A New SNP-Genotyping Resource for Cowpea and Its Deployment for Breeding

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Introduction

Cowpea (Vigna unguiculata L. Walp.), native to Africa and a member of the Fabaceae family, is a primary source of protein for millions of people in Sub-Saharan Africa and other parts of the developing world. It is grown for fresh and dry grains, edible pods and leaves, or used for hay or forage. Until recently cowpea has lacked genomic resources to support modern breeding approaches. We previously developed a 1,536 GoldenGate assay, which enabled construction of a ~1100 SNP genetic linkage map (Muchero et al. 2009, Lucas et al. 2011). Many of these SNPs have been used in other genotyping platforms by the cowpea breeding community.

Here we present the development of an Illumina “60k” iSelect BeadChip as part of the “USAID Feed the Future Innovation Lab for Climate Resilient Cowpea”. This new genotyping resource supports higher-density genetic mapping, pedigree validation, germplasm characterization and marker-assisted breeding of cowpea. Primary target traits for Burkina Faso, Ghana, Nigeria, Senegal, China and California include drought and heat tolerance, seed qualities, yield, and resistance to Macrophomina phaseolina, aphids, flower thrips, pod-sucking bugs and Striga.

SNP Discovery Panel

SNPs for the iSelect assay were discovered using whole-genome shotgun sequencing of 37 cowpea accessions including breeding lines & landraces relevant to Africa, China and the USA (Table 1). They represent the geographic, genetic, and phenotypic diversity relative to the breeding objectives (Figure 2; Table 1).

Processing steps include the following:

- BWA alignment to reference genome (draft, unpublished).
- SNPs called with SAMTools, SGSautoSNP, FreeBayes. ~1.5 million SNPs were common to all three.
- Selected 56,719 SNPs for 60,000 iSelect BeadAssays. The majority of these SNPs target cowpea sequences corresponding to 20,788 gene models of Phaseolus vulgaris (common bean), a close relative of the genus Vigna. 1,163 GoldenGate SNPs were included.
- 51,128 SNPs are represented on the iSelect. It became available on July 2014. Contact Illumina for availability.

Initial Results

- DNAs are primarily prepared from dry leaf tissue (Figure 3). Young leaves are collected and placed into a ziplock bag containing silica gel packs. Samples are shipped in this form to UCR for DNA isolation.
- A minimum DNA concentration of 50 ng/μl and a volume of 4 μl are required for iSelect genotyping. A wide range of DNA integrity is acceptable. For example, the four samples shown in Figure 4 were all equally successful, with approximately 0.8% missing data.
- 1,872 samples have been genotyped so far, including 10 RIL populations, breeding lines from West African programs, and diverse germplasm.
- More than 49,000 SNPs (96%) were informative. These SNPs can be used for allele mining and high density mapping, and can adapted to other genotyping platforms for a range of breeding applications.

Acknowledgments & References

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Table 1. The 37 accessions used for SNP discovery. The reference genome is underlined. UCR = University of California Riverside; DRA = Institut Sénégalais de Recherches Agricoles; INERA = Institut de l’Environnement et de Recherches Agricoles ; IITA = International Institute of Tropical Agriculture; INIA = Instituto de Investigación de Recursos Hídricos.

Figure 1. A cowpea farmer in Burkina Faso showing her harvested pods. Photo 12 Close, September 2014.

Figure 3. Young trifoliate leaf packed with silica gel to dry leaf tissue for DNA isolation.

Figure 4. Gel electrophoresis of genomic DNA from 4 cowpea accessions. M = 100 bp molecular marker. Note the difference in DNA integrity -- all are acceptable.

Figure 2. Principal component analysis (PCA) of 729 samples representing the diversity of cultivated cowpea and distribution of 35 accessions part of the SNP discovery panel. PCA was performed in TASSEL v.4.0 with data from 1,025 GoldenGate SNPs. Information of the cowpea accessions and genotypic data were available from Huynh et al. (2013).

Figure 5. GenotypeStudio display of typical data from an informative SNP.