ABSTRACT: 269

MARKER ASSISTED SELECTION FOR DEVELOPMENT OF SPECIALITY SOYBEAN IN INDIA

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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 \textit{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 \textit{Ti} locus on LGp A2, were validated in mapping populations generated from Indian soybean genotypes and PI542044 (donor for \textit{ti}), and are being utilized to transfer the \textit{ti} allele to 5 elite soybean varieties \textit{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 parental combinations are being leveraged for background selection. Linkage of Satt074 and Satt522 with the \textit{Lx2} gene was validated in the F\textsubscript{2} population generated from LSb1 x PI596540 (\textit{lx2} donor). A new SSR marker, Satt656, tightly linked with the \textit{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
Technology interventions for NICHE MARKET

- Off-flavour producing lipoxygenases
- Isoflavones
- Kunitz inhibitor
- Protein
- Starch
- Flatulence factors

Oil
Total SOYBEAN SEED PRODUCED (12.3 mt)

80% oil extraction

- Oil (18%)
- DOC (82%)

20%

8-10% for seed purpose

5-7% for food uses (soymilk, tofu, soynuts etc)

80% utilized domestically

- Exported
- Feed (80%)
- Food (20%)
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 BENEFITS
KUNITZ TRYPSIN INHIBITOR FREE

SOYBEAN VARIETIES

CONSTITUTES 80% of TOTAL TRYPSIN INHIBITOR ACTIVITY
Trypsin inhibitor (TI) responsible for the growth inhibition and pancreatic hypertrophy and hyperplasia in experimental animals.
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
## STRINGENT REQUIREMENTS FOR EXPORT:

<table>
<thead>
<tr>
<th>Soy products</th>
<th>TI content (mg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Toasted soymeal</td>
<td>5-8 mg</td>
</tr>
<tr>
<td>Toasted soy flour</td>
<td>5-8 mg</td>
</tr>
<tr>
<td>Soy protein concentrate</td>
<td>less than 4 mg</td>
</tr>
<tr>
<td>Soy nuggets</td>
<td>less than 1.25 mg</td>
</tr>
</tbody>
</table>

Heat treatment accounts **25%** of energy cost to soy processing.
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.

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’

![Image of gel electrophoresis showing KTI absent (-) and KTI present (+) bands](image)

**Satt 228** on LG-A2 showing linkage with KTI gene
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 LSb1 x PI542044

Population JS97-52 x PI542044
KUNITZ TYPSIN 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

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

PI542044: null KTI

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

DS97-12
JS97-52, NRC7
JS93-05
MACS 450
True F1s of JS97-52  PI 542044

5’ CTTTTGTGCTTCACCACCT 3’
R-5’GAATTCATCATCAGAAACTC3’
F<sub>1</sub> Plants NRC7  PI 542044 as confirmed by Satt228 and gene specific marker

N7-NRC7, NK-PI542044, F1- True F1

Gene Specific Marker

F-5’ CTTTTGTGCCTCACCACCT 3’  R-5’GAATTCATCATCAGAAACTC3
From the previous slide

<table>
<thead>
<tr>
<th>BREEDING LINE</th>
<th>STAGE</th>
<th>RPG content %</th>
</tr>
</thead>
<tbody>
<tr>
<td>JS97-52 x PI542044</td>
<td>BC₃F₁</td>
<td>96</td>
</tr>
<tr>
<td>NRC7 x PI542044</td>
<td>BC₃F₁</td>
<td>94</td>
</tr>
<tr>
<td>JS93-05 x PI542044</td>
<td>BC₃F₁</td>
<td>95</td>
</tr>
<tr>
<td>MACS450 x PI542044</td>
<td>BC₃F₁</td>
<td>96</td>
</tr>
<tr>
<td>DS97-12 x Pi542044</td>
<td>BC₃F₁</td>
<td>95</td>
</tr>
</tbody>
</table>
BC$_3$F$_1$ plants
I) JS97-52 x PI542044  II) NRC7 x PI542044
as confirmed with Satt228 and gene specific marker
BEANY FLAVOUR / OFF-FLAVOUR

LIPOXYGENASES

(1-2 % of total seed protein)

Hydroperoxides

O₂

OOH lyases

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

OFF-FLAVOUR causing volatile compounds

LINOLEIC (C18:2) AND LINOLENIC ACID (C18:3)
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.
Linkage map of *Lox 2* in segregating F$_2$ population

I) JS97-52 (*Lox2Lox2*) x PI596540 (*lox2 lox2*)

II) JS93-05 (*Lox2Lox2*) x PI596540 (*lox2 lox2*)
Sequences of primer specific to lox2

Forward  5’ - AAACCAGTAAGATAACAGCAGATG - 3’
Reverse   5’ - AATGGCTCAATCACCACGCT - 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 snacks. 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 forms – 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 *lox*2 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*. 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 μM 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 containing a thermocycler (model PTC100) and the reaction mixture (10 µl) contained 2 µl DNA (20 ng/µl), 1 µl PCR (10x) buffer, 1.1 µl MgCl₂ (25 mM), 0.1 µl dNTPs (25 mM), 0.4 µl each of forward and reverse SSR primers (30 ng/µl), 0.068 µl Taq DNA polymerase (3 U/µl) and 4.932 µl 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% metaphor 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

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Linkage map of *Lox1* and segregating F₂ population

I) LSb1 (*Lox1Lox1*)x PI408251 (*lox1 lox1*) and II) JS335 (*Lox1 Lox1*)xPI408251((*lox1 lox1*))
SELECTION OF HOMOZYGOUS RECESSIVE ($titi^{lx2lx2}$) USING SATT228 ON LGp A2 AND SATT656 ON LGp F
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)
- Linoleic acid (C18:2)
- α Linolenic acid (C18:3)

PUFA
Palmitic acid (C16:0) 11%
Stearic acid (C18:0)

Oleic acid (C18:1)
Linoleic acid (C18:2)
Linolenic acid (C18:3)

Oxidation 1 : 10.3 : 21.6
Regular Soy oil 24 53 7
Hydrogenated oil 63 15 3

TRANS FATS

Friday, April 05, 2013
A Presentation by Dr Vineet Kumar
FOR NO TRANS FATS IN SNACKS.....

HIGH OLEIC SOYBEAN

NON TRANSGENIC APPROACHES.....

>60

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

35% OLEIC ACID
<table>
<thead>
<tr>
<th>Traits</th>
<th>LSb1 (P1)</th>
<th>NRC7 (P2)</th>
<th>RIL (117 lines)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Min.</td>
<td>Max.</td>
<td></td>
</tr>
<tr>
<td>Isoflavones (µg/g)</td>
<td>1941.12</td>
<td>761.19</td>
<td>393.50</td>
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<tr>
<td>Diadzein (µg/g)</td>
<td>891.95</td>
<td>281.55</td>
<td>136.81</td>
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<tr>
<td>Genistein (µg/g)</td>
<td>735.77</td>
<td>365.58</td>
<td>110.58</td>
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<tr>
<td>Oleic Acid (%)</td>
<td>39.0</td>
<td>23.6</td>
<td>18.3</td>
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<tr>
<td>Linolenic Acid (%)</td>
<td>7.6</td>
<td>7.7</td>
<td>3.1</td>
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</table>
QTLs controlling ISOFLAVONES as assessed through RECOMBINANT INBRED LINES

<table>
<thead>
<tr>
<th>TRAIT</th>
<th>SSR MARKERS</th>
<th>LINKAGE GROUP</th>
<th>LOD</th>
<th>PERCENT CONTRIBUTION</th>
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<td>LOD</td>
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<tr>
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<td><strong>LINOLENIC ACID</strong></td>
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Thank you