A GENETIC LINKAGE MAP OF SSR MARKERS BASED ON A *GOSSYPIUM NELSONII* and *G. AUSTRALE* F_2 POPULATION

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Abstract

Simple sequence repeats (SSRs) represent a significant resource for constructing genetic maps and investigating phylogenetic relationships in crop species. The discovery and development of SSRs is a time consuming and expensive process. This investigation is a first attempt to utilize tetraploid species-derived primers to generate markers for mapping in diploid G genome cotton species. About one hundred individual F_2 plants, developed from the cross between *Gossypium nelsoni* and *G. australe*, were used to construct the linkage map. One allotetraploid (AD_1) *G. hirsutum* (TM-1) plant and one of its diploid ancestors, *G. raimondii* (D_5), were also included for comparative analysis. We developed a high-throughput multimix PCR assay for SSR markers separated using fluorescent-labeled capillary electrophoresis. Preliminary results demonstrated that out of 112 tetraploid-derived SSR primers screened, 86% of the primers amplified from the diploid G genome species, showing that flanking primer sequences are conserved in the diploid G and tetrploid AD_1 genome. DNA sequence information of a few randomly selected clones revealed that all of the clones contained SSR repeat motifs in the diploid species suggesting perhaps that SSR markers including the flanking primer sequences are conserved at the interspecific level in genomic DNA over several million years of evolution. About 85% of the primers showed polymorphism between the two diploid parent species. This indicated a great genetic diversity at molecular level between these two morphologically similar diploid parent species. 112 tetraploid derived SSR primers were used exhibiting 2-3 DNA markers/primer combination. Thirty-seven percent of SSRs in the G genome diploid parents amplified the same size of DNA fragment as in the tetraploid, (TM-1). This indicated that many of the tetraploid SSRs are conserved across diploid and tetraploid species. Results showed that *G. nelsonii* had more alleles similar to *G. hirsutum* compared to *G. australe*. Out of a total 36 markers, 19 exhibited codominant and 17 showed dominant segregation inheritance in the F_2 population. Twenty-five markers were linked to seven linkage groups covering a total map distance of 523 cM with an average of 21 cM/marker. A Chi-square test revealed that 8 markers on linkage map exhibited distorted segregation possibly due to interspecific genomic incompatibility between the parental species. Comparative analysis of DNA sequence results between *G. hirsutum* and the two parental species indicated that changes within a few selected clones have occurred during evolution in the following ways: 1) by increasing or decreasing the number of the repeat motif, 2) by single nucleotide mutation occurring within or outside of the repeat motif, 3) by inter conversion of a simple to a compound SSR motif and 4) by inversion of the repeat regions. These results suggested that tetraploid derived microsatellites will be useful in comparative genetic mapping of both tetraploid and diploid *Gossypium* species for evolutionary studies and in tracking the introgression of agriculturally important traits from exotic diploid and tetraploid germplasm sources.

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