

## **Regulation of nitrification in soil: Advances in integration of *Brachiaria* hybrids to intensify agriculture and to mitigate climate change**

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### **Introduction**

Higher rates of nitrification in soil facilitate nitrogen (N) losses from agricultural systems through nitrate-leaching and denitrification. Plants' ability to produce and release nitrification inhibitors from roots and suppress soil-nitrifier activity is termed 'biological nitrification inhibition' (BNI) (Subbarao *et al.*, 2015). Up to 70% of applied N-fertilizer is lost (via NO<sub>3</sub>-leaching and gaseous-N emissions) from agricultural systems and the annual economic loss from lost N-fertilizer is estimated at 90 US\$ billion. Previous research has indicated that *Brachiaria humidicola* (Bh), a tropical forage grass that is well adapted to infertile and waterlogged soils, has high capacity to inhibit nitrification in soil and reduce emissions of a highly potent greenhouse gas, nitrous oxide (N<sub>2</sub>O) (Subbarao *et al.*, 2009). CIAT has an on-going *Brachiaria* breeding program that generates interspecific (*B. decumbens*, *B. brizantha*, *B. ruziziensis*) and intraspecific (*Bh*) hybrids that combine several desirable attributes. An interinstitutional and multidisciplinary project was initiated in 2012 to integrate *Brachiaria* hybrids into crop-livestock systems of smallholders to improve livestock productivity and mitigate climate change by reducing nitrification in soil (Rao *et al.*, 2014). Here we report the major advances from the last three years of work from this project.

### **Materials and Methods**

The following methods were used to conduct the research activities that contributed to the outputs described below in the Results and Discussion section. Output 1: A set of 15 apomictic hybrids of *Bh* and 4 commercial grass cultivars that are commonly used in the area were evaluated with farmer participation to identify the selection criteria that producers take into account when choosing a new grass to be established on their farms. Output 2: Different phenotypic methods were applied to identify *Bh* hybrids with different levels of BNI. The project team has been testing, adapting and developing a number phenotyping tools to characterize differences in BNI ability among *Brachiaria* hybrids and also making progress to map quantitative trait loci (QTL) associated with BNI (Arango *et al.*, 2014; Moreta *et al.*, 2014). Output 3: Genotyping by Sequencing (GBS) technique has been applied to identify QTL associated with the BNI trait using single nucleotide polymorphism (SNP). Output 4: <sup>15</sup>N natural abundance in leaf tissue has been tested as an indicator of BNI activity under field and greenhouse conditions. Output 5: About 500 hundred on-farm surveys have been conducted in Nicaragua and Colombia aiming to identify recommendation domains for BNI technology in crop-livestock systems as well as to evaluate the potential economic benefits to farmers. The project also contributed to strengthen local capacity to evaluate BNI differences among grasses.

### **Results and Discussion**

**Output 1:** Rural livelihood benefits enhanced Farmer participatory evaluation was performed in two regions of Nicaragua (Camoapa and Nueva Guinea) and in the Piedmont region of the Llanos of Colombia with different *Bh* hybrids and commercial grasses used as checks. Colombian farmers identified six *Bh* hybrids (Bh08-405, Bh08-63, Bh08-418, Bh08-688, Bh08-1236 and Bh08-382) based on soil cover, biomass production, extent of flowering, leaf color, leaf size and palatability as superior to local checks. Farmer participatory evaluation in Nicaragua indicated that 14 *Bh* hybrids were well adapted and most of the hybrids produced greater dry matter yield than the commercial cultivar *B. brizantha* cv. Marandú.

**Output 2:** *Bh* hybrids with different levels of BNI identified Based on greenhouse evaluation of 118 apomictic hybrids of *Bh* in 2012, twelve contrasting *Bh* hybrids were selected with different BNI capacity and planted in the field in 2013 along with 8 checks at Corpoica-La Libertad station in the the Llanos of Colombia. Dry forage yield, forage quality, BNI

activity, and N use efficiency characteristics of the *Bh* hybrids and checks are being monitored to identify the best-bet hybrids.

**Output 3:** QTLs identified and molecular markers developed Approximately 3,400 genotyping-by-sequencing (GBS) derived SNPs and 150 transcriptome derived Kompetitive Allele Specific PCR (KASP) assays were used to create saturated genetic linkage maps of the parents (*Bh* CIAT26146 and *Bh* CIAT16888) of a biparental mapping population characterized for BNI. This is the first reasonably high-density linkage map ever generated in *Brachiaria* and should yield very interesting information about the genetic control of BNI as well as useful information about the base chromosome number of *Bh*. These draft linkage maps will be improved during 2015 with the addition of SSR and AFLP markers.

**Output 4:** Indicators of BNI activity developed A field trial was established in 2013 at Corpoica-La Libertad station and the effects of long-term *Bh* pastures (productive or degraded) with high BNI activity in soil on grain yield of subsequent crop of acid soil-adapted maize are being quantified. Results on grain yield among the N treatments showed a superior yield in the field sites where *Bh* pasture was a preceding land use compared with continuously cropped area. Nitrification rates in soil showed an inverse relation with grain yield, suggesting to some extent, a residual effect of the nitrification inhibitors released by *Bh* pastures to the soil over time, before planting the maize. Moreover, agronomic N use efficiency was markedly greater with *Bh* pasture sites than with the cropped area of maize with different rates of N application. Confirmatory results were observed in the second cycle of this experiment conducted in 2014 and a third cycle will be performed in 2015.

**Output 5:** Application domains of BNI technology identified, benefits assessed and local capacity strengthened To characterize the potential beneficiaries of the BNI technology, 137 livestock farms in Nicaragua and 337 farms Colombia were surveyed. Based on the collected information in Colombia, 55% of surveyed farms are clustered in 4 large groups according to the size of the farm, animals per unit of area, milk production and number of animals. Smallholders are predominant (more than 50%), and the principal variable to differentiate the cluster is animals per unit of area, indicating that technology is a key variable. There was no correlation between infrastructure and intensification and more than 90% of cattlemen do not practice cropping, which could cause some difficulties in adopting BNI technologies for crop-livestock integration in this region. But *Bh* hybrids that combine greater forage yield and forage quality with high BNI could contribute to greater livestock production. Extrapolation domains for potential adoption of BNI technology beyond the study areas are being estimated using data collected from two regions in Nicaragua and one region in Colombia.

## Conclusion

An interinstitutional and multidisciplinary research project funded by BMZ-GIZ, Germany is making progress in characterizing the natural phenomenon of BNI by developing new research tools and protocols for phenotyping BNI trait in *Brachiaria* grasses, identifying QTLs for BNI trait, and quantifying residual benefits of BNI on subsequent crop production. Early involvement of farmers has been a key component of the project to develop climate-smart crop-livestock systems for smallholders in the tropics.

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