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SCREENING OF RECOMBINANT INBRED LINE (RIL) POPULATION OF GROUNDNUT (*Arachis hypogaea* L.) AGAINST STEM ROT DISEASE INCIDENCE (*Sclerotium rolfsii*)

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ABSTRACT

Stem rot of groundnut, caused by the necrotrophic pathogen *Sclerotium rolfsii*is a devastating soil borne disease. Its incidence has been increasing in the hot and humid groundnut growing regions since the past decade. Chemical and cultural practices have been under practice to manage this disease. However, these measures cannotfully control the pod losses incurred by the crop, owing to the non-uniform distribution of the pathogen. Host resistance to the disease appears to be the most feasible solution to control the disease. In the present study, hundred genotypes of a groundnut RIL population (ICGV 91114 X ICGV 86590) along with resistant and susceptible checks have been screened during *Kharif,*2021 under artificially inoculated controlled conditions in poly-house. Disease was assessed using percent mortality which was recorded at 15, 30, 45 and 60 days after inoculation (DAI). Analysis of variance revealed significant differences among the genotypes for 45 and 60 DAI indicating that these could be the best scoring times. It was observed that most of the genotypes were susceptible to the disease, while a very few were resistant and moderately resistant. The genotypes ICGN 184776, ICGN 184806, ICGN 184849, ICGN 184784, ICGN 184783, ICGN 184836, ICGN 184809, ICGN 184811, ICGN 184812, ICGN 184846, ICGN 184768 and ICGN 184823 were found to have considerable resistance against the disease. They have the potential to be considered for selections to be used as parents in crossing programs for the transfer of resistance to cultivated breeding lines.

Keywords: Stem rot, resistance, groundnut, recombinant inbred line population.

Cultivated groundnut or peanut (*Arachis hypogaea* L.) is an important oil seed and food legume crop. It has originated in South America and is now grown in more than 100 countries all over the world. China, India, Nigeria, the United States, Senegal, Myanmar,Indonesia, Sudan, Argentina, Ghana, and Vietnam are the primary groundnut producing nations, accounting for 84% of global groundnut output (Pasupuleti *et al.,* 2013). In India,it is grown in the states Andhra Pradesh, Telangana, Gujarat, Karnataka and Tamil Nadu. Andhra Pradesh, Telangana and Gujarat account for more than half of the country's groundnut growing area (DGR Annual Report, 2013). Globally, it is cultivated over an area of 32.7 Mha with a production of 53.9 Mt leading to a productivity of 1.6 t/ha (FAOSTAT, 2021).

Groundnut is rich in its nutritional value. Kernels contain 40-54% oil, protein content of 22-36% and carbohydrate content of 10-20%. It is also high in B vitamins such as thiamine (B1), riboflavin (B2), niacin (B3), and tocopherol (Nagaraj, 1995). From 100 g of kernels, 564 K calories of energy is supplied (Jambunathan, 1991). The seeds are high in mono-unsaturated fatty acids and include several health-promoting minerals, antioxidants and vitamins (Pasupuleti *et al.,* 2013). Groundnut haulms serve as fodder for livestock. The crop has a variety of industrial applications, including food, feed, paints, lubricants and pesticides (Variath*et al.,* 2017). Being a legume crop, groundnut improves soil health and fertility by releasing N_2 and organic matter into soil (Alagirisamy, 2016).

Frequently, groundnut cultivation in India is suffering a considerable yield loss as a result of biotic and abiotic stresses, which are the main obstacles in achieving higher productivity (Divya Rani *et al.,* 2018). Among the biotic factors, seed and soil-borne diseases have been identified as the major constraints affecting groundnut production (Palaiah *et al.,* 2019). Stem rot disease, caused by the necrotrophic fungus*Sclerotium rolfsii*, is an emerging soil borne disease of groundnut.

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It is a serious limitation to groundnut production in many warm and humid countries (Bera *et al*., 2014). Groundnut yield loss due to stem rot disease typically ranges from 10-40%, but can exceedto 80% in heavily infected fields (Mehan *et al.,* 1995). *S. rolfsii* also causes indirect losses such as decrease in the dry weight and oil content of groundnut kernels as well as decrease in the quality of pod and fodder (Bera *et al*., 2014).

Chemical and cultural practises are the primary control strategies utilised to manage soil-borne diseases (Krishnakanth *et al*., 1999). The pathogen persistence in the soil and its vast host range frequently hinder the efficiency of chemical and cultural management of soil-borne diseases (Palaiah *et al*., 2019). Host plant resistance offers the best possible solution for controlling stem rot disease in groundnut. Growing resistant cultivars against stem rot disease is also a cost-effective, long-term strategy that fits well into integrated disease management (Divya Rani *et al.,* 2018). Earlier studies have been conducted to screen the groundnut genotypes for stem rot disease resistance identification by using artificial inoculation techniques (Divya Rani *et al.,* 2018; Bera *et al.,* 2014; Palaiah *et al.,* 2019). However, a highly resistant line against this disease has not yet been identified. The pathogen's non-uniform geographic distribution complicates large-scale screening for resistance in segregating populations under field conditions with natural or artificial infection. As a result, consistent data is difficult to be generatedwhen screened only under field conditions (Bera *et al.,* 2016a). To overcome the barriers faced under field screening conditions, it is also essential to screen for the same genotypes under controlled/glasshouse conditions. Hence, the present study has been conducted with the following objectives: To screen groundnut RIL population under controlled conditions in the poly-house, to identify groundnut genotypes resistant against stem rot disease.

MATERIAL AND METHODS

The plant material comprised of RIL population (ICGV 91114 X ICGV 86590) with 100 genotypes including parents and checks. ICGV 91114 is the stem rot susceptible parent. It is a Spanish bunch line with short duration. ICGV 86590 is the resistant parent. It is also a Spanish bunch line with medium duration and reported to be foliar disease resistant (Divya Rani *et al*., 2018). CS 319 and ICGV 86856 were used as the resistant checks whereas TMV-2 and GG-20 served as susceptible checks. The list of genotypes included in the present study is presented in Table 2. The experiment was conducted during *Kharif,* 2021 under controlled conditions of temperature (26 ± 2°C) and humidity (80-90%) in the poly-house (23.8 m x 6.1m) at International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru. The pots were arranged in a completely randomized block design (CRD), plastic pots 22.8 of centimetre were utilised. The pots were filled with sterilised soil mix (red soil and sand in 3:2 ratio) and five seeds were planted per pot (one pot per genotype in each replication).

S. rolfsii, the stem rot pathogen had been mass multiplied on sorghum grains (Bera *et al*., 2016a). The sorghum grains served as a medium for the pathogengrowth and multiplication. Each of the plants in the pots were artificially inoculated at 35 days after sowing through the application of the pathogen that had multiplied on the sorghum grains. After inoculation, the pots were immediately watered for two days. The pots were maintained at 26 ± 2°C and 90% RH until they were harvested. Regular irrigations were given. The number of plants in each pots (number of germinated plants) were recorded prior to inoculation. The stem rot disease progress was assessed in terms of number of dead plants after every 15 days starting from the day of inoculation. Plant mortality was used to measure the disease in terms of number of dead plants. Percent mortality was calculated using the formula:

Percent mortality = (Number of dead plants/total number of plants before inoculation) *100. Later, the plants were scored based on the disease scale given by Bera *et al.,* (2016b). The scale is denoted as:

Table1: Disease scale designated for the classification of genotypes

Mortality range (%)	Scoring
< 10%	Highly resistant (HR)
10 - 19%	Resistant (R)
20 - 29%	Moderately Resistant (MR)
≥ 30%	Susceptible (S)

To assess the variability among the genotypes, analysis of variance (ANOVA) was performed using R software (ver. 4.2.1).

RESULTS AND DISCUSSION

Stem rot of groundnut is a complex disease and host plant resistance is the most effective method of managing this disease. Identification of resistant lines is the initial step towards breeding for disease resistance. A total of 100 groundnut lines of the RIL population ICGV 91114 X ICGV 86590 along with the checks (ICGV 86856, CS 319, TMV-2, CS 319) were screened under poly-house conditions. The results of the present study are described herewith.

It was revealed from the present study that the stem rot disease severity increased gradually from 15 DAI to 60 DAI and genotypes exhibited significant variation in the disease incidence. Table 2 represents the mean percent mortality (PM) at 15,30,45,60 DAI. The mean PM was between 0-62.5% at 15 DAI, 0-65.7% at 30 DAI, 0-100% at 45 DAI and 7.14-100% at 60 DAI. The percent mortality at 60 DAI (final observation) was used to assess the number of resistant genotypes identified through the study based on the scale to score the genotypes (Bera *et al*., 2016b). Only one genotype showed PM <10% and was rated as highly resistant (HR), two genotypes showed PM between 10-19% and were rated as resistant (R). The PM range for ten

Table 2: Trait means for the genotypes under study

Note: PM- Percent mortality; DAI- Days after inoculation, RC- Resistant check, SC- Susceptible check, P1- Parent1, P2- Parent 2

S No.	GENOTYPES	PM 15 DAI	PM 30 DAI	PM 45 DAI	PM 60 DAI
1	CS 319 (RC)	10.00	34.29	48.57	48.57
2	GG 20 (SC)	0.00	22.50	45.00	57.50
3	ICGN 184767	0.00	27.14	39.29	44.29
4	ICGN 184768	16.67	26.67	26.67	26.67
5	ICGN 184770	16.67	29.17	29.17	45.83
6	ICGN 184771	10.00	30.00	50.00	60.00
7	ICGN 184772	8.33	35.00	70.00	90.00
8	ICGN 184773	10.00	10.00	32.50	45.00
9	ICGN 184774	0.00	33.33	33.33	33.33
10	ICGN 184776	0.00	7.14	7.14	7.14
11	ICGN 184779	22.50	32.50	55.00	55.00
12	ICGN 184781	7.14	28.57	42.86	64.29
13	ICGN 184783	0.00	22.50	22.50	22.50
14	ICGN 184784	0.00	10.00	20.00	20.00
15	ICGN 184785	0.00	20.00	40.00	40.00
16	ICGN 184786	0.00	45.83	54.17	66.67
17	ICGN 184787	7.14	38.57	62.86	70.00
18	ICGN 184788	0.00	25.00	43.75	50.00
19	ICGN 184789	48.57	65.71	92.86	92.86
20	ICGN 184790	0.00	7.14	21.43	71.43
21	ICGN 184791	0.00	43.33	53.33	53.33
22	ICGN 184792	12.50	41.67	83.33	91.67

S No.	GENOTYPES	PM 15 DAI	PM 30 DAI	PM 45 DAI	PM 60 DAI
23	ICGN 184793	0.00	10.00	22.50	32.50
24	ICGN 184794	14.29	33.93	54.46	54.46
25	ICGN 184795	16.67	43.33	53.33	83.33
26	ICGN 184796	10.00	32.50	55.00	55.00
27	ICGN 184797	0.00	35.71	57.14	71.43
28	ICGN 184799	10.00	28.33	38.33	46.67
29	ICGN 184800	0.00	24.29	31.43	41.43
30	ICGN 184801	0.00	10.00	20.00	30.00
31	ICGN 184802	12.50	25.00	50.00	62.50
32	ICGN 184803	0.00	25.00	47.22	55.56
33	ICGN 184804	0.00	58.33	75.00	100.00
34	ICGN 184805	0.00	37.50	60.00	60.00
35	ICGN 184806	12.50	12.50	12.50	12.50
36	ICGN 184807	10.00	10.00	32.50	55.00
37	ICGN 184808	0.00	20.00	30.00	30.00
38	ICGN 184809	12.50	12.50	25.00	25.00
39	ICGN 184810	0.00	45.00	55.00	87.50
40	ICGN 184811	0.00	0.00	12.50	25.00
41	ICGN 184812	0.00	25.00	25.00	25.00
42	ICGN 184815	0.00	55.00	73.33	73.33
43	ICGN 184816	0.00	7.14	28.57	100.00
44	ICGN 184817	0.00	0.00	50.00	75.00
45	ICGN 184818	0.00	10.00	10.00	75.00
46	ICGN 184819	12.50	25.00	35.00	47.50
47	ICGN 184820	16.67	36.67	46.67	46.67
48	ICGN 184821	0.00	28.57	42.86	50.00
49	ICGN 184822	0.00	20.00	30.00	52.50
50	ICGN 184823	7.14	14.29	29.76	29.76
51	ICGN 184825	0.00	35.00	70.00	70.00
52	ICGN 184826	22.50	45.00	70.00	80.00
53	ICGN 184827	16.67	50.00	75.00	83.33
54	ICGN 184828	0.00	30.00	48.33	48.33
55	ICGN 184829	10.00	10.00	10.00	50.00
56	ICGN 184830	0.00	30.95	61.90	78.57
57	ICGN 184831	0.00	10.00	20.00	45.00
58	ICGN 184832	20.00	50.00	60.00	70.00

S No.	GENOTYPES	PM 15 DAI	PM 30 DAI	PM 45 DAI	PM 60 DAI
59	ICGN 184833	10.00	10.00	20.00	30.00
60	ICGN 184834	21.43	26.98	26.98	45.24
61	ICGN 184835	18.33	38.33	56.67	56.67
62	ICGN 184836	8.33	8.33	25.00	25.00
63	ICGN 184837	0.00	41.67	41.67	41.67
64	ICGN 184838	0.00	50.00	70.00	90.00
65	ICGN 184839	62.50	62.50	75.00	75.00
66	ICGN 184841	0.00	40.00	60.00	70.00
67	ICGN 184842	12.50	33.33	41.67	75.00
68	ICGN 184843	16.67	25.00	51.67	51.67
69	ICGN 184844	0.00	39.29	58.93	73.21
70	ICGN 184845	0.00	7.14	13.39	64.29
71	ICGN 184846	0.00	8.33	16.67	25.00
72	ICGN 184847	8.33	22.62	38.10	38.10
73	ICGN 184848	16.67	55.56	69.44	75.00
74	ICGN 184849	0.00	0.00	0.00	12.50
75	ICGN 184850	13.39	32.14	45.54	52.68
76	ICGN 184851	0.00	41.43	58.57	58.57
77	ICGN 184852	0.00	18.75	37.50	68.75
78	ICGN 184853	0.00	16.67	16.67	33.33
79	ICGN 184854	0.00	35.00	43.33	81.67
80	ICGN 184856	0.00	25.00	50.00	50.00
81	ICGN 184857	0.00	29.76	60.71	75.00
82	ICGN 184858	0.00	51.25	83.75	90.00
83	ICGN 184859	0.00	60.00	80.00	100.00
84	ICGN 184860	0.00	16.67	66.67	66.67
85	ICGN 184861	8.33	33.33	50.00	58.33
86	ICGN 184863	16.67	58.33	70.83	83.33
87	ICGN 184864	25.00	50.00	100.00	100.00
88	ICGN 184865	0.00	28.33	38.33	46.67
89	ICGN 184866	0.00	41.67	66.67	83.33
90	ICGN 184867	0.00	7.14	7.14	31.43
91	ICGN 184868	0.00	21.43	41.07	53.57
92	ICGN 184869	10.00	32.50	55.00	55.00
93	ICGN 184870	12.50	29.17	70.83	70.83

genotypes was 20-29%, they were rated as moderately resistant (MR). Eighty-six genotypes of the hundred under study were rated as susceptible (S) with their PM e"30% (Table 3). This indicates that most of the genotypes were susceptible to the disease, while a very few were resistant and moderately resistant.

Stem rot of groundnut is an emerging disease among the groundnut growing regions. As mentioned, host plant resistance offers a long-term solution against the disease and no highly resistant breeding line has been identified to fight against this disease. Hence, screening of germplasm lines is an essential and initial step towards disease resistant breeding. It can be done in the field and/or glasshouse (controlled) conditions. The present study was conducted in a poly-house under controlled conditions. Screening for diverse genetic materials in the field conditions is a valuable selection approach for identifying truly resistant/tolerant genotypes (Guclu *et al*., 2020). Certain genotypes (ICG 12083) have showed field resistance but are less resistant in greenhouse experiments (Singh *et al.,*1997). To discover and characterise resistance components, promising genotypes should be investigated in field, micro plot and greenhouse conditions (Bera *et al.,* 2014). Hence the genotypes from the current study can be further evaluated for stem rot disease under field conditions to further confirm the truly resistant lines. After confirmations from the field and glasshouse studies, elite lines with significant resistance responses to the disease can be employed as parents in breeding programs to transfer this resistance into cultivable germplasm (Divya Rani *et al.,* 2018).

Table 3: Number of lines identified under each class based on percent mortality@60DAI (days after inoculation)

Number of genotypes	Mortality range	Scoring
1	<10%	HR
2	10-19%	R
10	20-29%	MR
86	≥30%	S

Similar results where a very few resistant lines and a large number of susceptible lines were identified have been reported by earlier researchers (Krishnakanth *et al*., 1999). The designations of the genotypes under each scoring category are represented in Table 4. The susceptible check TMV -2 showed PM of 75% whereas the resistant check ICGV 86856 showed PM of 20%. In comparison to the resistant parent ICGV 86590, a total of twelve genotypes recorded PM <30% at 60 DAI (Table 6 and Figure 1).

Analysis of variance was performed to assess the variation among the genotypes for the PM at 15,30,45 and 60 DAI. It was revealed from the ANOVA that the genotypes showed highly significant variation for PM at 45 DAI (1% level of significance). At 60 DAI, the genotypes showed a significant variation (5% Level of significance). There was no significant variation among the genotypes for PM at 15 and 30 DAI. Significant variations were observed among the genotypes at 45 and 60 DAI, this indicates that the PM observations taken at 45 and 60 DAI could be the best ones to assess the disease among the genotypes under study (Table 5). From the present study, the genotypes ICGN 184776 (HR); ICGN 184806, ICGN 184849 (R); ICGN 184784, ICGN 184783, ICGN 184836, ICGN 184809, ICGN 184811, ICGN 184812, ICGN 184846, ICGN 184768 and ICGN 184823 (MR) could be considered to be the best against the stem rot disease among all the genotypes under study.

CONCLUSION

Screening of 100 groundnut genotypes under poly-house conditions revealed that the genotypes were highly variable for the disease and the best time to score for the disease is at 45 and 60 days after inoculum application. It was observed from the study that most of the lines were susceptible to the disease. However, a set of lines with some degree of resistance could be identified. Since stem rot disease is highly

Table 4: The genotypes under each scoring category of disease score

	Percent mortality (%) at 60 DAI			
Classification	**<10%**	**10-19%**	**20-29%**	**≥ 30%**
Genotypes	ICGN 184776	ICGN 184806, ICGN 184849	ICGN 184784, ICGV 86856, ICGN 184783, ICGN 184836, ICGN 184809, ICGN 184811, ICGN 184812, ICGN 184846, ICGN 184768, ICGN 184823	ICGN 184801, ICGN 184808, ICGN 184833, ICGV 86590, ICGN 184867, ICGN 184793, ICGN 184774 ICGN 184853, ICGN 184847, ICGN 184785, ICGN 184800 ICGN 184837, ICGN 184767, ICGN 184773, ICGN 184831 ICGN 184834, ICGN 184770, ICGN 184799, ICGN 184820, ICGN 184865, ICGN 184819, ICGN 184828, CS 319 ICGN 184788, ICGN 184821, ICGN 184829, ICGN 184856, ICGN 184843, ICGN 184822, ICGN 184850, ICGN 184791, ICGN 184868, ICGN 184794, ICGN 184779, ICGN 184796, ICGN 184807, ICGN 184869, ICGN 184803, ICGN 184835, GG 20, ICGN 184861, ICGN 18485, ICGN 184771, ICGN 184805, ICGN 184802, ICGN 184781, ICGN 184845, ICGN 184860, ICGN 184786, ICGN 184852, ICGN 184787, ICGN 184825, ICGN 184832, ICGN 184841, ICGN 184871, ICGN 184870, ICGN 184797, ICGN 184790, ICGN 184844, ICGN 184815, ICGN 184817, ICGN 184818, ICGN 184839, ICGN 184842, ICGN 184848, ICGN 184857, TMV 2, ICGN 184830, ICGN 184826, ICGN 184854, ICGN 184795 ICGN 184827, ICGN 184863, ICGN 184873, ICGN 184866, ICGN 184810, ICGN 184874, ICGN 184772, ICGN 184838, ICGN 184858, ICGN 184792, ICGN 184789, ICGN 184804, ICGN 184816, ICGN 184859, ICGN 184864

Table 5: Analysis of variance (ANOVA) for the disease assessment traits

Note: PM – Percent mortality, DAI- days after inoculation, Rep- Replications, Df- degrees of freedom, Sum sq- sum of squares, Mean sq- mean sum of squares

'**'@1%LOS, '*'@5%LOS; LOS- level of significance

		Df	Sum Sq	Mean Sq	F value	Pr(>F)
PM15DAI	Rep	1	1.8	1.755	0.0098	0.9214
	Genotypes	96	20754.5	216.192	1.2049	0.1813
	Residuals	96	17224.5	179.422		
PM30DAI	Rep	1	38	38.43	0.0994	0.7532
	Genotypes	96	46783	487.32	1.2605	0.1293
	Residuals	96	37115	386.62		
PM45DAI	Rep	1	260	260.31	0.399	0.52908
	Genotypes	96	88802	925.02	1.418	0.04435**
	Residuals	96	62624	652.33		
PM60DAI	Rep	1	0	0.32	0.0004	0.9832
	Genotypes	96	95018	989.77	1.3724	0.06136*
	Residuals	96	69237	721.22		

Table 6: Best genotypes identified based on mean PM at 60 DAI in comparison to the parents and checks

GENOTYPES	PM@60 DAI
ICGV 86590(Parent2)	30.95
TMV 2 (Susceptible check)	75.00
ICGV 86856 (Resistant check)	20.00
ICGN 184776	7.14
ICGN 184806	12.50
ICGN 184849	12.50
ICGN 184784	20.00
ICGN 184783	22.50
ICGN 184836	25.00
ICGN 184809	25.00
ICGN 184811	25.00
ICGN 184812	25.00
ICGN 184846	25.00
ICGN 184768	26.67
ICGN 184823	29.76

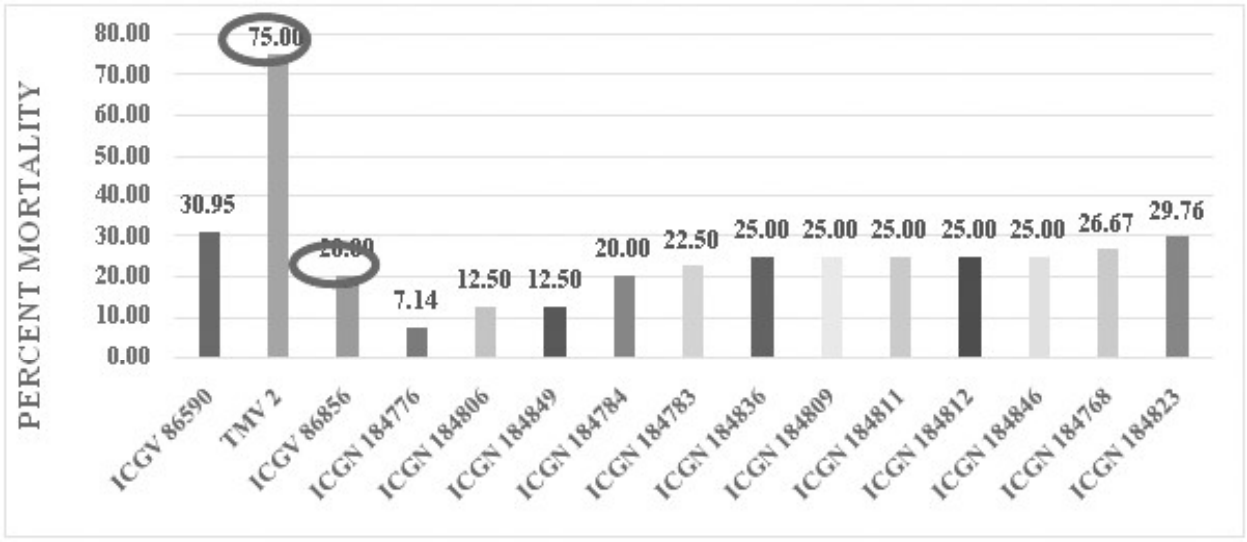

Figure 1. Representation of the best genotypes (PM at 60 DAI < 30%) in comparison to the parents and checks

variable under different environmental conditions, it is further required to screen these lines under field conditions to confirm the resistance levels. The identified lines after further confirmations could be used as parents in breeding programs to transfer the resistance trait to the cultivated varieties.

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