EFFECT OF SALINITY ON RHIZOBIUM-ROOT-HAIR INTERACTION, NODULATION AND GROWTH OF SOYBEAN

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The interaction of rhizobia with root-hairs in the rhizosphere of soybean was investigated under saline concentrations of 0–1.8%. At 1.0% NaCl, root-hairs showed little curling and deformation in response to rhizobial inoculation. At 1.5% or above, shrinkage of root-hairs was evident. The growth and multiplication of rhizobia declined rapidly when NaCl concentrations were increased from 0.2 to 0.8%. At higher concentrations, rhizobial growth was minimal. Nodulation was completely eliminated at concentrations of 1.2% NaCl or higher and increasing salinity caused a gradual continuous decline in soybean fresh weight and plant height. The failure of soybean to nodulate at high salinity was attributed to decreased rhizobial colonization and, at higher salinity, to shrinkage of root-hairs. Thus, the reduction in soybean growth under conditions of high salinity could only be partly accounted for by the reduction or failure in nodulation.

On a étudié l'interaction entre les rhizobiums et les radicelles du soja à des concentrations salines de 0–1.8% dans la rhizosphéere. A la dose de 1% de NaCl, les radicelles manifestent peu d'enroulement et de déformation à l'inoculation rhizobienne. A 1.5% ou plus, on note un ratatinement du chevelu. La croissance et la multiplication des rhizobiums diminuent rapidement lorsque les concentration de NaCl passent de 0.2 à 0.8%, au delà desquelles la croissance est minimale. La nodulation est complètement supprimée à des teneurs de 1.2% ou plus et l'accroissement de la salinité provoque une baisse graduelle du poids frais et de la taille des plants. L'incapacité du soja de noduler à des concentrations salines élevées est due à une baisse de la colonisation rhizobienne, et au ratatinement des radicelles à des teneurs encore plus fortes. La réduction de croissance du soja en sols halomorphes ne pourrait donc que partiellement s'expliquer par la réduction ou l'absence de nodulation.

High soil salinity often occurs on irrigated land where mineral salts in irrigation water are deposited into the soil over a period of time. Intensive cropping, which requires fertilizers, increases the problem. The majority of irrigated land is composed of coarse-textured soil in which water movement is rapid. Under these conditions water-soluble salts tend to accumulate at the root-soil interface and in the rhizosphere. Riley and Barber (1970) showed that salts, dissolved in the soil solution, moved to the root surface at a greater rate than the root could absorb them and were deposited around the roots. This high concentration of salts in the soil or irrigated lans could have a detrimental effect on plants.

Reduction in biomass and yield was correlated with increased soil salinity in many plants (Abel and McKenzie 1964; Gauch and Wadleigh 1944; Hayward and Wadleigh 1949). In legumes, such effects appear to be greater, possibly owing to a direct effect of salinity on nitrogen fixing ability (Munns et al. 1977). For example, in pea and mungbean, increasing levels of NaCl were associated with a decline in both nodulation and in nitrogen fixation rates (Steinborn and Roughley 1975).

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Environmental and ecological factors that affect nodulation and nodule efficiency have been extensively investigated (Nutman 1958). However, the effects of soil salinity on nodulation have not received much attention. Tolerance limit of different species of *Rhizobium* to NaCl varied widely from 0.5% for rhizobia of *Glycine javanica* (Wilson and Norris 1970), 0.50–0.72% for *R. trifolii* (Pillai and Sen 1966) and 3% for *R. meliloti* (Graham and Parker 1964; Moffett and Colwell 1968; Subba Rao et al. 1972). Similarly, different plant species also varied in their tolerance to salinity (Abel and McKenzie 1964; Gauch and Spurr 1944; Hayward and Wadleigh 1949). Therefore, each rhizobium-host combination should be studied independently.

The root-hair is the site of rhizobium infection. Therefore, if the effect of salinity on nodulation is to be elucidated, investigation should be focused on the interaction of rhizobium and root-hairs. This paper reports the effect of various levels of salt on plant growth and nodule formation in soybeans.

MATERIALS AND METHODS

Greenhouse Study

Seeds of soybean (*Glycine max* (L.) Merr. 'Amsoy') were surface-sterilized in 5% sodium hypochlorite for 5 min, rinsed with 70% ethanol and then with sterilized distilled water. Three seeds were sown in an 8-cm pot filled with white silica sand. Rhizobia (*Rhizobium japonicum*) were added to the sand at approximately 10^4 rhizobia/cm^3 sand. A total of 40 pots were prepared and divided into 10 groups. Upon germination of the seeds, each group was watered with a modified Hoagland solution (Johnson et al. 1957) containing 0–1.8% NaCl, in increments of 0.2%. Each treatment had four replications which were completely randomized. Free drainage was provided in the pots to maintain uniform salt concentration. All potted plants were maintained in a controlled environment at 21 ± 1°C with 14 h light at an intensity of 26 klx at bench level. Lighting was provided from cool-beam fluorescent lamps supplemented with incandescent bulbs.

One month after sowing, all plants were removed from the pots, washed three times in water and blotted dry on paper towels. Data on the number of root nodules, biomass, and height of the plants were taken.

The Effect of Salinity on Rhizobium in vitro

Liquid yeast-mannitol (YM) medium (Ham 1963), 50 mL/flask, was prepared in triplicate each with eleven 150-mL flasks. The salinity of the YM was adjusted with NaCl from 0 to 1.8% in increments of 0.2%. One millilitre of the rhizobium suspension with an optical density (OD) of 0.1 at 650 nm was added to each flask. After inoculation, the flasks were incubated in a 21 ± 1°C Gyrotory water-bath shaker (New Brunswick Instruments, New Brunswick, N.J. 08903) for 24 h. The growth rate of *R. japonicum* was assessed by the change of OD. Readings of OD were performed in a Beckman Spectronic-20 colorimeter.

Interaction of Rhizobium and Root-hair in Different Saline Concentrations

Sixteen pots of soybean were prepared as described above at 0, 1, 1.5 and 2% salinity. The root samples, 1 cm in length, cut 2 cm below the root base, were taken daily for 9 days and fixed overnight in 4% glutaraldehyde in 0.1 M phosphate buffer, pH 7.0. The samples were then washed with several changes in the same buffer for a total of 30 min, post-fixed in 2% OsO_4 for 2 h and finally dehydrated through a graded enthanol series. The samples were then passed through a graded series of enthanol-amyl acetate and critical-point-dried as described by Boyde and Wood (1969). The dried specimens were mounted, coated with C-Au mixture and examined in a Cambridge stereoscan S4 scanning electron microscope (SEM).

RESULTS

Greenhouse Study

This study provided an overview of the salinity effect on nodulation and plant growth. The latter was expressed in terms of fresh weight and plant height. The data are summarized in Fig. 1. Analysis of variance was performed to assess the relative importance of salinity on nodulation (Fig. 1A), fresh weight of root (Fig. 1B), top growth (Fig. 1C) and plant height (Fig. 1D). All four variables show significant differences. In addition, Duncan's multiple range

(DMR) tests were performed on the data in Fig. 1A–D. The results of these analyses are summarized in Fig. 1A–D using solid triangles and a dotted line to indicate a homogenous set.

Significant reduction in nodulation was noted in plants grown in 0.8 and 1.0% NaCl (Fig. 1A). Nodulation of soybean was completely inhibited at salinities of 1.2% NaCl or above. DMR test shows that nodulation in various NaCl concentrations falls into four homogenous subsets, i.e., 0–0.6%, 0.8%, 1.0% and 1.2–1.8% NaCl. Significant differences in nodulation were evident between each of the subsets at the 1% level.

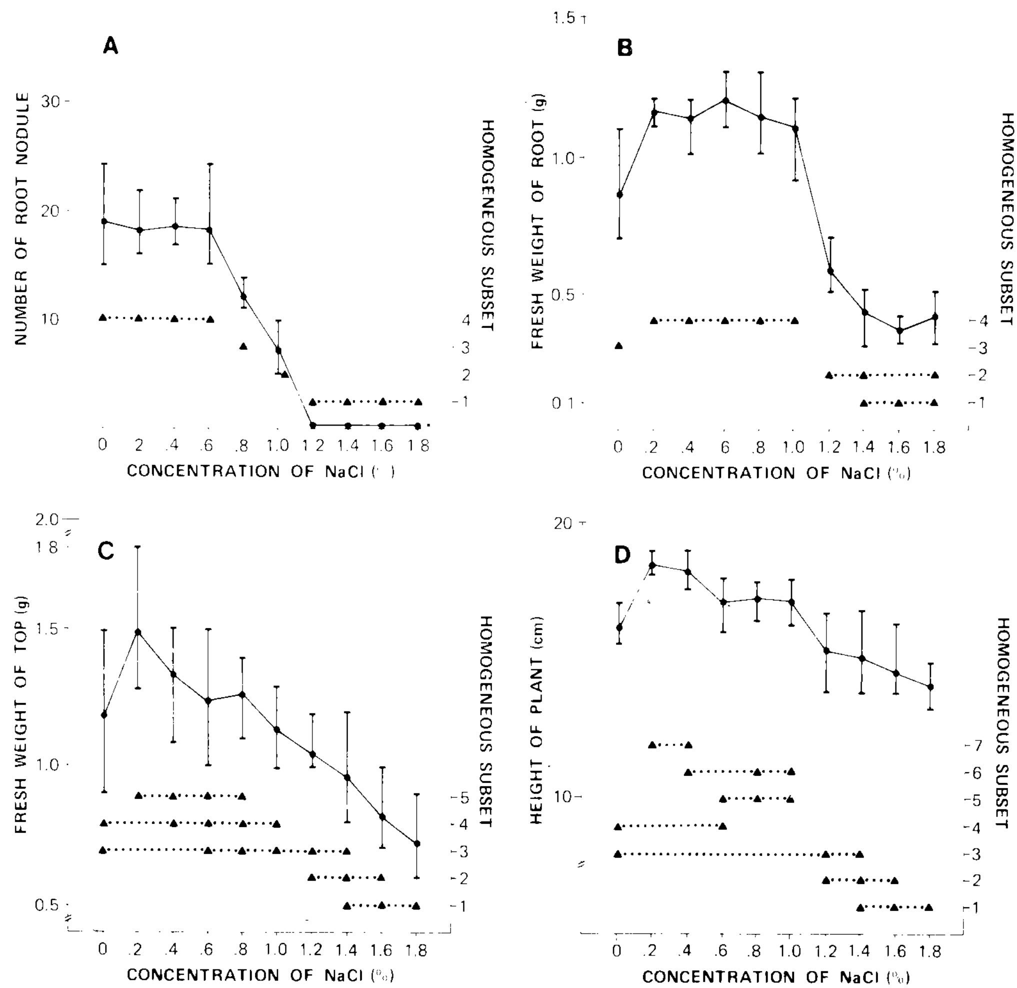

Fig. 1. Nodulation (A), fresh weight of root (B) and fresh weight of top (C) and height (D) of 1-mo-old soybean seedlings in different concentrations of saline. The homogenous subsets derived from Duncan's multiple range test are represented by solid triangles joined with a dotted line. The difference among the subsets are significant at 1% level.

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Figure 1B shows that the root weight increased significantly between salinity concentrations of 0 and 0.2% but not between 0.2% and 1.0% NaCl. Root weight decreased significantly at salinity concentrations between 1.0 and 1.2% NaCl. No difference in root weight reduction between salinities of 1.2 and 1.8% was observed.

Plant top weight showed considerable overlapping of the homogenous subsets (Fig. 1C). Although the top weight decreased gradually from 0.4% to 1.4% NaCl, the decrease was not significantly below the control. However, a significant increase and decrease in top weight in comparison to the control are evident at 0.2% and at 1.6–1.8% NaCl, respectively.

Plant height responded to increasing salt concentration in a similar way to fresh weight (Fig. 1C,D). Significant increase and decrease in plant height in comparison to the control (0% NaCl) are apparent in the plants grown in 0.1–0.8% and 1.6–1.8% NaCl, respectively.

Effect of Salinity on the Growth of Rhizobium

The growth of rhizobia declined rapidly as the concentration of NaCl increased from 0 to 0.8%. The growth was minimal in NaCl concentrations above 1.0% as indicated by the extreme low readings of OD (OD 660 nm < 0.1) (Fig. 2).

Interaction between Rhizobium and Root-hair

The SEM study was made on the samples collected daily for 9 days at four levels of salinity (0, 1.0, 1.2, and 2.0%). In general, the root-hairs increased in shrinkage and decreased in sensitivity to rhizobia. These phenomena were evident in the increased amount of root-hair shrinkage experienced in the increased NaCl concentration. This was exemplified in the samples taken 3 days after rhizobial inoculation as described below.

With 0.1% NaCl, the root hairs appeared to be slightly shrunken in comparison with the control (Fig. 3A) and appeared to be less curled and deformed (Fig. 3B). Conspicuous shrinkage of root-hairs was evident when the seedlings were irrigated with Hoagland's solution containing 1.5 or 2.0% NaCl (Fig. 3C,D). The root-hairs subjected to 2.0% NaCl showed more shrinkage than those subjected to 1.5% NaCl (compare Fig. 3C,D).

Since drastic differences in root-hair curling and deformation were observed in the seedlings grown in 0 and 1.0% NaCl, the surface interaction between rhizobium and root-hair of these two types of samples was further investigated. The results showed that at 0% NaCl, numerous rhizobia were attached to the root-hairs and the root-hairs were extensievely curled and deformed (Fig. 4B). A portion of a high magnification micrograph of Fig. 4B shows colonization of rhizobia in the areas of deformation (Fig. 4C). On the contrary, at 1.0% NaCl, very few rhizobia were attached to the root-hairs

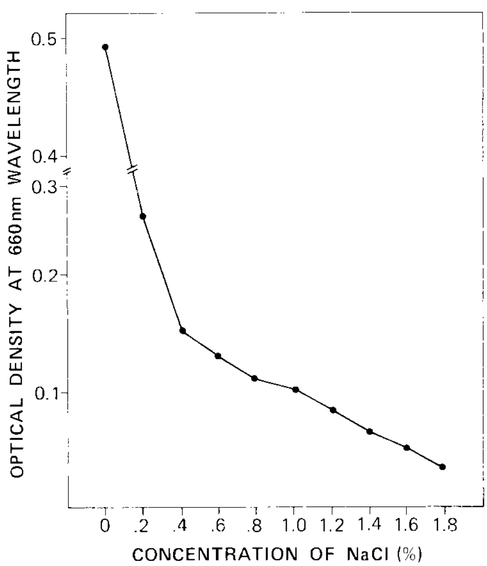

Fig. 2. Growth of *Rhizobium japonicum* in liquid yeast-mannitol medium with a range of NaCl concentrations. Readings were taken on 5 × diluted suspensions after 24 h of incubation at 21 ± 1°C.

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and very few curls and deformations were observed (Fig. 4D,E).

Since curling and deformation of root-hairs is known to occur as rhizobia infect the root-hairs (Nutman 1959), nodulation should be directly related to the amount of curling and deformation of the root-hairs as well as colonization of rhizobia on such deformed root-hairs. A series of SEM micrographs taken from samples of roots grown at four NaCl concentrations (0, 1.0, 1.5, and 2.0%) 9 days after rhizobial inoculation supported the relationship mentioned above. The micrograph of the control (0% NaCl) showed the presence of many well-developed nodule primordia (Fig. 5A).

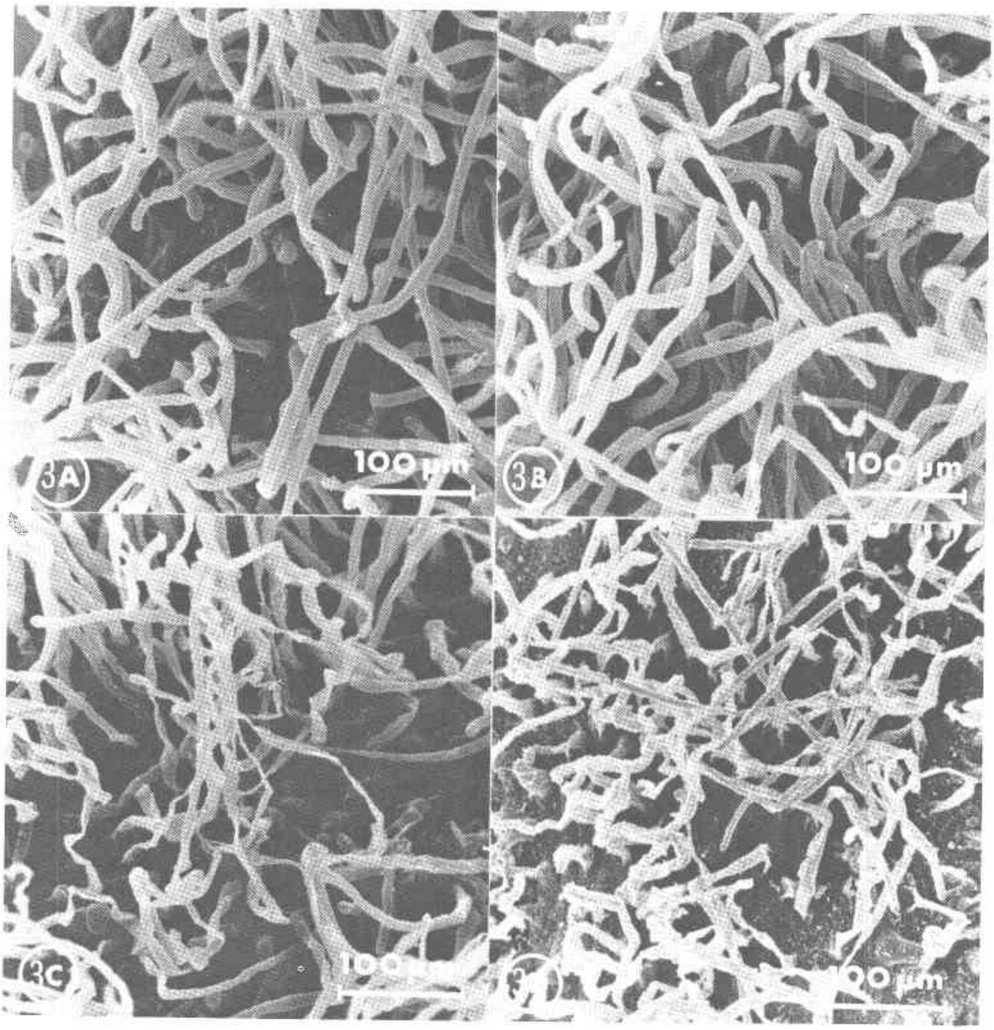

Fig. 3. Response of root-hair to four different NaCl concentrations i.e., 0 (A); 1.0 (B); 1.5 (C); and 2.0 (D), as visualized by scanning electron microscope. Samples were collected 3 days after rhizobial inoculations.

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At 1% NaCl, the nodule primordia were fewer and less developed compared to those of the control (Fig. 5B). However, as the concentration of NaCl reached 1.5 or 2.0%, few nodule primordia, if any, were observed (Fig. 5C,D).

DISCUSSION

As mentioned previously, the root-hair is the site of initial rhizobial infection (Raggio and Raggio 1962; Nutman 1959). Under normal circumstances, root-hairs respond to rhizobial colonization by curling and deformations which lead to infection (Hubbell 1970). Thus, a favorable rhizosphere environment is of vital importance to the root-hair-rhizobium interaction.

A favorable rhizosphere environment not only encourages the growth and multiplication of rhizobia but also ensures the healthy

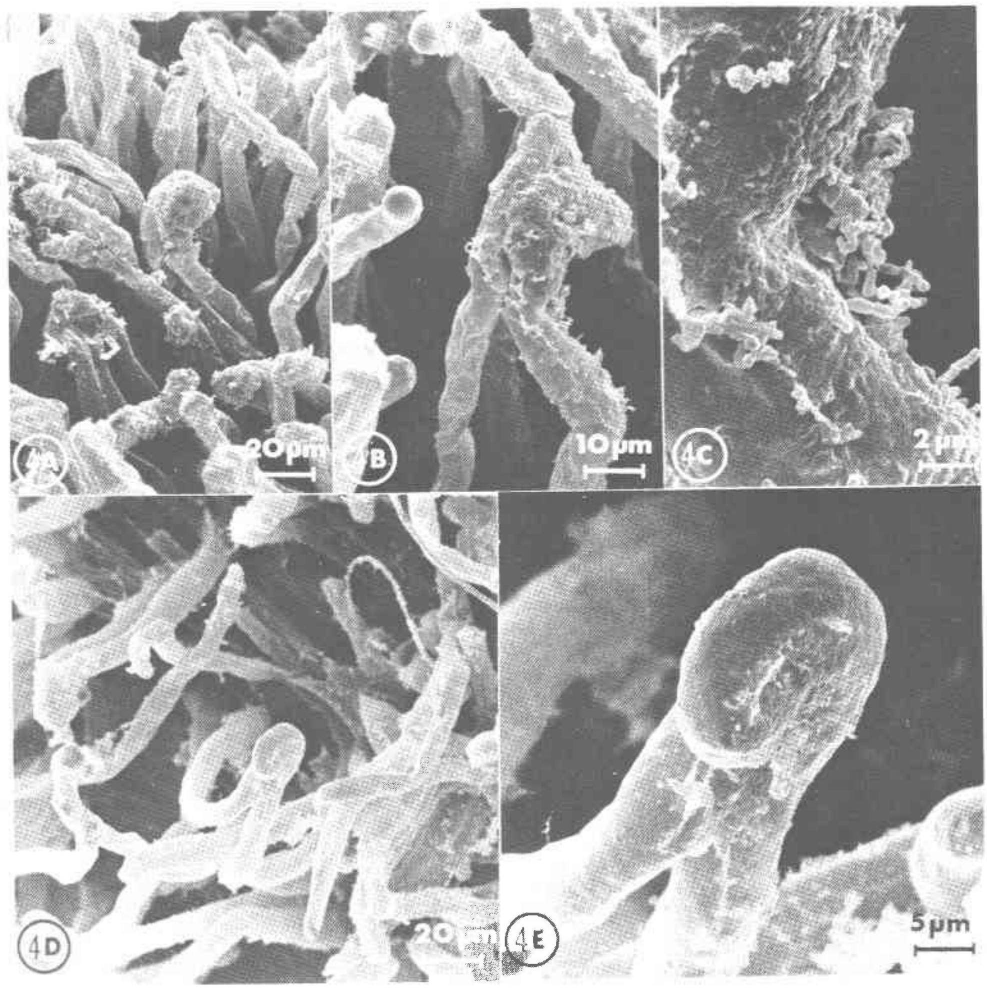

Fig. 4. High magnifications of the treatments in Fig. 3A,B show extent of curling, deformation and rhizobial colonization at 0% (Fig. 4A,B) and 1.0% (Fig. 4D) NaCl. Figures 4C and 4E are enlargements of Figs. 4B and 4D, respectively, revealing differences in rhizobial colonization at the sites of root-hair deformation.

development of root-hairs. The former increases the inoculum potential of rhizobia and the latter provides ample infection sites for the rhizobia. Therefore, conditions that promote root-hair and rhizobia growth and multiplication are generally favorable for nodulation (Nutman 1958). Conversely, factors which adversely affect the root-hair-rhizobium interaction directly or indirectly reduce nodulation. For example, shortage of soil moisture and increase in soil acidity adversely affect nodulation (Nutman 1958; Munns 1968). It was suspected that lack of soil moisture might adversely affect the suitability of root-hairs for rhizobial invasion and that high soil acidity decreased rhizobial growth and multiplication (Nutman 1958; Munns 1968). However, these claims were not experimentally substantiated. Similarly, reduction in nodulation

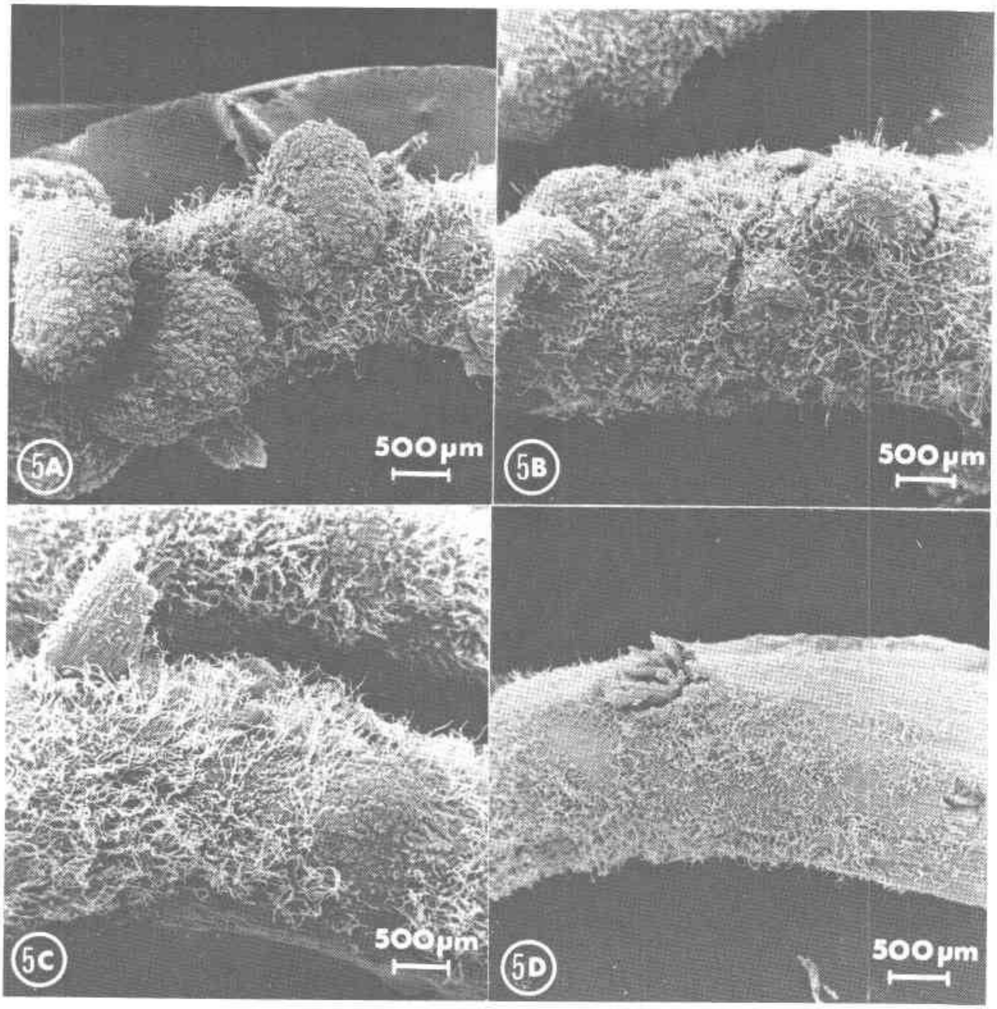

Fig. 5. Scanning micrographs of primary roots grown in four different NaCl concentrations, i.e., 0 (A); 1.0 (B); 1.5 (C); and 2.0% (D), 9 days after rhizobial inoculations. Note the different degrees of nodulation.

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under conditions of high salinity was observed in pea and mungbean (Steinborn and Roughley 1975), but the proper explanation was not provided.

The present study demonstrates that the reduction in nodulation, because of high salinity, was attributable to a combination of two factors: (a) decreased growth and multiplication of rhizobia (Fig. 2) and (b) decreased availability and susceptibility of root-hairs (Figs. 3 and 4). The former appeared to be more sensitive to the increased salinity than the latter because the rapid decrease in rhizobial growth started at 0.2% NaCl but the decrease in number and increase in plasmolysis of root-hairs occurred at 1% NaCl or above.

This work provides only partial elucidation for the reduced nodulation under various salinity conditions and shows only the net result of the salinity imposed rhizobium-root-hair interaction. These results should lay a foundation for future investigation into the mechanism of the reduced nodulation in soybeans under conditions of high salinity.

It is also apparent from the results that reduction in nodulation contributes to a reduction of plant growth; the extent of this interaction is not known. High salinity decreases plant growth due to reduction in uptake of minerals and water (Bernstein and Ogata 1966; Sprent 1972); however, high salinity may also inhibit nodule formation. Therefore, the question of soybean growth under saline conditions within rhizobial interference should be further studies so that a more exact attribution can be assessed separately against the reduction in nodulation and the reduction in uptake of nutrient and water.

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