Climate-smart crop-livestock systems for smallholders in the tropics: Integration of new forage hybrids to intensify agriculture and to mitigate climate change through regulation of nitrification in soil

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Introduction

It is widely recognized that less than 50% of applied nitrogen (N) fertilizer is recovered by crops, and based on current fertilizer prices the economic value of this "wasted N" globally is currently estimated as US$81 billion annually. Worse still, this "wasted" N has major effects on the environment (Subbarao *et al.* 2012). CIAT researchers and their collaborators in Japan reported a major breakthrough in managing N to benefit both agriculture and the environment (Subbarao *et al.* 2009). Termed "*Biological Nitrification Inhibition*" (BNI), this natural phenomenon has been the subject of long-term collaborative research that revealed the mechanism by which certain plants (and in particular the tropical pasture grass *B. humidicola*) naturally inhibit the conversion of N in the soil from a stable form to forms subject to leaching loss (NO_3) or to the potent greenhouse gas N_2O (Subbarao et al. 2012). *Brachiaria humidicola* which is well adapted to the low-nitrogen soils of South American savannas has shown high BNI-capacity among the tropical grasses tested (Subbarao *et al.* 2007). The major nitrification inhibitor in *Brachiaria* forage grasses is brachialactone, a cyclic diterpene (Subbarao *et al.* 2009). Reduction of N loss from the soil under a *B. humidicola* pasture has a direct and beneficial environmental effect. We hypothesize that this conservation of soil N will have an additional positive impact on a subsequent crop (*e.g.* maize). At present, recovery of fertilizer N and the impact on crop yield is not known. The main purpose of our inter-institutional and multi-disciplinary project, targeting small-scale farmers, is to develop the innovative approach of BNI using *B. humidicola* forage grass hybrids to realize sustainable economic and environmental benefits from integrated crop-livestock production systems.

Methods

The project is focused on five major outputs that will be accomplished through the development of new research tools and methodologies to test the BNI concept within a holistic agricultural context. These outputs include: (1) Rural livelihood benefits enhanced by involving small-scale farmers as decision makers and co-researchers in the integration of new *B. humidicola* hybrids in small-holder crop-livestock systems; (2) *Brachiaria humidicola* hybrids with different levels of BNI identified; (3) Quantitative trait loci (QTL) associated with the BNI trait identified and molecular markers developed for *B. humidicola* hybrid selection; (4) Indicators of BNI activity developed for use under field conditions based on the role of BNI in improving the efficiency of utilization of fertilizer nitrogen while reducing N_2O emissions from agricultural production systems; and (5) Application domains of BNI technology in crop-livestock systems identified, potential economic benefits assessed and local capacity to evaluate BNI strengthened. Research progress made by the project team will be presented.

Results

Output 1: Rural livelihood benefits enhanced

A set of 30 apomictic hybrids was transferred to partners in Nicaragua for agronomic evaluation with farmers in three regions (Camoapa, Nueva Guinea, El Rama). These hybrids were also made available to partners in Colombia for farmer participatory evaluation in the Piedmont

region of the Llanos of Colombia.

Output 2: B. humidicola *hybrids with different levels of BNI identified*

A greenhouse trial was established to evaluate phenotypic differences in BNI using 118 apomictic hybrids of *B. humidicola*. They are being evaluated for their growth and nutritive value, N uptake, N use efficiency and potential ability to inhibit nitrification (BNI index and ^{15}N natural abundance) in soil. Estimation of nitrification rates (mg NO_3/kg soil/day) and determination of soil microorganism populations through Real-Time PCR are currently ongoing to survey potential BNI function.

Output 3: *QTLs identified and molecular markers developed*

Genomic DNA from a *B. humidicola* biparental mapping population (CIAT 26146 x CIAT 16888) of 134 hybrids was extracted for genotyping purposes towards the generation of a high-density linkage map and identification of QTLs associated with the BNI trait. Genetic markers were identified from the above genotypes through Next-generation RNA sequencing (GS-FLX Plus and Illumina Hiseq 2000). More than 25000 molecular markers (SSR and SNPs) have been identified and at least 500 markers will be selected for polymorphism screening. Phenotyping efforts are underway to generate data for QTL analysis.

Output 4: *Indicators of BNI activity developed*

Stable carbon and N isotope analyses are being used to evaluate contrasting *B. humidicola* hybrids with different BNI capacity for their ability to release carbon in deeper soil layers, recover native and applied N and minimize leaching and gaseous losses of N in soil columns under greenhouse conditions. Natural abundance of ^{15}N was estimated in leaf samples of the *B. humidicola* biparental population (CIAT 26146 x CIAT 16888) to develop new indicators for evaluating BNI of contrasting hybrids under greenhouse and field conditions. A new analytical method based on high performance liquid chromatography (HPLC) is also under development for a precise detection and quantification of brachialactone. A bioassay using recombinant *Nitrosomonas* is being improved to detect BNI activity in root exudate samples. Sampling of nitrous oxide emissions is being adapted for large-scale screening of pot trials under greenhouse conditions. The residual value of the BNI function in long-term pastures (15-year-old) on N use efficiency and grain yield of subsequent crops (maize) is being determined along with the estimation of carbon footprints of different systems.

Output 5: *Application domains of BNI technology identified, benefits assessed and local capacity strengthened*

Extrapolation domains for potential adoption of BNI technology beyond the study areas are being estimated using data collected from local conditions in Nicaragua and Colombia. Spatial data sets, maps, demographic data, and land use information will be matched with farm-level economic surveys for further analysis. Public information available online is also being gathered from local institutions in Nicaragua and Colombia as complementary information. A survey was designed to collect information on farming systems to identify farming similarities through a microeconomic model for resource optimization.

Conclusions

The natural phenomenon of BNI is being characterized through an interinstitutional and multidisciplinary research project funded by BMZ-GIZ, Germany. The main aim was to develop new research tools and proven methodologies to detect BNI function to minimize N losses from crop-livestock systems. Farmer involvement is a key component of the project to ensure that the new forage germplasm is successfully integrated into existing crop-livestock systems in the face of climate change.

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