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Communication

Photooxidation of Plastids Inhibits Transcription of Nuclear Encoded Genes in Rye (*Secale cereale*)[1]

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ABSTRACT

Rye (*Secale cereale* cv Halo) seedlings treated with the herbicide Norflurazon SAN 9789 showed a reduced concentration of mRNA for the small subunit of ribulose-1,5-bisphosphate carboxylase and for the light-harvesting chlorophyll *a/b* protein. The inhibition of mRNA accumulation by Norflurazon occurred only in the presence of high light intensities and only after a period of days. The primary effect was an inhibition of the transcription rate that occurred within 1 day after exposure of the seedlings to light.

In higher plants, numerous genes are regulated by light (21, 32). Chloroplast development is light-dependent and requires interactions of the plastid and the nuclear genome (18). Many chloroplast proteins are nuclear-encoded; they are synthesized in the cytoplasm, transported into the chloroplast and, finally, processed into the mature polypeptide (3, 5–7, 10–12). The treatment of seedlings with NF,[2] a herbicide that blocks carotenoid synthesis, leads to the photooxidation of Chl under the influence of intense WL (1, 17). Chlorosis-inducing herbicides applied to seedlings in concentrations from 10 to 100 μM resulted in nearly 100% reduction of the carotenoid and 95 to 99% of the Chl contents in rye and wheat (15, 16), barley (22, 29), maize (24), mustard (17), pearl millet (19), and tomato (9). In the cotyledons of such seedlings, normal chloroplasts are no longer visible, and the thylakoid system of these chloroplasts is reduced to rudimentary membrane structures (17, 30). Typical chloroplast proteins such as LHCP and ribulose-1,5-bisphosphate carboxylase are no longer detectable, and almost no mRNA of these proteins is found (4, 23, 25, 27, 28). In contrast, cytosolic proteins such as phenylalanine ammonia-lyase, chalcone synthase, NAD-dependent malate dehydrogenase, and phytochrome (9, 30), as well as cytosolic mRNAs such as for phosphoenolpyruvate carboxylase (25), are not adversely affected by photodestruction of plastids. Plastidic factors might be necessary for light-dependent accumulation of SSU and LHCP mRNA (2, 27, 28). For LHCP mRNA it has been shown that transcription is inhibited in NF-treated barley seedlings grown under WL (2).

In this study a transcriptional inhibition similar to that found in barley was observed for LHCP mRNA in NF-treated rye seedlings. Furthermore, a reduction in the transcription rate of light-regulated SSU mRNA was also found. It appears very likely that in rye the transcription of light-regulated, nuclear-encoded mRNAs whose translation products are located in chloroplasts is dependent on intact plastids.

MATERIALS AND METHODS

Plant Material. Seeds of rye (*Secale cereale* cv Halo) (BayWa AG, Argelsried, FRG) were grown in absolute darkness at 27°C for 5 d in the absence or presence of 100 μM NF (Sandoz, Basel, Switzerland). Seedlings were then transferred to continuous WL (30 W/m^2). At different time intervals, plants were harvested for nuclei isolation or immediately frozen in liquid N_2 and stored at −70°C until use for RNA extraction.

RNA Extraction and Blotting. RNA was isolated and electrophoresed on agarose-formaldehyde gels as previously described (13). Non-stained RNA was transferred to Gene Screen (NEN, Dreieich, FRG) using the electroblot procedure. Hybridization was performed as described in the NEN manual with labeled riboprobes of anti-sense RNA for SSU and LHCP genes (13, 14).

***In Vitro* Transcription.** Nuclei were isolated as described (13), with a yield of about 7×10^6/g fresh weight. Transcription was performed in a total volume of 300 μL with 1 to 2×10^7 nuclei in the presence of 0.5 mM each ATP, GTP, and CTP, 50 mM Tris (pH 7.8), 33 mM KCl, 5 mM $MgCl_2$, 5 mM $MnCl_2$, 2.5 mM DTE, 12.5% glycerol (v/v), 100 μCi [α^{32}P]UTP (400 Ci/mmol; Amersham-Buchler, Braunschweig, FRG), and 15 units of ribonuclease inhibitor (Sigma, München, FRG). Transcription was stopped by the addition of 30 μL 100 mM Tris containing 50 mM EDTA and 10% SDS, 15 μL tRNA from yeast (2 mg/ml), and 3 μL UTP (120 mM). RNA isolation and slot blot hybridization with plasmids containing cDNA inserts of SSU (pFPB135) and LHCP (pFPB302) sequences have been previously described (13, 14). Briefly, after phenol/chloroform extraction, the RNA was ethanol precipitated, washed with 5% TCA, then washed with ethanol and, finally dried. The dried RNA was dissolved in 2 × SSC containing 50% formamide, 1% sarcosyl, 0.2% SDS, 5 × Denhardt solution, 2 mM EDTA, and 100 μg/mL salmon sperm DNA and hybridized with plasmid DNA (1 μg/slot), which was previously applied onto GeneScreen. The washed filters were autoradiographed and the autoradiograms were scanned using an electrophoresis scanner (Camag, Muttenz, Switzerland).

Pigment Analysis. Carotenoid extraction and assay was performed according to Davies (8) and Chl content was measured as described by Hippkins and Baker (20).

RESULTS

Nontreated and NF-treated rye seedlings grown in the dark for 5 d contained very low amounts, near the detection limit, of SSU and LHCP mRNA. Exposure to strong WL resulted in

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[2] Abbreviations: LHCP, light-harvesting Chl *a/b* protein; NF, Norflurazon SAN 9789; SSU, small subunit of ribulose-1,5-bisphosphate carboxylase; WL, white light.

increased concentrations of both mRNA species in untreated seedlings (Fig. 1). After longer exposure to WL, the concentrations of these mRNAs declined in untreated seedlings. NF-treated seedlings also showed an increase in the respective mRNA quantities as compared to the dark-grown controls (Fig. 1); however, after 2 d this mRNA amount declined and after 7 d WL the levels of SSU and LHCP mRNA in herbicide-treated seedlings corresponded to that in the dark controls (Fig. 1, lanes 1, 8). To determine the extent of plastidic damage by NF, the relative amount of plastid rRNA was estimated by ethidium bromide staining of RNA agarose-formaldehyde gels. Discrete plastid rRNA bands were not visible (Fig. 2); similar observations were made in NF-treated barley plastids (29). The absence of intact plastid rRNA indicated that chloroplasts have been destroyed (24, 30). Since a Chl-containing green tip was always present in whole NF-treated seedlings (Table I), the carotenoid-free hypocotyl segment was examined separately from the Chl-containing leaf tip in these experiments. After 2 d WL, SSU and LHCP mRNA were present in the bleached hypocotyl segment; however, these amounts were lower than in the untreated controls (Fig. 3A, lane 2). The green tips of both treated and untreated seedlings, on the other hand, contained approximately equal amounts of the two mRNA species (Fig. 3A, lane 1). After 6 d WL, the SSU and LHCP mRNA concentrations were strongly reduced in the hypocotyl of NF-treated seedlings compared to the control seedlings (Fig. 3B, lane 2). In contrast, the mRNA level of SSU and LHCP were the same in the green tips of both groups of seedlings (compare lane 1 of Fig. 3B). In addition, the NF-treated whole seedling now also showed a marked reduction of these mRNA species (Fig. 3B, lane 3).

Since a quantitation of RNA with labeled gene probes reflects only the steady state concentration of the specific messages, no decision regarding a transcriptional or post-transcriptional regulation of gene expression is possible. In order to discriminate between these two possibilities, the rate of transcription for SSU mRNA and LHCP mRNA was analyzed with nuclei that had been isolated from NF-treated and untreated seedlings. In etiolated rye seedlings, LHCP mRNA as well as SSU mRNA are under transcriptional control (Fig. 4, lanes 1 and 2) (13, 14). The transcription rate for SSU and LHCP mRNA in nuclei isolated from NF-treated seedlings grown under strong WL was reduced (Fig. 4, lanes 3 and 5). Densitometric scanning of the corresponding slot blots revealed for the SSU mRNA a 1.5-fold reduction and for the LHCP mRNA a 2.5-fold reduction after 1 d WL. After 3 d WL, the LHCP mRNA transcription rate in NF-treated seedlings was below the detection limit, and that for the SSU

Table I. *Chl and Carotenoid Content in NF-Treated Seedlings*

After 2 d of WL (30 W/m²) seedlings were cut into an uppermost green part (1 cm) and a bleached hypocotyl part.

Tissue	Chl		Carotenoid	
	−NF	+NF	−NF	+NF
	μg/g fresh wt			
Leaf tip	1480	1270	120	55
Hypocotyl	1290	140	66	

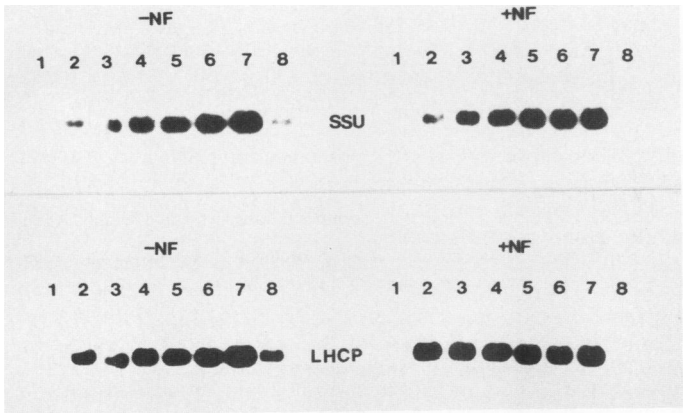

FIG. 1. Northern blotting of total RNA (5 μg) of rye seedlings. Hybridization was carried out with labeled SSU 'anti-sense' RNA (500,000 cpm) or LHCP 'anti-sense' RNA (500,000 cpm), respectively. Seedlings were grown in the absence or presence of NF for 5 d in darkness and then transferred to continuous WL (30 W/m²) for different times. 1 = dark control; 2 = 6 h; 3 = 12 h; 4 = 24 h; 5 = 36 h; 6 = 48 h; 7 = 77 h; 8 = 7 d.

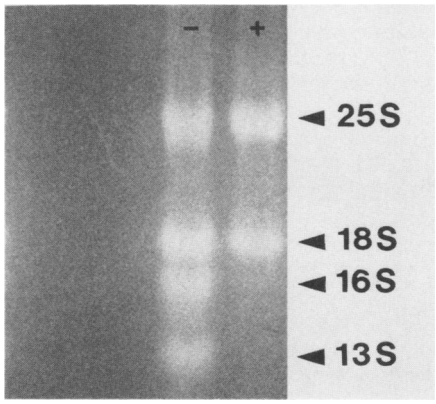

FIG. 2. Ethidium bromide-stained agarose gels of total RNA (5 μg) of the hypocotyl part of rye seedlings, grown in the absence (−) or presence (+) of NF under continuous WL (30 W/m²) for 6 d. Cytosol rRNA are 25S and 18S and plastid rRNA are 16S and 13S. rRNA 13S is a breakdown fragment of plastid 23S rRNA, due to the isolation procedure.

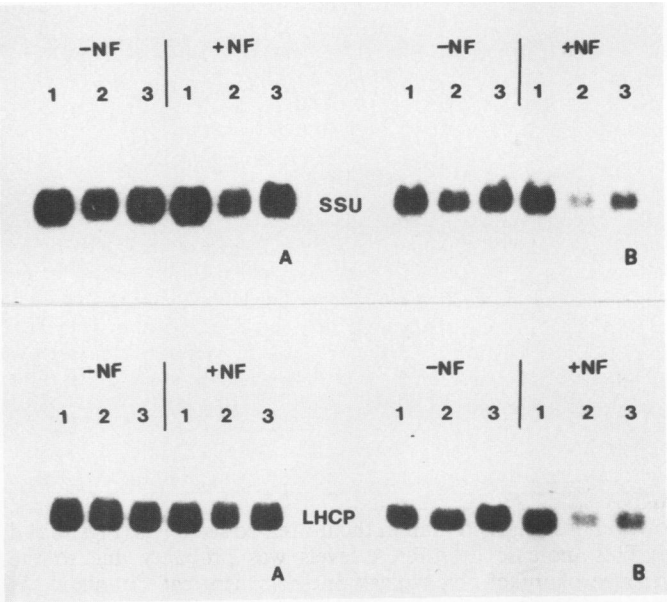

FIG. 3. Northern blotting of total RNA (10 μg) of rye seedlings. Hybridization was carried out with labeled SSU 'anti-sense' RNA (500,000 cpm) or LHCP 'anti-sense' RNA (500,000 cpm), respectively. Seedlings were grown in the absence or presence of NF for 4 d in darkness and then transferred under continuous WL (30 W/m²) for 2 d (A) or 6 d (B), respectively. 1 = green leaf tip (uppermost 1 cm section); 2 = lower hypocotyl part (virtually carotenoid-free in NF treated seedlings); 3 = whole seedling.

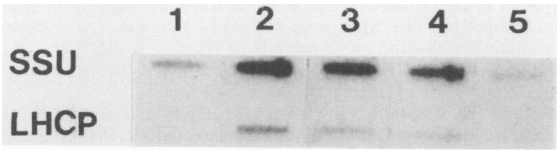

FIG. 4. Autoradiogram of slot blots of plasmid DNAs, complementary to SSU and LHCP mRNA, probed with equal amounts of *in vitro* labeled RNA transcripts of nuclei isolated from rye seedlings. Seedlings were grown for 5 d in darkness (1), for 4 d in darkness and 1 d under WL (30 W/m^2) (2, 3), or for 4 d in darkness and 3 d under WL (4, 5). Seedlings were grown in the absence (2, 4) or presence (3, 5) of 100 μM NF.

mRNA was reduced by a factor of 4.0 compared to the untreated seedlings (Fig. 4, lanes 4 and 5).

DISCUSSION

Treatment of plant seedlings with NF, under the influence of strong WL, resulted in damage to chloroplasts and a decrease of nuclear mRNAs whose translation products were located in the chloroplast (2, 4, 23, 25, 27, 28, 30). In NF-treated rye seedlings, a reduction of LHCP and SSU mRNA concentrations was found after 3 d of WL (Fig. 1, lane 7), and after 7 d of WL these mRNA concentrations corresponded to the dark controls (Fig. 1, lanes 1 and 8). This is in agreement with the effect of NF on LHCP mRNA levels in maize, barley, and mustard (2, 23, 27). Despite NF treatment, a concentration increase was measured for both mRNA species during the first 3 d of WL; this increase was, however, less marked than in the untreated seedlings (Figs. 1 and 3). This increase in mRNA levels was probably due to the presence of intact chloroplasts in the green leaf tip, since the levels of LHCP and SSU mRNA in the virtually carotenoid-free hypocotyl segments were lower than in the corresponding tissue of control seedlings (Fig. 3A, lane 2). In NF-treated barley, the LHCP mRNA concentration decreased after 12 h WL to the dark level (2); in maize, however, an elevated level as compared to the dark control was still measured after 8 d (23). In mustard, translatable LHCP and SSU mRNA were no longer found after 60 h WL (27). In mustard (31), maize (26, 33), and rye (13, 14), light affected the rate of increase and the amount to which these two mRNA species accumulated when compared to dark controls. Photooxidation in all these plants resulted in a decrease of the two mRNA levels; however, this decrease occurred with different kinetics, indicating a differential sensitivity to photooxidative damage or different mRNA stabilities in these plants.

In run-off studies with isolated nuclei, the transcription rate in NF-treated rye seedlings decreased after 1 d WL for both mRNA species studied and corresponded to the dark value after 3 d WL (Fig. 4, lanes 3 and 5). This clearly demonstrates an influence of plastids on nuclear gene transcription, rather than on mRNA stability. A similar effect was found for LHCP mRNA in barley (2). In contrast to rye, no effect of NF on SSU mRNA was found in barley. This could be accounted for by SSU mRNA not being light-regulated within 12 h after illumination in barley, whereas in rye a light and phytochrome control has been shown after just 30 min of illumination (2, 13, 14). As in maize and mustard, intact plastids were necessary in rye seedlings for the occurrence of these positively light-regulated nuclear mRNAs, whose translational products are located in chloroplasts (4, 25, 27, 28). Furthermore, as previously shown for LHCP mRNA in barley (2), the presented data indicated that under continuous white light intact plastids were necessary for the transcription of SSU and LHCP genes in rye.

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