Common Bean Coordinated Agricultural Project
“BeanCAP”
2012 Advisory Board Meeting at PAG XX

Marker development:
Perry Cregan & Qijian (Jian) Song

Presentation Outline

- Development of two Illumina iSelect 6,000 SNP GeneChips, BARCBEAN6K_1 and BARCBEAN6K_2
- Analysis of the 517 BeanCAP genotypes with the two Illumina GeneChips
- Analysis of the Stampede x Red Hawk mapping population with two Illumina GeneChips
- Design of a BeanCAP 6K SNP GeneChip for analysis of BeanCAP populations
- Bulked segregant analysis to define the position of the of the *sd* (slow darkening) locus and testing of SSR markers to identify slow darkening lines
- Release of 1575 selected SNP markers to the Generation Challenge Programme (GCP) of the Consultative Group on International Agricultural Research (CGIAR)
- Analysis of 94 Stampede x Red Hawk F5-derived lines with the two Illumina GeneChips and 270 SSR markers as part of a graduate student project

Two Illumina iSelect GeneChips for Single Nucleotide Polymorphism (SNP) Analysis

- SNPs were identified from the alignment of 19.6 billion bases of sequence data from 19 diverse common bean genotypes to the developing whole genome sequence of G19833

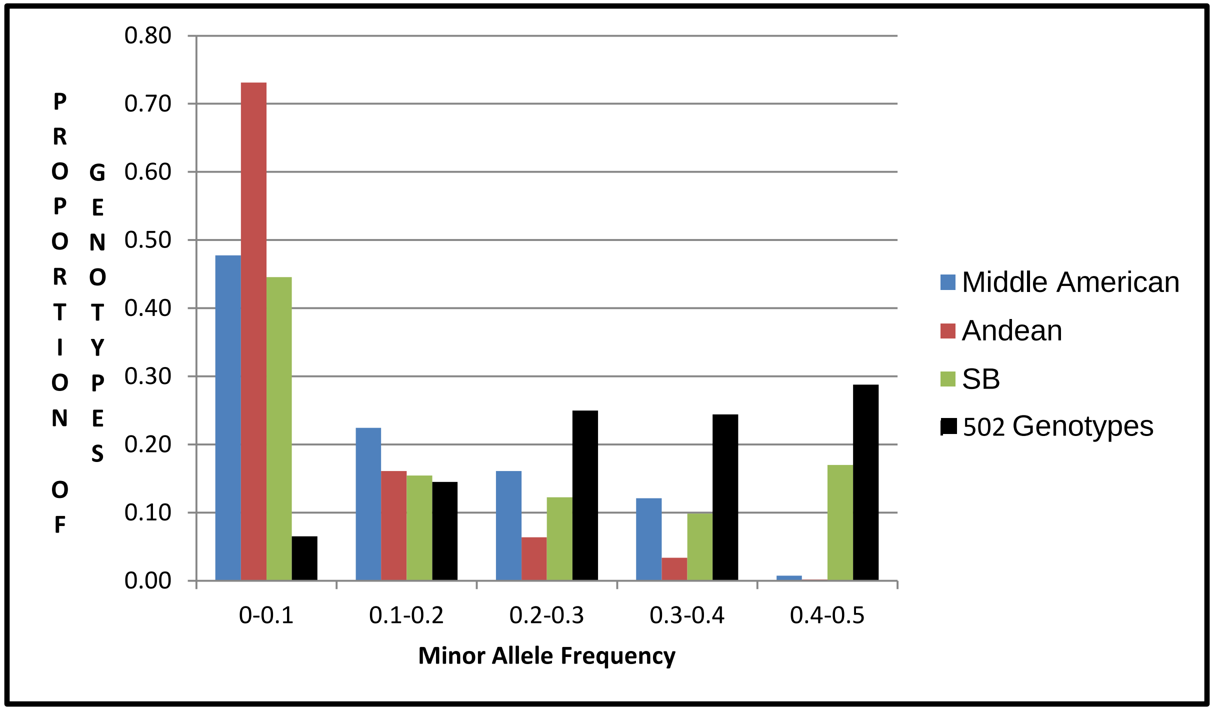
- BARCBEAN6K_1: Reliable allele call data for 5,188 loci on the BeanCAP genotypes

  BARCBEAN6K_2: Reliable allele call data for 5,265 loci on the BeanCAP genotypes

- Between the two GeneChips 6,533 SNPs were segregating in the Stampede x Red Hawk population

Minor Allele Frequencies of 10,543 SNPs Analyzed in 502 Common Bean Genotypes

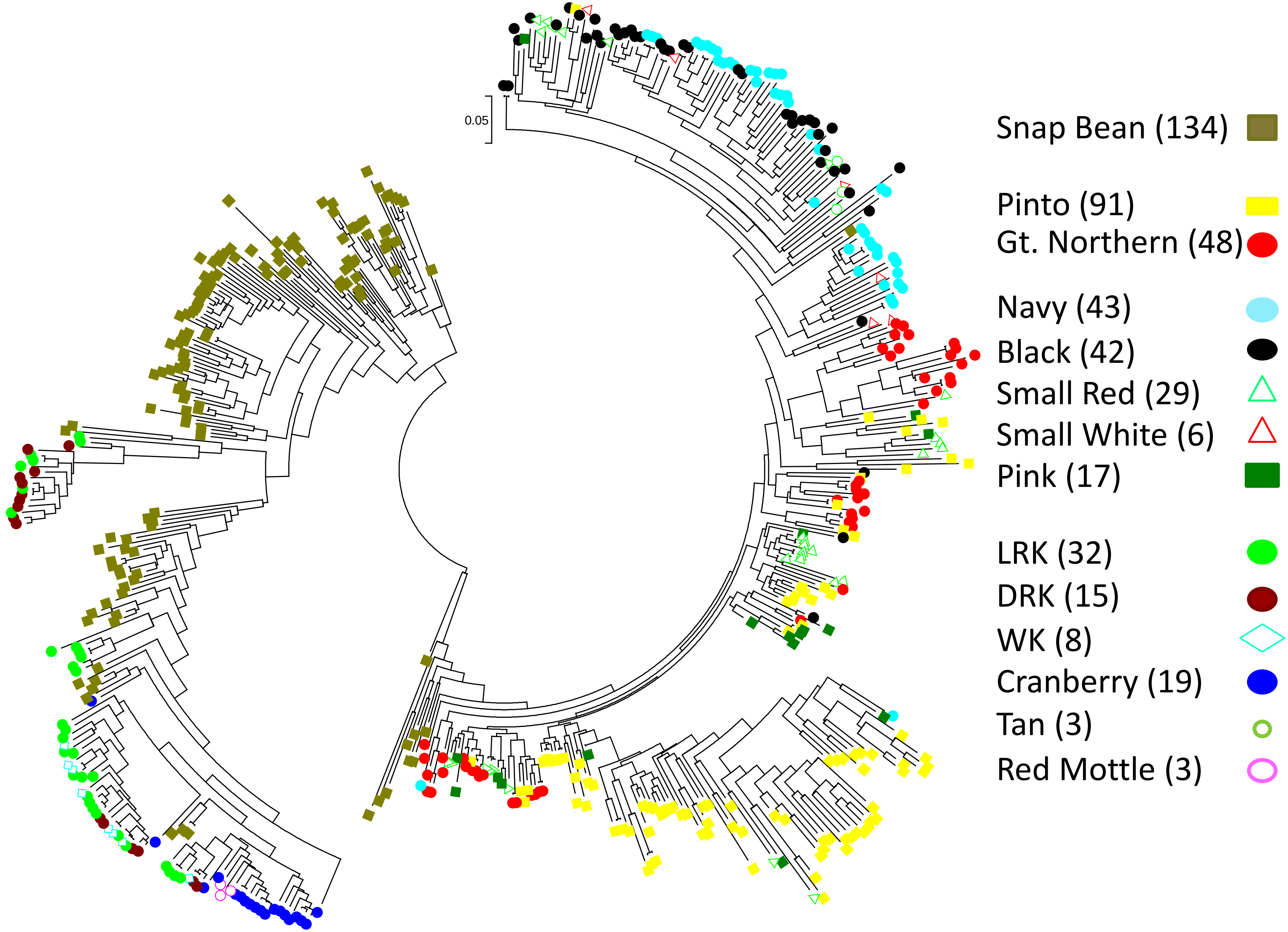
	Number of loci				Proportion of loci			
Minor Allele Frequency	**Middle American genotypes**	**Andean genotypes**	**Snap Bean genotypes**	**502 Genotypes**	**Middle American genotypes**	**Andean genotypes**	**Snap Bean genotypes**	**502 Genotypes**
0-0.1	**5,035**	**7,710**	**4,700**	**685**	**0.48**	**0.73**	**0.45**	**0.06**
0.1-0.2	**2,367**	**1,697**	**1,630**	**1528**	**0.22**	**0.16**	**0.15**	**0.14**
0.2-0.3	**1,699**	**671**	**1,292**	**2,634**	**0.16**	**0.06**	**0.12**	**0.25**
0.3-0.4	**1,275**	**354**	**1,039**	**2,573**	**0.12**	**0.03**	**0.10**	**0.24**
0.4-0.5	**77**	**21**	**1,792**	**3,033**	**0.01**	**0.00**	**0.17**	**0.29**
TOTAL	**10,543**	**10,543**	**10,543**	**10,543**	**1.00**	**1.00**	**1.00**	**1.00**



Number of genotypes in each market class and the number and proportion of the 10,453 SNP loci that were polymorphic within the genotypes of each market class

Gene Pool Race Market Class	No. of genotypes	No. of Polymorphic Loci	% of Loci polymorphic
Snap Bean	134	6230	59.6
Middle American			
Durango			
Pinto	91	5258	50.3
Great Northern	48	4338	41.5
Mesoamerican			
Navy	43	3805	36.4
Black	42	3449	33.0
Small Red	29	5091	48.7
Small White	6	4098	39.2
Pink	17	4756	45.5
Andean			
Nueva Granada			
LRK	32	3167	30.3
Cranberry	19	1495	14.3
DRK	15	2488	23.8
WK	8	1840	17.6

Neighbor Joining Tree – 502 BeanCAP Genotypes Analyzed with 10,154 SNPs



Observations Related to the Diversity Analysis of the 502 BeanCAP Genotypes

- There one or two genotypes from other minor market classes including: Andino, Black Mottle, Carioca, Cream, Flor de Mayo, Jacob's Cattle, Kidney, Otebo, Pink Cranberry, Yellow, and Yellow-Mayocoba. These were not included in the analysis.

- There were 8 genotypes that were analyzed twice. The duplicate analyses for 10,153 SNPs produced contradictory allele call data for 7 of the genotypes that disagreed on an average of 7.2 loci. In the case of the eighth genotype, BelMiNeb-RMR-4, there were 166 allele calls that disagreed.

- There were number 25 pairs of genotypes that gave identical allele calls for all loci.   In most cases these were genotypes in the same market class. In a few cases the similar allele call patterns were “surprising” e.g.,:

  115M(Black Rhino) & Cornell 49-242
  Royal Red (DRK) & Lisa (WK)
  USWA-12 (GN) & Bighorn (Pinto)
  Montcalm (DRK) & ND061106 (LRK)
  Tendercrop (Snap) & Gallatin 50 (Snap)

- We need to know the breeding program that produced each of the BeanCAP genotypes in order to order to complete the diversity analysis.

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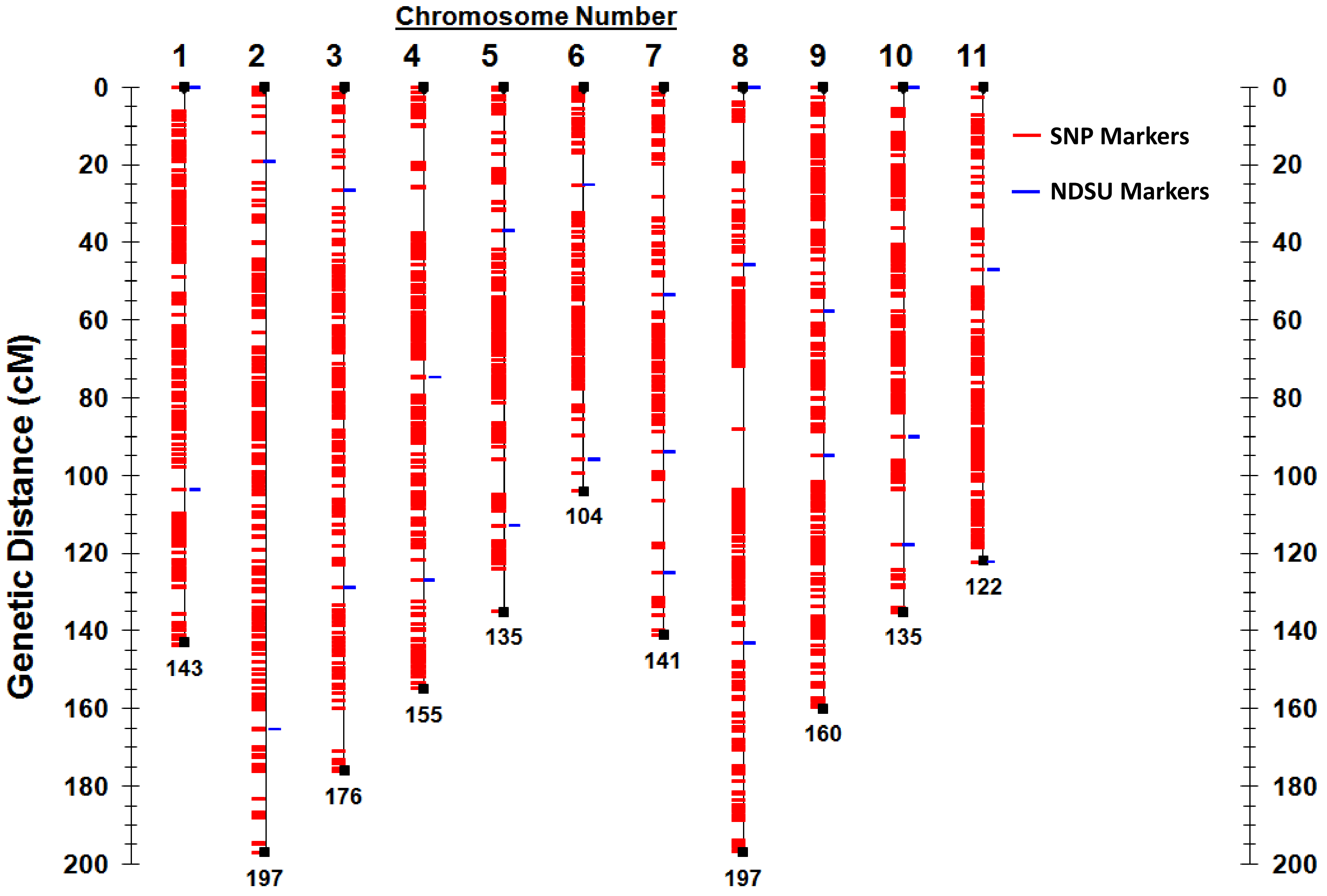
Genetic Mapping

Summary of the current status of the number of SNP markers mapped in the 288 F_2 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

Stampede x Red Hawk Genetic Map

7018 SNPs from BARCBEAN6K_1 and BARCBEAN6K_2 and OPA-1 + 25 NDSU Markers



Additional Genetic Mapping

- We have obtained more than 200 recombinant inbred lines of BAT93 x Jalo EEP558 from Embrapa, Brazil
- Based upon the comparison of BAT93 versus Jalo EEP558 from the analysis with BARCBEAN6K_1 and BARCBEAN6K_2 more than 2,000 additional SNPs can be mapped using the BAT93 x Jalo population
- The combined Stampede x Red Hawk and BAT93 x Jalo mapping populations will provide approximately 9,000 mapped SNPs
- The 9,000 mapped SNPs will be the source from which a set of 6,000 SNPs will be selected for the GeneChip that will be used for the genetic analysis of BeanCAP mapping populations

BeanCAP 6,000 SNP GeneChip Design

- **Select 3,500 SNPs targeted to the Middle American Genepool:**
  - High levels of polymorphism in the Durango, Mesoamerican and Guatemala races
  - Even distribution across the 11 linkage groups

- **Select 2,500 SNPs targeted to the Andean Genepool:**
  - High levels of polymorphism in the Nueva Granada race
  - Is there any concern about the race “Chile” and the race “Peru” ??

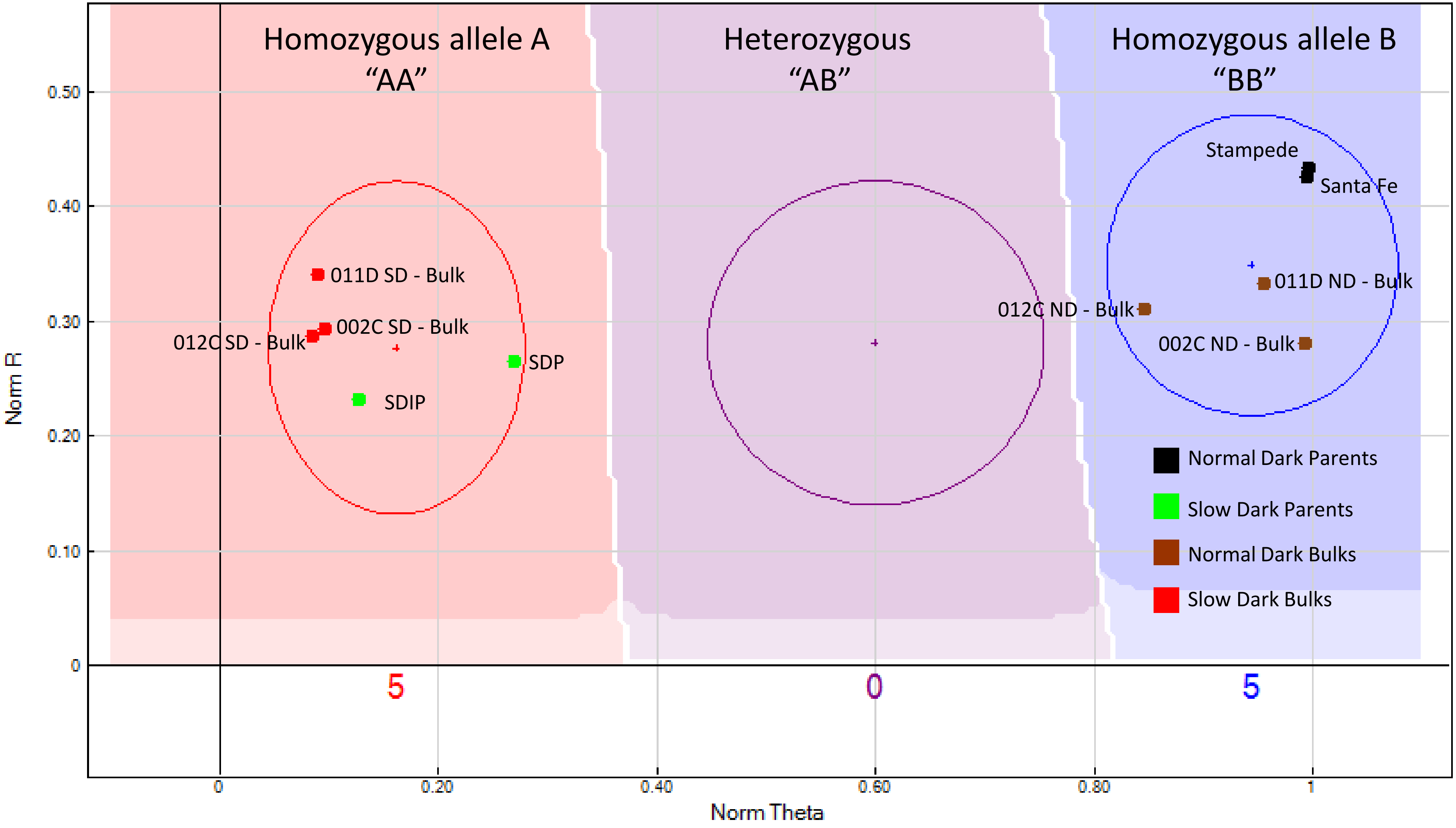
- **Snap beans:** A large proportion of the SNPs selected to the Middle American and Andean genepools will function well in the Snap bean germplasm

Bulked Segregant Analysis to Define the Position of the of the *sd* (Slow Darkening) locus

- **Genetic Materials:**
  - **from Phil Miklas,** USDA, ARS, Prosser, WA**:** Three RD (Regular Dark) x SD (Slow Darkening) crosses with a total of 159 F_2 progeny
  - **from Kristen Bett,** Univ. of Saskatchewan, Saskatoon, SK: RD x SD population consisting of 105 RILs
- **Accelerated Seed Coat Darkening Test:** Used to identify F_2 lines that were homozygous *sd/sd* or *Sd/Sd* to create three homozygous *sd/sd* and three homozygous *Sd/Sd* bulks
- **Bulked Segregant Analysis:** 1536 Illumina GoldenGate SNPs were used to analyze the parents and bulks

Bulked Segregant Analysis of Slow Darkening and Normal Darkening Parents and F_2 Bulks

SNP Locus BARC-PV-0000692



Bulked Segregant Analysis to Define the Position of the of the *sd* (Slow Darkening) locus

- The SNPs were associated with a sequence scaffold in the *P. vulgaris* whole genome sequence
- SSRs were identified in the sequence scaffold and PCR primers were designed to them
- Three SSRs distinguished the ND from the SD parents and were closed linked to the *sd* locus in the F_2 populations
- The SSRs were mapped in the RIL population and positioned the *sd* locus on bean Linkage Group 7
- The SSRs function well in marker assisted selection for the SD trait

Information on 1575 SNPs was forwarded to the Generation Challenge Programme of the CGIAR for use in developing country bean breeding programs. In collaboration with CIAT the SNPs were converted to the KBioscience KASPar SNP analysis platform

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- Press Release -

For immediate release to mark world food day on 16 October

Media Release – 14 October 2011

A boon to bean breeding in the tropics

Scaling to thousands: A first ever for beans, BeanCAP's landmark donation will greatly accelerate the quest for better beans

Graduate Student Project:

- **Analysis of 94 Stampede x Red Hawk F5-derived lines with the two Illumina GeneChips**
  - Experience with Illumina iSelect SNP assay system
  - Experience with the Illumina GenomeStudio software
- **Testing and mapping of SSR loci in the RIL population: A total of 270 SSR markers were mapped**
- **Genetic map development using JoinMap**