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Genetic comparison between natural and planted populations of *Pinus brutia* and *Cupressus sempervirens* in Syria

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Abstract: There is wide consensus that ongoing deforestation contributes to global warming and poses a threat to species diversity. Less understood is whether the practice of creating plantations might also erode genetic diversity and undermine the genetic structure of tree populations. We tested these hypotheses in natural and planted populations of *Pinus brutia* Ten. subsp. *brutia* and *Cupressus sempervirens* L. var. *horizontalis* (Mill.), 2 important forestry species in the Mediterranean region. We used plant material from 3 different bioclimatic regions in Syria. Using RAPD markers, we evaluated the genetic diversity and structure of 12 populations of *P. brutia* (6 natural, 6 planted) and 9 populations of *C. sempervirens* (3 natural, 6 planted). Expected heterozygosity (H_e) and percentage of polymorphic loci (*PPL*) were high in both species (*P. brutia*: H_e = 0.241, *PPL* = 81.2%; *C. sempervirens*: H_e = 0.241, *PPL* = 78.8%). In accordance with our assumptions, plantations of *P. brutia* manifested significant reduction in mean genetic diversity; this result, however, was not revealed in *C. sempervirens*. Analysis of molecular variance (AMOVA) demonstrated that the genetic structure of plantations differed from that of natural populations. Interestingly, plantations of both species harbored more genetic differentiation among them than natural populations. The partitions created by AMOVA also showed a significant differentiation between 2 groups, natural populations versus plantations in the 2 species, and among bioclimatic regions only in *C. sempervirens*. This result was corroborated by cluster analyses, which indicated a closer relationship among populations from the same geographic region. Genetic distance was positively related to geographic distance only in natural populations of *P. brutia*. Plantations in our research showed a significant reduction in genetic diversity, particularly in *P. brutia*, and stronger among-population genetic differentiation compared to natural populations. We recommend that forest management incorporates genetic diversity and differentiation as an important criterion for selecting appropriate tree stock material.

Key words: Deforestation, genetic differentiation, genetic diversity, genetic structure, plantations, Syria

1. Introduction

Forest ecosystems are of high ecological and socioeconomic significance. They provide timber for humans, create habitats for animals, regulate water and climate regimes, and stabilize soil (Pearce, 2001; Pak et al., 2010). Despite their significance, forest cover is being reduced on an annual basis by about 1% in some countries (World Resource Institute, 1994). The worldwide forest area of the year 2000 reached a loss of about 3.2% by 2010 (FAO, 2012).

Whereas the large scale of deforestation of natural vegetation is often discussed with regard to its effect on global warming (Bonan, 2008) and species diversity (Pandit et al., 2007), the subsequent effect of domestication on the composition of genetic diversity of economically important tree species has received little attention. Genetic diversity is crucial because it allows species to adapt to local conditions and evolve in new environments (Qian et al., 2001; Müller-Schärer et al., 2004; Koskela et al., 2007). However, genetic diversity is being impacted by natural and human factors. Therefore, management actions to ensure continued availability and existence of genetic variation in forestry species populations are a globally imperative issue (White et al., 2007).

In addition to deforestation and habitat fragmentation (Lise et al., 2007), the subsequent practice of creating plantations may also pose a threat to the genetic diversity of forestry species (Lefèvre, 2004). Globally, planted forests make up about 7% of the world's total forest area (FAO, 2011a). In contrast to natural populations, planted tree populations are specifically selected for their favorable traits, a process that may result in an increase in genetic diversity in the following generations and an undermining of local adaptation in native populations

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(Ledig, 1992). Moreover, the associated activities that are considered throughout tree management practices may lead to some losses of genetic variation in stocks used for artificial plantation (Edwards and El-Kassaby, 1996; El-Kassaby, 2000). While a low number of genetic parents can increase inbreeding among progeny (White et al., 2007), high genetic diversity and low differentiation among planted populations can result from large numbers of seed trees effectively contributing to the planted progenies (Gauli et al., 2009). With changing climate, genetic depletion through plantations and changes in populations' genetic structure can have detrimental consequences for the adaptive potential of tree populations to novel environments (Chen et al., 2003; Pandey et al., 2004).

Syria has experienced large-scale deforestation as a result of uncontrolled grazing, illegal logging, clearing for cultivation, and, in particular, fire events, which have increased significantly over the last few years (Nahal and Zahoueh, 2005; FAO, 2010). Today, only a few natural forest fragments remain, and forests occupy approximately 2.4% of the country's area, 57.3% of which are plantations (FAO, 2011b). To counteract the detrimental effects of deforestation, Syria has carried out extensive programs of reforestation and afforestation (Ghazal, 2008): 5 and 5920 ha year^{-1} was re- or afforested respectively between 1998 and 2002, and between 2003 and 2007, 6.5 and 12,600 ha year^{-1} was re- or afforested (FAO, 2010). Most notably, most operations of afforestation occurred in areas of low annual precipitation (<350 mm). The driving motivation behind these projects was to expand timber resources, protect watersheds, control soil erosion and desertification, and conserve biodiversity (FAO, 2002). However, despite such large-scale re- and afforestation programs, no information exists on how the practice of creating plantations might influence the genetic diversity and population genetic structure of important forestry species in Syria.

Our study focuses on 2 economically important conifer species in the Mediterranean region: *Pinus brutia* Ten. subsp. *brutia* (Brutia pine) and *Cupressus sempervirens* L. var. *horizontalis* (Mill.) Loudon (common cypress). We conducted our study with the following questions in mind: 1) Are planted populations genetically less diverse in terms of expected heterozygosity (H_e) and percentage of polymorphic loci (*PPL*) than natural populations? 2) Do planted populations exhibit more genetic differentiation among them than natural populations?

2. Materials and methods

2.1. Study region and study species

Syria borders the eastern edge of the Mediterranean Sea. It is characterized mostly by a Mediterranean climate and has substantial interannual and seasonal fluctuations (FAO, 1996). Its diverse vegetation reflects the influence of different soil types, as well as climatic, geological, and topographical heterogeneity. From a biogeographic perspective, most vegetation types in Syria are Mediterranean and Irano-Turanian (Ghazal, 2008). The coastal areas (Eu-Mediterranean humid) have a mild climate, while the interior areas (Eu-Mediterranean semiarid and Irano-Turanian) are rather continental, with cold winters where temperatures drop to below 0 °C and hot summers of above 40 °C (Ghazal, 2008).

We included samples from 3 different bioclimatic regions in our study (Table 1; Figure 1): 1) the Eu-Mediterranean humid region (Latakia, coastal area of western Syria); 2) the Eu-Mediterranean semiarid region (Aleppo governorate, Aleppo Mountain, also known as "Kurds Mountain", and the Manbij District, in northwestern and northern Syria, respectively); and 3) the Irano-Turanian region (Deir Ezzor city and Raqqa, both of which lie on the banks of the Euphrates River in eastern and north central Syria, respectively).

Pinus brutia and *C. sempervirens* are diploid monoecious tree species (White et al., 2007). Brutia pine flowers and pollinates between March and May (Boydak, 2004). Its seeds are winged and are dispersed by wind throughout the year, with maximum dispersal occurring in August (Ledig, 1998; Boydak, 2004). For common cypress, the first flowers appear after 3–4 years and the reproductive cycle develops over a period of 3 years with subsequent buds appearing from late spring to early summer (Valgimigli et al., 2005). Natural *P. brutia* forests in the Middle East region are classified into 3 vegetation types based on their bioclimatic stage: humid, subhumid, and semiarid (Nahal, 1983; Quézel, 1985). Brutia pine forests are regarded as one of the most picturesque of East Mediterranean coniferous forests (Zohary, 1973). *Cupressus sempervirens* L. var. *horizontalis* is considered to be the most common variety of *C. sempervirens* in cultivation (Tutin et al., 1964).

In Syria, Brutia pine forests grow naturally in a wide area in the western region, from sea level in the Bassit area up to 1100 m in the western slopes of the coastal mountains. In the Eu-Mediterranean humid stage, they are distributed as pure stands at 100–450 m a.s.l. in the Baer-Bassit Mountains of Latakia, while the natural forests of the Eu-Mediterranean semiarid region are found on Aleppo Mountain of northwestern Syria, at 200–900 m a.s.l. (Ghazal, 2008). The natural distribution of *C. sempervirens* in Syria is scattered and restricted to some locations in the coastal mountains, where there are almost-pure stands of this species, while in other locations it occurs in combination with *P. brutia* (Ghazal, 2008). In addition to being important for afforestation and as ornamental plantations across the Mediterranean region, the 2 studied species have also been artificially introduced

Table 1. Environmental variables and genetic diversity in *Pinus brutia* and *Cupressus sempervirens* populations.

Population	Population status	Bioclimatic region	Geographic data			Genetic diversity	
			Lat.	Long.	Alt.	H_e	*PPL*
Pinus brutia							
Latakia 1	Natural	1	35.776	35.902	143	0.253	80.6
Latakia 2	Natural	1	35.810	35.921	271	0.244	82.9
Latakia 3	Natural	1	35.815	35.972	425	0.266	85.2
Aleppo Mt 1	Natural	2	36.634	37.037	752	0.225	84
Aleppo Mt 2	Natural	2	36.493	36.678	560	0.246	82.9
Aleppo Mt 3	Natural	2	36.594	36.948	650	0.255	86.3
Mean						**0.248**	**83.7**
Aleppo 1	Planted	2	36.426	38.009	540	0.204	73.8
Aleppo 2	Panted	2	36.440	37.809	535	0.208	73.8
Aleppo 3	Planted	2	36.399	37.896	533	0.260	84
Deir Ezzor 1	Planted	3	35.406	40.059	238	0.226	80.6
Deir Ezzor 2	Planted	3	35.354	40.149	205	0.237	78.2
Raqqa	Planted	3	35.875	39.584	260	0.266	81.8
Mean						**0.234**	**78.7**
Overall mean						**0.241**	**81.2**
Cupressus sempervirens							
Latakia 1	Natural	1	35.887	35.898	130	0.219	69.8
Latakia 2	Natural	1	35.919	35.916	450	0.247	78.4
Latakia 3	Natural	1	35.823	35.962	539	0.265	85.3
Mean						**0.244**	**77.8**
Aleppo 1	Planted	2	36.426	38.009	540	0.239	81.9
Aleppo 2	Planted	2	36.440	37.809	535	0.229	75.8
Aleppo 3	Planted	2	36.508	37.962	474	0.240	80.1
Deir Ezzor 1	Planted	3	35.406	40.059	238	0.238	79.3
Deir Ezzor 2	Planted	3	35.347	40.152	205	0.242	79.3
Deir Ezzor 3	Planted	3	35.335	40.118	221	0.255	79.3
Mean						**0.241**	**79.3**
Overall mean						**0.241**	**78.8**

Abbreviations used: Bioclimatic region, 1 = Eu-Mediterranean humid, 2 = Eu-Mediterranean semiarid, 3 = Irano-Turanian; Geographical: Lat. = latitude (decimal degrees), Long. = longitude (decimal degrees), Alt. = altitude (m).

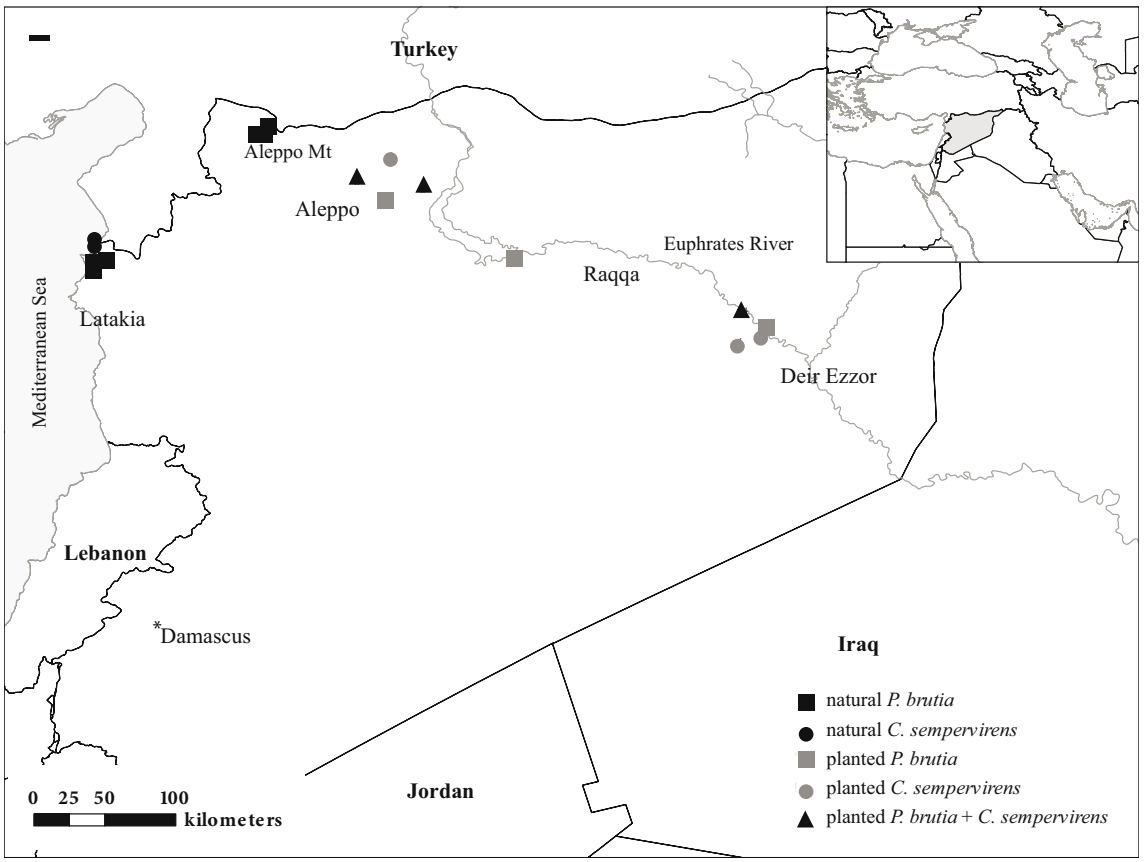

Figure 1. Locations of sampled populations of *Pinus brutia* and *Cupressus sempervirens* in Syria.

into climatically similar areas (Korol et al., 1997; Isik et al., 1999; Santini and Di Lonardo, 2000; Bagnoli et al., 2009). Widespread and landscape-forming, *P. brutia* and *C. sempervirens* are common native species in Syria that are also planted in afforestations far away from their natural range in different bioclimatic regions throughout the country.

2.2. Sampling of material

We sampled material from 12 populations of *P. brutia* (6 natural, 6 planted populations) and 9 populations of *C. sempervirens* (3 natural, 6 planted populations) in April and May of 2009 and 2010 (20 individuals per population), but for logistical reasons, only 12 individuals from each population were used in the experiment. The plantations in this study were afforested in areas out of the natural distribution range of the 2 species. Leaf material from a total of 144 *P. brutia* and 108 *C. sempervirens* individuals was sampled. Natural *P. brutia* populations were sampled from Latakia and Aleppo Mountain, while natural *C. sempervirens* populations were studied from Latakia. Material from planted populations of the 2 species was collected from the semiarid region of Aleppo (Manbij District) and the arid regions of Deir Ezzor and Raqqa. Distances between populations ranged from 3 to 426 km. The studied planted populations had been established in the 1980s. In Aleppo, seeds for plantations were originally obtained from plantations in Afrin, 40 km northwest of Aleppo, with rainfall of 450 mm year^{-1} (36°30′36″N, 36°52′4″E; Directorate of Forestry in Manbij District, Aleppo, Al-Khalaf, personal communication). The seed source for Deir Ezzor originated from plantations in Al-Hasaka, northeastern Syria, with rainfall of 250–300 mm year^{-1} (36°29′0″N, 40°45′0vE; Directorate of Forestry in Deir Ezzor, Al-Saleh, personal communication). We could not obtain any data on the genetic diversity of the aforementioned seed sources. All juvenile planted trees were subjected to a selection process in a forestry nursery, where weak individuals were discarded from the tree stock.

2.3. DNA extraction and RAPD-PCR analysis

We used random amplified polymorphic DNA (RAPD) markers in our study, which are the most widely used molecular marker type for forest trees (White et al., 2007). This marker system is easy to apply, as no prior DNA sequence information is needed for designing the polymerase chain reaction (PCR) primers (Williams et al., 1990). RAPD-PCR is a quick and reliable marker

method for producing data across the entire genome while requiring little plant material (Williams et al., 1990; Fritsch and Rieseberg, 1996). RAPD markers have been widely used to determine the genetic variation in natural or planted populations and also to compare natural populations with plantations (e.g., Li et al., 1999; Chen et al., 2003; Jeandroz et al., 2004; Kandemir et al., 2004; Li et al., 2005; İçgen et al., 2006; Lise et al., 2007).

Individual genomic DNA was extracted from 20 mg of silica gel-dried leaf tissue following the protocol described by QIAGEN (2000; DNeasy Plant Mini Kit). We screened 40 primers (Roth, Karlsruhe, Germany; sets A and D) using 3 samples each of the 2 species. We inspected the resulting amplification profiles for polymorphism, clarity, and reproducibility. A total of 4 and 5 primers were able to detect polymorphism among the samples of *P. brutia* and *C. sempervirens*, respectively: A07: GAAACGGGTG; A11: CAATCGCCGT; A15: TTCCGAACCC; D1: ACCGCGAAGG for *P. brutia*; and A04: AATCGGGCTG; A08: GTGACGTAGG; A09: GGGTAACGCC; A10: GTGATCGCAG; A17: GACCGCTTGT for *C. sempervirens*.

We carried out DNA amplifications in reaction volumes of 10 µL that contained 0.8 µL of DNA (10 ng μL^{-1}), 0.6 µL of the selected primer (10 pmol μL^{-1}; Roth), 1 µL of each dNTP (Peqlab, Erlangen, Germany), 1 µL of 10X buffer (Qbiogene, Illkirch, France), 0.1 µL of Taq Polymerase (5 U μL^{-1}, Qbiogene), and 6.5 µL of H_2O. PCR was carried out in an Eppendorf Mastercycler gradient that was programmed for the following temperature profile: preliminary denaturation of DNA at 94 °C for 2 min, followed by 36 cycles for 12 s at 94 °C, 45 s at 36 °C, and 120 s at 72 °C, with a final step of 7 min at 72 °C. The amplification products were separated by electrophoresis in 2% agarose gel with a Tris-acetate-EDTA buffer system at 50 V for 2 h and stained with ethidium bromide. DNA bands were envisaged by ultraviolet light and then documented using a video camera. Gel pictures were analyzed using the software RFLPSCAN PLUS Version 3.0 (Scanalytics, Rockville, MD, USA). Clear bands were used for the statistical analysis.

2.4. Data analysis

RAPD band patterns were scored as present (1) or absent (0) at each locus and entered into a matrix. In order to compare genetic diversity between natural and planted populations, we calculated expected H_e and *PPL*. Differences between estimated H_e and *PPL* values in natural and planted populations were tested with a t-test. We also performed an analysis of molecular variance for each species (AMOVA; Excoffier et al., 1992) to partition the genetic variation at different hierarchical levels. In a second AMOVA run, we split the data set into 2 groups, 1 made up of natural and 1 made up of planted populations. In addition, separate AMOVA runs were run for natural and planted populations. Finally, a run was carried out to test whether bioclimatic regions account for any partitioning in genetic variation. All mentioned analyses were performed in GenAlEx 6 (Peakall and Smouse, 2006).

To analyze and visualize the patterns of genetic distance among individuals, we calculated a principal coordinates analysis (PCoA). In order to detect any association between pairwise Φ_{st} genetic differentiation and geographic distance, we carried out a Mantel test (Mantel, 1967). We estimated gene flow among populations using the formula $Nm = 1/4\ (1/\Phi_{st} - 1$; Wright, 1951). The PCoA, Mantel test, and t-tests were conducted in the program R (R Core Development Team, 2011, v. 2.13.0). An unweighted pair group method with arithmetic mean (UPGMA) dendrogram of the 2 species was constructed based on the matrix of Nei's (1978) genetic distance for each species using the program SPLITSTREE (Huson and Bryant, 2006).

3. Results

3.1. *Pinus brutia*

3.1.1. Genetic diversity

The 4 selected primers yielded bands with high reproducibility, polymorphism, and clear resolution, and they produced 88 discernible bands ranging from 240 to 2500 bp in size. The total number of scored bands per primer was A07 (21), A11 (31), A15 (16), and D1 (20). In general, expected heterozygosity (mean = 0.241, range: 0.204–0.266) and percentage of polymorphic loci (mean = 81.2%, range: 73.8–86.3%) per population were relatively high (Table 1). In accordance with our expectations, planted Brutia pine populations showed significantly lower genetic diversity than natural populations in terms of H_e (mean = 0.248 vs. 0.234, t-test, df = 11, t = 3.305, P = 0.007), and *PPL* (mean = 83.7% vs. 78.7%, t-test, df = 11, t = 2.471, P = 0.031). Only one private band each was detected in a natural (Latakia 2) and planted (Deir Ezzor 1) population, respectively.

3.1.2. Genetic variance partitioning and differentiation

AMOVA conducted on all populations showed that the vast majority of genetic variation was found within populations (95%) and, to a much lesser extent, among populations (5%, see Table 2, Analysis 1). When the dataset was grouped into natural versus planted populations and into bioclimatic regions, most genetic variation was again found within populations (Table 2, Analyses 2 and 3). Similarly, when inspecting only natural populations, most molecular variance was found within populations (99%, Analysis 4). By contrast, planted populations in semiarid and arid regions retained only 91% of molecular variance within populations (Table 2, Analysis 5). AMOVA revealed a significant differentiation between natural populations

Table 2. Analysis of molecular variance (AMOVA) for *Pinus brutia*, performed for different sets of groups.

Source of variation	df	SS	Variance	Variance (%)	Φ statistics	P
Analysis 1: without groups						
Among populations	11	237.1	0.7	5%	$\Phi_{st} = 0.046$	0.001
Within populations	132	1804.1	13.7	95%		
Total	143	2041.2	14.3	100%		
Analysis 2: according to 2 groups (natural and planted)						
Among groups	1	28.1	0.1	1%	$\Phi_{ct} = 0.007$	0.011
Among populations	10	209.0	0.6	4%	$\Phi_{sc} = 0.042$	0.001
Within populations	132	1804.1	13.7	95%		
Total	143	2041.2	14.4	100%	$\Phi_{st} = 0.049$	0.001
Analysis 3: according to 3 bioclimatic regions (humid, semiarid, and arid)						
Among regions	2	48.8	0.1	1%	$\Phi_{ct} = 0.005$	0.052
Among populations	9	188.4	0.6	4%	$\Phi_{sc} = 0.042$	0.001
Within populations	132	1804.1	13.7	95%		
Total	143	2041.2	14.3	100%	$\Phi_{st} = 0.048$	0.001
Analysis 4: according to natural populations from humid and semiarid regions						
Among regions	1	20.8	0.2	1%	$\Phi_{ct} = 0.013$	0.012
Among populations	4	55.6	0.0	0%	$\Phi_{sc} = -0.001$	0.528
Within populations	66	931.8	14.1	99%		
Total	71	1008.2	14.3	100%	$\Phi_{st} = 0.012$	0.051
Analysis 5: according to planted populations from semiarid and arid regions						
Among regions	1	19.3	0.0	0%	$\Phi_{ct} = 0.018$	0.999
Among populations	4	133.3	1.2	9%	$\Phi_{sc} = 0.087$	0.001
Within populations	66	872.3	13.2	91%		
Total	71	1004.9	14.4	100%	$\Phi_{st} = 0.071$	0.001
Analysis 6: according to 2 groups (natural and planted) from semiarid region						
Among groups	1	19.4	0.0	0%	$\Phi_{ct} = 0.000$	0.661
Among populations	4	82.4	0.6	4%	$\Phi_{sc} = 0.043$	0.001
Within populations	66	882.3	13.4	96%		
Total	71	984. 1	14.0	100%	$\Phi_{st} = 0.041$	0.001

versus plantations as well as between the 2 regions to which the natural populations belong (Table 2, Analyses 2 and 4). We did not find any genetic differentiation among the bioclimatic regions studied or between the 2 groups, natural versus planted, from the same semiarid region (Table 2, Analyses 3 and 6).

In the PCoA, natural and planted populations were scattered across the ordination diagram, except for some samples from Latakia 1, 2, 3; Aleppo 1, 2, 3; and Deir Ezzor 1, 2, which formed weak clusters (Figure 2). Gene flow was estimated as $Nm = 4.9$.

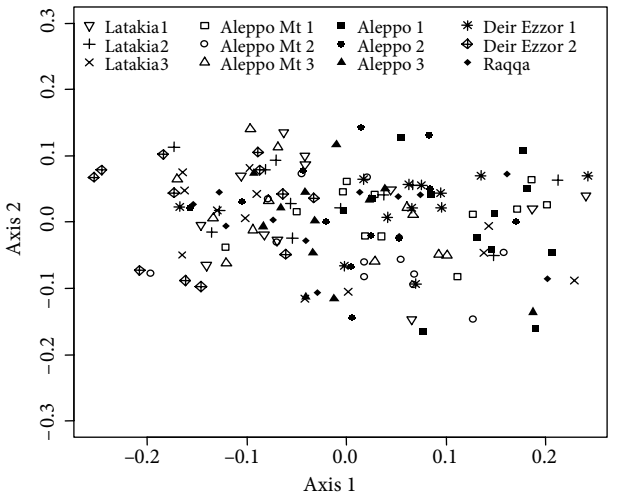

Figure 2. Principal coordinates analysis for Syrian *Pinus brutia* populations (explained variance: axis 1: 4.48%; axis 2: 3.33%).

The genetic distance ranged from 0.029 between the natural population Latakia 1 and the plantation of Raqqa to 0.129 between the planted populations Aleppo 1 and Deir Ezzor 2 (Table 3). The UPGMA dendrogram based on Nei's genetic distance (1978) did not clearly differentiate between natural and planted populations and did not show a clear geographical pattern (Figure 3). The Mantel test revealed no significant correlation between genetic and geographic distance among the overall

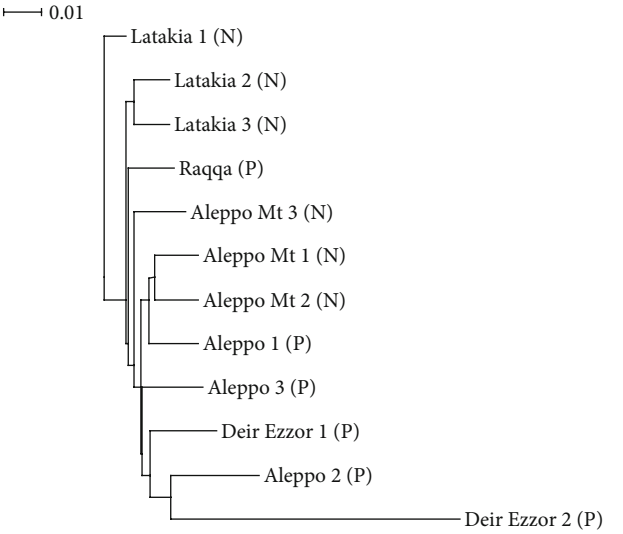

Figure 3. UPGMA dendrogram based on Nei's (1987) genetic distance among natural (N) and planted (P) populations of *Pinus brutia*.

Table 3. Matrix between Nei's genetic distance (pairwise Φ_{st} value; lower left triangle) and geographic distance (km; upper right triangle) among *Pinus brutia* populations.

	Latakia 1	Latakia 2	Latakia 3	Aleppo Mt 1	Aleppo Mt 2	Aleppo Mt 3	Aleppo 1	Aleppo 2	Aleppo 3	Deir Ezzor 1	Deir Ezzor 2	Raqqa
Latakia 1	0	4	8	143	139	134	216	173	212	417	423	313
Latakia 2	0.038	0	5	139	142	136	213	170	209	415	421	311
Latakia 3	0.038	0.030	0	134	138	132	208	165	204	410	416	305
Aleppo Mt 1	0.045	0.043	0.046	0	3	3	104	64	91	330	337	209
Aleppo Mt 2	0.038	0.047	0.052	0.031	0	6	103	64	89	329	336	208
Aleppo Mt 3	0.042	0.046	0.041	0.043	0.048	0	106	66	93	332	339	211
Aleppo 1	0.050	0.054	0.070	0.037	0.037	0.065	0	45	19	227	234	106
Aleppo 2	0.059	0.062	0.065	0.068	0.076	0.077	0.080	0	40	268	274	149
Aleppo 3	0.050	0.047	0.053	0.038	0.042	0.047	0.052	0.075	0	242	249	119
Deir Ezzor 1	0.045	0.059	0.060	0.037	0.052	0.059	0.045	0.048	0.053	0	7	125
Deir Ezzor 2	0.091	0.111	0.089	0.109	0.101	0.108	0.129	0.072	0.089	0.100	0	131
Raqqa	0.029	0.043	0.034	0.049	0.055	0.042	0.067	0.062	0.051	0.049	0.105	0

Brutia pine populations (r = 0.233, P = 0.072). Genetic distance was significantly associated with geographic distance in natural populations (r = 0.436, P = 0.031), but not in planted populations (r = –0.51, P = 0.565). In the Mediterranean semiarid region, genetic distance between natural and planted populations showed no association with geographic distance (r = 0.136, P = 0.179).

3.2. *Cupressus sempervirens*

3.2.1. Genetic diversity

The 5 selected primers produced band patterns with high reproducibility, polymorphism, and clarity. A total of 119 bands were observed; however, only 116 bands in the range between 250 and 2600 bp were scored. The number of scored bands per primer was A4 (19), A8 (18), A9 (21), A10 (29), and A17 (29). Gene diversity H_e was also high in *C. sempervirens* (mean = 0.241, range = 0.219–0.265), as was *PPL* (mean = 78.8%, range: 73.8–85.3%, Table 1). Contrary to our assumption, genetic diversity in *C. sempervirens* did not differ significantly between natural and planted populations (mean H_e = 0.244 vs. 0.241, t-test, df = 8, t = 1.501, P = 0.171; mean *PPL* = 77.8% vs. 79.3%, df = 8, t = 1.631, P = 0.141). There were no private bands except for 2, 1, and 3 private bands in Latakia 1, Latakia 2, and Latakia 3, respectively.

3.2.2. Genetic variance partitioning and differentiation

AMOVA showed that 97% of the genetic variation was found within populations, and only 3% among them (Table 4, Analysis 1). When the dataset was categorized according to natural versus planted populations and bioclimatic regions, all or the vast majority of molecular variance

Table 4. Analysis of molecular variance (AMOVA) for *Cupressus sempervirens*, performed for different sets of groups.

Source of variation	df	SS	Variance	Variance (%)	Φ statistics	P
Analysis 1: without groups						
Among populations	8	22.7	0.5	3%	Φ_{st} = 0.029	0.001
Within populations	99	16.6	16.6	97%		
Total	107	39.3	17.1	100%		
Analysis 2: according to 2 groups (natural and planted)						
Among groups	1	36.5	0.3	2%	Φ_{ct} = 0.019	0.001
Among populations	7	144.8	0.4	2%	Φ_{sc} = 0.020	0.001
Within populations	99	1646.0	16.6	96%		
Total	107	1827.3	17.3	100%	Φ_{st} = 0.039	0.001
Analysis 3: according to 3 bioclimatic regions (humid, semiarid, and arid)						
Among regions	2	63.8	0.3	2%	Φ_{ct} = 0.020	0.001
Among populations	6	117.4	0.2	1%	Φ_{sc} = 0.015	0.013
Within populations	99	1646.0	16.6	97%		
Total	107	1827.3	17.2	100%	Φ_{st} = 0.034	0.001
Analysis 4: according to natural populations from humid region						
Among populations	2	32.2	0.0	0%	Φ_{st} = 0.000	0.582
Within populations	33	550.5	16.7	100%		
Total	35	582.7	16.6	100%		
Analysis 5: according to planted populations from semiarid and arid regions						
Among regions	1	27.3	0.1	1%	Φ_{ct} = 0.010	0.044
Among populations	4	85.2	0.3	2%	Φ_{sc} = 0.023	0.004
Within populations	66	1095.5	16.5	97%		
Total	71	1208.1	17.1	100%	Φ_{st} = 0.033	0.001

occurred again within populations (Table 4, Analyses 2 and 3). When only natural populations were inspected, 100% of the molecular variance was found within populations, with no among-population differentiation (Table 4, Analysis 4). However, in the planted populations of the semiarid and arid regions, genetic variation within populations was 97% (Table 4, Analysis 5). AMOVA showed a significant differentiation between natural versus planted populations among the 3 studied bioclimatic regions and among semiarid and arid regions of planted populations (Table 4, Analyses 2, 3, and 5). Gene flow among all populations of Syrian common cypress was estimated as $Nm = 7.1$.

Natural and planted populations of *C. sempervirens* were spread throughout the PCoA ordination diagram, with no pattern that could be attributed to populations. The only exceptions were some samples of Latakia 1, 2, 3; Aleppo 1, 3; and Deir Ezzor 2, 3, which clustered in the vicinity of particular values (0.0 to 0.3 for Axis 1; –0.1 to 0.3 for Axis 2) (Figure 4).

Genetic distance ranged from 0.024 between the natural populations Latakia 2 and Latakia 3 to 0.068 between the natural population Latakia 2 and the plantation of Aleppo 2 (Table 5). In the UPGMA dendrogram, natural populations formed a cluster separated from plantations, and a clear geographic pattern was observed where Latakia (Eu-Mediterranean humid), Aleppo (Eu-Mediterranean semiarid), and Deir Ezzor (Irano-Turanian) formed separate clusters (Figure 5). A Mantel test carried out on all cypress populations showed no correlation between genetic and geographic distance (r = 0.023, P = 0.372). In natural and planted populations, genetic distance was not associated with geographic distance (natural populations: r = –0.168, P = 0.541; planted populations: r = 0.319, P = 0.123).

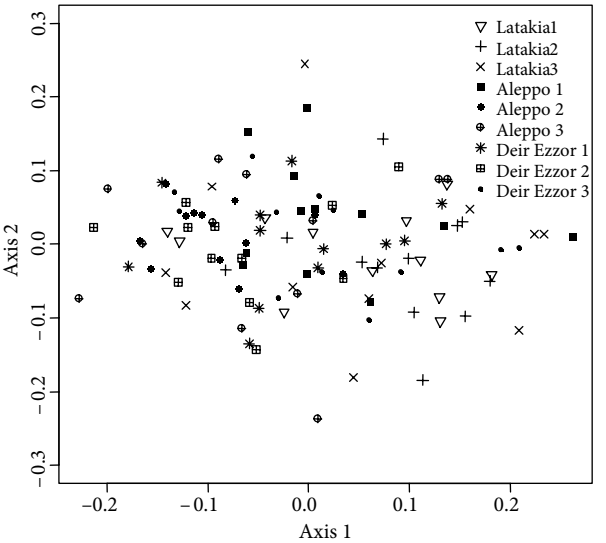

Figure 4. Principal coordinates analysis for Syrian *Cupressus sempervirens* populations (explained variance: axis 1: 5.52%; axis 2: 4.26%).

Table 5. Matrix between Nei's genetic distance (pairwise Φ_{st} value; lower left triangle) and geographic distance (km; upper right triangle) among *Cupressus sempervirens* populations.

	Latakia 1	Latakia 2	Latakia 3	Aleppo 1	Aleppo 2	Aleppo 3	Deir Ezzor 1	Deir Ezzor 2	Deir Ezzor 3
Latakia 1	0	4	9	214	170	173	419	425	426
Latakia 2	0.034	0	11	211	168	171	417	423	424
Latakia 3	0.037	0.024	0	209	165	169	411	417	418
Aleppo 1	0.053	0.039	0.046	0	45	43	227	234	231
Aleppo 2	0.064	0.068	0.060	0.053	0	5	268	274	272
Aleppo 3	0.044	0.051	0.056	0.054	0.041	0	268	274	272
Deir Ezzor 1	0.050	0.043	0.046	0.046	0.044	0.047	0	7	12
Deir Ezzor 2	0.036	0.046	0.037	0.050	0.037	0.036	0.031	0	13
Deir Ezzor 3	0.043	0.036	0.038	0.051	0.057	0.050	0.038	0.037	0

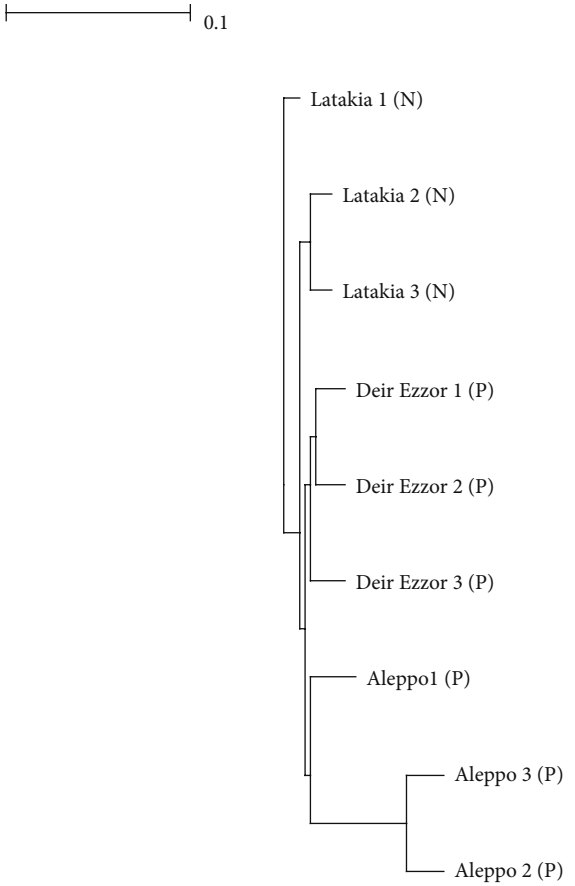

Figure 5. UPGMA dendrogram based on Nei's (1987) genetic distance among natural (N) and planted (P) populations of *Cupressus sempervirens*.

4. Discussion

4.1. Genetic diversity

Genetic diversity is an essential prerequisite for the long-term viability of economically important forestry species and can enable them to adapt to future environmental change (White et al., 2007). Overall, our study found high genetic diversity in *P. brutia* populations in Syria. These results were higher than those reported for *P. brutia* in an earlier RAPD study from Syria (H_e = 230, *PPL* = 57.6%; Choumane et al., 2004).

Genetic diversity in *C. sempervirens* populations was also high, which is in line with an earlier RAPD marker study (H_e = 0.286, *PPL* = 74%; Valgimigli et al., 2005). The high genetic diversity in both study species is not surprising. Conifer species are known to have high genetic diversity, which is usually explained by the fact that they are wind-pollinated (Changtragoon and Finkeldey, 1995; Ledig, 1998; Nybom and Bartish, 2000).

In accordance with our expectations, we detected significant differences in mean genetic diversity between natural and planted populations of *P. brutia*, where a significant loss of H_e and *PPL* was revealed in planted populations; this result, however, was not found in plantations of *C. sempervirens*. Our result is in accordance with that of Medri et al. (2003), who detected a reduction of RAPD polymorphism in managed populations in comparison to natural populations of *Araucaria angustifolia* (*PPL* = 72.5% vs. 82%, respectively). The loss of heterozygosity in Brutia pine plantations could be attributed to restricted gene flow among the distant planted populations in comparison to the natural populations (Dayanandan et al., 1999), and a potential inbreeding depression in the small and isolated plantations (Aldrich et al., 1998; Lowe et al., 2005). Moreover, this finding is reasonable given that seeds for planted populations originated from plantations and went through a selection process in the nursery (White et al., 2007), and it suggests that phenotypic selection throughout the consecutive stages of plantation establishment did not capture the same existing level of genetic diversity in natural populations of Brutia pine. Likewise, differences in genetic diversity between natural and planted populations were found in *Cupressus macrocarpa* (Kafton, 1977), *Picea abies* (Gömöry, 1992), Mediterranean cypress populations (Papageorgiou et al., 1994), *Picea glauca* × *engelmanni* (Stoehr and El-Kassaby 1997), and *Pinus contorta* (Thomas et al., 1999).

Our finding of no significant difference in genetic diversity between natural and planted populations in *C. sempervirens* is especially surprising given the aforementioned characteristics (e.g., small, isolated, and distant populations) of our plantations. We suspect in the case of *C. sempervirens* that the number of planted population generations is still too low to significantly lower genetic diversity in these plantations (Stefenon et al., 2008); such a decline could, however, still be manifested in future generations (Lowe et al., 2005). Indeed, a study by El-Kassaby and Ritland (1996) found that genetic diversity decreased only in the second planted progeny generation of *Pseudotsuga menziesii*. Moreover, although planted populations in our study originated from plantations, their ultimate primary origin was natural populations. This fact might have contributed to the persistent maintenance of genetic diversity in the studied plantations of *C. sempervirens* (Gauli et al., 2009).

Similarly, no difference in genetic diversity was found between natural and managed populations of *Picea glauca* and *Pinus banksiana* (Godt et al., 2001), *Picea abies* (Jeandroz et al., 2004), *Cupressus sempervirens* (Papageorgiou et al., 2005), *Pinus sylvestris* (Kosinska et al., 2007), and *Pinus roxburghii* (Gauli et al., 2009). Surprisingly, some studies have found similar or even higher levels of genetic diversity in seed orchards or plantations than in natural populations (Bergmann and

Ruetz, 1991; Chaisurisri and El-Kassaby, 1994; El-Kassaby and Ritland, 1996; Wellman et al., 2003; İçgen et al., 2006; Stefenon et al., 2008), which potentially highlights the importance of seed source in cultivation.

4.2. Genetic variance partitioning and differentiation

In pine species, the proportion of total genetic variation among populations is often <5% (Karhu, 2001). More generally, highly outcrossing long-lived perennial species are known to harbor very little genetic variation at the among-population level (Hamrick and Godt, 1989; Hamrick et al., 1993; Nybom, 2004). Among-population genetic variation has been found to be low for other conifer species (Yeh, 1988; Lagercrantz and Ryman, 1990; Semaan and Dodd, 2008; Gauli et al., 2009; Matsumoto et al., 2010). Our study has also shown that natural populations in particular had almost no among-population genetic variation (Hamrick and Godt, 1996; White et al., 2007).

However, the genetic structure of our plantations differed from natural populations, particularly in *P. brutia*. As a whole, genetic differentiation was more pronounced among planted than among natural Brutia pine populations, albeit by a small absolute difference (Φ_{st} = 0.071 vs. 0.012). In common cypress, planted populations also showed more genetic differentiation among them (Φ_{st} = 0.033 vs. 0). The very low or even absent genetic differentiation among natural populations indicates an efficient gene flow via pollen among populations, resulting in homogenization in the genetic structure of nearby populations (Díaz et al., 2001; Jeandroz et al., 2004).

The planted populations exhibited a stronger genetic differentiation among them than natural populations. Given that some RAPD markers might be affected by selection if they are of close linkage to loci that experience different selection pressure (Latta and Mitton, 1997), genetic differentiation can increase when the selection is strong (Le Corre and Kremer, 2003). We suspect that such a pattern of among-plantation genetic differentiation was created because unsuitable seedlings were discarded during the silvicultural selection process (Edwards and El-Kassaby, 1996; Korshikov et al., 2004; Stefenon et al., 2008). The likely fact that seeds originated from only a few populations, and that planted progenies were derived from a low number of seed trees, could also have led to a stronger genetic differentiation among plantations (El-Kassaby and Namkoong, 1994; Gauli et al., 2009). Furthermore, genetic differentiation could also reflect different seed source origins. The result that the 2 groups of natural and planted populations of *P. brutia* in the semiarid region harbored no genetic differentiation between them suggests that plantations maintained genetic information very similar to that of natural populations from the same geographic region (Stefenon et al., 2007; Stefenon et al., 2008; Gauli et al., 2009). In *P. brutia*, cluster analyses showed no clear geographic pattern; this finding was also demonstrated in previous studies on *P. brutia* (Kandemir et al., 2004; Lise et al., 2007). The cluster analyses revealed a clear relationship between populations and their geographic regions in *C. sempervirens*; a similar result was detected in a study by Valgimigli et al. (2005). This result is in accordance with the AMOVA analyses and suggests that populations within regions are more similar to each other than populations from different regions.

In our study, genetic distance among *P. brutia* populations was significantly associated with geographic distance only when the natural populations were analyzed (Díaz et al., 2001), not when the plantations were examined. Thus, for natural populations, genetic differentiation is most likely the result of past natural gene flow via pollen dispersal, which creates variations in genetic distance (Nybom and Bartish, 2000). Genetic and geographic distances were not correlated in either natural or planted *C. sempervirens* populations, a finding that was similarly observed in natural populations and plantations of *Pinus roxburghii* (Gauli et al., 2009). Such a result may be explained by 2 factors. First, it is possible that the geographic range of *C. sempervirens* was small enough in the past that distant populations were easily connected. Second, it is possible that seeds for plantations were collected from nearby sites.

In conclusion, this study indicates that RAPD-based markers are useful for assessing the changes in genetic diversity of natural and planted populations of *P. brutia* and *C. sempervirens*. Genetic diversity in natural and planted populations of our 2 species was found to be generally high. Our study showed that planted populations of *P. brutia*, in particular, exhibited a significant reduction in genetic diversity; this finding evidenced that the selection process during the ultimate domestication stages failed to capture the same level of genetic diversity that existed in natural populations of *P. brutia*. The genetic diversity in *C. sempervirens* was delivered to the plantations. We suspect that the low number of generations in planted populations and high gene flow among natural source populations prevented a significant decline of genetic diversity in *C. sempervirens* plantations. Plantations of both species maintained stronger among-population genetic differentiation compared to natural populations. The stronger genetic differentiation was probably due to the fact that the silvicultural selection process was performed only in a few populations. Thus, only a low number of seed trees contributed to planted progenies. An ongoing

silvicultural practice of using a few mother trees and selecting for the fittest progeny might influence these observed patterns in the next progeny generations and generate more distinct genetic differentiations among planted populations. Consequently, in order to maintain high levels of genetic diversity among afforested plantations, it is crucial to collect seeds from multiple and diverse populations and to forego severe selection procedures.

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