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Retention of Photoinduction of Cytosolic Enzymes in *aurea* Mutant of Tomato (*Lycopersicon esculentum*)[1]

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The tomato (*Lycopersicon esculentum* Mill.) *aurea* (*au*) mutant has been characterized as a phytochrome-deficient mutant lacking spectrally detectable phytochrome A in etiolated seedlings. Seedlings of *au* grown under red light (RL) lack phytochrome regulation of nuclear genes encoding plastidic proteins, possess ill-developed chloroplasts, and are slow to de-etiolate. In the present study, the effect of phytochrome deficiency on photoinduction of enzymes in etiolated *au* seedlings was investigated. The photoinduction of the cytosolic enzymes amylase and nitrate reductase (NR) and of the plastidic enzyme nitrite reductase (NiR) in *au* was compared with that in the isogenic wild-type (WT) tomato and the *high-pigment* (*hp*) mutant with exaggerated phytochrome response. In WT and *hp*, both brief RL pulses and continuous RL induced amylase, NR, and NiR activities, whereas in *au* no photoinduction of enzymes was observed with brief RL pulses, and continuous RL induced only amylase and NR activities. The time courses of photoinduction of NR and amylase in *au* under continuous RL followed patterns qualitatively similar to *hp* and WT. A blue-light pretreatment prior to continuous RL exposure was ineffective in inducing NiR activity in *au*. Only continuous white light could elicit a photoinduction of NiR in *au* seedlings. The norflurazon-triggered loss of photoinduction of NiR in WT and *hp* indicated that NiR photoinduction depended on chloroplast biogenesis. The results indicate that observed photoinduction of NR and amylase in *au* may be mediated by a residual phytochrome pool.

In higher plants the light environment is sensed by three photoreceptors: UV-B, blue/UV-A, and phytochrome. Of these, phytochrome is the only photoreceptor whose molecular identity is known (Quail, 1991). Phytochrome plays a prominent role in detecting light environment, alone or in conjunction with other photoreceptors, throughout the life history of plants. The molecular analysis of phytochrome genes in *Arabidopsis thaliana* has indicated that a small multigene family encodes phytochromes, of which at least five phytochrome genes, *PHYA* through *PHYE*, have been identified (Sharrock and Quail, 1989; Quail, 1991). The investigations on expression of *PHY* genes have established that the apoprotein of the photolabile phytochrome present in etiolated seedlings is encoded by the *PHYA* gene, whereas *PHYB* and *PHYC* encode the apoproteins of photostable phytochromes (Quail, 1991; Furuya, 1993). The presence of multiple phytochrome species has led to the suggestion that different phytochrome species may perform discrete functions via distinct signal-transduction pathways (Smith and Whitelam, 1990). Considering that in a light-grown plant all phytochrome species may function concurrently, mutants lacking one or more phytochrome species are valuable to distinguish the role played by different phytochrome species in regulating plant development (Reed et al., 1992).

Phytochrome-deficient mutants have been reported in *Arabidopsis*, tomato (*Lycopersicon esculentum*), sorghum, *Brassica*, and cucumber (Kendrick and Nagatani, 1991; Reed et al., 1992), and the relative functions of species of phytochrome in plant development have been inferred from observations of the physiological responses and studies on the photoregulation of gene expression in these mutants (Smith and Whitelam, 1990). Studies of *PHYB*-deficient mutants reveal that phytochrome B plays a major role in inhibition of hypocotyl elongation by RL, control of flowering, shade avoidance, and end-of-day far-red response (Nagatani et al., 1991; Somers et al., 1991; Childs et al., 1992; Devlin et al., 1992; López-Juez et al., 1992; Reed et al., 1993). Initial studies with *PHYA*-deficient mutants of *Arabidopsis* suggest that phytochrome A plays a major role in perception during the FR-triggered high-irradiance response in de-etiolating seedlings, but plays a minor role in mature plants after de-etiolation under WL (Nagatani et al., 1993; Parks and Quail, 1993; Whitelam et al., 1993).

The tomato *au* mutant possesses many features of chromophore-deficient *hy*1 and *hy*2 mutants of *Arabidopsis* (Parks and Quail, 1991): etiolated seedlings lack spectrally active phytochrome, possess ill-developed chloroplasts, and are slow to de-etiolate (Koornneef et al., 1985; Parks et al., 1987; Neuhaus et al., 1993). Etiolated seedlings of *au* show reduction in several phytochrome-mediated responses, such as RL-mediated induction of transcripts of nuclear-encoded plastidic proteins (Sharrock et al., 1988; Oelmüller et al., 1989) and inhibition of hypocotyl elongation (Adamse et al., 1988) and Chl synthesis (Ken-Dror and Horwitz, 1990). The deficiency of phytochrome in *au* seedlings has been exploited to develop single-cell assays for phytochrome-triggered signal transduction, where microinjection of oat phytochrome A

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Abbreviations: *au*, *aurea*; BL, blue light; FR, far-red light; *hp*, *high pigment*; NF, norflurazon; NiR, nitrite reductase; NR, nitrate reductase; PAL, phenylalanine ammonia lyase; RL, red light; WL, white light; WT, wild type.

into a single cell of an *au* hypocotyl restores photoinduced anthocyanin biosynthesis and chloroplast development in a cell-autonomous fashion (Neuhaus et al., 1993).

The molecular basis of the *au* mutation is not known, but it is not within the *PHYA* gene, because the *PHYA* gene in tomato maps to a chromosomal location distinct from the *au* locus (Sharrock et al., 1988). The etiolated seedlings of *au* possess both phytochrome A and phytochrome B apoproteins, but phytochrome A is spectrally inactive and is present at a level 20% of that in the WT (Sharma et al., 1993). In contrast, light-stable phytochromes (Adamse et al., 1988; López-Juez et al., 1990), such as phytochrome B (Sharma et al., 1993), and photoresponses pertaining to it, such as the end-of-day far-red response, can be detected in green seedlings and mature *au* plants (Adamse et al., 1988; López-Juez et al., 1990; Whitelam and Smith, 1991; Kerckhoffs et al., 1992). It is possible that *au* may be a chromophore mutant similar to the *hy1* and *hy2* mutants of *Arabidopsis* (Parks and Quail, 1991).

In view of the facts that photostable phytochrome and the responses triggered by it are observed in green tissues of *au*, it would be interesting to examine whether the RL-mediated induction of enzyme activities, seen typically during de-etiolation of seedlings, can be observed in etiolated *au* seedlings. Phytochrome induces synthesis of several cytosolic and plastidic enzymes during de-etiolation (Thompson and White, 1991). The photoinduction of enzymes such as NR, NiR (Rajasekhar and Oelmüller, 1987; Rajasekhar et al., 1988; Becker et al., 1992), PAL (Goud et al., 1991), and amylase (Manga and Sharma, 1988; Vally and Sharma, 1991) has been observed in many species. In the present study, we have compared photoinduction of the cytosolic enzymes NR and amylase and the plastidic enzyme NiR in the *au* mutant and WT tomato, in the *hp* mutant, which possesses amplified phytochrome responses (Peters et al., 1992), and in the *au,hp* double mutant. Here, we report that although the phytochrome-deficient *au* mutant retains photoinduction of the cytosolic enzymes NR and amylase, it lacks photoinduction of the plastidic enzyme NiR.

MATERIALS AND METHODS

Growth of Seedlings

Seeds of tomato (*Lycopersicon esculentum* Mill. cv Ailsa Craig) were raised at the Department of Genetics, Wageningen, The Netherlands. Isogenic WT, *hp*, *au*, and *au,hp* obtained as described by Adamse et al. (1989) were used in this study. Seeds were sown in transparent plastic boxes on 20 mL of 0.5% (w/v) agar support containing 5 mM potassium nitrate. Seedlings were grown at 25 ± 1°C in absolute darkness for 96 h and then transferred to continuous RL or WL. In the case of BL pretreatment, 84-h-old dark-grown seedlings were used. The light sources for RL (0.6 W m^{-2}), BL (0.13 W m^{-2}), and FR (3.5 W m^{-2}) were identical to those described by Manga and Sharma (1988).

NR Extraction and Assay

The procedure for the NR (EC 1.6.6.2) assay was essentially followed from Hageman and Reed (1980). Ten hypocotyls or pairs of cotyledons were homogenized in a precooled pestle and mortar on ice in 0.5 mL of extraction buffer (pH 7.8) containing 25 mM potassium phosphate, 1 mM Cys, 5 mM KNO_3, 5 mM EDTA, and 1% (w/v) BSA. The homogenate was centrifuged at 30,000*g* for 30 min at 4°C, and the clear supernatant was used for the assay. The NR assay was performed at 30°C for 1 h in an assay medium containing 200 μL of supernatant, 0.4 mM potassium phosphate buffer (pH 7.5), 0.15 mM KNO_3, and 35.2 μM NADH in a final volume of 0.5 mL. The reaction was terminated with 20 mM zinc acetate, and, after mixing, the tubes were centrifuged for 5 min at 3000*g*. The amount of nitrite formed in the supernatant was estimated by adding 1 mL of 0.2% (w/v) *N*-1-naphthyl ethylenediamine hydrochloride and 1 mL of 1% (w/v) sulphanilamide in 3 N HCl, and measuring the A_{540} (Snell and Snell, 1949).

NiR Extraction and Assay

Five hypocotyls or pairs of cotyledons were homogenized at 4°C with 0.5 mL of 0.1 M potassium phosphate buffer (pH 7.5) in a precooled pestle and mortar on ice, the homogenate was centrifuged at 30,000*g* for 30 min at 4°C, and the clear supernatant was used for the assay. The NiR (EC 1.7.7.1) assay was performed at 30°C for 1 h in an assay mixture containing 200 μL of extract, 0.04 mM potassium phosphate buffer (pH 7.5), 0.27 mM methyl viologen, 0.5 mM KNO_2, and 2.5 mM sodium dithionate in a final volume of 0.5 mL (Vega et al., 1980). The reaction was terminated by vigorous vortexing until the blue color disappeared; thereafter, the amount of nitrite utilized was estimated as described above for NR extraction and assay.

Amylase Extraction and Assay

Twenty hypocotyls or pairs of cotyledons were homogenized in a chilled pestle and mortar in 3 mL of buffer containing 0.1 M sodium acetate (pH 5) and 4 mM $CaCl_2$. The homogenate was centrifuged at 30,000*g* for 20 min at 4°C and the clear supernatant was used for assay. The assay was performed in a reaction medium containing 100 mM sodium acetate (pH 5.2), 4 mg/mL amylose, 4 mM $CaCl_2$, 1 mM sodium fluoride, and 500 μL of supernatant in a final volume of 4 mL (Vally and Sharma, 1991). The reaction was conducted for 2 h, and 500-μL aliquots were withdrawn at 1-h intervals. The amount of reducing sugars in the aliquots was estimated by adding an equal volume of dinitrosalicylic acid reagent (Bernfeld, 1955). After mixing, the samples were boiled for 5 min and diluted to 3 mL with distilled water. The increase in reducing sugars was determined by measuring A_{540} using maltose as standard.

Inhibitors

NF (0.06 mM) and sodium tungstate (2 mM) were used in different experiments. In the experiments where an inhibitor was applied from the time of sowing, the inhibitor was mixed with the agar before solidification. In other experiments, the required amount of an inhibitor was sprayed on the seedlings under a green safelight 2 h before the onset of light irradiation.

RESULTS

Photoinduction of Enzyme Activity

The involvement of phytochrome in the photoinduction of NR, NiR, and amylase activity in tomato seedlings is confirmed by the RL/FR reversibility experiments. Figure 1 shows that a brief pulse of RL significantly increased NR, NiR, and amylase activities in both cotyledons and hypocotyls of WT and *hp*. The effect of the RL pulse was significantly negated when it was followed by a FR pulse. In comparison with continuous RL irradiation, the effect of a brief RL pulse on photoinduction of enzymes was weak. In general, the magnitude of photoinduction of enzymes was higher in *hp* than in WT. By contrast, in *au* a brief RL pulse failed to induce activity of any of the above enzymes. On the other hand, continuous RL irradiation photoinduced only NR and amylase activities, but not NiR activity, in *au* (Fig. 1) and *au,hp* (data not shown).

Figure 2 shows the time course of NR activity under continuous RL. The continuous RL increased NR activity in cotyledons of WT, *hp*, and *au* after a lag of 8 h; thereafter, NR activity attained a peak at 24 h before declining gradually. However, at 48 h in WT and *hp*, NR activity was still higher than that in the respective dark controls (Fig. 2A). A comparison of enzyme activities in etiolated seedlings of mutants and WT (Fig. 1) reveals that, in general, the basal level of enzyme activities in *au* is less than that in WT, whereas in *hp* it is higher than that in WT. Taking into account the observed

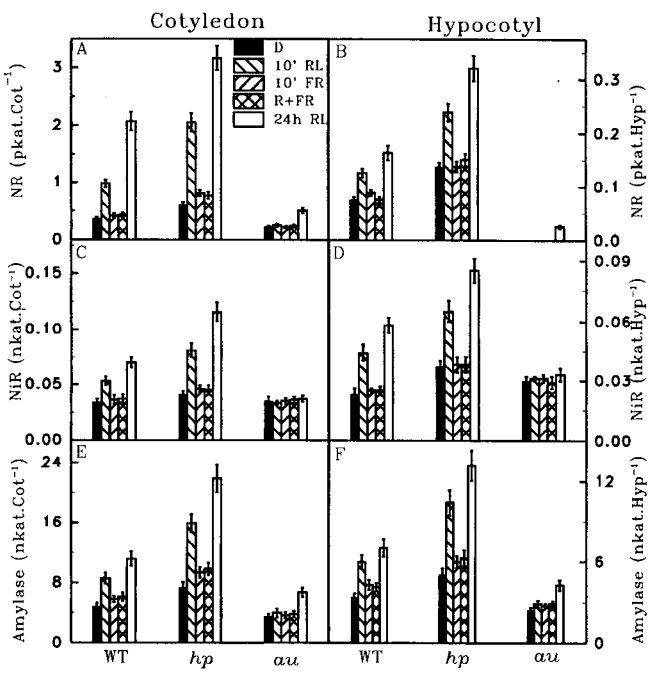

Figure 1. Effect of 10-min RL and FR irradiation on NR (A, B), NiR (C, D), and amylase (E, F) activities in cotyledons (A, C, E) and hypocotyls (B, D, F) of tomato. Seedlings were grown in darkness for 96 h from sowing and then were subjected to continuous RL, brief RL, or FR irradiation as described in the figure. The control seedlings remained in darkness (D). The enzyme activities were estimated at 120 h from sowing.

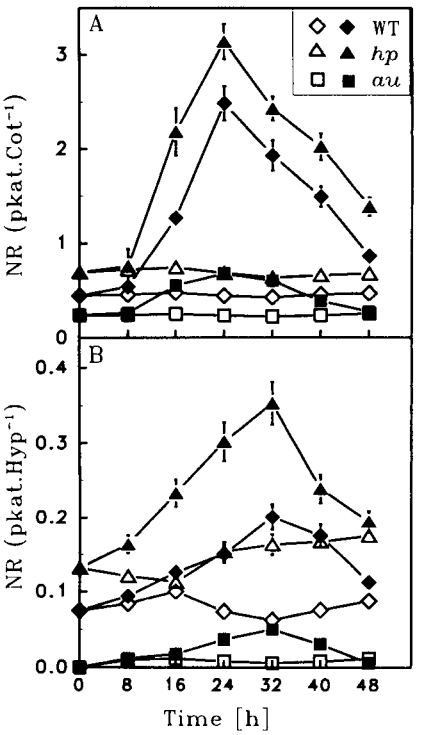

Figure 2. Time course of induction of NR activity in cotyledons (A) and hypocotyls (B) of tomato seedlings. Seedlings were grown up to 96 h from sowing in darkness and were then transferred to continuous RL (closed symbols). The control seedlings were maintained in continuous darkness (open symbols). ♦, ◇, WT; ▲, △, *hp*; ■, □, *au*.

difference in enzymic activities of etiolated mutant and WT seedlings, the relative efficiency of mutants and WT in responding to continuous RL was compared after calculating the fold stimulation of enzyme activity over the respective dark controls. Figure 2A shows that *au*, despite being deficient in phytochrome, retains a significant photoinduction of NR; moreover, the time course of NR increase in *au* cotyledons is similar to that in WT. Although the relative photoinduction of NR activity in *hp* was nearly equal to that in WT, the magnitude of photoinduction of NR in *au* was less than half of that in WT. In a fashion similar to that in the cotyledon, continuous RL induced NR activity in the hypocotyl, except that NR activity attained a peak at about 32 h after the onset of RL (Fig. 2B).

The time course of induction of NiR activity in cotyledons is very similar to that of NR. In WT and *hp* seedlings, RL-induced enhancement of NiR activity peaked at about 24 h (Fig. 3). A higher magnitude of induction of NiR was noticed in cotyledons of *hp* than in WT (Fig. 3A), whereas in hypocotyls the magnitude of NiR induction was nearly equal (Fig. 3B). However, no photoinduction of NiR activity was observed in *au* during the 48 h of continuous RL exposure in either cotyledons or hypocotyls.

The time course of amylase activity under continuous RL (Fig. 4) in mutants and WT was similar to that of NR. After a lag of 8 h, amylase activity peaked at about 24 h, before declining gradually. The time courses of amylase increase in

au cotyledons and hypocotyl were qualitatively similar to that of WT. In the *hp* mutant the amylase level increased more rapidly than in *au* or WT and attained a significantly higher level at 24 h.

Effect of BL/WL on Enzyme Activity

The effect of BL pretreatment on NR and NiR activity was investigated in both cotyledons and hypocotyls of tomato seedlings (Fig. 5). BL alone showed very little induction of NR activity (except in *hp* cotyledon). There was no significant difference in the photoinduction of NR activity between cotyledons treated with BL before RL and those irradiated with RL alone (Fig. 5A). Similar results were also obtained with hypocotyls (Fig. 5B). In comparison to NR, BL pretreatment induced NiR activity in WT and *hp* seedlings, and a 12-h BL pretreatment alone was as effective as a 24-h RL treatment (Fig. 5, C and D). Moreover, when BL treatment was followed by RL, there was no further increase in the magnitude of NiR induction. By contrast, in *au* and *au,hp* (data not shown) there was absolutely no induction of NiR activity with BL, RL, or BL followed by RL, except in seedlings transferred to continuous WL, where a 60% induction of NiR activity was observed (Fig. 5, C and D).

Effect of Inhibitors

The contribution of de novo synthesis versus activation in photoinduction of NR activity was examined by using sodium

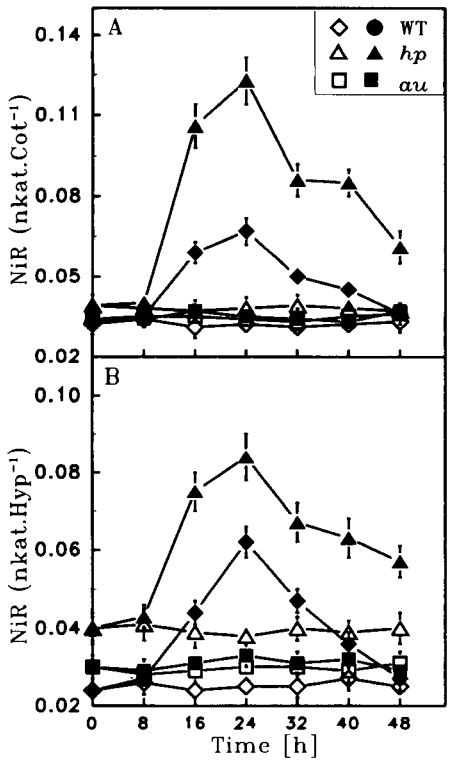

Figure 3. Time course of induction of NiR activity in cotyledons (A) and hypocotyls (B) of tomato seedlings. The experimental conditions and symbols are the same as those described in Figure 2.

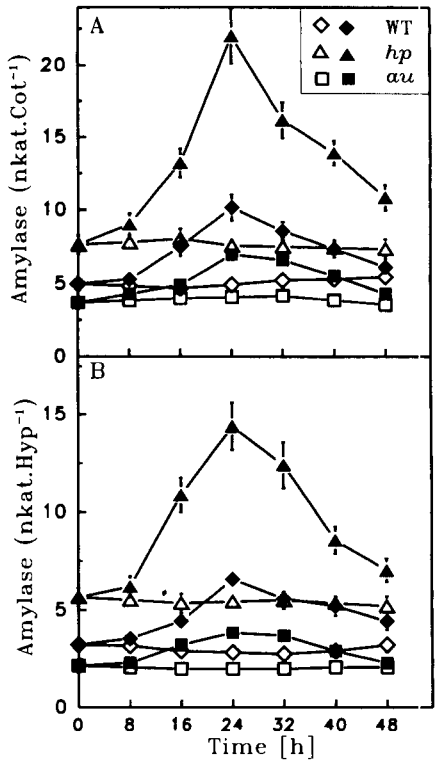

Figure 4. Time course of induction of amylase activity in cotyledons (A) and hypocotyls (B) of tomato seedlings. The experimental conditions and symbols are the same as those described in Figure 2.

tungstate. Tungstate renders newly synthesized NR protein inactive by replacing molybdenum with tungsten as a metal ion (Deng et al., 1989). Since NR and NiR in higher plants are coordinately induced (Faure et al., 1991), the consequence of loss of NR activity on NiR activity was also examined. In seedlings grown in tungstate from the time of sowing, NR activity was less than 5% of both dark- and RL-grown controls of WT and *hp*, and no NR activity could be detected in the *au* mutant (data not shown). In seedlings sprayed with tungstate 2 h before light treatment (Fig. 6), the photoinduction of NR in RL-grown seedlings was totally inhibited. The levels of NR activity in RL-grown tungstate-treated seedlings were close to dark levels in both cotyledons and hypocotyls (Fig. 6, A and B). The tungstate-mediated inhibition of NR had no significant effect on NiR activity in WT and *hp* seedlings (Fig. 6, C and D), but in *au* and *au,hp* (data not shown) seedlings the NiR level dropped below those of the respective controls.

In view of the spatial separation of NR and NiR, their localization being in cytosol and chloroplast, respectively (Rajasekhar and Oelmüller, 1987), the effect of NF-mediated loss of functional plastids (Oelmüller, 1989) on enzyme activity was also studied. In light-grown NF-treated seedlings, NR activity was only partially reduced in mutants and WT, except in the *au* hypocotyl, where NR activity could not be detected in the presence of NF (Fig. 7, A and B). In contrast to NR, photoinduction of NiR was completely abolished by NF treatment in WT and the *hp* mutant. Even in *au*, which lacks

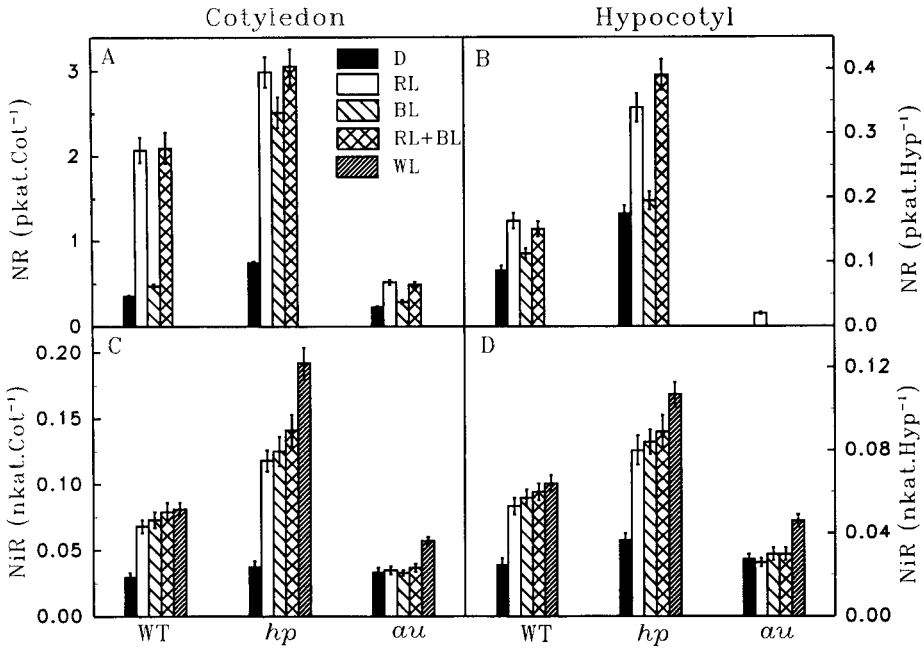

Figure 5. Effect of BL pretreatment on photoinduction of NR (A, B) and NiR (C, D) activity in cotyledons (A, C) and hypocotyls (B, D). Seedlings were grown in darkness for 84 h and were then irradiated with BL for 12 h. At the end of BL treatment seedlings were either returned to darkness (BL) or were irradiated with continuous RL (RL+BL). In case of the RL and WL treatments, seedlings were grown up to 96 h from sowing in darkness and were then transferred to the appropriate light. Control seedlings were maintained in continuous darkness (D). Enzyme activity was assayed at 120 h from the time of sowing in all samples. In seedlings irradiated with continuous WL, only NiR activity was estimated.

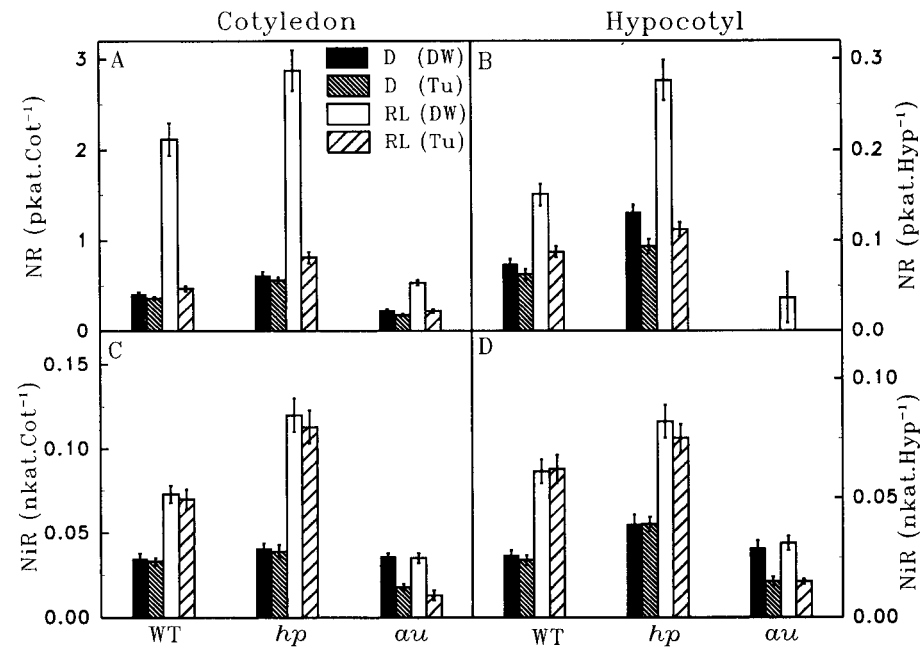

Figure 6. Effect of tungstate (Tu) on photoinduction of NR (A, B) and NiR (C, D) activity in cotyledons (A, C) and hypocotyls (B, D). Seedlings were grown in darkness for 96 h and then a set of seedlings was transferred to continuous RL. Seedlings were sprayed with tungstate (2 mM) under a green safelight 2 h prior to RL exposure. The control seedlings were similarly sprayed with distilled water (DW). NR and NiR activity were measured after 24 h of continuous RL.

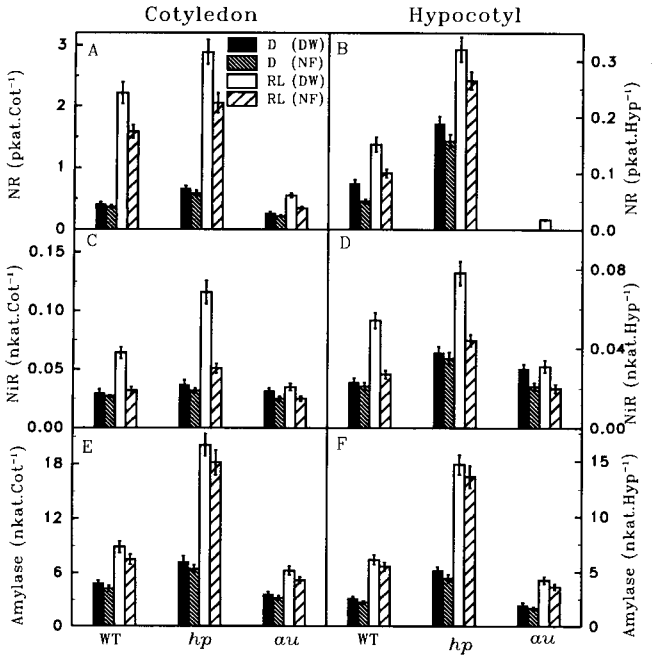

Figure 7. Effect of NF on photoinduction of NR (A, B), NiR (C, D), and amylase (E, F) activity in cotyledons (A, C, E) and hypocotyls (B, D, F). Seedlings were grown on agar support medium containing NF from the time of sowing, whereas controls were grown on medium without NF (DW). Seedlings were grown in darkness for 96 h, and then a set of seedlings was transferred to continuous RL. The control seedlings were maintained in darkness. Enzyme activity was measured after 24 h of continuous RL.

photoinduction of NiR, the level of NiR dropped below that of the respective controls (Fig. 7, C and D). In the case of amylase, NF treatment reduced the photoinduction of amylase in both mutants and WT seedlings only slightly (Fig. 7, E and F).

DISCUSSION

The results obtained with *au* in this study are in accord with the fact that the level of physiologically functional phytochrome is severely depleted in seedlings of this mutant. Although the etiolated *au* seedlings possessed a basal level of amylase, NR, and NiR activity, a brief RL pulse did not stimulate any of these enzymes (Fig. 1). The absence of RL-pulse-mediated enzyme induction in *au* is consistent with previous studies demonstrating that *au* shows little induction of photoresponses with RL pulses (Oelmüller et al., 1989; Oelmüller and Kendrick, 1991; Becker et al., 1992). Although *au* did not respond to brief RL pulses, it showed a stimulation of amylase and NR activity under continuous RL, in a fashion qualitatively similar to WT and *hp* seedlings. The time course of NR and amylase induction in *au* under continuous RL followed profiles similar to those in WT and *hp*, except that the magnitude of enzyme induction in *au* was considerably lower than in WT and *hp* (Figs. 2 and 4). Evidently, barring the absence of NiR photoinduction in *au*, the deficiency of phytochrome in *au*, or amplification of sensitivity to phytochrome in *hp* did not influence the profiles of enzyme induction, such as the duration of lag or the time required to attain peak induction of enzymes. The higher magnitudes of photoinduction of enzymes in *hp* seedlings are in conformity with the observed pleiotropic effect of *hp* mutation on amplification of phytochrome-regulated responses (Peters et al., 1992). By contrast, the observation that continuous RL stimulates amylase, NR (Fig. 1), and PAL activities (Goud et al., 1991) in *au* is at variance with earlier studies where continuous RL-mediated induction of several nuclear transcripts could not be detected in etiolated *au* seedlings (Sharrock et al., 1988; Oelmüller et al., 1989; Oelmüller and Kendrick, 1991).

The retention of continuous RL-mediated enzyme induction in *au* seedlings, despite severe reduction in the spectrally active phytochrome A level, indicates that *au* has a residual active phytochrome pool that regulates the above responses. Physiological experiments have shown that mature plants of *au* retain the end-of-day far-red response (Adamse et al., 1988; López-Juez et al., 1990) and shade-avoidance reactions (Whitelam and Smith, 1991; Kerckhoffs et al., 1992), which are assumed to be mediated by a photostable phytochrome. Since mutants deficient in phytochrome B apoprotein (López-Juez et al., 1992; Reed et al., 1992, 1993) lack the above photoresponses, it is assumed that phytochrome B is functional in mature *au* plants. Moreover, the elution profile of spectrally active phytochrome from green leaves of *au* was similar to that of phytochrome B, indicating that in mature *au* plants phytochrome B is spectrally active (Sharma et al., 1993). Considering the existence of spectrally active phytochrome in green *au* plants and the retention of phytochrome-triggered responses in *au* seedlings, it is plausible that the residual phytochrome pool of *au* seedlings may consist of photostable phytochromes. Since phytochrome in tomato is encoded by at least three genes (Hauser and Pratt, 1990), this pool may consist of one or more active phytochrome species. However, the relative proportions and functional contributions of phytochrome species constituting the phytochrome pool in *au* are not known.

Although it has been tacitly assumed that defective photoregulation in *au* arises from phytochrome deficiency, the pleiotropic nature of the *au* mutation cannot be ignored. Although it has been suggested that *au* is a phytochrome A-deficient mutant (Sharma et al., 1993), it is now apparent that the phytochrome A null mutant of *Arabidopsis* grown under WL displays a phenotype almost indistinguishable from WT (Nagatani et al., 1993; Parks and Quail, 1993; Whitelam et al., 1993). In contrast, *au* plants grown under normal daylight differ from WT, being pale green in color (López-Juez et al., 1990; Becker et al., 1992) and, unlike WT, possess an *agranal* chloroplast with a reduced number of thylakoid membranes (Koornneef et al., 1985). Such a pleiotropic effect on chloroplast development may complicate experimental interpretations. For instance, it has been shown that expression of nuclear genes like *CAB* and *RBCS* that encode plastidic proteins is closely associated with chloroplast development (Susek et al., 1993). In the present study, although photoinduction of cytosolic enzymes such as amylase and NR (Fig. 1) is present in *au*, a similar induction of plastidic proteins like NiR (Fig. 3) and of mRNA levels for other

plastidic proteins (Sharrock et al., 1988; Oelmüller and Kendrick, 1991) is not seen. The absence of photoinduction of NiR in *au*, even under continuous RL, is an intriguing observation. Becker et al. (1992) showed that although a brief RL pulse failed to elevate NiR and NR transcript levels in *au*, continuous RL elevated both NR and NiR transcripts. Whereas RL-mediated increase in NR transcript level (Becker et al., 1992) stimulated de novo synthesis of NR in *au* (Fig. 6), as revealed by tungstate-mediated inactivation of newly synthesized NR molecules (Deng et al., 1989), a similar increase in NiR transcript level in *au* is not accompanied by a stimulation in NiR enzyme level.

It is likely that the observed discrepancy between the RL-mediated increase in NiR transcript level and the absence of photoinduction of NiR activity may arise from a block in chloroplast development in *au*. Although phytochrome induction of NiR transcript (Becker et al., 1992) may not be tightly linked with chloroplast differentiation, because it is a plastidic enzyme, the expression of NiR activity is likely to be dependent on chloroplast development. Neuhaus et al. (1993) have unequivocally shown that in hypocotyl cells of etiolated *au* seedlings, plastid development is arrested at the level of proplastids, which do not even differentiate into etioplasts. Moreover, these proplastids do not transform to chloroplasts, even after a 48-h exposure to WL. It is plausible that the above delay in chloroplast development in *au* may in some way be responsible for the absence of photoinduction of NiR. The observation that NF-induced photooxidation of chloroplasts drastically reduces the NiR level in WT and *hp* indicates that chloroplast integrity is essential for photostimulation of NiR activity.

Since *au* mutants survive despite being deficient in phytochrome and having impaired chloroplast biogenesis, it is possible that a co-action by another photoreceptor during deetiolation may alleviate the adverse effects of phytochrome deficiency. For example, BL pretreatment of *au* restores phytochrome-mediated induction of nuclear transcripts encoding plastidic proteins (Oelmüller and Kendrick, 1991). Although NiR is a nuclear-encoded plastidic protein, a BL pretreatment of *au* did not induce NiR activity, whereas BL largely replaced RL-mediated NR and NiR induction in *hp* and WT. Only when *au* seedlings were exposed to continuous WL could a reduced level of NiR photoinduction be observed (Fig. 5). Likewise, photoinduction of *rbcs* transcripts in *au* was seen only under continuous WL (Sharrock et al., 1988). Probably a simultaneous operation of BL photoreceptor and residual phytochrome under continuous WL restores the photoinduction of NiR by stimulating chloroplast development in *au* seedlings.

The above discussion highlights the fact that although *au* retains photoinduction of enzymes that are possibly regulated by residual phytochrome, deficiency in NiR photoinduction may ensue from defective chloroplast development under RL. In view of the pleiotropic effects of the *au* mutation, a further biochemical and genetic analysis of this mutant is required.

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LITERATURE CITED

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