Genetic Variation in Wild Soybean (Glycine soja Sieb. & Zucc.) Populations Revealed with Simple Sequence Repeat Markers

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Introduction

Microsatellites or simple sequence repeats (SSRs) consist of tandemly repeated core sequences that often vary in repeat number and are flanked by conserved DNA sequences. (Maughan et al 1995). It is well-known that SSRs are ideal genetic markers in that they are (1) highly abundant and evenly distributed (2) highly polymorphic (3) codominant (4) rapidly typed via PCR and (5) very accessible to other laboratories via published primer sequences (Weber 1990; Saghai-Maroof et al 1994). The length polymorphisms of SSRs in soybean and wild soybean (Glycine soja) have been widely studied (Akkaya et al 1992; Jiang et al 1995; Akkaya et al 1995; Maughan et al 1995 and Powell et al 1996). However to our knowledge SSRs DNA marker has not yet been served as a genetic marker in the natural populations of wild soybean (Glycine soja Sieb. & Zucc.). The salt tolerance efficiencies of wild soybean individuals were evaluated by using the method in our previous work (Hu and Wang 1997; Wang et al. 1997) and several RAPD markers were found to be linked to the salt tolerance character of wild soybean (Zhang et al. 1999). In present paper we evaluated the applicability of SSRs as a source of genetic markers in salt resistance of wild soybean in two natural populations in saline conditions.

Materials and Methods

Total DNA was isolated from the leave of wild soybean seedlings as described by Stewart (1994). Primers were synthesized on an Oligo 1000 DNA synthesizer (Beckman Fullerton CA U.S.A..). PCR amplifications were performed with each of the 16 wild soybean individuals using primers to six (AT)n/(TA)n and two (ATT)n/(TAA)n SSR loci (Table 1). Reaction mixes contained 20ng wild soybean genomic DNA 0.4Î¼mol/L of 3' and 5' end primers 200Î¼M of the four deoxynucleoside triphosphates 1xPCR Buffer and 2.5UTaq DNA polymerase in a total volume of 25Î¼l. DNA amplification was conducted according to Jiang et al (1995) in the RapidcyclerTM (Idaho Technology Idaho U.S.A.). PCR products (1.0Î¼l/lane) were separated on 10% denaturing polyacrylamide gels with 8M Urea and 1xTBE at 200V(constant) using the Mini-Proteinâ…¡ Electrophoresis cell (Bio-Rad Lab. Hercules CA USA). Gels were detected by silver-staining (Bassam et al. 1991). Data presented in Table 2 were scored by visual observation.

The gene diversity was calculated as follow:

Gene Diversity = 1-Î£ Pij 2

Where Pij is the frequency of the jth allele for primer-pair i and is summed across patterns (Weir 1990).

Results and Discussion

Eight pairs of SSR oligonucleotide primers (Table 1.) were used to screen the length polymorphism of SSRs in the16 wild soybean individuals in two natural saline populations. Allele sizes were determined and listed in Table 2 17 alleles were identified with eight pairs of SSR primers (2.125 alleles per primers pair). To our surprise the values of gene diversity associated with each of the SSR markers were extremely low ranging from 0 to 0.3203 with a mean value of 0.2304 when calculated based upon the full set of 16 wild soybean individuals. Moreover in the case of SAT43 (AT)n and SATT1(ATT)n loci PCR amplification with all the individuals produced similar length products 151bp and 210bp respectively (Table 2.). In the 16 wild soybean individuals the five loci such as SAT1(AT)n SAT36(AT)n SAT43(AT)n SATT1(ATT)n and SATT2(ATT)n showed much lower levels of allelic length polymorphism than in the results demonstrated by Jiang et al (1995). In addition in the case of the SATT1(ATT)n locus the allele had an

estimated length of 210bp which was longer than that reported by Jiang et al(1995). In the case of SATT2(ATT)n locus even the smaller size of this allele(217bp) was also larger than the largest size demonstrated by Jiang et al(1995). Clearly Glycine soja Sieb. & Zucc. was not included in the work undertaken by Jiang et al(1995) and it is therefore not surprising that the allele size of the SATT1(ATT)n and SATT2(ATT)n loci in Glycine soja Sieb. & Zucc. were different from that reported by Jiang et al(1995). At the SOYABAB(AT)n locus the size of this allele ranging from 174bp to 206bp was smaller than that(290bp) predicted based upon the GeneBank sequence described as Akkaya et al(1992). The most interesting thing was that fewer ghosts bands (Edwards et al. 1991) or "stuttering" (Jiang et al. 1995) were found in the PCR products amplified with all the eight primers pairs whether di- or tri-nucleotide SSRs after detection by silver-staining.

In our cases no allelic length polymorphism of SSRs was found to be in correlation with the salt tolerant characters of the wild soybean in natural populations. Further research is needed to investigate the length polymorphism of the SSRs markers amplified with more pairs of primers.

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