

EFFECT OF pH ON THE GROWTH OF *MEDICAGO SATIVA* AND *LOTUS TENUIS*.

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Summary

Low soil pH limits the legume culture by effects on the plant, on the bacterium and on nodule formation and function (Rice et al. 1977). Alfalfa (*Medicago sativa*) fail to persist in moderately acid soils. The poor survival and multiplication of its symbiotic bacteria, *Rhizobium meliloti*, which has been reported as the most acid-sensitive of all the root-nodule bacteria (Brockwell et. al 1991, Pijnenborg et. al 1991), are the major cause for the poor growth of the plant under acid conditions. *Lotus tenuis* is more tolerant to low pH than alfalfa (Miñón et. al. 1990) and the *Rhizobium loti* strains show marked differences in their response to acidity, with strains grown at pH 4.5 (Wood et. al 1988).

The purpose of this work was determine the effects of pH on the growth of *M. sativa* and *L. tenuis* uninoculated and inoculated with appropriate rhizobia. *Rhizobium meliloti* strains B 58 and B 323 , *R. Loti* strain LL 56 were obtained from Instituto Nacional de tecnología Agropecuaria, INTA, Castelar, Argentina, and *R. loti* strain NZP 2037 was obtained from Instituto Nacional de Tecnología de Chascomús, INTECH, Chascomús, Argentina. The growth in culture media adjusted to pH 4.0, or 7.0. was determinate by absorbance at 550 nm and viable counts (cfu/ml).

Seeds of *L. tenuis* cv. Agroverónica and *M. sativa* cv. Florida were surface sterilized and germinated in water agar. Four seedlings were planted in 300 ml pots with washed sand, and inoculated immediately with approximately 10^7 cfu of a single strain of *Rhizobium*, which was previously grown to turbidity in broth. The pots were maintained in a controlled environment chamber with 25°C, 16 h day and 8 h night, and watered with 1/5 diluted solution Hoagland (1950). The solution was adjusted before watering to pH 4, 4.5, 5.0, 6.0, 6.5, 7.0 or 8.0 with HCl 0.1 N. In the inoculated treatment, no nitrogen was added to the rooting solution, in the uninoculated controls combined N (KNO₃ 0.75 g/l) was added. After 50 days the plants were removed from the sand and examined for nodules, dried for 48 h at 70°C and weighed. Analysis of variance was performed on data.

R. meliloti strains multiplied readily at the high pH value, but multiplication failed at the low pH value. In contrast, *R. loti* strains show no significative differences in growth at both pH. This results indicate that in liquid culture, loti rhizobia are tolerant to acidity at least at pH 4.0, whereas *meliloti* rhizobia are significantly affected by low pH stress.

The growth of *M. sativa* plants was markedly affected by low pH in both conditions of N supply. Total dry weight for the uninoculated plants provided with

mineral N was highly increased at pH values above 6.0, showing a poor growth at lower pH values. These results are in disagreement with Rice et al. (1977), who reported that the alfalfa plant is capable of successful growth at pH as low as 4.0 when combined nitrogen is supplied.

For the plants inoculated with their symbiotic bacteria the growth decreased at pH values below 6.0, showing differences among the two strains, with a poorer growth of the plants inoculated with the B58 strain. The loss of viability of *R. meliloti* at low pH is a major factor contributing to the poor growth of alfalfa (Rice et al. 1977). Although the ability of these strains to grow at acid pH in laboratory media, this doesn't indicate the ability of nodulating at the same pH value. These results suggest that in *M. sativa* cv. Florida the pH below 6.0 affects both the host plant growth and the nodule development

In contrast, uninoculated *L. tenuis* cv. Agroverónica showed no significant difference in its growth at all pH assayed. Under inoculated conditions, the growth depended upon the strain used. The plants inoculated with LL 56 almost doubled in growth that inoculated with NZP 2037. LL 56 strain grew at pH 4.0 or 7.0 without appreciable differences, and in inoculated assays behaved as highly effective.

As a conclusion, in alfalfa-*R. meliloti* symbiosis both the plant and the bacteria show a sensitivity to soil pH. Therefore, efforts to improve yields should be directed to search strains of *R. meliloti* with the ability to nodulate under acid conditions, as well as alfalfa varieties tolerant to low pH soils. On the other hand, in the *L. tenuis*-*R. loti* symbiosis, both plant and bacteria seem to have developed acid pH tolerance. According to our results, the infectiveness and effectiveness of the selected strain would be an important factor to be considered in this system.

References

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