



A USDA/NIFA Supported Project

**Common Bean Coordinated Agricultural Project
2012 Advisory Board Meeting
Thursday, January 12, 2012**

Crown Plaza
2270 Hotel Circle North, San Diego, CA
San Diego, California 92108 USA

Time	Topic
10:30 - 10:50 am	Introduction and General Update: Phil McClean
10:50 - 11:10 am	Multimedia Development: Phil McClean/Christina Johnson
11:10 - 11:40 pm	Breeding Education: Juan Osorno/Bradley Bisek /Carlos Urrea
11:40 - 12:00 pm	Outreach: Julie Garden-Robinson/Stacy Halvorson
12:00 - 12:20 pm	Database Applications: Paul Gepts/Dawei Lin
12:20 - 1:30 pm	Lunch
1:30 - 1:50 pm	2011-2012 Field Experiment: Jim Kelly/Juan Osorno/Phil Miklas
1:50 - 2:10 pm	Common Bean Sequencing Project: Phil McClean
2:10 - 2:30 pm	Marker Development/Genotyping: Perry Cregan/Qijian Song
2:30 - 2:50 pm	Marker Application: Phil McClean/Samira Moghaddam
2:50 - 3:20 pm	Nutrition Analysis: Mike Grusak/Jim Myers/Karen Cichy
3:20 - 3:35 pm	Break
3:35 - 4:00 pm	International Collaborations
4:00 - 4:30 pm	Committee Consultation
4:30 - 5:00 pm	Committee Oral Report



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BeanCAP Project Reports 2011 Activities

Annual Advisory Board Meeting
January 12, 2012
San Diego, California

BeanCAP
Common Bean Coordinated Agricultural Project
Progress Report and Work Plan
1/1/2011 – 12/31/2011

North Dakota State University
Julie Garden-Robinson, Phillip McClean, Juan Osorno

Progress During this Reporting Period (1/1/2011 – 12/31/2011)

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

Research

Within and between market class sequenced data was collected using Illumina GAI technology generated for eighteen genotypes representing the pinto, navy, black, Great Northern, and kidney market classes. The genotype sequence data was mined for indels >7nt, and the information was used to develop ~2,700 indel markers for both within and between market class genotyping. These markers were evaluated for within market class variation. We discovered the following number of market class specific indel markers: pinto, ~1000; light red kidney, ~550; black, ~200; ~navy, 150; and dark red kidney, ~140. All of the markers use the same amplification conditions. Combinations of markers, based on amplification product size, could be multiplexed.

Association mapping experiments were performed using the first set of SNP data on the core 196 genotypes. The phenotypes that were evaluated were two classic qualitative traits, colored vs. white seed, and stringless, and one quantitative trait, seed iron content. The color trait was only evaluated using genotypes from the Mesoamerican gene pool. The mixed linear model considered population structure (PCA, Structure) and relatedness as covariates. We were able to discover five linked SNP ($R^2=20-25\%$) associated with seed color on chromosome Pv07 near the *P* locus. We mapped eight significant loci linked to the genetic position of *St* on chromosome Pv02. We also discovered an SNP peak, yet to be mapped genetically, with a major effect on the stringless trait ($R^2=62\%$). In addition, two minor ($R^2=1.2\%$ each) QTL were discovered on Pv08. Finally, SNPs associated with seed Fe content were discovered on chromosomes Pv01 and Pv05. The locus on Pv01 maps near a gene for nicotianime synthase, a gene shown to be associated with iron metabolism in dicot plants.

Education

Hands-on Internships: This continues to be the most successful activity of the education program of the BeanCAP. During 2011, there were 19 students directly involved in the internships across the four institutions. Up today, a total a 40 students have been part of the internship program. Two of them are current graduate students in plant breeding and they both said that the internships were of key importance for their decision about getting into graduate school. At least one high school student started a B.S. in science (Biology) and she said that the

internship helped her to get more interest in nature and science. The program will continue in 2012 as planned.

High School Visits: An estimated of 170 high school students have been taught about plant breeding through different activities such as visits to high schools, reverse visits to breeding programs and laboratories, and internships. This is one of the most challenging activities and success it has been variable by institution, with Nebraska and Colorado being quite positive while activities have had limited success in Michigan and North Dakota. All institutions mentioned that making contacts with local schools and sparking interest in them is the most challenging part. Successful activities are mainly dictated by good one-to-one contacts with high school faculty. The addition of an education specialist into the project may be beneficial for these activities.

Facebook Group: The website continues to grow and generate its own feeding activity. We continuously add links and news related to plant breeding with the goal of generating discussion among the group members. We are finding ways to promote the group and get as many members as possible. The group is one to the public and can be found at: <http://www.facebook.com/groups/Plantbreeding?ap=1>.

Recruitment Documentary. An ~6:00 minute documentary was developed that features Norman Borlaug. The purpose of the documentary is to highlight how an individual plant breeder working in conjunction with others can have a significant impact on the economic well-being of a country. The documentary will be released onto the BeanCAP YouTube channel. The goal will be eventually use this documentary and package it with others that have been and will be to create a single ~30 minute documentary about the breeding that includes very general topics about how a plant breeding improves plants.

Extension

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, to encourage the public to incorporate more beans in their diet based on current recommendations, and to showcase the integrated efforts of the BeanCAP project. In 2011, Extension Service employees and 20 interns and student volunteers have promoted beans through face-to-face educational efforts, through media releases and through sharing web-based resources. In addition, we have made efforts to reach out to Extension and public health colleagues throughout the U.S. by participating in conference presentations and through an online survey with linked web-based materials.

The "Now Serving: Beans!" teaching kit for teens and adults developed in 2010-11 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. In 2011, the teaching kit was used by Family and Consumer Sciences teachers and Extension agents across North Dakota, and it was introduced to Extension agents

and specialists, dietitians and public health partners throughout the U.S. as a result of participation in conferences and through the survey described as follows.

A nationwide survey of Extension and public health educators was undertaken to determine knowledge and behavior of educators as well as perceptions of their clients' knowledge and intake of various types of beans. We also aimed to learn their preferred methods of acquiring new information about educational tools and the results of bean research. When the people responded to the survey, they were taken to the BeanCAP Extension website, where the "Now Serving: Beans!" teaching kit and other materials are linked.

As of December 2011, 732 Extension educators and specialists, dietitians and teachers from 43 states had participated in our online survey. About 99% reported serving beans in their homes, with 47% reporting serving beans at least one time per week. String beans (93%), kidney beans (81%), black beans (78%), and pinto beans (73%) were the most commonly served. The perceived usage among their clients was significantly lower. The professionals were aware of these health benefits associated with beans: heart health (92%), weight management (87%) and blood sugar management (69%). From a list of choices, the survey participants were asked to choose the top 3 preferred ways to learn about nutritionally- or flavor-enhanced bean varieties. Product samples (76%), email (64%), labels with approved health claims (43%), website (43%), and labels with an endorsement by a health organization (26%) ranked the highest. They indicated they were interested in a variety of materials to use in the promotion of the health benefits of beans. Brochures with recipes (76%), step-by-step recipes with photos (68%), lesson plans for adults (67%), tips to reduce intestinal comfort after eating beans (63%), lesson plans for children (54%), brochures detailing health benefits (50%) and online videos (43%) were the top-ranking choices.

NDSU Extension interns have helped develop and test bean recipes and have analyzed their nutritional content using a computer program. With assistance from the BeanCAP's video intern, we have filmed six recipe demonstrations featuring beans, which are available on the BeanCAP website as well as YouTube. Bean education was delivered to 250 people at a wellness fair, 200 people at the National Extension Association of Family and Consumer Sciences Annual Conference in New Mexico, 50 people at college research showcase, 90 people at the North Dakota Dietetic Association conference and 80 people at the North Dakota Nutrition Council. We collaborated with a colleague in Nebraska to produce a "Mixes in a Jar" holiday presentation, which includes a bean-based chili mix. At least 5,000 people have accessed the materials.

In the summer of 2011, "Spillin' the Beans", a curriculum highlighting the benefits of dry bean and snap bean consumption, gardening and nutrition was developed and piloted with 47 families, in collaboration with the USDA's Human Nutrition Research laboratory in Grand Forks. Targeting young children and their parents/caregivers, the curriculum was reviewed by early childhood educators, horticulture experts and nutrition specialists. The curriculum features hands-on activities, including bean sprouting, taste testing, art activities and stories about beans. According pre/post surveys, parents/caregivers increased their knowledge of beans as a source of natural antioxidants, fiber and folate. The use of canned beans significantly increased among families, specifically the use of black beans and Great Northern Beans. About 88% of parents reported reading the parent newsletter, 69% reported that their child talked about beans, 83% reported that their child talked about gardening and art activities, and 85% reported that their child talked about taste testing bean recipes. In 2012, we will package the materials, create a

“kid-approved” recipe handout and make the materials available for broad use in the state and across the United States

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

2700 indel markers developed for common bean

Market-class specific markers for the major market classes of common bean

Association mapping procedures defined for national association mapping experiment

Discovery of SNPs closely associated with the *P* and *St* qualitative trait loci and the seed iron content quantitative trait

Two student interns experienced the field of plant breeding

Animation depicting the trafficking of minerals through a plant developed

~Six minute animations depicting the impact of plant breeding on the economic well-being of a country

Plans for Upcoming Reporting Period (1/1/2012 – 12/30/2012)

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

Research

The primary goal this year will be complete the association mapping analyses of the many traits for which phenotypic data was collected from the national association mapping trial. This will include nutritional traits (minerals, fiber, protein, oil, phytate) and agronomic traits (days to flowering, days to maturity, seed weight, yield, and others).

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 3 of the plant breeding documentary series will be completed. This volume will focus on the use of genetic diversity to improve crops. The theme will be the long-term research efforts in wheat (1920s-2010s) to provide stable stem rust resistance.

Outreach

The will continue to promote beans as a healthy food. Several media formats. Those are currently being conceived. The animation project will focus on humans.

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.

- Association mapping of nutritional and agronomic traits of common bean
- Defining major loci associated with nutritional and agronomic traits of common bean
- Additional student interns will participate in plant breeding training
- New documentary focusing on the use of genetic diversity for crop improvement will be developed
- New animation focusing on human nutrition will be developed

BeanCAP
Common Bean Coordinated Agricultural Project
Progress Report and Work Plan
1/1/2011 – 12/31/2011

USDA, ARS, Beltsville, MD
Perry Cregan and Qijian Song

Progress During this Reporting Period (1/1/2011 – 12/31/2011)

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

Research

Analysis of Molecular Diversity Among Common Bean Genotypes: As previously reported, a total of 19.6 billion bases of “next generation” DNA sequence data was obtained from 19 common bean genotypes including the parents of the Stampede x Red Hawk and BAT93 x Jalo EEP558 mapping populations as well as 15 other genotypes. The 19 genotypes included representatives of the navy, black, pinto, dark red kidney, light red kidney, great northern and white kidney market classes. The sequence data were the basis of SNP discovery and the resulting SNPs were used in the design of two Illumina iSelect genechips. The two genechips, each with the potential to interrogate 6,000 SNPs, were used to analyze the 517 diverse BeanCAP genotypes. Reliable SNP allele call data were obtained for 5188 SNPs from the first Genechip (BARCBEAN6K_1) and 5265 SNPs from the second Genechip (BARCBEAN6K_2). High quality genotype data were obtained for 502 of the 517 BeanCAP lines which included 134 snap bean, 91 pinto, 48 great northern, 43 navy, 42 black, 32 light red kidney, 29 small red, 19 cranberry, 17 pink, 15 dark red kidney, 8 white kidney, 6 small white, 3 red mottle, and 3 tan genotypes as well as 12 additional lines from minor market classes.

The frequency of the least frequent of the two SNP alleles at a locus is referred to as the “minor allele frequency” (MAF) 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. An analysis of the SNP allele frequency data from the 10,453 SNPs for which high quality data were obtained indicated that, 73%, 48% and 45% of the loci had MAF less than 0.10 in the case of the Andean, Middle American and Snap Bean genotypes, respectively (Table 1). In contrast to the Middle American and Snap Bean market classes, there were a relatively small number of loci with MAF frequencies in the 0.2-0.3 and 0.3-0.4 categories in the Andean genotypes. Among the individual markets classes, as anticipated, the largest number of loci was polymorphic within the snap bean market class. The proportion of polymorphic loci ranged from only 14.3% in the case of the cranberry cultivars to 59.6% in the snap bean lines (Table 2). Further analysis of minor allele frequency showed that there were 3,051 loci with average MAF greater than 0.20 in the 276 lines in the combined Durango and Mesoamerican market classes. The MAF of these 3,051 in the 134 snap bean lines averaged 0.16 but among the 74 Andean lines the mean MAF was a very low 0.05. There were only 1046 SNPs with MAF above 0.20 in the Andean lines. The mean MAF of these SNPs was 0.09 among the Durango and Mesoamerican lines and 0.20 in the snap beans. These data clearly demonstrate the smaller genetic variability of the Andean versus the Middle American gene pools and the relatively high level of diversity among the snap beans for which there were 4123 loci with MAF > 0.20. These data suggest that in the selection of SNPs for an

Table 1. Numbers of SNPs in various minor allele frequency classes among the 502 genotypes from Mesoamerican, Andean, and Snap Bean market classes analyzed with the BARCBEAN6K_1 and BARCBEAN6K_2 Illumina genechips.

Minor Allele Frequency	Middle American genotypes	Andean genotypes	Snap Bean genotypes	502 Genotypes
0-0.1	5,035	7,710	4,700	685
0.1-0.2	2,367	1,697	1,630	1528
0.2-0.3	1,699	671	1,292	2,634
0.3-0.4	1,275	354	1,039	2,573
0.4-0.5	77	21	1,792	3,033
TOTAL	10,543	10,543	10,543	10,543

Table 2. Number of genotypes in each common bean market analyzed with the BARCBEAN6K_1 and BARCBEAN6K_2 Illumina iSelect genechips and the proportion of the 10,453 SNP loci for which reliable data were obtained that were polymorphic within the genotypes of each market class

Gene Pool		
Race	No. of genotypes	% of Loci polymorphic
Market Class		
Snap Bean	134	59.6
Middle American		
Durango		
Pinto	91	50.3
Great Northern	48	41.5
Mesoamerican		
Navy	43	36.4
Black	42	33.0
Small red	29	48.7
Small White	6	39.2
Pink	17	45.5
Andean		
Nueva Granada		
LRK	32	30.3
Cranberry	19	14.3
DRK	15	23.8
WK	8	17.6

Illumina genechip for use across all market classes it will particularly important to assure of that a sufficient number of the selected SNPs have high MAF in the Andean market classes. As observed previously, it again is apparent that the high level of diversity in snap beans suggests that any panel of SNPs is likely to have high levels of polymorphism in the snap bean market class.

Genetic Mapping in the Stampede x Red Hawk population: The BARCBEAN6K_1 and BARCBEAN6K_2 Illumina genechips were used to genotype DNA of the 288 F₂ lines of the Stampede x Red Hawk NDSU F₂ genetic mapping population. A total of 6533 SNPs were polymorphic in the population and provided high quality allele call data. The 6533 SNPs were mapped along 485 SNPs that were mapped in 2010 and 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 1665.5 centiMorgans in length (Table 3). The number of SNPs mapped per linkage group varied considerably ranging from 222 in linkage group 7 to 977 in linkage group 11. 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 hundreds of SNPs mapped in clusters that were only a few centiMorgans in length. These clusters were all located in heterochromatic regions where recombination is extremely low.

Table 3. Summary of the current status of the number of SNP markers mapped in the 288 F₂ lines of the NDSU Stampede x Red Hawk mapping population and the length in centiMorgans (cM) of the 11 *Phaseolus vulgaris* linkage groups

Linkage group	SNPs mapped in 2011	Previously mapped SNPs	Total SNPs	LG length in cM
1	760	73	833	143.7
2	518	48	566	197.0
3	531	55	586	175.9
4	708	47	755	154.8
5	666	29	695	135.1
6	199	28	227	103.8
7	192	30	222	141.1
8	824	38	862	196.8
9	449	41	490	159.7
10	763	42	805	135.4
11	923	54	977	122.3
TOTAL	6533	485	7018	1665.5

The application of Bulked Segregant Analysis (BSA) to discover the genome position of the slow darkening (*sd*) locus: Seed coat darkening in pinto bean occurs during prolonged storage and can result in significant loss in value. All current pinto bean cultivars are subject to darkening and these are referred to as regular darkening (RD) types. Dr. Phil Miklas developed three F₂ populations derived from crosses between RD and slow darkening (SD) lines. The progeny were phenotyped to identify homozygous SD and RD progeny and SD and RD bulks were created. The bulks as well as the SD and RN parents were screened with the 1536 SNPs of the original Illumina GoldenGate set of common bean SNPs. Two SNPs were found that differentiated the SD and RD parents and similarly differentiated the DNA bulks for all three populations. The common bean whole genome sequence scaffold in which the two SNPs were determined to reside was interrogated for simple sequence repeats (SSRs). Three of 12 SSRs from the SNP region distinguished between the SD and RD lines. The three SSRs, Pvsd-1157, Pvsd-1158, and Pvsd-0028 were observed to be tightly linked with the *sd* locus via genetic analysis of the F₂ populations. The SSRs were also assayed in a RD x SD recombinant inbred line (RIL) population and the *sd* gene was mapped to linkage group 7 by Kristin Bett, Department of Plant Sciences, University of Saskatchewan. The SSR markers will have utility for marker-assisted selection for the SD trait in pinto bean. This application of BSA is a clear demonstration of the usefulness of Illumina SNP detection for the rapid discovery of DNA markers that can be used in marker assisted selection for the genetic improvement of common bean.

Education:

Ms. Josiane Rodrigues, a graduate student from Brazil began work in our laboratory in January. She learned the procedures required to analyze SNP DNA markers using the Illumina iSelect genechips. A population of 94 F₅ RILs of the Stampede x Red Hawk population were obtained from Dr. Phil McClean at NDSU. Ms. Rodrigues genotyped these RILs with the BARCBEAN6K_1 and BARCBEAN6K_2 Illumina genechips. Following these analyses she used the Illumina GenomeStudio software to make the allele calls for the SNPs that were polymorphic in the RIL population. In addition, a set of SSR markers were selected from the developing common bean whole genome DNA sequence and Ms. Rodrigues analyzed the 94 RILs and parents with 270 SSRs. She then created a linkage map using MSTMAP software. The resulting map contains a total of 6776 loci.

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

- Developed and two 6,000 SNP Illumina iSelect genechips (BARCBEAN6K_1 and BARCBEAN6K_2)
- Analyzed the 288 F₂ lines and 94 RILs of the Stampede x Red Hawk mapping populations using the Illumina iSelect BARCBEAN6K_1 and BARCBEAN6K_2 genechips.
- Analyzed 517 common bean genotypes from 25 different market classes with 10,453 SNP DNA markers using the Illumina iSelect BARCBEAN6K_1 and BARCBEAN6K_2 genechips.
- Calculated minor allele frequency for 10,453 SNPs based upon the genotypes of the major market classes: snap bean, pinto, great northern, navy, black, light red kidney, small red, cranberry, pink, dark red kidney, white kidney and small white.

- Constructed a genetic linkage map derived from the analysis of 288 F2 lines from the Stampede x Red Hawk mapping population with 6533 SNP markers and unambiguously associated the resulting 11 linkage group with the 11 common bean linkage groups
- Used bulked segregant analysis to determine the position of the slow darkening gene (*sd*) and developed SSR markers that can be used to identify lines that carry the slow darkening trait.
- Mapped 270 new SSR markers to the common bean genetic map.

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

Additional Genetic mapping in 188 Recombinant Inbred Lines of a mapping population derived from BAT93 x Jalo EEP558: Of the 10,453 SNPs that produced reliable allele calls in the analysis of the 517 BeanCAP genotypes only 6,533 were segregating in the Stampede x Red Hawk F2 mapping population. This leaves 3,920 SNPs that are not genetically mapped. BAT93 and Jalo EEP558 were among the 517 BeanCAP genotypes that were analyzed with the Illumina iSelect BARCBEAN6K_1 and BARCBEAN6K_2 genechips. A comparison of the SNP allele calls for BAT93 and Jalo EEP558 indicated that of the 3,920 SNPs that were not polymorphic in the Stampede x Red Hawk population more than 2000 are polymorphic between BAT93 and Jalo EEP558. We recently received seeds from Embrapa, Brazil of a RIL population derived from a cross of BAT93 x Jalo EEP558. Plants of the population are currently growing in the greenhouse and DNA will be isolated from immature leaf tissue. A total of 188 BAT93 x Jalo EEP558 RILs will be genotyped using the BARCBEAN6K_1 and BARCBEAN6K_2 genechips. The resulting genotypic data will be used to create a second genetic map of the 11 common bean chromosomes using MSTMAP software. This map will be compared for consistency with the Stampede x Red Hawk map and will provide additional information needed to identify SNPs that will be used in the design of a 6,000 SNP Illumina iSelect Genechip that will be used to analyze segregating populations for the various BeanCAP breeders and geneticists.

Design of a 6,000 SNP Illumina iSelect Genechip: The current version of the Stampede x Red Hawk map contains a total of 7,018 mapped SNPs. Of these, 6,533 have been characterized on the 517 BeanCAP genotypes while for the remaining 485 there are only data from the first 192 BeanCAP genotypes. However, the genotypic data derived from the first 192 BeanCAP genotypes provide good information as to which loci are likely to be segregating in the various common bean market classes. With the addition of perhaps as many of 2,000 mapped SNPs that will be forthcoming from the analysis of the BAT93 x Jalo EEP558 RIL population described above we anticipate having approximately 8,500 loci with definitive linkage map positions and information related to their likely polymorphism in the various common bean market classes. Using this information 6,000 SNPs will be selected such that 3,500 would have a high likelihood of polymorphism in the Middle American market classes and 2,500 will be selected that would have a similarly high probability of polymorphism in the Andean market classes. Careful attention will be given in the selection process to identify SNPs that are evenly distributed across the 11 linkage groups. We normally anticipate that approximately 78-82% of the 6,000 markers on an Illumina iSelect Genechip will produce reliable allele call data. Based upon this success rate we would expect from 2,730 to 2,870 SNPs to have a high expectation of

polymorphism in crosses of Middle American genotypes and from 1,950 to 2050 SNPs to have a similarly high expectation of polymorphism in populations derived from crosses of Andean genotypes.

Analysis of 2000 genotypes nominated by BeanCAP breeders and geneticists: Once the design of the 6,000 SNP Illumina iSelect Genechip is available we will begin the genotypic analysis of populations that have been developed and are being characterized by the BeanCAP breeders and geneticists. We anticipate the genotypic analysis of at least 2,000 individual bean DNA samples during the next year.

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.

- Analysis of the 188 RILs of the Embrapa BAT93 x Jalo EEP558 population with the BARCBEAN6K_1 and BARCBEAN6K_2 genechips.
- Creation of a second common bean linkage map based upon the genotypic data obtained from the analysis of the BAT93 x Jalo EEP558 RIL population.
- Design of a 6000 SNP Illumina iSelect Genechip based upon 1) the genetic map positions of SNPs on the Stampede x Red Hawk and the BAT93 x Jalo EEP558 genetic maps and 2) the level of SNP polymorphism in the Middle American and the Andean gene pools based upon the SNP polymorphism obtained via analysis of the 517 diverse BeanCAP genotypes with the BARCBEAN6K_1 and BARCBEAN6K_2 genechips
- Analysis of at least 2000 genotypes nominated by the BeanCAP breeders and geneticists with the 6000 SNP Illumina iSelect genechip.

BeanCAP
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Progress Report and Work Plan
1/1/2011 – 12/31/2011

Michigan State University
James D. Kelly

Progress During this Reporting Period (1/1/2011 – 12/31/2011)

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

Research activities involved the planting, collection of field data on agronomic traits, and harvest of 300 dry bean genotypes chosen for study as part of the beancap genomic association mapping project. The two nurseries were planted under rainfed condition at Saginaw Valley Research and Extension Center at Frankenmuth, MI. One nursery had 100-entry race Mesoamerican genotypes and the other nursery had 200-entry Durango race genotypes. After harvest, yields, and seed weights were recorded and a sample of seed was shipped to USDA lab in Houston for mineral element analysis. A second nursery of 96 genotypes selected for tolerance to drought was evaluated in a drought prone soil at Montcalm MI. The site was selected to create drought as the soil type is very coarse textured McBride sandy loam. Plots were not supplied with any supplementary irrigation and experienced early season drought through late July. Seasonal rainfall was 202mm or approximately half normal 30-year average (430mm) for the region during the same period. Genotypes were planted in 6m rows, 0.5m apart. When fifty percent of the genotypes were flowering, five plants from each plot were excavated and data was recorded for: basal root angles, basal root branching in a three centimeter segment, number of basal roots, number of adventitious roots, adventitious root branching in a three centimeter segment, tap root diameter, tap root branching in a three centimeter segment, an overall root score, and dry weight of shoots and roots. We followed the 'shovelomics' protocol described at Penn State web site (<http://roots.psu.edu/en/node/945>). At the end of the season, plots were trimmed to 5m, plants were mechanically pulled using Pickett and total plant biomass was recorded before threshing. Throughout the growing season, other data taken included: flowering date, maturity date, lodging, height, seed weight and yield. Plot yields, harvest index and seed weights were determined. The root data will be compared with performance data for MI but from five other locations in the US where the same beancap trial was grown under drought stress. Seed phytic acid levels were measured colorimetrically with Wades reagent (ferric iron and sulfosalicylic acid) on 249 lines of the BeanCAP grown at two locations (East Lansing, MI and Prosser, WA) in 2010. 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. List of students and information was sent to Education Coordinator at NDSU.

Extension activities involved the maintenance 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:

Field agronomic and yield data were collected on 396 dry bean genotypes grown under normal rainfed conditions in Michigan. In addition data was collected on root rots of 96-entries grown under drought stress. Seed from all trials was sent to USDA lab in Houston for mineral element analysis. Two undergraduate students received training on aspects of lab and greenhouse activities of bean breeding during the fall 2010 and spring 2011 semesters. During this time the students were involved in aspects of DNA extraction, running SCAR markers, greenhouse crossing, disease inoculation, seed preparation for planting and harvesting under guidance of technical staff in the bean breeding program. Two high school students spent 8-weeks assisting a graduate student conduct field trials and learning the basis of both lab and field research on campus during the summer 2011 semester. High school students were under the direct supervision of a graduate student.

One hundred fifty-four Indel markers developed at NDSU to be polymorphic in the pinto bean market class were tested in three recombinant line populations at MSU. One population derived from cross of Matterhorn great northern with carioca bean from Brazil that produced pinto progeny showed a 44% level of polymorphism between the parents. Among the 124 Indel markers screened to date, 54 were polymorphic. The level of polymorphism was slightly higher than for SSR markers (37%) run on the same population. A small number of the Indel markers that appeared monomorphic on agarose gels proved to be polymorphic on polyacrylamide gels. In the other two pinto populations derived from crosses of two pinto breeding lines differing in reaction to white mold, only 42 markers have been screened to date. These were chosen from within the 72/154 Indel markers that were polymorphic between Type-II pintos at NDSU. We detected only a 26% level of polymorphism in AP630 population and 22% polymorphism in AP647 population. This is substantially lower than the 47% polymorphism reported between upright Type II pinto beans and 71% reported between Type II and Type III pinto beans data from NDSU. It is also lower than the 31-36% polymorphism detected with SSR markers in the same AP630 and AP647 populations.

Seed phytic acid data was collected on 249 genotypes grown at two locations. The average seed concentration of phytic acid phosphorus was 4.2 mg g^{-1} in both MI and WA and the range in WA was $2.6 - 6.5 \text{ mg g}^{-1}$ and in MI it was $2.7 - 6.0 \text{ mg g}^{-1}$. The correlation coefficient between the phytic acid phosphorus levels in the lines grown in the two locations was only 0.26. Pink beans had the lowest average phytic acid phosphorus levels in both MI and WA at 3.76 mg g^{-1} , which is 10.5% less than the average of all 249 lines.

Maintain and update the 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.

Plans for Upcoming Reporting Period (1/1/2012 – 6/30/2012)

- 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 a 96-entry field drought trial this coming summer in Michigan. The same trial that was grown in 2011 will be repeated in 2012 and root traits in addition to agronomic and yield data will be collected. Field data will be made available to other members of the beancap for use in association mapping studies. Continue to expand the utility of the beancap portal with input from beancap members as more information and data become available. Measure phytic acid content in the seed from the 2011 beancap field experiments.

- B. Deliverables. Continue training internship of undergraduate students in lab and greenhouse activities related to bean breeding. Data collected from field experiments on 96 bean genotypes that will be used in AM studies and phytic acid analyses.

BeanCAP
Common Bean Coordinated Agricultural Project
Progress Report
10/1/2010 – 09/30/2011

Colorado State University
Mark A. Brick and Henry Thompson

Overall objectives:

The overall objectives of research activities include development of research tools to better understand the genetic and genomic factors that define a nutritional crop. This objective will be accomplished by utilizing association mapping among 300 dry bean and 200 snap bean lines that will also be evaluated for nutritional characteristics and genomics experiments throughout the BeanCAP project. All of these lines will be genotyped and that data will be used for the statistical analyses to discover loci associated with the nutritional traits under study. The Colorado State University team has contributed pinto, white kidney and black cultivars to the list of field grown entries for these activities. The seed was sent to NDSU for to provide uniform and clean seed stocks to be increased in the greenhouse at NDSU.

Nutritional profiling on common bean germplasm.

In year 2, the project continued to develop and test the protocol for total dietary fiber (TDF) which includes soluble (SDF), insoluble, (IDF) and oligosaccharide (OLI) content based on the AOSA Codex 2009.01 method. The protocol was tested on cultivars representing all of the major market classes of common bean grown in the US. The seed for this protocol was grown under controlled greenhouse conditions in Idaho and Michigan and among a set of parents used for recombinant inbred line development. Results indicated significant difference among cultivars for SDF, IDF and OLI content. We evaluated 34 entries that represent parents from recombinant inbred line populations. Significant variation was found among entries for IDF, SDF, TDF and OLI. TDF varied from 14 % to 23% among entries. Significant differences were found in mean TDF between locations of seed production. The differences observed indicate a great deal of genetic diversity in fiber content in the species. In the evaluation, Andean beans were higher in SDF compared to Middle American beans, however sample size was small.

The content of total TDF found using the Codex 2009.01 method is higher than previously published fiber content for dry beans because the new Codex method includes oligosaccharides in the TDF content whereas the older method only included SDF and IDF. Oligosaccharide content averaged about 2.5 to 3% among the lines tested. IDF and SDF content were in the same range reported previously. The CSU team is prepared to conduct TDF analyses on the 300 dry bean lines grown at Fort Collins in 2011, and a subset of entries grown at Fargo, ND to estimate genotype x environment interaction in 2012 and 2013 and to use in association mapping studies and SNP discovery.

Total Phenolic (TP) assays were conducted on approximately 200 snap bean lines grown in Corvallis, OR in 2010 and 2011. The TP assay is derived from the work of Singleton and Rossi, (1965) based on the color reaction of phenolics with Folin-Ciocalteu Phenol reagent that absorbs at 765nm. Sample extracts were compared to a gallic acid standard curve. Total phenolics, in general, is a measure of reducing capacity through electron transfer reactions, expressed as gallic

acid equivalents in mg/g. Frozen pods were sent by Dr. Jim Myers to CSU for phenolic assay in early 2011. Phenolic content varied almost 5 fold from 0.30 to 1.4 mg/g among snap bean cultivars. In general pole beans and heirloom beans were highest in TP content. A manuscript is in preparation to report the results. This data will also be used to find associations with SNP markers generated by NDSU and collaborators on the Bean CAP project.

Design and implementation of young plant breeder training program. This objective is to train young students for a career in plant breeding. In the 2010-11 budget year, we employed two undergraduates and two high school students that participated in all phases of the BeanCAP research program and dry bean breeding. Undergraduate interns Sonya Hueftle and Griffin Carpenter worked 10 hours per week during the school year and 40 hours per week during the summer academic semester. Both students presented results of their research projects at the annual meeting of the ASA/CSSA/SSSA meetings in San Antonio, TX. Griffin Carpenter was awarded first place in the Student Division in the category scientific presentations. High School interns Alyssa Bollig and Keera Brown worked 20 hours per week during the summer. They participated in all phases of the Bean CAP research activities and dry bean breeding project throughout the summer.

B. Deliverables and outcomes achieved during this reporting period.

Outcomes:

1. Research

- Data collected from replicated field trials on 300 dry bean lines at Fort Collins for seed yield, seed weight, days to flowering, days to maturity, lodging, and growth habit to determine if these traits may be correlated with nutritional traits and association mapping in years 3 and 4.
- Evaluated more than 100 dry bean lines for TDF
- Evaluated more than 200 snap bean lines for phenolic content
- Supervised and trained a research associate to conduct phenolic and TDF assays on bean lines.
- Maintained and supervised lab space and equipment for phenolic and TDF assays for nutritional components in bean.

2. Training

- Supervised and trained two undergraduate interns on the Dry Bean Breeding Project.
- Supervised and trained two high school interns during summer 2011 on the Dry Bean Breeding Project.
- Supervised and trained a research associate on analysis using the AOSA Codex 2009.1 method for dietary fiber and colorimetric phenolic assay.
- Presented the experiences of summer interns from year 1 at two local high schools, with approximately 120 contacts present.
- Presented three reports at scientific meeting regarding Bean CAP activities.
- Student Hanna Walters presented experiences as an undergraduate student intern at annual directors of the BeanCAP in San Diego, CA.
- Student Griffin Carpenter presented results and participated in the undergraduate student scientific paper competition at the ASA/CSSA annual meeting in San Antonio Texas. See abstract title in publications section.

- Student Soni Hueftle presented results and participated in the undergraduate student scientific paper competition at the ASA/CSSA annual meeting in San Antonio Texas. See abstract title in publications section.

Plans for Upcoming Reporting Period (10/1/2011 – 09/30/2012)

Nutritional profiling common bean germplasm:

At the end of year 3, the project will have analyzed more than 400 dry bean genotypes grown in Fort Collins for IDF, SDF, and OLI, a subset of 50 entries grown at Fargo ND. These data will allow the genomics section to discover SNP profiles that are associated with each of the components of TDF. In year 3, we will submit two manuscripts on the genetic diversity of phenolic compounds in snap bean and TDF in dry bean.

Association Mapping:

Colorado State University will contribute to the Association Mapping experiment by providing TDF data on all dry bean genotypes produced in replicated trials at Fort Collins in year 2. Association Mapping statistical analyses will also be performed to discover those loci associated with each trait.

Implementation of young plant breeder training program. During year three, two additional undergraduates and two high school students will be hired to work and train on plant breeding projects in the Department of Soil and Crop Sciences with emphasis on dry bean breeding. Site visits will be made to local and regional high schools with the undergraduates sharing their experiences and participating in presentations on the role of plant breeders in a global society. Follow up visits will be made to the high schools that provided the interns for 2011-12 with interaction with the biology teachers at each site. The replacement high school trainees will be interviewed for year 4 of the project.

B. Deliverables and outcomes that will be achieved during this reporting period.

Nutritional profiling common bean germplasm:

- Fiber content will be determined on 400 bean lines grown at two locations.
- The research associate will be supervises to conduct all nutritional assays.
- Two peer reviewed publications on fiber and phenolic content of beans assayed in year 2.

Design and implementation of young plant breeder training program:

- Supervise and train two undergraduate interns on the Dry Bean Breeding Project.
- Supervise and train two high school interns during summer 2011 on the Dry Bean Breeding Project.
- Supervise a research associate and lab assistant on analysis using the AOSA Codex 2009.1 method for dietary fiber and colorimetric phenolic assay.
- Present the experiences of summer interns from year 1 at two local high schools, with approximately 120 contacts present.
- Present three reports at scientific meeting regarding Bean CAP activities.
- Assign research project to both undergraduate student interns to be presented at the national meeting of ASA/CSSA/SSSA to be held in Cincinnati, OH.

Abstracts and publications in 2011

Brick, L.A., M.A. Brick*, D. Echeverria, H.J. Thompson and A.Kleintop. 2011. Variation for dietary fiber content in dry edible beans. Oral Presentation at the Western Society of Crop Science Meetings, June 20-22, 2011, Laramie, WY. *Presenter. Published abstract in American Society of Agronomy Abstracts, 2011, Madison, WI.

Brick, L.A., D. Echeverria, M.A. Brick. And H.J. Thompson 2011. Dietary fiber content of dry edible beans. Colorado Bean News Vol. 24 (2):6-7.

Brick, L.A., M.A. Brick, D. Echeverria, H. Thompson and A. Kleintop. 2011. Dietary Fiber Content of Dry Edible Beans based on the 2009.01 method. Annu. Rep. Bean Improv. Coop. 54:4-5.

Brick, L.A., M.A. Brick*, D. Echeverria, H.J. Thompson and A.Kleintop. 2011. Variation for dietary fiber content in dry edible beans. Oral Presentation at the biennial meeting of the Bean Improvement Cooperative. October 3- November 2, San Juan, Puerto Rico.

Carpenter, G., M.A. Brick, B. Ogg, and K. Cichy. 2011. Comparison of Seed Coat Luster in a Recombinant Inbred Line of Shiny and Opaque Black beans. Agronomy Abstracts. Presented at the annual meeting of Crop Science Society of America, October 16-20, 2011 San Antonio, TX.

Hueftle, S., P.R. Byrne, and M.A. Brick. 2011. Molecular Markers Linked to Quantitative Trait Loci For Resistance to *Fusarium* Wilt in Common Bean. Agronomy Abstracts. Presented at the annual meeting of Crop Science Society of America, October 16-20, 2011 San Antonio, TX.

BeanCAP
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Progress Report and Work Plan
1/1/2011 – 12/31/2011

University of Nebraska-Lincoln
Carlos A. Urrea

Progress During this Reporting Period (1/1/2011 – 12/31/2011)

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

Hosted 29 members of the Nebraska LEAD Tour on April 5. I talked about dry bean breeding activities in western Nebraska. The BeanCAP program was discussed.

Talked to 50 undergraduate students about the dry bean breeding program and BeanCAP opportunities at the Western Community College international week on April 21.

Three high school students joined the BeanCAP internship program between May and August. Students were involved in all dry bean breeding activities.

From August up to date one undergraduate student from Western Nebraska Community College is working under the BeanCAP internship.

BeanCAP activities and opportunities were discussed during the University of Nebraska-Expo Field day on July 26.

BeanCAP activities and opportunities were discussed with 132 people on August 16, 17, and 18 during the Crop Management Workshop at the Panhandle Res. & Ext. Center, Scottsbluff, NE.

Discussed BeanCAP experiments during the Nebraska Dry Bean Growers Field day on August 23. About 150 growers attended.

About 60 students extracted DNA and were exposed to the BeanCAP activities during the Scottsbluff Youth Day on September 15.

About 20 people attending Leadership Scotts Bluff, INC on October 21 were informed about the BeanCAP activities.

Conducted 4 BeanCAP experiments at Mitchell, NE in replicated trials. A total of 453 cultivars/germplasm were tested under normal conditions. Of those, 108 were from the Middle America, 200 from Jalisco/Durango, and 49 from The Andean center of origin/domestication. A set of 100 lines was also tested under drought conditions where irrigation was stopped at flowering stage. Data on the agronomics including days to flowering and maturity, plant height, lodging, seed yield, and 100-seed weight was collected. Statistical analysis is under way.

A sample of 200 g from each plot of the replicated trials described above were sent to the Children's Nutrition Research Center for nutritional analysis.

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

Some Scottsbluff/Gering high school students expressed their interest to work during summer 2012 for the dry bean breeding program.

Three undergraduate students and four high school students participated in the internship and were involved in all 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.

Seven articles were published in local newspaper and in the Bean Bag newsletter as follows:

- Hansen, S. 2011. Bean counting isn't all that boring. StarHerald, Scottsbluff, NE April 13, pages 1 & 3.
- Hansen, S. 2011. Panhandle is latest stop on international guest for excellence. StarHerald, Scottsbluff, NE. May 29, page 3 & 4.
- Hansen, S. 2011. Program: BeanCAP gives students a chance to learn about dry edible bean breeding. StarHerald, Scottsbluff, NE. June 26, pages 1 & 2.
- Hansen, S. 2011. Learning leaf language. StarHerald, Scottsbluff, NE. August 14, pages 1 & 2.
- Otsdiek, D. 2011. UNL bean plots part of worldwide effort to improve crops. StarHerald, Scottsbluff, NE. September 18, page 1.
- Otsdiek, D. 2011. UNL bean plots part of worldwide effort to improve crops. The Business Farmer. September 30, pages 6 & 7.
- Urrea, C.A. 2011. UNL dry bean breeding program. The Bean Bag. 29(4):2.

Plans for Upcoming Reporting Period (1/1/2012 – 12/30/2012)

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 2012. 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.

Set up a of couple experiments on Genetics for the Biology class at Scottsbluff High School.

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

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 working in dry bean breeding with their classmates.

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1/1/2011 – 12/31/2011

University of California, Davis
Paul Gepts

Progress During this Reporting Period (1/1/2011 – 12/31/2011)

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

The responsibility of the UC Davis component is to continue developing the genome database PhaseolusGenes: <http://phaseolusgenes.bioinformatics.ucdavis.edu/>. This genome database, unlike other, larger databases, is focused primarily on marker information and discovery. It seeks to help breeders identify existing markers and develop new markers in specific regions of the genome. To achieve this goal, the availability of DNA sequence is a pre-requisite. In addition, these DNA sequences have to be mapped, either physically or genetically. Physical mapping is achieved by placing individual sequences against the whole-genome sequence (WGS), whereas genetic mapping relies on segregation and linkage analysis of sequence-based markers in mapping populations.

While awaiting the public release of the two major *P. vulgaris* WGS (Andean and Mesoamerican by U.S. and Ibero-American groups, respectively), the soybean WGS has been used as soybean is an evolutionary close relative of common bean as both species are members of the Phaseoleae tribe of the Fabaceae. While there are disadvantages to this approach (divergence between the two species estimated at ~ 20 MYA; tetraploidization followed by diploidization around 14 MYA), a substantial amount of synteny has been documented between the two genera (McClellan et al. 2010; Galeano et al. 2011). This residual synteny allows for mutual marker and trait discovery. Hence, even after the release of the two *P. vulgaris* WGSs, the soybean WGS will remain a feature of the *PhaseolusGenes* database.

During this reporting period, we have continued accumulating existing marker data as well as newly published markers. As a consequence, there are now 2370 markers (~1540 last year) to which should be added 118,619 sequences containing an SSR motif. The latter sequences resulted from an independently funded effort to sequence methyl-filtrated DNA of the BAT93 bean line. We have also continued adding QTLs to the searchable database, which now includes 1176 entries (925 last year). The Genome Browser representation using the soybean WGS as anchor now includes several groups of tracks (from top to bottom in <http://gb.genomecenter.ucdavis.edu/cgi-bin/hgTracks?clade=plant&org=G.+max&db=glyMax1&position=Gm01>): a) *P. vulgaris* BAT93 1x sequencing of methyl-filtrated genomic DNA, including a sequence assembly; b) *P. vulgaris* SSR-containing sequences, including the top two hits, when available, against the soybean genome for some 120,000 sequences; c) *P. vulgaris* markers, including SCARs, SSRs, and STSs; d) *P. vulgaris* ESTs; e) *P. vulgaris* ESTs; e) *Vigna unguiculata* (cowpea) 1x methyl-

filtrated reads and assembly, as well as ESTs; f) *Glycine max* (soybean) gene models, old and recent duplication blocks, and synteny blocks with *Medicago*.

The CMap representation will include six molecular linkage maps for bean, including Bean1 [BAT93 x Jalo EEP558 (BJ population), Mesoamerican x Andean; McClean 2007; from LIS: www.comparative-legumes.org; 450 markers, mainly g STS markers], Bean2 (BJ population; Navarro Gomes and Gepts, unpubl. res.; STS markers, including g, Leg, Bng, and D markers; 485 markers), Bean3 (DOR364 x BAT477, intra-gene-pool, Mesoamerican; Galeano et al. 2011; 291 markers, mainly SSRs), Bean4 (Dorado364 x G19833, Mesoamerican x Andean; Galeano et al. 2011; 499 markers), Bean 5 (BJ population; Galeano et al. 2011; 424 markers, mainly SNPs), and Bean6 (“consensus” map, with 1010 markers).

Two tutorials have been developed, one to illustrate a search by keyword (for markers, QTLs, traits) and the other a search by DNA sequence. These tutorials are presented as web page with side-by-side instructions and screenshots. I am a member of the Legume Information System (LIS) working group, which provides advice to the LIS investigators. This activity is useful because, eventually, PhaseolusGenes will establish closer links with LIS following a model in which hardware/software issues are maintained by LIS and data curation by the Phaseolus community.

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

- a) We have completed incorporating historical mapping and QTL data prior to this project, into the database, and where possible, have linked those markers to available soybean sequence data. From now on, we expect to add currently developed markers as they become available.
- b) We have used and are using the database to map genes for agronomic traits and identify new markers as illustrated by Gonçalves-Vidigal et al. (2011) and Miklas et al. (2011).
- c) Develop a legume genome database – PhaseolusGenes – that is interoperable with other legume database

Plans for Upcoming Reporting Period (1/1/2011 – 6/30/2012)

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

- a) Start incorporating all BeanCAP project phenotypic and marker information into the database.
- b) Hyperlink the CMap element of PhaseolusGenes with the searchable database and the genome browser.
- c) Start incorporating SNP markers into PhaseolusGenes and – depending on availability – the WGSs of *P. vulgaris*
- d) Initiate placement of QTL regions onto the CMap instance of PhaseolusGenes
- e) Continue collaborating with other legume databases and the Legume Information System to assure interoperability.

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.

- a) Increased functionality of PhaseolusGenes
- b) Improved presentation of QTL information in PhaseolusGenes
- c) Improved cross-linking with other legume databases

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1/1/2010 – 12/31/2010

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.

BeanCAP
Common Bean Coordinated Agricultural Project
Progress Report and Work Plan
1/1/2011 – 6/30/2012

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

Progress During this Reporting Period (1/1/2011 – 12/31/2011)

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. During the current reporting period, we completed the mineral analyses of 142 snap bean entries (times 3 field replicates), sent by cooperator Jim Myers (Oregon State). Variations in the concentrations of both macro- and microelements were identified; ranges were ~2- to 2.5-fold for Cu, Fe, K, Mg, P, S, and Zn; ~3-fold for Ca; ~4-fold for Ni; and ~5-fold for Mn. Average values (mg/g DW) for the macroelements were: Ca, 5.47; K, 18.46; Mg, 2.75; P, 3.31; and S, 1.65; average values ($\mu\text{g/g DW}$) for the microelements were: Cu, 4.57; Fe, 75.87; Mn, 26.62; Ni, 4.46; and Zn, 28.69. Values for Co, Mo, and Se were below detection limits for most of the samples.

We completed the bulk sample grinding (150 gm samples) of 248 entries received (in November 2010) from both Michigan (cooperator Jim Kelly) and Washington State (cooperator Phil Miklas). Once ground, these samples were split and sent to (1) North Dakota State University (NDSU; Mehmet Tulbek; 100 gm) for protein %, fat %, and crude fiber % analyses and (2) Michigan State University (MSU, Karen Cichy; 15 gm) for phytate analyses. Data received back from these cooperators indicated broad ranges for all nutritional components, with combined average values (DW basis) for MI- and WA-grown beans of: protein %, 24.84, fat %, 1.51, crude fiber %, 4.28, and phytate (mg/g), 4.19. In Houston, we completed the acid digestion and elemental analyses (inductively coupled plasma – optical emission spectroscopy) of all MI-grown samples; analysis of the WA-grown samples is still in progress. For the MI-grown entries (248), average values (mg/g DW) for the seed macroelements were: Ca, 1.61; K, 13.91; Mg, 1.82; P, 4.44; and S, 2.09; average values ($\mu\text{g/g DW}$) for the seed microelements were: Cu, 9.24; Fe, 69.21; Mn, 13.90; Ni, 2.50; Se, 0.45; and Zn, 48.59. Values for Co and Mo were below detection limits for most of the samples.

In addition to the compositional analyses noted above, we also processed and completed the Fe bioavailability assays with the 2010 WA-grown dry bean entries (248 samples) using the *in vitro* Caco-2 cell culture method. Ground samples were mixed with water (1 part bean powder to 4 parts water) and cooked for 30 minutes at 121°C in a Primus Steam Sterilizer. Once cool, they were transferred to a -20°C freezer for at least 24 hrs. The samples were then lyophilized for 72 hrs. Once out of the freeze dryer, they were ground in a mortar and pestle and stored at 4°C until

the iron bioavailability experiments. This involved mixing aliquots of cooked bean sample with a known quantity of Fe, digesting this using an *in vitro* digestion (a mix of digestive enzymes), then feeding this 'food' to the Caco-2 cells. Ferritin formation in the cells (after 24 h) was used as a proxy for Fe bioavailability potential; a control sample of ground and cooked chickpea was run with each set of cells and was used to normalize the data in each assay. Our results (248 entries; average of 5 replicates/entry) indicate that the normalized Fe bioavailability values demonstrated a 2.7-fold range.

Finally, in November and December of 2011, we began receiving the dry bean samples grown by various cooperators in the summer of 2011. These included the replicated field trials of the BeanCAP 300 entries, as well as the replicated field trials of the 100 entry subset grown in irrigated and non-irrigated fields. Samples received thus far include those from WA (~ 384 samples; Miklas); ID (~ 384 samples; Despain); and NE (~900 samples; Urrea). We have begun grinding these samples with stainless steel coffee grinders, as before, and will bar code all samples to maintain quality control over the sample identities. We also have spoken with cooperators at NDSU and MSU to confirm the amount of ground sample to send to these labs for their compositional analyses (60 gm and 8 gm, respectively).

Education

Nothing to report.

Outreach

Nothing to report.

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

- Completed sample grinding and distribution of ~500 dry bean sub-samples to cooperators for compositional analyses.
- Completed elemental analyses on ~450 snap bean samples and ~250 dry bean samples.
- Attended BeanCAP annual meeting at PAG XIX.
- Completed Caco-2 studies to assess iron bioavailability potential of diverse dry bean samples (248 entries).
- Coordinated the collection of and verified the values for seed protein %, fat %, crude fiber %, and phytate (mg/g DW), generated by cooperators.
- Initiated the grinding of 2011 BeanCAP entries received from several cooperators.

Plans for Upcoming Reporting Period (1/1/2012 – 6/30/2012)

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

Research

Elemental analyses will be completed on remaining dry bean samples received from Pullman (2010). A manuscript will be developed, focusing on the nutrient diversity identified in the WA- and MI-grown samples. Grinding of all dry bean samples received from Michigan, Idaho, Nebraska, North Dakota, Puerto Rico, and Washington State 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 a subset of the dry bean samples, once all material has been ground, and once we have fully analyzed the results with the 2010 Prosser samples.

Education

No specific plans.

Outreach

Continue to offer assistance with the Mineral Nutrition animations, especially the next videos 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 ~4000 dry bean sub-samples to cooperators.
- Complete elemental analyses on ~4000 dry bean samples.
- Complete Caco-2 studies to assess iron bioavailability potential on a subset of the dry bean samples.
- Completion of manuscripts on nutrient diversity in snap bean entries and dry bean entries.
- Attend BeanCAP annual meeting at PAG XX.
- Assist with animation package for BeanCAP web presence.

BeanCAP
Common Bean Coordinated Agricultural Project
Progress Report and Work Plan
1/1/2011 – 6/30/2012

Oregon State University
James R. Myers

Introduction

The objectives for snap beans are similar, 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).

We are working with 150 cultivars provided by private and public breeding programs. 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 used in this year's analyses was increased 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 on meter between rows while bush beans were planted in 5 meter plots with 0.75 meters between rows. Plants were fertilized with N:P:K:S 12:29:10:10 at 450 lb/A banded in the row and were grown under sprinkler irrigation throughout the season. Data were taken on: flower color, days to green pod maturity, pod length and string length, pod color (measured with a chromameter), growth habit, plant height, internode length, lodging score, and 100 seed weight. Data are being summarized in preparation for association mapping. Progress with the nutritional analyses are reported below.

Phenolics

Dr. Mark Brick at Colorado State University completed analysis of total phenolics from samples collected in 2010. Levels ranged from 0.30 to about 1.32 mg/100g FWt (a fourfold difference). Highest in total phenolics were several of the pole beans, including 'Blue Peter Pole', 'Kentucky Wonder', 'Royal Burgundy' and 'Trail of Tears'. The bush bean with the highest level was 'Tendergreen' with 1.11 mg/100g Fwt. We collected a subset of pods this year which were frozen (-20C) and sent to Dr. Mark Brick at Colorado State University for total phenolics analysis. The objective was to determine if G x E interaction for phenolic content might be present; none was detected.

Minerals

In 2010, we provided dried pod samples to Dr. Mike Grusak for mineral analysis. The data were received back and have incorporated into the dataset for to be used for association mapping. At last year's board meeting, it was decided that we should provide seeds of the snap bean cultivars to Dr. Grusak for mineral analysis. Accordingly, dry seed was harvested from all plots and approximately 50g of each cultivar (x 3 reps) was provided to Dr. Grusak in October. One

concern with this material is that we observed K deficiency in some plots in this trial, which may influence mineral analysis results. The K deficiency apparently resulted from an unusually cool early growing season which influenced nutrient uptake in the plots especially since fertilizer was banded within the row.

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 -80°C until HPLC analysis. A total of 450 samples (150 lines, 3 reps each) were extracted, and an additional 9 duplicate samples (3 lines, 3 reps each) were harvested and processed immediately to compare fresh versus 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. A total of 455 samples were assayed using HPLC and the data analyzed for vitamin C concentration. The range of values were observed to be from 0.087 to 22.685 mg/100g, with an average of 2.611 mg/100g. The low average value appears to be the result of lower than expected readings for many of the samples. We believe this variation may occur in the extraction process, where temperature and oxidation can affect the stability of vitamin C. It has been recommended that an increase in the antioxidant level in the extraction buffer and maintaining samples at colder temperatures during and after homogenization will improve results in the future.

Carotenoids

Four hundred fifty samples were stored at -80°C prior to freeze drying for subsequent carotenoid extractions. After freeze drying the samples were milled to a fine powder using a sample mill and stored at -20°C. A small-scale solvent-based extraction protocol has been developed that enables us to measure total carotenoid content using UV-spectroscopy. With this method is possible to extract and measure between 24 and 32 samples per day. We have tested this protocol on several samples and will continue processing the remainder pending replacement of critical lab equipment.

Pod fiber analysis

Analysis of pod fiber was completed during the spring of 2011. Pod fiber ranged from 0.02 to 0.51% with many of the romano and blue lake types having lowest fiber. Data have been integrated into the master spreadsheet for association mapping.

In summary, the work is proceeding as expected with the exception of the vitamin C and carotenoid analyses. However, this should be completed early in 2012.



A USDA/NIFA Supported Project

Conference Calls

2011



A USDA/NIFA Supported Project

Minutes of April 7, 2011 BeanCAP Conference Call

Participants: Perry Cregan, Paul Gepts, Mike Grusak, Stacy Halvorson, Dave Hyten, Jim Kelly, Phil McClean, Juan Osorno, Qijian Song

1. Phil McClean reported that the response to the Advisory Committee report was sent to Fred Bliss of the committee and Ed Kaleikau (USDA/NIFA). The McClean lab has been using the data from the first 1536 SNP chip to test different association mapping models. The goal is to settle on several models before the field and lab data for the AM panel arrives. The first indel markers for mapping within the pinto marker class are being tested.
2. Mike Grusak reported that the 154 snap bean pod samples from Jim Myer's replicated trial have been fully analyzed for minerals. The data showed a good range (2x-5x) which is consistent with other reports. Jim Myers reported that the fiber analysis on these samples is now complete. The dry bean samples from Miklas and Kelly were received by Grusak, and all samples were ground. These samples were then distributed to Cichy and Tulbek for additional nutritional analysis. The Kelly samples have been analyzed for mineral content and the data is being analyzed.
4. Perry Cregan reported that the 6K SNP chip has been delivered. The first initial test will be with the core 192 sample previously screened using the 1536 SNP chip. We should expect that 75-80% of the SNP will give good data. McClean will get DNA from the Stampede x Redhawk population for mapping purposes to support the sequencing project. Cregan reported that 1500 SSRs, identified from the sequencing of 18 genotypes, are being tested on 16 diverse (10 Mesoamerican, 6 Andean) genotypes.
5. Paul Gepts reported that that *in silico* information is becoming available for SSR. 160,000 SSR have been defined from sequence information. Primers were defined for this set. Included in the database are the following data for the currently available SSRs: primer Tm, sequence of fragment from which SSR was derived, repeat sequence, number of repeats, source of information, URL of publication.
6. Jim Kelly reported that the seed has been distributed for the 2011 national field trial. Seminis has performed all of the seed treatments, packaging, and delivering. The trial with 300 genotypes (258 Mesoamerican; 42 Andean) will be grown at Michigan, Nebraska, North Dakota, and Colorado. The final MS Excel file is being shared for the development of field books. Issues regarding field design (lattice or other) are being resolved. The drought trial (100 genotypes) will be performed in Colorado, Idaho, Nebraska, and Washington using irrigated vs. non-irrigated treatments for comparison. Trials will also be performed in North Dakota and Michigan. Those trials will depend upon rain/no rain conditions.

7. Juan Osorno reported that nearly all positions have been filled for the internship program this summer.

8. Stacy Halvorson reported that regional and national presentations will be made regarding the extension component of the BeanCAP project. Featured will be the bean bingo game. Pilot studies are underway to introduce pre-K children to beans as a food. Included will be a bean growing activity.

9. The next conference call is tentatively scheduled for Thursday, May 5 at 11 am (east), 10 am (central), and 8 am (west).



A USDA/NIFA Supported Project

Minutes of May 5, 2011 BeanCAP Conference Call

Participants: Perry Cregan, Julie Garden-Robinson, Mike Grusak, Phil McClean, Jim Myers, Juan Osorno

1. Phil McClean reported that he is waiting for instructions regarding the renewal. He will alert everyone to the process once he receives that information. The McClean lab is continuing to evaluate multiple statistical models to develop one that best accounts for structure within the BeanCAP genotypes. Traits that are being tested include color/no color (P locus), string/stringless (St locus), and the seed iron data from Kelly's 2010 grow out. The second animation is nearing completion. Artwork for the second documentary is being developed.
2. Mike Grusak reported that the Miklas field grown lines (large subset of those grown by Seminis) are nearing to be ready for IPC analysis. Those materials are in the analysis pipeline. The CaCo2 cell line experiments are underway, and results are anticipated in the next 8-10 weeks. Mike, Phil, and Juan will communicate to develop a method of receiving phenotypic data that will then be incorporated into spreadsheets to be used for the association mapping analyses.
3. Julie Garden-Robinson reported that a poster describing the BeanCAP extension activities was accepted for the National Extension Family and Consumer Sciences conference. The project information will be disseminated to ~500 individuals. New artwork was developed, and that will be available for others in the project for their posters. Two new videos were developed that feature beans and bean recipes. A pre-K project will soon be launched to provide kids the opportunity to grow beans (along with other crops) in a community garden. The end goal is to develop a curriculum for students to become more involved with their foods, with beans being one of the focuses. Jim Myers and Juan Osorno will help Julie by providing seed for the garden project.
4. Jim Myers reported that that the pod mineral data has been received. That data is being evaluated currently. Fiber data has been collected for those materials. HPLC and spectrophotometric procedures are being considered for the carotenoid characterization. One possible route is to use the spectrophotometric procedure for total carotenoid evaluation on the full set and then use a subset for the HPLC analysis. The full BeanCAP snap trial has been planted (n=150). Those will be grown to seed to test for mineral content.
5. Juan Osorno reported that the field trial is set. It will consist of 300 Mesoamerican cultivars grown in ND, MI, NE, and CO. It will be planted in an incomplete block design. Because of the severe population structure for common bean, it was decided to restrict the trial to Mesoamerican cultivars. A side trial of 47 Andean lines will also be grown.

6. Perry Cregan reported that the 6K SNP chip has been delivered, and data has been collected for the 192 genotype set. The chip consists of 5399 SNPs, and a success rate of 87% (=4697 SNPs) has been observed. This is a high success rate. Decisions will be made based on the 192 set data regarding the SNP data sets to be used for mapping other genotypes. Phil will get F5:6 Stampede x Redhawk lines to Perry for mapping purposes. The raw sequencing data from the 18 cultivars (Illumina 150bp, paired-end) will be available soon.



A USDA/NIFA Supported Project

Minutes of September 27, 2011 BeanCAP Conference Call

Participants: Perry Cregan, Paul Gepts, Mike Grusak, Phil McClean, Jim Myers, Juan Osorno, Qijian Song

1. The final two years of the project have been approved. NDSU is in the process of creating the subawards.
2. Fred Bliss resigned from the Advisory Committee. Steve Beebe was mentioned as a potential alternative.
3. Perry Cregan announced that 1575 SNPs were released to the Generation Challenge Program. They will work with KBioscience to develop a set of primers that is appropriate for their technology. That will then be made available world-wide.
4. Phil McClean announced that the first set of 180 indel markers for the pinto market class have been released. These are based on Buckskin, Sierra, and Stampede polymorphisms. An additional 1034 were developed using *in silico* approach. 22 were confirmed using a collection of genotypes.
5. The genome sequence of G19833 is in the final assembly.
6. Carlos Urrea will be invited to attend the 2012 Advisory Committee meeting. Juan Osorno will recruit a student from his program to attend.
7. Mike Grusak, Juan Osorno, Jim Myers, and Phil McClean will discuss the format for data submission for project analysis.
8. Perry Cregan reported that ~5000 SNP markers have been designed and 85% are functioning properly. A decision regarding the format used for SNP screening (one OPA set for all market classes vs. market-class specific OPAs) is pending pricing from Illumina.
9. Perry Cregan will see if the Genome Studio software is freely available. If so, individual labs will look into performing their own SNP evaluations. This has worked successfully with soybean researchers.
10. Paul Gepts reported that QTL data continues to be integrated into the PhaseolusGenes database. They are investigating the SolCAP QTL model for display purposes.

11. Jim Myers has completed the nutritional data analysis for snap beans. This includes: fiber, minerals, flavonoids, vitamin C, and carotenoids. Materials have also been packaged and sent to Mike Grusak for mineral evaluations.

12. Mike Grusak reported that mineral evaluations and CaCo₂ cell tests are on-going.

13. Juan Osorno noted that the Stampede x Redhawk is showing many morphological differences that can be mapped. He also reported that the drought trials in WA, ID, and NE are looking very good. The ND trial was abandoned because of excessive rain.