NODULATION OF *Medicago sativa* IN SOLUTION CULTURE

V. CALCIUM AND pH REQUIREMENTS DURING INFECTION

by D. N. MUNNS

Department of Soils and Plant Nutrition, University of California, Davis

**SUMMARY**

Calcium and hydrogen ions interacted on nodulation. Increasing acidity from pH 5.6 to pH 4.8 increased the calcium concentration required to nodulate 50% of the plants, from 0.1 mM to 6 mM. Calcium concentration below 0.2 mM or pH below 4.8 inhibited nodulation at all tested levels of the other variable. Root extension and root-hair production were insufficiently affected by calcium or pH to explain reductions in nodule numbers.

Initiation of infection, the most acid-sensitive stage of the nodulation process, was also the most calcium-demanding stage at pH 5.2. Once infections were initiated, infection threads still developed and nodules still grew despite transfer of the plants to solutions too low in calcium to have permitted infection to begin. Pretreatments at 0.5 mM and 8 mM calcium at pH 5.2 before inoculation had no significantly different effects on nodulation.

Observations on root-hair distribution suggest that developing nodules can suppress further infection by suppressing the emergence of root hairs on newly developing roots.

**INTRODUCTION**

Interaction between hydrogen and calcium ions on root-nodulation has been studied in the legumes *Glycine max* and *Trifolium subterraneum*\(^1\). For both species there is a range of calcium concentrations and pH over which nodulation requires higher calcium the lower the pH, or, conversely, higher pH the lower the calcium concentration. These effects are not explained by limitations on the growth of nodule bacteria or host plant: pH and calcium specifically affect the nodulation process itself\(^5\). The first two experiments in this paper confirm and amplify these findings for *Medicago sativa*, in solution culture.
In *M. sativa* the most acid-sensitive stage of the nodulation process comes during initiation of the infection, before the infection thread appears in the root hair. Infection initiates rapidly when the pH of the nutrient solution is raised from an inhibitory level, but will then proceed through later stages unhindered by a subsequent drop in pH. Initiation of infection may likewise be the most calcium-demanding stage of the nodulation process. This possibility is tested in a third experiment.

**EXPERIMENTAL PROCEDURES**

The procedures differed little from those described in detail previously, except in the use of (a) variety Lahontan instead of Hunter River, (b) higher temperatures, (c) smaller cultures with fewer plants, and (d) varying concentrations of CaCl₂ as an experimental variable.

**Greenhouse procedure**

The experiments were done in continuously aerated nitrate-free nutrient solutions, without asepsis. Temperatures varied diurnally between 18 and 25°C in the air, and between 18 and 30°C in the culture solutions. The basal solutions contained micronutrients and were 1 mM in K₂SO₄, MgSO₄, and KH₂PO₄.

Calcium was added as CaCl₂ in experiments 1 and 3. Experiment 2 consisted of a comparison of CaCl₂ with CaSO₄ and MgCl₂, to check the propriety of referring to effects of CaCl₂ as effects of calcium. Calcium concentrations were checked by atomic absorption analysis at the end of each experiment. Calcium concentrations needed no adjustments during the growth period. The pH was checked and adjusted daily with KOH or H₂SO₄.

Each replicate of each treatment consisted of a vessel containing 3 liters of solution and 15 seedlings. The seedlings were transplanted into these vessels when 7 days old, having been raised in solutions which had the basal composition plus 3 mM Ca(NO₃)₂.

Inoculum, added 1 or 2 days after transplanting, was 0.5 ml of a 3-day-old broth culture of *Rhizobium meliloti* strain U 45, containing 10¹⁰ cells per ml.

**Observations**

Plants subsampled daily from control cultures were examined to determine when infection threads first appeared. Cultures were inspected daily to determine root lengths, to determine when nodules first appeared, and thereafter to estimate nodule numbers. (Estimates of nodulation in the greenhouse tended to be low and variable). At harvest, 8 days after inoculation, nodule counts and distribution were recorded in the laboratory for each plant. In experiment 1, there were additional microscope observations on root hair distribution. All data presented are averages.