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A Temporarily Red Light-Insensitive Mutant of Tomato Lacks a Light-Stable, B-Like Phytochrome[1]

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We have selected four recessive mutants in tomato (*Lycopersicon esculentum* Mill.) that, under continuous red light (R), have long hypocotyls and small cotyledons compared to wild type (WT), a phenotype typical of phytochrome B (phyB) mutants of other species. These mutants, which are allelic, are only insensitive to R during the first 2 days upon transition from darkness to R, and therefore we propose the gene symbol *tri* (temporarily red light insensitive). White light-grown mutant plants have a more elongated growth habit than that of the WT. An immunochemically and spectrophotometrically detectable phyB-like polypeptide detectable in the WT is absent or below detection limits in the tri^1 mutant. In contrast to the absence of an elongation growth response to far-red light (FR) given at the end of the daily photoperiod (EODFR) in all phyB-deficient mutants so far characterized, the tri^1 mutant responds to EODFR treatment. The tri^1 mutant also shows a strong response to supplementary daytime far-red light. We propose that the phyB-like phytochrome deficient in the *tri* mutants plays a major role during de-etiolation and that other light-stable phytochromes can regulate the EODFR and shade-avoidance responses in tomato.

The R/FR-absorbing phytochrome photoreceptor system plays a leading role in the regulation of development throughout the life cycle of plants. Examples of the light-mediated processes that it influences are seed germination, de-etiolation (inhibition of hypocotyl growth, opening of the apical hook, expansion of the cotyledons, development of chloroplasts, accumulation of anthocyanin), shade avoidance, and induction of flowering (Kendrick and Kronenberg, 1994).

In *Arabidopsis thaliana* the phytochrome family consists of at least five different genes referred to as *PHYA* through *PHYE*, which encode apophytochrome PHYA through PHYE and form holophytochrome phyA through phyE after insertion of the chromophore, respectively (Quail, 1994). Recent research (Hauser et al., 1994; Pratt, 1995) reports the presence of an even more complex gene family in tomato (*Lycopersicon esculentum* Mill.). Mutants deficient in one specific type of phytochrome are needed if we are to unravel the roles of the different phytochrome species in photomorphogenesis. So far, three types of phytochrome mutants have been characterized: (a) Mutants that are thought to be deficient in all types of phytochrome are probably caused by a defect in the biosynthesis of the common phytochrome chromophore, like the *hy1* and *hy2* mutants of *Arabidopsis* (Parks and Quail, 1991) and the *pew* mutants of *Nicotiana plumbaginifolia* (Kraepiel et al., 1994). In tomato the *aurea* (*au*) and *yellow green-2* (*yg-2*) mutants (Koornneef et al., 1985) are possible candidates for tomato chromophore mutants (Sharma et al., 1993; Van Tuinen et al., 1995). (b) PhyA-deficient mutants have been reported in Arabidopsis (Dehesh et al., 1993; Nagatani et al., 1993; Parks and Quail, 1993; Whitelam et al., 1993) and more recently in tomato (Van Tuinen et al., 1995). (c) Mutants that lack a light-stable, phyB-like protein have been reported in several species, including cucumber (López-Juez et al., 1992), *Brassica rapa* (Devlin et al., 1992), and Arabidopsis. Only in the case of the Arabidopsis *hy3* (=*phyB*) mutant has it been proven that the mutation is located in the *PHYB* gene itself (Reed et al., 1993). The phyB-deficient mutants are characterized by their failure to de-etiolate in continuous R, resulting in a long hypocotyl and small cotyledons, the absence of an EODFR response, an elongated stature, and a slightly reduced Chl content when grown in WL.

In view of the large number of phytochrome genes in tomato, there is a need for more type-specific phytochrome mutants to enable the physiological roles of the different phytochromes to be elucidated. The fact that phyB-deficient mutants already described in other species have a common phenotype has enabled us to screen for phyB-deficient mutants in tomato under WL and continuous R. This paper presents the isolation and characterization of such mutants in tomato.

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Abbreviations: B, blue light; D, dark; EODFR, end-of-day far-red light; FR, far-red light; GT, tomato breeding line GT; LFR, low fluence response; MM, tomato cv Moneymaker; phyA through phyE, phytochrome A through phytochrome E; R, red light; WL, white light; WT, wild type.

MATERIALS AND METHODS

Plant Material

Mutants were obtained by treating seeds of tomato (*Lycopersicon esculentum* Mill.) MM and GT with ethyl methanesulfonate for 24 h in darkness at 25°C (Koornneef et al., 1990). The M_2 seed groups were screened for mutants with phenotypes deviating from WT in WL and broad-band R and B. One mutant with an elongated phenotype in WL, due to somaclonal variation, was also isolated in the progeny of plants regenerated from tissue culture described by Van den Bulk et al. (1990).

Genetic Characterization

Seedlings used in all types of genetic analyses were grown for 7 d after emergence under continuous R (3 μmol m^{-2} s^{-1}). Segregation ratios were determined by counting the number of seedlings with a WT or mutant hypocotyl length. The mutants obtained were tested for allelism versus nonallelism on the basis of noncomplementation versus complementation to WT phenotype in the F_1 plants. The subsequent F_2 generation was retested in R and confirmed the F_1 data.

Growth of Plants for Phytochrome Assays

Seeds of the *tri*^{1} mutant and WT were briefly surface sterilized under WL with a 1% (v/v) solution of commercial bleach for 3 min and then washed thoroughly with running tap water. Seeds were then sown on 0.6% (w/v) agar medium containing 0.46 g L^{-1} of Murashige-Skoog salts (GIBCO-BRL) in plant tissue culture containers obtained from Flow Laboratories (McLean, VA). Seedlings were grown at 25°C for 4 d either in darkness or irradiated with R (20 μmol m^{-2} s^{-1}; white fluorescent tubes [FL20S.W.SDL.NU; National, Tokyo, Japan] filtered through 3-mm red acrylic [Shinkolite A102; Mitsibutsi Rayon, Tokyo]) for 4 h prior to harvest. The upper 1 cm of the hypocotyls, including the cotyledons, were harvested under a dim-green safelight after gently removing any remaining seedcoats. For in vivo spectrophotometry the samples were collected on ice and used immediately. The samples for immunoblotting were frozen in liquid nitrogen and stored at −80°C before analysis.

Plants were also grown in pots containing a 2:3 (v/v) granular clay-based compost:vermiculite mixture in a phytotron (Koitotron KG-206HL-D, Koito, Tokyo) at 25°C with a daily regime of 16-h white fluorescent light (150 μmol m^{-2} s^{-1})/8 h of darkness. Twenty-one days after sowing, leaf samples were harvested (second and third leaves, only leaflets were used), frozen in liquid nitrogen, and stored at −80°C before extraction for immunoblotting.

In Vivo Phytochrome Spectrophotometry

For the spectrophotometric measurements of phytochrome, about 0.4 g of tissue (collected from 40 seedlings) were gently packed into a custom-built, stainless-steel cuvette with glass windows (10 mm in diameter and about a 4-mm path length), and the phytochrome content was measured as the difference in *A* between 730 and 800 nm [$\Delta(\Delta A)$] in a dual-wavelength spectrophotometer (model 557; Hitachi, Tokyo, Japan), which was equipped with an actinic irradiation unit for photoconverting the sample with saturating irradiations of R (30 s) and FR (60 s).

Phytochrome Extraction and Immunoblotting

About 0.2 g (collected from 20 seedlings) of frozen material was homogenized just before use in a microfuge tube at 4°C using a homogenizer fitting the tube at full speed for 1 min after adding 20 mg of insoluble PVP in 0.2 mL of extraction buffer (100 mM Tris-HCl [pH 8.3], 50% [v/v] ethylene glycol, 140 mM ammonium sulfate, 56 mM 2-mercaptoethanol, 20 mM sodium bisulfate, 10 mM EDTA, 4 mM PMSF, 4 mM iodoacetamide), which was adjusted to 1 μg mL^{-1} pepstatin A, 2 μg mL^{-1} aprotinin, and 2 μg mL^{-1} leupeptin. The homogenate was centrifuged at 0°C for 15 min at 18,000*g* in a microfuge. The supernatant was mixed directly with 2× standard concentration SDS-sample buffer (Laemmli, 1970) and dissolved at 100°C for 2 min. Then 5 μL was immediately used for the SDS-PAGE and the remainder was stored at −20°C for further analysis.

About 0.5 g of frozen leaves were homogenized after adding 50 mg of insoluble PVP in 0.5 mL of extraction buffer, using a blender (Physcotron, Niti-on Co., Tokyo, Japan) at full speed for 1 min. The homogenate was centrifuged at 0°C for 15 min at 15,000*g*. The supernatant was collected and polyethyleneimine was added to a final concentration of 0.1%. The extract was vortexed and centrifuged for 10 min at 12,000*g*. The supernatant was precipitated by adding 0.725 volumes of saturated ammonium sulfate solution. The precipitate was collected by centrifugation at 12,000*g* for 15 min, directly resuspended into SDS-sample buffer, and dissolved at 100°C for 2 min, 4 μL was directly used for the SDS-PAGE, and the remainder was stored at −20°C for further analysis.

Proteins were electrophoresed in 6.5% SDS-polyacrylamide gels, using prestained molecular mass standards (SDS-7B markers, Sigma). The apparent molecular masses of these prestained markers were recalibrated using high molecular mass standards (SDS-6H markers, Sigma) and then electroblotted onto a nylon filter (FineBlott; Atto, Tokyo, Japan) in 100 mM Tris-HCl, 192 mM Gly, and 20% (v/v) methanol. The membranes were blocked in a series of Tris-HCl buffer-saline-Tween solutions, all containing 20 mM Tris-HCl, pH 7.5, and varying Tween-20 and NaCl concentrations: 2% (v/v) Tween and 500 mM NaCl for 3 min; 0.05% (v/v) Tween and 500 mM NaCl for 10 min; 0.05% (v/v) Tween and 150 mM NaCl for 3 min. Incubation with the primary antibody was in 20 mM Tris-HCl, pH 7.5, 150 mM NaCl, and 1% (w/v) fat-free milk powder. The monoclonal anti-PHYA and anti-PHYB antibodies used were mAP5 (Nagatani et al., 1985) and mAT1 (López-Juez et al., 1992) in dilutions of 2 μg mL^{-1} and a 1:1 dilution of hybridoma culture supernatant, respectively. The incubation was at room temperature for 2 h, after washing three times with Tris-HCl buffer-saline-Tween, as at the end of the blocking, and membranes were incubated with a 1:5000 dilution of goat anti-mouse IgG conjugated to alkaline phosphatase (Protoblot kit; Promega) for 45 min, washed, and stained for alkaline phosphatase according to the manufacturer's instructions.

Pretreatment of the Seeds

To obtain a higher germination percentage, the seeds used in the EODFR, pulse, delayed R, and broad-band light experiments, as well as those used for genetic analysis, were pretreated before the final sowing. The seeds were therefore sown in 9 × 9-cm plastic boxes on one layer of thick, absorbent paper (T300–45 mm, Schut B.V., Heelsum, The Netherlands), moistened with 7.4 mL of germination buffer (0.01 M $NaH_2PO_4.H_2O$, 0.01 M $K_2HPO_4.3H_2O$, 5 mM KNO_3, pH 7.5), and placed in a darkroom at 25°C. After 2 d of pretreatment, the seeds were planted out under a dim-green safelight in trays filled with a mixture of potting compost and sand (volume ratio 3:1).

Continuous Broad-Band Light Experiment

Pretreated seeds sown in trays were incubated in D for 72 h at 25°C. The irradiation with continuous B, R, and FR (3 μmol m^{-2} s^{-1}) was started just before the seedlings emerged through the soil surface. The length of 20 hypocotyls was measured daily for 7 d with a ruler under a dim-green safelight. In addition, the hypocotyl length of seedlings grown in D was also measured daily. At the end of the experiment, the seedlings (four replicates) were used for the determination of anthocyanin in hypocotyls and Chl in cotyledons and for the measurement of cotyledon area.

For the determination of anthocyanin, samples of five hypocotyls were extracted with 1.2 mL of acidified (1% [w/v] HCl) methanol for 24 h in D with shaking. A Folch partitioning (Folch et al., 1957) was performed by adding 0.9 mL of H_2O and 2.4 mL of chloroform to the extracts and centrifuging it for 30 min at 3600 rpm. The A_{535} of the top phase was determined with a Beckman DU-64 spectrophotometer.

The cotyledon area was measured with a leaf-analysis system (Skye Instruments Ltd, Powys, UK). For Chl extraction, samples of 10 cotyledons were weighed, placed in glass tubes, immersed in a 100 times excess volume of *N,N*-dimethylformamide (w/v) (Moran, 1982), and incubated in D for 24 h. The A_{647} and A_{664} of the extracts were measured, and Chl content was calculated on a fresh weight basis using the equations published by Inskeep and Bloom (1985).

Pulse Experiment

Pretreated seeds sown in trays were incubated in D for 48 h at 25°C. Pulses of R (3 min, 10 μmol m^{-2} s^{-1}) or R immediately followed by FR (6 min, 13 μmol m^{-2} s^{-1}), both saturating for attaining phytochrome photoequilibrium, were given every 4 h beginning at the time of emergence of the first seedlings. During the pulse irradiation, every seedling was marked on emergence, enabling the measurement of hypocotyl growth of each seedling after the appropriate number of pulses (6, 12, 18, or 24). For each treatment, 15 to 30 seedlings were measured.

Delayed R Experiment

Pretreated seeds sown in trays were either placed in D or in a continuous R cabinet at 25°C. After 72 h all seedlings that had just emerged were marked (d 1) and measured daily for 7 d with a ruler under a dim-green safelight. In addition, after the measurement of seedlings grown in D at d 1 and d 3, some were transferred to continuous R (1 d D → R; 3 d D → R). For each treatment, 10 to 25 seedlings were measured.

EODFR Experiment

Pretreated seeds sown in trays were grown for 12 d in a phytotron with a daily irradiation schedule of 16 h of WL (PAR, 190 μmol m^{-2} s^{-1})/8 h of D at 25°C and RH of 65 to 70%. At d 13 the seedlings were transplanted into 10-cm square plastic pots, and after transfer to cabinets at d 16, they were allowed to adjust to the lower level of WL (PAR, 125 μmol m^{-2} s^{-1}) for 1 d before the start of the experimental treatment. Plants were then selected for uniform height, and after the daily WL period they received an immediate 20 min of FR irradiation (4.6 μmol m^{-2} s^{-1}). The controls were grown in a similar cabinet and received no FR irradiation. Plant height (six plants per treatment) was measured during a 15-d EODFR treatment.

Supplementary Daytime FR Experiment

Seedlings of the *tri*^{1} mutant and its isogenic WT GT were raised from seed at 25°C in a potting compost/sand mixture in a 16-h WL (PAR, 170 μmol m^{-2} s^{-1})/8-h D cycle for 7 d. The plants were then transplanted into 10-cm diameter pots and transferred to a cabinet with the same cycle but higher irradiance (PAR, 250 μmol m^{-2} s^{-1}) that had a R:FR photon ratio of 6.90. After 18 d the plants were transferred to two cabinets with a similar light/dark cycle, one of which had additional FR, which is not photosynthetically active and reduces the R:FR photon ratio to 0.13. All other environmental conditions within the cabinets were identical, with a 16-h photoperiod at a constant temperature of 25°C day/night and RH of 70%. Plant height (six plants per treatment) was measured during a 6-d period of light treatment.

Light Sources

The broad-band B, R, and FR cabinets used for the screening of mutants, the broad-band and delayed-R experiments, and genetic analysis were the same as described by Koornneef et al. (1980).

For the pulse experiment, R was obtained from light-emitting diodes (NLS01, 660-nm peak, half-bandwidth 25 nm, Nijssen Light Division BV, Wageningen, The Netherlands), whereas the FR source was the same as that described by Koornneef et al. (1980).

The EODFR experiment was carried out in cabinets described earlier by Joustra (1970). WL was obtained from Philips TL40/33 fluorescent tubes. FR was provided by Sylvania F48T12/232/VHO tubes wrapped with one layer of dark-green and one layer of primary-red filter (Lee, Flashlight Sales BV, Utrecht, The Netherlands).

The fluence rates and exposure times used are given in the description of each experiment. All light measurements were made using a LI1800/12 spectroradiometer (Li-Cor, Lincoln, NE)

RESULTS AND DISCUSSION

Mutant Isolation and Genetic Characterization

The M_2 populations of tomato derived from ethyl methanesulfonate-treated seeds were screened under WL (GT background) or continuous B and R (MM background). Two independently induced mutants, C66 and B10, were selected for their slightly longer hypocotyls in WL in the M_2 generation derived from 1650 M_1 plants (experiments II and III in Koornneef et al., 1990). A third mutant, sc72, was isolated because of its longer hypocotyl in WL as a somaclonal variant in experiments described by Van den Bulk et al. (1990), which involved testing of 1052 progenies of regenerated MM plants. The fourth mutant, 2–19ARL, was selected for its longer hypocotyl under continuous broad-band R in M_2 material that was described by Van Tuinen et al. (1995). In broad-band spectral study experiments, all the mutants showed a reduced hypocotyl growth inhibition in R. Genetic complementation analysis showed that the four mutants were allelic. Since the mutants are insensitive to R only during the first 2 d of R treatment (see Fig. 7), we propose the gene symbol *tri* (temporarily red light insensitive) for these mutants. The different alleles have been numbered in order of isolation, i.e. *tri*^{1} = C66; *tri*^{2} = B10; *tri*^{3} = sc72; and *tri*^{4} = 2–19ARL. Under continuous R the progeny of selfed F_1 plants from the cross between the new mutant lines and the WT parent segregated in a 3:1 (χ^2 = 0.92, $P > 0.05$ for the F_2 WT × *tri*^{1} [Fig. 1]) ratio of normal to elongated hypocotyls and normal to small cotyledons expected for a monogenic recessive mutation. Figure 1 shows that the hypocotyl length of the heterozygote F_1 is slightly longer than that of the WT parent. This partial dominance of the mutation is a feature expected for a rate-limiting component, such as a photoreceptor, and has previously been observed for the phyB-deficient *hy3* (Koornneef et al., 1980) and the phyA-deficient *fhy2* (= *phyA*) (Whitelam et al., 1993) mutants of Arabidopsis, the *fri* mutants of tomato (Van Tuinen et al., 1995), and the *ma*$_3^R$ mutant of *Sorghum bicolor*, which lacks a phytochrome that predominates in green tissue (Childs et al., 1992; Foster et al., 1994).

Phenotypes of the *tri* Mutants

Under broad-band spectral light sources we have examined hypocotyl length, cotyledon area, and anthocyanin and Chl content for plants homozygous for the *tri* mutation. There is no difference between the WT and *tri*^{1} and *tri*^{3} mutants in D, and under FR. In B the hypocotyls of the *tri*^{1} and *tri*^{3} mutants are slightly elongated compared to their respective WT. In R, however, the mutants are characterized by a longer hypocotyl, less anthocyanin, and smaller, darker-green cotyledons than the WT (Figs. 2 and 3). There is an inverse relationship between cotyledon area and Chl content expressed on a fresh weight basis, suggesting that total Chl production is little influenced by the mutation. Figure 3 also shows that anthocyanin accumulation and cotyledon area are both affected by genetic background. However, the *tri*^{1} and *tri*^{3} mutant phenotypes are qualitatively similar.

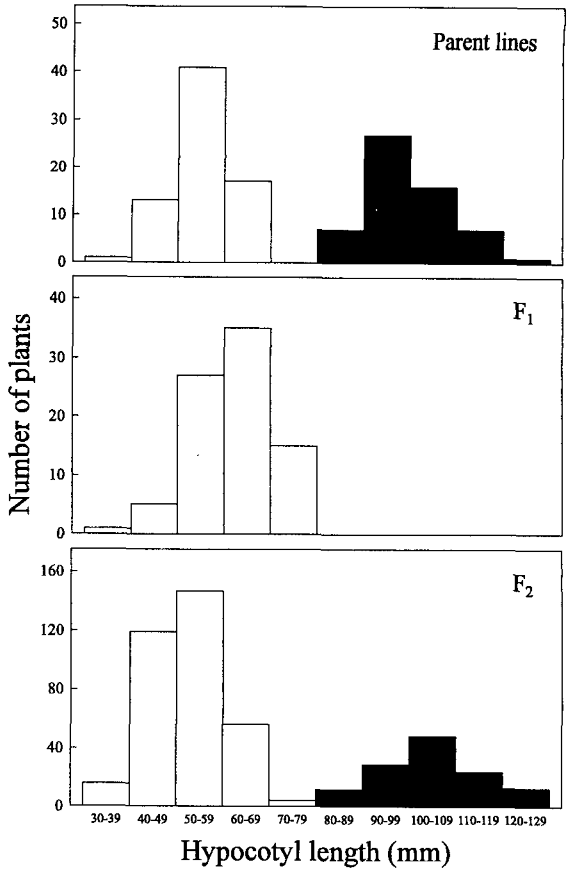

Figure 1. Frequency distribution of hypocotyl length of tomato seedlings of the WT (open bars), *tri*^{1} mutant (filled bars), and F_1 and F_2 generations after 7 d of continuous R (3 μmol m^{-2} s^{-1}).

When grown in a 16-h WL/8-h D cycle, the hypocotyl of the *tri*^{1} mutant is slightly elongated at the seedling stage (Fig. 4). The difference in height between the *tri* mutants and their isogenic WTs becomes more apparent with age, and the young immature leaves possess less anthocyanin (data not shown).

Mutants with a similar phenotype in R, but that exhibit hypocotyl inhibition by other wavelengths, have been shown to be phyB deficient in Arabidopsis (Nagatani et al., 1991; Reed et al., 1993) or to lack a phyB-like phytochrome, as in the cucumber *lh* mutant (López-Juez et al., 1992) and the *Brassica ein* mutant (Devlin et al., 1992).

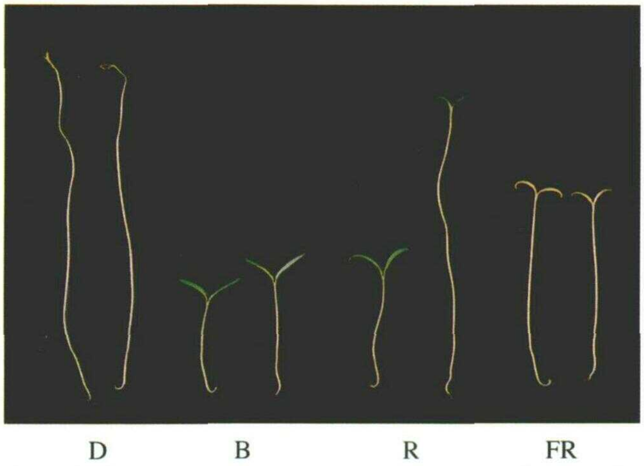

Figure 2. The phenotype of tomato seedlings grown for 7 d after emergence in D and continuous broad-band B, R, and FR of 3 µmol m^{-2} s^{-1}. For each treatment the seedling on the left is the wild type and that on the right is the *tri*^{1} mutant.

Immunochemical and in Vivo Spectrophotometrical Analysis of Phytochrome

In extracts of etiolated seedlings of the *tri*^{1} mutant, an immunochemically detectable phyB-like polypeptide (PHYB), readily detectable in WT extracts, is absent or below detection limits, yet it contains WT levels of phyA apoprotein (Fig. 5). Spectrophotometric analysis showed that the light-labile phyA pool is depleted after 4 h of R (Van Tuinen et al., 1995). The difference in $\Delta(\Delta A)$ between the WT and the reduced level observed in the *tri*^{1} mutant presumably represents the lack of a phyB-like, stable apoprotein in the *tri*^{1} mutant. These results resemble those found by Peters et al. (1991) for the cucumber *lh* mutant, which also lacks a phyB-like apoprotein (López-Juez et al., 1992).

Immunoblot analysis of WL-grown tissue (Fig. 6) revealed that the phyB-like apoprotein is not only absent in etiolated mutant seedlings, but remains absent in 3-week-old plants.

LFR Experiments

Phytochrome not only exists in multiple types, but also works via different modes: the LFR, which is R/FR reversible, and a high-irradiance response, which is irradiance and duration dependent (Mancinelli, 1994).

The phyB-like-deficient *tri* mutants, in contrast to the phyA-deficient *fri* mutants, show little hypocotyl growth inhibition in continuous broad-band R (Fig. 3). Since spectrophotometric analysis has shown that the phyA pool is depleted after 4 h of R (Van Tuinen et al., 1995), phyB and/or other light-stable-type phytochrome(s) must play the major role in growth inhibition under continuous R. We tested the involvement of an LFR in hypocotyl growth inhibition with pulses of R or R immediately followed by FR (both saturating for phytochrome photoconversion) given every 4 h on hypocotyl growth inhibition. Figure 7 shows that the *tri*^{1} mutant is insensitive to R only during the first 2 d of pulse treatment. Thereafter the inhibitory effect of R on hypocotyl elongation growth and FR reversibility are retained in the *tri* mutants. Since the phyB-like phytochrome is still below detection limits in older WL-grown plants of the *tri*^{1} mutant (Fig. 6), the temporal appearance of responsiveness to R cannot be explained by a delay in appearance of the phyB-like phytochrome.

Delayed R Experiments

To test whether the 2-d period of insensitivity to R for hypocotyl growth inhibition of the *tri* mutant depends on a

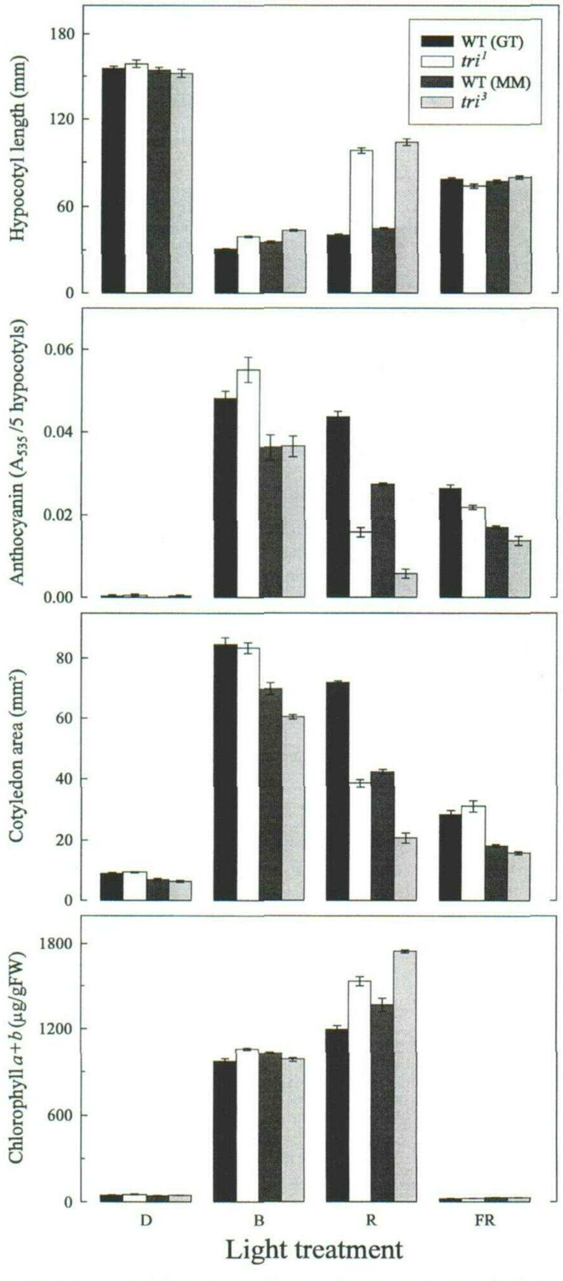

Figure 3. Hypocotyl length, anthocyanin content, cotyledon area, and Chl content of tomato WT and *tri*^{1}- and *tri*^{3}-mutant seedlings after 7 d of continuous D, B, R, or FR of 3 µmol m^{-2} s^{-1}. The mean hypocotyl length of 20 seedlings from each light treatment is plotted. Error bars represent the SE.

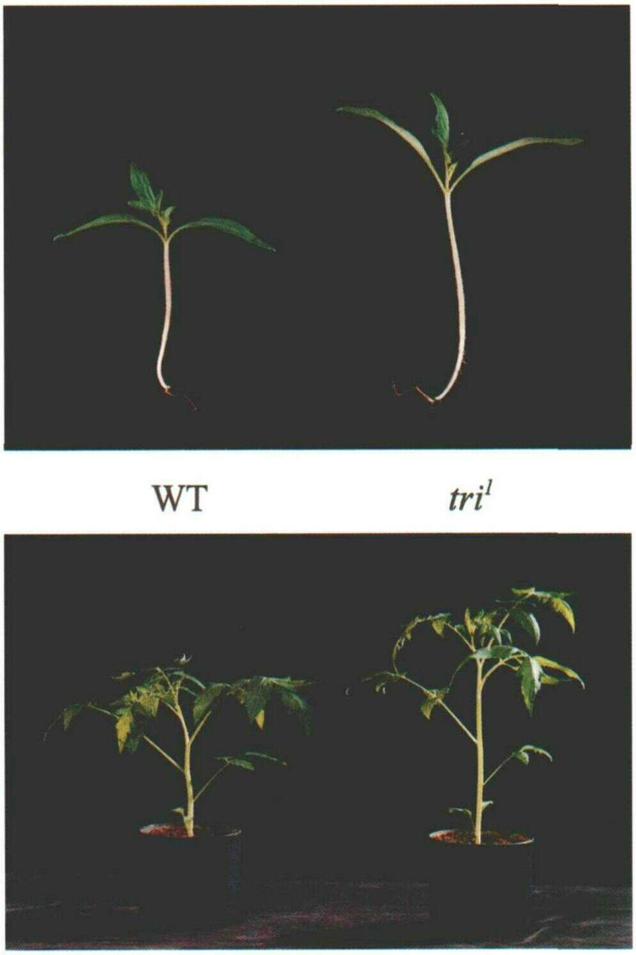

Figure 4. WT and tri^1-mutant tomato seedlings grown for 7 (top) or 28 (bottom) d in a 16-h WL (PAR, 175 and 100 μmol m^{-2} s^{-1}, respectively)/8-h D cycle at 25°C.

temporal pattern of development or the time after a transfer from D, seedlings were grown in D or continuous R, or were grown and kept in D for 1, 2 (data not shown), or 3 d after emergence before transfer to continuous R. The WT exhibits a significant response to R within 24 h after transfer from D to R, whereas the tri^1 mutant stays insensitive to R for 2 d after the transfer from D to R (Fig. 8) irrespective of the length of the preceding D period.

Responses to End-of-Day and Supplementary Daytime FR

The tri^1 mutant responds to EODFR treatment with an increase in plant height that is quantitatively similar to WT, although the absolute height of the mutant is somewhat greater (Fig. 9).

Both WL-grown WT and tri^1 mutant plants also show a typical promotion of elongation growth in response to supplementary FR during the daily photoperiod (Fig. 10). The response is apparently slightly less in the tri^1 mutant, but this could be due to attainment of the maximal growth possible under these conditions.

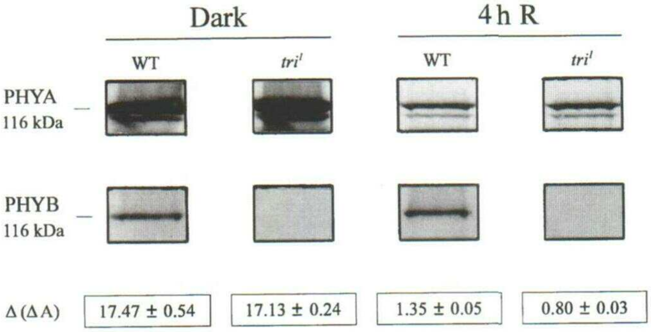

Figure 5. Immunoblot detection of phyA and phyB polypeptide (PHYA and PHYB, respectively) and in vivo measurement of spectral activity of phytochrome in WT and tri^1-mutant seedlings. Dark-grown 4-d-old seedlings or seedlings of the same age exposed to 4 h of R were used for the detection of PHYA and PHYB, with monoclonal antibodies mAP5 and mAT1, respectively, in crude extracts. The phytochrome content was measured using a dual-wavelength spectrophotometer and is expressed as $\Delta(\Delta A)$/40 hypocotyl sections.

The Role of PhyB in Tomato

The tri^1 mutant of tomato resembles the *hy3* (= *phyB*) mutant of Arabidopsis (Reed et al., 1993), the cucumber *lh* mutant (Adamse et al., 1987; López-Juez et al., 1992), and the *Brassica ein* mutant (Devlin et al., 1992) in such characteristics as a longer hypocotyl, reduced anthocyanin content, and smaller cotyledons in continuous broad-band R (Fig. 3) and the absence of a phyB-like apoprotein compared to the WT (Fig. 5).

In contrast to the *hy3*, *ein*, and *lh* mutants, in which the inhibition of hypocotyl growth and cotyledon expansion in R is essentially lost, the tri^1 mutant is insensitive to R only during the first 2 d upon transition from D to R (Figs. 7 and 8). This results in a phenotype in continuous R with longer hypocotyls and smaller cotyledons, but less extreme, for instance, than that of the almost completely R- and FR-blind tomato *au* mutant (Koornneef et al., 1985).

The EODFR response (Fig. 9) and the effect of supplementary daytime FR (Fig. 10), commonly accepted to be regulated by phyB (Adamse et al., 1987; Devlin et al., 1992; López-Juez et al., 1992; Reed et al., 1993), however, are present in the tri^1 mutant. Preliminary experiments indicate that another phyB-mediated response, simulated phototropism as a result of covering one of the cotyledons with aluminum foil, is also present in the *tri* mutant (results not shown). The fact that the *tri* mutant, which lacks a phyB-like apoprotein, is only temporarily insensitive to R and still responds to EODFR and supplementary daytime FR

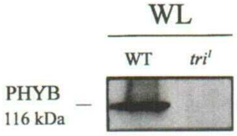

Figure 6. Immunoblot detection of phyB polypeptide (PHYB) with monoclonal antibody mAT1 in crude extracts of 21-d-old WT and tri^1 mutant plants. Plants were grown in a phytotron at 25°C with a daily regime of 16 h of WL (PAR, 150 μmol m^{-2} s^{-1}) and 8 h of D.

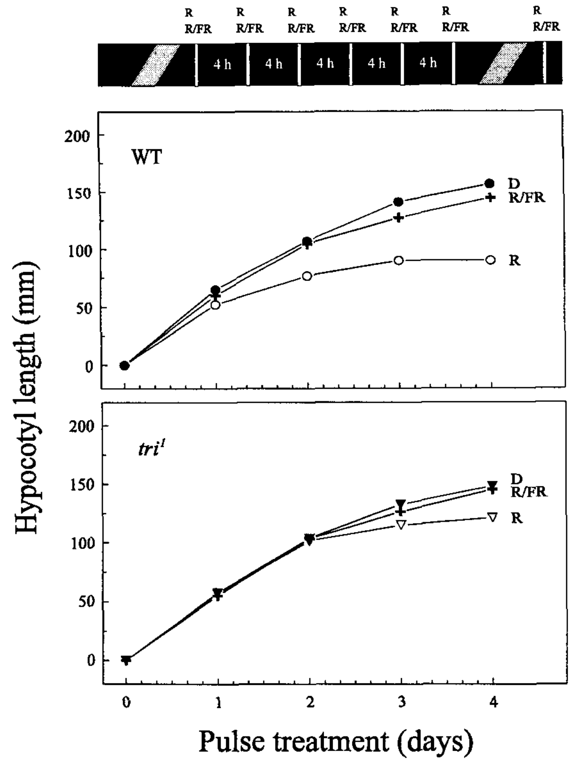

Figure 7. Hypocotyl length of WT and the *tri*^{1} mutant seedlings treated with pulses of R or R immediately followed by FR and a D control. The R and FR pulses (both saturating for phytochrome photoconversion) were repeated every 4 h from the time of emergence. The SE in all cases was smaller than the symbols used.

treatment distinguishes this mutant from previously described phyB-deficient mutants in other species (Whitelam and Smith, 1991). The temporal insensitivity to R can be explained by assuming that other stable phytochromes can perform physiological functions similar to the phyB-like phytochrome absent in the *tri*^{1} mutant. The identification of multiple phytochrome genes, including two phyB-like genes, in tomato (Hauser et al., 1994; Pratt, 1995) implies that this might be possible. However, the complete insensitivity of the *tri* mutants to R in the 2 d after the transition from D to R could suggest that at this stage the other phytochrome genes are not expressed or do not function. We have eliminated the possibility that the appearance of sensitivity is due to a delayed appearance of the phyB-like phytochrome in the *tri*^{1} mutant, since it is still below detection limits in light-grown plants.

The *tri* mutants are the first examples of mutants that indicate that a process thought to be regulated by phyB exclusively seems, at least in tomato, to involve more than one light-stable type of phytochrome. It is interesting to note that the PHYB antibody used in this study (mAT1) was the same as that which detected the absence of a phyB-like apoprotein in the cucumber *lh* mutant (López-Juez et al., 1992), again supporting the difference between tomato and other species. It should be noted, however, that the apparent insensitivity to supplementary FR on flowering of the *hy3* mutants is not found when they are studied

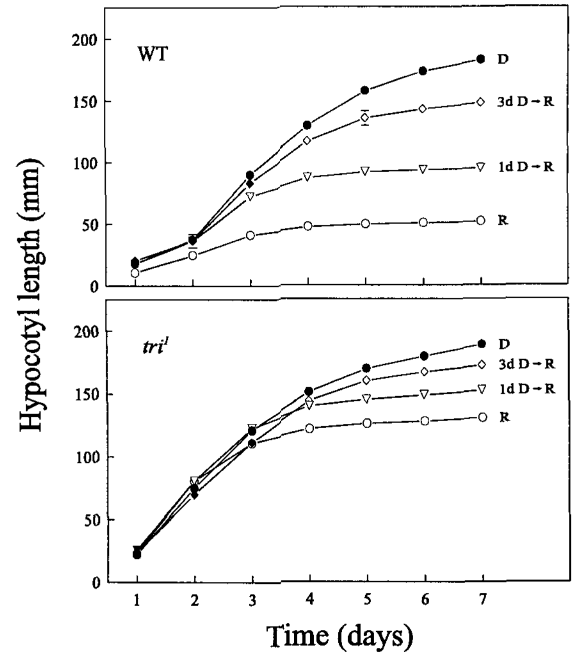

Figure 8. Hypocotyl length of WT and the *tri*^{1} mutant seedlings. Seedlings were grown for 7 d in D (●), continuous R (3 μmol m^{-2} s^{-1}, ○), or transferred to R (open symbols) after a D period of 1 (1d, ▽) or 3 d (3d, ◇).

in a more extreme genetic, and therefore a more sensitive, background (Halliday et al., 1994), which has also been explained by the proposal of the action of other light-stable phytochromes. A more detailed molecular analysis of the different phytochrome genes in the *tri* mutants is required for the ultimate proof of the relationship between the physiological defects of the *tri* mutants and a specific phytochrome gene. A detailed analysis of the various phy-

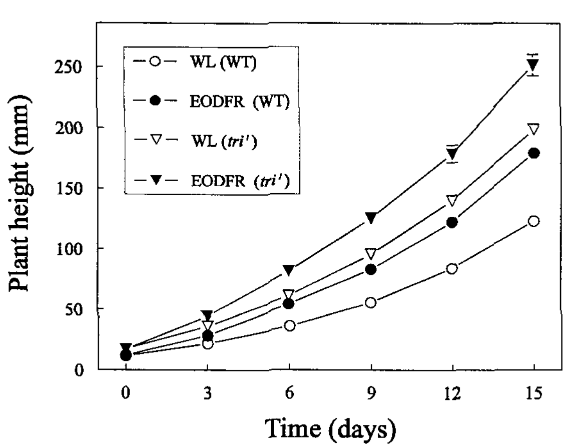

Figure 9. Plant height of WT and *tri*^{1} mutant plants. After the 16-h daily WL (PAR, 125 μmol m^{-2} s^{-1}) period, plants were either submitted to an immediate 8-h D period or given a 20-min FR pulse before the D period. Plant height was measured every 3rd d during a 15-d period of daily cycles with an EODFR treatment. Error bars represent the SE.

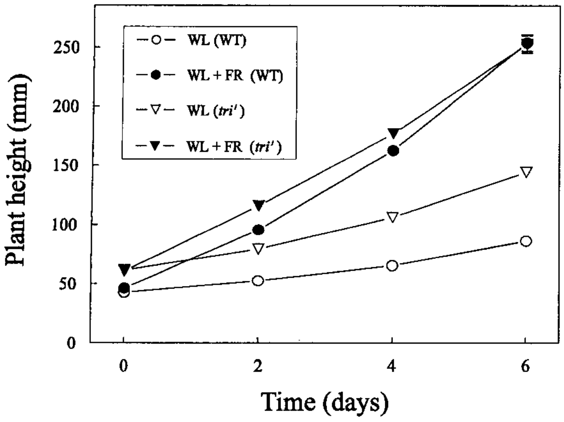

Figure 10. Plant height of WT and *tri*^{1} mutant plants under conditions of 16-h WL (PAR, 250 μmol m^{-2} s^{-1})/8-h D or the same WL photoperiod with supplementary FR (WL + FR). Error bars represent the SE.

tochrome-induced processes, using well-characterized mutants and double mutants lacking specific phytochrome types, will be essential for our full understanding of light-controlled plant development.

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