HIGH POTENTIAL OF TRAP MARKERS IN SUNFLOWER GENOME MAPPING

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Abstract

We constructed a linkage map with a set of 129 RILs (recombinant inbred lines) derived from the cross of 83HR4 x RHA345. Twenty oligos, selected from 61 oligos used in a preliminary screening between the two parental lines for the higher level of polymorphism and three oligos of published SSR (simple sequence repeat) were used as fixed primers in combination with six infrared fluorescence dye-labeled arbitrary primers in the TRAP reactions. All the fixed primers were designed against sunflower EST (expressed sequence tag) sequences. One hundred and seventy-six polymorphic markers were generated with 38 primer combinations in 23 PCR reactions with an average of 4.6 markers per primer combination. A linkage map was constructed with the MapMaker program using a LOD score of 5.0 and maximum recombination fraction of 0.25. The resulting map consisted of 160 markers in 17 linkage groups plus four pairs of linked markers, and covered a total length of 1140 centiMorgans (cM). These TRAP markers are well distributed throughout the genome; the number of markers per linkage group ranged from three to 28 and the average interval between two markers is 9 cM. Since TRAP can detect a high level of polymorphic markers that segregate in expected genetic ratios, it will be useful as a high throughput mapping method to develop ultra-high density linkage maps and tag important agronomic traits in segregating populations.

Introduction

During the last decade, about a dozen genetic linkage maps have been constructed for the sunflower genome. These included the maps comprising the first generation of molecular

markers—RFLP (restriction fragment length polymorphism) markers (Berry et al. 1995, 1996, 1997; Gentzbittel et al. 1995, 1999; Jan et al. 1998), the second generation of PCR-based multiple copy markers—RAPD (random amplified polymorphic DNA), AFLP (amplified fragment length polymorphism) markers (Rieseberg et al. 1993; Peerbolte and Peleman 1996; Gedil et al. 2001), DALP (direct amplification of length polymorphism) (Langar et al. 2002), and SSR (simple sequence repeat), the best class of the amplification-based markers (Tang et al. 2002). In this report, we describe the construction of a sunflower linkage map of a new generation of markers, TRAP (target region amplification polymorphism) markers, which are generated with PCR primers designed against the sunflower EST (expressed sequence tag) sequences.

Materials and Methods

Segregating Population. One hundred and twenty-nine RILs (recombinant inbred lines) derived from the cross of 83HR4 x RHA345 were used in the current study. This cross was made to investigate the oleic content in the sunflower seed oil. DNA samples were prepared from RI lines of the F8 generation in France and shipped to Fargo, ND, for TRAP analysis.

TRAP Marker Generation. TRAP marker generation was conducted by following the protocol of Hu and Vick (2003). In addition to the sunflower EST-derived oligos, three published SSR primers (Tang et al., 2002) were used in the TRAP reaction (Table 1). The names of the fixed primer correspond to the EST sequence ID in the Compositae Genomics Database hosted by the University of California at Davis. Sixteen of them are sunflower ESTs (QHxxxxx), four are lettuce ESTs (QGxxxx), three are SSR primers (ORSxxxx) and one is from a sunflower gene encoding for acetolactate synthase.

Nomenclature of the TRAP Markers. The name of each scored marker consists of two parts: the code for the primer combination and the fragment size in base pairs. The primer combination part begins with the primer combination code followed by the numeral 7 or 8, indicating the IR dyes of the labeled arbitrary primers which generate the 700 nm or 800 nm image. Since the majority of the TRAP fragments are in the range of 40 to 1000, we use the last three digits of the marker name to indicate the size. For example, Marker ABA017096 is a 96 bp fragment amplified by primer combination ABA01 scored from the 700 nm image.

Data Analysis. The size of each segregating marker was determined by the SAGA Genotyping software (Li-Cor Biosciences*) and the markers were manually scored as 1 (present), 0 (absent) and – (not amplified or missing). A simple chi-square test was carried out for each locus for the 1:1 expected segregating ratio using the "Data Analysis" package embedded in the Excel program of Microsoft Office.

Map construction was performed with the Macintosh version of MapMaker (Lander et al. 1987). A LOD score of 5.0 and a maximum recombination fraction of 0.25 were used when grouping the linked markers. The "First order" command was used to order the markers in each linkage group. The Kosambi mapping function (Kosambi, 1944) was used to convert the recombination value into genetic distance (cM).

Table 1. Primers and primer combinations used for TRAP analysis.

Primer combination code	Fixed primers	Sequences (5' to 3')	Arbitrary primer IR dye 700	labeled with IR dye 800
ABA01	QHF6H21L	ACAGGAAAAGCCTGTCAC	Sa12-700	Ga5-800
ABA02	QHG17L13R	AAGGATTCGGACAAACAT	Sa12-700	Ga5-800
ABA03	ALSR1	CATATTGAGGCTTTGGCATTC	Sa12-700	Ga5-800
ABA04	QHA10B18a	GCAAACAAGTTCCTGGCT	Sa12-700	Ga5-800
ABA05	QHA10B18b	GTTTGCCTTTAAGAACCG	Sa12-700	Ga5-800
ABA06	QHG14I14F1	CGTGGAAGCATCTAGACA	Sa12-700	Ga5-800
ABA07	QHA12P24b	CTCCAGTCTGACCCGTTG	Trap03-700	Sa17-800
ABA08	QHA13J07a	CGATCTAGAATCCAAGCC	Trap03-700	Sa17-800
ABA09	QHA14H20a	CGAATCTCCACTAAACCC	Trap03-700	Sa17-800
ABA10	QHA20I01a	CCGAGTTGGTATGCTTGT	Trap03-700	Sa17-800
ABA11	QHA21B09a	TGTCATTCAATTCGGTGC	Trap03-700	Sa17-800
ABA12	QHB37D06a	ATCAGTTCATTAGGGCAC	Trap03-700	Sa17-800
ABA13	QHB14G14b	AATCTCAAGGACAAAAGG	Trap03-700	Sa17-800
ABA14	QHB18I19b	CGTTTATTTCCTCGCCTC	Trap03-700	Sa17-800
ABA15	QHB18F12b	TCTTCAGTTTGGATAGGC	Trap03-700	Sa17-800
ABA16	ORS323R	GCCGGAGGATTAGAGGAGTT	Trap03-700	Sa17-800
ABA17	ORS333R	ATATTAAGTTTTGGTTTTAGCCAGAA	Trap03-700	Sa17-800
ABA18	ORS523R	GGATGAAAGATATGACCTGGATG	Trap03-700	Sa17-800
Lac02	QHF6H21R	CTGCTGCTGTTGAAGTTG	Sa12-700	Ga5-800
Lac03	QHG17L13L	TGGCTGTTTGAACACTTT	Sa12-700	Ga5-800
Lac05	QGA7H07L	TGTTCATGTTCCTTGCAT	Sa4-700	Ga3-800
Lac06	QGA7H07R	CACCATTGGCTTCCATAG	Sa4-700	Ga3-800
Lac07	QGB9J18L	TGGACTTCAACCAAGACA	Sa4-700	Ga3-800
	Arbitrary primers			
	Sa12-700	TTCTAGGTAATCCAACAACA	IR dye 700	
	Ga5-800	GGAACCAAACACATGAAGA	IR dye 800	
	Trap03-700	CGTAGCGCGTCAATTATG	IR dye 700	
	Sa17-800	ATAAGAATCAGCAGACGCAT	IR dye 800	
	Sa4-700	TTCTTCTTCCCTGGACACAAA	IR dye 700	
	Ga3-800	TCATCTCAAACCATCTACAC	IR dye 800	

Results and Discussion

Each of the 64 primer combinations detected polymorphism between the two parental lines (83HR4 and RHA345) in the preliminary screening. The 24 fixed primers were selected for their ability to amplify multiple polymorphic markers to map the 129 RI lines. Three gels

were needed to run one set of PCR since 48 samples can be loaded on one gel. We scored only the markers that were well resolved in all three gels of the same set. Figure 1 shows 16 polymorphic markers amplified by a single primer combination. A total of 176 markers were scored from 38 primer combinations, and the average was 4.6 segregating markers per primer combination.

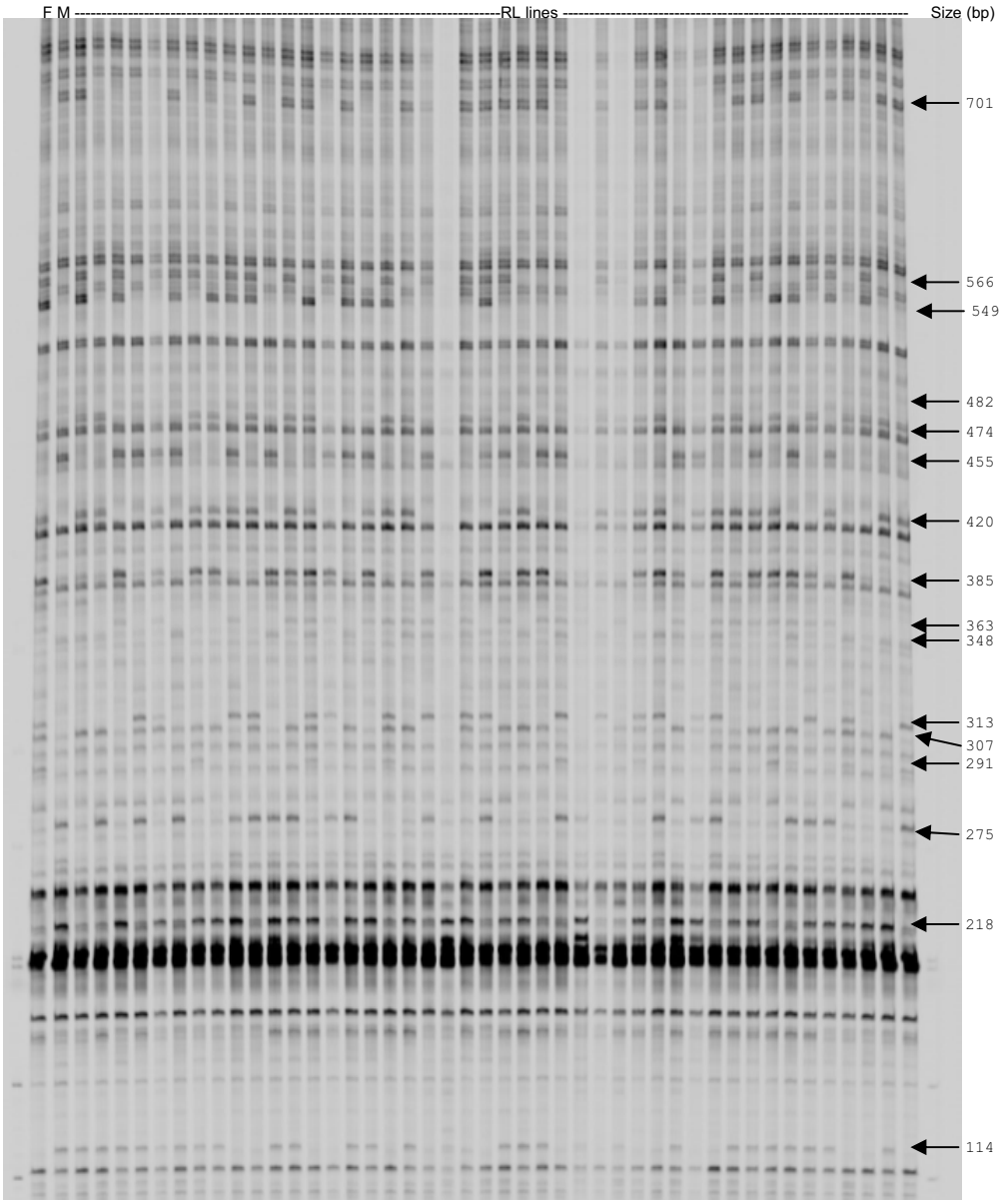

Figure 1. TRAP markers amplified by primer combination ABA167. Sixteen polymorphic markers were scored.

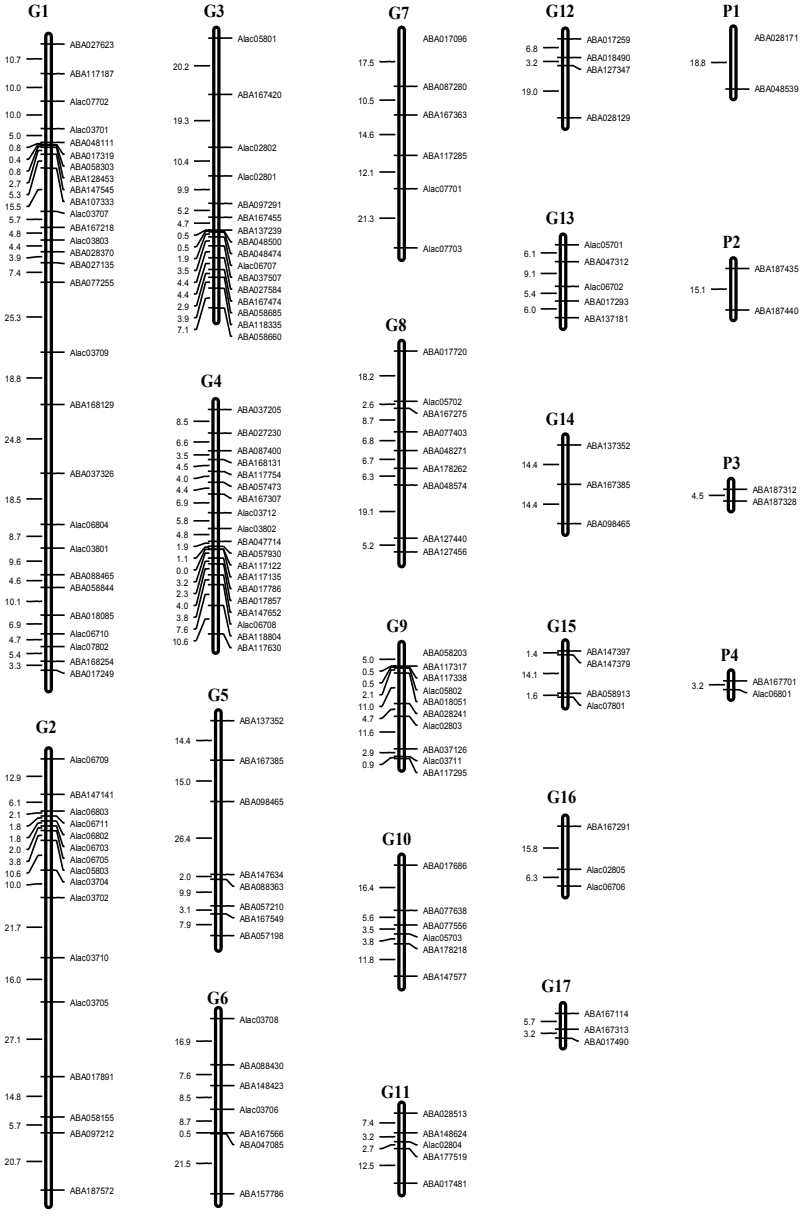

Figure 2. The TRAP marker linkage map of sunflower constructed from 129 recombinant inbred lines derived from the cross of 83HR4 x RHA345. The 160 linked markers were generated with 50 primer combinations in 24 PCR reactions. Seventeen linkage groups are labeled G1 through G17 (arranged by length) and 4 linkage pairs P1 to P4.

Each of the two parents contributed approximately equal numbers of alleles to the RI population. There are 84 markers from the female (scored as present) and 92 markers from the male ($X^2_{1:1}$=0.364, p>0.5). For individual markers, 151 of the 176 markers fit the expected 1:1 ratio. Of the 25 markers that deviated from the normal segregation, 13 showed more alleles from the female and 12 had more alleles from the male. Three of these markers fit the 3:1 ratio, implying possible duplication of the amplified fragments in the genome.

MapMaker program grouped 160 markers into 17 linkage groups and four pairs. It covered a total length of 1140 cM using a LOD score of 5.0 and a maximum recombination fraction of 0.25. The number of markers per linkage group varied from three to 28, and the cM per linkage group ranged from 8.9 to 228. The interval between two linked markers varied between 0 and 26.4 cM with an average of 9.0 cM (Figure 2).

The TRAP markers are generated as easily as RAPD markers. The number of markers per PCR reaction in some primer combinations is comparable to that of AFLP. For the current study, the data were collected in a relatively short period of about one month. The fact that the TRAP markers were distributed more or less evenly throughout the genome (Figure 2) with no obvious clusters suggested that the fixed primers played a more important role in detecting polymorphism than the arbitrary primers, since only six arbitrary primers were used. Taking into account that over 70,000 sunflower ESTs are available to the public and each of the ESTs can be used to design a fixed primer, the TRAP technique could be used to generate sufficient numbers of markers to construct ultra-high density linkage maps for fine mapping QTLs (quantitative trait loci) and tagging genes governing important agronomic traits in the sunflower crop.

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*****Mention of trade names or commercial products in this publication is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture.

References

Berry, S. T., Leon, A. J., Peerbolte, R., Challis, P., Livini, C., Jones, R., and Feingold, S. 1997. Presentation of the Advanta sunflower RFLP linkage map for public research. *In*: Proc. 19th Sunflower Res. Workshop, Fargo, North Dakota, USA. p. 113-118.

Berry, S.T., Leon, A. J., Hanfrey, C. C., Challis, P., Burkholz, A., Barnes, S. R., Rufener, G. K., Lee, M., and Caligari, P. D. S. 1995. Molecular marker analysis of *Helianthus annuus* L. 2. Construction of an RFLP linkage map for cultivated sunflower. Theor. Appl. Genet. 91:195-199.

Gedil, M. A., Wye, C., Berry, S. T., Seger, B., Peleman, J., Jones, R., Leon, A., Slabaugh, M. B., and Knapp, S. J. 2001. An integrated RFLP-AFLP linkage map for cultivated sunflower. Genome. 44:213-221.

Gentzbittel, L., Mestries, E., Mouzeyar, S., Mazeyrat, F., Badaoui, S., Vear, F., Tourvieille de Labrouhe, D., and Nicolas, P. 1999. A composite map of expressed sequences and phenotypic traits of the sunflower (*Helianthus annuus* L.) genome. Theor. Appl. Genet. 99:218-234.

Gentzbittel, L., Vear, F., Zhang, Y. X., Berville, A., and Nicolas, P. 1995. Development of a consensus RFLP linkage map of cultivated sunflower (*Helianthus annuus* L.). Theor. Appl. Genet. 90:1079-1086.

Hu, J., and Vick, B. A. 2003. TRAP target region amplification polymorphism, a novel marker technique for plant genotyping. Plant Mol. Biol. Reporter 21:289-294.

Jan, C. C., Vick, B. A., Miller, J. F., Kahler, A. L., and Butler, E. T. 1998. Construction of an RFLP linkage map for cultivated sunflower. Theor. Appl. Genet. 96:15-22.

Knapp, S. J., Berry, S., and Rieseberg, L. H. 2001. Genetic mapping in sunflowers. *In*: Phillips, R. L., Vasil, I. K. (eds) DNA markers in plants. Kluwer, Dordrecht, the Netherlands. p. 379-403.

Kosambi, D. D. 1944. The estimation of map distance from recombination value. Ann. Eugen. 12:172-175.

Lacombe, S. and Bervillé, A. 2001. A dominant mutation for high oleic acid content in sunflower (*Helianthus annuus* L.) seed oil is genetically linked to a single oleate-desaturase RFLP locus. Mol. Breed. 8:129-137.

Lander, E. S., Green, P., Abrahamson, J., Barlow, A., Daly, M. J., Lincoln, S. E., and Newburg, L. 1987. MAPMAKER: an interactive computer package for constructing primary genetic linkage maps of experimental and natural populations. Genomics 1: 174-181.

Peerbolte, R. P., and Peleman, J. 1996. The CARTISOL sunflower RFLP map (146 loci) extended with 291 AFLP markers. *In*: Proc 18 Sunflower Res. Forum, Fargo, North Dakota. p. 174-178.

Rieseberg, L. H., Choi, H., Chan, R., and Spore, C. 1993. Genomic map of a diploid hybrid species. Heredity. 70:285-283.

Tang, S., Yu, J.-K., Slabaugh, M. B., Shintani, D. K. and Knapp, S. J. 2002. Simple sequence repeat map of the sunflower genome. Theor. Appl. Genet. 91:195-199.