EFFECT OF AGE AND NITRATE CONCENTRATION ON NITRATE REDUCTASE ACTIVITY IN DIFFERENT PARTS OF CORCHORUS CAPSULARIS L. SEEDLINGS

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Abstract

Response of Corchorus capsularis L. seedlings to various concentration of nitrate were evaluated in terms of variation in nitrate reductase activity, total nitrogen, fresh weight, dry weight and leaf size. An increased activity of nitrate reductase was observed up to 13 days of seedling growth. Maximum activity was found in leaves whereas stem exhibited no significant effect of nitrate concentration on enzyme level at different stages of growth. An appreciable increase in total nitrogen by exogenous supply of nitrate was also encountered whereas in nitrogen starved seedlings it declined after 13 days. Fresh and dry weight of seedlings were less affected with increasing concentration of nitrate. Leaf size increased when seedlings were grown in 10mM KNO₃ solution and decreased in 0mM solution.

Introduction

Nitrate reductase (E.C. 1.6.1.) an enzyme complex, which catalyzes the reduction of nitrate to nitrite has been demonstrated in algae (Ahmed & Spiller, 1975), fungi (Subramanian & Sorger, 1972) and higher plants (Beever & Hageman, 1969). Numerous studies relating to its distribution in higher plants show that this enzyme has been detected in aleurone cells of barley (Ferrari & Varner, 1970), scutellum of corn (Huckleby & Elsaer, 1969), germinating seedlings of cotton (Radin, 1974), cotton cotyledons (Purvis & Tischler, 1976) and in attached or excised root systems of many plants (Wallace & Pate, 1965). Abundent nitrate reductase is reported in apple leaves as well as in roots (Klepper & Hageman, 1969). Although nitrate reductase activity could be detected in all parts of a plant yet barring few exceptions (Weissman, 1972) the bulk of nitrate is reduced in the leaves of higher plants (Beever & Hageman, 1969; Srivastava, 1975).

The factors which control the in vivo activity of nitrate reductase include age of plant (Amindari, et al, 1978), exogenous nitrate concentration (Klepper et al, 1971); pH of the incubating medium (Jaworski, 1971); concentration of chemical used for enhancing cellular permeability (Ferrari & Varner, 1970; Jaworski, 1971); and necessity of strict anaerobiosis for maximum nitrite production (Ferrari & Varner, 1970; Jaworski, 1971).

The age of the plant and exogenous nitrate concentration appears to have a significant effect on enzyme levels (Amindari et al, 1978, Klepper et al, 1971). Plants growing
with nitrate frequently exhibit higher activity of nitrate reductase than those growing with other nitrogen source (Ferguson & Knypl, 1974). Harper & Hageman (1972) have found that enzyme activity was high in early seedlings of soybean and declines as the plant gradually advances to maturity. Oaks et al. 1972. observed a more rapid decay of nitrate reductase in mature primary roots than in young root tip cells of maize.

The present investigation demonstrates the occurrence of nitrate reductase in Corchorus capsularis and the effect of age and nitrate concentration on the development and distribution of nitrate reductase in different parts of jute seedlings is reported.

Materials and Methods

Jute seedlings (Corchorus capsularis L.) were surface sterilized with 0.2% HgCl₂ solution and germinated in sand moistened with modified half strength Hoagland solution containing 0.5 and 10 mM nitrate. In nitrogen free medium (complete medium minus nitrogen source), KNO₃ & Ca(NO₃)₂ were replaced by KCl & CaCl₂. The final pH of the growth medium was adjusted to 6. Plants were grown in controlled environmental chambers for 16 days in 16 hours photoperiods and 8 hours dark period at 90°F and 80°F, respectively. Light intensity of 7000 Lux was supplied by cool white fluorescent tubes supplemented with incandescent lamps. Plant parts were collected at 7, 9, 11, 13 and 16 day interval for the measurement of leaf size, fresh weight, dry weight, total nitrogen and nitrate reductase activity.

Fresh plants were collected each day for fresh weight determination, they were dried in oven at 80°C for 48 hours and dry weights recorded. For the measurement of leaf size, leaves were drawn on a paper of even thickness. The sketched area was cut and

Fig. 1. Effect of nitrate on fresh weight of jute seedlings of different ages grown in half strength Hoagland’s solution containing 0 mM to 10 mM KNO₃.
weighed. Area of leaves were calculated by dividing the weight of sketched paper with the weight of one sq.cm. paper.

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\text{Area} = \frac{\text{Weight of sketched paper}}{\text{Weight of one sq.cm. paper}}.
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For nitrate reductase assay, 500 mg of fresh plant material was incubated in Thunberg tube containing 80mM phosphate buffer of pH 7.4, 50mM KNO₃ and 2.5% n-propanol (v/v) in a total volume of 10ml. The samples were vacuum intitrated and incubated in dark at 25 ± 1°C for 30 min. (Srivastava, 1975). The amount of nitrite produced was measured in a manner described by Snell & Snell (1949). Total organic nitrogen contents in dried plant material was assayed by nesslerization after microkjeldahl digestion (McKanzee & Wallace, 1953).

Results

**Effect of nitrate on the growth of seedlings of different ages.**

Nitrate showed a promoting effect on the growth of seedlings. Fresh weight and dry weights increased with increasing concentrations of nitrate (Fig. 1 & Fig.2 ) Significant increase in leaf size were observed when plants were supplied with 5 and 10mM KNO₃ (Fig. 3).

![Fig. 2. Effect of nitrate on dry weight of jute seedlings of different ages grown in half strength Hoagland's solution containing 0mM to 10 mM KNO₃](image)

**Effect of nitrate and age on nitrate reductase activity.**

Nitrate reductase activity was measured in different parts of the seedlings grown in media having 0, 5 and 10mM KNO₃ from 7th to 16th days. The enzyme levels increased as nitrate concentration increased from 0 to 10mM (Fig. 4a-c).
Fig. 3. Effect of nitrate on leaf size of jute seedlings of different ages grown in half strength Hoagland's solution containing 0mM to 10 mM KNO₃.

It was observed that leaves had more enzyme activity than stem and root. Enzyme levels of root and leaf increased up to 11 and 13 days then it started to decline, but in stem it was more or less constant at all stages of growth.

Effect of nitrate concentration on nitrogen contents of seedlings.

The effect of different concentrations of nitrate on total nitrogen contents in root, stem and leaf are shown in Fig. 5a-c. When seedlings were grown in media having 5 and 10 mM KNO₃, total nitrogen contents of primary leaves increased linearly with age whereas in nitrogen free medium (0mM KNO₃) it declined after 13 days. A supply of exogenous nitrate caused an appreciable increase of total nitrogen in leaves. No significant change in root and stem were observed throughout the growth period.

Discussion

The supply of exogenous nitrate in the growth medium increases nitrate reductase activity and total nitrogen contents in jute seedlings. Most of the activity of nitrate reductase was found in leaves as compared to stem and root. This may be due to the translocation of nitrate from root to leaves for its reduction and incorporation in amino acids for the synthesis of proteins.

During the early phase of seedling growth the enzyme activity was low. This decreased activity may be due to the availability of organic nitrogen from the cotyledons. It has been observed that the enzyme activity increased rapidly in bean seedlings devoid of cotyledons as compared to seedlings with cotyledons. (Srivastava, 1975).

The hydrolysis of seed proteins during early stages of germination results in the production of amino acids (Danielson, 1951) and some of the amino acids have been
Fig. 4. Effect of nitrate and age on nitrate reductase activity in root, stem and primary leaves of jute seedlings grown in:

a) Nitrogen free medium.
b) Half strength Hoagland’s solution containing 5 mM KNO₃.
c) Half strength Hoagland’s solution containing 10 mM KNO₃.

shown to inhibit or repress nitrate reductase activity in cultured tobacco cells (Filner, 1965), Neurospora crassa (Subramanian & Sorger, 1972) and Chlorella fusca (Abdullah & Ahmed, 1975).

Fig. 5. Effect of nitrate on total nitrogen content in root, stem and primary leaves of jute seedlings grown in:

a) Nitrogen starved condition.
b) Half strength Hoagland’s solution containing 5 mM KNO₃.
c) Half strength Hoagland’s solution containing 10 mM KNO₃.
The decline in total nitrogen contents in leaves after 13 days in nitrogen starved conditions and a linear increase in its level due to the supply of exogenous nitrate (5 & 10 mM KNO₃) suggests that cotyledon nitrogen supports the leaf requirement upto this stage.

The decrease in enzyme activity after 13 days may be due to a decrease in enzyme levels, breakdown of enzyme protein or reduction in the protein synthesizing capacity of plant during maturation. These conclusions are consistent with the earlier observations of Travis & Key (1971).

References


