



RESEARCH ARTICLE

Morphological and biochemical characterisation of seed coat traits in response to mitigate pre-harvest sprouting in soybean (*Glycine max* (L.) Merr.) genotypes

Thota Joseph Raju^{1*}, S N Vasudevan², S R Doddagoudar³, Suma T C⁴, Pooja S K⁵, Harish M S⁶, Sarita Pardhan⁷, Parashivamurthy¹, Geetha Govind⁸ & Kartoori Saisanthosh⁹

¹Department of Seed Science and Technology, University of Agricultural Sciences, Bangalore 560 065, Karnataka, India

²Department of Seed Science and Technology, Alva's Institute of Agricultural Sciences and Technology, Mijar, Moodbidr 574 225, Karnataka, India

³Department of Seed Science and Technology, University of Agricultural Sciences, Raichur 584 104, Karnataka, India

⁴Department of Plant Physiology, University of Agricultural Sciences, Raichur 584 104, Karnataka, India

⁵Department of Agricultural Biotechnology, University of Agricultural Sciences, Bangalore 560 065, Karnataka, India

⁶Department of Seed Science and Technology, Chaudhary Charan Singh Haryana Agricultural University, Bawal 123 501, Haryana, India

⁷Department of Seed Science and Technology, Odisha University of Agriculture and Technology (OUAT), Bhubaneswar 751 003, Orissa, India

⁸Department of Crop Physiology, University of Agricultural Sciences, Bangalore 560 065, Karnataka, India

⁹Department of Seed Science and Technology, University of Agricultural Sciences, Dharwad 580 085, Karnataka, India

*Correspondence email - josephrajuthota@gmail.com

Received: 25 April 2026; Accepted: 12 June 2026; Available online: Version 1.0: 28 July 2026

Cite this article: Raju TJ, Vasudevan SN, Doddagoudar SR, Suma TC, Pooja SK, Harish MS, Sarita P, Parashivamurthy, Geetha G, Kartoori S. Morphological and biochemical characterisation of seed coat traits in response to mitigate pre-harvest sprouting in soybean (*Glycine max* (L.) Merr.) genotypes. Plant Science Today. 2026; 13(sp5): 1-13. <https://doi.org/10.14719/pst.15234>

Abstract

Seed coat integrity (porosity, colour and thickness) and structure (hilum, strophiole and micropyle) explain, predict and even allow genotypes resistant to pre-harvest sprouting (PHS) and field weathering under certain environmental conditions. This study examined how morphological and biochemical seed coat characteristics and their role in mitigating PHS in 96 soybean genotypes. All traits showed significant genetic variation, with broad-sense heritability ranging from 0.336 to 0.611. The principal component analysis (PCA) revealed that the first two components explained 62.50 % of total variation. Seed coat proportion correlated strongly with peroxidase activity (POD) ($r=0.511$) and negatively with water absorption capacity (WAC) ($r=-0.533$). Seeds had black (4 genotypes) and yellow (92 genotypes) seed coats and grey (5 genotypes) and brown (91 genotypes) seed hilums. Physical and biochemical characterisation varied in 96 genotypes, including Birsa Soya 1, Kalitur, Pune 14, Pune 30 (lack colour) and MAUS 61-2 (yellow colour) which were found resistant to field weathering due to hard seed coat (HSC) and seed colour. Black and brown testa genotypes are resistant to field weathering because they have the highest percentage proportion of seed coat, seed thickness (ST) and minimum seed coat permeability than yellow genotypes, which have a delicate seed coat, minimum mechanical strength and maximum seed coat permeability. These findings provide useful selection criteria for breeding soybean cultivars with improved resistance to PHS under humid tropical environments.

Keywords: genetic variation; hard seededness; pre-harvest sprouting; principal component analysis; seed dormancy

Introduction

Soybean (*Glycine max* (L.) Merr.) produces 25 % of vegetable oil worldwide and provide protein (40–42 %), oil (20–22 %) and other nutrients to humans and animals (1). The average yield is 181 MT and a productivity of 2297 kg/ha on 78.6 Mha worldwide. Despite being a new crop in India, it covers 12.69 Mha yields 15.27 MT and 1203 kg/ha. Karnataka grows 0.44 Mha of it, producing 0.45 MT and 1020 kg/ha (2). Diseases, pests, drought, nutritional deficits and pre-harvest sprouting (PHS) impair soybean seed yield and quality. Among them, fungal diseases and PHS most affect seed yield and quality (3). Pre-harvest sprouting occurs between physiological and harvestable maturity leads to severe quality loss in the soybean seeds. The soybean seed is susceptible to field weathering, which shortens its lifespan and causes mechanical

damages (4). Normally seed production of soybean is undertaken during kharif season in Karnataka districts (Bidar, Haveri and Dharwad). However, due to unusual heavy rains during harvesting, more than 50 % of the seeds start germinating on mother plant (vivipary), resulting in poor seed quality and failure to meet the Indian minimum seed certification standards for germination (70 %). This causes a shortage of quality seeds.

Pre-harvest sprouting is a complex quantitative trait regulated by seed dormancy, endogenous hormones (abscisic acid (ABA), gibberellic acid (GA3), indole acetic acid (IAA) and seed coat permeability (3, 5, 6). While weak dormancy favours and leave seeds vulnerable to PHS under high humidity. A primary defence mechanism against PHS is hard seededness or hard testa (7, 8), a physical dormancy driven by the deposition of

phenolics, suberin and lignin in the seed coat's palisade layer, which effectively blocks water imbibition and resists mechanical damage (9, 10). Hard seededness in legumes is influenced by various mechanisms, including temperature and habitat changes, phenolic content, catechol oxidase activity, epicatechin levels and β -1,3-glucans deposition on neck region of plasmodesmata (11–13). Hard seededness, which prevents seeds from germinating by not absorbing water, increases PHS tolerance (14). This structural impermeability is highly correlated with PHS tolerance across multiple legumes and cereals (5, 15, 16). Consequently, incorporating hard seeded trait, often sourced from wild progenitors into cultivated varieties is a critical strategy for mitigating weather-induced seed damage and ensuring high-quality seed production in humid tropical regions (17, 18).

Pre-harvest sprouting tolerance soybean germplasms have been identified and studied in relation to morphological traits and their direct and indirect impacts on PHS (19–21). Genotypes with improved membrane integrity and seed coat strength is essential to develop varieties withstanding field weathering. In soybean breeding, controlled deterioration methods with molecular markers efficiently assess field weathering resistance in soybean breeding initiatives (22, 23). To achieve soybean seed quality requirements, the optimum methods for producing quality seeds under untimely rains at harvest must be found. Thus, identifying ways to mitigate PHS and screening 96 soybean genotypes for handedness that resist PHS will benefit the seed industry and farming community, which directly or indirectly affect field weathering.

Materials and Methods

Plant materials

A total of 96 soybean accessions (68 released varieties and 28 initial variety trials including check (JS 335) procured from Agriculture Research Station, Bidar, University of Agricultural Sciences, Raichur (UASR) and Zonal Agricultural Research Station (ZARS), University of Agricultural Sciences, Bangalore (UASB)) obtained were assessed for morphological and biochemical traits in the current study (Tables 1 and 2).

Experimental conditions

Screening of 96 accessions was conducted during the year 2021 at the Laboratory of Department of Seed Science and Technology, UASR. The accessions were analysed by following complete randomised design by maintaining room temperature (20–25 °C) and humidity (24–35 %).

Traits studied

The observations were recorded for 11 seed physical traits on 5 randomly selected seeds for each genotype and the mean data of the five seeds were considered for final statistical analysis, such as seed length (SL; mm), seed breadth (SB; mm), seed thickness (ST; mm), seed size (SS; mm³), seed coat colour (SCC), seed coat thickness excluding hilum (SCT; %), seed coat lusture (SCL), seed hilum colour (SHC), percent proportion of seed coat (PPSC; %), test weight (TW; g) and water absorption capacity (WAC; %). Biochemical traits such as total protein content (TPC; %), electrical conductivity (EC; dS cm⁻¹) and peroxidase activity (POD; mmol⁻¹min⁻¹g⁻¹).

Physical characters

Traits such as SL, SW and ST were measured from 5 randomly selected seeds with digital grain Verniercaliper (24) and measurements were repeated three times and taken the average. The SCC for all the accessions including SHC and SCL were compared with the Royal Horticultural Society's (RHS) colour chart, by visual assessment under natural day light. The SCT excluding hilum (mm) was measured by taking percentage of the fraction of seed coat which was calculated as the ratio of the seed coat to the total seed. Further 100 seeds sample were dried (5 hr at 60 °C), weighed and watered for 15 hr. The seed coat was dried (5 hr at 60 °C) without seeds. Five randomly selected seeds were measured for SCT with digital grain verniercaliper. The % of seed coat is estimated by carefully removing 20 seed coats in 2 replications with forceps after gently stroking seeds between muslin cloth (25).

$$\% \text{ of seed coat} = \frac{\text{Proportion of seed coat weight}}{\text{Seed coat weight}} \times 100 \quad (\text{Eq. 1})$$

Randomly picked seeds (100 in quantity) were weighed for TW. The WAC was evaluated by soaking ten grams of healthy seeds in distilled water at room temperature for 10 hr (26).

$$\text{WAC} = \frac{W_1 - W_0}{W_0} \times 100 \quad (\text{Eq. 2})$$

where W_0 = seed weight and W_1 = soaked weight

Biochemical traits

The EC of seed leachate was assessed (27). The POD was evaluated by measuring the increase in optical density resulting from the oxidation of guaiacol to tetra-guaiacol (28). The TPC using UV-Vis spectrophotometer and was expressed in % (29).

Data analysis

The final statistical analysis used average data. R software was used for analysis of variance (ANOVA) to assess genotypic variation on trait diversity. Correlation analyses were performed using R software (<http://CRAN.R-project.org/package=Hmisc>). Principal component analysis (PCA) was used to classify genotypes, identify variation patterns and evaluate trait significance within each component. For this, a correlation matrix was used. This study used R 4.1 for PCA analysis.

Results

Under lab conditions, 96 accessions were tested for 11 characteristics. The mean value and variation of the attributes were calculated using statistical analysis of phenotypic data.

Performance of accessions for seed physical and biochemical traits

Physical traits

Physical and biochemical properties of 96 soybean genotypes are shown in Tables 1 and 2. Seed length ranged from 6.38 to 9.21 mm for soybean genotypes. The genotype BNS 5 had the shortest SL (6.38 mm), followed by SL 96 (9.21 mm) and PUNE 30 (9.09 mm). Seed breadth dimensions were 5.50–7.44 mm. The genotypes AGS 48 and BNS 5 had the lowest SB (5.50 and 5.54 mm respectively) and IVT 38 had the highest SB (7.44 mm). Seed thickness was 4.33–6.46 mm. The genotypes BNS 5 and AGS 48 had lower ST

(4.33 and 4.35 mm) than HARASOYA (6.46 mm). Seed sizes ranged from 152.96 to 397.87 mm³. The genotype HARASOYA has the largest SS (397.87 mm³), followed by SL 96 (393.32 mm³) and BNS 5 (152.96 mm³). Only black and yellow seed coats were found in this investigation. Four genotypes (BIRSA SOYA 1, KALITUR, PUNE 30 and PUNE 14) had black seed coats, while 92 had yellow. Percent proportion of seed coat varied from 5.15 % to 11.53 % among soybean genotypes. Percent proportion of seed coat was greatest (11.53) for genotype PUNE 30, followed by KALITUR (11.29), PUNE 14 (11.21), BIRSA SOYA 1 (10.46), MAUS 61-2 (10.36) and PUNE 39 (10.31). The lowest seed coat proportion was 5.15 % for genotype IVT 38. The soybean genotypes showed significant diversity in TW, ranging from 7.71 g to 24.12 g, with a mean of 15.51 g. The seed groups were bold (> 12 g), medium (10–12 g) and tiny (< 10 g). Genotypes (89) were bold-seeded, KALITUR, BNS 5 and AGS 48 were small and 107-4, IVT 20, JS 90-41 and IVT 8 were intermediate. Seed coat thickness excluding hilum variation among soybean genotypes was also considerable from 0.084 to 0.199 mm. The genotype JS 93-05 had the thickest seed coat (0.199 mm), followed by IVT 11, BIRSA SOYA 1, AGS 48, 107-4 and KALITUR, while HARASOYA had the thinnest (0.084 mm). The mean WAC of 96 genotypes was 118.91 %, ranging from 81.18 % to 196.81 % (Table 1). Water absorption was lowest in genotypes PUNE 30 (82.30 %) and KALITUR (87.47 %) and highest in ASRB 1 (196.81 %). AGS-25, BIRSA SOYA 1, KALITUR, KDS 726 and SL 96 had grey seed hilums, while the rest were brown (Table 1).

Biochemical traits

Seed biochemical properties like TPC, EC and POD are shown in the Table 2. The genotypes had TPC ranging from 32.05 to 44.93 %. PK 1029 had the highest TPC (44.93 %), followed by SL 979 (43.68 %) and SL 958 (40.83 %). The genotype MAUS 61-2 had the lowest (32.05 %). Seed leachate EC ranged from 0.468 to 1.087 dS cm⁻¹. The genotypes BIRSA SOYA 1 and PUNE 14 had the lowest EC (0.468 and 0.470 dS cm⁻¹ respectively), whereas BRAGG had the greatest EC (1.087). The mean POD was 4.482 mmol min⁻¹g⁻¹, ranging from 1.997 to 6.046. Peroxidase activity was highest in BIRSA SOYA 1 (6.046 mmol min⁻¹ g⁻¹) and lowest in 104-31 (1.997 mmol min⁻¹ g⁻¹).

Analysis of variance and summary statistics

The ANOVA test showed significant findings for morphological and biochemical variables for GCVs ranging 4.254 to 18.726 % demonstrating significant variability suited for trait enhancement by selection (Table 3). The greater broad-sense heritability (h²) values for POD (0.611), WAC (0.395), seed coat ratio (SCR) (0.368) and TPC (0.336) and negative broad-heritability estimates observed for SS (-0.0007), TW (-0.037) and SCT (-0.052) (Table 4).

Trait correlation

Seed size correlated positively with TW ($r = 0.781$) and EC ($r = 0.443$) and strong negative connection with SCT ($r = -0.269$). Seed size did not correlate with PPSC, WAR, TPC, EC or POD. Percent proportion of seed coat and POD were positively correlated ($r = 0.511$). The PPSC was negatively correlated with WAC ($r = -0.533$) and EC ($r = -0.550$). The TW correlated positively with SS ($r = 0.781$) and EC ($r = 0.632$). In this investigation, SW did not correlate with PPSC, coat thickness (CT), WAC, TPC, or POD. Seed coat thickness was negatively correlated with SS ($r = -0.269$) and EC ($r = -0.267$). This study suggests that PPSC, TW, WAC, TPC and POD may not affect SCT. Water absorption capacity was negatively correlated with PPSC and POD

($r = -0.469$) and positively correlated with EC ($r = 0.386$). Electric conductivity (EC) correlated positively with SS ($r = 0.443$), TW ($r = 0.367$) and EC ($r = 0.386$). Electric conductivity was negatively correlated with PPSC ($r = -0.550$) and SCT ($r = -0.267$) (Fig. 1; Table 5).

Principal component analysis

Principal component analysis was performed independently for 11 characteristics (9 seed morphological and 2 biochemical), SCC and SHC. Principal component analysis showed that PC1 and PC2 explained 40.00 % and 22.5 % of the variability respectively and 62.50 % of the total variation (Fig. 2). Other PCA studies on soybean traits show that the first two components account for 60–65 % of the total variability, indicating their importance in distinguishing soybean genotypes based on seed characteristics. The PCA biplot angles showed a substantial positive association between SL, SB, ST, TW and EC. The traits PPSC, SCT, TPC and POD also correlated well. Morphological features determined the PC1 axis, with substantial positive loadings for SL, SB, SS and TW. The co-segregation of these features allows genotypes to be distinguished by seed physical dimensions. Conversely, PC2 has biochemical and permeability features such as SCT, TPC, PPSC and POD. The secondary axis of variation is mostly independent of SS. Genetics were clearly distinguished along the PC1 axis by seed physical features. The genotypes HARASOYA, IVT 38, SL 96, PUNE 30, BRAGG and ANKUR have greater SL, SB, SS, ST and TW values (PC1). Worse PC1 values were related with worse morphological qualities in genotypes AGS 48, BNS 5, 107-4 and IVT 8.

The genotypes PUNE 30, KALITUR, PUNE 14 and BIRSOYA clustered around the PPSC vector, indicating increased seed coat permeability proportions, according to biplot analysis. Genotypes JS 93-05, IT 11 and BIRSOYA had higher SCT, while ASRB 1, AVKS 1 and IVT 4 had higher WAC. PK1029, SL 979 and SL 958 had higher TPC and BRAGG, IVT 28 and IVT 9 had higher EC. The POD was greater for genotypes 104-31, 107-4 and AGS 48. Interestingly, genotypes PUNE 30 and KALITUR performed well in physical (SS and PPSC) and biochemical (high POD, low EC) characteristics, suggesting mechanical and biochemical resistance mechanisms. Genotypes AVKS 4, AVKS 6, IVT 38, IVT 40, PS 1225, HARASOYA, MAUS 61-2, KALITUR, PUNE 30, NRC 21, MAUS 81, PUNE 14 and BIRSOYA 1 separated from the origin, indicating that PPSC, SCT, WAC, TPC, EC and POD differentiated these accessions.

Discussion

The present study extensively examined the seed coat morphological and biochemical features of 96 soybean genotypes to uncover PHS resistance mechanisms. A substantial genetic variation across physical and biochemical traits, moderate-to-high heritability estimates and significant trait correlations show that seed coat integrity acts as a multifactorial barrier to premature seed germination in humid field conditions.

Seed coat architecture and physical resistance to pre-harvest sprouting

The difference in PPSC and ST (0.084–0.299 mm) represents mechanical protection and water impermeability. The black-seeded genotypes (Birsa soya 1, Kalitpur, Pune 14 and Pune 30) had higher SCP values (10.46–11.53 %) than the yellow-seeded ones, supporting the literature that black seed coats have thicker palisade layers and greater mechanical strength (25). The outermost structural

Table 1. Mean performance of seed morphological characteristics of soybean genotypes

Sl. No.	Genotypes	Seed length (mm)	Seed breadth (mm)	Seed thickness (mm)	Seed size (mm ³)	Seed coat colour	Seed coat thickness excluding hilum (%)	Seed coat lusture	Seed hilum colour	Percent proportion of seed coat (%)	Test weight (g)	Water absorption capacity (%)
1	104-31	7.09	6.28	4.91	218.69	Yellow	0.180	Shiny	Brown	5.53	15.45	134.41
2	107-4	6.75	5.83	4.59	180.88	Yellow	0.194	Shiny	Brown	6.18	11.66	112.45
3	AGS 48	6.45	5.50	4.35	156.96	Yellow	0.195	Shiny	Brown	5.33	7.71	138.70
4	AGS-25	7.57	6.54	5.44	270.04	Yellow	0.137	Shiny	Grey	5.61	15.43	129.53
5	ANKUR	8.20	6.98	6.00	343.81	Yellow	0.098	Shiny	Brown	5.53	19.79	150.18
6	ASRB 1	7.55	6.26	5.16	244.18	Yellow	0.157	Shiny	Brown	5.53	14.55	196.81
7	AVKS 1	7.78	6.60	5.94	310.45	Yellow	0.114	Shiny	Brown	5.89	16.46	195.41
8	AVKS 2	7.35	6.47	5.57	264.53	Yellow	0.136	Shiny	Brown	8.45	16.70	131.09
9	AVKS 3	7.25	6.22	5.28	238.29	Yellow	0.154	Shiny	Brown	5.33	15.00	144.87
10	AVKS 4	7.56	6.59	5.55	276.70	Yellow	0.126	Shiny	Brown	5.22	17.82	136.47
11	AVKS 6	7.04	6.33	5.33	237.64	Yellow	0.159	Shiny	Brown	5.22	15.25	144.50
12	BIRSA SOYA 1	7.67	6.26	5.12	233.39	Black	0.196	Shiny	Grey	10.46	15.62	92.14
13	BNS 5	6.38	5.54	4.33	152.96	Yellow	0.091	Shiny	Brown	6.88	9.31	141.77
14	BRAGG	8.11	7.13	6.20	358.39	Yellow	0.184	Shiny	Brown	5.81	23.19	138.88
15	CAT 3293	7.02	6.13	5.21	224.85	Yellow	0.133	Shiny	Brown	7.35	14.47	124.45
16	CAT 44	7.57	6.34	5.68	272.37	Yellow	0.129	Shiny	Brown	5.27	17.12	134.72
17	DS 3106	7.48	6.44	5.83	280.66	Yellow	0.128	Shiny	Brown	8.12	16.61	121.00
18	DSB 21	7.66	6.37	5.32	260.00	Yellow	0.140	Shiny	Brown	6.23	15.22	132.76
19	DSB 23-2	7.46	6.38	5.41	257.10	Yellow	0.148	Shiny	Brown	7.09	14.80	124.87
20	DSB 25	7.56	6.03	5.19	236.44	Yellow	0.154	Shiny	Brown	7.94	12.91	124.70
21	DSB 27	7.27	6.18	5.11	230.00	Yellow	0.179	Shiny	Brown	8.97	14.28	128.60
22	DSB 28-3	7.75	6.58	5.34	272.28	Yellow	0.139	Shiny	Brown	9.12	14.91	137.64
23	DSB 31	7.76	6.60	5.14	263.26	Yellow	0.138	Shiny	Brown	8.75	15.69	128.70
24	DSB 32	7.08	6.53	5.49	253.80	Yellow	0.149	Shiny	Brown	10.15	15.26	113.10
25	DSB 33	7.13	6.42	5.43	248.88	Yellow	0.154	Shiny	Brown	8.75	14.02	140.40
26	DSB 34	7.10	6.46	5.45	249.94	Yellow	0.151	Shiny	Brown	10.12	15.59	124.78
27	DURGA	7.53	6.07	5.14	235.23	Yellow	0.158	Shiny	Brown	9.30	13.97	120.18
28	EC 1720617	7.70	6.48	5.40	269.47	Yellow	0.135	Shiny	Brown	9.42	15.77	142.49
29	EC 241780	7.28	6.07	4.79	211.84	Yellow	0.183	Shiny	Brown	9.39	12.63	136.02
30	EC 58705	7.84	6.52	5.47	279.79	Yellow	0.127	Shiny	Brown	10.11	18.65	112.45
31	HARASOYA	8.41	7.32	6.46	397.87	Green	0.084	Shiny	Brown	8.69	23.29	139.42
32	IVT 3	7.10	6.50	5.47	252.61	Yellow	0.144	Shiny	Brown	6.87	14.31	98.74
33	IVT 4	7.53	6.61	5.24	261.05	Yellow	0.143	Shiny	Brown	6.65	14.79	162.02
34	IVT 6	7.26	5.97	4.89	212.41	Yellow	0.138	Shiny	Brown	7.04	12.54	130.78
35	IVT 8	6.79	5.83	4.90	193.85	Yellow	0.181	Shiny	Brown	5.61	10.25	127.97
36	IVT 9	7.60	6.68	6.20	314.98	Yellow	0.161	Shiny	Brown	5.89	18.99	136.87
37	IVT 11	7.62	6.26	5.38	256.62	Yellow	0.198	Shiny	Brown	5.84	15.16	138.94
38	IVT 13	7.11	6.78	5.75	277.10	Yellow	0.112	Shiny	Brown	7.59	17.66	139.35
39	IVT 14	7.71	6.42	5.03	249.07	Yellow	0.146	Shiny	Brown	7.52	16.06	112.45
40	IVT 17	7.96	6.61	5.14	270.20	Yellow	0.123	Shiny	Brown	7.63	15.54	108.74
41	IVT 18	7.38	6.37	5.28	248.22	Yellow	0.152	Shiny	Brown	7.41	15.22	154.15
42	IVT 19	7.74	6.41	5.48	271.49	Yellow	0.139	Shiny	Brown	7.50	15.58	108.48
43	IVT 20	6.82	6.11	5.14	214.36	Yellow	0.156	Shiny	Brown	7.56	11.33	130.39
44	IVT 22	7.12	6.23	5.11	226.61	Yellow	0.135	Shiny	Brown	8.83	12.74	126.84
45	IVT 25	7.19	6.12	5.36	235.69	Yellow	0.187	Shiny	Brown	9.34	13.87	126.49
46	IVT 26	7.54	6.68	5.42	273.20	Yellow	0.181	Shiny	Brown	6.61	17.35	123.64
47	IVT 28	8.29	6.81	5.66	319.72	Yellow	0.157	Shiny	Brown	6.18	19.58	100.40
48	IVT 29	7.90	6.31	5.19	258.80	Yellow	0.134	Shiny	Brown	8.60	14.27	121.45
49	IVT 31	7.71	7.11	6.10	334.88	Yellow	0.110	Shiny	Brown	5.89	18.38	138.74
50	IVT 33	7.69	6.34	5.13	250.02	Yellow	0.143	Shiny	Brown	6.85	13.42	133.80

51	IVT 34	7.28	6.32	5.19	239.10	Yellow	0.101	Shiny	Brown	6.65	14.98	139.21
52	IVT 35	8.06	6.73	5.52	299.81	Yellow	0.149	Shiny	Brown	6.35	16.99	112.45
53	IVT 36	7.67	6.16	4.88	231.05	Yellow	0.159	Shiny	Brown	10.04	12.92	100.24
54	IVT 37	7.96	6.72	5.46	291.77	Yellow	0.117	Shiny	Brown	6.01	16.27	123.45
55	IVT 38	8.20	7.44	6.26	381.53	Yellow	0.172	Shiny	Brown	5.15	24.12	106.45
56	IVT 39	7.78	6.49	5.15	260.26	Yellow	0.125	Shiny	Brown	7.63	14.63	121.54
57	IVT 40	7.45	6.40	5.30	253.43	Yellow	0.089	Shiny	Brown	9.92	15.72	130.79
58	IVT 41	7.75	6.53	5.44	275.76	Yellow	0.137	Shiny	Brown	6.35	15.24	112.45
59	IVT 43	7.61	7.07	6.00	322.83	Yellow	0.143	Shiny	Brown	9.90	19.69	103.21
60	JS 20-34	7.52	6.41	5.49	264.70	Yellow	0.128	Shiny	Brown	6.85	15.83	121.45
61	NRC 12	7.55	6.35	5.30	254.20	Yellow	0.108	Shiny	Brown	8.91	14.76	112.04
62	JS 335	7.64	6.63	5.82	294.45	Yellow	0.134	Shiny	Black	9.87	15.62	132.12
63	JS 7105	7.81	6.45	5.50	277.16	Yellow	0.147	Shiny	Brown	7.27	15.13	115.00
64	JS 90-41	6.78	6.16	5.01	209.50	Yellow	0.128	Shiny	Brown	10.21	11.32	100.89
65	JS 93-05	7.42	6.53	5.17	250.17	Yellow	0.199	Shiny	Brown	10.18	15.62	93.04
66	JS 95 60	7.68	6.67	5.30	271.25	Yellow	0.148	Shiny	Brown	9.85	15.62	102.45
67	JS 97-52	7.32	5.98	4.83	211.32	Yellow	0.136	Shiny	Brown	8.63	15.62	90.49
68	KALITUR	8.01	6.52	4.85	253.16	Yellow	0.192	Shiny	Grey	11.29	14.20	87.47
69	KDS 726	8.23	6.65	5.40	295.31	Yellow	0.121	Shiny	Grey	6.01	15.62	125.17
70	MACS 1410	7.55	6.22	5.23	246.33	Yellow	0.159	Shiny	Brown	10.17	15.62	95.80
71	MACS 1460	7.20	6.39	5.19	238.63	Yellow	0.151	Shiny	Brown	6.23	15.62	121.45
72	MACS 1488	8.24	6.71	5.26	291.23	Yellow	0.126	Shiny	Brown	7.64	15.62	108.10
73	MACS 450	7.46	6.36	5.55	263.34	Yellow	0.137	Shiny	Brown	8.65	15.62	113.37
74	MAUS 158	7.49	6.22	5.27	246.17	Yellow	0.154	Shiny	Brown	9.06	15.62	97.29
75	MAUS 162	7.60	6.49	5.26	259.74	Yellow	0.142	Shiny	Brown	9.12	15.62	114.91
76	MAUS 2	8.02	6.76	5.68	308.22	Yellow	0.119	Shiny	Brown	8.47	15.62	106.81
77	MAUS 61-2	7.66	6.60	5.53	279.35	Yellow	0.129	Shiny	Brown	10.36	15.62	89.97
78	MAUS 71	7.77	6.62	5.35	275.21	Yellow	0.131	Shiny	Brown	9.34	15.62	95.45
79	MAUS 81	7.51	6.37	5.23	250.57	Yellow	0.149	Shiny	Brown	6.61	15.62	128.74
80	NRC 21	7.70	6.41	5.55	273.77	Yellow	0.131	Shiny	Brown	8.57	15.62	121.02
81	NRC 37	7.17	6.34	5.22	237.63	Yellow	0.161	Shiny	Brown	7.29	15.62	114.79
82	NRC 86	7.40	6.19	5.12	234.68	Yellow	0.124	Shiny	Brown	9.62	15.62	130.21
83	PK 472	7.45	6.52	5.91	287.51	Yellow	0.189	Shiny	Brown	7.16	16.52	124.04
84	PK 1029	7.88	6.46	5.65	274.23	Yellow	0.118	Shiny	Brown	10.27	15.62	134.87
85	PS 1225	7.69	6.69	5.91	304.07	Yellow	0.086	Shiny	Brown	9.37	15.62	112.45
86	PUNE 30	9.09	7.18	5.66	369.83	Black	0.145	Shiny	Brown	11.53	15.62	82.30
87	PUNE 14	8.06	6.56	5.09	269.17	Black	0.178	Shiny	Brown	11.21	15.62	94.14
88	PUNE 39	7.57	6.59	5.38	268.63	Yellow	0.136	Shiny	Brown	10.31	15.62	104.87
89	RKS 18	7.44	6.25	5.34	248.51	Yellow	0.157	Shiny	Brown	10.29	15.62	96.32
90	RSC 1046	7.44	6.42	5.19	262.79	Yellow	0.140	Shiny	Brown	9.52	15.62	102.48
91	SL 955	7.19	6.27	5.21	234.86	Yellow	0.129	Shiny	Brown	5.81	16.22	103.21
92	SL 958	7.51	6.10	5.16	244.02	Yellow	0.154	Shiny	Brown	8.43	15.62	101.75
93	SL 96	9.21	7.30	5.85	393.32	Yellow	0.191	Shiny	Grey	9.58	15.62	101.12
94	SL 979	7.27	7.07	6.11	344.97	Yellow	0.096	Shiny	Brown	8.74	15.62	116.09
95	SR 2401	7.98	6.25	5.03	241.89	Yellow	0.153	Shiny	Brown	9.43	15.62	96.88
96	VLS 59	7.70	6.93	5.94	354.35	Yellow	0.094	Shiny	Brown	8.02	15.62	95.12
Mean		7.566	6.461	5.378	265.535		0.144			7.969	15.514	118.91
Variance		0.447	0.341	0.384	44.243		0.027			1.733	2.384	23.26
Standard Deviation (S.D.)		0.2	0.116	0.148	1.975		0.001			3.002	5.681	20.73
Minimum		6.38	5.5	4.33	152.96		0.084			5.15	7.71	81.18
Maximum		9.21	7.44	6.46	397.87		0.199			11.53	24.12	196.81
Range		2.83	1.94	2.13	244.91		0.115			6.38	16.41	115.63

Table 2. Mean performance of seed biochemical characteristics of soybean genotypes

SI. No.	Genotypes	Total protein content (%)	Electrical conductivity (dS cm ⁻¹)	Peroxidase activity (mmol min ⁻¹ g ⁻¹)
1	104-31	36.23	0.614	1.997
2	107-4	35.14	0.391	2.223
3	AGS 48	35.78	0.624	3.745
4	AGS-25	36.79	0.662	2.668
5	ANKUR	35.89	0.637	2.994
6	ASRB 1	36.23	0.628	2.003
7	AVKS 1	35.14	0.751	3.445
8	AVKS 2	35.78	0.617	2.335
9	AVKS 3	36.12	0.674	2.667
10	AVKS 4	35.78	0.642	2.089
11	AVKS 6	35.89	0.612	2.997
12	BIRSA SOYA 1	39.12	0.168	6.046
13	BNS 5	36.23	0.376	3.398
14	BRAGG	37.64	0.787	4.547
15	CAT 3293	36.79	0.461	3.716
16	CAT 44	35.89	0.634	4.008
17	DS 3106	35.89	0.661	3.007
18	DSB 21	36.79	0.618	3.426
19	DSB 23-2	35.89	0.641	3.917
20	DSB 25	35.78	0.531	3.201
21	DSB 27	36.12	0.469	3.217
22	DSB 28-3	35.78	0.663	3.619
23	DSB 31	35.78	0.625	4.971
24	DSB 32	36.12	0.255	3.853
25	DSB 33	35.78	0.574	4.109
26	DSB 34	36.79	0.240	3.318
27	DURGA	35.89	0.478	4.306
28	EC 1720617	35.89	0.678	3.743
29	EC 241780	36.23	0.387	3.957
30	EC 58705	35.14	0.265	3.453
31	HARASOYA	35.78	0.741	3.114
32	IVT 3	35.78	0.568	4.219
33	IVT 4	36.45	0.645	3.654
34	IVT 6	36.74	0.419	3.901
35	IVT 8	36.01	0.361	4.009
36	IVT 9	35.12	0.763	3.587
37	IVT 11	36.45	0.619	3.856
38	IVT 13	36.74	0.612	3.301
39	IVT 14	36.74	0.568	3.217
40	IVT 17	35.98	0.607	3.619
41	IVT 18	36.74	0.541	4.971
42	IVT 19	36.01	0.611	3.853
43	IVT 20	35.12	0.417	4.109
44	IVT 22	35.12	0.475	3.318
45	IVT 25	36.45	0.535	4.306
46	IVT 26	36.01	0.659	3.743
47	IVT 28	35.12	0.787	3.957
48	IVT 29	36.74	0.634	3.453
49	IVT 31	35.98	0.727	3.338
50	IVT 33	36.74	0.559	3.958

51	IVT 34	36.74	0.537	5.121
52	IVT 35	35.98	0.728	4.201
53	IVT 36	36.74	0.275	4.354
54	IVT 37	36.01	0.645	3.458
55	IVT 38	35.12	0.667	4.788
56	IVT 39	35.12	0.621	4.012
57	IVT 40	36.45	0.594	4.198
58	IVT 41	36.74	0.687	3.712
59	IVT 43	36.74	0.724	5.840
60	JS 20-34	35.98	0.633	5.732
61	NRC 12	36.74	0.607	5.811
62	JS 335	37.23	0.694	6.046
63	JS 7105	35.12	0.643	5.757
64	JS 90-41	39.93	0.260	5.759
65	JS 93-05	35.98	0.260	5.779
66	JS 95 60	36.74	0.609	5.840
67	JS 97-52	36.01	0.397	5.765
68	KALITUR	36.12	0.172	3.412
69	KDS 726	35.12	0.719	5.805
70	MACS 1410	35.89	0.255	5.779
71	MACS 1460	35.12	0.531	5.814
72	MACS 1488	36.74	0.678	5.756
73	MACS 450	35.98	0.687	5.765
74	MAUS 158	36.74	0.527	5.805
75	MAUS 162	36.01	0.654	5.983
76	MAUS 2	33.22	0.734	6.046
77	MAUS 61-2	32.05	0.171	5.935
78	MAUS 71	35.51	0.653	6.017
79	MAUS 81	32.25	0.574	5.960
80	NRC 21	33.05	0.654	5.962
81	NRC 37	35.89	0.527	5.983
82	NRC 86	36.04	0.457	5.969
83	PK 472	36.23	0.653	4.301
84	PK 1029	44.93	0.265	5.962
85	PS 1225	38.09	0.714	6.011
86	PUNE 30	36.74	0.171	6.046
87	PUNE 14	35.98	0.170	5.935
88	PUNE 39	36.74	0.265	5.969
89	RKS 18	36.74	0.260	6.011
90	RSC 1046	36.74	0.633	5.983
91	SL 955	36.74	0.414	3.209
92	SL 958	40.83	0.534	5.983
93	SL 96	35.12	0.717	5.983
94	SL 979	43.68	0.752	5.969
95	SR 2401	36.12	0.549	6.011
96	VLS 59	35.78	0.726	6.245
Mean		36.27	0.848	4.482
Variance		1.65	0.468	1.248
Standard Deviation (S.D.)		2.73	0.028	1.56
Minimum		32.05	0.468	1.997
Maximum		44.93	1.087	6.245
Range		12.88	0.619	4.248

Table 3. Analysis of variance for morphological and biochemical traits

Sl. No.	Traits / Variance	SS		MSS		F ratio	p value	SEm	CD (1 %)	CV (%)
		Gen df (95)	Error Df (384)	Gen	Error					
1	Seed length (SL)	38.869	73.777	0.409	0.192	2.129	0.000 ***	0.196	0.717	5.793
2	Seed breadth (SB)	21.991	46.184	0.231	0.120	1.925	0.000 ***	0.155	0.567	5.369
3	Seed thickness (ST)	19.922	59.128	0.209	0.154	1.362	0.0230 *	0.176	0.642	7.297
4	Seed size (SS)	183553.5	768863.2	1932.14	2002.24	0.9650	0.5739	20.112	-	16.852
5	Percent proportion of seed coat (PPSC)	712.465	735.297	7.499	1.9148	3.9166	0.000 ***	0.619	2.266	17.364
6	Test weight (TW)	495.049	2439.74	5.211	6.354	0.8202	0.8780	1.127	-	16.315
7	Seed coat thickness (SCT)	0.055	0.296	0.0005	0.0007	0.7548	0.9507	0.012	-	19.306
8	Water absorption content (WAC)	102043.8	96738.97	1074.15	251.92	4.2638	0.000 ***	7.098	25.986	13.105
9	Total protein content (TPC)	797.001	913.951	8.389	2.3801	3.5249	0.000 ***	0.689	2.525	4.254
10	Electrical conductivity (EC)	3.910	9.678	0.0411	0.0252	1.6332	0.0007 ***	0.071	0.259	18.723
11	Peroxidase activity (POD)	503.676	229.807	5.302	0.598	8.859	0.0000 ***	0.346	1.266	17.298

*: significance at 0.0005 probability level and ***: significance at 0.0001 probability level; SS: sum of square; MSS: mean sum of square; EMSS: error mean sum of square; Gen: genotypes; SEm: standard error of mean; CD (1 %): critical difference at 1 %; CV: coefficient of variation.

Table 4. Genetic variability parameters for morphological and biochemical traits

Sl. No.	Traits	Mean	Range		Coefficient of variance		h ² _{bs}	GA	GAM
			Max	Min	GCV	PCV			
1	Seed length (SL)	7.5665	9.5324	6.1899	2.754	6.414	0.184	0.236	3.121
2	Seed breadth (SB)	6.4592	7.6956	5.3305	2.309	5.845	0.156	0.156	2.408
3	Seed thickness (ST)	5.3773	6.6889	4.1962	1.963	7.557	0.068	0.072	1.347
4	Seed size (SS)	265.5348	411.7952	148.3712	1.410	16.792	-0.007	-0.830	-0.313
5	Seed coat ratio (SCR)	7.9686	11.9335	4.9960	13.262	21.850	0.368	1.694	21.252
6	Test weight (TW)	15.4498	24.9594	7.4766	3.094	16.019	-0.037	-0.244	-1.578
7	Coat thickness (CT)	0.1438	0.2060	0.0815	4.275	18.827	-0.052	-0.004	-2.563
8	Water absorption content (WAC)	121.1166	203.7035	79.8295	10.588	16.847	0.395	21.276	17.566
9	Total protein content (TPC)	36.2675	46.5026	31.0837	3.023	5.218	0.336	1.677	4.623
10	Electrical conductivity (EC)	0.8480	1.1250	0.4544	6.663	19.873	0.112	0.050	5.897
11	Peroxidase activity (POD)	4.4723	6.2581	1.9371	21.687	27.741	0.611	2.002	44.759

Max: maximum; Min: minimum; GCV: genotypic coefficient of variance; PCV: phenotypic coefficient of variance; h²_{bs}: heritability (broad sense); GA: genetic advance; GAM: genetic advance as percentage of mean.

Table 5. Correlation analysis between physical and biochemical traits in soybean genotypes

Traits	SS	PPSC	TW	SCT	WAC	TPC	EC	POD
SS	1	0.022	0.781**	-0.269**	-0.079	0.001	0.443**	0.176
PPSC	0.022	1	-0.098	0.003	-0.533**	0.198	-0.550**	0.511**
TW	0.781**	-0.098	1	-0.168	-0.023	-0.044	0.367**	0.032
SCT	-0.269**	-0.003	0.168	1	-0.142	0.111	-0.267**	-0.066
WAC	-0.079	-0.533**	-0.023	-0.142	1	-0.025	0.386**	-0.552**
TPC	0.001	0.198	-0.044	-0.111	-0.025	1	-0.128	0.145
EC	0.443**	-0.550**	0.367**	-0.267**	0.386**	-0.128	1	-0.148
POD	0.176	0.511**	0.032	-0.066	-0.552**	0.145	-0.148	1

** : significance at 0.001 level; SS: seed size; PPSC: percent proportion of seed coat; TW: test weight; SCT: seed coat thickness excluding hilum; WAC: water absorption capacity; TPC: total protein content; EC: electrical conductivity; POD: peroxidase activity.

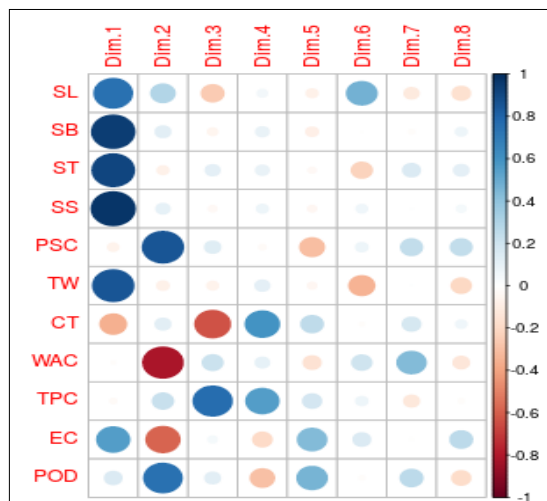


Fig. 1. Correlation analysis between morphological and biochemical traits among 96 soybean genotypes. SL: seed length; SB: seed breadth; ST: seed thickness; SS: seed size; PPSC: percent proportion of seed coat; TW: test weight; CT: seed coat thickness excluding hilum; WAC: water absorption capacity; TPC: total protein content; EC: electrical conductivity; POD: peroxidase activity.

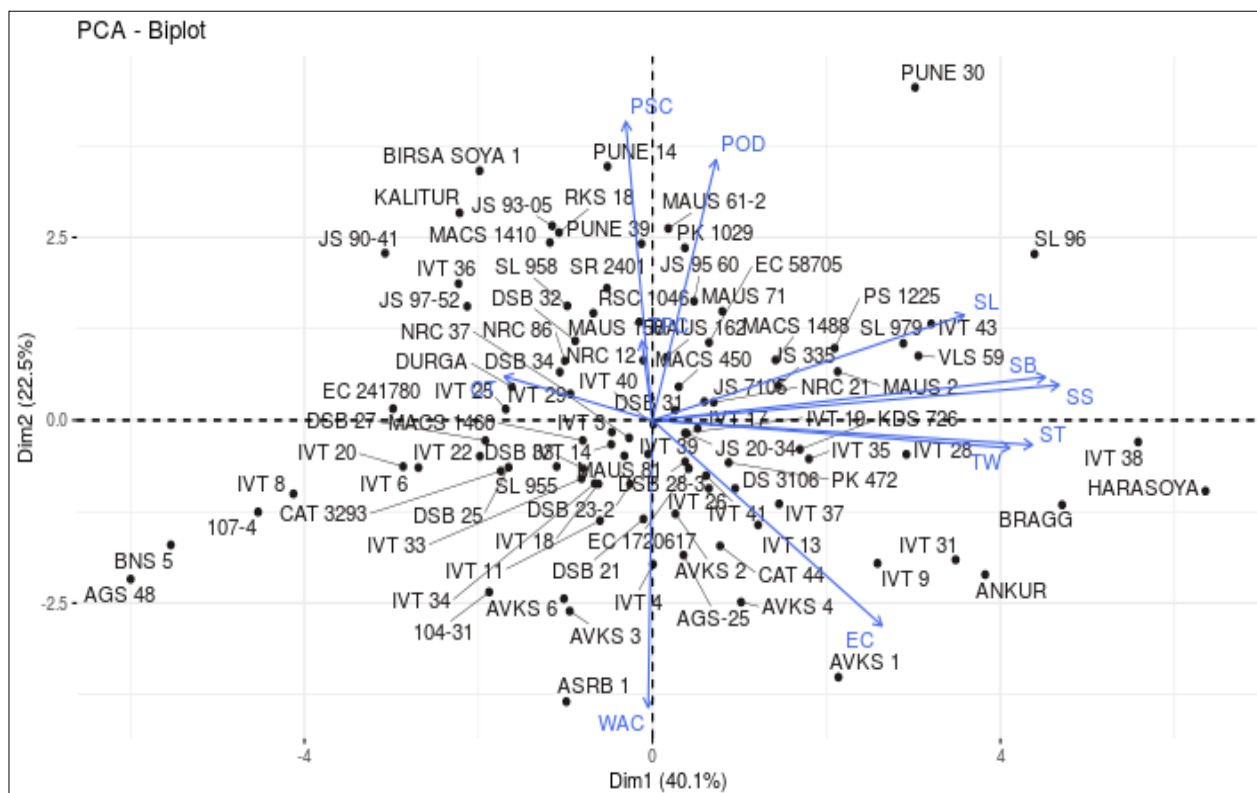


Fig. 2. Biplot generated from principal component analysis of traits measured in 96 genotypes of soybean.

component, the palisade layer cuticle, determines seed coat permeability. Genotypes with mechanically robust cuticles prevent water infiltration through micro-cracks that form under moisture stress. This physical architecture reduces water absorption (WAC: 82.30 % in Pune 30 vs. 196.81 % in ASRB 1), which is bad for PHS tolerance breeding. The inverse correlation between PPSC and WAC ($r = -0.533$) suggests that a larger seed coat mass limits water absorption, preventing premature germination during harvest (30–32).

Grey hilums in several genotypes (AGS-25, BIRSA SOYA 1, KALITUR, KDS 726 and SL 96) may indicate an altered seed coat structure or composition, altering water permeability and HSC. Previous studies have demonstrated that hilum pigmentation and colour variation can represent seed coat biochemical and structural changes that affect water uptake and dormancy. In soybean, changes in cuticle integrity and lignin content around the hilum region have been linked to altered permeability and increased resistance to PHS, which is notable since black seed coats are associated with physical dormancy and hard seed coat (HSC) traits (33). The broad-sense heritability of seed coat morphological features ($h^2 = 0.368$ for PPSC) suggests significant additive genetic influences and predictable inheritance in segregating populations, making them useful as breeding program selection criteria. Biochemical defence and permeability are both influenced by genetics. The SS, TW and SCT analysed using multivariate couldn't be detected by this method and indicate that there is no detectable variation of genetic origin based on the physical measurement of the trait. Mechanistically, lack of detectable genetic variance may be caused by the strong sensitivity of plants to their micro-environment during maternal filling phases of seed-formation. Seed weight and volume are primarily determined during this time by moisture concentration in the soil and the amount of nutrients allocated for seed development and the position of the developing pod relative to the surrounding canopy of leaves to all contributing to a large amount of residual variability masking the existing narrow range of

seed genetic diversity for traits like ST. The variation associated with the mechanical extraction of a seed coat and subsequent digital measurement of SCT is at least 84 to 199 microns, creating significant variability around the narrow baseline difference in seed genetic diversity found in cultivated seed stocks, due to this dramatic decoupling of seed traits from genetic variance, whirling for field weathering (i.e., developing outer layer of seed) and seedling resistance (i.e., PHS) should focus on selecting seeds that contain high levels of genetic determinacy through highly heritable physiological (i.e., POD) and biochemical (i.e., WAC) properties, rather than gross seed traits such as physical dimensions. In particular, the GCV (18.726 % for SS) exceeds the environmental coefficient, indicating that morphological seed coat variation is genetically controlled and responsive to selection and these studies emphasise structural seed features such SCT, PPSC and SCL.

Biochemical markers of membrane integrity for pre-harvest sprouting

Seed leachate EC ($0.468\text{--}1.087\text{ dS cm}^{-1}$) negatively correlates with cell membrane integrity and seed vigour. The genotypes Birsa soya 1 and Pune 1, the most PHS-resistant genotypes, had the lowest EC values (0.468 and 0.470 dS cm^{-1}), indicating superior membrane stability and minimal solute leakage during imbibition (34, 35). The negative association between PPSC and EC ($r = -0.550$) shows that physically strong seed coverings with larger proportions constrain initial water uptake, slowing cell membrane reconfiguration and lowering electrolyte leakage into the seed soaking media. This mechanistic insight explains why genotypes with higher seed coat proportion have lower EC values and the physical barrier delay allows more orderly membrane reorganisation upon hydration, preserving cellular integrity during pre-harvest weathering. Lower EC values improve membrane integrity and seed vigour due to reduced solute leakage, which is essential for PHS resistance.

The biochemical variable with the highest heritability was POD. The resistant genotypes (Birsa soya 1, Pune 30 and Maus 2)

showed significantly higher POD activity. Peroxidase catalyses guaiacol oxidation and regulates reactive oxygen species (ROS) homeostasis during seed imbibition as a constitutive antioxidant. Percent proportion of seed coat and POD are positively correlated suggesting genotypes with superior seed coat architecture invest in biochemical defence through POD upregulation, may be due to coordinated expression of phenolic biosynthesis and antioxidant enzyme genes in seed coat tissues, both protective mechanisms via overlapping genetic pathways. Peroxidase's high heritability makes it ideal for indirect selection via molecular markers, eliminating the need for laborious enzymatic assays in regular screening and is related to withstanding water related stresses and protects from oxidative damage and is very advantageous trait for PHS resistance (36–38). The seeds TPC ranged from 32.05–44.93 %, reflecting its nutritional reserve and metabolic capacity, with moderate heritability ($h^2 = 0.336$) and demonstrated fewer significant trait correlations in the multivariate analysis, but resistant genotypes (PK 1029: 44.93 %; SL 979: 43.68 %) may contribute to sustained antioxidant enzyme synthesis, cellular homeostasis and protein accumulation during pre-harvest weathering stress and has been identified for photosynthetic efficiency and UV radiation tolerance, indicating its genetic resilience (39–41). The findings reveal these features genetic diversity and potential to improve seed quality and resilience (42). Though its involvement in PHS resistance complex and context-dependent, genetic and environmental factors drive such variability in protein content (43, 44). This study's biochemical traits illuminate soybean PHS resistance mechanism. Pre-harvest sprouting, an abiotic stress, can impair soybean production and quality. Genotypes with favourable biochemical profiles can contribute to breed PHS-resistant cultivars (31, 45, 46).

Genetic architecture and principal component analysis

Principal component analysis showed clusters of traits that synergistically confers PHS resistance explaining 62.5 % of overall phenotypic variation through PC1 (40.0 %) and PC2 (22.5 %), identified clusters of traits acting synergistically to confer PHS resistance. The significant correlation between SL, SB, SS and TW (loading on PC1) indicate these morphological traits co-segregate, facilitating simultaneous selection of larger-seeded, higher-vigour genotypes. Conversely, PC2 loading emphasised the biochemical and permeability traits (SCT, TPC, PPSC and POD) revealing a secondary axis of genetic variation independent of SS per se (47). This bipartite genetic structure requires breeding strategy to balance selection for larger seeds (PC1) and hardness (PC2) to avoid unintended outcomes. Genotypes (PUNE 30 and KALITUR) were high performing across both physical (SS and PPSC) and biochemical traits (low EC, high POD), suggesting that integrated resistance combining mechanical strength and biochemical defence is achievable within cultivated soybean germplasm, despite the traditional seed vigour and dormancy trade-off. Principal component analysis showed genotypic differentiation between HARSOYA, IVT 38, SL 96, PUNE 30, BRAGG and ANKUR, which were clearly distinguished from those with lower values (AGS 48, BNS 5, 107-4 and IVT 8) for SL, SB, SS, ST and TW. This difference is essential for selecting genotypes with desired seed traits for breeding goals (48).

Several genotypes clustered around trait vectors, indicating exceptional trait performance. The genotypes PUNE 30, KALITUR, PUNE 14 and BIRSOYA grouped around the PPSC vector, indicating increased seed coat proportions. Similarly, JS 93-05, IT 11 and BIRSOYA were associated with higher SCT, while ASRB 1, AVKS 1 and

IVT 4 showed increased WAC. The genotypes PK1029, SL 979 and SL 958 had higher TPC, while BRAGG, IVT 28 and IVT 9 had higher EC. The genotypes 104-31, 107-4 and AGS 48 increased POD. Percent proportion of seed coat, SCT, WAC, TPC, EC and POD genotypes were further distinguished by PC2 loading values. Genotypes (AVKS 4, AVKS 6, IVT 38, IVT 40, PS 1225, HARSOYA, MAUS 61-2, KALITUR, PUNE 30, NRC 21, MAUS 81, PUNE 14 and BIRSOYA 1) far from the vector origin had lower trait values. Previous studies support this distribution and its pattern helps breeders select genotypes with certain trait combinations for PHS resistance (47–49).

Correlation structure and indirect selection strategy

Strong correlations in the study give predictive frameworks for indirect selection, especially when direct PHS field evaluations are logistically hindered. The negative connection between PPSC and WAC ($r = -0.533$) makes seed coat proportion a valid indicator of water impermeability. Breeders might target genotypes with PPSC ≥ 10 % for additional PHS screening. Seed coat features regulate water intake and membrane integrity, which are crucial to PHS resistance. The positive correlation between PPSC and POD ($r = 0.511$) suggests that single-trait phenotyping (POD assay) in diverse germplasm populations could efficiently identify subsets enriched for HSC architecture, reducing screening volume for downstream field PHS evaluation. In soybean seed coat tissues, especially in sub-epidermal hourglass cells, POD enzymes concentrate in considerable numbers to protect against oxidative damage. This enzyme scavenges ROS produced during seed imbibition, which might cause premature germination. Unlike traditional breeding assumptions, hard seededness is not mechanically linked to large-seed morphology when SS and PHS-relevant variables (PPSC, WAC at $p > 0.05$) are uncorrelated. This decoupling allows breeding programs to select for both hardness (essential for PHS mitigation) and larger seeds (desired for agronomic uniformity and seedling vigour), removing a major constraint (48, 50).

Implications for soybean breeding under humid tropical climates

Pre-harvest sprouting tolerant phenotypes exist in accessible soybean germplasm, particularly among cultivated varieties adapted to sub-humid and humid Indian agro ecologies, as shown by the identification of Birsas soya 1, Kalitpur, Pune 14, Pune 30 and Maus 61-2 genotypes that resist field weathering. Resistance is linked to black or dark brown seed coat colour, raised SCP, limited water absorption, low electrolyte leakage and increased POD activity, forming a physiological "hard seed coat syndrome" that can guide parental line selection in crossover programs. Peroxidase activity has high heritability ($h^2 = 0.611$), indicating predictable selection response. The complex nature of PHS tolerance requires multi-trait breeding due to distinct genetic and metabolic components. Selecting for higher PPSC, reduced WAC, low EC and strong POD activity may improve field performance over single-trait optimisation.

Conclusion

Evaluation of 96 soybean genotypes showed that pre-harvest sprouting (PHS) resistance is governed by a synergistic "hard seed coat syndrome," combining physical barriers such as reduced water absorption with biochemical resilience through

higher peroxidase (POD) activity and lower electrolyte leakage. These traits exhibited moderate-to-high heritability and were independent of SS, making them reliable indicators for indirect selection without compromising yield. Genotypes such as Birsya Soya 1, Kalitur, Pune 14, Pune 30 and MAUS 61-2 were identified as valuable donor lines for breeding climate-resilient, PHS-tolerant soybean varieties. High-density genome-wide association studies (GWAS), transcriptome profiling and multi-year / multi-location field trials are necessary to identify quantitative trait loci (QTLs) related to seed coat impermeability, antioxidant defence mechanisms, physical dormancy and post-harvest seed vigour under different agro-ecological conditions. Marker-assisted backcrossing (MABC) with functional markers, multispectral imaging and automated seed coat porosity assessments will accelerate the introgression and evaluation of precocious germination (PHS) resistant alleles into superior cultivars while minimising the linkage drag. With climate variability becoming more severe, multi-trait pre-breeding strategies are vital for producing sustainable seed quality, as well as establishing stable seed supply chains.

Acknowledgements

The authors thank the Department of Seed Science and Technology and Agricultural Microbiology, College of Agriculture, University of Agricultural Sciences, Raichur; Main Agricultural Research Station, University of Agricultural Sciences, Raichur and All India Coordinated Research Project, Soybean, University of Agricultural Sciences, Bangalore. The authors also thank the University of Agricultural Sciences, Raichur for financial assistance to Dr. Thota Joseph Raju in the form of fellowship. The authors acknowledge the technical help from Mr. Thimmappa in field work.

Authors' contributions

TJR contributed to conceptualisation, data curation, formal analysis, investigation, methodology, resources, software, validation, visualisation, writing of the original draft and manuscript review and editing. SNV contributed to conceptualisation, formal analysis, funding acquisition, investigation, methodology, project administration, resources, supervision, validation, writing of the original draft and manuscript review and editing. SRD contributed to conceptualisation, funding acquisition, investigation, project administration, resources and supervision. STC contributed to manuscript writing, review and editing. PSK contributed to data curation, formal analysis, software and writing of the original draft. HMS, SP, P, GG and KS contributed to manuscript writing, review and editing. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interest to declare.

Ethical issues: None

References

- Archana P, Taware SP, Oak MD, Tamhankar SA, Rao VS. Improvement of oil quality in soybean (*Glycine max* (L.) Merrill) by mutation breeding. *J Am Oil Chem Soc.* 2007;84:1117–24. <https://doi.org/10.1007/s11746-007-1146-1>
- Ministry of Agriculture & Farmers Welfare. Final estimates of production of major agricultural crops for 2024–25. Government of India. <https://agricoop.gov.in/en/StatEstimates>
- Yang R, Harrison M, Yang YC, Wang S, Sergey M, Zhao H, et al. Pre-harvest sprouting in cereals: global incidence, impacts and mitigation strategies. *Field Crops Res.* 2025;333:110111. <https://doi.org/10.1016/j.fcr.2025.110111>
- Bhatia VS, Bhatnagar PS, Joshi OP. Screening of Indian soybean genotypes for seed longevity as affected by field weathering. *Soybean Genetics Newsletter.* 1996;23:102–4.
- Nautiya PC, Bandyopadhyay A, Zala PV. *In situ* sprouting and regulation of fresh seed dormancy in Spanish type groundnut (*Arachis hypogaea* L. ssp. *fastigiata* var. *vulgaris*). *Field Crops Res.* 2001;70:233–41. [https://doi.org/10.1016/S0378-4290\(01\)00143-5](https://doi.org/10.1016/S0378-4290(01)00143-5)
- Sohn SI, Pandian S, Kumar TS, Zoclanclounon YAB, Muthuramalingam P, Shilpha J, et al. Seed dormancy and pre-harvest sprouting in rice: an updated overview. *Int J Mol Sci.* 2021;22:11804. <https://doi.org/10.3390/ijms222111804>
- Kueneman EA, Dassou S. Screening methodology for resistance to field weathering of soybean seed. *Crop Sci.* 1982.
- Ahmad S, Khulbe RK, Roy D. Evaluation of mungbean (*Vigna radiata*) germplasm for pre-harvest sprouting tolerance. *Legume Res.* 2014;37:259–63. <https://doi.org/10.5958/j.0976-0571.37.3.039>
- Alvarez PJC, Krzyzanowski FC, Mandarino JMG, Franca NJB. Relationship between soybean seed coat lignin content and resistance to mechanical damage. *Seed Sci Technol.* 1997;25:209–14.
- Carbonell SAM, Krzyzanowski FC. The pendulum test for screening soybean genotypes for seeds resistant to mechanical damage. *Seed Sci Technol.* 1995;23:331–9.
- Petr S, Vanessa V, Matthew WB, Ales S, Richard DT. The role of the testa during development and in establishment of dormancy of the legume seed. *Front Plant Sci.* 2014;5:351. <https://doi.org/10.3389/fpls.2014.00351>
- Zhou S, Sekizaki H, Yang Z, Sawa S, Pan J. Phenolics in the seed coat of wild soybean (*Glycine soja*) and their significance for seed hardness and seed germination. *J Agric Food Chem.* 2010;58:10972–8. <https://doi.org/10.1021/jf102694k>
- Finch-Savage W, Leubner-Metzger G. Seed dormancy and the control of germination. *New Phytol.* 2006;171:501–23. <https://doi.org/10.1111/j.1469-8137.2006.01787.x>
- Gao X, Hu CH, Li HZ, Yao YJ, Meng M, Dong J, et al. Factors affecting pre-harvest sprouting resistance in wheat (*Triticum aestivum* L.): a review. *J Anim Plant Sci.* 2013;23:556–65.
- Sunayana R, Ramendra NS, Raj NSD. Variation in seed dormancy and α -amylase activity in Indian rice (*Oryza sativa*) accessions. *Indian J Agric Sci.* 2013;83:56–62.
- Singh A, Khulbe RK, Panwar RK. Evaluation of blackgram (*Vigna mungo*) germplasm for pre-harvesting sprouting tolerance. *J Food Legumes.* 2012;25:183–6.
- Dorian Q, Robin A. Seed dispersal and crop domestication: shattering, germination and seasonality in evolution under cultivation. *Annu Plant Rev.* 2009;38:238–95.
- Humphry ME, Lambrides CJ, Chapman SC, Aitken EAB, Imrie BC, Lawn RJ. Relationships between hard-seededness and seed weight in mungbean assessed by QTL analysis. *Plant Breed.* 2005;124:292–8. <https://doi.org/10.1111/j.1439-0523.2005.01084.x>
- Kumar G, Praveen M, Pallavi N, Swapna, Shahana F, Reddy GE, Rakesh G. Genetic variability and correlation studies for pre-harvest sprouting tolerance and associated traits in soybean (*Glycine max* L. Merrill). *Curr J Appl Sci Technol.* 2021;40(4):1–10. <https://doi.org/10.1007/s11746-007-1146-1>

- doi.org/10.9734/cjast/2021/v40i431290
20. Rani R, Raza G, Ashfaq H, Rizwan M, Shimelis H, Tung MH, Arif M. Analysis of genotype × environment interactions for agronomic traits of soybean (*Glycine max* (L.) Merr.) using association mapping. *Front Genet.* 2023;13:1090994. <https://doi.org/10.3389/fgene.2022.1090994>
 21. Fufa WG, Nepir G. Assessment of genetic variability, heritability and genetic advance in soybean (*Glycine max* (L.) Merrill) genotypes at Assosa, western Ethiopia. *Asian J Biol Sci.* 2025;18:83–91. <https://doi.org/10.3923/ajbs.2025.83.92>
 22. Raju JT, Vasudevan SN, Basavae Gowda SR, Doddagoudar TC, Suma B, Kisan Beladhadri RV. SSR markers linked to hard seediness and electrolyte leakage in soybeans. *Pharma Innov.* 2022;11:617–20.
 23. Changrong Y, Sripichitt P, Sunanta J, Vipha H, Arom S. Modifying controlled deterioration for evaluating field weathering resistance of soybean. *Kasetsart J Nat Sci.* 2007;41(2).
 24. Alexander HM, Cummings CL, Khan L, Snow A. Seed size variation and predation of seeds produced by wild and crop-wild sunflowers. *Ann Bot.* 2001;88:623–7. <https://doi.org/10.2307/2657061>
 25. Egli DB, TeKrony DM, Wiralaga RA. Effect of soybean seed vigor and size on seedling growth. *J Seed Tech.* 1990;14(1):1–12. <http://www.jstor.org/stable/23432656>
 26. Ragus LN. Variation in water absorbing capacity and other seed characters of soybean (*Glycine max* (L.) Merr.). *Philipp J Crop Sci.* 1986;11:195–201.
 27. Presley JT. Relation of protoplast permeability to cotton seed viability and predisposition to seedling disease. *Plant Dis Rep.* 1958;42:852. <https://www.cabidigitallibrary.org/doi/full/10.5555/19581102834>
 28. Castillo MDP, Stenstrom J, Ander P. Determination of manganese peroxidase activity with 3-methyl-2-benzothiazolinone and 3-(dimethylamino) benzoic acid. *Ann Chem Sci Res.* 1994;218:399–404. <https://doi.org/10.1006/abio.1994.1198>
 29. Nnenna EO, Kalu CM, Nnorom C. Estimation of protein content and amino acid compositions in selected plant samples using UV-Vis spectrophotometric method. *Am J Food Sci Health.* 2016;3:41–6.
 30. MA F. Cracks in the palisade cuticle of soybean seed coats correlate with their permeability to water. *Ann Bot.* 2004;94:213–28. <https://doi.org/10.1093/aob/mch133>
 31. Sun Y, Gong Y. Research advances on the hard seediness trait of soybean and the underlying regulatory mechanisms. *Front Plant Sci.* 2024;15:1419962. <https://doi.org/10.3389/fpls.2024.1419962>
 32. Zhang L, Jia R, Liu L, Shen W, Fang Z, Zhou B, Liu B. Seed coat colour and structure are related to the seed dormancy and overwintering ability of crop-to-wild hybrid soybean. *Ann Bot Plants.* 2023;15:eplad081. <https://doi.org/10.1093/aobpla/plad081>
 33. Bewley JD, Bradford KJ, Hilhorst HWM, Nonogaki H. *Seeds: Physiology of Development, Germination and Dormancy.* 3rd ed. Springer; 2013. <https://doi.org/10.1007/978-1-4614-4693-4>
 34. Salinas R, Roque MC, Celina B, Vilma B, Ana MY. Electrical conductivity of soybean seed cultivars and adjusted models of leakage curves along the time. *Rev Caatinga.* 2010;23:1–7.
 35. Cavalcante JA, Gadotti GI, Pinheiro RM, Silva RNO, de Oliveira FK, de Moraes DM. Vigor and anaerobic metabolism of soybean seeds evaluated by ethanol test. *J Seed Sci.* 2023;45:e202345007. <https://doi.org/10.1590/2317-1545v45e263825>
 36. Gillikin JW, Graham JS. Purification and developmental analysis of the major anionic peroxidase from the seed coat of *Glycine max*. *Plant Physiol.* 1991;96:214–20. <https://doi.org/10.1104/pp.96.1.214>
 37. Capeleti I, Bonini EA, Ferrarese MDLL. Lignin content and peroxidase activity in soybean seed coat susceptible and resistant to mechanical damage. *Acta Physiol Plant.* 2005;27:103–8. <https://doi.org/10.1007/s11738-005-0042-2>
 38. Ranathunge K, Shao S, Qutob D. Properties of the soybean seed coat cuticle change during development. *Planta.* 2010;231:1171–88. <https://doi.org/10.1007/s00425-010-1118-9>
 39. Guo B, Sun L, Jiang S, Ren H, Sun R, Wei Z, et al. Soybean genetic resources contributing to sustainable protein production. *Theor Appl Genet.* 2022;135:4095–121. <https://doi.org/10.1007/s00122-022-04222-9>
 40. Kholmurodova G, Gulchekhra T, Murod R, Rano Y. Analysis of protein and oil content in seeds of soybean collection varieties. *E3S Web Conf.* 2022;377:03016. <https://doi.org/10.1051/e3sconf/202337703016>
 41. Baroniya SS, Jumrani K, Baroniya M, Guruprasad KN, Landi M, Kataria S. Intraspecific variation in photosynthetic efficiency in soybean (*Glycine max* L.) varieties towards solar ultraviolet radiations. *Photosynthetica.* 2022;61:203–14. <https://doi.org/10.32615/ps.2022.048>
 42. Mishra N, Jiang C, Chen L, Paul A, Chatterjee A, Shen G. Achieving abiotic stress tolerance in plants through antioxidative defense mechanisms. *Front Plant Sci.* 2023;14:1110622. <https://doi.org/10.3389/fpls.2023.1110622>
 43. Wilson RF. Seed composition. In: Boerma HR, Specht JE, editors. *Soybeans: Improvement, Production and Uses.* 3rd ed. ASA, CSSA and SSSA; 2004. p. 621–68. <https://doi.org/10.2134/agronmonogr16.3ed.c13>
 44. Panthee DR, Pantalone VR, West DR, Saxton AM, Sams CE. Quantitative trait loci for seed protein and oil concentration and seed size in soybean. *Crop Sci.* 2005;45:2015–22. <https://doi.org/10.2135/cropsci2004.0720>
 45. Rao PS, Madhulety TY, Ankaiah R, Volet SR. Morpho-physiological trait variation of pre-harvest sprouting tolerance to simulated rain in mungbean (*Vigna radiata*). *Indian J Agric Sci.* 2023;93:269–73. <https://doi.org/10.56093/ijas.v93i3.128735>
 46. Gupta S, Aski M, Mishra GP, Yadav PS, Tripathi K, Lal SK, et al. Genetic variation for tolerance to pre-harvest sprouting in mungbean (*Vigna radiata*) genotypes. *PeerJ.* 2024;12:e17609. <https://doi.org/10.7717/peerj.17609>
 47. Pinheiro DT, dos Santos Dias DCF, Silva JM, Gama GFV, León MJZ, da Silva LJ. Assessment of the tolerance of soybean seeds to weathering deterioration in the pre-harvest phase by multivariate analysis. *J Seed Sci.* 2022;44:e202244035. <https://doi.org/10.1590/2317-1545v44e263001>
 48. Abati J, Zucareli C, Brzezinski C, Lopes I, Krzyzanowski F, Cardoso M, Henning F. Water absorption and storage tolerance of soybean seeds with contrasting seed coat characteristics. *Acta Sci Agron.* 2022;44:e53096. <https://doi.org/10.4025/actasciagron.v44i1.53096>
 49. Yunuskhonov MSh, Jaynakov ZL, Abdurazakova. Soybean seed peroxidase. *J Propulsion Technol.* 2023;44:4. <https://doi.org/10.52783/tjpt.v44.i4.969>
 50. Soppe WJJ, Bentsink L. Seed dormancy back on track; its definition and regulation by DOG1. *New Phytol.* 2020;228:816–9. <https://doi.org/10.1111/nph.16592>

Additional information

Peer review: Publisher thanks Sectional Editor and the other anonymous reviewers for their contribution to the peer review of this work.

Reprints & permissions information is available at https://horizonpublishing.com/journals/index.php/PST/open_access_policy

Publisher's Note: Horizon e-Publishing Group remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Indexing: Plant Science Today, published by Horizon e-Publishing Group, is covered by Scopus, Web of Science, BIOSIS Previews, Clarivate Analytics, NAAS, UGC Care, etc. See https://horizonpublishing.com/journals/index.php/PST/indexing_abstracting

Copyright: © The Author(s). This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution and reproduction in any medium, provided the

original author and source are credited (<https://creativecommons.org/licenses/by/4.0/>)

Publisher information: Plant Science Today is published by HORIZON e-Publishing Group with support from Empirion Publishers Private Limited, Thiruvananthapuram, India.