Soybean Seed Lipoxygenase Genes: Molecular Characterization and Development of Molecular Marker Assays

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Introduction

Soybean food products have flavor and aroma issues

Several soybean-containing food products have grassy and beany flavor and aroma due to the action of Lipoxygenase 1, 2 and 3 enzymes.

Off-flavors result from the oxidation of Polyunsaturated Fatty Acids (PUFA) by Lipoxygenases!: PUFA + O₂ + Lipoxygenases = I don't like soybean food!!

Materials & Methods

DNA isolation

(96 single and triple mutant individuals)

PCR

Sequence analysis

(96 single and triple mutant individuals)

Sybr green assay for Lox1

Simple probe assays for Lox2 and Lox3

Genotyping

(96 single and triple mutant individuals)

Molecular marker assays

(results specific for causative mutations)

Results

Genetic mutations in Lox1 and Lox3 genes

Genetic recombination in triple mutant genotype

Genotyping

74 bp deletion, Y565

Lox1+c (Jinpumkong 2)

Lox2 (PI 86023)

Lox3+ (PI 4049251)

Genotypic segregation in triple mutant genotype: Lox1,c, Lox2 and Lox3 alleles

Identified seed lox mutant alleles

Together with the previously described mutation in Lox2 gene there are now a total of five identified lox mutant alleles

Lox1 mutants

Lox2 mutants

Lox3 mutants

Triple Lox mutants

Molecular marker assays

Perfectly distinguished the mutant lox1-c, lox2-a and lox3-a from the wild type alleles. Since these markers are associated to the causative mutations there is no chance to get false positives due to recombination. Additional advantages are:

- Easy to perform
- Rapid
- Distinguish heterozygote from homozygote individuals (co-dominant)
- Fewer samples required
- Repeatable

There was a perfect association between genotype of parental plants and Lox phenotype of progeny seeds as shown in the table below

Conclusions

We identified two independent null mutations responsible for the Lox1-free phenotype.

We also found the molecular basis of the null mutation at Lox3 loci.

We determined that genetic recombination within the Lox1 locus broke the tight linkage in repulsion phase between Lox1 and Lox3 loci in Jinpumkong 2.

We developed co-dominant molecular markers perfectly associated with the causative mutations for Lox1, Lox2 and Lox3 genes.

These molecular markers will allow breeders the easy and rapid introgression of the Lipoxygenase-free trait in their soybean varieties.