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Morphological, cytological and biochemical characterization of soybean germplasm

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ABSTRACT

Soybean oil is used in many foods, industrial and fuel products, and soybean meal is incorporated into animal feed. The variation in the quality and quantity of these products is basically dependent on the genetic diversity of soybean germplasm, which was evolved from the dispersion of the cultivated soybean domesticated by the Chinese farmers. The dispersion of soybean germplasm was affected by many factors including regional adaptation and selection. A wide range of markers have been used for evaluating the genetic diversity of the cultivated and wild relative of soybean extends from morphological characters to molecular ones. In this review we focused on morphological, cytological and biochemical markers. Soybean accessions from different collections exhibited a wide range of phenotypic variation for morphological and yield traits. This variation was successful in determining: (1) the gene pool of different collections of accessions, and (2) genotypes that tolerate drought stress and other stress which is the major factor that limiting soybean yield. The variation in genome size was pronounced in Chinese germplasm collected from diverse geographic locations. It has been ranged from 40 to 0%. This wide range is highly reproducible. SDS-PAGE was efficiently used for identification of various genotypes of wild soybean at the inter- and intra-specific levels. Allozyme markers have been used in soybean to evaluate genetic diversity in accessions from diverse geographic regions and the alleles specific to regional population.

KEYWORDS

Morphological characters;
Cytological traits;
Protein content;
Electrophoretic pattern;
Isozymes.

INTRODUCTION

The genes are the blueprint of the genetic material that translates into products. The plant breeding is one of traditional ways that transfer genes to yield improvement that feeds the expanding human populations. Genetically improved plant crops supply food for most humans, animals and other organisms. About 60 percent of the human population directly or indirectly depends in their living on agriculture. Unfortunatly, food production is population driven, that is, as we produce more food, the human population becomes larger and the demand for increased yield creates an open spiral of greater impact on the land[1]. Germplasm is the source of the genetic potential of living organisms, including plants and animals. The genetic variations in germplasm

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allows plant crops including soybean to adapt to changing environmental conditions. However, no one individual of any plant species contains all the genetic diversity of that species. That is mean that the total genetic diversity is represented only in populations made up of many individuals grown along the distribution range of the species. What is known the genepool of the species. The genetic potential represented in a genepool is the foundation for our crop plants in agriculture. Any degredation for the diversity of any plant crops narrows community's scope to respond to new problems and opportunities[2]. The problems we except to face in future are new plant diseases or pests, climatic change due to the greenhouse effect, and so forth. Therefore, the conservation of genetic resources of plant crops including soybean must be on the top agenda of polyciy makers, agriculturists, scholars and non governmental organization around the world.

SOYBEAN CLASSIFICATION

There are two subgenera under the genus, *Glycine* Willd.: *Glycine* and *Soja*. The subgenus, *Soja* (Moench) F.J. Herm., includes the cultivated soybean, *Glycine max* (L.) Merr., and the wild soybean, *Glycine soja* Sieb. & Zucc. Both species are annual. *Glycine soja* is the wild ancestor of *Glycine max* and grows wild in each of China, Japan, Korea, Taiwan and Russia[3]. The subgenus *Glycine* consists of at least 16 wild perennial species: for example, *Glycine canescens* F.J. Herm. and *G. tomentella* Hayata, both found in Australia and Papua New Guinea[4]. Beside *G. max* and *G. soja* in subgenus Soja, there is *G. gracilis* which is an intermediate form, first proposed as a new species by[5]. This form has numerous characteristics between *G. max* and *G. soja*. Based on data from morphology[6], cytogenetics[7], phytoalexins[8], restriction endonuclease fragment analysis of mitochondrial DNA[9], ribosomal RNA[10], chloroplast DNA[11] and sequences from the ITS (internal transcribed spacer) region of nuclear ribosomal DNA[12], *G. max* and *G. soja* form the primary gene pool for the cultivated soybean. Sexual compatibility provides direct evidence of the genomic relationship between these species. Singh and Hymowitz[13] conducted pachytene chromosome analysis of fertile F1 hybrids between.

ORIGIN

Soybean is originated in China and has been planted for over 3000 years. It has been grown in Korea and Japan for more than 2,000 years. These three countries are thus considered as major sources of soybean germplasm. It was domesticated in Korea and Japan from the wild annual species *Glycine soja*.

At one time it was thought that *G. gracilis* would have an important role in determining the origin of *G. max*. Fukuda[14] suggested that differentiation from the wild to the cultivated forms involved *G. gracilis* in the following manner: *G. soja* - *G. gracilis* - *G. max*. However, Hymowitz[15] pointed out that *G. gracilis*, rather than being an intermediate step from *G. soja* to *G. max*, might actually be a hybridization product of *G. max* and *G. soja*. This hypothesis was supported by Broich and Palmer[16] based on the results from the study of frequency and distribution of 10 loci among *G. max*, *G. soja* and *G. gracilis*. The distribution of *G.soja* is limited to China, Japan, Korea and the Far East area of Russia in East Asia. The evidences that China is the origin and main center of diversity of soybean are (1) the distribution of *G.soja* in China is the most extensive in terms of the numbers and diversity of types; (2) China has the earliest written records of soybean cultivation, about 4500 years ago; (3) soybean has been found in unearthed artifacts; (4) soybeans cultivated in different countries in the world were introduced directly or indirectly from China; and (5) the pronunciation of the word of soybean in many countries is about the same as the Chinese 'Shu'; for instance, it is pronounced 'soya' in England, 'soy' in the USA, and in other languages. Although, the origin of soybean cultivation may be China, scholars have different viewpoints on the original areas of soybean domestication[14,15,17-20].

GERMPLASM CHARACTERIZATION

Germplasm characterization is an important operation for a gene bank. The value of the germplasm collection depends upon the availability of information relative to the accessions. Morphological, agronomic, biochemical and molecular traits as well as reaction to biotic and abiotic stresses that are known to be in the

individual accessions increase the importance of the germplasm. Moreover, systematic description leads to a more efficient use of germplasm in the collection

Characterization of soybean germplasm based on morphological traits

As we know, phenotypic traits are controlled by genes and affected by environment, but large numbers of accessions can adapt to environments. The phenotypic data has more polymorphism in genetic diversity and reveal genetic variation indirectly. On the contrary, the molecular data reveal genetic variation directly, but fewer markers have less polymorphism. It is very difficult to obtain molecular data for a large number of accessions that has enough polymorphism to show the genetic diversity of germplasm. So, the morphological traits are the suitable and practical tools for studying the genetic diversity on large numbers of accessions. Variation in shape of plants has always been an important means of (1) distinguishing individuals; (2) controlling seed production; and (3) identifying the negative traits those effects on yield, the genetic diversity centers of annual wild soybean and the soybean lines resistance to pod shatter, drought, pests or disease[21,22]. The studied soybean germplasm exhibited a wide range of phenotypic variation for yield traits. It also showed that soybean developing stages had close association with agronomic traits as well as yield and yield components.

The genetic variability in 131 accessions of edamame soybeans (the Japanese name for a type of vegetable soybean eaten at the immature R6 stage) was analyzed using phenotypic traits e. g. maturity information, testa color, and 100-seed weight for breeding new edamame lines resistance to pod shatter[23]. The 131 accessions include 108 Japanese edamame, 11 Chinese maodou, 8 WSU breeding lines, 2 US edamame and 2 US grain soybeans. The obtained results indicated that Edamame genetic diversity was generally clustered around maturity groups and testa color. It was also reported that the genetic diversity among the Japanese edamame cultivars was narrow, compared to Chinese maodou; Japanese edamame and Chinese maodou soybeans may have different genetic pools.

Soybean genotypes, which exhibit genetic diversity in root system developmental plasticity in response to water deficits in order to enable physiological and genetic analyses of the regulatory mechanisms involved, were identified[24]. These genotypes can tolerate drought stress which is the major factor that limiting soybean yield. The results showed substantial genetic diversity in the capacity for increased lateral root development (number and total length of roots produced) and in the responses of overall root and shoot growth under water deficit conditions.

Pod shape is one of the important descriptors for evaluating soybean genetic resources[25,26]. Truong *et al.*[27] tested the applicability of elliptic Fourier method for evaluating genetic diversity of pod shape in 20 soybean (*Glycine max* L. Merrill) genotypes. They concluded that principal component scores based on elliptic Fourier descriptors yield seemed to be useful in quantitative parameters not only for evaluating soybean pod shape in a soybean breeding program but also for describing pod shape for evaluating soybean germplasm.

The genetic diversity was evaluated for genotypes of soybean based on the yield-related traits[21,22]. It has been reported that differences among genotypes for all the characters were highly significant and the grain yield was positively and significantly correlated with number of pods per plant. The selection for the character had positive direct effect on yield. However, some traits had negative direct effects on yield, such as the leaf area, first pod height, days to 50% flowering, days to flowering completion, days to maturity, plant height, oil content and protein content. Rajput *et al.*[28] observed considerable genetic variability among 36 diverse soybean varieties for plant height, pods plant^{-1} and grain yield plant^{-1}, exhibiting high heritability and genetic advance for pods plant^{-1} and branches plant^{-1}, and suggesting intensive selection for these characters to increase yield. Mehetre *et al.*[29] showed highly significant differences among the genotypes for the eight yield related traits. The genotypes were grouped into ten clusters. The cluster pattern revealed that there was no association between genetic diversity and geographic distribution. Vollmann *et al.*[30] conducted field experiments to study the variations in different genotypes of soybean and revealed that grain yield and environmental variations had more effects on seed size than days to flowering, days to maturity, and oil content. It was also noted that environmental covariates of grain yield and protein content were highly positively correlated.

Bhartiya *et al.*[31] studied genetic divergence for yield and different yield contributing traits in 282 black

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soybean accessions collected from different eco geographic regions of the world. Based on non-hierarchical euclidean cluster analysis all accessions were grouped into 9 clusters, indicating high genetic variability among the accessions. Cluster 1 showed minimum mean value for days to 50% flowering, plant height and days to maturity. So this cluster can be very useful to develop early maturing genotypes. Cluster IX exhibited maximum mean value for pod length, 100 seed weight and seed yield per plant. So, from yield point of view, this cluster can be used to develop high yielding as well as high grain weight genotypes. Inter cluster distance was found maximum between cluster IV and IX. Hence genotypes from these clusters can be used in hybridization to get desirable recombinants. Accessions VBS 25, VBS 48 from cluster VII and VBS161, VBS152 from cluster VIII found as exceptionally superior donor which can be used in multiple crossing programmes to get transgressive segregants for desirable traits.

Tyagi and Sethi[32] estimated the genetic distance for 40 genotypes of soybean collected from different states of India and abroad using D2 statistics. These genotypes were grouped into six clusters. The analysis further indicated that the genotypes of common geographical origin or same location were grouped into different clusters, suggesting a lack of relationship between genetic and geographical diversity. The highest inter-cluster distance was observed between II and IV followed by II and VI may serve as potential parents for hybridization.

Khan et al.[33] estimated the genetic variability in 20 different soybean genotypes using agronomic traits. The results of analysis revealed that all the characters were significantly affected due to various soybean genotypes. The genotypes Zane, Black hack, Bragg and Menlin remained the best among 20 lines studied in term of bearing pods, high grain wt and yield production. It was suggested that these superior lines may be focused and involved in future breeding programme for development of new high yielding soybean varieties.

Characterization of soybean germplasm based on karyological traits

Genetic diversity based on genome size among and within plant species has been well documented in the literature[34-37]. The variation was pronounced in Chinese germplasm collected from diverse geographic locations. It was attributed to the environmental factors[38,39], cell size, minimum generation time, cell division rate and growth rate[40,41] and polypoid species, in species with large seeds, and habits type[42].

Reports of genome size variation in soybean [*Glycine max* (L.)] have ranged from 40 to 0%[43]. This wide range is highly reproducible and has resulted in doubts of the existence of intra-specific DNA variation in soybean. Rayburn *et al*.[43] determined genome size of 18 soybean lines, selected on the basis of diversity of origin, by flow cytometry. They found that genome size variation between these lines was at approximately 4%. This amount of DNA variation is lower than was originally reported[45-48]. Doerschug *et al*.[49] is the first to determine genome size of soybean, upon examining 11 soybean lines, reporting over a 40% variation in nuclear DNA content. Graham *et al*.[48] observed a 15% variation among soybean cultivars while Rayburn *et al*.[36] reported a 12% variation among 90 Chinese soybean introductions. Chung *et al*.[42] observed among 12 soybean strains a 4.6% DNA content variation. Yamamota and Nagato[50] stated about 60% variation, while Hammatt *et al*.[45] reported that the variation of genome size in 14 different *Glycine* species from different parts of the world was approximately 58%. These results indicated that the variability between DNA content was varied between the different scholars. The wide variation in genome size between soybean germplasm makes these accessions good candidates for crop improvement.

Greilhuber and Obermayer[51] reinvestigated the genome size of cultivars reported a 1.15-fold difference in genome size between them and a positive correlation of genome size and maturity group, using DAPI and ethidium bromide flow cytometry and Feulgen densitometry. Their analysis revealed no reproducible genome size differences between these cultivars with this technique and correlation with maturity group was not confirmed. The previously claimed statistical significance of such a correlation was found to result from only one exceedingly low DNA value of an early maturing cultivar, which, according to their data, is not different from the others. Furthermore ten accessions (five allegedly ranking high and five low) were reinvestigated for genome size using propidium iodide flow cytometry and Feulgen densitometry[52]. Using flow cytometry, the maximum difference between accessions was 1.018-

fold (non-significant); the difference between the means of the high-ranking and low-ranking group was 1.002-fold (non-significant). With Feulgen densitometry, the maximum difference between accessions was 1.034-fold (non-significantcant). This data suggest genome size constancy, confirming the previous one, in terms of cytometric evidence, for the Chinese soybean accessions in question. Likewise, no reasonable evidence was obtained for a difference between Chinese and American soybeans.

Xu *et al*.[53] investigated the DNA density of the embryo, cotyledon, and seed coat of each soybean from 15 soybean cultivars, and evaluated the impacts of variations of tissue DNA density and nuclear DNA content in soybean lines on GMO quantification. The results have shown that DNA densities and DNA quantity ratios among the various tissues of soybean are significantly different from each other and have insignificant influence on the transgenic copy number and therefore on GMO quantification. Nuclear DNA content of soybean is different from cultivar to cultivar. Results show that variation of nuclear DNA content in soybean lines has a great impact on the accurate determination of GMO. In some extreme situations, the deviation amplitude can reach 26%, which is intolerable for accurate determination.

Characterization of soybean germplasm based on biochemical traits

The genetic markers have made possible a more accurate evaluation of the genetic and environmental components of variation. The biochemical markers are ones of the interesting measures of genetic diversity. They include protein techniques and isozymes. The protein techniques are practical and reliable methods for cultivars and species identification because seed storage proteins are largely independent of environmental fluctuation[54-57]. They are less expensive as compared to DNA Genetic markers. SDS-PAGE is one of these techniques, widely used to describe seed protein diversity of crop germplasm[58-65]. Genetic diversity and the pattern of variation in soybean germplasm have been evaluated with seed proteins[66-70]. SDS-PAGE[71] and discontinuous polyacrylamide slab gel electrophoresis[72-74] were used very successfully in evaluating the genetic diversity and identifying soybean (*Glycine max*) cultivars. Malik *et al*.[69] evaluated the genetic variation in 92 accessions of soybean collected from five different geographical regions using the electrophoretic patterns of seed proteins. The accessions from various sources differed considerably, indicating that there is no definite relationship between genetic diversity and geographic diversity. Similar results were reported by (Ghafoor *et al*.[75]. Salimi *et al*[76] assayed the genetic variation of seed protein by SDS-PAGE for 19 genotypes of soybean (*Glycine max*). The results of factor analysis for SDS-PAGE revealed that 5 independent factors explained 78.018% of variations in the studied genotypes. The construced dendrogram classified the evaluated genotypes into 7 groups. On the basis of SDS-PAGE, 20 reproducible bands were used for analysis of the genetic diversity in the evaluated genotypes. 60% of these bands were polymorphic, indicating the successful use of SDS-PAGE in assaying the genetic diversity in soybean germplasm.

Based on the results of Barakat and Malik *et al*.and Salimi et al.[69,76,77]. SDS-PAGE can be used effeciently for identification of various genotypes of wild soybean at the inter and intra-specific levels[78-82]. 2-D electrophoresis can be used to characterize the genotypes exhibited similar banding patterns[83,84].

Allozyme markers have been used in soybean to evaluate genetic diversity in accessions from diverse geographic regions[85-87], in wild soybean in natural populations from China, Japan and South Korea[88-91], and in Asian soybean populations[92-94]. From an analysis of the Kunitz trypsin inhibitor (*Ti*) and beta-amylase isozyme (*Sp1 = Amy3*),[95-98] defined seven soybean germplasm pools in Asia: (1) northeast China and the USSR, (2) central and south China, (3) Korea, (4) Japan, (5) Taiwan and south Asia, (6) north India and Nepal and (7) central India. Hirata *et al*.[93] compared the genetic variation at 16 isozyme of 781 Japanese accessions with the genetic variations of 158 Koren and 94 Chinese accessions, detecting a number of region-specific alleles that discriminated Japanese from Chinese accessions. The presence of alleles specific to the Japanese population suggested that the present Japanese soybean population was not solely a subset of the Chinese population.

Valentini[99] used polymorphism levels in "- and β-esterase loci from leaf tissues of Brazilian soybean cultivars for the analysis of population genetic diversity and structure, and to investigate relationships between conventional and genetically modified cultivars. The genetic

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basis of the conventional cultivars was found to be broader than that of genetically modified cultivars. Higher genetic identity was detected between plants of conventional and genetically modified cultivars.

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