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The role of light in the induction of nitrate reductase activity in etiolated shoots of *Triticum vulgare*

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ABSTRACT

The etiolated shoots were exposed to a constant intensity of light (5000 lux supplied by a bank of white, cool, fluorescent lights) in the presence of optimal concentration of the substrate – inducer (20mM $KNO_{3)}$. When the etiolated shoots were exposed to light and 20mM KNO_3 simultaneously, there was a progressive synthesis of photosynthetic pigments and induction of NR, assayed by the in vivo method under optimal assay conditions, with time of exposure (0,4,8,12, and 24 hours). The amount of Chl b synthesis, compared to that of Chl a, was less throughout the time of exposure to light. Chl a/b remained constant up to 8 hours of exposure to light and decreased slightly thereafter. A positive correlation was observed between the amount of total chlorophyll and the magnitude of *in vivo* NR activity throughout the 24 hours period. Significantly, by the 24th hour of greening, the amount of NR activity in the 3-day-old greening shoots was 1.75 micromole NO_2 produced per hour per gram fresh weight, a value almost equal to that of 7-day-old green leaves induced under similar conditions. Etiolated shoots kept in darkness throughout the NR induction period did not show any enzyme activity, indicating that even in the presence of optimal external nitrate, the NR-inducer; light is still and perquisite for optimal induction of NR activity.

Key words: Etiolated Shoots, Photosynthetic Pigments, Substrate Inducer, *Triticum Vulgare*
Abbreviations: NR: Nitrate Reductase, Chl: Chlorophyll

INTRODUCTION

Light as an environmental factor affecting enzyme activity in plants is widely recognized. Light has been reported to play a complex and varied role in nitrate assimilation in that it.

- Stimulates nitrate uptake.[1-3]
- Enhances transfer of nitrate from the vacuolar (storage) pool to the easily accessible cytoplasmic (metabolic) pool in the cells[4].
- Promotes synthesis of nitrate reductase[5].
- Activates the pre-existing enzyme[6].
- Increase the accessibility of the enzyme to nitrate via phytochrome – mediated membrane changes and /or other phytochorme effects[7].
- Provides the reductant via photosynthesis[8-9].
- However, nitrate reductase was detected in dark-grown corn seedlings[10] and Chlorella[11], etiolated barely leaves[12], radish seedlings[13], and etiolated wheat seedlings[14].

The role of light in the reduction of nitrate has been considered indirect. In addition, the nitrate reductase complex from higher plants specifically requires NADH as an electron donor. For this reason, a direct involvement of chloroplast reaction in nitrate reduction is thought to be improbable. The extent of dependence of nitrate reduction on chloroplast development and metabolism therefore need to be explored.

MATERIAL AND METHODS

Seed material

Wheat (*Triticum vulgare)* seeds of Punjab variety were obtained from local seed stores.

Cultivation of wheat seedlings

Healthy seeds of wheat were surface – sterilized in 4% (v/v) sodium hypochlorite for 5 min, thoroughly washed several times in tap water and subsequently soaked for 3 hours in distilled water. The seeds were germinated in Petri dishes lined with a course filter paper (Kalpi) in distilled water in dark before being exposed to 20 mM KNO_3 under a constant illumination of 5000 lux supplied by a bank of white, cool, fluorescent lamps.

Harvest of seedlings

Unless otherwise stated, uniformly growing seven-day-old green seedlings were harvested at least 4 hours after exposure to light. For experiments involving etiolated seedlings, 3-day-old etiolated shoots raised in distilled water in dark were exposed to light and 20mM KNO_3 simultaneously.

Induction of Nitrate reductase (NR) and assay of *in vivo* NR activity

Nitrate reductase was induced in green. Seedlings and etiolated shoots with 20mMKNO_3 under constant illumination.

Unless otherwise mentioned, the standard, infiltration medium (2ml to 5ml depending on the experiment) for the in vivo NR assay was composed of

-100mM KH_2 PO_4 – KOH, pH 7.5
-100mM KNO_3
-1% butanol
-0.1% Triton X – 100

Leaf segments (1mm) equivalent to 0.1-0.2g fresh weight were incubated in dark at room temperature (30^{0}C) in 20ml glass vials with air-tight caps containing either 2ml (for greening shoots) or 5ml (for green leaf segments) infiltration medium. The contents were periodically shaken. After 1 hour incubation, 0.2ml to 0.5ml of the infiltration medium was removed for nitrite analysis. The nitrate reductase activity was expressed as µ moles nitrite produced per hour per gram fresh weight.

Extraction and estimation of chlorophyll and carotenoids

Shoot segments (100mg) were ground with a chilled pestle and mortar in diffuse light in 80% cold acetone and the homogenate was centrifuged at 3,000 × g for 2min. Aliquots of 5ml of 80% cold acetone were added to the pellet and pigments were extracted in dark and cold till the pellet was non-green. The supernatants were pooled and protected from light prior to estimation of chlorophyll and carotenoids in a UV-visible spectrophotometer (Systronics, Model 118).
The concentration of chlorophyll was measured according to[15] Arnon (1949)

$$\text{Chl a (mg/l)} = (12.7 \times A_{663}) - (2.69 \times A_{645})$$
$$\text{Chl b (mg/l)} = (22.9 \times A_{645}) - (4.68 \times A_{645})$$
$$\text{Total chlorophyll (mg/l)} = (20.2 \times A_{645}) + (8.02 \times A_{663})$$

The concentration of carotenoids was measured at 473 nm according to Goodwin (1954)

$$E_{1Cm}^{1\%} = 2{,}500$$

Nitrate (NO_2^-) estimation

To nitrite solution or a known volume of the infiltration medium containing nitrite, 1ml of 1% (w/v) sulphanilamide reagent prepared in 3N HCL and 1ml of 0.02% (w/v) N – (1 – naphthyl) ethylendiamine dihydrochloride reagent were added in quick succession and the contents were thoroughly mixed. The color was allowed to develop for 15 min prior to reading at 540nm in a UV-visible spectrophotometer (Systronics, Model 118)

The amount of nitrite formed was calculated based on

$$E_{1Cm}^{1mM} \text{ Nitrite complex (540nm)} = 55$$

RESULTS

The role of light in the induction and activity of nitrate reductase activity has been reported to be indirect[17]. In the present investigation the relationship between light, the level of photosynthetic pigment synthesis indicative of extent of chloroplast development and nitrate reductase induction was investigated in 3-day-old etiolated shoots raised in distilled water. When these

Table 1: Effect of greening on pigment synthesis in 3-day-old etiolated shoots exposed to light +20 mm KNO_3 simultaneously

S.No.	Hrs. of greening	CHL.A	CHL.B	CHL. A/b	Total CHL.	Carotenoids
1	0	0.00	0.00	0.00	0.00	Not estimated
2	4	50.6	16.1	3.15	66.7	29
3	8	103.8	32.8	3.17	136.6	53
4	12	114.0	40.0	2.8	154.0	67
5	24	311.0	131.0	2.38	442.0	121

Table 2: Effect of greeting on induction of NR activity in 3-day-old etiolated shoots exposed to light + 20mm KNO_3 simultaneously

S. No.	Hrs. of Greening	µ moles of NO_2^- formed /Hr/ gram fresh Wt
1	0	0.00
2	4	0.19
3	8	0.32
4	12	0.65
5	24	1.75

etiolated shoots were exposed to light and 20 mM KNO_3 simultaneously, there was a photosynthetic pigments and induction of nitrate reductase activity with time of exposure to light and nitrate. The amount of enzyme activity was related to the level of total chlorophyll (Table 1& 2) and no enzyme activity was observed in the etiolated shoots induced in darkness with 20 mM KNO_3.

DISCUSSION

NR activity is the rate – limiting step in the process of nitrate reduction to ammonia[18]. Several workers have recommended the use of intact tissue (in vivo) assay of NR[19-20]. The in vivo assay described in this study is a simple and rapid way of assaying NR activity in the leaves of Punjab wheat.

The observed correlation between the increase in the levels of photosynthetic pigments and induction of nitrate reductase activity with the time of exposure of etiolated shoots to light under NR – inducing conditions indicates that induction and activity of nitrate reductase depends on the extent of chlorophyll synthesis and therefore chloroplast development. Such a dependence of induction and activity of NR on chloroplast development was shown in pigment – deficient leaves[21] and mustard seedlings[23] and wheat seedlings under bleaching and non-bleaching condtions[24-25]. The effects of light on NR induction and activity have been shown to include stimulation of *de novo* synthesis, activation of the enzyme, increased transcription of the NR genes by light absorbed by chlorophyll[26-27].

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