Mol Gen Genet (1995) 246:133–141

ORIGINAL PAPER

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Far-red light-insensitive, phytochrome A-deficient mutants of tomato

Received: 9 June 1994 / Accepted: 20 July 1994

Abstract We have selected two recessive mutants of tomato with slightly longer hypocotyls than the wild type, one under low fluence rate (3 μmol/m²/s) red light (R) and the other under low fluence rate blue light. These two mutants were shown to be allelic and further analysis revealed that hypocotyl growth was totally insensitive to far-red light (FR). We propose the gene symbol *fri* (far-red light insensitive) for this locus and have mapped it on chromosome 10. Immunochemically detectable phytochrome A polypeptide is essentially absent in the *fri* mutants as is the bulk spectrophotometrically detectable labile phytochrome pool in etiolated seedlings. A phytochrome B-like polypeptide is present in normal amounts and a small stable phytochrome pool can be readily detected by spectrophotometry in the *fri* mutants. Inhibition of hypocotyl growth by a R pulse given every 4 h is quantitatively similar in the *fri* mutants and wild type and the effect is to a large extent reversible if R pulses are followed immediately by a FR pulse. After 7 days in darkness, both *fri* mutants and the wild type become green on transfer to white light, but after 7 days in FR, the wild-type seedlings that have expanded their cotyledons lose their capacity to green in white light, while the *fri* mutants de-etiolate. Adult plants of the *fri* mutants show retarded growth and are prone to wilting, but exhibit a normal elongation response to FR given at the end of the daily photoperiod. The inhibition of seed germination by continuous FR exhibited by the wild type is normal in the *fri* mutants. It is proposed that these *fri* mutants are putative phytochrome A mutants which have normal pools of other phytochromes.

Key words *Lycopersicon* · Photomorphogenesis
Physiological mutants · De-etiolation

Communicated by A. Kondorosi

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Introduction

The control of plant development by light involves different groups of photoreceptors: those that absorb in the blue light (B), UV-A, UV-B, and the red light (R)/far-red light (FR)-absorbing phytochromes. Together they control processes of seedling development such as inhibition of hypocotyl growth, opening of the apical hook, expansion of the cotyledons and the accumulation of anthocyanin. Mutants deficient in one of these photoreceptors can be used to reveal the contribution of the various photoreceptors and their interaction in the regulation of photomorphogenic responses.

In *Arabidopsis thaliana*, the isolation and characterization of the phytochrome-deficient mutants (reviewed by Koornneef and Kendrick 1994), the *blu* mutants (Liscum and Hangarter 1991), and the evidence that the *HY4* gene encodes a protein with characteristics of a B photoreceptor (Ahmad and Cashmore 1993), proves that mutants for different photoreceptors can be isolated and are useful tools for dissection of the complex network of light-regulated responses in photomorphogenesis. Furthermore, Young et al. (1992) using the *blu* and *hy6* mutants and the *blu,hy6* double mutant found that the UV-A photosensory system caused inhibition of hypocotyl elongation by a process independent of the phytochrome and B photosensory systems. The fact that phototropism and B inhibition of hypocotyl elongation are genetically separable (Liscum et al. 1992) in-

dicates that these processes may be controlled by different B photoreceptors. Not only the B photoreceptor system but also the R/FR-absorbing phytochrome system is more complex than initially thought. For instance, in *Arabidopsis* the phytochrome family consists of at least five different genes referred to as *PHYA–E*, which encode apophytochrome PHYA–E, and form holophytochrome phyA–E, respectively, after insertion of the chromophore (Quail 1994). To study the role of each phytochrome species in photomorphogenesis, mutants are needed which are deficient in one specific type of phytochrome. So far, three types of phytochrome mutants have been characterized. Mutants deficient in phyB have been reported in several species, including *Arabidopsis* (Reed et al. 1993), cucumber (López-Juez et al. 1992) and *Brassica rapa* (Devlin et al. 1992), whereas mutants lacking phyA have only more recently been characterized in *Arabidopsis* (Dehesh et al. 1993; Nagatani et al. 1993; Parks and Quail 1993; Whitelam et al. 1993). The third type of mutant, represented by the *hy1* and *hy2* mutants of *Arabidopsis* (Parks and Quail 1991) and the recently described *pew* mutants of *Nicotiana plumbaginifolia* (Kraepiel et al. 1994), are apparently deficient in the biosynthesis of the phytochrome chromophore.

In tomato, which is an attractive model system because of its favourable size for physiological experiments, the extensively studied *aurea* mutant has been considered a phyA-deficient mutant. However, there are indications that this mutant might be a chromophore mutant (Sharma et al. 1993; A. van Tuinen, unpublished data). Since all phytochromes share the same chromophore, a chromophore mutant will presumably be deficient in all types of phytochrome. In an endeavour to search for type-specific phytochrome mutants in tomato, we have screened for mutants at different wavelengths in an attempt to avoid escape of mutants with minor phenotypic effects in white light (W). In this paper we present the isolation and characterization of a mutant deficient in immunologically and spectrophotometrically active phyA.

Materials and methods

Plant material

Mutants were obtained by treating seeds of tomato (*Lycopersicon esculentum* Mill.) cv. Moneymaker (MM) with ethyl methanesulphonate (EMS) for 24 h in darkness at 25°C (Koornneef et al. 1990). The population of M_1 plants was divided into groups of approximately ten plants and, from each group, M_2 seeds were harvested. The M_2 seed groups were screened for mutants with phenotypes deviating from wild type (WT) in broad band R and B.

Genetic characterization

Seedlings used in all types of genetic analyses were grown for 7 days after emergence under continuous FR (3 μmol/m²/s). Segregation ratios were determined by counting the number of seedlings with WT and mutant hypocotyl length. The mutants obtained were tested for allelism versus non-allelism on the basis of non-complementation versus complementation to the WT phenotype in the F_1 plants.

Linkage analysis was done in F_2 populations derived from the cross of the *fri*[2] mutant with linkage marker stock LA780, obtained from C. M. Rick and homozygous recessive for markers *yv*, *c*, *h*, *ag*, *r* and *j*, but WT for the *u* gene (U^+) for which the cv. MM is recessive. Most of the Fri^+ seedlings did not survive the transition to W after the 7-day FR period necessary for the identification of *fri* seedlings. The recombination percentages are based on the ratio of *fri* plants recessive for a given marker, as a fraction of the total number of *fri* seedlings tested (b) and estimated with the following formulae:

$$r_R = 100\sqrt{b} \qquad r_C = 1 - r_R$$

$$s_r = \frac{100}{2\sqrt{b}}\sqrt{\frac{b(1-b)}{n}}$$

where

n = total number of plants; r_R = estimate of recombination percentage for repulsion phase, r_C for coupling phase; s_r = standard deviation of r.

Map positions were calculated from the estimated recombination percentages using the computer program JOINMAP described by Stam (1993).

Growth of plants for phytochrome assays

Seeds of the *fri*[1] mutant and WT were briefly incubated in W with a 1% (v/v) solution of commercial bleach for 3 min and then washed thoroughly with running tap water. Seeds were sown on 0.6% (w/v) agar medium containing 0.46 g/l of Murashige-Skoog salts (Gibco, Gaithersburg, Md., USA) in plant tissue culture containers obtained from Flow Laboratories (McLean, Va., USA). Seedlings were grown at 25°C for 4 days either in darkness or irradiated with R (20 μmol/m²/s; 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, was harvested under 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.

In vivo phytochrome spectrophotometry

For the spectrophotometric measurements of phytochrome, about 0.4 g tissue (collected from 40 seedlings) was gently packed into a custom-built stainless steel cuvette with glass windows (10 mm in diameter and about 4 mm path length) and the phytochrome content was measured in a dual-wavelength spectrophotometer (model 557; Hitachi, Tokyo, Japan) using 730 and 800 nm measuring beams, 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) frozen material was homogenized after adding 20 mg insoluble polyvinylpyrrolidone in 0.2 ml extraction buffer (100 mM TRIS-HCl pH 8.3, 50% v/v ethylene glycol, 140 mM ammonium sulphate, 56 mM 2-mercaptoethanol, 20 mM sodium bisulphate, 10 mM EDTA, 4 mM phenylmethylsulphonyl fluoride, 4 mM iodoacetamide), which was adjusted to 1 μg/ml pepstatin A, 2 μg/ml aprotinin and 2 μg/ml leupeptin just before use in a microcentrifuge tube at 4°C, using

an homogenizer fitting the tube at full speed for 1 min. The homogenate was centrifuged at 0°C for 15 min at 18000 × *g* in a microcentrifuge. The supernatant was mixed directly with 2 × SDS-sample buffer (Laemmli 1970) and dissolved at 100°C for 2 min. Then, 5 µl was immediately used for 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, St. Louis, Mo., USA). The apparent molecular mass of these prestained markers was 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 glycine and 20% (v/v) methanol. The membranes were blocked in a series of TRIS-HCl buffer-saline Tween (TBST) 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. 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 TBST, as at the end of the blocking; membranes were incubated with a 1:5000 dilution of goat anti-mouse IgG conjugated to alkaline phosphatase (Protoblot kit; Promega, Madison, Wis., USA) 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 end-of-day FR (EODFR), pulse, and broad-band light experiments, and in the genetic analysis were pretreated before the final sowing. The seeds were sown in 9 × 9 cm refrigerator boxes on one layer of thick filter paper (T300-45 mm, Schut, Heelsum, The Netherlands) moistened with 7.4 ml 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 wooden boxes. The boxes were put in a darkroom with a temperature of 25°C and relative humidity of 50–60%. After 2 days pretreatment, the seeds were planted out under dim green safe light in trays (30 × 20 × 5 cm) filled with a mixture of potting compost and sand (ratio 3:1) for the genetic analysis, pulse and broad-band light experiments.

Continuous broad-band light experiment

Pretreated seeds sown in trays were incubated in darkness (D) for 72 h at 25°C. The irradiation with continuous B, R and FR (3 µmol/m^2/s) was started just before the seedlings emerged through the soil surface. The length of each hypocotyl was measured daily for 7 days with a ruler under dim green safe light. In addition, the hypocotyl length of plants grown in absolute D for the duration of the experiment was measured.

De-etiolation experiments

The pretreated WT, *fri*^{1} and *fri*^{2} seeds were sown in trays and incubated in D for 56 h at 25°C. At that time the first seedlings started to emerge and the trays were either kept in D or transferred to FR (3 µmol/m^2/s). Seedlings emerging during the following 12 h period were marked, allowed to grow for 7 days and then transferred to W [photosynthetically active irradiance (PAR, 400–700 nm), 110 µmol/m^2/s). At 6, 12, 24 and 48 h after transfer to W, cotyledon area and chlorophyll content of the cotyledons were measured as indicators of de-etiolation.

Cotyledon area was measured with the leaf analysis system of Skye Instruments (Powys, UK). Chlorophyll was extracted using a method adapted from that described by Hiscox and Israelstam (1979). Samples of ten cotyledons were weighed, placed in glass tubes and incubated in D for 24 h at 65°C with 5 ml DMSO and assayed immediately after cooling to room temperature. Chlorophyll content was calculated using the equations for ethanol published by Lichtenthaler and Wellbrun (1983).

Pulse experiment

Pretreated seeds sown in trays were incubated in D for 48 h at 25°C. Pulses of R (3 min, 7.8 µmol/m^2/s) or R immediately followed by FR (6 min, 6.7 µmol/m^2/s), 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 emerging seedling was marked, enabling the measurement of hypocotyl growth of each seedling after the appropriate number of pulses (6, 12, 18 or 24).

End-of-day far-red light experiments

Pretreated seeds were sown in 7 × 7 × 8 cm plastic pots filled with a mixture of potting compost and sand (ratio 3:1) and grown for 12 days in a phytotron with a daily irradiation schedule of 16 h W (PAR, 160 µmol/m^2/s)/8 h D at 25°C and relative humidity of 65–70%. At day 13, the plants were transferred to cabinets and allowed to adjust to the lower level of W (PAR, 60 µmol/m^2/s) for 4 days. Plants were then selected for uniform height and after the daily W period received an immediate 20 min FR irradiation (4.6 µmol/m^2/s) before the D period. The controls were grown in an identical cabinet and received no FR irradiation. Plant height was measured after 12 days of EODFR treatment.

Light sources

The broad-band B and R cabinets used in the initial screen for mutants, together with the FR cabinets used in the additional broad-band spectrum scan of the newly isolated mutants, were the same as those described by Koornneef et al. (1980).

The genetic analysis, broad-band, pulse, EODFR and germination experiments were carried out in new light cabinets at Wageningen. The lamp compartment provides space for five fluorescent tube fittings with reflectors which are controlled by a continuously variable dimmer unit and time switches (Nijssen Light Division, Wageningen, The Netherlands). These cabinets were used for irradiation with W, B, R, and FR using the tubes and filters described below. The fluence rates and exposure times used are given in the description of each experiment.

W was obtained from Philips TLD36/84 fluorescent tubes; the B source was the same as described in Koornneef et al. (1980); R was obtained by filtering the light from Philips TL40/103339 fluorescent tubes through two layers of primary red filter (Lee, Flashlight Sales, Utrecht, The Netherlands); FR continuous broad-band: Sylvania F48T12/232/HO with one layer of primary red and one layer of dark green filter (Lee). For pulse and EODFR experiments, Sylvania F48T12/232/VHO tubes were wrapped with one layer of dark green and one layer primary red filter (Lee). All light measurements were made using a LI1800/12 spectroradiometer (Li-cor, Lincoln, Neb., USA).

Results and discussion

Mutant isolation and genetic characterization

The M_2 populations of tomato derived from EMS-treated seeds were screened under continuous B and R. Two

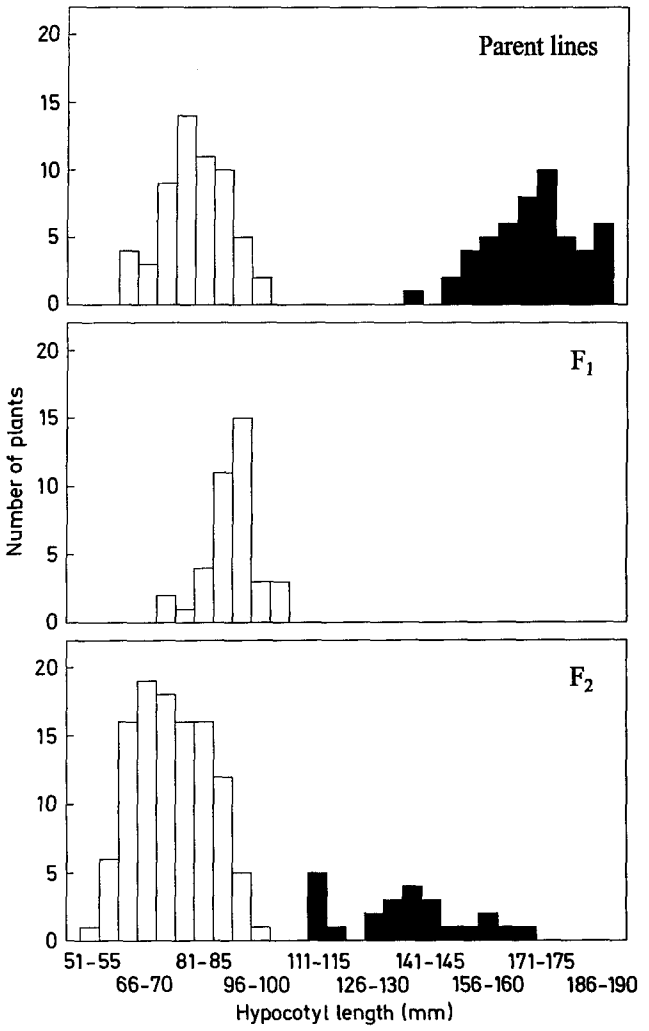


Fig. 1 Frequency distribution of hypocotyl length of tomato seedlings of the wild type (cv. Moneymaker; MM; *open bars*), *fri*^{2} mutant (*filled bars*), F_1 and F_2 generations after 7 days of continuous far-red light (3 μmol/m^2/s)

independently induced mutants, 1-7RL and 1-17BL, were selected for their slightly longer hypocotyls in R and B, respectively. In a broad-band spectral scan both mutants, unlike the WT, were shown to be completely insensitive to FR. We therefore propose the gene symbol *fri* (far-red insensitive) for these mutants. Genetic complementation analysis showed that the two mutants were allelic. 1-7RL is more extreme than 1-17BL and we refer to them as *fri*^{1} and *fri*^{2}, respectively. Under continuous FR the progeny of selfed F_1 plants from the cross between the new mutant lines and the WT parent segregated in a 3:1 ($\chi^2 = 3.59$, $P > 0.05$) ratio of normal to elongated hypocotyls expected for a monogenic recessive mutation. Data for *fri*^{2} are given in Fig. 1.

Phenotypes of the *fri*^{1} and *fri*^{2} mutants

When grown in FR, plants homozygous for the *fri*^{1} and *fri*^{2} mutations do not differ from plants grown in D. The hypocotyls are elongated, the apical hooks closed and the cotyledons are unexpanded. The WT plants grown in FR exhibit less hypocotyl growth inhibition than in B and R, and the cotyledons remain yellow, but are fully expanded. Compared to WT the hypocotyl of the *fri*^{1} mutant is slightly elongated in B and R (Figs. 2, 3).

When grown in a 16 h W/8 h D cycle for 28 days, the *fri* mutants are phenotypically barely distinguishable from WT, apart from their very slightly retarded growth (Fig. 4). This retarded growth becomes more obvious in plants grown in the greenhouse and the *fri* mutant plants wilt strongly on sunny days. Preliminary data indicate that detached leaves of *fri* mutant plants do not show an enhanced water loss, as is found in abscisic acid-deficient mutants where this is due to their inability to close their stomata (data not shown).

Mutants with a similar D phenotype in FR, but which exhibit hypocotyl inhibition by other wavelengths, have been shown to be phyA mutants in *Arabidopsis* (Nagatani et al. 1993; Parks and Quail 1993; Whitelam et al. 1993).

Mapping

Recent research indicates the presence of at least five *phy* genes in tomato, at least three showing sequence homology to previously characterized *phy* genes in *Arabidopsis* (Hauser et al. 1994). None of these genes has yet been mapped to a specific chromosome in tomato. However, Sharrock et al. (1988), using an RFLP for a phytochrome-coding sequence from *Arabidopsis*, which they presumed was *phyA*, mapped this locus on chromosome 10. Since the phenotypic characteristics of the *fri* mutants, as shown in Figs. 2 and 3, resemble those of the phyA-deficient mutants of *Arabidopsis* (Nagatani et al. 1993; Parks and Quail 1993; Whitelam et al. 1993), linkage analysis was performed in F_2 populations derived from crosses of the *fri* mutants with a tester line homozygous recessive for several morphological markers on chromosome 10. Significant linkage was detected with all chromosome 10 markers used. Estimates of recombination percentages and map positions calculated from these data are given in Fig. 5.

If the *fri* mutants are shown to be phyA mutants, like those in *Arabidopsis*, then the *PHYA* gene of tomato is located on chromosome 10, a prediction confirmed by recent experiments (A. van Tuinen, unpublished data).

Immunochemical and in vivo spectrophotometrical analysis of phytochrome

In extracts of etiolated seedlings of both *fri* mutants, immunochemically detectable phyA polypeptide (PHYA) is essentially absent, yet both contain WT levels of a PHYB-like apoprotein. Results for the *fri*^{1} mutant are given in Fig. 6, but similar results were obtained for *fri*^{2} (data not shown). The very faint staining de-

Fig. 2 Phenotype exhibited by tomato seedlings grown for 7 days after emergence in darkness (*D*) and continuous broad band blue (*B*), red (*R*) and far-red (*FR*) light of 3 μmol/m²/s. For each treatment, the seedling on the left is the wild type (WT, cv. MM) and on the right is the *fri*[1] mutant

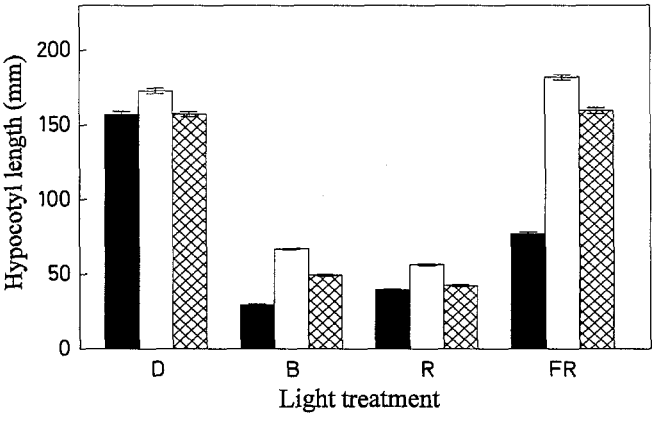


Fig. 3 Hypocotyl length of tomato wild-type (WT, cv. MM, *filled bars*), *fri*[1] (*open bars*) and *fri*[2] (*hatched bars*) mutant seedlings after 7 days continuous darkness (D), blue (B), red (R) or far-red (FR) light of 3 μmol/m²/s. The mean hypocotyl length of at least 25 seedlings from each light treatment is plotted. Error bars represent the SE of the means

tectable in the *fri* mutants (estimated as less than 1% of WT band by dilution studies) is quite likely due to the fact that the antibody used for detection of PHYA, which was raised against pea PHYA, recognizes other minor phytochrome species. Immunoblot analysis with specific antibodies raised against tomato PHYA when available will prove whether the *fri* mutants are slightly leaky or not.

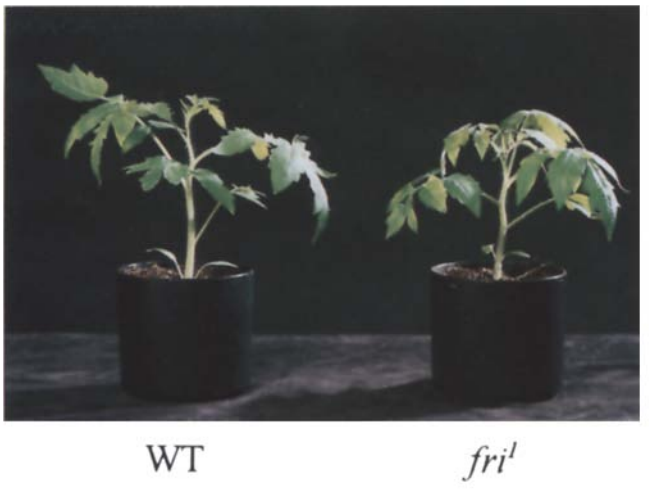


Fig. 4 Wild-type (WT) and *fri*[1] mutant tomato seedlings grown for 28 days in a 16 h W [photosynthetically active irradiance (PAR, 400–700 nm):100 μmol/m²/s]/8 h dark (*D*) cycle at 25°C

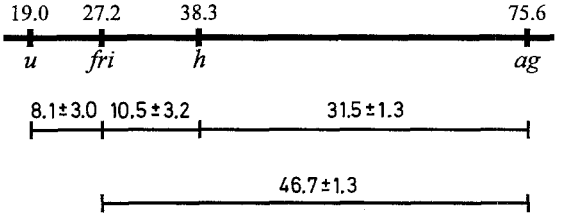


Fig. 5 Location of the *fri* locus on tomato chromosome 10 and estimates of recombination percentages between the *fri* locus and morphological markers specific for chromosome 10. The map position of *u* is that on the linkage map published by Tanksley (1993)

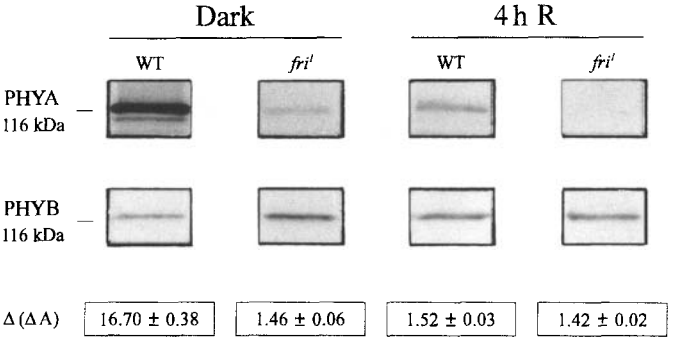
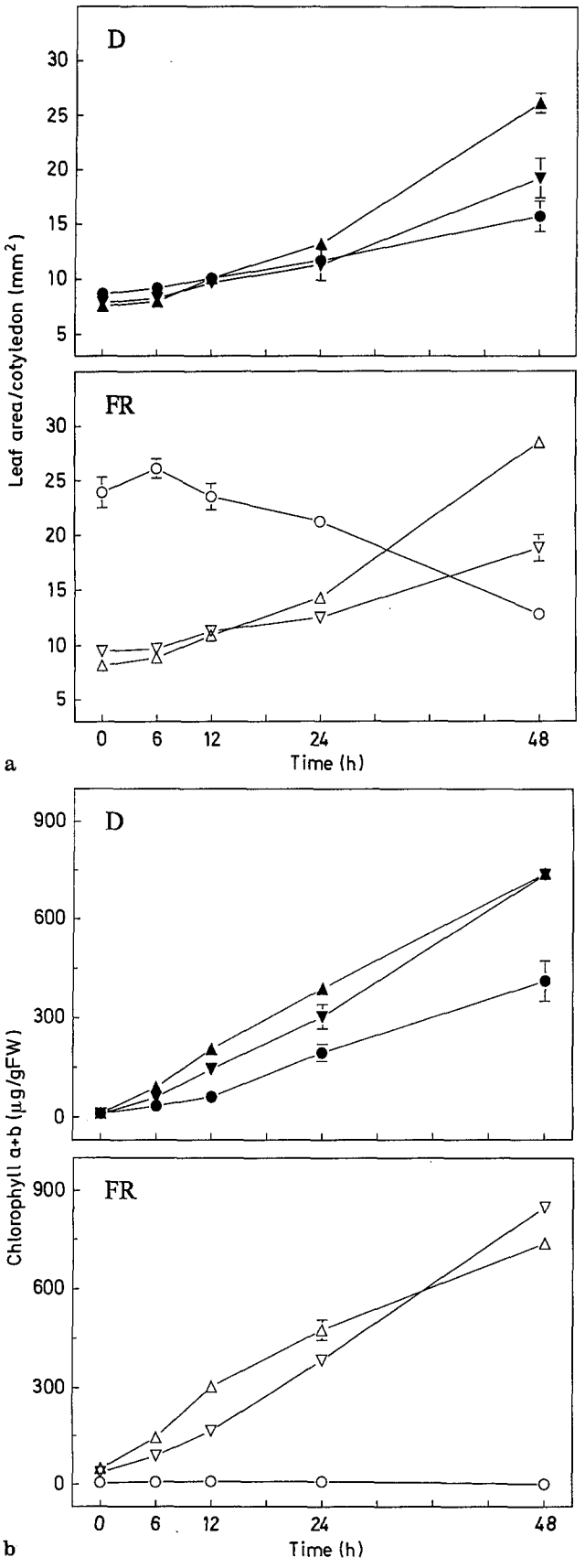


Fig. 6 Immunoblot detection of phytochrome A and B polypeptides (PHYA and PHYB, respectively) and in vivo measurement of spectral activity of phytochrome in wild-type (WT, cv MM) and *fri*[1] mutant seedlings. Dark-grown seedlings of 4-day-old or seedlings of the same age exposed to 4 h of red light (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 Δ (ΔA)/40 seedlings

Spectrophotometric analysis showed that total phytochrome in the WT decreases during a 4 h R irradiation to the low level present in the *fri* mutants (Fig. 6). However, the low level of total phytochrome in the *fri* mutants is not susceptible to destruction during this 4 h irradiation with R, indicating that it is light-stable.



Physiological characterization

De-etiolation

To obtain more detailed information about the resemblance between FR- and D-grown *fri* mutant seedlings, leaf area per cotyledon and chlorophyll content (characteristics of the de-etiolation process) were measured at different times following transfer of D and FR pretreated seedlings to W. Figure 7 shows that the de-etiolation of the *fri* mutants follows the same kinetics, regardless of the pretreatment given. Cotyledons of the *fri* mutants expand in W to a greater extent than those of the WT and have a higher chlorophyll content expressed on a per g fresh weight basis. In contrast to the *fri* mutants, the WT is unable to green after a pretreatment with FR and the fully expanded cotyledons lose their ability to produce chlorophyll, shrivel up and eventually the seedlings die. The *fri* mutants are blind to FR for the cotyledon expansion, but exhibit efficient chlorophyll biosynthesis and cotyledon expansion upon transfer to W.

Low fluence response for hypocotyl growth

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

In continuous low fluence broad-band R, the hypocotyl growth of the *fri* mutants is only slightly less inhibited than the WT (Figs. 2, 3). Spectrophotometric analysis showed that the PHYA pool is depleted after 4 h R (Fig. 6). This suggests the involvement of phyB and/or other stable pool type phytochrome(s) in the LFR of hypocotyl growth inhibition. It is therefore expected that the phyA-deficient *fri* mutants display normal R/FR reversibility for hypocotyl growth inhibition. To test this hypothesis the effects of pulses of R or R immediately followed by FR (both saturating for phytochrome photoconversion) given every 4 h on hypocotyl growth inhibition were investigated (Fig. 8). As expected, the R/FR reversibility of the hypocotyl growth inhibition is retained in the *fri* mutants. The fact that the *au* mutant is more or less blind to R (Koornneef et al. 1985) and it is probably deficient in all types of phytochrome, gives an additional indication of the in-

Fig. 7 Leaf area (**a**) and chlorophyll content (**b**) of cotyledons of wild-type (WT, cv MM, *circles*) and the *fri*[1] (*inverted triangles*) and *fri*[2] (*triangles*) mutant seedlings measured 0, 6 12, 24 and 48 h after transfer to continuous white light [*W*, (PAR, 400–700 nm):120 µmol/m²/s]. The seedlings were pretreated with a 7 day period of darkness (D, *closed symbols*) or continuous far-red light (FR, 3 µmol/m²/s, *open symbols*). Error bars represent the SE of the mean

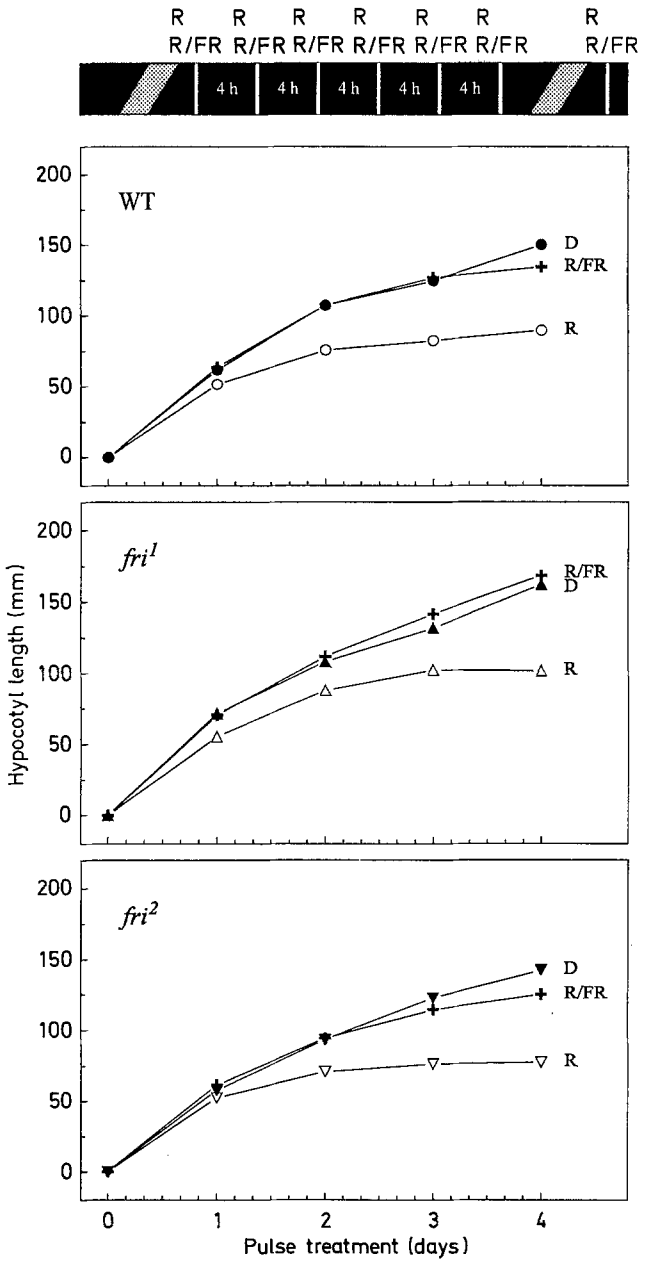


Fig. 8 Hypocotyl length of wild-type (WT, cv. MM) and the *fri*[1] and *fri*[2] mutant seedlings treated with pulses of red light (R) or R immediately followed by far-red light (FR) and a dark (D) control. The R and FR pulses (both saturating for phytochrome photoconversion) were repeated every 4 h from the time of emergence. The SE in all cases was smaller than the symbols used

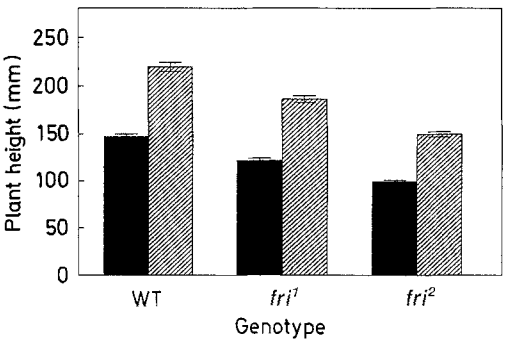


Fig. 9 Plant height of 28-day-old wild-type (WT, cv. MM) and *fri*[1] and *fri*[2] mutant plants. After the 16 h daily white period (PAR, 400–700 nm:60 μmol/m²/s), plants were either submitted to an immediate 8 h dark (*D*) period (*filled bars*) or given a 20 min FR pulse before the D period (*hatched bars*). Plant height was measured after 12 daily cycles with this end-of-day far-red light treatment. Error bars represent the SE of the mean

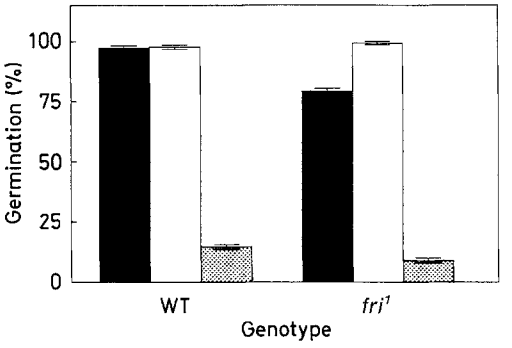


Fig. 10 Final germination percentage of wild-type (WT, cv. MM) and *fri*[1] mutant seeds in darkness (*filled bars*), continuous red light (*open bars*) or far-red light (*shaded bars*). Error bars represent the SE of the mean

volvement of phyB and/or other phytochromes in the photocontrol of hypocotyl growth inhibition.

End-of-day far-red light response

The immunoblot analysis showed that D-grown seedlings, as well as W-grown *fri* mutant plants have WT levels of a PHYB-like apoprotein. Because all mutants characterized as phyB deficient, i.e. *Arabidopsis hy3* (Reed et al. 1993), cucumber *lh* (López-Juez et al. 1992) and *Brassica ein* (Devlin et al. 1992), lack the EODFR response, it is commonly accepted that this response is mediated by phyB. The *fri* mutants respond to EODFR treatment with an increase in plant height qualitatively similar to WT (Fig. 9). This suggests that phyB functions normally and, moreover, that phyA has little or no influence on stem elongation in W-grown tomato plants.

Germination experiments

Unlike the *au* mutant (Koornneef et al. 1985), seeds of the *fri*[1] mutant which germinate in darkness are inhibited by continuous FR (Fig. 10). We conclude that the phytochrome involved in this inhibition is not phyA.

The role of phytochrome A in tomato

Phytochrome A-deficient mutants of tomato resemble the phyA mutants of *Arabidopsis*. In both species the absence of a FR-HIR for hypocotyl inhibition and the

rather normal phenotype in W are the most obvious characteristics. The effectiveness of phyA in the WT in continuous FR is explained by the lability of the FR-absorbing form of phyA (PfrA), the steady-state level of which is lower in FR, resulting in a higher integrated level of Pfr being maintained than in R (Mancinelli 1994).

In the *fri* mutant, FR is still able to photoconvert phytochrome (deplete Pfr), as shown in the EODFR, LFR and germination experiments in which the *fri* mutants resemble WT. These responses are therefore apparently mediated by other phytochromes, whose effectiveness is determined by the photoequilibrium between the R-absorbing form of phytochrome (Pr) and Pfr at any particular wavelength, meaning that R is more effective than FR. In contrast to the *Arabidopsis* phyA mutants, the tomato *fri* mutants show a slightly reduced sensitivity to B and R, which allowed their initial selection as mutants at those wavelengths. The explanation for the R effect is that enough PfrA is presumably present during the prolonged exposure to contribute to the inhibition by R. The B effect can be explained because in tomato the effect of B is mediated via the phytochrome system (Mohr 1994), whereas in *Arabidopsis* B acts independently (Koornneef et al. 1980; Young et al. 1992). The most striking conclusion we can draw from the phenotypes of the *Arabidopsis* phyA mutants and the putative tomato phyA mutants reported here is the apparent absence of a role of this phytochrome species in light-grown plants (Fig. 4). While young *fri* mutant plants grown in the phytotron are only slightly retarded compared to the WT, the older plants in the greenhouse exhibit strong wilting on sunny days, which presumably accounts for their slower growth. The normal green leaf colour and growth habit of the *fri* mutants in general is in strong contrast to the phenotype of the *au* and yellow-green-2 (*yg-2*) mutants of tomato, which at both the seedling and adult plant stage are characterized by an elongated and pale green phenotype. We now assume that these effects, formerly attributed exclusively to the phyA deficiency of these mutants (Adamse et al. 1988), might be due to the deficiency of other phytochrome types and/or other effects of a defect in tetrapyrrole biosynthesis.

Acknowledgements This research was supported by grants to A.v.T. and L.H.J.K. from the Foundation for Life Sciences (SLW) formerly the Foundation for Biological Research (BION) which is subsidized by the Netherlands Organization for Scientific Research (NWO). We thank Corrie Hanhart and Mariëlle Schreuder for technical assistance, Willem Tonk for assistance in the design and construction of the broad-band light sources at Wageningen, Martijn van Eeden for the data of the germination experiment, Enrique López-Juez for assistance with Western blotting and Dr. S. Yoshida for use of greenhouse facilities at Riken.

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