A Preliminary Genetic Linkage Map of Soybean Using an Intraspecific Cross of Two Cultivars: 'Peking' and 'Lee'

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Abstract

A total of 64 EcoR I (+3) and Mse I (+3) primer combinations were used to generate amplified fragment length polymorphisms in a soybean (Glycine max L. Merr.) mapping population that consisted of 116 F2 plants from a cross between two cultivars: 'Peking' and 'Lee'. One hundred eleven amplified fragment length polymorphism markers were developed from 30 primer pairs. A molecular map consisting of 74 amplified fragment length polymorphisms, 2 simple sequence repeats, and one phenotypic trait (RcsPeking, resistance to Cercospora sojina), has been constructed. The map defined 599.8 cM of the soybean genome comprising 15 linkage groups. The average interval between these markers was 11.9 cM. An additional 37 polymorphic markers remained unlinked.

Introduction

Genetic linkage mapping has proven to be a powerful tool for gene localization, gene isolation, marker-assisted selection and evolutionary

studies. Molecular maps have been constructed in many higher plants (Bert et al. 1999; Chen and Foolad 1999; Lu et al. 1998; Paillard et al. 1996; Prince et al. 1993; Qi et al. 1998). Soybean chromosomes have been mapped in detail by restriction fragment length polymorphism (RFLP) (Lorenzen et al. 1995; Shoemaker and Specht 1995), simple sequence repeat (SSR) (Akkaya et al. 1995), and amplified fragment length polymorphism (AFLP) analyses (Keim et al. 1997). Molecular and classical genetic linkage groups have also been integrated (Cregan et al. 1999).

Peking was first identified as resistant material to soybean cyst nematode, Heterodera glycines Ichinohe (Ross and Brim 1957). It has been extensively used as a genetic resource in soybean breeding. Previous studies indicated that this cultivar was also resistant to reniform nematode, Rotylenchulus reniformis (Rebois et al. 1968), peanut mottle virus (Demski and Kuhn 1975), soybean dwarf disease (Tanimura and Tamada 1976), soybean stem canker (Diaporthe phaseolorum f. sp. caulivora) (Keeling 1982), and frogeye leaf spot (Cercospora sojina) (Baker et al. 1999). Moreover it is tolerant to Phytophthora megasperma races 2 and 11 and to Pseudomonas syringae pv. Glycinea (Pacumbaba and Sapra 1984). DNA genetic markers linked to soybean cyst nematode resistance have been found in Peking (Concibido et al. 1997; Mahalingam and Skorupska 1995; Qiu et al. 1999; Skorupska et al. 1994).

In this paper we present the genetic linkage map obtained from the cross of Peking X Lee. This will provide information for localizing and isolating resistance genes from Peking.

Materials and methods

Plant Materials

We developed an F2 segregating population of 116 individuals from a cross between two soybean cultivars, Peking and Lee. The F1 hybrids were developed in July 1996. The F1 plants were grown in the greenhouse during the following winter and were selfed to produce F2 seeds. F2 plants were grown in the field during 1997 and used as a source of leaf material for DNA extraction. F2 individuals were classified as resistant or susceptible to frogeye leaf spot according to the methods of Phillips and Boerma (1981).

Sample collection and DNA preparation

We collected young leaves from Peking Lee and each F2 plant. All leaf samples were placed on ice in the field and during transportation to the laboratory and were subsequently stored at -80 Â°C until processed. Genomic DNA was isolated from the leaf samples using the urea-based procedure (Chen and Dellaporta 1994). The DNA was purified and quantified according to the methods of Qiu et al. (1995) and then stored at -20 Â°C.

AFLP and SSR analysis

We conducted AFLP analysis according to the methods of Vos et al. (1995). AFLP® Analysis System I kit (Life Technologies Gathersburg MD) was used except that eight selective EcoR I primers were replaced by IRD 700 Dye-labeled primers (LI-COR Inc. Lincoln NE) in the final PCR amplification. SSR analysis was conducted according to the protocol of Diwan and Cregan (1997) with the exception of using IRD 700 Dye-labeled forward primer (LI-COR). All reactions were performed using a PTC-100™ Peltler-effect Thermal Cycler (MJ Research Inc. Watertown MA).

Gel analysis

Following the amplification reactions the PCR products were mixed with 3 μl of formamide stop/loading buffer (95% formamide 20 mM EDTA pH 8.0 and bromo phenol blue) denatured at 94°C for 3 min and then chilled immediately to 4 °C using a PTC-100 Peltler-effect Thermal Cycler. Each sample (0.8 μl for AFLP analysis and 1.5 μl for SSR analysis) was loaded onto an 8% Long ranger gel (FMC Corporation Rockland ME) using an 8-channel Hamilton syringe (Hamilton Company Reno NV). IRD-labeled molecular markers (LI-COR) were loaded in two lanes as a standard. The gel was cast using the LI-COR 25-cm (for AFLP analysis) or 18 cm (for SSR analysis) gel apparatus and electrophoresis was performed at constant power (35 W for AFLP analysis and 25 W for SSR analysis) using the Long ReadIR 4200 DNA Sequencer (LI-COR). Data were collected by BaseimageIR v4.0 software package (LI-COR). Automated band calling and size determination were performed using Gene ImageIR Software v3.0 (LI-COR).

Nomenclature of AFLP markers

AFLP markers were designated according to the letters of the three selective

nucleotide extension at the 3' end of the EcoR I + 3 primer followed by the three letters of the Mse I + 3 primers and the size of markers produced by the combination of the primers. Amplification products with a size below 100 nucleotides were omitted from further analysis.

Data analysis

We analyzed the data using the Kosambi map function of Mapmaker/Exp v 3.0 (Lander et al. 1987) to develop a linkage map for this population. The mapping analysis was conducted using a 3.0 minimum LOD (logarithm of the odds ratio) score and 50 cM maximum distance.

Results and discussion

A single pair of enzyme combinations (EcoR I and Mse I) was used to generate the AFLP data. The eight EcoR I (+3) and the eight Mse I (+3) primers gave 64 different combinations. The 64 primer pairs and three SSR markers were used to survey the polymorphisms in the parents Peking and Lee. Two of the three SSR markers could detect polymorphisms between the two parents. Thirty (47%) of the AFLP primer pairs produced approximately 1 658 amplified DNA bands ranging in size from 100 to 800 bp and detected polymorphic bands ranging in number from 1 to 9 with an average of three per primer combination. The number of bands generated by each primer combination ranged from 29 to 81 (Table 1). The rest of the AFLP primer combinations (53%) could not detect polymorphisms between Peking and Lee. These results indicated that the parental genotypes in this study showed a low frequency of polymorphic AFLP markers. Peking and Lee were well-adapted cultivars in China and the Southern USA respectively. Keim et al. (1992) found that a lower frequency of polymorphic AFLP markers would be expected among adapted germplasms based on RFLP marker results. Maughan et al. (1996) also indicated the relatively low genetic diversity present in cultivated soybean. The lower frequency of polymorphisms in the intraspecific cross has made it difficult to construct a genetic map. However it has the advantage in genetic analysis of agronomic traits such as yield and disease resistance which are difficult to evaluate in interspecific crosses.

The average number of observed AFLP fragments was highest for AT rich +3/+3 primers (Table 3). This is in agreement with the results of Keim et al.

(1997). However the frequency of polymorphic markers did not increase when a higher percentage of A + T was present in the primer combination in this study.

The entire F2 population was used to detect the possible markers and analyze segregation of polymorphism. Of the 176 AFLP polymorphic loci detected in the two parents 111 (63%) segregated as 3:1 presence/absence in the F2 population. These polymorphic amplified fragments were scored as AFLP markers (Table 1). Two SSR markers segregated as a 1:2:1 ratio among F2 individuals. The segregation of resistance to frogeye leaf spot showed a good fit to a 3:1 resistant/susceptible ratio (X2=1.15 0.25<p

A genetic linkage map consisting of 114 markers including 111 AFLP, two SSR, and one phenotypic trait (resistance to FLS) was constructed using Mapmaker software v3.0. Of the 114 markers 77 were assembled into 15 linkage groups (Table 2 Fig. 1). These markers covered 599.8 cM of the soybean genome with an average interval of 11.9 cM between adjacent markers. Thirty-seven markers remained unassigned to any linkage group. Two of three SSR markers which have been mapped to soybean linkage group J were put into this map. More work remains to be done to integrate this map with other maps.

Distorted segregation has been repeatedly observed in many plant species in molecular-marker linkage mapping. Lu et al. (1998) reported that about 15% of the AFLP markers deviated from the expected 3:1 or 1:2:1 segregation ratios in the K62-68 family of peach rootstocks. Xu et al. (1997) observed that 6.8 to 31.8% of the mapped marker loci showed segregation distortion. Paillard et al. (1996) found that 12% of the RFLP markers and 20% of the RAPD markers in a linkage map of a doubled-haploid population of Coffea canephora also showed segregation that deviated from the expected 1:1 ratio ($P<0.01$). Abnormal inheritance occurred with about 25% of AFLP markers observed in soybean (Prabhu and Gresshoff 1994). In our study about 37% of the AFLP polymorphic loci deviated from the expected 3:1 segregation ratio in the F2 population. This segregation distortion may be caused by a partial lethal-factor acting in the final generations (Cheng et al. 1998) such as expression of linked lethal genes either at the gametic or zygotic stage of development or by partial gametic selection in both the male and female sides.

Nonparental RAPD bands which were not amplified from either of the parental DNAs were first reported in offspring from both baboon and human CEPH pedigrees (Riedy et al. 1992). Explanations for nonparental bands include PCR artifacts (Jansen and Ledley 1990), genomic mutations (Jeffreys et al. 1988), contamination of sample (Davis et al. 1995) or reagents, or heteroduplex molecules formed between allelic sequences (Ayliffe et al. 1994). In our study 4 different primer combinations produced a total of four nonparental bands (the frequency is 0.022). They segregated as a 3:1 presence/absence ratio in the F2 population. Contamination of samples or reagents is unlikely given our control results and repeatability of these novel bands. It is clear that much additional work will be required before a complete understanding of why this occurs. We did not score the distorted segregation bands and nonparental bands as AFLP markers in this study.

The Peking cultivar will continue to be a useful genetic resource. We present here a genetic linkage map based on AFLP markers. This will provide additional information for soybean breeders who use this cultivar in their breeding program.

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