Development of High Density SNP-Based Linkage Map in Pearl Millet

Somashekhar Punnuri[1], Jason Wallace[2], Limei Liu[3], Peng Chee[3], Katie Hyma[4], Sharon Mitchel[4] , Charlotte Acharya[4], Joseph Knoll[5], Eric Obeng[1], Bharat Singh[1]

[1]Fort Valley State University, Fort Valley, GA,[2] Biotechnology Dept, Cornell University, NY, [3] University of Georgia, Tifton, GA, [4] Institute for Genomic Diversity, Cornell University, NY, [5] USDA-ARS, Tifton, GA.

ABSTRACT

Genome mapping studies are a prerequisite for tagging agronomicaly important traits. Genotyping-by-sequencing (GBS) is one such approach that results in genome-wide single nucleotide polymorphism (SNP) markers, even in species without a reference genome. We performed GBS analysis on one-hundred eighty seven recombinant inbred line (RIL) individuals derived from 99B (Tift 99B) as female parent and 99-17-1 (Tift 454) as male parent, along with a commercial hybrid line (Tiftgrain 102) derived from the same two parents. These samples were processed with ApeKI digestion, pooled in 96-plex, and sequenced on an Illumina HiSeq2000. Quality sequence reads from 179 RILs were processed with a reference-free pipeline, UNEAK. The final genetic map contained approximately 700 high-quality "core" SNPs across all seven chromosomes, plus an additional 37,000 SNPs anchored to these. This dense genetic map will facilitate quantitative trait mapping and marker-assisted selection for disease resistance and nematode resistance.

INTRODUCTION

Pearl Millet (*Cenchrus americanus* (L.) Morrone):

- ❖ Diploid, 7 chromosomes
- ❖ No reference genome
- ❖ Gluten-free food
- ❖ Rising demand for flour from certain ethnic groups
- ❖ Major constraints: nematodes, rust, blast

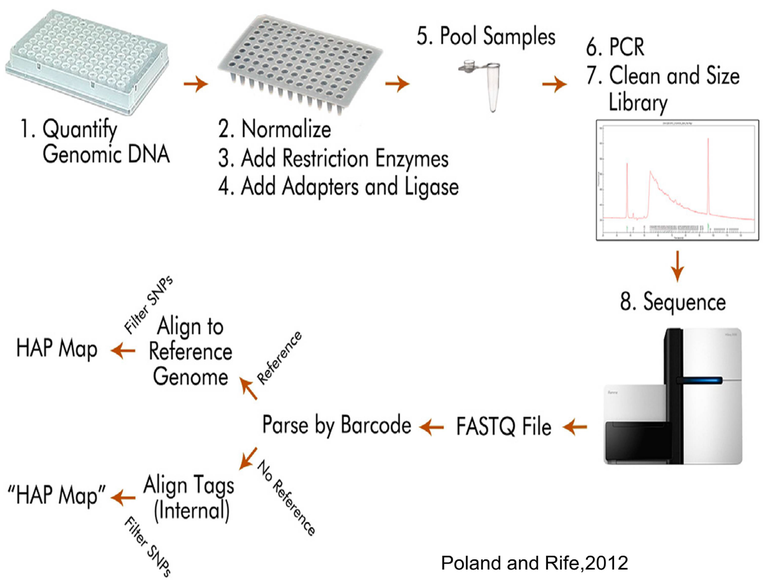
Mature pearl millet

Overall Goal: Develop high-yielding, adapted pearl millet cultivars resistant to nematodes and disease.

- **Specific Objective: Develop a high-density genetic map using GBS markers.**

Genotyping-by-sequencing (GBS):

- Rapid, inexpensive genotyping method
- Marker discovery and genotyping combined in a single step
- Does not require a reference genome



Poland and Rife,2012

MATERIALS & METHODS

Mapping population

- Tift 99B x Tift 454 (99-17-1)
- Make F1 cross, then self for 7 generations
- Developed by Jeff Wilson (USDA-ARS, Tifton GA)
- Currently being evaluated for leaf spot disease and other physiological traits at Fort Valley State University

The mapping population RILs growing in the field at Fort Valley State University.

Map development protocol

DNA isolated from greenhouse seedlings

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Genotyping-by-sequencing (GBS) marker development (Elshire *et al.* 2011)

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Marker discovery through reference-free UNEAK pipeline (Liu *et al.* 2013)

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SNPs filtered for quality calls using TASSEL (Bradbury *et al.* 2007)
Bad markers and lines removed

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Clustering SNPs into linkage groups using R software by hierarchical clustering

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Markers within groups ordered using MSTmap (Wu *et al.* 2008)

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Maps refined with R/qtl (Broman *et al.* 2003) and by manual inspection

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Use map to impute missing data

Iterate 2-3x

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High quality map with better marker order which covers all linkage groups in pearl millet

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Reduce map to non-redundant "core" markers

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Use core markers to anchor additional SNPs

CONTACT:

Somashekhar Punnuri
1005 State University Drive
Fort Valley State University
Fort Valley, GA 31030
478-825-6519 (Office)
punnuris@fvsu.edu (Email)

RESULTS

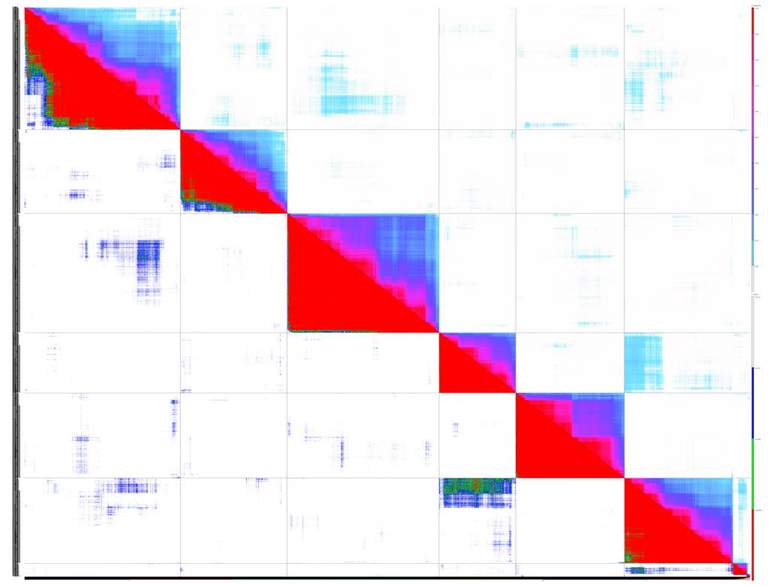

Figure 1. Linkage disequilibrium (LD) plot of the core 700 SNPs showing segregation into seven linkage groups. Color intensity indicates LD p-value (lower triangle) and r^2 (upper triangle).

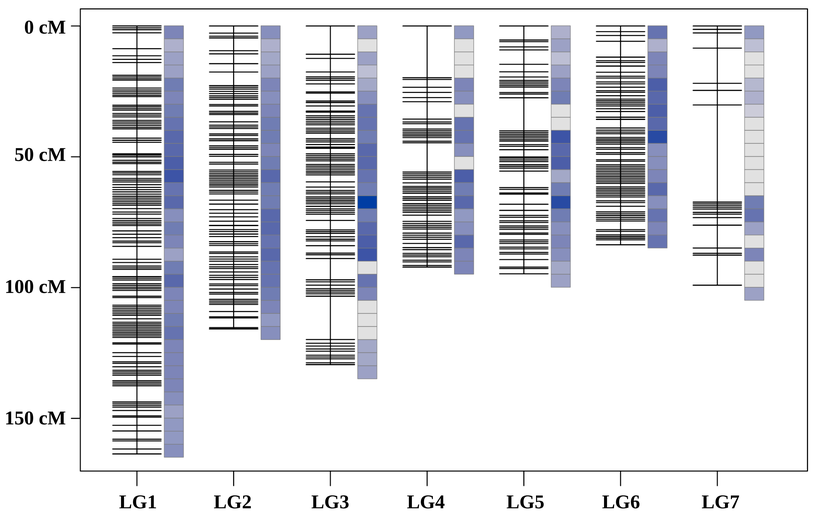

Figure 2. Genetic map, showing the locations of the core 700 SNPs (lines) and the density of 37,000 anchored SNPs (boxes). Color intensity for each 5 cM density bin is proportional to the log of total markers in that bin. Linkage group names are preliminary, pending correlation with existing maps.

CONCLUSIONS

- We generated a high-density SNP map for pearl millet that will be used to map traits in this population and inform other populations
- This is the first high-density SNP map in pearl millet
- GBS is an efficient method for generating *de novo* genetic maps in pearl millet
- One linkage group (7) is depleted for markers; it likely has a region of common descent around dwarfing gene *d2*.

ACKNOWLEDGEMENT

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