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Comparsion of Several Character between *Glycine soja* and *Glycine max* and Its Utilization in Soybean Breeding

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Abstract: Wild soybean (*Glycine soja*) could serve as important resources in soybean breeding for its aboundant genetic background. In the present study, wild and cultivated soybeans from Far-Eastern area of Russia and Northeast China were planted in Beijing, China, and compared their agronomic traits. We found that wild soybean had higher protein content (47.55 %) and lower oil content (12.91 %) compared with cultivated soybean. Besides, wild soybean had higher isoflavone content and better resistantce to soybean cyst nematode (SCN, *Heterodera glycines* Ichinohe). To select soybeans with better SCN resistance or higher isoflavone content, different hybride combination with the tested wild and cultivated soybean were made, and selected several hybrid progenies with desired traits. We also researched the natural hybridization between wild and cultivated soybean, and found it is possible to identify the true hybrids by analyzing the segregations patterns of flower and pod color of F_1 generation. The results suggestted that introduce relevant genes from wild soybean into cultivated soybean through hybridization is very useful for soybean breeding.

Key words: *Glycine soja*; *Glycine max*; Hybridization breeding; Soybean cyst nematode; Isoflavone

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野生与栽培大豆某些性状的比较及其在大豆育种中的利用

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摘 要:野生大豆由于丰富的遗传背景在大豆育种中具有重要的利用价值。选取来自俄罗斯远东地区和中国东北地区的野生大豆与栽培在中国北京种植,并对其农艺性状进行比较。与栽培大豆相比,野生大豆具有较高的蛋白质含量(47.55%),较低的脂肪含量(12.91%)。此外,野生大豆的异黄酮含量高并且具有较好的胞囊线虫抗性。将经过筛选的不同野生大豆与栽培大豆进行杂交,已经选育出一些具有高异黄酮含量和良好胞囊线虫抗性的大豆材料。同时研究了野生大豆与栽培大豆的天然杂交,发现通过分析 F_1 代花色和荚皮色的分离情况可以鉴定天然杂交种。结果证明通过杂交的方式将野生大豆中的目的基因导入栽培大豆进而提高大豆育种效率是切实可行的。

关键词:野生大豆;栽培大豆;杂交育种;大豆胞囊线虫;异黄酮

Wild soybean (*Glycine soja*) is the wild ancestor of cultivated soybean (*Glycine max*), which was an important resource for soybean breeding[1]. Soybean resources are the basis of soybean breeding and how to evaluate and explore their utilization deeply and thoroughly in soybean breeding remains the research hotspot [2]. After several years studies, Ala reported that wild and cultivated soybeans had an overlapped distribution in the Far Eastern area of Russia. Therefore, hybridization between wild and cultivated soybean resources could be used to choose desirable characters in morphology, biology and economy aspects[3-10].

Our previous studies showed that the allele genes of cultivated soybean were significantly lower than the wild ones[11]. The restricted genetic background maybe a severe limitation in improving seed yield of cultivated soybean[12]. Therefore, the study and utilization of wild soybean resources will faciliate the improvement of soybean germplasms. The objectives of this study were to ⅰ) compare of several interested agronomic traits, ⅱ) investigate the natural hybridization and, ⅲ) select SCN resistant or high isoflavone content soybean through the hybridization between wild and cultivated soybean.

1 Methods and materials

1.1 Characters of wild and cultivated soybeans

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the tested wild and cultivated soybeans (listed in Table 1 and Table 2), other soybean varieties were provided by Heihe Branch of Heilongjiang Academy of Agricultural Sciences (HAAS) and Soybean Institute of HAAS. All materials were planted on 15 July 2007, the traits including growth period, plant height, protein content, oil content, and pod number per plant, seed number per plant and 100-seed weight per plant were measured.

1.2 Studies of natural hybridization between wild and cultivated soybeans

Cultivated sobean with white flowers and wild soybean with purple flowers were used as female and male parents, respectively. Three combinations (F_4 131 × KZ 671, Heihe42 × KT156, Heihe27 × Khab 177-180) were adopted. Seed coat of wild soybean was pricked before planting. Wild and cultivated soybeans were planted in the same row, the flower and pod color of F_1 were recorded to single out the metromorphic hybrid and then caculated the natural crossing rate.

1.3 Breeding of soybean varieties resistant to *Heterodera glycines* Ichinohe

The resistance of collected soybean germplasm to soybean cyst nemedote (SCN) were determined according to Golden's method[12], and used the method from Riggs and Schmitt to classify the physiological strains [13], with Lee, Picket, Peking, PI88788 and PI90763 as the identification host. Several hybrid combinations from the tested germplasm were made; the offspring were selected with pedigree method and identified their SCN resistance.

1.4 Breeding of soybean varieties with high isoflavone content

Isoflavone content of soybean parents and hybrid progeny were evaluated by ultraviolet spectrophotometer. Firstly, soybeans were shattered and ground with a screen (80 mesh) and, then 20 mL ethanol (70%) was added to 50 mg samples. The samples were shaked with ultrasonic oscillator for 20 min and diluted with ethanol to 50 mL. The supernatant was centrifuged at 8,000 $r \cdot min^{-1}$ for 15 min, and the resulting supernatant was spectrophotoelectriced at 260 nm then calculated out the isoflavone content in the samples.

2 Results

2.1 Characters of wild and cultivated soybeans

We planted wild and cultivated soybeans in Chinese Academy of Agricultural Sciences experimental field and investigated the characters of wild soybean, including plant height, pods per plant, 100-seed weight, protein content, oil content and maturity (Table1). The wild soybeans from Far-Eastern area of Russia and Heihe area of Heilongjiang Province, China have a short growth duration when planted in Beijing, the early-mature, resistant to virus and SCN of wild soybean are all desired traits for soybean breeding.

Table 1 Characters of wild soybean in 2007

Variety	Plant height /cm	Pods per Plant	Seeds per Plant	100-seed weight/g	Protein content/%	Oil content /%	Growth duration/d
N1/2006	59.4	46.6	105.7	3.43	43.89	12.97	78
N6/2006	51.4	30.1	75.9	2.83	36.97	16.31	78
N7/2006	47.7	34.7	84.7	3.26	38.18	15.85	78
N8/2006	43.8	33.6	74.0	2.57	43.10	11.87	78
N9/2006	38.0	21.7	52.1	2.71	40.41	14.34	72
N12/2006	31.5	11.5	25.0	2.43	38.83	16.26	72
N13/2006	21.3	33.3	86.7	2.19	35.49	16.96	72
N14/2006	40.5	25.2	60.9	2.52	35.96	15.87	72
N19/2006	34.6	14.0	31.5	2.61	37.37	15.10	72
N22/2006	42.9	21.9	47.4	2.44	37.23	16.13	72
G. soja9873	24.4	-	-	2.22	41.96	11.47	72
KT156	54.2	13.8	27.0	-	40.99	13.47	-
KXa6177-180	57.7	24.8	66.3	-	32.8	19.56	-
K3671	52.4	14.8	51.7	-	36.85	16.75	-
Average	42.8	25.1	60.7	2.65	38.57	15.20	74

Table 2 Protein and oil content of wild soybeans in 2008

Variety	Protein content/%	Oil content /%
N2/2006	46.96	12.56
N3/2006	47.30	13.49
R-194	47.83	13.36
N1/2006	46.88	14.64
N5/2006	47.76	12.15
N6/2006	46.50	12.36
R-92	47.32	10.83
R-56	46.99	13.95
R-27	50.39	12.87
Average	47.55	12.91

To further study the quality characters of wild soybeans, the protein and oil contents were measured in 2008 (Table 2). The average protein and oil content was 47.55 % and 12.91%, and the maximum were 50.39% (R-27) and 14.64% (N1/2006), respectively. However, the average protein and oil contents in cultivated soybean were 38.07% and 19.44%, respectively (Table 3). The results indicated that wild soybean had higher protein content and lower oil content than cultivated soybeans. Therefore, wild soybean with higher protein content could be used in soybean high-protein breeding programs.

Table 3 Characters of cultivated soybeans

Variety	100-seed weight /g	Seed weight per plant /g	Plant height /cm	Pods per plant	Seeds per plant	Protein content/%	Oil content /%
Heihe 19	19.7	13.3	52.5	36.6	77.5	39.93	18.78
Nenfeng11	15.1	14.8	74.2	43.8	106.3	35.10	21.33
Heihe27	21.20	13.9	50.2	35.8	78.0	39.48	18.66
Kengfeng9	13.60	17.4	70.7	52.7	128.1	34.62	19.95
KP—109	17.70	14.5	69.1	42.8	93.6	38.54	19.34
Heihe42	18.30	17.9	58.0	48.3	106.8	38.40	20.01
Heihe34	16.00	14.0	49.0	51.0	100.4	41.13	18.70
KP—131	15.10	13.4	87.1	50.2	99.3	36.17	18.90
Beifeng11	19.20	14.4	51.1	41.1	90.0	39.24	19.35
Average	17.32	14.8	62.4	44.7	97.8	38.07	19.44

The average seed number per plant of cultivated soybean was 97.77, which was significant higher than that of wild soybeans (64.39). Besides, 100-seed weight of wild soybeans (2.65 g) was greatly lower than that of cultivated soybeans (17.32 g). And the yield of cultivated soybeans was much higher than that of wild soybean, the hybrid progeny of wild and cultivated soybeans could used as natto varieties.

2.2 Natural hybridization between wild and cultivated soybeans

The F_1 seeds of F_4 131 × KZ 671, Heihe42 × KT156 and Heihe27 × Khab 177-180 were planted in 2007, the true hybrids of natural hybridization were singled out by oberving the flower and pod color of F_1 plants. One F_1 plant of Heihe27 × Khab 177-180 had purple flower, suggested that it was true hybrid. The pod color of Heihe 42 and KT156 were yellow and dark brown, respectively, while pod color of one F_1 plant of Heihe 42 × KT156 showed dark brown, indicated that it is ture hybrid (Fig. 1). Generally, natural hybridization ratio between wild and cultivated soybeans was about 0.1%.

Fig. 1 Heihe 42 (*Glycine max*, yellow pod coat), KT156 (*Glycine soja*, dark brown pod coat) and natural hybridization F_1 plants between Heihe 42 × KT156 (dark brown pod coat)

Table 4 Plant types of F_1 from *G. max* × *G. soja*

Order	Hybridization combination	No. of F_1 Plant types	
		Cultivated types	Wild or middle types
1	F_1(Heihe27 × KZ 66/05)	0	2
2	F1(Zhonghuang 35 × JT-1)	0	1
3	F_1(Zhonghuang35 × *G. soja*. Khab 177-180)	0	7
4	F_1(Zhonghuang35 × KZ 66/05)(F1059)	3	6
5	F_1(Heihe27 × *G. soja*. Khab 177-180)	1	9
6	F_1(Heihe42 × *G. soja* KT156)	0	1
7	F_1(Zhonghuang35 × *G. soja* KT156)	0	1
8	F_1(F_4 131 × KZ 671)	16	0

There were 16 cultivated types and no middle or wild type in F_1(F_4 131 × KZ 671) combination, which indicated that this combination did not have natural hybridization. The other combinations in this test showed seperation in plant types and further observation still needed.

2.3 Breeding of soybean varieties resistant to SCN

PI437654 and cultivated soybean were used to bread Zhonghuang 26 which had high resistence to SCN. Besides, among the hybrid progenies five lines had middle resistance as Zaoshu 18 (CK-1) and five lines had high sensitivity as Lee(CK).

Table 5 Ability of resisitance to *Heterodera glycines* Ichinohe in new soybean varieties

Variety	Resistance index	Sensitivity	Variety	Resistance index	Sensitivity
Zaoshu18 (CK-1)	17.9	MR	Zhonghuang12	20.7	S
Lee(CK-2)	34.0	HS	Zhonghuang17	22.5	S
Zhonghuang26	0	I	Zhongzuo RN0811	24.0	S
Zhonghuang33	12.6	MR	Zhongzuo RN085	30.1	S
ZhongzuoRN086	12.6	MR	Zhongzuo RN084	34.3	HS
Zhongzuo RN082	14.4	MR	Zhongzuo RN087	34.8	HS
Zhonghuang35	17.1	MR	Zhongzuo RN089	35.5	HS
Zhongzuo RN083	18.5	MR	Zhongzuo RN088	36.2	HS
Zhongzuo RN0810	20.0	S	Zhongzuo RN081	41.3	HS

High sensitive-HS; Sensitive-S; Middle resistant-MR; Immune-I

We obtained Zhonghuang 26 and several other varieties by hybridized high SCN resistant with high yield varieties. And the results showed that PI437654, Huipizhiheidou and Yuanboheidou had high resistance to SCN, they can be used in soybean SCN resistant breeding.

2.4 Breeding of soybean varieties with high isoflavone content

Taking Zhangjiakouheidou as CK, the isoflavone content of 20 soybean germplasm could be divided into low, middle and high three groups with 1 000.0 mg · kg^{-1} and 2 000.0 mg · kg^{-1} as boundary (Table 6). The average isoflavone content of three group were 619.0, 1383.7 and 2 462.4 mg · kg^{-1}, respectively. The isoflavone content of JT-1 reached 3 273.3 mg · kg^{-1}. However, due to its tall stem and small seeds, JT-1 should be improved by other cultivated soybean varieties. From the results we concluded that black soybeans with small seeds tended to have higher isoflavone content.

To improve isoflavone content of cultivated soybean, we crossed JT-1 with high yield soybean Zhonghuang 35 and Zhonghuang 36, and the isoflavone content of some progeny lines reached 4000 mg · kg^{-1} (data not shown) so it is possible to select high isoflavone soybean varieties with better agronomic traits.

Table 6 Isoflavone content of collected soybean germplasm

Variety	Isoflavone content /mg · kg^{-1}	Variety	Isoflavone content /mg · kg^{-1}
Zhangjiakouheidou	1486.0	Xiaoheidou-4	1489.9
Zhonghei 2	220.5	Heidou-2	1492.3
Zhonghuang 17	433.4	Xiaoheidou-5	1510.2
Huaidou 1	612.0	Xiaoheidou-3	1547.0
98SM6003	755.0	Taekwang	1709.7
Heidou-1	810.2	Guangbiandou	1732.6
Zhonghuang 13	883.1	Zhonghei 1	2028.0
Heipiqing	1097.4	Xiaoheidou	2049.6
Zirongdou	1351.0	Yanxundou	2498.5
Zhonghei 3	1416.8	JT-1	3273.3
Xiaoheidou-2	1489.9		

3 Discussion

There are many differences between wild and cultivated soybeans in genetic background, especially in yield, protein content, oil content, isoflavone content and SCN resistance. Therefore, studying the genetic resources and utilizing desired traits of wild soybean are of great importance in soybean breeding[20,22]. For example, high protein content and early maturing characteristic of wild soybean could be used in soybean breeding studies. Besides, there are more than twenty thousand cultivated soybeans and thousands of wild soybean resources in China, however, the soybean resources that had been utilized was less than 10%[23]. Therefore, we should keep studying and utilizing these resources to breeding varieties that are more useful.

Natural hybridization was used as a new method in soybean breeding. Ala et al. had selected Dina, Aria, Neva and Tata four soybean varieties by hybridized with wild and cultivated soybean resources successfully[8-9]. However, this method did not used extensive enough in China. In this study, we used cultivated soybean with white flowers and wild soybean with purple flowers as female and male parents respectively and obtained natural hybrids successfully by analyzing the segregations patterns of flower and pod color of F_1 generation.

We had obtained Zhonghuang 26 that has high resistance to *Heterodera glycines* Ichinohe by hybridized Dan 8 with PI437654. Recently, we used Huipiheidou and Yuanboheidou hybridized with cultivated soybeans and obtained a set of soybean lines that had high resistance to *Heterodera glycines* Ichinohe[24,25].

Isoflavone was an important secondary metabolic compounds of soybean, and it plays essential roles in preventing certain diseases such as cancer[21]. Consequently, it was very important to increase the content of isoflavone in soybean varieties. We used JT-1, a soybean line with high isoflavone content to hybridize with high yield soybeans to obtain new varieties with both high isoflavone content and high yield. We have obtained several desired lines, and further field test and identification are still needed.

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