TEMPORAL GENETIC STRUCTURE OF IRANIAN POPULATIONS OF BEECH, FAGUS ORIENTALIS (FAGACEAE)

P. Salehi Shanjani, G. G. Vendramin & M. Calagari

Reforestation with autochthonous species should take into account the preservation of the temporal variability and the geographic structure of genetic diversity in forest species. In order to provide empirical data about the suitability of methods of sampling material, genetic comparison of 10 beech populations (at least 40 trees per population) and their progenies (seeds of 10 mother trees per population, each tree 7 seeds) were analysed using four highly polymorphic microsatellite loci. The allelic multiplicity was higher in seed samples than adult trees indicating gene flow from adjacent plant populations. The comparison for genetic diversity measures between adult trees and seed generation revealed no significant differences for allelic richness (*Na*), effective number of alleles (*Ne*), and number of rare alleles (*Nr*), neither observed (*Ho*) nor expected heterozygosity (*He*). Genetic differentiation in allelic frequencies between adult trees and seeds generation were rather low (*Fst* = 0.058). A close genetic relationship between adult trees from seed generation of each population, which revealed by un-weighted pair group method based on arithmetic average (UPGMA) and supported by an analysis of molecular variance (AMOVA), were detected. In this paper some aspects related to seed sampling were discussed.

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ساختار ژنتیکی زمانی جمعیت‌هایی از گونه راش (Fagus orientalis) در ایران

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در جنگاکاری با گونه های بومی می بایست حفاظت از گوناگونی زمانی و ساختار جغرافیایی تنوع ژنتیکی گونه‌های جنگلی در نظر گرفته شود. برای تهیه اطلاعات کاربردی در مورد کارآمدی روشهای جمع‌آوری نمونه، ترکیب ژنتیکی ده جمعیت راش (حداقل ٤٠ درخت در هر جمعیت) و نتاج آنها (بذور ١٠ درخت مادری در هر جمعیت، به میزان ٧ بذر از هر درخت) توسط چهار لوکوس میکروساتلایتی پلی‌مورف بررسی شد. تکثّر آللی در نمونه‌های بذر بیش از درختان بالغ بود که حاکی از وجود جریان ژن از جمعیتهای گیاهی مجاور است. مقایسه مقادیر تنوع ژنتیکی بین درختان بالغ و نسل بذری هیچ اختلافی را از نظر غنای آللی (Na)، تعداد موثر آللها (Ne)، تعداد آللهای نادر (Nr)، هتروزیگوزیتی مشاهده شده (Ho) و هتروزیگوزیتی مورد انتظار (He) نشان ندادند. تمایز ژنتیکی در فراوانی آللی بین درختان بالغ و نسل بذور نیز بسیار کم بود (Fst = ٠/٠٥٨). روابط ژنتیکی نزدیکی بین درختان بالغ و نسل بذری در هر جمعیت وجود داشت که بوسیله روش معدل گروهی (UPGMA) نشان داده شد و آنالیز واریانس ملکولی (AMOVA) نیز آن را تأیید نمود. در این پژوهش برخی ویژگیهای جمع‌آوری بذر مورد بحث قرار گرفت.

INTRODUCTION

Genetic variation is an important attribute of forest tree populations enabling them to survive spatial and temporal variations in environmental conditions. The genetic variation and its structure within and between populations are also important in conservation and management of genetic resources and in applications in breeding and silviculture (Brown 1978, Hattemer 1987,
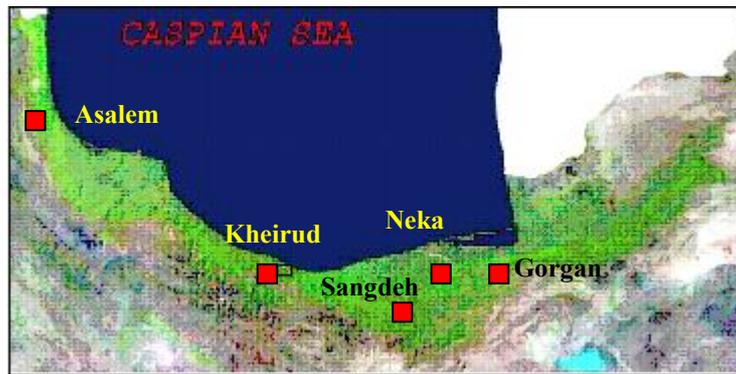
Fig. 1. Distribution of studied stands of *Fagus orientalis*.

Table1. Site characteristics of 10 Beech populations.

Region	Altitude (m)	Abbreviation	Latitude (N)	Longitude (E)	beech %	Canopy (%)
Gorgan	1400	G-1400	36º 41'	54º 05'	48	90
"	600	G-600	36º 42'	54º 06'	32	90
Neka	1400	N-1400	36º 22'	53º 33'	60	80
"	900	N-900	36'' 29'	53º 27'	72	90
Sangdeh	1400	S-1400	36º 03'	53º 14'	71	70
"	900	S-900	36º 06'	53º 16'	67	65
Kheirud	1200	K-1200	36º 32'	51º 39'	76	90
"	600	K-600	36º 35'	51º 33'	74	90
Asalem	1200	A-1200	37º 38'	48º 48'	42	90
"	600	A-600	37º 41'	48º 48'	37	70

Ziehe et al. 1989, Finkeldey 1993, Gregorius 1994). Genetic structure can also be an indicator of adaptation and adaptational potentials (Hattemer & Ziehe 1997, Ziehe et al. 1999). Restricted gene flow, disruptive selection, genetic drift and historical events are responsible for population genetic differentiation in space and in time (Levin and Kerster 1974). The extent and pattern of genetic diversity in forest trees are strongly influenced by their mating systems and the movement of genes (gene flow) between dispersed populations of the same species (Wang 2003).

   Oriental Beech (*Fagus orientalis* Lipsky) is a widespread, monoecious and wind-pollinated tree species. It belongs to the major forest tree species and is of importance in ecology and economy. The genetic variation of Hyrcanian beech populations has been investigated in few studies (Salehi Shanjani et al. 2002, 2004, 2008). Like many other tree species (Hamrick et al. 1992), beech trees reveal a high level of genetic variation at microsattelite and allozyme gene loci (Salehi Shanjani et al. 2002, 2008). Since its pollen can be dispersed over wide distances, genetic change can theoretically occur among very distant populations. Generally, a great intra-populational but relatively small inter-population variation is found in this species. The little differentiation of populations suggests that gene flow between populations is extensive. The comparisons of genetic structures of reproducing forest stands and the produced offspring in beech (*Fagus sylvatica* L.) indicated the occurrence of changes in genetic structures of population during reproduction (e.g. Müller-Starck & Ziehe 1991, Starke & Müller-Starck 1992, Hattemer et al. 1993, Müller-Starck 1996, Ziehe et al. 1998). In respect to developing interest in beech forests restoration, any study on temporal genetic variation of Oriental Beech was not been reported, which are information requirements for efficient sampling schemes for *ex situ* and *in situ* conservation programs. It is always desirable to capture as much genetic variation as possible, but the physical and financial limitations of the most *ex situ* and *in situ* conservation methods will determine what type and amount of genetic variation can be sampled and therefore conserved (Amaral et al. 2004). Hence, in this research, the genetic diversity and differentiation of adult trees and seed generation of different populations were compared to study gene flow between two studied generations and discussed on appropriate seed sampling strategy.

MATERIALS AND METHODS
Samples were taken from 10 natural beech populations covering a large part of the distribution range of Oriental Beech (*Fagus orientalis* Lipsky) in North of

Table 2. Characteristics of the 4 polymorphic nuclear microsatellite markers used for analysis of genetic diversity in the Iranian Beech populations.

Locus	Primer sequences 5'-3'	Annealing temp. (°C)	$MgCl_2$ concen.	Repeat	Observed allele No.
FS1-15	TCAAACCCAGTAAATTTCTCA GCCTCAATGAACTCAAAAAC	60	2.5	$(GA)_{26}$	26
FS1-03	CACAGCTTGACACATTCCAAC TGGTAAAGCACTTTTTCCCACT	60	1.5	$(GA)_{18}$	18
FS1-11	TGAATTCAATCATTTGACCATT C	63	2.5	$(GA)_{15}$	18
FS3-04	GGAAGGGTGCTTCAATTTGG AGATGCACCACTTCAAATTC TCTCCTCAGCAACATACCTC	60	1.5	$(GCT)_5(GTT)_3(GCT)_6$	6

Iran. The area (Beech forests) included are located on the northern slopes of Alborz Mountains, within an altitude of about 600-2000 m above sea level. They have formed a forest strip with 700 km length. Analyzed stands were chosen in order to maximally represent the ecological conditions. An overview of the investigated populations is shown in fig. 1 and table 1. For this purpose, five locations along the distribution area of Beech from west to east were identified and two populations in each region (at low and middle altitude) were selected. A total of 1155 Beech samples, including 490 bud samples from 10 sites, each with at least 40 trees. They were randomly chosen and separated by at least 30 m as adult trees. Altogether 665 seed samples (in each site, seeds from 10 mother trees, each with 7 individuals) were used (table 1, fig. 1).

Microsatellite analysis
DNA was isolated from dormant buds of trees and seed embryo (100 mg as starting material) using Nucleospin plant kit (Macherey Nagel, Germany). Four microsatellite markers (FS1-15, FS1-03, FS1-11 and FS3-04) described by Pastorelli et al. (2003) were amplified according to the following temperature profile: 5 min denaturation at 95°C followed by 30 cycles of 1 min denaturation at 95°C, 1 min annealing (table 2), 1 min extension at 72°C, with a final extension step of 8 min at 72°C. The PCR conditions for the six selected SSRs (final volume of 25 μl) were performed using 10ng of template DNA, 10× Amersham reaction buffer (500 mM KCl, 15 mM MgCl2 and 100mM Tris-HCl pH 9.0), $MgCl_2$ as in table 2, 0.2 mM dNTPs (Amersham), 0,4 μM of each primer, 1 U of Taq DNA polymerase (Amersham). The success of the amplification was confirmed on a 1.4% agarose gel. Amplified fragments were then multiplexed by size (mixed two by two) standards (50, 100, 150, 200, 250 and 300bp) were added to each mix

before loading onto Reprogel Long Read acrylamide gels (Amersham), and run on an automated sequencing machine (Alf Express, Amersham) at 1500V, 60 mA, 30W, 55°C. The results of the run were then analyzed with Fragment Manager 1.2 (Amersham).

Data analysis
Amplification reactions from all individuals were scored and the following statistics of genetic variation within different groups of Beech samples (seeds and adult trees bud) were computed as averages over loci using the GENAlEX 6 software (Peakal & Smouse, 2006): mean number of alleles per locus (*Na*), number of private alleles (*Nr*), effective number of alleles, (*Ne*); average heterozygosity, (*Ho*); and average expected heterozygosity (He) computed according to Nei (1978). An analysis of molecular variance (AMOVA) was performed using the GenAlEx 6 software (Peakall & Smouse 2006) in order to partition the genetic variation among species, among populations within species, and among individuals within populations (Schneider *et al.* 2000). The significance of each variance component was tested with permutation tests (Excoffier et al. 1992). Genetic distances were estimated according to Nei (1978), and principal coordinate analysis (PCO) (Gower 1966) and Unweighted Pair Group Method with Arithmetic mean (UPGMA) (Sneath & Sokal, 1973) analysis were performed. The UPGMA dendrogram was constructed with the MEGA 4 software (Tamura et al., 2007). Wright's *Fst* was used to estimate population differentiation. Mantel's test (1967) was used to assess the correlation between the calculated distance matrices and the test statistic tested for significance against 999 random permutations.

RESULTS
Microsatellite diversity
A total of 68 fragments were obtained from the four SSR primer pairs and all bands were polymorphic

Table 3. Levels of genetic diversity in adult trees and seed samples of 10 Beech populations.

Locus	Seeds						Trees					
	N	*Na*	*Ne*	*Ho*	*He*	*Fis*	*N*	*Na*	*Ne*	*Ho*	*He*	*Fis*
G-1400												
FS1-15	55	7.00	1.578	0.364	0.366	0.008	64	9.00	2.329	0.609	0.571	-0.068
FS3-04	56	2.00	1.194	0.179	0.163	-0.098	61	2.00	1.589	0.492	0.371	-0.326
FS1-11	56	8.00	2.302	0.696	0.566	-0.231	60	5.00	1.987	0.667	0.497	-0.342
FS1-03	56	9.00	3.486	0.607	0.713	0.149	64	8.00	1.670	0.359	0.401	0.105
Overall	*56*	*6.50*	*2.14*	*0.452*	*0.461*	*0.043*	*64*	*6.00*	*1.894*	*0.46*	*0.532*	*-0.158*
G-600												
FS1-15	76	11.00	3.539	0.645	0.717	0.101	51	12.00	3.040	0.706	0.671	-0.052
FS3-04	77	3.00	1.347	0.273	0.258	-0.059	51	3.00	1.525	0.392	0.344	-0.139
FS1-11	77	10.00	3.173	0.623	0.685	0.090	51	7.00	2.747	0.784	0.636	-0.233
FS1-03	74	10.00	3.457	0.716	0.711	-0.008	51	10.00	4.236	0.745	0.764	0.025
Overall	*77*	*8.50*	*2.879*	*0.593*	*0.564*	*0.031*	*51*	*8.00*	*2.887*	*0.604*	*0.657*	*-0.1*
N-1400												
FS1-15	70	13.00	4.651	0.686	0.785	0.126	50	11.00	3.861	0.980	0.741	-0.323
FS3-04	70	5.00	1.304	0.243	0.233	-0.042	50	4.00	1.432	0.360	0.302	-0.193
FS1-11	70	7.00	2.384	0.743	0.581	-0.279	49	7.00	2.542	0.633	0.607	-0.043
FS1-03	70	8.00	2.391	0.600	0.582	-0.031	50	10.00	3.023	0.680	0.669	-0.016
Overall	*70*	*8.25*	*2.683*	*0.545*	*0.568*	*-0.057*	*50*	*8.00*	*2.715*	*0.58*	*0.663*	*-0.144*
N-900												
FS1-15	70	11.00	3.417	0.600	0.707	0.152	48	13.00	4.692	0.604	0.787	0.232
FS3-04	70	3.00	1.364	0.286	0.267	-0.070	49	3.00	1.409	0.347	0.290	-0.196
FS1-11	70	8.00	2.257	0.614	0.557	-0.103	48	5.00	2.324	0.625	0.570	-0.097
FS1-03	69	9.00	3.661	0.681	0.727	0.063	48	8.00	3.165	0.729	0.684	-0.066
Overall	*70*	*7.75*	*2.675*	*0.565*	*0.545*	*0.01*	*49*	*7.25*	*2.897*	*0.583*	*0.576*	*-0.032*
S-1400												
FS1-15	70	12.00	2.276	0.586	0.561	-0.045	42	10.00	2.706	0.548	0.630	0.131
FS3-04	69	2.00	1.258	0.203	0.205	0.010	44	3.00	1.444	0.341	0.308	-0.108
FS1-11	63	13.00	2.488	0.762	0.598	-0.274	43	6.00	2.324	0.721	0.570	-0.265
FS1-03	67	11.00	2.873	0.567	0.652	0.130	44	9.00	3.138	0.682	0.681	-0.001
Overall	*70*	*9.50*	*2.224*	*0.504*	*0.529*	*-0.045*	*44*	*7.00*	*2.403*	*0.547*	*0.573*	*-0.061*
S-900												
FS1-15	70	11.00	3.977	0.657	0.749	0.122	53	17.00	4.998	0.792	0.800	0.009
FS3-04	70	3.00	1.274	0.186	0.215	0.136	48	3.00	1.182	0.125	0.154	0.190
FS1-11	69	8.00	2.284	0.725	0.562	-0.289	46	11.00	4.061	0.652	0.754	0.135
FS1-03	70	10.00	3.596	0.686	0.722	0.050	53	10.00	4.259	0.642	0.765	0.162
Overall	*70*	*8.00*	*2.783*	*0.562*	*0.563*	*0.005*	*53*	*10.25*	*3.625*	*0.618*	*0.553*	*0.124*
K-1200												
FS1-15	67	13.00	3.251	0.597	0.692	0.138	50	10.00	4.460	0.800	0.776	-0.031
FS3-04	67	3.00	1.363	0.254	0.267	0.048	46	4.00	1.446	0.304	0.309	0.014
FS1-11	65	8.00	2.671	0.754	0.626	-0.205	46	5.00	2.275	0.739	0.560	-0.319
FS1-03	68	10.00	4.650	0.676	0.785	0.138	49	12.00	6.744	0.776	0.852	0.089
Overall	*68*	*8.50*	*2.984*	*0.592*	*0.57*	*0.03*	*50*	*7.75*	*3.732*	*0.624*	*0.655*	*-0.062*
K-600												
FS1-15	56	14.00	4.015	0.804	0.751	-0.070	38	16.00	3.919	0.579	0.745	0.223
FS3-04	56	4.00	1.646	0.446	0.393	-0.137	38	2.00	1.532	0.289	0.347	0.167
FS1-11	56	11.00	2.271	0.714	0.560	-0.276	39	5.00	2.377	0.641	0.579	-0.107
FS1-03	56	10.00	2.906	0.625	0.656	0.047	39	11.00	2.881	0.615	0.653	0.057
Overall	*56*	*9.75*	*2.71*	*0.59*	*0.647*	*-0.106*	*39*	*8.50*	*2.677*	*0.581*	*0.531*	*0.085*
A-1200												
FS1-15	70	14.00	2.285	0.586	0.562	-0.041	49	11.00	2.736	0.653	0.635	-0.029
FS3-04	63	4.00	1.394	0.254	0.283	0.102	48	3.00	1.391	0.333	0.281	-0.186
FS1-11	65	11.00	2.070	0.631	0.517	-0.220	48	7.00	2.361	0.688	0.576	-0.193
FS1-03	70	8.00	2.305	0.557	0.566	0.016	49	11.00	2.621	0.653	0.618	-0.056
Overall	*70*	*9.25*	*2.014*	*0.482*	*0.507*	*-0.036*	*49*	*8.00*	*2.277*	*0.528*	*0.582*	*-0.116*
A-600												
FS1-15	56	10.00	3.681	0.696	0.728	0.044	39	9.00	3.865	0.410	0.741	0.447
FS3-04	56	4.00	1.451	0.304	0.311	0.023	39	3.00	1.330	0.256	0.248	-0.033
FS1-11	56	6.00	1.764	0.482	0.433	-0.113	39	10.00	2.347	0.692	0.574	-0.206
FS1-03	56	11.00	3.808	0.696	0.737	0.056	40	8.00	2.566	0.525	0.610	0.140
Overall	*56*	*7.75*	*2.676*	*0.552*	*0.545*	*0.002*	*40*	*7.50*	*2.527*	*0.543*	*0.471*	*0.087*
Overall		8.38	2.58	0.550	0.544	-0.019		7.83	2.76	0.580	0.567	-0.038

Table 4. Genetic differentiation (*Fst*) among Beech populations.

Comparison	N	*Fst*
Among all sample groups	20	0.058
Among all adult trees groups	10	0.055
Among all seeds groups	10	0.058
Pair-wise per site		
G-1400	2	0.040
G-600	2	0.006
N-1400	2	0.014
N-900	2	0.009
S-1400	2	0.005
S-900	2	0.012
K-1200	2	0.010
K-600	2	0.012
A-1200	2	0.005
K-600	2	0.014

across the genotypes. The number of alleles per locus ranged from 6 (FS3-04) to 26 (FS1-15), with an average of 17 alleles per locus (table 2). Rare alleles (defined as alleles with a frequency less than 1%) were identified at four loci, which had at least one allele unique to a genotype. Overall, seed germplasm showed higher number of bands compared to the adult tree samples (table 3).

Across all analyzed data sets of Beech (seeds and adult tree samples), the mean values of the *H*e indicate a considerable amount of genetic variation within each category (table 3). The seed samples had a gene diversity of 0.544 and an average of 8.38 alleles per locus, while the Adult trees data set had a gene diversity of 0.567 and an average of 7.38 alleles per locus, respectively. A comparison of the seed samples versus the adult trees, revealed a slightly lower diversity in the former set but not significant. A paired *t*-test for all 10 populations did not show significant differences between the seeds and adult trees data sets for *Na* ($P = 0.192$), *Ne* ($P = 0.108$), neither *Ho* ($P = 0.188$) nor *He* ($P = 0.126$).

Genetic differentiation
Several among-population analyses were performed. The differentiation among all seed and adult trees based on the microsatellite data set was low but differed significantly ($P < 0.001$) from zero (*Fst* = 0.058). The genetic differentiation within seed and adult trees samples was also low (*Fst*= 0.055, 0.058, $P < 0.001$ respectively). The pair-wise comparisons of the seed and adult trees within a site revealed even lower *Fst*-values (from 0.005 to 0.040) (table 4).

For the description of the differentiation pattern, the genetic distances between the analysed data sets of the

Beech (seeds versus adult trees) were calculated according to unbiased estimates of genetic distance. Genetic distances between populations were then used to perform principal coordinate analysis. The results of the PCO showed that on the basis of the first principal coordinate, which accounted for 68.36% of the total variation, the seeds and adult trees with origin from Asalem (A-1200, A-600) and Kkeirud (K-1200) were clearly separated from remained populations (fig. 2), suggesting valuable geographic information in our genetic data-set. The clinal pattern detected has been hypothesized to reflect past migration routes; we can not completely exclude a possible role of clinal selection in determining the observed geographic trends. Overall patterns of genetic differentiation were also examined using UPGMA analysis (fig. 3). The resulting tree had long terminal branches. Thus, it could be suggested that the seed and adult trees groups with origin of Asalem (A-1200, A-600) and Kkeirud (K-1200) were well differentiated. AMOVA analysis showed that the variation between the seeds and adult trees, between and within the populations accounted for 0%, 12%, and 88% of the total variation, respectively (table 5), which was in accordance with the *Fst*=5.8%, based on the Nei's gene diversity index. Also, results of AMOVA implied that 7 and 5% of genetic variation occurred among populations of seeds and adult trees, respectively, and most of the variation (87 and 88% of seeds and adult trees data sets, respectively) occurred within population (table 5). This was also in accordance with the *Fst* (5.5 and 4.8% among population of each of seeds and adult trees data sets, respectively) based on the Nei's gene diversity index.

Correlation coefficient among pair-wise genetic distance matrices generated by the different data sets

Principal Coordinates
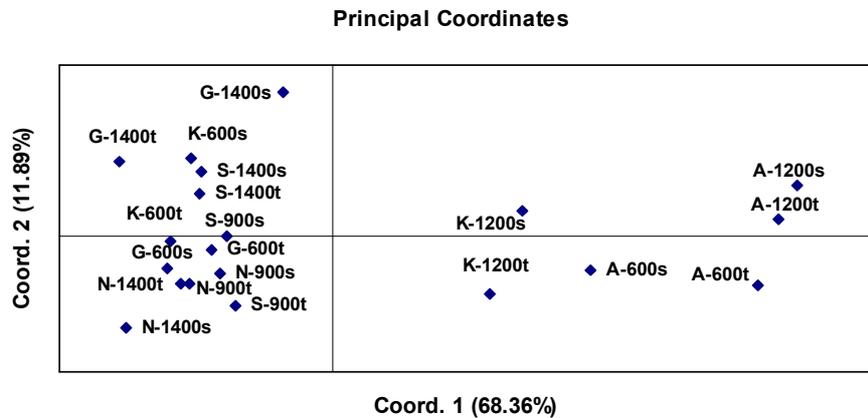
Fig. 2. Two dimensional graphs of adult trees (with suffix t) and seeds (with suffix s) groups of different Beech populations based on the ordination scores resulted from the principal coordinate analysis considering Nei's genetic distances.
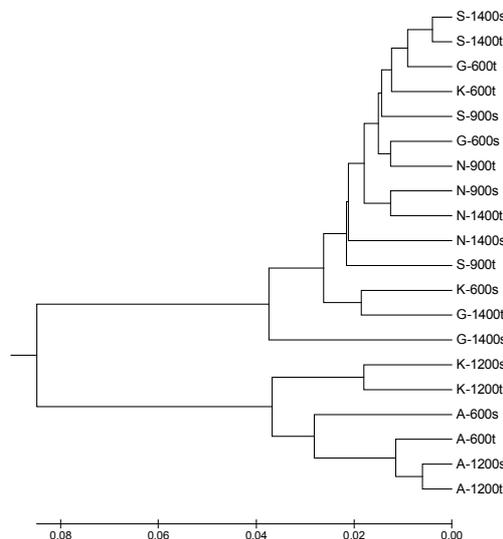
Fig. 3. Dendregram of the adult trees (with suffix t) and seeds (with suffix s) groups of different Beech populations produced by UPGMA cluster analysis.

Seeds vs adult trees

$y = 1.014x + 0.0036$
$R^2 = 0.7143**$
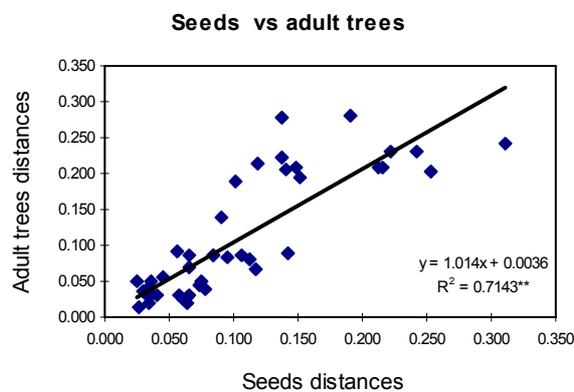
Fig. 4. Scatter plot of pair-wise seeds and adult trees distances of Beech.

Table 5. Analysis of molecular variance (AMOVA).

	Variance	d.f.	Sum of squares	Variance components	Meam of squares	Percentage of variation	P
Adult trees	Among Regions	4	113.606	0.201	28.401	7	0.001
	Among Pops./Regions	5	43.199	0.127	8.640	5	0.001
	Indiv./Within Pops.	480	1178.668	2.456	2.456	88	0.001
Seeds	Among Regions	4	139.239	0.148	34.810	6	0.001
	Among Pops./Regions	5	74.485	0.191	14.897	7	0.001
	Indiv./Within Pops.	655	1532.004	2.339	2.339	87	0.001
Seeds & Adult trees	Among generations	1	9.741	0.000	9.741	0	1.000
	Among pop./generations	18	370.529	0.316	20.585	12	0.001
	Within Pops	1135	2710.673	2.388	2.388	88	0.001

(seeds versus adult trees) were calculated using mantel's test (fig. 4), which showed a high correlation ($R^2 = 0.714$, P<0.01).

DISCUSSION
In the present study, we have compared the genetic diversity of Beech seeds and adult trees originating from 10 populations. The DNA marker technique (SSR) used was able to clearly discriminate the different Beech groups. According to our results, the mean values of the *H*e (0.555) indicate a considerable amount of genetic variation for revealing genetic diversity among populations. These values are considerably lower than that of the previous results (*He* = 0.8) obtained from European Beech stands, by Buiteveld et al. (2007). The pair-wise comparison revealed no differences in genetic diversity measures among the adult trees and selected seeds in every population. Therefore, it can be concluded that the selection of seeds from a few trees did not influence the level of genetic diversity at the genetic markers used. However, a few aspects should be taken into account. The allelic multiplicity in the seed generation is somehow higher than that in adult trees. Those values indicate that gene flow from neighbor stands is effective and resulted in an increase of allelic variants due to external pollen in seed generation as observed by Müller-Starck (1996), Levy and Neal (1999) and Wang (2003). Hamrick & Godt (1989) have pointed out that levels of genetic diversity within populations were influenced by several characteristics of the species. Seed dispersal, breeding system and geographic range all have predictive value. Under the conditions of small population size, genetic drift could lead to a rapid loss of alleles, particularly rare alleles. However, the high reproductive capability, high out-crossing rate and effective gene flow may have

counteracted this effect. Results of this study revealed no significant difference between seed-derived generation and adult trees for heterozygosity. Hence, the restricted amount of seeds used in the present study is not at all less variable than others and are, therefore, appropriate for natural regeneration.

The genetic differentiation in allelic frequencies between adult trees and seed-derived generation were rather low (*Fst* ranged from 0.05% to 0.4%) and significant only at few loci. This can be explanted by this fact that the two groups have the same genetic background in the sense that they originated from the same population. The genetic differentiation in allelic frequencies between seed trees and seed generation of European Beech are relatively similar to the results reported by Wang (2004, *Fst* = 0.4%), Ziehe et al. (1998, *Fst* = 2%) and Hattemer et al. (1993, 0.4%). Genetic differences between adult trees and seeds samples may be explained primarily as fertility selection and different degrees of self-fertilization (Ziehe et al., 1998).

Genetic relations between different groups of Beech genotypes (seeds versus adult trees) were tested using PCoA and UPGMA analysis. Scatter plots based on the SSR markers and combination of two data sets were developed in order to describe the relationships between the groups. We have noticed that seeds and adult tree groups of each population were placed in same clusters (fig. 2). The denderogram also showed same kind of clustering. The genetic relationship among the populations corresponds well to the geographical origin, in which populations Asalem and Kheirud classified in relatively different cluster than others. Similar results were reported for European Beech (Buiteveld et al. 2007) revealing that geographically different genotypes are quite different genetically as well. The eco-geographical separation of

the different Beech sample sets using SSRs can perhaps be explained by the polygenic inheritance of the adaptive traits to certain ecological conditions.

CONCLUSIONS

Increasing efforts are being made to rehabilitate degraded forests by Iranian forests services. Population genetic analysis in forest restoration practices with autochthonous species has important applied purposes. Among them are the preservation of genetic variability and reproductive potential, the evaluation of management strategies at the local level and the monitoring of contamination due to introduction of foreign material. Seed collection methods have considerable impact on the genetic quality of the seed. The gene-pool of the produced seeds should be fully represented in those harvested. Gene frequencies should not be unduly distorted, and the inbreeding depression should be at a minimum. It is clear that the genetic structure of the seeds is expected to have some similarity with the parent trees by which it is produced. However, it is certainly not identical because of external pollen flow, non-random mating and selection. The seeds collected in a stand do hardly have precisely the same genotypic structure as the parental adult trees (Hattemer et al. 1993). The theoretical conditions for genetic equilibrium in natural population are at best approximated.

According to our study, nuclear microsatellite markers are useful tools to investigate the genetic composition seed-lot harvests of Beech and to explore the processes linked to the harvest of genetic diversity from the natural populations. The same methodology could be applied to other autochthonous species used in ecosystem restoration. In comparison with adult generation, we have detected a slightly reduction in genetic diversity for the seed generations. This is probably an effect of an inappropriate seed strategy limited to a few trees (10 trees). In contrast to limited number of trees for seed harvesting, but suitable pattern of mother tree selection (at least 30 meters far from each other) the strong inbreeding was not detected. Further investigation would be carried out to verify this assumption and to discriminate the influences of different factors, and to check if genetic diversity increases because of more than one-year harvest. Janssen (2000) suggested that seed crop collections for conserving forest genetic resources and supplying forest enterprise with seed should be carried out in years of mast by considering as many as possible Beech trees that are distributed over the total plot.

The interest in assuring a broad genetic base to maintain the adaptive potential of forest systems supports the usefulness of the genetic data for planning specific sampling strategies and contamination control methods.

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