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Study on the Utilization of Aphid Resistant Character in Wild Soybean. I. Aphid Resistant Performance of F2 Generation from Crosses between Cultivated and Wild Soybeans.

Zhiqiang Sun Peizhan Tian Jian Wang
(*1nstitute of Soybean, Jilin Academy of Agricultural Sciences*)

Abstract Three crosses between 3 cultivated (*G. max*) and 2 aphid resistant wild soybean (*G. soja*) lines were made to study the inheritance of resistance and segregation patterns in F_2 generation with artificial inoculation techniques. The aphid resistance of F_2 plants showed continuous mono-peek distributions which are inclined towards the susceptible parents. Most F_2 plants were susceptible or highly susceptible. Only about 7.2 to 10.2% of the F_2 plants were resistant to soybean aphid. The segregations in 2 out of 3 crosses studied fitted the hypothesis that there are 2 independent gene pairs controlling the aphid resistant character of the wild soybean lines ($P > 0.50$). The segregation of the other cross also tended to fit the 2 major gene model. These results indicated that the aphid resistance of the wild parents might be controlled by 2 independent recessive genes and some other minor genes. Supposing that the aphid resistant character was quantitatively inherited, the overall inheritability estimated from the variances of parents and F_2 populations was between 85.5% and 97.0%. Despite of the high inheritability of aphid resistance, it is recommended that the selection of resistance should be postponed to later generations such as F_3 or F_4, because the scarcity of resistant genes may be recessively inherited, and the number of backcrosses made in each cycle of selection should be limited to avoid the resistant genes being lost.

Key words Wild soybean; Soybean aphid; Resistance segregation; Inheritance of resistance

The *Aphis glycines* is one of the most serious pests of soybeans in the soybean-producing areas in north China, causing great economic losses. It not only directly harms the soybean plants to seriously decrease yield, but also is the main vector of several kinds of viruses such as soybean mosaic virus, and increases the quantity of mottled

soybean kernels, which decreases grain quality. Chemical methods are often used for the prevention and cure of soybean aphid. The disadvantages of this method are the high costs of prevention and cure, development of insecticide resistance, killing of many natural enemies, severe environmental pollution and ecological damage. From the standpoint of long-term benefits, seed selection and using soybean seed with characteristics of aphid resistance or tolerance is the most economical and effective method of aphid management. Some research institutes have done the work of screening and selection of aphid resistant varieties since 1970. Zhen Feng *et al.* [1, 2] (sic) judged the aphid resistant characters of 2073 cultivated soybean entries. But no highly resistant entry was found except the Guoyu 98-4, Guoyu 100-4 etc. glabrous entries. Yiheng Fan [3] evaluated the resistant characters of 902 cultivated soybeans in the fields and found that only PingDingXian with cyan colored pod coat and DuLu bean had some resistant characters. Because of the lack of species resources with highly resistant characters, the glabrous entries usually were accompanied by limitations in shape, physiology and metabolism, which are undesirable in breeding.

Wild soybean is a closely related species of the cultivated soybean and is also one of the hosts of *A. glycines* [4] (sic). An aphid resistant species that could be used may exist among wild soybeans. Derong Yue *et al.* [2] performed aphid resistance filtering for about 1,000 wild and semi-wild soybean materials, and identified three highly resistant resources: wild 85-32, wild 85-39, wild 85-1. Their resistant characters were higher than the cultivated materials with resistant characters, establishing a foundation for aphid resistance breeding using wild soybean. The purpose of this research is to explore the potential and effective methods of transferring aphid resistant genes from wild to cultivated soybeans. This paper reports the aphid resistant segregation patterns and the inheritance rules of resistance in F_2 generation crossbred by the cultivated soybean x wild soybean.

Material and methods

In 1987, three cultivated soybeans (Jilin 20, Xuan 85-8 and Gong 7514-2) were used to crossbreed with the resistant wild soybeans Yie 85-32 and Yie 85-39 to produce three crossbreeding combinations: Gong 8794 (Jilin 20 × Yie 85-32), Gong 8795 (Xuan 85-8 × Yie 85-39), and Gong 8796 (Gong 7514 × Yie 85-32). In 1988, F_1 generation was planted in the Gongzhuling fields, then the false hybrids were removed and the seeds of F_1 plants were obtained. In 1989, the parents of three cross groups and F_2 population were planted in the screen houses. The size of the screening house was 20m x 5m x 2.1m with a capacity of 40 rows, each row space at 50cm and plant space of 15cm. Aphids were inoculated artificially on June 13. The inoculation method was to select plants on which there were some aphids from Jilin 21, then using small scissors, to remove some leaves with aphids on them so that 10~15 relatively identical aphids remained on each leaf. Then those leaves were pinned to the leaves of each plant that had not yet fully expanded. The aphid resistant characters were investigated on July 11. The resistant grading method was

based on the method introduced by Zhen Feng and Derong Yue *et al* [1,2]. The grading scale was as follows:

Grade 0: no aphid on the whole plants;

Grade 1: the plants develop normally with sporadic aphids. It is estimated that the number is less than 100;

Grade 2: the plants develop normally with some aphis on the top shoot and young leaves. It is estimated that the number is about 101~300;

Grade 3: the plants develop normally with a lot of aphids on the top shoot and young leaves. It is estimated that the number is about 301~800;

Grade 4: the leaf shrivels significantly, depressing the plants' development. Many aphids are scattered on the top shoot and young leaves. It is estimated that the number is about 801~1200;

Grade 5: the plants are stunted; the leaf surface appears moist and oily, the shoot and young leaves are bound together. The number of the aphids is very large; it is estimated that the number is more than 1200.

The following formula is used to calculate the infestation indices of the parents and F_2 population:

Infection index=($\sum$(infestation grade x no. of plant at relative grades)/(5 x total no. of plants)) x 100%

The proportion of resistance and segregation was calculated, and the number of resistant genes and inheritance of aphid resistance were estimated.

Research results

1. The average resistance of parents and F_2 generation

The grades of three crossed resistant parents were all around 1. They were found to be highly resistant, which was consistent with results of the previous report [2]. The average aphid infestation grades of the infested aphid parents were all greater than 4; they were highly infested. The average infested aphid grades and indices of F_2 generation were all between the two parents and inclined toward infested aphid parent, indicating aphid infestation characters expressed as incomplete dominance (Table 1).

Table 1 Average resistance of parents and F_2 generation

Crosses	Generations	Grades of resistance ($\overline{X} \pm S$)	Indices (%)
Gong 8749	P_1 Jilin 20	4.94 ± 0.23	98.9
	P_2 Yie 85-32	1.00 ± 0.00	20.0
	F_2	3.76 ± 0.94	75.1
Gong 8795	P_1 Xuan 85-8	4.41 ± 0.50	88.2
	P_2 Yie 85-39	1.00 ± 0.00	20.0
	F_2	3.88 ± 0.93	77.6
Gong 8796	P_1 Gong 7514-2	4.83 ± 0.39	96.6

	P_2 Yie 85-32	1.10 ± 0.38	22.0
	F_2	4.08 ± 1.02	81.6

2. The resistance distributions of the parents and F_2 individual plant

Most parents that were progenies of different cross-combinations recorded an infestation grade of 5 and few of them recorded 4. The resistant grades of wild soybean parents were all basically distributed at 1; nearly no parents were found to be immune and only few individual plants recorded grade 2. This indicates that resistant and infested parents are all pure-cross stable; aphid resistance is not affected by environmental conditions.

The aphid resistance of F_2 plants of the 3 crossbreeding combinations showed continuous mono-peak distributions. The distribution peaks of two combinations (Gong 8794 and Gong 8795) were at grade 4, but the distribution peak of Gong 8796 was at grade 5.

Table 2. Aphid resistance segregation ratios of crosses between cultivated (*G. Max*) and wild (*G. soja*) soybeans in F_2 generation

Crosses	Generations	Total No. of plants	Resistant*		Susceptible		Fitness**	
			No. of plants	Frequencies	No. of plants	Frequencies	X^2	P
8794	P_1	19	0	0.000	19	1.000	-	-
	P_2	14	14	1.000	0	0.000	-	-
	F_2	176	18	0.102	158	0.898	4.10	>0.025
8795	P_1	22	0	0.000	22	1.000	-	-
	P_2	17	17	1.000	0	0.000	-	-
	F_2	221	16	0.072	205	0.928	0.22	>0.500
8796	P_1	12	0	0.000	12	1.000	-	-
	P_2	22	22	1.000	0	0.000	-	-
	F_2	179	13	0.073	166	0.927	0.16	>0.500
8794+8796	F_2	355	31	0.087	324	0.912	3.32	>0.05

*: Plants with resistance scores of 0, 1 and 2 are considered to be resistant.

**: Ratio tested is 1 resistant: 15 susceptible.

The aphid resistance of the F_2 plants showed continuous mono-peak distributions that were inclined towards the susceptible parents. Most F_2 plants were susceptible or highly susceptible. Only about 7.2 to 10.2% of the F_2 plants were resistant to soybean aphids. The distributions of the F_2 individual plants with every kind of cross-breeding are all asymmetrical. An absolute majority of the individual plants distributed at grade 3~5, but very few distributed at grade 1 or 2. No individual was found to be immune (Fig. 1).

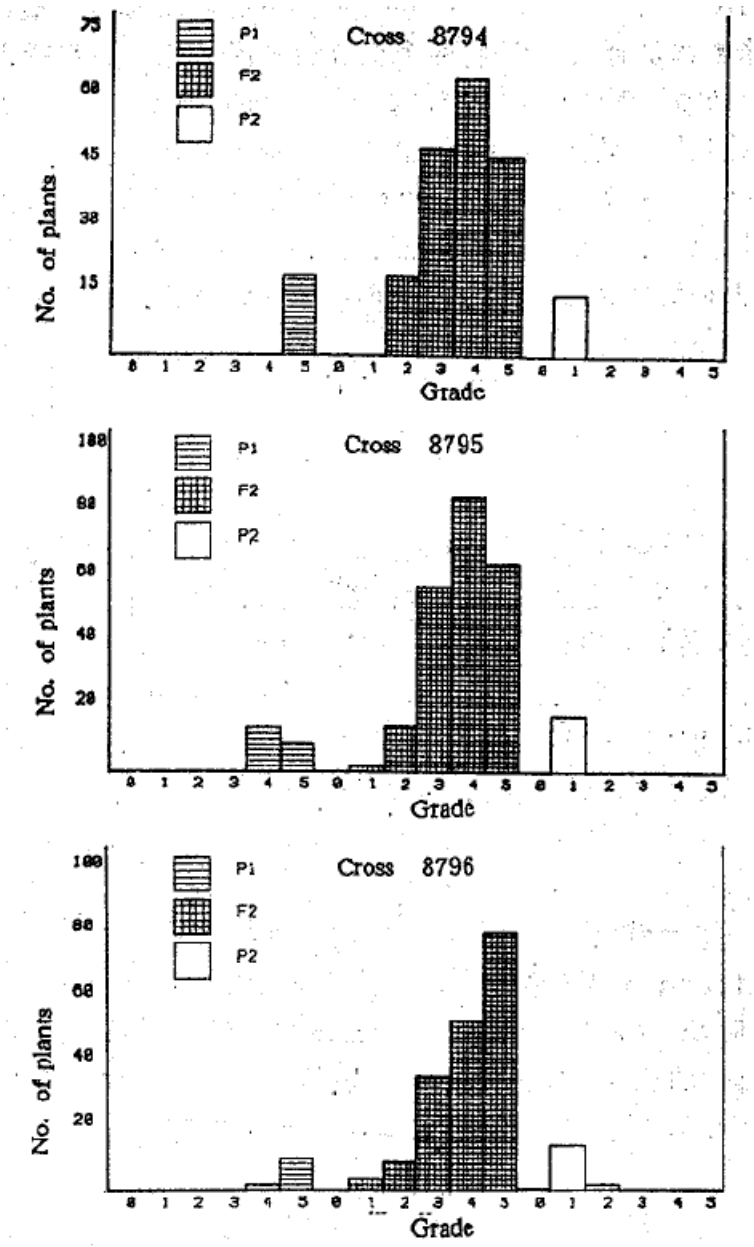

Fig. 1 Aphid resistance distribution of F_2 plants of three crosses between cultivated and aphid resistant wild soybeans

3. The segregation proportion of F_2 aphid resistant characters and the inheritance of resistance

According to the response of the resistant parents, grade 1 and 2 are regarded as resistant to aphid, grade 3, 4 and 5 as susceptible to aphid. And the individual plants in F_2 population are divided into two kinds: aphid resistant and susceptible. And the segregation proportion of aphid resistance were studied (Table 2.) The aphid resistant individuals segregated from the three crossbreeding combinations of Gong 8794, Gong 8795 and Gong 8796 take up about 10.2%, 7.2% and 7.3% of F_2

population respectively. The actual resistant segregation proportions are 1:8.8, 1:12.8 and 1:12.8 respectively. This indicates that the frequency of the aphid resistant individual plant appearing is very small; an absolute majority of the F_2 individual plants were aphid resistant.

In order to estimate and control the aphid resistance genes, some experimental materials were tested for adaptability. The results show that the segregations in 2 crosses studied (Gong 8795 and Gong 8796) fitted the hypothesis that there are 2 independent gene pairs controlling the aphid resistant character, the segregation proportions fitting the theoretical ratio ($P > 0.50$) of 1 resistant: 15 susceptible. The adaptability of Gong 9784 is a little worse ($0.05>P>0.025$), but obviously it fit the hypothesis of 2 gene pairs better compared with the hypothesis of 1 gene pair ($x^2 = 19.70$, $P<0.001$). Because the aphid resistant parents of Gong 8794 and Gong 8796 are all Yie 85-32, the F_2 material of the two crosses were merged to test fitness; the segregations of resistance still fit the theoretical value ($x^2 = 3.32$, $P>0.05$) of 1 resistant: 15 susceptible. Therefore, the aphid resistance of the Yie 85-32 and Yie 85-39 might be controlled by 2 independent major genes. The aphid susceptibility character was observed to be incomplete dominance, and the aphid resistance to be recessive. Because aphid resistance of the F_2 individual plants showed continuously asymmetrical distributions, the aphid resistance was still influenced by some minor genes. Supposing that the aphid resistant character was quantitatively inherited, the overall inheritability estimated from the materials in this research was 97.0%, 85.5% and 85.8% respectively. So it could be seen that the inheritability of aphid resistance is quite high, which supports the previous hypothesis that it was mainly a few major genes that controlled the aphid resistance.

Discussion

1. The crossing method of transferring wild aphid resistance genes: The wild soybean has a lot of disadvantageous characteristics such as rampancy, dehiscence (shattering), small seeds, hard grains, bad grain quality and virus susceptibility etc. We hope to introduce only the aphid resistant genes to the genetic background of the cultivated soybeans, without introducing other genes with bad characters. The method of single cross offspring selection used is helpful in the selection of aphid resistant characters; but the recovery of the selection made in each cycle to agricultural characters is limited. In order to increase the recovery speed of agricultural characters, a backcross-improvement method is usually used.

But with the extension of the number of times of backcrossing, the possibility of losing necessary aphid resistant genes increases. This is true especially in the case of resistant genes expressed as recessive. Therefore, the number of crosses made in each cycle of selection should be limited when using backcross methods; one crossbreed and one backcross are suitable; it should ensure more backcross seeds and correspondingly increase the offspring populations, providing more opportunities for selection.

2. Select the best generation of the aphid resistant characters: According to the results of this research, the aphid resistant characters of wild soybeans may be controlled by two recessive genes. The frequency of individual plants appearing that resist aphids is very small, so it is recommended that the selection generation be postponed to later generations. In theory, when the resistant characters are controlled by two recessive genes, the frequencies of individuals appearing with resistant characters in F_2, F_3 and F_4 populations are 6.25%, 14.06% and 19.14% respectively. The frequencies of individuals appearing with resistant characters in F_3 and F_4 populations are 2.3 and 3.1 times of that in F_2 population. Therefore, the selection is postponed to F_3 or F_4 populations. We could also consider the selection of other agricultural characteristics among many aphid resistant individuals so as to greatly increase selection efficiency.

3. The mechanism of aphid resistance: Wild soybeans differ greatly from cultivated soybeans on morphological, physiological, and ecological characteristics. If the difference is related to aphid resistant characters, it will cause some difficulty for utilization of wild soybean aphid resistant characters. The absolute majority of the individual plants are all inclined to wild soybeans in the F_2 generation crossbred by cultivated soybeans and wild soybeans. But the absolute majority of the individual plants in the F_2 generation were aphid resistant. Therefore, there may be no certain relationship between aphid resistant characters of wild soybeans and morphological characteristics such as rampancy, multi-ramification etc.

4. The method to transfer aphid resistant genes of wild soybeans: The recovery speed of agricultural characteristics of cultivated soybeans was slow when the traditional crossbreed and backcross methods were used to transfer aphid resistant genes. In order to overcome some bad characteristics, several cycles of crossbreeding and selection should be used; the cycle is very long. According to the results of this research, the aphid resistant characters of wild soybeans might be controlled by two major genes. Thus, a molecular inheritance operative technique is used to extract the DNA molecular segments and introduce them to the genetic background of the cultivated soybeans. The selection of aphid resistant characters for the offspring might be the effective way for transferring the aphid resistant genes of wild soybeans. We are currently exploring this area of research.

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