A DNA Marker for Columnar Growth Habit in Apple Contains a Simple Sequence Repeat

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ADDITIONAL INDEX WORDS. *Malus*, microsatellite, RAPD, SSR

ABSTRACT. The columnar mutation 'Wijcik McIntosh' has attracted much attention because of its compact growth habit, which is compatible with high-density plantings. Using bulked segregant analysis, we identified several randomly amplified polymorphic DNA (RAPD) markers that displayed a close linkage with the columnar locus (*Co*). The RAPD marker that displayed the closest linkage was end sequenced to develop a sequence tagged site for rapidly screening segregating populations. A simple sequence repeat (SSR) of (GA)₁₇ was identified within the DNA fragment. Four allelic forms, including an apparent null allele, could be distinguished among the cultivars tested. The null allele displayed close linkage with *Co* in two progenies, and we used this marker to identify the location of the gene on the apple linkage map.

Columnar habit in apple is caused by a mutation at the *Co* locus and was initially identified as a sport ('Wijcik' phenotype) of 'McIntosh' apple (Fisher, 1970). 'Wijcik McIntosh' has attracted much attention for its potential use in high-density plantings due to its columnar habit, reduced branching and plant height, and increased spurriness (Lapins, 1976). These characteristics make 'Wijcik' an important genetic resource for plant breeding research particularly for more compact cultivars (Kelsey, 1992). The columnar character is believed to be controlled by a dominant allele (Lapins, 1976), although in certain crosses the segregation ratio for columnar phenotype is not 1:1. Lapins (1976) suspected that modifiers may be involved in expression of columnar habit.

In many segregating progenies, columnar habit is difficult to identify in trees <2 years old. Therefore, identifying a DNA marker tightly linked to the *Co* locus would be valuable for early detection of trees carrying this gene to reduce the cost of planting, maintenance, and associated labor. More importantly, such a marker could be very useful for investigating factors producing the distorted segregation observed in certain crosses.

Linkage maps of 'Rome Beauty' and 'White Angel' have been developed based primarily on randomly amplified polymorphic DNA (RAPD) markers (Hemmat et al., 1994), and these maps have been used for identifying molecular markers linked to major genes controlling morphological traits (Lawson et al., 1995). Additional markers have been found for major genes controlling scab resistance (Hemmat et al., 1995) and fruit color in apple (Cheng et al., 1996) using bulked segregant analysis (BSA) (Michelmore et al., 1992).

Our objective was to identify a DNA marker for the columnar habit to screen rapidly progenies in the early seedling stage.

Materials and Methods

PLANT MATERIAL. Population 1 (126 plants) was derived from the cross 'Wijcik McIntosh' x NY 75441-67. Population 2 (173 plants) was derived from the cross 'Wijcik McIntosh' x NY 75441-58. Seedlings of population 1 were grown at the U.S. Dept. of Agriculture-Agricultural Research Service Plant Genetic Resources Unit, and seedlings of population 2 were grown at New York State Agricultural Experiment Station in Geneva. Plants were visually

categorized for their growth habit as columnar ('Wijcik' phenotype), standard, and intermediate. Seedlings were scored for their phenotypes after 1, 2, and 4 years of growth during winter when branches were defoliated. Plants possessing a columnar main shoot (with little difference in diameter between the top and the base of the main shoot) and secondary branches that had a narrow angle, almost parallel to the main shoot, were scored as columnar. Plants that did not exhibit any of the characteristics of 'Wijcik' and possessed many primary and secondary branches were scored as standard. Individuals with any combination of columnar and standard growth habit were categorized as intermediate.

DNA EXTRACTION AND ANALYSIS. DNA was extracted from leaf material and RAPD analysis was performed using the methods described by Hemmat et al. (1994). A thermocycler (PTC-100; MJ Research, Watertown, Mass.) was used for amplification. To identify RAPD markers that cosegregated with the columnar type, BSA (Michelmore et al., 1992) was performed using DNA samples from five plants with columnar and five with standard phenotypes. About 350 random 10-mer primers were used to screen the bulked samples. Primers were obtained from Operon Technologies (Alameda, Calif.), University of British Columbia (Vancouver, B.C.), and the Cornell Univ. (Ithaca, N.Y.) New York State Center for Advanced Technology, Biotechnology program. Primers that produced a RAPD fragment in one bulk but not in the other were used for screening the populations. To clone the identified DNA fragment, the fragment was cut from the agarose gel and soaked in 50 µL type I water for ≈4 h at room temperature. One microliter of this solution was used as template DNA to reamplify the RAPD fragment at 50 °C annealing temperature. The subsequent polymerase chain reaction (PCR) product was cloned into pCR II 3.9-kb vector (TA Cloning Kit, Invitrogen). Plasmid DNA was isolated and purified according to the Applied Biosystem Incorporated (ABI) protocol. M13 forward and reverse primers were used for end sequencing the RAPD marker.

Table 1. Presence or absence of OA11₁₀₀₀ in seedlings of 'Wijcik McIntosh' x NY 75441-67 (population 1).

Phenotype	No. trees	OA11 ₁₀₀₀	
		Present	Absent
Columnar	40	5	35
Intermediate	42	26	16
Standard	44	35	9

Received for publication 5 Aug. 1996. Accepted for publication 14 Jan. 1997. The cost of publishing this paper was defrayed in part by the payment of page charges. Under postal regulations, this paper therefore must be hereby marked *advertisement* solely to indicate this fact.

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Table 2. Joint segregation analysis between *Co* and linked markers in 'Wijcik McIntosh' x NY 75441-58 (population 2).

Loci	n	Genotype categories ^z				χ^2	r ^y
		0/0	0/1	1/0	1/1		
P255a/ <i>Co</i>	173	59	17	24	73	48	24 ± 3
SSR ^{co} / <i>Co</i>	166	7	82	74	3	128	6 ± 2
SSR ^{co} /P459z	165	8	81	67	9	104	10 ± 2
<i>Co</i> /P459z	169	71	10	5	83	115	8.9 ± 2

^zGenotype designation: 0 = standard growth habit or absence of marker fragment; 1 = columnar habit or presence of marker fragment.

^yRecombination frequency ± standard error.

To characterize the simple sequence repeat (SSR), which was identified in the initial sequencing of the RAPD marker, we designed forward and reverse primers to bracket the repeat. Genetic linkage maps of 'Wijcik McIntosh' and NY 75441-58 (Conner, 1996) were used to locate the SSR using LINKAGE-1 (Suiter, 1993).

Seven cultivars of 'Wijcik McIntosh', NY 75441-67, 'Empire', 'Esopus', 'Liberty', 'Robusta 5', and 'White Angel' were also examined for their amplified fragment-length polymorphism using the SSR primers. Amplification reaction and profile followed the methods described by Hemmat et al. (1994), except that the annealing temperature was increased to 59 °C. The reaction products were separated on a 2% gel (MetaPhor; FMC, Rockland, Maine) and stained with ethidium bromide.

Results

In population 1, almost one-third of the progeny had an intermediate branching habit. The segregation ratio for the phenotypes was 40 standard : 42 intermediate : 44 columnar. Initially, two extreme groups of columnar and standard were used for identifying markers. Primer OPA11 (5'-CAA TCG CCG T-3') amplified a 1-kb fragment, OA11₁₀₀₀ (Fig. 1), which correlated with the standard phenotype (Table 1).

Population 2 segregated equally for columnar and standard phenotypes. Both parents of population 2 generated a fragment of ≈1 kb in length when OPA11 was used as the primer. The fragment segregated 3:1 in the progeny (Table 2), suggesting that both

parents were heterozygous. Joint segregation analysis indicated a linkage between OA11₁₀₀₀ and the 'Wijcik' phenotype (see data for SSR in Table 3).

The 1025-bp sequence of the OA11₁₀₀₀ fragment (GenBank accession number U50187) contained a SSR of (GA)₁₇ (Fig. 1). Fasta (Pearson and Lipman, 1988) analyses found little similarity between the sequence and anything presently listed in GenBank.

A pair of primers (26 and 17 nucleotides long) specific to sequences flanking the SSR were designed (Fig. 1). The SSR primer produced three bands in progeny 2. These amplified fragments are referred to as SSR^{co}, with the individual fragments identified by size. Other apple cultivars were screened for SSR. Three different fragments were observed among the cultivars tested (Table 3) (Fig. 2). The RAPD marker cosegregated with SSR^{co}₁₆₅ band. No fragment in coupling with *Co* allele was observed.

Determining the precise linkage distance between SSR^{co} and *Co* was complicated due to the high frequency of intermediate branching types observed in population 1 (Table 1). To estimate recombination frequency between SSR^{co} and *Co* in this population, intermediate types were eliminated from our calculations. Using only the columnar and standard phenotypes and an expected ratio of 1:1:1:1, we obtained a 17% recombination frequency between *Co* and the marker.

In population 2, we were able to obtain a precise estimate of the distance between SSR^{co} and *Co* by taking advantage of a map already generated by Conner (1996). We used only markers P255a (1140-bp fragment), P459z (800-bp fragment), *Co*, and SSR^{co}.

Markers P255a and P459z were heterozygous only in the columnar parent and segregated 1:1. Our analysis established that *Co* was located between the flanking markers P255a and P459z on linkage group X of the McIntosh map. Calculated distances between *Co*/P255a was 24.0 cM and that for *Co*/P459z was 8.9 cM (Table 2). Joint segregation analysis of the SSR marker with each of the above markers and *Co* placed the fragment between *Co* and P255a ≈6 cM from *Co*.

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CAATCGCCGTTAAGCAAAGTAATAATCGGTAATTAACACAGATTTACAG      50
TTTCANGCGTNGATTCTTGTGTCATGCTATATAAATCACATAATCTAAA      100
TCTAATCGCATCANCTTAAGAAAAATCAGTTTCAGAAATCTCAAATGTCCAA      150
TTATTTACTACTAAAAATCGCTTCGAAAACCTCAAGAGATCATCAAAAAT      200
TCCAGAACGATAATCCCAAATTAATCATGTAAAAAAGGAGAAAGGCGTT      250
TACCTGAGAGAGCTCCAAACGCAGCGCTTGAGAGAGAGAGAGAGAGAGAG      300
AGAGAGAGAGAGAGAGGCAAAATGGACAAGTAGAAGGAGGGGAAGGGGAAGGCG      350
ACGCCCTGAAAAATCTAACACACAATTGTGACTTAGCCGCCGCGTTATCG      400
TATTTGACGTGGCGCACGCACAGTCAGCGTCCCGGCACAACTTCAATGA      450
CCAGTGACGACGAAGAATGAGTCAATCGTGCAGCGCCATGTGTGACGTGT      500
TTGTGATATTTCTTGTTCGCGATGTGAGCTGCGTGACGTGCTTGTGTTGA      550
TCCGCTTCGTTTCAACTCTTCTGACATTTGATATTTTTTTAGGGTATGAT      600
ATCCATACATCCTATATTATTTCTCACACATTTTTTTTTTAATTTTTTAA      650
CTGTCCGATTGGATGAATTAAGAATATCAACGAACAGAAATTATCAAAA      700
TGTATCTGAGAAGTAATATAGAATGCGTGGATAGTACACTCTTTTCTC      750
TTTTGCTCATTTTTCTTTCTAAGTGGCCCTGGTTTANTCTCTATTGGCC      800
CATCATGGAGAAAACCTTGGGTCGGCANTGTNGGGGCCATCCGGAAGGCC      850
ACATAAATGTCCCGCAGCAAAAATTCCTTATTCTATGTATTTTGGGA      900
CTCACCCTTCTGTTAGTTGGCAACCTTATATGGGTGTCCAGACTGAAG      950
AAATCTCCTTACGCAGGCGCCCGGAGTCCCGACACATGACTTTTCTTGTA      1000
CGGACTTTCCCAACCACGGCGATTG      1025

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Fig. 1. Sequence of the columnar marker (OA11₁₀₀₀, GenBank accession number: U50187) flanked by the forward and reverse randomly amplified polymorphic DNA primers (OPA11). The sequence revealed a simple sequence repeat (GA)₁₇ ≈280 bp from one of the priming ends. The two long primers (for SSR^{co}) are underlined.

Table 3. Segregation of SSR^{co} in different apple cultivars.

Cultivar	Genotype at SSR ^{co}
Wijcik McIntosh	165/null
NY 75441-67	165/180
Empire	165/-
Esopus	165/-
Liberty	155/165
Robusta 5	165/-
White Angel	165/-

Discussion

The DNA marker identified in this study permits the screening of very young seedlings for the presence of the *Co* gene with a high degree of accuracy (6% to 15% recombination). The actual recombination frequency between SSR^{co} and *Co* may be <5% because there are only 10.0 cM between SSR^{co} and P459z and *Co* lies between the two and is closer to SSR^{co}. The higher recombination rate between SSR^{co} and *Co* reported in our experiments appears to be due to interference with *Co* gene expression. Conner (1994) has identified a quantitative trait locus for branching habit on linkage group V (near B413). This locus may identify one of Lapins' (1976) modifier genes. The locus near B413 appears to be affecting expression of *Co* in population 1, and there were at least two trees in population 2 displaying an atypical phenotype. However for practical breeding programs, the observed recombination rate of 6% to 15% is probably the most useful estimate.

The map position of *Co* demonstrates that it is not the same locus as markers affecting branching habit in apple. Lawson et al. (1995) identified the recessive gene *tb*, which produces terminal bearing and branches with blind wood such as is exemplified by 'Rome Beauty'. The location of this gene is reported on group VI of apple linkage map. The *Co* gene appears to be on linkage group X of the 'Wijcik McIntosh' and NY 75441-58 maps (Conner, 1996) and displays independent assortment with markers on linkage group V and VI.

SSRs have displayed high levels of polymorphism even within relatively narrow germplasm. Thus, these repeats have been useful for developing widely applicable linkage maps for mouse (Love et al., 1990) and human genomes (Kwiatkowski et al., 1992). Similarly in plants, microsatellites and SSRs have displayed a higher degree of polymorphism than many other molecular markers (Thomas et al., 1993) and have been used for varietal identification in grape (*Vitis* spp.) (Thomas and Scott, 1993) and soybean

Fig. 2. Polymorphic characteristic of SSR^{co} in different apple cultivars. (left to right) Lane 1—100-bp DNA ladder; lane 2—'Wijcik McIntosh'; lane 3—NY 75441-67; lane 4—'Empire'; lane 5—'Esopus'; lane 6—'Liberty'; lane 7—'Robusta 5'; lane 8—'White Angel'.

[*Glycine max* (L.) Merr.] (Mahinur et al., 1992) and as anchors for genetic maps in soybean (Mahinur et al., 1992) and rice (*Oryza sativa*) (Wu and Tanksley, 1993).

SSR^{co} should serve as a practical marker for *Co* and as an important polymorphic anchor locus for apple genetics and variety identification. Among the seven cultivars analyzed using agarose gel, at least four alleles could be distinguished and many of the cultivars appeared to be heterozygous.

SSR^{co} represents only the second SSR marker that has been linked to a trait of interest to plant breeders, the other being a repeat linked to soybean mosaic virus resistance (*Rsv1*) in soybean (Yu et al., 1996).

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