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TECHNICAL NOTE

A set of 17 single nucleotide polymorphism (SNP) markers for European beech (*Fagus sylvatica* L.)

S. Seifert · B. Vornam · R. Finkeldey

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Abstract In view of the predicted changing environmental conditions, the question arises whether the important tree species European beech (*Fagus sylvatica* L.) will be adaptable to the future climate in Europe. Only few studies investigated the genetic background of drought stress tolerance in beech. In this study a set of 17 SNP (Single Nucleotide Polymorphism) markers selected from eight candidate genes potentially involved in drought stress response were developed. The polymorphisms of these markers were analysed in 50 adult trees from a population in Germany by using two multiplex sets (SNaPshot® Multiplex Kit (Applied Biosystems)). Observed and expected heterozygosity ranged from 0.060 to 0.520 and from 0.059 to 0.505, respectively. None of the loci showed a significant deviation from Hardy–Weinberg equilibrium.

Keywords Candidate gene · Drought stress · Climate change · SNP analysis · Adaptation

The forest tree species European beech (*Fagus sylvatica*) is one of the most important deciduous trees in Europe. The adaptation potential of beech to future environmental conditions is critically discussed in view of the predicted climate change (e.g., Ammer et al. 2005; Rennenberg et al. 2004). All climate change scenarios predict a warming for Europe during the next decades (EEA 2008; IPCC 2007). The overall precipitation is expected to change less, at least in the centre of the distribution of beech. However, less precipitation is forecasted for the summer months (EEA 2008; IPCC 2007). Thus, beech is expected to experience increasing drought stress in summer. The consequences of these changes for beech populations are largely unknown (e.g., Geßler et al. 2007).

The identification of candidate genes related to drought stress tolerance and the analysis of the variation within these genes is a first step to better understand the genetic basis for this trait. Single nucleotide polymorphisms (SNPs) are the most frequent type of variation found in DNA (Brookes 1999) and are valuable markers to study genetic variation and the genetic basis for adaptation of tree species (e.g., Gailing et al. 2009; Ingvarsson et al. 2008). We developed a set of 17 SNP markers for beech derived from eight different candidate genes which are putatively involved in drought stress tolerance.

The search for candidate genes was literature based (Seifert et al. 2012). After successful primer design for parts of the candidate genes, 18 different trees from six different populations were sequenced in order to analyse the variation within these genes (as described in Seifert et al. 2012). In order to avoid potential sequencing errors, only variation appearing in at least two different trees was identified as a potential SNP. In total, 17 SNPs in coding and non-coding regions of the genes (Table 1) were selected. Primers were designed according to the SNaPshot® Multiplex Kit (Applied Biosystems) by addition of nonhomologous polynucleotides (poly (dT)) of different lengths (Table 1) allowing the analysis of all SNPs in two multiplex reactions (Table 1). Primers were checked for self-annealing, dimer and hairpin formations using the program Oligo calc: Oligonucleotide Properties Calculator.

The SNPs were genotyped in one population in Northern Germany (N52 49.831 E10 18.985) comprising 50 individuals. Total DNA was extracted from leaves using the

S. Seifert (✉) · B. Vornam · R. Finkeldey
Faculty of Forest Sciences and Forest Ecology, Büsgen Institute, Forest Genetics and Forest Tree Breeding, Georg-August-University Göttingen, Büsgenweg 2, 37077 Göttingen, Germany
e-mail: sseifer@gwdg.de

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Table 1 Characterization of the 17 SNP markers with information about the related genes; forward/reverse: primer direction (important for the interpretation of the peaks)

Multiplex/ position	SNP name	Gene name	EMBL accession no.	5′ tail length—Primer sequence	forward/ reverse	variation	characteristic	H_o	H_e	P HWE
1/5	ALDH_1	*Aldehyde dehydrogenase*	FR774766	(T:38)—GGC AAC TAA CAC TAA CTG TAA CT	F	C/T	Non-coding	0.380	0.358	1.000
1/6	ALDH_2			(T:43)—GAT ACG AAG AGC AAA TTC CTC AC	F	C/A	Non-synonymous	0.400	0.407	1.000
1/7	ALDH_3			(T:51)—ACC CCA GCA GCA AGC CCA TA	R	C/G	Non-synonymous	0.080	0.078	1.000
1/8	ALDH_4			(T:56)—CGT GCA TTA CGA GTT GGG AC	F	T/A	Synonymous	0.080	0.078	1.000
1/1	IDH_1	*Isocitrate dehydrogenase*	FR796392 (part 1)	(T:21)—GGA CGT GAT GAG AAG AAA GAG TT	F	C/A	Synonymous	0.420	0.379	0.705
1/2	IDH_2		FR796392 (part 2)	(T:23)—CCA TGA AAA GTG AAG GAG GTT AT	F	G/A	Non-synonymous	0.200	0.182	1.000
1/3	IDH_3			(T:27)—GAT TTT TTA GCC CAA GGT TTG TAT	F	C/G	Non-coding	0.380	0.398	0.730
1/4	IDH_4			(T:35)—C AGC AAG ATT ACC AGC ACT GA	R	G/A	Synonymous	0.420	0.379	0.704
2/5	APX1_1	*Ascorbate peroxidase*	FR774767 (part 1)	(T:39)—CCA CCA TCT CTT ACG CCG ATT T	F	C/T	Synonymous	0.440	0.453	1.000
2/9	APX1_2			(T:63)—CAT GTC ATC GTG TCT TTA TCC TT	F	G/T	Non-coding	0.400	0.465	0.367
2/3	APX4_1	*Ascorbate peroxidase*	FR775801	(T:30)—CAG GTT AGC TCA TGC TAG ATA	R	G/T	Non-coding	0.260	0.228	0.579
2/6	APX4_2			(T:53)—G ATG TAG CAC AAG GTG CC	F	C/G	Non-synonymous	0.280	0.272	1.000
2/7	ERD	*Early responsive to dehydration*	FR775803	(T:50)—GCA AAG CGA ACG AGT TGA AAC AGA	R	G/A	Non-coding	0.480	0.505	0.784
2/1	Dhn_1	*Dehydrin*	FR772355	(T:21)—ACG GCA ACC GAG TTC GCA CT	F	C/G	Non-synonymous	0.520	0.498	0.781
2/2	Dhn_2			(T:24)—AGA AGA TAC CAG GTG TTG GGC A	F	C/G	Non-synonymous	0.280	0.272	1.000
2/8	GPX	*Glutathione peroxidase*	FR796394	(T:56)—CGA TAC CAT CCA TCA AAA CCT CA	F	C/T	Non-synonymous	0.060	0.059	1.000
2/4	PhyB	*Phytochrome B*	FR774765	(T:34)—CAA TCA TCC TAA CCC TAT TCT G	R	G/A	Synonymous	0.460	0.505	0.577

H_o Observed heterozygosity, H_e expected heterozygosity, *P HWE* exact *p* value for Hardy–Weinberg equilibrium test

DNeasy™ 96 Plant Kit (Qiagen, Hilden, Germany). The candidate gene fragments were amplified as described in Seifert et al. (2012). After amplification of the genes, the PCR products were cleaned using 1 unit Exonuclease I (Affymetrix, Santa Clara, USA) and 2.5 units SAP (Shrimp Alkaline Phosphatase; Affymetrix, Santa Clara, USA), 37 °C for one hour and 75 °C for 15 min.

SNaPshot® Multiplex Kit (Applied Biosystems) PCR amplifications were conducted in a 10 µl volume containing 5 µl of cleaned PCR product from the different genes, 5 µl Reaction Mix (SNaPshot® Multiplex Kit (Applied Biosystems)) and 0.2 µM of each primer. The PCR protocol consisted of 25 cycles of 96 °C for 10 s (denaturation), 50 °C for 5 s (annealing), and 60 °C for 30 s (extension). The PCR products were again cleaned using 1 unit SAP (Affymetrix, Santa Clara, USA). Preparations for the SNP analysis were done according to the protocol. SNP analyses were performed on an ABI PRISM® 3100*xl* Genetic Analyzer (Applied Biosystems) and scored according to the protocol. No automatic scoring was used.

All SNPs showed bi-allelic polymorphisms. The observed (H_o) and the expected (H_e) heterozygosities and fixation index (F) were calculated using Arlequin 3.11 (Excoffier et al. 2007) and GenAlEx 6.3 (Peakall and Smouse 2006). Deviation from Hardy–Weinberg equilibrium was tested locuswise using 100,000 steps in Markov chain and 1,000 dememorization steps with Arlequin 3.11 (Excoffier et al. 2007). Linkage disequilibrium was tested using 10,000 dememorization steps, 100 batches and 5,000 iterations per batch with GENEPOP 4.0.11 (Rousset 2008).

For the 17 SNP loci, the observed heterozygosity (H_o) varied from 0.06 to 0.52 with a mean of 0.326, while the expected heterozygosity (H_e) ranged from 0.059 to 0.505 with a mean of 0.324 (Table 1). No significant deviation from Hardy–Weinberg equilibrium was found. Significant linkage disequilibrium ($p < 5$ %) was detected for 14 SNP pairs ($p < 1$ % = seven pairs), six ($p < 1$ % = one pair) of them between fragments from different genes.

The markers described here are useful genomic tools to investigate drought stress tolerance of *F. sylvatica* in natural populations or in controlled drought stress experiments.

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