Effect of simulated acid rain on nodulation and nitrogen metabolism in *Vigna radiata* cultivars

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Abstract

Nodulation was inhibited in plants of green gram (*Vigna radiata*, cvs. ADT-1 and CO-5) exposed to different levels of simulated acid rain using a mixture of H$_2$SO$_4$, HNO$_3$ and HCl (6:3:1) of pH 2.5, 4.0 and 5.5 in comparison with control (pH 7.0). Protein content of leaves increased in cv. CO-5 but decreased in cv. ADT-1 whereas the nitrate content of leaves increased in cv. ADT-1 but lowered in cv. CO-5. Nitrate reductase activity was increased in the nodular roots of cv. ADT-1 but was decreased in leaves. In cv. CO-5 it was increased in leaves but was insignificantly reduced in the nodules at pH 2.5. The nodule nitrogenase activity increased at pH 4.0 and 2.5 in cv. ADT-1.

Additional key words: acid mixture, green gram, nitrate reductase, nitrogenase, proteins.

Introduction

Studies on the impact of acid rain on crop plants, mostly using sulphuric acid mists, have mainly concentrated on growth and yield. A few authors have reported the effect of simulated acid rain (SAR) on nitrogen metabolism, analysing the process of nodulation (Oden 1968, Gates and Muller 1979, McLaughlin and Shriner 1980, Shriner and Johnston 1981, Norby et al. 1986) and nitrate reductase activity (Muthuchelian et al. 1993, 1995).

Hence, experiments employing an acid mixture of sulphuric, nitric and hydrochloric acids were designed under the prevailing tropical conditions 1) to assess the effect of SAR on nodulation after *Rhizobium* inoculation into the soil, 2) to

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analyse the distribution of nitrogenous compounds, 3) to follow the activities of two key enzymes, nitrate reductase in leaves and roots, and nitrogenase in nodules, and 4) to assess the cultivar differences in responses, if any.

Materials and methods

Plants: Two green gram [Vigna radiata (L.) Wilczek] cultivars ADT-1 and CO-5 were raised in earthenware pots in greenhouse conditions [day/night temperature 36 ± 2/18 ± 2 °C; relative humidity 60 ± 5 %; photoperiod 12 - 14 h; maximum irradiance (PAR) 400 μmol(photon) m⁻² s⁻¹]. Ten-day-old seedlings in each pot were inoculated with 200 mg of the commercial preparation of Rhizobium (cowpea strain) inoculum suspended in 1 cm³ of water and poured on the surface of the soil as suggested by Shriner and Johnston (1981).

Treatment: Deionized double distilled water was adjusted to different pH levels (5.5, 4.0 and 2.5) using a diluted mixture of H₂SO₄, HNO₃ and HCl in the molar ratios of 6:3:1. Since the mean rain pH is around 7.0 in the tropics, glass distilled water, adjusted to pH 7.0 with 0.1 M NaOH served as control.

Starting from 15 d after sowing (DAS) plants were sprayed daily for 10 min for 10 d with acid mixture using a rain-generating device (Kohno and Kobayashi 1989) at an effective flow rate of 7.8 mm h⁻¹. The rain drop size ranged from 0.35 to 1.35 mm². Showers were applied to the top of the plant from a height of 1.2 m and no efforts were made to prevent the run off to soil.

Assessments were made at two stages, 5 and 15 d after beginning the treatment (DAT); stage 1 plants had received five showers while stage 2 corresponded to 5 d after termination of the treatment. Plants were carefully uprooted and the number of nodules were counted at 30, 40 and 50 DAS corresponding to 15, 25 and 35 DAT, respectively. Soluble proteins were estimated using Folin phenol reagent method (Lowry et al. 1951). Nitrate content was determined using naphthylamine salt-mixture (Woolley et al. 1960).

Nitrate reductase activity (NRA): In vivo NRA was assayed by the method of Jaworski (1971) with suitable modifications (Muthuchelian et al. 1993). Leaf discs/nodules (100 mg f.m.) were washed and placed in vials containing 5 cm³ of incubation medium prepared by mixing 0.1 M KNO₃ (1 cm³), 0.1 M phosphate buffer of pH 7.5 (3.75 cm³), 0.1 % of Triton X-100 (0.01 cm³) and 1 % propanol (0.25 cm³) and incubated in dark for 1 h at room temperature (28 ± 2 °C) with occasional shakings. Aliquots (0.2 cm³) from the incubated mixture were analysed for nitrite using 3 % sulphanilamide in 3 M HCl and 1 cm³ of 0.02 % n-(1-naphthyl ethylene-diamine dihydrochloride). After 15 min of incubation in darkness, the absorbance was read at 540 nm using a spectrophotometer (Shimadzu, Kiyoto, Japan).

Nitrogenase activity: Nodular nitrogenase activity was determined by the acetylene reduction technique (Stewart et al. 1967). Enzyme assay was carried out with 500 mg