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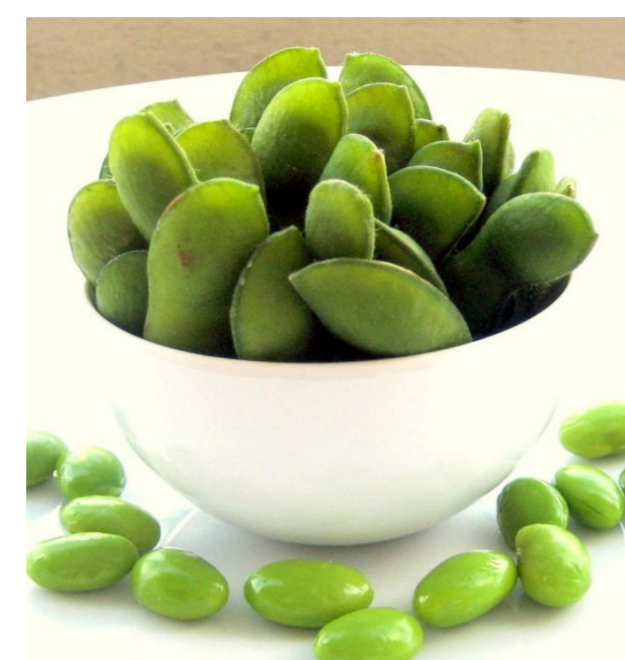
Several soybean-containing food products have grassy and beany flavor and aroma due to the action of Lipoxygenase 1, 2 and 3 enzymes.

Off-flavors result from the oxidation of Polyunsaturated Fatty Acids (PUFA) by Lipoxygenases!

PUFA + O_2 + Lipoxygenases = I don't like soybean food!!

Null recessive alleles for the three Lipoxygenase enzymes have been identified through genetic screening

Lipoxygenase 1 and 2 genes are in tight linkage, while Lipoxygenase 3 gene segregates independently of Lipoxygenase 1 and 2 loci

Lox2 Lox1 — Chromosome 13   Lox3 — Chromosome 15
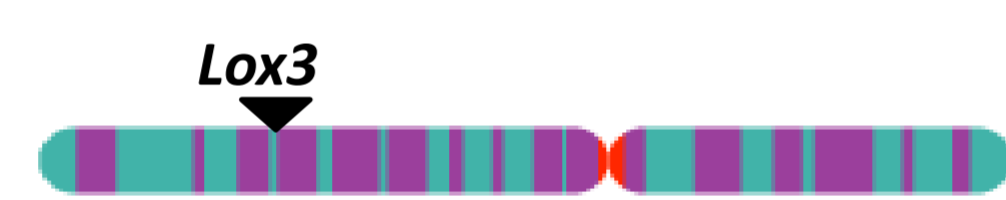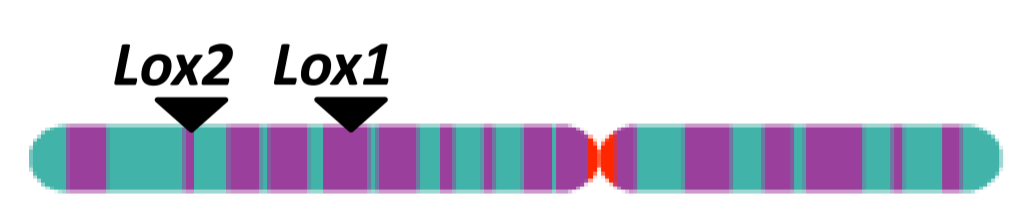
Lipoxygenase 1 and 2 genes are linked in repulsion phase. For many years it was IMPOSSIBLE to gather the three mutant alleles
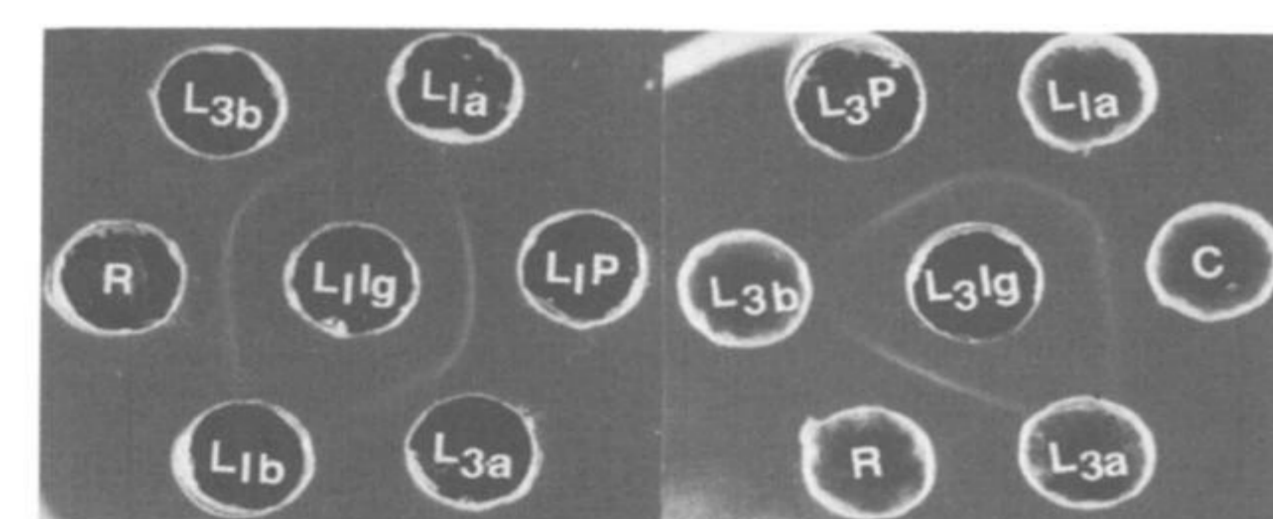
Molecular basis of null mutation in Lipoxygenase 2 was previously determined (Wang et al. 1994)

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L-2         527  H W L N T H A V I E P F I I A T N R H L  546
lox2 protein 527  * * * * * Q * * * * * * * * * * * * * *  546
L-1         499  * * * * * * * A M * * V * * * H * * *  509
L-3         518  * * * * * * * * V * * * * * * * * *  528
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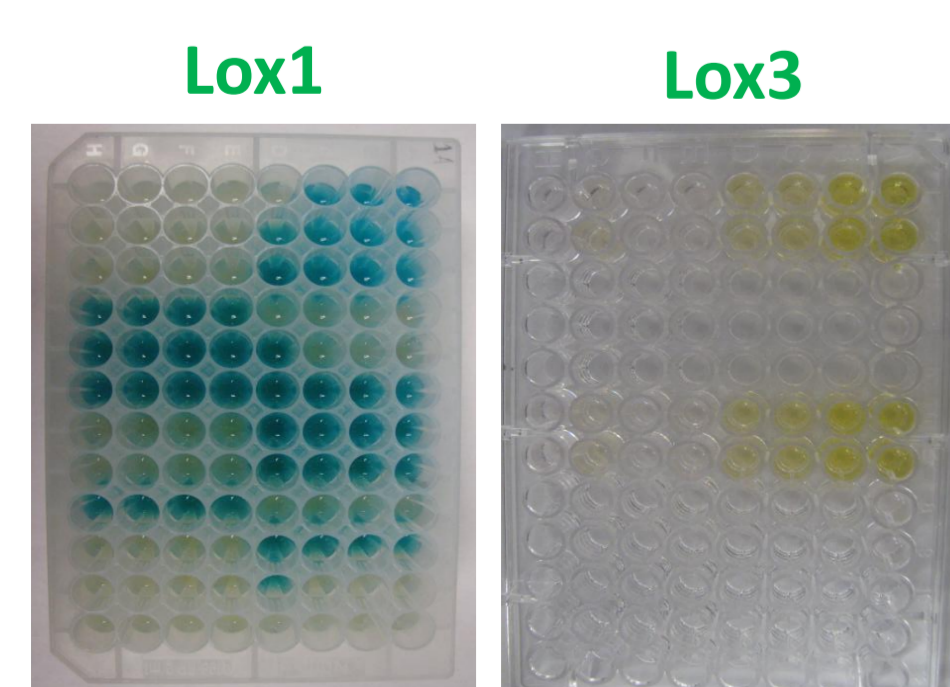### In the past, presence or absence of Lipoxygenases has been determined with methods such as:
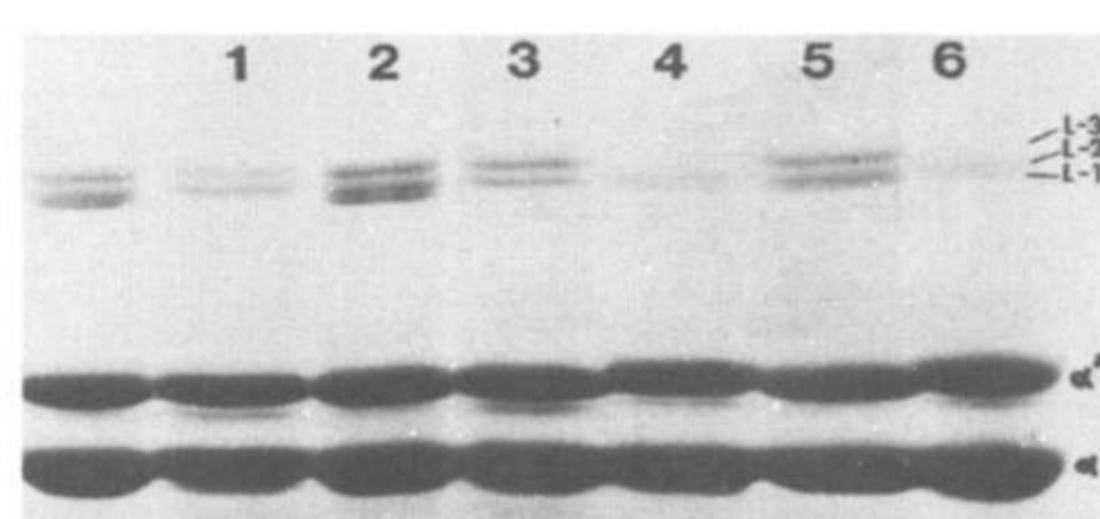
Single dimension SDS-PAGE   Immunological assays

Lox1   Lox3

Colorimetric assays

Disadvantages
- Laborious
- Time consuming
- Unable to distinguish heterozygote from homozygote wild type
- To determine genetic makeup of individuals, progeny test is required
- Consequently, more samples need to be analyzed.
- Repeatability issues

Objective

- **We wanted to determine the molecular basis of the null mutations in soybean Lox1 and Lox3 genes**
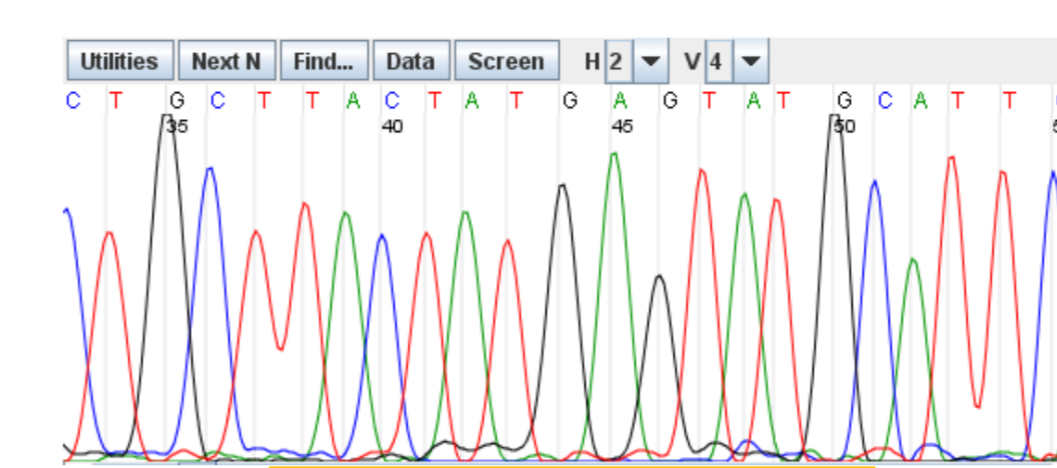- **In addition, we wanted to design molecular marker assays to accelerate breeding for the three soybean seed Lox genes**
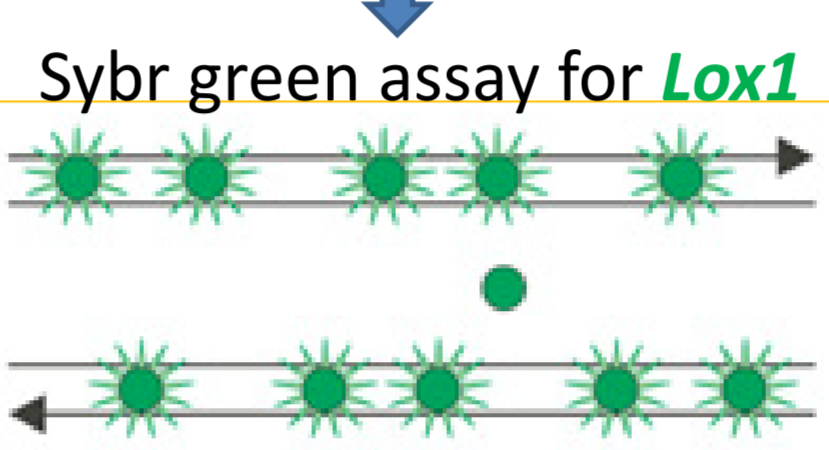
Materials & Methods

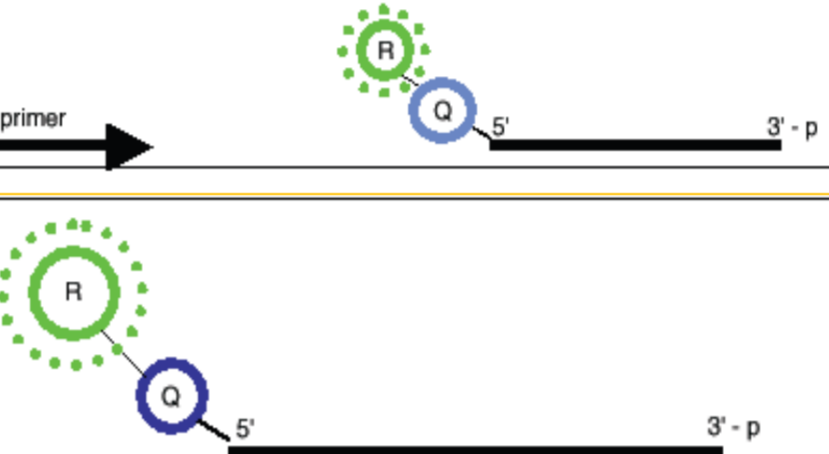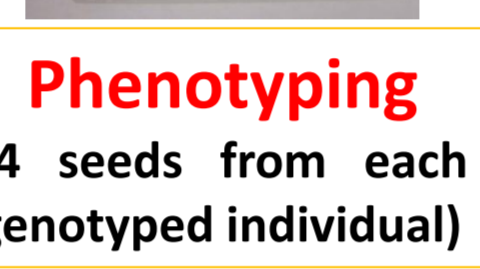**DNA isolation** (from Lox single and triple mutant individuals)

PCR

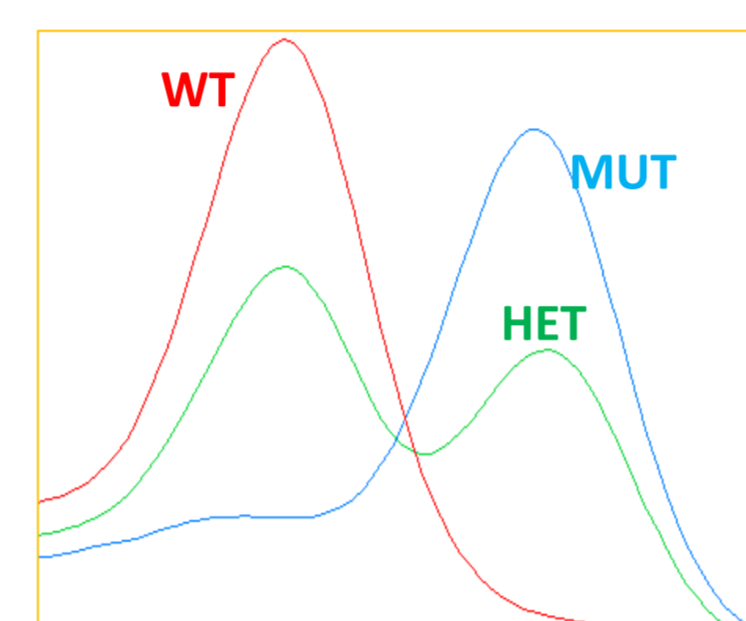**Sequence analysis** (and comparison with reference sequence)

Sybr green assay for Lox1

Simpleprobe assays for Lox2 and Lox3

Phenotyping (4 seeds from each genotyped individual)

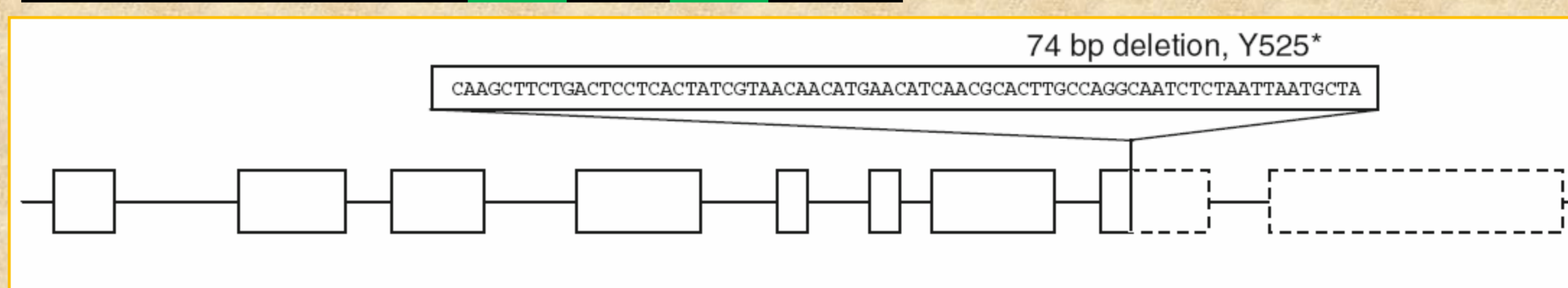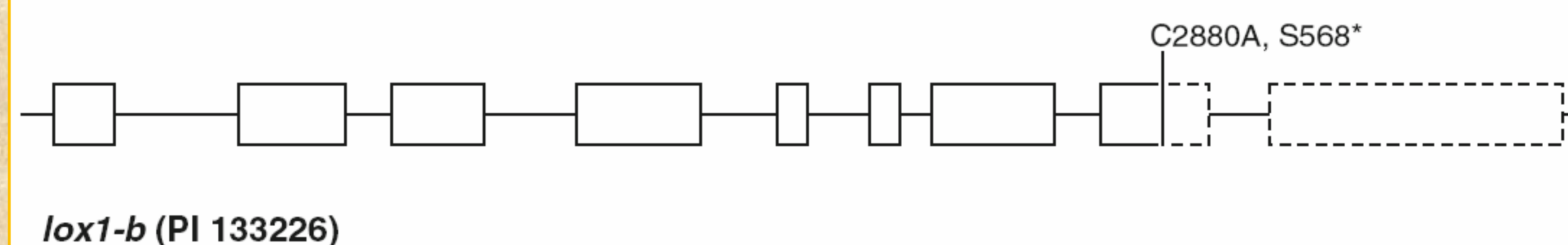**Genotyping** (169 individuals from two segregating populations)

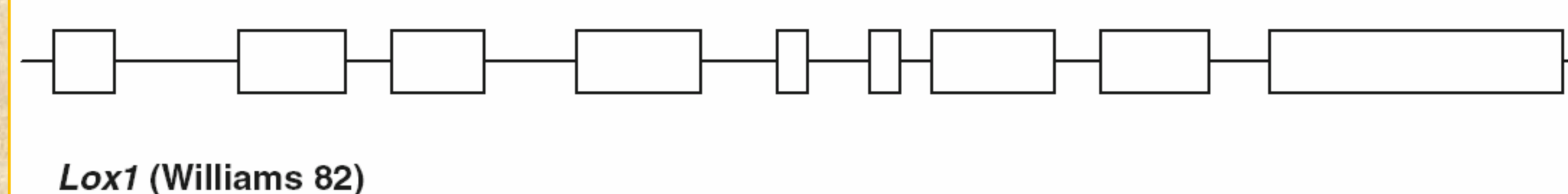**Molecular marker assays** (specific for causative mutations)

Results

Genetic mutations in Lox1 and Lox3 genes

74 bp deletion, Y525*

CAAGCTTCTGACTCCTCACTATCGTAACAACATGAACATCAACGCACTTGCCAGGCAATCTCTAATTAATGCTA
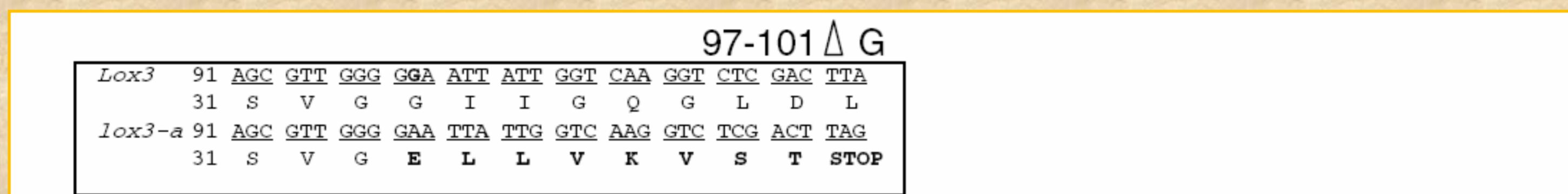

lox1-a (PI 408251)

C2880A, S568*

lox1-b (PI 133226)

Lox1 (Williams 82)

Two independent mutant alleles cause the lack of Lox1 in seeds of two lox1 mutant soy lines

97-101Δ G

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Lox3   91  AGC GTT GGG GGA ATT ATT GGT CAA GGT CTC GAC TTA
       31   S   V   G   G   I   I   G   Q   G   L   D   L
lox3-a 91  AGC GTT GGG GGA TTA TTG GTC AAG GTC TCG ACT TAG
       31   S   V   G   E   L   L   V   K   V   S   T  STOP
```
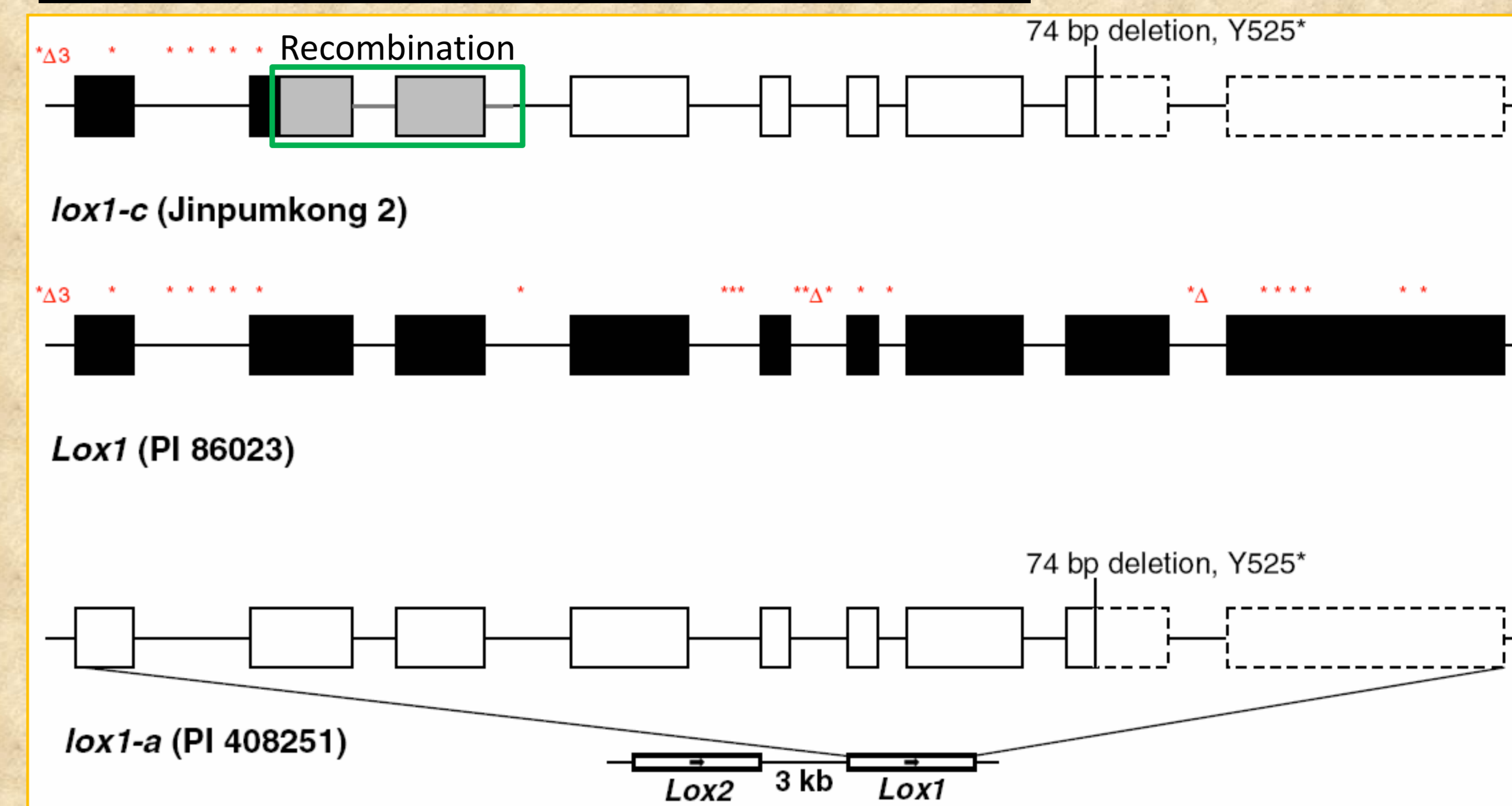

lox3-a (PI 205085, PI 417458)

Lox3 (Williams 82)

The same mutant allele causes the lack of Lox3 in seeds of two lox3 mutant soy lines

Acknowledgment

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Genetic recombination in triple mutant genotype

Recombination   74 bp deletion, Y525*

lox1-c (Jinpumkong 2)

Lox1 (PI 86023)

74 bp deletion, Y525*

Lox2   3 kb   Lox1

lox1-a (PI 408251)

Triple mutant genotype Jinpumkong 2 carries a novel mutant allele of Lox1 containing elements of both the PI 86023 Lox1 and the PI 408251 lox1 alleles. This is consistent with a recombination within the Lox1 locus that broke the linkage in repulsion to couple lox1 and lox2 mutant alleles from independent sources in a single line

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

Listing of mutant alleles at the three soybean seed Lox genes

	Genotype	Lox1	Lox2	Lox3	Reference
Lox1 mutants	PI 133226	lox1-b^{C2880A}	Lox2	Lox3	Lenis et al. 2010
	PI 408251	lox1-a^{Δ74bp}	Lox2	Lox3	Lenis et al. 2010
Lox2 mutant	PI 86023	Lox1	lox2-a^{T2849A}	Lox3	Wang et al. 1994
Lox3 mutants	PI 417458	Lox1	Lox2	lox3-a^{G101Δ}	Lenis et al. 2010
	PI 205085	Lox1	Lox2	lox3-a^{G101Δ}	Lenis et al. 2010
Triple Lox mutant	Jinpumkong 2	Lox1-c^{Δ74bp}	lox2-a^{T2849A}	lox3-a^{G101Δ}	Lenis et al. 2010

Blue and lower case font indicates mutant alleles
Red and uppercase font indicates wild type alleles

Molecular marker assays

Perfectly distinguished the mutant lox1-a/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)
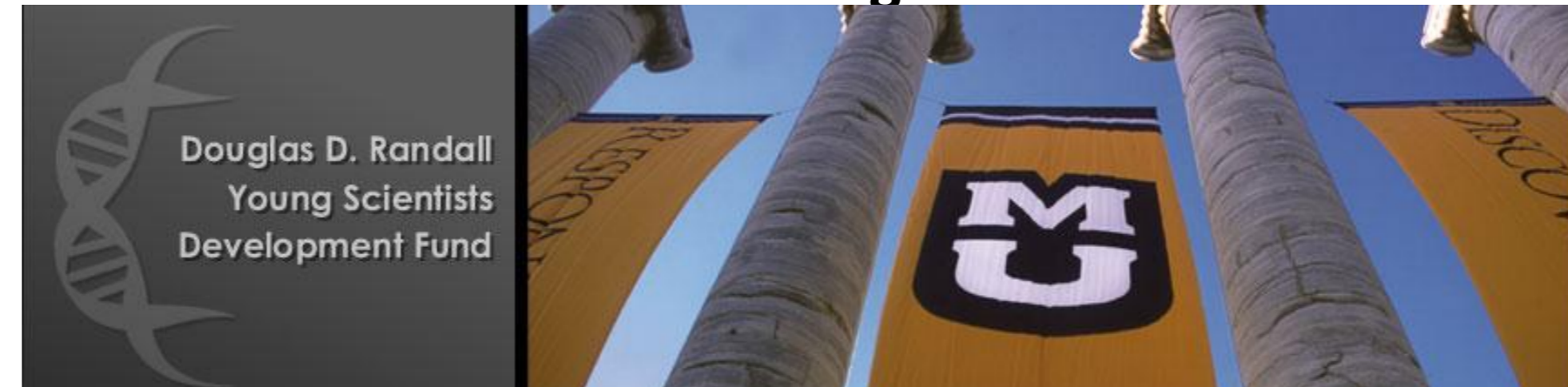- Fewer samples required
- Repeatable

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

Parents genotype		Progeny phenotype			
# of plants	Genotype	Lox1(+) Lox3(+)	Lox1(+) Lox3(-)	Lox1(-) Lox3(+)	Lox1(-) Lox3(-)
27	Lox1 Lox1 Lox3 Lox3	108	-	-	-
8	Lox1 Lox1 Lox3 lox3	28	4	-	-
32	Lox1 Lox1 lox3 lox3	-	128	-	-
2	Lox1 lox1 Lox3 Lox3	5	-	3	-
3	Lox1 lox1 Lox3 Lox3	12	-	-	-
10	Lox1 lox1 lox3 lox3	-	30	-	10
28	lox1 lox1 Lox3 Lox3	-	-	112	-
14	lox1 lox1 Lox3 lox3	-	-	40	16
45	lox1 lox1 lox3 lox3	-	-	-	180

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 Lox2 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.