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"Bridging the gap between increasing knowledge and decreasing resources"

Phenotyping of a Bi-Parental *Brachiaria humidicola* Population for its Biological Nitrification Inhibition Potential

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Abstract

Nitrification is an oxidation process, part of the larger nitrogen (N) cycle in the soil, and is mediated by microorganisms that transform ammonium (NH_4^+) to the water soluble nitrate (NO_3^-), producing nitrous oxide (N_2O, a potent greenhouse gas) as a by-product. Researchers at CIAT-Colombia, in collaboration with JIRCAS-Japan, reported that the tropical forage grass, *Brachiaria humidicola* (Bh), has the ability to inhibit the nitrification with chemical exudates from their roots into the soil. This capacity of *Brachiaria* grasses is known as biological nitrification inhibition (BNI) and this function could increase N efficiency in crop-livestock systems by improving recovery of applied N, while reducing NO_3^- leaching and N_2O emissions. Recently, we have been able to improve methodologies for more reliable quantification of the BNI trait in order to accelerate the process of identifying phenotypic differences in BNI ability.

Our aim is to quantify phenotypic differences in BNI capacity of a bi-parental hybrid population (n=134) of two Bh accessions with different BNI capacities, CIAT 26146 (medium to low BNI with sexual mode of reproduction) × CIAT 16888 (high BNI with apomictic mode of reproduction), in an attempt to identify QTLs (quantitative trait loci) associated with BNI trait. The phenotyping methodologies used were: 1) A bioassay using a recombinant *Nitrosomonas europaea* strain engineered to detect changes in nitrification *in vivo* in the presence of Bh root exudates; 2) A reliable and rapid soil incubation method to determine changes in nitrification in soil where Bh plants were grown; and 3) A molecular method where both DNA and RNA from soil nitrifying microbes are co-extracted. First, DNA is used to size the nitrifier populations as an indicator for the amount of nitrification inhibitors released by the Bh roots. Second, cDNA is synthesized using the RNA as template, to enable expression analysis of ammonia monooxygenase gene (responsible enzyme for NH_4^+ oxidation to NO_3^-) as a functional molecular marker for nitrification process. By using these different phenotyping methodologies we found high phenotypic variability in BNI capacity among hybrids. The importance of these results in regulating BNI function in *Brachiaria* will be discussed.

Keywords: Ammonia mono-oxygenase (amoA), brachialactone, *Brachiaria humidicola*, nitrous oxide, phenotyping, root exudates

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