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Identification of high-yielding and multi-disease resistant soybean lines for cultivation in the Northwestern Himalayan Region

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Abstract

Improving soybean yield, seed quality, and disease resistance is vital to meet the growing demand for dietary oil and protein while ensuring food security. The narrow genetic base of cultivated soybean necessitates hybridization to broaden genetic variability for crop improvement. However, limited information is available on the epistatic interactions for key soybean traits. In the present investigation, fifteen soybean lines were crossed with three testers (JS 335, Himso 1685, and their F_1 hybrid) using a triple test cross design to investigate the genetic control of yield, seed quality, and disease resistance traits, to identify superior parents and hybrids. Significant genetic variation was detected, with additive, dominance, and epistatic gene effects influencing most traits. Cross combination AS 40 × Himso 1685 and PS 1572 × JS 335 exhibited superior *per se* performance and favourable specific combining ability for yield and its component traits while Palam Early Soya 1, Hara Soya, EC 241778, and Him Soya emerged as strong general combiners. Hybrids Hara Soya × JS 335 and Palam Early Soya 1 × JS 335 showed substantial heterosis for earliness and yield. Himso 1685 and the hybrid JS 335 × Himso 1685 exhibited resistance to multiple diseases, including pod blight, frogeye leaf spot, brown spot, bacterial pustule, and powdery mildew. JS 335, EC 241778, Himso 1685, and Hara Soya × Himso 1685 recorded high oil and protein contents. Therefore, these parents and hybrids represent valuable genetic resources for developing high-yielding, nutritionally improved, and multi-disease-resistant soybean cultivars.

Introduction

Soybean is an exceptional legume, rich in high-quality protein (37–42%), ash (6%), carbohydrates (29%) and oil (17–24%), of which approximately 85% comprises polyunsaturated fatty acids along with diverse essential micro- and macronutrients (Painkra et al. 2017). India ranks fifth in soybean production but still lags in productivity (11.58 q/ha,(ICAR-Indian Institute of Soybean Research 2024) when compared with the global average of 34.09 q/ha (SoyStat 2024). Low soybean productivity mainly results from erratic monsoons, abiotic and biotic stresses, poor technology adoption, lack of quality high-yielding varieties, poor soil and nutrient management. Since its introduction, 130 soybean varieties have been developed in India for diverse agro-climatic conditions (Sivabharathi et al. 2020). However, only seven account for more than 80% of the total breeder seed production (Kumar and Naitha 2020). This reliance on a few varieties coupled with the self-pollinated nature has narrowed the genetic base, increased susceptibility to diseases and pests. Thus, high yielding, multi disease resistant soybean varieties are urgently needed to reduce the yield gap. In any crop breeding program, identifying elite parents for hybridization and selecting superior crosses is crucial to broaden the genetic variability for the selection of high-performing genotypes (Darwish 2007). Combining ability studies assist in these efforts (El Sayad et al. 2005). Although combining ability analysis is widely used for parent selection, it cannot fully explain the inheritance of complex quantitative traits because epistatic interactions can markedly affect their expression. Genetic analysis provides a method for assessing combining ability and uncovers the nature and scope of gene actions involved, aiding in the comprehension of the relative impacts of fixable and non-fixable gene effects in the inheritance of different traits (Hill et al. 1997). The most efficient and significant traits across different species are quantitative. Advances in quantitative genetics have provided a deeper insight into population structure by examining the genetic factors contributing to variation arising from allelic effects, non-allelic interactions, and epistatic interactions. According to (Bernardo 2008), the presence of epistasis can distort the estimates of additive and dominant genetic components, leading to inaccurate assessments of key genetic parameters like heritability and expected response to selection. Additionally, epistatic effects play a role in the genetic foundation of heterosis and inbreeding (Primomo et al. 2005). For autogamous species like soybean,

additive × additive epistatic effects are most crucial since their outcomes are predictable. Therefore, precise estimation of these components is essential for developing an effective breeding strategy. The triple test cross (TTC) method introduced by(Kearsey and Jinks 1968) and its subsequent adaptation by(Jinks et al. 1969) represent the most significant experimental design for investigating the genetic structure of populations, particularly well-suited for autogamous species (Jinks and Perkins 1970).(Pooni and Jinks 1976) showed that the TTC strategy was more effective than alternative approaches for identifying epistasis. Additionally, the method is not influenced by gene correlations, mating systems, or allelic frequencies. In recent years, several researchers(Segun and Ekundayo 2018; Jabeen and Chadha 2021; Anuradha et al. 2023; Soliman et al. 2024) have highlighted the significance of epistasis in various species for economically important traits. Modified TTC has successfully been applied to dissect gene action in soybean, but previous work done by (Rahangdale and Raut 2002; Barona et al. 2009, 2012; Rialch and Sharma 2020). have targeted either yield traits or a small set of agronomic features. An integrated analysis which simultaneously evaluates yield, seed quality, and multiple disease resistance remains largely unexplored. Therefore, this study aimed to estimate the combining ability, detect epistasis and heterosis to select economically desirable i.e. nutritionally improved, high-yielding, and multiple disease-resistant cross combinations thereby facilitating the generation of genetic variability and contributing to narrowing the yield gap.

Materials and Methods

2.1 Specific site, resources, and research layout

The study was conducted at the Experimental Farm of the Department of Genetics and Plant Breeding, CSK Himachal Pradesh Krishi Vishvavidyalaya, Palampur (32°09' N latitude, 76°55' E longitude;1290.8 m above mean sea level). The meteorological data recorded during *kharif* season 2021, 2022, and 2023 are presented in Supplementary Table 1. Triple test cross (TTC) progenies were developed following the modified TTC design proposed by (Jinks et al. 1969). The parental material used for generating the TTC progenies, along with their distinguishing characteristics, is presented in Table 1. During *kharif* 2021, F_1 seeds were produced by crossing JS 335 and Himso 1685. In the subsequent *kharif* 2022, three testers *viz*., JS 335, Himso 1685 and JS 335 × Himso 1685 (F_1) were used as male parents to cross with 15 soybean lines (female) to generate 45 TTC families. Further during *kharif* 2023, a total of 63 entries (15 lines + 3 testers + 45 TTC families) were evaluated for various quantitative traits in a randomized block design with three replications. Each genotype was grown in rows 1.5 m long, maintaining a spacing of 45 cm between rows and 10 cm between plants. The crop was grown following the recommended agronomic practices throughout the growing season.

Table 1
Soybean genotypes and their key features used in the hybridization program

Sr. No.	Genotypes	Parentage/Source	Distinctive feature
L_1	Palam Early Soya 1	JS 79–295 × Punjab No.-1	Early maturity and high yield
L_2	Hara Soya	Himso 1520 × Bragg	First culinary-purpose, Green seeded
L_3	Him Soya	JS-79-295 × Lee	Green round bold seeded, high protein content
L_4	KDS 1149	KDS-228 × Type-49	High yielding, high protein content
L_5	NRC 195	(NRC 79 × DS 97 − 84) × MACS 450	-
L_6	AS 40	G. Soy 1 × AMRS 472	Early maturity
L_7	Cat 411 A	Germplasm from IISR, Indore	Moderate yield, high oil content and maturity
L_8	AMS MB 5–18	Mutant of Bragg	Suitable for mechanical harvesting
L_9	Himso 1689	NRC 2008 × G 1–12	High yield with good adaptability
L_{10}	PS 1572	Promising line, GBPUAT, Pantnagar	Resistant to yellow mosaic virus and bacterial pustule
L_{11}	KDS 992	JS 93 − 05 × EC 241780	High yielding, high oil content
L_{12}	EC 241778	Exotic collection through IISR, Indore	Resistance to soybean rust, high oil content
L_{13}	DS 3163	DS-2205 × DS 12–13	High yielding, high protein content
L_{14}	RSC 10–52	NRC 37 × JS335	High yielding
L_{15}	PK 25	(KS 253 × KS 83) × NCS 40	-
T_1	JS 335	JS 78 − 77 × JS 71 − 05	Early maturity, high oil content and high yielding
T_2	Himso 1685	Himso 330 × Hardee	Green bold seeded, high yielding, multi-disease resistant
T_3	JS 335 × Himso 1685	JS 335 × Himso 1685	-

L=Line, T=Tester, IISR-Indian Institute of Soybean Research, Indore, Madhya Pradesh, India

Assessment of quantitative traits

Data on plant height (cm), number of nodes on the main stem, internode length (cm), branches per plant, pods per plant, seeds per pod, pod length (cm), 100-seed weight (g), biological yield per plant (g), seed yield per plant

(g), and harvest index (%) were recorded from five randomly selected competitive plants in each replication for every entry. Days to 50% flowering and days to 75% maturity were recorded on a plot basis.

Disease evaluation

The genotypes were evaluated for their response to prevalent diseases, including pod blight (*Colletotrichum truncatum*), frogeye leaf spot (*Cercospora sojina*), brown spot (*Septoria glycines*), bacterial pustule (*Xanthomonas axonopodis* pv. *glycines*) and powdery mildew (*Microsphaera diffusa*) under natural field conditions. Disease severity was assessed using the standard 0–9 disease rating scale recommended by (Stonehouse 1994; AICRP 2022).

Biochemical estimation

The oven-dried seeds of sixty-three soybean genotypes were finely ground and analyzed for oil content (%), crude protein content (%), phosphorus (%), potassium (%) and micronutrients *viz*., iron (ppm), manganese (ppm), zinc (ppm) and copper (ppm). Oil content was determined by Soxhlet method, crude protein content by Micro-Kjeldahl method (AOAC 2005), phosphorus by the colorimeter method given by (Jackson 1967), potassium by flame photometry (Black 1965) and Fe, Zn, Mn and Cu by atomic absorption spectroscopy following di-acid digestion (Jackson 1967).

Statistical analysis

The experimental data were subjected to analysis of variance (ANOVA) following the procedure of (Panse and Sukhatme 1984) and further analysed by RStudio software. Epistatic interactions, additive and dominance genetic components were analysed using the TNAUSTAT statistical package (Manivannan 2014). Combining ability analysis was carried out using R software. Heterobeltiosis and standard heterosis were estimated using GRAPES software version 1.1.0 (Gopinath et al. 2020), and their significance was tested using the *t*-test. Pearson's correlation analysis was performed to determine the relationships among combining ability effects, heterosis, and F_1 performance. Clustered heat maps were generated using RStudio software. For disease assessment per cent disease index (PDI) was calculated by following (McKinney 1923). All the biochemical parameters were analysed by 'Z' test.

Results and Discussion

Triple test cross analysis

The triple test cross analysis (Supplementary Table 2) showed significant variation among parents and progenies for all the studied traits, indicating the presence of substantial genetic variability. Significant total epistasis was observed for all traits except days to 75% maturity, demonstrating the widespread involvement of non-allelic gene interactions in the inheritance of agro-morphological traits (Table 2). Significant additive × additive (i) effects were observed only for 75% maturity and pods per plant suggesting that these interactions are fixable through selection and can be effectively exploited in breeding programmes. On the contrary, significant (*j* + *l*)-type epistasis was detected for all traits except days to 75% maturity, indicating the predominance of additive × dominance and dominance × dominance interactions for most traits. This suggests that these traits are

expressed through interactions among multiple loci rather than individual gene effects alone, so selecting directly in early generations is less effective. Positive and significant correlations (r) for days to 50% flowering suggested the predominance of increasing alleles, whereas negative correlations for most of the remaining traits indicated a greater contribution of decreasing (recessive) alleles. Overall, the genetic parameter estimates demonstrated that additive, dominance, and epistatic gene actions jointly governed the inheritance of yield and related traits. The predominance of both additive and non-additive gene effects suggests that biparental mating or intermating in early segregating generations could facilitate the accumulation of favourable alleles while maintaining sufficient genetic variability for selection. Although previous research based on diallel (De Oliveira et al. 2014; Tesfaye et al. 2020) partial diallel (Bagateli et al. 2020), and line × tester (Nagarajan et al. 2017) primarily emphasized additive and dominance effects, the present study further demonstrates the usefulness of the triple test cross design in detecting epistatic interactions that contribute to the inheritance of complex quantitative traits.

Combining ability analysis

Combining ability i.e. general combining ability and specific combining ability is vital for selection in breeding programs (Sprague and Tatum 1942). Analysis of variance showed significant differences among crosses for all traits, except seeds per pod, which showed only marginal significance (Table 3). Line effects were significant for thirteen traits, whereas tester effects were significant for most traits except days to 50% flowering, plant height, branches per plant, pod length, and 100-seed weight. Line × tester interactions were significant for eleven traits, highlighting the predominance of non-additive gene action for these traits. GCA estimates identified Palam Early Soya 1, Hara Soya, EC 241778 and Him Soya as superior general combiners for several yield traits, including earliness and maturity (Supplementary Table 3). Their favourable GCA effects indicate a high frequency of desirable additive alleles, making them valuable parents for soybean improvement programmes. List of superior parents and hybrids for various traits in soybean is given in Table 4.

Cross performance depends on parental *per se* performance, GCA, SCA, and heterosis. None of single combination exhibited superior SCA effects for all traits (Supplementary Table 4). Among the crosses, Palam Early Soya 1 × JS 335 showed significant negative SCA effects for earliness despite involving a good × average GCA combination, suggesting the possibility of obtaining desirable transgressive segregants. Five crosses, namely AS 40 × Himso 1685 (average × good), PS 1572 × JS 335 (good × good), AMS MB 5-18 × JS 335 (poor × good), EC 241778 × Himso 1685 (good × good), and Him Soya × Himso 1685 (good × good), recorded highly significant positive SCA effects for seed yield per plant, indicating their potential for developing superior recombinants.

Crosses with good × good GCA are expected to possess favourable additive effects along with additive × additive (i-type) epistasis, facilitating the fixation of superior alleles in advanced generations. Crosses involving poor × good combiners may benefit from favourable additive effects contributed by the superior parent together with complementary epistatic interactions, thereby offering opportunities for selecting desirable transgressive segregants. In contrast, favourable SCA effects observed in crosses involving poor general combiners generally reflect the predominance of dominance and epistatic interactions rather than additive gene action.

Overall, the predominance of dominance × dominance epistatic interactions for yield traits suggests that breeding approaches such as biparental mating or reciprocal recurrent selection, followed by pedigree selection,

would be effective for exploiting and retaining favourable non-additive gene combinations. Earlier, (Bhateria et al. 2006; Bisht and Paul 2020; Thakur and Paul 2022)) have also reported a similar pattern.

Table 2 Analysis of variance for epistasis detection in soybean traits

		Mean sum of squares					
Source of variation		**Total epistasis**	**i-type interaction**	**(j+l) type interaction**	**Epistasis × replication**	**i-type × replication**	**(j+l) type × replication**
Traits	**df**	**15**	**1**	**14**	**30**	**2**	**28**
Days to 50% flowering		53.49*	41.09	54.37*	17.42	10.27	17.93
Days to 75% maturity		89.56	352.80*	70.75	59.24	88.20	57.18
Plant height (cm)		523.03*	13.33	38.41*	36.07	3.33	38.41
Nodes on main stem		12.49*	0.81	13.32*	4.70	0.20	5.01
Internode length (cm)		4.65*	0.01	4.50*	0.52	0.002	0.55
Branches per plant		6.40*	0.09	6.85*	1.62	0.02	1.74
Pods per plant		383.28*	803.15*	353.29*	66.53	200.79	56.93
Seeds per pod		0.87*	0.05	0.92*	0.35	0.01	0.37
Pod length (cm)		0.84*	0.01	0.90*	0.23	0.02	0.25
100-seed weight (g)		49.17*	2.95	52.47*	7.12	0.73	7.58
Biological yield per plant (g)		275.01*	0.30	294.73*	33.61	0.08	36.01
Seed yield per plant (g)		69.65*	12.32	73.74*	5.63	3.08	5.81
Harvest index (%)		526.95*	36.52	561.98*	41.08	9.13	43.36

*Significance at P ≤ 0.05

Table 3 Variance analysis of combining ability and contributions of lines, testers and their interaction in soybean

Source of variation	Replication	Crosses	Lines (c)	Testers (c)	Lines × testers	Error	Proportional contribution (%)		
df	2	29	14	1	14	58	Lines	Testers	Lines × testers
Traits									
Days to 50% flowering	29.47	24.22*	35.09*	16.90	13.88*	5.44	69.94	2.41	27.65
Days to 75% maturity	72.41	37.19*	45.53*	205.51*	16.82	16.73	59.10	19.06	21.84
Plant height (cm)	95.33	102.51*	152.25*	14.66	59.03*	10.09	71.71	0.49	27.80
Nodes on main stem	25.83	4.79*	4.54*	8.95*	4.73*	1.19	45.79	6.45	47.76
Internode length (cm)	1.14	0.96*	1.50*	0.87*	0.44*	0.17	74.82	3.10	22.08
Branches per plant	2.37	2.29*	1.88*	0.54	2.82*	0.30	39.70	0.81	59.49
Pods per plant	114.34	218.73*	217.21*	139.55*	225.91*	17.39	47.94	2.20	49.86
Seeds per pod	3.00	0.14†	0.16†	0.12†	0.12†	0.09	54.46	2.96	42.58
Pod length (cm)	0.13	0.22*	0.36*	0.26	0.07	0.08	80.54	4.10	15.36
100-seed weight (g)	3.12	12.67*	20.61*	0.003	5.64*	1.28	78.51	0.001	21.49
Biological yield per plant (g)	24.53	83.75*	99.15*	198.56*	60.16*	9.26	57.15	8.17	34.68
Seed yield per plant (g)	1.28	14.05*	18.88*	8.21*	9.64*	0.93	64.85	2.02	33.13
Harvest index (%)	0.81	79.87*	102.07*	150.85*	52.59*	8.34	61.70	6.51	31.79

† Significance at P ≤ 0.10, *Significance at P ≤ 0.05, df= degree of freedom

Table 4 Superior parents and hybrids for various traits in soybean

Traits	Superior parent (s)	Superior cross combination (s)
Days to 50% flowering	Palam Early Soya 1, Hara Soya	Palam Early Soya 1 × JS 335
Days to 75% maturity	Palam Early Soya 1, JS 335	-
Plant height (cm)	Palam Early Soya 1, Hara Soya,	Palam Early Soya 1 × JS 335
		RSC 10-52 × JS 335
Nodes on main stem	AS 40, EC 241778	Palam Early Soya 1 × JS 335
Internode length (cm)	Hara Soya, Him Soya	Hara Soya × Himso 1685
Branches per plant	Hara Soya, DS 3163	Hara Soya × Himso 1685
		Cat 411A × Himso 1685
		Himso 1689 × JS 335
Pods per plant	NRC 195, JS 335	Palam Early Soya 1 × JS 335
		DS 3163 × JS 335
Seeds per pod	Himso 1689	Himso 1689 × JS 335
Pod length (cm)	Hara Soya, EC 241778	-
100-seed weight (g)	Palam Early Soya 1, Hara Soya	Him Soya × JS 335
		Cat 411A × JS 335
Biological yield per plant (g)	JS 335	DS 3163 × JS 335
		KDS 1149 × Himso 1685
Seed yield per plant (g)	Himso 1685	AS 40 × Himso 1685
		PS 1572 × JS 335
Harvest index (%)	Himso 1685	AS 40 × Himso 1685
		AMS MB 5-18 × JS 335

Heterosis

Heterobeltiosis (heterosis over the better parent) and standard heterosis (heterosis over the standard check) of the 45 soybean cross combinations are presented in Figure 1, while the range and frequency of significant desirable heterotic effects for each trait are summarized in Table 5. Positive heterosis was considered desirable for all traits except days to 50% flowering and days to 75% maturity, where negative heterotic effects indicate earliness.

Among all the hybrids, Hara Soya × JS 335, Palam Early Soya 1 × JS 335, and Himso 1689 × JS 335 consistently exhibited significant desirable heterosis for most yield-contributing traits, including plant height, nodes on the main stem, branches per plant, pods per plant, seeds per pod, 100-seed weight, and biological yield per plant,

indicating their potential as promising hybrids for yield improvement. For earliness, cross combinations *viz*., Hara Soya × JS 335, Palam Early Soya 1 × JS 335, Hara Soya × Himso 1685, and PS 1572 × JS 335 recorded significant negative heterobeltiosis and standard heterosis for flowering and maturity, indicating their suitability for developing short-duration soybean cultivars adapted to diverse production environments.

The predominance of desirable heterosis together with their significant SCA effects observed indicates that dominance and epistatic interactions played an important role in hybrid vigour and thus the involvement of favourable non-additive gene effects governing yield and its related traits. Although soybean is predominantly self-pollinated, these superior hybrids provide an excellent source for recovering transgressive segregants in later generations. Similar observations have been reported in soybean by (Ren et al. 2024; Meena et al. 2024)

Table 5 Cross combinations for each trait exhibiting significant desirable heterosis

Traits	Heterosis	Range	Mean	Number of crosses	Positive Heterosis
Days to 50% flowering	HBP	-14.29 to 3.39	-1.59	18	3
	HSC	-20.92 to -3.57	-4.59	28	0
Days to 75% maturity	HBP	-5.90 to 3.40	-0.85	1	10
	HSC	-7.02 to 3.09	-1.47	4	8
Plant height (cm)	HBP	-36.18 to 23.48	-0.03	5	7
	HSC	-38.90 to -3.92	-21.41	None	0
Nodes on main stem	HBP	-25.33 to 26.60	0.21	1	14
	HSC	-25.33 to 18.72	0.37	None	13
Internode length (cm)	HBP	-48.97 to 8.14	-6.39	None	2
	HSC	-41.88 to -0.28	-5.96	None	0
Branches per plant	HBP	-31.30 to 34.47	-4.84	1	3
	HSC	-29.17 to 34.47	-8.00	1	2
Pods per plant	HBP	-35.73 to 38.19	-1.63	2	6
	HSC	-14.69 to 93.25	5.32	12	25
Seeds per pod	HBP	-27.60 to 13.64	-7.36	None	1
	HSC	-17.29 to18.15	-8.76	1	2
Pod length (cm)	HBP	-23.67 to 6.18	-4.20	None	2
	HSC	-23.67 to -0.63	-6.28	None	0
100-seed weight (g)	HBP	-40.79 to 34.94	-3.52	3	3
	HSC	-45.75 to 0.35	-2.34	None	1
Biological yield per plant (g)	HBP	-45.32 to 16.98	2.31	1	7
	HSC	-45.32 to -7.97	-7.97	None	0

Seed yield per plant (g)	HBP	-46.64 to 5.92	-0.44	None	4
	HSC	-46.64 to 5.33	-1.47	None	4
Harvest index (%)	HBP	-39.08 to 49.74	0.72	4	10
	HSC	-21.60 to 49.74	-1.15	8	17

Abbreviations: HBP: heterosis over better parent and HSH: heterosis over standard check.

Quality parameters

Eight biochemical parameters, namely oil, crude protein, potassium (K), phosphorus (P), and the micronutrients iron (Fe), zinc (Zn), manganese (Mn), and copper (Cu), were evaluated in 63 soybean genotypes. Among the parental lines, EC 241778, JS 335, Himso 1685 and KDS 992 recorded superior oil content, whereas DS 3163, KDS 1149, Himso 1689 and PK 25 exhibited high crude protein content. Among the hybrids, Him Soya × Himso 1685, PS 1572 × Himso 1685, AS 40 × JS 335 and KDS 992 × JS 335 were superior for oil, while Hara Soya × Himso 1685, EC 241778 × JS 335, KDS 1149 × JS 335 and EC 241778 × (JS 335 × Himso 1685) exhibited high crude protein content. The oil and protein contents were higher than those reported by (Filho et al. 2001), (Rodrigues et al. 2014), (Flafsman et al. 2019) and (Šarcevic et al. 2022), possibly due to genotypic and environmental differences. Seed quality plays a crucial role in seedling establishment, nitrogen fixation, and crop productivity, while inadequate seed nutrient reserves can hinder early growth and reduce crop yield (Carvalho et al. 2017; Vargas et al. 2018).

Mean phosphorus and potassium contents were 0.47 ± 0.01% and 2.15 ± 0.15%, respectively, with maximum values recorded in Palam Early Soya 1 × JS 335 (0.700%) and Cat 411A (6.45%). Mean Fe, Mn, Zn, and Cu contents were 83.76 ± 1.70, 56.12 ± 1.53, 48.35 ± 1.51, and 12.77 ± 0.28 ppm, with the highest concentrations observed in Cat 411A (Fe), RSC 10-52 (Mn), Himso 1689 × Himso 1685 (Zn), and DS 3163 × JS 335 (Cu).

The substantial variation observed for oil, protein, and mineral nutrients among the parental lines and hybrids reflects the diverse genetic constitution of the evaluated genotypes and demonstrates considerable scope for improving seed nutritional quality through breeding. Oil and protein contents are complex quantitative traits influenced by the efficiency of assimilate partitioning and seed storage metabolism. Similarly, the observed differences in macro- and micronutrient concentrations suggests the presence of exploitable genetic variability for nutrient uptake, translocation, and accumulation in seeds.

In conclusion, among the evaluated genotypes, Cat 411A emerged as the best multi-trait parent, followed by KDS 992, DS 3163, and Himso 1685, while Himso 1689 × Himso 1685 was the most promising hybrid for multiple nutritional traits. The superior parents and hybrid combinations for individual biochemical traits are presented in Table 6. Genotypes with higher mineral content can be used as donor parents in biofortification programs to enhance soybean nutritional quality while retaining desirable traits.

Table 6 Superior parental lines and hybrid combinations identified for biochemical traits in soybean

Biochemical parameters	Superior parental donors	Superior hybrid combinations
Oil (%)	EC 241778, JS 335, Himso 1685, KDS 992	Him Soya × Himso 1685, PS 1572 × Himso 1685, AS 40 × JS 335, KDS 992 × JS 335
Crude protein (%)	DS 3163, KDS 1149, Himso 1689, PK 25	Hara Soya × Himso 1685, EC 241778 × JS 335, KDS 1149 × JS 335, EC 241778 × (JS 335 × Himso 1685)
Potassium (%)	Cat 411A, AMS MB 5-18, AS 40, NRC 195	Palam Early Soya 1 × JS 335, Palam Early Soya 1 × (JS 335 × Himso 1685), Palam Early Soya 1 × Himso 1685, Himso 1689 × Himso 1685
Phosphorus (%)	KDS 992, Cat 411A, JS 335, DS 3163	Palam Early Soya 1 × JS 335, Cat 411A × Himso 1685, EC 241778 × Himso 1685, Hara Soya × JS 335
Fe (ppm)	Cat 411A, NRC 195, AS 40, RSC 10-52	AMS MB 5-18 × Himso 1685, DS 3163 × Himso 1685, AMS MB 5-18 × (JS 335 × Himso 1685), PS 1572 × Himso 1685
Mn (ppm)	RSC 10-52, Cat 411A, Hara Soya, AS 40	AS 40 × JS 335, AS 40 × Himso 1685, PS 1572 × JS 335, Himso 1689 × Himso 1685
Zn (ppm)	RSC 10-52	Himso 1689 × Himso 1685, AMS MB 5-18 × Himso 1685, AS 40 × JS 335, Himso 1689 × (JS 335 × Himso 1685)
Cu (ppm)	Hara Soya, RSC 10-52, Him Soya, EC 241778	DS 3163 × JS 335, DS 3163 × (JS 335 × Himso 1685), DS 3163 × Himso 1685, AMS MB 5-18 × JS 335

Disease assessments

Soybean production is severely constrained by fungal and bacterial diseases making the identification of resistant genotypes a key objective in soybean improvement. The disease response of 63 soybean genotypes was evaluated for pod blight, frogeye leaf spot, brown spot, powdery mildew and bacterial pustule under natural field conditions, and the mean percent disease index is presented in Supplementary Table 5. Considerable variation in disease reaction was observed among the evaluated genotypes. Among the parental lines, Himso 1685 exhibited the resistance to frogeye leaf spot, pod blight, bacterial pustule, brown spot, and powdery mildew. Among the hybrids, JS 335 × Himso 1685 showed resistance to pod blight, frogeye leaf spot, and brown spot, indicating successful transmission of disease resistance from the donor parent. The multi-disease resistant parents and hybrids identified in this study are summarized in Table 7.

For pod blight, Nataraj et al. (2020) identified five highly resistant accessions (AKSS 67, EC 538828, EC 34372, EC 457254, and Karune) from 225 soybean germplasm lines, whereas (Bhatt et al. 2022)) reported that none of the 40 released soybean varieties evaluated were resistant to this disease. Similarly, resistance to frogeye leaf spot has been reported in 16 genotypes by (Khati et al. 2007), 15 genotypes by (Das et al. 2017), and five highly resistant lines by (Sharma et al. 2023) from a collection of 200 soybean accessions. In contrast to these studies, which primarily focused on resistance to a single disease, the present investigation identified Himso 1685 as a valuable source of broad-spectrum resistance against multiple soybean diseases. Additionally, the cross-

combination JS 335 × Himso 1685 combined resistance to three important diseases, highlighting its potential as a promising donor for pyramiding disease resistance in soybean breeding programmes.

Although earlier reports on simultaneous screening for multiple diseases are limited, Singh et al. (2014) emphasized the importance of identifying genotypes with broad-spectrum resistance to ensure stable crop performance under diverse disease pressures. Therefore, these findings suggests that the multi-disease resistant parent Himso 1685, together with the resistant hybrid JS 335 × Himso 1685, represents valuable breeding material for developing high-yielding soybean cultivars with durable and broad-spectrum disease resistance.

Table 7 Resistant soybean parental lines and hybrids for disease response

Disease	Resistance level	Superior parental donor(s)	Superior hybrid combination(s)
Pod blight	Resistant	Himso 1685	JS 335 × Himso 1685
Frogeye leaf spot	Highly resistant	Himso 1685	JS 335 × Himso 1685
Brown spot	Resistant	-	JS 335 × Himso 1685
	Moderately resistant	EC 241778	Him Soya × Himso 1685, NRC 195 × JS 335, AS 40 × JS 335, PS 1572 × JS 335, PS 1572 × Himso 1685, KDS 992 × Himso 1685, DS 3163 × JS 335, DS 3163 × Himso 1685, RSC 10-52 × JS 335, RSC 10-52 × Himso 1685, PK 25 × Himso 1685
Bacterial pustule	Highly resistant	Himso 1685	-
	Moderately resistant	PS 1572, EC 241778, RSC 10-52, PK 25	Representative hybrids: Palam Early Soya 1 × JS 335, Hara Soya × JS 335, Him Soya × Himso 1685, KDS 992 × Himso 1685, DS 3163 × Himso 1685, JS 335 × Himso 1685
Powdery mildew	Resistant	Himso 1685	-
	Moderately resistant	Palam Early Soya 1, Hara Soya, KDS 1149, KDS 992, EC 241778, PK 25, JS 335	Representative hybrids: Palam Early Soya 1 × Himso 1685, Him Soya × JS 335, KDS 1149 × JS 335, NRC 195 × JS 335, KDS 992 × Himso 1685, EC 241778 × Himso 1685, DS 3163 × JS 335, JS 335 × Himso 1685

Conclusion

The present study revealed that additive, non-additive, and epistatic gene effects collectively govern the inheritance of yield and related traits in soybean, emphasizing the need for trait-specific breeding strategies. Among the parental lines, Himso 1685, JS 335, and DS 3163 were identified as promising general combiners for several yield related, quality, and disease resistance traits. The crosses namely AS 40 × Himso 1685, Hara Soya × JS 335 and Palam Early Soya 1 × JS 335 and exhibited desirable specific combining ability and significant heterosis, highlighting its potential for developing superior segregating populations. Moreover, Cat 411A emerged as a valuable donor for enhanced nutritional quality, while Himso 1685 combined high nutritional value with

multiple disease resistance, making it an excellent parent for soybean improvement along with JS 335 × Himso 1685 which exhibited broad-spectrum disease resistance. Overall, the elite parents and cross combinations identified in this study provide valuable genetic resources for developing high-yielding, nutritionally improved, and disease-resistant soybean cultivars through the selection of superior transgressive segregants and their subsequent utilization in soybean breeding programmes.

Declarations

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Ethics declaration: Not applicable.

Data Availability: All data generated during this study are included in this published article and its supplementary information files.

Conflict of interest: The authors have no competing interests to declare that are relevant to the content of this article.

Consent to participate

Not applicable. This study did not involve human participants.

Consent to publish

Not applicable. This study does not contain any individual person's data in any form.

Ethics and consent to participate

The plant materials used in present study were obtained from cultivated sources and handled in accordance with applicable institutional and national guidelines. No human participants or animals were involved in this study.

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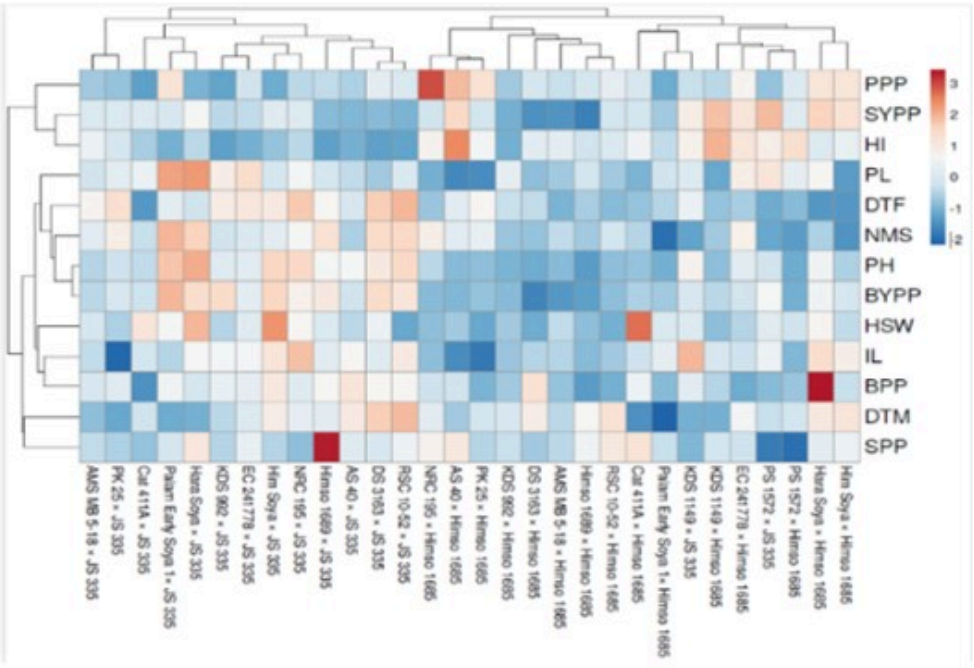
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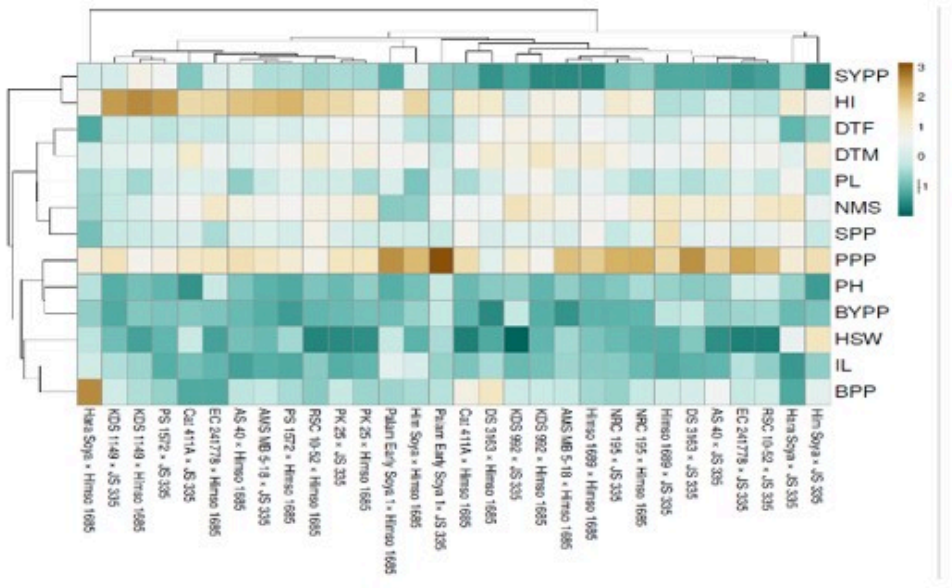
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Figures



a) Heterosis over better parent (HBP%)



(b) Heterosis over standard check (HSC%)

Figure 1

Heat map depicting heterobeltiosis (HBP) and standard heterosis (HSC) for yield and yield-related traits in 45 soybean cross combinations

DTF- Days to 50% flowering, DTM-Days to 75% maturity, PH- Plant height, NMS-Nodes on main stem, IL-Internode length, BPP- Branches per plant, PPP- Pods per plant, SPP-Seeds per pod, PL-Pod length, HSW-100-seed weight, BYPP-Biological yield per plant, SYPP- Seed yield per plant, HI- Harvest index

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