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Genetic diversity in *Populus nigra* plantations from west of Iran

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Abstract. In order to adopt strategies for forest conservation and development, it is necessary to estimate the amount and distribution of genetic diversity in existing populations of poplar in Iran. In this study, the genetic diversity between eight stands of *Populus nigra* established in Kermanshah province was evaluated on the basis of molecular and morphological markers. To amplify microsatellite loci (WPMS09, WPMS16 and WPMS18), DNA extraction from young and fresh leaveswas done. Various conditions of the PCR assay were examined and to evaluate the morphological variation of the morphological characters leaves (consist of 19 traits) were measured. In addition, height growth was measured, to evaluate the growth function of the stands in homogeneous conditions. Genetic diversity in term of polymorphic loci was 0%, because three investigated microsatellite loci were monomorphic. The total number of alleles for 3 microsatellite loci was 6 (*na* = 2, *ne* = 2, *heo* = 1, *hee* = 0.51). Genetic identity based on Nei was 100%, so genetic distance was 0%. The whole sampled trees represented the same thus the genotype. No significant differences between the mean values of all morphological characters and height growth were revealed. Observed genetic similarity gave indication that same ramets had been selected to plant in poplar plantation established in Kermanshah province. These results suggest the need for an initial evaluation of the genetic diversity in selected ramets for planting in plantation to avoid repetition.
Keywords genetic inventory, microsatellite markers, leaf traits, height growth, *Populus nigra*, Iran.

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Introduction

Hyrcanian forests (established in the north of Iran), are relics from the Tertiary and they are rich in species including rare, threatened and endemic species (over 80 trees and 50 shrubs species are recognized)(Mohajer 2006). They are the only commercial forests in Iran and because of their importance and for achieving sustainable forestry, it is essential that an alternative source for wood production have been appreciated. Poplar plantations can be considered as an appropriate alternative, due to its properties, like wide geographical distribution in Iran, fast growth, ease of propagation and good economical revenue. The genus *Populus* L. (Salicaceae), in addition to its value for wood products, provides a range of ecological services, including carbon sequestration, bioremediation, nutrient cycling, and biofiltration (Brenner et al. 2004, Taylor 2002). Because of these, *Populus* species, especially *Populus nigra,* have been planted in Iran for many years, one of the main regions of *Populus nigra* cutivations is being Kermanshah province (Nori et al, 2008). In Iran, genetic diversity of *Populus nigra* stands has been endangered by vegetative propagation, because the reduction of the level of genetic diversity (Asadi et al. 2005). Genetic diversity is a basic necessity for species, to evolve in their changing environment. The basic prerequisite for genetic improvement and protection of genetic resources is the study of genetic variability (Bakshi & Konnert 2011). It is essential to assess the amount and distribution of genetic diversity in existing black poplar stands in this region. Molecular genetic is a keystone to evaluate the genetic diversity of *P. nigra* populations (Gaudet et al. 2008). Microsatellite markers or simple sequence repeats (SSRs) are co-dominant molecular markers, and the most appropriate markers for population genetic studies and clone identification (Suvanto & Latva-Karjanmaa 2005). Molecular markers have been applied to assess genetic diversity of natural or breeding poplar populations (Lefevre et al. 2002, Suvanto & Latva-Karjanmaa 2005, Li et al. 2006, Smulders et al. 2008b). These are also useful for differentiating and detecting clones, cultivars, varieties, hybrids and species (Chen et al. 2007, Jianming et al. 2007, Smulders et al. 2008a), investigating gene flow (Fossati et al. 2003, Imbert & lefevre 2003, Rathmacher et al. 2010), detecting probable introgression (Krystufek et al. 2002) and for investigating mating systems (Smulders et al. 2002, Vanden Broeck et al. 2002).

In addition to the molecular markers, there are other markers used to estimate diversity, such as the morphological markers. In the same environment, it is possible to assess the genetic diversity based on leaf characteristics (Storme et al. 2002). There are some studies on the development of morphological markers for assessing diversity in poplar populations (Lopez et al. 2004, Asadi et al. 2005, Ballian et al. 2006). These markers can be used as tools for defining *in situ* conservation units in endangered or marginal populations (Lopez et al. 2004) and for separating various populations (Calagari et al. 2006).

In this study, both microsatellite and morphological markers were used and this is the first study of assessing genetic variation through these markers in *Populus nigra* cutivations established in Kermanshah province of Iran. The aims of present investigation are: (1) to assess the genetic diversity of *Populus nigra* plantations established in Kermanshah province of Iran on the basis of microsatellite markers (2) to determine the morphological variation in leaf characters and the growth potential of the selected stands based on height growth. This paper provides a framework for a future research regarding the evaluation of the genetic diversity in poplar plantations established in Iran. Furthermore, this study aims to provide information for the development of the most appropriate strategy for the conservation of these poplar plantations.

Materials and methods

Site description

Kermanshah province is located on 34°23'N/ 47°03' E, northwest Iran. The average elevation is 1200 m above sea level. Kermanshah is a mountainous area, with mean annual rainfall ranging 400 to 500 mm. Mean annual temperature of 14° C and

Plant material and experimental design

A total of 24 individuals of *P. nigra* (3 individuals per stand) were sampled from 8 artificial stands in Kermanshah province. Sampled stands separated by a distance of more than 3.5 km, in order to retain independence. Location of Kermanshah province and position of selected stands are shown in Figure 1, geographical and soil characteristics of the sampling stands are shown in Table 1. Cuttings of the sampled trees were collected during the winter. In order to have a homogeneous material, the cuttings were grown in the nursery for one growing season. Following the successful propagation, they were established in a field trial, as a randomized complete block design with 3 replications.

Microsatellites analysis

For DNA extraction, young, not fully expanded, leaves were collected for each ramet, a total of 24 ramets: 3 ramets per stand (8 stands in total), frozen immediately in liquid nitrogen and stored at -20˚C. DNA extraction was done with Sarkosyl method (Apples 1997). DNA concentration was determined by agarose gel electrophoresis and ethidium bromide staining. Samples were analyzed on 3 microsatellite loci: WPMS09, WPMS16, WPMS18 (Van der Schoot et al. 2000, Smulders et al. 2001) to provide multilocus genotype for each individual (Table 2). In order to optimize the PCR assay and to create large amounts of a desired product, seven protocols and two thermal profiles were examined (Table 3). The first protocol (protocol no. 1, Table 3) was based on other findings (Van der Schoot et al. 2000, Smulders et al. 2001, Asadi et al. 2005), but for reason of producing allelic patterns with a poor quality, some modifications in the volumes of PCR reaction components were made

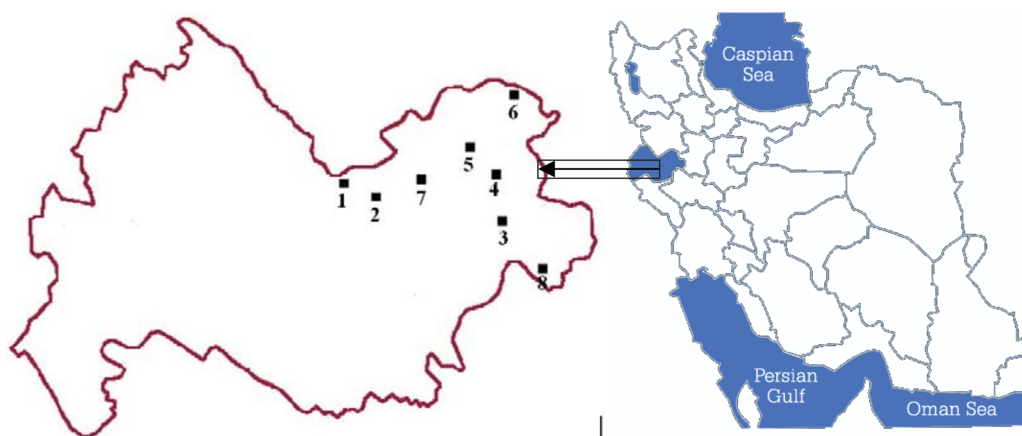

Figure 1 Location of Kermanshah province in Iran (right) and position of selected stands (■) in Kermanshah province (left).

Table 1 The geographical and soil characteristics of the sampling stands

Stand code	Sample size (m²)	Altitude (m)	pH of paste	Organic carbon (%)	Total nitrogen (%)	Sand (%)	Silt (%)	Clay (%)	Soil texture
1	11300	1325	7.1	3.3	0.3	8	44	48	Silt-Clay
2	11080	1400	7.3	2.1	0.2	29	44	27	Loam
3	10120	1720	7.6	1.2	0.1	19	38	43	Clay
4	9520	1680	7.6	2.2	0.2	18	39	43	Clay
5	11800	1690	7.5	2.9	0.3	13	45	42	Silt-Clay
6	11960	1850	7.6	1.1	0.1	10	46	44	Silt-Clay
7	6040	1390	7.5	2.1	0.2	24	44	32	Clay-Loam
8	6100	1460	7.8	1.3	0.1	32	33	35	Clay-Loam

Table 2 Characterization of the microsatellite markers

Reference	Quality of patterns in *P. nigra*	Expected product length	Allelic range	Primer sequences (forward, reverse)	Repeat	Locus
Van der Schoot et al. (2000)	1	295	246-298	F: 5' –CTGCTTGCTACCGTGG R: 5'-AAGCAATTTGGGTCTGA GTATCTG-3'	(GT)21 (GA)24	WPMS09
Smulders et al. (2001)	1	158	131-166	F: 5'-CTCGTACTATTTCCGATG TGACC-3' R:5'-AGATTATTAGGTGGGCCA AGGACT-3'	(GTC)8 (ATCCTC)5	WPMS16
Smulders et al. (2001)	1	245	217-253	F: 5'-CTTCACTAGGACATAGCA GCATC-3' R: 5'-CACCAGAGTCATCACC AGTTATTG-3'	(GTG)13	WPMS18

Note: [1] - Quality 1, weak stutter bands, well scorable

experimentally, to improve the product yield and quality (protocols no. 2 to no. 7, Table 3). The amplification products were separated in a 1.5% high-resolution agarose gel, for picking the best protocol. Because of producing high yields of desired PCR product, the seventh protocol was known as the best one, so it was applied to do PCR for all extracted DNA. The amplified PCR products were separated on 6 % w/v denaturing polyacrylamide gels (National Diagnostics).

Morphological analysis

In middle August 9, fully expanded leaves in the central part of per ramet (a total of 24 ramets, 3 ramets per stand) were randomly collected and pressed (a total of 216 leaves). The following leaf morphological characters were measured manually: the angle between the second lower lateral vein and midrib (β), the angle between the first lower lateral vein and midrib (α), the angle at 10% of leaf length (*A10*), the angle at 25% of the leaf length (*A25*), the apex

Table 3 Concentrates and volumes of PCR reaction components for seven protocols and steps of two thermal profiles used in PCR reaction

Variables	Template DNA		Taq DNA polymerase		dNTP		Primer		$MgCl_2$		Buffer		Water	Total volume	Amplification condition[2]
Concentrates & volumes protocols	C ng	V µL	C unit	V µL	C mM	V µL	C pmol/µL	V µL	C mM	V µL	C x	V µL	V µL	V µL	
1	4	8.00	250	0.10	25	0.20	2	0.8	25	1.5	10	2.50	11.10	25	A
2	4	8.00	250	0.10	25	0.50	2	0.8	25	1.5	10	2.50	10.80	25	A
3	4	8.00	250	0.10	25	0.20	2	2.0	25	1.5	10	2.50	8.70	25	A
4	4	8.00	250	0.10	25	0.50	2	2.0	25	1.5	10	2.50	8.40	25	A
5	4	8.00	50	2.00	25	0.42	2	7.0	25	7.0	10	3.58	0.00	35	B
6	4	8.46	250	0.34	25	0.36	4	6.0	25	2.4	10	3.00	3.44	30	B
7	4	8.00	500	0.06	25	0.08	2	2.0	100	0.3	10	2.00	5.56	20	B

angle (*AA*), the base angle (*BA*), the leaf length (*LL*), the leaf width (*LW*), distance between the leaf widest part and the leaf base (*DBW*), the ratio between leaf length and leaf width (*LR*), the petiole length (*LP*), the ratio between petiole length and leaf length (*PR*), the leaf width at 50% of leaf length (*BW50*), the leaf width at 90% of leaf length (*BW90*), the leaf width at 1 cm from the leaf tip (*LT*), the leaf area (*LA*), the number of teeth on the middle one third of the leaf edge (*NT*), the depth of teeth on middle one third of the leaf edge (*DT*), the number of veins (*NV*). Presentation of the measured leaf morphological traits were shown in Figures 2, 3 and 4.

The height from ground level to the base of the apical bud on the terminal shoot (*H*) was measured on 1 June and 1 September, in order to measure height growth.

Data analysis

For the data analysis in the molecular study, an alphabetical letter was assigned to each allele. Alphabet was started with the uppercase *A* letter for the largest one. This process avoids the need to estimate the exact size in terms of base pairs of each allele; moreover the data recorded can be directly used for population studies when a software package such as POPGENE 1.32 is employed (Fossati et al. 2005).

The genetic parameters (average number of observed alleles, expected alleles, observed heterozygosity, expected heterozygosity, genetic identity based on Nei and proportion of sample required to represent all genotypes) were obtained via POPGENE 1.32 software.

In order to compare the means of morphologiacal traits and the height growth of every population, an analysis of variance was done using MSTATC computer software. To summarize the variability of different morphological traits, as well as the relationships between traits, a multivariate analysis (Principal Component Analysis-PCA) was performed using SPSS 16. After Varimax axis rotation, component scores and PC loadings were determined.

Results

Analysis of molecular markers

Results of employing different PCR protocols showed that four protocols (1 to 4) did not produce any bands. Two protocols (5 & 6) produced some faint unspecific bands but PCR protocol 7 gave best results without any unspecific bands. The analysis of three loci used in the microsatellite analysis indicated heterozygosity in all produced bands but all loci were monomorphic (Figure 5) so this analysis

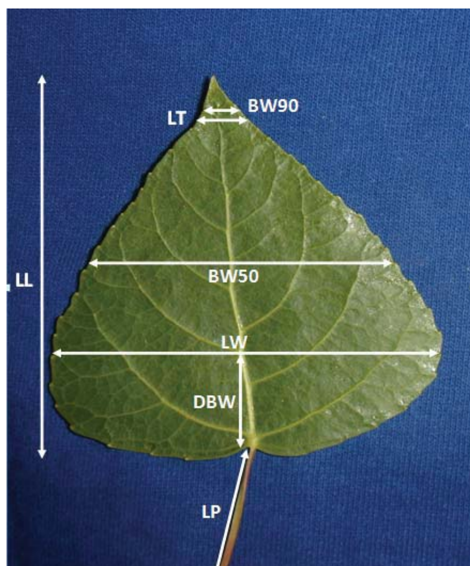

Figure 2 Presentation of the assessed leaf parameters: length - (*LL*), leaf width - (*LW*), leaf width at 50% of leaf length - (*BW50*), leaf width at 90% of leaf length - (*BW90*), distance between the leaf widest part and the leaf base - (*DBW*) leaf width at 1 cm from the leaf tip - (*LT*) and petiole length - (*LP*)

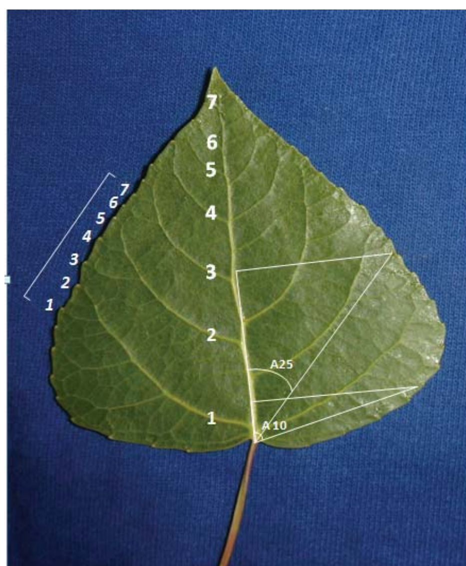

Figure 3 Presentation of the assessed leaf parameters: number of veins - (*NV*), angle at 25% of leaf length - (*A25*), angle at 10% of leaf length - (*A10*) and number of teeth on the middle one third of the leaf edge - (*NT*) (Italic number). The depth of the these teeth (*DT*) is a measured trait that hasnot shown in this figure

identified one genotype in all sampled trees of 8 stands. Proportion of sample required to represent all genotypes was 0.04 (1 genotype out of 24 trees).

The average number of observed alleles (*na*), expected alleles (*ne*), observed heterozygosity (*heo*) and expected heterozygosity (*hee*) are shown in Table 4. Genetic identity, based on Nei, was 100%, so the genetic distance was 0%. The genetic diversity assessed in terms of the number of alleles per locus, was low. The total number of alleles for 3 microsatellite loci was 6: *M* and *b* alleles in WPMS09 locus, *D* and *F* alleles in WPMS16 locus *I* and *J* alleles in WPMS18 locus. These results are in contrast to the results of several researches which have shown a higher frequency of alleles in *Populus nigra* stands, such as the work of Cottrell et al. (2002) which based on the three microsatellite loci (WPMS09, WPMS16 and WPMS18, the same loci as the loci used in the present study) revealed 15 alleles: *Z*, *W*, *Q*, *B*, *T*, *X*, *e* and a alleles in WPMS09 locus, *E*, *G* and *H* alleles in WPMS16 locus and *G*, *D*, *H* and *I* alleles in WPMS18 locus and based on these alleles revealed 15 different genotypes whereas in the present study was shown that all sampled trees represent a genotype.

Analysis of morphological traits

The results of taking the measurements of leaf morphological characters indicated that leaf area (*LA*) varied from 39.3 cm² (in stand K3) to 12.1 cm² (in stand K3). Leaf length (*LL*) ranged from 8.5 cm (in stand K3) to 5.1 cm (in

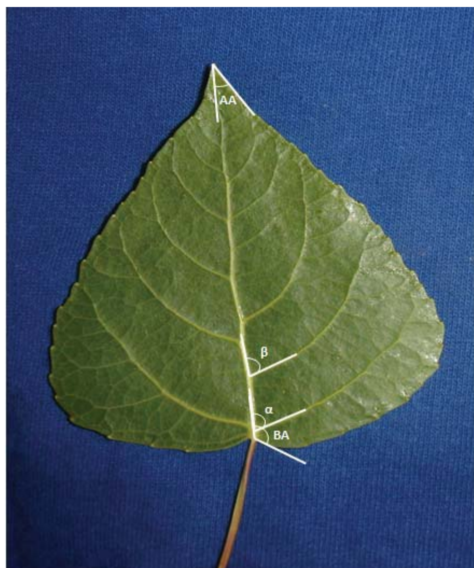

Figure 4 Presentation of the assessed leaf parameters: apex angle - (*AA*), base angle - (*BA*), angle between the first lower lateral vein and midrib - (α), and angle between the second lower lateral vein and midrib - (β). Note: It is not possible to show these morphological traits in a figure: ratio between petiol length and leaf length - (*PR*), ratio between leaf length and leaf width - (*LR*) and leaf area - (*LA*)

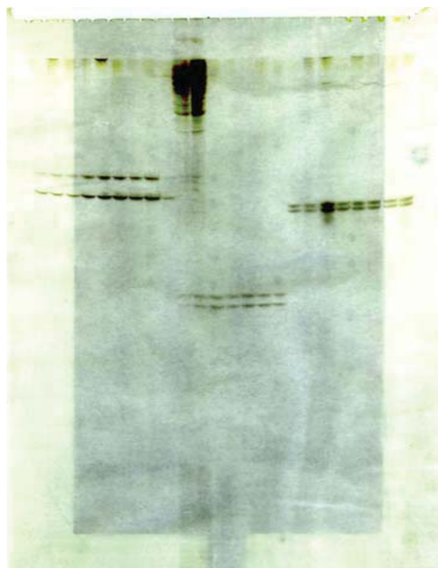

Figure 5 Revealed bands at the 3 different loci: WPMS09 (on the left), WPMS16 (on the mid) and WPMS18 (on the right)

Table 4 The mean value of observed and expected homo - and heterozygosities across all loci

Locus	Sample size	*na*	*ne*	*heo*	*hee*
WPMS09	48	2	2	1	0.51
WPMS16	48	2	2	1	0.51
WPMS18	48	2	2	1	0.51

stand K3). Leaf width (*LW*) ranged from 7.6 cm (in stand K8) to 3.9 cm (in stand K3). Petiole length (*LP*) varied from 4.0 cm (in stand K8) to 2.6 cm (in stand K3). Ratio between petiole length and leaf length (*PR*) varied from 0.5 (in stands K7 & K2) to 0.4 (in stand K8). Ratio between leaf length and leaf width (*LR*) varied from 1.4 (in stand K1) to 1.0 (in stand K8). Angle between the second lower lateral vein and midrib (β) ranged from 63.7 (in stand K3) to 50.0 (in stand K4). Angle between the first lower lateral vein and midrib (α) ranged from 63.9 (in stand K8) to 45.9 (in stand K7). Angle at 10% of leaf length (*A10*) ranged from 76.7 (in stand K8) to 63.3 (in stand K3). Angle at 25% of leaf length (*A25*) ranged from 63.0 (in stand K8) to 53.3 (in stand K3). Apex angle (*AA*) ranged from 45.1 (in stand K1) to 14.7 (in stand K6). Base angle (*BA*) ranged from 110.6 (in stands K8 & K3) to 61.1 (in stand K7). The largest and the smallest number of teeth on the middle one third of the leaf edge (*NT*) were

seen in stand K7 (21.1) and K3 (8.2), respectively. Depth of teeth on middle one third of the leaf edge (*DT*) varied from 2.4 cm (in stand K6) to 0.7 cm (in stand K6). Leaf width at 1 cm from the leaf tip (*LT*) ranged from 2.0 cm (in stand K1) to 0.9 cm (in stand K7). Distance between the leaf widest part and the leaf base (*DBW*) varied from 2.5 cm (in stand K7) to 1.5 cm (in stand K6). The largest and the smallest number of veins (*NV*) were seen in stand K8 (12.9) and K2 (9.4), respectively. Leaf width at 50% of leaf length (*BW50*) varied from 6.6 cm (in stand K8) to 3.2 cm (in stand K3) and leaf width at 90% of leaf length (*BW90*) varied from 1.2 cm (in stand K1) to 0.5 cm (in stand K8). The Bar charts of the means of different foliar traits in the eight stands are shown in Table 5. The results of using analysis of variance showed no significant differences between mean values of the eight stands for all of the leaf morphological character (Table 6).

As shown in Table 7 (the correlations between morphological traits) leaf width at 50% of leaf length (*BW50*) and angle at 25% of leaf length (*A25*) correlated with 14 traits. Leaf width (*LW*), ratio between leaf length and leaf width (*LR*) and angle at 10% of leaf length (*A10*) correlated with 13 traits. Number of veins (*NV*) correlated with 12 traits. Ratio between petiole length and leaf length (*PR*) and leaf area (*LA*) correlated with 11 traits. Petiole length (*LP*) correlated with 10 traits. Leaf length (*LL*) correlated with 9 traits. Leaf width at 90% of leaf length (*BW90*), leaf tip (*LT*) and angle between the second lower lateral vein and midrib (β) correlated with 8 traits. Apex angle (*AA*) and leaf width at 1 cm from the base angle (*BA*) correlated with 7 traits. Angle between the first lower lateral vein and midrib (α) correlated with 6 traits. Number of teeth on the middle one third of the leaf edge (*NT*) and depth of teeth on middle one third of the leaf edge (*DT*) correlated with 5 traits. Distance between the leaf widest part and the

Table 5 The means and standard deviation (*SD*) of different foliar traits in the eight stands of *Populus nigra*

Stands	1		2		3		4		5		6		7		8	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
LL	6.38	0.39	6.40	0.32	6.50	0.89	6.75	0.70	6.88	0.61	6.53	0.78	6.62	0.61	6.62	0.92
BW50	4.54	0.62	4.35	0.35	4.46	0.84	4.69	0.78	4.68	0.62	4.46	0.86	4.59	0.67	4.68	1.06
BW90	0.80	0.24	0.72	0.10	0.74	0.16	0.76	0.15	0.71	0.09	0.68	0.20	0.77	0.21	0.77	0.21
LT	1.42	0.30	1.27	0.16	1.34	0.16	1.28	0.17	1.18	0.08	1.21	0.25	1.35	0.30	1.33	0.26
LW	5.24	0.59	5.09	0.41	5.21	0.99	5.63	0.88	5.64	0.78	5.29	1.04	5.37	0.76	5.48	1.20
DBW	1.97	0.17	1.92	0.18	1.94	0.17	1.92	0.21	1.96	0.19	1.97	0.27	2.02	0.24	1.95	0.20
LP	3.21	0.28	3.17	0.14	3.21	0.37	3.44	0.33	3.41	0.25	3.18	0.36	3.31	0.35	3.26	0.37
NT	11.31	0.71	11.91	1.01	11.25	1.33	11.21	1.20	11.36	1.37	11.52	1.22	12.41	3.42	11.68	1.94
A10	70.96	3.50	69.36	2.70	69.46	3.43	71.43	2.77	71.75	1.98	70.20	3.31	69.94	3.13	70.72	4.79
A25	58.19	2.15	56.42	1.57	56.85	1.63	57.83	1.76	57.43	1.90	56.72	2.04	56.83	1.71	58.43	2.43
α	57.00	2.49	55.32	1.96	56.72	4.25	55.49	3.19	57.31	1.60	56.56	3.11	56.22	4.39	57.17	4.19
β	59.46	2.36	59.30	1.89	58.62	3.54	58.17	3.79	57.96	2.33	58.67	2.71	58.41	2.17	59.06	1.21
AA	29.49	8.24	28.52	5.17	27.02	5.25	31.68	5.92	25.90	4.22	25.37	7.79	30.86	8.05	31.53	6.65
BA	93.65	8.17	89.85	6.60	89.84	8.31	90.74	8.38	93.67	8.37	89.58	9.86	87.70	11.43	91.36	10.40
LA	20.82	3.49	19.94	2.67	21.61	7.44	23.28	6.30	23.71	5.15	21.11	7.26	22.12	5.35	22.80	8.15
NV	10.70	0.80	10.78	0.85	10.63	0.94	10.84	0.43	10.72	0.62	10.57	0.77	10.70	0.83	10.91	1.01
DT	1.08	0.27	1.25	0.21	1.07	0.29	1.23	0.41	1.21	0.36	1.13	0.53	1.09	0.23	1.28	0.48
LR	1.23	0.11	1.27	0.05	1.27	0.06	1.21	0.07	1.24	0.08	1.26	0.10	1.25	0.08	1.24	0.10
PR	0.51	0.03	0.50	0.02	0.50	0.03	0.51	0.03	0.50	0.03	0.49	0.03	0.50	0.02	0.50	0.03

Table 6 Inter-stands variability of the black poplar leaf morphological characters and height growth (*F*-value)

Morphological and growth characters							
characters	*β*	*α*	*A10*	*A25*	*AA*	*BA*	*LL*
F- value	0.55^{ns}	0.73^{ns}	1.66^{ns}	1.98^{ns}	1.14^{ns}	0.84^{ns}	0.77^{ns}
characters	*LW*	*DBW*	*LR*	*LP*	*PR*	*BW50*	*BW90*
F- value	0.75^{ns}	0.27^{ns}	0.76^{ns}	1.53^{ns}	0.41^{ns}	0.40^{ns}	0.57^{ns}
characters	*LT*	*LA*	*NT*	*DT*	*NV*	*H*	
F- value	1.38^{ns}	0.63^{ns}	0.63^{ns}	0.74^{ns}	0.39^{ns}	0.67^{ns}	

leaf base (*DBW*) correlated with 4 traits.

Principal component analysis reduced 19 input variables to 5 PCs, explaining 95/75% of the total variance. The relationship of the original variables with PCs is indicated by PC loadings (Table 8). Communality values for all factors were greater than 0.90 with the exception of ratio between leaf length and leaf width (*LR*) and base angle (*BA*). Scores in PC1 were positively related to petiole length (*LP*), leaf length (*LL*), leaf width (*LW*), leaf area (*LA*), leaf width at 50% of leaf length (*BW50*) and angle at 10% of leaf length (*A10*) as well as negatively related to angle between the second lower lateral vein and midrib (*β*) and ratio between leaf length and leaf width (*LR*). Petiole length (*LP*) was the more heavily weighted variable in PC1. Scores in PC2 were positively related to leaf width at 90% of leaf length (*BW90*), leaf width at 1 cm from the leaf tip (*LT*), apex angle (*AA*), ratio between petiole length and leaf length (*PR*) and angle at 25% of leaf length (*A25*). Scores in PC3 were positively related to number of teeth on the middle one third of the leaf edge (*NT*) and distance between the leaf widest part and the leaf base (*DBW*) as well as negatively related to base angle (*BA*). Scores in PC4 were positively related to depth of teeth on middle one third of the leaf edge (*DT*), number of veins (*NV*). Scores in PC5 were positively related to angle between the first lower lateral vein and midrib (*α*). Plant height ranged from 84.9 cm (in stand K8) to 61.4 cm (in stand K6). There were no significant differences between mean values of height growth in all stands.

Discussion

The two marker systems (leaf morphology and microsatellites) to assess the genetic variation of *Populus nigra* plantations established in Kermanshah province of Iran showed similar results. That is, the analyzed 8 stands do not contain an appreciable amount of genetic diversity.

Morphological analysis revealed no significant variation between poplar plantations (Table 5). In spite of these results, several other authors (Ballian et al. 2006, Gebhardt et al. 2002, Kajba & Romanic 2002) revealed significant differences between populations of *P. nigra* based on leaf morphological characters. Principal component analysis indicated that variation in all input variables was explained by 5 principal components. There are relationships among the whole of morphological traits (Table 6). This may show that similar genetic factors controls the studied leaf traits. The finding relationships between morphological traits are in agreement with othev findings Safavi 2011, which has shown a high positive correlation between leaf width (*LW*) and petiole length (*LP*) in *Populus euramericana* and *Populus deltoides*.

The usefulness of leaf morphology for evaluating genetic diversity within populations is not obvious yet. Some studies have already shown that it is possible to estimate

Table 7 Pearson coefficient of correlation between pairs of leaf morphological traits. (leaf length - *LL*, leaf width at 50% of leaf length - *BW50*, leaf width at 90% of leaf length - *BW90*, leaf width at 1 cm from the leaf tip - *LT*, leaf width - *LW*, distance between the leaf widest part and the leaf base - *DBW*, petiole length - *LP*, number of teeth on the middle one third of the leaf edge - *NT*, angle at 10% of leaf length - *A10*, angle at 25% of leaf length - *A25*, angle between the first lower lateral vein and midrib - α, and angle between the second lower lateral vein and midrib - β, apex angle - *AA*, base angle - *BA*, number of veins - *NV*, depth of teeth on middle one third of the leaf edge - *DT*, ratio between leaf length and leaf width - *LR*, ratio between petiole length and leaf length - *PR*, and leaf area - *LA*

	LL	*BW50*	*BW90*	*LT*	*LW*	*DBW*	*LP*	*NT*	*A10*	*A25*	α	β	*AA*	*BA*	*NV*	*DT*	*LR*	*PR*	*LA*
LL	1																		
BW50	0.79**	1																	
BW90	-0.21	0.34*	1																
LT	-0.55**	-0.01	0.91**	1															
LW	0.93**	0.93 **	0.02	-0.34*	1														
DBW	0.06	0.12	0.13	0.17	0.00	1													
LP	0.91**	0.85**	0.10	-0.28	0.93**	0.00	1												
NT	-0.13	-0.20	0.10	0.12	-0.25	0.59**	-0.17	1											
A10	0.69**	0.84**	0.10	-0.22	0.86**	-0.04	0.76**	-0.47**	1										
A25	0.21	0.73**	0.62**	0.40*	0.52**	-0.12	0.36*	-0.40*	0.69**	1									
α	0.20	0.39*	0.08	0.07	0.25	0.38*	0.01	-0.27	0.37*	0.47**	1								
β	-0.89**	-0.54**	0.35*	0.57**	-0.73**	-0.14	-0.79**	0.11	-0.44**	0.14	-0.03	1							
AA	0.01	0.44**	0.80**	0.61**	0.23	-0.04	0.29	0.29	0.13	0.53**	-0.30	0.17	1						
BA	0.18	0.39*	0.13	-0.02	0.34*	-0.24	0.23	-0.65**	0.72**	0.65**	0.55**	0.10	-0.15	1					
NV	0.25	0.51**	0.49**	0.20	0.41*	-0.40*	0.40*	0.08	0.33*	0.60**	-0.16	0.12	0.78**	0.19	1				
DT	0.37*	0.29	-0.16	-0.41*	0.40*	-0.55**	0.31	0.01	0.31	0.25	-0.23	-0.05	0.29	0.18	0.78**	1			
LR	-0.49**	-0.80**	-0.39*	-0.08	-0.76**	0.16	-0.72**	0.40*	-0.88**	-0.77**	-0.06	0.27	-0.50**	-0.52**	-0.51**	-0.30	1		
PR	0.23	0.50**	0.67**	0.45**	0.40*	-0.20	0.60**	-0.30	0.45**	0.48**	-0.23	-0.20	0.62**	0.25	0.40*	-0.04	-0.71**	1	
LA	0.94**	0.92**	0.06	-0.28	0.96**	0.05	0.89**	-0.23	0.74**	0.48**	0.36*	-0.77**	0.20	0.27	0.37*	0.31	-0.60**	0.36*	1

Table 8 Principal components (PC) solution of 19 leaf morphological traits. The loadings are shown in gray scale; the communalities were determined after Varimax axis rotation

Variable	PC1	PC2	PC3	PC4	PC5	Communality
LP	0.96	0.14	-0.10	0.11		0.98
LL	0.96	-0.21		0.13		0.99
LW	0.95		-0.11	0.21	0.17	0.99
LA	0.94			0.13	0.21	0.94
β	-0.89	0.32		0.22	0.21	0.98
bw50	0.85	0.34		0.18	0.34	0.99
A10	0.74	0.13	-0.42	0.15	0.39	0.91
LR	-0.63	-0.48	0.42	-0.22	-0.12	0.87
bw90		0.98			0.13	0.98
LT	-0.38	0.89		-0.18	0.11	0.98
AA	0.15	0.85	0.21	0.41	-0.18	0.99
PR	0.42	0.74	-0.39		-0.29	0.97
A25	0.29	0.60	-0.34	0.31	0.57	0.97
NT	-0.13		0.94	0.11	-0.16	0.94
DBW	0.14		0.70	-0.53	0.35	0.93
BA	0.16		-0.67	0.13	0.62	0.88
DT	0.23	-0.15		0.96		0.99
NV	0.23	0.48		0.84		0.98
α	0.14			-0.23	0.93	0.94
Explained variance (%)	35/26	57/21	70/50	83/75	95/75	

the genetic diversity based on leaf traits and this marker is a useful tool in genetic variation studies (Alba et al. 2002, Storme et al. 2002). Safavi (2011) revealed also a significant variation among poplar genotypes for some morphological traits such as leaf width (*LW*) and petiole length (*LP*) and high heritability for the traits such as leaf length (*LL*), leaf width (*LW*) and petiole length (*LP*). On the other hand, it has been shown morphological markers is not useful for estimating genetic diversity because the plants with the same genetic composition (clones), grown in different field tests differ significantly for this characters, therefore the differences in morphology of the samples do not have a genetic basis (van Dom et al. 2002). In present research the result of using molecular markers confirmed the result of using leaf morphological characters, so this marker can be used as easy and cheap marker to find out about genetic diversity initially. In fact morphological and molecular information does not cancel each other out, but they support each other and one of the two cannot fully substitute the other (Fossati et al. 2005).

On the basis of microsatellite markers, genetic diversity in term of polymorphic loci was 0%. There are several studies that have been shown high level of genotypic diversity in poplar population based on microsatellite markers (Storme et al. 2002, Smulders et al. 2002, van Dam 2002), although it is not easy to compare different genetical studies because of the factors that may affect the findings such as number and kind of loci. Proportion of sample required to represent all genotypes was 0.04. This is comparable to the obtained value in the work of Cottrell et al. (2002) which was 0.21. Whereas they expressed this value is the lowest proportion in comparison the results of other researches which had been shown almost every sampled tree represented a unique genotype.

The total number of alleles for 3 microsatel-

lite loci was 6 (Table 4). This value is comparable to the estimates obtained for *P.nigra* using the same microsatellite loci (7 allels in studied populations established in Iran, Asadi et al. 2005). In total whole sampled trees have the same genotype. These results with the result of morphological markers showed the level of genetic diversity present in the *P. nigra* populations established in Iran is low. In a population of a cross–pollinating species, sexually propagated individuals usually have a certain genetic distance from each other. Sister plants may share between 0% and 100% of the alleles they inherit from their parents. But only few couples will have a genetic similarity above 0.90, while a genetic similarity above 0.95 is extremely rare. Vegetative propagation leads to a genetic similarity of 1.00. Therefore, the distinction between clonal plants and sexually derived genotypes will, in principle, be clear (Smulder et al. 2002). Based on this finding, it can be assumed that poplar populations in Kermanshah province have been propagated of identical clones because of observed genetic identity (100%). So, the trees showed the same patterns in height growth and leaf morphological characters. Low level of genotypic diversity in poplar population has been indicated in some studies (Arens et al. 1998, Cottrell et al. 2002, Barsoum et al. 2004, Brundu et al. 2008, Smulders et al. 2008b).

For a long-term survival of poplar populations having sufficient genetic diversity is of great significance. Considering the strong vegetative reproduction ability of poplars, the individual cuttings sampled from a population should be separated by a distance great enough (Peng et al. 2005). To avoid the decrease of genetic diversity in poplar population established in Iran, it is essential to control clonal reproduction. For this purpose molecular markers are useful tools that be able to establish the extent of clonal propagation, while the morphological markers can be used as cheap diagnostic markers in early identification of clones for further selection (Lopez et al. 2004).

Conclusions

In this study, using the molecular and morphological markers it was revealed the low level of genetic variation in *P. nigra* stands in Kermanshah province of Iran. Monomorphics was shown at 3 investigated microsatellite loci and there were no significant differences between mean values of all morphological characters. These results indicate that studied stands are not in a good state of conservation. Since poplar plantations are threaten with environmental pressures which cause harmful effects on the growth and productivity of crops, genetic variation is essential for the long-term survival in these situations. For conservation strategy at least two conclusions can be drawn from these results: the first recommendation would be to avoid sampling the same ramets for planting in a poplar plantation. The second recommendation would be to increase the genetic richness of exiting population through providing conditions for sexual reproduction and planting seedlings with different genomes.

In conclusion, efforts should be made to optimize the level of genetic diversity in poplar populations established in Kermanshah province, in order to allow them to survive and reproduce under changing environmental conditions. This is a primary step to make effective use of these resources possible.

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References

Alba N.C., Maestro D., Gundez M.D., Notivol E., 2002. Advances in the preservation of genetic resources in *Populus nigra* L. in Spain. In: van Dam B.C., Bor-

dacs S., (eds.), Towards the: Generic diversity in river populations of european black poplar: implications for riparian ecosystem management. Proceedings of an international symposium held Csiszàr Nyomda, Hungary, pp. 125-136.

Apples R., 1997. DNA technology. A master class in new technologies for the measurement of biodiversity. The University Pertanian Malaysia, Serdang, Malaysia.

Arens P., Coops H., Jansen J., Vosman B., 1998. Molecular genetic analysis of black poplar (*Populus nigra* L.) along Dutch rivers. Molecular Ecology 7: 11-18.

Asadi F., Naderi-Shahab M.A., Mirzaie-Nodoushan H., 2005. Identity and genetic diversity of populus species clones using microsatellite marker. Pajouhesh Sazandegi 66: 49-55 (In Persian).

Bakshi M., Konnert M., 2011. Genetic diversity and differentiation through isozymes in natural populations of *Pinus wallichiana* A.B. Jacks (Blue Pine) in India. Annals of Forest Research 54: 23-37.

Ballian D., Kajba D., Idzojtic M., 2006. Morphological diversity of hairy european black poplar (*Populus nigra* subsp. caudina) in Bosnia and Herzegovina. Оригинални научни рад 5: 13-22.

Barsoum N., Muller E., Skot L., 2004. Variations in levels of clonality among *Populus nigra* L. stands of different ages. Evolutionary Ecology 18: 601-624.

Brenner AM., Busov VB., Strauss SH., 2004. Poplar genome sequence: functional genomics in an ecologically dominant plant species. Trends in Plant Sciemces 9: 49-56.

Brundu G., Lupi R., Zapelli I., Fossati T., Patrignani G., Camarda I., Sala F., Castiglione S., 2008. The Origin of Clonal Diversity and Structure of Populus alba in Sardinia: Evidence from Nuclear and Plastid Microsatellite Markers. Annals of Botany 102: 997-1006.

Calagari M., Modirrahmati A.R., Asadi F., 2006. Morphological variation in leaf trait of *Populus euphratica* Oliv. natural populations. International journal of Agriculture and Biology 6: 754-758.

Chen K., Peng Y., Wang Y., Korpelainen H. Li C., 2007. Genetic relationships among poplar species in section Tacamahaca (*Populus* L.) from western Sichusn, China. Plant Sicence 172: 196-203.

Cottrell J.E., Tabbener H.E., Forrest G.I., 2002. Distribution of variation in british black poplar: the role of human management. In: van Dam B.C., Bordacs S., (eds.), Towards the: Generic diversity in river populations of european black poplar: implications for riparian ecosystem management. Proceedings of an international symposium held Csiszàr Nyomda, Hungary, pp. 73-84.

Fossati T., Zapelli I. Bisoffi S., 2005. Genetic relationship and clonal identity in a collection of commercially relevant poplar cultivars assessed by AFLP and SSR. Tree Genetic & Genomes 1: 11-19.

Fossati T., Grassi F., Sala F. Castiglione S., 2003. Moleculiar analysis of natural population of *Populus nigra* L. intermingled with cultivated hybrids. Molecular Ecology 12: 2033-2043.

Gaudet M., Jorge V., Paolucci I., Beritognolo I., Scarascia Mugnozza G., Sabatti M. 2008. Genetic linkage maps of *Populus nigra* L. including AFLPs, SSRs, SNPs, and sex trait. Tree Genetics & Genomes 4: 25-36.

Gebhardt K., Pohland A., Vornam, B., 2002. Genetic inventory of black poplar population in the Upper Rhine Floodplains: conclution for conservation of an endangered plant species. In: van Dam B.C., Bordacs S., (eds.), Towards the generic diversity in river populations of european black poplar: implications for riparian ecosystem management. Proceedings of an international symposium held Csiszàr Nyomda, Hungary, pp. 145-156.

Jianming G., Shougong Z., Liwang Q., Yong Z., Chunguo W., ruiyang C., Wenqin S., 2007. AFLP fingerprinting of elite varieties (clones) from the genus Populus. Frontiers of Forestry in China 2: 204-209.

Kajba D., Romanic B., 2002. Morphological leaf variability of the european black poplar (*Populus nigra* L.) in natural populations in the Drava River Basin in Croatia. In: van Dam B.C., Bordacs S., (eds.), Towards the: Generic diversity in river populations of european black poplar: implications for riparian ecosystem management. Proceedings of an international symposium held Csiszàr Nyomda, Hungary, pp. 221-227.

Khan I.A., Awan F.S., Ahmad A., Khan A.A., 2004. A Modified mini-prep method for economical and rapid extraction of genomic DNA in plants. Plant Molecular Biology Reporter 22: 89a-89e.

Krystufek V., Fluch S., Burg K., 2002. Artificial yet natural: colonization of an artificial island in the river Danube in Vienna. . In: van Dam B.C., Bordacs S., (eds.), Towards the: Generic diversity in river populations of european black poplar: implications for riparian ecosystem management. Proceedings of an international symposium held Csiszàr Nyomda, Hungary, pp. 137-144.

Lefevre F., Achard P., Azasis D., Smulder M.J.M., Van der Schoot J., Bovenschen J., Ivens B., Storme V., Fluch S., Krystufek V., Castiglione S., 2002. Distribution of *Populus nigra* genetic diversity within France and its consequences for ex-situ conservation strategy. In: van Dam B.C., Bordacs S., (eds.), Towards the generic diversity in river populations of european black poplar: implications for riparian ecosystem management. Proceedings of an international symposium held Csiszàr Nyomda, Hungary, pp. 85-91.

Li S., Zhang Bo., Yin C., Pan H., Minren H., 2006. Analysis of genetic diversity of *Populus deltoides* germplasm by SSRs. International Poplar Symposium, China.

Lopez D.H., Sierra U.R., Cristobal M.D., 2004. A comparison of isozyme and morphological markers to assess the within population variation in small populations of European aspen (*Populus tremula* L.) in Spain. Silvae Genetica 53: 227-233.

Mohajer M.R., 2006. Silviculture, 2th edition, Tehran University, Tehran (In Persian).

Nori F., Modir-Rahmati A., Asadi F., Khoda-karami Y., Khan-Hassani M., 2008. Investigation on growth char-

acteristics and wood production of 12 poplar clones open crown *Populus delto*ides and *Populus euramerican*. Proceedings of the Second National Congress On Polar and Potential Use in Poplar Plantation, pp. 129-139.

Peng Y.H., Lu Z.X., Chen K., Luukkanen O., Korpelainen H., Li C.Y., 2005. Population genetic survey of *Populus cathayana* originating from southeastern Qinghai-Tibetan plateau of China based on SSR markers. Silvae Genetica 54: 116-122.

Rathmacher G., Niggemann M., Köhnen M., Ziegenhagen1 B., Bialozyt R., 2010. Short-distance gene flow in *Populus nigra* L. accounts for small-scale spatial genetic structures: implications for in situ conservation measures. Conservation Genetics 11: 1327-1338.

Safavi S.M., 2011. Estimation of genetic parameters related to morphological traits in poplar clones. American Journal of Scientific Research 27: 105-110.

Smulders M.J.M., Beringen R., Volosyanchuk R., Vanden Broeck A., Van der Schoot J., Arens P., Vosman B., 2008a. Natural hybridisation between Populus nigra L. and P. xcanadensis Moench. Hybrid offspring competes for nichesalong the Rhine river in the Netherlands. Tree Genetics & Genomes 4: 663-675.

Smulders M.J.M., Cottrell JE., Lefèvre F., Van der Schoot J., Arens P., Vosman B., Tabbener HE., Grassi F., Fossati T., Castiglione S., Krystufek V., Fluch S., Burg K., Vornam B., Pohl A., Gebhardt K., Alba N., Agúndez D., Maestro C., Notivol E., Volosyanchuk R., Pospíšková M., Bordács S., Bovenschen J., van Dam BC., Koelewijn HP., Halfmaerten D., Ivens B., van Slycken J., Vanden Broeck A., Storme V., Boerjan W., 2008b. Structure of the genetic diversity in black poplar (*Populus nigra* L.) populations across European river systems: consequences for conservation and restoration. Forest Ecology and Management 255: 1388-1399.

Smulders M.J.M., Van der Schoot J., Ivens B., Storme V., Castiglione S., Grassi F., Fossati T., Bovenschen J., van Dam BC., Vosman B., 2002. Clonal propagation in black poplar (*Populus nigra*). In: van Dam B.C., Bordacs S., (eds.), Towards the generic diversity in river populations of European black poplar: implications for riparian ecosystem management. Proceedings of an international symposium held Csiszàr Nyomda, Hungary, pp. 39-52.

Smulders M.J.M., Van der Schoot J., Arens P., Vosman B., 2001. Trinucleotide repeat microsatellite markers for black poplar (*Populus nigra* L). Molecular Ecology 1: 188-190.

Storme V., Vanden Broeck A.H., Ivens B., Smulders M.J.M., Halfmaerten D., Van Slycken J., Boerjan, W., 2002. Ex situ conservation of black poplar in Belgium, the margin of the geographical distribution area of the species. In: van Dam B.C., Bordacs S., (eds.), Towards the generic diversity in river populations of european black poplar: implications for riparian ecosystem management. Proceedings of an international symposium held Csiszàr Nyomda, Hungary, pp. 61-72.

Suvanto LI., Latva-Karjanmaa T., 2005. Clone identification and clonal structure of the European aspen (*Populus tremula* L.). Molecular Ecology 14: 2851-2860.

Taylor G., 2002. *Populus:* arabidopsis for forestry. Do we need a model tree? Annals of Botany 90: 681-689.

Vanden Broeck A.H., Storme V., Cox K., Quataert P., Ivens B., Hostyn V., Halfmaerten D., Boerjan W., Van Slycken, J., 2002. Mating system of *Populus nigra* in a mixed poplar sand composed of *P. nigra*, *P. deltoids* and *P*.x *euramericana*. In: van Dam B.C., Bordacs S., (eds.), Towards the generic diversity in river populations of european black poplar: implications for riparian ecosystem management. Proceedings of an international symposium held Csiszàr Nyomda, Hungary, pp. 53-60.

Van Dam B.C., 2002. EUROPOP: Genetic diversity in river population of european black poplar for evalution of biodiversity, conservation strategies, nature development and genetic improvement. In: van Dam B.C., Bordacs S., (eds.), Towards the generic diversity in river populations of european black poplar: implications for riparian ecosystem management. Proceedings of an international symposium held Csiszàr Nyomda, Hungary, pp. 15-32.

Van der Schoot J., Pospíškova M., Vosman B., Smulders M.J.M., 2000. Development and characterization of microsatellite markers in black poplar (*Populus nigra* L). Theoretical and Applied Genetics 101: 317-322.