

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

KHURSHID SATTAR AND JAMIL AHMED

*Department of Botany, University of Karachi, Karachi-32.*

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 upto 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  $\text{KNO}_3$  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 (Beevers & 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 (Hucklesby & Elsner, 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). Abundant 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 (Beevers & 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%  $\text{HgCl}_2$  solution and germinated in sand moistened with modified half strength Hoagland solution containing 0, 5 and 10mM nitrate. In nitrogen free medium (complete medium minus nitrogen source).  $\text{KNO}_3$  &  $\text{Ca}(\text{NO}_3)_2$  were replaced by  $\text{KCl}$  &  $\text{CaCl}_2$ . 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  $90^\circ\text{F}$  and  $80^\circ\text{F}$ , 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  $80^\circ\text{C}$  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

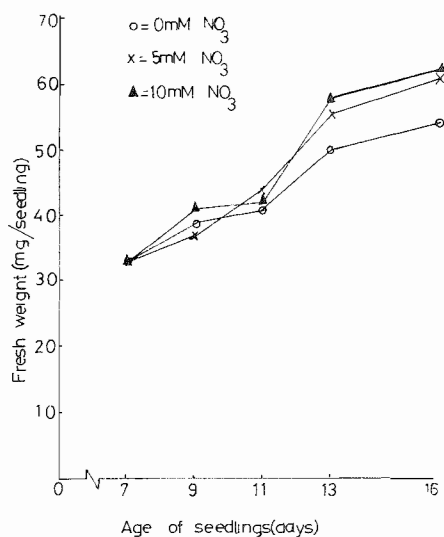


Fig. 1. Effect of nitrate on fresh weight of jute seedlings of different ages grown in half strength Hoagland's solution containing 0mM to 10 mM  $\text{KNO}_3$ .

weighed. Area of leaves were calculated by dividing the weight of sketched paper with the weight of one sq.cm. paper.

$$\text{Area} = \frac{\text{Weight of sketched paper}}{\text{Weight of one sq.cm. paper.}}$$

For nitrate reductase assay, 500 mg of fresh plant material was incubated in Thunberg tube containing 80mM phosphate buffer of pH 7.4, 50mM  $\text{KNO}_3$  and 2.5% n-propanol (v/v) in a total volume of 10ml. The samples were vacuum infiltrated and incubated in dark at  $25 \pm 1^\circ\text{C}$  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  $\text{KNO}_3$  (Fig. 3).

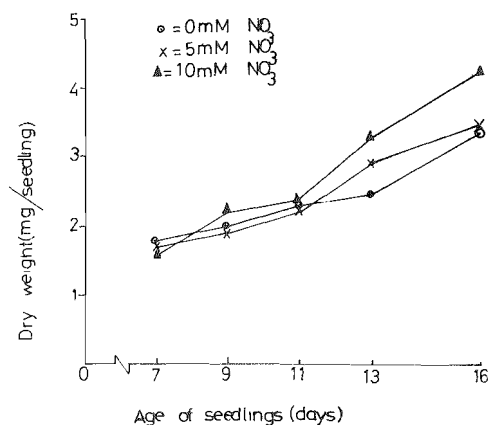


Fig. 2. Effect of nitrate on dry weight of jute seedlings of different ages grown in half strength Hoagland's solution containing 0mM to 10 mM  $\text{KNO}_3$

### *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  $\text{KNO}_3$  from 7th to 16th days. The enzyme levels increased as nitrate concentration increased from 0 to 10mM (Fig. 4 a-c).

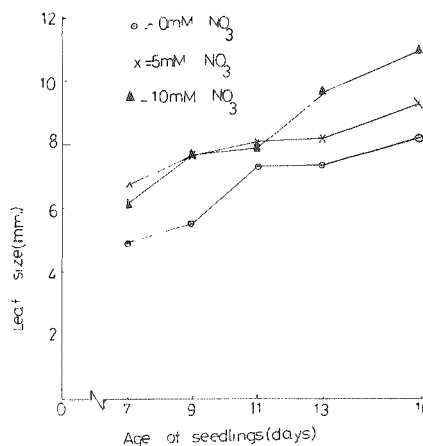


Fig. 3. Effect of nitrate on leaf size of jute seedlings of different ages grown in half strength Hoagland's solution containing 0mM to 10 mM  $\text{KNO}_3$ .

It was observed that leaves had more enzyme activity than stem and root. Enzyme levels of root and leaf increased upto 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 10mM  $\text{KNO}_3$ , total nitrogen contents of primary leaves increased linearly with age whereas in nitrogen free medium (0mM  $\text{KNO}_3$ ) 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

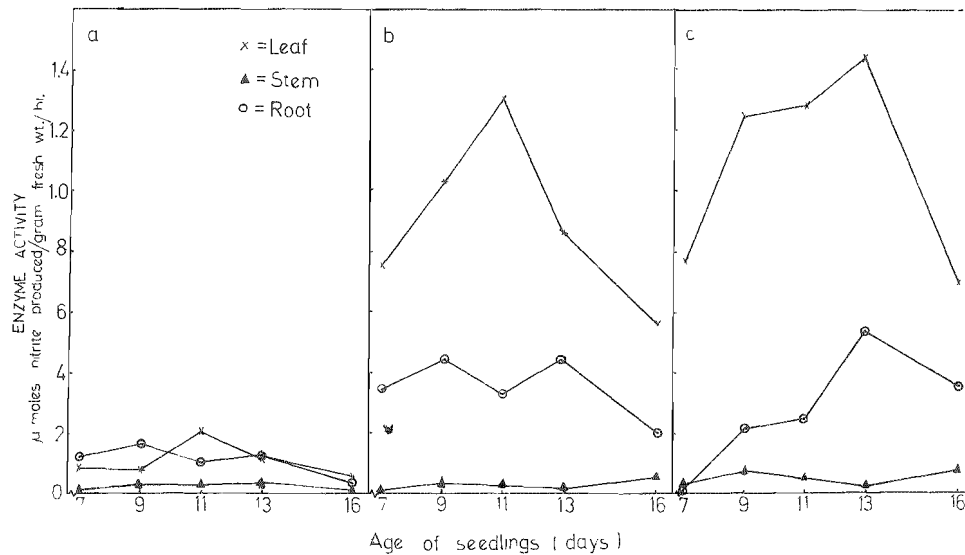


Fig. 4. Effect of nitrate and age on nitrate reductase activity in root, stem and primary leaves of jute seedlings grown in:

- Nitrogen free medium.
- Half strength Hoagland's solution containing 5 mM  $\text{KNO}_3$ .
- Half strength Hoagland's solution containing 10 mM  $\text{KNO}_3$ .

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).

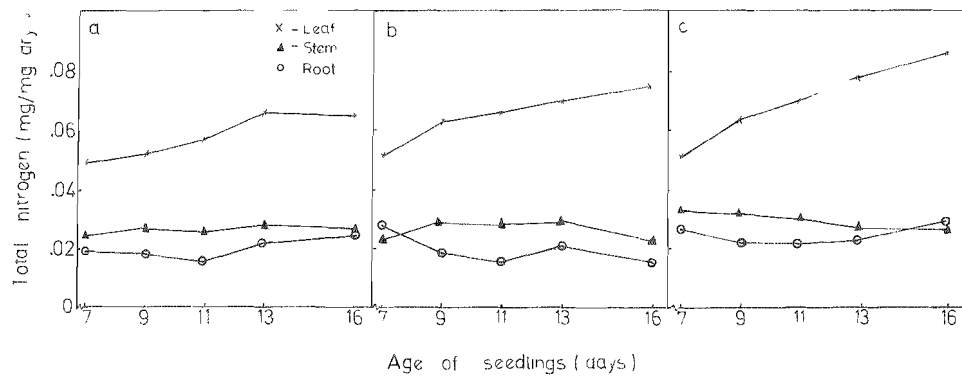


Fig. 5. Effect of nitrate on total nitrogen content in root, stem and primary leaves of jute seedlings grown in:

- Nitrogen starved condition.
- Half strength Hoagland's solution containing 5 mM  $\text{KNO}_3$ .
- Half strength Hoagland's solution containing 10 mM  $\text{KNO}_3$ .

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 & 10mM  $\text{KNO}_3$ ) 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

- Abdullah, Z. and J. Ahmed, 1975 Regulation of nitrate assimilation by amino acids in *Chlorella*. *Plan & Cell Physiol.*
- Ahmed, J. and H. Spiller. 1976. Purification and some properties of nitrate reductase from *Ankistrodesmus braunii*. *ibid.*, 17 : 1-10.
- Amindari, S., M.A. Dirr and W.E. Splittoesser. 1978. Induction of nitrate reductase in Pumpkin seedlings. *ibid.* 19: 299-302.
- Beevers, L. and R.H. Hageman, 1969. Nitrate reduction in higher plants. *Ann. Rev. Pl. Physiol*, 20: 495-522.
- Danielson, C.E. 1951. The breakdown of high molecular reserve proteins of pea during germination. *Acta Chem. Scand.* 5: 551-554.
- Ferguson, A.R. and Knypl, J.S. 1974. Specificity of induction of nitrate reductase in plants. In *Plant analysis and fertilizer problems* Edited by Wehrman. Vol. 1 pp. 101-109 German Society of Plant Nutrition.
- Ferrari, T.E. and J.E. Varner, 1970. Control of nitrate reductase activity in barley aleurone layers. *Proc. Nat. Acad. Sci. U.S.A.*, 65: 729-736.
- Filner, P. 1916. Regulation of nitrate reductase in cultured tobacco cells. *Biochem. Biophys. Acta.*, 118: 299-310.
- Harper, J.E. and R.H. Hageman, 1972. Canopy and seasonal profiles of nitrate reductase in soybeans (*Glycine max* L. Merr.). *Plant Physiol.*, 49: 146-154.
- Hucklesby, D.P. and J.E. Elsner, 1969. Nitrate and nitrite reductase in extracts of corn Scutellum. XI. *Intern. Botanical Congr. Abstracts.* p. 96.
- Jaworski, E.G. 1971. Nitrate reductase assay in intact plant tissues. *Biochem. Biophys. Res. Commun.*, 43 : 1274-1279.
- Klepper, L. and R.H. Hageman., 1969. The occurrence of nitrate reductase in apple leaves., *Plant Physiol* 44: 110-114.
- Klepper, L., D. Flesher and R.H. Hageman, 1971. Generation of reduced nicotinamide adenine dinucleotide for nitrate reduction in green leaves. *Ibid.*, 48 : 580-590.

- McKanzee, H.A. and H.S. Wallace. 1953. The Kjeldahl determination of nitrogen, a critical study of digestion condition, temperature, catalyst and oxidizing agent. *Aust. J. Chem.*, 7 : 55-70.
- Oaks, A., W. Wallace and D. Stevens. 1972. Synthesis and turnover of nitrate reductase in corn roots. *Plant Physiol.*, 50 : 649-654.
- Purvis, A.C. and C.R. Tischler 1976. *In vitro* studies of nitrate reductase activity in cotton cotyledon. *ibid.*, 58 : 95-99.
- Radin, J. W. 1974. Distribution and development of nitrate reductase activity in germinating cotton seedlings. *ibid.*, 53 : 458-463
- Srivastava, H.S. 1975. Distribution of nitrate reductase in ageing bean seedlings. *Plant & Cell Physiol.*, 16 : 995-999.
- Snell, F.E. and C.T. Snell 1949. Colorimetric methods of analysis 3rd. Edn. Vol. 11 pp. 785-807. Van. Nostrand. Company. Inc. Toronto, New York., London.
- Subramanian, K.N. and G.J. Sorger. 1972. Regulation of nitrate reductase in *Neurospora crassa*. Stability *in vivo*. *J. Bacteriol.*, 110 : 538-546.
- Travis, R.L., and J.L. Key. 1971. Correlation between polyribosome level and the ability to induce nitrate reductase in dark green corn seedlings. *Plant Physiol.*, 48 : 617-620.
- Wallace, W. and J.D. Pate. 1965. Nitrate reductase in field pea (*Pisum arvense* L.). *Ann. Bot.*, 29 : 655-671.
- Weissman, G.S. 1972. Influence of ammonium and nitrate nutrition on enzymatic activity in soybean and sunflower *Plant Physiol.*, 49: 138-141.