

Note

Marker assisted accelerated introgression of null allele of kunitz trypsin inhibitor in soybean

Vineet Kumar*, Anita Rani, Reena Rawal and Vaishali Mourya

ICAR-Directorate of Soybean Research, Khandwa Road, Madhya Pradesh, India, 452001

Development of kunitz trypsin inhibitor (KTI)-free soybean is crucial for soy-food industry as the heat inactivation employed to inactivate the anti-nutritional factor in regular soybean incurs extra cost and affects protein solubility. In the presented work, a null allele of KTI from PI542044 was introgressed into cultivar ‘JS97-52’ (recurrent parent) through marker assisted backcrossing. Foreground selection in BC₁F₂, BC₂F₂ and BC₃F₂ was carried out using the null allele-specific marker in tandem with SSR marker Satt228, tightly linked with a trypsin inhibitor *Ti* locus. Background selection in null allele-carrying plants through 106 polymorphic SSR markers across the genome led to the identification of 9 KTI-free lines exhibiting 98.6% average recurrent parent genome content (RPGC) after three backcrosses, which otherwise had required 5–6 backcrosses through conventional method. Introgressed lines (ILs) were free from KTI and yielded at par with recurrent parent. Reduction of 68.8–83.5% in trypsin inhibitor content (TIC) in ILs compared to the recurrent parent (‘JS97-52’) was attributed to the elimination of KTI.

Key Words: kunitz trypsin inhibitor, introgression, simple sequence repeat marker, soybean.

Introduction

Consumption of soy food has been reported to stave off broad spectrum of health complications *viz.* atherosclerosis, breast cancer, oral cancer, diabetes, osteoporosis, obesity, gall bladder stone (Ali *et al.* 2004, Clemente *et al.* 2013, Gilbert and Liu 2013, Steinberg 2007), attributed to the presence of numerous nutraceutical components *viz.* isoflavones, tocopherols, Bowman-Birk factor, saponins, lecithin *et.c.* in soybean seed. More importantly, being the economical source of basic nutrients *viz.* protein, essential fatty acids, minerals *et.c.*, soybean can mitigate mal- and under-nutrition in developing countries including India. Despite these virtues, utilization of total global soybean produce in food uses is minuscule, with only 17 million tonne of the grain being utilized for tofu and unfermented products (Soybean Market Scan 2012). In India, barely 10% of total soybean produce is processed into soy foods (TAAS 2014). One of the several constraints in acceptance of soy foods is the high concentration of trypsin inhibitor in soybean compared to other legumes (Guillamon *et al.* 2008). Trypsin inhibitor in its active form has been found to be responsible for the growth inhibition, pancreatic hypertrophy and hyper-

plasia in experimental animals (Isanga and Zhang 2008, Liner 1994). In fermented products like *miso*, *tempeh*, *nato*, which are integral components of traditional diet in Asian countries, this anti-nutritional factor is reduced considerably due to the action of proteases released from the microorganisms employed in fermentation (Anderson and Wolf 1995). Similarly, in products prepared from germinated soybean seedlings, TIC is low due to the release of proteases during sprouting (Kumar *et al.* 2006). However, soy products derived from fermentation and sprouts are not in much use in several countries including India. Trypsin inhibitor activity in soybean seed is ascribed to two protease inhibitors *viz.* KTI (21 kD polypeptide) and Bowman Birk factor (8 kD polypeptide). KTI protein is thermo-labile due to presence of just 2 disulfide bridges and is responsible for the anti-nutritional effects of trypsin inhibitor. On the contrary, Bowman-Birk, with 7 disulfide bridges in its tertiary structure is a heat stable molecule, and more importantly, several studies have shown it possessing anti-carcinogenic properties *in vitro* and *in vivo* model systems (Clemente *et al.* 2013, Magee *et al.* 2012). KTI in unfermented products is reduced drastically in preparations entailing moist heating at 90–100°C for at least 20 min. However, thermal inactivation of this anti-nutrient has its own shortfalls. Residual activity of this anti-nutrient persists in the final products depending upon the initial endogenous level in the raw material, temperature and time of heating (Friedman and

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*Corresponding author (e-mail: vineetkshni@yahoo.com)

Brandon 2001, Savage *et al.* 1995). Moreover, thermal treatment required to inactivate 90% of the protease inhibitor negatively affects the protein efficiency ratio by rendering essential amino acids, such as lysine and cystine, biologically unavailable (Rackis 1974) and causes decline in protein solubility (Anderson 1992). Moreover, the heat treatment is cost-ineffective, as the soy processing industries has to incur extra expenditure on energy. In India, the most convenient mode of incorporation of soybean in daily diet to avail its afore-mentioned health benefits is through fortifying 9 parts of wheat flour with one part of soy flour to prepare soy-fortified *chapatti*—thin-flattened wheat flour bread, which is the mainstay of traditional diet across the country. For this purpose, at house hold level, wheat grains are blended with soybean, which is recommended to be pre-boiled for 20 min followed by air-drying for ensuring inactivation of KTI, and the mixture is milled to prepare soy-fortified flour for making *chapatti*. At household level, this time-consuming and cumbersome step of pre-treatment of soybean grain is either skipped; else, commercially available soy flour is mixed with wheat flour in the required proportion (1:9). Stringent norms for TIC in several soy products *viz.* soymeal, soy concentrate, soy isolate are in practice in the international trading (Huisman and Tolman 1992, NOPA 1997). However, in the absence of regulatory upper safe limit of this anti-nutrient, the primary soy products (soymilk, tofu, nuts etc) in domestic market may contain high level of TIC due to faulty processing (Gilani *et al.* 2012). Therefore, availability of soybean genotypes genetically free from KTI in the market is critical to boost soybean utilization in food uses for ensuring nutritional security.

Presence of KTI is governed by a single dominant gene (*Ti*), while the absence is attributed to the recessive form (*titi*). Genotypes with a null allele of KTI have been identified in soybean germplasm (Bernard *et al.* 1991). Further, SSR marker Satt228 has been reported to be at a distance of 0–3.7 cM from the *Ti* locus (Kim *et al.* 2006). Reliability of these SSR markers was also ascertained by carrying out validation in the mapping population generated using Indian soybean genotypes as the recipient parent (*TiT*) and PI542044 (*titi*) as the donor for the null allele (Rani *et al.* 2011). Further, a gene-specific marker has also been designed from the null allele of KTI from genotype PI157440 (de Moraes *et al.* 2006); and has been deployed in identification of plants carrying the null allele of KTI derived from PI542044 (Kumar *et al.* 2013). In the wake of identification of afore-mentioned SSR markers tightly linked with the *Ti* locus and the designing of gene-specific marker, it is possible to introgress the null allele of KTI into high yielding soybean cultivars through marker assisted backcrossing (MABC). MABC is an expeditious process over the conventional backcrossing and has already been employed for introgression of useful traits in soybean (Kim *et al.* 2008, Zhu *et al.* 2007). The present study was undertaken aiming at the introgression of the null allele of KTI in the genetic background of a high yielding Indian soybean cultivar *viz.*

‘JS97-52’ by MABC approach, involving three backcrosses. Foreground selection for the target allele (*titi*) was carried out using gene specific marker in combination with linked SSR marker (Satt228), while background of JS97-52 was recovered using polymorphic SSR markers spanning across the genome.

Materials and Methods

Recipient genotype

Indian soybean variety ‘JS97-52’ was selected for the introgression of null allele of KTI. The variety was developed from cross PK327 × L129 at Jawahar Lal Nehru Krishi Vidyalaya, an Agricultural University located at Jabalpur (Madhya Pradesh), and is recommended for cultivation in Central India, which is the hub of soybean cultivation and processing. ‘JS97-52’ is tolerant to wide array of abiotic and biotic stress, especially to water-logging conditions. The genotype attains harvest maturity in 105–110 days, with a yield potential of 3.0 tonne per ha. However, like all other soybean varieties released so far for cultivation under All Indian Co-ordinated Research Project on Soybean, it contains the KTI polypeptide.

Donor genotype

Soybean accession PI542044 (Maturity Group III), also known as ‘kunitz soybean’, which has the null allele of KTI was developed from ‘Williams 82’ × PI157440 through 5 backcrossing at Illinois Agricultural Experimentation Station and USDA-ARS and is a near-isogenic line of ‘Williams 82’, differing from the latter in the *Ti* genotype (Bernard *et al.* 1991). The accession was procured from United States Department of Agriculture (USDA). In agro-climatic conditions of Central India, this accession exhibited poor agronomic performance with regards to germination and plant architecture *viz.* height, branching and yield components. However, the absence of KTI in its seeds was the trait exploited in breeding programme focusing on development of food-grade soybean in India.

Molecular markers, DNA isolation and PCR amplification

Synthesis of oligonucleotides of markers required for foreground selection *viz.* gene-specific marker (Forward 5’CTTTTGTGCCTTCACCCACCT-3, Reverse 5’GAATTCATCATCAGAAACTCTA3’) (de Moraes *et al.* 2006) and the linked SSR marker Satt228 (Forward 5’TCATAACGT AAGAGATAATAAAACT3’, Reverse 5’CATTATAAGAAA ACGTGCTAAAGAG3’) ; and for background selection across the genome was outsourced to M/S Sigma Chemicals, Bangalore. Genomic DNA was isolated from the finely ground young leaf tissues following cetyl trimethyl ammonium bromide procedure (Doyle and Doyle 1990). Purification of DNA was done through phenol: chloroform: isoamyl alcohol method. DNA quality was tested on 0.8% agarose gel. DNA concentration was quantified through spectrophotometric method. Quantified DNA was suitably diluted

for genotyping work. PCR amplification was carried out using thermocycler (Make LifePro Bioer). The reaction mixture (10 μ l) contained 2 μ l DNA (25 ng/ μ l), 1 μ l PCR 10 \times buffer, 1.1 μ l MgCl₂ (25 mM), 0.1 μ l dNTPs (25 mM), 0.4 μ l each forward and reverse SSR primers (30 ng/ μ l), 0.068 μ l *Taq* DNA polymerase (3 units/ μ l) and 4.932 μ l distilled water. Initially, DNA was denatured at 94°C for 2 min followed by 30 cycles each consisting of denaturation at 94°C for 1 min, primer annealing at 50°C for 2 min, primer elongation at 72°C for 3 min. Final elongation was carried out at 72°C for 10 min. Amplified products were resolved on 3% rezophore agarose gel.

Backcrossing

Due to the significant difference in days-to-flowering of recurrent parent 'JS97-52' (48 days) and the donor parent PI542044 (28 days), at the latitude of experimentation site (22°44'N, 75°50'E), staggered sowing was done in the field/pots to synchronize the flowering of the male plants with the initiation of buds in the recurrent parent 'JS97-52' to effect crosses. First filial backcross generation of the cross 'JS97-52' \times PI542044 was developed by spraying pollens from the true F₁ plants derived from 'JS97-52' \times PI542044 on the stigmas of 'JS97-52' after opening the buds between 7.30 to 9.00 A.M. The subsequent backcrosses (BC₂F₁ and BC₃F₁) were effected by transferring the pollens from foreground-selected BC₁F₂ and BC₂F₂ plants on the stigma of the buds of recurrent parent ('JS97-52'). Selected BC₁F₁, BC₂F₁, BC₃F₁ were selfed to obtain backcrossed F₂ generation segregating for the target allele (*ti*).

Determination of RPGC

For assessing the RPGC for an individual plant surveyed through a set of SSR markers (*loci*), score '1' was given to heterozygous locus (H), while the locus homozygous for recurrent parent (A) was allotted a score of '2'. No score was allotted to SSR marker exhibiting homozygosity for the donor parent (B). Percent RPGC was calculated by summing up the score of an individual plant given for all the *loci* divided by total number of alleles (2 \times number of SSR markers surveyed).

Phenotyping

The seeds from homozygous recessive plants (*titi*) as identified by the linked SSR marker Satt228 were phenotyped electrophoretically for confirming absence of KTI polypeptide. The finely ground seed flour was incubated in 1 ml Tris-HCl buffer (pH 8.0) for 30 min. and centrifuged. Equal volumes of supernatant and 5 \times sample buffer containing 50% v/v glycerol, 1.96% v/v mercaptoethanol, 0.05% bromophenol dye and 1 M Tris-HCl (pH 6.8) were loaded on 10% acrylamide gel in vertical electrophoresis unit (Model SE 600 Ruby[®], GE Healthcare) and run at 35 mA for 2 h. Gels were stained overnight in 0.25% aqueous solution of coomassie brilliant blue (R-250) in methanol, water and glacial acetic acid in 45:45:10 ratio, respectively; fol-

lowed by destaining in methanol, water and glacial acetic acid (45:45:10) solution. Standard trypsin inhibitor protein (21.0 kD) procured from M/S Sigma Aldrich, Bangalore was run in a separate lane for identification of KTI polypeptide.

Estimation of TIC

Trypsin inhibitor content in the soybean seeds was estimated following the method given by Hamerstrand *et al.* (1981).

Field trial

Breeding lines of 'JS97-52' introgressed with the null allele of KTI were raised along with the recurrent parent in three replicates in the random block design in the fields of ICAR-Directorate of Soybean Research, Indore during the *monsoon* season of 2014 for assessing the yield. Each introgressed line was planted by hand in a three-row plot with 3 m length, 45 cm row-to-row distance and 5 cm plant-to-plant distance. Nitrogen, phosphorus and potassium were applied at the rates of 20, 40, 60 kg/ha, respectively, and 1000 g seeds were treated with 1 g mixture of bavistin and thiram (1:1) before sowing.

Statistical analysis

Trypsin inhibitor content and the yield for each introgressed line were assessed in triplicate. The statistical significance between the values for yield was assessed using analysis of variance ($P < 0.05$) by SPSS evaluation version 14.

Results

Table 1 details the scheme of introgression of the null allele of KTI from PI542044 (*titi*) in elite soybean variety 'JS97-52' (*TiT*) through foreground and background selection. 'JS97-52' was crossed with PI542044 to obtain F₁ plants. Subsequently, three backcrosses in tandem with selfing after each backcross were performed. Foreground selection for identification of target plants in BC₁F₁, BC₂F₁, BC₃F₁ generation was carried out using gene-specific marker and in BC₃F₂ employing gene-specific marker in tandem with linked marker Satt228.

Foreground and background selection

KTI positive (*TiT*) genotype 'JS97-52' on crossing with donor genotype 'PI542044' (*titi*) generated 55 seeds. F₁ plants were screened with null allele specific marker, before the initiation of buds, resulted into identification of 12 true F₁ plants. Pollens from true F₁ plants were transferred on the stigma of plants of 'JS97-52' (recurrent parent). BC₁F₁ seeds so obtained were sown, and after foreground selection using the null allele-specific marker, plants with *Titi* genotype were carried till maturity. BC₁F₂ generation raised were subjected to foreground selection for the identification of homozygous recessive (*titi*) plants at 10–15 days' seedling stage. For this purpose, initially, all the seedlings were

Table 1. Details of foreground and background selection of backcross generations

| Generation | Selection | No. of plants screened | No. of plants confirmed (<i>Titi/titi</i>) | Plants surveyed for background selection | No. of primers for background selection | Plants selected based on high % RPGC* |
|--------------------------------|--|------------------------|--|--|---|---------------------------------------|
| F ₁ | – | 55 | 12 (<i>Titi</i>) | – | – | – |
| BC ₁ F ₁ | Foreground for <i>Titi</i> plants | 35 | 5 (<i>Titi</i>) | 5 | – | – |
| BC ₁ F ₂ | Foreground for <i>titi</i> plants and I1st background | 399 | 75 (<i>titi</i>) | 35 | 106 | 9 (76.88–80.66) |
| BC ₂ F ₁ | Foreground for <i>Titi</i> plants | 118 | 24 (<i>Titi</i>) | 24 | – | 24 |
| BC ₂ F ₂ | Foreground for <i>titi</i> plants and IIInd background | 2044 | 450 (<i>titi</i>) | 51 | 21–30 | 12 (93.39–94.33) |
| BC ₃ F ₁ | Foreground for <i>Titi</i> plants and IIIrd background | 104 | 42 (<i>Titi</i>) | 42 | 8–12 | 3 (98.11–99.05) |
| BC ₃ F ₂ | Foreground for <i>titi</i> plants and IVth background | 700 | 169 (<i>titi</i>) | 169 | 3–4 | 9 (97.64–99.05) |

* RPGC recovery does not include SSR marker Satt228 tightly linked to *Ti* locus employed for foreground selection. Values given in the parenthesis indicate % RPGC.

screened using the null allele-specific primer. This primer amplified both homozygous (*titi*) and heterozygous (*Titi*) individuals. To distinguish *titi* and *Titi* genotypes, genomic DNA of the seedlings found to harbour null allele of KTI was amplified using linked marker Satt228. This led to the identification of 75 homozygous recessive (*titi*) plants. Of these, 35 plants morphologically similar to recurrent parent were subjected to background selection using 106 polymorphic SSR markers (uniformly spaced across the genome), of the 210 *loci* identified to be polymorphic for recipient and donor parent in parental polymorphism survey through 440 SSR markers (data unpublished). This resulted in the identification of 9 plants with RPGC in the range of 76.9–80.7%. Pollens from these selected plants were used for effecting crossing with recurrent parent to advance to second backcross generation. Twenty four foreground-selected (through the null allele-specific marker) BC₂F₁ plants were selfed to obtain BC₂F₂ seeds. BC₂F₂ population was first screened through the null allele-specific marker. Plants identified carrying null allele of KTI (*Titi/titi*) were subsequently genotyped using linked SSR markers Satt228. This led to the identification of 450 homozygous recessive (*titi*) plants. Of these, 51 plants, which showed recurrent parent-like morphological characters in pre-reproductive phase, were subjected to background selection (IIInd background selection) using 21–30 polymorphic markers, which showed either heterozygosity or fixation towards donor parent (PI542044) genome in BC₁F₂ generation. This resulted in the identification of 12 plants with RPGC more than 93%. Pollens from these plants were used to effect third backcross generation. Foreground selection of BC₃F₁ generation (104 plants) led to the identification of 42 true BC₃F₁ plants (*Titi*), all of which were subjected to background selection using (8–12 markers), leading to the identification of 20 plants with recurrent genome content in the range of 95.3–99.1%. Three plants exhibiting more than 98% were selfed to obtain BC₃F₂ seeds. Screening of BC₃F₂ generation (700 plants) using the null allele-specific marker and Satt228 resulted in the identification of 169 homozygous recessive (*titi*) plants. Of these, plants whose parents were heterozygous at 3–4

loci in BC₃F₁ generation were surveyed with the corresponding SSR markers. Some of these BC₃F₂ plants showed recovery at these *loci*. A chromosomal segment spanning a length of 16.4 cM starting from Satt409 to Satt429 including *Ti* locus was inherited from donor plant in the initial backcross generations. Though Satt228, being tightly linked, was inherited from donor parent in all the selected plants in BC₃F₃. The linkage between Satt429 and *Ti* locus was broken in all the plants and one of the selected plants recovered Satt409 from the recurrent parent. Finally, we identified 9 soybean lines (BC₃F_{2,3} seeds) viz. DSRJKTIF-1, DSRJKTIF-2, DSRJKTIF-3, DSRJKTIF-4, DSRJKTIF-5, DSRJKTIF-6, DSRJKTIF-7, DSRJKTIF-8, DSRJKTIF-9, which showed background recovery of 97.6 to 99.1% and yielded at par with ‘JS97-52’.

Confirmation for absence of KTI in ILs

Fig. 1 shows the absence of KTI polypeptide in the lanes corresponding to the ILs and the donor parent (PI542044), however, the lane corresponding to recurrent parent (‘JS97-52’) showed the presence of KTI polypeptide. Quantitatively, TIC which was 87.4 mg per gram of defatted flour in the

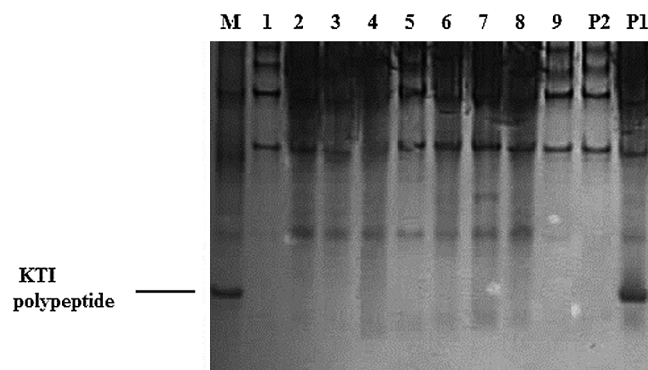


Fig. 1. Confirmation for the absence of KTI polypeptide in BC₃F_{2,3} lines (*titi*) of the cross ‘JS97-52’ × PI542044 on 10% PAGE, where P1, P2 and M denote ‘JS97-52’ (KTI +ve) and PI542044 (KTI –ve) genotypes and marker for KTI polypeptide, respectively. Lanes 1–9 represent the introgressed lines DSRJKTIF-1 to DSRJKTIF-9, respectively.

Table 2. Assessment of ILs* in comparison to recipient variety 'JS97-52' for yield and TIC

| Lines BC ₃ F ₃ | Yield (tonnes per ha) | TIC (mg/g defatted flour) | % TIC reduction (compared to 'JS97-52') |
|--------------------------------------|--------------------------|---------------------------------|---|
| JS97-52 | 3.60 ± 0.83 | 87.4 ± 4.61 | – |
| PI542044 | 1.20 ± 0.24 | 22.5 ± 1.53 | – |
| DSRJKTIF-1 | 3.74 ± 0.64 | 18.5 ± 0.86 | 78.83 |
| DSRJKTIF-2 | 3.41 ± 0.93 | 24.3 ± 1.30 | 72.19 |
| DSRJKTIF-3 | 3.62 ± 0.77 | 26.1 ± 1.42 | 70.13 |
| DSRJKTIF-4 | 3.44 ± 0.41 | 15.6 ± 0.86 | 82.15 |
| DSRJKTIF-5 | 3.56 ± 0.42 | 27.3 ± 1.43 | 68.76 |
| DSRJKTIF-6 | 3.60 ± 0.35 | 25.2 ± 1.76 | 71.16 |
| DSRJKTIF-7 | 3.68 ± 0.43 | 18.1 ± 1.34 | 79.29 |
| DSRJKTIF-8 | 3.39 ± 0.32 | 15.3 ± 0.78 | 82.49 |
| DSRJKTIF-9 | 3.66 ± 0.79 | 14.4 ± 0.91 | 83.52 |

* DSRJKIF-1 to DSJKTIF-9 denote ILs.

seeds of 'JS97-52' was found to be reduced to 14.4–27.3 mg per gram of defatted flour in 9 introgressed lines free from KTI (**Table 2**).

Assessment for yield

Seeds (BC₃F_{2,3}) of 9 introgressed lines were sown in the field in 2014 along with the recurrent parent in three replications in random block design for estimating the yield potential. All the introgressed lines reached harvest maturity in 105–110 days and yielded at par with the recurrent parent 'JS97-52' (**Table 2**).

Discussion

Given the importance of KTI-free soybean seeds in soy food and soymeal industry in reducing the cost of processing, a breeding program focusing on the genetic elimination of the kunitz trypsin inhibitor has been initiated in several countries. In Serbia, two KTI-free soybean genotypes viz. 'Laura' and 'Launa' have been developed (Peric *et al.* 2004). In India, 2 KTI-free soybean genotypes, namely, 'NRC101' and 'NRC102' have been recently developed and registered with ICAR-National Bureau of Plant Genetic Resources, India (Rani *et al.* 2010). But these two genotypes have yield potential of only 2 tonne per ha. In the presented work, the null allele of KTI was introgressed from PI542044, a *titi* genotype, in high yielding soybean variety 'JS97-52' by MABC approach. Gene specific-marker was deployed for confirmation of *Titi* genotypes in F₁, BC₁F₁, BC₂F₁ and BC₃F₁ generation; while the gene-specific marker in tandem with the linked SSR marker Satt228 was employed in identification of target plants (*titi*) in BC₁F₂, BC₂F₂ and BC₃F₂ generation. The gene-specific marker could help in selection of plants carrying the null allele at *Ti* locus without distinguishing the homozygous/heterozygous (*titi/Titi*) state, while codominant linked marker Satt228 could spot on target homozygous recessive (*titi*) plants. Background selection performed in BC₁F₂, BC₂F₂ and BC₃F₁ facilitated the expeditious recovery of recurrent par-

ent ('JS97-52') genome. After 3 backcrosses, the recurrent-parent like 9 selected ILs contained RPGC in the range of 97.6–99.1% with average value of 98.6%, which would have been possible only after 5–6 backcrosses through conventional method. Thus, marker assisted background selection could accelerate the recovery of RPG by three backcross generations. TIC in these lines reduced in the range of 68.8–83.5% (**Table 2**), which can be attributed to the elimination of KTI. The percentage reduction in TIC in these introgressed lines was higher than the magnitude of reduction (50%) reported in KTI free soybean variety 'Laura' developed in Serbia (Peric *et al.* 2004). The remaining content of trypsin inhibitor in ILs in our study as well as KTI free soybean variety 'Laura' developed by Peric *et al.* (2004) may be attributed to the Bowman-Birk protease inhibitor. This molecule which is heat stable, in contrast to KTI polypeptide, due to 7 disulfide linkages is not detrimental to human health. Instead, it has been reported to possess anti-oral cancer properties (Clemente *et al.* 2013) and being marketed for its nutraceutical value. In the backdrop of genetic variability for concentration of Bowman-Birk reported in soybean recently (Arefrad *et al.* 2013), varying level of residual trypsin inhibitor in introgressed lines may be attributed to the genotypic variation in the contents of Bowman-Birk protease inhibitor. More importantly, yield of these ILs was at par with the recurrent parent. A multi-location trial of these lines, under All India Co-ordinated Research Project on Soybean, can lead to their release as varieties, which would be first-ever KTI-free soybean varieties developed through marker assisted backcrossing.

Conclusion

In brief, in the present investigation, introgression of the null allele of KTI into a high yielding soybean cultivar 'JS97-52' was accelerated by deploying marker-assisted foreground and background selection. The alternate conventional backcross method would have taken 5–6 backcrosses, which has been achieved in only 3 backcrosses using MABC. The KTI-free breeding lines developed in this work would be very useful for industries, manufacturing primary soy products like tofu, soy milk, soy-nuts, which presently use regular soybean seeds, containing KTI, as the initial raw material; and have to expend considerably in lowering TIC. Further, the benefits of these lines can be availed by soy feed industry as well, where the raw grains of these KTI-free breeding lines can be supplemented directly in the ration for non-ruminants.

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