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Exploring genetic diversity coupled with multiple disease resistance in soybean

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Abstract
Genetic diversity was assessed among one hundred sixty-five soybean accessions for eleven significant yield attributing traits and their ability for resistance to multiple diseases under the field conditions. The experimental location is one of the hotspots for significant soybean diseases. Due to the virtual susceptibility to all or some of the diseases, more than sixty genotypes could not survive. Genetic analysis was carried out with the remaining 100 genotypes. The principal components analysis revealed that PC1, PC2, PC3 and PC4 have eigenvalues higher than the unity explained total variability of 76.59% among soybean genotypes attributable to seed yield. While screening 165 genotypes, only four genotypes, namely UPSL 152, DCB 137, JSM 232, EC 350664, were identified as high resistance to Yellow mosaic virus, Charcoal rot and Aerial blight diseases and can be used in any improvement programme attributed to higher yield and resistance to this significant soybean disease.

Keywords: Soybean, PCA, aerial blight, charcoal rot, YMV, resistance

Introduction
Soybean (*Glycine max* (L.) Merrill) is the most valuable eco-friendly leguminous oilseed crop globally. The soybean seed is rich in protein (40 percent) and oil (20 percent). India is among the top five soybean producer nations where soybean is mainly grown under rainfed conditions, significantly affected by several biotic and abiotic stresses. Madhya Pradesh alone contributes more than fifty percent of the area and production of the country's soybean.
The diversity present in the genotypes is an essential tool for crop improvement as diverse parents produce high heterotic effects (Tiwari *et al.* 1982; Mian and Bahl 1989, Burton and Brownie 2006) [14, 11, 6]. Genetic diversity can be revealed with different agro morphological traits and molecular markers (Kachare *et al.*, 2020; Tiwari *et al.* 2019) [7, 15]. Principal Component Analysis (PC) divides the total variance into several factors. Classification of genotypes according to agronomic characteristics made using multi-factor techniques can shorten the period and the cost of improving the yield.
Presently, soybean diseases like charcoal rot (CR), Yellow Mosaic Virus (YMV), and Rhizoctonia aerial blight (RAB) are severely affecting the soybean crop with considerable yield losses in major soybean growing regions of India. RAB (*Rhizoctonia solani)* causes significant yield loss (Wrather *et al.*, 2010) [17] under humid fields (Yang *et al.*, 1990) [18]. It can be recognised by grey to reddish brown irregular water-soaked lesions coupled with web of mycelium (aerial hyphae) and small dark brown Sclerotia of fungus on affected trifoliate (Amrate *et al.*, 2021) [4]. Charcoal rot (*Macrophomina phaseolina*) is very severe disease causes plant mortality and significant high yield reduction under dry field conditions (Luna *et al.* 2017; Wrather *et al.*, 2010) [9, 17]. It is usually affects soybean crops at reproductive stages and can be recognized by the greyish black appearance of lower stem and root tissue due to formation of numerous black microsclerotia of pathogenic fungus (Luna *et al.* 2017) [9]. Most Indian soybean varieties are entirely susceptible to charcoal rot disease and can cause as high as 92.6 % yield reduction (Amrate *et al.*, 2019) [2]. Yellow mosaic is a begomovirus disease (*Mungbean yellow mosaic India virus*) transmitted by *Bemisia tabaci* (Usharani *et al.*, 2004; Kumar *et al.*, 2014) [16, 8]. YMV disease is characterized with the presence of contrast yellow mottles on leaves and can cause heavy yield losses(>80.0 percent) in soybean upon severe infection at early stage of growth (Amrate *et al.*, 2020) [3].

The present soybean varieties in India have a narrow genetic base. The deployment of multiple disease resistance under high yielding background is an effective strategy for enhancing soybean productivity and sustainability. Therefore, realizing the importance of the problem, an investigation was undertaken to assess genetic divergence for resistance for multiple diseases (YMV, CR and RAB) along with crucial traits in a panel of diverse soybean germplasm.

Materials and Methods

An experiment comprising one hundred sixty-five diverse soybean germplasm was carried out at the All India Coordinated Research Project on soybean, JN Agriculture University, Jabalpur, India (Latitude: 23º14 N, Longitude: 79º56 E, Altitude: 411.5 m) during *Kharif* 2019. The experiment was laid down in augmented plot design in 4 m rows with 50 cm row to row and 6-8 cm plant to plant distance. Five released varieties (JS 20-34, JS 335, JS 20-98, JS 95-60 and NRC 86) were used as check. The present experimental location is designated as a hotspot for charcoal rot (CR), Yellow mosaic virus (YMV) and Rhizoctonia aerial blight (RAB) diseases by the AICRP on Soybean. Genetic divergence analysis was undertaken for eleven significant yield attributing traits of soybean. The observations were recorded for days to flower initiation, days to maturity, plant height (cm), number of primary branches/plant, number of nodes/plant, number of pods/plant, number of seeds/pod, seed yield/plant (g), 100 seeds weight (g), biological yield per plant (g) and harvest index (%) from five randomly selected healthy plants from each genotype and the principal component analysis was worked out.

Diseases scoring and resistant reaction

Each genotype was critically observed for appearance, incidence and severity of diseases throughout the cropping season, and final reactions were given based on the highest disease severity. Disease/resistance reaction was assigned to each genotype for RAB, CR and YMV based on percent disease index (PDI), percentage mortality and Coefficient of infection, respectively (Table 1) (Amrate *et al.*, 2018) [1].

Table 1: Disease/resistance reaction for Aerial blight (RAB), Charcoal rot (CR) and Yellow mosaic virus (YMV) based on percent disease index (PDI), percentage mortality and Coefficient of infection (CI)

Aerial blight/Charcoal rot		Yellow mosaic	
PDI/Mortality %	**Reaction**	**Coefficient infection (CI)**	**Reaction**
0.0	Absolutely resistant (AR)	0-4	Highly resistant (HR)
>0-1	Highly resistant (HR)	5-9	Resistant (R)
>1 to 10	Moderately resistant (MR)	10-19	Moderately resistant (MR)
>10to 25	Moderately susceptible (MS)	20-39	Moderately susceptible (MS)
>25 to 50	Susceptible (S)	40-69	Susceptible (S)
>50	Highly susceptible (HS)	70-100	Highly susceptible (HS)

Results and discussion

Genetic divergence

As some genotypes were ultimately killed due to complex and high disease pressure, only 100 genotypes were included in the genetic divergence analysis of eleven traits. PCA provides a roadmap for reducing complex data set to a lower dimension to sometimes hidden, simplified structures that often underlies it. In the present investigation, out of twelve, only six principal components (PCs) exhibited more than 0.5 Eigenvalue (Table 2) and showed about 87.78% variability among the traits studied. The first principal component recorded the highest variation (40.380 %). Semi curve line obtained after the sixth PC with slight variation observed in each PC indicated that maximum variation was found in the first PC; therefore, selection for characters under the first PC may be desirable. The dispersion of genotypes in biplot indicated the presence of fair amount of genetic diversity (Figure 1). The genotypes closer to each other had little or no differences with respect to seed yield. Therefore, based on the homogeneity existing in the groups, it was possible to make a second selection focusing on seed yield.

Table 2: Eigen values, percentage of total variation and cumulative percentage for corresponding traits in soybean genotypes

Characters	Principal Component (PC)	Eigen value	Variability %	Cumulative %
Days to flower initiation	PC 1	4.442	40.380	40.380
Days to maturity	PC 2	1.852	16.833	57.213
Plant height	PC 3	1.371	12.464	69.676
No. of primary branches/plant	PC 4	0.761	6.917	76.593
No. of nodes/plant	PC 5	0.649	5.900	82.493
No. of pods/plant	PC 6	0.582	5.287	87.780
No. of seeds/plant	PC 7	0.491	4.462	92.242
100 seed weight	PC 8	0.432	3.926	96.168
Biological yield/plant	PC 9	0.310	2.817	98.985
Harvest index	PC 10	0.090	0.821	99.806
Seed yield/plant	PC 11	0.021	0.194	100.00

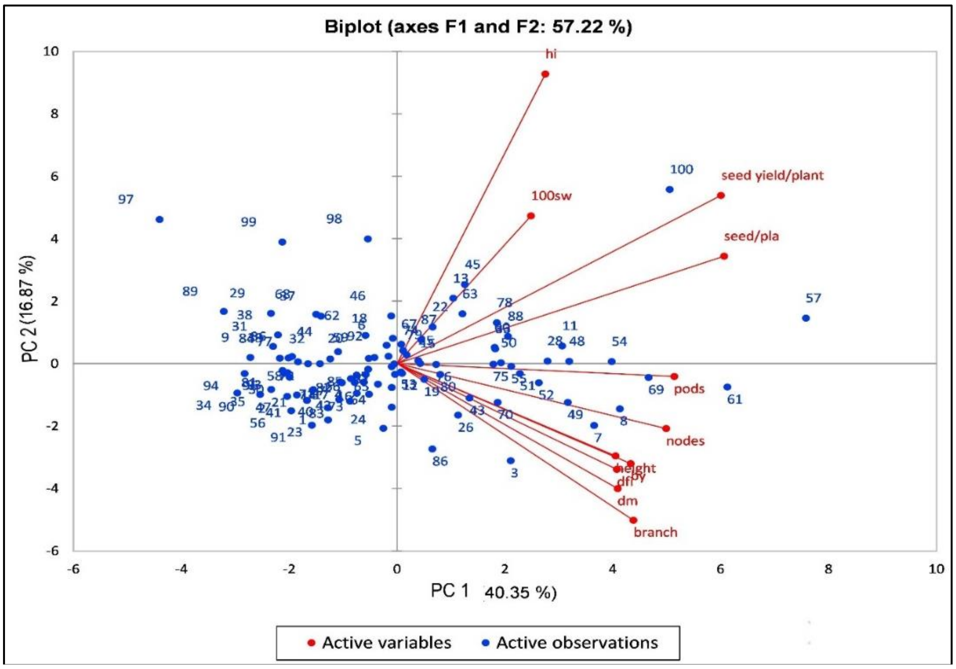

Fig 1: Biplot of PC1 and PC2

Rotated component matrix (Table 3) revealed that from the six PC selected; only the first four PCs represent maximum variability (76.59%); hence, the traits falling in these PCs might be given due importance in the soybean breeding programme. Results revealed that the first principal component (PC1), which accounted for the highest variation (40.380%), was mainly related to yield and its contributing traits such as days to flower initiation, days to maturity, number of pods per plant, number of nodes per plant, number of seeds per plant, biological yield per plant, and seed yield/plant. The second principal component (PC2) consisted of the harvest index. The third principal component was related to 100 seed weight, and the fourth principal component with plant height.

Table 3: Rotated component matrix for different traits of soybean genotypes

Traits	Principal Components			
	PC1	PC2	PC3	PC4
Days to flower initiation	0.572	-0.307	-0.357	-0.036
Days to maturity	0.574	-0.361	0.368	0.055
Plant height	0.569	-0.267	-0.364	0.596
No. of primary branches/plant	0.616	-0.453	0.129	0.058
No. of nodes/pod	0.700	-0.188	-0.197	0.147
No. of pods/plant	0.721	-0.036	-0.213	-0.393
No. of seeds/plant	0.852	0.306	-0.211	-0.241
Biological yield/plant	0.347	0.433	0.671	0.325
100 seed weight	0.608	-0.288	0.567	-0.208
Harvest index	0.384	0.842	-0.213	0.118
Seed yield/plant	0.841	0.491	0.114	-0.037

Similar results were obtained by Miladinovic *et al.* (2006) [12] reported three principal components in his investigation. Smith *et al.* (1995) [13] conducted average linkage cluster and principal component analysis and reported the utility of these results in preserving and utilising germplasm. Broschat (1979) [5] considered PCA as a powerful technique for data reduction which removes interrelationships among components.

Resistance to YMV, RAB and CR disease

The severe symptoms of YMV, RAB and CR appeared during the second fortnight of July, August and September, respectively (Figure 3, A-D). Incidence and severity of CR and RAB were wildly higher than YMV disease in all the genotypes. The screening result also revealed that different genotypes were reacted differently for all three diseases (Table 4). Out of one hundred sixty-five, only eleven were found to be highly resistant to RAB, forty-eight for CR and ninety-three genotypes were highly resistant for YMV disease, respectively. Others genotypes have shown moderate resistance to susceptible reactions for all three diseases.

Ten genotypes were found to be highly resistant against charcoal rot plus aerial blight, fifteen for charcoal rot plus YMV and four for aerial blight plus YMV. Only four genotypes, namely UPSL 152, DCB 137, JSM 232, EC 350664, were identified as high resistance to all three diseases (Table 5). Different levels of disease resistance in soybean genotypes were also reported by Mengistu *et al.* (2013) [10] against charcoal rot using root and stem severity index and identified four genotypes with higher resistance levels. Kumar *et al.* (2014) [8] identified resistant and susceptible genotypes for YMV under field conditions. In contrast, Amrate *et al.* (2018) [1] identified seventeen genotypes with higher-level resistance to charcoal rot, aerial blight and YMV under Madhya Pradesh conditions.

Table 4: Disease reaction of 165 diverse soybean genotypes for YMV, CR and RAB diseases

Genotype	Reaction			Genotype	Reaction		
	YMV	CR	RAB		YMV	CR	RAB
VP1199*	R	HS	MS	PKS 7*	HR	HS	MR
K-53	R	MR	MS	JSM 285	R	AR	HR
EC-389153	R	AR	MR	EC 33872B	HR	MS	S
UPSL 152	HR	AR	HR	G91*	MS	HS	MS
EC 389154C*	R	S	MS	SQL 1	R	AR	MS
UPSL 72*	HR	MS	S	JSM 232	HR	AR	HR
UPSM 9*	HR	MS	S	JSM 222	MR	AR	MR
UPSM 20*	HR	S	MS	JSM 245	MR	AR	MS
EC 389179B	MS	S	MR	EC 39513*	S	HS	MR
UPSL 652*	MS	S	MS	EC 39743*	AR	HS	MS
UPSM 77	MS	MR	MR	TG X1835-10F*	R	S	MS
EC 391181	S	AR	MS	EPS 1B*	R	HS	MS
VGM 70	MR	MR	MS	EC 116343*	R	HS	S
UPSM 57*	MS	HS	MR	MACS 1045*	HR	HS	MS
VLS 75	S	MR	MR	G47*	MS	HS	MR
EC 391316	MS	MR	MS	JS 20-32	MR	AR	MR
RVS 2006-21*	R	MR	S	SL (E)1	MS	AR	MS
JS 97-52	R	MR	MR	PK 701	HR	AR	MS
EC 391346*	MS	AR	MR	PK 1038	MS	AR	MR
NRC 2007A	S	MR	MR	RKS 54	MS	AR	HR
NRC 80-1	MS	MR	MS	EC 33940	AR	AR	MS
JS 20-01	MR	MS	MS	RKS-45*	R	HS	MR
EC 396065	R	MS	MS	MAUS-128*	MR	HS	MS
EC 251682*	MR	MR	S	G2225	R	AR	HR
TGX 840-4-E*	HR	MS	S	JS 20-29	HR	MS	MS
EC 251541*	MR	MR	MS	TNAU 20023*	MR	HS	MR
EC 393224*	MS	S	MS	G4P15	HR	AR	MR
EC 340506B*	HR	MS	S	GP 465	HR	HS	MR
EC 393231*	R	MS	MS	TGX 849-D-13-4	HR	AR	MR
EC 251516*	HR	HS	MS	JSM 227	MR	AR	MR
G 2258*	HR	HS	MS	JS 20-96	R	AR	MR
DS 91-3*	MR	MS	S	TG X 849-813	R	AR	MR
DCB 137	HR	AR	HR	KB-17	R	MR	MR
EC 457214	R	MS	MS	KDS 256	HR	AR	MR
DE 201	HR	HS	MR	LEE-54	R	MR	MR
EC 381884*	HR	HS	MR	JS 96-31	R	MR	MR
EC 457336	HR	HS	MR	TNAU 20024	R	MR	MR
AGS 12	MR	AR	HR	SQL 37	MR	AR	MS
F4P20	MR	MR	HR	WT 88	MR	AR	MS
SL 794*	HR	S	MS	TNAU 20022	S	MR	MR
EC 103336	HR	MS	S	EC 528640	HR	MR	MS
NRC 84	MR	MR	MS	EC 538830	MR	MR	MR
EC 106998	HR	MS	MS	PK 258	MS	AR	MR
SL 752	MR	AR	MR	EC 468597	R	MR	MR
EC 109540	MR	AR	S	EC 572160	MR	S	MR
UGM 75	R	AR	MR	SL 682	MS	MS	MR
EC 113778*	R	MR	MS	PI 204336	MS	AR	MR
MACS 303	MS	AR	HR	UGM 70	S	AR	MR
NRC 2006M	MR	AR	MR	PS 931Q8*	S	HS	MR
EC16213	MR	AR	MS	LEE 96	S	HS	MR
EC 23001*	MR	MS	MS	TG X 1488-9-1D	MS	MR	MR
EC 15961*	R	MR	S	LEE 53*	S	HS	MR
EC 173325*	R	MR	S	TG X 702-4-8*	MS	HS	MR
PSL 6*	MS	S	MR	GP566	S	MR	S
JS 20-52	MR	S	MS	JS 98-11*	S	S	MS
SL 525*	MS	S	MR	PS1421	HR	HS	MR
EC 242004*	AR	HS	MR	VLS 11	HR	AR	MR
EC242072*	AR	HS	MS	PS 1467	MR	AR	HR
PS 1475	MR	AR	MR	EC 350664	HR	AR	HR
NRC 37	MS	MR	MS	EC 377883B	HR	AR	MS
EC 242111*	HR	AR	MS	JS 20-76	MR	AR	MR
MACS 171	R	HS	MR	GC 84051-32-1	HR	S	MS
SL 2951	MS	HS	MS	PK 431337*	HR	MS	S
AGS 164*	R	S	MS	EC 390977*	MR	S	MS
EC 251501*	HR	S	S	JS 20-86*	R	S	S

MACS 1259*	HR	S	MS	JS 20-50	R	MS	MS
AGS 174*	MR	HS	MS	MACS-7102	MR	MS	MR
EC 251876*	HR	S	MS	11-5E(Z-2)*	R	HS	MS
SL 432*	HR	HS	MR	20-40B(Z-9)	HR	MR	S
LEE 75*	MR	HS	MS	20-148 (Z-15)	HR	MS	S
EC 389099	HR	AR	MS	23-10B(Z-17)	HR	AR	S
JS 20-69	HR	MR	MS	23-11A(Z-18)	R	MR	MS
JSM 195	R	MS	MR	14-11B(Z-19)*	HR	MR	MR
EC 291397	HR	MR	MS	20-14C(Z-22)*	HR	MS	MR
PS 1336	HR	MS	MR	23-16C(Z-24)*	MR	MR	S
EC 294003	HR	MR	MR	23-16 C(Z-23)*	R	MR	MS
EC 291451	HR	MS	MR	DS 3106*	HR	MS	MS
EC 24091	HR	S	MR	JS 20-34 (C)	MR	AR	MR
EC 291453*	MR	AR	MR	JS 335 (C)	MS	MR	MS
EC 309537*	HR	MR	S	JS 20-98 (C)	HR	AR	MR
EC 30967A*	HR	MR	MR	JS 95-60 (C)*	MR	HS	MS
RSC 14	MR	MS	MS	NRC86 (C)	MR	MR	MR
EC 325103	HR	AR	MR				

*****Genotype died completely due complex of diseases.

Table 5: Soybean genotypes exhibiting dual and multiple disease resistance

Diseases	Absolute/Highly resistant lines	Total
Charcoal rot +Aerial blight	MACS 303, UPSL 152, DCB 137, AGS 12, RKS 54, PS 1467, EC 350664, G2225, JSM 285, JSM 232	10
Charcoal rot + YMV	UPSL 152, DCB 137, EC 389099, EC 325103, JSM 232, TGX 849-D-13-4, G4P15, KDS 256, VLS 11, JS 20-98 (C), 23-10B(Z-17), EC 350664, EC 377883B, PK 701, EC 33940	15
Aerial blight + YMV	UPSL 152, DCB 137, JSM 232, EC 350664	04
Charcoal rot + Aerial blight + Yellow Mosaic Viruses	UPSL 152, DCB 137, JSM 232, EC 350664	04

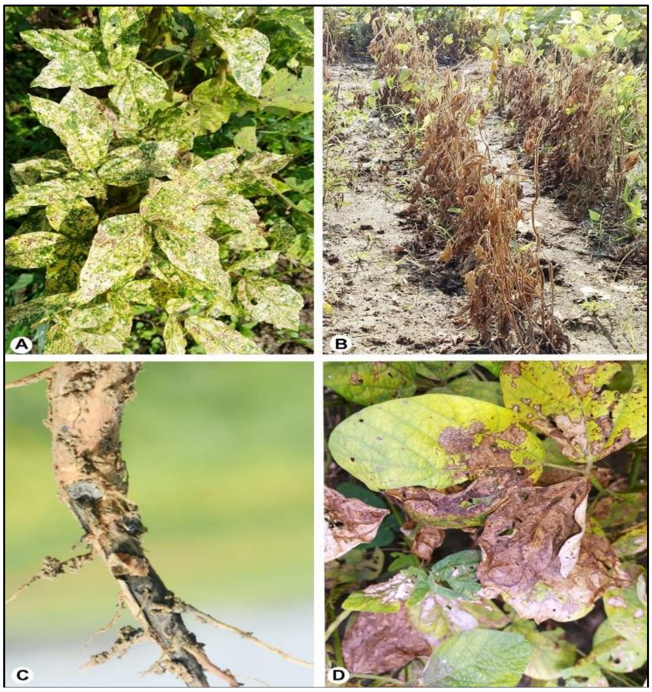

Fig 2: Disease incidence

Conclusion

The principal components analysis detected the vast genetic diversity among the studied genotypes for seed yield. These results indicate an excellent opportunity to improve soybean yield through wide hybridization by crossing genotypes in the farthest clusters. Field screening for multiple disease resistance revealed that the genotypes UPSL 152, DCB 137, JSM 232, EC 350664 were highly resistant against biotic stresses and, thus, can be further utilized as superior parents/donors in future breeding programmes. The diverse genotypes coupled with multiple disease resistance may be helpful in further varietal improvement.

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