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RAPD Loci for Seed Protein and Oil Content in Soybean (*Glycine max*)

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ABSTRACT

Seed protein and oil content is an important trait in the soybean. Both seed protein and oil content in this plant species is inherited quantitatively. A 68-plant F_2 segregation population derived from a mating between Mercury and PI 467.468 was evaluated with random amplified polymorphic DNA (RAPD) markers to identify QTL related to seed protein and oil content. Marker OPB12 was found to be associated with differences in seed protein content. Four markers, OPA09b, OPM07b, OPC14, and OPN11b had highly significant effects on seed oil content. By interval mapping, the interval between marker OPK3c and OPQ1b on linkage group 13 contained a QTL that explained 25.7% variation for seed oil content.

Key word: QTL, RAPD, seed protein, seed oil, soybean.

INTRODUCTION

Soybean is grown primarily for the protein and oil processed from its seed (Smith and Huyser, 1987). Both protein and oil content are quantitatively inherited in soybean. Molecular markers based upon DNA polymorphism have greatly simplified the genetic analysis of quantitative traits, providing a reliable and extensive framework of qualitative markers to which quantitative trait loci (QTL) can be linked (Stuber, 1989). Molecular markers have been used to identify QTL for seed protein and oil content in soybean by numerous authors (Diers et al., 1992a; Mansur et al., 1993; Lee et al., 1996). These authors used RFLP markers to identify QTL for seed protein and oil content. No RAPD markers were used in QTL mapping study for seed protein and oil content. The purpose of this research was to use a particular molecular marker known as randomly amplified polymorphic DNA (RAPD) to detect and map quantitative trait loci for seed protein and oil content in soybean.

MATERIALS AND METHODS

Plant materials

The study was conducted with a F_2 plant population derived from a mating between the cultivar 'Mercury' (female parent) and the plant introduction 'PI 417.468' (male parent). A random sample of F_2 seeds were planted on 17 May 1995 in the University of Nebraska-Lincoln, USA East Campus field nursery in a bordered block of six 25-seed 0.75 m x 0.90 m plots. After emergence, 110 randomly chosen F_2 plants were tagged and numbered prior to DNA extraction.

DNA isolation and Marker assays

Total genomic DNA was isolated from the 68 F_2 plants that emerged in the field and from the two parents grown previously in the greenhouse. Young leaves were collected from each F_2 plant and parent plant, and the leaf samples were immediately lyophilized, before being stored at -20° C. DNA was isolated from finely ground leaf tissue by means of a modified CTAB procedure (Saghai Maroof et al., 1984). The procedures of RAPD

marker analysis have been described elsewhere (Chung and Specht, 1997).

Linkage mapping and QTL mapping

A linkage map of RAPD markers was constructed by applying the computer program MAPMAKER v. 3.0 (Lander et al., 1987) to the marker data obtained from F_2 plants. A random sample of the F_3 seed of each F_2 plant was drawn for an analysis of seed protein and oil content. The protein and oil content in seed was estimated by using NIR grain analyzer (INFRATEC 1255 Food & Feed Analyzer 1255). The two estimates were averaged to obtain a mean seed protein and mean seed oil content for use in QTL mapping. Single-factor analysis of variance was performed for each marker locus to discern the effect of the two alleles on seed protein and oil content, using PROC GLM Software (SAS Institute, Inc.). An F-test significance level of $P<0.01$ was chosen for declaring that a marker was linked to a seed protein and oil content QTL. An interval mapping technique (Lander and Botstein, 1989), implemented by means of the computer program MAPMAKER-QTL v. 1.1 (Lincoln et al. 1992) was conducted on the seed protein and oil content data of F_2 generations. A LOD score of 2.0 was chosen as the threshold for declaring the presence of a QTL in any given interval between two adjacent markers.

RESULTS AND DISCUSSION

Approximately 13% of the 1,000 RAPD primers that were tested produced polymorphic DNA fragment differences between "Mercury" and "PI 467.468". Most of the polymorphic bands were inherited in a Mendelian fashion. However, 18 of the bands displayed segregation distortion at the 5% probability level. A genetic map was constructed from the 156 segregating markers. Of the 156 markers, 113 were found to be genetically linked and formed 29 linkage groups, with 43 markers unlinked. The linkage map spanned 1,043 cM across all 29 linkage groups, with markers separated by an average distance of 9.2 cM.

The results of single factor analysis are presented in Table 1. These markers are presumably linked to QTLs for seed protein and oil content. Marker OPB12 was found to be associated with differences in seed protein content. Four markers, OPA09b, OPM07b, OPC14, and OPN11b had highly significant effects on seed oil content.

Using the QTL log-likelihood scans of MAPMAKER-QTL, QTL was detected for seed oil content. No QTLs were detected for seed protein content. Localization of the QTLs for seed oil content are presented in Figure 1. The interval between marker OPK3c and OPQ1b on linkage group 13 contained a QTL that explained 25.7% variation for seed oil content.

In this study, RAPD technique was used to construct

Table 1. Single factor ANOVA between the marker locus and QTLs for seed protein and oil content

Marker name	F- test Value	probility	Means for F_2 plants grouped by amplicon type: (+)	(-)	Linkage group
	Seed protein content		——— g Kg^{-1} ———		
OPB12	8.088	0.0060	40.71	41.90	unl*
	Seed oil content		——— g Kg^{-1} ———		
OPA9b	8.067	0.0061	20.98	20.25	3
OPM7b	12.337	0.0008	21.03	20.16	3
OPC14	11.863	0.0010	20.68	22.53	unl
OPN11b	10.173	0.0022	21.00	20.17	unl

*) Unlinked marker.

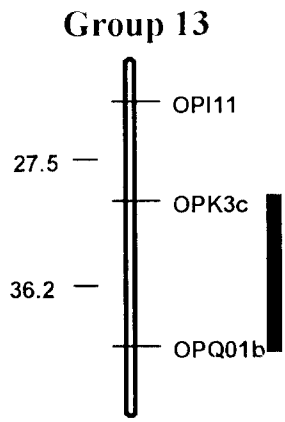

Figure 1. Map location of the QTLs detected for seed oil content using interval analysis with a LOD>2.0 threshold criterion. Marker loci names are on the right and map distances are on the left. The solid bars on the right denote a QTL region exceeding LOD 2.0.

a genetic linkage map and to identify QTLs for seed protein and oil content. Most of RAPD markers segregated in a completely dominant fashion. A soybean linkage map consisting of one-hundred thirteen markers was constructed in this study. The twenty-nine linkage groups spanned 1,043 cM, and the markers were separated by an average distance of 9.2 cM. While many markers (forty three) remain unlinked, this is likely due to the relatively small population size (68 F_2 progeny) and the inability to differentiate between homozygotes and heterozygotes in those F_2 genotypes, which possess two and one, respectively, copies of the RAPD amplicon. However, this study demonstrated that the RAPD marker technique is a convenient and rapid means of identifying QTLs controlling important quantitative traits in soybean. We believe it has substantial potential for manipulating those traits in soybean genetics and breeding.

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