SIMPLE SEQUENCE REPEAT LENGTH POLYMORPHISMS AMONG ELITE INBRED LINES OF SUNFLOWER: ANALYSIS OF PUBLIC SECTOR CARTISOL III MARKERS

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SUMMARY

The number of high-throughput, sequence-based DNA markers for cultivated sunflower (*Helianthus annuus* L.) is presently limited. Our aims were to develop two hundred or more simple sequence repeat (SSR) markers from a genomic DNA library enriched for several SSR motifs and screen a sample of 10 elite inbred lines for SSR length polymorphisms. We sequenced 980 clones, found 540 unique repeats, and designed and screened oligonucleotide primers for 359 unique repeats (primer pairs could not be designed for the other 181 repeats). The genotyping assays were performed on an ABI377 automated DNA sequencer using fluorescently end-labelled forward primers and 'pig- tailed' reverse primers. Two-hundred and sixty-five primer pairs produced scoreable genotypes, while 94 primer pairs either failed to amplify DNA or produced unscoreable genotypes. Seventy-one percent of the markers (181 out of 256) were polymorphic among the inbred lines tested. Polymorphic information contents (*PIC*) ranged from 0.00 to 0.93 with a mean of 0.57 (excluding monomorphic markers). Forty-one SSRs had *PIC* scores between 0.70 and 0.93. There was no correlation ($\rho = 0.0$) between *n* and *PIC* and there were no significant differences among motifs for *n* or *PIC*. These markers increase the supply of high-throughput, sequence-based markers for molecular breeding and genomics research in sunflower.

SSR MARKER DEVELOPMENT

SSR markers were developed by mass sequencing genomic clones from an SSR-enriched library of cultivated sunflower. An overview of the marker development process is presented in Table 1. We developed ~265 functional SSR markers from the DNA sequences of 980 genomic clones. Although repeats were found in 73% of the clones (717 out of 980), 177 of the clone sequences were redundant. There were 378 di-nucleotides, 110 tri-nucleotides, and 52 tetra-nucleotides among unique repeats (540 total) with four or more repeats (*n*).

Table 1. Summary of sunflower simple sequence repeat marker development process.

Stage	Number
Clones Sequenced	980
Repeats Found	717
Unique Repeats Found	540
Primer Pairs Designed and Tested	359

Analyses of the unique repeats (540 total) were performed to design primers for amplifying the SSRs by PCR. Primers could not be designed for 181 of the unique repeats because the repeats were positioned too close to the ends of the cloned fragments (the flanking sequences were too short to design one of the primers). The primer pairs were tested by assaying PCR amplicons on agarose and polyacrylamide gels. The agarose gels were used to check amplicon lengths and numbers. Polyacrylamide gel assays were performed on an ABI377 automated DNA sequencer using fluorescently end-labelled forward primers and pig-tailed (Brownstein et al. 1996). Three-fourths of the primers tested (~265 out of 359) produced clean PCR products on agarose gels and scoreable SSR genotypes on polyacrylamide gels.

SSR LENGTH POLYMORPHISMS AMONG ELITE INBRED LINES

SSRs were screened for length polymorphisms among 10 inbred lines of cultivated sunflower (HA89, HA292, HA370, HA372, HA383, HA821, RHA274, RHA280, RHA377, and RHA801). Among markers developed from SSRs with five or more reference allele repeats ($n \geq 5$), 185 were di-nucleotides, 31 were tri-nucleotides, and 10 were tetra-nucleotides. Seventy-one percent of the markers tested (161 out of 226) were polymorphic among the lines tested (Fig. 1). The distribution of *PIC* scores was similar for di-, tri-, and tetra-nucleotide repeats. The tri-nucleotide markers in this sample were significantly more polymorphic than the tri-nucleotide markers described by Gedil et al. (2000) (both sets of markers were screened for length polymorphisms using the same set of inbred lines).

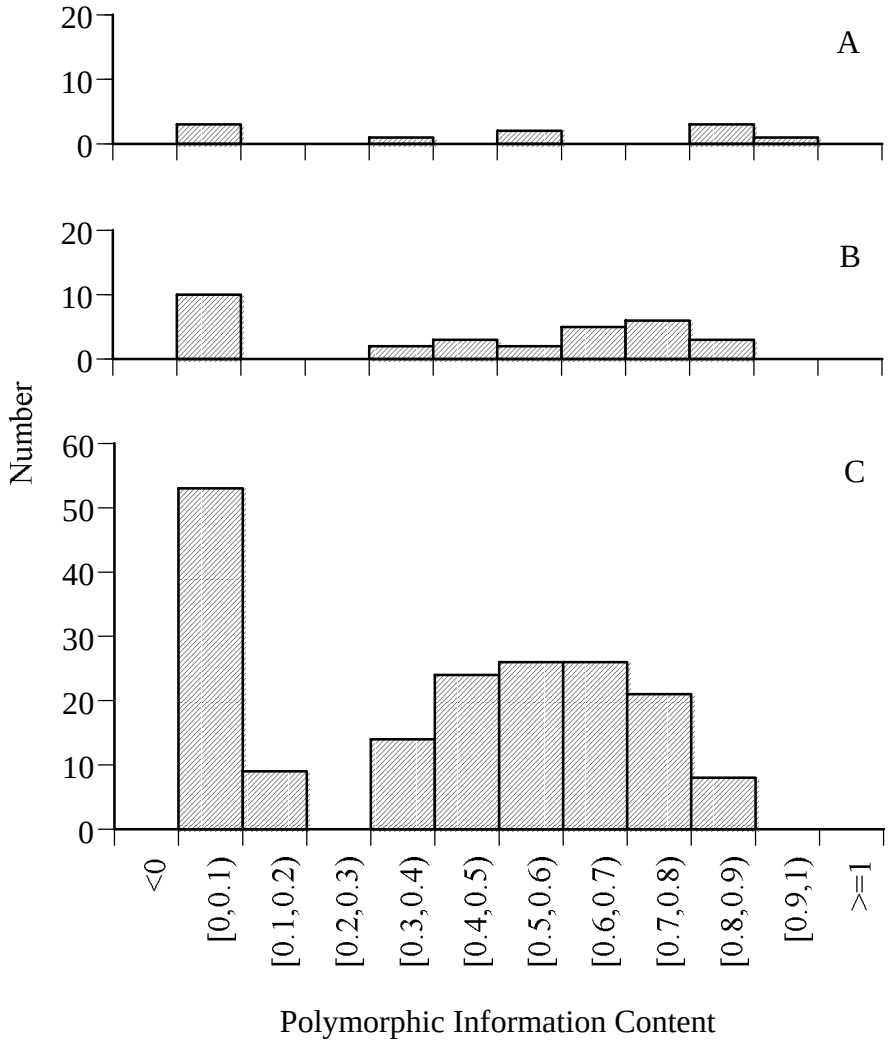

Figure 1. The distribution of polymorphic information contents for 185 di-nucleotide (A), 31 tri-nucleotide (B), and 10 tetra-nucleotide (C) repeat markers screened for length polymorphisms among 10 inbred lines of cultivated sunflower (*Helianthus annuus* L.).

The mean *PIC* for the SSR markers assayed thus far, excluding monomorphic markers, was 0.57. SSRs seem to be slightly more polymorphic than RFLPs in sunflower (Berry et al., 1995; Gentzbittel et al., 1995; Jan et al., 1997). Several highly polymorphic SSR markers were developed—41 SSRs had *PIC* scores between 0.70 and 0.93.

The subset of markers (56 oligonucleotide primer pairs) developed from reference allele sequences harboring $n = 4$ repeats have not yet been screened for polymorphisms. Originally, we did not design primers for $n = 4$ repeats because shorter repeats are known to be less polymorphic; however, because there was no correlation between n and *PIC* for reference alleles with $n \geq 5$ (Fig. 2), analyses of SSR markers developed from sequences harboring unique $n = 4$ repeats seemed warranted and are underway.

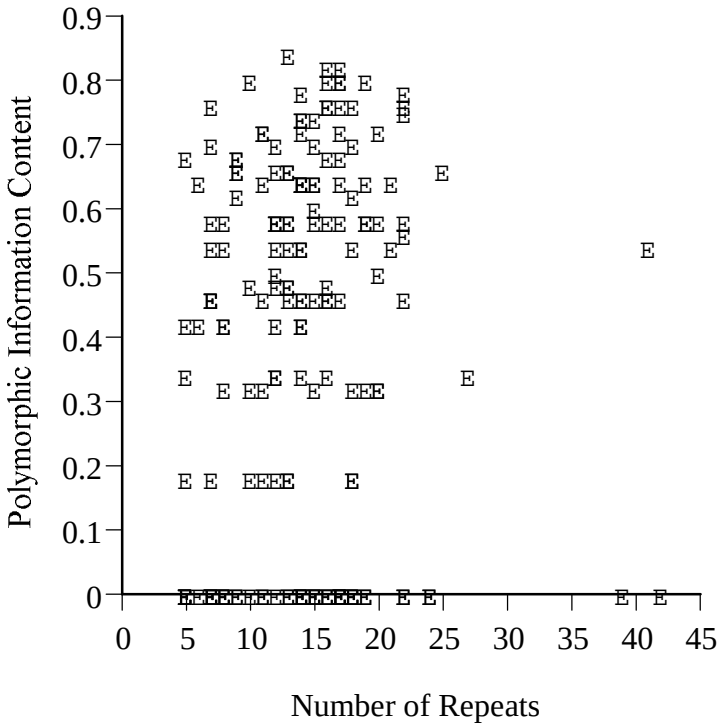

Figure 2. Scatter plot of reference allele repeat length (number of repeats) versus polymorphic information content (*PIC*) for 185 di-nucleotide, 31 tri-nucleotide, and 10 tetra-nucleotide repeat markers screened for length polymorphisms among 10 inbred lines of cultivated sunflower (*Helianthus annuus* L.).

LITERATURE CITED

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