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AMMONIUM ENHANCES THE NITRATE INDUCED NITRATE REDUCTASE ACTIVITY IN COCONUT *(COCOS NUCIFERA* L.) LEAVES*

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ABSTRACT

Induction of nitrate reductase (NR) activity by nitrate in leaves of low and high yielding coconut palms was markedly different, requiring different levels of nitrate for optimum induction. Ammonium enhanced induction of NR activity by nitrate, under both light and dark conditions. Nitrate uptake was also enhanced in the presence of ammonium in the induction medium. It is postulated that ammonium might bring about the activation of NR by binding to an allosteric site on the enzyme in addition to its role in inducing NR synthesis.

INTRODUCTION

Nitrate reductase (NR) catalyzes the rate-limiting reaction in the reductive conversion of nitrate to ammonium in all higher plants. Nitrate has been shown to induce this enzyme in nearly all systems studied (Beevers and Hageman, 1969). Since ammonium is the end-product of the reaction sequence, it may be expected to exert inhibitory effect on NR. However, the effect of ammonium on NR activity is reported to vary widely ranging from no effect (Beevers *et al.*, 1965; Ingle *et al.*, 1966; Schrader and Hageman, 1967; Oaks *et al.*, 1977) to inhibition (Townsed, 1970; Smith and Thompson, 1971; Radin, 1975; Orebanjo and Stewart, 1975) and even stimulation (Bayley *et al.*, 1972; Mohanty and Fletcher, 1976).

The nitrogen requirement of the coconut palm is 500 g annually, This is given in the form of urea granules twice a year. Urea undergoes rapid hydrolysis to ammonium in the soil, although the time required for the complete conversion to nitrate takes several days, depending upon the soil conditions. The coconut roots are, therefore, exposed to high concen- trations of ammonium.

The present study was taken up to understand the effect of variable concentrations of ammonium on the NR activity and nitrate uptake in excised leaves of coconut.

MATERIALS AND METHODS

Leaf samples for the study were collected from adult palms of West Coast Tall cultivar (WCT) growing in the Institute campus under scientific management. Leaflets from the middle portion on either side of the rachis of leaf no. 14 were wiped clean and incubated with their cut ends dipped in various induction media under subdued light. Samples were

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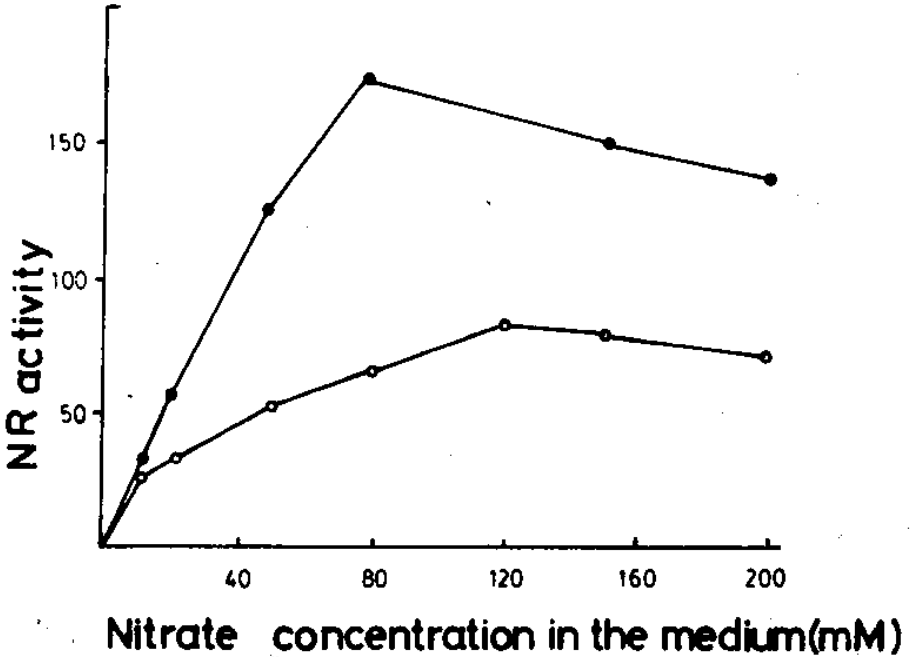

Fig. 1. Effect of increasing nitrate concentration on the leaf NR activity in high (●–●) and low (○–○) yielders.

drawn out at regular intervals for analysis.

Nitrate reductase was assayed *in vivo* by a modified method of Joworski (1971) as described by Shivashankar and Rajagopal (1983).

Nitrate uptake by leaves was determined by floating one gram of the leaf discs on medium containing nitrate for 24 h. The nitrate that disappeared from the medium was determined by spectrophotometry (Cawse, 1967).

RESULTS AND DISCUSSION

Effect of nitrate concentration: The course of enzyme induction at increasing levels of nitrate in the medium is presented in Fig. 1. The induction of NR activity by nitrate in the leaves of high yielders was markedly higher than that in low yielders. The nitrate concentration required for the maximum induction was much less (80 mM) for high yielders than that for the low yielders (140 mM). Applying Michaelis-Menten postulates, it is found that the Km for high yielders is relatively lower than that of low yielders. Since, the Km is a measure of the affinity of the enzymes towards nitrate, it is obvious that the high yielders are able to utilize the available nitrate better than the low yielders. Although, the response of roots to similar conditions has not been studied and the existing soil nitrate levels may be different from the experimental conditions, the results do provide an important insight into the pattern of nitrate utilization, as the level of enzyme is dependent within limits, upon the concentration and rate of supply of the substrate to the tissue.

Effect of ammonium and benzyladenine (BA): Inclusion of ammonium in the induction medium containing nitrate enhanced NR activity up to 6 hours and

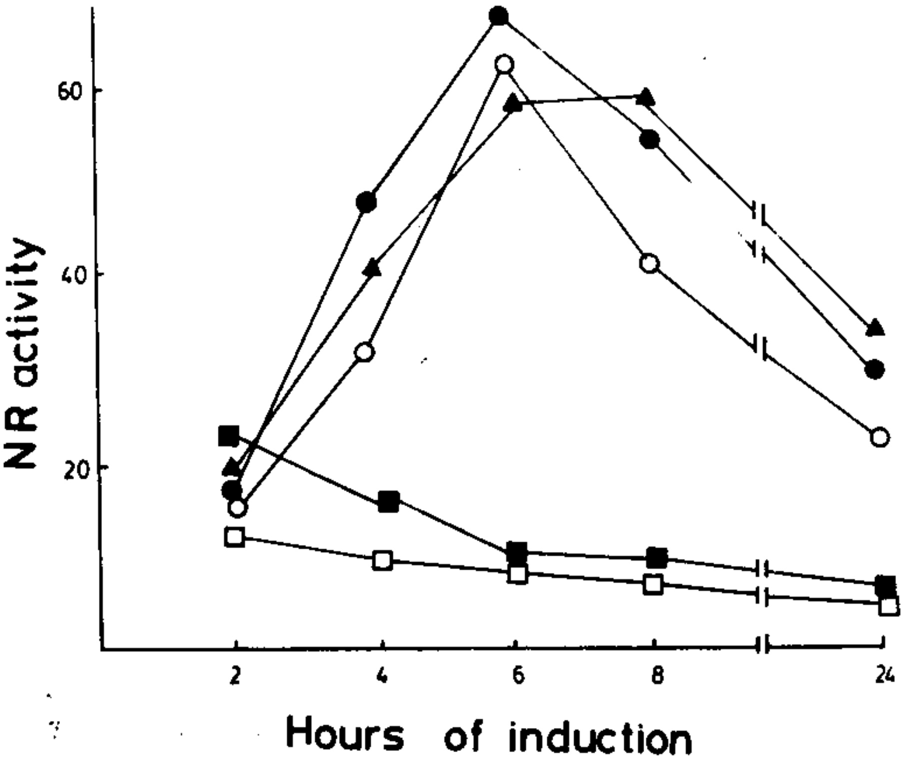

Fig. 2. Effect of ammonium on the induction of NR activity. Nitrate control (▲) Nitrate + 1 mM NH_4 (○), Ammonium nitrate (●), Ammonium alone (□) and water (■).

reached levels higher than those obtainable by nitrate alone (Fig 2). The enhancement was greater with ammonium nitrate than that containing nitrate and 100 μM ammonium. However, in the presence of only ammonium, the NR activity was far too low and declined continuously with lapse of time. The NR activity in others also was reduced at the end of 24h, as compared to the nitrate control.

BA also greatly enhanced the *in vivo* NR activity. The level of activity with nitrate was only marginally higher than that with BA alone. When BA was used along with nitrate, there was a further enhancement of enzyme activity, thus showing the additive effect of BA on NR activity (Fig 3).

The results show that ammonium enhanced the induction of NR by nitrate. Hirchberg *et al.* (1972) showed that NR protein synthesized *de novo* in response to cytokinins. Subsequently Peterson and Miller (1976) demonstrated that exogenously supplied ammonium increases the biosynthesis of cytokinins in *Vinca rosea*. The present findings when viewed in the light of these reports show that ammonium might be stimulating NR activity by induction of synthesis of NR protein via the biosynthesis of cytokinins.

In another experiment in which graded levels of ammonium was used in the assay medium containing increasing concentrations of nitrate, it was found that NR activity measured at the end of one hour was considerably higher than those containing either nitrate or ammonium only. A Lineweaver Burk plot

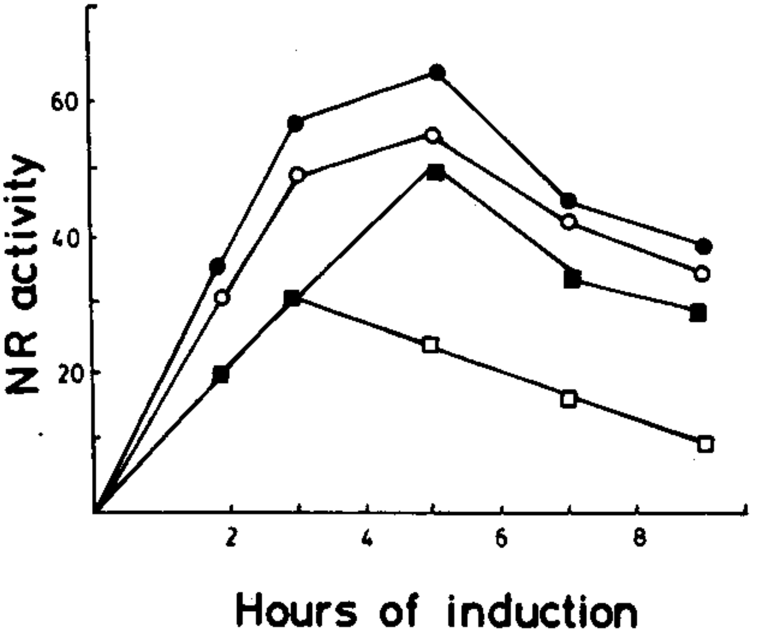

Fig. 3. Effect of benzyladenine on NR activity. (●) BA + Nitrate; (○) Nitrate (■) BA and (□) Transferred from BA medium to water.

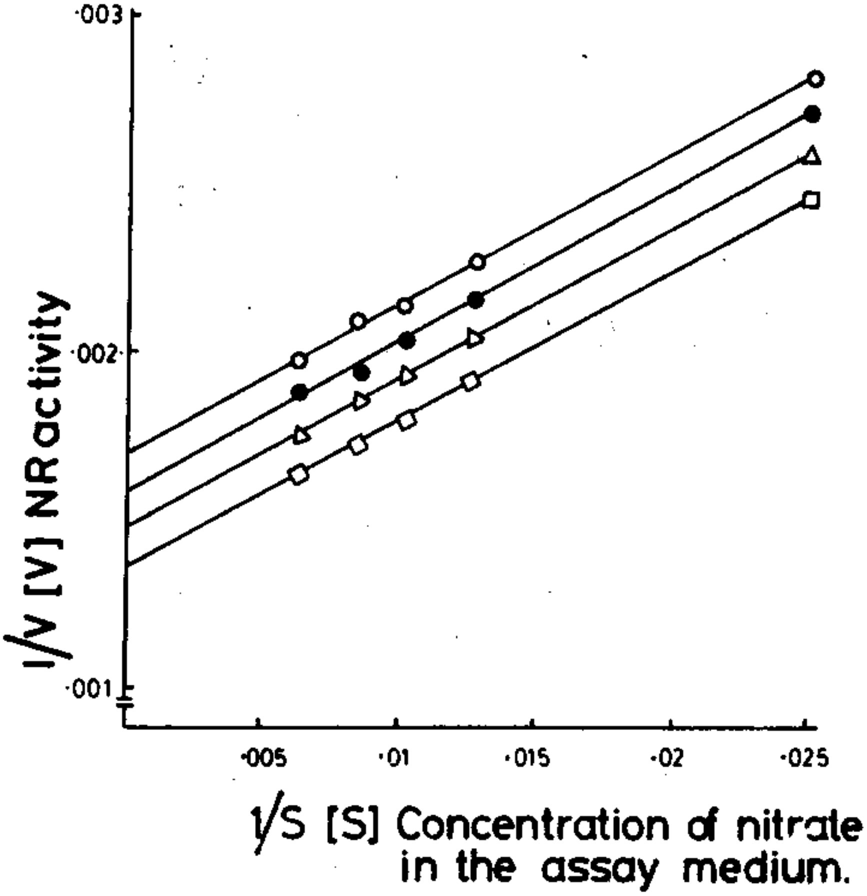

Fig. 4. Lineweaver-Burk plots for the acitity measured in nitrate medium containing graded levels of ammonium. No ammonium (○), 40 μM ammonium (●), 100 μM ammonium (△) and 200 μM ammonium (□).

Table I. *Nitrate uptake by excised leaf discs.*

Incubation medium	Nitrate taken up at the end of 24h. (μ moles. g^{-1} fresh wt.)
0.5 mM KNO_3	4.46
0.5 mM KNO_3 + μM NH_4	6.82
0.5 mM KNO_3 + 100 μM NH_4	6.89
0.5 mM KNO_3 + μM NH_4	4.76

gave paralled lines with constant slope and increasing Vo. The degree of stimulation increased with increasing concentrations of ammonium in the medium. However, when only ammonium was used with no exogenously supplied nitrate, NR activity was less than half of that in the nitrate control. The lowered Km in presence of both nitrate and ammonium (Fig. 4) indicates that ammonium might also bind to an allosteric site on the NR protein to bring about the activation.

Ammonium upto a concentration of 100 μM in the medium also enhances the rate of nitrate uptake by excised leaves. (Table I). Thus, the results show that the induction of NR by low concentrations of ammonium and inhibition at higher levels thus provides the plant with an efficient mechanism to utilize both the predominant forms of soil nitrogen and also to regulate the input of reduced N in the cell.

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