyB A. thaliana*, an LDP, is also photoperiod sensitive (Goto et al., 1991). Night-breaks as well as a reduced R/FR ratio can hasten flowering in *phyB A. thaliana* (Halliday et al., 1994; Reed et al., 1994). These observations have been taken to mean that, although phyB is involved in photoperiodism, it may work in a fashion completely separate from a clock-related mechanism (Reed et al., 1994). It is particularly interesting and unexpected to find that a phyB-deficient LDP and a putative phyB-deficient SDP are similarly affected in their photoperiodic sensitivity.

Photoperiod strongly affected flowering in ma_3^R sorghum but only slightly altered internode elongation. Normally when the cultivars used here are grown under intermediate daylengths (10–14 h), internode elongation follows floral initiation (data not shown). Under the longest daylengths used here, the internodes of ma_3^R plants elongated before floral initiation (Tables I and II). As is usual, there was essentially no internode elongation in Ma_3 plants within the 30 d of this experiment. Two of the tenets of our ongoing research project have been that high GA concentrations due to the ma_3^R allele cause ma_3^R sorghum to be taller and to flower early (Pao and Morgan, 1986b; Morgan and Quinby, 1987). However, when grown in very long days, floral initiation is apparently uncoupled from GA synthesis, because excessive internode elongation may occur in ma_3^R sorghum before floral initiation (Tables I and II). Morgan and Quinby (1987) found that exogenous GA_3 hastened floral initiation but not flowering in non-ma_3^R sorghum. They suggested that, although GAs can accelerate floral initiation, a separate SD signal is required to allow panicle development to proceed. Our data support this suggestion and also imply that GA metabolism is not controlled by photoperiodism but may be linked more directly to control by phytochrome, or if GA synthesis is regulated via photoperiodism, that regulation must somehow be potentiated by phytochrome.

Recently, phyB mutants have been discovered not to exhibit an R-HIR inhibition of hypocotyl elongation but to respond normally to FR (McCormac et al., 1993). Previously, a single fluence rate of R (4.35 μmol m^{-2} s^{-1}) was found not to cause mesocotyl inhibition in ma_3^R sorghum (Childs et al., 1991). Here R did induce some mesocotyl inhibition in ma_3^R sorghum (Fig. 3). However, although Ma_3 sorghum demonstrated an R-HIR, ma_3^R sorghum had the same level of mesocotyl inhibition regardless of the fluence rate used and thus failed to display an R-HIR. Both Ma_3 and ma_3^R seedlings were equally inhibited by a rela-

tively high fluence rate of FR. Because *phyB A. thaliana* also lacks an R-HIR but does respond to FR, the present findings further strengthen our initial identification of the 123-kD sorghum phytochrome as a phyB.

The fact that R did induce some mesocotyl inhibition in ma_3^R sorghum indicates that at least two R-mediated responses control mesocotyl inhibition in sorghum. Although not observed previously (Childs et al., 1991), the more sensitive response may be either a low-fluence or very-low-fluence phytochrome response that was saturated in both genotypes in the experiment reported here, or it may be that a second HIR response that is present in ma_3^R sorghum and was saturated at the lowest fluence rate used here. To allow selection for uniformity in the earlier report, individual seedlings were germinated within test tubes sealed with corks (Childs et al., 1991). Although the seedlings appeared healthy, they may have been stunted. In preliminary tests for the R-HIR experiment here, seedlings germinated within test tubes did not grow as well as seedlings germinated in soil (data not shown). The more vigorous seedlings used in this experiment may have been able to display the more sensitive response that less hardy seedlings germinated in test tubes could not display.

The experiments presented here were designed to further our understanding of the role of 123-kD phytochrome in photoperiodism by determining whether ma_3^R sorghum contains a functional biological clock. Lhcb and RbcS mRNA levels were found to cycle in a circadian manner, indicating the presence of a biological clock and that 123-kD phytochrome is not necessary for entrainment of that biological clock. In contrast to earlier reports, ma_3^R sorghum was also determined to be photoperiodic, although the photoperiod sensitivity of ma_3^R plants is greatly reduced compared to that of non-ma_3^R plants. Thus, 123-kD phytochrome probably affects photoperiod sensitivity by some other mechanism than the entrainment of the biological clock. The tremendous elongation growth of the internodes of ma_3^R sorghum was not greatly affected by different photoperiod treatments, although flowering was delayed. This indicates that, although GAs are involved in flowering, there are other photoperiod-sensitive factors that are also required for floral initiation. Additionally, ma_3^R sorghum does not express an R-HIR inhibition of mesocotyl elongation. This is a characteristic of phyB-deficient mutants and further strengthens the position that light-stable, green tissue-predominant, 123-kD sorghum phytochrome is a phyB.

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LITERATURE CITED

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