- MIURA, M., M. KUBOTA, S. KURINOBU and A. WATANABE (2006): Clone identification of Hinoki cypress plus-tree using SSR markers in Kanto breeding region. *Kanto J For Res* **57**: 135–136.
- MIURA, M. and A. WATANABE (2007): Clone identification of Hinoki cypress plus-tree using 7 SSR markers and detection of contaminated clones in a breeding orchard. *Kanto J For Res* **58**: 245–246.
- MORIGUCHI, Y., N. TANI, S. ITOO, F. KANEHIRA, K. TANAKA, H. YOMOGIDA, H. TAIRA and Y. TSUMURA (2005): Gene flow and mating system in five *Cryptomeria japonica* D. Don seed orchards as revealed by analysis of microsatellite markers. *Tree Genetics and Genomes* **1**: 174–183.
- NAKAO, Y., H. IWATA, A. MATSUMOTO, Y. TSUMURA and N. TOMARU (2001): Highly polymorphic microsatellite markers in hinoki (*Chamaecyparis obtusa*). *Can J For Res* **31**: 2248–2251.
- RAYMOND, M. and F. ROUSSET (1995): GENEPOP (version 1.2): population genetics software for exact tests and ecumenicism. *J Hered* **86**: 248–249.
- ROZEN, S. and H. J. SKALETSKY (2000): Primer3 on the WWW for general users and for biologist programmers, pp. 365–386. *In*: Bioinformatics Methods and Protocols: Methods in Molecular Biology, edited by S. KRAWETZ and S. MISNER. Humana Press, Totowa/NJ.
- SCHUELKE, M. (2000): An economic method for the fluorescent labeling of PCR fragments. *Nature Biotechnology* **18**: 233–234.
- SHIRAIISHI, S., H. KAMINAKA and N. OHYAMA (1987): Genetic variation and differentiation recognized at two allozyme loci in Hinoki (*Chamaecyparis obtusa*). *J Jpn For Soc* **69**: 88–93 (in Japanese with English summary).
- SMOUSE, P. E., R. PEAKALL and E. GONZALES (2008): A heterogeneity test for fine-scale genetic structure. *Mol Ecol* **17**: 3389–3400.
- TAKAHASHI, T., N. TANI, H. TAIRA and Y. TSUMURA (2005): Microsatellite markers reveal high allelic variation in natural populations of *Cryptomeria japonica* near refugial areas of the last glacial period. *J Plant Res* **118**: 83–90.
- TSUMURA, Y., A. MATSUMOTO, N. TANI, T. UJINO-IHARA, T. KADO, H. IWATA and K. UCHIDA (2007): Genetic diversity and the genetic structure of natural populations of *Chamaecyparis obtusa*: implications for management and conservation. *Heredity* **99**: 161–172.
- UCHIDA, K., N. TOMARU, T. TOMARU, C. YAMAMOTO and K. OHBA (1997): Allozyme variation in natural populations of hinoki, *Chamaecyparis obtusa* and its comparison with hinoki plus-trees selected from artificial stands. *Breed Sci* **47**: 7–14.

Short Note: Cross-Species Amplification and Characterization of *Pinus* Chloroplast Microsatellite Markers in *Cedrus deodara* Roxb.

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(Received 18th December 2009)

Abstract

The study reports the transferability of chloroplast microsatellite markers developed for *Pinus* species to *Cedrus deodara*. A total of 49 primer pairs (both nuclear and chloroplast) of *Pinus* species were tested in *C. deodara* out of which 21 chloroplast primers showed positive amplification and 20 were found polymorphic. The primers were screened on 100 adult trees of two natural populations of *C. deodara*. Using twenty cpSSR primers, a total of 64 variants were found which combined in 70 different haplotypes. The total haplotype diversity in two populations was 0.860 and 0.876 with a mean of 0.868. These sets of markers can further be used for population genetic studies and characterization in

C. deodara for which no cpSSR markers have been reported till date.

Key words: Cross-amplification, cpSSRs, *Cedrus deodara*, microsatellite, genetic diversity.

Cedrus deodara (deodar), also known as Indian cedar is the most important among the Indian conifers. It is an important timber species of Western Himalayas at elevation ranging from 1200 m to 3050 m (TIWARI, 1994). It covers an area of about 0.2 million hectares in India mainly in the states of Jammu & Kashmir, Himachal Pradesh and Uttarakhand. The species is in great demand for its timber and had been subjected to exploitation for centuries. Natural deodar forests are under great pressure not only due to human impact but also due to the changing weather conditions. In spite of the importance of *C. deodara* forests, limited information is available on their basic biology and genetic variability.

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As a part of our studies on population genetic analysis of deodar forests of western Himalayas, we report here the results aimed at detecting and testing microsatellites in *C. deodara*. Microsatellites, or simple sequence repeats (SSRs), show many advantages since they are co-dominantly inherited and are generally highly polymorphic. Furthermore, their transferability

between closely related species makes them useful for the genetic studies of related species (WEISING *et al.*, 2005). Microsatellites could be of great utility not only in determining the levels of genetic diversity but also in unraveling the genetic structure of the populations and in estimating the levels of gene flow (RAO *et al.*, 2006).

Table 1. – Microsatellite (chloroplast) primer pair sequences, annealing temperature (T_m), product size and repeat motifs that showed positive amplification in *Cedrus deodara*.

S.No.	Locus	Sequence (5'-3')	Annealing temperature (°C)	Product size range (bp)	Repeat motif
1	Pt15169*	F: CTTGGATGGAATAGCAGCC	58.0	120-126	(C) ₈ (T) ₈ A(T) ₈
		R: GGAAGGGCATTAAAGTCATTA			
2	Pt26081*	F: CCCGTATCCAGATATACTTCCA	58.0	112-118	(T) ₁₄
		R: TGGTTTGATTTCATTCGTTTCAT			
3	Pt30204*	F: TCATAGCGGAAGATCCTCTTT	58.0	131-143	(A) ₁₂ (G) ₁₀
		R: CGGATTGATCCTAACCATAACC			
4	Pt36480*	F: TTTTGGCTTACAAAATAAAAGAGG	58.0	145-152	(T) ₁₁
		R: AAA'TTCTTAAAGAAGGAAGAGCA			
5	Pt71936*	F: TTCATTGGAAAATACACTAGCCC	58.0	148-149	(T) ₁₆
		R: AAAACCGTACATGAGAT'TCCC			
6	Pt109567*	F: TATTATCGAACACGAGAATAATCC	58.0	115-126	(T)11G(T)3
		R: TCACTGTCACTCTACAAAACCG			
7	Pt107148*	F: GTTCATTCCGGATCCTTAAAA	58.0	119-122	(T) ₁₀
		R: GTACTTTCCTTCAGCCAATCTG			
8	Pt102584*	F: TTCATGTAATTCCCAGATCCA	58.0	127-127	(T) ₁₁
		R: CATTATGTGCGCGATAATTTC			
9	Pt41093*	F: TCCCGAAAATACTAAAAAAGCA	58.0	76-82	(T) ₁₁
		R: CTCATTGTTGAACTCATCGAGA			
10	Pt45002*	F: AAGTTGGATTTTACCCAGGTG	58.0	160-163	(T) ₁₅
		R: GAACAAGAGGATTTT'TCTCATACA			
11	Pt51873*	F: AATCTTTCTACGGAACGGA	58.0	86-92	(T) ₁₀
		R: ATCA'TTTTGCTATGCAATGA			
12	Pt63718*	F: CACAAAAGGATTTT'TTTCAGTG	58.0	89-98	(T) ₉
		R: CGACGTGAGTAAGAATGGTTG			
13	Pt79951*	F: CTTTTGT'TTTCACAAATTGCA	58.0	141-149	(T) ₉
		R: ACATCTATCTCCCATATCGGC			
14	Pt87268*	F: GCCAGGGAAAATCGTAGG	58.0	165-178	(T)10C(T)5
		R: AGACGATTAGACATCCAACCC			
15	Pt110048*	F: TAAGGGGACTAGAGCAGGCTA	58.0	88-92	(T) ₉
		R: T'TCGATATTGAACCTTGGACA			
16	PCP1289^	F: TCCTGGTTCAGAAAATGGAG	58.5	113-117	(T) ₁₇
		R: TAATTGGTTCAGAAATGGCG			
17	PCP9434^	F: AAACGTACGTAGATGCCATGG	58.5	131-142	(A) ₁₀
		R: GCGGTATGAGGGAAGAAGC			
18	PCP26106^	F: AATCCGACAAAAAAGATTTCGG	58.5	131-149	(A) ₁₄
		R: GCTCCATTTACGTGGTTG			
19	PCP36567^	F: AAAAGAGGAGGAAAAACACCTT	58.5	115-118	(T) ₁₁
		R: AAGAGCAGACAAGTAAGGGGC			
20	PCP45071^	F: ACTGGTCTGATCGACCCAAT	58.5	147-152	(T) ₁₅
		R: TTCTACACTTGCAGAAACCC			
21	PCP100842^	F: TCAATACAAATGATGGGAGTCG	58.5	146-162	(A) ₁₂
		R: TTTTGCCATATCCTGAAACTCC			

Adopted from *VENDRAMIN et al. (1996), ^PROVAN et al. (1999)

Table 2. – Haplotype frequency (sorted by overall abundance) in two populations of *Cedrus deodara*. In the first column the allele size at each of the twenty cpSSR loci studied. In the last column overall frequency of haplotypes is reported.

Haplotype																				Haplotype label	Populations		All	
																					Jgeshwar	Champawat		
120	112	131	143	148	115	119	127	76	160	86	89	141	165	88	113	142	135	118	152	159	1	0.043	0.000	0.022
120	112	131	143	148	120	122	127	76	160	86	89	141	165	88	113	145	135	118	152	159	2	0.043	0.000	0.022
120	112	131	143	148	120	122	127	76	160	86	89	141	165	88	113	140	135	118	147	159	3	0.348	0.315	0.332
120	112	131	143	148	120	122	127	76	160	86	89	141	165	88	113	140	137	118	152	159	4	0.000	0.011	0.005
120	112	131	143	148	120	122	127	76	160	86	89	141	165	88	113	137	135	118	152	162	5	0.054	0.000	0.027
120	112	131	143	148	120	122	127	76	160	86	89	141	165	88	113	137	135	118	152	159	6	0.011	0.000	0.005
120	112	131	143	148	120	122	127	76	160	86	89	141	165	88	113	140	135	118	152	159	7	0.011	0.000	0.005
120	112	131	143	148	120	122	127	76	160	86	89	141	165	88	117	137	135	118	152	159	8	0.033	0.000	0.016
120	112	131	143	148	120	122	127	76	160	86	89	141	165	88	117	137	135	118	152	155	9	0.022	0.000	0.011
120	112	131	143	148	120	122	127	76	160	86	89	141	165	88	117	140	135	118	152	162	10	0.000	0.011	0.005
120	112	131	143	148	120	122	127	76	160	86	89	141	165	88	117	145	135	118	152	162	11	0.000	0.011	0.005
120	112	131	143	148	120	122	127	76	160	86	89	141	165	88	117	145	137	118	152	155	12	0.000	0.011	0.005
120	112	131	143	148	120	122	127	76	160	86	89	141	165	88	117	145	135	118	152	159	13	0.000	0.011	0.005
120	112	131	143	148	120	122	127	76	160	86	89	141	165	88	117	145	135	118	147	162	14	0.033	0.000	0.016
120	112	131	143	148	120	122	127	76	160	86	89	141	165	88	117	145	135	118	152	162	15	0.076	0.000	0.038
120	112	131	143	148	120	122	127	76	160	86	89	141	165	88	113	145	135	118	152	162	16	0.000	0.087	0.043
120	112	131	143	148	120	122	127	76	160	86	89	141	165	92	113	145	135	118	152	162	17	0.000	0.011	0.005
120	112	131	143	148	120	122	127	76	160	86	89	141	165	92	113	140	135	118	152	162	18	0.011	0.000	0.005
120	112	131	143	148	120	122	127	76	160	86	89	141	165	92	113	137	135	118	152	162	19	0.011	0.000	0.005
120	112	131	143	148	122	119	127	76	160	86	89	141	165	92	113	145	135	118	152	159	20	0.000	0.011	0.005
120	112	131	143	148	122	119	127	76	160	86	89	141	165	92	117	145	135	118	147	155	21	0.000	0.011	0.005
120	112	131	143	148	122	119	127	76	160	86	89	141	165	92	117	145	135	118	147	155	22	0.000	0.011	0.005
120	112	131	143	148	122	119	127	76	160	86	89	141	165	92	117	145	137	118	147	155	23	0.033	0.000	0.016
120	112	131	143	148	122	119	127	76	160	86	89	141	165	92	117	140	137	118	147	155	24	0.011	0.000	0.005
120	112	131	143	148	122	119	127	76	160	92	89	141	165	88	117	140	137	118	147	155	25	0.022	0.000	0.011
120	112	131	143	148	122	119	127	76	160	92	89	141	165	88	117	140	137	118	147	162	26	0.011	0.000	0.005
120	112	131	143	148	122	119	127	76	160	92	89	141	165	88	113	140	137	118	147	155	27	0.000	0.022	0.011
120	112	131	143	148	122	119	127	76	160	92	89	141	165	88	113	145	137	118	147	155	28	0.000	0.022	0.011
120	112	131	143	148	122	119	127	76	160	92	89	141	165	88	113	145	135	118	147	155	29	0.011	0.000	0.005
120	112	131	143	148	122	119	127	76	160	92	89	141	165	88	113	145	135	118	152	155	30	0.011	0.000	0.005
120	112	131	143	148	122	119	127	76	160	92	89	141	165	88	113	145	135	118	152	159	31	0.000	0.011	0.005
120	112	131	143	148	122	119	127	76	160	86	89	141	165	92	113	145	137	118	152	162	32	0.087	0.098	0.092
120	112	131	143	148	122	119	127	76	160	86	89	141	165	92	113	145	137	118	152	162	33	0.011	0.000	0.005
120	112	131	143	148	122	119	127	76	160	86	89	141	165	92	113	145	135	118	147	162	34	0.011	0.000	0.005
120	112	131	143	148	122	119	127	76	160	86	89	141	165	92	113	145	135	118	152	159	35	0.000	0.011	0.005
120	112	131	143	148	122	119	127	76	160	86	89	141	170	92	113	145	135	118	152	159	36	0.011	0.000	0.005
120	112	131	143	148	122	119	127	76	160	86	89	145	170	92	113	145	135	118	152	159	37	0.011	0.000	0.005
120	112	131	143	148	122	119	127	76	160	86	89	145	170	88	113	145	135	118	152	159	38	0.000	0.022	0.011
120	112	131	143	148	122	119	127	76	160	86	89	145	170	88	117	145	135	118	152	159	39	0.000	0.022	0.011
120	112	131	143	148	122	119	127	76	160	86	89	145	176	88	117	145	135	118	152	159	40	0.011	0.000	0.005
120	112	131	143	148	122	119	127	76	160	86	89	145	176	88	117	140	135	118	152	159	41	0.000	0.022	0.011
120	112	131	152	148	122	119	127	76	160	86	89	145	176	88	117	140	137	118	147	159	42	0.000	0.011	0.005
120	112	131	152	148	122	119	127	80	160	86	89	145	176	88	117	140	137	118	147	162	43	0.000	0.011	0.005
120	112	131	152	148	122	119	127	80	160	86	89	145	178	88	117	140	137	118	147	162	44	0.000	0.011	0.005
120	112	131	152	148	122	119	127	80	160	86	89	145	178	88	113	140	137	118	147	162	45	0.011	0.000	0.005
120	112	131	152	148	122	119	127	80	160	86	89	145	178	92	113	140	137	118	147	162	46	0.022	0.000	0.011
120	112	131	152	148	122	119	127	80	160	86	89	141	178	92	113	140	137	118	147	162	47	0.011	0.000	0.005
123	116	131	143	148	122	119	127	80	160	86	89	141	165	88	117	145	135	118	152	162	48	0.000	0.011	0.005
123	116	131	143	148	122	119	127	80	160	86	89	141	165	88	117	145	137	118	152	155	49	0.011	0.000	0.005
123	116	131	143	148	122	119	127	80	160	86	89	141	165	88	117	145	135	118	152	159	50	0.011	0.000	0.005
123	116	131	143	148	126	119	127	80	160	86	89	141	165	88	117	145								

The development of simple sequence repeats (SSRs) from genomic DNA is costly, labor-intensive and time-consuming process (CHABANE *et al.*, 2005). Moreover, SSR discovery from genomic libraries has proved problematic in conifers, with a low return for effort (RAJORA *et al.*, 2001; HODGETTS *et al.*, 2001). This is deemed to be because of the large size and repetitive nature of the conifer genome. Hence, microsatellite transfer across the species is a valued methodology. It has been shown that closely related species are more likely to share microsatellite-priming sites than more distantly related ones, but it is possible to transfer microsatellite primers even from distantly related species (LORIEUX *et al.*, 2000).

We examined the transferability of cpSSRs and nuclear SSRs from *C. atlantica*, *Pinus thunbergii*, *P. resinosa*, *P. taeda* and *P. sylvestris* to *C. deodara* and their characteristics in *C. deodara*. Earlier CHAIB *et al.* (2006), FADY *et al.* (2003), FADY *et al.* (2008) and TERRAB *et al.* (2006) demonstrated that pine cpSSRs Pt15169, Pt63718, Pt71936, Pt87268, Pt26081, Pt36480 and Pt110048 can be amplified in *Cedrus*. They were not tested in *C. deodara*, but this was a good indication that their transferability to *C. deodara* can be possible. Here we report the successful transfer of primer pairs designed for the amplification of microsatellite loci in different *Pinus* species to a member of the same family but separate genus, *C. deodara*. This is the first report wherein cpSSR marker sets have been reported for *C. deodara*, an important Himalayan conifer.

The individuals tested belonged to two natural populations viz. Champawat forest division (latitude 39°20'N, longitude 80°07'E, altitude 1992 m) & Jageshwar forest division (latitude 39°38'N, longitude 79°51'E, altitude 1667 m) of *C. deodara* from field collection in Uttarakhand. Samples of DNA from the needles were extracted following CTAB protocol (DOYLE and DOYLE, 1990). The extracted DNA was quantified and diluted to 30 ng/µl for further use in amplification reactions.

For amplification in *C. deodara* a total of 49 primer pairs (both nuclear and chloroplast) from *C. atlantica* (CHAIB *et al.*, 2006), *P. thunbergii* (VENDRAMIN *et al.*, 1996), *P. resinosa* (BOYS *et al.*, 2005), *P. taeda* (ZHOU *et al.*, 2002) and *P. sylvestris* (PROVAN *et al.*, 1999) were tested. Loci were initially screened for polymorphism by running PCR products for four individuals on polyacrylamide gel (8%) and stained with ethidium bromide (500 µg/Litre). PCR amplification was carried out at 95°C, 5 min for initial denaturation, followed by 30 cycles of denaturing at 94°C for 1 min, primers annealing for 1 min, extension at 72°C for 1 min and then the final extension/termination at 72°C for 8 min. The PCR reaction mixture contained 30 ng template DNA, 0.2 mM of each dNTPs (Bangalore Genei, Bangalore, India), 3.0 mM of MgCl₂, 0.2 µM of each of primer pair and 1 unit of *Taq* DNA Polymerase (Bangalore Genei, Bangalore, India) in a total volume of 15 µl. Amplification was considered positive when the primers yielded specific PCR products of expected size without multiple bands.

Out of the 49 primers tested, only 21 chloroplast primer pairs yielded amplification products of expected size in *C. deodara* and were selected for fluorescent labelling with 6-FAM and HEX at the forward primers. The labelled primers were further screened for polymorphisms using 100 samples collected from the two natural populations. For genotyping, the PCR products were electrophoresed along with GeneScan ROX 400 internal size standard on an ABI prism 3100 Genetic Analyser (Applied Biosystems). Allele sizes were assigned against the internal size standard and individuals were genotyped using GeneScan analysis software and Genotyper software version 3.7 (Applied Biosystems).

As chloroplast has haploid genomes that are paternally inherited in pines (NEALE and SEDEROFF, 1989; WATANO *et al.*, 1996), hence for the diversity measure, the chloroplast genome was regarded as a single nonrecombining locus and repeat length differences between individuals were scored as haplotypic variants over all cpSSR sites. The effective number of haplotypes for each population was calculated as $ne = 1/(\sum p_i^2)$, as well as the unbiased haplotype diversity as $He = [n/(n-1)](1 - \sum p_i^2)$, where p_i is the frequency of i -th haplotype and n is the number of individuals analysed (NEI, 1987; AVISE, 1994; VENDRAMIN *et al.*, 1998).

Screening for polymorphism indicated that except for one (Pt102584) the other twenty loci were polymorphic. The characteristics of cpSSRs in *C. deodara* are summarized in Table 1. The twenty cpSSR loci gave a total of 64 different variants among the 100 individuals of two populations surveyed. The 64 variants were combined into 70 different haplotypes, the frequency distribution of which is reported in Table 2. Only two haplotypes were common to the two populations. The primer Pt102584 though yielded a sharp amplification but was found monomorphic hence can be discarded for further studies. The total haplotype diversity (He) in two populations was 0.860 (Jageshwar) and 0.876 (Champawat) with a mean of 0.868, which is comparable to the haplotypic diversity reported in *Pinus pinaster* (VENDRAMIN *et al.*, 1998) and in Eastern North American *Abies* (CLARK *et al.*, 2000). CHAIB *et al.* (2006) tested the transferability of nSSRs developed in *C. atlantica* to *C. deodara* and reported four nSSR out of six to work in a single *C. deodara* sample. However, none of these nSSR worked in our 100 test samples representing two populations of *C. deodara*. QIAO *et al.* (2007) indicated that there are reasons to believe that transferability might be more difficult from *C. atlantica* to *C. deodara* than other species of the genus because of their distant phylogenetic relationship. The less transferability of genomic SSRs has also been reported earlier by CHAGNE *et al.* (2004) in pines. The percentage of chloroplast microsatellites successfully transferred from *P. thunbergii* and *P. sylvestris* was more as compared to the nuclear microsatellites transferred from *P. taeda* and *P. resinosa* in which either no amplification or very poor amplification was observed. The identified chloroplast microsatellite loci are currently being applied to survey the genetic diversity and unraveling the genetic structure of the populations of *C. deodara* forests of western Himalaya.

Acknowledgements

The authors are grateful to Director, Forest Research Institute, Dehradun for providing facilities and Indian Council of Forestry Research and Education, Dehradun for financial support for conducting this research.

References

- AVISE, J. C. (1994): Molecular markers, natural history and evolution. Chapman and Hall, New York.
- BOYS, J., M. CHERRY and S. DAYANANDAN (2005): Microsatellite analysis reveals genetically distinct population of Red Pine (*Pinus resinosa*, Pinaceae). *American Journal of Botany* **92**(5): 833–841.
- CHABANE, K., G. A. ABLETT and G. M. CORDEIRO (2005): EST versus genomic derived microsatellite markers for genotyping wild and cultivated barley. *Genetic Resources and Crop Evolution* **52**: 903–909.
- CHAIB, J., S. DANAN, B. JOAUD, L. S. HAGEN, F. LEFÈVRE and B. FADY (2006): Identification and characterization of nuclear microsatellites in Mediterranean cedars (*Cedrus* sp.). *Molecular Ecology Notes* **6**: 840–842.
- CHAGNÉ, D., P. CHAUMEIL, A. RAMBOER, C. COLLADA, A. GUEVARA, M. T. CERVERA, G. G. VENDRAMIN, V. GARCIA, J.-M. FRIGERIO, C. ECHT, T. RICHARDSON and C. PLOMION (2004): Cross-species transferability and mapping of genomic and cDNA SSRs in pines. *Theoretical and Applied Genetics* **109**(6): 1204–1214.
- CLARK, M. C., T. R. WENTWORTH and D. M. O'MALLEY (2000): Genetic discontinuity revealed by chloroplast microsatellites in Eastern North American *Abies* (Pinaceae). *American Journal of Botany* **87**(6): 774–782.
- DOYLE, J. J. and J. L. DOYLE (1990): Isolation of plant DNA from fresh tissue. *Focus* **12**: 13–15.
- FADY, B., F. LEFÈVRE, M. REYNAUD, G. G. VENDRAMIN, M. BOU DAGHER-KHARRAT, M. ANZIDEI, R. PASTORELLI, A. SAVOURÉ and M. BARITEAU (2003): Gene flow among different taxonomic units: evidence from nuclear and cytoplasmic markers in *Cedrus* plantation forests. *Theoretical and Applied Genetics* **107**(6): 1132–1138.
- FADY, B., F. LEFÈVRE, G. G. VENDRAMIN, A. AMBERT, C. RÉGNIER and M. BARITEAU (2008): Genetic consequences of past climate and human impact on eastern Mediterranean *Cedrus libani* forests. Implications for their conservation. *Conservation Genetics* **9**: 85–95.
- HODGETTS, R. B., M. A. ALEKSIUK, A. BROWN, C. CLARKE, E. MACDONALD, S. NADEEM and D. KHASA (2001): Development of microsatellite markers for white spruce (*Picea glauca*) and related species. *Theoretical and Applied Genetics* **102**: 1252–1258.
- NEALE, D. B. and R. R. SEDEROFF (1989): Paternal inheritance of chloroplast DNA and maternal inheritance of mitochondrial DNA in loblolly pine. *Theoretical and Applied Genetics* **77**: 212–216.
- NEI, M. (1987). Molecular evolutionary genetics. Columbia University Press, New York.
- LOURIEX, M., M. N. NDJONDJOP and A. GHESAUIERE (2000): A first interspecific *Oryza sativa* & *Oryza glaberrima* microsatellite-based genetic linkage map. *Theoretical and Applied Genetics* **100**: 593–601.
- PROVAN, J., N. SORANZO, N. J. WILSON, D. B. GOLDSTEIN and W. POWELL (1999): A low mutation rate for chloroplast microsatellites. *Genetics* **153**: 943–947.
- QIAO, C. Y., J. H. RAN, Y. LI and X. Q. WANG (2007): Phylogeny and Biogeography of *Cedrus* (Pinaceae) Inferred from Sequences of Seven Paternal Chloroplast and Maternal Mitochondrial DNA Regions. *Annals of Botany* **100**: 573–580.
- RAJORA, O. P., M. H. RAHMAN, S. DAYANANDAN and A. MOSSELER (2001): Isolation, characterization, inheritance and linkage of microsatellite DNA markers in white spruce (*Picea glauca*) and their usefulness in other spruce species. *Molecular and General Genetics* **264**: 871–882.
- RAO NAGESWARA, M., B. T. RAMESHA, G. RAVIKANTH, K. N. GANESHAIAH and R. UMA SHAANKER (2006): Cross-species amplification of coconut microsatellite markers in rattans. *Silvae Genetica* **56**(6): 282–286.
- TERRAB, A., O. PAUN, S. TALAVERA, K. TREMETSBERGER, M. ARISTA and T. F. STUESSY (2006): Genetic diversity and population structure in natural populations of Moroccan Atlas cedar (*Cedrus atlantica*; Pinaceae) determined with cpSSR markers. *American Journal of Botany* **93**: 1274–1280.
- TIWARI, D. N. (1994): A monograph on Chir Pine (*Pinus roxburghii* SARG). Indian Council of Forestry Research & Education, Dehradun, 311 p.
- VENDRAMIN, G. G., L. R. LELLI, P. ROSSI and M. MORGANTE (1996): A set of primers for the amplification of 20 chloroplast microsatellites in *Pinaceae*. *Molecular Ecology* **5**: 595–598.
- VENDRAMIN, G. G., M. ANZIDEI, A. MADAGHIELE and G. BUCCI (1998): Distribution of genetic diversity in *Pinus pinaster* Ait. as revealed by chloroplast microsatellites. *Theoretical and Applied Genetics* **97**: 456–463.
- WATANO, Y., M. IMAZU and T. SHIMIZU (1996): Spatial distribution of cpDNA and mtDNA haplotypes in a hybrid zone between *Pinus pumila* and *P. parviflora* var. *pentaphylla* (Pinaceae). *Journal of Plant Research* **109**: 403–408.
- WEISING, K., H. NYBOM and K. WOLFF (2005): DNA Fingerprinting in Plants Principles, Methods, and Applications. 2nd edn. Taylor & Francis Group, CRS Press, New York.
- ZHOU, Y., T. BUI, L. D. AUCLAND and C. G. WILLIAMS (2002): Under-methylated DNA as a source of microsatellites from a conifer genome. *Genome* **45**: 91–99.