INFLUENCE OF SUCROSE, GLUCOSE, AND MALATE ON NITRATE REDUCTASE ACTIVITY IN BROAD BEAN UNDER LIGHT/DARK CONDITIONS

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An investigation to study the diurnal variation in the activity of leaf nitrate reductase in broad bean has been carried out. The result showed a relatively higher rate of NR activity in light than in dark. Dark NR activity was increased by sucrose, glucose and malate. The effect of nitrate on light and dark NR activities was also studied. The light and dark NR activities registered an increment up to 63 and 100% respectively indicating that NR activity can be induced even in the dark.

Key Words : Diurnal variation, Nitrate reductase, NR activity.

Nitrogen is central to the growth and development of all crop plants required in large amounts. It is taken up from the soil in the form of nitrate and reduced to ammonium by the enzymes nitrate reductase (NR, EC 1.6. 6.1) and nitrite reductase (NIR, EC 1.7.7.1) (Beevers and Hagemen, 1969). It is well established that the appearance of these enzymes is induced by nitrate and it is generally believed that NR is cytosolic while NIR is plastidic (Schuster and Mohr, 1990). The NADH required for the nitrate reduction in the cytosol could be provided in multiple ways, either by photosynthetic electron transport via malate oxaloacetate or triosephosphate 3-3-phosphoglycerate shuttles or from mitochondrial substrate oxidation via malate - oxaloacetate shuttle (Ebbighausen et al., 1985; Heincke et al., 1991). Since NIR needs reduced ferredoxin, which is provided by PSI, its activity is essentially light dependent (Aslam et al., 1979).

The influence of light on nitrate assimilation has been investigated quite extensively (Singh and Sawhney, 1993). NR is extremely labile enzyme and regulated by many environmental factors, availability of nitrate and light being most important (Hewitt, 1966; Solomonsen and Barber, 1990; Redinbaugh and Campbell, 1991). High NR activity under light condition and low NR activity under dark condition have been reported in Squash and in a wide range of higher plants (Lillo, 1994). Recent findings show that nitrate nutrition enhances NR gene transcription (Melzer et al., 1989) and even in the dark nitrate nutrition induces NR expression in tomato and tobacco (Galangau et al., 1988). It has also been suggested that glucose or sucrose can replace light in eliciting the increase in NR mRNA (Lillo, 1994). However, inter-and intra-species differences exit with respect to the plants ability to assimilate nitrate in the dark (Reed et al., 1993; Kaim et al., 1991). Hence, the present work is aimed to investigate the influence of sucrose, glucose and malate on NR activity in broad bean plants under light/dark conditions.

MATERIALS AND METHODS

Broad bean (Vicia faba L.) seeds (obtained from Horticulture Research Station, Ooty) were raised in pots containing garden soil under natural conditions. The plants were irrigated at two days intervals. For the experiments, leaf samples of 30 days old plants were used. Sampling with an interval of 3 hour were taken for a period of 24 hours i.e. 6 a.m. to 6 a.m. Detached leaves were thoroughly washed, vinced with distilled water, blotted on filter paper and cut into small discs. These were vacuum infiltrated with 0.05 M KNO₃ in 0.1 M phosphate buffer pH 7.5. The effect of sucrose, glucose and malate on NR activity was studied by adding them in the infiltration medium at concentration of 1.0 mM. The effect of NO₃⁻ on NR activity was also studied by vacuum infiltrating the leaf discs with 0.05, 0.1, 0.15 or 0.2 M KNO₃ in 0.1 M Phosphate buffer pH 7.5 or with buffer only.

In vivo NR activity was assayed as described by Hageman & Hucklesby (1971).
RESULTS

NR activity in broad bean leaves was higher during the day period, the maximum being at 12.00 pm. Thereafter, the activity declined gradually and the lowest NR activity was observed at 12.00 a.m. in control (Table 1). The reduction between day time activity and night time activity was 71% in control. The percentage of decrease in NR activity in the leaves infiltrated with sucrose, glucose and malate was 19.1, 16.3 and 8.8% respectively. When the leaves collected from plants under light and dark conditions were infiltrated with varying concentrations of KN03, an increase in NR activity was observed in both the cases. In light leaves NR activity was maximal in the presence of 0.1 M KN03 whereas in dark leaves the maximal activity was observed at 0.15 M KN03 concentration (Table 2). However, the stimulatory effect of nitrate on NR activity was only to a minor extent when compared with that of sucrose, glucose and malate (Table 1 and 2).

DISCUSSION

Our results indicated that in broad bean leaves nitrate reduction takes place at relatively high rates during the light period (Table 1). Since NR appears to be in the cytoplasm, NADH for nitrate reduction in the light is probably generated via a shuttle where reduced metabolites coming from the chloroplasts are used to reduce NAD in the cytoplasm (Campbell, 1988). The current evidence indicates that light stimulates de novo synthesis, as well as activation of higher plant NR at the protein level (Lillo, 1994). It has also been demonstrated in spinach leaf that NR is very rapidly inactivated by sudden darkening avoiding an accumulation of the toxic nitrite in the cells (Riens and Heldt, 1992).

**Table 1. In vivo nitrate reductase activity in broad bean leaves under light/dark conditions - Stimulatory effect of sucrose, glucose and malate.**

<table>
<thead>
<tr>
<th>Additive</th>
<th>6 hr</th>
<th>9 hr</th>
<th>12 hr</th>
<th>15 hr</th>
<th>18 hr</th>
<th>21 hr</th>
<th>24 hr</th>
<th>3 hr</th>
<th>6 hr</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>2.3</td>
<td>5.8</td>
<td>8.2</td>
<td>8.0</td>
<td>7.1</td>
<td>3.1</td>
<td>2.3</td>
<td>2.3</td>
<td>2.3</td>
</tr>
<tr>
<td>Sucrose</td>
<td>7.2</td>
<td>7.4</td>
<td>8.9</td>
<td>8.7</td>
<td>8.5</td>
<td>8.1</td>
<td>7.4</td>
<td>7.2</td>
<td>7.2</td>
</tr>
<tr>
<td>Glucose</td>
<td>7.7</td>
<td>8.0</td>
<td>9.2</td>
<td>9.0</td>
<td>8.5</td>
<td>8.0</td>
<td>7.8</td>
<td>7.8</td>
<td>7.8</td>
</tr>
<tr>
<td>Malate</td>
<td>10.3</td>
<td>10.7</td>
<td>11.3</td>
<td>11.1</td>
<td>10.8</td>
<td>10.3</td>
<td>10.3</td>
<td>10.3</td>
<td>10.3</td>
</tr>
</tbody>
</table>

It has been suggested that carbohydrates may supply the metabolic energy for the reduction of nitrate under such conditions (Aslam and Huffaker, 1984).

Table 1 demonstrates that the decrease in dark NR activity was minimal when the leaves were infiltrated with sucrose, glucose and malate. In green leaves, the light effects are mediated through photosynthetically active light by products of the Calvin cycle (Lillo, 1994). Evidence for this is from experiments showing that sucrose or glucose could replace light in the induction of NR mRNA in *Arabidopsis* leaves (Cheng *et al.*, 1992). The lowest reduction in dark NR activity was observed in leaves infiltrated with malate. Relationship between the malate and nitrate metabolism is well documented (Abdin *et al.*, 1993). The close association between the levels of endogenous malate and NR activity for certain period of time during the diurnal cycle shows that malate probably supports nitrate reduction (Deng *et al.*, 1989).

Dark leaves infiltrated with 0.15 M KN03 exhibited an increment in NR activity by 100% compared to control which was infiltrated with buffer only (Table 2). Similar findings have been reported in tomato and tobacco plants (Galangau *et al.*, 1988). This could be due to the induction of NR enzyme even in the dark, since the enzyme is substrate inducible (Melzer *et al.*, 1989). When light leaves were infiltrated with KN03, NR activity increased by 63% compared to control. This decrease in percentage of light NR activity compared to dark NR activity could be due to negative feedback effect exerted by some metabolites from nitrate assimilation or by the adverse effect of elevated...
Influence of sucrose, glucose and malate on Nitrate temperature during the light period (Beavers and Hageman, 1969; Lillo, 1994).

REFERENCES


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