Changes in nitrogen metabolism of *Vigna radiata* in response to elevated CO₂

A.C. SRIVASTAVA*, M. PAL** and U.K. SENGUPTA**

Division of Biochemistry and Plant Physiology, SKUAST, RS Pura, Jammu-181102, India*
Division of Plant Physiology, Indian Agricultural Research Institute, New Delhi-110012, India**

Abstract

With the aim to determine the effects of CO₂ on nitrogen metabolism mungbean (*Vigna radiata*) plants were grown from seedling emergence to maturity inside open top chambers under ambient CO₂ (CA, 350 ± 25 μmol mol⁻¹) and elevated CO₂ (CE, 600 ± 50 μmol mol⁻¹) concentrations at the Indian Agricultural Research Institute, New Delhi. Leaflet blades of the same physiological age were sampled at 20, 35 and 50 d after germination. Total nitrogen concentration in dry mass was consistently lower under CE than in CA. Non-protein nitrogen and protein nitrogen were also decreased under CE. Total soluble protein content also decreased up to 35 d after germination under CE. However, a 27 % increase in protein content at 50 d after germination due to CE was observed. A significant decrease in total free amino acid under CE at 20 d after germination was observed. CE also brought about a remarkable decrease in the activity of nitrate reductase in leaves at 20 d after germination but increase at 35 d and 50 d after germination. Nitrogenase activity increased at all growth stages due to CE. Although total harvested leaves of CE plants accumulated more nitrogen, the relative amount of nitrogen on a percentage basis was low, probably due to a comparatively greater accumulation of sugars in the leaves of CE plants.

*Additional key words:* mungbean, nitrate reductase, nitrogen fixation, protein content.

Introduction

Earlier studies indicated that CO₂ enrichment of C₃ plants can result in accumulation of starch and other non-structural polysaccharides in the leaves and increase in total biomass of the plants (Warrick and Gifford 1986, Sharma and Sengupta 1990, Ulman et al. 2000). Enhanced growth and photosynthetic rate under elevated CO₂ is also reported in mungbean (Gifford et al. 1985) and soybean (Griffin and Luo 1999).

Carbon and nitrogen metabolism are linked in several ways. At the cellular level, uptake of nitrate, reduction of NO₃⁻ and incorporation of ammonium into amino acids and protein are dependent on carbon metabolism for the production of energy, reductant and carbon skeletons. Photosynthetic rate of a plant, on the other hand, is influenced by the nitrogen status (Evans 1989).

Some of the earlier experiments indicated that CO₂ enrichment lowered the concentration of nitrogen in leaves and altered the distribution pattern of nitrogen within the plant (Larigauderie et al. 1988, Hocking and Meyer 1985, Upreti and Rabha 1999). It has been shown that less enzyme (Rubisco) and leaf protein nitrogen are required to produce a given amount of dry matter (Warrick and Gifford 1986, Allen et al. 1988). Consequently, CO₂ enrichment may improve the nitrogen use efficiency of C₃ crop species (Schmitt and Edwards 1981, Goudriaan and de Ruiter 1983), weeds (Hocking and Meyer 1985) and grasses (Larigauderie et al. 1988).

In contrast to the above findings, plants accumulated more nitrogen in wheat under elevated CO₂ conditions but the proportional increase in the nitrogen content was not as great as dry matter (Hocking and Meyer 1991). Nitrogen metabolism in legumes under high CO₂ will be...
Materials and methods

Plants and treatments: Mungbean (Vigna radiata (L.) Wilczek cv. PS16) was grown in pots at the Plant Physiology Division, Indian Agricultural Research Institute, New Delhi (India) from 10 April, 1999 to 18 June, 1999. In the experiment, a modified open top chamber (OTC), as described by Rogers et al. (1983) was developed to study crop responses to elevated CO₂. The height and diameter of the OTC was 1.8 m and 1.6 m, respectively. One set of plants was grown under ambient CO₂ (CA, 350 ± 25 μmol mol⁻¹) and other group of plants was grown under elevated CO₂ (CE, 600 ± 50 μmol mol⁻¹) in a naturally lit OTC. A total of 16 pots were used in each chamber for the treatment. The concentration of CO₂ in the chamber was monitored by an infra red gas analyser (LicOR 6200, Lincoln, USA).

Leaflet blades of the same physiological age were sampled in May - June, 1999 at 20, 35 and 50 d after germination for analyzing amino acids, protein, nitrate reductase and total nitrogen. Dry matter of leaves was determined after drying in an oven at 80 °C for 4 h and then at 60 °C till a constant mass using an electronic balance (Sartorius 1212 MP, Göttingen, Germany).

Assay of nitrate reductase activity (EC 1.6.6.1): Three samples of 5 leaves of the same physiological age were collected at midday from both treatments and carried to the laboratory in an ice bucket. An in vivo procedure was used (Klepper et al. 1971), in which fresh leaves were finely chopped in a cold room at 3 °C and 0.3 g subsamples transferred to flasks containing 2.5 cm² of 60 mM potassium nitrate, 2.5 cm³ of 0.1 M phosphate buffer (pH 7.5) and 0.1M n-propanol. The solution was infiltrated into the leaf tissue by creating a vacuum in the tubes using a vacuum pump. The infiltration was done for 5 min and then the infiltrated material was kept for incubation at 35 °C for 20 min; boiling the reaction mixture on a hot plate for 2 min stopped the reaction. A volume of 0.3 cm³ was taken in test tubes with 3 replicates to assay NO₃⁻. The absorbance was recorded at 553 nm by using spectrophotometer Spectronic-20 (Bausch and Lamb, Rochester, USA).

Total nitrogen concentration: Non-protein and protein nitrogen portions in the form of reduced nitrogen in the trichloro acetic acid (TCA) preserved leaf material were determined by using N-Kjeltech Auto 1030 analyzer, following the procedure detailed in TECATOR manual, 1987 (TECATOR Company, Haganas, Sweden).

Leaf samples were collected randomly between plants in polyethylene bags and 0.5 g of leaf from each sample was preserved in 10 cm³ of 10 % TCA. Homogenized leaves were centrifuged twice at 20 124 g for 10 min. Supernatant (non-protein) and residues (protein nitrogen) were subjected to digestion and distillation.

Total soluble protein and amino acids: Leaflets blades were chopped into small pieces and 2 g of leaf material was homogenized in an extraction buffer of approximately 0.5 M Tris-HCl (hydroxymethyl) amino ethane HCl buffer (pH 6.8). The homogenate was centrifuged at 20 124 g for 10 min at 4 °C. Soluble protein in the supernatant was measured by the method of Lowry et al. (1951).

For the estimation of free amino acids, 0.5 g of leaf material from each treatment was homogenized with 10 cm³ distilled water. The supernatant was used as a crude extract for amino acid assay. Free amino acid amounts were estimated following the method described by Lee and Takahashi (1966).

Nitrogenase activity in root nodules: Total nitrogenase activity (TNA) of the plants was assayed by the acetylene reduction technique (Hardy et al. 1968). Nodulated root systems were carefully uprooted, washed clean of soil, separated from the shoot, and incubated in the dark for 60 min in plastic bottles with 10 cm³ acetylene. The manipulation of the root system and detachment from the shoot probably resulted in lower values of TNA than would have been obtained from the intact plant (Wheeler et al. 1978), but errors due to differential gas diffusion through the soil were thereby avoided. Ethylene production was measured on a Perkin Elmer Model 2000, (Boston, USA) gas chromatograph. An external ethylene standard was used. The nodules were removed from the roots, dried at 70 °C for 4 h and then at 60 °C till a constant mass and the dry mass was recorded.