RAPD Markers Associated with Salt Tolerance In Wild Soybean Populations

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Introduction

Salinity is a major environmental constraint to crop production in the arid and semi-arid regions of the world (Greenway and Munns 1980). Most crop plants including wild soybeans are sensitive to salt stress. Sources of salt tolerance have been identified among some related wild species. These genetic resources could potentially be used to improve salt tolerance of soybean cultivars.

One approach to facilitate the selection and breeding for complex traits such as salt tolerance is the identification and utilization of simply inherited genetic markers that are genetically associated with the trait of interest (Tanksley 1993; Foolad et al., 1995). In previous study, we employed isozymes and RAPDs as genetic markers to identify the relationship between the markers and salt-tolerance. Little correlation was found (Wang et al., 1997). The present investigation was conducted to further examine randomly amplified polymorphism DNA (RAPD) markers associated with salt tolerance and therefore to study the molecular mechanisms of adaptation of wild soybean to changing saline conditions.

Materials and methods

Plant material:

The wild soybean populations No.3 and No.4 (Glycine soja Sieb et Zucc.) selected in the experiment survive in saline conditions near the Yellow River Delta (Wang et al., 1997).

DNA extraction

Total DNA was extracted from leaf tissue of each selected individual as described by Stewart (1994).

RAPD Analysis

Eleven random 10-mer primers (Operon TM Technologies, Alameda. Calif.) were used for polymerase chain reaction (PCR) amplification and identification of polymorphic markers. Amplification for RAPD was carried out in 25ul volumes containing 20mMTris-HCl, pH 8.0; 2.0mM MgCl(2); 200uM each of dATP, dCTP, dGTP and dTTP (Huamei company); 1.2ng/ul primer; 20ng template DNA and 1.0 unit of Taq DNA polymerase (Institute of Genetics, China Academy of Sciences). Amplification was performed in an Idaho Technology thermal cycler. The PCR cycle profile was: one cycle of 5 min at 95°C for predenaturing; 45 cycles of 1 min at 94°C, 1 min at 35°C, 2 min at 72°C for denaturing, annealing and primer extension respectively; one cycle of 10 min at 72°C for final extension. Amplification products (3ul) were separated on 3.5 % polyacrylamide gels and detected by silver staining (Bassam et al., 1991) as described in our previous report (Hu et al., 1997).

Results and discussion

Genetic diversity as revealed by RAPDs

Using 11 primers, 148 polymorphic and 75 monomorphic distinct bands (66.3% polymorphism) were revealed in wild soybean populations of No. 3; While 93 polymorphic and 102 monomorphic distinct bands (47.6 polymorphism) were revealed in those of No. 4. The salt tolerance of No.3 is higher than that of No.4 has already been confirmed through physiological data (Wang et al., 1997). But whether the polymorphisms and salt-tolerance are inevitably related demands further study and discussion. By the Neighbor Joining (NJ) tree methods in the Molecular Evolutionary Genetic Analysis

(MEGA) (Kumr et al., 1993), a dendrogram based on average linkage cluster analysis was generated from the RAPD data. The dendrogram shows clustering of genotypes according to their location. It is useful for us to extensively study the evolutionary relationships among and within the populations and to further explore the molecular origin about the mechanisms on adaptation of wild soybean populations to saline conditions.

Association of RAPDs with salt tolerance

Of the 148 polymorphic RAPD bands, 6 were significantly associated with salt tolerance. They are OPF05(213), OPF19(4361), OPF19(1727), OPF19(14000), OPF19(700), OPH02(1350). These markers are present in all the salt-tolerant individuals selected in the study while absent in all the salt-sensitive ones; as for the middle salt-tolerant ones, only some markers are present. The results indicate that there perhaps exists certain relationship between the molecular markers and salt-tolerance. Further study is required to check whether the markers are closely related to salt tolerance and potentially use these genetic resources to improve salt tolerance of modern soybean cultivars.

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