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Genetic and physiological differences of European beech provenances (*F. sylvatica* L.) exposed to drought stress

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ABSTRACT

Prolonged summer droughts constitute to a major risk for the cultivation of beech in Central Europe. Therefore the identification of beech ecotypes that cope with growth conditions expected in future is highly desirable for forest practitioners. In a pot experiment under controlled conditions, the influence of longterm water deprivation on growth, root/shoot ratio, nitrogen (N)- and carbon (C) assimilation of seedlings from 6 European beech (*Fagus sylvatica*) provenances, originating from Central Europe (Germany), the Balkan Peninsula (Croatia), and south-east Europe (Bulgaria, Greece) were examined. Genetic diversity and relationships between the provenances were analysed by molecular markers such as nuclear EST microsatellites (EST-SSRs), SNPs and chloroplast microsatellites. Genetic diversity within provenances was high and highlighted close relationships between plants from Greece, Croatia and Germany, whereas beech from Bavaria (Germany) seemed to be admixed with genotypes from Bulgaria. Significant changes in fine root $\delta^{13}C$ and C/N ratio as well as the intrinsic Water Use Efficiency (iWUE) demonstrate a better adaptability to future environmental conditions of beech ecotypes genetically related to the Greek beech population.

1. Introduction

European beech (*Fagus sylvatica* L.) forests cover a large continuous geographic range in Europe. They are stocking on moderately dry to moderately wet soils at a spatial distribution that is limited by drought in the south (Ellenberg, 1996). In total, beech covers about 12 million hectares of forest area in Central Europe and, thus, is ecologically and economically one of the most important broad-leaved tree species (Schraml and Rennenberg, 2002; Rennenberg et al., 2004). European beech is a monoecious, wind-pollinated and allogamous heavy-fruit tree species with long-distance seed dispersal and, hence, gene flow mediated by birds.

Considering future climate changes (IPCC, 2013) with expected prolonged summer drought and increasing mean annual air temperature (Geßler et al., 2007; Mayer et al., 2005; Rennenberg et al., 2004) serious risks have been prognosticated for the cultivation of common beech for southern Germany, especially if growing on shallow, calcareous soils with low water holding capacity, thereby lacking sufficient water supply during summer. This prognosis was based on fundamental knowledge on drought and heat sensitivity of beech (Ellenberg, 1996). These environmental factors impair water relations, nitrogen nutrition, and carbon metabolism of both, adult trees and saplings (Ciais et al., 2005; Fotelli et al., 2001; Leuschner et al., 2001; Peuke et al., 2002; Rose et al., 2009) and ultimately result in reduced competitiveness of beech in forest ecosystems (Fotelli et al., 2004; Peuke and Rennenberg, 2004; Rennenberg et al., 2006). Despite these risks, beech has been promoted by forest practitioners for economic and social reasons. In Baden-Württemberg (Germany), for example, the contribution of beech to total woodland is planned to be raised to 30% in the long-term (Moosmayer, 2002; Schraml and Volz, 2004).

Thus, the identification of beech ecotypes that may be able to cope with future growth conditions appears highly desirable (Meier and Leuschner, 2008; Nielsen and Jørgensen, 2003; Rose et al., 2009; Schraml and Rennenberg, 2002). For this purpose, information about beech populations with high genetic potential

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to adapt to changing growth conditions seems to be essential, since such populations are supposed to be able to survive under extreme climate/weather conditions. In this context, the investigation of refugial beech populations in southern Europe and their comparison with populations in Central Europe are thought to be a useful approach (Geßler et al., 2007; Rennenberg et al., 2004). Previous studies based on palaeobotanical and genetic data such as isozymes and chloroplast markers (Magri et al., 2006) presented scenarios about past distributions and postglacial spread of beech in Europe. In this context isozyme and molecular markers showed genetic variation under the influence of environmental factors (Bilela et al., 2012; Kramer et al., 2010). In addition, genetic variation within and differentiation between beech populations from different European regions was shown in a number of studies using allozymes and molecular genetic markers (Comps et al., 2001, 1991, 1990; Dounavi et al., 2010; Gailing and Wuehlisch, 2004; Hatziskakis et al., 2009; Magri et al., 2006; Pastorelli et al., 2003; Rajendra et al., 2014; Vornam et al., 2004). However, there are only a few studies on the molecular basis of adaptive genetic diversity of beech (Seifert et al., 2012).

In the present study, adaptive genetic variability and genetic relationships between six beech populations from Germany, Greece, Bulgaria and Croatia are studied. Physiological data has been derived from a drought stress experiment under controlled conditions to point out short- and longterm effects of water shortage on beech sapling from the investigated European provenances. The main goals are: (a) to study whether the genetic pool of south European provenances, as better adapted to limited water availability, is different from this of German provenances and (b) to investigate whether south European provenances physiologically better cope with drought stress than German ones. By linking the genetic and physiological results, conclusions about the adaptive potential of south European and German provenances in view of climate change can been drawn.

2. Materials and methods

2.1. Selection of beech provenances

Two beech provenances from Germany (Illertissen in Bavaria: 48°11′32″N, 10°11′53″E; Neidenstein in Baden-Württemberg: 49°19′N, 8°56′E), one from Bulgaria (Kotel: 42°51′59″N, 26°26′40″E) and one from Greece (Paikos: 40°58′N, 22°20′E) were used in a controlled drought stress experiment in summer 2012 to be tested for drought sensitivity. In 2013 the same experiment was repeated with two additional Croatian provenances (Zagreb: 45°53′1″N, 15°55′17″E, Gospic: 44°32′54″N, 15°7′34″E) (Fig. 1). In order to compare the results of the two experiments, the same provenance from Baden-Württemberg (Germany) used in the first year was used in the second year as well.

The provenances can be divided into three groups: (a) originating from temperate climatic conditions (Illertissen in Bavaria und Neidenstein in Baden-Württemberg, Germany), (b) originating from Balkan regions (Kotel in Bulgaria, Gospic and Zagreb in Croatia) (Table 1), whereas (c) beeches from Paikos (Greece) are representing the species' south-eastern distribution limit in Europe (Fotelli et al., 2009; Nahm et al., 2006).

2.2. Plant material

Controlled drought experiments were conducted in summers 2012 and 2013 in the greenhouses of the Forest Research Institute Baden Württemberg (Freiburg, Germany) and the Croatian Forest Research Institute (Jastrebarsko, Croatia), respectively. In both years, plants from the same provenance from Neidenstein (Baden-Württemberg, Germany) were used to assure the comparability of the results between the two years. The experiments were conducted using the same protocols. The plants were transferred to plastic pots (10.7 cm × 10.7 cm), containing the same soil substrate (Einheitserde, Spezial, Gebr. Patzer GmbH & Co. KG, Sinntal-Altengronau, Germany). Before starting the experiment, all plants were irrigated with 50 ml of tap water three times a week. Weeds were removed regularly to avoid competition for water, nutrients and light. Holes in the bottom of the pots allowed for drainage of excess irrigation water.

2.3. Experimental design

After the first period of leaf and shoot tip growth was completed in 2012 and 2013, respectively, 50 seedlings from each provenance were randomly separated into two groups, i.e. (a) control and (b) drought stress. Control plants were continued to be watered sufficiently as described above, drought stress was applied to the other group of plants by withholding the water supply for 21 (Bulgarian provenance) and 28 days (all other provenances).

At the beginning of the drought treatment (day 0) 4 extra plants of each provenance were randomly harvested as a control. To determine short and long term effects of drought on beech, 5 control and 5 drought stressed trees of each provenance were harvested randomly 5, 9, 14 (short term) and 21 and 28 days (long term) after the beginning of the drought treatment.

2.4. Genetic analysis

Leaves of 50 individuals (stressed plants) from each provenance were sampled and total DNA was extracted using the DNeasy Plant Mini Kit (Qiagen, Hilden, Germany). Six chloroplast microsatellites, namely ccmp2, ccmp4, ccmp6, ccmp7, ccmp10 (Weising and Gardner, 1999), and μkk3 (Deguilloux et al., 2003) were used to study haplotype differentiation of each provenance. Genetic differentiation on gene-based EST (Expressed Sequence Tag) microsatellite markers were studied (FcC00468, FcC00483, FcC00927, FcC01009, FcC00730, FcC01877, FcC02208, FcC03095 and FcC03300) (Ueno et al., 2009) to identify differences on the genetic potential of the different beech provenances. PCR (Polymerase Chain Reaction) amplifications of the EST-SSR (simple sequence repeats) markers were performed using fluorescent labelled primers in a mixture containing 1× HotStart reaction buffer (Eppendorf, Hamburg, Germany), 0.2 μM of each primer, and 10–20 ng/μl of template DNA for a 10 μl reaction volume. After an initial denaturation step for 15 min at 95 °C, the amplification reaction was carried out for 25 cycles as follows: 95 °C for 30 s, 55 °C for 90 s and 72 °C for 30 s. Final elongation was at 72 °C for 10 min. For the chloroplast microsatellites the reaction was changed as follows: the amplification was performed in 30 cycles, with 1 min annealing time and a temperature of 57 °C for ccmp2, ccmp4, ccmp6, ccmp7, ccmp10 or 55 °C for μkk3. The PCR reactions were conducted on a gradient PCR cycler (PTC 200, MJResearch, St Bruno, Canada) after optimization of the annealing temperature. The quality of the PCR products was controlled in a 1.3% agarose gel.

Alleles were scored by means of capillary electrophoresis using an ABI PRISM-3130xl genetic analyser (Applied Biosystems, Foster City, USA). Fragment analysis was carried out by using the software GeneMapper v4.0 (Applied Biosystems, Foster City, USA).

Additionally, SNPs (single nucleotide polymorphism) were identified and eight trees from each provenance were genotyped. The following five genes were sequenced: Gen 53 (S-adenosyl-L-homocysteine hydrolase, developed in collaboration with FVA-Freiburg und Prof. Dr. Vendramin, CNR, Florenz, IT), ALDH (aldehyde dehydrogenase), APX4_1 (ascorbate-peroxidase), DHN (dehydrin) (all three from Seifert et al., 2012), and Gen_8983_2