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SSR Markers For Assessing The Hybrid Nature Of Two High Yielding Mulberry Varieties

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Abstract

The mulberry silkworm, *Bombyx mori* L. (Lepidoptera: Bombycidae) is a monophagous insect that feeds exclusively on Mulberry (*Morus spp.*) foliage for its nutrition and produces the natural proteinous silk. Hence, a two pronged approach of equal importance to develop improved silk moth genotypes as well as their feeds has to be adopted by both silk worm and mulberry breeders. The challenge in Mulberry breeding is deciphering suitable markers, and to link those with important agronomic traits so that those can be helpful for early screening of promising recombinants in Marker Assisted Selection / Breeding (MAS/MAB) program. Reports of molecular characterization of various genotypes of Mulberry are plentiful but attempt to linking those with traits of interest is relatively scanty. The present work reports use of *Morus* specific SSRs to check the hybrid nature of two high yielding Mulberry varieties in the backdrop of their respective parents. These genotypes maintained in the repository of Central Sericulture Research and Training Institute (CSR&TI) at Berhampore, WB, India are unique since they constitute of parents and hybrids of two consecutively successful hybridization programmes, wherein the hybrid of the first crossing programme (itself a high yielding genotype, widely grown by the Mulberry growers in several parts of India) was further crossed with an open pollinated selection of a landrace to develop even superior Mulberry genotype, which is awaiting release as specific accession. Furthermore, the segregation patterns of the SSR markers are also being screened in the mapping population with the ultimate objective of identification of association between agro morphological traits with molecular markers.

1. Introduction

Mulberry (*Morus* spp. L., family: Moraceae) has prime importance in the sericulture industry since its foliage is the only natural feed of the silkworm *Bombyx mori* L. Therefore, the quantity and quality of leaf production has a direct bearing on the productivity of silk. Like most of the tree crops Mulberry is extremely heterogeneous and out breeding in nature. This complexity of Mulberry genetics often becomes compounded when the genotypes developed are categorized through leaf related morphological or physiological parameters since it is the major point of interest from the perspective of sericulture industry. Furthermore, leaf, a plastic vegetative structure, being the sole criterion of intense human selection, characterization on the basis of reproductive descriptors often remains poorly worked out. A series of high yielding Mulberry genotypes have been selected, developed and released till date by Central Sericulture Research and Training Institute (CSR&TI) at Berhampore, suitable for different agro climatic conditions and the 'hunt' for developing even superior genotypes is on, particularly targeting towards several quantitative traits such as leaf retention capacity, leaf size and weight, total biomass, resistance to pest and diseases, tolerance to drought, salinity, and cold stress. The horizontal expansion of Moriculture has succeeded as traditional methods of plant breeding have made significant contributions to Mulberry improvement by developing several varieties with desirable agronomic traits which have been released for commercial exploitation by the farmers.

The challenge in Mulberry breeding is deciphering suitable markers, be it morphological or molecular, and to link those with important agronomic traits so that those can be helpful for early screening of promising recombinants in Marker Assisted Selection / Breeding (MAS/MAB) program (Arora et al. 2013). In the backdrop of these, the present study centers around molecular characterization of five selected genotypes of mulberry using *Morus* specific SSR (Short/Simple Sequence Repeat) markers. These five genotypes maintained in the repository of Central Sericulture Research and Training Institute (CSR&TI) at Berhampore, WB, India are exclusive since they constitute of parents and hybrids of two successive as well as successful hybridization programmes, wherein the hybrid of the first crossing programme (itself a high yielding genotype, widely grown by the mulberry growers in several parts of India) was further crossed with an open pollinated selection of a landrace to develop even superior mulberry genotype, which is awaiting release as specific mulberry accession.

2. Experimental and Observations

2.1 Plant materials and Methods

Five genotypes of mulberry, viz. C776, V1, S30, Gen1 and Kajli (OP) were used as study materials. All the five genotypes are in the repository of Central Sericulture Research and Training Institute (CSR&TI) at Berhampore, WB, India. These five genotypes comprise of candidates for two successive crossing programmes: The first one – C776 (♂), V1 (H), S30 (♀); and the second – V1 (♂), Gen1 (H) and Kajli (OP)

(♀).Genomic DNA was isolated from newly emerging sprouts using Qiaquick DNeasy plant minikit (Qiagen) following the manufacturer's protocol. The DNA was subsequently quantified using Nanodrop Spectrophotometer (Thermo Scientific) and resolved in 1.0% (w/v) agarose gel to ascertain the quality of DNA. Mulberry specific SSR primers have been worked out following the reference (Venkateswarlu et al. 2006). The flanking primers for amplifying the target SSRs are as follows:

Table1. SSR primers

Primer No.	Sequence and repeat Motifs	Particulars of SSRs - expected size range of alleles (bp)
P-1	F: 5'-GCCGTGTACCAGTGGAGTTTGCA-3' R: 5'-TGACCGTTTCTTCCACTTTACCTAATG-3', Repeat Motif – $(GTT)_6(GTT)_4$	181–209
P-2	F: 5'-CGTGGGGCTTAGGCTGAGTAGAGG-3' R: 5'-CACCACCACTACTTCTCTTCTTCCAG-3', Repeat Motif – $(GTT)_{11}$	163–206
P-3	F:5'-GGGTTGGGTAGATGGGCTTATGTTA-3' R**:**5'-CCCTATTAACTTTTTGGTCACCTCTA-3' Repeat Motif – $(GA)_{33}$	107-221
P-4	F:5'-GGTCAAGCGCTCCAGAGAAAAG-3' R:5'GGTGCAGAGGATGAAAGATGAGGT -3' Repeat Motif – $(GAA)_6$	112-146
P-5	F: 5'- CCCCCTGCAATGCCCTCTTTC -3' R**:**5'-TGGGCGAGGCAGGGAAGATTC-3' Repeat Motif – $(CCA)_8$	134-166
P-6	F: 5'-TCCTTAGGTTTTTGGGGTCTGTTTACAT-3' R: 5'-CCTCATTCTCCTTTCACTTATTGTTG-3', Repeat Motif – $(GT)_{15}$	119-181

2.2 Results

All the six micro satellite primers studied worked leading to identification of total 36 alleles to analyze the allelic difference among the five genotypes under study. The number of alleles varied in the range 3-9. Primer-2 revealed least number of alleles (three) while Primer-1 resulted in maximum number (nine) of alleles per locus The expected heterozygosity (H*e*) ranged from 0.578 to 0.865 with mean H*e* being 0.771. The minimum H*e* (0.578) was observed in case of Primer-2, while the maximum (0.865) was noted for Primer-1. The Polymorphism Information Content (PIC) value ranged from 0.486 to 0.850 with mean value of 0.732. Primer-1 represented highest PIC value (0.850) followed by Primer-5 (0.811).

▪ *Representative gel photograph of SSR analysis*. Mulberry specific Micro satellite (SSR) marker (Primer combination P-1).

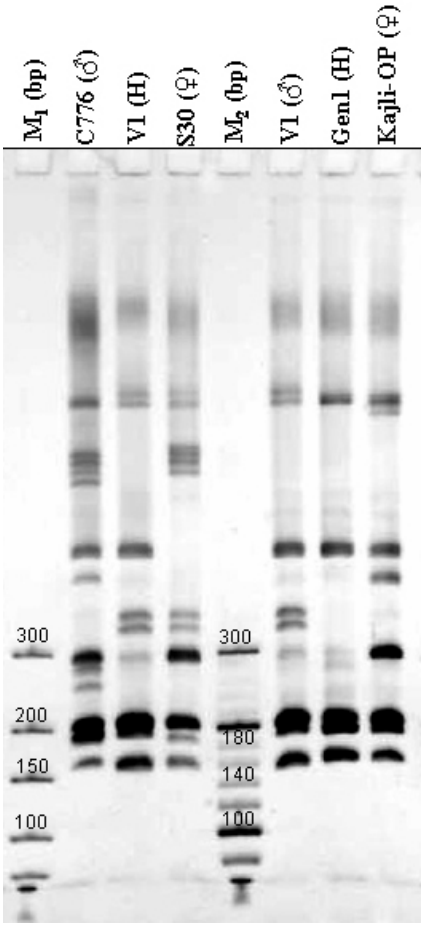

Figure1. Denaturing Urea - PAGE (6%)

3. Conclusions

Understanding the importance of development of a linkage / QTL map of mulberry, Aggarwal and Udaykumar (2004) isolated and characterized six novel micro satellite markers for mulberry (*Morus indica*). Venkateswarlu et al. (2006) later adopted an innovative two-way pseudo testcross mapping strategy based on those SSR markers along with some dominant ones. Since these six SSR markers with all probability are the only of its kind reported till date, the present work was also carried out with those. The considerably high values of expected heterozygosity and polymorphic information content in almost all the markers tested resulted in some unique allelic profiles in parents and hybrids, where some amplicons were specific to the hybrids and some were passed on to the hybrids from either of the parents.

Furthermore, the hybrid nature of two genotypes (V1 and Gen1) was validated with the aforesaid markers along with the reported mulberry specific SSR markers. Future study on co segregation of these molecular markers with agro morphological

traits in the breeding population will contribute to the development of a linkage / QTL map of mulberry.

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