BeanCAP Project Reports
2010 Activities

Annual Advisory Board Meeting
January 15, 2011
San Diego, California

BeanCAP
Common Bean Coordinated Agricultural Project
Progress Report and Work Plan
1/1/2010 – 12/31/2010

North Dakota State University
Phil McClean, Juan Osorno, Julie Garden-Robinson, Christina Johnson

Progress During this Reporting Period (1/1/2010 – 12/31/2010)

A. Describe your research, education, and/or outreach activities completed in this reporting period

Research

The primary goal of this year's research activity was the development of markers that can be used in a low volume breeding and/or genetics program. We took two approaches:

1) design marker assays, using the SNP marker data to develop KASPar SNP Genotyping System (KBiosciences), that are market-class specific; and
2) test the feasibility of codominant indel markers.

KASPar primers were designed based on the SNPs discovered by the Cregan lab. The approach was to first look for SNP loci that distinguished market classes. These were chosen from the first P. vulgaris OPA set that was used by the Cregan lab screen 192 modern breeding lines. That data was used to select primers that were variable in the most number of market class. In many cases, a specific SNP was useful in at least two market classes. Currently, we have developed 101 SNP markers. Across all market classes, we have at least two markers on each chromosome. The only exception is Pv3. At this point in time, we are ahead of over deliverable schedule to develop 22 market class specific markers in the first year.

Education

Hands-on internships:

The ongoing hands-on internships occurring at four institutions (CSU, MSU, NDSU, and UNL) have proven to be the most successful way to spark interest in the students exposed. A total of 18 students (high school and undergraduates) across all institutions have work helping in the everyday activities of a breeding program with the goal of letting the students to learn by practical experiences and not just in a passive manner. Most interns have expressed that their views about agriculture and science have changed after this experiences. In addition, some interns have said that they are more aware of the impact that plant breeders can make into the food chain and also have a better understanding of all the work involved in a breeding/genetics effort. This is of critical importance since the BeanCAP early recruitment program is conscious

that not all interns will become plant breeders; however, we are creating more awareness in the young students and hopefully, they will make more educated decisions in the future.

High school visits:

The high school visits have been successful in some areas while it has been more difficult in others. For example Nebraska and Colorado have been quite active in this area and had received a lot of interest and attention from the schools. Several schools have been visited by the plant breeders and also interns that have participated in the program had the opportunity to share their experiences with their fellow students. Reverse visits have been very successful in Nebraska, where groups of high school students have visited the breeding program at Scottsbluff and had the opportunity to see all the components and infrastructure needed. In general, these activities have been highly depending of finding motivated teachers and administrators at high schools whom have a serious interest in exposing their students to different areas and career options. In order to overcome the lack of interest in urban schools in North Dakota, efforts have been focused on rural schools as well as the already established 4-H programs which currently work on exposing young students into agricultural aspects. They have expressed a lot of interest to work with the BeanCAP and some activities are currently being planned for 2011.

Facebook group website:

A Facebook website named "Plant Breeding Fan Group" has been created and all the students that have been part of the internship program were invited to join the group. This Facebook group will be a mirror for much of the material already available in the education section of the BeanCAP website, so the material can be easily found by Facebook users as most young students already spend a significant amount of time in this social network application. Another advantage of having a Facebook group is the possibility of having blogs, conversations, and discussions about topics related to plant breeding and agriculture in general. Unfortunately, very few students have joined and more promotion may be needed.

Recruitment Documentary

A 4:30 minute documentary was developed that features the reason that a person would want to be a plant breeder. The focus is on the role plant breeding plays in alleviating world hunger. This is documentary is targeted to a general audience. The development plan of this documentary and others to follow will follow a consistent theme. As these development, it may be possible to create a single ~30 minute documentary about the breeding that includes very general discussions about how a plant breeding improves plants.

Outreach

Promotion of Beans as a Healthy Food

The Extension portion of the BeanCAP project seeks to educate consumers of all ages about the nutritional benefits of beans and to encourage the public to incorporate more beans in their diet based on current recommendations. This past year, the Extension Service has been

actively promoting beans in communities across North Dakota through web-based resources and face-to-face educational efforts. The BeanCAP Extension web page has resources for educators and consumers. The links include USDA resources, elementary-school level lesson plans, and resources for health professionals about the role of beans in a healthful diet.

The "Now Serving: Beans!" teaching kit for teens and adults provides nutritional facts about beans and ideas for adding them to menus. The teaching kit includes an interactive Powerpoint, handout summarizing the lesson, recipe ideas, a bingo game and evaluation tools. This teaching kit, along with training, has been provided to more than 150 Family and Consumer Science teachers and Extension agents across North Dakota. The program presently is being evaluated and will be marketed as a free web-based resource for nutrition educators nationwide.

NDSU Extension interns have helped develop and test bean recipes and have analyzed their nutritional content using a computer program. We recently filmed a recipe demonstration featuring beans, which is available on the website as well as YouTube. Additional consumer videos focusing on bean-based recipe preparation are forthcoming. We currently are developing a bean lesson for preschool age children and their parents with help from the Center for Child Development at NDSU. In addition, a survey to determine awareness of the nutritional content of beans and to determine awareness of plant breeding as a career is in development.

Animations

Animations were also developed this year. In particular we focused on 1) the plant molecular biology systems that interacts with the soil chemistry reactions to make minerals available; and 2) the biochemical and molecular biology steps that are involved in the movement of minerals into the plant and eventually into the leaf and seed. These will be distributed via the project WWW site, the VCell Animation WWW site, and YouTube.

Agricultural Documentary Footage

In the course of our documentary development, we have filmed over four hours of footage of agricultural settings, agricultural activities, food settings, and human/food interactions. These are being collated in packages of "good footage". These will be offered to public organizations free-of-charge.

Plans for Upcoming Reporting Period (1/1/2011 – 6/30/2011)

A. Describe your research, education, and/or outreach activities for the upcoming reporting period

Research

Marker development will continue. This year we will continue to convert SNP markers to KASPr markers. As the SNP set is created and mapped, we will in tandem also develop the medium through-put KASPr markersIn addition, as the sequence data for each of the market

classes becomes available, it will be mined for within-market class indels. Preference will first be given to those indels that map near the SNPs that will make up our Illumina OPA sets.

Education

The traineeship programs will continue. Students this year will be asked to document their activities on Facebook to provide others with a glimpse of what a career in plant breeding and genetics consists of. Recruitment will focus on groups such as 4H that have constituents from an agricultural background. Visits to high schools will also continue.

Volume 2 of the plant breeding documentary series will be completed. This volume will focus on activities that lead to the development of semi-dwarf wheat and rice. It will use Norman Borlaug as a focal point

Outreach

The project will continue to promote beans as a healthy food using several media formats. Those are currently being conceived. The animation project will focus on humans and the flow of minerals through the body.

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USDA-ARS- Prosser, WA
Phillip Miklas

Progress During this Reporting Period (1/1/2010 – 12/31/2010)

A. Describe your research, education, and/or outreach activities completed in this reporting period

Research:
We (Miklas, Kelly and others) solicited 403 dry bean lines from public and private breeders for preliminary inclusion in the AM population. Each breeder was asked to submit 100 seeds of each line/cultivar to Juan Osorno. Juan subsequently sent a 25-seed sample to Ken Kmiecik for greenhouse increase of all 403 BeanCAP lines. Here at Prosser we obtained enough remnant seed from the greenhouse increase (Ken Kmiecik) to grow out 300 of the BeanCAP lines. Adequate seed was increased from 247 of the lines. Participated with Jim Kelly in establishing a drought nursery which included 100 BeanCAP lines. We sent 200 seeds for 85 of 100 BeanCAP lines in the drought nursery to Tim Porch. We sent 150 grams of 247 BeanCAP lines to Mike Grusak, and 100 seeds of the same 247 lines to Karen Cichy for nutritional analysis. I made a trip to Filer, ID, to inspect the field increase (Ken Kmiecik) of the 403 BeanCAP lines.

Education: A graduate student assisted with the increase and shipment of the BeanCAP lines, and by doing so became more familiar with dry bean diversity, market types, and agronomic traits.

B. List the deliverables and outcomes achieved during this reporting period
We increased seed for 300 BeanCAP lines.
We shipped seed of the BeanCAP lines increased in WA to others for nutritional analysis.
A graduate student received training in seed increase and trait measurement.

Plans for Upcoming Reporting Period (1/1/2011 – 6/30/2011)

A. Describe your research, education, and/or outreach activities for the upcoming reporting period

Research:
We will plant the drought nursery (100 BeanCAP lines) across two replications and treatments in the field in 2011 in Othello, WA. We will collect yield, seed weight, maturity, and other traits. This work will be conducted by a graduate student.

We will plant another increase of the BeanCAP lines for maintaining seed for use by others.

B. List the deliverables and outcomes that will be achieved during this reporting period. This will be the benchmarks for progress during this upcoming period.

Response to drought will be measured for 100 BeanCAP lines for inclusion in AM analysis.
BeanCAP dry bean lines will again be increased in the field.
Graduate student training.

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Colorado State University
Mark A. Brick and Henry Thompson

Progress During this Reporting Period (1/1/2010 – 12/31/2010)

A. Describe your research, education, and/or outreach activities completed in this reporting period

Research: We adapted the 2010 Association of Analytical Chemists (AOAC) approved method to evaluate dietary fiber (DF) for dry edible bean. This method evaluates soluble dietary fiber (SDF), insoluble dietary fiber (IDF), total dietary fiber (TDF), as well as oligiosaccharide content. The AOAC method to measure fiber was updated in 2010 to ensure that dietary fiber and other carbohydrates are accurately measured and allows for the measurement of nondigestible oligiosacharides. The key steps in the method are: a) incubation of sample with pancreatic α-amylase and amyloglucosidase to effect hydrolysis of non-RS; b) the denaturation of protein by incubation of a sample at 100°C; c) the adjustment of pH to ~4.3 and precipitation of high molecular weight soluble dietary fiber; d) the recovery of high molecular weight soluble dietary fiber (SDF) and insoluble dietary fiber (IDF); and d) the analysis of low molecular weight soluble dietary fiber using HPLC. We first evaluated uncooked dry bean seed, however uncooked beans produced highly gelatinous material that did not filter properly. After we cooked the bean samples for the analysis, the procedure worked very well. We first tested pinto, black, and great northern beans grown in Colorado during the 2008 and 2009 crop year. IDF, SDF and TF differed among cultivars and mean IDF and SDF differed for crop year. We then evaluated 34 of the dry bean lines in the Bean CAP from greenhouse produced seed in Michigan and Idaho. The lines were chosen to represent 5 races of common bean produced in two environments. IDF among the lines varied from 10.4 to 16.4 % ($P< 0.001$), SDF varied from 3 to 10 % ($P<0.001$) and TDF varied from 16 to 23% ($P<0.001$). The assay for oligiosacharides utilizes HPLC and has not been perfected to date. We hope to have it perfected in 2011 to evaluate a subset of the 300 dry bean lines.

We also started to develop the assay for total polyphenolic (TP) content on dry bean. The assay is based on the color reaction of phenolics with Folin-Ciocalteu Phenol reagent that absorbs at 765nm. Total phenolics, in general, is a measure of reducing capacity through electron transfer reactions, expressed as gallic acid equivalents. The assay should be ready to evaluate the 300 dry bean and ~150 green bean entries in 2011.

Education: We hired three high school interns and two undergraduate Colorado State University interns in 2010 to enhance the number of people exposed to plant breeding as a career. The interns were exposed to all phases of the dry bean breeding project (field, greenhouse, and

laboratory), in addition to full day experiences with sugar beet, wheat and corn breeding projects in the area. Undergraduate intern Hannah Walters conducted research on heirloom bean varieties in 2010 and presented her results in the student division at the American Society of Agronomy meeting in Long Beach, CA, Nov 1-4, 2010, citing the Bean CAP as supporting her internship. We interviewed four undergraduate interns for 2011 and hired two for 2011. We also developed relationships with two high schools to recruit high school interns for 2011.

Outreach:We presented a lecture on plant breeding as a career to Ridgeview Classical School in Fort Collins (approximately 85 students in attendance). We also hosted a campus visit for 20 high school students in the spring 2010 to expose them to molecular, greenhouse and field breeding activities on the Dry Bean Breeding Project. These activities were published on the College of Agriculture web site: http://www.agsci.colostate.edu/news/e-connection_summer10/DryBeans.html published in the SOGES newsletter distributed to faculty and sponsors from CSU. Two of the high school interns will present their experiences to the biology students at Poudre High School in spring 2011.

B. List the deliverables and outcomes achieved during this reporting period.

Established the protocol for the analysis of dietary fiber in the Thompson lab. Evaluated 34 dry bean entries for soluble, insoluble and total fiber. Genetic variation was much higher than expected (~50%) for all three variables. There was a significant mean difference between the two environments that were used to grow the seed. Initial evaluations for Polyphenolilc content was initiated .

Conducted training programs for two high school and two undergraduate interns. One undergraduate intern is planning to attend graduate school in plant breeding, the remaining students are undecided.

Plans for Upcoming Reporting Period (1/1/2011 – 6/30/2011)

A. Describe your research, education, and/or outreach activities for the upcoming reporting period

During the first half of 2011, we will continue to develop the assay for low molecular weight soluble oligiosaccharides and sucrose. This will require setting up the HPLC protocol and develop standard curves for quantification.

We will plant a replicated regional trial with 300 dry bean genotypes in a field nursery at Fort Collins to collect agronomic and phenotypic data for association mapping studies, and generate seed for nutritional analysis. The field trial will be planted in late May using project resources.

Hire two high school interns for training. Employ two undergraduate students as plant breeding interns. We will make at least two high school site visits to discuss plant breeding as a career.

Plans for Upcoming Reporting Period (6/30/2011-12/31/2011)

Research:
Continue to collect agronomic and nutritional performance data for ~300 dry bean lines grown under field conditions. The data will be used for association mapping.
Conduct fiber analysis on a subset of the 300 dry bean lines. Conduct polyphenolic analysis on 300 dry bean and 150 snap bean lines.

Teaching: Two undergradutate interns and two high school interns will continue to work on the dry bean breeding project. The high school interns will return in the fall to their respective high schools to present their experiences to their fellow students.

We plan to publish one manuscript on fiber in dry bean by the end of this period.

Outreach: Develop a web site at Colorado State University to present the student activities. Publish activities on Bean CAP and local websites.

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USDA, ARS, Beltsville, MD
Perry Cregan and David Hyten

Progress During this Reporting Period (1/1/2010 – 12/31/2010)

A. Describe the research, education, and/or outreach activities completed in this reporting period

Research

Analysis of Molecular Diversity Among Common Bean Genotypes: A set of 1536 single nucleotide polymorphisms (SNPs) had previously been developed using SNPs that were discovered via sequence analysis of polymerase chain reaction (PCR) products amplified from a set of common bean lines and from the analysis of DNA sequence obtained via the use of "next generation" (Roche 454 and Illumina Genome Analyzer) DNA sequencing technology. The set of SNPs was used to provide a molecular genetic analysis of a diverse set of 192 common bean genotypes using the Illumina GoldenGate assay system. The genotypes were selected by the various BeanCAP collaborators and consisted mainly of cultivars developed and released from breeding programs in private industry, State Universities and the USDA, Agricultural Research Service. The numbers of genotypes from the Mesoamerican market classes were as follows: 21 Black, 28 Navy, 29 Great Northern, 60 Pinto, and 2 Small White. The numbers from the Andean market classes included 19 Light Red Kidney, 9 Dark Red Kidney and 4 White Kidney. Twenty Snap Bean genotypes were also included in the analysis. Of the 1536 SNPs included in the Illumina GoldenGate assay, 1159 yielded high quality allele calls. Using these molecular marker data a neighbor joining tree was constructed to define the genetic relationships among the genotypes. The tree is available on the BeanCAP website and shows the separation of the genotypes into two major groups one consisting of the genotypes in the Mesoamerican market classes and the other the genotypes in the Andean market classes. In contrast, the Snap Bean genotypes were present in both of the major groups.

The frequency of the least frequent of the two SNP alleles at a locus is referred to as the "minor allele frequency" and is a measure of the usefulness of a SNP in a given set of germplasm. Generally, a minor allele frequency of greater than 0.10 is desirable. A further analysis of the SNP allele frequency data from the 1159 SNP loci indicated that in the case of both the Mesoamerican and Andean market classes over two-thirds of the SNPs had minor allele frequencies less than 0.10 suggesting that they would have little use in these market classes (Table 1). In contrast, only 25% of the 1159 SNPs had minor allele frequencies of 0.10 or less in the Snap Beans. These data were quite useful in terms of defining how best to proceed with subsequent SNP discovery in *Phaseolus vulgaris*. It was apparent that it would be important to specifically target market class specific SNPs in the Mesoamerican and Andean market classes in subsequent SNP discovery and selection. Likewise, it was apparent that most SNPs, including those that were informative in the Mesoamerican and/or Andean market classes would be useful in the Snap Bean market class. Thus, there is no need for SNP discovery that is specifically targeted to the Snap Bean market class.

Table 1. Numbers of SNPs in various minor allele frequency classes among the 192 genotypes from Mesoamerican, Andean, and Snap Bean market classes analyzed with the Illumina GoldenGate assay.

Minor Allele Frequency	Meso american genotypes	Andean genotypes	Snap Bean genotypes	192_Genotypes
0-0.1	782	868	292	61
0.1-0.2	89	99	271	220
0.2-0.3	97	106	243	599
0.3-0.4	105	34	133	141
0.4-0.5	84	52	220	138
TOTAL	1157	1159	1159	1159

Genetic Mapping in the Stampede x Red Hawk population: The same set of 1536 SNPs used in the Illumina GoldenGate analysis of the 192 diverse genotypes was used to genotype DNA of 288 F2 lines of the Stampede x Red Hawk genetic mapping population. A total of 651 SNPs were polymorphic in the population and provided high quality allele call data. The 651 SNPs were mapped along with 25 markers that had previously been mapped in the Stampede x Red Hawk population at NDSU. These 25 markers were specifically chosen because they mapped to the 11 *Phaseolus vulgaris* linkage groups and thereby allowed the identification of the 11 *Phaseolus vulgaris* linkage groups. The resulting genetic map with the anticipated 11 linkage groups was 924 centiMorgans in length. One interesting feature of the map was the distribution of the SNP markers across the 11 linkage groups. In a number of linkage groups there were sets of 15 to more than 30 SNPs mapped in clusters that were only 1-3 centiMorgans in length. The assumption is that these are SNPs in heterochromatic regions where recombination is extremely low. Thus, there is little recombination distance between such SNPs. Because no whole genome sequence of *Phaseolus vulgaris* was available when the 1536 SNPs were selected it was not possible to select SNPs based upon their physical position along the 11 chromosomes. Thus, it was not possible to distinguish between SNPs derived from euchromatic or heterochromatic regions.

DNA Sequence Analysis Using the Illumina Genome Analyzer: Genomic DNA was isolated from leaf tissue of the 19 common bean genotypes (Table 2). The first 17 genotypes were selected to include at least two genotypes from the major market classes with the exception of the Snap Bean market class. In the case of the Light Red Kidney, Navy and Pinto market classes three genotypes were used. The last two genotypes, BAT93 and Jalo EEP558 were included because they are the parents of important genetic mapping populations from UC Davis and Embrapa. The genotypes within each market class were selected because they identified the greatest numbers of polymorphic SNPs as determined by the analysis of the 192 genotypes described above. The genomic DNA was fragmented using a DNA fragmentase (New England Biolabs) followed by size selection to isolate fragments in the 250 to 400 bp size range. The DNA was end repaired, an A nucleotide was added to the 3' ends of the fragments and indexed adaptors (barcodes) were ligated to the fragment ends. The indexes were used so that more than

one genotype could be sequenced in each of the seven lanes of the Illumina Genome Analyzer "flow cell". The DNA was PCR amplified to obtain sufficient DNA for analysis on the Illumina Genome Analyzer. Paired-end sequencing was used to obtain 115 bp of sequence from each end of the genomic fragments. The Illumina Off-Line Basecaller V1.8 software was used for base calling and demultiplexing. Approximately, 19.6 billion bases of DNA sequence data were obtained. The paired-end sequence reads from each genotype were aligned to the 14x *Phaseolus vulgaris* genome sequence of G19833. Following alignment, the positions, alternative alleles, the read coverage and the sequence quality at the site of potential SNPs was assessed. The preliminary analysis of theses sequence data discovered more than 1.4 million high quality SNPs.

Table 2: Common bean genotypes used for single nucleotide polymorphism (SNP) discovery via "next generation" sequence analysis on the Illumina Genome Analyzer.

Cultivar	Market Class
Redhawk	Dark Red Kidney
Fiero	Dark Red Kidney
California Early LRK	Light Red Kidney
Kardinal	Light Red Kidney
Lark	Light Red Kidney
UC_White_Kidney	White Kidney
Cornell_49_242	Black
T-39	Black
UI_906	Black
Laker	Navy
C-20	Navy
Michelite	Navy
Buckskin	Pinto
Stampede	Pinto
Sierra	Pinto
Gemini	Great Northern
Matterhorn	Great Northern
BAT93	Bayo
Jalo EEP558	Canário

Education

Mr. Tristan Werner, a senior in the Plant Science and Landscape Architecture Department at the University of Maryland, College Park began work in the laboratory in early October. He learned the procedures for the isolation of DNA required for whole genome DNA sequence analysis and for PCR amplification for the detection of genetic markers. He also learned to amplify and determine relative allele sizes of simple sequence repeat (SSR) markers via agarose gel electrophoresis. For this latter analysis he amplified and analyzed SSR loci from the genotypes listed in Table 2. Mr. Werner also learned how to cross common bean under the guidance of Dr. Talo Pastor-Corrales.

B. List the deliverables and outcomes achieved during this reporting period

- Analyzed 192 common bean genotypes from nine different market classes with 1159 SNP DNA markers using the Illumina GoldenGate assay system.
- Constructed a neighbor joining tree and a summary of allele frequency data based upon the analysis of the 192 genotypes with 1159 SNP markers. Summaries of these analyses are available on the BeanCAP website.
- Constructed a genetic map derived from the analysis of 288 F2 lines from the Stampede x Red Hawk mapping population with 651 SNP markers and unambiguously associated the resulting 11 linkage group with the 11 common bean linkage groups
- Obtained 19.6 billion bases of DNA sequence data from 19 common bean genotypes which included genotypes from the important market classes
- Conducted a preliminary analysis of the 19.6 billion bases of DNA sequence data and discovered more than 1.4 million potential SNPs

Plans for Upcoming Reporting Period (1/1/2011 – 6/30/2011)

A. Describe your research, education, and/or outreach activities for the upcoming reporting period

Design of an Illumina Infinium Genechip with 6000 SNPs: Using the 19.6 billion bases of DNA sequence data from 19 diverse common bean genotypes, plus additional Illumina Genome Analyzer sequence that will be completed before the end of 2010, we will complete the selection of 6000 SNPs to be used in the 6000 SNP Illumina Infinium Genechip. Following base calling using the Illumina Off-Line Basecaller the sequence reads of the 19 genotypes will each be individually aligned to the 14x genome sequence of G19833. The alignments will identify the position and the alleles at each potential SNP position in each comparison of a given genotype with the G19833 sequence. The read coverage and the quality score of the aligned reads at each SNP position will then be determined. The individual genotype SNP files will be merged and SNPs that are ambiguous in any one genotype (two alleles present) will be eliminated. This serves to eliminate paralogous "SNPs". Subsequently, the SNP allele at each locus in all genotypes will be determined. SNPs will be filtered to eliminate SNPs that are less than 25bp from each other and to eliminate SNPs with non-specific flanking sequences. From these, SNPs will be selected for inclusion in the 6000 SNP Illumina Infinium genechip from G19833 sequence scaffolds greater than 25kbp in length and will be evenly spaced along each scaffold. Additional and very important SNP selection criteria will include 1.) the evaluation of polymorphism within market classes and between market classes and 2.) the position of selected SNPs in euchromatic vs. heterochromatic DNA. The latter criterion is important because a higher SNP density in required in euchromatic regions where recombination is higher and gene density is greater. In order to distinguish heterochromatic and euchromatic regions, the sequence scaffolds from the G19833 genome sequence are being aligned to the whole soybean genome sequence. Based upon the assumption of synteny between the two genomes those scaffolds aligned to the euchromatic regions in soybean will be categorized as euchromatic and those scaffolds aligned to heterochromatic regions will be categorized as heterochromatic. Additional analysis of the gene and transposon content of the G19833 scaffolds will be used to further identify euchromatic vs. heterochromatic regions. In addition, the recombination rate along the

11 linkage groups was calculated in order to identify regions with extremely low or zero recombination rate. Sequences flanking the SNPs which are positioned in the regions of low recombination will be mapped to the 14x *Phaseolus vulgaris* genome sequence of G19833 and the corresponding scaffolds will be determined. The scaffolds identified in this fashion are most likely from heterochromatic regions. Following these analyses, SNPs with maximal polymorphism will be selected such that the predicted SNP density will be 5 times greater in euchromatic versus heterochromatic regions.

Genetic Analyses using the Illumina Infinium Genechip with 6000 SNPs: The 6000 SNP genechip will be used to analyze the 288 lines of the Stampede x Red Hawk population, 67 lines of the UC Davis BAT93 x Jalo EEP558 population as well as 300 lines of the Embrapa BAT93 x Jalo EEP558 population. The allele call data from these mapping populations will be combined with the previous data from the analyses with the 1536 GoldenGate SNPs for the purpose of creating a dense genetic map using JoinMap analysis. After the analysis of the mapping populations, sufficient reagents will remain to genotype 400 additional genotypes. It will be necessary for the BeanCAP collaborators to select a diverse set of genotypes that are representative of the diversity within each of the major common bean market classes. The 192 genotypes that were analyzed with the set of 1536 Illumina GoldenGate SNPs would likely be included among this set of 400 genotypes.

Selection of 768 GoldenGate SNPs for analysis of breeding populations derived from the Mesoamerican market classes: Based upon genetic map position and the minor allele frequency, 768 SNPs will be selected from the 6000 SNPs in the Illumina Infinium genechip. SNPs will be chosen based upon 1.) high minor allele frequency in each of the Mesoamerican and the Snap Bean market classes and 2.) even distribution (based upon recombination distance) across the 11 linkage groups.

Selection of 768 GoldenGate SNPs for analysis of breeding populations derived from the Andean market classes. Based upon genetic map position and the minor allele frequency, 768 SNPs will be selected from the 6000 SNPs in the Illumina Infinium genechip. SNPs will be chosen based upon 1.) high minor allele frequency in each of the Andean and the Snap Bean market classes and 2.) even distribution (based upon recombination distance) across the 11 linkage groups.

Analysis of 2000 genotypes nominated by BeanCAP breeders and geneticists: It will be important to determine as early as possible the number of genotypes from the Mesoamerican market classes and the Andean market classes so that appropriate quantities of the two sets of 768 GoldenGate SNPs are ordered to match the requirements of the BeanCAP breeders and geneticists. The 2000 nominated genotypes will then be analyzed with the appropriate 768 GoldenGate set of SNPs.

B. List the deliverables and outcomes that will be achieved during this reporting period. This will be the benchmarks for progress during this upcoming period.

- Design of a 6000 SNP Illumina Infinium genechip based upon DNA sequence analysis of 19 selected common bean genotypes

- Analysis of mapping populations and a set of 400 additional diverse genotypes with the 6000 SNP Illumina Infinium genechip
- Creation of a updated genetic map using the data from the 6000 SNP Illumina Infinium genechip along with previously obtained mapping data
- Design of a 768 SNP GoldenGate genechip specifically targeted to the Mesoamerican market classes
- Design of a 768 SNP GoldenGate genechip specifically targeted to the Andean market classes
- Analysis of 2000 genotypes nominated by the BeanCAP breeders and geneticists with one of the two 768 SNP GoldenGate genechips

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USDA/ARS Children's Nutrition Research Center, Houston, TX
Michael A. Grusak

Progress During this Reporting Period (10/1/2010 – 12/31/2010)

A. Describe your research, education, and/or outreach activities completed in this reporting period.

Research

Our major activities in this project are to analyze mineral concentrations and iron bioavailability potential in the dry bean and snap bean samples that have been (and will be) shipped to us by other cooperators. As of this reporting period, we received dried tissue samples for 142 snap bean entries (times 3 field replicates) in late September, 2010, from cooperator Jim Myers (Oregon State). These entries were ground and processed (acid digested) for the analysis of elemental composition using ICP-OES (inductively coupled plasma – optical emission spectroscopy). At present, we have analyzed about one-third of the sample digests (including at least one field replicate of 105 of the entries) and should have the remainder completed by early to mid January, 2011. Preliminary assessment of the entries analyzed thus far (105 of 142) indicates variation in both macro- and microelements, with preliminary ranges of: ~2- to 2.5-fold for Ca, K, Mg, Ni, P, S, and Zn; ~3-fold for Cu and Fe; ~5-fold for Se; and ~7-fold for Mn. These ranges may expand slightly once all the entries and field reps have been analyzed. Nonetheless, the current values show significant variation across the genotypes, which bodes well for future association mapping efforts directed towards these mineral traits.

In late November 2010, we received ~150 gram seed samples of 248 dry bean entries grown in field plots in Michigan (from cooperator Jim Kelly) and ~150 gram samples of the same 248 entries grown in field plots in Washington State (from cooperator Phil Miklas). We are currently grinding these samples in stainless steel mills. From these bulks, 100 grams of each entry (times two locations) will be sent to Mehmet Tulbek (NDSU) for protein, fiber, and oil analysis, and 15 grams of each will be sent to Karen Cichy (USDA, MSU) for phytate analysis. We have completed the grinding of about one-half of the samples from Michigan and have started to digest these for subsequent elemental analysis. We anticipate sending the 100 g and 15 g sub-samples from each location to cooperators in mid to late January 2011.

A bar code scanner, label printer, and software were purchased this past reporting year. These are now being used to label all the samples and sub-samples for our long-term storage and for better identification when samples are sent to cooperators.

Education

Nothing to report.

Outreach
I provided expertise and feedback on the content of the animated Mineral Nutrition video, which focused on plant iron deficiency and the processes involved in iron acquisition from soil to the interior of plant roots.

B. List the deliverables and outcomes achieved during this reporting period

- A bar code scanner, label maker, and software were purchased and placed into operation for sample tracking.
- Received and processed ~450 snap bean samples for elemental analysis.
- Completed the elemental analysis of ~150 snap bean samples using ICP-OES.
- Received 248 dry bean samples from each of two locations and began grinding for subsequent nutrient analyses in Houston and in cooperator's labs.
- Provided feedback during the development of the animated Mineral Nutrition video.

Plans for Upcoming Reporting Period (1/1/2011 – 6/30/2011)

A. Describe your research, education, and/or outreach activities for the upcoming reporting period.

Research
Elemental analyses will be completed on remaining snap bean samples received in 2010, which are already prepared for ICP-OES analysis. Grinding of Michigan and Washington State grown dry bean samples will be completed; sub-samples will be sent to cooperators for protein, fiber, and oil, or for phytate analyses. The same samples will be digested and analyzed for elemental composition in Houston. Caco-2 studies, to assess iron bioavailability potential, will be conducted with dry bean samples once all material has been ground.

Education
No specific plans.

Outreach
Continue to offer assistance with the Mineral Nutrition animations, especially the next video to be focused on the movement of iron from roots to various parts of the plant. My background in plant physiology, mineral nutrition, and root and seed biology should be useful in this effort.

B. List the deliverables and outcomes that will be achieved during this reporting period. This will be the benchmarks for progress during this upcoming period.

- Complete sample grinding and distribution of dry bean sub-samples to cooperators.
- Complete elemental analyses on ~450 snap bean samples and ~500 dry bean samples.
- Complete Caco-2 studies to assess iron bioavailability potential of diverse dry bean samples.
- Work with cooperators to draft manuscripts on nutrient diversity in snap bean entries and dry bean entries.
- Attend BeanCAP annual meeting at PAG XIX.
- Assist with animation package for BeanCAP web presence.

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University of Nebraska-Lincoln
Carlos A. Urrea

Progress During this Reporting Period (1/1/2010 – 12/31/2010)
A. Describe your research, education, and/or outreach activities completed in this reporting period

Twelve genotypes were provided for the BeanCAP nutritional analysis as follows: 5 great northern, 6 pintos, and 1 navy.

Hosted 115 Big Red Road Show students from Lincoln on March 19. I talked about breeding dry beans for western Nebraska. Career opportunities in Plant Breeding were emphasized.

Presentation to 60 VALTS students at the greenhouse facilities on April 28 and 29. I talked about dry bean diversity and how to create variability in beans. I also talked about career opportunities in the Agronomy and Horticulture emphasizing Plant Breeding.

Hosted 40 members of the Nebraska LEAD Tour on March 30. I talked about dry bean breeding activities in western Nebraska. BeanCAP activities were discussed.

Western Nebraska Community College located in Scottsbluff, NE, was contacted to identify potential undergraduate students to be working during the 2010 school year on bean breeding activities.

Two sets of Western Nebraska Community College undergraduate students joined the program from January-May 2010 and May-to date, respectively.

One Scottsbluff High School student joined the program from May-August, 2010.

B. List the deliverables and outcomes achieved during this reporting period

Some Scottsbluff/Gering high school students expressed their interest to work during summer 2011 for the dry bean breeding program.

The students were involved in all dry bean breeding activities including seed preparation, randomization, planting, scoring for diseases, plant selection, harvesting, cleaning seeds, entering data, and data analysis. They were also involved in greenhouse disease screening and scoring. We screened for common blight, bacterial wilt, bacterial brown spot, and bean common rust. The students were able to create variability through hybridization. Most of their hybrids were successful.

Four articles were published in local newspaper and in the Bean Bag as follows:
Hansen, Sandra. 2010. New program aimed at recruiting young scientists. StarHerald. March 13, page 1.
Hansen, Sandra. 2010. Hands-on experiences fill lives of WNCC students. StarHerald. March 13, page 2.
Hansen, Sandra. 2010. Summer is the best time to learn. StarHerald. August 22, page 2-3.
Butterfield, Sandra. 2010. BeanCAP project underway at panhandle station. The Bean Bag 28(1):15-16.

Plans for Upcoming Reporting Period (1/1/2011 – 12/30/2011)

A. Describe your research, education, and/or outreach activities for the upcoming reporting period

Two undergraduate students will be carrying out projects in the dry bean breeding program. They will be involved in bacterial wilt screening and fingerprinting bean breeding lines to molecular DNA markers. They will also learn how to create variability through hybridization.

One or two high school students will be involved in preparation of dry bean trials to be planted during summer 2011. They will help to prepare seeds, randomize experiments, layout experiments, and help plant those trials. They will also learn about plant phenology and disease ratings.

Visit Scottsbluff, Gering, and Mitchell, NE High Schools to talk about career opportunities in plant breeding.

Visit at least one High School in Wyoming to talk about career opportunities in plant breeding activities.

B. List the deliverables and outcomes that will be achieved during this reporting period. This will be the benchmarks for progress during this upcoming period.

Plant breeding presentations and materials will be prepared for the high school visits.

Students will be asked to complete their assignments. A written report will be expected by each student involve in the project.

High school students will share their experiences on working in dry bean breeding with their classmates.

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Oregon State University
James R. Myers

Progress During this Reporting Period (1/1/2010 – 12/31/2010)

B. Describe your research, education, and/or outreach activities completed in this reporting period

Introduction

Under the Bean CAP, snap beans have a similar set of objectives to dry beans, but are evaluated for a different suite of traits. Because the immature pod is the portion of the plant of greatest interest, the phenotypic data collection is focused on this organ. Traits of interest include morphology (length, thickness, cross-sectional shape, height, width, fiber and suture strings), and nutritional qualities (flavonoids and phenolics, minerals, vitamin C and carotenoids).

At the initiation of the project, public and private breeders provided input to identify 150 snap bean cultivars. These included 11 pole (vining) as well as 139 bush types representing all different classes of snap beans and covering a range from heirlooms to contemporary commercial types. These classes come from both Andean and Mesoamerican centers of domestication and include the flat podded romanos, and round podded fresh market and processing, wax, bush blue lake and extra fine European types. Seed was provided by the OSU snap bean breeding program, various private seed companies, and the USDA-NPGS plant introduction collection. Seed was increased in the greenhouse in Oregon and by Seminis Vegetable Company in Idaho during the winter of 2010. Further increase was made by Semins in the field at Filer, Idaho.

Field trial

The OSU program planted the 150 snap bean cultivars in three reps in a field at the Vegetable Research Farm near Corvallis, OR. Pole beans were grown in 5 meter plots on trellises spaced two meters between rows while bush beans were planted in 5 meter plots with 0.75 meters between rows. Plants were grown under drip irrigation to minimize mineral contamination of pods. Data were taken on plant height and maturity in the field. Pods were hand-picked at harvest maturity (determined by when a cultivar had pods within certain sieve sizes, each target sieve distribution being specific to that variety). Pods were divided among six subsamples: Samples of approximately five grams were frozen (-20C) and sent to Dr. Mark Brick at Colorado State University for determination of total phenolics. Fifty gram samples were dried at 30C and these were mailed to Dr. Mike Grusek for mineral analysis. Another set (~50 g samples) were blanched and frozen (-30C) for fiber analysis by Brian Yorgey at OSU. A fourth set of samples of about 10 g each were frozen (-80C) for carotenoid analysis and a fifth set of 10 gram samples were processed fresh for vitamin C analysis. The sixth set of samples consisting

of 10 pods was used to acquire pod measurements. These included presence of suture strings, pod and beak length, pod height and width, cavity length and width, and a visual estimate of fiber. Data are being summarized in preparation for association mapping. Progress with the nutritional analyses are reported below.

Phenolics and Minerals

Dr. Brick will report on progress in preparing and analyzing these samples for phenolics. Dr. Grusak reports that all snap bean samples have been ground and digested and that ICP analysis has been run on about 150 of the 450 samples. The data reveals about a 2 to 2.5-fold-range in Ca, K, Mg, Ni, P, S, Zn, approximately 3-fold range for Cu, Fe, about 5-fold range for Se, and a 7-fold-range for Mn. The ranges appear appropriate based on previously published work.

Vitamin C

Five grams of each sample were homogenized in potassium phosphate buffer (0.5%, pH2.5, plus 0.5g/L dithiothreitol) using a Tekmar homogenizer. After centrifugation 2 ml aliquots were stored at -80ºC until HPLC analysis. A total of 450 samples (150 lines, 3 reps each) were extracted, and an additional 18 duplicate samples (3 lines, 3 reps each) were harvested and processed immediately to compare fresh verses cold storage vitamin C levels. Two of the lines were evaluated by HPLC analysis to check vitamin C content. The concentration of vitamin C in the cold storage samples were found to range from 1.34 to 3.78 mg/100g tissue (whole pod with seeds). This is about 10 fold lower than reported in the USDA Nutrition Database for uncooked green beans, but may reflect the inclusion of seeds with pods.

Carotenoids

Forty-five of the samples stored at -80C have been further processed by freeze drying for subsequent carotenoid extractions, with the remainder pending freeze drying as facilities become available. Solvent-based carotenoid extraction protocols will be evaluated for determining total carotenoid content.

Pod fiber analysis

We have analyzed 85 out of the 150 beans lines (in duplicate). We encountered problems in repeatability in early samples, but these have been ironed out. Apparently, the method of thawing of the frozen samples has an effect on reproducibility. We are processing about 8 lines a week with part time student help.

In summary, the work is proceeding as expected with nutritional data analysis expected to be completed by the end of the fiscal year.

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Michigan State University
James D. Kelly

Progress During this Reporting Period (1/1/2010 – 12/31/2010)

A. Describe your research, education, and/or outreach activities completed in this reporting period

Research activities involved the assembly and seed multiplication of 400 dry bean genotypes chosen for study as part of the beancap genomic mapping project.

Education activities involved training of both undergraduate and high school students in lab, greenhouse and field research activities related to bean breeding as part of the beancap project.

Extension activities involved the development of the beancap portal to share information being generated by beancap members with the general public interested in bean research, nutrition, and plant breeding training.

B. Deliverables: Two undergraduate students received training on aspects of lab and greenhouse activities of bean breeding during the spring 2010 semester. Two high school students spent 8-weeks assisting a graduate student conduct field trials and learning the basis of field research on campus during the summer semester. In the fall one new undergraduate student joined our group and the other returning student continue to work 10 hours/week during the semester and were involved in aspects of DNA extraction, running SCAR markers, greenhouse crossing and disease inoculation under guidance of technical staff in the bean breeding program.

Developed an operational BeanCAP portal www.beancap.org where the public and beancap members can access information related to the project. Members provide information on research, training and extension activities which is posted and accessible through this site.

In coordination with Dr. Miklas over 400 dry bean genotypes from public and private sector breeding programs in North America were assembled for inclusion in the beancap genomics mapping project. Colleagues in the private sector agreed to the terms that research information generated on their material would be publically available. Continue to coordinate with other beancap members on the choice of genotypes going into the different phenotyping field testing and nutritional analyses and genotyping experiments.

At East Lansing a sample of the 400 genotypes was multiplied in the greenhouse during spring semester. The same group (with exception of those lost to poor germination, or photoperiod sensitivity) was grown in the field in 2010 and 375 lines were harvested in the fall. Seed quantities ranged from minimum amounts due to lack of adaption of some tropical entries

(killing frost on Oct 5) to over 1-pound samples from most of entries grown in single row plots. No major disease problems were encountered as the seed was greenhouse produced. A subsample of entries from the greenhouse was sent to Colorado State for fiber analysis; a second larger sample – 250 genotypes was sent to Houston to initiate the nutritional analysis of the seed.

Plans for Upcoming Reporting Period (1/1/2011 – 6/30/2011)

A. Describe your research, education, and/or outreach activities for the upcoming reporting period

Plan to continue the training of undergraduate students in lab, greenhouse research activities related to bean breeding. We also plan to recruit undergraduate students for field activities during the summer.

Preparation and planting of two major field studies this coming summer in Michigan. The Association Mapping - AM Population will include 300 genotypes for phenotyping under local conditions. An additional study with 100 genotypes will evaluate aspects of drought on different characteristics.

Continue to expand the utility of the beancap portal with input from beancap members as more information and data become available.

B. Deliverables. Continue training internship of undergraduate students in lab and greenhouse activities related to bean breeding. Data collected from field experiments on 300 bean genotypes that will be used in AM studies.

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University of California, Davis
Paul Gepts and Steve Temple

Progress During this Reporting Period (1/1/2010 – 12/31/2010)

C. Describe your research, education, and/or outreach activities completed in this reporting period

Research

Objective 1: We extracted DNA from 180 dry and 20 green bean lines (cultivars or advanced breeding lines), representing U.S. bean germplasm and chosen by the BeanCAP project. Seeds were sent to us by Juan Orozco (NDSU) and were planted in the greenhouse. Following lyophilization of young leaf samples, DNA was extracted using Qiagen columns. The concentration of DNA was checked and the quality of the extracted DNA was verified by electrophoresis. DNA samples were then sent to P. Cregan and D. Hyten (USDA-ARS, Beltsville) for SNP analysis.

Objective 3:

1) We have continued collating marker information, principally microsatellite marker information. This has required downloading information from GenBank, but also contacting individual authors to obtain complementary information about the markers they developed. The marker database now includes ~1540 markers. These consist of 795 SSR markers, 633 STS markers 76 SCAR markers and 38 gene-based markers. About half of these markers have been mapped genetically; the other half, mostly microsatellite markers, remains to be mapped.
2) The 1x methyl-filtrated sequence of the BAT93 line (obtained through funding from the Kirkhouse Trust) was analyzed for the presence of microsatellite motifs with assistance from the Bioinformatics Core Facility of the UC Davis Genome Center (Dr. Dawei Lin). Three algorithms to discover such motifs –SSRIT, QDD, and SSR Finder – were applied. They differed in both the number and actual microsatellite identified, with the greatest similarity observed between SSRIT (164,391) and QDD (118,610) compared to SSRFinder (47,317). Microsatellites identified by all three algorithms numbered 21,825. Regardless of the algorithm, about 70% of the SSR-containing sequences had at least one BLAST hit in the soybean whole-genome sequence. Three new tracks – one per algorithm – have been added to the GBrowse version of the PhaseolusGenes database.
3) A survey of the QTL literature of common bean was conducted to collate information to included into the database. Overall, 888 QTLs (trait x location x year) have been identified. Traits include disease resistances (e.g., common bacterial blight, anthracnose), abiotic stress (low P, high Al), mineral content and uptake, green pod quality, and plant

development. The information resulting from this survey is now up to date and has been provided to the bioinformatics group. Discussions are ongoing as to the best format to display this information.

4) Comparison of major gene and QTL traits with those for soybean and grasses (Gramene) shows that there is little overlap in terms of ontology. Thus, Phaseolus will have to develop its own ontology.

D. List the deliverables and outcomes achieved during this reporting period

Deliverables:

a) DNA samples of 200 bean varieties representing a broad cross-section of common bean germplasm in the U.S.
b) Updating of searchable marker table and GBrowse implementations in the PhaseolusGenes database (http://phaseolusgenes.bioinformatics.ucdavis.edu/search/)
c) Collation of QTL information

Outcome:

a) Large number of potential SSR sequences now available for mapping

Outreach

a) Presentation to the UC Grain Legume Workgroup, February 2010

Plans for Upcoming Reporting Period (1/1/2011 – 6/30/2011)

B. Describe your research, education, and/or outreach activities for the upcoming reporting period

Research

1) Integrate BAT93xJalo EEP558 (BJ) segregation data from different sources and build a new, denser molecular linkage map based on the current framework of "g" markers.
2) Fully integrate the SSR information into the PhaseolusGenes database: collapse the current three tracks into one, link to information such as repeat type and length, primers and Tm, and synteny with soybean
3) Develop a system for QTL display [CMap based on integration in 1)], linked to QTL specific data (markers, R^2)
4) Integration of phenotypic and genotypic data into PhaseolusGenes database
5) Continuation of DNA extraction for BeanCAP project.

Outreach

1) Presentations to bean groups: UC Workgroup, W2150, BIC
2) Workshop to illustrate the function of PhaseolusGenes

C. List the deliverables and outcomes that will be achieved during this reporting period. This will be the benchmarks for progress during this upcoming period.

1) Improved PhaseolusGenes database: broader coverage of data,user-friendliness
2) DNA samples for BeanCAP project