Development and Implementation of Robust Molecular Markers and Genetic Improvement of Common and Tepary Beans to Increase Grain Legume Production in Central America and Haiti (S01.A4)

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Project Problem Statement and Justification
During the past 30 years, most of the growth in bean production in Central America and the Caribbean has occurred in the lowlands (< 1000 m), especially in the more humid regions. This project addresses several biotic and abiotic constraints often encountered in the tropical lowlands. The presence of BGYMV (Bean common mosaic necrosis virus) and BCMNV (Bean common mosaic necrosis virus) in the Caribbean, Central America, and southeastern Mexico make the selection for resistance to these viruses priority breeding objectives. Legume Innovation Lab plant breeders have developed and released black bean lines, such as *DPC-40*, *XRAV-40-4*, and *MEN-2201-64ML* that combine resistance to BCMNV and BGYMV. Small red bean breeding lines with the same combination of traits for disease resistance are currently being developed at Zamorano. Greater levels of common bacterial blight (CBB) and web blight (WB) resistance are needed for beans produced in warm and humid lowland regions, such as the Petén in Guatemala. Resistance to these diseases also permits increased production of beans in Central America during the first growing season, when rainfall is more abundant and reliable. This project's plant breeders have developed Middle American and Andean bean breeding lines with different combinations of resistance to diseases (CBB, rust, angular leaf spot ALS, WB, and root rot), pests (bruchids, leafhoppers) and tolerance to edaphic constraints (low N soils, high temperature). This project will use these elite breeding lines as the base for the continued improvement of beans for our target countries.

There are regions and/or growing seasons in Central America and Haiti that are too hot and/or dry to produce common beans. The tepary bean (*P. acutifolius*) is a potential alternative grain legume for these stressful environments. Farmers on the Pacific coast of Central America and some countries of Africa already produce tepary beans on a limited scale. In addition to heat and drought tolerance, there are tepary beans with high levels of resistance to common bacterial blight, bruchids and other important traits. Resistance to BCMV and BGYMV as well as larger seed size and improved agronomic traits would increase the potential adoption of tepary beans. Interspecific crosses with common beans will be used to introgress these traits into tepary beans. This effort represents the first systematic attempt to genetically improve tepary beans.

Bean breeders were early adopters of marker-assisted selection (MAS) to identify lines with desired combinations of traits. This resulted in increased efficiency in the development of improved bean breeding lines. There are, however, molecular markers available for a limited number of traits. Others are only effective in a specific gene pool. Therefore, there is a need to develop new or more robust markers, particularly for traits of economic importance to bean breeding programs in the tropics. Recent advances by the BeanCAP project, led by North Dakota State University, in sequencing the bean genome and the

development of an SNP array, will facilitate the mapping and development of molecular markers for traits of economic importance, while breeder friendly indel markers are a broadly applicable technology. The availability of phenotypic data in appropriate populations is a major factor limiting the development of these markers. This Legume Innovation Lab project will assist this effort through the development of the populations and information needed to identify the more robust markers. Dr. Phil McClean at NDSU will lead the collaborative effort to develop improved molecular markers.

There is an urgent need to strengthen the capacity of bean programs in Central America and the Caribbean to conduct research and to independently develop, release, and disseminate improved cultivars. This project will provide MS and PhD degree training in plant breeding and genetics and conduct informal workshops dealing with research techniques to enable national bean programs to contribute to the genetic improvement of beans for Central America and the Caribbean.

Objectives

1. Genetic improvement of common and tepary beans for Central America and Haiti
2. Develop and implement robust molecular markers for disease resistance genes
3. Strengthen the capacity of bean programs in Central America and the Caribbean to conduct research and to develop, release, and disseminate improved bean cultivars.

Research Approach and Methods

Conventional plant breeding techniques and marker-assisted selection are being used by project scientists to develop common bean cultivars and tepary bean breeding lines with enhanced levels of disease and pest resistance and greater tolerance to abiotic stresses. Regional performance trials are conducted in collaboration with national bean research programs and CIAT. Testing in different Central American and Caribbean countries provides additional information concerning the potential performance of breeding lines and expands the potential impact of the research supported by the Legume Innovation Lab. Interspecific populations will be developed to introgress BGYMV- and BCMNV-resistance from common bean to tepary bean.

The BeanCAP project developed a suite of approximately 3000 indel markers distributed across all common bean chromosomes that are codominant and designed to be functional with a single experimental condition (PCR protocol). The power of these markers is that they are simple to implement and thus completely portable in all laboratories and are amenable to multiplexing with suites of markers. Multiplexing reduces the cost of genotyping an individual line. The release of the common bean whole genome assembled sequence allows for precise localization of each of these markers. We will search the published literature and communicate with breeders, geneticists, and pathologists in other Legume Innovation Lab projects to identify genetic materials with contrasting phenotypes (e.g., resistance and susceptibility for the specific disease). Once the location of the marker is determined, it will then be compared to the indel database to discover 30 indel markers that straddle the physical location of the marker. Those indel markers will be used in PCR amplification to determine which one acts as a definitive marker that is unambiguous in its predictive power. NDSU and USDA/ARS scientists will collaborate to determine the potential use of *P. vulgaris* Indels for tepary genetic analysis and mapping

Anticipated Achievements and Outputs

- Release and dissemination in the lowlands of Central America and the Caribbean of black and small red bean cultivars with BGYMV and BCMV (Bean common mosaic virus) resistance and greater tolerance to low soil fertility.

- Release and dissemination in the lowlands of Central America and the Caribbean black, white, and Andean bean breeding lines with resistance to bruchids, BGYMV, BCMV, and BCMNV.
- Release and dissemination of lowland black and white bean breeding lines with resistance to BGYMV, BCMV, BCMNV, and rust.
- Release of yellow and red mottled bean lines with resistance to BGYMV, BCMNV, and BCMV.
- New bioinformatic-based approach to facilitate marker development.
- Release of tepary bean lines with virus resistance and improved agronomic traits.
- Indel markers for traits of economic importance that will facilitate the selection of bean lines with the desired combination of traits.
- Technical personnel in Central America and the Caribbean with greater capacity to produce reliable and repeatable results from field trials and to develop and release improved cultivars.
- Graduate degree training in plant breeding of students from Central America and the Caribbean

Projected Developmental Outcomes

Several improved (black, small red, red mottled, and yellow) bean germplasm lines and cultivars are expected to be released in Central America and the Caribbean during the next five years. This Legume Innovation Laboratory project will continue, in collaboration with CIAT, to support bean research network activities in Central America and the Caribbean. Collaborative activities such as the regional performance nurseries will help to extend the impact of this project through the release of improved cultivars throughout the region. Disease and pest resistance and greater tolerance to abiotic stress of improved cultivars should increase or produce more stable bean yields in Central America and the Caribbean. The BCMNV resistant Andean bean lines and tepary bean breeding lines developed by this project will be shared with Legume Innovation Lab and Feed the Future projects working in Africa.

The development of robust indel markers for traits of economic importance should improve the efficiency of bean breeding programs. Multiplexing indel markers would permit simultaneous screening for multiple traits. Bean lines having desirable genotypes can be identified in earlier generations. Disease resistance genes can be combined without the need to screen lines with specific isolates or races of pathogens. These indel markers should have worldwide utility.

Contributions to Institutional Capacity Building

- In-service training will be provided at NDSU for Legume Innovation Laboratory scientists to review recent advances in sequencing the bean genome and the utilization of SNP arrays to develop indel markers for traits of economic importance.
- Workshops will be held at Zamorano to train technical personnel concerning bean research techniques with the goal of improving the quality of field research. Topics will include the development and management of field trials, breeding and selection methods, field evaluation techniques, research with *Rhizobium*, participatory plant breeding, production of basic seed stocks, and agroecological techniques.
- Undergraduate students at Zamorano will be provided opportunities to participate in bean research activities related to Legume Innovation Lab project objectives.
- M.S. degree training will be completed at the UPR by Ana Vargas (Nicaragua).
- Ph.D. degree training at NDSU of two bean researchers from Central America, the Caribbean, or Africa will be initiated. Both students will be trained in the use of conventional and molecular techniques.