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Genetic Regulation of Development in *Sorghum bicolor*

VI. The ma_3^R Allele Results in Abnormal Phytochrome Physiology

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ABSTRACT

Physiological processes controlled by phytochrome were examined in three near-isogenic genotypes of *Sorghum bicolor*, differing at the allele of the third maturity gene locus. Seedlings of 58M ($ma_3^Rma_3^R$) did not show phytochrome control of anthocyanin synthesis. In contrast, seedlings of 90M (ma_3ma_3) and 100M (Ma_3Ma_3) demonstrated reduced anthocyanin synthesis after treatment with far red and reversal of the far red effect by red. De-etiolation of 48-hour-old 90M and 100M dark-grown seedlings occurred with 48 hours of continuous red. Dark-grown 58M seedlings did not de-etiolate with continuous red treatment. Treatment of seedlings with gibberellic acid or tetcyclacis, a gibberellin synthesis inhibitor, did not alter anthocyanin synthesis. Levels of chlorophyll and anthocyanin were lower in light-grown 58M seedlings than in 90M and 100M. Etiolated seedlings of all three genotypes have similar amounts of photoreversible phytochrome. Crude protein extracts from etiolated seedlings were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and transferred to nitrocellulose. Phytochrome was visualized with Pea-25, a monoclonal antibody directed to phytochrome from etiolated peas. The samples from all three genotypes contained approximately equivalent amounts of a prominent, immunostaining band at 126 kD. However, the sample from 58M did not show a fainter, secondary band at 123 kD that was present in 90M and 100M. The identity and importance of this secondary band at 123 kD is unknown. We propose that 58M is a phytochrome-related mutant that contains normal amounts of photoreversible phytochrome and normal phytochrome protein when grown in the dark.

Sorghum (*Sorghum bicolor* [L.] Moench) is a quantitative SD plant (10, 29). Although sorghum originated in the tropics, it is now grown at latitudes far removed from the equator. This is possible only because mutations have been recognized in genes that reduce the plant's photoperiodic sensitivity and thus allow flowering under LDs. These genes are referred to as maturity genes and have been described (22, 23).

The milo group of sorghum has three known maturity genes (21–23). Eleven near-isogenic milo sorghum genotypes with homozygous maturity genes have been prepared to aid the study of sorghum development. The third maturity locus has three known alleles: Ma_3, ma_3, and ma_3^R. Genotypes without the ma_3^R allele vary in their photoperiodic sensitivity. However, genotypes containing ma_3^R are essentially photoperiod insensitive and flower early under a wide variety of photoperiods (18).

The ma_3^R allele also confers morphological differences to sorghum when compared with the actions of Ma_3 or ma_3. Sorghum seedlings with ma_3^R are taller and have longer leaf sheaths, longer and narrower leaf blades, less leaf area, fewer tillers, and greater dry weight than Ma_3 or ma_3 genotypes (3, 18). The ma_3^R phenotype can be mimicked by exogenous applications of GA_3 to Ma_3 or ma_3 genotypes (3, 19). Additionally, treatment of ma_3^R plants with a GA[1] biosynthesis inhibitor, tetcyclacis, will evoke a phenotype like Ma_3 or ma_3 sorghum (3). In fact the ma_3^R allele results in increased levels of GAs in sorghum, and this higher concentration of GAs has been proposed to be part or all of the cause of the ma_3^R phenotype (3).

The ma_3^R allele is pleiotropic in its effect on sorghum. Although elevated GA levels in ma_3^R sorghum can account for many of the phenotypic traits of ma_3^R plants, it is not clear whether altered GA metabolism is the primary site of action of ma_3^R. Gibberellin metabolism has been shown to be controlled by phytochrome in pea (*Pisum sativum* L.) (4). Another possible explanation of the ma_3^R allele is that it drastically alters the phytochrome physiology of the plant, and this results secondarily in abnormal GA metabolism. The experiments reported here compare the phytochrome system of an ma_3^R genotype with non-ma_3^R genotypes.

MATERIALS AND METHODS

Plant Material

Three near-isogenic genotypes of *Sorghum bicolor* (L.) Moench differing only in the allele at the third maturity gene locus were used: 100M (Ma_3Ma_3), 90M (ma_3ma_3), and 58M (ma_3^R ma_3^R). In some experiments an additional genotype was used, A.Tx399 × R.Tx430. The alleles of the maturity genes of this hybrid were unknown to us, but it did not contain ma_3^R.

Light Sources

FR light was obtained by filtering the output of a 60-W incandescent lamp through a CBS FR 750 filter (Carolina

[1] Abbreviations: GA, gibberellin; FR, far red; R, red; WL, white light; BL, blue light.

Biological Supply, Burlington, NC). R light was obtained from a 22-W Gro-Lux fluorescent lamp and a CBS red 650 filter. Fluence rates of 1.78 (690–780 nm) and 4.35 μmol m^{-2} s^{-1} (600–700 nm) were measured for the FR and R lights, respectively, with a Li-Cor 1800 Spectroradiometer (Lincoln, NE). The wavelength ranges used to calculate these fluence rates were previously found to be the physiologically active ranges in sorghum (9).

Phytochrome Control of Anthocyanin Synthesis

Seeds were germinated in darkness on moist filter paper in Petri dishes. Three-day-old seedlings were exposed to WL for 4 h. Seedlings were then treated under a green safelight with 12.2 min FR or 12.2 min FR followed by 5 min R. As a control, some seedlings were handled under a green safelight but not treated with either FR or R (dark treated). After the dark, FR, or FR + R treatments, the seedlings were returned to darkness for 24 h. Mesocotyls were then excised, massed, and extracted with 5 mL of 1% (v/v) HCl in methanol for 24 h at 4°C to remove anthocyanins. Absorbances at 530 nm were measured with a Beckman DU 40 spectrophotometer (Palo Alto, CA) (9). Five seedlings were used for each extraction; the experiment was replicated five times.

Growth Regulator Influence on Anthocyanin Synthesis

Solutions of GA_3 (Sigma, St Louis, MO) and tetcyclacis (BASF, Ludwigshafen, Germany) were made in liquid Murashige-Skoog tissue culture medium (1.8% sucrose, pH 5.2).

Method 1

Three-day-old seedlings, germinated as above, were placed in WL for 4 h. The shoots of these seedlings were then excised, placed on filter paper moistened with treatment solutions in Petri dishes, and returned to darkness for 24 h. The seed and root were removed to assure that no stored GAs were released for use in the shoot. Anthocyanins were extracted from mesocotyls and measured as above.

Method 2

Shoots were excised from 3-d-old seedlings and placed in Petri dishes with filter paper moistened with treatment solutions. The Petri dishes were then placed under WL for 24 h. This method resembles that used in a previous study (27).

For each method, five shoots were used for each treatment, and each treatment was replicated three times.

Effects of Continuous Red Light on Seedling Growth

Individual seedlings were germinated and grown between the glass wall of a test tube and a piece of rolled-up, wetted filter paper. Forty-eight-hour-old, dark-grown seedlings were selected for uniformity with the aid of a green safelight and either placed in front of a horizontal R light source or returned to darkness. After an additional 48 h, the mesocotyl, coleoptile, and first leaf were measured. This experiment was replicated three times using five seedlings for each trial.

Pigment Content of Green Tissues

Plants were grown under a 12-h photoperiod for 14 d in a growth chamber as described previously (3). Seed were overplanted and thinned to five plants for each 20-cm pot. All of the leaf blades from a pot were harvested, frozen at −70°C, lyophilized, and macerated. Chl was extracted with DMSO (11), and Chl content was estimated by the equation, $C = (20.2)A_{645} + (8.02)A_{663}$, which gives a measure of Chl in mg/L (2). Anthocyanins were extracted with 1% (v/v) HCl in methanol for 24 h at 4°C. Anthocyanin content was estimated by using the equation, $A_{530} - (0.25)A_{657}$, which compensates for the contribution of Chl to the absorbance at 530 nm (24). This experiment was performed once with six replicates.

Photoreversible Phytochrome Measurement

Seedlings were germinated in darkness on absorbent paper at 25°C and 100% humidity. Beginning on the fourth day after sowing, shoots >3 cm in height were harvested daily, placed in a humid chamber, and stored at 2 to 4°C until enough tissue was collected for measurement. This method of collecting tissue was used because germination was not uniform. By harvesting shoots of similar size, they were considered to be of similar morphological age. Entire shoots were chopped into segments of 1 to 2 mm and gently packed into a vertical light-path cuvette with a 0.33-cm^2 cross-sectional area. Photoreversibility measurements were made with a custom-built dual wavelength spectrophotometer at 666 and 728 nm.

Phytochrome Immunoblot

Seedlings were germinated as for the photoreversibility measurements. Shoots were harvested, immediately frozen in liquid N_2, and stored at −80°C. Shoots were accumulated until enough were available for lyophilization and homogenization. Tissue was extracted with boiling SDS-sample buffer (45 mg tissue to 1 mL buffer) for 5 min, cooled, and clarified by centrifugation (7). An aliquot of each sample was electrophoresed in a 7.5 to 15% linear gradient SDS-polyacrylamide gel (14). Protein was electrotransferred to nitrocellulose and immunostained with Pea-25, a monoclonal antibody directed to phytochrome from etiolated pea shoots (7, 8).

RESULTS AND DISCUSSION

The recent findings that ma_3^R-containing sorghum seedlings have higher endogenous levels of GAs than non-ma_3^R seedlings initiated this study of the phytochrome physiology of the same genotypes (3). GA metabolism is known to be regulated by phytochrome (4). Other processes influenced by phytochrome were examined here.

Anthocyanin synthesis in sorghum seedlings is regulated by phytochrome (9, 17). Figure 1 shows that 90M (ma_3), 100M (Ma_3), and A.Tx399 × R.Tx430 all demonstrated normal phytochrome control of anthocyanin synthesis. FR inhibited anthocyanin production compared with dark controls, and R reversed the inhibition back to control levels. Phytochrome regulation of anthocyanin synthesis did not occur in 58M (ma_3^R) seedlings, which produced little anthocyanin under

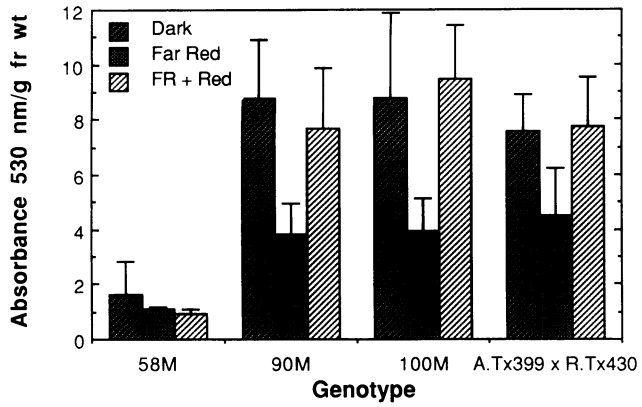

Figure 1. Phytochrome control of anthocyanin synthesis in sorghum seedling mesocotyls. Three-day-old etiolated seedlings were placed in WL for 4 h, treated with FR or FR + R, and returned to darkness for 24 h. Anthocyanins were extracted from five mesocotyls with 5 mL 1% HCl in methanol for 24 h at 4°C. Absorbance measurements were taken at 530 nm.

any of the treatment conditions (Fig. 1). However, this observation is by itself not sufficient proof that the ma_3^R allele alters the phytochrome physiology of sorghum because anthocyanin synthesis in sorghum seedlings can be reduced by high GA concentrations (27). Because light-grown 58M plants do contain elevated concentrations of GAs (3), it is possible that elevated GA levels inhibited anthocyanin accumulation in 58M seedlings. This possibility was tested by applying exogenous GA_3 to 100M seedlings or by applying the GA synthesis inhibitor tetcyclasis to 58M seedlings. Two methods were used to test for GA influence over anthocyanin synthesis in these two genotypes. Method 1 was comparable to the procedure used in the FR/R reversibility experiment above. Method 2 was like a proven design used previously (27).

Table I. *Effect of Tetcyclacis on Anthocyanin Content of 58M Sorghum Seedlings*

Method 1. Five 3-d-old etiolated 58M sorghum seedlings were placed in WL for 6 h. The shoots were excised, placed in treatment solutions, and returned to darkness. After 24 h anthocyanins were extracted from mesocotyls with 1% HCl in methanol at 4°C for 24 h. Method 2. The shoots from five 3-d-old etiolated 58M sorghum seedlings were excised and placed in treatment solutions under WL. After 24 h anthocyanins were extracted and measured as in Method 1.

Tetcyclacis Concentration	Anthocyanin Content[a, b]	
	Method 1	Method 2
M		
0	0.2 ± 0.0 a	1.5 ± 0.5 a
10^{-9}	0.3 ± 0.1 a	1.4 ± 0.5 a
10^{-7}	0.1 ± 0.0 a	1.9 ± 0.3 a
10^{-5}	0.2 ± 0.0 a	1.2 ± 0.4 a, b
10^{-3}	0.2 ± 0.1 a	0.2 ± 0.2 b

[a] Values are A_{530}·g dry wt^{-1} ± SD. [b] Means followed by the same letter are not declared different by Tukey's studentized range test at the 0.05 level of probability.

Table II. *Effect of GA_3 on Anthocyanin Content of 100M Sorghum Seedlings*

Method 1. Five 3-d-old etiolated 100M sorghum seedlings were placed in WL for 6 h. The shoots were excised, placed in treatment solutions, and returned to darkness. After 24 h anthocyanins were extracted from mesocotyls with 1% HCl in methanol at 4°C for 24 h. Method 2. The shoots from five 3-d-old etiolated 100M sorghum seedlings were excised and placed in treatment solutions under WL. After 24 h anthocyanins were extracted and measured as in Method 1.

GA_3 Concentration	Anthocyanin Content[a, b]	
	Method 1	Method 2
M		
0	1.4 ± 0.4 a	0.7 ± 0.3 a
10^{-6}	1.9 ± 0.6 a	2.9 ± 1.3 a
10^{-4}	1.0 ± 0.2 a	1.4 ± 1.1 a
10^{-2}	1.4 ± 0.5 a	1.6 ± 1.4 a

[a] Values are A_{530}·g dry wt^{-1} ± SD. [b] Means followed by the same letter are not declared different by Tukey's studentized range test at the 0.05 level of probability.

Anthocyanin synthesis in 58M was influenced by 1 mM tetcyclacis only when Method 2 was used (Table I). The inhibition observed at this high concentration of tetcyclacis was probably due to nonspecific physiological effects; it was much higher than the 50 nM tetcyclacis found to alter growth in light-grown 58M plants (3). In contrast with earlier results (27), GA_3 was not found to affect anthocyanin synthesis in 100M seedlings (Table II). The difference between GA_3 influence on anthocyanin production reported here and reported previously (27) may be that entire mesocotyls (>25 mm) were used in this experiment but only the upper 7 mm of the mesocotyl was used in the earlier work (27). Furthermore, in the previous work the GA synthesis inhibitor, (2-chloroethyl) trimethylammonium chloride, also decreased anthocyanin production (27). Thus, in the initial study, both excess exogenous GA_3 (10 mM) and high levels of 2-chloroethyl) trimethylammonium chloride (20 mM) inhibited anthocyanin synthesis (27). The role of GAs in anthocyanin synthesis remains unclear. The present results do not suggest that elevated levels of GAs in 58M inhibit anthocyanin production but that some aspect of phytochrome physiology may be the cause of the abnormal behavior of 58M.

Anthocyanin synthesis in sorghum seedlings is regulated by phytochrome only after satisfaction of an initial BL/UV requirement (9, 17). In this experiment (Fig. 1), the WL fulfilled the BL/UV requirement (9). Thus, the abnormal behavior of 58M may be interpreted as being due to either dysfunctional phytochrome or disfunctional BL/UV receptor. To distinguish between these two possibilities, de-etiolation of seedlings by R was investigated. De-etiolation is a phytochrome-controlled response that is not dependent on BL/UV (15). When 48-h-old etiolated seedlings of 90M, 100M, and A.Tx399 × R.Tx430 were irradiated with 48 h of R, mesocotyl growth was strongly inhibited and first leaf elongation was stimulated compared with seedlings grown in darkness for 96 h (Fig. 2). Coleoptile growth was not significantly affected. When 48-h-old etiolated 58M seedlings were treated with 48

h of R, they were unaffected and showed growth identical with dark-grown controls (Fig. 2). The dark-grown seedlings of all genotypes were indistinguishable (Fig. 2). Although the R source used here did not cause 58M seedlings to de-etiolate, when 58M is grown in environmental growth chambers or in the field it does de-etiolate (18, 19, 23). The combination of the lack of FR/R control of anthocyanin production and the failure of R to cause de-etiolation in 58M seedlings indicates that the ma_3^R allele causes a lesion in the phytochrome system in sorghum. Given that 58M seedlings do de-etiolate in WL, this lesion is either leaky or can be bypassed by an alternative photomorphogenic system.

Anthocyanin and Chl contents in green tissues are also regulated by phytochrome (5, 9, 24). Leaves of 14-d-old 58M plants contained significantly lower levels of both of these pigments than did leaves of 90M, 100M, or A.Tx399 × R.Tx430 sorghum (Table III). Thus, 58M sorghum apparently displays abnormal phytochrome responses in both green and dark-grown tissues.

All of the abnormal phytochrome responses of 58M may be explained by an absence of phytochrome, dysfunctional phytochrome, or a blockage in a phytochrome signal transduction pathway. Phytochrome in the four genotypes used here was characterized in two ways. First, the phytochrome content of etiolated shoots was analyzed by photoreversibility measurements. Etiolated seedlings of all four genotypes contained similar amounts of photoreversible phytochrome (Table IV). Second, crude protein extracts from etiolated shoots were electrophoresed in a linear gradient SDS-polyacrylamide gel, transferred to nitrocellulose, and immunostained with the monoclonal antibody Pea-25. A main immunostaining band at approximately 126 kD was found for full-size phytochrome (Fig. 3). All genotypes have approximately equal amounts of this full-size phytochrome. However, a second fainter band at approximately 123 kD is missing from the sample from 58M (Fig. 3). The other three sorghum genotypes all have this band in approximately equal amounts.

Table III. *Chl and Anthocyanin Contents of 14-d-Old Sorghum Leaves*

Chl was extracted from lyophilized leaf tissue with DMSO at 60°C for 24 h. Anthocyanin was extracted from lyophilized leaf tissue with 1% HCl in methanol at 4°C for 24 h.

Genotype	Chl Content[a, b]	Anthocyanin Content[b, c]
58M	10.5 ± 0.3 a	10.9 ± 1.6 a
90M	15.2 ± 0.7 b	18.0 ± 1.6 b
100M	15.5 ± 0.4 b	16.9 ± 2.7 b
A.Tx399 × RTx430	16.9 ± 0.5 c	18.4 ± 1.6 b

[a] Values are mg·g dry wt^{-1} ± SD. [b] Means followed by the same letter are not declared different by Tukey's studentized range test at the 0.05 level of probability. [c] Values are $(A_{530} - (0.25)A_{657})$·g dry wt^{-1} ± SD.

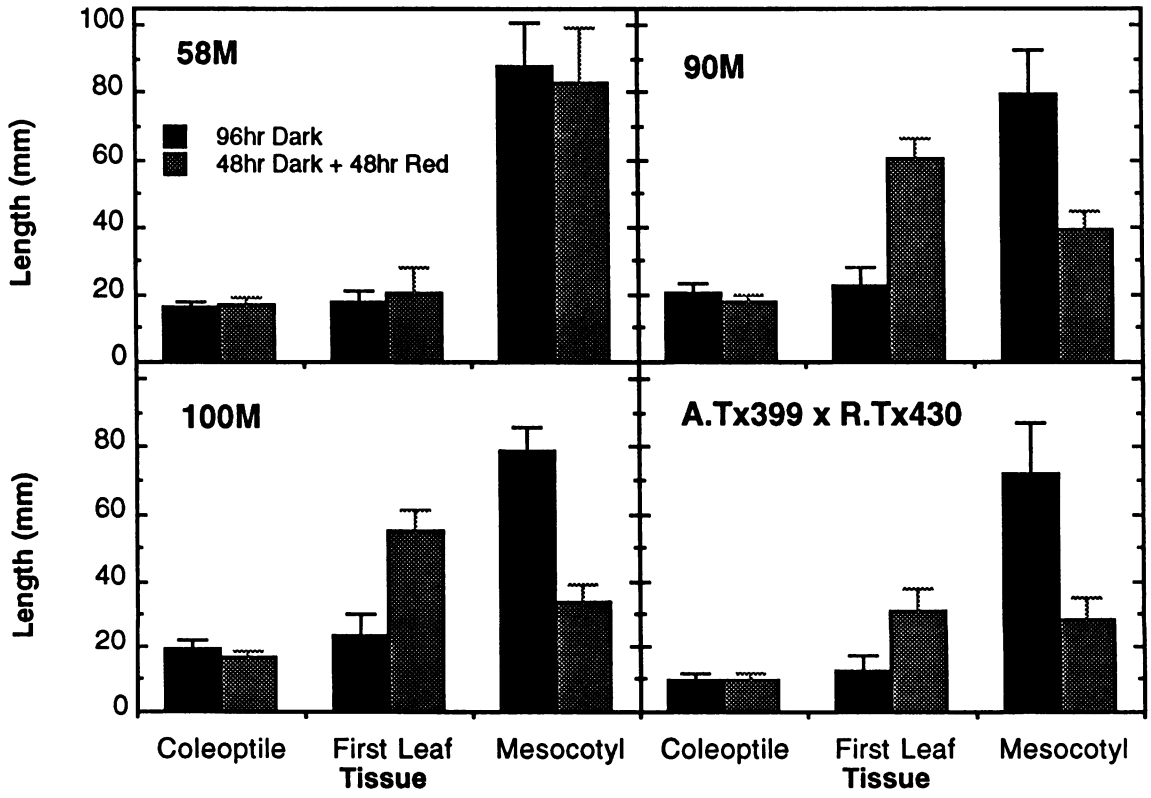

Figure 2. Growth of dark-grown and R-treated sorghum seedlings. Seeds were germinated in test tubes. After 48 h etiolated seedlings were placed in front of R (4.35 μmol m^{-2} s^{-1}) for 48 h or returned to darkness for 48 h. The genotypes examined were 58M (A), 90M (B), 100M (C), and ATx399 × RTx430 (D).

Table IV. *Photoreversible Phytochrome Content of Etiolated Sorghum Seedlings*

Etiolated shoots were harvested, cut into segments of 1 to 2 mm, and gently packed into a vertical light-path cuvette with a 0.33-cm^2 cross-sectional area. Photoreversible phytochrome content was determined with a custom-built dual wavelength spectrophotometer at 666 and 728 nm. Phytochrome content is expressed as $\Delta\Delta A \pm SE \times 10^3$. Sample sizes are expressed in mg.

Genotype	Phytochrome Content (Repetitions)	Sample Size (No. of shoots)
58M	33.5 ± 2.9 (3)	240–288 (5–7)
90M	36.1 ± 3.2 (4)	260–273 (6–8)
100M	39.1 ± 6.8 (6)	255–291 (7–10)
A.Tx399 × RTx430	30.8 ± 2.9 (4)	255–291 (5–8)

A standard of hydroxyapatite-purified phytochrome from etiolated oats was stained by Pea-25 and has a main band at approximately 124 kD and secondary bands at 114 and 107 kD (Fig. 3). The meaning of the missing band from the protein extract from 58M is unclear. Such secondary bands of lower mol wt have been demonstrated to be degradation products of full-length phytochrome (26). Alternatively, monoclonal antibodies have been developed that bind only to particular types of phytochrome (12, 25). In those studies, secondary bands have been shown to be separate, less abundant types of phytochrome.

Pea-25, the monoclonal antibody used here, has proven to bind to the phytochromes from a wide range of species (8) and to at least some of the less abundant types (7, 28). There is no precedent indicating that Pea-25 recognizes a non-phytochrome polypeptide in crude extracts of etiolated or light-grown angiosperm tissues. Thus, because samples used here for immunoblot assay were prepared in a way that minimizes or even eliminates posthomogenization artifacts (26), it is possible that the 123-kD band is one of the lesser abundant types of phytochrome that predominate in light-grown tissues (26). Because these types of phytochrome might also be present in etiolated tissue, it is feasible to anticipate that 58M sorghum may have apparently normal amounts of the predominant phytochrome in etiolated tissue but might be missing one or more of the phytochromes that predominate in light-grown tissue. A preliminary phytochrome extraction experiment with de-etiolated tissue from the hybrid A.Tx399 × R.Tx430 indicates that the 126-kD band decreases and the 123-kD band remains constant with de-etiolation. This is consistent with the possibility that the 123-kD band corresponds to a light-predominant phytochrome. However, present data do not permit a firm conclusion to be reached concerning the nature of the missing 123-kD band from 58M sorghum.

Several phytochrome mutants have been described. The *au* mutant of tomato (*Lycopersicon esculentum*) (13), the *hy* mutants of *Arabidopsis thaliana* (6), and the *lh* mutant of cucumber (*Cucumis sativus* L.) (1) were all isolated because of their greater hypocotyl growth compared with wild types. All of these mutants have abnormal phytochrome physiologies. The cause of the *au* tomato mutation has been ascribed to missing phytochrome apoprotein (13). The *hy*1, *hy*2, and *hy*6 mutants of *A. thaliana* contain normal levels of phytochrome apoprotein but reduced levels of photoreversible phytochrome (6, 20). Mature tissues of cucumbers homozygous for *lh* have been reported to have less photoreversibly detectable phytochrome in de-etiolated tissues than do wild-type plants (1). In a more recent study in which cotyledon tissue was used, green and etiolated *lh* cucumber seedlings were found to have normal amounts of both photoreversible phytochrome and immunologically detectable phytochrome protein. However, in preliminary work mentioned in that text (16), older leaves of *lh* cucumber were found to have less spectrophotochemically detectable phytochrome than wild-type cucumber (16). Our initial results indicate that ma_3^R sorghum is most like *lh* cucumber in that both have apparently wild-type amounts of phytochrome in etiolated tissues. More work is needed to fully compare these mutants.

We propose that the ma_3^R allele is a phytochrome-related mutation. Phytochrome-controlled processes, including anthocyanin synthesis, de-etiolation, and Chl content, are altered by the ma_3^R allele. This mutation does not appear to affect the phytochrome protein or phytochrome photoreversibility that predominates in etiolated tissue. However, the significance of the missing immunodetectable peptide at 123 kD remains an important question. Although seedlings with ma_3^R elongate more quickly than non-ma_3^R sorghum (18), the ma_3^R mutation was not initially isolated because of greater elongation as were the phytochrome mutants from other species. The ma_3^R allele was initially of interest because it causes sorghum to be essentially nonphotoperiodic and to flower very early (18, 23). The allele is also involved in altered GA metabolism (3). Because we failed by GA_3 treatment to manipulate anthocyanin synthesis, a phytochrome-mediated process, it appears that ma_3^R represents a lesion in the phytchrome system and that this lesion deregulates GA biosynthesis, allowing higher levels to accumulate. The result of the enhanced GA concentrations is altered morphology. The validity of this hypothesis and the question of whether non-

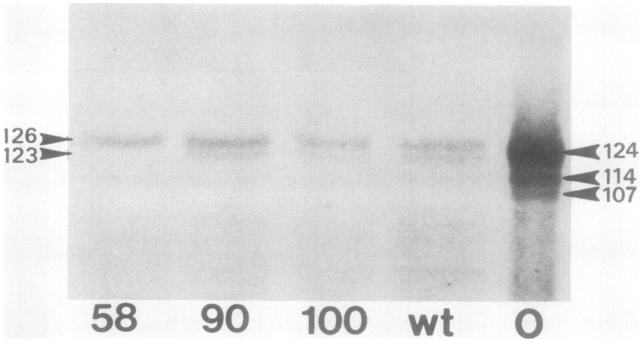

Figure 3. Immunoblot of SDS-sample buffer extracts of lyophilized etiolated sorghum shoots following electrophoresis in a 7.5 to 15% linear gradient polyacrylamide gel. Lanes received 5 μL of extract from 58M (58), 90M (90), 100M (100), or ATx399 × RTx430 (wt). As a reference, one lane received 25 ng of hydroxyapatite-purified phytochrome from etiolated oats (O). Following electrophoresis and electroblotting to nitrocellulose, polypeptides were immunostained with 1 μg/mL of monoclonal antibody Pea-25. Arrowsheads, M_r (in thousands) of immunostained bands.

photoperiodic flowering in ma_3^R genotypes is due to altered or absent phytochrome or to altered GA levels are currently being studied.

LITERATURE CITED

1. **Adamse P, Jaspers PAPM, Bakker JA, Kendrick RE, Koornneef M** (1988) Photophysiology and phytochrome content of long-hypocotyl mutant and wild-type cucumber seedlings. Plant Physiol **87:** 264–268
2. **Arnon DI** (1949) Copper enzymes in isolated chloroplasts. Polyphenoloxidases in *Beta vulgaris*. Plant Physiol **24:** 1–15
3. **Beall FD, Morgan PW, Mander LN, Miller FR, Babb KH** (1990) Genetic regulation of development in *Sorghum bicolor*. V. The ma_3^R allele results in gibberellin enrichment. Plant Physiol **95:** 116–125
4. **Campell BR, Bonner BA** (1986) Evidence for phytochrome regulation of gibberellin A20 3β-hydroxylation in shoots of dwarf (*lele*) *Pisum sativum* L. Plant Physiol **82:** 909–915
5. **Casal JJ, Aphalo PJ** (1989) Phytochrome control of chlorophyll content in mature attached leaves of *Petunia axillaris*. Ann Bot **63:** 595–598
6. **Chory J, Peto CA, Ashbaugh M, Saganich R, Pratt L, Ausubel F** (1989) Different roles for phytochrome in etiolated and green plants deduced from characterization of *Arabidopsis thaliana* mutants. Plant Cell **1:** 867–880
7. **Cordonnier M-M, Greppin H, Pratt LH** (1986) Phytochrome from green *Avena* shoots characterized with monoclonal antibody to phytochrome from etiolated *Pisum* shoots. Biochemistry **25:** 7657–7666
8. **Cordonnier M-M, Greppin H, Pratt LH** (1986) Identification of a highly conserved domain on phytochrome from angiosperms to algae. Plant Physiol **80:** 982–987
9. **Downs RJ, Siegleman HW** (1963) Photocontrol of anthocyanin synthesis in milo seedlings. Plant Physiol **38:** 25–30
10. **Garner WW, Allard HA** (1923) Further studies in photoperiodism, the response of the plant to relative length of day and night. J Agric Res **23:** 871–920
11. **Hiscox JD, Israelstam GF** (1979) A method for the extraction of chlorophyll from leaf tissue without maceration. Can J Bot **57:** 1332–1334
12. **Konomi K, Abe H, Furuya M** (1987) Changes in the content of phytochrome I and II apoproteins in embryonic axes of pea seeds during imbibition. Plant Cell Physiol **28:** 1443–1451
13. **Koornneef M, Cone JW, Dekens RG, O'Hearne-Robers EG, Spruit CJP, Kendrick RE** (1985) Photomorphogenic responses of long-hypocotyl mutants of tomato. J Plant Physiol **120:** 153–165
14. **Laemmli UK** (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature **227:** 680–685
15. **Mandoli DF, Briggs WR** (1981) Phytochrome control of two low-irradiance responses in etiolated oat seedlings. Plant Physiol **67:** 733–739
16. **Nagatani A, Kendrick RE, Koornneef M, Furuya M** (1989) Partial characterization of phytochrome I and II in etiolated and de-etiolated tissues of a photomorphogenetic mutant (*lh*) of cucumber (*Cucumis sativus* L.) and its isogenic wild type. Plant Cell Physiol **30:** 685–690
17. **Oelmüller R, Mohr H** (1985) Mode of coaction between blue/UV light and light absorbed by phytochrome in light-mediated anthocyanin formation in the milo (*Sorghum vulgare* Pers.) seedling. Proc Natl Acad Sci USA **82:** 6124–6128
18. **Pao CI, Morgan PW** (1986) Genetic regulation of development in *Sorghum bicolor*. I. Role of the maturity genes. Plant Physiol **82:** 575–580
19. **Pao CI, Morgan PW** (1986) Genetic regulation of development in *Sorghum bicolor*. II. Effect of the ma_3^R allele mimicked by GA_3. Plant Physiol **82:** 581–584
20. **Parks BM, Shanklin J, Koornneef M, Kendrick RE, Quail PH** (1989) Immunochemically detectable phytochrome is present at normal levels but is photochemically nonfunctional in the *hy*1 and *hy*2 long hypocotyl mutants of *Arabodopsis*. Plant Mol Biol **12:** 425–437
21. **Quinby JR** (1967) The maturity genes of sorghum. *In* AG Norman, ed, *Advances in Agronomy*, Vol 19. Academic Press, New York, pp 267–305
22. **Quinby JR, Karper RE** (1945) The inheritance of three genes that influence time of floral initiation and maturity date in milo. J Agron **37:** 916–936
23. **Quinby JR, Karper RE** (1961) Inheritance of duration of growth in the milo group of sorghum. Crop Sci **1:** 8–10
24. **Rabino I, Mancinelli AL** (1986) Light, temperature, and anthocyanin production. Plant Physiol **81:** 922–924
25. **Tokuhisa JG, Daniels SM, Quail PH** (1985) Phytochrome in green tissue: spectral and immunochemical evidence for two distinct molecular species of phytochrome in light grown *Avena sativa* L. Planta **164:** 321–332
26. **Vierstra RD, Cordonnier MM, Pratt LH, Quail PH** (1984) Native phytochrome: immunoblot analysis of relative molecular mass and *in-vitro* proteolytic degradation for several plant species. Planta **160:** 521–528
27. **Vince D** (1968) Growth and anthocyanin synthesis in excised *Sorghum* internodes. I. Effects of growth regulating substances. Planta **82:** 261–279
28. **Wang Y-C, Stewart SJ, Cordonnier M-M, Pratt LH** (1991) *Avena sativa* L. contains three phytochromes, only one of which is abundant in etiolated tissue. Planta **184:** 96–104
29. **Williams EA, Morgan PW** (1979) Floral initiation in sorghum hastened by gibberellic acid and far-red light. Planta **145:** 269–272