Rice transgenic plants overexpressing avp1 gene exhibit differential proton pumps activation and a related nitric oxide synthesis

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Two transgenic lines of rice, AVP1-7 and AVP1-10, overexpressing the gene encoding the vacuolar H<sup>+</sup>-PPase were used to study the involvement of this enzyme and of the plasma membrane H<sup>+</sup>-ATPase in the apoplastic NO synthesis. Rice plants were grown in soil during five month and afterward they were collected and treated in hydroponic media containing inhibitors of the nitrate reductase and NO synthase enzymes. Adventitious roots were selected and processed by cell fractionation in order to isolated plasma membranes enriched vesicles or sliced to analyze the NO synthesis by measuring the DAF fluorescence. Both AVP1-7 and AVP1-10 exhibited an induction of both H<sup>+</sup>-pumps at the plasma membranes. However, these H<sup>+</sup>-pumps were further stimulated by the treatment with the inhibitors of NO synthesize enzymes, including the wide type line, which exhibited an activation lower than that found in the transgenic lines. On the other hand, the inhibitor's treatment lead to a strong decrease of the NO fluorescent signal, whereas no guenching was detected for the DAF fluorescence of transgenic lines. These results may suggest that activations of H<sup>+</sup>-pumps in the plasma membrane would be a key element of a non-enzymatic mechanism by which NO could be synthesized upon the pump-dependent apoplast acidification.

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