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Determination of Brachialactone Release Mechanism in *B. humidicola* by Root Exudate Profiling under Different Elicitors

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Abstract

The ability of plants to suppress soil nitrification through the release of nitrification inhibitors from the roots is termed 'biological nitrification inhibition' (BNI). The tropical forage grass *Brachiaria humidicola* (Bh) has particularly caught attention in this respect due to its ability to release a root exudate termed "brachialactone" that deters both ammonia monooxygenase (AMO) and hydroxylamine oxidoreductase (HAO) enzymatic pathways in nitrifying organisms such as *Nitrosomonas europaea*. What remains unknown is if brachialactone is exuded in the rhizosphere via an active, regulated transport or through passive release mechanisms (diffusion / root turnover). Taking this as a starting point, we studied the exudation pattern of brachialactone in hydroponically grown Bh under the effect of contrasting nutritional nitrogen forms and different pH regimes. Contrary to the previous hydroponic studies, exudation patterns were studied in the full nutrient trap solutions and plants were exposed to the exudate collection solution for only 4 hours. Through this approach we have minimised the plasma membrane damage due to osmotic stress or pH shifts to the greatest possible extent. The trial included six treatment combinations with three different nitrogen sources (i.e. ammonium & nitrate, sole ammonium and sole nitrate) and two levels of pH (i.e. 4.8 and 6). Subsequent to root exudate collection, trap solutions were analysed for brachialactone, ammonium/nitrate levels, sugars, amino acids and carboxylates. To elucidate whether brachialactone is released by means of a regulated process, i.e. demonstrates a unique response to the different treatment combinations, its exudation levels were compared to the exudation levels of major primary metabolites. Our study confirmed that the nutritional N form has a significant effect on brachialactone exudation ($p = 0.0001$) and pH has a synergistic effect on its release($p = 0.0417$). We have found that brachialactone exudation is correlated with ammonium uptake but does not seem to be linked to the exudation of any of the other primary metabolites.

Keywords: Biological nitrification inhibition, brachialactone, primary metabolites, release mechanism, root exudate profiling

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