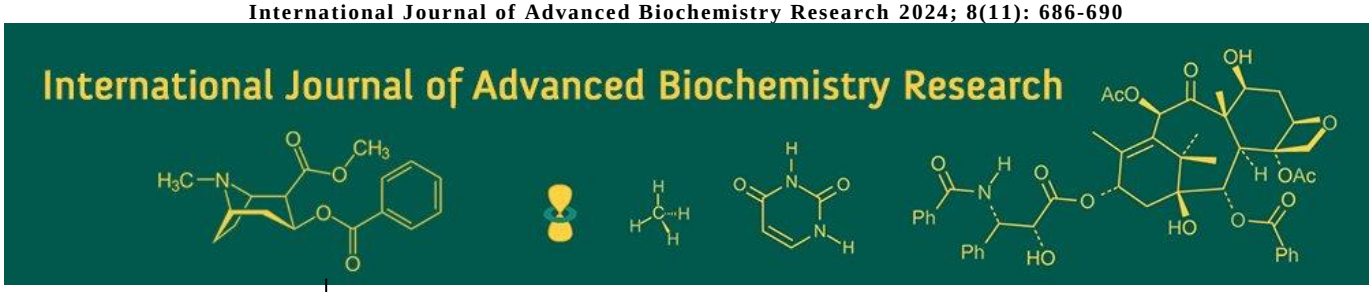


ISSN Print: 2617-4693
ISSN Online: 2617-4707
IJABR 2024; 8(11): 686-690
www.biochemjournal.com
Received: 02-09-2024
Accepted: 12-10-2024

Bhagyashri Suresh Gavande
Biotechnology Centre, Post Graduate Institute, Department of Agriculture Botany, Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola, Maharashtra, India

Pravin V Jadhav
Biotechnology Centre, Post Graduate Institute, Department of Agriculture Botany, Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola, Maharashtra, India

Umesh D Shinde
Biotechnology Centre, Post Graduate Institute, Department of Agriculture Botany, Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola, Maharashtra, India

Akanksha Jadhav
Biotechnology Centre, Post Graduate Institute, Department of Agriculture Botany, Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola, Maharashtra, India

Sagar L Zanjal
Biotechnology Centre, Post Graduate Institute, Department of Agriculture Botany, Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola, Maharashtra, India

MP Moharil
Biotechnology Centre, Post Graduate Institute, Department of Agriculture Botany, Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola, Maharashtra, India

SS Nichal
Regional Research Centre, Amravati, Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola, Maharashtra, India

SS Mane
Head, Department of Plant Pathology, College of Agriculture, Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola, Maharashtra, India

Vineet Kumar
Indian Institute of Soybean Research, Indore, Madhya Pradesh, India

Corresponding Author:
Pravin V Jadhav
Biotechnology Centre, Post Graduate Institute, Department of Agriculture Botany, Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola, Maharashtra, India

Genetic and molecular characterization of Null KTI allele in backcrossed soybean populations

Bhagyashri Suresh Gavande, Pravin V Jadhav, Umesh D Shinde, Akanksha Jadhav, Sagar L Zanjal, MP Moharil, SS Nichal, SS Mane and Vineet Kumar

DOI: https://doi.org/10.33545/26174693.2024.v8.i11i.2939

Abstract
Soybean (*Glycine max*), a nutrient-rich crop, is valued for its high protein and essential fatty acid content, alongside bioactive compounds beneficial to human health. Despite its advantages, soybean's anti-nutritional factors, especially Kunitz trypsin inhibitor (KTI) and Bowman-Birk inhibitor (BBI), limit protein digestibility and nutrient absorption, necessitating genetic modifications for improved utilization. This study utilizes marker-assisted backcrossing (MABC) to introduce a null allele for the KTI gene into high-yielding, charcoal rot-resistant soybean genotypes AMS-MB-5-18 and AMS-MB-5-19, with NRC-101 and NRC-127 as donor parents. A BC_1F_4 population derived from these crosses was analysed to identify lines free of KTI while retaining desirable agronomic traits. Marker-assisted background selection involved 63 SSR markers to monitor recurrent parent genome content (RPGC), analyzed using GGT (v.2.0) software. Results showed a progressive increase in RPGC, reaching an average of 84% in BC_1F_4 lines, with top plants achieving up to 85.18%. Screening confirmed the successful introgression of the null KTI allele in multiple plants across generations, with 8 plants in the BC_1F_4 population tested, yielding 5 individuals free of the KTI peptide. These findings demonstrate efficient genomic recovery and anti-nutritional factor reduction in improved soybean lines, advancing their suitability for human consumption and agricultural resilience in Indian soybean breeding programs.

Keywords: Soybean, KTI, Marker assisted backcross breeding, Null KTI allele, RPGC

Introduction
Soybean, known as the "Golden Bean" or "Miracle Crop," is a key food commodity and an affordable protein source (Ghorpade *et al*., 2022) [10]. It provides about 40% protein, 20% fats (mostly unsaturated), and is rich in essential minerals like calcium, magnesium, potassium, and iron. Soybeans also contain bioactive compounds- such as vitamins, phenols, and isoflavones—with antioxidant properties (Agyenim-Boateng *et al*., 2023) [1]. Soybean oil, high in polyunsaturated fats (61%), contains linoleic and linolenic acids that support heart health by lowering cholesterol and promoting eicosanoid production (Kahraman, 2017) [12]. Despite being low in methionine, soy's high lysine levels complement cereal proteins (Sharma *et al*., 2014) [24]. Commonly used in Asian cuisine for fermented and non-fermented products, soy consumption is linked to reduced risks of heart disease, cancer, diabetes, and obesity (Ali *et al*., 2004; Clemente *et al*., 2013; Steinberg, 2007) [2, 8, 25].
Soybeans are rich in nutrients but contain anti-nutritional factors that limit their nutrient availability. Heat-sensitive compounds, such as protease inhibitors and lectins, can be neutralized by cooking, but heat-resistant compounds like non-starch polysaccharides (NSP) and oligosaccharides persist and may cause digestive issues (Bueno *et al*., 2018) [5]. Soybeans contain significant trypsin inhibitors, notably the Kunitz Trypsin Inhibitor (KTI) and the Bowman-Birk Inhibitor (BBI). BBI, a small protein of about 8 kDa, inhibits trypsin and chymotrypsin and has shown anti-cancer potential in animal studies (Mittal *et al*., 2021) [20]. KTI, a larger inhibitor at 21.5 kDa, effectively binds trypsin, impeding protein digestion, and has been associated with pancreatic hypertrophy in animals. KTI is thermally stable due to hydrophobic interactions and disulfide bridges, driving demand for soybean varieties lacking this inhibitor due to its adverse effects (Embaby, 2010; Roychaudhuri *et al*., 2004; Salim *et al*., 2023) [9, 22, 23].

Soybean, while protein-rich, contains anti-nutritional factors that must be deactivated for safe consumption and nutrient retention. In India, soybean breeding advancements have produced high-yield, disease-resistant varieties, suitable for rainfed farming and resilient against charcoal rot. Introducing a null allele for the Kunitz trypsin inhibitor (KTI) through Marker-Assisted Backcrossing (MABC) helps minimize these anti-nutritional elements, supported by cost-effective SSR markers. This approach enables early genetic screening for efficient breeding cycles (Taneja & Upadhyay, 2021) [27].

Charcoal rot disease significantly reduces soybean yield, affecting major producing states in India (Amrate *et al*., 2023) [4]. Efforts to breed Kunitz trypsin inhibitor (KTI)-free varieties, which improve soybean's nutritional profile, have led to the development of NRC-101 and NRC-127 by IISR, using a null allele from PI 542044 (Bulatova *et al*., 2019; Kumar *et al*., 2019) [6, 14]. The current study employs marker-assisted selection to incorporate this allele into high-yielding, charcoal rot-resistant genotypes, AMS-MB-5-18 and AMS-MB-5-19, for improved agricultural resilience.

Materials and Methods

The study focuses on four soybean genotypes, specifically with AMS-MB-5-18 and AMS-MB-5-19 serving as recurrent parents, and NRC-101 and NRC-127 as donor parents. It includes BC_1F_4 population derived from different parental combinations. All seeds were sourced from the Biotechnology Centre, Department of Agricultural Botany, Dr. Panjabrao Deshmukh Krishi Vidyapeeth in Akola.

The Null KTI allele-specific marker (Alves de Moraes *et al*., 2006; Kumar *et al*., 2015) [3, 16] was used for foreground selection, with 63 SSR markers used for background recovery analysis through PCR and PAGE scoring. DNA was extracted from finely ground young leaf tissues using the cetyl trimethyl ammonium bromide (CTAB) method with minor adjustments. The DNA was then purified using a chloroform: isoamyl alcohol extraction procedure. Its quality was checked by running it on a 0.8% agarose gel. The DNA was diluted to the appropriate concentration for genotyping. PCR amplification was performed using an Applied Biosystems thermal cycler. The reaction mixture (10 µl) contained 1 µl of DNA (30-40 ng/µl), 5 µl of 2X master mix, 3 µl of water, and 0.5 µl each of the forward and reverse primers (10 pmol). For the Null KTI allele-specific primer, the PCR conditions were: initial denaturation at 94 °C for 2 minutes, followed by 32 cycles of denaturation at 94 °C for 1 minute, primer annealing at 53°C for 2 minutes, and elongation at 72 °C for 3 minutes. A final extension step was done at 72 °C for 10 minutes. The PCR products were separated on a 3% metaphor agarose gel. Thermal Profile for PCR background selection the DNA was first heated to 94 °C for 3 minutes to denature it. Then, 32 cycles were run, each consisting of: Denaturation at 94 °C for 30 seconds; Annealing at a temperature between 45 °C and 65 °C for 45 seconds; Extension at 72 °C for 1 minute. After the cycles, a final extension was done at 72 °C for 10 minutes. The PCR products were analysed by electrophoresis on a 6% polyacrylamide gel (PAGE).

Determining RPGC (Recurrent Parent Genome Content)

To measure the RPGC in individual plants, SSR markers were used. Bands that matched the recurrent parent (AMS-MB-5-18, AMS-MB-5-19) were scored as "A," while bands similar to the donor parent (NRC-101, NRC-127) were scored as "B." Progeny with both types of bands were scored as "H" for heterozygous. The marker data was analysed using Graphical Genotyper (GGT version 2.0) software.

Phenotyping

Seeds identified as Null KTI allele introgressed were confirmed using a null KTI allele specific marker. To do this, 33 mg of ground seed flour was mixed with 1 ml of distilled water and incubated overnight on a shaker. After incubation, the sample was centrifuged at 1000 rpm for 15 minutes. Then, 30 µl of the supernatant was mixed with 30 µl of dye (containing bromophenol blue) and loaded onto a gel. The gel used had 5% stacking and 12% separating acrylamide. It was run at 35 mA for 2 hours in a vertical electrophoresis unit. After electrophoresis, the gel was stained overnight in a solution of Coomassie Brilliant Blue (R-250) mixed with methanol, distilled water, and acetic acid (45:45:10). The gel was destained in a solution containing methanol, distilled water and glacial acetic acid in the ratio of 45:45:10. A standard 21.0 kD trypsin inhibitor protein was used in a separate lane to help identify the KTI peptide.

Results

The Marker-assisted backcrossing program aimed at improving soybean with resistance to Charcoal rot and null KTI (Kunitz trypsin inhibitor) alleles involved crossing mutant soybean genotypes (AMS-MB-5-18 and AMS-MB-5-19) with donor genotypes NRC-101 and NRC-127. The F1 plants from four cross combinations (A, B, C, D) were confirmed for hybridity using a null KTI marker. In Kharif 2021, BC_1F_1 plants were developed and 24 out of 39 were positive for the null KTI allele, with varying recurrent parent genome recovery (RPGC) from 46.29% to 79.41%. In Kharif 2022, 259 BC_1F_2 lines were screened, and 23 plants were confirmed for null KTI introgression. RPGC ranged from 68.15% to 77.99% across different crosses. Further selection in BC_2F_1 and BC_2F_2 generations identified additional plants with null KTI introgression. In the off-season of 2022, BC_1F_3 seeds were raised, with 13 plants showing positive introgression of the null KTI allele. Subsequent analysis revealed high RPGC, with cross A achieving 80.28% and cross B reaching 81.79%, indicating successful introgression and recurrent parent genome recovery.

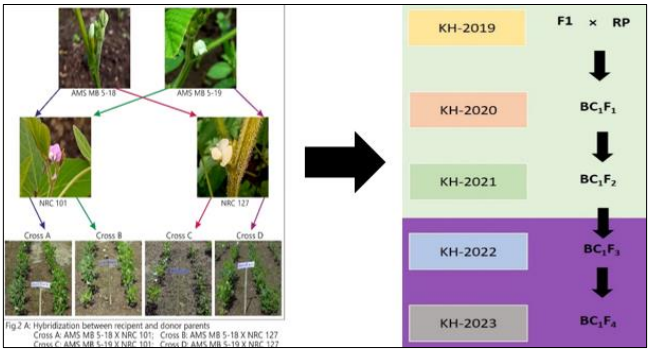
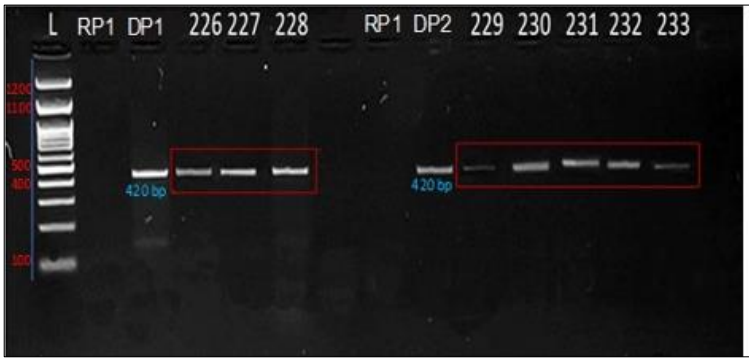


Fig 1: Diagrammatic representation of research programme

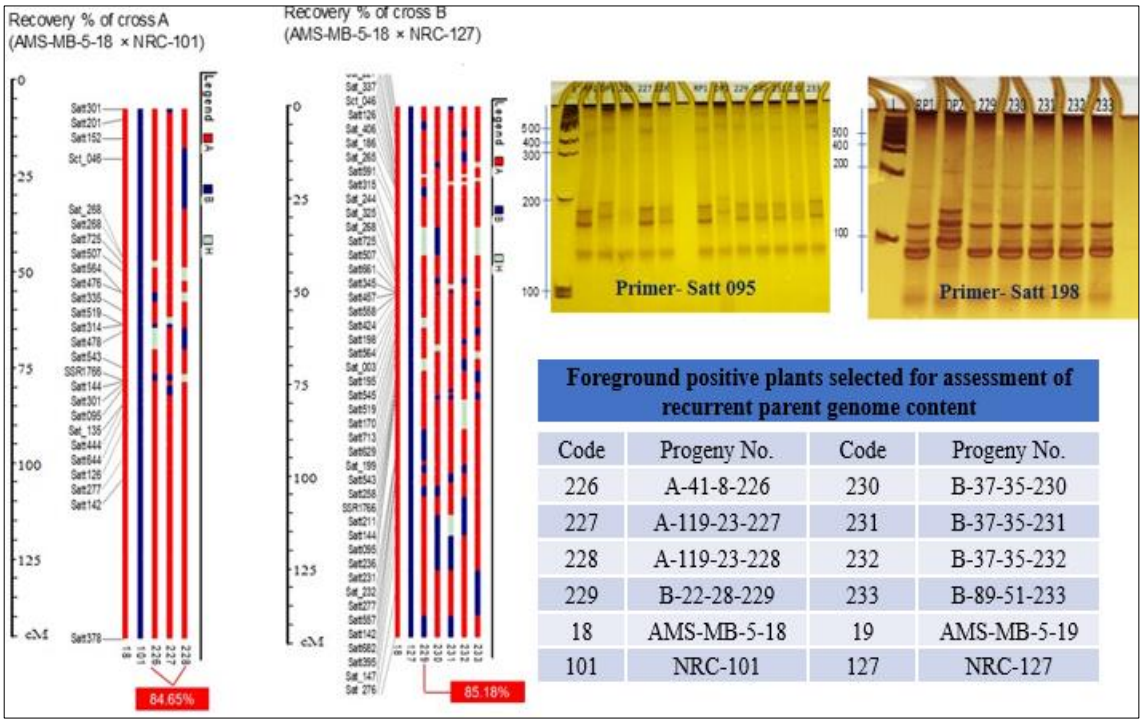
The introgression of the null KTI allele into the segregants of BC_1F_3 was confirmed during the Kharif- 2022 and Rabi-2022 season, and these BC_1F_3 plants were subsequently used for the continuation of the breeding program. The BC_1F_4 generation was harvested from individual plants of the BC_1F_3 population. In the Kharif-2023 season, the BC_1F_4 population was grown and confirmed for the presence of the null KTI allele using a linked marker. In the BC_1F_4 generation, a total of 8 lines were sown, of which 5 germinated. A total of 8 plants (3 from Cross A and 5 from Cross B) of the BC_1F_4 population were raised in Kharif-2023 and screened for the introgression of the null KTI allele. All 8 plants were found to be positive for the introgression of the null KTI allele (Fig.2).



Parental combination	Plants selected for foreground screening	
RP1 - AMS-MB-5-18	Code	Progeny No.
DP1 – NRC- 101	226	A-41-8-226
DP2 – NRC- 127	227	A-119-23-227
	228	A-119-23-228
	229	B-22-28-229
	230	B-37-35-230
L- Ladder	231	B-37-35-231
	232	B-37-35-232
	233	B-89-51-233

Fig 2: Foreground selection of BC_1F_4 plants using Null KTI allele specific marker

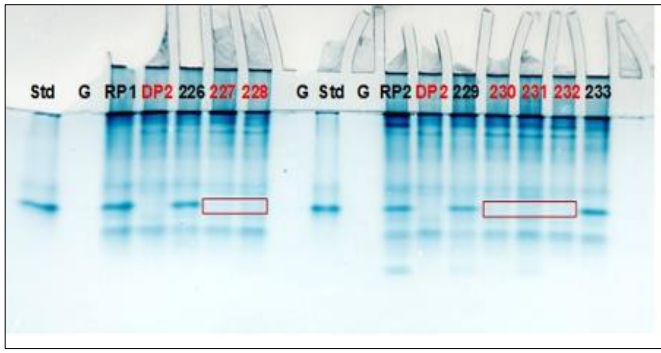
In the BC_1F_4 population, eight plants (three from Cross A and five from Cross B) exhibited positive introgression of the null KTI allele. For Cross A, 26 polymorphic markers were screened across progenies and parents (AMS-MB-5-18 and NRC-101). The marker data, recorded in a tri-scoring matrix, was analyzed using GGT (v.2.0) software. The average genome recovery in Cross A was 83.97%, with the highest recovery observed in plants 226 and 228 (84.65%). In Cross B, 54 polymorphic markers were screened with parents (AMS-MB-5-18 and NRC-127), and data was processed similarly. Cross B progenies showed an average recurrent parent genome recovery of 84.07%, with plant 229 exhibiting the highest recovery at 85.18%. These results indicate significant genomic recovery and successful introgression of the null KTI allele in both crosses.



Foreground positive plants selected for assessment of recurrent parent genome content			
Code	Progeny No.	Code	Progeny No.
226	A-41-8-226	230	B-37-35-230
227	A-119-23-227	231	B-37-35-231
228	A-119-23-228	232	B-37-35-232
229	B-22-28-229	233	B-89-51-233
18	AMS-MB-5-18	19	AMS-MB-5-19
101	NRC-101	127	NRC-127

Fig 3: Recurrent Parent Genome Recovery (RPGC) in BC_1F_4 population

In the BC_1F_4 population, a total of 8 plants were screened for the absence of the KTI peptide. In Cross A, 3 plants were screened, of which 2 tested negative for the presence of the KTI peptide. In Cross B, 5 plants were screened, and 3 of them were negative for the KTI peptide.



Code	Plants selected for foreground screening	
Std –Standard G –Gap RP1: AMS-MB-5-18 RP2: AMS-MB-5-19 DP2: NRC-127	Code	Progeny No.
	226	A-41-8-226
	227	A-119-23-227
	228	A-119-23-228
	229	B-22-28-229
	230	B-37-35-230
	231	B-37-35-231
	232	B-37-35-232
	233	B-89-51-233

Fig 4: Qualitative characterization of $BC_1F_{4:5}$ seeds

Discussion

This study successfully demonstrates the use of Marker-Assisted Backcross (MAB) breeding to incorporate the KTI null allele in selected charcoal rot-resistant soybean genotypes (AMS-MB-5-18 and AMS-MB-5-19) from Dr. Panjabrao Deshmukh Krishi Vidyapeeth. The presence of the null allele, which eliminates the Kunitz trypsin inhibitor (KTI), a major anti-nutritional factor, has been shown to significantly enhance the nutritional quality of soybean without altering other beneficial traits, offering a promising route for developing soybean lines suitable for monogastric animal feed (Kumar *et al*., 2015; Maranna *et al*., 2016; Talukdar *et al*., 2014) [16, 18, 26]. By employing molecular markers for precise allele tracking, the study follows previous successful introgression studies that report substantial recovery of the recurrent parent genome (RPG) using selective SSR markers linked with KTI-related traits, and the findings align with the phenotypic data that reveal reduced KTI content in selected progenies (Bulatova *et al*., 2019; Kumar *et al*., 2020; Rani *et al*., 2023) [6, 15, 21]. Foreground selection through the null allele-specific marker and the SSR marker Satt228, tightly linked with the Ti locus, allowed efficient identification of progeny carrying the desirable allele. Previous research indicates that the tight linkage of Satt228 within 0-3.7 cM to the Ti locus (Kim *et al*., 2006) [13] is critical in ensuring that the KTI-free trait remains stable in subsequent generations. In line with findings from Kumar *et al*. (2015) [16], foreground and background selections conducted in this study exhibited a high recovery rate of RPG, with the SSR marker analysis indicating up to 98% RPGC recovery, which is comparable to rates seen in advanced backcrossing and indicates reduced linkage drag, often observed in non-MAB breeding methods.The removal of KTI improves digestibility for non-ruminants by reducing anti-nutritional interference with proteolytic enzymes, especially trypsin, critical for protein breakdown in monogastric animals. In addition, the null allele's monogenic nature facilitates its stable inheritance, making it a viable trait for integration into diverse soybean varieties with minimal risk of recombination loss (Kumar *et al*., 2019; Mishra *et al*., 2021) [14, 19].

Notably, the protein gel electrophoresis results confirm the absence of KTI in the seeds, indicating successful introgression, which is consistent with work by Talukdar *et al*., (2014) [26] and Kumar *et al*., (2018) [17], who achieved similar reductions in trypsin inhibitor content. Our findings also illustrate the genetic diversity present within the SSR markers used, which aligns with the work of Jadhav *et al*., (2023) [11], who observed allelic frequencies with polymorphic SSR markers across different parental combinations. The use of the SSR markers not only allowed efficient selection but also provided insight into the diversity and structural variability within the soybean genome that can aid future breeding programs.

Further studies could explore enhancing the agronomic traits associated with KTI-free soybeans by combining the null KTI allele with other beneficial traits like disease resistance and drought tolerance, optimizing them through MAB. Additionally, examining the effect of environmental variables on the stability of KTI-null alleles could provide valuable data for commercial deployment. These findings contribute to broader applications of molecular markers in breeding programs, promoting both nutritional enhancement and genetic diversity in soybeans, which could support both food security and sustainability goals in developing nations (Chukwu *et al*., 2020) [7].

Conclusion

This study confirms that MAS is an effective approach for integrating the KTI-null allele into charcoal rot-resistant soybean genotypes, marking a step forward in the development of high-protein, nutritionally enhanced soybean lines suitable for broader applications. Through targeted breeding and marker-assisted selection, soybean varieties can be developed to provide sustainable and nutritive feed options, meeting the demands of both human and animal nutrition.

Acknowledgement

Financial support from the DBT, Govt. of India and experimental facility from Biotechnology Centre, Department of Agricultural Botany, Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola, is duly acknowledged and authors are grateful to ICAR-Indian Institute of Soybean Research, Indore for providing experimental seed material required for the present investigation.

References

1. Agyenim-Boateng KG, Zhang S, Zhang S, Khattak AN, Shaibu A, Abdelghany AM, *et al*. The nutritional composition of the vegetable soybean (Maodou) and its potential in combatting malnutrition. Front Nutr. 2023;9:1034115.
2. Ali AA, Velasquez MT, Hansen CT, Mohamed AI, Bhathena SJ. Effects of soybean isoflavones, probiotics, and their interactions on lipid metabolism and endocrine system in an animal model of obesity and diabetes. J Nutr Biochem. 2004;15(10):583-590.
3. Alves de Moraes RM, Bastos Soares TC, Colombo LR, Spegiorin Salla MF, Gomes de Almeida Barros J, Deniz Piovesan N, *et al*. Assisted selection by specific DNA markers for genetic elimination of the kunitz trypsin

inhibitor and lectin in soybean seeds. Euphytica. 2006;149:221-226.
4. Amrate PK, Shrivastava MK, Bhale MS, Agrawal N, Kumawat G, Shivakumar M, *et al*. Identification and genetic diversity analysis of high-yielding charcoal rot resistant soybean genotypes. Sci Rep. 2023;13(1):8905. [doi:10.1038/s41598-023-35688-3562]
5. Bueno RD, Borges LL, God PIVG, Piovesan ND, Teixeira AI, Cruz CD, *et al*. Quantification of anti-nutritional factors and their correlations with protein and oil in soybeans. An Acad Bras Cienc. 2018;90(1):205-217.
6. Bulatova K, Mazkirat S, Didorenko S, Babissekova D, Kudaibergenov M, Alchinbayeva P, *et al*. Trypsin inhibitor assessment with biochemical and molecular markers in a soybean germplasm collection and hybrid populations for seed quality improvement. Agronomy. 2019;9(2):76.
7. Chukwu SC, Rafii MY, Ramlee SI, Ismail SI, Oladosu Y, Muhammad I, *et al*. Recovery of recurrent parent genome in a marker-assisted backcrossing against rice blast and blight infections using functional markers and SSRs. Plants. 2020;9(11):1411.
8. Clemente A, Marín-Manzano MDC, Arques MDC, Domoney C. Bowman-Birk inhibitors from legumes: utilisation in disease prevention and therapy. Bioactive Peptides in Health and Disease. Tech-Open Access Publisher; c2013. p. 23-44.
9. Embaby HE. Effect of heat treatments on certain antinutrients and *in vitro* protein digestibility of peanut and sesame seeds. Food Sci Technol Res. 2010;17(1):31-38.
10. Ghorpade BB, Salokhe SS, Ghuge VG, Adsul AT. Molecular characterization of different soybean [*Glycine max* (L). Merril] genotypes by RAPD Markers. J Biotechnol Bioinform Res. 2022;SRC/JBBR-156. [doi:10.47363/JBBR/2022]
11. Jadhav P, Torop E, Nagrale S, Moharil M, Shinde U, Pranita T, *et al*. Genome-Wide Microsatellite Survey and Introgression of Null KTI Allele in Charcoal Rot Resistant Soybean.
12. Kahraman A. Nutritional value and foliar fertilization in soybean. J Elementol. 2017;22(1).
13. Kim MS, Park MJ, Jeong WH, Nam KC, Chung JI. SSR marker tightly linked to the Ti locus in soybean [*Glycine max* (L.) Merr.]. Euphytica. 2006;152:361-366.
14. Kumar V, Rani A, Mittal P, Shuaib M. Kunitz trypsin inhibitor in soybean: Contribution to total trypsin inhibitor activity as a function of genotype and fate during processing. J Food Meas Charact. 2019;13(2):1583-90.
15. Kumar V, Rani A, Mourya V, Rawal R. NRC 127: First Kunitz Trypsin Inhibitor Free Indian Soybean Variety Developed through Marker Assisted Backcrossing. Soybean Res. 2020;5:98.
16. Kumar V, Rani A, Rawal R, Mourya V. Marker assisted accelerated introgression of null allele of kunitz trypsin inhibitor in soybean. Breed Sci. 2015;65(5):447-52.
17. Kumar V, Rani A, Shuaib M, Mittal P. Comparative assessment of trypsin inhibitor vis-à-vis Kunitz trypsin inhibitor and Bowman-Birk inhibitor activities in soybean. Food Anal Methods. 2018;11(9):2431-7.
18. Maranna S, Verma K, Talukdar A, Lal SK, Kumar A, Mukherjee K. Introgression of null allele of Kunitz trypsin inhibitor through marker-assisted backcross breeding in soybean (*Glycine max* L. Merr.). BMC Genet. 2016;17:1-9.
19. Mishra N, Tripathi MK, Tiwari S, Tripathi N, Gupta N, Sharma A, *et al*. Evaluation of diversity among soybean genotypes via yield attributing traits and SSR molecular markers. Curr J Appl Sci Technol. 2021;40(20):9-24.
20. Mittal P, Kumar V, Anita R, Gokhale SM. Bowman-Birk inhibitor in soybean: genetic variability in relation to total trypsin inhibitor activity and elimination of Kunitz trypsin inhibitor. Not Sci Biol. 2021;13(1):10836.
21. Rani R, Raza G, Tung MH, Rizwan M, Ashfaq H, Shimelis H, *et al*. Genetic diversity and population structure analysis in cultivated soybean (*Glycine max* [L.] Merr.) using SSR and EST-SSR markers. PLoS One. 2023;18(5)
22. Roychaudhuri R, Sarath G, Zeece M, Markwell J. Stability of the allergenic soybean Kunitz trypsin inhibitor. Biochim Biophys Acta. 2004;1699(1-2):207-12.
23. Salim R, Nehvi IB, Mir RA, Tyagi A, Ali S, Bhat OM. A review on anti-nutritional factors: Unraveling the natural gateways to human health. Front Nutr. 2023;10:1215873.
24. Sharma S, Kaur M, Goyal R, Gill BS. Physical characteristics and nutritional composition of some new soybean (*Glycine max* (L.) Merrill) genotypes. J Food Sci Technol. 2014;51:551-7.
25. Steinberg FM. Soybeans or soymilk: does it make a difference for cardiovascular protection? Does it even matter? Am J Clin Nutr. 2007;85(4):927-8.
26. Talukdar A, Shivakumar M, Verma K, Kumar A, Mukherjee K, Lal SK. Genetic elimination of Kunitz trypsin inhibitors (KTI) from DS9712, an Indian soybean variety. Indian J Genet Plant Breed. 2014;74(Suppl.):608-11.
27. Taneja M, Upadhyay SK. An overview of annexins in plants. Calcium Transport Elements in Plants. 2021;8(3):171-191. [doi:10.1016/B978-0-12-821792-4.00009-6]