Exploring the Genetic Basis of Root Mucilage in Cowpea *(Vigna unguiculata* (L.) Walp.)

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Introduction

Cowpea *(Vigna unguiculata* (L.) Walp.) is a member of the legume family, Fabaceae. Cowpea varieties are vital agricultural crops in arid and semi-arid environments in Africa, Asia, India, and Central America. Cowpea sold in the United States is commonly known as black eyed peas. Breeding for specific cowpea varieties depends on environmental conditions where phenotypic traits may relate to drought tolerance, and resistance to fungal and bacterial pathogens, aphids, nematodes, viruses, and many others. The agricultural production of a variety with desirable traits is an important economic consideration that ultimately determines yield and quality of a harvest.

The cowpea genome research group at the University of California Riverside recently developed a high density single nucleotide polymorphism (SNP) genetic linkage map (Muchero et al. 2009) and coupled this genetic map to a 1 OX-coverage physical map of the cowpea genome. These new genome resources together with knowledge of synteny between the genomes of cowpea and fully sequenced reference legumes soybean *(Glycine max* (L.) Merr.) and *Medicago truncatula,* have been utilized to genetically and physically map phenotypic traits. One of the most visually striking of these mapped traits is the production of a high amount of root mucilage.

Root mucilage is of importance to plant-microbe interactions in the rhizosphere of cowpea and other legumes, and may hinder the uptake of aluminum from the soil (Knee et al. 2001). A region of the cowpea genome bearing a major gene controlling root mucilage has been identified using a recombinant inbred line mapping population. Within this region, several candidate genes exist that may also be responsible in seed mucilage production in G. *max* and Arabidopsis (Li et. al 2009).

The objectives of this study include phenotyping diverse germplasm for the mucilage trait, testing one or more candidate genes for polymorphisms, and the chemical quantitation and characterization of root mucilage. Phenotypes of several hundred cowpea germplasm accessions were compared to their existing SNP haplotypes spanning the region of interest to categorize haplotypes containing different alleles for the root mucilage trait. The gene sequences of different alleles for two candidate genes were determined for comparison between lines. Root mucilage will evenutally be analyzed for protein and carbohydrate content and specifically for arabinogalactans and proteins (Moody et al. 1988).

Results

Phenotyping Diverse Germplasm

The phenotyping of 373 cowpea lines from the UCR germplasm collection revealed only one additional line that produces high amounts of root mucilage (UCR3944), numerous lines that have a moderate amount of mucilage, and an abundance of lines that have low mucilage. Phenotypic scoring for the mucilage trait lead to the desire to genotype lines that exhibit the characteristic of high mucilage (UCR232) and low mucilage (CB- 27). The mucilage trait is also evident in near isogenic lines derived from these parents, exhibiting high mucilage (BB-33) and low mucilage (BB-3).

Genetic Inquiry

The genotyping of these lines has lead to the discovery of polymorphism within a region marked by a SNP in a gene annotated as phenazine biosynthesis. Within this region, there are other genes with potential involvement in mucilage production. These candidate genes are annotated as extracellular dermal glycoprotein (EDGP), and transparent testa S (TIS), based on synteny between cowpea and annotations from G. *max* and Arabidopsis. Inquiry into the DNA sequences for these candidate genes began with oligonucleotide design. Primers were designed based on the partial sequences available for these genes from cowpea. Two sets of PCR primers were chosen for each candidate gene. The starting and ending base of each set were different between the two sets of primers for each gene. An initial polymerase

chain reaction was run at 53°C with only the near isogenic lines, and produced multiple bands. A second run of polymerase chain reaction included the near isogenic lines and the parents, was run at 56°C and produced prominent amplicons of the desired base lengths: 500 bases for TIS, and SOD bases for EDGP. The second set of primers for each gene produced fewer and cleaner bands. The product of polymerase chain reaction from these desired lanes was subject to an E-Gel apparatus, which allowed the extraction of the bands of interest from the E-Gel. The products of interest were run on a 1 % Agarose gel in 0.5 TBE buffer, to determine the accuracy of the bands but a problem occurred with the gel. The products were run again with a new gel and provided quality results. These products were submitted for Sanger Dideoxy Sequencing.

Chemical Analysis

Cowpea seeds were germinated on glass microfiber discs in petri dishes for the collection for root mucilage for chemical analysis. After 3 days the rootlets of the seedlings grew in a random fashion and were re-orientated for the rootlet to grow parallel with the filter paper. After 2-3 days the seedlings continued to grow upwards toward the condensation on the roof of the petri dish, and subsequently dried out and died. Arabidopsis mutants were germinated on glass microfiber filter paper discs and the rootlets grew into the filter paper, inhibiting the visual observation of root mucilage.

Discussion

Considerations for future work

The phenotyping diverse germplasm revealed one additional line with this rare trait of copious root mucilage and this line should be genotyped for comparison to the polymorphism observed between UCR232 and CB27. The sequencing data obtained for the candidate genes did not reveal any polymorph isms between the parents or the near isogenic lines, and deriving additional sequence data from these genes may help to determine whether these genes playa role in determining the phenotype. The portion of the project pertaining to the chemical analysis of root mucilage did not proceed very far. After the cowpea seeds dried out the consensus was to germinate sterile seeds in petri dishes with the glass fiber discs on both the bottom and the top of seeds, allowing the root mucilage to potentially adhere to both the top and bottom discs, as well as providing adequate water in the germination process. The problem with the Arabidopsis seeds germinating into the glass fiber may be overcome by germination on a water agar solution, allowing for the visually observation of root mucilage.

Genomics assisted plant breeding

The use of genomic resources, genetic tools, and principally the application of molecular biology to plant breeding has provided monumental achievements in terms of valuable crop selection. The applicability and use of a phenotype with a copious amount of root mucilage may be revealed in the future.' With plant breeders and farmers facing unpredictable environmental changes, a diversity of cowpea varieties is an invaluable resource. The use of marker assisted breeding programs in agriculture production may help solve some of the most problematic causes of plant mortality.

Experimental Procedures

Phenotypic Scoring for Mucilage Trait

A random sample of 373 cowpea lines from the germplasm were germinated in a plastic pouch that contains a paper wick that allows for visual observation of root growth without disrupting the seedlings. Rootlets were stored upright in folders, in the greenhouse, and observed after 4 days with an approximate length of 4-5 cm. Rootlets were scored with observable criteria including high mucilage, some mucilage, and low mucilage.

Genetic Inquiry

The DNA from recombinant inbred lines Big Buff and CB-27 and near isogenic lines BB-3 and BB-33 have previously been extracted from leaf tissue. The concentration of DNA in these samples was estimated via a nanodrop spectrophotometer. Primers were designed based on gene sequences of genes annotated as Transparent Testa 8 (TT8) and Extracellular Dermal Glycoprotein (EDGP) from G. *max,* derived from syntenic relationships between V. *unguiculata* and G. *max*.

Chemical Analysis of Mucilage

Cowpea seeds were surface sterilized with 1 % EtOH for 1 min, 6% Bleach (v/v) for 10 min, with 3x 10 min sequential washes, and antibiotics chloramphenicol (60j..Jg/ml), streptomycin (100 j..Jg/ml), cefotoxime (100 j..Jg/ml), and ampicilin (100 j..Jg/ml), at 24°C for 24 hours on a rotary shaker and subsequently washes 2 times for 10-15 min each. Glass-fibre filter discs were sterilized in commercial bleach for 5 min and washed with ultra purified sterilized water. Cowpea seeds were germinated on Whatman Glass Microfiber filter papers (GF/A, 125mm diameter) in petri dishes and placed in an incubator. At the onset of germination, the seedlings roots were growing upwards and were reoriented for the roots to grow parallel with the filter paper for mucilage adherence. After 4 days the roots had continued to grow upwards towards the condensation and inherently dried out. Experiment was repeated with a glass fibre disc on both the bottom and the top of the seeds in the petri dishes. Seeds were also grown in modified pouches with the paper wick removed and the glass fiber discs stapled to the holding fold at the top of the paper wick insert.

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