**Appendix 3**

**Related, Current and Previous Work**

Additional background information is provided for those research areas outlined in the W-3150 proposal.

Common bacterial blight. (CA, NE, ND, PR, WA). Markers linked with the CBB QTL/genes facilitate MAS in combination with traditional breeding, i.e., direct selection following pathogen inoculation (Duncan et al., 2012; Viteri et al., 2014b) leading to the development and release of cultivars and breeding lines with higher levels of resistance (Beaver et al., 2008, 2010; Brick et al., 2011; Miklas et al., 2011; Osorno et al., 2013). However, there is evidence that highest levels of resistance still require direct selection involving pathogen inoculation (Duncan et al., 2012). Fine-mapping the resistance conditioned by SU91 has narrowed the genomic region for the QTL but has yet to reveal the candidate gene (Shi et al., 2012).

Halo blight/Bacterial Brown Spot. (CA, NE, ND, WA). The seed borne nature of halo blight greatly limits the ability to conduct winter nurseries in other countries including Chile and New Zealand. Bacterial brown spot, caused by Pseudomonas syringae pv. syringae (Pss), can be the predominant bacterial disease in some years in the Northern Great Plains. Both USDA-GRIN and CIAT’s dry bean core collections were screened against PSM 5 isolate of Pss. From the USDA-GRIN Core Collection, 11.8% (50), 16% (68), and 72.2% (306) showed resistant, intermediate, and susceptible reactions to the bacterial brown spot isolate PSM 5, respectively (Urrea and Harveson, 2012). From CIAT’s Core Collection, 9.5% (128), 20.5% (277), and 70.0% (948) showed resistant, intermediate, and susceptible reactions to PSM 5 isolate, respectively (Urrea and Harveson, 2012).

Bacterial Wilt. (CA, NE, ND, WA). Previously reported resistant great northern ‘Emerson’ and PI 165078 (Coyne and Schuster, 1976), were consistently resistant in Colorado tests to yellow, orange, and purple isolates of the bacterial wilt pathogen (Schwartz et al., 2010, Urrea and Harveson, 2014). PI 325691, a wild common bean accession from Mexico, was identified as a source of bacterial wilt resistance, but has a small seed size that is commercially unacceptable (Urrea and Harveson, 2014). Inheritance of resistance has been variable (quantitative, recessive) in earlier research (Coyne and Schuster, 1971); but there is no additional information on inheritance in recent cultivars and lines such as Resolute (great northern), Agrinto (pinto) and Early Rose (pink), with resistance to bacterial wilt (Huang et al., 2007).

Anthracnose. (MI, ND). Fourteen independent loci, Co-1 to Co-14, conditioning resistance that mapped to the seven chromosomes Pv01, Pv02, Pv03, Pv04, Pv07, Pv08 and Pv11 were described in addition to six other genes Co-u, Co-v, Co-w, Co-x, Co-y and Co-z, some of which have not been fully characterized or mapped to independent loci.The Co-x gene was recently fine mapped to Pv01, independent of the Co-1 locus, and to a syntenic region, located at one end of Glycine max chromosome 18 that carries Rhg1, a major gene conditioning resistance to soybean cyst nematode (Richard et al., 2014). Anthracnose resistance is dominant at all loci except the co-8 locus, and multiple alleles have been identified at the Co-1, Co-3, Co-4, and Co-5 loci. More recently, additional dominant resistance genes Co-15 and Co-16 have been identified on Pv04 in the Brazilian Andean landraces, Corinthiano and Crioulo 159 (Sousa et al., submitted; G. K. Coimbra, personal communication); and three other anthracnose resistance sources in Andean landraces, Paloma, Amendoim Cavalo and Jalo Pintado await additional confirmation (Castro et al., 2014).

Quantitative resistance loci (QRL) conditioning resistance to 3 races of C. lindemuthianum were mapped in Brazilian carioca cultivar ICA-UNA and two major effect QRLs coincided with two previously characterized major genes Co-u and Co-5 located on Pv02 and Pv07, respectively (Oblessuc et al., 2014). In addition, the colocalization of the major Co-1 gene with the Phg-1 gene conditioning resistance to angular leaf spot was confirmed on Pv01 (Gonçalves-Vidigal et al., 2011). Co-segregation of the Co-10 (renamed Co-34) gene with the Phg-ON (renamed Phg-3) for angular leaf spot in the black bean cultivar Ouro Negro was also confirmed on Pv04 (Gonçalves-Vidigal et al., 2013). A major resistance cluster consisting of the Co-3 locus with three alleles, one formerly known as Co-9 (renamed Co-33), Co-10 (renamed Co-34) and newly proposed genes Co-15 and Co-16, are all located on Pv04 along with Ur-5, Ur-14, and Phg-3 genes for rust and angular leaf spot.

Root rots. (CO, MI, NE, ND, OR, PR, WA). Some research has been conducted on Fusarium root rot isolates, but little is known about the prevalence and aggressiveness of four subspecies (anastomosis groups) of Rhizoctonia solani found on bean. Venegas (2008) reported that of 59 R. solani isolates from Nebraskan bean fields, AG-4 and AG-2-2 were the most frequent. Crops grown in rotation with beans, such as sugar beets, are also hosts for R. solani. Cramer et al. (2003) found low genetic diversity among 166 isolates of the Fusarium wilt pathogen, Fusarium oxysporum Schltdl. f.sp. phaseoli, from the U.S. Central High Plains. Webb et al. (2013) reported that some isolates of Fusarium wilt recovered from sugar beet were also pathogenic to other crops including dry bean.

Observed variability necessitates continued evaluation of the changing pathogen population and identification of sources of resistance (Singh and Schwartz, 2010). Fusarium root rot resistance has been difficult to pinpoint even though heritability estimates in breeding line FR266 ranged from 0.48 to 0.71 (Schneider et al., 2001). Conversely, resistance to Fusarium wilt in race Durango dry beans CO 33142 and 'Fisher' were controlled by a single dominant gene, whereas polygenic control (h2 ranged from 0.25 to 0.60) was found for resistance in race Mesoamerica cultivars Rio Tibagi and Jamapa (Cross et al., 2000; Velasquez and Schwartz, 2000). A 55 of Mesoamerica race possesses a QTL on Pv10 which accounted for 63.5% of the variance for Fusarium wilt resistance observed in a RIL population (Fall et al., 2001). A major gene on Pv07, likely the P locus conditioning seed color, controlled the emergence rate in crosses for resistance to Pythium ultimum (Campa et al., 2010).

Rust. (CO, MD, MI, NE, ND, PR, WA). Disease resistance is the most cost-effective strategy to manage the bean rust disease; however, the effective implementation of this strategy could be severely affected by the appearance of new virulent races of the bean rust pathogen. To date, ten dominant rust resistance genes, from Ur-3 to Ur-14, have been named and mapped to six chromosomes, Pv01, Pv04, Pv06, Pv07, Pv08, and Pv11 (Kelly et al., 1996; Alzate-Marin et al., 2003; 2004; Liebenberg et al., 2006; Souza et al., 2011). Rust resistance genes are present on Pv04 and Pv11within clusters of R genes conditioning resistance to other pathogens.

A number of dry and snap cultivars with resistance to rust have been developed since 2008. These include ABC-Weihing (Mutlu et al., 2008b), CO 46348 (Brick et al., 2008), Coyne (Urrea et al., 2009a), snap bean cultivars combining resistance to rust and heat tolerance (Wasonga et al., 2010), Croissant and Long’s Peak (Brick et al., 2011), and Stampede (Osorno et al., 2010) with resistance to the new races 22-2 and 20-3 (Pastor-Corrales et al., 2011). More recently, a multi-collaborative project led by the University of Puerto Rico and including ARS-Beltsville, ARS-Mayaguez, the Zamorano University in Honduras, and the Departments (Ministry) of Agriculture from the Dominican Republic and Haiti, has resulted in the registration of two white seeded bean lines (PR0806-80 and PR0806-81) with broad resistance to rust, Bean common mosaic virus (BCMV), Bean common mosaic necrosis virus (BCMNV) and Bean golden yellow mosaic virus (BGYMV).

White mold. (CO, ID, MI, ND, NY, NE, OR, WA, WI). The critical importance of disease avoidance due to plant architecture and resistance to lodging in breeding for WM resistance was reestablished (Miklas et al., 2013). Common beans with combined partial physiological resistance and disease avoidance lessen the need for fungicides to control WM (Miklas et al., 2013). Fewer chemical applications contribute to a safer environment and exert less pressure on the pathogen to develop fungicide resistance. The identification of numerous QTL (Soule et al., 2011), some with major and robust effect across populations and environments, is helping to understand the complexity of partial resistance, plan for marker-assisted breeding (Miklas, 2007), and direct breeders to combine independent sources of resistance (Singh et al., 2014), often through multi-parent gamete selection (Teran and Singh, 2009). Inconsistent results between evaluations in grower fields and research trials, between field and greenhouse trials, and across major bean production regions have been observed (Otto-Hanson and Steadman, 2007). The BWMN has been used to collect and compare pathogen isolates which has helped to standardize methods to evaluate breeding lines across field and greenhouse screening tests for reaction to white mold (Otto-Hanson et al., 2011; Jhala et al., 2014). Nonetheless, screening methods still need improvement. Preliminary studies using metabolomics will also aid to understand the diseases mechanisms, and consequently, to develop improved genotypes.

Abiotic Stresses (MI, NE, NY, PR, WA):

Drought. Early genetic studies found that drought tolerance, measured as yield, was an additive trait that interacts with the environment (White et al., 1994). A wide range of heritabilities (0.09 to 0.80) were reported depending on environmental conditions and market class of the bean lines evaluated (Schneider et al., 1997; Singh, 1995). The development of a collaborative drought nursery will play a key role in identifying broad drought adaptation and rapidly integrating new sources of tolerance into breeding programs. The integration of SNP markers that allow for the development of dense genetic maps through GBS and the BeanCAP SNP chip, and techniques such as association and QTL mapping, will allow for more precise identification of regions associated with drought tolerance.

Heat tolerance. Due to the coincidence of high temperature stress with other abiotic and biotic stresses, such as bacterial blight and drought, breeding work has focused on the pyramiding of multiple stress tolerance in several major market classes and on the elucidation of mechanisms related to heat tolerance.

Characterization/Utilization of exotic germplasm. (CA, CO, MD, MI, NE, PR, WA). The traits that distinguish wild and domesticated beans have been described (Gepts and Debouck, 1991). They include growth habit, seed dispersal and dormancy, pod and seed size, pigmentation, and photoperiod sensitivity. Alleles and QTL controlling these traits have been identified and mapped (Koinange et al., 1996), and should facilitate exotic germplasm conversion, including St for pod string, a QTL for seed dormancy, fin for growth habit, and ppd and hr for insensitivity to photoperiod, and sd that slows the postharvest darkening of the seed coat for some market classes.

Applied Genomics (CA, MD, MI, ND, NE, OR, PR, WA):

Genotyping-by-sequencing (GBS). GBS is an alternative method to SNP genotyping. The traditional GBS methodology uses a single-enzyme digestion to reduce complexity prior to bar-coding multiple samples into a single sequencing library (Elshire et al., 2011). While straight-forward, this reduction step may skew the SNP distribution to the euchromatic regions on the ends of the chromosome. Therefore, potentially useful SNPs within the pericentromeric region will be underrepresented. Utilizing this approach, and employing the methylation sensitive enzyme ApeKI, 53,356 SNPs were discovered in a panel of 380 snap bean genotypes. Across the genome, 70% (37,281) of those SNPs were discovered in peritelomeric regions, and 30% (16,075) were discovered in pericentromeric regions.

Other methodologies include using a two enzyme system which can capture additional sequence variability, and is also available for GBS. Without a careful selection of restriction enzyme(s), this two-enzyme system can also skew the SNP identification to the euchromatic region. These two traditional methods look at the size distribution of fragments without considering the restriction site distribution. Recently, scripts from the Setaria genome project have become available that optimize the selection of enzymes for the two enzyme system (Schroder, personal communication). Utilizing 1) a two-enzyme system along with careful selection of enzyme based on restriction enzyme distribution and 2) a SNP calling pipeline that adjusts for difference between sequencing identity between the samples and the Andean reference, 46,603 SNPs were identified among 170 Middle American cultivars.

PhaseolusGenes marker database. During the development of the PhaseolusGenes database, DNA sequencing technology continued to evolve and, particularly, to become cheaper. Hence, multiple genotypes within and across species could be re-sequenced through the application of genotyping-by-sequencing protocols. The marker of choice for high-throughput analyses became SNPs, not only because of their sheer number, but because they allowed genome-wide analysis of haplotype diversity and linkage disequilibrium. These markers can be easily converted into Indel markers which require only basic equipment allowing breeding programs with limited resources to exploit them.

Epigenomics. Epigenetics deals with a change in molecular or morphological phenotype of an organism without alteration of the nucleotide sequence. Epigenetic mechanisms drive a myriad of physiological, developmental, and genetic processes in organisms from plants to microbes to humans. Epigenetic mechanisms appear to be prevalent in modulating plant development, as well as, physiological responses to environmental cues, and for transposable element silencing. These changes in phenotype are mediated by epigenetic marks, such as nucleotide or chromatin modifications, which can be propagated through mitotic cell divisions, and in some cases, through meiosis and multiple generations. Fundamental knowledge gained from epigenetic research has not yet been fully exploited for the improvement of crop species, specifically as related to legumes such as common bean. Considering that little is known about the interaction of the environment and epigenome in the physiological development of common bean, the common bean community should direct some efforts towards understanding the interaction between the abiotic and biotic stresses and the epigenome of common bean.

A foundation to the epigenome is laid by covalent modifications of nucleotide bases, the best understood modification being cytosine methylation. The next level of epigenetic regulation involves packaging of DNA in nucleosomes of different composition and the differential modification of the constituent histone proteins. The third level of epigenetic information is specified in the complement of non-coding RNA molecules, such as the ‘small RNA species, siRNA, miRNA and piRNA. Plants are particularly adapted for passing on de novo epigenetic modifications, as they produce germline cells late in development. Plant responses to biotic and abiotic stresses also involve epigenetic mechanisms (Chinnusamy and Zhu, 2009). Flooding in rice was found to induce histone modifications of two genes, ADH1 and PDC1 (Ismail et al., 2009) and cold stress in maize induces a reduction in cytosine methylation of the ZmMI1 locus (Chinnusamy and Zhu, 2009). These epigenetic modifications can return to the basal level after the stress is removed, or perhaps might persist to become a ‘stress memory’ that might influence stress adaptation in subsequent generations (Chinnusamy and Zhu, 2009). We propose to focus some work in this proposal in developing methods for better understanding the effects of histone modification and DNA methylation in common bean, in addition to understanding and adapting methods for bioinformatics analysis of the resultant data. A large number of histone modifications that affect either the expression or suppression of genes by differential histone DNA interactions are known in mammalian research.

Ayyappan and Kalavacharla (unpublished) and Kalavacharla et al. (2014) developed a chromatin immunoprecipitation (ChIP) protocol in five common bean genotypes (G19833, Sierra, Olathe, BAT 93 and Jalo EEP558), and soybean. Using antibodies to two known histone modification marks, H3K12ac (activation mark) and H3K9me2 (repression mark), the resulting histone-DNA complexes were isolated and after removal of the bound histones, the DNA was sequenced by Illumina sequencing (ChIP-seq).

Health and Nutrition (CA, CO, IA, IL, MD, MI, MO, ND, NE, TX, WA):

Nutritional value. A recent review on the nutritional aspects of bean consumption (Hayat et al., 2014) lists numerous qualities, such as: high protein (Siddiq et al., 2010); high amounts of starch, dietary fiber, minerals, and vitamins (Kutos et al., 2003; Costa et al., 2006); an array of healthy phytochemicals (Beninger and Hosfield, 2003; Choung et al., 2003; Aparicio-Fernandez et al., 2005; Granito et al., 2008; Lin et al., 2008); and an association with health benefits such as reduced cardiovascular disease, the prevention of diabetes, and even the prevention of cancer (Jenkins, 2007; Chung et al., 2008; Thompson et al., 2008; 2009).

Preliminary evaluation using molecular markers shows nuña beans as genetically distinct from the common dry beans tested, as well as having two rather distinctive gene pools within the nuña type. Characterization of nuña sequences has the potential as an untapped gene pool for the future improvement of the common bean as well. A recent AFLP analysis of 35 nuña accessions from the NPGS (not published) shows nuñas clustering separately from the eight common dry beans tested from different market classes, and to have two separate clusters within their class. There was a broad range in protein, extractable and non-extractable phenolics, and phytate comparable to the range found in the common dry bean market classes.

Processing Quality/Flavor. Snap beans, which are consumed as immature ovaries, are a significant source of soluble fiber, carotenoids, flavonoids, and vitamins (Anderson and Bridges, 1988; Bureau and Bushway, 1986; Favell, 1998; Granado et al., 1992; Hertog et al., 1992; Judith and Vollendorf, 1993; USDA, 2010). Many important aromatic compounds that contribute to the unique flavor of snap beans have been identified, including 1-octen-3-ol, cis-3-hexenal, cis-3-Hexen-1-ol, and trans-2-hexenal (de Lumen et al., 1978). In snap beans, variation in total sugar ranges from 0.6% to 5.2% of fresh weight with individual ranges for glucose, fructose, and sucrose of 0.23% to 1.38%, 0.25% to 1.78%, and 0.1% to 0.78% of fresh weight, respectively (Lee et al., 1970; USDA, 2010).

Canned beans are an affordable nutrient dense food that could be added to a meal to fulfill recommended dietary requirements for vegetables and/or meat (Matthews et al., 2011). An estimated 90% of navy beans, and 45 % of pinto beans, are sold in cans (USDA-ERS, 2010). Many factors influence Americans food choices and taste is the most important followed by cost, convenience, and nutrition (Glanz et al., 1998). How beans hold up during the canning process plays a big role in determining the adoption of a variety.

Color retention is an integral component of canning quality in beans and is probably most important in black beans. Anthocyanins are a class of flavonoids present in the seed coat and responsible for the black color. Specific anthocyanins found in black beans include delphinidin 3-glucoside, petunidin 3-glucoside, and malvidin 3-glucoside (Takeoka et al., 1997). Since anthocyanins are water soluble, they readily leach out of seed during soaking and thermal processing. This is a processing quality issue that affects consumer acceptance of beans such that the cooked or canned product no longer appears black, but instead a shade of brown. There is significant genetic variability for color leaching in black bean, but the factors involved in the control of this trait have only been partially elucidated.

Health Effects. Beyond their nutritive benefits, beans are rich in phytochemicals with health promoting activity. Luthria and Pastor-Corrales (2006) found ranges from 19.1 – 48.3 mg/100g total phenolic acid content in an analysis of 15 bean cultivars from 10 market classes. While seed color is thought to be an indicator of antioxidant activity (Beninger and Hosfield, 1999), associations may not be straightforward. Beninger and Hosfield (2003) found red kidney and yellow brown seeds to have the highest antioxidant activity, followed by browns, then blacks, with white seed having the least; while in contrast, Luthria and Pastor-Corrales (2006) found the navy bean “Vista” to be highest in phenolic acid content, with dark red kidney “Red Hawk” and the cranberry bean “Taylor Cranberry” to be lowest.

Sustainable and Profitable Agricultural Systems (CO, MD, MI, ND, NE, OR, PA, PR, WA):

Integrated Pest and Disease Management (IPM). IPM control measures must be compatible with production systems, marketing objectives, and consumer preferences. Because it generally is easier and more advantageous to limit damage by pests than to eliminate them, crop sanitation always should be practiced. Methods include crop rotation; selection of resistant varieties; use of appropriate cultural practices to reduce plant stress and/or not to favor pest spread and development; soil fumigation; and pesticide treatment of seed, soil and foliage. Foliage protection, especially during the last few weeks of crop development, is important to achieve acceptable seed size, yield and quality.

Improved fixation, acquisition and utilization of nitrogen. The production of organic beans has become an important niche market in some regions in the U.S. (Heilig and Kelly, 2012). Hirel et al. (2011) reported that less than 50% of the N applied as fertilizer in intensive cropping systems is utilized by plants. Researchers have identified root architecture traits in common beans associated with improved P acquisition (Beebe et al., 2006b) and tolerance to terminal drought (Frahm et al., 2004), while additional research on root traits associated with nodulation and the acquisition of soil nitrogen is needed. Under these conditions bean cultivars need healthy root systems capable of scavenging available N in the soil.

Many of the W-2150 researchers are active participants in USAID-funded Feed the Future (FtF) projects that are evaluating a wide array of bean germplasm under abiotic stress, including low soil N and P. Results from trials conducted by FtF projects should help to identify bean germplasm with potential value to the W-2150 objective of increasing N acquisition and utilization. Because root health is an essential trait for the efficient acquisition of soil nutrients and water, the W-3150 breeding objective to increase root rot resistance in beans will contribute to the achievement of the goals of objective 3.

Development of beans with improved BNF has been slow. Research was intitiated recently at Michigan State University to develop black beans with enhanced biological nitrogen fixation (Heilig and Kelly, 2014). Several lines of the Puebla 152/Zorro mapping population which combined the enhanced BNF characteristics of Puebla 152 with the improved agronomic traits of the black bean Zorro were identified and phenotyped for root and shoot traits (Ibarra-Perez et al., 2014). In addition to lines with enhanced BNF, two regions of co-localizing QTL were discovered, one on Pv01 and one on Pv08 which point to a relationship between BNF ability and partitioning (Heilig and Kelly, 2014). US-international collaborations, led by Researchers at the University of Puerto Rico and USDA-ARS with support from FtF projects, identified a group of Andean bean lines with greater nodulation, and developed small red and black bean breeding lines for Central America with greater BNF using recurrent selection. These efforts provide the W-3150 project with access to bean breeding lines that should contribute to the genetic improvement of biological nitrogen fixation.