ACTA SOCIETATIS
BOTANICORUM POLONIAE
Vol. XLV, nr 1—2
1976

The role of light in the inducation of nitrate reductase and nitrite reductase in cucumber seedlings

J. BUCZEK

Department of Plant Physiology, Institute of Botany, Wrocław University

(Received: September 11, 1975)

Abstract

The activity of nitrate reductase (NR) and nitrite reductase (NiR) was investigated *in vivo* and *in vitro* in the roots and NR activity in 3-day-old cotyledons of cucumber seedlings. NR activity in the roots appears almost immediately after addition of nitrate ions to the induction medium, whereas, in the cotyledones NR induction is delayed. In general light enhances NR activity in the cotyledons and depresses it in the roots in experiments of short duration. Etiolation of the cotyledons reduces NR activity in the roots and leads to disappearance of the activity of this enzyme in the cotyledons, whereas the NR activity of roots kept in darkness, after transfer of the etiolated plants to light, increases threefold. In roots growing in darkness a delay in NiR induction is observed, while in those growing in ligth it occurs at the same time as NR induction. Chlormaphenicol (CAP), cycloheximide (CHI) and actinomycin D (ACM) applied at the beginning of the period of seedling induction with initrates inhibit NR activity in the cotyledons, whereas in the roots only CHI and ACM exert such an effect. To sum up, NR is synthesized in cucumber roots and cotyledons de novo on the cytoplasmic polyribosomes, and light per se is not indispensable for this synthesis, but it has an indirect influence on the activity level of NR and NiR both in the roots and the cotyledons.

INTRODUCTION

Nitrate reductase is an enzyme induced by the substrate, nitrate ion (for review see Beevers and Hageman, 1969). In the green tissues of numerous plant species light beside nitrates plays an important role. Lately, however, Travis and Key (1971) and Knypl (1973) reported that light is not indispensable for NR induction. On the other hand, in the leaves of a number of plant species growing in darkness nitrates do not induce NR activity beyond the low endogenous level, unless the plants

are illuminated (Chen and Ries, 1969, Travis et al., 1970a and b). The results of the above quoted authors point to an indirect role of light in the induction of NR, and the function of light may consist either in the supply of photosynthesis products as energy for enzyme induction (Travis and Key, 1971) or light may be indispensable for the production of electron donors for nitrate and nitrate reduction (Klepper et al., 1971).

Thus the role of light is particularly important as regards nitrite reductase. It is believed that NiR is localized in the chloroplasts (Ritenour et al., 1967; Dalling et al., 1972; Magalhaes et al., 1974) and it utilizes ferrodoxin reduced in light for the reduction of nitrites (Hewitt and Betts, 1963; Losada et al., 1963; Joy and Hageman, 1966). Recently, however, it has been found that NR can be induced in darkness in tobacco plants (Lips and Roth-Bejerano, 1969) and in the roots of many plants (Smith and Thompson, 1971; Wallace, 1973; Radin, 1974), whereas NiR was extracted from the roots of numerous species (Sanderson and Cocking, 1974; Miflin, Dalling et al., 1972, 1973). Thus the role of light in the induction of NR and NiR activity requires further elucidation.

The aim of the present study was to establish the development and distribution of nitrate and nitrite reductase in the roots and cotyledons of 3-day-old cucumber seedlings after induction by means of the substrate and to compare certain characteristic properties of these enzymes in the examined parts of young seedlings growing under various light conditions.

MATERIAL AND METHODS

Plant cultures

Cucumis sativus L. seeds of the variety Monastyrski were germinated in darkness at 27° C for 2 days on Petri dishes lined with moist filter paper. Uniform seedlings were chosen and transplanted onto cheesecloth stretched over glass crystallizers filled with medium without nitrates. The nutrient solution contained macro- and microelements (mg/liter): KH_2PO_4 — 8.0; KCl — 14.8; $CaCl_2 \cdot 6\ H_2O$ — 6.9; $MgSO_4 \cdot 7\ H_2O$ — 12.8; Fe-citrate — 0.8; $MnSO_4 \cdot 5\ H_2O$ — 0.65; H_2MoO_4 — 0.002 and H_3BO_3 — 0.003. pH of the solution was adjusted to 6.0. The seedlings grew in this medium for 3 days at 25° C under different light conditions: either the whole seedlings (shoots and roots) were exposed to continuous fluorescent light (4000 lux) or the whole seedlings were kept in continuous darkness or else the shoots were under light and the roots in darkness. In the latter case the crystallizers and cheesecloth were covered with aluminium foil to keep out the light.

Enzyme induction in whole seedlings

For NR induction by nitrates the nitrate-free medium was removed after 3 days of seedling growth and the cyrstallizers were filled with the induction solution. It contained the same elements as the medium without nitrates, with the difference that, instead of KCl, 101.1 mg of KNO_3 was added. The light conditions were the same as during the 3-day growth of the seedlings. The cotyledons and roots were collected after 24 h or at 2-h intervals and NR and NiR activity in them was investigated. NR activity induction in the presence of inhibitors was examined in intact seedlings. CHI, CAP and ACM were introduced into the induction medium at the beginning of induction with nitrates.

Enzyme induction in cut off roots

For measuring NR induction in cut off roots intact seedlings were grown on medium without nitrates or under light or else the shoots were kept under light and the roots in darknes. After 3 days the roots were cut 10 mm below the root neck, washed and transferred to Petri dishes (5 roots per dish) lined with Whatman No. 1 filter paper moistened with induction medium and 10 μg/ml chloramphenicol as antiseptic. Induction was run under light or in darkness at 25° C. At 2-h intervals the roots were washed, weighed and examined for NR activity.

In vivo measurement of NR activity

For determination of NR activity *in vivo* the earlier described method (B u c z e k, 1973) was adapted. The plant tissues (on the average 5-cotyledons or 5 roots each time) were placed in 25-ml conical flasks containing 1 ml of incubation medium per each 100 mg of fresh tissue. The incubation medium contained 0.1 M KNO_3, 0.1 M K-phosphate buffer (pH 7.0—7.5), 1 per cent (v/v) 1-propanol and 0.001 per cent w/v) chloramphenicol as antiseptic. The whole procedure was performed at 0°—3° C. All the manipulations with roots or cotyledons growing in darkness were done under weak green light. The conical flasks were then incubated at 33° C in darkness for 2 h with shaking from time to time. After incubation a certain amount of the solution was taken from each beaker and analysed for nitrite content. To each collected sample 1 ml of 1 per cent (w/v) sulphanilamide in 1 n HCl and 1 ml of 0.01 per cent (w/v) N-1-naphthyl--ethylenediamine dihydrochloride was added. The whole was made up with bidistilled water to 5 ml and mixed thoroughly. After 30 min absorption was read at 540 nm. The nitrite content was calculated from the standard curve. NR activity was expressed as μmoles of NO_2^- released per 100 mg

fresh tissue weight per hour. Each value in the tables and diagrams represents a mean of 4-6 replications, each experiment was replicated at least three times.

In vitro investigation of NR and NiR activity

The seedlings grew under light for 3 days on a medium free of nitrates or the shoots were kept under light and the roots in darkness, and then transferred to medium with nitrates (0 time). The roots were cut off after 0, 2, 6 and 24 h, washed and immediately examined for NR and NiR activity. For NR activity determination 1 g of fresh root mass was ground with a 5-fold amount (by weight) of 0.05 M Tris. HCl, pH 7.8, containing 1 mM cystein and 0.3 mM EDTA. For determination of NiR activity cystein hydrochloride was added instead of cystein. The ground mass was pressed out through 4 layers of cheesecloth into plastic centrifuge test tubes and the filtrate was centrifuged for 20 min at 20 000 g. All these operations were performed at 0°—3° C, with the use of cooled mortar and reagents. The supernatant solution was used for determination of NR and NiR activity and protein, nitrate and nitrite contents.

NR activity was measured by determining the amount of nitrites formed. The procedure was based on the method described by H a g e m a n et al. (1960) with certain modifications. The mixture contained in its final volume of 2 ml one milliliter phosphate buffer (0.1 M, pH 7.5), 0.2 ml of 0.1 M KNO_3, 0.5 ml of 1 mM $NADH_2$, 0.2 ml of enzyme extract and 0.1 ml of bidistilled water. After 30 min of incubation at 27° C the reaction was stopped by addition of zinc acetate (50 μmoles/ml of mixture). The test tubes were placed in a refrigerator for 10 min and centrifuged afterwards for 5 min at 10 000 g. After centrifugation to each milliliter of the reaction mixture 1 ml of the diazo-coupling reagent was added and the whole was made up with water to 5 ml. The amount of nitrites formed was measured by the method described above (Investigations *in vivo*). NR activity was expressed in μmoles of nitrites formed per 1 mg of protein per hour (specific activity) or by the number of μmoles of nitrites form ed per 100 mg fresh tissue weight per hour (total activity).

NiR activity was measured by the method described by J o y and H a g e m a n (1966) with certain modifications. The mixture in the final volume of 3 ml contained 1 ml of 0.05 M K-phosphate buffer (pH 7.5), 0.5 ml of 1 mM $NaNO_2$, 0.5 ml of enzyme extract, 0.5 ml of 2 mM benzyl viologen and 0.5 ml of $Na_2S_2O_4$ (32 mg sodium dithionite in 10 ml of 0.1 M $NaHCO_3$). The reaction was run in 5-ml bottles. For starting the reaction dithionite was added carefully to avoid effervescence of the liquid. Then the bottles were closed wih plastic corks, shaken thorougly and incubated for 3 min at 27° C. The reaction was stopped by passing an air flux through the reaction mixture at least 30 seconds after the disappearance of the

blue colouring due to the reduced benzyl viologen. In the control bottles an air flux was passed a moment zero immediately after addition of sodium dithionite. Further procedure was the same as in NR activity determination. NiR activity was expressed as the decrease (in μmoles) of nitrites per 1 mg of protein per hour or as the decrease (in μmoles) of nitrites per 100 mg fresh weight per hour.

The protein content in both enzyme extracts was determined after Lowry et al. (1951). Nitrite and nitrate content in the extraction solution was determined by Woolley et al., method (1960). Reagents. CHI, CAP and ACM (Sigma). NADH + H^+ (Reanal, Hungary), benzyl viologen (Koch-Light Laboratories Ltd.), Tris, bovine albumin and cystein (Serva). Other reagents were products of the Polish Reagents Laboratories, Gliwice.

RESULTS

The preliminary experiments (Fig. 1) concerned NR activity in roots, hypocotyls and cotyledons at various concentrations of the substrate (NO_3^-) in the incubation medium. Fig. 2 shows the influence of various pH of the incubation medium on the enzyme activity. The cotyledons and hypocotyls in both experiments grew under light and the roots remained continuously in darknes.

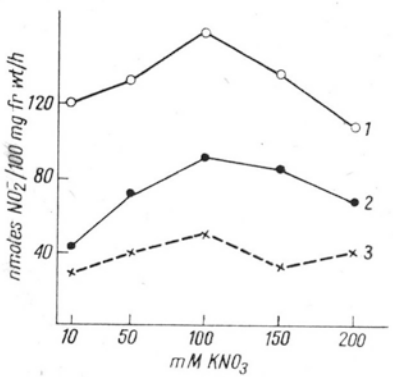

Fig. 1. Effect of nitrate concentration in incubation medium on nitrate reductase activity in cucumber seedlings
1 — roots; 2 — cotyledons; 3 — hypocotyls

As shown in Fig. 1, *in vivo* maximum enzyme activity was induced by nitrates in a 100 mM concentration, although NR activity may be relatively high at 10 mM in the incubation medium. Distribution of enzyme activity in the particular organs of cucumber seedlings was different after 24-h induction and showed high values in the roots, moderate in the cotyledons and very low ones in the hypocotyls (Figs 1 and 2). Optimum pH for roots and hypocotyls in the incubation medium was 7.0 and for the

cotyledons 7.5 (Fig. 2). All further experiments *in vivo* were performed in medium containing 100 mM KNO_3 at pH 7.0 for roots and 7.5 for cotyledons.

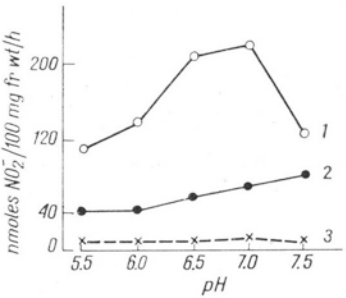

Fig. 2. Effect of pH of incubation medium on nitrate reductase activity in cucumber seedlings
1 — roots; 2 — cotyledons; 3 — hypocotyls

The time course of NR induction in various treatments of the seedlings with light and darkness is shown in Fig. 3.

The seedlings grew for 3 days without nitrates in the medium and at time 0 nitrate solution was added under various light and darkness conditions. NR activity was measured at 2-h intervals *in vivo*. As seen from Fig. 3, NR induction in roots is strongly stimulated in darkness (Fig. 3b and d), and much weaker when the roots are exposed to light (Fig. 3c). The continuous growth of the seedlings in darkness does not affect enzyme induction, but it reduces NR activity (Fig. 3c and d). On the other hand, darkness depresses NR activity reducing it almost to zero. These results indicate that NR induction in roots is independent of light, but light has an indirect influence on the reduction rate. Moreover, enzyme induction in the cotyledons shows a delay of about 2-4 h as compared with that in the roots which is immediate, reaching maximum about 6-8 h after the beginning of induction. After 24 h NR activity in the roots decreases to a constant level. In the cotyledons, on the other hand, NR activity rises continuously for 6—8 h and reaches maximum after 24 h.

The immediate reaction of roots to nitrates is shown in Fig. 4. Intact seedlings grew for 3 days in a medium without nitrates under light or in darknes or else the roots were kept in darkness and the shoots in light. Then the cotyledons and roots were cut off in one group of seedlings and placed in beakers with nitrates and NR induction was measured in vivo at 30-min intervals.

The remaining seedlings continued to grow on the nitrate-containing medium and after 24 h of induction NR activity under various light conditions was measured.

The data in Fig. 4 indicate that NR induction in the roots is not directly dependent on light, but that light has a certain influence on the NR activity level in these organs. It seems that light acts indirectly on the NR activity in the roots through the cotyledons. The highest NR activity was observed in the roots when the cotyledons were exposed to

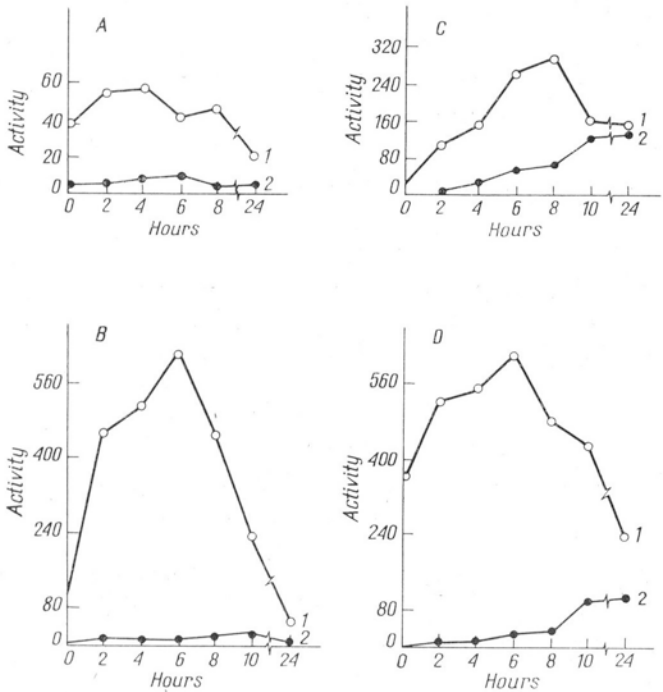

Fig. 3. Effect of light and dark on the development of NR activity in roots and cotyledons of 3 days old cucumber seedlings

The seedlings were grown 3 days on solution without nitrates and at O time in solution with nitrate ions. The NR activity was assayed seperately in roots (1 — open circles) and cotyledons (2 — solid circles) in 2 hrs intervals. The activity is expressed as μmoles of NO_2^- formed per 100 mg of freesh weight per hour

A — Effect of dark on the induction of NR in roots and cotyledons growing constantly in the dark. Induction of enzyme were performed in the dark.

B — Effect of dark on the induction of NR in roots and cotyledons growing 3 days in light before induction. Induction of enzyme were performed in the dark.

C — Effect of light on the induction of NR in roots and cotyledons growing 3 days on constant light. Induction of enzyme were performed in the light.

D — Effect of dark (roots) and light (cotyledons) on the induction of NR in roots and cotyledons. The roots were grown 3 days in constant darcness and the cotyledons in the light. The induction of NR in roots were performed in the dark and in cotyledons in the ligth conditions respectively.

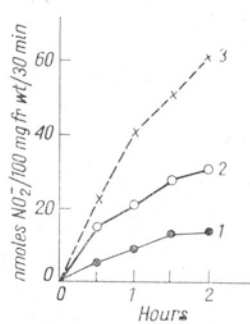

Fig. 4. Effect of light or dark pretreatment of cucumber seedlings on rate of nitrate reductase induction in roots

1 — etiolated seedlings; 2 — light grown seedlings; 3 — ligth grown shoots and dark grown roots.

The further explanation in the text.

light and the roots grew in darkness. When both cotyledons and roots were exposed to light during growth, the NR activity in the roots was reduced by one half. Lack of light during growth of the cucumber seedlings, however, depressed NR activity, but had no inhibitory effect on enzyme induction in the roots. It is noteworthy that at the same time no NR activity was noted in any of the above mentioned combinations in the cotyledons.

The immediate reaction of roots to nitrates may suggest that NR is a constitutional enzyme in cucumber roots. The results compiled in Table 1 indicate, however, that NR is induced by the nitrate substrate both in the roots and cotyledons of cucumbe seedlings.

Table 1

The activity of nitrate reductase in intact cotyledons and roots of cucumber seedlings

Induction medium	Incubation medium	Nitrate reductase activity μmoles NO_2^- /100 mg fr wt/h	
		cotyledons	roots
KNO_3	KNO_3	128.70	169.52
KCl	KNO_3	105.31	158.96
KCl	KCl	0.0	0.0

The shoots of cucumber seedlings were grown 3 days under light and the roots in the dark in a nutrient solution without nitrates, subsequently 24 h in induction medium with 0.1 M KNO_3 or with 0.1 M KCl. The NR activity was measured *in vivo* after 24 h induction in medium with 0.1 M KNO_3 or 0.1 M KCl.

Table 2 presents the results concerning NR activity values in the roots and cotyledons of cucumber seedlings after 24-h enzyme induction with nitrates under various light conditions.

The highest NR activity was observed when the roots grew in darkness and the cotyledons under light, or at least were illuminated during induction (Table 2). A similar NR activity value in the roots was found when etiolated seedlings were exposed to light during the period of induction. When NR induction in etiolated plants was run in darkness, nitrate reduction in the roots occurred at a low rate. These results indicate that light affects the NR activity in roots, probably by activating or mobilizing some factor in the cotyledons.

The indispensability of cotyledons for a high NR activity in the roots is confirmed by the results plotted in Fig. 5. One group of intact seedlings grew for 3 days without nitrates under light. In the second group the roots were protected from light while the cotyledons were exposed. After

Table 2

The rate of nitrate reductase activity in roots and cotyledons cucumber seedlings after 24 h induction of enzyme with nitrates under different light-darkness conditions

Seedlings	Treatment		Nitrate reductase activity nmoles NO_2^- /100 mg fr wt/h	
	growth 3 days	induction 1 day	cotyledons	roots
Shoots	darkness	darkness	8,86	47.33
Roots	darkness	darkness		
Shoots	light	darkness	14.44	85.00
Roots	light	darkness		
Shoots	darkness	light	34.99	112.00
Roots	darkness	light		
Shoots	darkness	light	34.95	145.93
Roots	darkness	darkness		
Shoots	light	light	51.11	151.29
Roots	darkness	darkness		
Shoots	light	light	61.55	87.87
Roots	light	light		

3 days the roots were cut off, washed and placed on Petri dishes with induction medium. The dishes were kept in the dark or under light and NR activity was measured *in vivo* at 2-h intervals. As shown by the

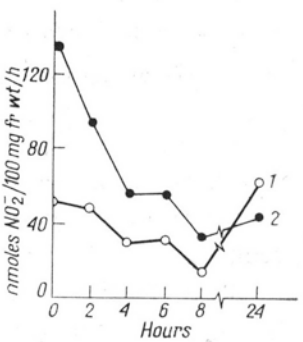

Fig. 5. Kinetic of nitrate reductase induction in detached cucumber roots floated in incubation medium in light (1 — open circles) and dark (2 — solid circles)

results (Fig. 5), with time the NR activity decreases continuously in roots exposed to light as well as in those kept in darkness. It was observed, however, that light after 24 h raises the NR activity level in the cut off roots, whereas in roots kept in the dark the activity level remains more or less constant.

The diagram in Fig. 6 shows the influence of protein and RNA syntethesis inhibitors on NR induction in roots in the presence of NO^{-}_{3} ions and inhibitors. The roots of intact seedlings after 3-day growth or under light-light or else light-darkness conditions were cut and placed in beakers filled with nitrates and inhibitors. NR activity was measured *in vivo* at half hour intervals. As seen from the data in Fig. 6a and b, CHI in a 10^{-1} M concentration inhibited in about 40 per cent immediate induction of NR activity in roots of cucumber seedlings, both of those under light and those growing in the dark. The roots growing under light, however,

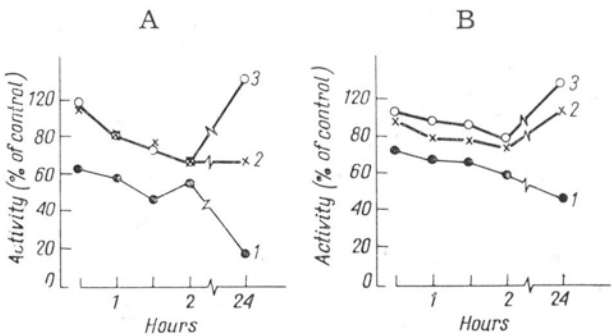

Fig. 6. Effect of RNA and protein synthesis inhibitors on the induction of nitrate reductase by nitrate in roots of cucumber seedlings grown 3 days in the absence of nitrates in the light (A) and in the dark (B). At O time the roots were excissed, placed into the flask with nitrates and inhibitors and the activity of NR was measured in vivo assays

A — The whole seedlings were grown 3 days in constant light; B — The roots of seedlings were grown 3 days in the dark and the shoots in the light
1 — 10^{-4} M cycloheximide; 2 — 2 — 10^{-5} M actinomycin D; 3 — 10^{-3} M chloramphenicol

were more sensitive to this inhibitor. The influence of CAP and ACM was les pronounced than that of CHI, but significant for NR induction in roots.

Table 3 shows the influence of the tested inhibitors on NR activity in the roots and cotyledons after 24-h induction of intact seedlings with nitrates in the presence of inhibitors. After a lapse of 24 h from the beginning of induction with 10^{-4} M CHI, NR activity in the roots growing exposed to light was completely inhibited and in 50 per cent in roots growing in the dark, whereas the influence of CAP was slightly stimulating. The effect of ACM on NR activity after 24-h growth of roots under light or in darkness in the presence of nitrates varied widely. The results (Fig. 6 and Table 3) show that ACM inhibits in about 30 per cent NR activity in

roots growing under light, whereas in roots kept in the dark the inhibitory effect of the antibiotic was very weak. It should be mentioned that all the tested inhibitors practically inhibited NR activity in the cotyledons after 24 h of induction with nitrates (Table 3).

Table 3

Effect of RNA and protein synthesis inhibitors on nitrate reductase activity of intact cotyledons and roots of cucumber seedlings

The activity of enzyme was assayed *in vivo* after 24 h induction of NR by nitrates and with inhibitors

Inhibitors	Conditions of growth and induction			
	light-darkness[a]		light-light[b]	
	cotyledons	roots	cotyledons	roots
	μmoles NO_2^- per 100 mg of freesh weight per hour			
Controls	27.59	228.35	19.01	188.35
Cycloheximide 10^{-4}M	3.92	110.69	1.31	30.99
Chloramphenicol 10^{-3}M	4.39	247.25	5.63	209.27
Actinomycin D 10^{-5}M	4.12	202.09	4.94	125.00

[a] — The shoots were grown and induced on light and the roots in dark.

[b] — Whole seedlings were grown and induced on light.

Since the results obtained by the in vivo method may be burdened with certain errors, the influence of light and darkness on induction and NR and NiR activity in cucumber seedlings was also investigated in vitro. The cotyledons of intact seedlings grew under continuous light, whereas the roots were either exposed or not. The results are presented in Table 4. Roots induced in darkness were capable of a two times more intensive nitrate reduction than those growing under light after 2 h of induction. A similar effect was observed after 6 h from the beginning of induction. After 24 h, however, the differences between roots exposed to light and those growing in darkness gradually disappeared. It was also found that after 2-h induction with nitrates the roots growing under light reduced both nitrates and nitrites, whereas those kept in darkness did not show NiR activity. The activity of the latter only appears in roots kept in the dark in the following hours, but it is always markedly lower than in roots growing under light.

Accumulation of NO_3^- in roots and cotyledons after placing the roots in the induction medium is shown in Table 5. As seen, the roots accumulated rapidly nitrate ions independently of the light conditions, whereas in the cotyledons nitrates appeared only as late as after 4 h of induction.

Table 4

Induction of nitrate reductase (NR) and nitrite reductase (NiR) by nitrates in roots of intact cucumber seedlings grown in light or darkness conditions

The activity of enzymes was assayed *in vitro*

Conditions of growth and induction of enzymes in roots	Hours after induction	NR activity		NiR activity		Nitrate and nitrite content (μg) upper g fr. wt.		Protein mg per 1 g fr. wt.
		total	specific	total	specific			
		μmoles NO_2^- produced per		μmoles NO_2^- reduced per				
		100 mg fr. wt. per h	1 mg protein per h	100 mg fr. wt. per h	1 mg protein per h	NO_3^-	NO_2^-	
Light	0	0.0	0.0	0.0	0.0	0.0	0.0	2.42
Dark	0	0.0	0.0	0.0	0.0	0.0	0.0	2.01
Light	2	21.56	129.58	59.41	273.01	6,87	1.72	2.18
Dark	2	53.49	263.65	0.0	0.0	2.14	3.39	2.31
Light	6	54.81	380.59	237.46	1173.33	93,55	3.91	1.96
Dark	6	91.98	533.24	126.05	740.16	69.79	5.52	1.75
Light	24	63.58	380.72	204.40	903.88	280.76	2.76	2.53
Dark	24	54.33	420.87	134.85	705.20	138.31	3.45	1.87

Table 5

Nitrate and nitrite content in cotyledons and roots growing in the light or the roots in darkness and cotyledons in light

Part of seedling	Condition of growth and induction	Hours after induction with nitrates									
		2		4		6		8		24	
		NO_3^-	NO_2^-	NO_3^-	NO_2^-	NO_3^-	NO_2^-	NO_3^-	NO_2^-	NO_3^-	NO_2^-
		ug per g freesh weight									
Cotyledons	light	0.0	0.0	4.0	21.3	2.7	113.7	2.8	181.3	2.0	111.4
Roots	light	8.1	52.3	8.5	120.8	6.9	159,8	5.6	177.0	5.8	130.8
Roots	darkness	3.7	24.6	11.5	87.4	9.2	92.5	6.3	137.7	6.7	72.5

The lower content of nitrate ions in roots growing in the dark may be due to the higher activity of NR than in roots growing under lihgt (Table 4, Fig. 3a and b).

DISCUSSION

In agreement with the earlier papers of Beevers et al. (1965), Oji and Izawa (1969); Sluiter-Scholten (1973) and Knypl (1975) the present results indicate that NR synthesis in intact green tissues (cotyledons) of cucumber seedlings occurs on the cytoplasmic polyribosomes. This supposition results from the fact that NR is almost completely inhibited by CHI (Table 3) — the inhibitor of protein synthesis on cytoplasmic polyribosomes. The fact that NR activity in the cotyledons is sensitive to ACM suggests that the enzyme is probably synthesized de novo. The inhibition noted in the present experiments (Table 3) of NR activity in the cotyledons by CAP — the inhibitor of protein synthesis on the chloroplast ribosomes may indicate that a certain development of chloroplasts is necessary before the enzyme forms in the cytoplasm (Sluiter-Scholten, 1973). The latter hypothesis is confirmed by the present experiments with etiolated seedlings (Table 2 and Fig. 3a).

Experiments with the inhibitors, ACM, CHI and CAP (Fig. 6) performed with intact roots of cucumber seedlings showed that in roots only CHI inhibits completely enzyme induction and activity. Kinetic studies with CAP performed on roots demonstrated that this inhibitor reduces NR induction in roots growing under light, but has no effect on those growing in the dark. After 24-h growth of roots in an induction medium with nitrates and CAP, a weak stimulating influence of CAP on NR activity is observed. These results conform with the studies of Sluiter-Scholten (1973) who recently reported that CAP inhibits NR induction only

at the beginning of the period og greening of etiolated tissues, but after 24-h exposure to light has no more effect. The data here presented thus suggest that de novo synthesis of NR in 3-day-old roots is dependent of the activity of cytoplasmic polyribosomes.

The relatively weak inhibition of NR induction by ACM in the roots, according to the earlier communications of Oaks et al. (1972) may be an indication of the presence in cucumber roots of an inactivating system which is more sensitive to ACM than the NR-inducing system. Another supposition concerns the existence in cucumber roots of an inactive NR proenzyme like that found for instance in *Chlorella* (Vennesland and Jetschmann, 1971; Morano et al., 1972). It would seem, however, on the basis of the data here presented and the earlier experiments of Smith and Thompson (1971); Oaks et al. (1972) and Radin (1974) that NR induction by nitrates in the roots of a number of plant species is the result of de novo protein synthesis.

The data shown in Fig. 4 distinctly indicate that, after addition of nitrate ions to the induction medium, NR induction in intact roots of cucumber seedlings is almost immediate, however, in the green tissues (cotyledons) the delay in NR induction reaches about 2 h. This difference may be explained by the different accumulation time for nitrate ions in roots and cotyledons (Table 5). This supposition is in agreement with the investigations of Smith and Thompson (1971) who concluded that roots are capable of a faster absorption of nitrates than are other tissues. Moreover, since NR synthesis is induced by the substrate, nitrate, the appearance of the enzyme in the cotyledons is dependent on the time of nitrate translocation to the cotyledons.

In conformity with the investigations of Beevers et al. (1965) Travis et al. (1970a and b), Travis and Key (1971), the present results show that light per se is not indispensable for NR induction either in green, non-green or etiolated cucumber seedlings. It would seem, however, that light has a distinct influence on NR activity and the high rate of enzyme turnover in the cotyledons and leaves. This supposition results from the fact that NR activity markedly decreases when intact 3-day-old green cucumber cotyledons are transferred to darkness during the induction period, whereas enzyme synthesis is not inhibited. It was also found that NR synthesis may occur both in roots and in cotyledons when nitrates are supplied to etiolated seedlings, but the NR activity rate is at this time distinctly lowered. It is possible that, when cucumber seedlings are grown in darkness the NR activity remains at the same level until the carbohydrates are exhausted. Then it begins to decline (Alsam et al., 1973).

Very interesting are the latest discoveries of Travis et al. (1970) and Travis and Key (1971) that light is indispensable for activition and maintenance of cytoplasmic protein synthesis. These authors, namely,

found that the cytoplasmic polyribosomes level is dependent on light and the disappearance of polyribosomes is positively correlated with the fall of NR activity in tissues growing in darkness.

In the light of the here presented results it is difficult to explain the influence of darkness on the activation level of NR in cucumber roots. This influence is observed both in vivo and in vitro. Moreover, it has been found that light induces NR in roots, while there is a delay in the induction of NiR in roots growing in the dark.

The influence of light on NR activity in roots cannot as yet be satisfactorily elucidated. It is possible that the differences in the NR activity level in roots induced during exposure to light or in darkness may consist the competition for electron donor formation for NR and NiR. Certain experimental data concerning this supposition have been reported by Ferrari and Varner (1970), Klepper et al. (1971) and Radin (1973). The above quoted authors point out that nitrate reduction in vivo is determined rather by the production of the reducing power than by NR itself. It may be that the system of physiological electron donors for NR and NiR in roots growing under light determines the activity level of these enzymes, whereas in roots growing in the dark no such competition occurs because in experiments of short duration NiR is not formed.

REFERENCES

Alsam M., Huffaker R. C., and Travis R. L., 1973. Plant Physiol. 52: 137—141.

Beevers L., and Hageman R. H., 1969, An Rev. Plant Physiol. 20: 495—582.

Beevers L., Schrader L. E., Flesher D., and Hageman R. H., 1965, Plant Physiol. 40: 691—698.

Buczek J., Acta Soc. Bot. Pol. 42: 223—232.

Chen T. M., and Ries A., 1969. Can J. Bot. 47: 341—343.

Dalling M. J., Hucklesby D. P., Hageman R. H., 1973. Plant Physiol. 51: 481—484.

Dalling M. J., Tolbert N. E., and Hageman R. H., 1972, Biochim. Biophys. Acta 283: 505—512.

Ferrari T. E., and Varner J., 1970. Proc. Nat. Acad. U. S. A. 65: 729—735.

Hageman R. H., and Flesher D., 1960. Plant Physiol. 35: 700—708.

Hewitt E. J., and Betts G. F., 1963, Biochem J. 89: 20.

Hewitt E. J., and Betts G. F., 1963. Biochem. J. 89. 20.

Joy K. W., and Hageman R. H., 1966. Biochem. J., 100: 263—273.

Klepper L., Flesher D., and Hageman R. H., 1971. Plant Physiol. 48: 580—590.

Knypl J. S., 1973. Z. Pflanzenphysiol. 70: 1—11.

Lips S. H., and Roth-Bejerano N., 1969. Science N. Y. 66: 109.

Losada M., Paneque A., Remirez J. M., and Del Campo F. F., 1963. Biochem. Biophys. Res. Commun. 10: 298—303.

Lowry O. H., Rosebrough N. J., Farr A. L., and Randall R. J., 1951, Biol. Chem. 193: 265—275.

Magalhaes A. C., Neyra C. A., and Hageman R. H., 1975. Plant Physiol. 53: 411—415.
Miflin B. J., 1970, Planta, 93: 160—170.
Moreno C. G., Aparicio R. J., Palacián E., and Losada M., 1972, FEBS Letters 26: 11-13.
Oaks A., Wallace W., and Stevens D., 1972, Plant Physiol. 50: 649-654.
Oji Y., and Izawa G., 1969, Plant and Cell Physiol. 10: 665-674.
Radin J. W., 1974, Plant Physiol. 53: 458-469.
Ritenour G. L., Joy K. W. Bunning J., and Hageman R. H., 1967, Plant Physiol. 42: 233-237.
Sanderson G. W., and Cocking E. C., 1974, Plant Physiol. 39: 423-431.
Sluiters-Scholten C. M. Th., 1973, Planta, 113: 229-240.
Smith S. W., and Thompson J. F., 1971, Plant Physiol. 48: 219-223.
Travis R. L., and Key J. L., 1971, Plant Physiol. 48: 617-620.
Travis R. L., Huffaker R. C., and Key J. L., 1970a, Plant Physiol. 46: 800-805.
Travis R. L., Jordan W. R., and Huffaker R. C., 1970b, Physiol. Plantarum, 23: 678-685.
Vennesland B., and Jetschmann C., 1971, Biochim. Biophys. Acta, 227: 554-564.
Wallace W., 1973, Plant Physiol. 52: 197-201.
Woolley J. T., Hicks G. P., and Hageman R. H., 1960, J. Biol. Chem. 66: 375-400.

Author's address
Doc. dr J. Buczek
Institute of Botany, University of Wrocław,
ul. Kanonia 6/8; 50-328 Wrocław; Poland

Rola światła i ciemności w indukowaniu reduktazy azotanowej i reduktazy azotynowej w siewkach ogórka

Streszczenie

Badano metodą *in vivo* i *in vitro* aktywność reduktazy azotanowej (NR) i reduktazy azotynowej (NiR) w korzeniach oraz aktywność NR w liścieniach trzydniowych siewek ogórka. Aktywność NR w korzeniach pojawia się niemal natychmiast po dodaniu jonów azotanowych do medium indukcyjnego, podczas gdy w liścieniach obserwuje się zwłokę w indukcji NR. Ogólnie światło wzmaga aktywność NR w liścieniach i obniża ją w korzeniach w krótkotrwałych doświadczeniach. Etiolacja liścieni powoduje obniżenie aktywności NR w korzeniach i prowadzi do zaniku aktywności enzymu w liścieniach, jednakże aktywność NR wzmaga się trzykrotnie w korzeniach trzymanych w ciemności po przeniesieniu etiolowanych liścieni do światła. W korzeniach rosnących w ciemności obserwuje się zwłokę w indukcji NiR, podczas gdy w korzeniach rosnących na świetle indukcja NiR zachodzi w tym samym czasie co indukcja NR. Chloramfenikol (CAP) cykloheksimid (CHI) i actynomycyna D (ACM) podane na początku okresu indukcji siewek azotanami, hamują aktywność NR w liścieniach, podczas gdy w korzeniach tylko CHI i ACM hamują aktywność NR. W konkluzji NR w korzeniach i liścieniach ogórka jest syntetyzowana de novo na polirybosomach cytoplazmatycznych, a światło per se nie jest konieczne dla syntezy, ale ma pośredni wpływ na poziom aktywności NR i NiR zarówno w korzeniach, jak liścieniach.