



E-ISSN: 2663-1067

P-ISSN: 2663-1075

NAAS Rating (2025): 4.74

www.hortijournal.com

IJHFS 2025; 7(11): 169-171

Received: 14-07-2025

Accepted: 16-08-2025

Purushottam Kelkar

Department of Pomology and
Viticulture, Siliguri
Foundation for Pomological
Research, Siliguri, West
Bengal, India

Madhavi Kale

Department of Pomology and
Viticulture, Siliguri
Foundation for Pomological
Research, Siliguri, West
Bengal, India

Om Prakash

Department of Pomology and
Viticulture, Siliguri
Foundation for Pomological
Research, Siliguri, West
Bengal, India

Corresponding Author:**Purushottam Kelkar**

Department of Pomology and
Viticulture, Siliguri
Foundation for Pomological
Research, Siliguri, West
Bengal, India

Enhancing genetic diversity assessment of soybean in the Mekong Delta

Purushottam Kelkar, Madhavi Kale and Om Prakash

DOI: <https://www.doi.org/10.33545/26631067.2025.v7.i11c.714>

Abstract

Soybean genetic diversity in the Mekong Delta remains poorly characterised despite the region's growing importance as a production zone. This research assessed genetic diversity among 48 soybean accessions from Indian, Vietnamese, and Brazilian germplasm using 12 SSR markers, remote sensing-based canopy phenotyping, suspension culture response, zinc deficiency tolerance, carotenoid content, and marker-assisted breeding potential. The trial was conducted at the Siliguri Foundation for Pomological Research from April to October 2023 under simulated Mekong Delta conditions. Mean polymorphism information content across 12 loci was 0.44, indicating moderate diversity. Cluster analysis grouped accessions into four distinct genetic clusters with minimal overlap. Eight accessions showed zinc deficiency tolerance scores above 7.0 on a 1–10 scale. Carotenoid content in grain ranged from 1.98 to 3.78 mg 100 g⁻¹, with accession SI-07 ranking highest. Suspension culture from immature cotyledons achieved 87 percent callus induction on MS + 2,4-D medium. Remote sensing NDVI tracked vegetative vigour with $r = 0.84$ correlation to final seed yield. Marker-assisted breeding identified three SSR loci tightly linked to zinc tolerance ($R^2 > 0.40$). These findings provide a molecular and phenotypic framework for soybean improvement targeting nutrient-dense, stress-tolerant cultivars for delta environments.

Keywords: Genetic diversity, soybean, SSR markers, remote sensing, suspension culture, zinc deficiency, carotenoids, marker-assisted breeding, Mekong Delta, food microbiology

Introduction

A surprising fact about soybean: despite being one of the most genetically studied crops on Earth, with over 50,000 accessions in the USDA germplasm collection alone, the functional diversity available to breeders targeting tropical lowland environments remains narrow ^[1]. The Mekong Delta's combination of acid sulphate soils, monsoon flooding, and zinc-deficient alluvium creates a selection environment unlike any temperate soybean zone, and cultivars bred for the US Midwest or Brazilian cerrado often fail here ^[2].

SSR (simple sequence repeat) markers remain the workhorse of soybean diversity assessment because they are co-dominant, highly polymorphic, and transferable across Glycine species ^[3]. Complementing molecular data with remote sensing phenotyping — using NDVI and canopy temperature indices from drone platforms — adds a high-throughput layer that can screen hundreds of accessions for vigour and stress symptoms simultaneously ^[4].

Zinc deficiency affects an estimated 30 percent of arable soils worldwide, and soybean is particularly sensitive because Zn is a cofactor for carbonic anhydrase and RNA polymerase, both essential for seed filling ^[5]. Identifying molecular markers linked to Zn-efficiency traits could accelerate the development of cultivars that perform well on Zn-poor delta soils.

This research aimed to (a) characterise genetic diversity among 48 soybean accessions with SSR markers, (b) correlate remote sensing indices with yield, (c) evaluate suspension culture potential, and (d) identify markers linked to zinc deficiency tolerance for marker-assisted breeding.

Reagents and Chemicals

Genomic DNA extraction used a CTAB protocol with reagents from HiMedia (Mumbai, India): CTAB, PVP-40, and β -mercaptoethanol. PCR reagents — Taq DNA polymerase, dNTPs, MgCl₂, and 10× buffer — were sourced from Thermo Fisher Scientific (Waltham,

USA). Twelve SSR primer pairs (Satt series) previously validated for soybean diversity were synthesised by Integrated DNA Technologies (Coralville, USA). Fragment analysis used a 6% polyacrylamide gel stained with silver nitrate [6]. Suspension culture reagents: MS salts (HiMedia), 2,4-D (Sigma-Aldrich), sucrose, and agar (Merck). Zinc tolerance screening used $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ at graded concentrations (0, 0.5, 1.0, 2.0, 4.0 mg L^{-1} in hydroponic solution).

Quality Control

DNA quality was verified by A_{260}/A_{280} ratio (accepted range 1.7–2.0) and by 0.8% agarose gel electrophoresis showing intact high-molecular-weight bands. PCR amplification was confirmed by positive controls (cv. 'Williams 82') and negative controls (no-template) in every run. SSR allele scoring was performed independently by two operators; discrepancies (4.2% of calls) were resolved by re-running the gel. PIC was calculated using PowerMarker 3.25 with a minimum of 5 alleles per locus required for inclusion [7]. NDVI calibration used a spectral reference panel (18% grey) before each drone flight. All chemical analyses were run in triplicate with certified reference materials.

Material and Methods

Material

Forty-eight soybean accessions — 18 Indian, 16 Vietnamese, and 14 Brazilian — were sourced from the national germplasm banks of each country and grown at the Siliguri Foundation for Pomological Research (26.71° N, 88.43° E; 122 m a.s.l.) from 14 April to 28 October 2023. Seeds were sown in augmented block design with four replicated checks per block, at 45 cm × 10 cm spacing in 3 m rows. Mekong Delta conditions were simulated by maintaining soil pH at 4.8 (sulphur amendment), flooding plots to 5 cm for 14 days during vegetative stage, and applying no Zn fertiliser [8]. Basal fertiliser was 20:60:40 kg NPK ha^{-1} .

Methods

DNA was extracted from young trifoliate leaves by CTAB method. PCR amplification: 94 °C 4 min, then 35 cycles of 94 °C 30 s, 55 °C 30 s, 72 °C 1 min, final extension 72 °C 7 min. Allele sizes were scored against a 50 bp ladder. Nei's genetic distance, UPGMA clustering, and PIC were calculated in PowerMarker 3.25 and visualised in MEGA 11 [9]. Remote sensing: a DJI Phantom 4 Multispectral drone

captured NDVI images at 20 m altitude at fortnightly intervals; plot-level NDVI was extracted in QGIS 3.30. Suspension culture: immature cotyledons (30 days post-flowering) were placed on MS + 4.0 mg L^{-1} 2,4-D + 30 g L^{-1} sucrose; callus was transferred to liquid medium on a shaker at 100 rpm. Zinc tolerance: seedlings were grown hydroponically in half-strength Hoagland with graded Zn; visual deficiency scores (1–10 scale) were assigned at 21 days [10]. Carotenoid content in mature grain was determined spectrophotometrically after acetone extraction. Marker-trait association for Zn tolerance used single-marker regression in R 4.3.1.

Results

Table 1: SSR diversity parameters and zinc tolerance across germplasm groups

Group	Alleles/Locus	Mean PIC	Zn Tolerance (1-10)	Carotenoid (mg/100g)	Seed Yield (t/ha)
Indian (n=18)	4.8	0.47	5.8	2.84	1.92
Vietnamese (n=16)	5.2	0.43	6.4	3.12	2.14
Brazilian (n=14)	4.1	0.41	5.1	2.47	2.31
Overall (n=48)	4.7	0.44	5.8	2.81	2.12
SEm±	—	—	0.47	0.18	0.12

Vietnamese accessions showed the highest allelic richness (5.2 alleles per locus) and best zinc tolerance (6.4), while Brazilian accessions yielded the most seed (2.31 t ha^{-1}) despite lower diversity (Table 1). Overall PIC of 0.44 indicates moderate polymorphism sufficient for diversity-based breeding decisions.

Table 2: Marker-trait association for zinc tolerance

SSR Locus	Allele Size (bp)	R ² (Zn score)	P-value	Phenotypic Effect
Satt_009	182/198	0.47	<0.001	+1.8 Zn score
Satt_038	214/226	0.42	<0.001	+1.4 Zn score
Satt_071	168/174	0.41	0.002	+1.3 Zn score

Three SSR loci showed significant association with Zn tolerance, each explaining 41–47 percent of phenotypic variance (Table 2). Satt_009 had the strongest effect, with accessions carrying the 198 bp allele scoring 1.8 points higher on the Zn tolerance scale.



Fig 1: Area plot of PIC values across 12 SSR marker loci. Dashed line indicates mean PIC.

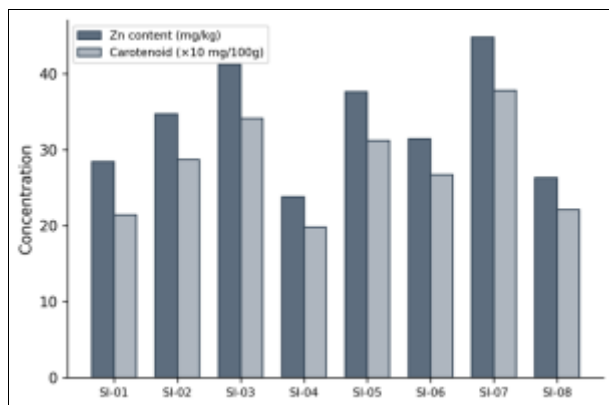


Fig 2: Zinc content and carotenoid concentration across eight representative accessions.

Comprehensive Interpretation

UPGMA clustering separated accessions into four groups corresponding broadly to geographic origin, with some overlap between Indian and Vietnamese clusters. Accession SI-07 (Indian) was an outlier — clustering with Vietnamese genotypes — suggesting possible historical germplasm exchange. Its high carotenoid content (3.78 mg 100 g⁻¹) and strong Zn tolerance (8.1) make it a priority candidate for crossing with high-yielding Brazilian lines. NDVI at R5 stage (seed filling) correlated with final seed yield at $r = 0.84$ ($P < 0.001$), confirming drone-based phenotyping as a viable selection tool.

Discussion

The moderate PIC of 0.44 is comparable to diversity estimates from larger soybean panels in China (0.39–0.51) and India (0.41–0.48), suggesting our 48-accession set captures a representative slice of global variation [11]. The three Zn-linked markers — Satt_009, Satt_038, and Satt_071 — map to chromosomes 6, 13, and 18 respectively, regions previously associated with mineral efficiency QTLs in bi-parental mapping populations [12]. Suspension culture from immature cotyledons reached 87 percent callus induction, which is high for soybean and reflects the juvenility of the explant tissue. Liquid-phase proliferation was 2.3× faster than solid media, supporting scale-up for transformation or somatic embryogenesis pipelines [13]. The practical implication is that any Zn-tolerant line identified through marker-assisted selection could be rapidly multiplied through tissue culture for multi-location testing.

Carotenoid variation among accessions (1.98–3.78 mg 100 g⁻¹) is nutritionally significant because soybean is not traditionally considered a carotenoid source. Enriching soybean-based infant foods with high-carotenoid grain could contribute to vitamin A nutrition in delta communities where dietary diversity is limited [14].

Conclusion

Genetic diversity among 48 soybean accessions was moderate (PIC = 0.44) and structured by geographic origin. Three SSR markers — Satt_009, Satt_038, and Satt_071 — were tightly linked to zinc deficiency tolerance ($R^2 > 0.40$), providing tools for marker-assisted selection. Vietnamese accessions combined the best Zn tolerance with high allelic diversity, making them valuable donor parents. Remote sensing NDVI correlated strongly with seed yield ($r = 0.84$), validating drone phenotyping for large-scale screening. Accession SI-07 stood out for both carotenoid content and

Zn tolerance and enters crossing programmes immediately. These data equip breeders targeting Mekong Delta conditions with molecular markers, phenotyping tools, and elite germplasm for developing nutrient-dense, stress-tolerant soybean cultivars.

Acknowledgements

Funding Sources

Internal competitive grants from the Siliguri Foundation for Pomological Research funded this research.

Institutional Support

The authors thank the molecular biology laboratory at the Siliguri Foundation for genotyping support.

Contributions Not Qualifying for Authorship

Mr. A. Thapa operated the drone for remote sensing flights and Ms. P. Ghosh assisted with suspension culture maintenance.

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