NITRATE REDUCTASE ACTIVITY IN CORN SEEDLINGS AS AFFECTED BY LIGHT AND NITRATE CONTENT OF NUTRIENT MEDIA[1, 2]

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The requirement of light for the reduction of nitrate by higher plants has been under investigation for some time. Burstrom (2) obtained evidence that wheat leaves would reduce nitrate in the light but not in the dark. He concluded that nitrate reduction was directly linked to a photochemical reaction, or that it occurred concomitantly with the fixation and reduction of carbon dioxide. In contrast, Delwiche (5) used isotopic nitrogen to demonstrate that tobacco plants metabolize nitrate or ammonia in the dark as well as in the light. Other workers (7, 13) have demonstrated that nitrate and hyponitrite are metabolized both in dark and light but that in light the rate is accelerated.

The extraction, purification, and characterization of a pyridine nucleotide-nitrate reductase from soybean leaves and the occurrence of this enzyme in other higher plant species was reported in 1953 (6). Other aspects of nitrate reductase metabolism and characteristics have been published by Nason (14), Nicholas and Stevens (15), and Cheniae and Evans (4).

In 1956 experiments were initiated on the Agronomy South Farm, Urbana, to determine the effect of shade, both artificial and self-(competitive plant) shading on the nitrate metabolism of corn plants. It was concluded from these experiments (12) that nitrate accumulated in plants grown under conditions of low light intensity and that nitrate metabolism in two lines of corn (Hy2 × Oh7 and WF9 × C103) were differentially affected by the shade treatments. Candella et al (3) demonstrated that cauliflower plants slowly lost their nitrate reductase activity when placed in the dark. The nitrate reductase activity returned rapidly when the plants were returned to light.

The experiments described in this report were carried out to determine effects of light and nitrate supply on the activity of nitrate reductase in young corn plants.

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MATERIALS AND METHODS

PLANT MATERIAL: The shoots of corn (*Zea mays* L.) seedlings or the deribbed leaves of older plants of the two hybrids (WF9 × C103 and Hy2 × Oh7)[3] were used as source material. Shoot and leaf tissue were used because the level of nitrate reductase activity was lower (80 %) in the root extracts. WF9 × C103 seeds were larger (0.31 g/seed) than Hy2 × Oh7 (0.23 g/seed) and normally produced a larger more vigorous seedling and vegetative plant. These two lines were selected because their agronomic characteristics were known (12).

SAMPLING AND EXTRACTION OF ENZYMES: The shoot portion or leaves were removed from four to six plants and composited to form a sample from each treatment. The sample was immersed immediately in cold (2° C) deionized water and carried to the laboratory. The shoots or deribbed leaves were blotted dry, weighed, cut into small pieces, and ground in an Omnimixer at maximum speed for 2 minutes. The grinding medium was 0.1 M Tris,[4] 0.01 M cysteine, and 0.0003 M EDTA at a pH of between 7.3 and 7.8 (adjusted with HCl). The precise pH was determined experimentally for each kind of tissue so that the homogenate would have a pH of approximately 7.0 after grinding. Four ml of cold (2° C) grinding medium were added for each gram of tissue. The homogenate was pressed through cheesecloth and centrifuged[5] for 15 minutes at 20,000 G. The supernatant liquid was decanted through glass wool and assayed. The homogenates and extracts were kept cold (3–5° C) throughout. The assays were completed within 2 to 3 hours after sampling.

[3] The seeds were obtained from the Illinois Seed Producers Association through the courtesy of Mr. F. S. Ingersoll. The seeds were selected for uniformity of size and appearance.

[4] The following abbreviations are used: Tris for tris (hydroxymethyl) aminomethane; EDTA for ethylenediaminetetraacetic acid; TPD, triosephosphate dehydrogenase; and DPN and TPN for di- and tri-phosphopyridine nucleotides, respectively.

[5] All centrifugations were carried out in refrigerated centrifuges.

ASSAYS: The nitrate reductase was measured by a modification of the method described by Evans and Nason (6). The assay mixture contained 1.0 ml of 0.1 M potassium phosphate buffer, 0.2 ml of 0.1 M KNO_3, 0.5 ml of 1.36×10^{-3} M DPNH, 0.2 ml of enzyme extract, and deionized water to bring volume to 2.0 ml. The assay was initiated by addition of, first, DPNH and immediately thereafter the enzyme extract. The mixture was incubated at 27° C for 15 minutes and the reaction stopped by adding 1 ml of 1 % w/v sulfanilamide in 1.5 N HCl. N-(1 napthyl) ethylene diamine hydrochloride reagent was added (1 ml of 0.02 % w/v) and the contents mixed by inverting the tubes. The color was allowed to develop for 5 minutes before centrifuging at 1,500 G for 10 minutes to remove the turbidity. The absorbancy was determined by reading each sample against its own blank (complete except for DPNH) in a Beckman DU spectrophotometer at 540 mμ. Nitrate reductase extracted from corn, like that extracted from germinating wheat (16) is DPN-specific. Adding flavin nucleotide to the assay mixture did not enhance the activity and therefore it was not used. Enzyme extracts that were heated for 3 minutes in boiling water were inactive. The production of nitrite was linear with enzyme extract added over the range 0.05 to 0.6 ml. Hydroxylamine hydrochloride at the concentration suggested by Evans and Nason (6) was not used because it inhibited the activity of the enzyme. Nitrite which was added as potassium nitrite was lost from supplemental blanks that were complete except for DPNH. Although this loss of nitrite was relatively constant and non-enzymatic, it was not used as a correction factor. The activity of the nitrate reductase was expressed as μm KNO_2 formed per hour per gram fresh weight or per shoot.

Triosephosphate dehydrogenase activities were determined by a procedure previously described (9). The DPN-dependent TPD was selected as a representative enzyme, to determine if prolonged darkness inactivates enzymes in general. The TPN-dependent TPD was selected because it has been shown (8) to possess certain light-dependent characteristics.

Nitrate content was estimated by the method of Wooley et al (18). The protein content of the extracts was determined by Nesslerizing the 5 % trichloroacetic acid precipitable material (17). Bovine serum albumin was used for the reference protein.

Single determinations were made on each sample. Replication was obtained by repeating the experiment with separate lots of material.

CULTURAL TECHNIQUES AND EXPERIMENTAL PROCEDURES: *Experiment I.* The plants were grown in fertile soil in 6-inch clay pots under normal greenhouse conditions. Ten seeds were planted per pot and the plants thinned to five within 10 days. Adequate soil moisture was maintained with deionized water. Dark treatments were initiated when the plants were 21 days old, by transferring the plants to a darkened basement and placing the pots under a large (2′ × 2′ × 3′) box. Compressed air was bled into the box at the rate of 5 l/min to prevent excessive accumulation of CO_2 and moisture. The temperature varied from 24 to 28° C. A 5-watt red light bulb provided light at sampling time, otherwise the plants were in complete darkness.

Experiment II. Cultural techniques were the same as used in experiment I.

Variation in light intensity was obtained by placing the plants under box frames (30″ × 60″ × 30″) that were covered with Saran screen of varying mesh. The percentage of light transmitted was measured with a Weston (Model 603) light meter. When this series of experiments was conducted the greenhouse was covered with whitewash. This coating reduced the light intensity incident at the top of the shade structures and the unshaded plants to 6,000 to 7,000 ft-c on clear days at noon. The two shade treatments, which further reduced the light intensity 25 and 90 %, were applied 6 days after the seeds were planted.

Experiment III. Seedling material used in this experiment was grown in 250 g Terra-Lite a commercial expanded vermiculite in 3-quart Pyrex utility dishes. Forty seeds were planted in each dish. The vermiculite was wetted at planting time with 10^{-4} M $CaCl_2$ or No. 1 Hoagland solution (11) at the rate of 470 ml/100 g vermiculite. Deionized water or nutrient solution was added as needed to maintain the initial moisture level.

The material was grown in a controlled environmental chamber regulated at 22 to 23° C and 65 to 75 % relative humidity. Light was supplied by an overhead bank of 30 cool white, 72-inch fluorescent tubes and supplemented with twelve 60-watt incandescent bulbs. Incident light intensity (measured with a Weston light meter) at the top of the plants was approximately 1,750 ft-c.

Three sets of treatments were used. In set one, the seedlings were supplied with a No. 1 Hoagland solution and were germinated and grown in complete darkness. Darkness was obtained by covering with an aerated but light tight box.

A second set of seedlings was handled in an identical manner until the seedlings were 8 days old. At this time they were transferred into the light (1,750 ft-c, continuous). A third set of seeds was watered with a modified Hoagland solution that contained no nitrate (equimolar amounts of sulfate salts were substituted) and germinated in the light (1,750 ft-c—16 hr day). After the 8th day this third set was illuminated, continuously, (1,750 ft-c).

The plants were harvested and assayed at the initiation of illumination and after 5 and 26 hours of continuous illumination for sets two and three. Plants in set one (continuous dark) were harvested at the same time intervals to obtain material of comparable age.

Experiment IV. The cultural techniques were the same as in experiment III.

The following treatments were used. Half of the material was supplied with No. 1 Hoagland solution and the remainder with a modified "no nitrogen"

Hoagland solution. All plants were germinated in the dark for 8 days. On the 8th day all plants were transferred into the light and at the same time 12.5 mm of nitrate were added to each utility dish that held "no nitrate" plants. This was the same amount of nitrate that had been supplied initially in the complete Hoagland media.

Experiment V. Cultural techniques were the same as in experiment I, except that quartz sand and Hoagland No. 1 nutrient solution, with micro-nutrient supplement, were used for the nutrient medium. Excess solution was added at each watering and permitted to drain from the pots. The pots were flushed with tap water periodically to prevent excess salt accumulation. Equimolar quantities of K_2SO_4 or $CaSO_4$ were substituted for KNO_3 and $Ca(NO_3)_2$ in the nutrient solutions to obtain the differential nitrogen treatments.

Experimental Results

Experiment I. This experiment was conducted to determine the effect of complete darkness on the activity of nitrate reductase and triosephosphate dehydrogenases and the water soluble protein content of young corn.

The results are shown in figure 1 (A, B, C, and D). The initial level of activity of the enzymes and protein content was established by sampling on 2 successive days prior to the dark treatment. The nitrate reductase dropped to less than 10 % of the original level of activity in both hybrids within 48 hours after the plants were placed in the dark. Nitrate reductase was not detectable in the extracts prepared from the WF9 × C103 seedlings after 96 hours of darkness and was present only in trace amounts in extracts from Hy2 × Oh7 material.

An increase in nitrate reductase was detectable within 2 hours after the plants were returned to full sunlight in the greenhouse after 65 hours of darkness. Thirty hours after removal of the plants from the dark the activity of nitrate reductase had returned to 60 % of the initial level of activity. Approximately one-third of this 30-hour period was in darkness (night). Some leaves on the plants were damaged after the plants were transferred back to the light. The damaged leaves were removed from the plants

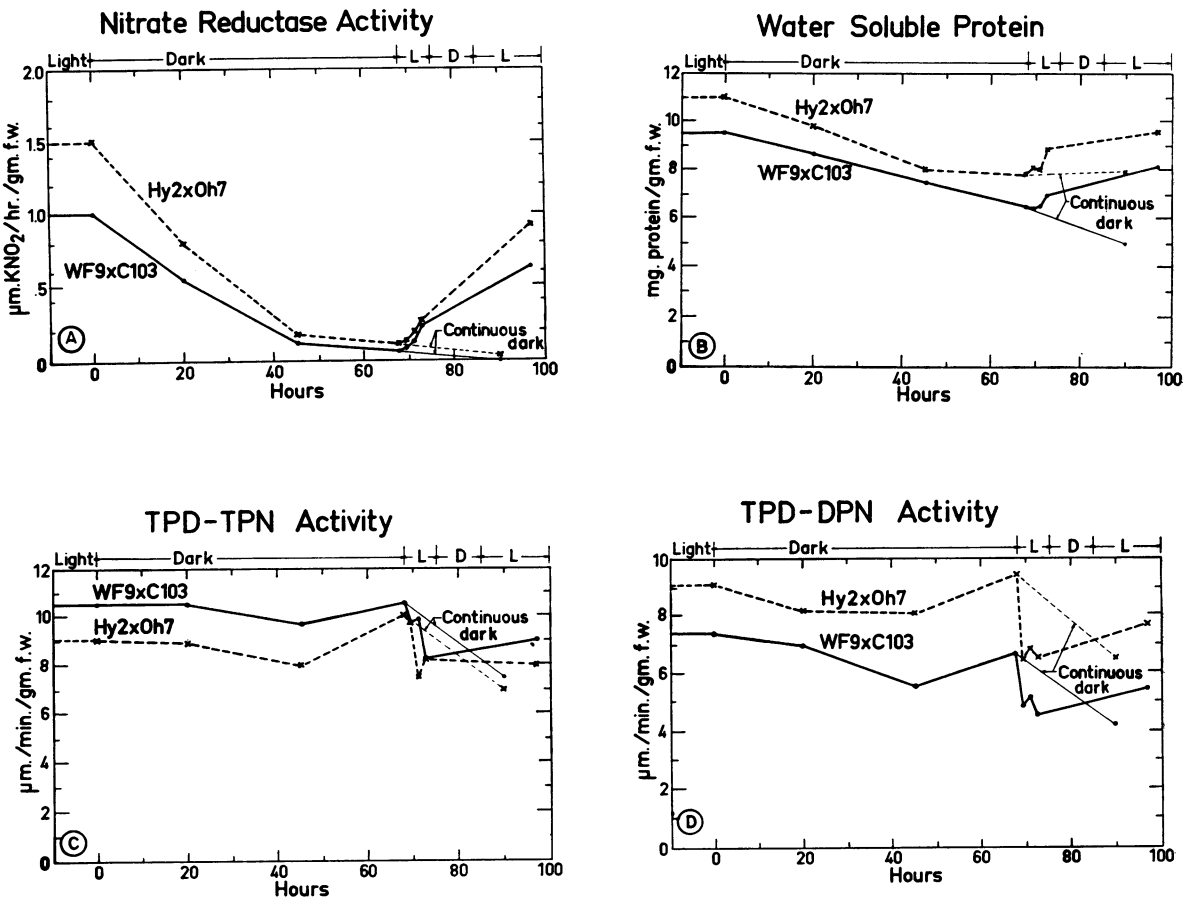

Fig. 1. Effect of dark treatment on the nitrate reductase (A) and triosephosphate dehydrogenases activities TPN (C) and DPN (D) and the protein content (B) of young corn plants.

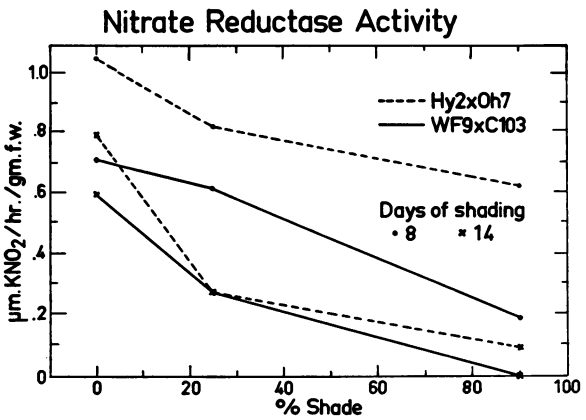

FIG. 2. Effect of shading on the nitrate reductase activity of young corn plants. (Assays made on leaf extracts).

and discarded. Plants that were transferred to the light after 96 hours of darkness did not survive.

Water-soluble protein content of the plants decreased during the dark treatment and increased when the plants were returned to the light as shown in figure 1-B. The changes in protein content of the plant correlated positively with the changes in nitrate reductase activity [fig 1 (A, B)] obtained with the light and dark treatments.

The DPN- and TPN-dependent triosephosphate dehydrogenases were not affected to any great extent by the alternate treatments of light and dark [fig 1 (C, D)]. The TPN-dependent TPD activity was consistently higher in the WF9 × C103 hybrid than in the Hy2 × Oh7 hybrid, while the converse was observed for the DPN-dependent TPD. Additional work is contemplated to determine if these are genetic characteristics of these hybrids. The TPD activity per unit of protein with both coenzymes increased throughout the first 72 hours of darkness but decreased during the next 24 hours. The increase in specific activity during the first 72 hours is attributed to the decrease in total protein with no accompanying decrease in TPD.

Experiment II. The effect of various levels of shade and duration of shade treatment on nitrate reductase activity, growth, and water soluble protein content of corn seedlings was determined. Figure 2 shows that the nitrate reductase activity decreased roughly in proportion to the amount of shading. Prolonging the shade treatment caused further decrease in enzymatic activity. After 14 days little or no nitrate reductase could be detected in either the Hy2 × Oh7 or WF9 × C103 plants grown under the 90 % shade structure.

TABLE I

EFFECT OF SHADING ON FRESH WEIGHT AND PROTEIN CONTENT OF YOUNG CORN SEEDLINGS

TREATMENTS % SHADE	FRESH WT G/SHOOT*		PROTEIN MG/SHOOT**	
	DAYS SHADED			
	8 DAYS	14 DAYS	8 DAYS	14 DAYS
	Hy2 × Oh7			
0	1.98	6.00	17.2	44.5
25	1.54	5.00	10.9	30.5
90	1.06	1.90	11.5	8.4
	WF9 × C103			
0	3.86	9.50	29.7	77.0
25	2.44	6.50	21.7	42.0
90	1.65	2.50	7.6	11.5

* Total portion of plant above ground level.
** Leaves only. The same as used for the nitrate reductase activity.

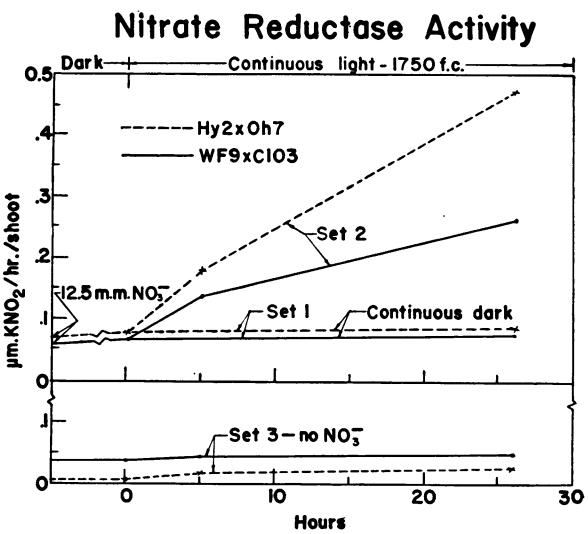

FIG. 3. Nitrate reductase activity of young corn seedlings grown under various light and dark treatments with and without nitrate. Set 1. Seedlings grown in continuous dark with 12.5 mm nitrate per dish. Set 2. Seedlings grown same as set 1 except that they were transferred to continuous light (1,750 ft-c) when 192 hours old. Set 3. Seedling grown in light (1,750 ft-c-16-hour day) without nitrate and transferred to continuous light (1,750 ft-c) when 192 hours old.

As shown in table I, growth (fresh wt) of the plants was also reduced, roughly in proportion to the amount of shading. The protein content per shoot (table I) was affected in a similar manner, especially after 14 days of shade treatment. There is a positive correlation between the nitrate reductase (fig 2) and the protein content and growth (table I) of these plants.

Experiment III. Figure 3 shows the effect of continuous dark and light and dark→light treatments on nitrate reductase activity in corn seedlings grown

TABLE II

COMPARISON OF FRESH WEIGHT AND WATER SOLUBLE-PROTEIN CONTENT OF YOUNG CORN SEEDLINGS GROWN UNDER VARIOUS LIGHT AND DARK TREATMENTS WITH AND WITHOUT NITRATE

TREATMENT AGE (HRS)	FRESH WT G/SHOOT*			PROTEIN MG/SHOOT*		
	192	197	218	192	197	218
			Hy2 × *Oh7*			
Set 1** + NO_3^- Dark	0.52	0.60	0.79	2.8	2.9	3.2
Set 2*** + NO_3^- Dark→Light	0.51	0.63	0.78	2.9	2.9	4.5
Set 3† − NO_3^- Light→Light	0.44	0.48	0.50	2.1	2.2	2.6
			WF9 × *C103*			
Set 1** + NO_3^- Dark	0.62	0.74	0.82	3.7	3.8	3.8
Set 2*** + NO_3^- Dark→Light	0.63	0.69	0.84	3.8	3.8	6.2
Set 3† − NO_3^- Light→Light	0.62	0.64	0.62	3.4	4.3	3.9

* Total portion of plant above ground.
** Seedlings grown in continuous dark with 12.5 mm nitrate per dish.
*** Seedlings grown same as set 1 except transferred to continuous light (1,750 ft-c) when 192 hours old.
† Seedlings grown in light (1,750 ft-c—16-hour day) without nitrate and transferred to continuous light (1,750 ft-c) when 192 hours old.

TABLE III

EFFECTS OF LIGHT AND NITRATE ADDITION TIME ON GROWTH AND PROTEIN CONTENT OF 2 CORN HYBRIDS

LIGHT TREATMENT	FRESH WT G/SHOOT*		PROTEIN MG/SHOOT*	
	$+NO_3$**	−, $+NO_3$***	$+NO_3$**	−, $+NO_3$***
		Hy2 × *Oh7*		
Dark—8 days	0.75	0.59	3.1	2.6
Light—hours				
4	0.73	0.60	3.1	2.3
8	0.77	0.61	3.2	2.4
28	0.92	0.67	5.2	3.6
55	0.90	0.70	5.9	4.2
		WF9 × *C103*		
Dark—8 days	0.89	0.68	4.2	3.4
Light—hours				
4	0.90	0.70	4.6	3.1
8	0.95	0.70	5.1	3.5
28	0.98	0.75	6.2	4.7
55	1.26	0.88	9.2	6.8

* Total portion of plant above ground level.
** Plants were germinated in vermiculite wetted with Hoagland solution. Deionized water was added as needed thereafter.
*** Plants were germinated in vermiculite wetted with a modified Hoagland solution devoid of nitrogen. When the plants were transferred into the light, 12.5 mm of nitrate was added to each dish. Deionized water was added as needed, throughout.

with and without nitrate. Both nitrate and light are necessary for the formation of nitrate reductase in quantities required by plants for normal growth.

Other experiments have shown that plants grown without nitrate in the greenhouse under normal light cycles have a level of nitrate reductase activity comparable to that shown for the "no-NO_3^- plants" (fig 3), however after 7 or 8 days even this low level of nitrate reductase activity could not be detected.

The data show that the dark-grown seedlings are capable of reducing nitrate at a slow rate (0.06 to 0.07 μm KNO_2/hr/shoot). This suggests that the light effect is indirect. Fresh weight and water soluble protein content of these plants are given in table II.

There was a positive correlation between the nitrate reductase activity and protein content of the plants. A comparison of the protein content (table II) with the nitrate reductase activity (fig 3), suggests that a portion of the increase in nitrate reductase activity was the result of synthesis.

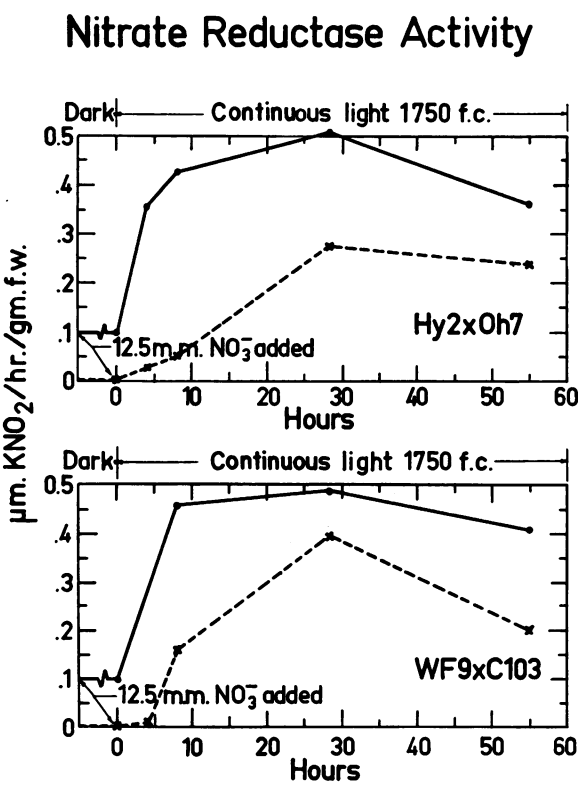

FIG. 4. Effect of light and time of nitrate addition on the nitrate reductase activity of young corn shoots. Solid lines indicate the nitrate reductase activity in seedlings germinated in the dark with nitrate (Hoagland solution) and transferred into the light at zero time. Dotted lines indicate the nitrate reductase activity in seedlings germinated in the dark without nitrate (modified Hoagland solution) but supplied with nitrate at the time of transfer into the light.

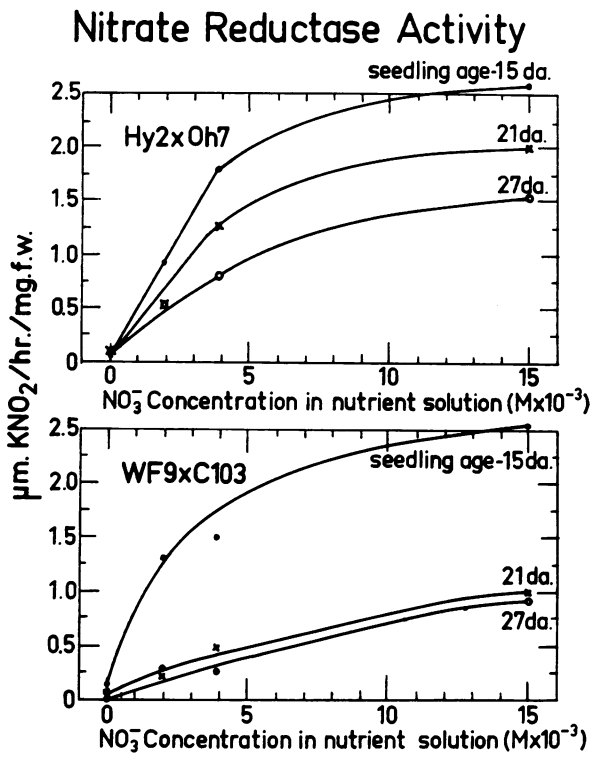

FIG. 5. Nitrate reductase activity of young corn shoots grown in media that contained various concentrations of nitrate.

Experiment IV. Studies were made concerning the rate of synthesis of nitrate reductase in corn seedlings upon exposure to light. Typical results are presented in figure 4 and table III. The hybrid WF9 × C103 formed nitrate reductase more rapidly than hybrid Hy2 × Oh7 (fig 4) when the nitrate was added at the same time the plants were placed in the light. This response is attributed to the more vigorous seedling growth, and ability to accumulate ions that is characteristic of WF9 × C103.

Experiment V. Nitrate reductase activity was increased in corn seedlings as the concentration of nitrate was increased in the nutrient solution. The results of one set of treatments are shown in figure 5.

In general, there was a positive correlation between the growth (fresh wt) or protein content of the plants (table IV) and the nitrate reductase activity (fig 5). The WF9 × C103 plants grown in the medium supplied with 0.0038 M nitrate were chlorotic and stunted. Although no reason is known for the aberrant growth, the effect was reflected in the fresh weight, protein and nitrate content of these plants (table IV).

Similar results were obtained with plants grown in vermiculite cultures and supplied with varied concentrations of nitrate.

TABLE IV

GROWTH, PROTEIN, AND NITRATE CONTENT OF YOUNG CORN SHOOTS AS AFFECTED BY VARIOUS CONCENTRATIONS OF NITRATE IN NUTRIENT MEDIA

SEEDLING AGE DAYS	Hy2 × Oh7			WF9 × C103		
	15	21	27	15	21	27
	Fresh wt (g/shoot)*					
Conc. NO_3^- in media M						
0.0	0.35	0.51	0.67	0.75	1.17	2.01
0.0019	0.40	1.28	2.03	0.95	2.40	5.31
0.0038	0.53	1.35	2.40	0.84	1.56	3.15
0.015	0.56	1.50	3.07	0.90	2.41	5.60
	Protein (mg/shoot)					
0.0	1.7	1.6	2.2	4.5	4.2	4.8
0.0019	3.4	6.3	12.6	8.3	10.8	25.5
0.0038	4.7	9.8	16.1	7.2	10.9	14.8
0.015	5.5	13.4	20.6	8.6	17.4	35.3
	NO_3^- N (ppm/g ft wt)					
0.0	N.D.**	N.D.	N.D.	N.D.	N.D.	N.D.
0.0019	N.D.	N.D.	26	310	tr	140
0.0038	340	250	270	310	15	75
0.015	1,240	740	1,200	1,580	730	1,540

* Total portion of plant above ground level.
** Not detectable.

DISCUSSION

At present no explanation can be given for the loss of nitrate reductase activity in the plants placed in the dark (Expt I). The loss of protein (fig 1–B) and the accumulation of nitrate (data to be presented elsewhere) when the plants are placed in the dark, argues that this loss of enzyme activity occurs in vivo and is not an artifact of isolation. Two possible causal factors are the accumulation in the dark of an inhibitory nitrogenous metabolite and the oxidation of the active sulfhydryl group(s) (6) on the enzyme. Some information has been obtained on the former. Plant material grown in the dark was mixed with an equal portion of material exposed to normal greenhouse light prior to grinding. Assays made on extracts prepared in this manner did not indicate the presence of an inhibitor in the dark-grown material of either Hy2 × Oh7 or WF9 × C103. Similar results were obtained by mixing of extracts prepared separately from the light- and dark-grown materials. Adding hydroxylamine to the assay in final concentrations of 10^{-4} and 10^{-3} M caused an inhibition of nitrate reductase activity of 25 and 78 %, respectively. It is doubtful if hydroxylamine would ever reach these concentrations in plant material as a natural metabolite. Ammonia as ammonium sulfate, asparagine, and glutamine (10^{-3} M) did not inhibit nitrate reductase activity.

Under dark conditions the oxidation-reduction systems of the plant may shift toward oxidation thereby inactivating certain enzyme systems. The return to the light should reverse this process which in turn may reduce an inactive, oxidized enzyme. Unless some specific oxidizable-reducible compound or localization of nitrate reductase in the tissue is envisaged, this postulate is untenable because of the stability of the sulfhydyl-active triosephosphate dehydrogenases under similar conditions (fig 1–C and 1–D).

No adequate explanation can be given for the decrease in nitrate reductase at the last sampling (55 hrs light, fig 4, Expt IV). The decrease in nitrate reductase with age of plants grown in vermiculite was observed repeatedly under varied environmental conditions. Although corn seedlings in vermiculite are vigorous and appear healthy up to periods of 12 to 14 days, growth thereafter is extremely slow and the plant becomes spindly. The use of greater amounts of vermiculite per plant, aeration, or modification of nutrient supply did not change this pattern of growth.

In experiment V, the loss of nitrate reductase activity with seedling age is attributed, in part, to the proportionate increase in stem tissue. The expanded leaves have the highest level of nitrate reductase. In these assays the entire shoot portion was homogenized, thereby increasing the stem to leaf ratio.

Other environmental factors, such as excessively high salt content in the tissue for prolonged periods, may also reduce nitrate reductase activity by some unknown mechanism. The nitrate concentration in the tissue grown in Hoagland solution (0.015 M nitrate) (table IV) is well in excess of 0.02 M that has been reported by Evans and Nason (6) to be inhibitory

(75%) to the photosynthetic reduction of TPN. Since these plants were not subdivided into leaf and stalk portion, the relative nitrate content in the individual parts is not known. In general, the stalk portion of corn plants is higher (5–20-fold) in nitrate content than the leaves (10). However, these high concentrations of nitrate (table IV) did not suppress growth and protein synthesis in comparison with the effects obtained with media containing lower levels of nitrate. This does not preclude the possibility that uniform application of intermediate levels of nitrate-containing media might produce larger plants.

In general, agronomists have observed that corn plants accumulate nitrate (tissue tests) under various conditions of environmental stress. Conditions of droughty or water-logged soil, reduced light or darkness, as well as deficiencies in phosphate and iron, have been associated with high nitrate accumulation in corn leaves (Pers. communic. from Prof. Edward H. Tyner, Agronomy Dept., Univ. of Ill., Urbana). It is postulated that conditions of environmental stress activate some biological control mechanism that inhibits nitrate reductase activity which in turn results in the accumulation of nitrate in the plants.

It is commonly accepted that nitrates are not toxic to plants while the intermediates from nitrite to ammonia are toxic within limits. Corn plants grown under high nitrogen fertility accumulate appreciable quantities of the amides, glutamine, and asparagine (10). According to Prianischnikov, amide formation (asparagine) is a mechanism for detoxification of ammonia (1). It is proposed that nitrate reductase and its biological control mechanism provide the plant with a second system for the regulation and control of toxic nitrogenous products. The question arises as to whether the differing abilities of various corn varieties to reduce nitrate under artificial or competitive plant shade (12) may be related to the relative proportions and effectiveness of these two protective systems, present in each hybrid.

The data presented in figures 1 to 5, permit the conclusion that the level of nitrate reductase activity (computed on basis of activity/g fresh wt, mg of protein, or shoot) was higher in the hybrid Hy2 × Oh7 than it was in the hybrid WF9 × C103. Assays made throughout the growing season on Hy2 × Oh7 plants grown under field conditions also support this conclusion. This higher level of nitrate reductase activity in the Hy2 × Oh7 strain was associated with a higher content of water soluble protein when the plants were grown under field, greenhouse, or constant temperature chamber conditions. Assays made on field-grown corn plants show that nitrate content correlates negatively with the nitrate reductase activity. Knipmeyer (12) showed that WF9 × C103 plants accumulated more nitrate when shaded than did the Hy2 × Oh7 plants. The consistent differences between these two strains in nitrate metabolism are attributed to the genetic characteristics of each strain.

Summary

Experiments were carried out to determine the effect of light and nitrate supply on the activity of nitrate reductase in two single cross corn hybrids (Hy2 × Oh7 and WF9 × C103). Plants were grown in the greenhouse or in a constant temperature room in soil, sand, or vermiculite cultures.

Young corn plants placed in complete darkness for 48 hours lost 90% of their nitrate reductase activity. The activity was quickly restored when the plants were returned to the light. In other experiments, corn plants were grown under artificial shade in the greenhouse. The nitrate reductase activity in these plants decreased roughly in proportion to the amount of shading. It was shown that both light and nitrate are necessary for the formation of nitrate reductase in quantities required by the plant for normal growth. Corn seedlings germinated in the dark in Hoagland solution showed a fourfold increase in nitrate reductase within 8 hours after they were transferred into the light. Plants that were germinated in the dark without nitrate, and given nitrate at the same time they were transferred to the light, showed a slower rate of development of nitrate reductase activity. Greenhouse experiments in which the nitrate content of nutrient media was varied showed that the level of nitrate reductase activity was dependent in part upon the substrate concentration.

There was a positive correlation between the nitrate reductase activity and the growth and protein content of both strains. This correlation supports the contention that the nitrate reductase activities measured are a reflection of the in vivo activities.

Shoots and leaves of the hybrid Hy2 × Oh7 have a higher level of nitrate reductase activity and protein content than similar tissue from WF9 × C103 plants.

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OXIDATION AND PHOSPHORYLATION BY MITOCHONDRIA FROM GREEN STEMS [1, 2, 3]

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Etiolated plants or very young seedlings generally are used as a source of mitochondrial preparations (5, 7). For example, Millerd et al (9), who apparently were first to isolate mitochondria capable of phosphorylation from plants, used etiolated mung bean seedlings. It is desirable for various reasons to start with green plants, but satisfactory preparations have been difficult to obtain (2). Recently, however, preparations oxidizing tricarboxylic acid cycle intermediates were prepared from green leaves (4, 6, 12, 14, 15) and phosphorylation was demonstrated (14, 15). In the present work, particulate fractions were obtained from green stems of tomato; the preparations were superior in several respects to those from etiolated tomato plants (11) and from green leaves (12). Ratios of phosphorus esterified to oxygen consumed were about 3.0. A hexokinase-glucose trapping system was required for phosphorylation. The preparation was relatively insensitive to 2, 4-dinitrophenol (DNP). Catalytic amounts of Krebs cycle acids were required for pyruvate oxidation. High activities of mitochondria from stems make these preparations useful for further experimentation, such as determination of biochemical changes associated with disease development (Wu, Lung-Chi and R. P. Scheffer. 1960. Comparative activities of mitochondria from Fusarium-infected and healthy tomato plants. In manuscript).

MATERIALS AND METHODS

Tomato plants of the variety, Bonny Best, were grown in the greenhouse in peat-sand mixtures and were fertilized with a suitable concentration of a complete nutrient solution. Greenhouse temperature was held at a minimum of 20° C. Plants were grown during the fall and winter for 4 to 6 weeks before harvesting, at which time they were 5 to 12 inches high. Stems were excised above the cotyledons and leaves were removed. The top 4 to 6 inches of long stems was used, unless indicated otherwise.

Pierpoint's procedure (12) was slightly modified for isolation of mitochondria. Stems were cut into pieces about one centimeter long and were chilled at 2° C for 10 minutes. The pieces were then placed in a mortar with half their weight of acid-washed sea sand and with a volume of extraction fluid twice the weight of the stems (ml–g). The material was ground vigorously for 2 minutes, then filtered through six layers of cheesecloth. The pH of the extraction medium did not change while grinding stems.

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