

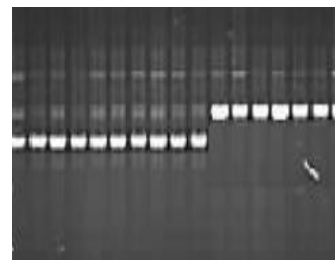
MARKER ASSISTED SELECTION FOR DEVELOPMENT OF SPECIALITY SOYBEAN IN INDIA

KUMAR V AND RANI A

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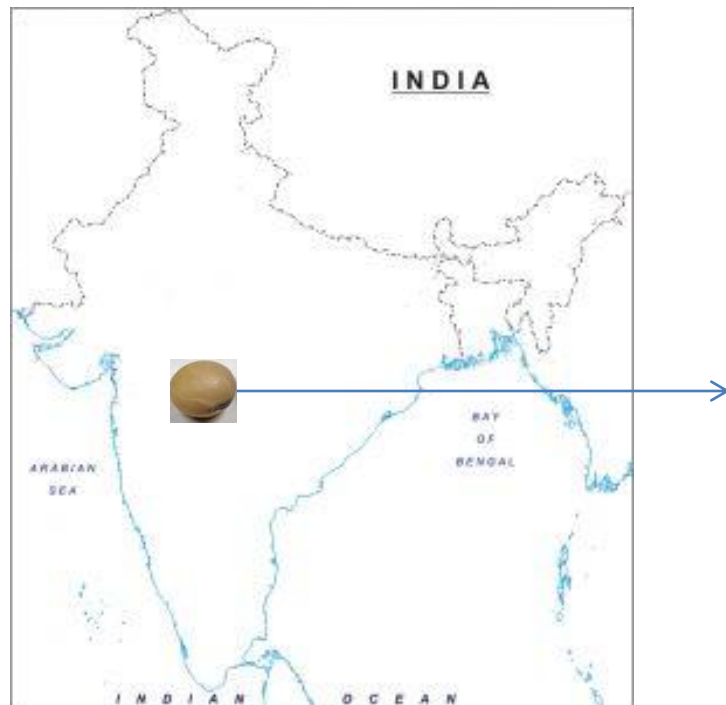
Most of the Indian soybean varieties had yield as the major criterion for their release. Speciality soybeans, which possess special traits, are becoming an increasingly important commodity in the soy trade to address niche-specific requirements. Soybean genotypes with high oil content and high oleic acid are in demand for their higher yield of oil and enhanced shelf life sans *trans* fats. High protein soymeal fetches a premium in the poultry industry while kunitz trypsin inhibitor and lipoxygenase-free soybeans are sought for soyfood processing units. Soybeans with low phytic acid and low oligosaccharides are required for both the soy food and feed sectors. High isoflavones soybeans are a much-sought-after commodity for nutraceutical applications. In our laboratory, we have undertaken marker assisted introgression of null alleles of undesirable monogenic traits into high yielding soybean varieties and identification of QTLs associated with quantitative traits like oil, oleic acid, protein, and isoflavones. We have done exhaustive screening for low phytic acid, low oligosaccharides and high starch soybean genotypes. The Kunitz Trypsin Inhibitor (KTI) and lipoxygenase-2, controlled by single dominant genes, are major deterrents in enhancing the utilization of soybean in food uses. The former constitutes 80% of the total trypsin inhibitor activity, while the latter is the principal contributor to off-flavor. Though both are heat labile, heat treatment is not only cost ineffective but also affects protein solubility. Therefore, development of KTI- as well as lipoxygenase-2-free soybean varieties is critical to boost utilization of soybean in food uses. SSR markers Satt409 and Satt228 reported to be tightly linked with the *Ti* locus on LGp A2, were validated in mapping populations generated from Indian soybean genotypes and PI542044 (donor for *ti*), and are being utilized to transfer the *ti* allele to 5 elite soybean varieties viz., NRC7, JS97-52, MACS450, DS93-05 and DS97-12 through marker-assisted backcross selection under the aegis of DBT, Government of India. A parental polymorphism survey using 484 SSR markers spanning across 20 LGs revealed 44.0-48% polymorphism in 5 parental combinations. One hundred and ten SSR markers found to be polymorphic for all the 5 parental combinations are being leveraged for background selection. Linkage of Satt074 and Satt522 with the *Lx2* gene was validated in the F₂ population generated from LSb1 x PI596540 (*lx2* donor). A new SSR marker, Satt656, tightly linked with the *Lx2* locus was identified in our laboratory. Parental polymorphism was surveyed for 3 popular soybean cultivars JS97-52, JS335 and JS93-05 in combination with PI596540 using linked SSR markers Satt074, Satt522 and Satt656 and SSR markers across the genome for foreground and background selection, respectively.



MARKER ASSISTED SELECTION FOR DEVELOPMENT OF SPECIALTY SOYBEAN IN INDIA

by VINEET KUMAR, PhD





DIRECTORATE OF SOYBEAN RESEARCH
(INDIAN COUNCIL OF AGRICULTURAL RESEARCH)
INDORE, M.P., 452001, INDIA



VEGETABLE OIL

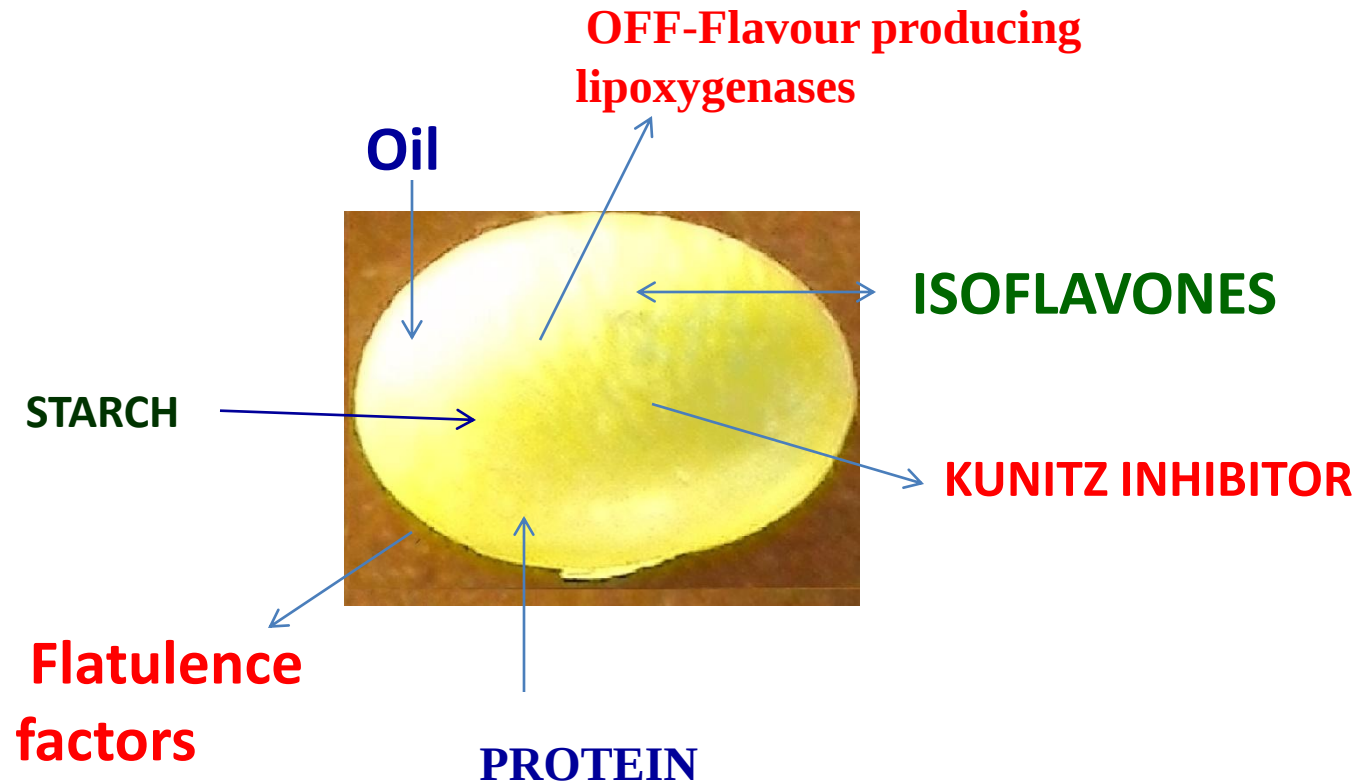


SOY MEAL

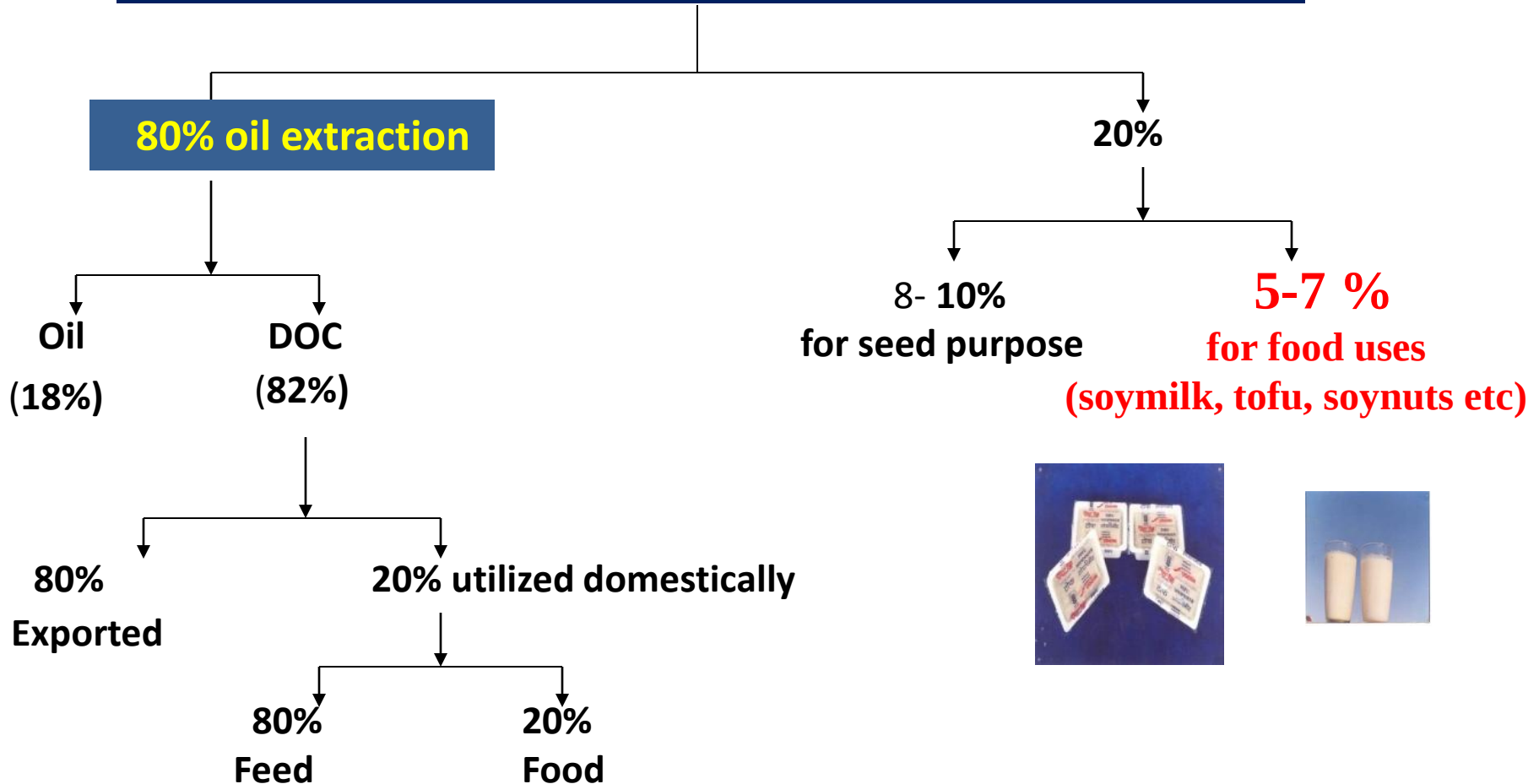
SOY FOOD

NUTRACEUTICALS

Technology interventions for NICHE MARKET



Total SOYBEAN SEED PRODUCED (12.3 mt)

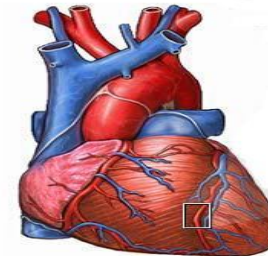
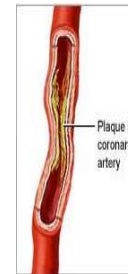


HEALTH PROFILE

Mal- and under-nutrition still rampant



Country is 'diabetes capital of the world'
(4% population)



Atherosclerosis: 64 million by 2015

Osteoporosis : 1 out of every 3 women after the age 45

Obesity : 5% of the population



Breast and Uterus cancer rising

Renewed emphasis for inclusion of soy-based products in
daily diet of the people

WHY SOY PRODUCTS MISSING IN INDIAN KITCHEN.....



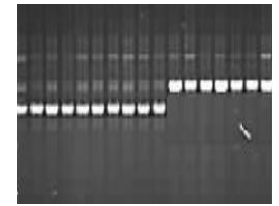
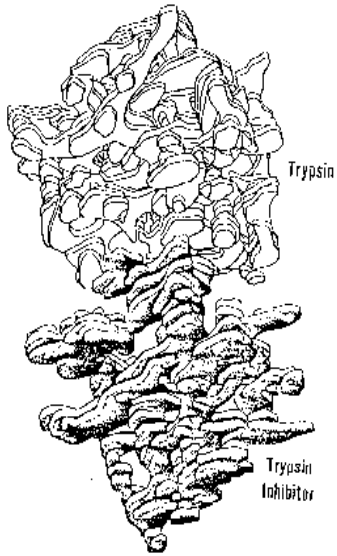
BEANY FLAVOUR

PROTEASE INHIBITORS

FLATULENCE

**POOR OXIDATIVE
STABILITY OF OIL
FRACTION**

**LACK OF
AWARENESS
ABOUT HEALTH
BENIFITS**



KUNITZ TRYPSIN INHIBITOR **FREE**



**CONSTITUTES 80% of TOTAL TRYPSIN
INHIBITOR ACTIVITY**

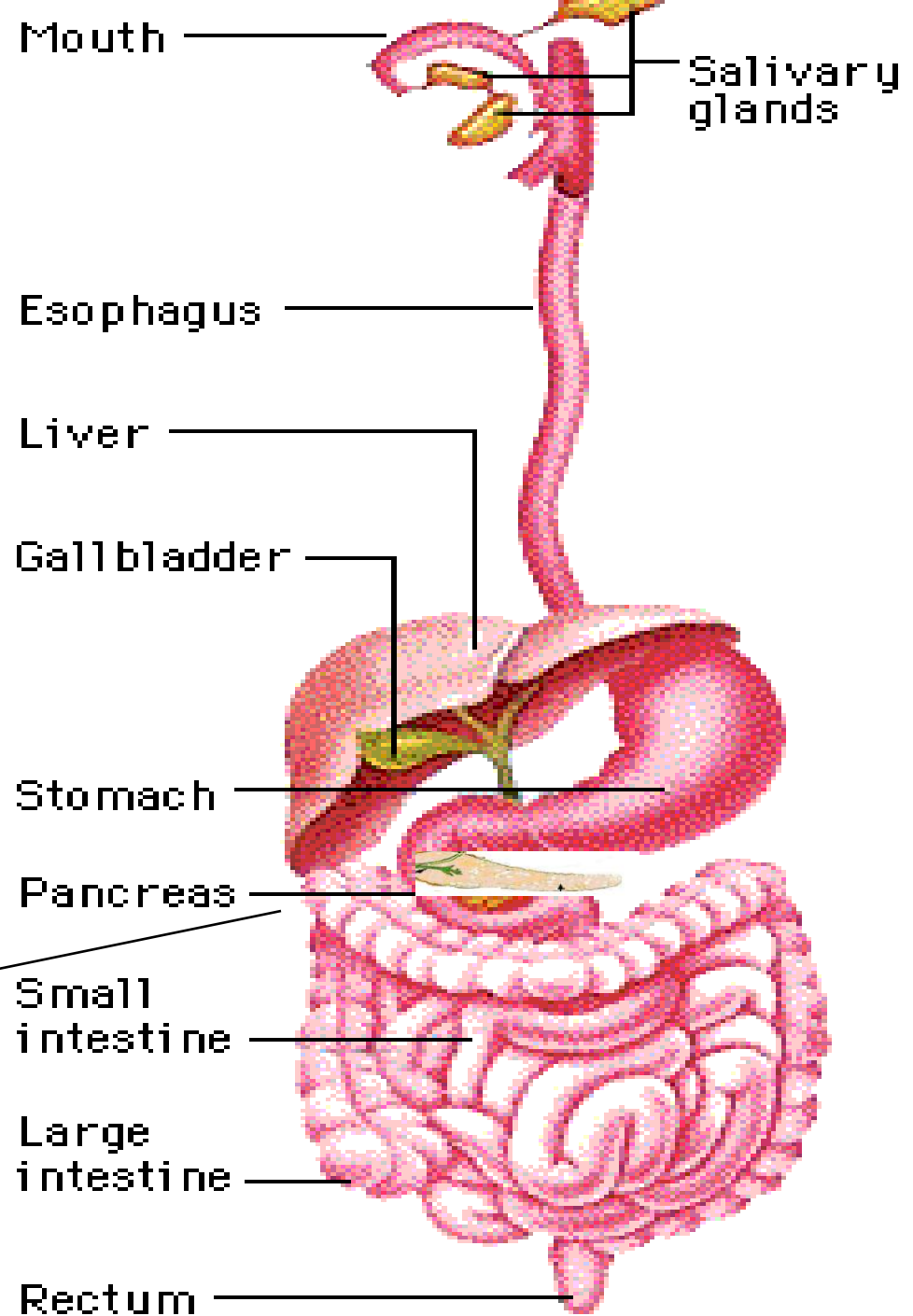
Trypsin inhibitor (TI)

responsible for the growth inhibition and pancreatic hypertrophy and hyperplasia in experimental animals.

TRYPSIN



TRYPSIN INHIBITOR





TRYPSIN INHIBITOR in soybean

KUNITZ TRYPSIN INHIBITOR (21kDa)

Bowman-birk factor (8 kDa) : Anti carcinogenic properties



Soybean kunitz trypsin inhibitor complex with trypsin

WHY KUNITZ TRYPSIN INHIBITOR FREE SOYBEAN in INDIA ?



GRINDING WITH WHEAT (1:9) FOR PREPARING *Chapati* FLOUR. **BOILING FOR 20 min. FOR MAX. INACTIVATION OF KTI (prior to grinding)**



SOY FORTIFIED CHAPATI FOR NUTRITIONAL SECURITY

contd from previous slide....



STRINGENT REQUIREMENTS FOR EXPORT:

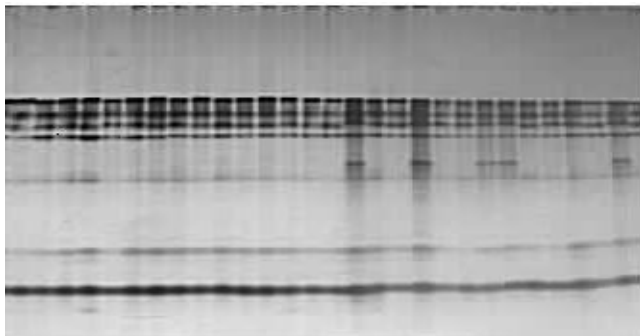
Soy products	TI content (mg/g)
Toasted soymeal	5-8 mg
Toasted soy flour	5-8 mg
Soy protein concentrate	less than 4 mg
Soy nuggets	less than 1.25 mg



Heat treatment accounts 25% of energy cost to soy processing

Contd. from the previous slide....

KTI
band

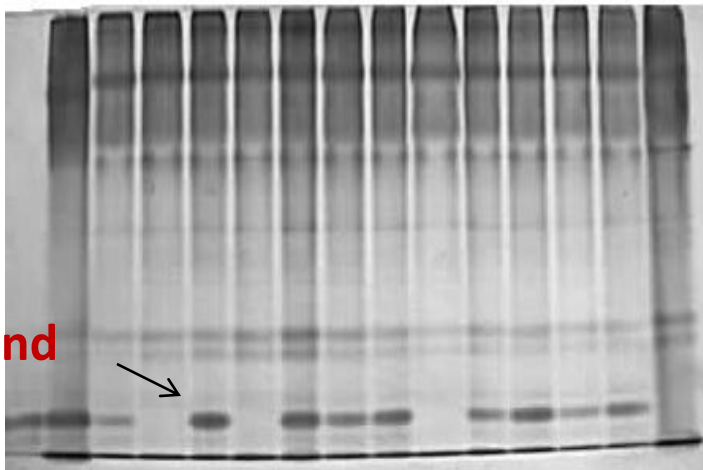


All the released Indian soybean varieties tested electrophoretically and none of them found to be free from KTI. Ranged from 42-110 mg/g of soy flour



Donor of null **kunitz trypsin inhibitor** allele 'PI542044' was procured from USDA at Directorate of Soybean Research, India

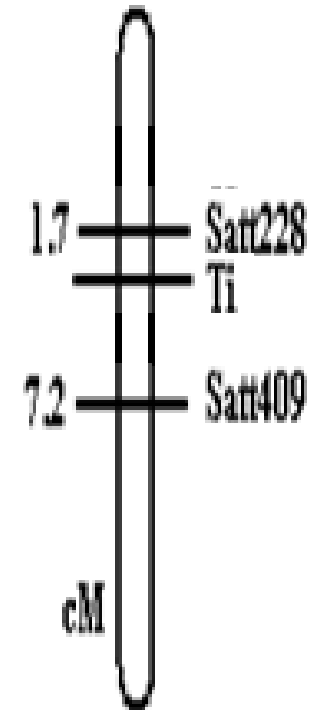
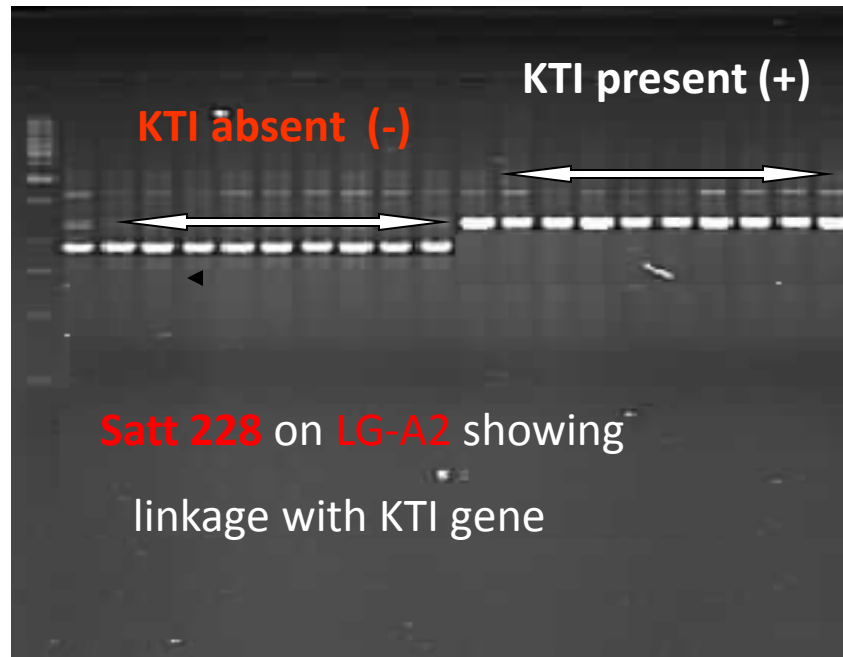
KTI band



Presence or absence of KTI band in early segregating population of Samrat x PI542044



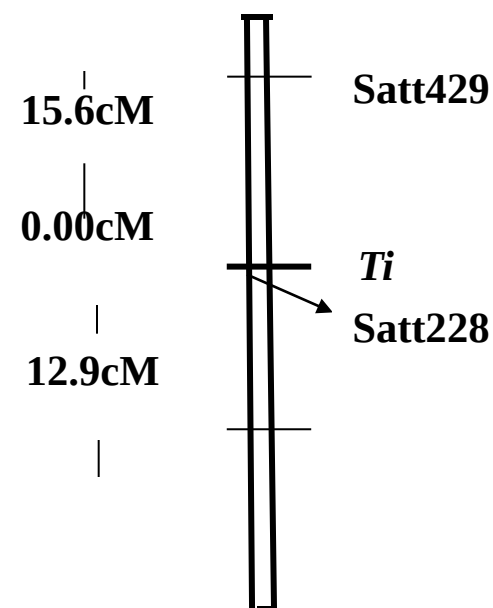
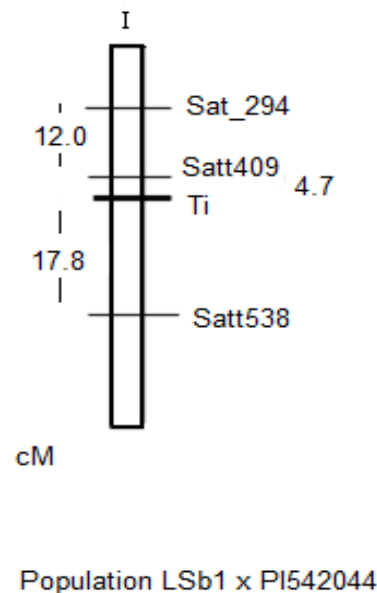
Satt228 marker, reported to be tightly linked with *Ti* locus (Kim *et al.* 2006) for **kunitz trypsin inhibitor**, has been validated in our laboratory by developing mapping population between Indian soybean ‘Samrat’ and ‘PI542044’



Validation of SSR markers linked to null kunitz trypsin inhibitor allele in Indian soybean [*Glycine max* (L.) Merr.] population

Anita Rani • Vineet Kumar • Vaishali Mourya •
R. K. Singh • S. M. Husain

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Population JS97-52 x PI542044

KUNITZ TRYPSIN INHIBITOR FREE GENOTYPES ...

NRC101

EARLY MATURITY : 87 days
YIELD : 2.4 t/ha

ANNEXURE 1b

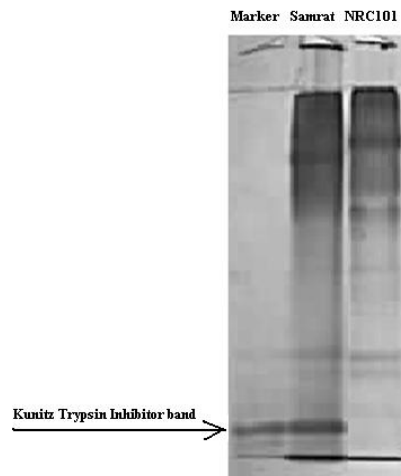


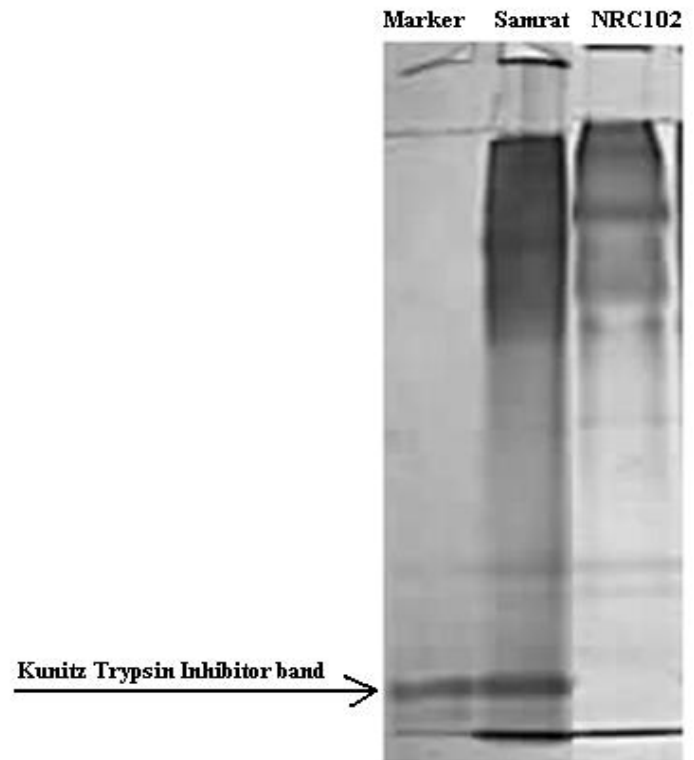
Figure 1: Native PAGE showing absence of Kunitz Trypsin Inhibitor band in NRC101

Contd.....

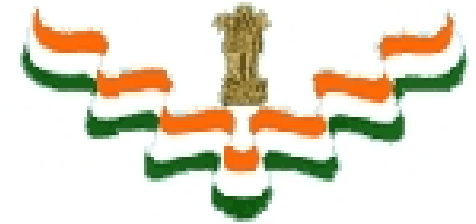
NRC102

Early maturity : 81 days

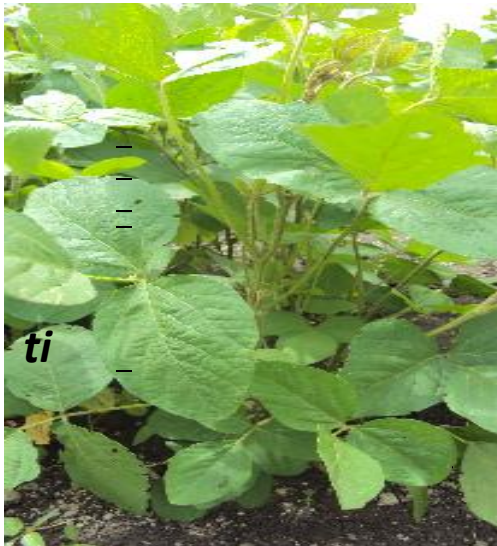
Yield : 2.1 t/ha



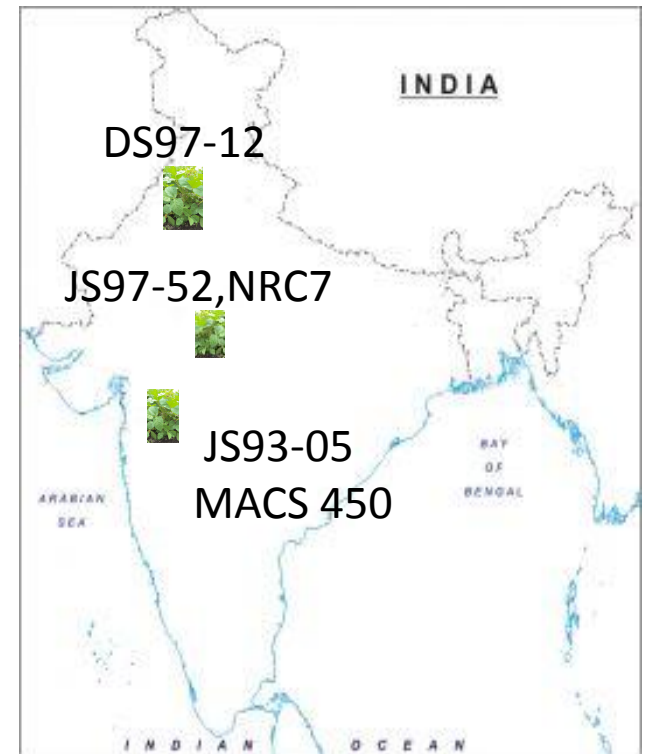
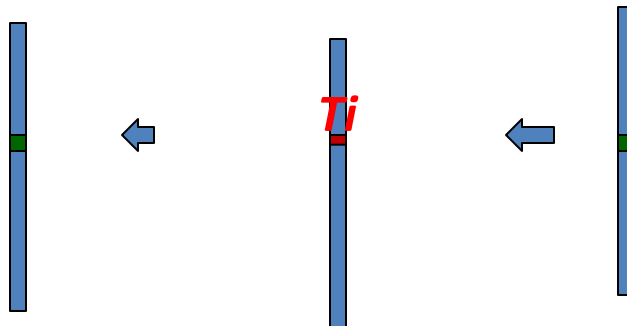
MARKER ASSISTED INTROGRESSION OF NULL ALLELE OF KTI INTO POPULAR INDIAN SOYBEAN VARIETIES



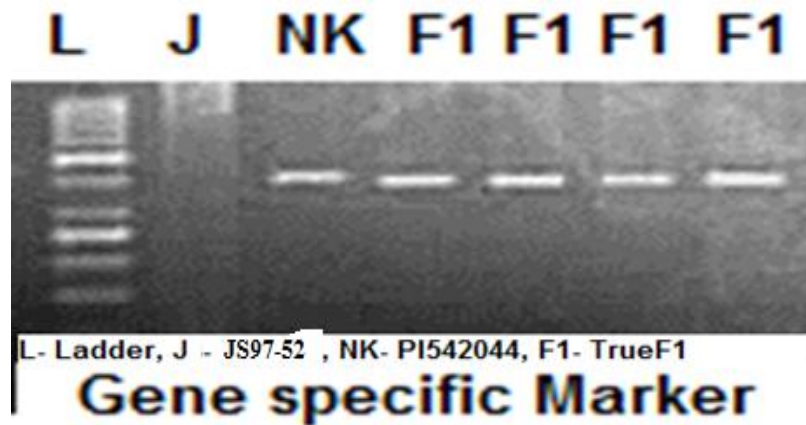
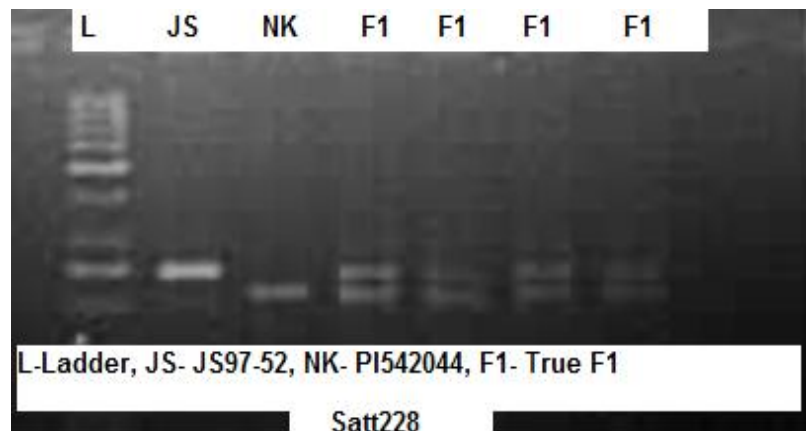
Department of Biotechnology
Ministry of Science & Technology
Govt. of India



PI542044: null KTI

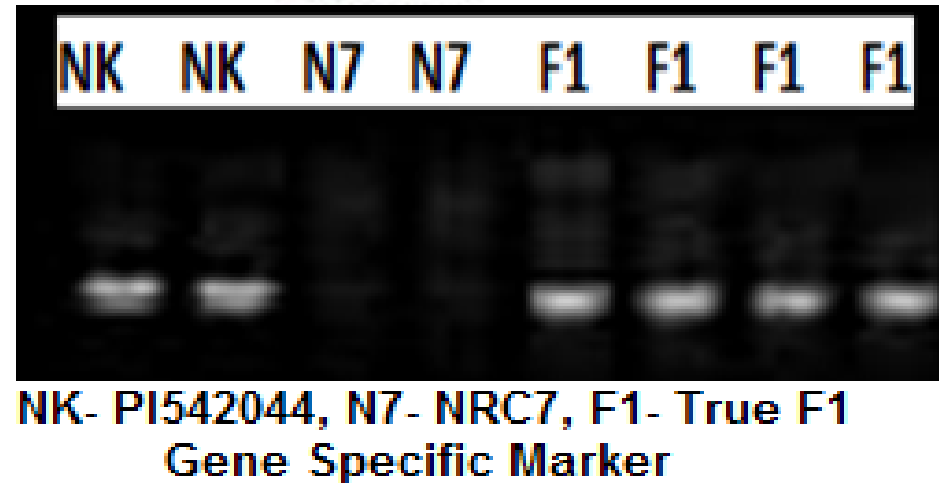
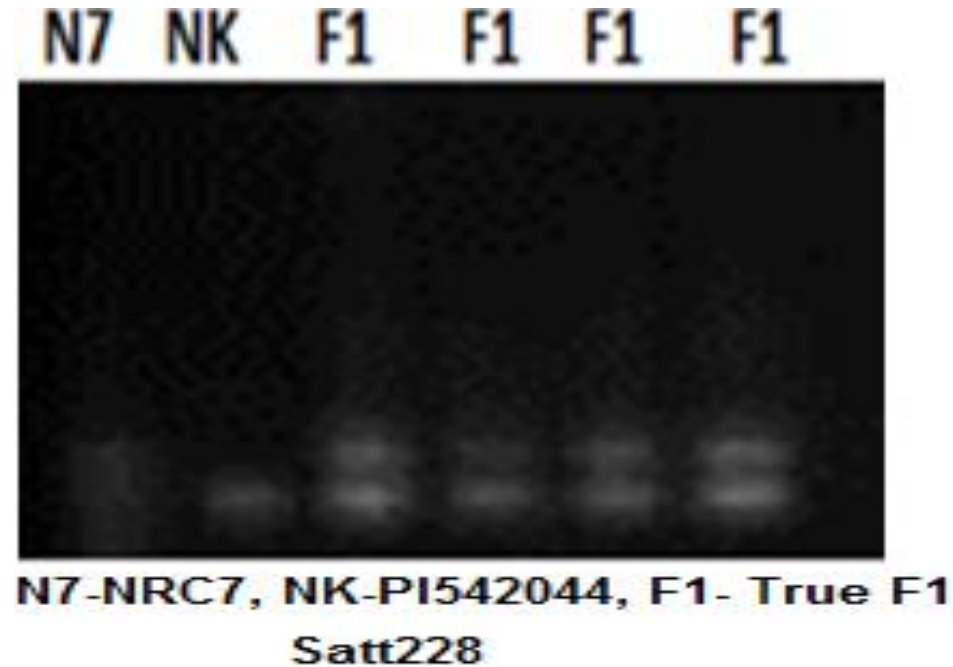


True F1s of JS97-52 PI 542044



5' CTTTTGTGCCTTCACCACCT 3'
R-5'GAATTCATCATCAGAAACTC3'

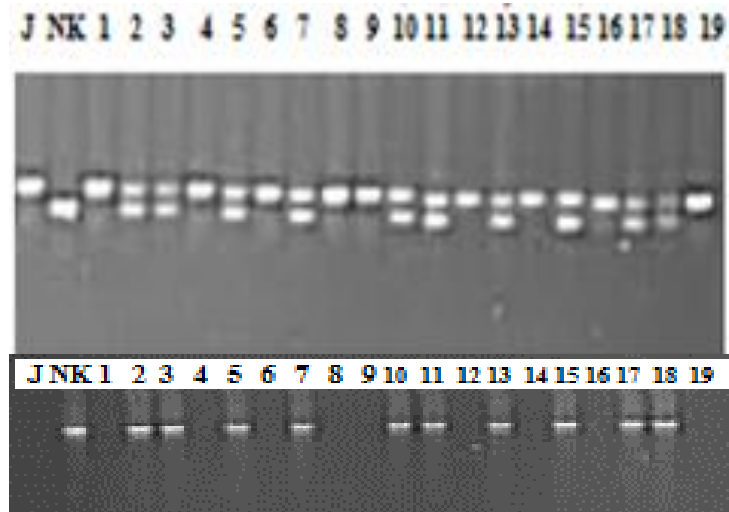
F₁ Plants NRC7 PI 542044 as confirmed by Satt228 and gene specific marker



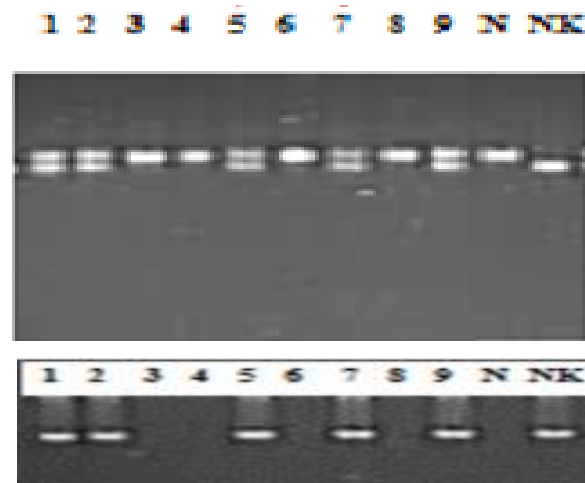
F-5' CTTTTGTGCCTTCACCACT 3' R-
5'GAATTCATCATCAGAACTC3

From the previous slide.....

BREEDING LINE	STAGE	RPG content % (titi)
JS97-52 xPI542044	BC₃F₁	96
NRC7 x PI542044	BC₃F₁	94
JS93-05x PI542044	BC₃F₁	95
MACS450xPI542044	BC₃F₁	96
DS97-12 xPi542044	BC₃F₁	95



I



II

BC₃F₁ plants

I) JS97-52 x PI542044

II) NRC7 x PI542044

as confirmed with Satt228 and gene specific marker

BEANY FLAVOUR / OFF-FLAVOUR



O_2

LIPOXYGENASES

(1-2 % of total seed protein)

Hydroperoxides

OOH lyases

ALDEHYDES (e.g. **Hexanal**), KETONES, ALCOHOLS

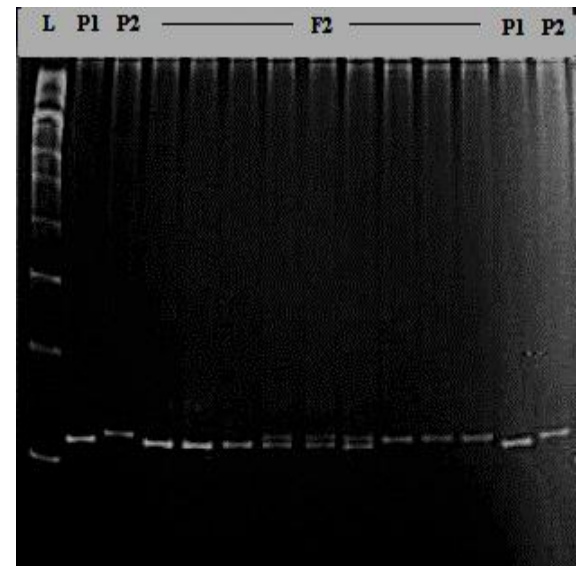
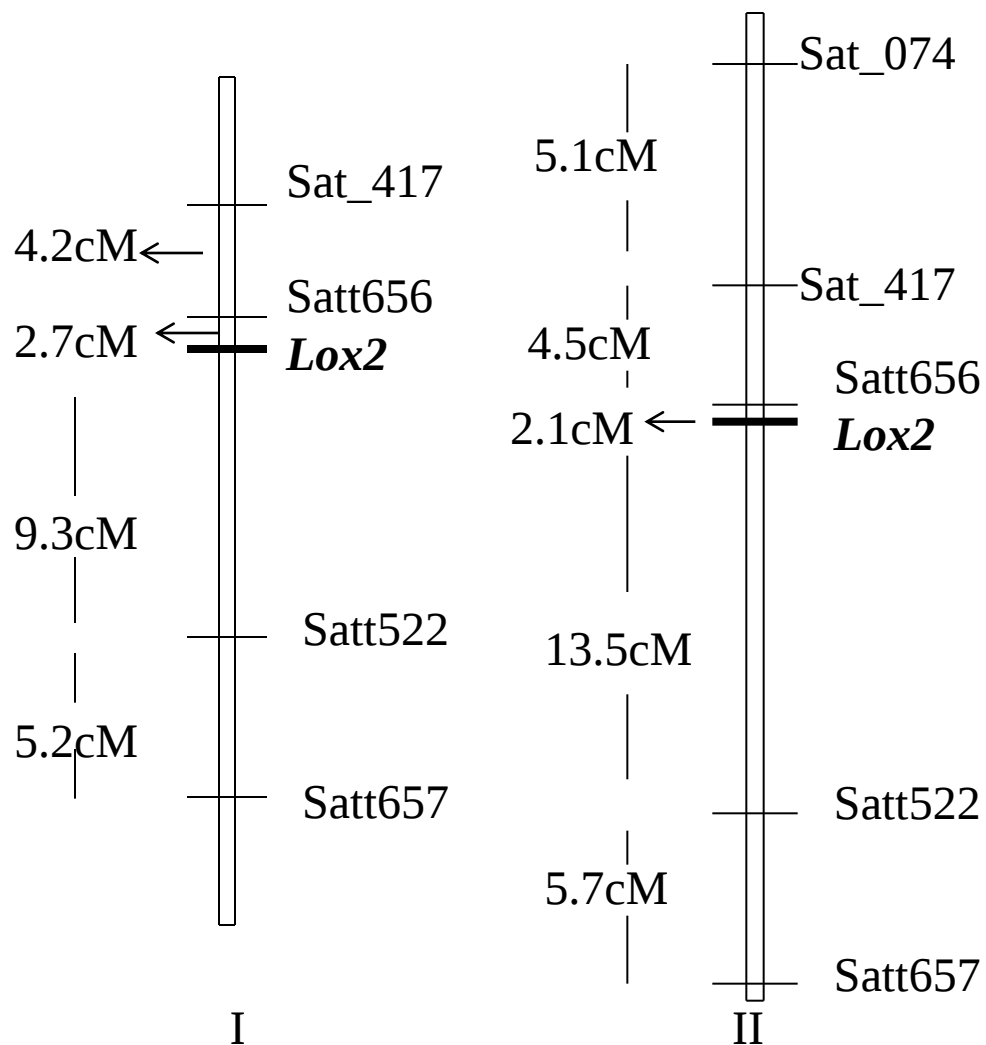
OFF-FLAVOUR causing volatile compounds

Contd from previous slide....

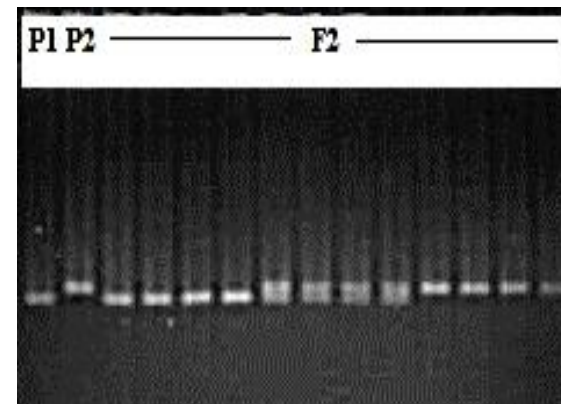
 Presence of each isozyme is controlled by single dominant gene : *Lx1*, *Lx2* and *Lx3*, respectively

 Absence of each of these isozyme is due to single null allele : *lx1*, *lx2* and *lx3*, respectively which are recessive to *Lx1*, *Lx2* and *Lx3*, respectively

 *Lx2* is the principal contributor to the off flavor



I



II

Linkage map of *Lox2* in segregating F₂ population

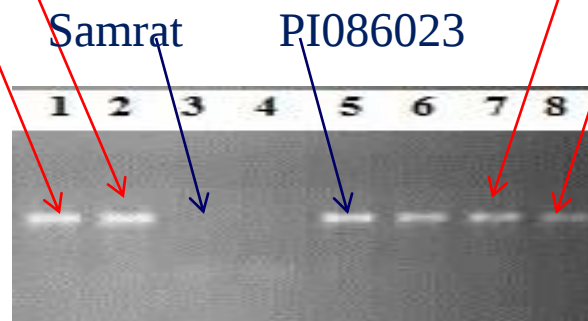
I) JS97-52 (*Lox2Lox2*) x PI596540 (*lox2 lox2*) II) JS93-05 (*Lox2Lox2*) x PI596540 (*lox2 lox2*)



NRC109

NRC110

Lipoxygenase -2 free soybean varieties



Sequences of primer specific to *lox2*

Forward 5' - AAACCAGTAAGATAACAGCAGATG - 3'

Reverse 5' - AATGGCTCAATCACCGCT - 3'

Lipoxygenase-2-free-Indian soybean (*Glycine max* L.) genotypes

Numerous nutraceutical components present in soybean are reported to check the onset of killer diseases like diabetes, estrogen deficiency-induced cancer, atherosclerosis, etc. Also its richness in basic nutrients which can fight the diseases originating from mal and/or under nutrition have made soybean the 'functional food of the century'. Despite these virtues, barely 5–7% of the soybean seeds produced in India is processed for soyfood and snacks¹. The off-flavour associated with soy products is one of the major deterrents in the widespread acceptance of soyfood. This is due to the hexanal compounds released by the catalytic oxidation of polyunsaturated fatty acids in the oil fraction seed lipoxygenase enzyme in soybean. When the seeds are subjected to grinding for making any soy product, lipoxygenase comes in contact with its substrate (polyunsaturated fatty acids) and thereby triggers the catalytic oxidation in the presence of oxygen. In fact, lipoxygenase in soybean seed exists in three isozymic forms² – lipoxygenase-1, lipoxygenase-2 and

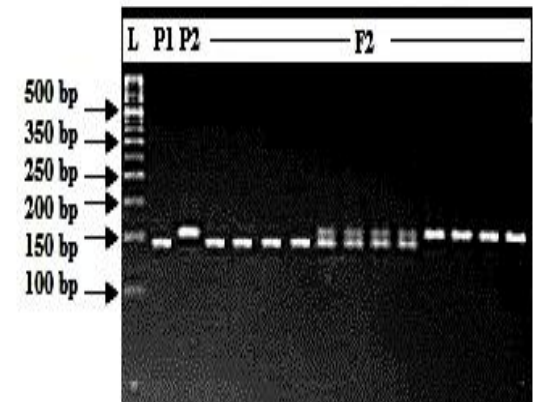
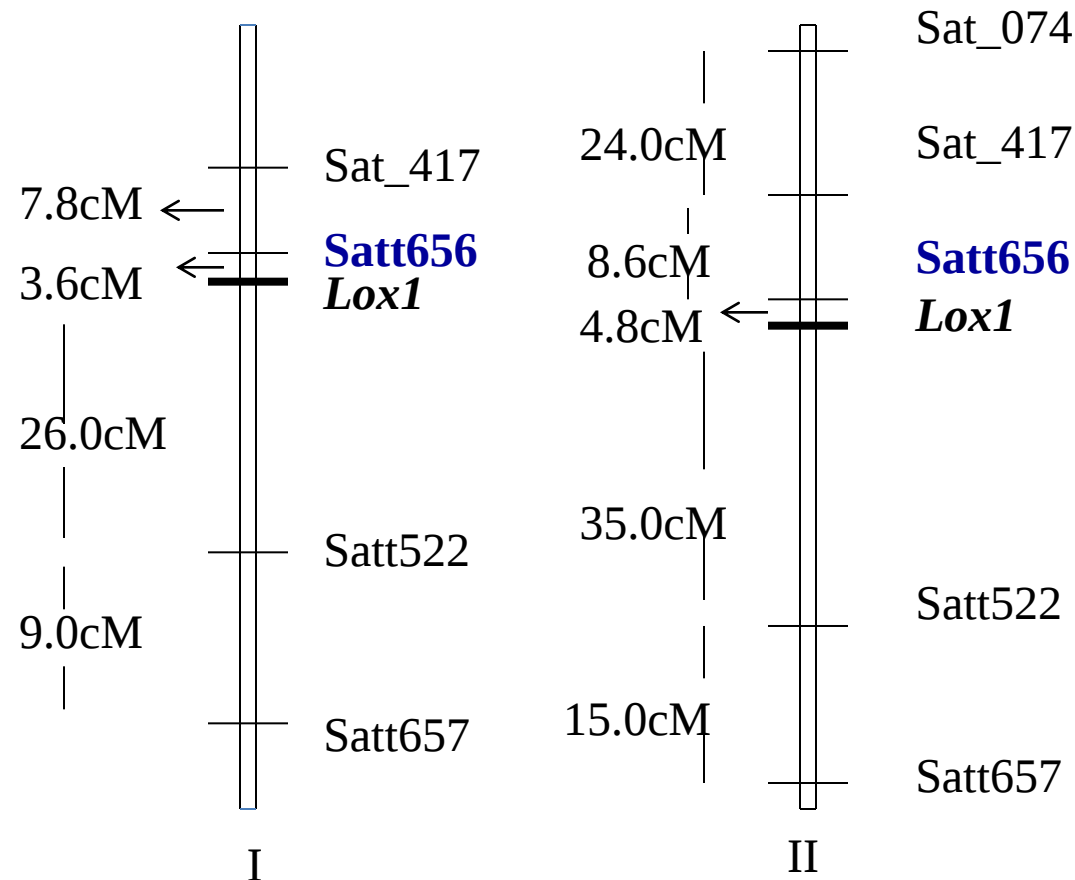
bean Research, Indore. Here we report the successful development of lipoxygenase-2-free genotypes NRC109 and NRC110 by crossing varieties Samrat and PI086023. Samrat is a farmers' variety of soybean cultivated widely in Central India. PI086023 is a source of *lox2* allele which was procured from the United States Department of Agriculture. Its plant type is agronomically poor and not adaptable to Indian conditions. The crossing programme was carried out up to F7 generation. The selection for null lipoxygenase-2 plants in each generation was made by rapid assay in the seeds, according to Suda *et al.*⁷. Briefly, 2.5 mg soybean seed flour was mixed with 0.5 ml of distilled water by mild stirring and kept for 3–10 min and mixed with 2 ml dye substrate. The dye substrate stock was prepared by mixing 154 mg dithiothreitol, 25 ml 200 mM sodium phosphate buffer (pH 6.0), 5 ml 100 μ M methylene blue, 5 ml 10 mM sodium linoleate substrate and 5 ml acetone. Change in colour after 3 min was recorded visually. In the test tubes con-

in a thermocycler (model PTC100) and the reaction mixture (10 μ l) contained 2 μ l DNA (20 ng/ μ l), 1 μ l PCR (10 $\times$) buffer, 1.1 μ l $MgCl_2$ (25 mM), 0.1 μ l dNTPs (25 mM), 0.4 μ l each of forward and reverse SSR primers (30 ng/ μ l), 0.068 μ l Taq DNA polymerase (3 U/ μ l) and 4.932 μ l distilled water. Initially, DNA was denatured at 94°C for 1 min followed by 30 cycles, each cycle comprising denaturation at 94°C for 2 min, primer annealing at 68°C for 2 min and primer elongation at 72°C for 3 min. Finally, elongation was carried out at 72°C for 10 min. The PCR products were resolved on 3% metaphore gel. Amplicons of 769 bp size were observed in NRC109 and NRC110, similar to the donor parent PI086023 (Figure 1) and no amplicon was seen in Samrat, the lipoxygenase-2-positive parent. This confirmed the transfer of null allele of lipoxygenase-2 in NRC109 and NRC110. There was minor difference in plant height and flowering time between NRC109 and NRC110 and both the varieties bore purple flowers. NRC109 yielded seeds with yellow seed

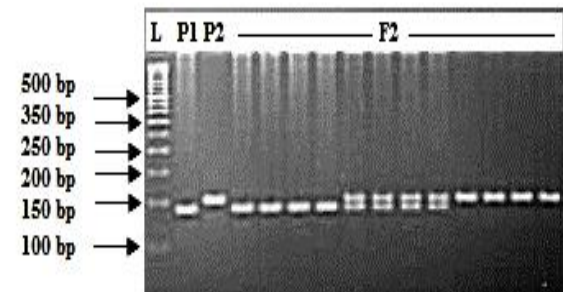
Identification of simple sequence repeat markers linked to lipoxygenase-1 gene in soybean

Anita Rani • Vineet Kumar • Reena Rawal

Received: 28 July 2012 / Accepted: 30 November 2012
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I



II

Linkage map of *Lox1* and segregating F₂ population

I) LSb1 (*Lox1Lox1*)x PI408251 (*lox1 lox1*) and II) JS335 (*Lox1 Lox1*)xPI408251(*(lox1 lox1)*)

MARKER ASSISTED STACKING OF NULL KUNITZ TRYPSIN INHIBITOR WITH NULL LIPOXYGENASE-2...

JS 97-52 x PI542044 (**titi**)



F₁ x JS97-52



BC₁F₁ (Satt228, Satt409)



BC₃F₂

JS 97-52 x PI596540 (**lx2lx2**)



F₁ x JS97-52



BC₁F₁ (Satt656)



BC₃F₂

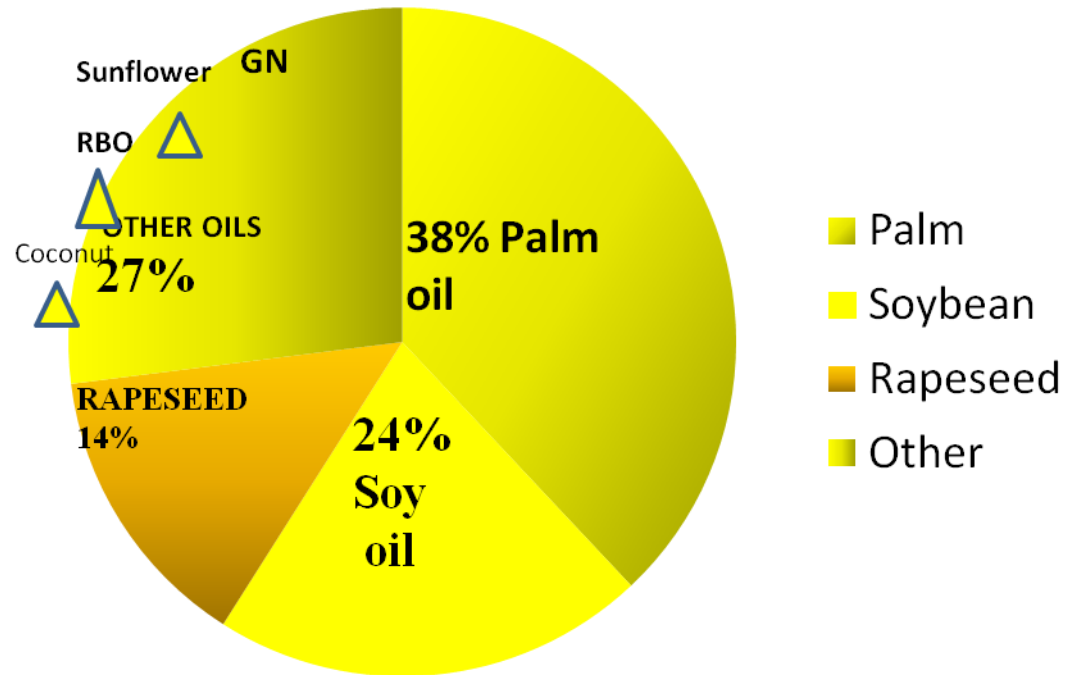
F₁



F₂

SELECTION OF HOMOZYGOUS RECESSIVE (**titilx2lx2**)
USING SATT228 ON LGp A2 AND SATT656 ON LGp F

Edible oil consumption



EDIBLE OIL CONSUMPTION
(18 mt)
IN INDIA

Shelf life of VEGETABLE Oils is function of Fatty acid composition



Palmitic acid (C16:0)

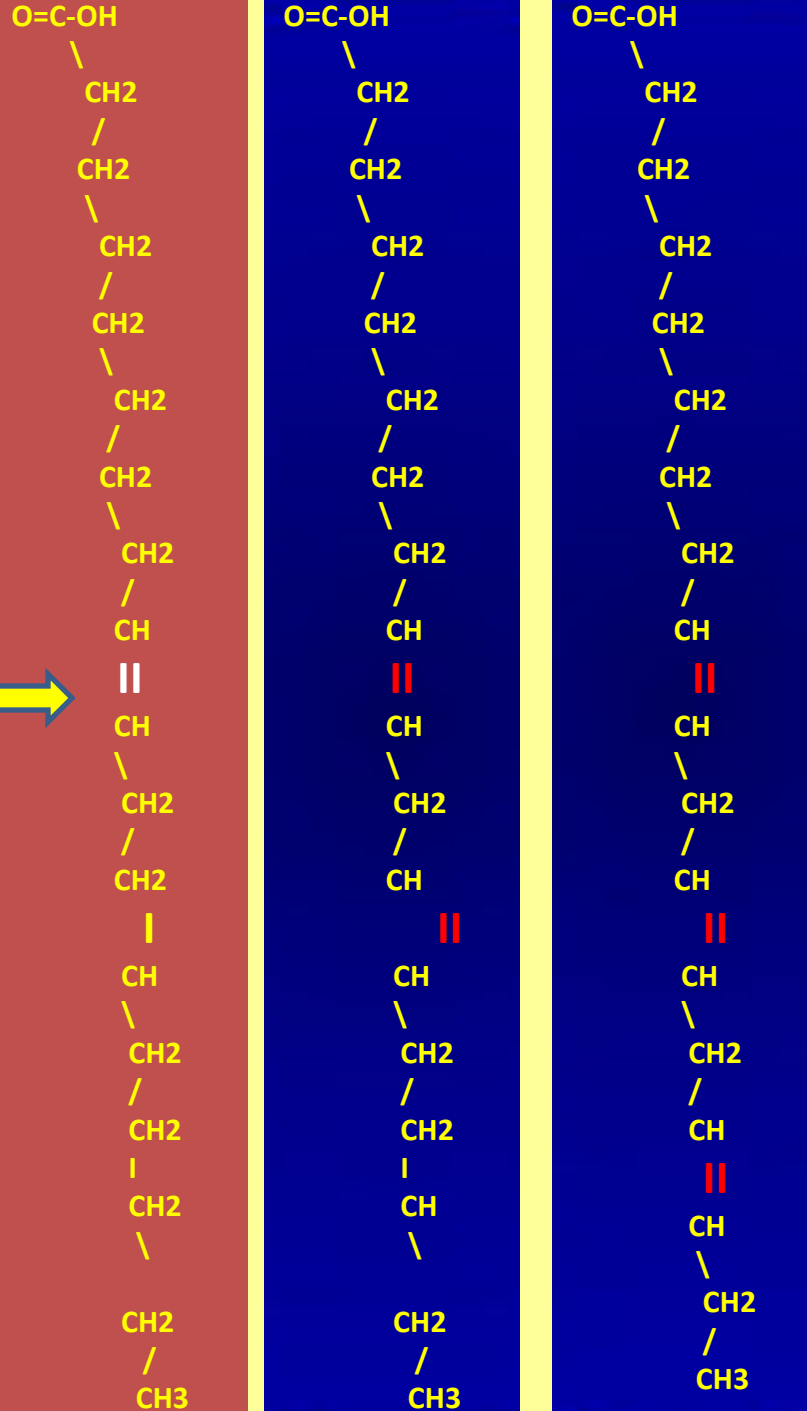
Stearic (C18:0)

Oleic acid (C18:1) **M**

Linoleic acid (C18:2)

PUFA

α Linolenic acid (C18:3)



**Palmitic
(C16:0)
11%**

**Stearic
acid
(C18:0)**



**Oleic acid
(C18:1)**



**Linoleic
(C18:2)**



**Linolenic
(C18:3)**



Oxidation

1



:

10.3



:

21.6



**Regular
Soy oil**

24

53

7

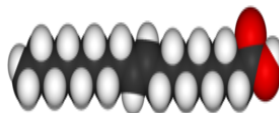
Hdgen oil

63

15

3

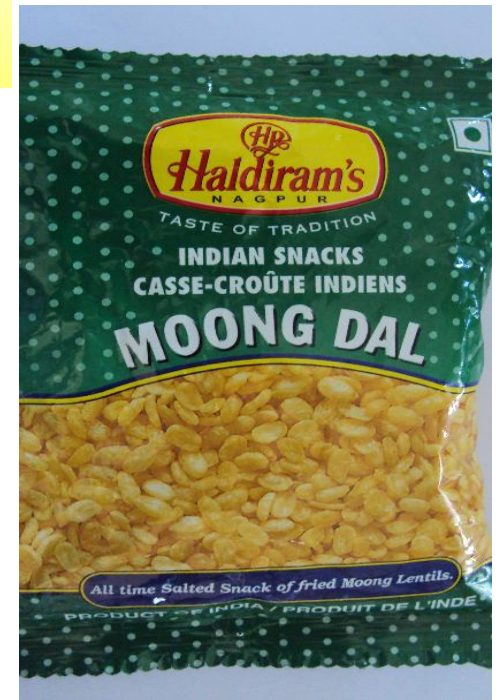
TRANS FATS



ELAEDIC ACID

FOR NO TRANS FATS IN SNACKS.....

HIGH OLEIC SOYBEAN



Nutritional Values Per 100 g	
1. Total Fat	23.30g
Saturated Fatty Acids	3.58g
Monounsaturated Fatty Acids	9.76g
Polyunsaturated Fatty Acids	7.26g
Trans. Fatty Acids	0g
2. Protein	20.55g
3. Carbohydrate	47.16g
4. Fiber	1.53g
5. Cholesterol	Nil
6. Calories	480 Kcal

*These are approximate values



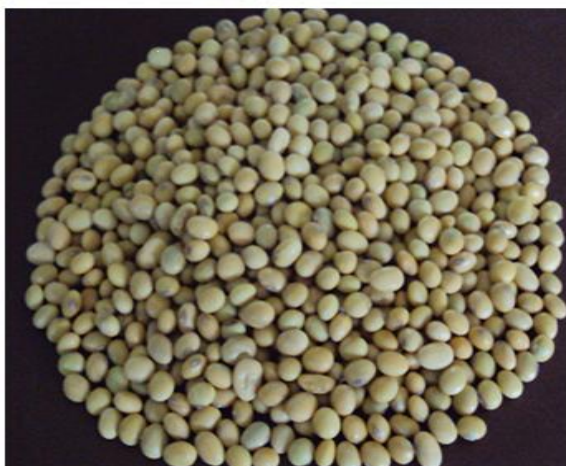
▲
NON TRANSGENIC
APPROACHES.....



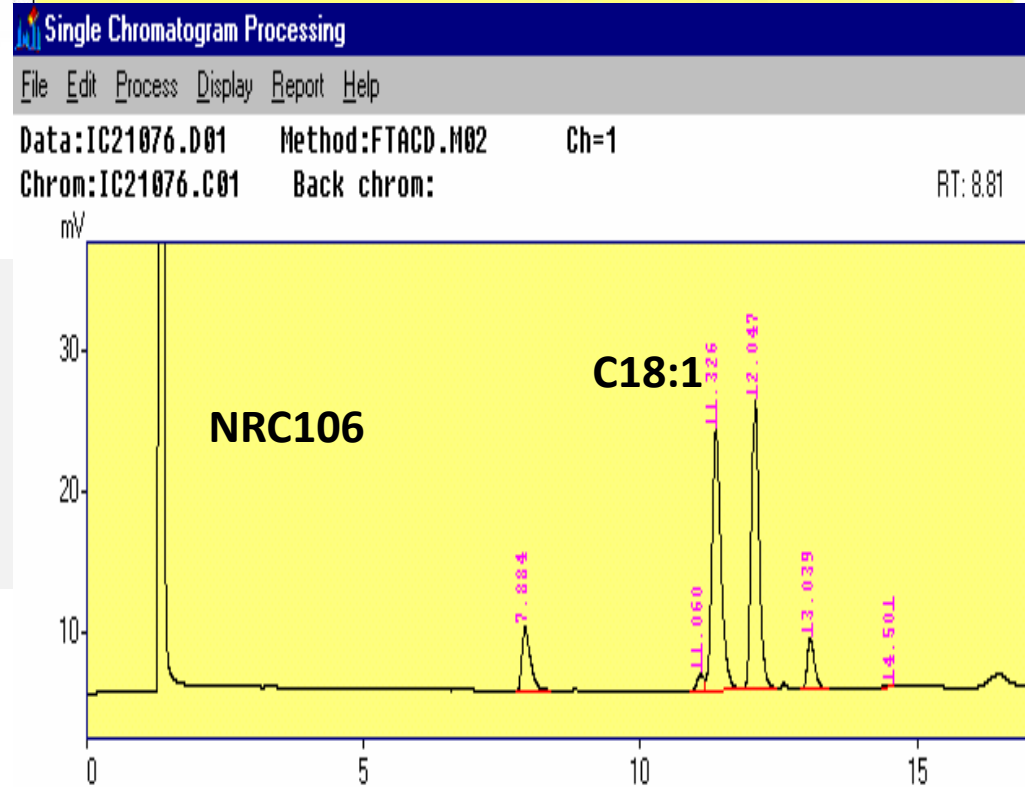
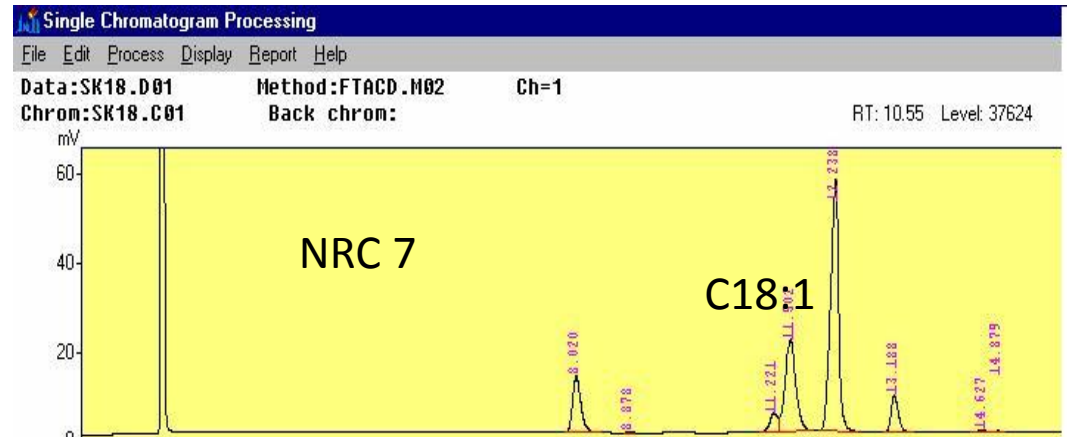
OLEIC ACID

High OLEIC INDIAN SOYBEAN (42% oleic acid)

IC210;M: 98 days
Yield: 3.0 t /ha



NRC106
92 d
Yield :
3.2 t/ha





35% OLEIC ACID

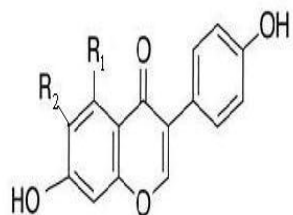


**Developed from the lab sought
by Indian Tobacco Company
Ltd for commercialization.....**

PHENOTYPIC VARIATION FOR QUALITY TRAITS IN RECOMBINANT INBRED LINES DEVELOPED FROM.....

Traits	LSb1 (P1)	NRC7 (P2)	RIL (117 lines)	
			Min.	Max.
Total Isoflavones (µg/g)	1941.12	761.19	393.50	2147.95
Diadzein (µg/g)	891.95	281.55	136.81	819.72
Genistein (µg/g)	735.77	365.58	110.58	1049.84
Oleic Acid (%)	39.0	23.6	18.3	61.9
Linolenic Acid (%)	7.6	7.7	3.1	9.5

	Daidzein	Genistein	Glycitein
R1	H	OH	H
R2	H	H	OCH3



**HIGH ISOFLAVONES soy
AS EXCELLENT RAW MATERIAL
FOR NUTRACEUTICAL
MARKET.....**



**QTLs controlling ISOFLAVONES *as assessed through*
RECOMBINANT INBRED LINES**

TRAIT	SSR MARKERS	LINKAGE GROUP	LOD	PERCENT CONTRIBUTION
DAIDZEIN	Sat_304	B2	3.02	8.6
	Sat_131	G	3.24	9.5
	Satt564	G	3.21	9.3
GENISTEIN	Satt564	G	3.37	13.1

QTLs CONTROLLING <i>OLEIC ACID</i> & <i>LINOLENIC ACID</i> as assessed in RIL population derived from Indian soybean LSb1 x NRC7				
	SSR MARKERS	LINKAGE GROUP	LOD	PERCENT CONTRIBUTION
OLEIC ACID	Satt276	A1	3.37	10
	Satt128	B1	3.04	8.6
	Satt_042	C2	3.9	12
	Sat_131	G	3.14	9.1
	Satt564	G	3.39	10.0
	Satt513	L	3.3	9.7
LINOLENIC ACID	Satt128	B1	3.64	11.0
	Satt577	B2	3.72	11.4
	AI856416	D1b	3.35	9.9
	Satt570	G	3.42	14.1
	Satt_131	G	5.18	20.5

Thank you

