

Detection of genetic identity for hybrid *Populus x canadensis* trees along the Seyhan River

Seyhan Nehri boyunca yayılan hibrit *Populus x canadensis* ağaçlarının genetik kimliğinin açığa çıkarılması

Asiye ULUĞ¹ 

¹Kafkas Üniversitesi, Fen Edebiyat Fakültesi, Biyoloji Bölümü, Merkez, Kars

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Sorumlu yazar / Corresponding author

Asiye ULUĞ

e-mail: duru36@gmail.com

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Abstract

Populus x canadensis hybrids play an important role for the commercial poplar cultivation with good growth performance and adaptability in the world. Hybrid clones introduced the different region of Turkey replaced the native stands of *Populus nigra* gradually. Along the Seyhan River there are many mature and young hybrid poplar trees despite of less number of *P. nigra* as a result of hybrid plantations growing near the river. To detect the genetic identity of hybrid trees, 17 microsatellite markers were studied. Out of ten hybrid trees, seven trees had the different genotype. From the results of current study it is seen that hybrid poplar species reproduce at larger distances from the plantation sites and colonize riparian habitat thereby competing for resources with the native *P. nigra* species and threaten the genetic diversity of the species with genetic pollution via introgression. From the field trip observation and obtained genetic results, conservation of the native *P. nigra* trees is an important requirements to prevent the reduction of effective population size and population density. Establishing more seed sources of black poplar over the river stretch could provide the establishment of mainly pure black poplar seedlings nearby the parental stands.

Özet

Populus x canadensis melezleri, iyi büyüme performansı ve adaptasyonu ile dünyadaki ticari kavak yetiştiriciliğinde önemli bir rol oynamaktadır. Türkiye'nin farklı bölgelerine yayılan hibrit klonlar, *Populus nigra* 'nın yerli meşcerelerinin yerini aşamalı olarak almıştır. Seyhan nehrine yakın bölgede yetiştirilen hibrit plantasyonların bir sonucu olarak nehir boyunca çok sayıda olgun ve genç melez kavak ağaçlarının olmasına rağmen daha az sayıda *P. nigra* görülmüştür. Melez ağaçların genetik kimliğini tespit etmek için 17 mikrosatellit markör ile çalışılmıştır. On hibrit ağacın yedisi farklı bir genotipe sahiptir. Mevcut çalışmanın sonuçları, melez kavak türlerinin plantasyon alanlarından daha uzak mesafelerde çoğaldığını ve nehir kıyısındaki habitatı kolonize ederek yerli türlerle doğal kaynaklar için rekabet ettiğini ve *P. nigra*'nın genetik çeşitliliğini genetik kirlenme ile tehdit ettiğini göstermektedir. Saha çalışmalarında ve elde edilen genetik sonuçlardan, yerli *P. nigra* ağaçlarının korunması, etkin popülasyon büyüklüğünün ve popülasyon yoğunluğunun azalmasını önlemek için önemli bir gerekliliktir. Nehir boyunca daha fazla karakavak tohum kaynağı oluşturmak, ana meşcerelerin yakınında çoğunlukla saf karakavak fidanlarının çoğalmasını sağlayabilir.

INTRODUCTION

Poplar trees are ecologically and economically important plants growing naturally different regions of Turkey. With their wide geographic distribution and ability to adapt to different environmental conditions, poplar species have been used model organism in many genetic and breeding studies. *Populus nigra* L. has an important role in poplar breeding programs contributing to many successful hybrids (Frison et al. 1994). Produced hybrids are used for wood production, windbreaks, ornamental and landscape purposes (Smulders et al. 2008a).

The hybrid (*P. x canadensis*) of *P. nigra* and *Populus deltoides* L. with good growth performance, adaptability and strong hybrid vigor has been selected for commercial cultivation in the world (Smulders et al. 2008b). *Populus x canadensis* has been propagated in Turkey since 1960s (Toplu 2005). Hybrid trees indicated extraordinary success. After many field trials, several *P. x canadensis* and *P. deltoides* clones were selected for cultivation in different regions of Turkey. Samsun and I-214 clones were used in poplar plantations of Marmara and Blacksea regions. For the Southern region, the first trial included 40 hybrid trees were planted in Adana and Şanlıurfa. Seven of 40 clones were found successfully grown. The second trial was performed with those seven clones and

I-214 control clone. After 13 years, 83.011.015 hybrid clone showed high growth performance (Toplu 2001). This clone was produced commercially in Adana and Şanlıurfa plantations. There are many large poplar plantations composed of *P. nigra* and *P. deltoides* and *P. x canadensis* cultivars in Turkey to meet the need of economy (Tunçtaner 1991).

In a field trip for a different project with *P. nigra*, we observed that along the Seyhan River there are many hybrid poplar trees despite of less number of *P. nigra*. In addition to mature trees, high level of seedlings were observed extensively. The occurrence of seedlings and mature hybrid trees could be explained by hybrid plantations growing near the Seyhan River. As a result of close proximity of hybrid plantation to the river, the transport of viable branches, trunks or cuttings from hybrid trees could be relatively frequent. The harvest and deposit of harvested wood material of hybrid trees are common in riparian areas. The population could be naturally grown by dispersal of hybrid trees' seed or vegetative material via wind and water. Also, another option is that this population could be introduced by local farmers. For both conditions, growing seedlings could possibly start to naturalize along the river. It is known that there is a concern for the conservation of native *P. nigra* populations as a result of absence of apparent fertilisation barriers between *P. nigra* and *P. x canadensis* hybrid and spontaneous hybridization between them (Cagelli and Lefevre 1995, Heinze 1998b). Also, the cultivated or naturally grown hybrid trees can produce viable seeds and propagate sexually (Smulders et al. 2008b). The high distribution of hybrid offspring prevent growth of *P. nigra* seedlings because hybrid

clones viability in juvenile stands is better than *P. nigra* seedlings. Also a small number of *P. x canadensis* clones could intercross with *P. nigra* and threaten the genetic diversity with introgression. (Heinze 2008).

To detect the genetic identity of morphologically identified hybrid trees, whether all trees are clones of 83.011.015 hybrid tree propagated in close proximity or not, establish the extent of genetic variation, the occurrence of sexual or clonal reproduction and dispersal characteristic of hybrid poplar trees, 17 microsatellite markers were studied with ten hybrid trees sampled across the Seyhan River. Determining the genetic identity is crucial for the understanding population dynamic of hybrids and conservation of native *P. nigra* trees.

MATERIAL AND METHODS

Plant Material

Ten morphologically identified hybrid poplar trees were sampled with at least 500m distance to avoid sampling of ramets of the same genotype along the Seyhan River. The study site was on natural mixed stand composed of mainly *Salix alba*, *P. x canadensis* and less *P. nigra*. Near the hybrid trees, there were no *P. nigra* trees or seedlings. The presence of *P. nigra* is usually reduced to linear formations or scattered individual trees. Mature and young trees were selected from the upper, middle, and lower sections of the river to obtain information about the reproduction and dispersal type of hybrid poplar (Table 1). Young leaves were collected from each mature and seedling trees for microsatellite analysis and stored in silica gel.

Table 1. Sampling and genotype information about ten *P. x canadensis* trees

Sample name	Age of tree	Sampling location from the river	Name of different genotypes
H1	Mature	Upstream	A
H2	Seedling	Upstream	A
H3	Mature	Upstream	B
H4	Seedling	Upstream	C
H5	Seedling	Middle	C
H6	Mature	Middle	C
H7	Seedling	Middle	D
H8	Mature	Downstream	E
H9	Seedling	Downstream	F
H10	Mature	Downstream	G

Amplification of Loci

Nuclear DNA was extracted from young leaf samples with Doyle and Doyle (1990) CTAB extraction method. To detect the genetic identity and genetic diversity of the population, 17 nuclear microsatellite loci were selected from Smulders et al. (2001), Wu et al. (2008), and the International Populus Genome Consortium (IPGC) SSR Resource (2016, http://www.ornl.gov/sci/ipgc/ssr_resouce.htm). Out of 17 loci, five loci (WPMS09, WPMS18, WPMS20, PMGC014 and PMGC2163) are known as including diagnostic alleles for *P. deltoides* and for *P. x canadensis*. 20- μ l total volume composed of 4 μ l 5 $\times$ HOT FIREPol Blend Master Mix (Solis Biodyne, Estonia), 0.5 μ l each fluorescently tagged primer pair, 10 ng template DNA was used for the polymerase chain reaction for all loci. The PCR protocols described by Van Der Schoot et al. (2000) and Smulders et al. (2001) with different annealing temperature between 55 and 60 °C were applied. Microsatellite genotyping was performed by Capillary Electrophoresis using ABI PRISM 310 Genetic Analyzer (Applied Biosystems) in BM Laboratory Systems Facilities in Ankara. The allele sizes of the studied microsatellite loci were examined by Peak Scanner v2.0 (Applied Biosystems) with the GeneScan 400 size standard.

Analysis of Genetic Data

Genetic identity and genetic diversity structure of the studied population were determined by GENALEX 6.503 software (Peakall and Smouse 2012). The number of

alleles, observed (H_o), expected heterozygosity (H_e) and F statistics were calculated for individual loci and whole population.

RESULTS AND DISCUSSION

The results of microsatellite genotyping of ten trees indicated that some of the sampled seedlings from the upstream, middle and downstream parts of the river were found as clones of mature trees. Some mature trees had more than one clone. Out of ten trees, seven trees indicated different multilocus genotype (Supplementary Material).

Even though, there is an overlapping allelic range for *P. nigra* and *P. deltoides* at most of the studied loci (Çiftçi and Kaya 2019), the obtained microsatellite data confirm the morphological identification of hybrid trees with the presence of species-diagnostic alleles reported in former studies by Liesebach et al. (2010), Fossati et al. (2003) and Khasa et al. (2005) for PMGC14, PMGC2163, and WPMS20 loci (Table 3. For PMGC14 locus, the diagnostic alleles 190 and 195 were observed for clones of *P. x canadensis*. For PMGC2163 locus, species-specific alleles 186 was present in the clones of *P. x canadensis*. For WPMS09 and WPMS18 loci, we did not observe any diagnostic alleles. The diagnostic alleles had frequency value ranged between 0.43 and 0.50. It was found that there were one or two bp differences in allele size at some loci detected in different laboratory settings and the current study.

Table 2. Phyllotaxis ratios in some North East Anatolian taxa. + rare species, *: endemic species

Loci with diagnostic alleles	Allele size (bp)	Allele size (bp)	Allele size (bp)	Allele size (bp)	Allele size (bp)
	Liesebach et al. (2009)	Fossati et al. (2003)	Khasa et al. (2005)	Van den Broeck et al. 2021	Current study
WPMS09	-	234		232	-
WPMS18	-	220		-	-
PMGC14	192-195	194		192-198	190-195
PMGC21	188	-	185-187	185	186
WPMS20	-	224	-	200	-

Two reference hybrid trees from the study of Vanden Broeck et al. (2021) were compared with the studied hybrid trees to confirm the fragment length and diagnostic alleles. For the common loci, studied trees and

reference trees had generally same alleles for *P. nigra* and *P. deltoides*. For PMGC14 and PMGC2163 loci, hybrid trees had the **190** and **186** diagnostic alleles of *P. deltoides*, respectively (Table 3). In addition to

morphological identification, multilocus genotyping indicated that the studied trees were belongs to different hybrid class (Table 3).

Tablo 3. Comparison of Multilocus genotypes of ten hybrid poplar trees with I-214 and Leipzig clones of *P. deltoides* (Lieseback et al. 2009) at seven SSR loci (Pd *P. deltoides*, Pn *P. nigra*. Unique alleles for *P. deltoides* and *P. nigra* are bold.)

Loci name	WPMS05		WPMS09		WPMS14		WPMS15		WPMS20		PMGC14		PMGC21	
Tree name	<i>P.d</i>	<i>P.n</i>	<i>P.d</i>	<i>P.n</i>	<i>P.d</i>	<i>P.n</i>	<i>P.d</i>	<i>P.n</i>	<i>P.d</i>	<i>P.n</i>	<i>P.d</i>	<i>P.n</i>	<i>P.d</i>	<i>P.n</i>
H1	286	290	250	250	234	252	183	195	222	228	190	211	0	0
H2	286	290	250	250	234	252	183	195	222	228	190	211	186	242
H3	286	290	250	250	234	252	0	0	0	0	190	211	0	0
H4	286	290	250	250	234	252	183	195	222	228	190	211	186	242
H5	286	290	250	250	234	252	183	195	222	228	190	211	0	0
H6	286	290	250	250	234	252	183	195	222	228	190	211	186	256
H7	278	278	245	274	210	242	183	195	222	234	195	199	186	256
H8	286	290	250	250	234	252	183	195	222	228	190	211	0	0
H9	286	290	250	250	234	252	183	195	222	228	190	211	186	242
H10	278	286	250	250	234	252	183	195	222	228	195	199	186	242
<i>I-214</i>	286	290	235	249	233	250	197	215	222	228	192	210	188	241
<i>Leipzig</i>	280	290	267	267	241	256	197	215	222	234	195	210	188	241

DISCUSSION and CONCLUSION

The genetic identity of morphologically identified hybrid *P. x canadensis* trees were detected along the Seyhan River. It is found that out of ten trees, seven trees have different genotypes. Even though, it was expected that the sampled trees could be coming from the cultivated hybrid plantation and mainly clones of the 83.011.015 tree, the occurrence of different hybrid genotype could be explained by natural or human assisted hybrid tree propagation. Hybrid trees along the Seyhan River could be introduced by local farmers however there is a evidence that some of these trees were completely naturally grown dispersed by seed or prapagule from the plantation. Those trees were inside of river where human cannot reach.

The observed identical multilocus genotypes along the Seyhan River were shared by some of the mature and seedling trees. Generally mature and seedling trees which found in close to each other had same genotype. Those seedlings can reproduce by vegetative propagation naturally by root suckers of the mature trees and clonal propagation by farmers and breeders (Barsoum et

al. 2004). The seedlings with different genotype have a large number of allele in common with the adult trees. These could be the main source of seed contribution from mature trees. As the seedlings H7 had unique alleles at all loci, except WPMS15 locus that were absent in adult trees (Supplementary Material). Also H8 and H9 have some unique alleles. These trees were probably introduced from elsewhere.

The results of microsatellite genotyping indicated that morphologically identified hybrid trees result from a cross of *P. nigra* and *P. deltoides*. Two reference hybrid trees from the study of Van den Broeck et al. (2021) were compared with the studied hybrid trees to confirm the fragment lenght and diagnostic alleles. For the common loci, studied trees and reference trees had generally same alleles for *P. nigra* and *P. deltoides*. I-214, commercial clones of *P. canadensis* has same allele with hybrid trees at five loci. It is concluded that studied hybrids could be offspring or clones of I-214 tree which propagated in the near plantation.

Most of the loci where the two inbred parents possess different alleles were heterozygous. It is known that

plants of a single-cross hybrid becoming heterozygous are more vigorous than the parental inbred plants as a result of coverage of detrimental alleles by dominant alleles. (Timberlake 2013). F₁ crosses should be found which are capable of producing higher yield over all commercial or native varieties. (Negm 2020). Even though, most of the trees were seen as F₁ hybrid since all the alleles typical of *P. deltoides* were in the heterozygote state with other allele being typical of pure *P. nigra*, for the WPMS04, WPMS09, WPMS12 and WPMS18 loci, most of the trees possess same alleles for two inbred parents, homozygous. This can be explained by back-cross of clones to parents. The detected hybrids were found to be derived from a direct mating between cultivated *P. x canadensis* clones and named as F₂. This result indicates that cultivated or naturally grown hybrid in the commercial plantations or riparian area produce seeds. It was reported that there are no apparent crossing barriers for gene flow between cultivated hybrid poplars and native European black poplar (Vanden Broeck et al. 2004; Smulders et al. 2008b). Similarly, Smulders et al. (2008b) reported that 14 of 42 young poplar trees sampled along the Rhine River in Netherlands were F₂ or backcross hybrids.

From the results of current study, it is seen that hybrid poplar species reproduce at larger distances from the plantation sites and colonize riparian habitat thereby competing for resources with the native species. (Van den Broeck et al. 2021). The occurrence of seedling recruitment coupled with observed vegetative propagation of from mature trees and effective dispersal of seed or vegetative material from hybrid plantations may greatly increase the likelihood of local establishment of hybrid trees along the river. These hybrid plants could adapt the riparian habitat and promote the successful colonization. Increasing number of hybrid poplar will reduce black poplar reproductive success along the Seyhan River. Similar to the results of Smulders et al. (2008b), occurrence of low quantity of European black poplar seedlings along the Seyhan River could be explained that high numbers of hybrid seedlings outcompete and suppress *P. nigra* seedlings to be established in the same habitat along the river ecosystems (Heinze 2008). *P. nigra* is endangered species in Europe and Türkiye as a result of exposing to

introgression from cultivars of *P. canadensis*. From the field trip observation and obtained genetic results, conservation of the natural *P. nigra* trees is an important requirements to prevent the effective population size and population density. Establishing more seed sources of black poplar over the river stretch could guarantee the establishment of mainly pure black poplar seedlings nearby the parental stands.

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