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ROLE OF NITRATE, AMMONIUM ION AND NADH ON THE NITRATE REDUCTASE IN LEAVES OF BLACK PEPPER

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ABSTRACT

The study conducted to understand the effect of nitrate and ammonium on Nitrate Reductase (NR) showed that the NR activity was induced by nitrate alone in the medium and these induction was enhanced by the addition of ammonium. Whereas, induction medium with ammonium alone inhibited the activity. Inclusion of NADH in the assay medium reduced the activity partially but, without nitrate little or no activity was obtained.

Key words: Black Pepper, Nitrate Reductase, Nitrate, Ammonium, NADH.

INTRODUCTION

The reductuon of nitrate to ammonium within the cells of higher plants occurs in two steps catalysed by the enzyme Nitrate Reductase (NR) and Nitrite Reductase. Nitrate reductase is considered to be a limiting factor for growth and development and its activity is mainly controlled by nitrate (Beevers and Hageman, 1969). Ammonium is the end product of the reaction sequence, its effect on NR varied from no effect (Beevers et. al., 1965) to inhibition (Orebanjo and Stewart, 1975). Induction of the NR activity by ammonium was also reported (Mohanty and Fletcher, 1976).

Nitrogen is given to black pepper in the form of urea and it undergoes rapid hydrolysis to ammonium in the soil, but the complete conversion of ammonium to nitrate takes long time. So the roots of pepper plants are exposed to a mixture of ammonium and nitrate. Therefore, an experiment was conducted to understand the role of ammonium and nitrate on the NR in the excised leaves of black paper.

MATERIAL AND METHODS

Rooted cuttings of Black Pepper (*Piper nigrum* L. cv. Karimunda) were planted in 30 cm earthen pots containing soil, sand and cowdung in the ratio 1:1:1. Youngest mature leaves were taken when plants were 3 months old, having five to six leaves to conduct the experiment. These leaves were wiped clean and placed with their cut ends in 20 ml of media containing 10 mM ammonium, 100 mM nitrate or both. The leaves were removed at desired intervals from the inductuon media, rinsed twice with deionised water and blotted dry. NR activity was assayed by modified method of Joworski (1971)

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as described by Shaukathali and Ramadasan (1995). To study the effect of added NADH, the physiological electron donor for NR, on NR activity 0.1% NADH was added to the assay medium just prior to the infiltration. The samples were drawn out at regular intervals for the NR assay. The NR activity is expressed as n.mol nitrite produced hr^{-1} g^{-1} fresh weight. The experiments were repeated and to similar trends, but the results from only a single experiment is reported here.

RESULTS AND DISCUSSION

The data are presented in text figures 1 and 2. The NR activity decreased with respect to the progress of time in induction media without nitrate. Whereas in the case of induction media with nitrate gave increasing trend up to 8 hours and there after it declined (fig.1). It showed that nitrate induced the NR activity . These results were also in agreement with the results of Beevers et. al (1969) who concluded that substrate induction of NR in maize leaves. Oaks et.al. (1982) reported that these induction of the NR activity was due to the conversion of inactive NR-protein to active form by nitrate. Inclusion of ammonium in the induction medium containing nitrate enhanced NR activity up to 8 hours

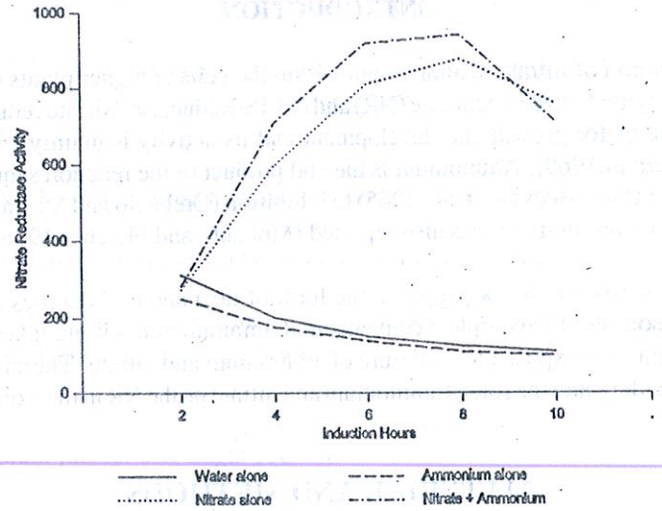

Fig. 1. Effect of Nitrate and Ammonium on NR Activity

and attained levels higher than those obtained by nitrate alone (fig.1). However, ammonium alone, gave lowest activity than others. The above results show that ammonium enhanced the nitrate induced NR activity. The results are in confirmation with those of Shivashankar and Kasthuribai (1989). They viewed that ammonium might be stimulating NR activity by induction of synthesis of NR-protein via the biosynthesis of cytokinin. Losad et.al. (1970) found that NR activity is inhibited by ammonium

alone in *Chlorella,* whereas it is induced by ammonium with nitrate in the medium .

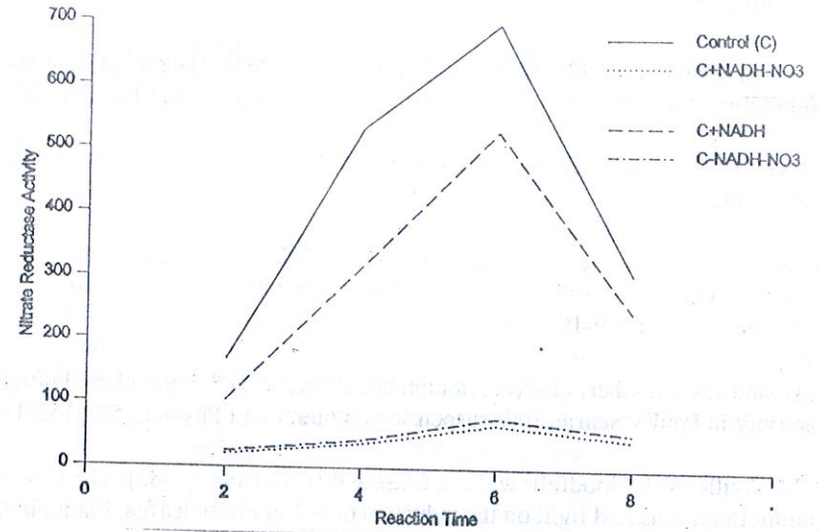

Fig.2 Effect of added NADH on NR Activity

Study conducted to understand the effect of added NADH on NR activity gives an inhibitive effect with nitrate in the medium, NADH addition to the assay medium without nitrate gives negligible activity. These results are contrary to the results of Shivashankar and Ramadasan (1983) in coconut leaf. The NR from wheat leaves is inactivated by pretreatment with NADH, in the absence of nitrate and in the presence of nitrate it was inhibited partially by NADH (Aryan et.al., 1983). The above results support our findings.

Thus, it may be concluded that the NR activity is induced by nitrate alone in the induction medium and these induction was enhanced by the addition of ammonium. Inclusion of NADH in the standared assay medium reduced the NR activity partially but, without nitrate little or no activity was observed.

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