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Technique and Method for Resistance Evaluation of Wild Soybean (*Glycine soja*) to Soybean Aphids

DERONG YUE, SHOUGUI GUO, YULIAN SHAN
(*Jilin* Academy of Agricultural Sciences, China)

ABSTRACT Visual evaluation is a convenient and efficient way to identify and grade the aphid resistance of *Glycine soja*. Inoculating aphids to net covered plants can greatly increase the aphid numbers in the tested plants and improve the reliability of resistant evaluation results. The needed aphids could be reproduced in advance on soybean plants. The responses of wild soybean to aphid infestation were the same during both the seedling and flowering stages.

In order to establish an accurate and convenient method for identification of aphid resistance in *Glycine soja*, we conducted research work on aphid resistance of wild soybean as well as studied the methods for aphid resistance identification since 1984. There is no such work in the literatures both in China and abroad. We referenced to the identification methods for aphid resistance used for other crops during our research, and conducted our study on the following four aspects.

1. The comparison between counting method and estimation method

There are many counting aphid methods, which include direct count, time (second)-counting, volume-counting methods etc. Nevertheless, none of these methods meets the requirements for fast screening of large amount of materials. By referencing to the aphid resistant identification method for cultivated soybean, we visually evaluated every wild soybean plant for its severity of infestation, and then converted the severity of infestation into damage index by the formula below. Five resistance grades were defined according to the damage index of the tested materials: I highly resistance, II resistance, III normal, IV susceptible, and V highly susceptible.

$$\text{Damage index} = \frac{\sum(\text{severity grade of infestation} \times \text{plant number at such severity level})}{4 \times \text{total plant numbers}} \times 100$$

According to the characteristics of aphid infestation on wild soybean, we classified the severity of aphid infestation into five grades by evaluating both the appearance of the infested plants and the aphid numbers in the plant:

Grade 0: Plant grows normally and no aphid is found;

Grade 1: Plant grows normally, yet a few aphids are found (aphid number < 100);

Grade 2: Plant shows normal growth with many aphids on the tender stems and leaves (aphid numbers are from 101 to 300);

Grade 3: leaves slightly curl with some honeydew on surface, and aphid numbers are about 301-800;

Grade 4: plants are dwarfish, leaves severely curl and turn yellow, and aphid number is over 801.

In 1985, we conducted an experiment in a climate controllable chamber and identified the resistant grade of 17 wild soybean materials by both calculating method and visual method.

The 17 wild soybeans as 85-13, 85-19 etc. were sowed in a climate controllable chamber on September 5. Each material had five plants. The day/night temperatures were 25°C/20°C, and the illumination time was 15 hours/day. When the first compound leaf stretched out, five aphids at similar development stage were inoculated on each plant. The resistant grades of the tested materials were evaluated by the two methods described above on October 10. Results are shown in Table 1.

Data in Table 1 showed that the resistant grades of the tested materials evaluated by the two methods were essentially consistent. The resistant grades of nine materials were the same by both calculating and visual evaluation. Seven materials had a difference of one resistant grade when evaluated by the two methods. Only one material showed a difference of two resistant grades between the two evaluation methods

Table 1. Resistant grades of 17 materials by the two methods (1985, *Gongzhulin*g)

Material No.	Calculated resistant grade	Visual resistant grade
85-13	V	V
85-39	III	I
85-32	I	I
85-36	IV	IV
85-19	IV	IV
85-2	V	V
85-4	I	II
85-1	I	II
85-34	II	II
85-14	II	III
85-18	III	IV
85-31	I	II
85-17	V	V
85-15	III	III
85-9	III	III
85-38	II	I
85-16	III	IV

2. The reproduction of source aphids

The major insect pest of *G. soja* in *Gongzhuling* region, China, is *Aphis glycines*. Because wild soybean has less tender stems and leaves for aphid to feed on, and it is more difficult to grow wild soybean, thus, it is not practical to propagate *A. glycines* on wild soybean and use them as source of inoculation. Then, reproduction of large amount of *A. glycines* beforehand on cultivated soybean in green house is an effective way to deal with the aphid source problem. Therefore, an experiment was carried out in 1984 to see if *A. glycines* had partiality for food.

Thirty wild soybean plants of the same variety with similar size were grown for the experiment. Two adjacent plants were treated as a comparison group. One plant was inoculated with five apterous nymphs from wild soybean, the other one inoculated with nymphs from cultivated soybean. Fifteen replicates were conducted. Each plant was covered by a 40 mesh net cage of 25 cm in diameter and 40 cm height. The number of survival aphids on every plant was examined 15 days after inoculation and listed in Table 2.

Table 2. Survival aphids on wild soybean 15 days after inoculation of aphids from different sources (1984, *Gongzhuling*)

Group	Aphid from wild soybean (No./plant)	Aphid from cultivated soybean (No./plant)	d
1	97	11	86
2	56	36	-7
3	58	13	45
4	109	300	-191
5	3	56	-53
6	11	278	-267
7	38	105	-67
8	102	101	1
9	320	418	-98
10	68	12	56
11	214	8	206
12	127	15	112
13	109	118	-9
14	104	185	-81
15	3	121	-118

The t test was used to examine the pairing data in Table 2:
$t = \alpha/s\alpha = 0.825$, when V=14, $t_{0.01} = 2.997$. since $t = 0.825 < t_{0.01}(2.997)$, $\mu_d = 0$, thus, there is no significant difference in the final aphid numbers 15 days after the tested wild soybean plants were inoculated with aphids from either wild soybean or cultivated soybean plants.

3. Creating a favorable conditions for severe aphid infestation

In general year, *A. glycines* in *Gongzhuling* region, China, does not cause heavy infestation in *Glycine soja* neither naturally nor with artificial inoculation of aphids, especially not so in years with plenty of rain in June and July. Thus, it not only makes it difficult to distinguish the resistant grade of wild soybean materials, but also affects the accuracy of evaluation. In order to solve this problem, the aphid resistance of 16 wild soybean materials was evaluated under three different conditions in 1986.

The three treatments were as follows: 1) evaluated in net covered space with inoculated aphids; 2) evaluated in open fields with inoculation of aphids; 3) evaluated in open fields without inoculation of aphids. The materials in all three conditions were the same. Every material was sowed in one row of two-meters long. Moreover, ten plants were reserved (only seven plants for condition 1). There were four replicates for condition 3 and no replicate for the other two conditions. All of the materials were sowed in fields in early May. The size of net house in treatment 1 was 10×5.2×1.8 meters and the net material was 40-mesh white nylon gauze. In treatment 1, aphids, natural enemies and other leaf-feeding pests were removed from the plants before the inoculation of aphids. Each plant was inoculated with 20 equally developed apterous nymph aphids right after covered with the net using clipping leaf method (June 18). In treatment 2, the inoculation procedure and time were the same as in treatment 1. The damage indices of wild soybean in the three treatments were identified on July 10, 1986 (Table 3).

Table 3. Damage indices of tested materials under three different conditions (1986, *Gongzhuling* region)

Serial No.	Treatment 1	Treatment 2	Treatment 3
85-16	58.33	50.00	47.7
85-39	30.56	25.00	25.7
85-1	31.25	25.00	29.7
85-13	100.00	50.00	48.6
85-4	35.71	33.33	27.3
85-31	65.63	25.00	25.7
85-40	65.63	32.14	31.4
85-38	40.63	25.00	29.6
85-9	95.83	57.14	46.6
85-29	71.43	37.50	32.0
85-32	25.00	25.00	25.0
85-14	78.13	25.00	25.6
85-20	83.33	33.33	26.1
85-18	100.00	67.86	47.5
85-34	65.00	29.17	30.2
85-2	97.50	50.00	46.5

The results were as follows.

1. Damage indices of tested materials under conditions 1 had a wider distribution range than those under conditions 2 or 3. The damage indices of the 16 materials under condition 1 ranged from 25 to 100, while those under conditions 2 and 3 only ranged from 25 to 67.86 and from 25 to 48.6 respectively. Obviously, it would be more difficult to distinguish between mediate resistant, neutral type and susceptible materials under conditions 2 and 3. The resistance of highly resistant materials and the susceptibility of susceptible materials were also not very distinct under conditions 2 and 3.

2. The damage index orders of all the tested materials under all three conditions were accordant, which indicated that the covering net did not change the aphid resistance or the susceptibility of the tested materials.

4. Aphid resistance of wild soybean at different growth stages

In 1986, in order to clarify whether wild soybean has the same resistance or susceptibility to *A. glycines* during the seedling stage and the blooming stage, we conducted an evaluation experiment using five materials with similar growth durations. These materials were sowed at different dates but inoculated at the same day with aphids. After repeated experiments on these materials, their aphid resistance or susceptibility has been understood.

The tested materials were sowed on May 25, June 5 and June 17 in small plastic pan with one plant per pan and four pans per material. The experiment was performed in a big net covered chamber. Every plant was inoculated with ten nymphs on June 23. The developing stages of materials at the time of inoculation were as follows: seven to eight compound leaves on the caulis (sowed on May 25); one to two compound leaves on the caulis (sowed on June 5); no fully expanded euphylla yet (sowed on June 17). The aphid number on each plant was surveyed on July 5. At this time, the developing stages of the tested materials were 12-13 compound leaves on caulis (blossomed), 5-6 compound leaves on caulis, and two compound leaves on caulis respectively.

The results showed that the order of aphid resistance grades for each tested material sowed at different time were basically consistent. The materials 85-9 and 85-16 were highly-susceptibility to aphids. Although the ranking positions of these two materials changed a little with different sowing dates, their aphid number was always the top two. Material 85-32 was highly resistant to aphid and its aphid number was the lowest for all the three sowing dates (Table 4).

Table 4. Average aphid numbers per plant sowed at different dates (1986, *Gongzhuling* region)

Serial number	Resistance grade	May 25		June 5		June 17	
		number/plant	sequence	number/plant	sequence	number/plant	sequence
85-9	HS	232.5	2	225.3	1	135.0	1
85-16	HS	307.5	1	144.0	2	134.0	2
85-38	R	155.0	3	113.3	3	55.3	3
85-39	HR	92.3	4	88.0	4	90.0	4
85-32	HR	40.1	5	16.8	5	30.0	5

∗ HR, high resistance; R, resistance; HS, high susceptibility.

5. Results and discussion

Visually evaluating the damage severity and converting it into the susceptibility index is a convenient and reliable technique for identifying the aphid-resistant grade of wild soybean. This technique can handle large amount of materials in a short period and is advantageous over all the other methods in aphid resistant evaluation of wild soybean. The results are the same as those by the direct counting method.

When there is no standard resistance or sensitivity material, the comparability of the resistance grade differentiated by damage index is not good among different years. This may be overcome by adding reference materials or increasing replicates.

In addition, before any artificial aphid-rearing method is developed, reproduce aphids on cultivated soybean in greenhouse before hand is an effective way to solve the aphid source problem for aphid resistant research on wild soybean. There is no significant difference between the reproductive rates of *A. glycines* grown on cultivated soybean and on wild soybean.

In order to increase the aphid number on wild soybean plants and improve the accuracy of

aphid resistant evaluation, some primary screened resistance materials could be evaluated further by inoculating aphids in net covered chambers. The aphid resistance or susceptibility grade of the tested materials does not change while evaluated in net covered chambers. It is practicable to identify aphid resistance of wild soybean by inoculating aphids at the seedling stage in net chamber, because the aphid resistance or susceptibility of wild soybean at both seedling and blooming stages is similar.

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