Identification of Naturally Occurring and Irradiation-induced Mutant GBSSI Alleles of Cassava in a Heterozygous Genetic Background

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INTRODUCTION

The globalization of economies has meant a search for local crops that are competitive and will preserve local agriculture. Economic surveys in the past ten years in cassava growing regions of Africa, Asia and Latin America have revealed that cassava is an important factor in the improved livelihoods of the rural population of these regions (Nweke et al 2001; Kawano 2001; Ceballos 2002). A change in starch quality, for example the elimination of amylose (waxy starch), via a natural, GMO or irradiation-assisted knockout of the GBSS I gene, implies access to new markets for cassava growers. For most rural communities, a better standard of living depends on increasing income from their crop harvest.

Mutagenesis has been applied extensively in the production of novel phenotypes in crop species (Van Harten 1998). Seeds as well as vegetative parts of crops have been irradiated. However a mutant gene that has a recessive mode of action, such as the GBSSI gene, cannot be detected in a heterozygous background and requires inbreeding to reveal the desired trait. But it is unrealistic to inbreed the thousands of irradiated plants and molecular techniques are required to detect mutants in the heterozygous state. We describe here the development of molecular techniques for detecting irradiation or natural mutants of the GBSSI gene. The project will take advantage of simultaneous research, currently under way, that involves the production of inbred cassava lines.

MATERIALS AND METHODS

Plant materials for this project were obtained from irradiated sexual seeds from cassava genotypes tolerant to inbreeding (Fig1). About 2000 sexual seeds were shipped to IAEA for irradiation, using gamma rays (a Cobalt-60 source), 1000 seeds, and fast neutrons, 1000 seeds. The level of irradiation with gamma rays was 200Gy. They were sent back to CIAT a and germinated in seedling nursery and transferred to the field, about the 1600 seeds could be transferred to the field, the other seeds suffered from lethal mutations that affected their ability to germinate.

The heterozygous nature of cassava implies that mutations in a recessive gene will not be observed in the M_0 phenotype. There is therefore a need to self the M_0 plants to permit identification of the recessive mutants. However, the task of selfing thousands of plants is beyond capacity at CIAT and a selection of mutants for genes of interest needs to be first be carried out to identify mutants. Molecular methods that can detect insertions/deletions (INDELS) and point mutations are being adapted to detect the mutants, they include PCR amplification, gel electrophoresis, and more recently DNA Targeted Induced Local Lesions In Genomes (DNA TILLING) developed for *Arabidopsis* (Fig. 2). PCR amplification was by primers designed to amplify the full length GBSSI gene as well as 3' and 5' ends of the gene.

At 10 months after planting, the plants from the irradiated seeds that have been shown by molecular analysis to be mutated in the GBSSI gene will be selected and evaluated for its ability to produce flowers. Plants that flower will be cloned and planted the following year in a clonal observation trial fashion of 10 plants per genotype. Plants in the clonal observation trial above will be selfed to obtain the M_1 (S_0) generation. The seeds from the M1 (S_0 generation) will again be established in the field at CIAT and other key target environments and thoroughly evaluated for the waxy trait.

Figure 1. Pollinated flowers that produced fruits and eventually seeds of cassava, plant material source for irradiation with gamma rays and fast neutrons.

RESULTS AND DISCUSSION

The project to identify mutant GBSSI gene is in its preliminary stages having just begun January this year. DNA isolation from the 1600 plants obtained from the 2000 irradiated sexual seeds have just been concluded and PCR amplification and agarose gel analysis initiated. The GBSSI gene in all the above plants will be checked for deletions/insertions by PCR amplification with primers that amplify the full length gene and gel eletrophor4esis. Next the samples will be analyzed by DNA TILLING to detect point mutations. A draw back of this method is the detection of new mutations in a heterozygous genetic background, we intend to get around this by DNA TILLING on family basis.

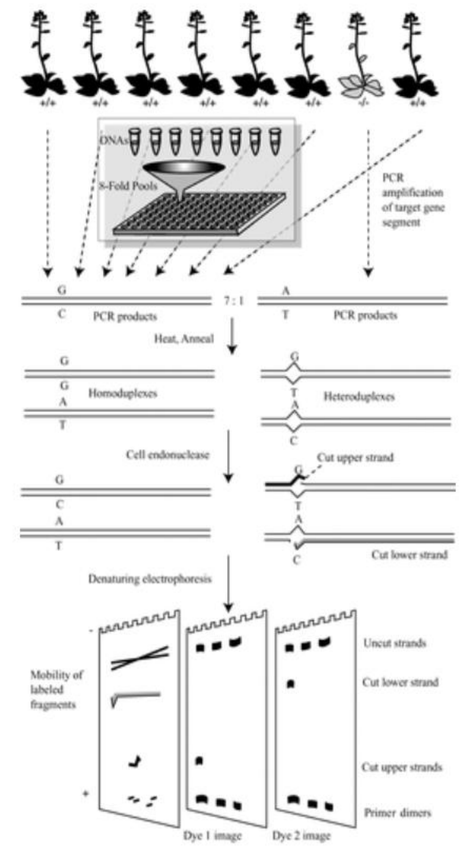

Figure 2. Formation of heteroduplexes in pooled DNA for high-throughput TILLING. The DNAs of eight individuals are pooled in each of 96 wells of a plate. Amplification of a target locus in a pool (i.e., a well of a plate) containing DNA of a mutant (either +/ or /) will result in heterogeneous products, which upon melting and reannealing will form homo- and heteroduplexes. CELI cuts mismatched sites and produces shorter strands that are detected differentially on a sequencing gel, owing to asymmetric 5' labeling with infrared dyes of different colors.

Cortesy of Steven Henikoff and Luca Comai. Single-Nucleotide Mutations for Plant Functional Genomics. Ann. Rev. Plant. Biol 2003. 54:375-401

CONCLUSIONS AND ONGOING WORK

This project seeks to use novel methods of mutagenesis, conventional plant breeding and molecular genetic analysis to identify cassava genotypes with value added traits. It will take advantage of the recently initiated research to produce inbred cassava germplasm. The project will also use tools of genomics to track down genes responsible for the above traits, markers associated to these genes can be used to efficiently move these genes around the different cassava gene pools defined by agro-ecologies. The new methods will not only accelerate the production of improved germplasm but also be a model for the development of other traits of interest to the market and farmer.

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