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BRIEF COMMUNICATION

Influence of salinity and abscisic acid on the O_2 uptake by N_2-fixing nodules of common bean

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Abstract

The effects of NaCl and ABA on the respiration of N_2-fixing nodules were analysed in common bean (*Phaseolus vulgaris*) inoculated with *Rhizobium tropici* the reference strain CIAT899. Shoot and nodule growth was more inhibited by NaCl than root growth. The O_2 uptake by nodulated roots at 21 kPa O_2 was significantly inhibited by salinity. Raising pO_2 stimulated nodule respiration more under NaCl treatment than for the control, although it did not compensate totally for the inhibitory effect of NaCl. Short NaCl application was less destructive than long term application. Also, the external application of ABA inhibited nodule respiration, and this inhibition was partly compensated by raising pO_2.

Additional key words: oxygen diffusion, *Phaseolus vulgaris*, *Rhizobiaceae*.

Salinity is one of the major agricultural limitations in the arid and semi-arid regions, especially in the Mediterranean basin where symbiotic N_2 fixation contributes to the soil texture improvement (Drevon *et al.* 2001). Unfortunately, N_2 fixation can be extremely sensitive to salinity (Bekki *et al.* 1987, Serraj *et al.* 1994). Thus, the necessity to develop salt-tolerant symbioses has long been emphasised (Sprent and Zahran 1988). Although the success of such an approach would require the improvement of both partners of the symbiosis, it is generally concluded that rhizobia are relatively more salt-tolerant than the corresponding host-legumes (Jebara *et al.* 2001b). Moreover, most legume species have been found to be either sensitive or only moderately tolerant to salinity although considerable variability in salt tolerance has been reported among and within legume species (Delgado *et al.* 1994).

The mechanisms of N_2-fixation inhibition by salinity are still poorly understood. Sprent and Zahran (1988) reported that NaCl inhibits the expansion and curling of root-hairs and reduces the number of nodules in faba bean. According to Bekki *et al.* (1987) nodule activity would be less affected by salinity than plant growth and nodulation. A rapid NaCl-induced decrease was observed in the acetylene reduction activity of excised soybean nodules, even though the later may already have reduced activity (Sprent 1972). This was confirmed with intact soybean nodulated-roots and attributed to a decrease in nodule permeability to O_2 diffusion (Serraj *et al.* 1994). It is well established that an oxygen limitation is associated with nitrogenase inhibition by various environmental limitations including nitrate application (Vessy *et al.* 1988), drought (Serraj and Sinclair 1996) and phloem-sap deprivation (Hartwig *et al.* 1987). Several hypotheses have been recently proposed for the regulation of the nodule permeability to oxygen by environmental factors, involving accumulation of glycoproteins (Iannetta *et al.* 1995), osmoelectric regulation (Denison and Kinraide 1995) or osmocontractible cells (Drevon *et al.* 1995) in the nodule inner-cortex.

During growth under salinity, endogenous abscisic acid (ABA) content increase in various tissues, including nodules (Irekti and Drevon 2003). ABA is an essential mediator in triggering the plant responses to mechanical

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Abbreviations: ABA - abscisic acid; CONR - consumption of oxygen by nodulated-root; COP - critical oxygen pressure.

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damage and to adverse environmental stimuli, though ABA-independent pathways may also be involved (Leung and Giraudat 1998, Zeevaart and Creelman 2001). Substantial evidence supports the proposition that ABA levels limit water loss through transpiration by reducing stomatal aperture. So, a positive effects of ABA application were observed after rehydration of French bean leaves during water stress (Pospíšilová and Bat'ková 2004).

In this work, our objective was to test the effect of the NaCl treatment on the nodule O_2 supply and address whether this diffusion is affected by an external application of ABA in the nodulated-root rhizosphere.

The common bean (*Phaseolus vulgaris* L. cv. BAT477) seeds (supplied by S. Beebe, CIAT; Colombia) were surface sterilized, inoculated with the reference strain *Rhizobium tropici* CIAT899 and germinated on *Perlite*. Seven days after sowing (DAS), the plant seedlings were transferred to 1 dm^3 serum bottles and reinoculated by the supply of 1 cm^3 of the above inoculant directly into the nutrient solution (Jebara *et al.* 2001a). During the first 15 d, *i.e.*, before nodule emergence, the nutrient solution was complemented with 2 mM urea. After this period, the plants were transferred to a new nutrient solution with 1 mM urea for the next 2 weeks. At this stage (20 DAS), the solution was renewed; and the plants were exposed to salinity by adding NaCl (final concentration 50 mM) to the growth medium. Thereafter the solution was renewed each 2 weeks. Dry matter of shoots, roots and nodules under salt stress was 3.89 ± 0.17, 1.98 ± 0.08 and 0.36 ± 0.01 g $plant^{-1}$ at 42 DAS, compared to 7.59 ± 0.21, 2.29 ± 0.09 and 0.67 ± 0.02 g $plant^{-1}$ for the control, respectively (Jebara and Drevon 2001).

The NaCl + ABA applications were made at flowering stage (35 to 42 DAS) by gently adding these compounds to the nutrient solution with a syringe to reach final concentrations of 50 and 100 µM, respectively, avoiding any plant perturbation. ABA (100 µM) was used after preliminary test including 1 and 10 µM ABA (data not shown). Indeed only 100 µM ABA induced a rapid decrease in nodule respiration although it exceeds the ABA concentrations in nodule and root tissues. Salt or ABA treatments were stopped by completely renewing the nutrient solution.

The measurement of nodulated-root respiration was performed during flowering when first pods were 2 cm long, (for detail see Jebara and Drevon 2001). Briefly, the day before measurements, the level of the nutrient solution was lowered to one third of the volume of the culture bottle. Therefore, the majority of nodules were in direct contact with the gaseous phase during measurement. The circulation of the gaseous phase in the circuit from the nodulated-root environment through the oxymeter was driven by a peristaltic pump with a flow of 400 cm^3 min^{-1}. For each successive confinement with an initial pO_2 of 21, 25, 30, 35, 40, 45, 50 kPa O_2, the CONR could be calculated every 20 min as follows: CONR = [(initial - final) pO_2] × [V/22.4] × [60/t] with t - duration between initial and final O_2 measurement approximating 20 min; pO_2 - partial pressure of O_2 [kPa]; V- volume of gas phase [dm^3]; 22.4 [dm^3] - volume of 1 mol pure gas. However, the change of pO2 in the experiment was above the critical pO_2 for the root respiration, the later being below 8 kPa O_2 (Jebara and Drevon 2001).

The plants were harvested after the gas-exchange measurements at 42 - 49 DAS. Dried nodules were sieved into 5 corresponding classes of mean diameter (± 0.1 mm) as described in details by Jebara *et al.* (2001a). Nodules smaller than 1 mm were not counted since they were difficult to distinguish from root primordia. Moreover, their contribution to nodule mass was negligible. Assuming that nodules were spheres, the nodule surface area was computed.

The values were compared at $P < 0.05$ by *ANOVA* analysis of variance and the standard deviation of the means was used to determine the significance of differences in symbiotic effectiveness. Statistics of multiple regressions were computed with *Statistica* software.

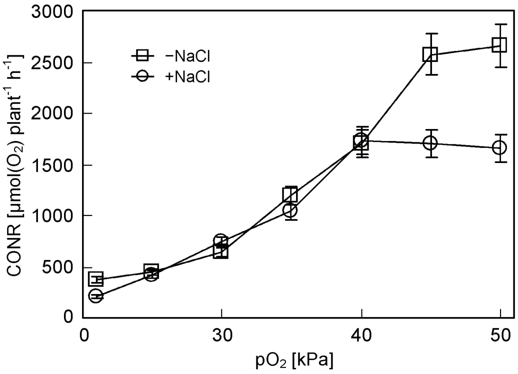

Fig. 1. Effect of rhizosphere pO_2 on *in situ* measurements of nodulated-root O_2 uptake of the symbiosis *P. vulgaris* BAT477 × *R. tropici* CIAT899 grown in a glasshouse without (*squares*) or with 50 mM NaCl (*circles*). Means ± SD of 6 replicates per treatment, plants were 42 to 45-d-old. Mean values of O_2 uptake at 21 kPa O_2 were 372 ± 103 and 213 ± 57 µmol $plant^{-1}$ h^{-1} for control and treated plants, respectively.

To observe the effect of salinity on the nodule conductance to O_2 diffusion, we compared nodulated-root O_2 consumption (CONR) as a function of external pO_2, for plants grown either under control conditions or with 50 mM NaCl. The raising pO_2 in the environment induced an immediate stimulation of CONR (Fig. 1). This stimulation was significantly higher for the treated plants than for the control ones, and reach at 40 kPa eight times initial value at 21kPa whereas the control did not exceed five times. Nevertheless, the slope of linear regression of CONR (µmol h^{-1}) as a function of external O_2 concentration within the 21 - 40 kPa O_2 was not significantly different for control and treated plants with values of 0.53 ± 0.07 and 0.51 ± 0.05 mm^3 h^{-1}, respectively. The critical oxygen pressure (COP = the value of pO_2 above which there was no more CONR stimulation) was close to 40 $kPaO_2$ with NaCl whereas it was above 50 $kPaO_2$ for control ($P < 0.05$). Thus, at 40 $kPaO_2$, the NaCl-treated plants and the controls expressed similar CONR.

In order to compute the nodule conductance, *i.e.* the nodule permeability per nodule surface unit, the above value corresponding to the O_2 permeability for the whole nodule population per plant, was divided by the total nodule surface area. NaCl decreased significantly the number of nodules smaller than 2.1 mm in diameter. The NaCl also decreased the number of nodules larger than 4.5 mm. Thus the proportion of middle-size nodules was not affected by salinity (Fig. 2).

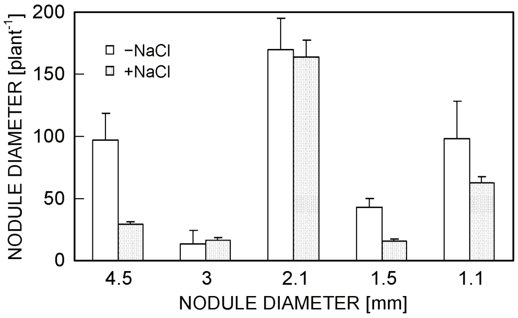

Fig. 2. Relative distribution of fresh nodule size per mean nodule diameter (± 0.30 mm) of the symbiosis *P. vulgaris* BAT477 × *R. tropici* CIAT899 grown in a glasshouse. Means ± SD of 6 replicates of 42 to 45-d-old plants.

The nodule surface area decreased significantly from 120 ± 18 cm^2 $plant^{-1}$ in control plants; to 50 ± 6 cm^2 $plant^{-1}$ in plants grown with NaCl. As a consequence, the nodule conductance was significantly higher under salinity, with values of 4.4 ± 0.3 and 10.4 ± 0.7 $\mu m\ s^{-1}$ for control and salt-treated plants, respectively.

The CONR decreased rapidly after the application of NaCl (Fig. 3*A*). Thereafter, it immediately increased transiently to 140 % of the control-plant CONR, and subsequently decreased to a level equal to that of the control at the beginning of the dark phase. When NaCl was removed the next day, the previously treated plants show a lower CONR than the control. Therefore, they recovered after 24 h a similar CONR to the control.

Since ABA is closely related with the response of plants to NaCl, and since the change in nodule permeability is postulated to be osmoregulated, we investigated how CONR was affected by the 100 µM ABA application at the beginning of the light-phase steady-state. Thus, ABA induced an immediate decrease in CONR which maintained at 50 % of the control value for the next 6 h (Fig. 3*B*). This was followed by a new decrease which was concomitant with that of the control prior to the end of the light period. The transfer to an ABA-free solution the next day induced a large increase in CONR. However, this was followed by a remarkable fall of CONR which stabilised at 80 % of the control after 24 h (Fig. 3).

The effect of raising pO_2 on the CONR was compared during the steady state observed a 4 h after the

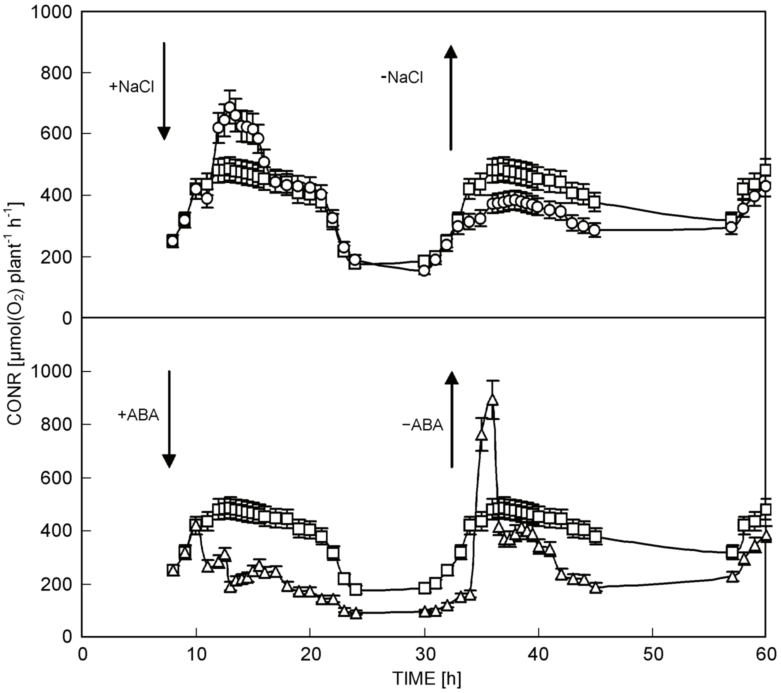

Fig. 3. Kinetics of response of O_2 uptake by nodulated-roots of the symbiosis *P. vulgaris* BAT477 × *R. tropici* CIAT899 to an application of 50 mM NaCl (*circles*), or 100 µM ABA (*triangles*) in comparison with control (*squares*). Means ± SD of 6 replicates of 42 to 45-d-old plants. Values were adjusted with diurnal cycle.

applications (Fig. 3). So, the CONR, expressed in proportion of that at 21kPa O_2, was less stimulated by raising O_2 for ABA than for NaCl or control treatments (Fig. 4). The nodule conductance was 30 and 12 % lower for ABA and NaCl, respectively, than for control (4.4 ± 0.3 µm s^{-1}). The COP, extrapolated from curvilinear regression models of CONR as a function of pO_2 was higher for ABA than for NaCl or control treatments. Thus, nodule respiration appeared to be saturated by O_2 at higher pO_2 after ABA than after the short NaCl application which was less inhibited than long term NaCl effects.

The increase in nodule conductance to O_2 diffusion under salinity, as computed from data in Fig. 1 and 2, suggests an effect of NaCl on the nitrogenase-linked nodule respiration. Since nodulation is decreased under salinity (Fig. 2), this effect may correspond to a relatively higher N demand per individual nodule. This result has similarities with the one previously observed for a rhizobia-legume symbiosis under phosphorous deficiency (Ribbet and Drevon 1995). The unknown signal might be a nitrogenous compound in phloem (Hartwig and Trommler 2001), or some of photosynthates (Gonzalez *et al.* 2001).

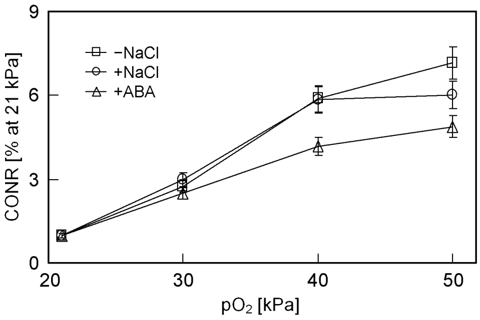

Fig. 4. Effect of rhizosphere pO_2 on *in situ* measurements of nodulated-root O_2 uptake of the symbiosis *P. vulgaris* BAT477 × *R. tropici* CIAT899 control (*squares*), with 50 mM NaCl (*circles*) or 100 µM ABA (*triangles*). Means ± SD of 5 replicates per treatment of 42 to 45-d-old plants.

The fact that the CONR under NaCl did not reach that of the control, whatever the pO_2 (Fig. 1) suggests that another factor than O_2 may contribute to the limitation of nodule activity under salinity as previously argued by Serraj and Sinclair (1996). Interestingly, the CONR response to raising pO_2 after NaCl application on common bean nodules (Fig. 3*A*) was comparable with that previously observed with soybean nodules (Serraj *et al.* 1994).

The relative similarity of CONR response to raising pO_2 after application of NaCl or ABA (Fig. 4) is consistent with the previous observation of an increase in nodule content of ABA after NaCl application (Irekti and Drevon 2003). This is consistent with an osmoregulation of nodule permeability to O_2 controlled by contraction/expansion of cells in the nodule inner-cortex (Drevon *et al.* 1995). The latter may be subjected to a similar mechanism as the one described for the ABA-induced contraction of guard cells. Alternatively, the decrease in nodule conductance after ABA application may be the result of an accumulation of 19-kDa proline rich proteins in the plasma membrane as described by Koike *et al.* (1997). Conversely, the sudden increase in CONR after removal of ABA (Fig. 3*B*) might be due to expansion of inner-cortex cells, like the expansion of guard-cell associated with the stomatal aperture after ABA suppression (Leung and Giraudat 1998). However, the removal of ABA or NaCl may be the source of a mechanical stress to which the nodule conductance has been shown to be sensitive (Minchin 1997).

In conclusion, the results presented here are consistent with an ABA mediated effect of NaCl on nodule permeability. However, the inhibition of the respiration linked to nodule N_2 fixation in the presence of 50 mM NaCl appears to involve other factors. Therefore, more work is needed to test whether this ABA-mediated effect could be completely reversed by raising pO_2 with less salinity, and whether it is affected by the rhizobial micropartner, since some rhizobial strains have been shown to affect the tolerance of the symbiosis to NaCl (Jebara *et al.* 2001b, Ben Abdelkhalek *et al.* 2003).

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