ACID TOLERANCE OF *LOTUS TENUIS-RHIZOBIUM LOTI* IN LABORATORY MEDIA.

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Soil acidity determines a low efficiency of the Rhizobium-legume symbiosis and the need to select acid-tolerant symbiotic associations. The degree of pH tolerance depends upon the bacterial strains and the plant species involved. The bacteria can be more sensitive to low pH than their legume host, so that their ability to colonize acid soils is limited by the effects of acidity on their survival and growth (O'Hara et al, 1994).

The symbiotic association between ***Lotus tenuis*** and ***Rhizobium loti*** is successfully used on heavy and alkaline soils and could be used also under acid conditions. Rhizobium strains which nodulate ***Lotus*** sp. show marked differences in their response to acidity (Wood et al, 1988) but little is known about the effect of soil acidity on ***Lotus tenuis***.

Our aim is to determine the response of ***Lotus tenuis*** cv Chajá and several ***Rhizobium loti*** strains to acid pH in order to use them under this conditions.

Bacterial strains and growth media.

Rhizobium loti strains LL22, LL32, LL54, LL55 were provided by the Instituto Nacional de Tecnología Agropecuaria-INTA-Castelar, Buenos Aires, Argentina, and A1 was isolated by our laboratory. Cultures were maintained at 4°C on yeast extract mannitol agar (YEMA, Vincent 1970) slopes and grown in yeast extract mannitol broth (YEMB, Vincent 1970) before using. Cultures were inoculated (ca 10^5 viable cells/ml) into flasks of YEMB adjusted to pH 4.0, 5.0, 6.0 or 7.0 with 0.1 N ClH or NaOH before autoclaving. Flasks were held at 30°C for 48 hours on a rotary shaker (150 rpm) and samples were withdrawn aseptically for the determination of absorbance at 600 nm. Each experiment was performed in triplicate.

Growth of *Lotus tenuis* in rooting solution with N

Seeds of ***Lotus tenuis*** cv Chajá were surface sterilized, washed and germinated on water agar for 2 days at 22°C before being aseptically transferred to tubes (190 x 19 mm) containing 25 ml of sterile rooting solutions, adjusted to pH 4.0, 4.5, 5.0, 6.0 or 7.0. The rooting solution has the following composition:

Solution A : KNO_3 0.1 g; $MgSO_4$. $7H_2O$ 0.2g; $CaCl_2$ 0.25g; FeEDTA, 10 ml of a solution containing 0.8g disodium ethylenediamine tetra acetate, 3.0 ml of a 10% solution of $FeCl_3$ and 1 l of distilled water; trace elements (B 0.5mg; Zn 0.05mg; Mo 0.05mg; Cu 0.02mg); deionised water 900ml. The solution was adjusted to the required pH value with diluted HCl or NaOH. Solution B : For pH 4.0, 4.5, 5.0 or 6.0 media 100ml of a mixture of citric acid 0.1M and Na_2HPO_4 0.2M; for pH 7.0 medium 100ml of Tris-HCl 0.1M and Na_2HPO_4 0.2M. The two solutions were sterilized separately by autoclaving at 121°C for 15 min. and then combined aseptically to set the required pH.
Seedlings (1 per tube) were supported by a roll of filter paper and the tubes were closed with cotton wool plugs. The tubes were maintained in a controlled environment chamber with 25°C, 16 h day and 8 h night. After 50 days the plants were removed from the rooting solution, dried for 48 h at 80°C and weighed. Four replicates were included per treatment.

Nodulation and growth of *Lotus tenuis* in N-free rooting solution

Nodulation was assayed in the same conditions as described above except no nitrogen was added to the rooting solution (KCl 0.1 g instead of KNO_3) and the seedlings were inoculated with ca 10^7 cfu

of a single strain that were grown in YEMB. After 50 days the plants were removed from the rooting solution, the nodules were counted and then the plants were dried and weighted. There were four replicates per strain and pH.
Analysis of variance was performed on data.

Results and Discussion

All 5 strains tested grew at pH 5 or above (Fig. 1). No strain grew at pH below 5.

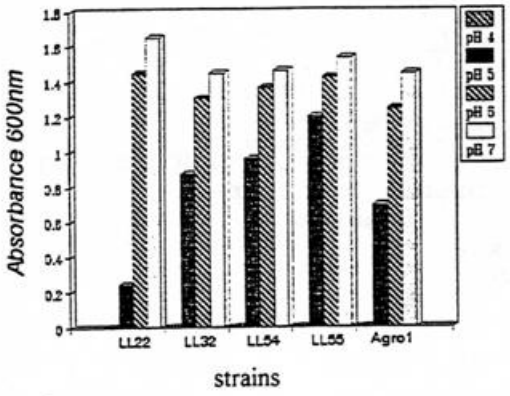

Fig.1: Growth of ***Rhizobium loti*** in YEMB media at different pH.

The growth of ***Lotus tenuis*** in rooting solution with nitrogen at the different pH values showed no significant differences. This result indicates that ***Lotus tenuis*** cv Chajá is very acid tolerant when it grows in rooting solution with mineral nitrogen.

However, when inoculated, ***Lotus tenuis*** cv.Chajá in nitrogen-free rooting solution significant differences ($p<0.05$) were observed among the strains at the different pH tested. The plants inoculated with LL22 showed the highest growth at pH 4.0 and 7.0 (Fig. 2). The strain LL22 formed a significantly higher number of nodules ($p<0.05$) than the other ***R.loti*** strains at the lowest pH value. The nitrogen fixation by ***Lotus tenuis*** was strongly affected by the medium pH, and the pH tolerance was dependent upon the bacterial strains.

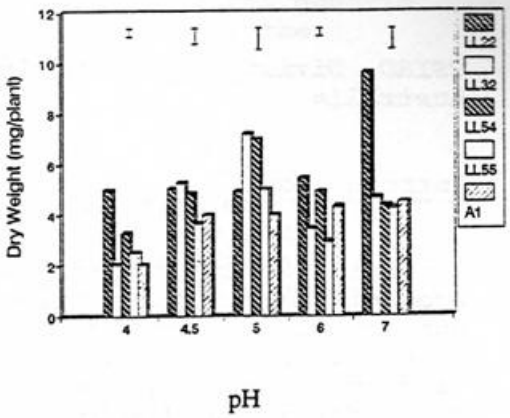

Fig.2: Growth of ***Lotus tenuis*** cv Chajá in rooting solution at different pH, inoculated with ***Rhizobium loti*** strains.

These results indicate that for the ***R.loti*** strains tested in these experiments there is no relation between the ability to grow in acid medium and to nodulate in a rooting solution at the same pH value, and the growth in liquid culture is not an indicator of nodulating ability under acid soil conditions.

References

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Wood M, Cooper J E and Bjourson A J. (1988). Plant and Soil 107: 227-231.