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## A promising multiple disease resistant genotype in soybean

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### Abstract

In the present study, the segregating material developed from four crosses were stabilized and screened for five years (2016-2020) at two hotspots Dharwad and Ludhiana for resistance to rust and Yellow mosaic disease (YMD) respectively by Shuttle breeding. Among four crosses involved, two highly resistant varieties for rust and YMD, cross SL 979 x DSb 21 exhibited resistant reaction to both the diseases. In the said crosses one promising line *viz.*, DLSb 1 (SL 979 x DSb 21) with maturity duration 90-95 days. The line recorded 14.3 per cent higher yield (2264 kg/ha) over the best check DSb 21 (1980 kg/ha) in station trial over the years (2019 and 2020). Therefore, the soybean line DLSb 1 was developed for multiple disease resistant and tested under Co-ordinated trial (IVT) during *kharif* 2020 across all zones of India and secured second rank under Central zone for yield and it exhibited moderately resistant reaction to rust at Dharwad and highly resistant reaction to YMD at hotspots *viz.*, Ludhiana, Delhi and Jabalpur. The line exhibited 9.8 per cent higher yield (2658 kg/ha) over the check JS 20-116 (2420 kg/ha) in Central Zone. This line Dharwad Ludhiana Soybean 1 (DLSb 1) will be useful for future plant breeding programme in the development of variety for multiple disease resistance.

**Keywords:** DLSb 1, rust, YMD, resistance reaction, shuttle breeding

### Introduction

Soybean is the Numero Uno oilseed crop in the country. Soybean ranks first as one of the oilseed crop both in area and production in India. In India, it occupies an area of 12.12 million hectare with the production of 13.58 million tonnes and productivity of 1125 kg/ha (Anon, 2021) [2]. In Karnataka, soybean is grown over an area of 3.31 lakh ha with a production of 3.70 lakh tonnes and productivity of about 1124 kg/ha (SOPA, 2021) [19]. The failure in harnessing the yield potential of released varieties has been ascribed to several biotic and abiotic factors. Of the biotic factors, rust in southern parts of India and yellow mosaic disease in northern and central parts of India has been reported to cause significant yield loss in soybean.

### Rust

Among the diseases of soybean, soybean rust incited by pathogen *Phakopsora pachyrhizi* (Syd.) is a major disease and reported to cause an yield loss from 20-80 percent (Bromfield, 1976) [4]. Soybean rust reduces yield through premature defoliation, less number of filled pods and by reducing the seed size. It also lowers the quality of seed produced. The severity of loss and the particular components of yield affected depend primarily on the time of disease onset and the intensity of disease at particular growth stages of the crop (Bromfield, 1984) [5]. Also known as Asian rust, this fungal infection can defoliate soybean fields rapidly, often resulting in severe and sometimes total loss. The disease appeared suddenly in epiphytotic form during *kharif* 1994-95 and caused substantial yield losses particularly in Northern parts of Karnataka, Maharashtra and Madhya Pradesh (Anahosur *et al.*, 1995) [1]. Now, it has become a major constraint for the soybean production particularly in northern Karnataka and southern parts of Maharashtra. Two germplasm lines *viz.*, EC 241778 and EC 241780 of soybean as promising source of resistance to rust caused by *Phakopsora pachyrhizi* after rigorous screening of 982 germplasm lines. But these two germplasm lines are highly susceptible to bacterial pustule and soybean mosaic virus (SMV) with long maturity duration (110 days) (Patil *et al.*, 2004) [10].

### Yellow Mosaic Disease (YMD)

Soybean, is also attacked by YMD, which is known to be caused by *Mungbean Yellow Mosaic*

*Virus* (MYMV) and *Mungbean Yellow Mosaic India Virus* (MYMIV). Nucleotide sequence of the virus isolated from YMD infected soybean plants from northern and central India showed 89% similarity with MYMIV and was designated as soybean isolate of MYMIV (MYMIV-[Sb]) (Usharani *et al.*, 2004) [17]. MYMIV is the most prevalent virus infecting soybean in northern and central India. YMD is a widespread and very destructive disease of soybean. The disease can be identified by scattered yellow spots, produced in indefinite bands along the major veins of soybean leaves. On matured leaves, rusty necrotic spots will appear. In severe infection, mottling and crinkling of leaves can also be observed. These affected plants bear less number of flowers and pods, leading to decrease in yield and oil. Yellow mosaic virus has been reported to cause significant yield loss in soybean in North India in early 70s (Suteri, 1974) [16] when the magnitude of the loss due to the disease was reported to be as high as 80% (Nene *et al.*, 1972) [18].

Since 1970s, YMD is posing a major threat to soybean cultivation and is reported to spread throughout India in alarming proportions (Varma and Malathi, 2003) [18]. Earlier, this disease was confined to northwest plains of India, but its sudden outbreak in central India in 2015 and further spread to south India shows that it has potential to spread to other soybean growing countries. In soybean, YMD causes 15%–75% yield losses (Sharma *et al.*, 2014) [11]. None of the varieties of central India which is the hub of soybean cultivation is resistant to this disease. It is therefore imperative to introgress YMD resistance in elite, widely adopted soybean varieties.

This geminivirus belongs to genus *Begomovirus* and family *Geminiviridae* with bipartite genome and is transmitted by whitefly (*Bemisia tabaci*). The YMD can be controlled by reducing whitefly population using various insecticides, but it is not a very effective control measure and may lead to severe resurgence of the pest. Also, it adds to the economic burden on the farmers due to additional costs of repeated insecticidal sprays. Moreover, complete elimination of whitefly using insecticides is not possible. Therefore, use of resistant varieties is an economical as well as ecologically friendly

method to manage this disease (Bhanu *et al.*, 2019) [3]. There are two sources of YMD resistance in soybean. One source is the wild progenitor (*Glycine soja*) of soybean (Singh *et al.*, 1974a) [13], (Singh *et al.*, 1974b) [14], while the other source is from the cultivated gene pool (Singh and Mallick, 1978) [15]. For efficient use of these sources in resistance breeding, development of yellow mosaic virus resistant varieties for these specific soybean growing regions remains a challenge for the plant breeders. Gaurav Khosla *et al.*, 2021 [6] first reported in soybean indicating role of two genes with inhibitory gene action for YMD resistance and mapped two QTLs on two different chromosomes, namely, chromosome 2 and chromosome 6. On chromosome 6, one major QTL was flanked by Satt281 and Sat\_076 having LOD score of 44.47 and explained 27.0% variation. On chromosome 2, found one major QTL flanked by markers BARCSOYSSR\_02\_0423-BARCSOYSSR\_02\_0425.

Due to severity of YMD on soybean in Athani, Raibagh and Chikkodi taluks of Belagavi of Karnataka District during 2015, which is also a rust prone area, an urgent need to develop multiple disease resistant genotypes. In this regard it was necessary to combat disease severity, AICRP on Soybean, University of Agricultural Sciences, Dharwad attempted hybridization work to develop soybean genotypes having multiple disease resistance against rust and YMD across environments and seasons by Shuttle breeding.

### Materials and Methods

DLSb 1 was developed from a cross between SL 979 x DSb 21. SL 979 (a YMD resistant line received from Punjab Agricultural University, Ludhiana) and DSb 21 (a ruling variety resistant to rust with high yield potentiality) developed at AICRP on Soybean, Main Agricultural Research Station, Dharwad.

### Hybridization

The hybridization was carried out using two rust and two YMD resistant parents in order to incorporate disease resistance genes into a single background to develop a variety (Table 1).

**Table 1:** Salient features of parents

Sl. No.	Variety	Pedigree	Duration (days)	Yield potential (q/ha)	Disease reaction
1	DSb 21	JS 335 x EC 241778	90-95	30-32	Highly resistant to rust. Recommended for Southern zone (Karnataka, Tamil Nadu, Telangana, Andhra Pradesh and southern parts of Maharashtra States)
2	DSb 23	JS 335 x EC 241780	95-100	32-35	Highly resistant to rust. Recommended for Southern Zone (Karnataka, Tamil Nadu, Telangana, Andhra Pradesh and southern parts of Maharashtra States)
3	SL 958	SL 525 x SL 706	130-135	20-25	Resistant to YMV and SMV. Recommended for Northern Plain Zone (NPZ)
4	SL 979	SL 525 x DS 98-14	125-130	23-25	Resistant to yellow mosaic disease and soybean mosaic virus. Recommended for Northern Plain Zone (NPZ) (Punjab, Haryana, Delhi, UP (Except Bundelkhand) and Bihar states of India)

Four crosses between parental lines were effected at the experimental field of AICRP on Soybean, MARS, Dharwad during *kharif* 2016. During summer 2017 True F<sub>1</sub>'s were identified and screened again at Dharwad. During *kharif* 2017 F<sub>2</sub>'s were screened for rust at Dharwad and YMD at Ludhiana simultaneously. During summer 2018, F<sub>3</sub>'s were advanced to F<sub>4</sub>'s. Screened for rust at Dharwad and again for YMD screening carried out at Ludhiana during *kharif* 2018 for F<sub>4</sub>'s. The selections were made for rust resistance in F<sub>5</sub>'s from the segregating generations of four crosses. Among the several selections in segregating populations of above crosses, a promising line DLSb 1 (SL 979 X DSb 21) was identified as

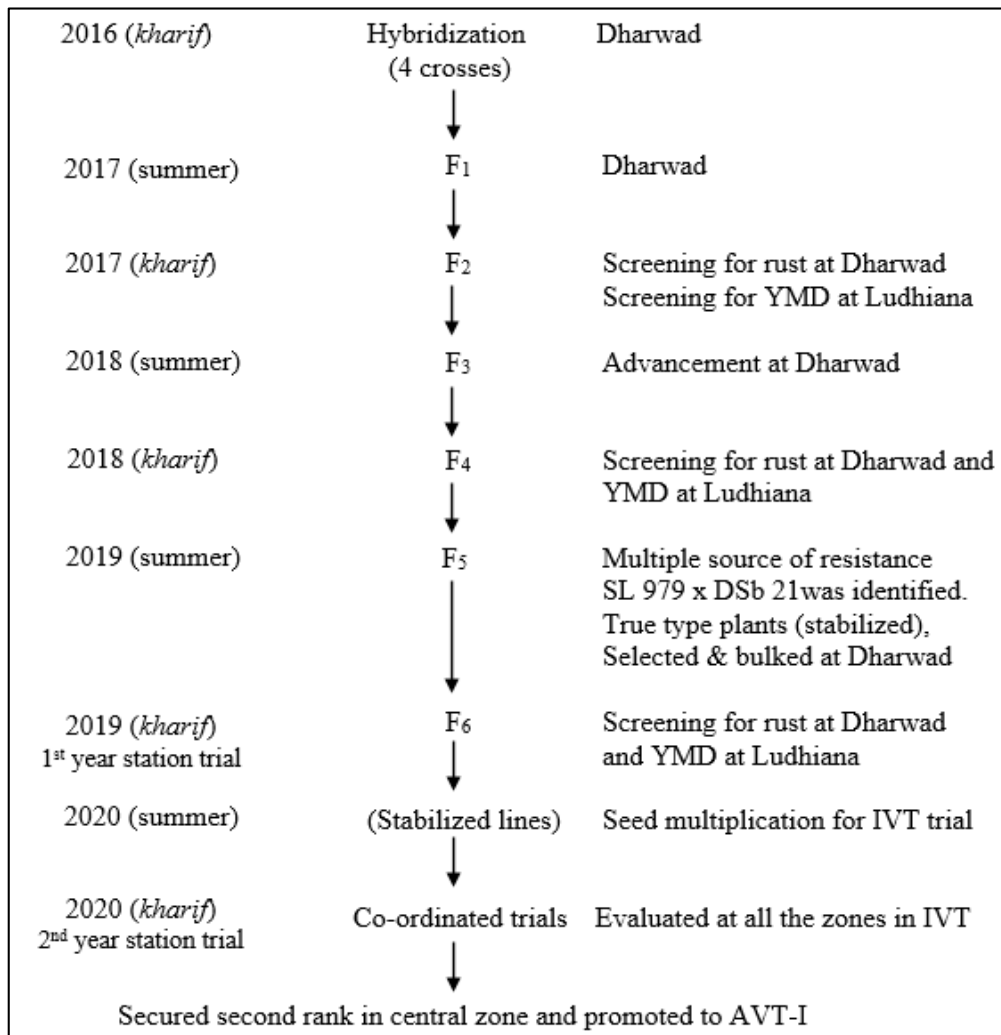
multiple source of resistance to both rust and YMD at Dharwad and Ludhiana. During *kharif* 2019 stabilized lines were screened for YMD at Ludhiana and simultaneously for rust at Dharwad. In Summer 2020 promising line (SL 979 x DSb 21) was evaluated and further multiplied and entered into All India Co-ordinated trials as contributing entry from Dharwad in Initial Varietal Trial 2020 for yield as well reaction to pests and diseases. Based on yield performance at various test locations, DLSb 1 was secured second rank in Central Zone of India.

In addition to this, yield data of two years, station trials were statistically analysed according to Panse and Sukhatme (1978)

[9]. Perusal of Figure 1 shows various steps involved in the development of DLSb 1 by Shuttle breeding.

**Generation advancement (F<sub>1</sub>-F<sub>5</sub>):** A single row of 4 m length was planted with F<sub>0</sub> hybrid seed. Each line was raised in one

row of 4 m length with a spacing of 45 x 10 cm. Planting was continued upto F<sub>5</sub> generation with negative selection of undesirable diseased plants under natural infection conditions. The seed from true-to-type plants were harvested and bulked. Progeny of DLSb 1 was selected in F<sub>5</sub> generation.



**Fig 1:** Development of rust and YMD resistant genotype (DLSb 1) by Shuttle breeding

**Planting of yield trials:** A total of ten promising rust resistant genotypes along with two resistant and one highly susceptible check was consecutively evaluated for two years in station trial during *kharif* 2019 and 2020. The crop was raised under recommended package of practices along with prophylactic protection measures. The experiment was laid out in RCBD with three replications. The row to row distance was 30 cm. The length of row was 4m with 6 rows. Two standard checks (DSb 21 as rust resistant check and JS 335 as highly

susceptible check) were included in experiment for comparison. Disease reaction was separately recorded.

**Disease severity assessment**

**Severity of rust reaction**

The severity of rust was scored between 65-90 days after sowing based on percent leaf area infected by using 0-9 scale (Mayee and Datar, 1986) [7] (Table 2).

**Table 2:** Disease rating scale

Disease grade	Description	Disease Reaction	
0	<1% infection	Absolute Resistant	AR
1	1-10% of leaf area infected	Highly resistant	HR
3	11-25% of leaf area infected	Moderately resistant	MR
5	26-50% of leaf area infected	Moderately susceptible	MS
7	50-75% of leaf area infected	Susceptible	S
9	>75% of leaf area infected	Highly susceptible	HS

**Severity of yellow mosaic disease:** To quantify the disease severity, calculations were made accordingly (Singh and Singh, 2000) [12]. The coefficient of infection (CI) was calculated

by multiplying the per cent disease incidence to the response value assigned for each severity grade. Thus, the coefficient values combine the amount of infection and its severity.



**Table 3:** Scale for classifying reaction of YMD (Singh and Singh, 2000) [12]

Severity grade	Symptoms	Response value	Coefficient of infection (CI)	Disease Reaction
0	Symptoms absent	0	0-4	Highly resistant (HR)
1	Very mild symptoms upto 25% leaves	0.25	5-9	Resistant (R)
2	Appearance of symptoms in 26-50% leaves	0.50	10-19	Moderately resistant (MR)
3	Appearance of symptoms in 51-75% leaves	0.75	20-39	Moderately susceptible (MS)
4	Severe disease infection in symptoms (> 75% leaves)	1.00	40-69	Susceptible (S)
			70-100	Highly susceptible (HS)

### Results and Discussion

Initial screening of ten promising rust resistant genotypes including two rust resistant (DSb 21 and DSb 23) and one highly susceptible check (JS 335) was consecutively evaluated for two years in station trial during *kharif* 2019 and 2020 under natural epiphytotic conditions at hotspot for rust at Dharwad revealed that only DSb 39 (2325 kg/ha), DLSb 1 (2264 kg/ha), and DSb 34 (2209 kg/ha), recorded higher

mean yield of 17.4%, 14.3% and 11.5% respectively over check DSb 21 (1980 kg/ha) (Table 4). All the genotypes recorded grade 1 (Highly resistant) except DSb 31 with grade 3 (moderately resistant) whereas JS 335 exhibited grade 9 (Highly susceptible) reaction during *kharif* 2019. Whereas during *kharif* 2020 only two genotypes DSb 34 and DSb 39 exhibited highly resistant reaction along with check DSb 23 compared to highly susceptible check JS 335.

**Table 4:** Performance of DLSb 1 for grain yield (kg/ha) over two years along with rust resistant reaction

Sl. No.	Genotypes	Grain yield (kg/ha)		Mean	Reaction to rust resistant	
		2019-20	2020-21		2019-20	2020-21
1	DSb 31	2272	1032	1652	3 (MR)	3 (MR)
2	DSb 32	2175	1346	1761	1 (HR)	3 (MR)
3	DSb 33	2468	1348	1908	1 (HR)	3 (MR)
4	DSb 34	2832	1586	2209	1 (HR)	1 (HR)
5	DSb 38	2336	1230	1783	1 (HR)	3 (MR)
6	DSb 39	3107	1543	2325	1 (HR)	1 (HR)
7	DSb 40	2175	1379	1777	1 (HR)	3 (MR)
8	DSb 42	2363	1173	1768	1 (HR)	3 (MR)
9	DSb 43	2175	1528	1852	1 (HR)	3 (MR)
10	DLSb 1	2950	1578	2264	1 (HR)	3 (MR)
11	DSb 21 (C)	2601	1358	1980	1 (HR)	3 (MR)
12	DSb 23 (C)	2807	1654	2231	1 (HR)	1 (HR)
13	JS 335 (C)	2006	988	1497	9 (HS)	9 (HS)
	SEm	0.11	0.08			
	CD (0.05)	468	336			
	CV (%)	11.32	14.43			

Thirty eight soybean genotypes along with three national checks were evaluated for grain yield and disease reaction under initial varietal trial (IVT) for all zones during *kharif* 2020. Among thirty eight genotypes DLSb 1 (2658 kg/ha) secured second rank in grain yield under Central zone with 9.8% higher grain yield over best check JS 20-116 (2420 kg/ha) followed by BAUS 96-17 (2625 kg/ha). With regard to Amreli and Anand centres, DLSb 1 ranked first for grain yield (3012 kg/ha and 2864 kg/ha) over best check JS 20-116 (3383 and 2420 kg/ha) respectively with moderately resistant reaction to rust at hotspot Dharwad (Table 5). With respect to Yellow mosaic disease virus, at all the three recognized hotspots *viz.*, Ludhiana, Delhi and Jabalpur, DLSb 1 exhibited highly resistant reaction for YMD with disease severity grade 0. Figure 2a and 2b depicting DLSb 1 resistant reaction of rust with highly susceptible check JS 335 at Dharwad and DLSb 1 resistant reaction of YMD with susceptible line VLS 101 at Ludhiana *respectively*. Based on the yield potentiality along with disease reaction for rust and YMD, the genotype DLSb 1 has been promoted to Advanced Varietal Trial-I (AVT-I) under Central Zone of India during *kharif* 2021. From the

above results, it is apparent that the resistant genotype DLSb 1 possesses a major gene which is responsible for imparting resistance to both rust and yellow mosaic virus with high yield potentiality.



**DL Sb 1**  
(Rust resistant line)      **JS 335**  
(Rust susceptible line)

**Fig 2a:** Evaluation of rust resistant line DLSb 1 at UAS, Dharwad



**DL Sb 1**  
(YMD Resistant line)

**VLS 101**  
(YMD Susceptible line)

**Fig 2b:** Evaluation of YMD resistant line DLSb 1 at PAU, Ludhiana

**Table 5:** Performance and disease reaction of DLSb 1 in Coordinated trial in Central Zone during *kharif* 2020

Sl. No.	Varieties	Yield (Kg/ha)*			Mean	Rank	Rust#	YMD#			
		Amreli	Anand	Lok Bharti				Dharwad	Ludhiana	Delhi	Jabalpur
1	DSb 38	272	1012	395	560	XXXV	MR	-	-	HR	
2	DS 3105	1802	1580	1506	1629	XXVIII	MS	HR	HR	HR	
3	CAUM 52	2272	1679	2444	2132	XV	MS	MR	HS	HR	
4	JS 22-11	2272	2049	2173	2165	XIII	MS	MR	MR	HR	
5	DLSb 2	2074	1383	1975	1811	XXIV	MR	HR	HS	MR	
6	RVSM 2012-11	2346	2049	2123	2173	XII	MS	HR	HR	HR	
7	RSC 11-39	2914	2296	1827	2346	VI	S	MR	HS	HR	
8	AS-15	2346	1679	1457	1827	XXIII	-	-	-	-	
9	PS 1664	1358	1531	1506	1465	XXX	MS	MR	HS	MS	
10	MACS 1520 (C)	2815	1605	1457	1959	XX	-	-	-	-	
11	HIMSO 1691	2346	1704	1959	2003	XIX	MS	MR	S	HR	
12	JS 22-14	2963	2370	1679	2337	VII	MS	MR	HS	HR	
13	DS 3144	2346	2568	1728	2214	X	S	HR	HR	MR	
14	DLSb 1	3012	2864	2099	2658	II	MR	HR	HR	HR	
15	NRC 128	2370	2148	1259	1926	XXI	MS	HR	HR	HR	
16	VLS 101	2247	2543	1407	2066	XVIII	MS	MS	HS	R	
17	RSC 11-35	2864	2272	1481	2206	XI	S	MS	HS	HR	
18	PS 1661	1877	2074	1309	1753	XXV	S	HR	HR	HR	
19	Himso- 1692	1506	2519	1111	1712	XXVI	S	S	HS	R	
20	JS 20-116 (C)	3383	2420	1457	2420	IV	-	-	-	-	
21	RVS 2012-10	1802	2519	2049	2123	XVII	MS	HR	MR	HR	
22	PS 1670	1679	1630	765	1358	XXXII	S	HR	HR	HR	
23	NRC 109	2321	2049	1383	1918	XXII	MS	S	HS	R	
24	MAUS 806	1630	1654	1679	1654	XXVII	MR	HR	HR	MR	
25	RVS 2011-10	2469	2741	3210	2807	I	MS	S	MR	R	
26	MAUS 768	2543	2198	2519	2420	V	-	-	-	-	
27	ASb 36	1111	247	1160	839	XXXIV	MS	MR	HS	MS	
28	ASb 9	272	543	642	486	XXXVII	MS	HR	-	MR	
29	AUKS 207	395	272	123	263	XL	-	-	-	-	
30	NRC 86 (C)	2815	2099	1877	2264	IX	-	-	-	-	
31	AUKS 206	123	321	321	255	XLI	-	-	-	-	
32	MACS 1701	2074	2049	2296	2140	XIV	MS	S	HS	MS	
33	KDS 1096	99	1284	123	502	XXXVI	MS	S	-	MS	
34	MACS 1691	99	815	173	362	XXXIX	MS	S	-	HR	
35	KDS 1144	2074	1481	1160	1572	XXIX	MS	-	HS	MS	
36	BAUS 96-17	2543	1926	3407	2625	III	MS	HR	HS	S	
37	BAUS 31-17	2296	1951	2568	2272	VIII	MS	HR	HS	MS	
38	TS 20-5	74	790	272	379	XXXVIII	MS	HR	-	R	
39	SL 1212	1432	1506	815	1251	XXXIII	MS	HR	HR	HR	
40	SL 1250	1160	1531	1679	1457	XXXI	MS	HR	HR	R	
41	DS 1312	2247	2173	1975	2132	XVI	MS	HR	HR	MS	
	Mean	1869.34	1759.12	1525.37			HS (JS 335)	HS (JS 335)	-	-	
	CD (0.05)	370.37	271.6	320.99							
	CV (%)	11.79	9.82	12.98							

\* Data from only Amreli, Anand and Lok Bharti (Hot spot) centres were considered, rest of the centre data were rejected due to high CV/<1000kg/ha yield.

# Recognized hot spots for respective diseases.

## Conclusion

The findings of the study will pave the way for mapping the genes for YMD and rust resistance with linked molecular markers. The identified line DLSb 1 will act as starting material for developing improved lines with combined source of rust and YMD resistance imparting genes would be utilized in further hybridization programme for breeding disease resistance improvement in Soybean.

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