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A Decade Study of Chinese Wild Soybean (*Glycine. soja*)

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Abstract

Wild soybeans, *Glycine. soja,* originated in China. Chinese wild soybean resources make up 90% of all global soybean resources. In the past decade, Chinese scholars have achieved distinction in interdisciplinary soybean research. Some of those researches hold leading positions worldwide. The study of wild soybeans has entered a new era. This paper provides a comprehensive review of wild soybean research in China, including distribution and growth conditions, morphological development, the biological, physiological, biochemical, cytogenetic and quality characteristics, resistance to virus and disease, hybridization, and research achievements in the areas of origin, evolution and classification. The direction for future research studies are also indicated in this paper.

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The wild soybean (*Glycine. soja sieb. et Zucc*.) is the direct ancestor of the cultivated soybean (*G. max* (L.) Merrill). It is exclusively distributed in the east of Asia, including China, North Korea, Japan and the far eastern region of the Soviet Union. In the early 1940's, Chinese scholars Zhenglin Ding [1], Xingdong Sun [2] and Jinling Wang et al [3] started to observe and study the wild soybean. In the 1970's, Jinling Wang et al [4,5] carried out research on photosensitive types of wild soybean in China and made important observations. In 1978 the Jilin Academy of Agricultural Science surveyed wild soybeans in Jilin province [6]. They collected 800 samples and found many new genotypes. This survey was highly evaluated among soybean researchers. A national survey of wild soybean was held in 1979-1981 and 5,000 accessions were collected from 24 provinces [7]. The number of collected accessions was 9 times those that were collected worldwide from the U.S.A. (approximately 500). This survey greatly enhanced the world soybean germplasm accessions and paved the path for the future soybean research.

In the ten-year study of wild soybeans, Chinese researchers first screened for the significant priority of high protein content and strong adaptability [7,8]. They produced erect type hybrids with high protein content for seed production [9]. The geographical distribution of the wild soybean was clarified and both the ecotypes with abnormal cytological [10] and biochemical [11] characteristics were found. The photo-temperature ecological zones of wild soybean in China were theoretically defined [12]. As a result of multi-disciplinary studies of wild and cultivated soybeans, new reasons were found to support the hypothesis that soybeans originated in the Yellow River valley [13]. It was further proven that wild soybeans evolved into cultivated soybeans by various means. Researchers offered viewpoints on the classification of *Soja* subgenus [14]. A great quantity of important research papers have been published nationally and internationally. Research on wild soybeans in China is at the cutting edge. The following section will give a thorough description of its achievements.

Distribution and growth condition

The annual wild soybean is widely distributed in China. Its distribution covers the region from 24° N to 53° N and from 97° E to 135° E. The vertical distribution ranges from 0 to 2650 altitudes. The Tahe County, Helongjiang (52° 55'N) is the northernmost point of the distribution; The zone from Wusuli River Valley along Hainan Island to northern Taiwan is the easternmost distribution; Jiangtai County, along Yellow River Valley, Gansu (52° 55' N) the most northwest point of the distribution; in the southwest, it is distributed at Yaan County, Sichuan to Chayu County and Tibet; the southernmost distribution reaches the 24° N. The vertical distribution is from sea level to 2650 m above sea level in the Ninglang Autopolitics County [7], Yunnan. The distribution density is higher in northern China, where 75% of all varieties are located north of 5° N. The climactic factors restricting wild soybean are: 1) annual accumulated temperature (>10° C) lower than 1700° C or higher than 7000° C; 2) highest monthly average temperature lower than 19° C; 3) and frostless period shorter than 80 days; 4) annual precipitation less than 350 mm [15].

Wild soybeans prefer warmth, light and neutral pH soil. It generally grows along rivers, lakes and ditches. It also can be found growing on saline soils and at the seashore. The seed has strong cold hardiness and can survive through the winter when the lowest temperature is below 35° C (sic.).

Morphometrics, Developmental and Ecological Characteristics

Typical wild soybean is an herbaceous annual with thin stems. It has the following characteristics: spreading; differentiation of stems and branching not obvious; strongly twined; individual plant heights range from more than 10 mm to above 6 meters; trifoliate leaf; the shape and size of the leaflet differ greatly; the smallest leaflet is about 1.5 cm long and 0.7 cm wide; the size of the large leaflet is similar to that of the cultivated soybean leaf; the flower is very small and color in most wild genotypes is purple; not all flowers produce pods; the pod is 2 cm long and bears 1 to 4 seeds. The 100-kernel weight is 1-3 g. Most of the seeds are black and covered by a seed coat. The hilum is black or brown, and the cotyledon is yellow. The tap root system can reach 50 cm long [7]. The genotypes with larger seeds (3 to 8g per 100 seeds or heavier), bigger stem and larger leaflets are generally called half-wild. They are also called half-cultivated or intermediate soybeans.

The growth period of soybean crop varies significantly with location. It takes 80 to 210 days from seed emergence to plant maturation. Generally the maturation period becomes shorter from South to North. The difference is substantial in the period between seed germination and anthesis, which ranges from 40 to 150 days or so. The reproductive period is mainly influenced by the photoperiod and temperature [12,15]. It is shown that no matter where the wild soybean is planted, the reproductive period will be longer than that of its low-latitude origin [17,18].

Observations of different photoperiods and temperatures demonstrate that: (1) wild soybeans are all short-day plants, no matter where they are cultivated. There is a significant negative correlation between the requirements for daylight and the latitude of the originally cultivated area. The number of days (FD) (whose photoperiod is 13.5 hours) between the seed germination and bloom of the materials from the area of 24°N to 53°N latitude has an exponential curve relation with the latitude of the species' area of origin. The equation of the exponential curve is: $Y=6128^{x} \times 0.8468 + 23$(Y: FD value, X: latitude). The turning point of the curve is around 35°N. (2) If we used the daytime temperature/nighttime temperature 20/20°C as a standard, the high daytime temperature (30/20°C) will accelerate the blossoming, the low nighttime temperature 20/10°C will delay flowering. An increased difference between daytime and nighttime temperatures (30/10°C) can enhance the flowering of high latitude varieties (to the north of 40°C) and defer the blossoming of the low latitude varieties. Generally, the varieties in the high latitude area have strong adaptability to high day temperature and low night temperature; the varieties in the low latitude areas have the opposite tendency. The main soybean area is distributed between 35°N to 40°N. Compared with 30/20°C, the high temperature of 35/25°C can delay the flowering of most wild soybean types [19]. (3) The comparison of FD value of the varieties that are located in the same latitude is: high sea-level < low sea-level and inland < on shore. (4) Under some temperature and light conditions, the varieties in the high latitude will produce malformed pods; though the materials in the low latitude will delay podding. (5) According to the above results and 292 survey reports on the procreation and morphometric characteristics of wild soybeans in China, we can divide the Chinese wild soybean into 7 ecological zones. Considerating the 32 survey reports from Japan, 14 survey reports from North Korea and 5 survey reports from Soviet Union, the world wild soybean can be divided into 7 zones and 13 sub zones. The wild soybean is the extra-tropical species in the non-arid region of east Asia. In addition, Ying Li analyzed the relationship between the wild soybean and ecological conditions in Shanxi province, and indicated that the latitude and sea level of the area of origin have negative correlation with stalk height, branching and tiller, number of nodes, pods per plant, seeds per plant, and 100 kernel weight. The temperature in the area of origin has a positive correlation with the above factors. Within Shanxi, Jilin and Shaanxi provinces, local ecological zones of wild soybean have been identified.

Physiological and bio-chemical characteristics

Huan Sun et al collected 1694 wild soybean samples nationwide and tested the enzyme activity of the peroxide of the seed coat. All the results showed high enzyme activity. The electrophoresis analysis of Ti^a frequency of 339 samples' seeds protein trypsin proteinase was 80.8%, the frequency of 205 medium type samples was 89.9% [24] and the frequency of 102 cultivated soybean samples was 98.8%. The Ti^a gene type was first found in the wild soybean. According to the hypocotyl sulphate isozyme enzyme spectra analysis of 65 soybean samples of different evolutionary stages, it was found that intermediate soybean type had 6 spectra bands (A1, A2, C1, C2, C3, C4) [25]. These frequencies were between those of wild soybean and cultivated soybean. Acidic phosphatase and malic dehydrogenase have exhibited similar phenomena. Huan Sun et al. measured fluorescence reaction of wild soybean tender roots using 1653 materials collected from all over the country, and 88% of them had strong fluorescence reactions.

The chlorophyll contents of 74 samples of wild soybean functional leaves collected from all over the country were measured during anthisis [9]. It was illustrated that the chlorophyll content was significantly correlated to the latitude of area of origin. The unit chlorophyll content in fresh wild soybean leaf was higher than that in the cultivated soybean. However the chlorophyll a/b ratio was smaller than that of the cultivated one [26]. By studing the photo spectrum of wild soybean chlorophyll and exciton energy shift, it was found that three fluorescence emission photo spectrum bands that were shown at the low temperature (77 k) were from different pigment protein compounds [27].

During the time of anthisis and podding, the photosynthetic rates of wild soybean, intermediate soybean and cultivated soybean (100 units) were measured. The results were: wild soybean < intermediate soybean < cultivated soybean [9]. The photo saturation point value of wild soybean (40,000 1x) was lower than that of the cultivated soybean (60,000 1x). The photosynthetic rate of wild soybean under blue-green light was relatively higher than that of the cultivated soybean [28,29].

The nitrogen fixation activity of wild soybean was generally lower than that of cultivated soybean. The nitrogen fixation activity of some intermediate soybeans in the late reproductive period was obviously higher than that of cultivated soybeans. The fast-growing rhizobia strains had been separated from the wild soybean somewhere in China. The fast-growing rhizobia strain was mainly located in Shanxi, Henan and Ningxia provinces. Different sera types of fast-growing rhizobia were also identified.

Quality characteristics

The average protein content of 1695 wild soybean samples collected from all over the country was 44.38± 3.10%; The average protein content of 128 intermediate soybean samples was 46.80± 3.18%; The average protein content of 1635 cultivated soybean samples was 42.15± 3.19%; The distribution range (in latitude) of the soybean with high-protein content genotypes was between 30-32^o N and north of 43^o N. The protein content of the highest protein content reaches 55.37% [8]. With the SDS acrylanide gel electrophoresis analysis of 127 wild and intermediate types and 82 cultivated types, it was found that there was mobility deformation in some sub units of wild soybean's seed protein, especially the deformation of β sub units is larger than that of the cultivated soybean [30]. Bao Xu et al. found more than ten different genotypes that separated α globulins rapidly on electrophoresis. The 7S globulin was separated from the intermediate soybean and cultivated soybean. It was found that the sulphur-containing amino acids content of certain intermediate soybean was three times as that of the cultivated soybean [31]. Three globulins, 11S, 7S and 2S, were separated from all three groups, the wild, intermediate and cultivated soybeans. The 11S protein content was found to increase with evolutionary status. But the 7S showed the opposite tendency [32]. Bao Xu et al measured the 11S/7S ratio of the wild soybean and intermediate soybean, which were collected from throughout the country. The ratio was significantly correlated to the latitude of the area of origin (r=0.5689). A special geneotype whose 11S/7S ratio was as high as 4.4 was also found.

Forty-one wild soybean protein samples were analyzed for their amino acid constitution (g/16g N). The glutamic acid content was highest, the aspartic acid content second highest, followed by arginine, leusine and lysine. Others were lower than 5%. The aspartic acid and phenylalanine contents of cultivated soybean were a little higher than those of wild soybean. The histidine and arginine contents were a little bit lower than those of wild soybean [33]. The sulphur-containing amino acids content of the wild soybean with high protein (above 40%) was generally low, and the deformation coefficient was large. There was no correlation between the contents of sulphur-containing amino acids and protein [34].

Bao Xu et al. reported that the average fat content of 1598 wild soybean samples collected from all over the country was 9.94±1.59%. The average fat content of 118 intermediate soybean samples was 13.65±2.49% and that of 1595 cultivated soybean samples was 19.05±1.21%. The trends of protein content were the opposite. The composition of the wild soybean fat acid measured in 74 samples collected across the country was: linoleic acid (18:2): 55-56%, linolenic acid (18:3): 14-15%, oleic acid (18:1): 12-15%, palmitic acid (16:0): 11-12%, and stearic acid (18:0): 3-4%. The linoleic acid content of wild soybean was higher than that of the intermediate soybean; the latter was higher than that of cultivated soybean. The trend in oleic acid content was the opposite [35]. With the analysis of 127 samples of wild soybean, intermediate soybean and cultivated soybean, there were 95 wild soybean samples whose linolenic acid content was significantly positively correlated with the 100 kernel weight (r = -0.3362**) and negatively correlated with the place of origin latitude (r = -0.4181**). Wild soybean area of origin latitude was also significantly negatively correlated with stearic acid (r = -6504**) and palmitic acid (r = -253*) contents [36]. Bingchang Zhuang et al found that with the increase of daytime and nighttime temperatures, the linolenic acid content in wild soybean would decrease and the palmitic acid content would increase [37]. The ^{13}C nuclear magnetic resonance apparatus was ideal for analyzing the total components of the wild soybean seed [38].

Resistance to virus and diseases

Under natural conditions, no Soja viruses were found infecting wild soybean. However, when the wild soybean was cultivated, different symptoms of diseases were seen. The diagnoses indicated that the virus source of wild soybean's virus disease is the same as the virus source SMV of the cultivated soybean [39]. On the basis of field screening, 300 less-infected samples were selected for artificial inoculation screening. They showed different levels of resistance. The sample with the strongest resistance cannot compete with cultivated soybean samples [9], which had strong resistance. In the field screening of disease-resistance to *Heterodera glycines*, no highly resistant samples were found [40]. In the field screening for resistance to *Cerocospora sojin*, some immune samples were found [41].

On the basis of field screening of resistance of wild soybean to *A. glycines*, Derong Yue et al selected 30 highly resistant lines from 1500 lines collected throughout the country. By screening in different controlled conditions such as cube net cage, climate-controlled cube cage for three years, 2 samples with high-resistance were selected. Their resistance was higher than the strongest among the cultivated soybeans. In field observations in Heilongjiang, the wild soybean's resistance to *Grapholitha glycinivorella* was tested, and defoliation was lower than that of cultivated soybeans [42].

Cytogenetic characteristics and hybridization utilization

132 samples of wild and intermediate soybeans were selected from different areas for karyotype analysis of root-tip cell chromosome. The basic result is 2n=40=32 m + 6 Sm + 2 Smsat (m is the centromere, Sm is the second centromere, Smsat is the chromosome with satellite). Valuable seed material with 4-chromosome with satellites was discovered for the first time [10]. It was a distinguished finding in the research on soybean ploidy evolution. Jianbo Wang [43] carried out the analysis of the comparative karyotype of three sub-species of *Soja* and found certain differences.

Observing the pollen abortion of 64 combinations of wild and cultivated soybean F1 hybrids, Huan Sun et al calculated that chromosome translocation frequency was 83%. Many scholars carried out a detailed research on the hybridization and inheritance phenomenon among wild, intermediate and cultivated soybeans [44-54]. The following are some of the significant results:
(1) Inheritance of agronomic characteristics such as shoot height, number of branches, pod development, seed shape index and ratio of seed and stem follow the additive-dominant-superposition model. But the size of the shoot, vegetative growth duration, reproductive duration, 100-kernel weight and weight of single seed did not follow the above model. The inheritance of crop duration is strongest, followed by shoot height, 100 kernel weight, seed shape index and yield. Then, following size of main stem and ratio of seed to stem, the seed number per plant and branching were weakest in inheritance. (2) The inheritance of protein content followed the additive-dominant-superposition model. The inheritability was strong. It was correlated to the branching and duration of reproductive period. There was little correlation with other characteristics. (3) Parent selection was the key to the success of interspecific hybridization. In order to overcome the wild character of spreading, a short soybean, with one dominant dwarf allele and several minor genes, was selected as parent, achieving significant results. Using soybean with limited podding also achieved good results. Selecting the parent with larger 100-kernel weight can greatly increase the output of hybrids. To produce a descendent with yellow seed coat, species with yellow-brown coat should be selected in the pre-generation for backcrossing, so that the output of the yellow-brown seed coat type can increase significantly. When hybridizing the intermediate soybean with cultivated soybean, it was easy to obtain the type with middle- or small-sized seed. (4) The selective backcrossing in F3-generation can keep the wild soybean's character of high protein content. Besides, it is efficient in increasing the 100 kernel weight and raising the

frequency of yellow seed coat gene type. But random backcrossing in F1 generations always greatly decreased the occurrence of genes such as high protein content.

Through interspecific hybridization we obtained some intermediate material that had high protein content (45-50%) and also obtained a group of medium- and small-sized seeds. Some of them have already been released for production. Past assumptions that "wild soybean is difficult to breed" have been changed by the above experiences.

Moreover, there is significant progress in the field of cytogenetics. Using the *Agrobacterium tumefaciens* to cultivate tumors, we screened tumor-inducing materials from these soybean varieties. After tissue culturing, the virus-free callus is separated from the tumor. Upon testing, it is shown that part of it contained T-DNA. Through liquid cultivation, the T-DNA cell line was set up and it established a basis for gene transfer [55]. Similar results were also attained by Yuyu Jian et al. [56].

The researchers studied the structure of protein storing gene in the wild soybean seed , compared them with the cultivated soybean, and set up cDNA lines of both the wild soybean G.Soja SH and cotyledon of cultivated soybeans [57]. The researchers obtained seedlings from the callus of the wild soybean's lower hypocotyls [58].

The origination, evolution and classification of soybean

It is well known that soybeans originated in China. There are many hypotheses about where in China soybeans first appeared: northeast, middle north, central middle, south of the watershed of Yangtze River, and multiple originating centers etc. [4,59,60]. By comparisons of wild and cultivated soybean distributed all over China, and with research methods from ecology, quality chemistry and seed protein electrophoresis analysis, it can be concluded that soybean mainly originated from the middle down-stream area of the Yellow River, whose central area was around 35 ^{o} latitude. Shuen Wang supported the conclusion that soybean originated in the watershed of the Yellow River with evidence from ancient documents, evolutionary characteristics and so on. According to the distribution of wild soybeans and the numbers of abnormal types, soybean originated from Shanxi province, Shaanxi province and Jilin province. However, ancient documents supported the viewpoint that soybean originated from Hebei province.

There are different opinions about the evolution and classification of *G. Soja*. A majority of taxonomists support the a division between *G. soja* and *G. max* [63], but Skvovtzow (1943) indicated there should be an intermediate type between *G. soja* and *G.max*. Hymowitz indicated that the intermediate type was the natural hybridization of G. soja and G. max.

During the national wild soybean survey, many new characteristics were found, such as white flowers, elongate leaf, long inflorescence, limited pod numbers, yellow, green, and brown seed coats, blue pod color leaves, without the membranous coat, different leaf shapes and sizes, and different seed sizes, etc. Especially in areas where soybean was never cultivated before, many intermediate types between the wild soybean and cultivated soybean were found. They were even established in large areas. Without a doubt, the evolution and classification of *G. soja* will continue to raise academic interest and discussion.

1. Agronomic characteristics

Early in 1940's, Jinling Wang indicated that seed size, flowering, and stem diameter were the most important indicators to judge the evolutionary levels of soybean. Shizhen Shu et al compared the main characteristics of 30 wild soybean lines (1.6 g / 100 seeds), 30 intermediate lines 5.4 g) and 30 cultivated lines (15.1 g). The results were similar, showing yield as the most important evolutionary character, especially the 100 kernel weight. If seed size is larger, the pod and leaf will be larger, the pods and seeds per plant will be greater and plant height and branching will be lower. Bao Xu simultaneously compared wild soybean (1 g per 100 seeds), intermediate soybean (about 3 g), semi-cultivated soybean (about 5 g) and cultivated soybean (about 20 g) at the same altitude (45^{o} N). The continuously changing characteristics appearing during the evolutionary process of wild—intermediate—semi-cultivated—cultivated are: flower counts per single plant, pod count, seed count and weight; the height and size of stem, the progression and extent of branching and tiller, the duration of vegetative growth and reproductive growth, time of flowering (beginning to end), time of podding (beginning to end), size and weight of 100 seeds during time of maturity and 100 kernel weight. All flower pod shedding rates were between 43-47%, and harvest rates were all above 55%.

2. Photoperiod and temperature characteristics.

The sensitivity levels of photoperiod and day-night temperature to soybean plant growth and development are: wild type > mediate type > cultivated type [16,19,66] .

3. Physiological and bio-chemical characteristics.

Ti[a] frequency of seeds protein trypsin proteinase enzyme activity of the peroxide of the seed coat, the frequency of hypocotyl sulphate isozyme enzyme spectra and the frequency of acidic phosphatase and malic dehydrogenase all have the charateristics of continuous mutation developed from wild to intermediate to cultivated [24,25]. The performance of ultra-fluorine dismutase will dramatically decline with the evolution [67]. During the duration of seed emergence, the decrease rate of fat content, linolenic acid content and protein composition of 7S globulin's α α' subunit all declined in the rate as the sequence of wild > intermediate> cultivated [68,69].

4. Quality chemical characters.

Xu Bao et al. reports that protein content is: wild > intermediate> cultivated, and the fat acid has the opposite trend. Linolenic acid content is: wild > intermediate> cultivated and the olic acid has the opposite trend [35].

Some characteristics of the intermediate soybean are similar to those of the wild soybean, some are similar to those of the cultivated soybean and other characteristics are higher or lower than the value of those wild soybean or cultivated soybean. This phenomena shows that there are multiple possibilities that wild soybean evolves into cultivated soybean by many ways [14,68,70].

In recent years there are some new opinions indicated about the classification of the subunit of the *G. Soja* according to botanical characters. For example, Liaoning province indicated that beside *G. max* there should be two types, one varied type and 3 varied species [71]; Jilin province indicated that beside *G. max,* there should be two types and one varied species [10]; Heilongjiang province indicates that besides *G. max* there should be only one type under which is divided into 2 varied types and 7 varied species [40]; Shannxi province indicated that besides *G. soja* there should be 6 varied types, 1 sub varied type, four varied species and six sub varied species [23] etc.

The future study of wild soybeans

In the past decade, great discoveries have been achieved in research on the Chinese wild soybean. In this era of biological engineering, the quantity of attained gene sources and the depth and breadth of research will principally determine strategies for development of new varieties. Thorough research into the plentiful resources of wild soybean in China will not only help enhance productivity by settling the global issue of demand for high-quality protein, but will also offer keys to the research into origination, evolution and classification of soybeans. It is of great interest to science and sociology. China is the home of soybean. It strengthens advanced soybean research, explores soybean resources we have, establishes soybean research centers, advancing Chinese soybean cultivation techniques and contributes to world soybean research and production. These are required responsibilities of biological researchers in China.

To accelerate the pace of study, we need to unify research directions and materials, facilitate cooperation between multiple academies and research units and take advantage of modern biology technology [72]. The following work should be our focus:

1) Botanical characteristics study: including cooperative research on different soybean evolution types;
2) Research on wild soybean population ecology;
3) Identification of critical economic characteristics;
4) Research on inheritance between varieties;
5) Research on soybean origin, evolution and classification.

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