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Study of Glycine Soja Resistance to Aphis glycines. I. Screening of Resistant Varieties*

Derong Yue[1] Shougui Guo[2] Yulian Shan[2]
([1]*Institute of Plant Protection, Jilin Academy of Agricultural Sciences*)
([2]*Institute of Soybean, Jilin Academy of Agricultural Sciences*)

Abstract

A total of 985 wild and semi-wild soybean accessions were field screened for resistance to the aphid. The investigation involved inoculating *A. glycines* in cage houses, and was carried out for two years. We identified that three wild soybeans 85-32, 85-39 and 85-1 had resistance characters on *A. glycines*, whose resistance level was identified to be "High Resistance (HR)". The aphid resistance characters in these three materials were higher than those found in cultivated soybean materials. The damage index difference significance level test and the observed growth rate of aphis populations also further demonstrated an obvious difference in the resistance characters between the wild soybeans and *A. glycines*; simultaneously, they validated the aphid resistance characters in the above three materials.

Aphis glycines is one of the most serious pests of soybeans in northern China, especially in the northeastern soybean-producing areas, causing huge losses. The soybean loss per year usually makes up 5 – 10%, and it can reach above 30% in years of mass outbreaks. *A. glycines* is also the main transmission vector of soybean mosaic virus. Screening for aphid resistance and its sources began after the 1970s in our country. But among cultivated soybeans, no source of high resistance to *A. glycines* was found except several glabrous soybean varieties with certain aphid resistance characters. Wild soybeans are one of *A. glycines'* hosts [2]. Wild soybean resources are very abundant in our country. The large-scale germplasm resource investigation and collections were started at the end of 1970s and the beginning of the 1980s[3]. Many scientists have performed extensive research related to physiological biochemistry, pattern ecological characteristics and genetics in recent years and achieved significant progress [1]. But there is little research on aphid resistance characters of wild soybeans in our country, and no formal

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research has been reported so far. The purpose of this research was to screen out the genotypes of high resistance from wild soybean resources and carry out research on the mechanisms and genetic aspects of resistance. This paper reports the first part of this research, namely, the results of screening for resistance.

Investigation materials and identification methods

1. Preliminary field screening

Test materials: 932 wild soybeans and 53 semi-wild soybeans collected by the materials group, Soybean Institute, Jilin Academy of Agricultural Sciences. Materials were planted along one row with a row length of 5 meters, and plant and row spacings of each. The investigation was conducted during the optimal outbreak period (July 7-8) of *A. glycines*. Damage levels on each plant and the number of plants on which there were *A. glycines* were investigated, and the damage index could be calculated using the following formula:

Damage index=($\sum$(representative value of damage level * plant number at relative levels)/(4*total plant number investigated))*100%

Then, according to the damage index distribution range of all materials, the resistance characters of each material were divided into five levels: Highly Resistant (HR), Resistant (R), Moderately Resistance (M), Susceptible (S), High Susceptible (HS).

The plant infection level-grading standard is as follows:

Representative value of level	Plant exterior response and aphid population
Level 0	The plants develop normally with no aphids on whole plants;
Level 1	The plants develop normally with sporadic aphids (c. less than 100)
Level 2	The plants develop almost normally with some aphids on the top caulicle and young leave (about 101~300);
Level 3	Honey dew appears on the leaf surfaces; the leaves are a little curled; many aphids are scattered on the caulicle and young leaves (about 301~800);
Level 4	The plants are dwarfish, the leaves change to yellow and are very frizzy, the aphid amount is above 801.

The preliminary screening in fields was done in 1984.

2. Artificial inoculation screening

Test materials: In 1985, artificial inoculation screening was carried out. The soybean material genotypes used were 31 85-1s with high resistance etc. in the preliminary field screening and 9 genotypes with high susceptibility such as 85-18s etc. as a contrast. In

1986, the inoculation screening test materials were 10 85-1s that exhibited high resistance and aphid resistant genotypes from the inoculation screening from the last year and 5 control genotypes that exhibited high susceptibility such as 85-18s; 8 cultivated soybeans that exhibited aphid resistance in fields such as SunWuXiaoBaiMei and ZhongShengLuo etc. were also used.

Test methods: Sowing was done in early May. Each genotype was planted in one row with a plant spacing of 20cm and row spacing of 60cm; there were 10 plants in each row and there was no replication. When the plants grew to 20cm height, then bamboo poles were used to support them. All test and control materials were covered under a large net cage covering an area of 40 *mu* [666.7m^2]; the size of the cage house was 20m long x 5.4m wide x 1.8m high. All aphids, natural enemies of aphids, and other pests on plants were removed manually before the net was fixed. The leaf-clipping method was used to inoculate aphids until the second compound leaf in the plants fully unfurled. All aphids were mass-cultured in advance on Jiunong No. 9 cultivated soybeans; 20 *A. glycines* nymphs that developed almost uniformly were inoculated on each plant. The damage levels and number of plants on which there were *A. glycines* were investigated every 5 days after inoculation, and the damage indices were calculated. Then, based on the highest damage index of the genotypes, the levels could be identified while referring to the susceptible checks.

A non-buckling net screening was conducted in 1986. This was a replicated field experiment in which a cage structure without any buckling device was used. Each genotype was planted on one row with a plant spacing of 45cm and row spacing of 60cm; there were 10 plants in each row and they were replicated four times. The design was in randomized blocks. The aphid inoculation period and the numbers were the same as the buckling net inoculation screening. The damage levels and the plant number on which there were *A. glycines* were observed on July 7-8, and the damage indices were calculated. Analysis of variance was done after the damage indices of each material and each replicate were transformed by $\sin^{-1}$.

3. Observations of growth rate of aphid populations on different materials

Materials: Highly resistant genotypes, 85-39 and 85-32; resistant genotype, 85-38; and highly susceptible genotype, 85-16.

Methods: The above materials were planted in glass tubes with a length of 5cm and diameter of 4cm. One end of the tube was sealed using copper mesh, and wet absorbent-cotton was laid under the mesh to plant the seeds within. The other end was sealed using nylon yarn with 40 *mu* to prevent aphids from escaping. One variety was planted in each tube, replicated four times. 5 young aphids that had developed almost consistently were inoculated on each plant after the true leaves appeared. The aphid source for inoculation was the offspring that had been reproduced by the same aphid on the Jiunong No. 9 cultivated soybeans. The aphid amount was investigated daily the second day after inoculation. The experiment

was carried out under the conditions of 12 hours illumination and 25℃ (day temperature)/ 20℃ (night temperature) in an artificial climate box.

Results and discussion

1. Preliminary field screening

Table 1 shows that the aphid resistant and susceptible materials had almost the same number as the highly resistant and highly susceptible materials in the fields. And almost half of the genotypes exhibited moderate resistance or susceptibility.

Table 1 Preliminary screening results of 985 wild and semi-wild *A. glycines*in fields (Gongzhuling, 1984)

Resistance level / Unit number / Item	HR	R	M	S	HS	Total
Unit number	36	217	435	256	41	985
Percentage	3.7	22.0	44.2	26.0	4.2	100.0

Table 2 Distribution table of wild and semi-wild soybeans' preliminary screening in fields (Gongzhuling, 1984)

Resistance level	Wild soybeans		semi-wild soybeans	
	Unit number	Percentage (%)	Unit number	Percentage (%)
HR	36	3.9	0	0.0
R	216	23.2	1	1.9
M	430	46.1	5	9.4
S	232	24.9	24	45.3
HS	18	1.9	23	43.4
Total	932	160.0	53	100.0

Aphid density in the experimental space was relatively low and had no consistency under preliminary field screening conditions, which might cause some materials to generate pseudo-resistance phenomena. It is generally believed that preliminary screening might only remove many non-resistant character materials, but without ascertaining the resistant characters of materials

Among the 53 semi-wild soybeans in the preliminary field screening, there were no high resistant materials; 1 resistant material; and 47 susceptible and highly susceptible aphid materials, which made up 88.7% of the total amount. The resistance level distribution of the semi-wild soybeans was distinctly different from that of the wild soybeans (see Table 2). This seems to indicate that the aphid resistance level of this middle evolutionary type between wild and cultivated soybeans --semi-wild soybeans-- has a declining trend compared

with that of wild soybeans.

2. Artificial inoculation screening

In the cage house screening of 1985, 6 out of 31 genotypes that showed high resistance in preliminary field screening continued to exhibit high resistance: 85-1, 85-4, 85-29, 85-39, 85-32 and 85-38. Four out of 31 materials were aphid resistant: 85-31, 85-20, 85-40 and 85-34. The total of these two kinds were 10, which marked 32.3% of the total genotypes. The 21 remaining materials were moderately resistant (12), susceptible (1), or highly susceptible (8) respectively (see table 3).

Table 3 Response of 31 wild soybeans to inoculating aphid in cage houses (Gongzhuling, 1985)

Material No.	Damage index (%)	Resistance level	Preliminary field screening level	Material No.	Damage index (%)	Resistance level	Preliminary field screening level
85-1	50.0	HR	HR	85-21	60.7	M	HR
85-2	100.0	HS	HS	85-22	85.1	HS	HS
85-3	55.0	M	HS	85-23	65.0	M	HR
85-4	47.0	HR	HR	85-24	100.0	HS	HR
85-5	100.0	HS	HR	85-25	100.0	HS	HS
85-6	65.0	M	HR	85-26	72.2	M	HR
85-7	65.0	M	HR	85-27	60.1	M	HR
85-8	65.0	M	HR	85-28	60.1	M	HS
85-9	100.0	HS	HR	85-29	45.0	HR	HR
85-10	100.0	HS	HR	85-30	100.0	HS	HS
85-11	100.0	HS	HR	85-31	50.1	R	HR
85-12	72.5	M	HR	85-32	44.4	HR	HR
85-13	100.0	HS	HS	85-33	62.5	M	HR
85-14	62.5	M	HR	85-34	52.7	R	HR
85-15	100.0	HS	HR	85-35	100.0	HS	HR
85-16	100.0	HS	HR	85-36	62.5	M	HR
85-17	100.0	HS	HS	85-37	75.0	M	HR
85-18	82.5	S	HS	85-38	41.6	HR	HR
85-19	77.5	S	HR	85-39	37.5	HR	HR
85-20	57.5	R	HR	85-40	52.5	R	HR

Note: Resistance level range:

Resistance level	HR	R	M	S	HS
Damage index range	0.0-50.0	50.1-60.0	60.1-75.0	75.1-85.0	85.1-100.0

The conditions of an aphid outbreak were created using buckled net [4]. The aphid density in the cage house was even higher than that in an outbreak year under

natural field conditions, which made the different values for damage indices of various resistant materials increase. In the experiment, the lowest damage index was 37.5% and the highest damage index was 100.0%; the difference value was 62.5% (see Table 3). In this case, the resistance characters and sense characters are easily identified. Seen from the exterior appearance of the plants, although there were few aphids parasitizing aphid resistant materials, they grew normally. The aphid susceptible materials stopped growing, and their leaves were curled and crimpled when there was high-density aphid infestation. Also, their leaf surfaces were black gray due to mildews.

In the cage house inoculating aphid identification in 1986, the damage indices of partial materials increased compared with those in the previous year. The five control lines that were observed to be aphid susceptible in two years were almost consistent (see Table 4), which perhaps resulted from the situation where aphid density in the cage house was too high in 1986. Three high resistance and aphid resistance materials 85-32, 85-38 and 85-39 were sown by plant series in 1986. Totally 22 rows made up over half the total cage house area. But these three materials were sown into only three rows in 1985 (the area of the cage house being the same as in 1986). When highly resistant materials were selectively grown in an area, more aphids might infest some materials with relatively low resistant characters and increase the damage indices.

Table 4 Response of 10 wild soybean to *A. glycines* in cage house (Gongzhuling, 1986)

Material No.	Damage index (%)	Resistance level	Resistance level in 1985
85-1	43.8	HR	HR
85-4	50.1	R	HR
85-29	79.2	S	HR
85-39	44.0	HR	HR
85-32	43.9	HR	HR
85-38	56.6	R	HR
85-31	50.1	R	R
85-20	75.0	M	R
85-40	88.9	HS	R
85-34	69.4	M	R
85-16	78.6	HS	HS
85-13	100.0	HS	HS
85-9	100.0	HS	HS
85-18	100.0	HS	HS
85-2	97.5	HS	HS
SunWuXiaoBaiMei	100.0	HS	
LvZhaDou	100.0	HS	
AnDongFuShou	100.0	HS	
ZheDa455	100.0	HS	
ZhongShengLuo	100.0	HS	

GuoYu98-2	110.0	HS	
GuoYu98-4	55.5	R	
GuoYu100-4	55.5	R	

Note: Resistance level range is the same as that in 1985. The Chinese words in the table are the names of the cultivated soybeans.

Among the 8 cultivated soybeans that were found to be aphid resistant in fields, only Guoyu98-4 and Guoyu100-4 were still resistant. All others were seen to be highly susceptible in the cage house inoculation experiment (see Table 4).

Based on the above results, we think the number of genes that could resist *A. glycines* in the wild soybeans is higher than that in the cultivated soybeans. The Soybean Institute, Jilin Academy of Agricultural Sciences evaluated aphid resistant characters of 2073 cultivated soybeans; only 10 soybean varieties were aphid resistant, and none was highly resistant. Only 2 varieties were still aphid resistant after 8 out of 10 varieties were subjected to inoculation experiments repeatedly. The selection rate of the aphid resistant and highly resistant materials was no greater than 0.19%, and the resistance level was also relatively low. In our experiment, the selection rate of the aphid resistant and resistant highly materials of wild soybeans was 0.65, and the resistance character level was also higher than that of cultivated soybeans.

We summarized the results of the cage house inoculation experiment in two years and with reference to the preliminary field screening, the generally evaluated resistance levels of the following 10 materials are as follows (see Table 5).

Table 5 Comprehensive evaluation of 10 wild soybeans for aphid resistance (1986)

Material No.	Cage houses investigation in 1985		Cage houses investigation in 1986		Overall resistance level	Preliminary filtering resistance level in 1984
	Damage index (%)	Resistance level	Damage index (%)	Resistance level		
85-1	50.0	HR	43.8	HR	HR	HR
85-4	47.5	HR	50.1	R	R	HR
85-29	45.0	HR	79.2	S	S	HR
85-39	37.5	HR	44.0	HR	HR	HR
85-32	44.4	HR	43.9	HR	HR	HR
85-38	41.6	HR	56.6	R	R	HR
85-31	50.1	R	50.1	R	R	HR
85-20	57.5	R	75.0	M	M	HR
85-40	52.5	R	88.9	HS	HS	HR
85-34	52.7	R	69.4	M	M	HR

The overall resistance level [column 6] was evaluated using the experiment on

which the damage index was the highest.

In the replicated non-buckled cage inoculation experiment in fields, the outbreak of aphids was very low. Although the damage indices of the highly resistant materials were distinctly different from those of the non-highly resistant materials, the difference in the damage indices among the non-highly resistant materials was not remarkable (see Table 6). The damage indices of the highly resistant and aphid resistant materials in the non-highly resistant materials were usually lower than those of other kinds of materials, which were basically consistent with the results of the cage house experiment (see Table 6).

Table 6 Mean comparison of 15 wild soybeans' damage indices (Gongzhuling, 1986)

Material No.	Damage index (%)	Significance level (5%)	Overall resistance level	Remark
85-13	48.6	a	HS	1. The damage indices are the average values of 4 repetitions. 2. In ANOVA $F_{0.01}=2.48$; Processing: F=7.7; Repetition: F=0.39;
85-16	47.7	a	HS	
85-18	47.5	a	HS	
85-9	46.6	a	HS	
85-2	46.2	a	HS	
85-29	32.0	b	M	
85-40	31.6	b	S	
85-34	30.2	b	M	
85-38	30.0	b	R	
85-1	29.7	b	HR	
85-4	27.3	b	R	
85-20	26.1	b	M	
85-39	25.7	b	HR	
85-31	25.7	b	R	
85-32	25.0	b	HR	

3. The growth rate observation of *A. glycines* populations on different materials

The population growth rate of aphids that parasitized seedlings of susceptible materials was obviously higher than that on the aphid resistant materials. The average aphid population on each plant was 23 after the susceptible accession 85-16 was inoculated for 9 days. In the same period, it was 1.8 on 85-39, 4.5 on 85-38 and 5.3 on 85-32 (see Table 7).

Table 7 Growth rate of *A. glycines* on different soybean accessions (Gongzhuling, 1986)

Day number / Plant number / No.	1	3	5	7	9	11	13	15	17	19
85-39	3.2	1.8	1.3	2.0	1.8	2.3	1.3	3.3	1.5	2.5
85-38	3.3	2.0	3.5	3.3	4.5	5.8	7.3	7.3	7.3	5.0

85-32	3.0	2.3	1.0	3.0	5.3	3.0	3.3	3.3	3.3	4.7
85-16	5.3	7.7	8.0	14.0	23.0	23.3	20.3	13.0	22.7	33.0

The aphid resistant level observed on wild soybean in the seedling period was almost consistent with the resistant characters of fully-grown plants. The above results also further validated the results of the cage house and the aphid inoculation experiment in fields.

Conclusions

1. The responses of different wild soybean materials to damage from *A. glycines* were different from one other. The damage indices of the aphid resistant materials were obviously lower than those of the susceptible materials. The highest difference value between these two groups of material damage indices reached 62.5% under high-density aphid infestation in the cage house. The growth rate observation results of the *A. glycines* also validated the existence of such differences.

2. Three wild soybeans -- 85-39, 85-32 and 85-13 -- had very high aphid resistance characters, which had been identified as Highly Resistant in our grading method of resistance levels. Their resistance character levels were also higher than those of the aphid resistant materials that were screened from the current cultivated soybeans. In addition, three wild soybeans -- 85-38, 85-31 and 85-4 -- also had some resistant characters, which could be identified as aphid resistant.

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