Planta (1992) 187:381–387

Planta

Coaction of light, nitrate and a plastidic factor in controlling nitrite-reductase gene expression in tobacco

A. Neininger [1], J. Kronenberger [2], and H. Mohr [1] *

[1] Biologisches Institut II der Universität, Schänzlestrasse 1, W-7800 Freiburg, Federal Republic of Germany
[2] Laboratoire de Biologie Cellulaire, Institut National de la Recherche Agronomique, F-78026 Versailles Cedex, France

Received 19 November 1991; accepted 3 February 1992

Abstract. Nitrite reductase (NIR; EC 1.7.7.1) – a key enzyme of nitrate reduction – is known to be induced by nitrate and light. In the present study with tobacco (*Nicotiana tabacum* L.) seedlings the dependency of NIR gene expression on nitrate, light and a plastidic factor was investigated to establish the nature of the coaction between these controlling factors. A cDNA clone coding for tobacco plastidic NIR was available as a probe. The major results were as follows: (i) The light effect on the appearance of NIR occurred predominantly through phytochrome. However, a specific blue-light effect was also involved. (ii) There was no effect of light on NIR appearance in the absence of nitrate while light exerted a strong effect when nitrate was provided. (iii) Anion-exchange chromatography revealed only a single form of NIR. While experiments involving plastid photooxidation indicated that this NIR is plastidic, a small residual level could not be eliminated by photooxidation. (iv) Northern blot analysis of NIR-transcript levels indicated that a low transcript level existed in the absence of nitrate and light; however, this level appeared to be increased slightly by light (in the absence of nitrate) and by nitrate (in the absence of light). A high transcript level was detected only when light as well as nitrate were provided. A low level was found when the plastids were damaged by photooxidation. It is concluded that plastidic NIR gene expression in tobacco requires positive control by a plastidic factor. Moreover, a synergistic action of phytochrome and nitrate is required to bring about a high transcript level. As found previously with mustard and spinach seedlings, there is no quantitative relationship between the transcript level and the rate of enzyme synthesis.

Key words: Gene expression – Nitrate – Nitrate reductase (control) – Phytochrome – *Nicotiana* (nitrite reductase gene expression)

Introduction

Nitrite reductase (NIR; EC 1.7.7.1), the second enzyme in the nitrate-assimilation pathway, catalyzes the reduction of nitrite to ammonia. It is well established that the enzyme is nuclear encoded and that the appearance of this enzyme in plant material is controlled by nitrate and light (for reviews see Rajasekhar and Oelmüller 1987; Redinbaugh and Campbell 1991), and requires positive control by a plastidic factor (Schuster and Mohr 1990; Seith et al. 1991).

In previous work on the regulation of gene expression of plastidic NIR, conspicuous differences were found in our laboratory between mustard (*Sinapis alba* L.) and spinach (*Spinacia oleracea* L.) seedlings (Schuster and Mohr 1990; Seith et al. 1991). In mustard, NIR synthesis depends on nitrate while in spinach a considerable level of enzyme appears in the absence of nitrate in light as well as in darkness. In spinach, the action of light (phytochrome) was multiplicatively superimposed on the action of nitrate, indicating the independent action of both factors, while in mustard a strong synergism between nitrate and light (phytochrome) was observed. At the level of mRNA, light (operating via phytochrome), but not nitrate, stimulated the steady-state level of NIR mRNA in mustard, while in the case of spinach the steady-state level of NIR mRNA was only affected by nitrate. The two species behaved identically only with respect to dependence on the plastidic factor: intact plastids (i.e. positive control by the putative plastidic factor, for a review see Oelmüller 1989) are a prerequisite for NIR gene expression.

Regarding the site of action of light (phytochrome) and nitrate on gene expression of nuclear-encoded, plas-

* To whom correspondence should be addressed

Abbreviations and symbol: B = blue light; D = darkness; NF = Norflurazon; NIR = nitrite reductase (EC 1.7.7.1); R = red light; φ = Pfr/Ptot = far-red absorbing form of phytochrome/total phytochrome

tidic NIR it was concluded that in mustard phytochrome controls the transcript level while nitrate exerts its control post-transcriptionally (Schuster and Mohr 1990). On the other hand, in spinach nitrate determines the transcript level while the action of light (phytochrome) is strictly post-transcriptional (Seith et al. 1991). The data show that control of gene expression can differ widely in different species even though the pertinent enzyme occupies the same functional role (plastidic NIR) and the regulatory factors (nitrate, phytochrome) are the same.

In the present study, control of NIR gene expression by light, nitrate and the plastidic factor was investigated in seedlings of tobacco for the following reasons: (i) In order to find rules it is necessary to know how other species behave. Tobacco was selected since it is unrelated to either mustard or spinach, and a copy-DNA (cDNA) probe was available. (ii) It was planned to study control of NIR gene expression in transgenic tobacco which contains the NIR promotor from spinach fused to a reporter gene. To this end, the regulatory pattern of wild-type tobacco had to be known. In the case that spinach and tobacco behaved differently the crucial question could be addressed of whether the NIR promoter from spinach (fused to a reporter gene and introduced in tobacco) responds to nitrate and phytochrome in accordance with spinach (donor) or in accordance with tobacco (acceptor). In the present paper we report the results obtained with wild-type tobacco.

Material and methods

Plant material and growth conditions. Seeds of *Nicotiana tabacum* L., cv. Coker 176 were a gift from Prof. H. Sandermann and Dr. E. Back (GSF Munich, FRG). Seeds of other cultivars were purchased from the Landesanstalt für Pflanzenbau Forchheim (Rheinstetten, FRG). Since the different cultivars responded in our experiments in principally the same way, data obtained with different cultivars were combined in the present paper.

Seeds were sown in transparent plastic containers ($10 \cdot 10 \cdot 6\ cm^3$) with a perforated bottom filled with 200 ml perlite (Agriperl; Perlite Dämmstoffe GmbH, Dortmund, FRG; size of granules 1–3 mm). The perlite was saturated with the appropriate solution (7.5 mM K_2SO_4, 15 mM KNO_3), and 64 seeds per container, soaked in the pertinent solution for 30 min, were sown at equal distances on the flattened surface of the perlite. The containers were then covered with lids and the seedlings were grown at $25 \pm 0.5°$ C.

To obtain a high synchrony of germination, resulting in a homogeneous population of seedlings, the seeds received a weak 5-min red light (R, 0.68 $W \cdot m^{-2}$) pulse 24 h after sowing.

To prevent accumulation of carotenoids, tobacco seedlings were grown in the presence of $1 \cdot 10^{-6}$ M of the herbicide Norflurazon (NF; SAN 9789) dissolved in the corresponding solution from sowing onwards (Reiß et al. 1983).

Light treatments. For long-term light treatments, standardized light fields (as described by Mohr and Drumm-Herrel 1981) were used: red light (R, λ_{max} at 658 nm, half-bandwidth 24 nm, fluence rate 6.8 $W \cdot m^{-2}$ and 0.68 $W \cdot m^{-2}$ respectively, $\varphi_R = 0.8$), blue light (B, λ_{max} at 450 nm, half-bandwidth 45 nm, fluence rate 10 $W \cdot m^{-2}$ $\varphi_B = 0.4$).

For dichromatic light treatments and experiments with different fluence rates, the same equipment was used as described in Fernbach and Mohr (1991) and Drumm-Herrel and Mohr (1991).

Estimates of the Pfr/Ptot (ratio of far-red-absorbing form of phytochrome to total phytochrome) photoequilibrium at different wavelengths (φ-values) are based on the in-vitro absorbance spectra for 124-kDa *Avena* phytochrome published by Vierstra and Quail (1983) and on the phytochrome-photoconversion rate constants published by Kelly and Lagarias (1985). The particular software was provided by Dr. J. Hughes and B. Sturm (Biologisches Institut II, Freiburg, FRG).

Enzyme assay and chromatography. Crude extracts of tobacco cotyledons were prepared and NIR was assayed according to Schuster et al. (1987) with slight modifications.

Characterization of NIR by anion-exchange chromatography was performed as follows: 320 pairs of cotyledons were homogenized with 8 ml extraction medium (50 mM Tris-HCl, 3 mM EDTA, pH = 8.0) containing 0.3 mg Dowex (1.2–400). After removing low-molecular-weight substances with Sephadex G 25, adding of 7 mg bovine serum albumin (BSA) and clearing the eluate with a membrane filter (Millipore, Molsheim, France; pore size 0.22 µm), the prepared extract was loaded onto an anion-exchange column (Mono Q HR 5/5) attached to a fast protein liquid chromatography system (FPLC; Pharmacia, Freiburg, FRG), previously equilibrated with buffer A (20 mM bis-Tris-propane, pH = 6.5; 5% glycerol; 0.4% betaine; 0.1% 2-mercaptoethanol). The column was washed with 20 bed-volumes of buffer A. Elution was carried out with a linear gradient of 0–500 mM NaCl in buffer B (= buffer A plus 1 M NaCl). Fractions of 1 ml were collected at a flow rate of 1 $ml \cdot min^{-1}$. Recovery of NIR activity was of the order of 95% as compared with the total activity applied in the original volume of extract.

Isolation of RNA and Northern blot analysis. Ribonucleic acid was extracted from whole tobacco seedlings using an extraction buffer containing 50 mM Tris-HCl, 2.5 mM $MgCl_2$, 1% (w/v) NaCl, 2% (w/v) triisopropylnaphthalene sulfonic acid, pH = 7.6. After phenol/chloroform (1:1, v/v) extraction, nucleic acids were precipitated. Final DNA contamination was removed by selectively pelleting the RNA in 2.5 M LiCl. Northern blot analysis was performed according to Maniatis et al. (1982).

The NIR cDNA (see Faure et al. 1991, for details) was labelled with [^{35}S]dCTP using random primer (Feinberg and Vogelstein 1983) to a specific activity of $3 \cdot 10^8$–$4 \cdot 10^8$ $cpm \cdot \mu g^{-1}$.

For quantification, silver grains were eluted from the autoradiograms according to Suissa (1983).

Statistics. All values are means of three to six independent experiments. Estimates of the standard error are of the order of 5–10% for NIR assays and 10–15% for quantification of the Northern blots.

The data for NIR chromatography are based on three to four independent experiments. The results obtained in the different runs were always the same in principle and differed only slightly in detail. Thus, the elution profiles selected for the figures are representative of those obtained in the independent runs.

Results

Does blue light (B) control NIR synthesis independently of phytochrome? The usual light-pulse experiments to satisfy the operational criteria for the involvement of phytochrome indicate that phytochrome is involved in the R-mediated response (Table 1). While the effect of three R pulses on NIR activity was quite strong (19.1 pkat · pair of cotyledons^{-1} compared with the dark level, 14.4), a 48-h R treatment (6.8 $W \cdot m^{-2}$) led to a considerably higher enzyme level (25.3). Since the R effect was fluence-rate-dependent and far-red light (FR) exerted a similar effect it is concluded that the High Irradiance

Table 1. Effect of different light treatments on NIR activity isolated from the cotyledons of tobacco seedlings (Coker 64/323 S) grown with 15 mM KNO_3 in the substrate. Before the light treatments (onset at 7 d after sowing), seedlings were kept in complete darkness. Cotyledons were harvested and assayed at 9 d after sowing. R, red light; FR, far-red light; B, blue light; RG9, long-wavelength far-red light

Light treatment	NIR activity (pkat·pair of cotyledons^{-1})
Dark control	14.4±0.3
3·(5 min R+12 h D)	19.1±0.5
3·(10 min RG9+12 h D)	15.8±0.8
3·(5 min R+10 min RG9+12 h D)	14.9±0.9
RG9 (20 W·m^{-2})	20.2±0.7
FR (0.35 W·m^{-2})	22.0±1.0
FR (3.5 W·m^{-2})	24.1±0.1
FR (20 W·m^{-2})	24.6±0.4
R (0.68 W·m^{-2})	20.8±0.5
R (6.8 W·m^{-2})	25.3±1.0
R (20 W·m^{-2})	27.8±0.9
B (1 W·m^{-2})	23.6±0.7
B (10 W·m^{-2})	31.2±1.5
B (10 W·m^{-2})+RG9 (20 W·m^{-2})	32.5±0.9
B (10 W·m^{-2})+R (20 W·m^{-2})	31.0±0.7
B (1 W·m^{-2})+RG9 (25 W·m^{-2})	25.0±0.7

Table 2. Effect of a blue-light (B) pretreatment on the effectiveness of long-wavelength far-red (RG9) or red (R) light in controlling the appearance of NIR activity in the cotyledons of tobacco seedlings (Coker 64/323 S). Seedlings were irradiated between 7 and 9 d after sowing with RG9 or R either with or without a 1-d B pretreatment. Before the light treatment, seedlings were kept in darkness (D). Control experiments were carried out as indicated

Light treatment	NIR activity (pkat·pair of cotyledons^{-1})	
		Increase in RG9 or R
7 d D	16.0±0.4	
6 d D+1 d B	20.6±1.2	
6 d D+1 d B+2 d RG9	25.5±1.0	4.9
7 d D+2 d RG9	20.2±1.3	4.2
6 d D+1 d B+2 d R	29.5±2.7	8.9
7 d D+2 d R	25.3±1.0	9.3
6 d D+3 d R	25.9±1.2	
6 d D+3 d B	35.6±0.8	

Reaction (HIR) of phytochrome is predominantly involved, as one might expect in the case of a dicotyledonous seedling (for a review see Mohr 1984). The fact that the HIR was found to be strongly expressed even in long-wavelength far-red light (RG9-light, $\varphi_{RG9} < 0.01$) indicates a very high sensitivity of the response to phytochrome.

Blue light was found to be particularly effective, and this effect was not reduced in experiments with dichromatic light (simultaneous application of B/RG9-light to establish a low Pfr/Ptot ratio). In cases where the HIR in RG9-light is non-existent or low, this technique has been applied successfully (cucumber: Gaba et al. 1984; Drumm-Herrel and Mohr 1991; pine: Fernbach and Mohr 1990; Elmlinger and Mohr 1991) to show that the action of B on seedling development is not independent of phytochrome but is related to the level of Pfr even in long-term light.

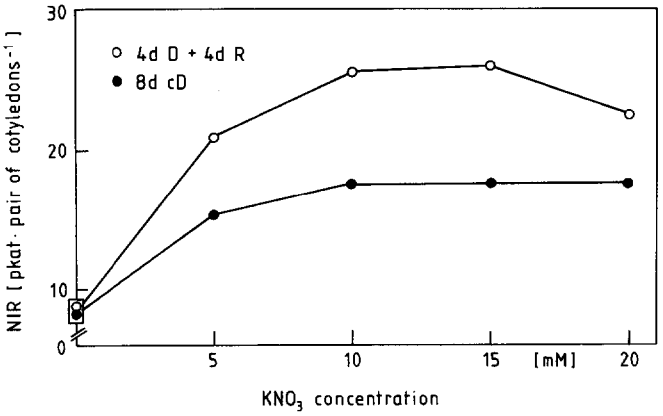

Fig. 1. Nitrate-response curves for NIR appearance in tobacco cotyledons (Coker 176). Seedlings were grown with different KNO_3 concentrations in the substrate either in continuous darkness (*cD*) or in darkness (*D*) followed by red light (*R*) between 4 and 8 d after sowing. Seedlings were harvested for assay 8 d after sowing

In the present case, simultaneous application of strong RG9-light to lower the Pfr/Ptot ratio established by B did not reduce the response. Rather it appears that the action of B is stimulated by simultaneously provided RG9-light (Table 1).

The conclusion that B and light absorbed by phytochrome may act independently of each other in tobacco was confirmed in experiments where B and RG9-light (or R) were applied in sequence (Table 2). The increase in the enzyme level caused by R or RG9-light was virtually the same irrespective of B treatment. There was no amplification of Pfr responsiveness by B as noticed with other species (for a review see Mohr 1986). On the basis of these data it is concluded that in tobacco B stimulates NIR synthesis independently of phytochrome.

Response to nitrate. The appearance of NIR in tobacco seedling cotyledons was affected by externally applied nitrate. The concentration-effect curves in light and darkness (D) show that the promotive effect was saturated at 15 mM in the medium (Fig. 1). This is the same range was previously found in spinach (Seith et al. 1991). However, since in tobacco (Coker 176) light has no effect on NIR appearance in the absence of nitrate, one curve cannot be transformed to the other by a constant factor (as in spinach, Seith et al. 1991). This means that the actions of light and nitrate are not independent of each other (as they are in the case of a multiplicative relationship); rather an interaction between nitrate and light is indicated by the data. As an example, if red light were to affect transcription only in the presence of nitrate, this type of interaction would result.

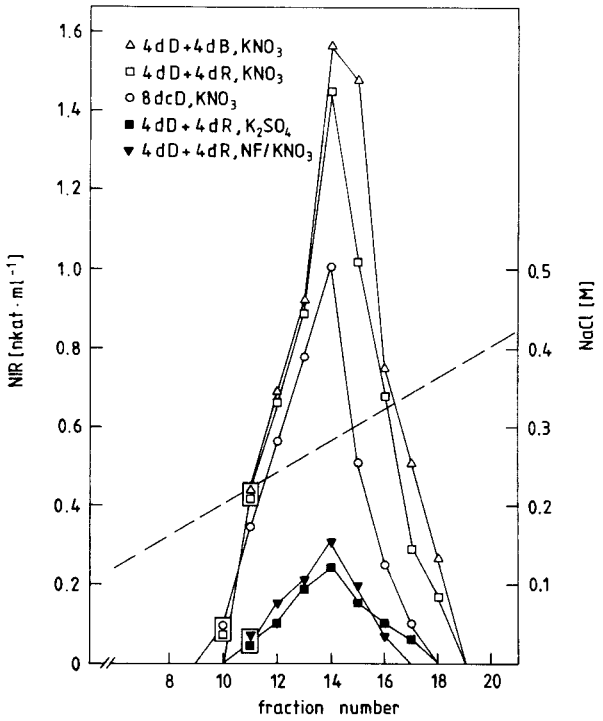

Fig. 2. Elution profile of NIR activity from extracts of tobacco seedling cotyledons (Coker 140). Seedlings were grown as indicated either with 7.5 mM K_2SO_4 (■) or 15 mM KNO_3 (△, □, ○) or a solution containing KNO_3 (15 mM) plus Norflurazon (NF, $1 \cdot 10^{-6}$ M). Separation was carried out by anion-exchange chromatography (FPLC System, Mono Q HR 5/5 column) as described in *Material and methods*. In all cases the elution profile exhibits a single peak of activity at 280 mM NaCl

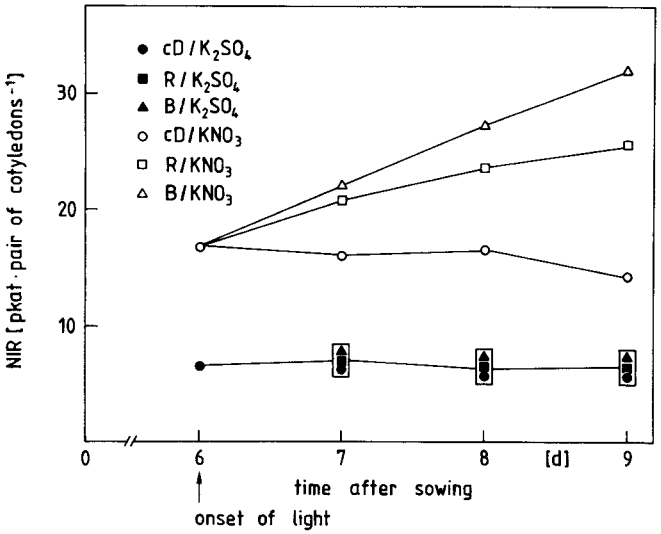

Fig. 3. Time courses of NIR activity isolated from the cotyledons of tobacco seedlings (Coker 176) between 6 and 9 d after sowing. The seedlings were grown in darkness (*cD*) or in red (*R*) or blue (*B*) light in the absence (●, ▲, ■) or presence (○, △, □) of nitrate in the substrate. During the first 6 d after sowing all seedlings were kept in darkness

Test for isoforms. Anion-exchange chromatography (Mono Q HR 5/5-column, FPLC) revealed a single, almost symmetrical peak of NIR activity under all experimental conditions (Fig. 2). Even with a very flat salt gradient, total NIR eluted within a few fractions. There was no activity below fraction 10 and beyond fraction 18. This result is compatible with the assumption that in tobacco (as in spinach, see Seith et al. 1991) there is only a single form of NIR.

Figure 2 also shows that the appearance of NIR in response to light and nitrate depends on the integrity of the plastids. When the plastids were damaged by photooxidation in R in the presence of NF (▼) the NIR level was dramatically reduced.

At a concentration of $1 \cdot 10^{-6}$ M, NF inhibits synthesis of coloured carotenoids almost completely (Oelmüller et al. 1988). In NF-treated seedlings, chlorophyll is no longer protected by the carotenoids against photooxidation, and the plastids become rapidly and heavily damaged in R while the cytosolic functions are not adversely affected and morphogenesis of seedlings proceeds normally (Reiß et al. 1983; for details see Oelmüller et al. 1988).

The small residual NIR level in NF-treated seedlings cannot be attributed to the low NF concentration applied since $5 \cdot 10^{-6}$ M NF led to the same result. Moreover, the NF-treated seedlings were totally bleached, i.e. completely devoid of chlorophyll and carotenoids. We cannot exclude the possibility that a cytosolic isoform of NIR with very similar properties comes into play as observed previously in the case of mustard (Schuster and Mohr 1990).

Time course of NIR levels in tobacco cotyledons. Nitrate (15 mM) was applied at the time of sowing while the onset of light was at 6 d after sowing. By that time the NIR level induced by nitrate in D was fully established (○) and a high competence to respond to light had developed (Fig. 3).

It was found that even in D and in the absence of nitrate a considerable level of NIR was established (●). This level could not be affected by light in the absence of nitrate. This result is totally different from what we have previously observed with spinach cotyledons (Seith et al. 1991) where the level of NIR was strongly increased by light even in the absence of exogenous (and indigenous) nitrate.

As a response to light the NIR level increased strongly. In agreement with Table 1 B (applied at the same photon fluence rate, 35 $\mu mol \cdot m^{-2} \cdot s^{-1}$, as R) had a stronger effect than one might have expected if phytochrome were the only photoreceptor. In this regard the tobacco seedling differs from the spinach seedling where R and B, applied at the same photon fluence rate, had the same effect.

Northern blot analysis. In order to compare the levels of NIR with the amount of NIR mRNA under identical experimental conditions the level of NIR transcripts was measured via Northern blot hybridization 8 d after sowing (see Fig. 3). The resulting autoradiographs (Fig. 4) show a single prominent band, as expected from Fig. 2.

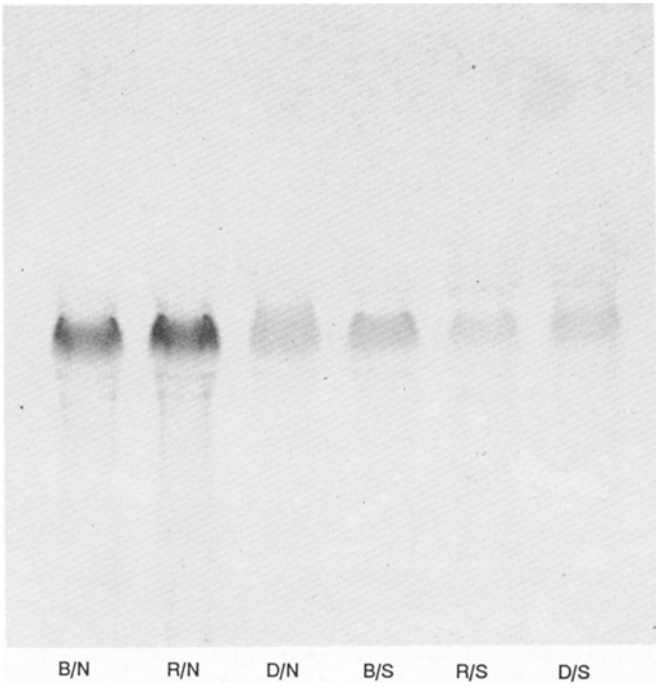

Fig. 4. Northern blot analysis of NIR-mRNA levels. Total RNA was extracted from tobacco (Coker 176) seedlings 8 d after sowing. Each lane was loaded with 20 µg of RNA. Seedlings were kept for 8 d in complete darkness (*D*) or kept in darkness for 6 d after sowing and then transferred to red (*R*) or blue (*B*) light for 2 additional days. *S*, K_2SO_4 (7.5 mM) in the medium; *N*, KNO_3 (15 mM) in the medium.

Table 3. Quantification of NIR-mRNA levels after Northern blot analysis (three independent experiments). Experimental treatments were the same as in Fig. 4 (D, darkness; R, red light; B, blue light). Quantification was performed according to Suissa (1983)

Treatment of seedlings	Relative transcript level (%)
8 d D/K_2SO_4	100
6 d D+2 d R/K_2SO_4	123±13
6 d D+2 d B/K_2SO_4	125±22
8 d D/KNO_3	126±13
6 d D+2 d R/KNO_3	209±29
6 d D+2 d B/KNO_3	214±10

The level of NIR mRNA was strongly increased by the simultaneous application of nitrate and light.

Quantification of the Northern blots (Table 3) indicated a doubling of the transcript level in the presence of light and nitrate. Light (in the absence of nitrate) and nitrate (in the absence of light) perhaps stimulated the transcript level slightly, but the response was not significant: a high transcript level was found only when light as well as nitrate were available. No specific B effect, i.e. a B effect which could not be attributed to phytochrome, was found.

When the plastids were photo-damaged in the presence of NF the level of NIR transcripts dropped to the level for D plus nitrate (D/N, Fig. 5). In principle, this

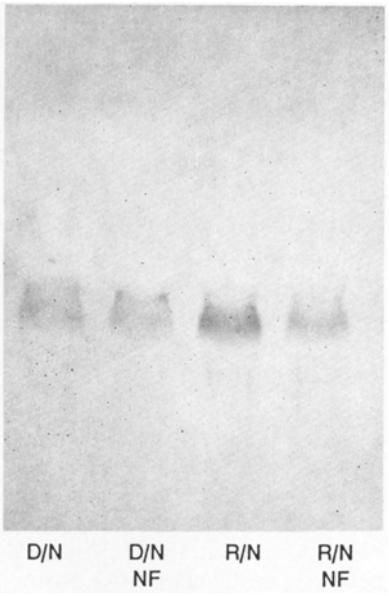

Fig. 5. Northern blot analysis of NIR-mRNA levels, performed as in Fig. 4. Seedlings were kept for 8 d in complete darkness (*D*) or kept in darkness for 6 d and then transferred to red light (*R*) for 2 additional days. *N*, KNO_3 (15 mM) in the medium; *NF*, Norflurazon ($1 \cdot 10^{-6}$ M) in the medium

result was as expected since NIR is a nuclear-encoded, plastidic protein. However, the residual level was somewhat higher than expected from previous experience with mustard (Schuster and Mohr 1990) and spinach (Seith et al. 1991).

Discussion

The goal of the present study with wild-type tobacco seedlings was to define the factors which control NIR gene expression in this species, and thus to create the basis for the use of transgenic tobacco seedlings. As pointed out in the *Introduction* the eventual aim of the current series of investigations has been to find a plant which indeed expresses plastidic NIR under the control of nitrate and light but *differently* from spinach. Moreover, this plant must be transformable with an NIR-promoter from spinach fused to a reporter gene. The tobacco seedling fulfills these requirements.

Regarding control of NIR gene expression in wild-type plants, the differences between mustard, spinach and tobacco (Schuster and Mohr 1990; Seith et al. 1991; the present paper) can be summarized as follows: (i) In mustard we observed a strong synergism between nitrate and light in enyzme synthesis while the transcript level was unaffected by nitrate but was determined by phytochrome. (ii) In spinach we observed a considerable effect of light on the enzyme level even in the absence of nitrate while the transcript level was unaffected by light and determined by nitrate. In spinach the action of light (phytochrome on the NIR level was multiplicatively superimposed on the action of nitrate, indicating that each factor acted independently. (iii) In tobacco

(Coker 176) we observed no light effect on the enzyme level in the absence of nitrate while a strong synergistic action of light was seen in the presence of nitrate. The determination of the transcript level was characterized by a synergism between nitrate and phytochrome, i.e. both factors coact at the level of transcription.

Thus, in tobacco a coaction of nitrate and light (phytochrome) is required to bring about a higher transcript level, while in mustard the transcript level was found to be determined by phytochrome alone and in spinach by nitrate alone. It is obvious that in different plants phytochrome and nitrate control NIR gene expression differently. Only the dependence on the plastidic factor appears to be the same in all cases studied so far. As shown previously, with other plastidic proteins encoded in the nucleus, transcription depends on a positive control by a putative plastidic factor (Batschauer et al. 1986; Börner et al. 1986; Oelmüller and Mohr 1986; Burgess and Taylor 1988; Ernst and Schefbeck 1988). In its absence, transcription of those nuclear genes whose protein products are destined for the plastids is turned off, and transcription no longer responds to Pfr and nitrate.

Neither Pfr nor the NO_3^- ion are likely to interact directly with the NIR promotor. Rather, it is expected that DNA-binding proteins (trans-acting factors) will mediate this process. In the case of phytochrome the identification of (so far) two light-regulated trans-acting factors has been achieved through gel retardation and footprinting studies (for a recent discussion see Jenkins 1991). In the case of NO_3^- it has been suggested that a constitutive 'NO_3^- sensor' protein detects the presence of environmental NO_3^-. When NO_3^- binds to the sensor the protein is converted to an 'active' form which might act on the NIR-promoter (for a recent discussion see Redinbaugh and Campbell 1991).

As noticed previously (Schuster et al. 1988; Seith et al. 1991) there is no quantitative relationship between the transcript level and the rate of enzyme synthesis. It was concluded that regulation at the transcript level is only coarse and does not determine quantitatively the output at the level of the protein which itself is light-controlled. This applies also to tobacco NIR gene expression: while B has no specific effect on the transcript level, this spectral range exerts a considerable effect on enzyme appearance which cannot be attributed to phytochrome.

This research was supported by a grant from the Deutsche Forschungsgemeinschaft (SFB 206). We thank Dr. M. Caboche (Laboratoire de Biologie Cellulaire, Institut National de la Recherche Agronomique, F-78026, Versailles Cedex, France) for his interest and support.

References

Batschauer, A., Mösinger, E., Kreuz, K., Dorr, J., Apel, K. (1986) The implication of a plastid-derived factor in the transcriptional control of nuclear genes encoding the light-harvesting chlorophyll a/b protein. Eur. J. Biochem. **154**, 625–634

Börner, T., Mendel, R.R., Schiemann, J. (1986) Nitrate reductase is not accumulated in chloroplast-ribosome-deficient mutants of higher plants. Planta **169**, 202–207

Burgess, D.G., Taylor, W.C. (1988) The chloroplast affects the transcrition of a nuclear gene family. Mol. Gen. Genet. **214**, 89–96

Drumm-Herrel, H., Mohr, H. (1991) Involvement of phytochrome in light control of stem elongation in cucumber (*Cucumis sativus* L.) seedlings. Photochem. Photobiol. **53**, 539–544

Elmlinger, M.W., Mohr, H. (1991) Coaction of blue/ultraviolet-A light and light absorbed by phytochrome in controlling the appearance of ferredoxin-dependent glutamate synthase in the Scots pine (*Pinus sylvestris* L.) seedling. Planta **183**, 374–380

Ernst, D., Schefbeck, K. (1988) Photooxidation of plastids inhibits transcription of nuclear-encoded genes in rye (*Secale cereale*). Plant Physiol. **88**, 255–258

Faure, J.-D., Vincentz, M., Kronenberger, J., Caboche, M. (1991) Co-regulated expression of nitrate and nitrite reductases. Plant J. **1**, 107–113

Feinberg, A.P., Vogelstein, B. (1983) A technique for radiolabelling DNA restriction endonuclease fragments to high specific activity. Anal. Biochem. **132**, 6–13

Fernbach, E., Mohr, H. (1990) Coaction of blue/UV-A light and light absorbed by phytochrome in controlling growth of pine (*Pinus sylvestris* L.) seedlings. Planta **180**, 212–216

Gaba, V., Black, M., Attridge, T.H. (1984) Photocontrol of hypocotyl elongation in de-etiolated *Cucumis sativus* – Long term, fluence rate dependent responses to blue light. Plant Physiol. **74**, 897–900

Jenkins, G.I. (1991) Photoregulation of plant gene expression. In: Developmental regulation of plant gene expression, pp. 1–41, Grierson, D., ed. Blackie, Glasgow London

Kelly, J.M., Lagarias, C.J. (1985) Photochemistry of 124-kilodalton phytochrome under constant illumination in vitro. Biochemistry **24**, 6003–6010

Maniatis, T., Fritsch, E.F., Sambrook, J. (1982) Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York

Mohr, H. (1984) Criteria for photoreceptor involvement. In: Methods in photomorphogenesis, pp. 13–42, Smith, H., Holmes, M.G., eds. Academic Press, London

Mohr, H. (1986) Coaction between pigment systems. In: Photomorphogenesis in plants, pp. 547–564, Kendrick, R.E., Kronenberg, G.H.M., eds. Nijhoff, Dordrecht, The Netherlands

Mohr, H., Drumm-Herrel, H. (1981) Interaction between blue/UV light and light operating through phytochrome in higher plants. In: Plants and the daylight spectrum, pp. 423–441, Smith, H., ed. Academic Press, London

Oelmüller, R. (1989) Photooxidative destruction of chloroplasts and its effect on nuclear gene expression and extraplastidic enzyme levels. Photochem. Photobiol. **49**, 229–239

Oelmüller, R., Mohr, H. (1986) Photooxidative destruction of chloroplasts and its consequences for expression of nuclear genes. Planta **167**, 106–113

Oelmüller, R., Schuster, C., Mohr, H. (1988) Physiological characterization of a plastidic signal required for nitrate-induced appearance of nitrate and nitrite reductases. Planta **174**, 75–83

Rajasekhar, V.K., Oelmüller, R. (1987) Regulation of induction of nitrate reductase and nitrite reductase in higher plants. Mini review. Physiol. Plant. **71**, 517–521

Redinbaugh, M.G., Campbell, W.H. (1991) Higher plant responses to environmental nitrate. Mini review. Physiol. Plant. **82**, 640–650

Reiß, T., Bergfeld, R., Link, G., Thien, W., Mohr, H. (1983) Photooxidative destruction of chloroplasts and its consequences of cytosolic enzyme levels and plant development. Planta **159**, 518–528

Schuster, C., Mohr, H. (1990) Appearance of nitrite-reductase mRNA in mustard seedling cotyledons is regulated by phytochrome. Planta **181**, 327–334

Schuster, C., Oelmüller, R., Mohr, H. (1987) Signal storage in phytochrome action on nitrate-mediated induction of nitrate and nitrite reductases in mustard seedling cotyledons. Planta **171**, 136–143

Schuster, C., Oelmüller, R., Mohr, H. (1988) Control by phytochrome of the appearance of ribulose-1,5-bisphosphate carboxylase and the mRNA for its small subunit. Planta **174**, 426–432

Seith, B., Schuster, C., Mohr, H. (1991) Coaction of light, nitrate and a plastidic factor in controlling nitrite-reductase gene expression in spinach. Planta **184**, 74–80

Suissa, M. (1983) Spectrophotometric quantitation of silver grains eluted from autoradiograms. Anal. Biochem. **133**, 511–514

Vierstra, R.D., Quail, P.H. (1983) Purification and initial characterization of 124 kilodalton phytochrome from *Avena*. Biochemistry **22**, 2498–2505