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UPTAKE OF NITRATE AND AMMONIUM ION BY CELL SUSPENSION CULTURES OF *VIGNA RADIATA*

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Abstract

A study was conducted to examine the effect of pH on the uptake of NH_4^+ and NO_3^- by the cell suspension culture of *Vigna radiata*. Uptake of NH_4^+ was faster than NO_3^- under all conditions of the medium whereas uptake of NH_4^+ and NO_3^- both were affected by the pH of the medium. Maximum uptake of NH_4^+ was observed on pH 4.5. Uptake of NO_3^- was faster in the culture having pH 7.0. It is therefore concluded that pH of the medium influence the uptake of both NH_4^+ and NO_3^- in the cell suspension culture of *Vigna radiata*.

Introduction

Photoautotrophic cell cultures offer a useful experimental system for studies in plant physiology. Suspension cultures have been proposed as an alternative model for studying cellular biology of ion transport and assimilation (Miflin & Lea, 1982). The assimilatory reduction of nitrate by plants is a fundamental biological process in which a highly oxidized form of inorganic nitrogen is reduced to ammonia. The presence of nitrate in the external solution induces the *de novo* synthesis of protein that facilitates movement of nitrate in cytoplasm of cell (McClure *et al*., 1987). After nitrate is taken up into the cell, it can be reduced to nitrite and then to ammonia which ultimately converted into amino acids (Hoff *et al*., 1994). The rate of Nitrate reduction might also be affected by ammonium (Guerrero *et al*., 1981). The majority of cell suspension cultures, however, require both nitrogenous ions for optimal growth, although ammonia is only needed at low concentration as compared to nitrate (Doughall, 1977).

Nitrogen fixation in cell cultures of *Vigna radiata* (Gupta *et al*., 1984) and enzymatic studies on suspension and callus cultures (Singh & Singh, 1984) has been reported. The present study was conducted to find out the uptake of NO_3^- and NH_4^+ by cell suspension cultures of *Vigna radiata*, when both NH_4^+ and NO_3^- were present in the medium under different pH conditions.

Materials and Methods

The certified seeds of *Vigna radiata* obtained from the National Agriculture Research Council (NARC) Islamabad were germinated under *in vitro* condition on plain agar medium. Callus was initiated on MS medium (Murashige & Skoog, 1962) containing 2,4-D (0.2m g/l), IAA (0.25 mg/l) and Kin (0.25 mg/l) using hypocotyls explants taken from germinating seedlings. Method described by Harris *et al*., (1988) was used to prepare cell suspension culture from callus. Ten g healthy and friable callus was

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transferred into 250 ml Erlenmeyer flask containing 100 ml liquid MS medium containing same hormonal combination as described for callus formation. It was placed on rotary shaker adjusted at 100 rpm. Cells were harvested under aseptic condition by centrifugation at 10000 rpm for 20 minutes and added to 20ml fresh media with varying pH @ 0.5 g/ml. Cell culture was placed on a shaker in growth room at 27 ºC±1. Uptake of NO_3^- and NH_4^+ was determined from the depletion of these ions from the media. After every 12 hours, 2.0 ml of culture was taken from the flask and centrifuged at 20000 rpm for 20 minutes. Clear supernatant was used to determine ammonium and nitrate ions. Nitrate was determined by the method of Winkleman *et al*., (1990) and ammonium was determined by using Martinek (1969) method.

Results and Discussion

Cell suspension cultures have been extensively used by many researchers for investigation of various physiological processes (Lillo *et al*., 1996; Peters *et al*., 1995; Macduff, 1997). It provides a source of homogenous cells exposed to uniform distribution of chemicals, environment and response which is representative of the majority of cells.

NH_4^+ ion uptake by cell suspension culture of *Vigna radiata* at different pH is shown in Fig. 1. NH_4^+ uptake was higher at acidic pH and influx gradually decreases when pH of the medium was adjusted towards basic. NH_4^+ ion concentration in MS medium was 5.53 mMol and after 60 hours the concentration was 3.39 and 3.36 at pH 4.5 and 5.0 respectively. Steiner & Dougall (1995) also observed 25% high NH_4^+ uptake rate at pH 4.5 than at 5.5. NO_3^- endogenous depletion was less as compared to NH_4^+ as shown in Fig. 2. After 60 hours maximum uptake was observed at neutral pH (7.0). When medium pH varied to acidic or basic, uptake of NO_3^- also decreased. Glass *et al*., 1990 working with barley also found that pH in the range of 4.5 to 7.5 caused insignificant effect on NO_3^- influx, however pH optima was between 4.5 to 6.0. McClure *et al*., (1992) stated that increased transport activity at acidic pH is compatible with H^+ ion transport mechanism where more than one H^+ ion moves across the membrane NO_3^-.

Comparative analysis of both the figures shows that NH_4^+ ions influx was higher as compared to influx of NO_3^-. Dougall (1977) reported that majority of cell suspension culture requires both NO_3^- and NH_4^+ for optimum nitrogen metabolism although NH_4^+ is only needed at low concentrations compared to NO_3^-. But presence of growth regulators, environmental conditions and nitrogen source also effect on uptake of NH_4^+ and NO_3^- and their concentration in the medium too. Mackown *et al*., (1982) found that NH_4^+, amino acid and urea also exerted inhibitory effect on NO_3^- absorption and suggested that the NO_3^- uptake is directly related to its concentration in the medium. While Clarkson, (1986) reported that NO_3^- uptake rates are strongly influenced by the environment in which tissue were grown and that the plant is able to closely regulate the influx and efflux of NO_3^- across the cell membrane (Deane-Drummond, 1990).

Present study also indicates that *Vigna radiata* cell suspension cultures take up both NH_4^+ and NO_3^- ions simultaneously.

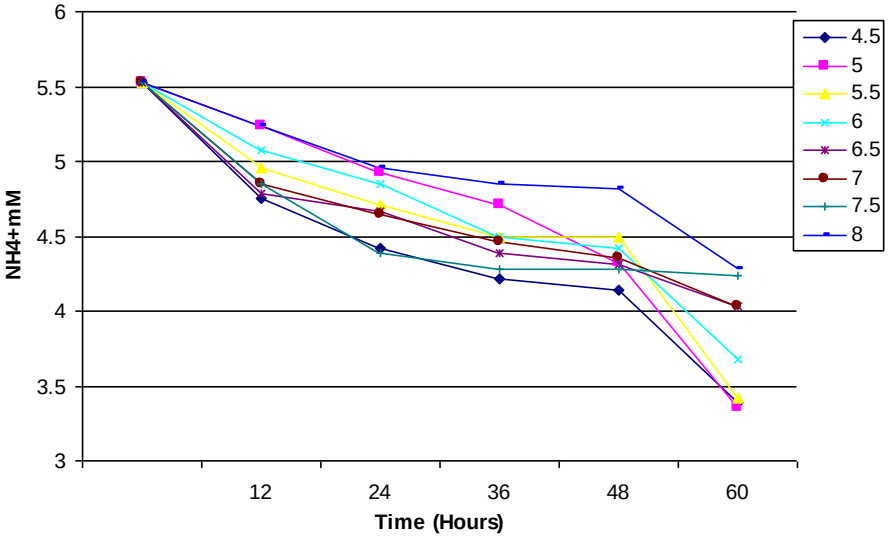

Fig. 1. Effect of pH on the depletion of NH_4^+ in the cell suspension culture of *Vigna radiata*.

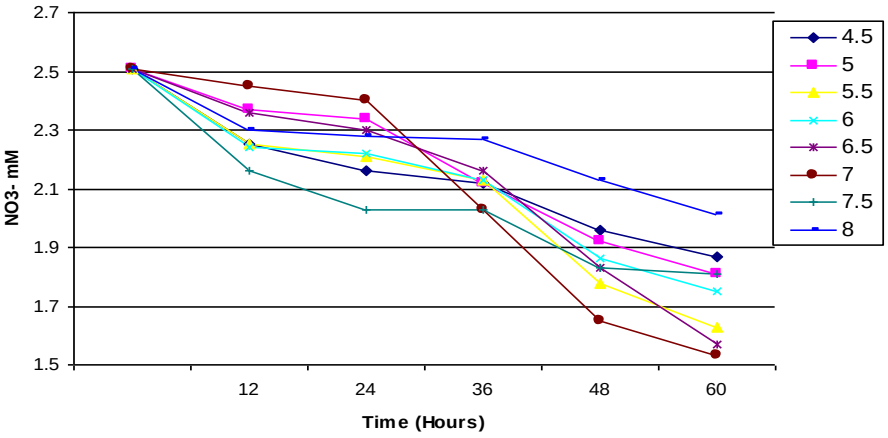

Fig. 2. Effect of pH on the depletion of NO_3^- in the cell suspension culture of *Vigna radiata*.

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