



A USDA/NIFA Supported Project

**Common Bean Coordinated Agricultural Project
2013 Advisory Board Meeting
Thursday, January 10, 2013**

Handlery Hotel San Diego
950 Hotel Circle North
San Diego, California 92108 USA

Time	Topic
10:30 - 10:50 am	BeanCAP and Sequencing Project Synergy: Phil McClean
10:50 - 11:10 am	Multimedia Development: Phil McClean/Christina Johnson
11:10 - 11:50 pm	Breeding Education: Juan Osorno/Scout Wilson
11:50 - 12:10 pm	Outreach: Julie Garden-Robinson
12:10 - 12:30 pm	Database Applications: Paul Gepts
12:20 - 1:30 pm	Lunch
1:30 - 1:50 pm	Snap Bean Analysis: Jim Myers
1:50 - 2:00 pm	Phenotyping, 2013 Plans: Jim Kelly
2:00 - 2:20 pm	Drought Stress Analysis: Tim Porch
2:30 - 2:50 pm	Marker Development/Genotyping: Perry Cregan
2:50 - 3:10 pm	Association Mapping: Samira Moghaddam
3:10 - 3:30 pm	Nutrition Analysis: Mike Grusak/Jim Myers
3:30 - 3:45 pm	Break
3:45 - 4:00 pm	Committee Consultation
4:00 - 4:15 pm	Committee Oral Report



A USDA/NIFA Supported Project

BeanCAP Project Reports 2012 Activities

Annual Advisory Board Meeting
January 9, 2013
San Diego, California

BeanCAP
Common Bean Coordinated Agricultural Project
Progress Report and Work Plan
1/1/2012 – 12/31/2013

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

Progress During this Reporting Period (1/1/2012 – 12/31/2012)

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

Research

Data from a large national association mapping (AM) trial (n=300; locations = 4) was analyzed using multiple statistical models that account for population structure and genotype relatedness. Using the GAPIT software environment, we analyzed seven agronomic traits: days to flower, days to maturity, growth habit, lodging, plant height, seed weight, and seed yield. A total of 30 significant peaks were noted for these traits, and one region one Pv01 appeared to affect multiple traits. In a separate trial, eleven root traits, many related to basal root traits known to affect performance, were evaluated. A total of 37 significant peaks were observed. For both of these analyses, significant factors were noted on all chromosomes. A subset of the national trial was grown under normal and terminal drought conditions. For some traits, the same loci were significant under both conditions. Additionally, many of the same loci were significant under normal conditions in this trial (n=96; locations = 5) and in the national trial with three times the number of genotypes.

The original set of ~10,200 SNP markers were used by Dr. Cregan's team to genotype an F2 and recombinant bred population and develop a dense genetic map of common bean. This map was integral to the assembly of the common bean genome sequence data into chromosomal level scaffolds. Using the mapping coordinates along with a large suite of genotypes (>500) representing the major common bean marker classes, our group measured linkage disequilibrium (LD) across the genome. LD was highly dependent on the population level evaluated. For example, across the entire collection, that represented genotypes from Mesoamerican and Andean origin, LD decayed to $r^2 < 0.5$ within 100kb. In contrast, when LD was analyzed within a genepool, these distance were at the megabase level. LD also differed between chromosomes at any population level.

A set of >2800 indel markers was developed and released to bean breeding and genetics community. These were developed as a gel-based, medium-throughput alternative to the SNP system developed by the project. In addition, they were developed to provide markers specific to a common bean market class of importance to the USDA dry bean economy. The main features of the indel marker collection are: a single, common reaction condition; gel-based screening capability; and market class specificity. The BeanCAP WWW site (<http://www.beancap.org>) is serving as the portal for the project. Shared results are released there prior to publication. For

example, all of the indel marker information is available pre-publication (www.beancap.org/Indel-Markers-bean-NDSU.xls).

High and low recombination regions were defined in common bean by merging the Version 1.0 release of the common bean genome and the high-density genetic map developed by Dr. Cregan's team. The following table summarizes the data for various regions of the common bean genome.

Table 1. Physical and genetic boundaries of the common bean genome in relationship to gene density.

Chromosome	Recomb level	Physical boundaries		Genetic boundaries		Kbp/cM	# of Genes	# Genes/Mb
		Start	End	Start	End			
Pv01	High	0.29	7.48	0.00	24.62	292.0	611	85.0
	Low	8.05	37.77	25.50	29.21	8,010.8	727	24.5
	High	37.79	52.18	29.21	84.03	262.5	1,356	94.2
Pv02	High	0.00	4.59	0.00	47.77	96.0	492	107.3
	Low	5.24	25.81	49.31	54.92	3,666.7	794	38.6
	High	27.19	49.03	58.29	127.60	315.1	2,052	94.0
Pv03	High	0.11	5.89	0.21	38.86	149.5	489	84.6
	Low	6.37	32.02	40.52	49.84	2,752.1	817	31.9
	High	32.29	52.55	50.60	116.94	305.4	1,667	82.3
Pv04	High	0.03	5.70	0.00	37.99	149.2	489	86.3
	Low	5.98	39.52	38.92	48.87	3,370.9	743	22.2
	High	40.77	45.79	51.95	94.01	119.4	557	111.0
Pv05	High	0.01	5.17	0.00	43.49	118.6	468	90.8
	Low	5.17	33.02	44.04	50.69	4,188.0	667	23.9
	High	33.61	40.24	51.99	90.80	170.8	728	109.8
Pv06	Low	0.04	14.43	0.00	2.36	6,095.8	364	25.3
	High	17.08	31.97	6.76	70.76	232.7	1,857	124.7
Pv07	High	0.06	10.22	0.00	51.64	196.7	970	95.5
	Low	10.72	40.72	52.11	56.92	6,234.4	766	25.5
	High	41.80	51.59	59.54	104.27	218.9	1,076	109.9
Pv08	High	0.13	7.82	6.73	51.97	170.0	815	106.0
	Low	8.01	49.14	52.33	61.96	4,271.0	1,062	25.8
	High	49.96	59.63	63.51	114.01	191.5	1,056	109.2
Pv09	Low	0.13	7.70	0.00	3.22	2,350.2	367	48.5
	High	8.75	37.40	5.71	94.60	322.3	2,265	79.1
Pv10	High	0.06	7.87	1.23	8.37	1,093.8	498	63.8
	Low	8.08	34.92	8.56	10.78	12,090.1	463	17.3
	High	35.06	43.21	11.16	60.17	166.3	698	85.6
Pv11	High	0.02	8.86	0.00	51.42	172.0	899	101.7
	Low	9.28	41.12	52.04	57.39	5,951.4	660	20.7
	High	41.32	50.20	57.77	78.55	427.3	610	68.7

Education

Mini-documentaries that feature various aspects of plant breeding have been released via the BeanCAP@NDSU YouTube channel (<http://www.youtube.com/user/ndsubeancap>). These

include the following topics: Plant Breeding and Food Security (http://www.youtube.com/watch?v=7zPK_GI03N0&feature=plcp); Norman Borlaug and the Green Revolution (<http://www.youtube.com/watch?v=Lg9-HTtgFOk&feature=plcp>); Genetic Variation and the Story of Stem Rust (<http://www.youtube.com/watch?v=kd6B706ByZg&feature=plcp>); and Bean Plant Architecture (http://www.youtube.com/watch?v=wf_nOs7DP-o&feature=plcp). These were developed to augment the Plant Breeding Education efforts.

Two plant breeding mini-documentaries were used in an on-line survey with two sections of a Genetics course (junior level) to determine students' knowledge of plant breeding and their career aspirations in plant breeding before and after viewing the documentaries. Analysis showed that students exhibited a significantly increased knowledge of the key concepts presented in the short videos. Answers to qualitative questions indicated that the statistics knowledge of world hunger and the role of plant breeding was "eye-opening". 10% of students "somewhat agreed", "agreed" or "strongly agreed" that at some point in the future they plan to enroll in a plant-breeding program.

School visits continued this year with visits to two high schools in Fargo. Total attendance at those two visits was ~75 students. Two summer students and one full time student were recruited into the program this past year.

Outreach

A preschool gardening curriculum was developed and piloted with 13 families at the NDSU Center for Child Development as part of a "Junior Master Gardener" program. This project expands on the "Spilling the Beans" Preschool Project completed in 2011. The "*Now Serving: Beans*" lesson plan was developed. All of the materials related to this are available at: <http://www.beancap.org/Extension.cfm>. It was tested in multiple classrooms and was well received. Multiple bean based recipes were released in written and video format. See the BeanCAP @ NDSU YouTube Channel to view the videos.

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

- Indel marker set: these new markers meet the project goal of developing markers for important US market-classes.
- Association mapping of agronomic traits: this provides a comprehensive look at the loci of importance for performance of cultivars in the Mesoamerican gene pool.
- Linkage disequilibrium measures across multiple population types: this informs breeders and geneticist about the marker density they should be considering when working with a specific type of common bean population.
- Physical/genetic distance relationships: this result informs geneticists on the marker and population size requirements that will be necessary for the development of a markers to a specific trait relative to the gene's position in the genome.

- Three mini-documentaries were developed that feature several features of plant breeding: this learning tool points to relevance to plant breeding from a social and economic aspects and can be used in educational settings to help recruit students into the plant breeding field.
- A preschool gardening curriculum that features common bean: this expands the understanding of beans and their importance for nutrition to a new, younger audience.

Plans for Upcoming Reporting Period (1/1/2013 – 6/30/2013)

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

Research

We will continue the association mapping analyses with a focus on the nutritional traits (minerals, fiber, protein, oil, phytate) data as it arrives.

Education

The traineeship programs will continue. High school visits will continue. Additional volumes of the plant breeding mini-documentary series will be developed. Currently in the pipeline are one that features graduate students' perspectives on breeding and their perceptions regarding the future of the profession. The second volume will focus on the general steps a plant breeder makes in developing a variety. The third volume will focus on the rationale that a plant breeder brings to decision making during a pedigree breeding program that is popular for common bean breeding programs.

Outreach

Beans will continue to be promoted as a healthy food. Several media formats are currently being conceived.

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 results that focus on nutritional traits
- Student intern training
- High school recruitment
- Three mini-documentaries focusing on plant breeding
- Extension tools that promote the nutritional benefits of common bean

BeanCAP
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Progress Report and Work Plan
1/1/2012 – 12/31/2012

Michigan State University
James D. Kelly
Karen A. Cichy

Progress during this Reporting Period (1/1/2012 – 12/31/2012)

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 and root traits, and harvest of 96 dry bean genotypes chosen for study as part of the beancap genomic association mapping project on drought. The two experiments were planted side-by-side with and without supplemental irrigation at the Saginaw Valley Research and Extension Center at Frankenmuth, MI. Plots not supplied with any supplementary irrigation experienced early season drought through late July. Seasonal rainfall (June-Sept.) was 259mm or 54mm below the 30-year average (313mm) 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 (576) 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 one step rod weeder 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 between treatments and compared with performance under drought stress.

We have completed analysis of phytic acid levels in 950 bean samples from the 2011 field season. An additional 300 lines have been prepared for phytic acid analysis. These lines are part of a drought experiment grown in 8 locations throughout the U.S consisting of 4,000 total samples. The average phytic acid phosphorus level of the lines we have analyzed thus far is 4.86 mg g⁻¹. Phytic acid phosphorus levels ranged from a low of 2.49 mg g⁻¹ to a high of 7.17 mg g⁻¹. We compared the phytic acid levels of 94 lines grown in drought stressed and non drought stressed conditions and found significant genotype and environment effects, but no significant genotype x environment interaction. Mean phytic acid phosphorus levels were higher under drought stress at 5.15 mg g⁻¹ vs. 4.80 mg g⁻¹ in non drought stress conditions. A negative correlation was identified between cooking time and phytic acid levels in a screening of 100 bean lines (r= -0.45, p<.0001). This suggests that attempting to lower phytic acid levels to improve micronutrient bioavailability may have unforeseen consequences on culinary quality.

Undergraduate students trained in the program included Cynthia Amstutz, Lucas Costanza, Mary Harris and Yusong Mu and more complete information on their projects 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 four 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 150 Indel markers screened to date, 54 were polymorphic. The level of polymorphism (27%) was slightly lower than for SSR markers (32%) 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. The indel markers were combined with polymorphic SSR markers to produce a genetic map (1386 cM) of the population on which a major QTL for resistance to leafhopper was confirmed on bean chromosome Pv07. Other QTL identified on four other chromosomes were novel and could be used in marker-assisted breeding for resistance to leafhoppers. In a second RIL population (SEA5 x CAL 96) constructed to study drought in Andean beans, a small group of 32 indels were selected to complete the mapping of chromosomes Pv01, Pv04, Pv07. Only 12 indel were polymorphic (32%) and these were combined with SSR markers to construct a genetic map (1031 cM) of the population. A major QTL for yield and seed size expressed under drought was identified on Pv11. Most other QTL identified in the study were minor and should be verified. QTL analysis was conducted on two pinto populations derived from crosses of two pinto breeding lines differing in reaction to white mold. A total of 154 Indel markers were screened from within the 72/154 Indel markers that were polymorphic between Type-II pintos at NDSU. We detected only a 20% 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 54% polymorphism detected with SSR markers in the same AP630 and AP647 populations. A genetic linkage (727 cM) was constructed in the AP639 population using both Indel and SSR markers and a major QTL for white mold resistance on Pv02 and QTL on Pv07 were confirmed. A unique QTL for seed yield under white mold pressure was detected on Pv05 near the Indel marker NDSUind-Pt0251. Another Indel marker NDSUInd-Pt013 on Pv07 near the WM QTL was detected and may be easier to genotype than the current SSR markers being used in marker assisted selection.

Identified a bean line (CDC Whitecap) with nearly 50% less phytic acid than the average levels found in bean seed. Drought stress increased seed phytic acid phosphorus levels by seven percent.

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 (1/1/2013 – 6/30/2013)

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 the same 96-entry field drought trial this coming summer in Michigan. The same trial that was grown under drought and non stress will be repeated in 2013 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. Analysis of SNP data performed on five RIL populations segregating for reaction to drought, white mold, potato leafhopper pest and expression of nitrogen fixation will be conducted to confirm QTL mapping previously conducted with SSR and Indel markers. Continue to expand the utility of the beancap portal with input from beancap members as more information and data become available.

Continue phytic acid analysis on 3,050 bean samples from the 2011 growing season. This data will be analyzed to determine the genotype and environment influence on phytic acid levels. It will also be used for association mapping. Continue to evaluate the link between phytic acid and cooking time in beans,

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. Information on the genotype, environment, and genotype x environment interaction of bean seed phytic acid levels will be available. Identification of bean lines with low phytic acid levels to be used in breeding lines with superior mineral bioavailability.

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1/1/2012 – 12/31/2013

James R. Myers
Oregon State University

Progress during this Reporting Period (1/1/2012 – 12/31/2012)

A. Research, education, and/or outreach activities completed in this reporting period

The OSU program focused on data compilation and completing analysis of remaining carotenoid samples for the snap bean panel. Data for vitamin C and carotenoids has been assembled and is ready for combination with other phenotypic data. We also supplied seed of the snap bean panel to Mike Grusek for analysis of seed mineral content. The snap bean panel was not grown in the field for nutritional or phenotypic data acquisition. However, we did plant 139 entries of the snap bean panel (vining types excluded) in a white mold (*Sclerotinia sclerotiorum*) field screening nursery and collected data on disease incidence and severity, and on traits associated with disease avoidance. Data has been provided to NDSU for association mapping. DNA of a green bean x dry bean recombinant inbred population segregating for Fusarium root rot resistance was prepared and sent to Perry Cregan for genotyping with the 10K Illumina SNP chip. The data received from Cregan's lab is being used to construct a genetic map and map QTL for root rot resistance.

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

Phenotypic data from 2010 and 2011 for the snap bean panel has been compiled and provided to NDSU.

C. List publications (refereed, non-refereed, meeting abstracts)

None

D. List all personnel associated with the project.

Joel Davis (faculty research assistant)
Christina Hagerty (graduate research assistant)
Ceely Will (undergraduate, senior research thesis project)

Plans for Upcoming Reporting Period (1/1/2013 – 6/30/2013)

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

We plan to provide DNA samples of additional mapping populations and advanced breeding lines to the Cregan lab for genotyping. We will also cooperate with the NDSU group to conduct association mapping studies on the phenotypic and nutritional data sets.

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.

We plan to develop several manuscripts based on the association mapping studies of snap bean phenotypic and nutritional traits. These will include phenolics and associated color traits, carotenoids and vitamin C, flavor and fiber traits, pod and plant traits, white mold disease, and root rot QTL mapping.

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1/1/2012 – 12/31/2012

University of California, Davis
Paul Gepts

Progress during this Reporting Period (1/1/2012 – 12/31/2012)

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

Development of the PhaseolusGenes database

This past year has seen a major shift in the development of the PhaseolusGenes database with the development of a whole-genome sequence (WGS) for *Phaseolus vulgaris*. This sequence, developed by the companion project to the BeanCAP, was obtained from accession G19833 (Andean gene pool; race Peru) and consists of 11 pseudomolecules and has been made available with conditions on the Phytozome web site (www.phytozome.net). Co-directors of that project are Scott Jackson, Jeremy Schmutz, and Phil McClean.

Up until now, the genome browser in the PhaseolusGenes database was anchored onto the soybean whole-genome sequence (Schmutz et al. 2010), also available from the Phytozome web site. Because of close phylogenetic relationships at the legume sub-family level (Papilionoideae tribe Phaseoleae; Gepts et al. 2005), the two genomes show synteny over large chromosome blocks (McClean et al. 2010), making the anchoring of common bean sequences possible on the soybean genome. There were nevertheless some difficulties that were dealt with. First, the soybean genome is allotetraploid (some 15 Mya; Schlueter et al. 2004; Shoemaker et al. 2006) whereas the common bean genome is diploid. As a consequence, soybean is an allotetraploid. For every Phaseolus sequence, one should expect two soybean sequences, barring deletions or further duplications since the polyploidization event. Additional chromosomal differences arose during the diploidization phase to assure bivalent pairing in the newly formed tetraploid. Thus, although one expects an overall background of synteny between these related legumes (Hougaard et al. 2008; McClean et al. 2010), repeated rearrangements could limit the scope of this synteny to smaller chromosomal segments. From the viewpoint of the BeanCAP project, however, microsynteny remained the most important focus in order to identify tightly linked markers, either for de novo marker development or for the identification of alternative markers. These difficulties would be eliminated with the anchoring of the genome browser on a common bean sequence.

This was achieved by downloading the sequence information from Phytozome. Substantial additional discussion ensued at UC Davis as to how to manage two WGS in the same database. Because of the benefits provided by the two sequences, it was decided to develop a second genome browser instance based on the bean WGS, in parallel with the earlier instance based on the soybean WGS. Thus, PhaseolusGenes users now can choose to place their sequences by

g2303

Type: Marker | Browser links: (1) (2) (3) | CMap link

Reference 871
Id
Marker g2303

DescriptionUTR3; Best A thaliana hit: At3g29301;Annotation (inherited from A thaliana) : expansion, putative (E05);False REFSEQ De GenBank accession : E510097;Forward primer : GGGGCGAATCAGGTCACCA;Reverse primer : GGTTTAGGACAACTAATGAGAGTGATACCGT result : -.Polymorphic populations : A55/G122;Source : Genomic; feature_aliases : Pu-23038;

Sequence CTATTGCTCTGAAAGTGAATTTTCTGATGGATTTTGAATGAAAGGTAAGGTTCC
 AGAAGAAGGGGGGAATCAGGTCACCACTCAATGCTCATTACACTCAACTAAGTCCCTT
 GTGACTAATGTTGAAAGTCTGCTGATGATGCAATTCCTGGCCTCAAAGGTTCAAGGAGT
 AGATGGCAAGCTATCTCAAGGATTTGGGGCAAACTGGCAGAGTAATTCCTACTTTAAT
 GGACAGATCTCTCTTTTGGTACCAGCAAGTGAATGGGGGAGTGTCTCTCAATAAAT
 CTCCACCAAGCAAGTGGTCTCTGGCAAACTGACTGGAAGGCTATTCTCTACTAAC
 CAACCTTACATGGTACTACTCTACTTA

Blast this sequence (FASTA file):
 P. vulgaris v.1.0 (Anderson:G19833) --- Soybean

Organism phaseolus vulgaris

Genbank E510097
Code
Forward Primer GGGGCGAATCAGGTCACCA
Reverse Primer GGTTTAGGACAACTAATGAGAGTGATACCGTGT

Linkage 4
Group
Reference McConnell et al. 2010

Hyperlink 1. <http://www.biomedcentral.com/2473-2164/13/194>
 2. http://lis.comparative-legumes.org/cgi-bin/cmap/viewer?mapMenu=&map.FeatureMenu=&map.coordMenu=&map.displayMenu=&map.advancedMenu=&map.ref_species_acc=Pv&map.ref_map_set_s=&selected=54%27&+Map&map.prev_ref_species_acc=Pv&map.prev_ref_map_set_acc=0&map.highlight=&map.pixel_height=&map.image_tsz=png&map.da
 3. <http://www.springerlink.com/content/0v4672336m024038/>

Figure 1. Screen image of the PhaseolusGenes marker database showing links (surrounded by green ellipse) to the *Phaseolus vulgaris* and soybean genome browser instances of this database.

BLAST against the bean or soybean WGS (Fig. 1). In turn, these links lead to a pre-calculated or *de novo* BLAST analysis to either of the two genome browser instances.

In turn, the two genome browsers include different types of information (“tracks”) (Tables 1 and 2). Although the track arrangement of the soybean instance is stable for now (except for normal future changes as the need arises), the track arrangement for the common bean instance is still being changed pending further discussions and as the need arises. BLASTing of all Phaseolus sequences has been accomplished at a markedly lower threshold value against the common bean WGS (e-20) than the soybean WGS (e-4). The justification is that there is overall stronger homology against sequences of the species than sequences of a different genus.

Concurrently with the re-building of the PhaseolusGenes database, additional information and markers have been added to the marker database. These include: Addition of the first SNP markers to the database: Hyten et al. 2010 (n = 3487) Addition of indels developed by the McClean group (n = 2687). These markers have already been added to the *P. vulgaris* genome browser; however, display in the actual searchable marker database is forthcoming.

Table 2. Tracks included in the soybean genome browser instance of PhaseolusGenes

Track name	Description	Source
centromere	Soybean centromere	www.soybase.org
pericentromere	Soybean pericentromere	www.soybase.org
Phaseolus vulgaris sequences		
Pv_ssr_predictions	Alignments of <i>P. vulgaris</i> cv. BAT93 reads containing predicted SSRs (QDD, SSRIT, SSRFinder)	Current
Pv_BAT93_assembly	All <i>P. vulgaris</i> sequences resulting from the 1X methyl-filtrated dideoxy sequencing	Current
Bat93_hsp_evalue	Average (-log) Evalues of HSPs over sliding window (1000bp)	Current
Bat93_hsp_evalue	Average (-log) Evalues of HSPs over sliding window (100000bp)	Current
Pv BAT93 alignments	<i>P. vulgaris</i> cv. BAT93 read alignments to <i>G. max</i>	Current
Phaseolus vulgaris tracks		
scar_markers	SCAR markers from PhaseolusGenes	Current
ssr_markers	SSR markers from PhaseolusGenes	Current
sts_markers	STS markers from PhaseolusGenes	Current
pv_jcvi_2	<i>P. vulgaris</i> EST (common bean, JCVI)	http://plantta.jcvi.org/ cgi- bin/plantta_release.pl
pv_mcclean	<i>P. vulgaris</i> EST (common bean, McClean)	www.soybase.org
pv_bac_clones	<i>P. vulgaris</i> BAC clones	www.soybase.org
Phaseolus coccineus tracks		
phaseolus_coccineus_2	<i>P. coccineus</i> EST (runner bean, JCVI)	http://plantta.jcvi.org/ cgi- bin/plantta_release.pl
cowpea_assembly	<i>V. unguiculata</i> (cowpea) GSR assembly	Timko et al. 2008
cowpea_gsr_reads	<i>V. unguiculata</i> (cowpea) GSR reads	Timko et al. 2008
vigna_unguiculata	<i>V. unguiculata</i> EST (cowpea, JCVI)	http://plantta.jcvi.org/

Table 2. Continued

Track name	Source	Reference
<i>Glycine max</i> (Soybase) tracks		
glyma1	<i>G. max</i> gene models	www.phytozome.org
old_duplication_blocks	Old duplication blocks (soy-soy 58 Mya)	www.soybase.org
recent_duplication_blocks	Recent duplication blocks (soy-soy 13 Mya)	www.soybase.org
syteny_blocks_to_Medicago	Syteny blocks (with <i>Medicago</i>)	www.soybase.org

Plans for Upcoming Reporting Period (1/1/2013 – 6/30/2013)

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

1. BLASTing of 1x methyl-filtrated BAT93 (Mesoamerican) sequencing produced by Sanger sequencing against the existing common bean whole-genome sequencing (based on the Andean G19833 accession). Whenever the two other WGS become available (Iberia-American project: on Mesoamerican accession BAT93; Ontario, Canada, project on Mesoamerican accession OAC Rex, introgressed with *P. acutifolius* germplasm).
2. Addition of newer SNPs as these become available: BeanCAP SNP platform (n = ?), Blair et al. 2012 (n = 768), Galeano et al. 2012 (n = 173), as well as those that will arise from sequence comparisons between the Andean and Mesoamerican WGS comparisons
3. Addition of reduced representation or re-sequencing efforts. This includes the first RAD (restriction-associated DNA) lima bean sequences, developed at UC Davis, and any other sequences that will be published
4. Place QTLs on the CMap instance of PhaseolusGenes; they are already included in the marker database.
5. Speed up the initial search.

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BeanCAP
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Progress Report and Work Plan
1/1/2012 – 12/31/2012

University of Nebraska-Lincoln
Carlos A. Urrea

Progress During this Reporting Period (1/1/2012 – 12/31/2012)

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

On January 10, during the Nebraska Dry Bean Growers Association day, I informed about the BeanCAP activities including the experiments on drought to 200 dry bean growers.

On January 12, I was invited to the BeanCAP Advisory and Steering Committee meeting at San Diego, CA. I talked about Nebraska BeanCAP activities.

On February 9 and 10, I was invited to the Scottsbluff High School Biology class to talk about Mendelian genetics. About 128 students were informed about BeanCAP activities. High students were encouraged to visit the dry bean breeding program.

On March 3, during the Rocky Mountain Bean Dealers Association Meeting at Englewood, CO, I informed about the BeanCAP activities in Nebraska.

On March 6, during the Stateline Producers Cooperative meeting, I informed about the BeanCAP activities to 100 dry bean growers.

On April 3, we hosted 31 members of the Nebraska LEAD Tour. We talked about dry bean breeding activities in western Nebraska. The BeanCAP program was discussed.

On June, I was invited to an International Workshop on Drought to Colorado State University to discuss findings on drought. BeanCAP experiments were discussed. Sixteen students participated in the workshop.

On June 30, we hosted a Chinese Food Science Trade Team. Skye Martin (BeanCAP) student cooked beans in a Matson Cooker and explained the experiment to five people.

One undergraduate student joined the BeanCAP internship program between May and August. Students were involved in all dry bean breeding activities.

From August up to date two undergraduate students from Western Nebraska Community College are working under the BeanCAP internship.

On August 23, I discussed BeanCAP experiment on drought during the Nebraska Dry Bean Growers Field day. About 130 growers attended.

On May 22, 40 people attending the Road Scholar Tour were informed about the BeanCAP activities.

On September 15 and 16, we participated in the University of Nebraska Expo Day hosted by the Farm and Ranch Museum in Gering NE. About 400 people visited our booth displays. Scout Wilson (BeanCAP student) had some hands on how to extract DNA from strawberries and talked about the BeanCAP activities.

I conducted one BeanCAP experiment at Mitchell, NE in replicated trial. Stampede/Red Hawk RIL mapping population was tested under drought and non-drought conditions. For the non-drought experiment, irrigation was stopped at flowering stage. Data on growth habit, days to flowering and maturity, seed yield, and 100-seed weight was collected.

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

Some Scottsbluff/Gering high school students expressed their interest to work during summer 2013 for the dry bean breeding program.

Three undergraduate 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 bacterial blight, bacterial wilt, bacterial brown spot, and bean common mosaic virus. Cooking test and molecular work in the lab was part of the training. The students were able to create variability through hybridization. Most of their combinations were truly hybrids.

Three articles were published in local newspaper as follows:

- Hansen, S. 2012. Bean fields first step to molecular lab studies for local teenager. StarHerald, Scottsbluff, NE. May 27, 2012.
- Otsdiek, D. 2012. Developing drought tolerance is one goal of dry bean breeding program. StarHerald, Scottsbluff, NE. May 27, 2012.
- Hansen, S. 2012. Dry bean take area student on educational journeys. StarHerald, Scottsbluff, NE. November 25, 2012.

Plans for Upcoming Reporting Period (1/1/2013 – 12/30/2013)

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

Two to three undergraduate students will be carrying out projects in the dry bean breeding program. They will be involved in bacterial wilt and bacterial brown spot screening and

fingerprinting bean breeding lines to molecular 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 2013. 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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1/1/2012 – 12/31/2013

Colorado State University
Mark A. Brick, Henry Thompson, and Dimas Echeverria

Overall objectives:

The overall objectives of research activities include development of research tools to better understand genetic and genomic factors that control nutritional and agronomic traits in dry edible beans. At Colorado State University, we focused on evaluating dry bean lines for soluble dietary fiber (SDF), insoluble dietary fiber (IDF), total dietary fiber (TDF), and oligosaccharide content as well as conduct a replicated field trial at Fort Collins to measure agronomic traits and yield in a diverse set of dry edible bean lines/cultivars. These objectives will be accomplished by utilizing association mapping among 300 dry bean and 200 snap bean lines for nutritional characteristics and agronomic traits. All of the lines will be genotyped using SNP markers and that data will be used for the statistical analyses to discover loci associated with the nutritional traits under study.

Progress during this Reporting Period (1/1/2012 – 12/31/2012)

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

In year 3, the project at CSU focused on two major projects. First, the seed yield and agronomic data collected in 2011 was compiled and incorporated into a spreadsheet then sent to NDSU for compilation, statistical analysis and sharing with other BeanCAP cooperators. Second, we applied the 2009.01 CODEX protocol to determine soluble dietary fiber (SDF), insoluble dietary fiber, total dietary fiber (TDF), and oligosaccharide (OLI) content on 75 % of the samples from replicates 1 and 2 of entries grown in Fort Collins in 2011. The assay takes three days to complete and we can only conduct 6 assays per day, so the entire year was spent running the assay bean entries. Results suggest significant difference among cultivars for SDF, IDF, TDF and OLI content and TDF varied from approximately 14 % to 23% among entries. The differences among entries indicate a great deal of genetic diversity in fiber content in the species.

We also developed a modified protocol to determine oligosaccharide content of bean entries. This involved a modified HPLC technique than the Codex 2009.01 method. The new technique is more efficient and better estimates individual components of oligosaccharides in dry bean. Preliminary estimates for oligosaccharide content are approximately 2.5 to 3% among the lines tested. In 2013, we will evaluate two replicates for all 300 entries OLI content to provide a complete estimate of TDF 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 and to use in association mapping studies and SNP discovery.

Total phenolic (TP) content was continued on 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.

B. Deliverables and outcomes achieved during this reporting period.

1. Research

- Data compiled 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. The data set was sent to Juan Osorno in Jan 2012 to determine if these traits may be correlated with nutritional traits and association mapping in years 3 and 4.
- Evaluated two replicates for more than 250 dry bean lines for IDF, SDF and TDF
- Evaluated additional snap bean lines for phenolic content
- Supervised a research associate to develop phenolic and fiber 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 (Emily Troxell and Donny Hodgkinson) on the Dry Bean Breeding Project.
- Supervised and trained one high school intern (Nathan Pohl) during summer 2012 on the Dry Bean Breeding Project.
- Trained a research associate on a modified analysis for oligosaccharide content using the AOAC Codex 2009.01 as a starting point.
- High School intern presented the experiences of his summer interns at his high school.
- Presented two reports at scientific meeting regarding Bean CAP activities.
- Undergraduate student intern Donny Hodgkinson was selected as a Golden Opportunity Scholar at the Tri-societies meeting in Cincinnati, OH.
- Former undergraduate intern, Hanna Walters, entered graduate school at Washington State University with a focus on breeding crops for organic systems
- Former undergraduate intern, Soni Hueftle, was awarded highest recognition for her presentation at the Colorado State University Undergraduate Student Research competition in May 2012.

C. List publications (refereed, non-refereed, meeting abstracts)

Non-refereed:

Kleintop, A.E., D. Echeverria, L.A. Brick, M.A. Brick, and H.J. Thompson. 2012. Variation in total dietary fiber content in dry edible bean cultivar/lines. *Annu. Rep. Bean Improv. Coop.* 55: 57-58.

Meeting Abstracts:

Brick, M.A., A. Kleintop, D. Echeverria, and H.J. Thompson. 2012. Variation in Total Dietary Fiber Content among Dry Edible Bean Cultivars. Abstract Western Society of Crop Science Annual Meeting, July 11-13, Pullman, WA.

Kleintop, A.E., D. Echeverria, M.A. Brick, and H.J. Thompson. 2012. Dietary Fiber and Oligosaccharide Content in a Diverse Collection of Dry Edible Beans. Abstract Crop Science Society of America Annual Meeting, October 20-25, Cincinnati, OH.

D. List all personnel associated with the project.

Mark A. Brick (PI)

Henry Thompson (Co-PI)

Dimas Echeverria (Research Associate)

Adrienne Kleintop (4 months as Research Associate)

Plans for Upcoming Reporting Period (1/1/2013 – 6/30/2013)

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

In 2013, we will complete the analysis of soluble dietary fiber, insoluble dietary fiber, total dietary fiber, and oligosaccharide content for the 300 BeanCAP lines grown in replicated field trials at Fort Collins and a subset of lines grown in North Dakota to estimate GXE and contribute to discovery of SNP markers associated with these traits. Two refereed publications will be submitted on methods to evaluate fiber content in dry beans and phenolic content in snap beans. Analysis of SNP marker discovery will be initiated upon completion of lab analysis. At least two publications are scheduled to be developed from the results of this work

Education activities will include training of two additional undergraduate interns and one high school intern in methods of plant breeding.

Outreach will include presentations at the Crop Science Society of American and the Bean Improvement Cooperative meetings in 2013. One popular press article will be published in Colorado.

2013 will be complete all objectives that were stated in the proposal and completion of data analysis and preparation of manuscripts for publication.

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

Research

- Completion of analysis of 300 Bean CAP lines/cultivars for IDF, SDF, TDF and oligosaccharide content
- Submission of one manuscript on snap bean lines for phenolic content

- Submission of one manuscript on revised methods for fiber content in dry beans using the Codex 2009.01 method.
- Preparation of two manuscripts on variation in fiber content and associations with SNP markers in dry bean.

Training

- Supervised and train two undergraduate interns on the Dry Bean Breeding Project.
- Supervise and train one high school intern on the Dry Bean Breeding.

Outreach

- Publish one popular press article on the health benefits of dry beans
- Report the results of fiber, oligosachharide content of dry beans
- Report the results of phenolic content in snap beans.

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

Progress During this Reporting Period (1/1/2012 – 12/31/2012)

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

Research

Design of Illumina Infinium Genechip BARCBEAN6K_3: A total of 6,000 SNPs were selected for inclusion in the BARCBEAN6K_3 beadchip. These SNPs were selected from the BARCBEAN6K_1 and BARCBEAN6K_2 beadchips that had been developed and tested previously at the USDA, ARS, Beltsville, MD. The BARCBEAN6K_1 and BARCBEAN6K_2 beadchips were used for genotyping the recombinant inbred lines (RILs) in the Stampede × Red Hawk and the BAT93 × Jalo EEP558 mapping populations and for genotyping a diverse group of more than 500 common bean accessions that represented a wide diversity of common bean market classes. Based on the genetic mapping data and the analysis of the diverse common bean accessions, a total of 10,039 SNPs remained after eliminating SNPs with a minor allele frequency (MAF) <5%. We further compared the allele calls for the 10,039 SNPs in all the accessions and SNPs with the same allele call across all accessions fell into 4,622 groups. One SNP was selected from each of the 4622 groups. An additional 199 SNPs were selected from these groups if they mapped to a different position than the SNP that had been selected from that group. The remaining 1179 SNPs were selected based upon polymorphism within the Durango, Mesoamerican and Guatemalan races of the Middle American gene pool. Among the final set of 6000 SNPs, a total of 5921 and 5107 SNPs were polymorphic within at least one market class or two or more market classes, respectively. Among the 6,000 SNPs, 5262 distinguished the Andean vs. Middle American gene pools; 3495 were polymorphic within the Andean gene pool; 2837 were polymorphic in both the Middle American and Andean gene pools, and 4480, 4699, and 4795 SNPs which were polymorphic within the Mesoamerican, Guatemalan and Durango races, respectively.

The design of the BARCBEAN6K_3 beadchip was completed in July 2012 and enough beadchips were ordered from Illumina to analyze 8256 common bean DNAs. A total of 17 public common bean researchers at university and ARS locations across the country were included in the purchase either as part of the BeanCAP project or as part of the Feed the Future Initiative. In addition, two companies working on common bean breeding and genetics were included in the order for the BARCBEAN6K_3 beadchip.

The BARCBEAN6K_3 beadchip as received from Illumina Inc. contains a total of 5398 bead types which means it contains viable assays for 90.0% of the 6,000 SNPs that were submitted to Illumina for the design of the beadchip. Illumina guarantees that 80% of the submitted SNPs will be present on a beadchip. Thus, we were quite pleased with the 90.0% number. In addition, the analyses we have conducted with the BARCBEAN6K_3 beadchip have produced excellent

data. One of the companies that participated in the purchase of the BARCBEAN6K_3 beadchip was BioDiagnostics Inc. in River Falls, WI. Dr. Pegadaraju Venkatramana at BioDiagnostics, Inc. who has worked with Illumina Infinium beadchips for various plant species was quite pleased with the BARCBEAN6K_3 beadchip. He stated the following: "...we ran the BeanCAP chip on a first set of 47 samples, data looks fabulous. All the SNPs were auto called and clustered perfectly. There were only 100 of those that needed manual calling. We are used to running a wide range of chips in various crops. But never had such a positive experience before."

Analyses of common bean DNA using the BARCBEAN6K_3 beadchip: To date a total of 960 common bean DNAs have been analyzed. These analyses include the following:

1. Completed the analysis of 192 common bean DNA samples from Dr. Jim Myers at Oregon State University. The samples included 173 from one population and 19 from another. The allele calling was completed separately on the two sets of samples.
2. Completed the analysis of 480 common bean DNA samples from Dr. Jim Kelly at Michigan State University. The samples were derived from four different populations as well as a population of advanced breeding lines.
3. Completed the analysis of 288 common bean DNA samples from Dr. Phil Miklas, USDA-ARS; Vegetable and Forage Crop Research, Prosser, WA. These samples were derived from two populations and produced excellent quality genotype data.

Determination of the genome positions of common bean rust resistance genes: In collaborative research with Dr. M. Pastor Corrales crosses of three common bean genotypes that carry new resistance genes to common bean rust (*Uromyces appendiculatus*) were made and F₂ seed was produced. The crosses used the rust susceptible genotype Pinto 114 as the female parent crossed with the new resistance sources PI310762, PI260418 and CNC. A minimum of 120 F₂ plants of each cross were grown in the greenhouse at Beltsville, MD. Immature leaf tissue was collected from each F₂ plant, lyophilized and DNA was isolated from each. The F₂'s of Pinto 114 x PI310762 were inoculated with *U. appendiculatus* races 67, 84, 105 and 108. The F₂'s of Pinto 114 x PI260418 were inoculated with races 38, 72, 89 and 105 and the F₂'s of Pinto 114 x CNC were inoculated with races 53, 67, 73 and 108. In each of the three crosses the rust infection phenotypes for each of the four races were identical on each F₂ plant and the ratio of resistant to susceptible plants was approximately 3:1. Three bulks of nine susceptible F₂ plants each were created using the progeny from the Pinto 114 x PI310762. A similar set of three bulks of nine susceptible F₂ plants were created from both the Pinto 114 x PI260418 cross and the Pinto 114 x CNC cross. Bulked segregant analysis was used via analysis with the BARCBEAN6K_1 Illumina beadchip of the nine bulk DNAs along with DNA of Pinto 114, PI310762, PI260418 and CNC. The analysis of the resulting genotype data identified SNP markers that distinguished the parents and for which the SNP allele call for the bulks was identical to the susceptible parent Pinto 114. A set of simple sequence repeat (SSR) markers was selected using the common bean whole genome sequence from the same DNA sequence scaffolds in which the SNPs identified by the bulked segregant analysis resided. These SSRs are

now being used to genotype the F₂ plants of the three populations to verify co-segregation of the marker alleles with resistance vs. susceptibility.

Determination of the genome positions of soybean rust resistance genes in common bean: In collaborative research with Dr. Marcial Pastor Corrales and Dr. Reid Frederick, a cross of common bean genotypes Mexico 309 x CNC was made and F₂ seed was produced. Mexico 309 is susceptible to soybean rust (*Phakopsora pachyrhizi*) and CNC is resistant. A total of 250 F₂ progeny along with the parents Mexico 309 and CNC were grown in the greenhouse USDA-ARS, Foreign Disease-Weed Science Research Unit at Ft. Detrick, MD. Immature leaf tissue was collected from each F₂ plant, lyophilized and DNA was isolated from each. The plants were subsequently inoculated with soybean rust isolates TW72-1, TW80-2, BZ01-1, PG01-2, TH01-1 and ZM01-1. Two susceptible bulks were created and the DNA of the bulks and the Mexico 309 and CNC parents were analyzed with the BARCBEAN6K_1 Illumina Infinium beadchip. The analysis of the resulting genotype data identified SNP markers that distinguished the parents and for which the SNP allele calls for the bulks was similar to the susceptible parent Mexico 309. A set of SSR markers are being selected that reside in close proximity to the SNP markers using the common bean whole genome sequence. These SSR markers will be used to genotype the F₂ plants to verify co-segregation of the marker alleles with resistance vs. susceptibility.

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

- Developed the BARCBEAN6K_3 Illumina Infinium beadchip that contained 5398 SNPs selected to cover the common bean genome with high levels of polymorphism
- Analyzed 960 common bean DNA samples from three different BeanCAP cooperators using the BARCBEAN6K_3 beadchip
- Used bulked segregant analysis with the BARCBEAN6K_1 beadchip to find the genome position of new genes conditioning resistance to common bean rust (*Uromyces appendiculatus*)
- Used bulked segregant analysis with the BARCBEAN6K_1 beadchip to find the genome position of new genes conditioning resistance to soybean rust (*Phakopsora pachyrhizi*) in common bean

C. List publications (refereed, non-refereed, meeting abstracts)

None

D. List all personnel associated with the project.

Dr. Perry Cregan, Research Geneticist, Soybean Genomics and Improvement Laboratory, USDA, ARS, Beltsville, MD

Dr. Qijian Song, Research Geneticist, Soybean Genomics and Improvement Laboratory, USDA, ARS, Beltsville, MD

Dr. Gaofeng Jia, Visiting Scientist, Soybean Genomics and Improvement Laboratory, USDA, ARS, Beltsville, MD

Mr. Charles Quigley, Support Scientist, Soybean Genomics and Improvement Laboratory, USDA, ARS, Beltsville, MD

Plans for Upcoming Reporting Period (1/1/2013 – 9/30/2013)

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

Analysis of at least 2000 genotypes nominated by BeanCAP breeders and geneticists: The Illumina BARCBEAN6K_3 beadchip will be used in 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 common bean DNA samples during the next year. An important aspect of this research is the need for consistently high quality DNA to provide successful genotypic analysis. We will work closely with the BeanCAP collaborators and their staffs to ensure consistently high quality DNA.

Provide Illumina GenomeStudio software to the BeanCAP breeders and geneticists: The Illumina GenomeStudio software is used to do the “allele calling” of the genotypes analyzed using the Illumina Infinium beadchip. The allele calling is based upon the fluorescence produced as a result of the single base extension analysis of the thousands of SNPs being analyzed in each DNA sample. The fluorescence signals from each SNP with each genotype must be observed and in some cases manually adjusted using the GenomeStudio software. This is an important and necessary process for obtaining maximally useful genotypic data. It is also a process that should be understood by those who will be working with the genotypic data. In the near future we will purchase the GenomeStudio software for all the BeanCAP breeders and geneticists so that it is available in their laboratories. To provide training in the use of the software, Illumina Inc. has agreed to provide a webinar in which the basic aspects of GenomeStudio are demonstrated to the BeanCAP collaborators and their associates.

Identification of SSR markers for use in marker assisted selection to select for resistance to common bean rust genes contained in PI310762, PI260418 and CNC: As described above, bulked segregant analysis has been used to identify SNPs in regions of the common bean genome that putatively contain the genes associated with the common bean rust resistance in PI310762, PI260418 and CNC. A total of 37 SSR markers have been identified on the sequence scaffolds of the C19833 whole genome sequence in which the aforementioned SNPs are located. The F₂ plants from the crosses of PI310762, PI260418 and CNC with Pinto 114 that have been phenotyped for rust resistance will be analyzed with these SSR markers to identify SSRs that co-segregate with rust resistance and which therefore can be used in MAS to identify genotypes that carry the highly effective rust resistance alleles that are present in PI310762, PI260418 and CNC.

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

- Analysis of at least 2000 genotypes nominated by the BeanCAP breeders and geneticists with the BARCBEAN6K_3 Illumina iSelect beadchip

- Provide copies of the Illumina GenomeStudio software to the BeanCAP breeders and geneticists as well as training in the use of use of the software for the analysis of the genotypic data obtained from the analysis of common bean DNA with the BARCBEAN6K_3 beadchip
- Identify useful SSR markers for use in MAS for the rust resistance alleles contained in the rust resistance sources PI310762, PI260418 and CNC

BeanCAP
Common Bean Coordinated Agricultural Project
Progress Report and Work Plan
1/1/2012 – 12/31/2013

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

Progress During this Reporting Period (1/1/2012 – 12/31/2012)

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 shipped to us by other cooperators. During the current reporting period, we completed the acid digestion and elemental analyses (inductively coupled plasma – optical emission spectroscopy) of the initial trial entries (248) grown in WA in 2010. Last year, we had completed the analyses of these same entries (248) grown in MI in 2010. 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 B, Co, and Mo were below detection limits for most of the samples. For the WA-grown entries (248), average values (mg/g DW) for the seed macroelements were: Ca, 1.96; K, 14.94; Mg, 1.83; P, 4.87; and S, 2.31; average values ($\mu\text{g/g DW}$) for the seed microelements were: B, 13.19; Co, 0.15; Cu, 9.36; Fe, 77.24; Mn, 16.03; Mo, 1.67; Ni, 3.03; and Zn, 34.57. Values for Se were below detection limits for most of the samples. It is worth noting that we also analyzed for arsenic (As) in all of the samples, but no sample showed a detectable level of As (all readings were below our detection limits).

Throughout the early months of 2012, we received all of the dry bean samples grown by various cooperators in the summer of 2011, amounting to over 4000 samples. These included the replicated field trials of the BeanCAP 300 entries, as well as the replicated field trials of the 96 entry subset grown in irrigated and non-irrigated fields. Samples received thus far include those from CO (~600 samples; Brick); MI (~792 samples; Kelly); ND (~610 samples; Osorno); WA (~376 samples; Miklas); ID (~384 samples; Despain); PR (~384 samples; Porch); and NE (~900 samples; Urrea). We have completed the grinding of 3100 of the samples, using an Udy Mill, and have bar coded all samples to maintain quality control over the sample identities. From bulk quantities of ~100 gm seeds for each sample, we generated 60 gm of sample for protein/fat/crude fiber analysis (samples shipped to NDSU), 10 gm of sample for phytate analysis (samples shipped to MSU), and ~30 gm of sample for our mineral analyses. As of December 2012, ~3100 ground samples have been shipped to each of the two cooperators at NDSU and MSU.

Finally, throughout the year, we have been working our way through the digestions and ICP-OES mineral analyses of the 2011 field samples, analyzing for several minerals (Ca, Mg, K, P, S, B, Co, Cu, Fe, Mn, Mo, Ni, Se, Zn, and As). Of the 3100 samples currently ground and

homogenized, we have completed the mineral analysis of ~2100. Analytical results are being reviewed, all calculations are being double-checked, and all data will be made available to cooperators and interested individuals in the coming months.

Education

Nothing to report.

Outreach

Nothing to report.

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

- Completed sample grinding and shipment of ~2600 dry bean sub-samples to cooperators for compositional analyses.
- Completed digestions and elemental analyses on ~2100 dry bean samples.
- Attended BeanCAP annual meeting at PAG XX.

Plans for Upcoming Reporting Period (1/1/2013 – 6/30/2013)

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

Research

Grinding of the remaining (~1000) dry bean samples received from 2011 field trials will be completed; sub-samples will be sent to cooperators for protein, fiber, and oil, or for phytate analyses. Elemental analyses will be continued on the remaining dry bean samples already ground, and soon to be ground; a second technician has been hired to help with the completion of this labor-intensive work. A manuscript will be developed, focusing on the nutrient diversity identified in the WA- and MI-grown samples from 2010.

Education

No specific plans.

Outreach

Continue to offer assistance with the Mineral Nutrition animations. 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 the final ~1000 dry bean sub-samples to cooperators.
- By June 2013, complete elemental analyses on another ~1000 dry bean samples.
- Completion of manuscripts on nutrient diversity in snap bean entries and dry bean entries.
- Attend BeanCAP annual meeting at PAG XXI.
- Assist with animation package for BeanCAP web presence.