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An exotic germplasm line EC 242104 with resistance to Asian soybean rust

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Abstract
Asian soybean rust caused by *Phakospora pachyrhizi* Syd. is one of the most important constraints to soybean production worldwide. Though there are many effective management practices are there to manage this disease, breeding for resistance is the most effective method. A first step in breeding for resistance will be screening and identification of stable resistant germplasm. Hence, an attempt was made to identify the new sources by screening 144 exotic germplasm lines along with resistant and susceptible checks at University of Agricultural Sciences, Dharwad during kharif 2015. Among them, EC 242104 recorded disease grade 1(8.89%) with highly resistant reaction. Further, evaluation of 22 selected lines with different resistant reaction, under natural epiphytotic condition at two hotspots for rust *viz*., Dharwad and Ugarkhurd during *kharif* 2016 lead to confirmation of highly resistant reaction of EC 242104 with grade 1. EC 242104 would serve as a source of resistant genes for incorporation into high yielding and adapted cultivars in crop improvement programmes.

Keywords: Asian **s**oybean **r**ust, *Phakospora pachyrhizi*, resistant source, exotic germplasm

Introduction
Soybean, an important oilseed crop ranks first both in area and production 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) [15]. Outbreak of diseases and insect pests is a major impediment in achieving higher productivity. Among the diseases of soybean, soybean rust incited by pathogen *Phakopsora pachyrhizi*, is major disease and reported to cause a yield loss of 20-80 percent (Bromfield, 1976) [3]. Soybean rust reduces yield through premature defoliation, decreasing the number of filled pods and by reducing the weight of seeds. 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) [4]. Apparently it is able to travel great distances *via* wind-borne spores. Also known as Asian rust, this fungal infection can defoliate soybean fields rapidly, often resulting in severe and sometimes total crop 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. Currently, the primary form of control is based on the use of fungicides of different classes and mode of action (Miles *et al*., 2007) [9]. The continuous use of these chemical fungicides may pose problem of development of resistance to pathogen in addition to health hazards. Therefore, development of high yielding rust resistant varieties is of prime importance. Scientists opined that solely breeding cannot solve soybean rust problem until a highly resistant or immune cultivar is available (Chan, 1977) [5].
The first step in host plant resistance will be the identification of stable resistant lines through screening available germplasm. Earlier study (Patil *et al*., 2004) [11] identified two soybean germplasm lines *viz*., EC 241778 and EC 241780 as promising sources 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). In this regard, it was necessary to identify new sources for resistance to soybean rust. Hence, the present study was aimed at screening exotic germplasm lines against *Phakospora pachyrhizi* resistance across different environments and

years to identify potential and stable sources of rust resistance.

Materials and Methods

Experimental material and layout

A set of 144 exotic germplasm lines along with highly resistant checks *viz*., DSb 21, EC 241780 and EC 241778 and highly susceptible check JS 335 collected from different geographical regions *viz*., AVRDC (Taiwan), ICAR-IISR (Indore), ARI (Pune), NBPGR (New Delhi) and JNKVV (Jabalpur). The experiment was executed in augmented block design (Federer, 1956) [6]. Each line was raised in one row of 5 m length with a spacing of 45 x 10 cm during *kharif* 2015 at Main Agricultural Research Station, University of Agricultural Sciences, Dharwad. The screening was carried out under rust prone condition (unprotected condition) without any fungicidal spray. Based on resistance reaction, twenty two lines were selected and further evaluated to confirm their resistance along with checks under natural epiphytotic condition at two hotspots for rust *viz*., Dharwad and Ugarkhurd. Each line was raised in one row of 5 m length with a spacing of 45 x 10 cm in two replications during *kharif* 2016. The lines were evaluated for productivity parameters (number of branches per plant, number of pods per plant, 100 seed weight, days to maturity and seed yield per plant) along with disease reaction at Dharwad location.

Inoculum preparation

The leaves from rust infected fields were collected and soaked overnight. In the morning uredospores were oozed out and the uredospore suspension was sprayed on all the entries at 45 and 55 days after sowing. In addition to this, leaf stapling technique was also followed.

Rust severity assessment

Type of the lesions and count for the number of lesions

Type of lesions may be either reddish brown or tan colour. Reddish Brown lesions may produce few urediospores, whereas Tan lesions may produce numerous urediospores. Based on the colour of lesions, the lines were scored either as resistant or susceptible (Bromfield, 1984) [4], (Pham *et al*., 2009) [12] and (Sharadha and Jahagirdar, 2015) [13]. The count of the number of lesions was taken per cm^2 of infected leaves from mid-vein and both sides of mid-vein. The lesion colour on the infected leaves was recorded in the form of Reddish Brown (resistant) and Tan (susceptible). The number of lesions per cm^2 square of infected leaves was recorded using a magnifying glass.

Severity of rust reaction and statistical analysis

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 1). Per cent disease index (PDI) was calculated and the data was analyzed using standard statistical procedures.

Table 1: Disease rating scale

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

$$\text{Per cent disease index (PDI)} = \frac{\text{Sum of numerical ratings}}{\text{Total number of leaves scored}} \times \frac{100}{\text{Maximum grade}}$$

Rate of development of disease (r)

The rate of development of disease (r) at different intervals was also calculated by following formula given by (Vander Plank, 1963) [14].

$$r = \frac{2.3}{t_2 - t_1}\left[\log\frac{X_2}{1-X_2} - \log\frac{X_1}{1-X_1}\right]$$

Where,

r = Apparent rate of infection or spread

X_1 = Per cent disease index at time t_1

X_2 = Per cent disease index at time t_2

t_2- t_1 = Time interval in days between the two consecutive observations

Area under disease progress curve (AUDPC)

Area under disease progress curve is an important feature associated with disease resistance. It is the area of graph under the line that depicts the progress of epidemics and is calculated using the formula by (Wilcoxson, 1975) [16].

$$AUDPC = \sum_{i=1}^{n-1} \frac{y_i + y_{i+1}}{2} \times (t_{i+1} - t_i)$$

Y_i and $Y_{i+1}$are the disease scores done at t_i and t_{i+1} time intervals.

Results and Discussion

Initial screening of 144 exotic germplasm lines including resistant and susceptible checks under rust prone conditions revealed that only one line EC 242104 (8.89%) with resistant checks *viz.*, DSb 21 (8.89%), EC241780 (8.89%) and EC 241778 (8.89%) scored disease grade 1 and was highly resistant. Nine lines *viz.*, EC 391336 (20.00%), EC 385243, EC 333934, EC 308334, EC 287754, EC 100031, EC 14476 with 24.44%, EC 250578 and EC 15966 with 20 % disease incidence recorded disease grade 3 and were classified as moderately resistant (Table 2 & Fig.1) and they also showed same reaction when screened under natural epiphytotic condition at two hotspots for rust *viz.*, Dharwad and Ugarkhurd during *kharif* 2016.

In general, the lines with a low initial per cent disease index invariably resulted with a low terminal disease index. PDI status at different intervals observed in lines EC 242104 with resistance checks DSb 21 and EC 241780 recorded 6.67 % at 65 DAS and 8.89 % at 85 DAS and EC 241778 recorded 4.44 % at 65 DAS and 8.89 % at 85 DAS followed by EC 391336 and EC 15966 which recorded 8.89 % at 65 DAS and 20.0 % at 85 DAS.

The apparent rate of infection was calculated and this has been widely used in identification of genotypes with low rate of disease development. The range of 'r' values among 144 germplasm lines ranged from 0.009 to 0.169 indicating the importance of infection rate in spreading the rust disease. The low average 'r' values indicate less rate of infection compared to higher values. Based on apparent rate of infection, germplasm lines EC 3251 (0.009) followed by EC 391346, EC 14426 (0.011), EC 685252 (0.017), EC 242104, DSb 21 and EC 241780 with (0.031) recorded lower 'r' values

indicating the rate of infection in these lines is very slow. Whereas germplasm lines EC 33917 (0.169) followed by EC 37937 (0.151), EC 95291 (0.124) and EC 95815 (0.114) recorded higher 'r' values indicating fast spread of disease in these lines.

The check JS 335 (0.074) and germplasm lines *viz*., EC 685250 (0.054), EC 39219 (0.054) and EC 242105 (0.054) having low apparent rate of infection actually recorded high disease infection at their early growth stage however infection rate was low. The germplasm lines EC 385243 (0.037), EC 391336 (0.047) and EC 308334 (0.060) having high apparent rate of infection registered very low level of disease infection at their early crop growth stage. However, once the infection started spread of the disease was fast. These results indicate the low apparent rate of infection which did not indicate the resistant level of the genotypes. The calculated 'r' values varied and at times they did not remain consistent for a given genotype and also did not show a particular trend in general. These observations are in agreement (Wilcoxson *et al.*, 1975) [16], (Nargund, 1989) [10] who have pointed out that 'r' values are not useful criteria for selecting the genotype, 'r' values indicate the progressive development of diseases and help in categorizing as slow or fast rusters. However, it can be used in studying the disease development in different genetic background.

The Area under Disease Progress Curve (AUDPC) revealed a wide variation among the different lines at different intervals. Among, the lines tested, the highest average AUDPC value was observed in the lines *viz*., JS 335 (944.44) followed by EC 685250 (855.55), EC 94625, EC 685255, EC 685252 (833.33). While, the least average AUDPC value was recorded in lines EC 241778 (66.67) followed by DSb 21, EC 242104, EC 241780 (83.33) and EC 15966, EC 391336 (150.00).

The exotic lines were screened in the field conditions for lesions count per cm^2 on mid-vein and both the sides of mid-vein of the infected leaves after 65 days of sowing. The line EC 242104 (5.0) followed by DSb 21 (6.87), EC 241780 (7.56), EC 241778 (8.73), EC 287754 (8.65) and EC 391336 (9.21) recorded least number of lesions while JS 335 (42.80) followed by EC 389178 (41.53), EC 917258 (38.20), EC 457406 (37.85) and EC 389400 (37.27) recorded highest lesion count. The lines EC 242104, DSb 21, EC 241780 and EC 241778 showed resistant reaction in the form of reddish brown reaction while dark tan colour appearance of lesions was shown by JS 335 signifying high susceptibility to rust. Earlier findings of (Miles *et al.*, 2003) [8], (Sharadha and Jahagirdar, 2015) [13] also indicated that the reddish brown lesion type is considered to be a resistant lesion type when compared to a fully susceptible tan lesion.

Table 2: Rust severity assessment in selected exotic germplasm lines during *kharif* 2015 at Dharwad

Sl. No.	Germplasm lines	PDI/ DAS			Grade (0-9 Scale)	Reaction	No. of lesions/ cm^2	Type of lesion	AUDPC/ DAS		Rate of spread 'r'/ DAS		Average 'r'
		65	75	85					65-75	75-85	65-75	75-85	
1	EC 100031	20.00	24.44	24.44	3	MR	9.23	RB	222.22	244.44	0.026	0.026	0.026
2	EC 14426	24.44	24.44	28.89	5	MS	19.27	TAN	244.44	266.67	0.000	0.023	0.011
3	EC 14476	15.56	24.44	24.44	3	MR	10.14	RB	200.00	244.44	0.056	0.056	0.056
4	EC 15966	8.89	15.56	20.00	3	MR	10.25	RB	122.22	177.78	0.063	0.031	0.047
5	EC 242104	6.67	8.89	8.89	1	HR	5.00	RB	77.78	88.89	0.031	0.031	0.031
6	EC 242105	77.78	82.22	91.11	9	HS	26.60	TAN	800.00	866.67	0.028	0.079	0.054
7	EC 250578	13.33	15.56	20.00	3	MR	9.82	RB	144.44	177.78	0.018	0.031	0.024
8	EC 287754	13.33	24.44	24.44	3	MR	8.65	RB	188.89	244.44	0.074	0.074	0.074
9	EC 308334	8.89	20.00	24.44	3	MR	9.23	RB	144.44	222.22	0.094	0.026	0.060
10	EC 3251	42.22	42.22	46.67	5	MS	20.32	TAN	422.22	444.44	0.000	0.018	0.009
11	EC 33917	42.22	64.44	95.56	9	HS	31.00	TAN	533.33	800.00	0.091	0.247	0.169
12	EC 333934	20.00	24.44	24.44	3	MR	9.45	RB	222.22	244.44	0.026	0.026	0.026
13	EC 37937	51.11	73.33	95.56	9	HS	26.33	TAN	622.22	844.44	0.097	0.205	0.151
14	EC 385243	13.33	20.00	24.44	3	MR	9.89	RB	166.67	222.22	0.048	0.026	0.037
15	EC 389178	55.56	68.89	73.33	7	S	41.53	TAN	622.22	711.11	0.057	0.022	0.039
16	EC 389400	77.78	82.22	86.67	9	HS	37.27	TAN	800.00	844.44	0.028	0.034	0.031
17	EC 39219	77.78	82.22	91.11	9	HS	32.67	TAN	800.00	866.67	0.028	0.079	0.054
18	EC 391336	8.89	15.56	20.00	3	MR	9.21	RB	122.22	177.78	0.063	0.031	0.047
19	EC 391346	68.89	68.89	73.33	7	S	22.20	TAN	688.89	711.11	0.000	0.022	0.011
20	EC 457406	73.33	82.22	86.67	9	HS	37.85	TAN	777.78	844.44	0.052	0.034	0.043
21	EC 685250	77.78	86.67	91.11	9	HS	30.07	TAN	822.22	888.89	0.062	0.045	0.054
22	EC 685252	82.22	82.22	86.67	9	HS	34.60	TAN	822.22	844.44	0.000	0.034	0.017
23	EC 685255	73.33	82.22	95.56	9	HS	23.87	TAN	777.78	888.89	0.052	0.153	0.103
24	EC 917258	73.33	77.78	91.11	9	HS	38.20	TAN	755.56	844.44	0.024	0.107	0.066
25	EC 94625	68.89	86.67	91.11	9	HS	32.53	TAN	777.78	888.89	0.108	0.045	0.077
26	EC 95291	64.44	82.22	95.56	9	HS	29.60	TAN	733.33	888.89	0.094	0.153	0.124
27	EC 95815	68.89	82.22	95.56	9	HS	36.33	TAN	755.56	888.89	0.074	0.153	0.114
28	EC 241778 (RC)	4.44	6.67	8.89	1	HR	8.73	RB	55.56	77.78	0.043	0.031	0.037
29	EC 241780 (RC)	6.67	8.89	8.89	1	HR	7.56	RB	77.78	88.89	0.031	0.031	0.031
30	DSb 21 (RC)	6.67	8.89	8.89	1	HR	6.87	RB	77.78	88.89	0.031	0.031	0.031
31	JS 335 (SC)	91.11	95.56	95.56	9	HS	42.80	TAN	933.33	955.56	0.074	0.074	0.074

*RC-Resistant check, SC- Susceptible check

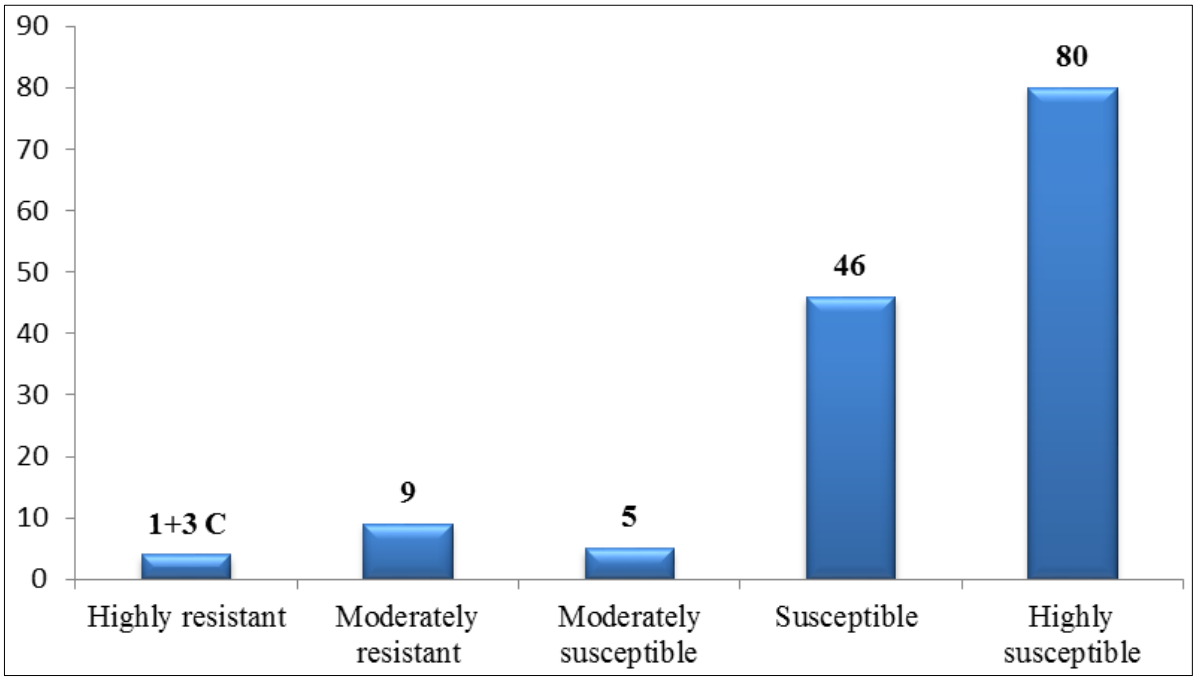

Fig 1: Disease severity for rust in soybean 144 exotic germplasm lines during *kharif* 2015 at Dharwad

Reaction of germplasm lines during *kharif* 2016 at Dharwad and Ugarkhurd

Based on the degree of rust resistance during *kharif* 2015, 22 promising lines were selected and evaluated to confirm their resistance reaction along with resistant checks (DSb 21, EC 241780 and EC 241778) and highly susceptible check (JS 335) under natural epiphytotic condition at hotspots *viz.*, Dharwad and Ugarkhurd during *kharif* 2016 (Table 3). Scoring of the disease was done by using 0-9 scale between 65 to 85 days after sowing based on percent leaf area infected at both the locations. Observations on productivity parameters (number of branches per plant, number of pods per plant, 100 seed weight, days to maturity and seed yield per plant) were recorded on five randomly selected plants.

Table 3: Disease severity for rust in soybean exotic germplasm lines with yield attributes during *kharif* 2016 at Dharwad and Ugarkhurd

Sl. No.	Genotypes	Disease scoring (0-9)				Yield attributing traits				
		Dharwad		Ugarkhurd						
		Grade	Reaction	Grade	Reaction	DM	NBP	NPP	100 SW	SYP
1	EC 14426	3	MR	3	MR	90	4.0	46.0	11.79	11.48
2	EC 15966	3	MR	3	MR	90	3.8	52.0	12.80	10.12
3	EC 16119	7	S	7	S	93	3.4	39.6	9.20	8.66
4	EC 33922	5	MS	5	MS	88	6.0	43.2	11.60	9.68
5	EC 100031	3	MR	3	MR	95	3.2	44.8	12.70	8.44
6	EC 118420	5	MS	5	MS	90	3.4	43.2	11.85	9.42
7	EC 149988	7	S	7	S	89	4.4	39.2	10.80	9.18
8	EC 175529	5	MS	5	MS	89	8.0	53.4	12.05	10.40
9	EC 221329	5	MS	5	MS	88	5.0	40.6	11.89	8.38
10	EC 242104	1	HR	1	HR	93	4.6	52.4	13.90	11.60
11	EC 250578	3	MR	3	MR	95	5.0	67.2	12.57	11.32
12	EC 251358	5	MS	5	MS	89	4.6	39.2	11.00	9.58
13	EC 251401	5	MS	5	MS	89	3.8	49.4	12.40	9.74
14	EC 257754	3	MR	3	MR	85	3.4	38.0	12.39	10.96
15	EC 308334	3	MR	3	MR	93	7.2	53.8	10.48	11.08
16	EC 325101	5	MS	5	MS	88	3.6	52.8	11.80	9.60
17	EC 325102	5	MS	5	MS	95	4.8	45.8	12.00	10.46
18	EC 333909	7	S	7	S	93	5.4	55.4	11.90	10.84
19	EC 333934	3	MR	3	MR	91	4.2	41.2	12.79	9.10
20	EC 385243	3	MR	3	MR	90	8.6	54.6	12.80	10.52
21	EC 391158	5	MS	5	MS	92	7.2	33.0	12.00	8.24
22	EC 391336	3	MR	3	MR	91	4.4	39.6	12.80	10.28
23	EC241778 (RC)	1	HR	1	HR	102	5.4	48.4	13.90	10.60
24	EC241780 (RC)	1	HR	1	HR	101	4.4	44.2	14.10	9.80
25	DSb 21 (RC)	1	HR	1	HR	92	4.4	54.8	14.00	15.72
26	JS 335 (SC)	9	HS	9	HS	84	3.4	43.4	8.10	9.30

*RC-Resistant check, SC- Susceptible check

DFF- Days to 50 % flowering; DM- Days to maturity; NBP- Number of branches per plant;
NPP- Number of pods per plant; 100 SW- 100 Seed weight (g); SYP- Seed yield per plant (g).

Only one line EC 242104 and resistant checks *viz.*, DSb 21, EC 241780, EC 241778 recorded grade 1 (highly resistant); nine lines with grade 3 (moderately resistant); nine lines with grade 5 (moderately susceptible); three lines with grade 7 (susceptible) whereas JS 335 exhibited highly susceptible reaction (grade 9). The resistant reaction of this line along with highly susceptible check JS 335 is depicted in Plate 1 in evaluation at Dharwad during Kharif 2016 and in large scale evaluation at Dharwad during *kharif*, 2016 (Plate 2).

In addition to rust resistance exhibited by EC 242104, it is early maturing (90-95 days) with more number of pods (52.4) compared to earlier reported rust resistant lines *viz.*, EC 241780 and EC 241778 which mature in 100-110 days with 44.2 and 48.4 pods, respectively. It recorded seed yield per plant of 11.60 g followed by EC 14426 (11.48 g), EC 250578 (11.32 g), EC 308334 (11.08 g) compared to the susceptible check JS 335 (9.30 g). Owing to its rust resistance nature EC 242104 recorded 13.90 g 100 seed weight compared to check JS 335 (8.10 g) wherein the reduced seed size is mainly due to its rust susceptibility. The identified exotic line EC 242104 is highly resistant to rust with earliness and on par seed yield. Hence, this line EC 242104 would be utilized as donor parent in future breeding programmes for development of rust resistant genotypes by gene pyramiding.

JS 335 (Highly susceptible) EC 242104 (Highly resistant)

Plate 1: Rust resistance reaction of EC 242104 with highly susceptible check JS 335 during *kharif* 2016 at Dharwad

JS 335 (Highly susceptible) EC 242104 (Highly resistant)

Plate 2: Rust resistant reaction of EC 242104 with highly susceptible check JS 335 in large scale plot at Dharwad (*kharif* 2016)

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