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INFLUENCE OF *AZOSPRIRILLUM BRASILENSE* ON ROOT MEMBRANES

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ABSTRACT

Inoculation of wheat and soybean plants with *A. brasilense* Cd reduced the membrane potential of root cells and increased proton efflux from the roots. Indirect evidence suggests that the continuous presence of the bacteria is not necessary to continue the response. Therefore, *A. brasilense* Cd may reduce the membrane potential of root cells and increase proton efflux by release of an unidentified bacterial signal(s).

INTRODUCTION

Azospirillum strains are bacteria that can positively affect plant growth and yield (2). However, the mechanism for growth promotion is not completely understood. One proposed mechanism is enhancement of mineral uptake by roots inoculated with *Azospirillum* (6). Mineral uptake in plants is directly related to root membrane activity. However, not all *A. brasilense* strains enhance mineral uptake (4).

Bashan *et al.* (3) studied the effect of root membrane activity in the presence of *A. brasilense* by measuring proton efflux from the roots. They found an enhancement of proton efflux from wheat roots inoculated with *A. brasilense*. In this study, we continue that work and investigate the influence of preincubation factors and timing on this interaction.

MATERIALS AND METHODS

Disinfected seeds were germinated and grown in hydroponic systems containing nutrient solution in a fully controlled growth chamber. Seedlings at ages ranging from 2 days (first root emergence) to 6-8 days (two leaves) were used. Three days after disinfection, the nutrient solution in the hydroponic systems was replaced by fresh nutrient solution supplemented with 10^6 CFU/ml *A. brasilense* Cd for 2-4 hr. Proton efflux activity was measured 16 hr after the inoculum was replaced with a fresh nutrient solution.

Starved bacteria were obtained by incubating washed *A. brasilense* Cd cells for 10 hr in potassium phosphate buffer. Heat treatment was accomplished by transferring a bacterial culture grown at 30 °C to 45 °C for 6 hr. Anaerobic stress

involved transferring aerobic cultures into jars containing helium for 6 hr. Antibiotic stress was accomplished by inserting filter-sterilized streptomycin sulphate at 500 mg/L into bacterial cultures followed by incubation for 6 hr. The streptomycin was washed from the bacterial cells before inoculation.

Bacteria were removed from root surfaces by mild sonication. Plants were then double washed in fresh nutrient solution. The original nutrient solution was immediately filter-sterilized and the plants were re-immersed in the original solution.

Continuous perfusion of the nutrient solution with fresh solution was carried out using a small pump. The overflow from the beaker containing inoculated plants was filtered via 0.45 μm filter. This resulted in the bacteria and roots being continuously washed, without the bacteria being removed from the root vicinity. The replaced solution was collected, its volume was reduced by rotoevaporator to its original volume and the amount of released protons was determined.

Proton efflux was measured in hydroponically grown seedlings after the bacterial removal procedure described above. The seedlings were transferred to a small beaker containing fresh nutrient solution and were incubated in a controlled-temperature water bath at 25 °C under constant illumination. The quantity of released protons was determined by titrating the nutrient solution with NaOH to its initial pH value.

RESULTS

Inoculation of wheat seedlings with *A. brasilense* Cd significantly increased the proton efflux of the roots starting 5 hr after inoculation compared with non-inoculated plants (Table 1). Bacterial cells themselves produce negligible proton reflux. Removal of the bacteria from the root surface at 2, 4 and 10 hr after inoculation did not affect proton extrusion, which remained similar to the proton efflux of inoculated roots having a permanent *A. brasilense* Cd population.

Proton efflux from roots after short exposure to *A. brasilense* Cd was directly related to the inoculation level and physiological status of the bacterial cells. Active bacteria, at an optimal level for inoculation (10^5-10^7 CFU/ml), produced the most proton efflux from the roots. Inoculation with dead cells, cell wall fragments, or several associative non-beneficial rhizosphere bacteria belonging to other genera (data not shown) did not enhance the proton efflux. Continuous perfusion of the nutrient solution of plants retained bacterial cells around roots but eliminated the proton efflux enhancement. Similar results were found with soybean seedlings treated similarly (data not shown).

Table 1. Enhancement of proton efflux in wheat roots by *A. brasilense* inoculation.

Treatment	Proton efflux after 10 hr (μmol H^+/g fresh wt./hr)
Non-inoculated plants	2.2±0.2[#]
Inoculated plants without bacterial removal (24 hr after inoculation)	4.8±1.4
Removal of bacteria 2 or 4 hr after inoculation	4.3±1.5
Short inoculation period (2 hr) with:	
10^3 CFU/ml	2.4±0.3
10^6 CFU/ml	4.2±1.3
$>10^8$ CFU/ml	8.5[*]
Inoculation with:	
starved bacteria	2.8±0.2
bacteria after heat treatment of 45°C	2.6±0.2
bacteria after anaerobic stress	3.2±0.1
bacteria after streptomycin stress (500 mg/L)	3.1±0.2
Continuous perfusion of inoculated roots	2.3±0.2

[*] Roots showed visible deformations
[#] SE

DISCUSSION

A. brasilense Cd enhanced proton efflux from wheat and soybean roots. If bacteria were removed from roots after a 2 hr incubation period, increased proton efflux was still noted. This is indirect evidence that the continuous presence of the bacteria was not necessary to continue the response. Such an induction response may partially explain the commonly observed phenomenon of positive plant growth responses continuing to be observed long after *Azospirillum* populations on roots have nearly disappeared.

We suggest that a short exposure of roots to an actively metabolizing *A. brasilense* strain increases proton efflux, probably through release of an unidentified bacterial signal(s).

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