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Microsatellite DNA and RAPD fingerprinting, identification and genetic relationships of hybrid poplar (*Populus x canadensis*) cultivars

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Abstract Microsatellite DNA markers of ten SSR loci and 248 RAPD loci (resolved by 26 RAPD primers) were used for DNA fingerprinting and differentiation of 17 widely grown *Populus x canadensis* syn. *Populus x euramericana* (interspecific *Populus deltoides* × *Populus nigra* hybrids) cultivars ('Baden 431', 'Blanc du Poitou', 'Canada Blanc', 'Dorskamp 925', 'Eugenei', 'Gelrica', 'Grandis', 'Heidemij', 'I-55/56', 'I-132/56', 'I-214', 'Jacometti', 'Ostia', 'Regenerata', 'Robusta', 'Steckby' and 'Zurich 03/3'), and determination of their genetic interrelationships. Informativeness of microsatellite and RAPD markers was also evaluated in comparison with allozyme markers for clone/cultivar identification in *P. x canadensis*. High microsatellite DNA and RAPD genetic diversity was observed in the sampled cultivars. All of the 17 *P. x canadensis* cultivars could be differentiated by their multilocus genotypes at four SSR loci, and were heterozygous for their parental species-specific alleles at the *PTR6* SSR locus. Except for 'Canada Blanc' and 'Ostia', which had identical RAPD patterns, all cultivars could also be differentiated by RAPD fingerprints produced by each of the two RAPD primers, OPA07 and OPB15. For microsatellites, the mean number of alleles, polymorphic information content, observed heterozygosity, observed number of genotypes and the number of cultivars with unique genotypes per locus was 5.2, 0.64, 0.67, 5.7 and 2.2, respectively. For RAPD markers, the number of haplotypes per locus, and the number of cultivars with unique RAPD profiles per locus were 1.06 and 0.72, respectively. Overall, microsatellite DNA markers were the most informative for DNA fingerprinting of *P. x canadensis* cultivars. On the per locus basis, microsatellites were about six-times more informative than RAPD markers and about nine-times more informative than allozyme markers. However, on the per primer basis, RAPD markers were more informative. The UPGMA cluster plots separated the 17 cultivars into two major groups based on their microsatellite genotypic similarities, and into three major groups based on their RAPD fragment similarities. Both the microsatellite and RAPD data suggest that the cultivars 'Baden 431', 'Heidemij', 'Robusta' and 'Steckby' are genetically closely related. The inter-cultivar genetic relationships from microsatellite DNA and RAPD markers were consistent with those observed from allozyme markers, and were in general agreement with their speculated origin. Microsatellite DNA and RAPD markers could be used for clone and cultivar identification, varietal control and registration, and stock handling in *P. x canadensis*.

Keywords Poplars · Simple sequence repeats · DNA fingerprinting · Clone/cultivar identification · Cultivar relationships

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Introduction

Accurate identification of clones, cultivars and varieties, and knowledge of their genetic interrelationships, are essential for breeding, varietal control and registration, stock handling and the protection of plant breeders' rights. *Populus* L. (poplar) (Salicaceae) species are fast-growing multipurpose tree species (FAO 1979). Many poplar species and their interspecific hybrids are suitable for poplar culture and intensive forest plantations (FAO 1979; Dickmann and Stuart 1983). Most of the poplar species and hybrids are cultivated clonally through vegetative propagation. The unit of cultivation and breeding in poplars is a clone, and the individual cultivars are normally represented by a single clone.

Populus x canadensis Moench (syn. *Populus x euramericana* (Dode) Guinier), commonly known as hybrid poplars, are interspecific hybrids between *Populus deltoides* Marsh. and *Populus nigra* L. (FAO 1979; Rajora and Zsuffa 1989). *Populus x canadensis* hybrids originated in France about 1750 (Rehder 1927) by natural hybridization of *P. deltoides* trees, introduced from North America, with the native *P. nigra* (Muhle Larsen 1960). Mitochondrial and chloroplast DNA analyses of a number of naturally originated *P. x canadensis* cultivars have suggested that their maternal parent was *P. deltoides* var. *deltoides* (Barrett et al. 1993; Rajora and Dancik 1995b). The earliest *P. x canadensis* cultivar 'Serotina' was described in 1755 (Houtzagers 1937). Subsequently, many other cultivars were described and cultivated, giving rise to poplar culture in the world (Zsuffa 1975).

Populus x canadensis cultivars are known for their fast growth, good form and ease of vegetative propagation, and are economically important in many countries (Zsuffa 1975). More than 90% of the cultivated poplars of the world are estimated to belong to *P. x canadensis* and their parental species *P. deltoides* and *P. nigra* (FAO 1979). Many poplar breeding programs have consequently focused on *P. deltoides* × *P. nigra* controlled crosses, resulting in a large number of cultivars and clones.

Traditionally, clone and cultivar identification in poplars has been attempted using a combination of morphological and phenological characteristics. The method adopted by the International Poplar Commission for identification, registration and certification of poplar clones is based on a total of 64 morphological, phenological and floral characteristics (IUPOV 1981). However, this method of clonal identification is difficult, ambiguous, time consuming and subjective. From the late 1980s, allozyme and RAPD markers have been successfully used for identification of clones and determination of their interrelationships in a number of poplar species (Rajora 1988, 1989a, b, c; Rajora and Zsuffa 1989; Rajora and Dancik 1992; Castiglione et al. 1993; Lin et al. 1994; Sigurdsson et al. 1995).

We have previously used allozymes to characterize widely cultivated 21 clones of 18 *P. x canadensis* cultivars and determined their genetic interrelationships (Rajora and Zsuffa 1989). However, all cultivars could not be uniquely fingerprinted from their allozyme genotypes at nine polymorphic loci. RAPD markers have also been used to characterize a small number of a different set of *P. x canadensis* cultivars (Castiglione et al. 1993; Lin et al. 1994) other than those examined in our previous (Rajora and Zsuffa 1989) or this study. Allozymes are limited by the amount of polymorphism and RAPD markers by their dominant nature and technical inconsistency. Microsatellite DNA or simple sequence repeat (SSR) markers, due to their hypervariability, codominance and high reproducibility, are ideal markers for clone/cultivar identification in poplars (Dayanandan et al. 1998; Rahman et al. 2000) and in a number of agricultural and horticultural plants (Russel et al. 1997; Bredemeijer et al. 1998; Gianfranceschi et al. 1998; Sanchez-Escribano et al. 1999; Tessier et al. 1999; Becher et al. 2000; Li et al. 2000).

The primary objective of this study was to use microsatellite DNA and RAPD markers for DNA fingerprinting of the same selected commercially grown *P. x canadensis* cultivars that we sampled earlier for allozyme fingerprinting (Rajora and Zsuffa 1989) and determine their molecular genetic interrelationships. We have used microsatellite DNA markers of ten SSR loci developed from *Populus tremuloides* Michx. (Dayanandan et al. 1998; Rahman et al. 2000) and RAPD markers of 248 loci resolved by 26 RAPD primers for DNA fingerprinting of 17 *P. x canadensis* cultivars and determining their genetic interrelationships. We have also evaluated and compared the informativeness of microsatellite and RAPD markers for clone/cultivar fingerprinting in *P. x canadensis*, and compared the results to those obtained previously from allozyme analysis (Rajora and Zsuffa 1989).

Materials and methods

Hybrid poplar cultivars

One clone each of 17 commercially grown cultivars of *Populus x canadensis* were sampled, namely: 'Baden 431' ('Rintheim'), 'Blanc du Poitou', 'Canada Blanc', 'Dorskamp 925', 'Eugenei' (Carolina poplar), 'Gelrica', 'Grandis', 'Heidemij', 'I-55/56', 'I-132/56', 'I-214', 'Jacometti', 'Ostia', 'Regenerata', 'Robusta', 'Steckby' and 'Zurich 03/3'. The information on the origin, source, description and accession codes for these cultivars is provided in Rajora and Zsuffa (1989). With only a few exceptions, each of these cultivars is represented by a single clone.

DNA extraction

Total genomic DNA was extracted from young leaves of each hybrid poplar cultivar as described in Rajora and Dancik (1995a).

Microsatellite DNA analysis

Markers of ten microsatellite DNA loci (*PTR1*, *PTR2*, *PTR3*, *PTR4*, *PTR5*, *PTR6*, *PTR7*, *PTR8*, *PTR12* and *PTR14*) developed from *P. tremuloides* (Dayanandan et al. 1998; Rahman et al. 2000) were used for DNA fingerprinting of the *P. x canadensis* cultivars. A touch-down protocol (Rahman et al. 2000) was used for PCR amplification of the microsatellites. The PCR reaction mix and gel-electrophoresis protocols were essentially the same as described in Rahman et al. (2000). Microsatellites were resolved by silver staining of the sequencing gels as described in Rajora et al. (2000). Microsatellite DNA analysis for each locus was repeated at least once and consistent results were obtained. Genotypes of individual *P. x canadensis* cultivars were determined from their allelic constitution.

RAPD analysis

RAPD analysis was performed using 26 Operon (Operon Technologies Inc., Alameda, Calif.) RAPD primers (Table 1) following the protocols described in Rajora (1999). RAPD analysis was repeated once for most of the primers to verify reproducibility, and consistent results were obtained. The RAPD fingerprints of indi-

Table 1 RAPD primers, and the number of RAPD loci and RAPD haplotypes observed in 17 *P. x canadensis* cultivars

Primer	No. loci	No. loci polymorphic	No. RAPD haplotypes observed	No. cultivars with unique RAPD profiles
OPA04	15	10	12	7
OPA07	15	13	16	15
OPA08	7	2	4	1
OPA10	14	7	10	7
OPA11	11	10	8	7
OPA13	11	7	8	5
OPA14	12	10	14	11
OPA15	9	6	12	9
OPA16	14	10	10	6
OPA17	10	9	15	14
OPB01	8	6	6	3
OPB04	10	8	11	7
OPB05	13	10	12	7
OPB11	13	12	15	13
OPB12	10	8	13	10
OPB13	4	3	5	2
OPB15	12	11	16	15
OPB18	7	5	7	3
OPB20	11	8	14	11
OPH02	3	2	2	0
OPH03	5	4	8	5
OPH07	5	5	8	5
OPH08	7	4	8	3
OPH09	8	5	9	4
OPH12	9	4	10	4
OPH20	7	6	8	4
Total	248	183	264	178
Mean (*primer*)	9.54	7.04	10.15	6.85
Mean (*loci*)			1.06	0.72

vidual clones were scored as 1 for the positive and 0 for the null phenotypes for each of the 248 RAPD markers resolved by the 26 primers (Table 1).

Genetic diversity, cultivar fingerprinting and marker informativeness

Single-locus and mutilocus microsatellite DNA genotypes and RAPD fragment profiles were determined for each cultivar. The number of different genotypes observed at each microsatellite locus and the number of different RAPD haplotypes produced by each RAPD primer among the 17 cultivars, were determined. The minimum number of microsatellite DNA loci and RAPD primers and loci required for the differentiation of all 17 *P. x canadensis* cultivars was determined. The number of cultivars with unique genotypes at a SSR locus and the number of cultivars with unique RAPD profiles for a single RAPD primer were determined. The polymorphic information content (PIC), and the number of alleles and heterozygosity observed at each of the ten SSR loci and means over all loci were calculated. The informativeness of the SSR and RAPD loci for fingerprinting and differentiation of *P. x canadensis* cultivars was determined by calculating the number of genotypes or RAPD haplotypes observed per locus and the number of clones with unique genotypes or RAPD profiles per locus.

Inter-cultivar genetic relationships

The genetic relationships among the cultivars were determined by calculating Jaccard's similarities (Sneath and Sokal 1973) from microsatellite DNA genotypes and RAPD profiles of individual cultivars by using NTSYS version 2.1software (Rohlf 2000). UPGMA cluster plots of the 17 cultivars were constructed from their RAPD fragments and SSR genotypic similarities for the microsatellite and RAPD data separately.

Results

Microsatellite DNA variation, and cultivar fingerprinting and identification

Nine of the ten SSR loci were polymorphic in the 17 cultivars (Table 2). The *PTR6* locus was monomorphic (Fig. 1). All of the 17 *P. x canadensis* cultivars were heterozygous for their parental (*P. deltoides* and *P. nigra*) species-specific alleles at this locus (Fig. 1). The number of alleles at a locus ranged from 2 to 8, with a mean of 5.2 alleles over all loci (Table 2). The polymorphic information content (PIC) of the SSR loci ranged from 0.424 to 0.813, with a mean of 0.638 over the ten loci (Table 2). The observed heterozygosity at a locus ranged from 0.29 to 1.00, with a mean of 0.67 over the ten loci (Table 2). The number of different microsatellite genotypes observed at a locus ranged from 1 to 9, with a mean of 5.7 over the ten loci, whereas the number of cultivars with unique genotypes at a locus ranged from 0 to 4, with a mean of 2.2 (Table 2; Fig. 2). *PTR7* was the most-informative locus for clonal fingerprinting and differentiation, with nine genotypes observed, of which four were unique among the17 cultivars (Fig. 2). *PTR2* and *PTR6* were the least-informative loci, with no unique genotypes detected among the 17 cultivars at these loci (Fig. 1; Table 2). All of the 17 cultivars could be uniquely identified by their multilocus genotypes at four SSR loci: *PTR4*, *PTR5*, *PTR7* and *PTR8*.

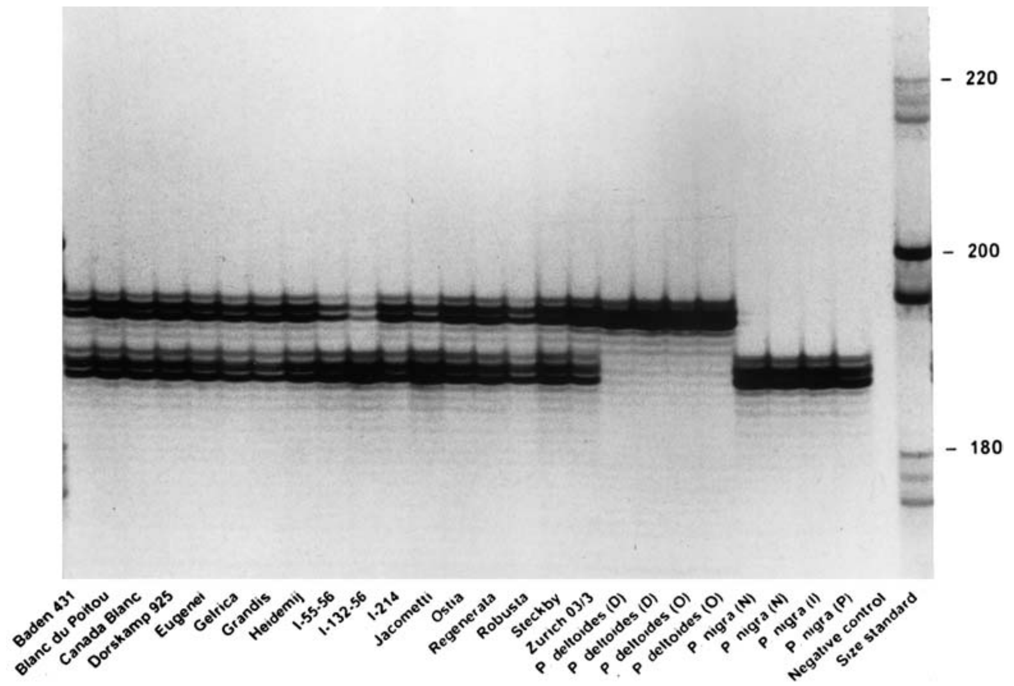

Fig. 1 Microsatellite DNA profiles of *P. x canadensis* cultivars, *P. deltoides* var. *deltoides* (D), *P. deltoides* var. *occidentalis* (O), *P. nigra* var. *nigra* (N), *P. nigra* var. *italica* (I) and *P. nigra* var. *plantierensis* (P) at the *PTR6* locus, demonstrating that all of the 17 *P. x canadensis* cultivars are heterozygous for their parental species-specific alleles at this locus

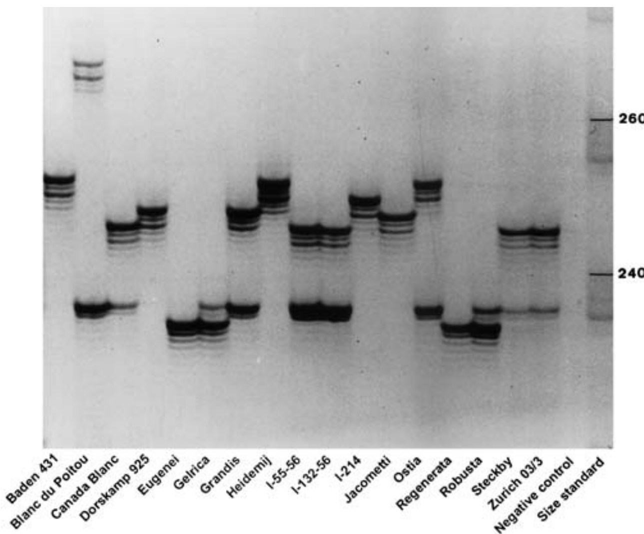

Fig. 2 Microsatellite DNA fingerprints of *P. x canadensis* cultivars at the *PTR7* locus

RAPD variation, and cultivar fingerprinting and identification

Two hundred and forty eight RAPD loci were consistently resolved by the 26 primers and were scored (Table 1). The number of RAPD loci resolved by one primer ranged from 3 to 15, with an average of 9.54 loci per primer (Table 1). Of the 248 RAPD loci, 183 were polymorphic (Table 1). The number of RAPD haplotypes produced by a single primer among the 17 cultivars ranged from 2 to 16, with a mean of 10.15 haplotypes per primer and 1.06 haplotypes per RAPD locus (Table 1). The number of unique RAPD fingerprints produced by a single primer among the 17 cultivars, i.e., the number of cultivars with unique RAPD fingerprints at RAPD loci resolved by a single primer, ranged from 0 to 15, with an average of 6.85 per primer and 0.72 per locus. With the exception of the cultivars 'Canada Blanc' and 'Ostia', all hybrid poplar cultivars could be uniquely fingerprinted by RAPD loci resolved by each of the primers OPA07 (Fig. 3) and OPB15. 'Canada

Table 2 Microsatellite DNA loci, number of alleles, polymorphic information content (PIC), heterozygosity and the number of genotypes observed in 17 *P. x canadensis* cultivars

Locus	No. alleles	PIC	Observed heterozygosity	No. observed genotypes	No. cultivars with unique genotypes
PTR1	7	0.709	1.00	4	1
PTR2	4	0.623	0.82	5	1
PTR3	5	0.616	0.29	6	2
PTR4	8	0.664	0.47	8	4
PTR5	6	0.730	0.88	6	2
PTR6	2	0.500	1.00	1	0
PTR7	7	0.813	0.59	9	4
PTR8	4	0.424	0.29	5	3
PTR12	5	0.599	0.88	5	2
PTR14	4	0.704	0.47	8	3
Mean	5.2	0.638	0.67	5.7	2.2

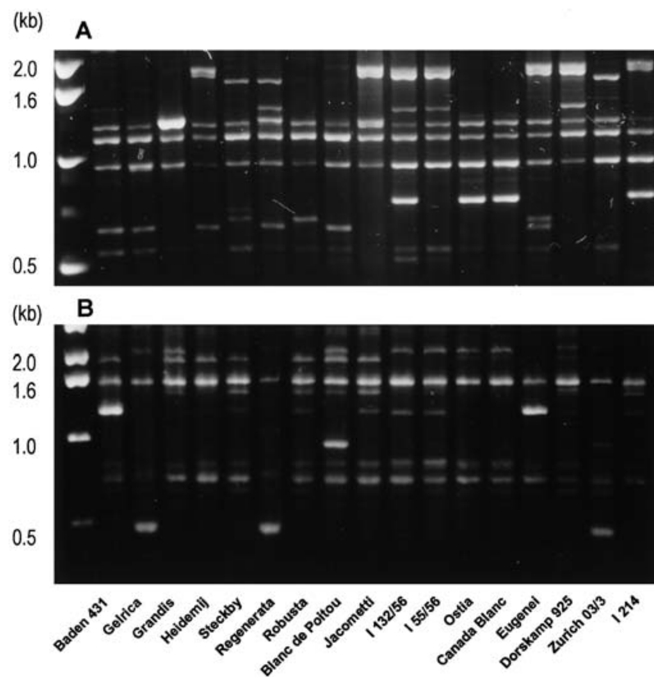

Fig. 3A, B RAPD fingerprints of *P. x canadensis* cultivars produced by the primers (**A**) OPA07 and (**B**) OPB12

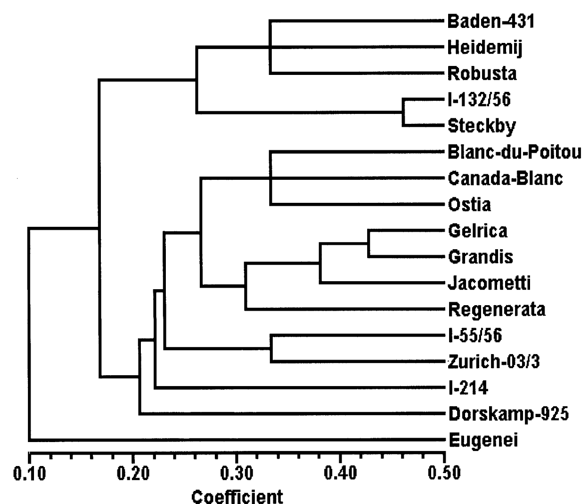

Fig. 4 UPGMA cluster plot of *P. x canadensis* cultivars based on Jaccard's similarities of their microsatellite DNA genotypes

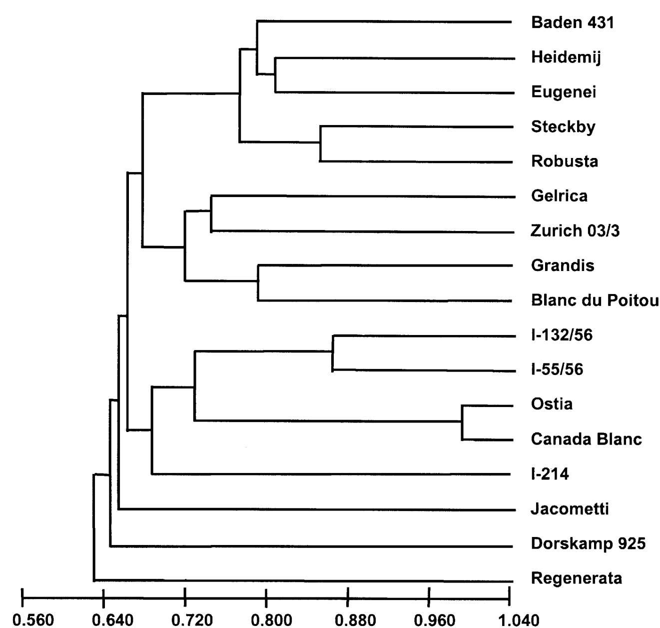

Fig. 5 UPGMA cluster plot of *P. x canadensis* cultivars based on their Jaccard's RAPD-fragment similarities

Blanc' and 'Ostia' showed identical RAPD profiles for all the 248 loci resolved by the 26 primers. The primers OPA07 and OPB15 were the most informative, whereas the primer OPH02 was the least informative for cultivar fingerprinting and differentiation.

Inter-cultivar genetic relationships

An UPGMA cluster plot of the 17 cultivars constructed from microsatellite DNA genotypic similarities (Jaccard) separated the 17 cultivars into two major groups, and the cultivar 'Eugenei' clustered separately from all other cultivars (Fig. 4). The first group consisted of cultivars 'Baden 431', 'Heidemij', 'Robusta', 'Steckby' and 'I-132/56' (Fig. 4). The remaining 12 cultivars formed the second group, which consisted of three subgroups. The first sub-group consisted of 'Blanc du Poitou', 'Canada Blanc' and 'Ostia', the second sub-group consisted of 'Gelrica', 'Grandis', 'Jacometti' and 'Regenerata', and the third sub-group consisted of 'I-55/56', 'Zurich 03/3', 'I-214' and 'Dorskamp 925'. Although the cultivars 'Canada Blanc' and 'Ostia' clustered in the same group, these were genetically distinct at each of the six microsatellite DNA loci: *PTR3*, *PTR4*, *PTR5*, *PTR7*, *PTR12* and *PTR14*. 'Eugenei' was the most distinct cultivar based on microsatellite DNA markers.

The genetic similarities observed among the cultivars from the RAPD marker data were higher than those observed from the microsatellite DNA marker data (Figs. 4 and 5). An UPGMA cluster plot of the cultivars constructed from their RAPD fragment similarities (Jaccard's) separated the 17 cultivars into three major groups of 14 cultivars, with the remaining three cultivars clustering independently (Fig. 5). The first group was formed by the cultivars 'Baden 431', 'Heidemij', 'Eugenei', 'Steckby' and 'Robusta', as was the case with four of these cultivars for the cluster plot based on the microsatellite DNA markers. The second group consisted of cultivars 'Gelrica', 'Zurich 03/3', 'Grandis' and 'Blanc du Poitou'. The third group consisted of cultivars 'I-132/56, 'I-55/56', 'Ostia', 'Canada Blanc' and 'I-214'. 'Canada Blanc' and 'Ostia' had a RAPD fragment similarity of 1.0, and consequently these cultivars clustered at this value. 'Jacometti', 'Dorskamp 925' and 'Regenerata' clustered individually. 'Regenerata' was the most distinct cultivar based on RAPD markers.

Discussion

Microsatellite DNA markers developed from *P. tremuloides* (Dayanandan et al. 1998; Rahman et al. 2000) could be used for genetic fingerprinting, identification and classification in *P. x canadensis*. All SSR primers resolved single locus patterns, and almost all microsatellite DNA loci showed high allelic diversity, polymorphic information content and observed heterozygosity in the studied *P. x canadensis* cultivars, suggesting their high informativeness.

Microsatellite genotype data for the *PTR6* locus confirms our earlier allozyme results (Rajora and Zsuffa 1989) that all of the 17 *P. x canadensis* cultivars sampled in this study are interspecific hybrids between *P. deltoides* and *P. nigra*, since all of the cultivars were heterozygous for the *P. deltoides* and *P. nigra* species-specific alleles at this locus. This was also the case with the cultivar 'Ostia', which originated from the seeds collected from a *P. deltoides* tree, and was sometimes assumed to be pure *P. deltoides* (Zufa 1960).

The results of our study clearly demonstrate that *P. x canadensis* cultivars could be uniquely identified based on their multilocus microsatellite genotypes and RAPD fingerprints. All of the 17 cultivars could be identified by their genotypes at four microsatellite DNA loci (*PTR4*, *PTR5*, *PTR7* and *PTR8*). With the exception of cultivars 'Canada Blanc' and 'Ostia', which shared the same RAPD profiles, all other *P. x canadensis* cultivars could be identified based on their RAPD fingerprints produced by each of the single RAPD primers OPA07 and OPB15. However, these two cultivars had distinct microsatellite DNA genotypes at each of the six SSR loci (*PTR3*, *PTR4*, *PTR5*, *PTR7*, *PTR12* and *PTR14*). 'Canada Blanc' was suspected as a re-named ramet of 'Ostia' (Zufa 1960). Our RAPD data supports this view since these cultivars had identical RAPD patterns at all the 248 RAPD loci. However, our microsatellite data clearly suggest that these two cultivars are genetically distinct and cannot be ramets of the same clone. 'Canada Blanc' and 'Ostia' showed identical patterns for 31 allozyme loci (Rajora and Zsuffa 1989) but had a distinct chloroplast genome separated by an addition of a restriction site in the chloroplast genome of 'Ostia' (Rajora and Dancik 1995c). Since the microsatellite DNA markers employed in the present study showed Mendelian inheritance (Rahman et al. 2000), the genetic differences observed between these two cultivars for the six microsatellite DNA loci most-likely reside in their nuclear genome.

Cultivars 'Regenerata' and 'Grandis' were suspected to represent a single clone (Zufa 1960). Our microsatellite DNA and RAPD data clearly confirm our earlier allozyme results (Rajora and Zsuffa 1989) that these cultivars represent two separate clones.

Several cultivars had unique genotypes at a single microsatellite locus by which they could be identified without resorting to their multilocus genotypes. Our results suggest that the microsatellite loci *PTR7*, *PTR4* and *PTR5*, and RAPD primers OPA07, OPB15, OPA17 and OPB11, are among the most-informative and useful for DNA fingerprinting and differentiation of *P. x canadensis* cultivars. In addition, RAPD primers OPA14, OPB12 and OPB20 are also highly informative. Our study suggests that on the per-locus basis, microsatellite DNA markers are (almost six-times) more informative than RAPD markers for cultivar fingerprinting and identification in *P. x canadensis*, whereas on the per-primer basis RAPD markers are more informative. However, RAPD markers suffer from the disadvantage of being dominant and technically inconsistent. On the other hand, microsatellites are not only codominant and highly informative markers but are also suitable for automated high-throughput analysis. The microsatellite DNA markers were also almost nine-times more informative (5.7 observed genotypes/polymorphic locus) than allozyme markers (0.65 observed genotypes per locus) (Rajora and Zsuffa 1989) for genetic fingerprinting and differentiation of the same hybrid poplar cultivars. Thus, microsatellite DNA markers are more informative than RAPD or allozyme markers for genetic fingerprinting in *P. x canadensis*. This was clearly demonstrated in the case of 'Canada Blanc' and 'Ostia', which could be differentiated by each of the six microsatellite loci, but could not be differentiated by the 248 RAPD markers resolved by 26 primers (this study) and 31 allozyme loci (Rajora and Zsuffa 1989). Microsatellite DNA markers were also found to be highly informative for differentiation of the clones in *P. tremuloides* (Dayanandan et al. 1998; Rahman et al. 2000) and cultivars in a number of crop and horticultural plants (Russel et al. 1997; Bredemeijer et al. 1998; Gianfranceschi et al. 1998; Sanchez-Escribano et al. 1999; Tessier et al. 1999; Becher et al. 2000; Li et al. 2000). This is the first report of the application of microsatellite DNA markers for hybrid poplar (*P. x canadensis*) cultivar fingerprinting and identification. Also, with the exception of 'I-214' (Castiglione et al. 1993; Lin et al. 1994), this is the first report for the application of RAPD markers for differentiation of the *P. x canadensis* cultivars studied here. Also, the RAPD markers that we used were more informative than those previously used for poplar clone fingerprinting (Castiglione et al. 1993; Lin et al. 1994; Sigurdsson et al. 1995).

The basic unit of breeding, propagation and cultivation in *P. x canadensis* and other poplar species is a clone. Thus, a cultivar is normally represented by a single clone (genotype). The microsatellite DNA genotyping and RAPD fingerprinting can be of great significance in cultivar/clone identification, varietal control and registration, and handling of planting and breeding stocks in *P. x canadensis*. This method of clonal identification is more reliable, precise, simple, fast and objective, and inexpensive as compared to the morphological-phenological approach (IUPOV 1981) and more informative than that based on allozyme markers (Rajora and Zsuffa 1989).

Both the microsatellite and RAPD data suggest high genetic diversity in the sampled *P. x canadensis* culti-

vars. This suggests a broad genetic base of these cultivars. Thus, these cultivars could be used for poplar cultivation without due concern about loosing genetic diversity in plantations of clonal mixtures of these cultivars.

The UPGMA clusters of the *P. x canadensis* cultivars (Figs. 4 and 5) portray their genetic interrelationships based on similarities of their microsatellite DNA genotypes and RAPD fragments. The genetic relationships observed among certain cultivars from the microsatellite and RAPD data are consistent with their historically known or speculated origin and (or) morphological relationships, as well as relationships observed from their allozyme analysis (Rajora and Zsuffa 1989). The genetic relationships observed between some clones in our study are noteworthy. Both the microsatellite and RAPD data suggest that the cultivars 'Baden 431', 'Heidemij', 'Robusta' and 'Steckby' are genetically closely related. These results are consistent with those obtained from allozyme analysis (Rajora and Zsuffa 1989). High RAPD genetic similarities between 'Robusta' and 'Eugenei' are consistent with their historically known origin, as well as from allozyme analysis (Rajora and Zsuffa 1989). 'Robusta' has been considered to have originated from a half-sib family of which 'Eugenei' was the pistillate parent (Houtzagers 1937). However, our microsatellite data suggest that 'Eugenei' is the most distinct from all other cultivars, including 'Robusta'. 'Gelrica' and 'Eugenei' cultivars were regarded as closely related to 'Blanc du Poitou' (Houtzagers 1937). However, both microsatellite and RAPD data suggest that 'Blanc du Poitou' is quite distinct from both 'Gelerica' and 'Eugenei' as was the case with allozyme analysis (Rajora and Zsuffa 1989). Our RAPD data suggest that the cultivars 'I-55/56' and 'I-132/56' are genetically closely related. These cultivars/clones were selected in Italy, and may have originated from the same or related family.

In conclusion, the *P. x canadensis* cultivars have high genetic diversity, and could be uniquely identified based on their multilocus microsatellite genotypes and RAPD fingerprints. Microsatellite DNA markers provide the most-informative markers for genetic fingerprinting of *P. x canadensis* cultivars, although some RAPD markers were also highly informative. Microsatellite and RAPD markers could be used for cultivar/clone identification, certification and registration and determining genetic relationships among cultivars/clones in *P. x canadensis*.

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