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Research

Identification of drought-sensitive beech ecotypes by physiological parameters

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Summary

• The effects of drought on European beech (*Fagus sylvatica*) were assessed in a pot experiment under controlled conditions.

• Plants from 11 autochthonous provenances originating from regions in Germany, which differed in annual precipitation, were exposed to a 3-wk drought period in a glasshouse after the first stage of shoot growth had been completed.

• Drought reduced the water content to 97% of control in leaves and axes and to 92% in the roots. A strong reduction of predawn water potential in roots and shoots, as well as on transpiration rate, was found. In the roots, the effect on water potential was the same for all provenances, but differences were observed in the shoot water potential. Leaf concentrations of abscisic acid (ABA), proline and sucrose increased in the drought-treated plants compared with the controls.

• Two extreme clusters from opposite climatic sites were identified by cluster analysis. A drought-sensitive cluster, originating from regions with high annual precipitation, had low water potential and transpiration rates, as well as high concentrations of fructose, ABA and proline after drought. Water potential and transpiration rates were less affected by drought in the other cluster, which comprised two provenances of relatively dry habitats, and concentrations of hexose, ABA and proline were low.

Key words: beech (*Fagus sylvatica*), provenances, drought stress, water potential, transpiration, osmoprotectants, abscisic acid.

Introduction

Global environmental conditions have changed rapidly over the last century as a result of human activities. One of these changes is the increase of atmospheric CO_2 by fossil fuel combustion, deforestation and biomass burning contributing to the glasshouse effect (Enquete-Komission, 1994; Saxe *et al.*, 1998; UNEP/IUC, 1999). Based on current trends, it is expected that within this century CO_2 concentrations will double and global temperature will rise by about 1–3.5°C (UNEP/IUC, 1999). As a consequence, precipitation and evaporation patterns will change and forests and other ecosystems will be exposed to drought and flooding events.

The increased frequency and severity of drought caused by climatic changes will affect plants directly through water depletion and indirectly by reduced nutrient uptake (Saxe *et al.*, 1998). Plants in a CO_2-enriched climate of the future might tolerate drought better through stomatal closure and/or decline in stomatal density (Bowes, 1993), but elevated CO_2 may also increase leaf area and thereby counteract the favourable effects of reduced stomatal aperture/density under water limitation (Saxe *et al.*, 1998).

For long-living plants, such as forest trees, the expected climatic changes will become relevant within the lifespan of an individual within the community. European beech (*Fagus sylvatica*) is one of the most important forest trees in central Europe and is known to be relatively drought sensitive (Ellenberg, 1992). Beech forests have mainly developed by natural regeneration and ecotypes have developed that are adapted to the local climatic conditions (Müller-Stark, 1997).

In the light of climate change, information on the drought-tolerance of ecotypes will be important for forest management and reafforestation.

Plants possess numerous mechanisms for responding to drought stress. A commonly used indicator for drought stress and water status in plants is the predawn water potential measured in different organs of the plants, mostly in leaves or small twigs (Fort *et al.*, 1997, 1998; Picon *et al.*, 1997; Cellier *et al.*, 1998; Tardieu & Simonneau, 1998; Thomas & Eamus, 1999; Schraml & Rennenberg, 2000; Fotelli *et al.*, 2001; Yao *et al.*, 2001). The resistance of trees to drought depends to a large extent on the capacity of the roots and stems for water uptake, transport to leaves and maintaining cellular turgor (Saxe *et al.*, 1998). Compatible solutes, also named osmoprotectants (saccharides, polyhydric compounds, amino acids and quaternary ammonium compounds) are involved in responses of plants to drought stress. These compounds raise osmotic pressure, maintain membrane integrity and stabilize proteins (Heuer, 1994; Hare *et al.*, 1998, 1999; McNeil *et al.*, 1999). The most frequent osmoprotectant, proline (Heuer, 1994), has been observed in beech trees (Schraml & Rennenberg, 2000). Abscisic acid (ABA) is a signal for drought stress and mediates general responses to drought (Cellier *et al.*, 1998; Hartung *et al.*, 1999). Differences in drought tolerance of species, lines, clones, cultivars or provenances have been attributed to the effects of drought on sugars, proline (Heuer, 1994; Hare *et al.*, 1999) and ABA (Volaire *et al.*, 1998).

In the present study, beech seedlings originating from autochthonous provenances in different climatic habitats in Germany were exposed in a pot experiment to drought treatment, after the first period of leaf and shoot tip growth had been completed. This experimental design was aimed to simulate a period of summer drought. Beech was chosen because it is the most important deciduous tree species in German forestry and is known to develop ecotypes adapted to climatic conditions in the habitat. The experiments were carried out to identify general effects of drought treatment on biometric parameters, water potential, transpiration, osmoprotectants and ABA in beech. Analysis of correlation and regression between the measured parameters should reveal possible mechanisms. With this approach we tested whether ecotypes of different drought sensitivity could be identified among the provenances and whether these differences could be attributed to the amount of rainfall in their original habitats.

Materials and Methods

Plant material and cultivation

Seeds of beech (*F. sylvatica* L) from different autochthonous provenances in Germany were used for the present study. The provenances were selected to represent large differences in annual precipitation in their growing region or habitat. Climatic data of the regions from which the provenances originate are given in Table 1. Six provenances originate from relatively dry habitats (< 680 mm rainfall yr^{-1}: 'red', 'kloe', 'small', 'goer', 'tbb' and 'bov'), two provenances from intermediate habitats ('black' and 'bad') and three from wet habitats (> 1150 mm rainfall yr^{-1}: 'sees', 'harz' and 'sont'). The beech nuts were provided by several official seed extractories/husking establishments in Germany: Niedersächsische Forstsaatgut-Beratungsstelle, Munster-Oerrel ('goer', 'bov', 'sees', and 'harz'); Forstsaatgut Beratungsstelle/Landesdarre Sachsen-Anhalt, Annaburg ('kloe'); Samendarre Mecklenburg-Vorpommern Forstamt Jatznick, Jatznick ('red' and 'small'); Bayerische Landesanstalt für forstliche Saat- und Pflanzenzucht, Teisendorf ('sont'); Staatsklenge Baden-Württemberg, Nagold ('tbb', 'black', and 'bad').

The beech nuts were surface-sterilized for 1 h with 0.1% Chinosol (Riedel de-Haen, Seelze, Germany) and soaked in continuously aerated water over night. For germination the

Table 1 Codes of provenances and climatic data of the region. The provenances are named by the forest district of harvest in federal state

Provenance	Latitude and longitude	Year of harvest	State	Precipitation (mm yr^{-1})	Temperature (°C)	Altitude (m above sea level)	Code
Tauberbischofsheim	09°39′ E 49°37′ N	1998	BW	650	8.5	400	tbb
Schwarzach	08°58′ E 49°22′ N	1998	BW	800	8.5	250	black
Bad Urach	09°21′ E 48°30′ N	1994	BW	890	7.0	730–810	bad
Sonthofen	10°17′ E 47°31′ N	1995	Bay.	1700	5.8	1050–1660	sont
Rothemühl	13°49′ E 53°35′ N	1998	Meck.	574	8.0	50	red
Lüttenhagen	13°25′ E 53°20′ N	1998	Meck.	599	7.5	110	small
Göhrde	10°52′ E 53°07′ N	1998	Nds	630	8.0	65	goer
Bovenden	09°56′ E 51°35′ N	1998	Nds	680	8.0	300–350	bov
Seesen	10°10′ E 51°52′ N	1998	Nds	1150	5.5	250–350	sees
Harz	10°20′ E 51°47′ N	1998	Nds	1400	4.5	600–650	harz
Klötze	11°09′ E 52°37′ N	1998	Sa.-An.	586	8.4	55	kloe

BW, Baden-Württemberg; Bay., Bayern/Bavaria; Meck., Mecklenburg-Vorpommern/Mecklenburg-Western Pomerania; Nds., Niedersachsen/Lower Saxonia; Sa.-An., Sachsen-Anhalt/Saxony-Anhalt.

seeds were placed in permanently wetted clay pots (without substrate) in darkness at 3–5°C. After 4 wks seeds that had developed radicles 5–10 mm long were transferred to pots in a glasshouse. Plastic containers, 17 cm diameter and 2 l volume, were filled with a mixture of five parts commercial potting soil (Floradur, Floragard GmbH, Oldenburg, Germany), five parts Perlite (Perligran G, Deutsche Perlite GmbH, Dortmund, Germany) and one part surface soil (v : v : v) from a natural beech stand near Freiburg. One seedling was planted in the middle of each pot and covered with a plastic Petri disk. After emergence of the cotyledons the young plant was covered for a further week with a small glass pot to avoid dehydration.

The pots were placed randomized in two growth chambers of a glasshouse. The plants were regularly rotated within each room and exchanged between the chambers in part every day to exclude gradients in climatic factors in the growth chambers. During an artificial light period of 16 h (starting at 07 : 00 h) initiated 2 wks after emerging, the plants were supplementally illuminated by mercury-vapour lamps (Osram HQL 400, Osram GmbH, München, Germany) at 200–250 µmol m^{-2} s^{-1}. The temperature was kept at 20 ± 5°C and the humidity at 40–60%. Every second day the pots were well watered with tap water and after the first month the plants were supplied every second week with a commercial fertilizer (0.3% Hakaphos Blau, Compo GmbH, Münster Germany).

Drought treatment

After 12 wks of cultivation in the glasshouse, drought treatment was initiated. At this time, the first period of leaf and shoot tip growth typical for beech had completed. The population of 20 plants per provenance was divided in two halves, controls and drought-treated, by selecting homogenous 'matched pairs' based on visual comparison. The controls were further watered, while water supply was omitted from the drought-treated plants. Owing to the limited volume in the pots it was necessary to add water to keep the field capacity at a minimum of about 20%, as required for relevant drought period, since the daily loss of water by evapotranspiration in the last 2 wks of drought treatments was, on average, 37.4 ± 0.5 g water d^{-1}. This quantity of water represented about 4% of the field capacity of the pots and, without continuously compensating for this loss, irreversible damage (necrosis of youngest leaves) would have occurred within a few days. Therefore, after 10 d every pot of the drought treatment was weighed and water was added to adjust the water content of the substrate to about 20 ± 5% (w : w). On average, 40–50 ml water were added every 1–2 d to compensate for water loss by transpiration and evaporation. The water content of the substrate was estimated by comparing the weights of pots with well watered pots and with pots dried for 3 wks at 30°C to constant weight $((pot - pot_{dry})/(pot_{wet} - pot_{dry})$ (%)). No water was supplied for 2 d before harvest.

Harvesting

Before harvest, the plants were transferred to a dark chamber before dawn, so that the plants could be harvested sequentially, each coming from dark conditions (06 : 30–10 : 00 h). The pots were weighed and, after measuring the diameter of the stems, the seedlings were cut at the epicotyl. The leaves were removed from the stem and total leaf area was determined (ΔT, AreaMeter, Delta-T Devices Ltd, Cambridge, UK). Shoot parts were carefully washed with deionized water, roots first rinsed with tap water and than cleaned from substrate particles in 100 mM sorbitol to avoid leaching of solutes. After weighing the plant parts were immediately frozen in liquid N_2 and stored at −80°C until analysis. Dry weight was evaluated after lyophilization and for further extractions the dried material was ground. Extraction and determination of solutes represent averages of the bulked leaf material of the whole seedling.

Water relations measurements

One day before harvest, the transpiration rates were measured by porometry using a Li-Cor 1600 steady-state porometer (Li-Cor Inc., Lincoln, NB, USA). Means of leaves three to five were used as averages for the entire plant. The measurements were carried out in the glasshouse chamber at local conditions between 12 : 00 h and 15 : 00 h.

Predawn water potential was measured in shoots and in roots during the harvesting procedure, after removing the bulk of substrate, using the Scholander pressure chamber (Scholander *et al.*, 1965).

The relative water content in the plant organs was calculated on the basis of difference in fresh to dry weight compared to fresh weight ((f. wt − d. wt) × f. wt^{-1} (%)).

Determination of sugars

Sugars were determined in aqueous extracts from lyophilized ground leaves 40 mg plant powder was mixed with 80 mg Polyvinylpolypyrrolldone (PVPP) (Sigma Chemie, Deisenhofen, Germany) to remove phenolic compounds during extraction. The dry material was extracted with 2.5 ml water for 1 h in an ice bath under continuous shaking. After boiling for 5 min the extracts were centrifuged for 10 min at 16 000 ***g*** to precipitate proteins. The clear supernatants were injected into an high-pressure liquid chromatography (HPLC) system Dionex DX 500 (Dionex, Idstein, Germany). Separation of sugars was achieved in a CarbonPac PA1 column (4 × 250 mm, Dionex) with 36 mM NaOH as mobile phase. Detection and quantification was performed with a pulsed amperometric detector (Electrochemical detector ED 40 Dionex, Idstein, Germany). Myo-inositol, fructose, glucose and sucrose were used as standards.

Determination of proline

Lyophilized ground plant material (50 mg) was extracted with 0.2 ml buffer containing 20 mM *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid (HEPES) (pH 7.0), 5 mM Ethyleneglycol-bis(2 aminoethyl) N, N, N′, N′-tetra acetic acid (EGTA), 10 mM NaF plus 1.25 ml chloroform–methanol (1.5 : 3.5, v : v). Samples were incubated for 30 min on ice. Water-soluble amino acid were extracted from this suspension twice with 0.2 ml distilled water. The combined aqueous phases were freeze-dried and resolved in 0.5 ml lithium citrate buffer (0.2 M, adjusted to pH 2.2 with HCl). After centrifugation (16 000 ***g*** for 5 min at 4°C) the aliquots of the clear supernatants were injected into an automated amino acid analyser (Biochrom, Pharmacia LKB, Freiburg, Germany). Amino compounds were separated on a PEEK column (Ultrapac 8 resin, Lithium 250 × 4.6 mm Biochrom, Pharmacia LKB) by a sequence of five different lithium citrate buffers (pH gradient from 2.80 to 3.55). The amino compounds plus ammonium were derivatized by ninhydrin (postcolumn derivatization) and the absorption of amino ninhydrin derivates was measured at 570 nm. Proline was identified and quantified by a commercial standard solution containing 39 amino compounds (Sigma Chemie).

Determination of ABA

Freeze-dried ground leaf material was extracted in 80% methanol. Extracts were passed through a Sep Pak C_{18} cartridge (Waters Coop., Milford, USA). Methanol was removed under reduced pressure and the aqueous residue partitioned three times against ethyl acetate at pH 3.0. The ethyl acetate of the combined organic fractions was removed under reduced pressure. The residue was taken up in TBS buffer (Tris buffered saline; 150 mol m^{-3} NaCl, 1 mol m^{-3} $MgCl_2$ and 50 mol m^{-3} Tris, pH 7.8) and subjected to an immunological ABA assay – enzyme-linked immunosorbent assay (ELISA) – as described by Peuke *et al.* (1994). The accuracy of the ELISA was verified in earlier investigations (Peuke *et al.*, 1994). Recoveries of ABA during purification procedures were checked routinely using radioactive ABA and found to be more than 95%.

Statistics

All statistical calculations were performed with SAS release 6.12 and 8.0 (SAS Institute Inc., Cary, NC, USA). Two-way analysis of variances (ANOVA model: 'drought (treatment)', 'provenance' and interactions 'drought × provenance') were performed by the GLM procedure.

Analysis of correlation was done with whole data set, divided into the two treatments or divided into provenances using the procedure CORR (K_p: Pearson correlation coefficient and *P*-value for H_0: $K_p = 0$).

To test for differing sensitivities of provenances to the drought treatment, analysis of regression was performed on predawn water potential. In the first step a complete regression model was composed with a number of potential effectors, which might possible affect water potential:

$$\begin{aligned}\mathrm{Wpot}_{\mathrm{shoot/root}} = {} & \text{intercept} + \log(\mathrm{Wcont}) + \text{transpiration} \\ & + \mathrm{DW}_{\mathrm{leaf/axis/root}} + \mathrm{rel.water}_{\mathrm{leaf/axis/root}} \\ & + \text{sugar} + \text{osmol}\end{aligned}$$

where $\mathrm{Wpot}_{\mathrm{shoot/root}}$ is the water potential in the shoots or roots, log(Wcont) is logarithm of the water content; transpiration is transpiration rate, $\mathrm{DW}_{\mathrm{leaf/axis/root}}$ is dry weight of the leaves, axes or roots, $\mathrm{rel.water}_{\mathrm{leaf/axis/root}}$ is the relative water content in the leaves, axes or roots, sugar is the concentration of sugars (sum of myo-inositol, fructose, glucose and sucrose) and osmol is osmolarity in water extract of leaf dry matter. The procedure REG was used for analysis of regression. Backwards selection eliminated effectors that were not significant (H_0 estimates = 0). In the second step, the resulting regression models were used to work out different sensitivities of water potential due to water status (water content or water potential in the root, respectively). In a linear model with provenance as class variable (procedure GLM of SAS), the homogeneity of slopes was tested model:

$$\begin{aligned}\mathrm{Wpot}_{\mathrm{root}} = {} & \log(\mathrm{Wcont}) + \text{provenance (class)} \\ & + \log(\mathrm{Wcont}) \times \text{provenance}\end{aligned}$$

and

$$\begin{aligned}\mathrm{Wpot}_{\mathrm{shoot}} = {} & \mathrm{Wpot}_{\mathrm{root}} + \mathrm{DW}_{\mathrm{leaf}} + \text{provenance (class)} \\ & + (\mathrm{Wpot}_{\mathrm{root}} \times \text{provenance}) \\ & + (\mathrm{DW}_{\mathrm{leaf}} \times \text{provenance})\end{aligned}$$

To assess the different pattern in behaviour of the provenances in response to drought, cluster analysis was performed with the procedure CLUST using the complete linkage of significantly affected parameters (control and drought-treated: relative water content in leaves, axes and roots, transpiration rate, predawn water potential in root and shoot, sucrose, proline and ABA concentration in leaf dry matter).

Results

Biometrical data

At the time of harvest, the mean water content of the substrate was 18.5 ± 0.3% in the drought treatment compared with 100 ± 0.2% in the controls $((\mathrm{pot} - \mathrm{pot}_{\mathrm{dry}})/(\mathrm{pot}_{\mathrm{wet}} - \mathrm{pot}_{\mathrm{dry}})$ (%)). No significant differences in water content of the substrates with different provenances were detected.

Drought had no effect on dry weight of roots, axes or leaves, but significant differences were observed between

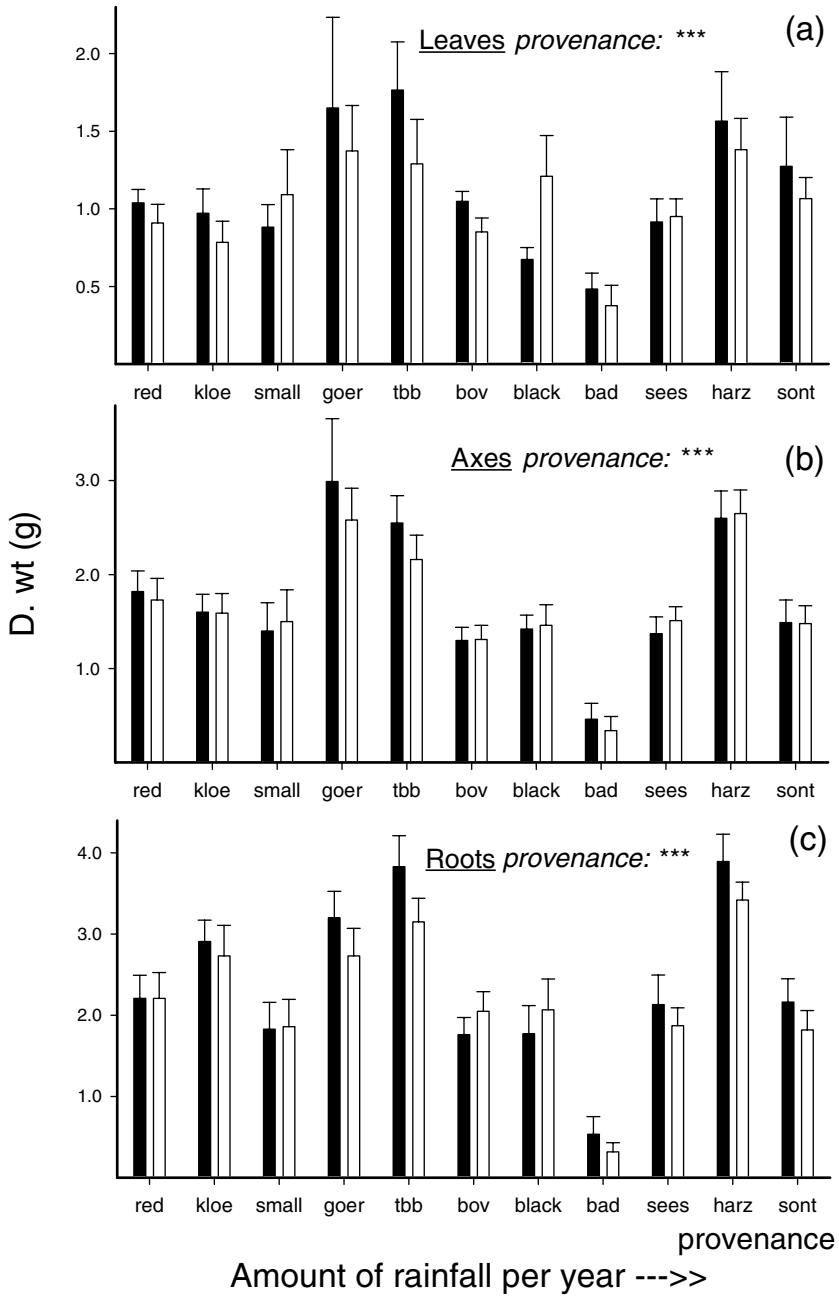

Fig. 1 Dry weight of (a) leaves (b) axes, and (c) roots in 4.5-month-old beech seedlings (*Fagus sylvatica*). After 1 months' germination the seedlings were cultivated in 2-l pots with a mixture of commercial potting soil–Perlite–forest surface soil (5 : 5 : 1) in a glasshouse chamber at 24°C, 40–60% humidity and 16 h artificial illumination (200–250 μmol m^{-2} s^{-1}). The drought treatment (empty columns) was effected by stopping watering the pots 3 wks before harvest and keeping them at a minimum of about 20 ± 5% water content. The controls (filled columns) were watered every second day to excess throughout. Values are means ± standard errors. The provenances are sorted in order of annual rainfall in the region. Significance of the main factors are indicated by asterisks and effects between treatments within a provenance with an asterisk at the columns.

provenances (Fig. 1). The mean dry weight of all plants was 5.20 ± 2.72 g, but there was a large variation between a group of provenances characterized by large plants ('goer', 'tbb' and 'harz': 7.27 ± 0.49 g, 7.38 ± 0.49 and 7.77 ± 0.49 g, respectively) and the provenance plants 'bad' characterized by smaller biornes (1.26 ± 0.59 g). The shoot-to-root ratio on a dry weight basis was not affected by drought treatment and was similar in all provenances, with exception of 'bad' (data not shown). While the shoot-to-root ratio varied between 0.90 ± 0.13 ('kloe') and 1.41 ± 0.13 ('goer') in most provenances, it was 2.92 ± 0.71 in the controls of 'bad' and was decreased significantly to 2.33 ± 0.25 by the drought treatment (data not shown).

The relative water content in the plant organs ((f. wt − d. wt) × f. wt^{-1} (%)) was significantly affected by both drought and provenance of the beech nuts (Fig. 2). Drought treatment reduced the relative water content in leaves and axes, to 97% (on average) of the control. The effect was strongest in the roots, where the relative water content was reduced to 92% of the controls because of drought. The drought effect on relative water content was significant for all provenances in the roots, but only for 'tbb' in the leaves and for 'black' and 'sees' in the axes (Fig. 2). The high relative water content in the organs of the very small provenance 'bad' was mainly responsible for the significant effect of provenances (0, homogenous group 'a').

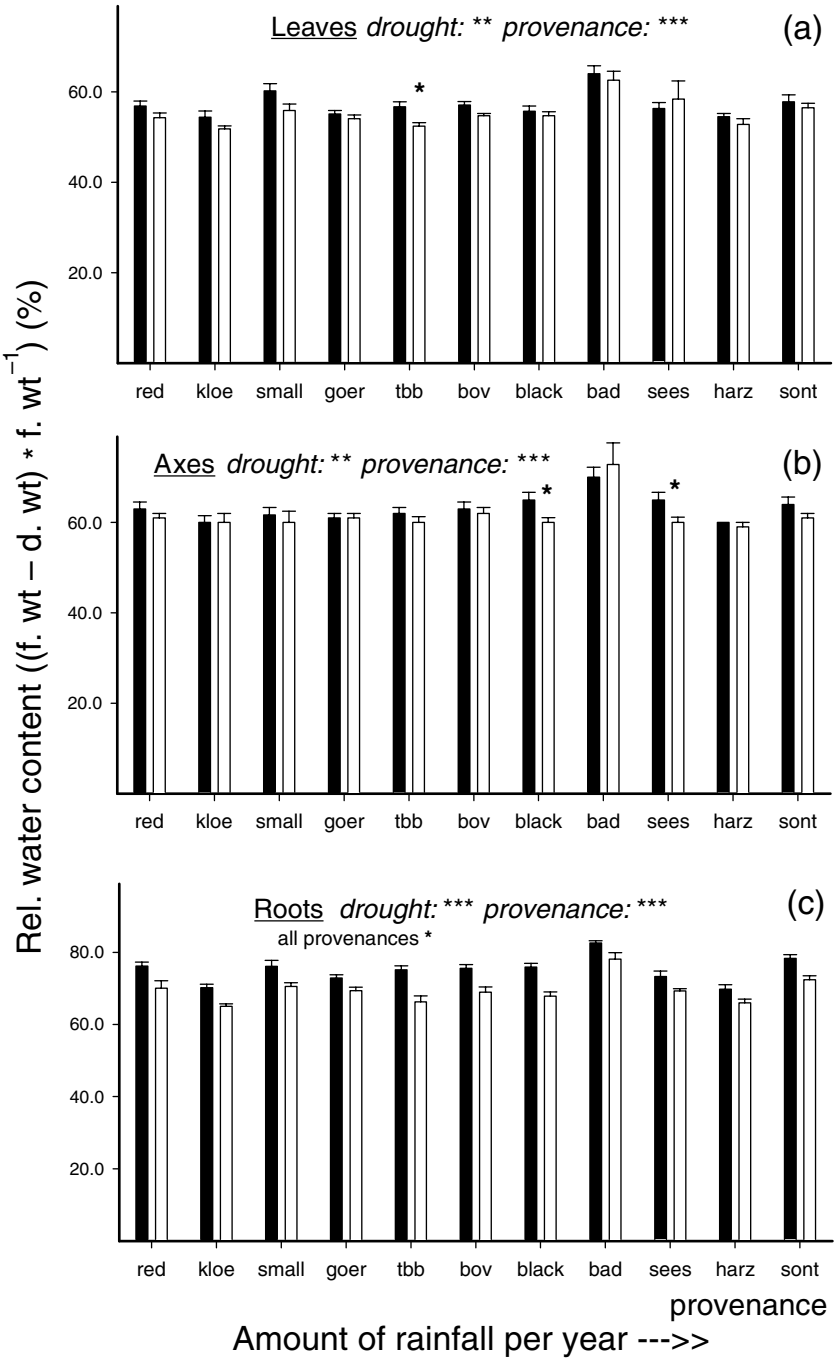

Fig. 2 Relative water content in (a) leaves (b) axes, and (c) roots in controls and drought-treated 4.5-month-old beech seedlings (*Fagus sylvatica*). After 1 months' germination the seedlings were cultivated in 2-l pots with a mixture of commercial potting soil–Perlite–forest surface soil (5 : 5 : 1) in a glasshouse chamber at 24°C, 40–60% humidity and 16 h artificial illumination (200–250 µmol m^{-2} s^{-1}). The drought treatment (empty columns) was effected by stopping watering the pots 3 wks before harvest and keeping them at a minimum of about 20 ± 5% water content. The controls (filled columns) were watered every second day to excess throughout. Values are means ± standard errors. The provenances are sorted in order of annual rainfall in the region. Significance of the main factors are indicated by asterisks and effects between treatments within a provenance with an asterisk at the columns.

Transpiration rates

Both drought and provenance affected transpiration rates (Fig. 3a). Drought reduced transpiration to about 0.6-fold of the control. This effect was significant for all provenances, except for 'kloe', 'tbb', and 'bov' from dry habitats. In these three provenances plus 'black', transpiration rates were already low under control conditions and only slightly affected by drought.

Predawn water potential

In control plants predawn water potential in shoots ranged between −0.20 ± 0.03 MPa ('black') and −0.28 ± 0.03 MPa ('tbb') and significantly declined at reduced water supply to −0.26 ± 0.2 ('bad') and −0.73 ± 0.11 ('sont') (Fig. 3b). The average effect of reduced water supply resulted in 2.2-fold lower water potential, compared with the control (−0.30 MPa) and was significant for all provenances except

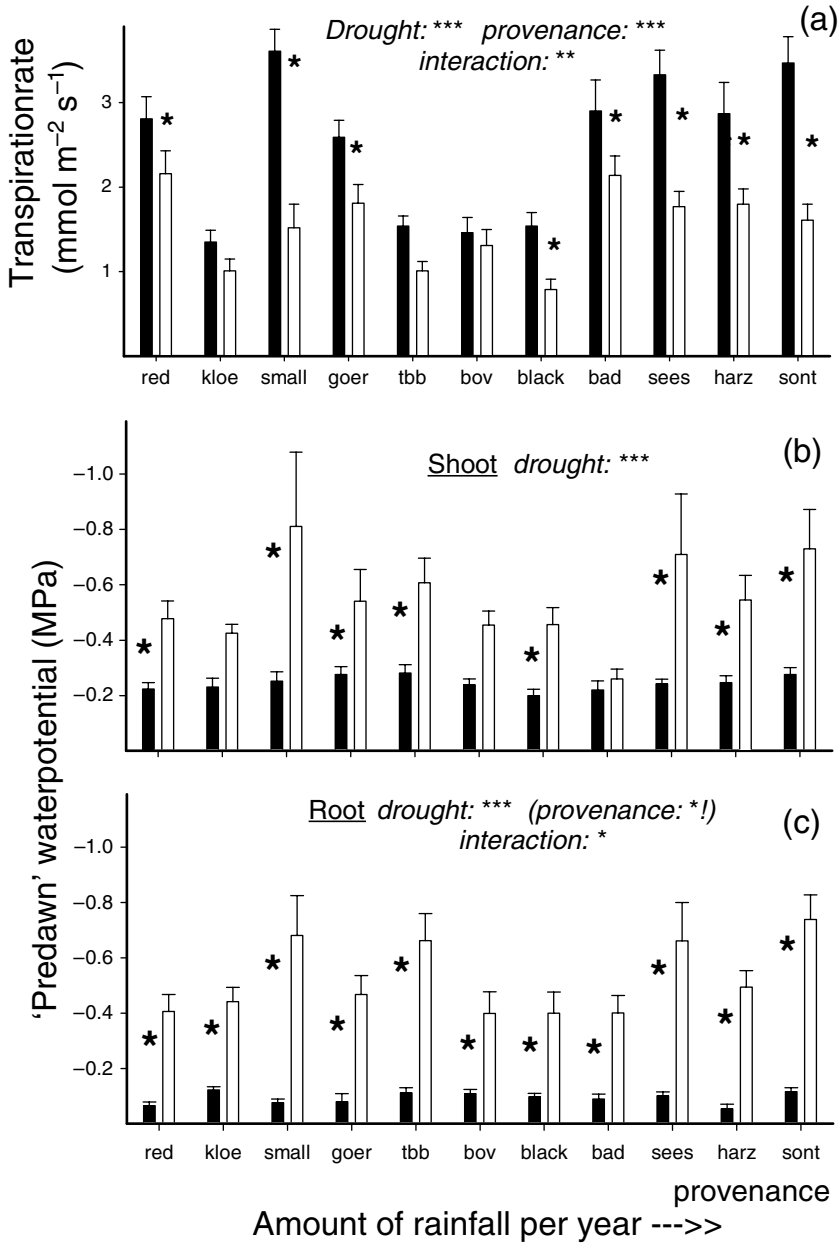

Fig. 3 Transpiration rates (a), 'predawn' water potential in (b) shoots and (c) roots of controls and drought treated 4.5-month-old-beech seedlings (*Fagus sylvatica*). After 1 months' germination the seedlings were cultivated in 2-l pots with a mixture of commercial potting soil–Perlite–forest surface soil (5 : 5 : 1) in a glasshouse chamber at 24°C, 40–60% humidity and 16 h artificial illumination (200–250 µmol m^{-2} s^{-1}). The drought treatment (empty columns) was effected by stopping watering the pots 3 wks before harvest and keeping them at a minimum of about 20 ± 5% water content. The controls (filled columns) were watered every second day to excess throughout. Values are means ± standard errors. The provenances are sorted in order of annual rainfall in the region. Significance of the main factors are indicated by asterisks and effects between treatments within a provenance with an asterisk at the columns.

'kloe', 'bov' and 'bad'. Significant differences between the provenances were not observed.

The effect of drought on the water potential in roots was much greater (5.6-fold lower than the control; −0.43 MPa) than in shoots (Fig. 3c). In control plants, the water potential ranged between −0.07 ± 0.02 ('small') and −0.12 ± 0.02 MPa ('sont'), and in treated plants between −0.40 ± 0.09 ('black' und 'bov') and −0.74 ± 0.09 MPa ('sont'). In all provenances the water potential was lowered significantly by drought.

Osmoprotectants

In aqueous extracts from beech leaves, sucrose (111 ± 3 µmol g^{-1} dry wt), glucose (97 ± 5 µmol g^{-1} dry wt), fructose (35 ± 2 µmol g^{-1} dry wt) and myo-inositol (71 ± 1 µmol g^{-1} dry wt) (means of all samples, respectively) were detected (Fig. 4). Only the concentration of sucrose was significantly affected by drought. On average, the concentration of sucrose was increased 1.2-fold by drought. However, this effect was not significant within a single provenance. The greatest differences between provenances were detected for the glucose concentrations, with a factor of about five: 39 µmol g^{-1} dry wt ('tbb'), compared to 194–196 µmol g^{-1} dry wt ('goer', 'harz', or 'red'). Provenances with low concentrations of hexoses contained high concentrations of sucrose (e.g. 'tbb', 'bov', 'kloe') and *vice versa*.

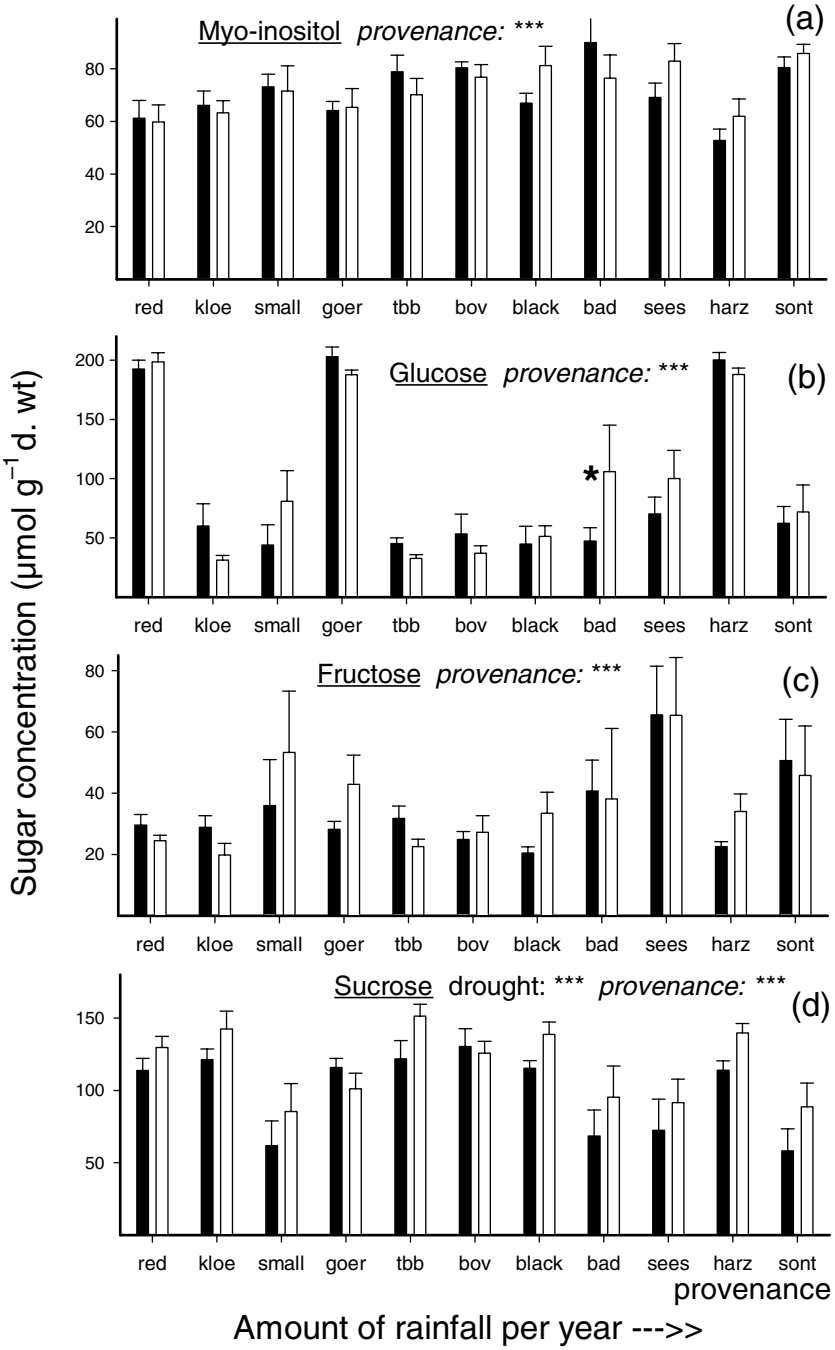

Fig. 4 Concentrations of sugars in the leaf dry matter of controls and drought-treated 4.5-month-old beech seedlings (*Fagus sylvatica* L). After 1 months' germination the seedlings were cultivated in 2-l pots with a mixture of commercial potting soil–Perlite–forest surface soil (5 : 5 : 1) in a glasshouse chamber at 24°C, 40–60% humidity and 16 h artificial illumination (200–250 µmol m^{-2} s^{-1}). The drought treatment (empty columns) was effected by stopping watering the pots 3 wks before harvest and keeping them at a minimum of about 20 ± 5% water content. The controls (filled columns) were watered every second day to excess throughout. Values are means ± standard errors. The provenances are sorted in order of annual rainfall in the region. Significance of the main factors are indicated by asterisks and effects between treatments within a provenance with an asterisk at the columns.

Under control conditions, the concentrations of proline per unit dry matter of leaves were relative similar in all provenances (1.02 ± 0.09 µmol g^{-1} dry wt, see Fig. 5b). Drought affected the concentrations of proline in the leaves (on average, 1.3-fold higher than the control) but the effects were different in the various provenances. While in 'small', 'bad' and 'sees' a significant increase in proline was observed, it significantly decreased in 'black'. The effect of provenance was statistically significant.

Abscisic acid

The concentrations of ABA in leaves were affected by drought as well as by provenance. One group of provenances ('kloe', 'tbb', 'bov' and 'black') contained low ABA concentrations in the leaves (approx. 200 pmol g^{-1} dry wt), which were not affected by drought treatment (Fig. 5a). The other provenances had higher concentrations of ABA, which increased owing to drought (up to 2190 ± 428 pmol g^{-1} dry

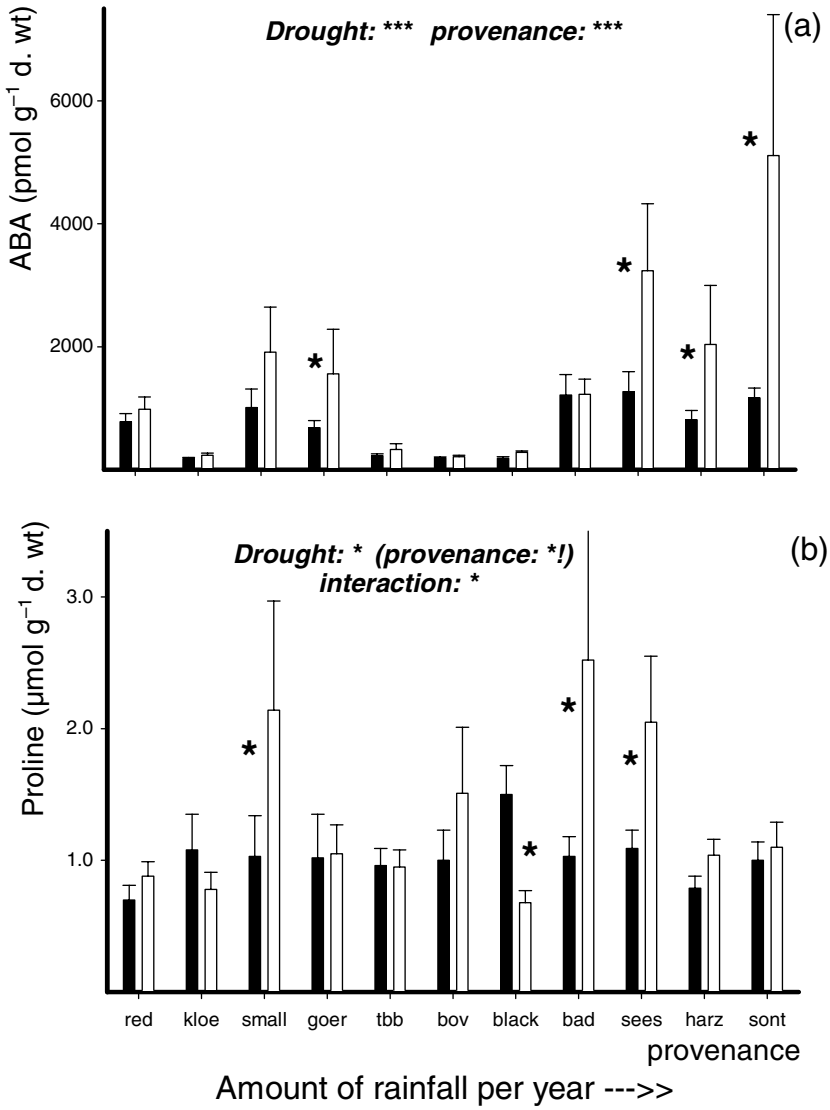

Fig. 5 Concentrations of abscisic acid (a) and proline (b) in the leaf dry matter of controls and drought treated 4.5-month-old-beech seedlings (*Fagus sylvatica* L). After 1 months' germination the seedlings were cultivated in 2-l pots with a mixture of commercial potting soil–Perlite–forest surface soil (5 : 5 : 1) in a glasshouse chamber at 24°C, 40–60% humidity and 16 h artificial illumination (200–250 µmol m^{-2} s^{-1}). The drought treatment (empty columns) was effected by stopping watering the pots 3 wks before harvest and keeping them at a minimum of about 20 ± 5% water content. The controls (filled columns) were watered every second day to excess throughout. Values are means ± standard errors. The provenances are sorted in order of annual rainfall in the region. Significance of the main factors are indicated by asterisks and effects between treatments within a provenance with an asterisk at the columns.

wt; 'sees'). For the entire data set the increase in ABA concentrations in the leaves was 1.7-fold by drought, but the effect was significant only in 'goer', 'sees', 'harz' and 'sont'.

Analysis of correlation and regression

The relationships of water content of the substrate to other parameters are shown in Fig. 6a–g. The data are not homogeneously distributed over the entire range of water content because of the experimental design ($0 < x = 1$ rel. units), but are arranged in two groups around 0.2 and 1.0 rel. units. Nevertheless, a fairly good correlation of water content to water potential in the roots ($K_p = 0.73$) and in the shoots ($K_p = 0.50$) was found. The correlation with the transpiration rates was just below $K_p = 0.5$. For the relationships of water potential of the shoot to other parameters (Fig. 6h–n), a good correlation with the water potential in the roots could be observed ($K_p = 0.83$). As indicated by Fig. 6c,d (and see Cellier *et al.*, 1998; Ray & Sinclair, 1998), the relationship between water content of the substrate and water potential approximates more to a logarithmic than a linear form (or possibly two or three linear phases). Shoot water potential also showed good correlation to ABA in the leaves ($K_p = -0.59$). Moreover, the dry weights of the plant organs correlated very well with each other, indicating coordinated growth, irrespective of drought treatment (data not shown).

The regression analysis for the water potential in the root was the result of backward selection in the model:

$$\mathrm{Wpot}_{root} = (-0.089 \pm 0.019) + (0.585 \pm 0.036) \times \log(\mathrm{Wcont})$$

($R^2 = 0.578$; $P < 0.0001$/ < 0.0001; $P_{total} < 0.0001$).

In the root only the field capacity of the substrate had a significant effect. For the shoot, the model resulted in a lower regression factor ($R^2 = 0.346$). However, if in the regression

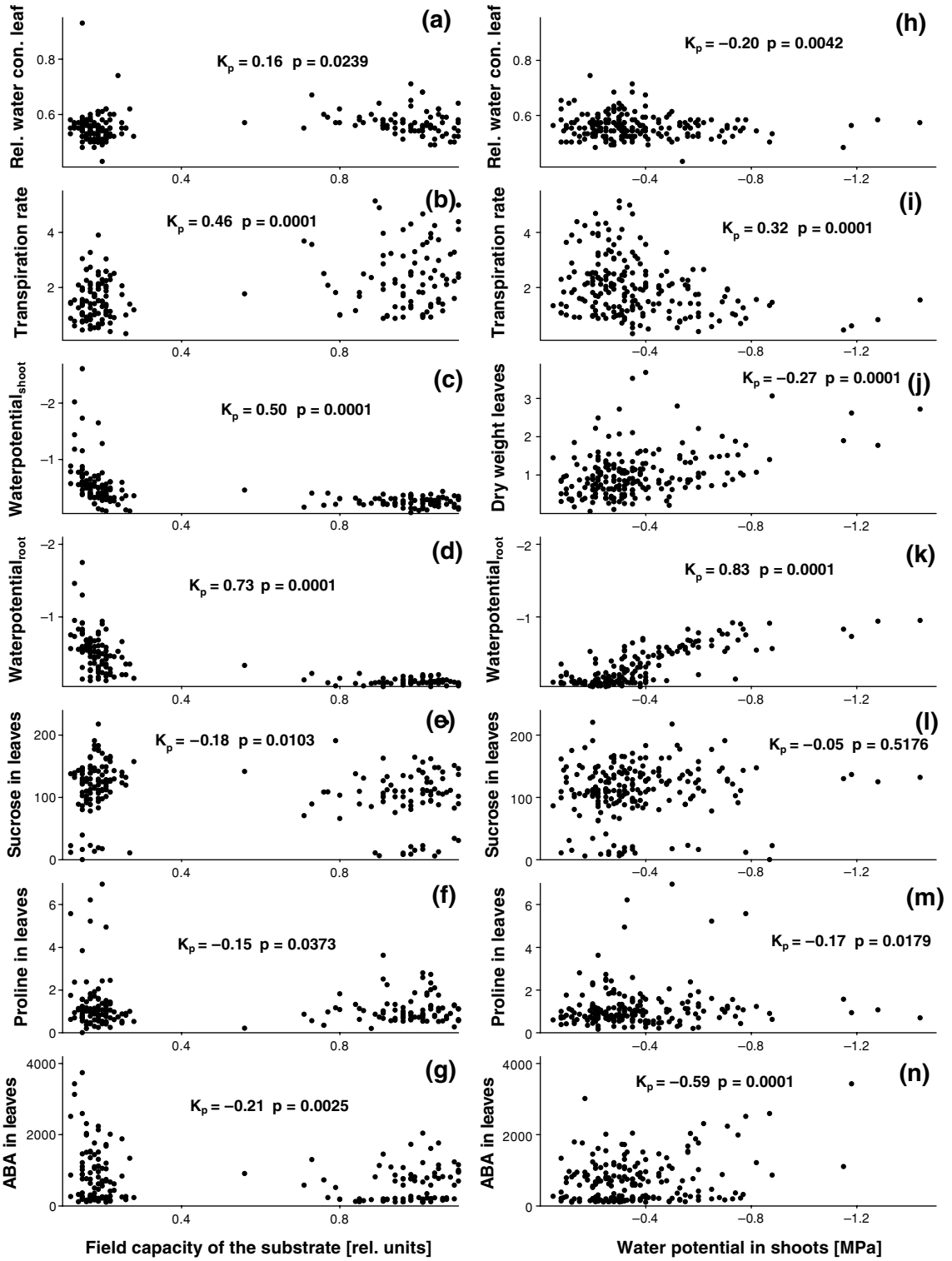

Fig. 6 Relationship in 4.5-month-old-beech seedlings (*Fagus sylvatica*) between water content of the substrate (relative units 0–1) and (a) relative water content in the leaves (relative units 0–1), (b) transpiration rate (mmol m^{-2} s^{-1}), (c) predawn water potential in shoots (MPa), (d) predawn water potential in roots (MPa), (e) sucrose concentration in the dry matter of leaves (μmol g^{-1} dry wt), (f) proline concentration in the dry matter of leaves (μmol g^{-1} dry wt), (g) abscisic acid (ABA) concentration in the dry matter of leaves (pmol g^{-1} dry wt), and water potential in shoots (MPa) with (h) relative water content in the leaves (relative units 0–1), (i) transpiration rate (mmol m^{-2} s^{-1}), (j) dry weights of leaves (g), (k) predawn water potential in roots (MPa), (l) sucrose concentration in the dry matter of leaves (μmol g^{-1} dry wt), (m) proline concentration in the dry matter of leaves (μmol g^{-1} dry wt), (n) ABA concentration in the dry matter of leaves (μmol g^{-1} dry wt). The Pearson correlation coefficients (K_p) and *P*-value for H_0: $K_p = 0$ are given. Some extreme values were omitted from the plots.

Table 2 Homogeneous groups of two way analysis of variances (ANOVA) calculated from data measured in 11 provenances of controls and drought-treated 4.5-month-old beech seedlings

	Homogeneous groups of multiple comparison										
Parameter	red	kloe	small	goer	tbb	bov	black	bad	sees	harz	sont
dry weight											
Leaf	a, b, c	a, b, c	a, b, c	b, c	b, c	a, b, c	a, b, c	a, b	a, b, c	b, c	a, b, c
Axis	b, c, d, e	b, d, e	a, b, c, d, e	c, d, e	b, c, d, e	a, b, d, e	a, b, d, e	a, b, e	a, b, d, e	c, d, e	a, b, d, e
Root	b, c, d, e	b, c, d, e, f	b, d, e	c, d, e, f	c, d, f	b, d, e	b, d, e	a	b, c, d, e	c, d, f	b, d, e
Relative water content											
Leaf	b, c	b, c	a, b, c	b, c	b, c	b, c	b, c	a	b, c	b, c	b, c
Axis	b	b	b	b	b	b	b	a	b	b	b
Root	b, c, e	c, d	b, c, e	b, c, d	b, d	b, c, e	b, c, e	a	b, c, d	c, d	b, e
Transpiration rate	a, c, d	b	a, d	a, c, d	b, c	b, c, d	b, c	a, d	a, d	a, d	a, d
Water potential											
Root	a	a	a	a	a	a	a	a	a	a	a
Shoot	a	a	a	a	a	a	a	a	a	a	a
myo-inositol	b, c, d, e, f	a, b, c, d, e, f	a, b, c, d, e, f, g	a, b, c, d, e, f, g	a, b, c, d, e, f	a, b, c, e, f, g	a, b, c, d, e, f, g	a, b, c, e, g	a, b, c, e, f, g	b, d, e, f	a, b, c, g
Glucose	b	a, c, d	a, c, d	b	a, d	a, c, d	a, c, d	a, c, d	a	b	a, c, d
Fructose	a, b	a, b	a, b, c	a, b, c	a, b	a, b	a, b	a, b, c	a, c	a, b	a, b, c
Sucrose	a, b, c, d, e	b, c, d	a, c, e	a, b, c, d, e	b, c, d	b, c, d	a, b, c, d	a, b, c, e	a, c, e	a, b, c, d	a, c, e
Abscisic acid	a, b, c, d, e	a, b, e	a, b, c, d, e	a, c, d, e	a, b, c, e	a, b, e	a, b, e	a, b, c, d, e	a, c, d	a, c, d	a, c, d
Proline	a	a	a	a	a	a	a	a	a	a	a

The calculations were performed with the GLM procedure of SAS release 6.12 (SAS Institute Inc.) with the following model: 'drought (treatment)', 'provenance' and 'drought (treatment) *provenance' (interactions). Homogenous groups of provenances for the multiple comparison (adjustment Tukey–Cramer) are indicated by letters. Significant effects of the main factors provenance (prov.), drought (treatment) and the interactions (prov. × drought) are given by an asterisk in the figures.

Table 3 Results of the regression analysis for the water potential in the shoots, as influenced by the water potential of the root and dry weight of leaves sliced for the provenances (Model: $Wpot_{shoot}$ = intercept + $Wpot_{root}$ + DW_{leaf})

Provenance	Intercept	Water potential root	Dry weight of leaves	Homogenous groups of slopes for water potential
red	−0.127 ± 0.078 ns	0.732 ± 0.117***	−0.053 ± 0.077 ns	a, b, c
kloe	−0.161 ± 0.068*	0.550 ± 0.123***	−0.014 ± 0.056 ns	a, b, c, d
small	0.180 ± 0.137 ns	0.881 ± 0.222**	−0.381 ± 0.153*	a, b, c, e
goer	−0.116 ± 0.060 ns	0.894 ± 0.151***	−0.032 ± 0.027 ns	a, b, c, e
tbb	−0.161 ± 0.092 ns	0.715 ± 0.116***	−0.013 ± 0.040 ns	a, b, c
bov	−0.219 ± 0.109 ns	0.496 ± 0.132**	0.006 ± 0.109 ns	a, b, c, d
black	−0.103 ± 0.076 ns	0.469 ± 0.195*	−0.125 ± 0.064 ns	a, b, c, d
bad	−0.141 ± 0.053*	0.186 ± 0.119 ns	−0.120 ± 0.078 ns	b, d
sees	−0.014 ± 0.126 ns	1.180 ± 0.120***	−0.014 ± 0.122 ns	c, e
harz	−0.071 ± 0.067 ns	0.796 ± 0.124***	−0.068 ± 0.038 ns	a, b, c, e
sont	−0.054 ± 0.096 ns	0.882 ± 0.129***	−0.091 ± 0.063 ns	a, b, c, e

The estimates of the slopes are given ± SE (H_0: estimate = 0; ns, not significant; : *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.). The homogeneity of the slopes for the different provenances was tested with the help of a general linear model (GLM) with provenances as class variable ($Wpot_{shoot}$ = intercept + $Wpot_{root}$ + DW_{leaf} + provenance (class) + $Wpot_{root}$ provenance + Dw_{leaf} × provenance). Homogenous groups for the slopes of the water potential in the roots are indicated by letters. No differences for the slopes of the dry weight of leaves were detected.

model for the water potential in the shoot the logarithm of water content was replaced by the water potential of the root (as an interface between water status in the rhizosphere and in the shoot) the model was a much better fit with respect to regression coefficient. In addition, the dry weight of the leaves (fitted better than d. wt of total shoot) was a significant effector in the model:

$$Wpot_{shoot} = -0.060 \pm 0.024 - (0.062 \pm 0.016) \times DW_{leaf} + (0.889 \pm 0.043) \times Wpot_{root}$$

($R^2 = 0.714$; $P = 0.013/ < 0.0001/0.0001$; $P_{total} < 0.0001$).

After introducing 'provenance' as a class variable in a general linear model, a significant interaction of provenances with water potential in the roots (effector) was found ($P = 0.0004$) only for the water potential in the shoots. In Table 3 the estimates of the regression analysis sliced for the provenances and the test on homogeneity of slopes are shown. In a number of provenances ('sees', 'harz', 'sont', 'small' and 'goer') relatively high slopes for the effect of water potential in the roots were found (0.796–1.18, homogenous group 'e') which were significantly different from another group (slopes 0.186–0.550, homogenous group 'd').

Discussion

Biometrical data, transpiration and predawn water potential

In the present experiment, beech seedlings were exposed to a 3-wk drought treatment which subsequently resulted in a homogenous water content of the substrate of 18.5% for all provenances used. The seedlings could respond to drought only by means of physiological and metabolic alterations but not by growth reactions, since the first phase of leaf and shoot tip growth was completed at the onset of drought treatment. This was confirmed by dry weight data which were not affected by the drought treatment. Other biometric data which were evaluated at harvest, such as leaf area or diameter of the epicotyl, were not affected by the drought treatment (data not shown).

The relative water content of the plant organs was clearly reduced by drought. Roots were more strongly affected than leaves and axis. The loss of water from tissue may lead to an increase in the concentration of osmotic solutes and result in a lower the osmotic potential of the plant (Heuer, 1994).

As reported repeatedly in earlier studies, transpiration rates were significantly decreased by drought (Fort *et al.*, 1997, 1998; Picon *et al.*, 1997; Cellier *et al.*, 1998; Ray & Sinclair, 1998; Tardieu & Simonneau, 1998; Thomas & Eamus, 1999; Augé *et al.*, 2000; Fotelli *et al.*, 2001; Yao *et al.*, 2001). The lowest transpiration rates were measured in provenances from dry regions (e.g. 'kloe' and 'tbb') and the highest in plants originating from wet habitats (e.g. 'sees' and 'sont'). Beech plants with limited water availability seem to use water more economically. The transpiration rates were not well correlated with other parameters (Fig. 6b,i; no correlation with ABA). This is in agreement with the findings by Augé *et al.* (2000). They compared several tree species and found that stomatal conductance correlated better with environmental parameters than with plant variables and correlated slightly better with hydraulic than with chemical variables within the plants. By contrast Bunce (1999) concluded that stomatal conductance is controlled more by leaf water potential rather than by root or soil signals. Yao *et al.* (2001) found, in partly dried split root experiments with bell pepper plants, that stomatal closure is controlled by hydraulic conditions. The situation may be very complicated and results and conclusions, moreover,

depend on the applied methods and on investigated species (Augé *et al.*, 2000), lines, cultivars or clones.

When the present data set was divided into the provenances, a good correlation was observed between water content and transpiration rate for provenances from prevailing wet habitats ($K_p > 0.7$): 'small', 'sont', 'sees' 'black'. Similarly, for the relation between transpiration and ABA, a fairly good negative correlation was detected for the sensitive provenance ('sont' $K_p = -0.56$; 'sees' $K_p = -0.63$). Tardieu and Simonneau (1998) distinguished plants species with isohydric behaviour (maize and poplar), maintaining a constant water potential and stomatal conductance close to death independent of soil water status, in contrast to anisohydric behaviour (sunflower and barley) with stronger correlation between water potential and stomatal conductance. This classification can be transferred with some restrictions to the present investigations: the provenances from wet habitats have an anisohydric behaviour.

The water potential in shoot and root was lowered significantly by drought. However, this effect was not as strong as expected from earlier experiments in beech (Schraml & Rennenberg, 2000; Fotelli *et al.*, 2001) as well as in oak (Fort *et al.*, 1997; Picon *et al.*, 1997) or *Eucalyptus* (Thomas & Eamus, 1999). The discrepancy between these experiments and the present study may be explained first by the small biomass of the plants and second by the experimental design, since a small amount of water was supplied during the drought period to maintain a low water content. Drought treatment reduced water potential more strongly in roots than in shoots, similar to the relative water content. Hsiao and Xu (2000) concluded that because of water stress, recovery in roots must occur more rapidly and efficiently than in shoots to allow further water uptake and growth.

The lowest water potentials were found in provenances with high annual rainfall (e.g. 'sont' and 'sees') and the highest in provenances with low rainfall ('bov' and 'kloe') after drought treatment. The induced water stress was obviously higher in provenances with high rainfall in the region of origin. The water potential in both root and shoot correlated with the water content of the substrate (Fig. 6c,d). This relationship was even stronger, dividing the data set into provenances for plants originating from dry habitats (e.g. 'bov', 'goer', 'kloe', 'small' and 'tbb', $K_p = 0.7–0.9$; data not shown). As expected, a good correlation was also found between shoot and root water potential ($K_p = 0.83$) (Fig. 6k).

The regression models for the root water potential detected dependency on the water availability only, whereas the shoot water potential depended also on the mass of leaves (dry wt).

The regression analysis revealed differences in the response of the water potential in the shoots due to the water potential in roots were observed, which can be partly related to the climatic conditions in the habitats with higher sensitivity (slopes approximately doubled) of provenances from wet habitats to lower water status (Table 3).

Sugars, proline and ABA

For sugars, a significant increase in sucrose (118%) due to the drought was observed. In the leaves of grasses an increase in sucrose and a decrease in hexoses was found (Volaire *et al.*, 1998), similar to culture of sweet potato cells (Wang *et al.*, 2001). By contrast, Picon *et al.* (1997) observed an increase in hexoses due to drought in *Quercus robur*. However, in both experiments, changes in sugar concentrations due water availability were observed (see also review by Hare *et al.*, 1998). A number of enzyme activities and gene expression on transcriptional level in sugar metabolism were affected by osmotic stress (Volaire *et al.*, 1998; Wang *et al.*, 2000), as well as other metabolic processes (Cellier *et al.*, 1998; Hare *et al.*, 1999), demonstrating the impact of sugars on the defence against osmotic stress.

Relatively low concentrations of hexoses were observed in a group of provenances ('kloe', 'tbb', 'bov' and 'black'), in part with drought-tolerant behaviour. In the remaining provenances, either glucose or fructose was high. In addition, there was obviously an antagonistic distribution of sugars: provenances with high concentrations of fructose or glucose had low concentration of sucrose and vice versa. This was confirmed by a negative correlation between sucrose and fructose ($K_p = -0.71$; $P < 0.001$) but not with glucose. Moreover, under control conditions the relative water content in leaves correlated positively with myo-inositol ($K_p = 0.58$; $P < 0.001$) and negatively with sucrose ($K_p = -0.52$; $P < 0.001$).

Proline concentration in the leaves was also increased owing to drought, but the effect was weak (1.3-fold compared with the control). It was significant only in three provenances ('small', 'bad' and 'sees'; the increase was by a factor of about 2) and the opposite effect was found in one provenance ('black'). Proline accumulation due to drought, or more generally osmotic stress, is a frequently observed response (Heuer, 1994; Volaire *et al.*, 1998; Hare *et al.*, 1999; Schraml & Rennenberg, 2000). However, the proline data did not correlate with other parameters in the whole data set or in controls, in the drought treatment before or after division into provenances. It is known that drought tolerance was found not to be always correlated with proline concentration (Heuer, 1994). Only in the provenance 'sees' from a wet habitat was a correlation for proline with transpiration ($K_p = -0.50$), water potential in the shoot ($K_p = -0.58$) and ABA concentration ($K_p = 0.55$) observed. This is not surprising since Hare *et al.* (1999), after reviewing a number of experiments, concluded that proline accumulation is mediated by both ABA-dependent and ABA-independent pathways.

After water potential, the concentration of ABA in the dry matter of leaves was most strongly affected by drought. Abscisic acid is known to be a stress signal in droughted plants (see review by Hartung *et al.*, 1999). It correlated negatively with the water potential in shoots ($K_p = -0.62$; $P < 0.0001$) in the whole data set. Such correlations were found only in the

drought treatment, demonstrating the importance of ABA in response to drought stress. Tardieu and Simonneau (1998) detected a correlation between water potential in the leaves and ABA in the xylem sap. Nevertheless, no correlations between ABA and water content or transpiration were observed in the present study. Abscisic acid correlated with the transpiration rates only for some provenances, originating from mainly wet habitats ('bad', 'sees', and 'sont'). In *Prunus dulcis* and *Eucalyptus*, Wartinger *et al.* (1990) and Thomas and Eamus (1999) also observed a correlation between ABA and predawn water potential in the leaves but, by contrast, also a correlation to stomatal conductance.

The concentration of ABA in the dry matter of leaves was relatively low in a group of provenances ('kloe', 'tbb', 'bov' and 'black'). In the remaining plants, ABA concentrations were remarkably higher and increased strongly by drought treatment, particularly in provenances from wet habitats ('sees', 'harz', 'sont' but also 'small' and 'goer'). Similarly, in drought stress-sensitive grasses, ABA concentrations in leaf bases were higher than in tolerant species (Volaire *et al.*, 1998). In sunflower, however, drought-induced ABA in the xylem was not correlated with drought tolerance; the stomata in different sunflower lines displayed similar sensitivity (Cellier *et al.*, 1998). In oak, ABA concentrations did not change in leaves or xylem sap, although stomatal conductance, leaf water potential, hydraulic conductance and leaf growth sharply decreased (Fort *et al.*, 1997). In a similar experiment in *Betula pendula* (Fort *et al.*, 1998), ABA in the xylem increased, while ABA flux was the same owing to a lower transpiration stream. Jia and Zhang (1999) concluded that stomatal response is affected more by ABA import via xylem than by accumulation in leaves (and see review of Hartung *et al.*, 1999). It was not possible to collect xylem sap because of the small size of the beech seedlings studied and the experimental design. The metabolic degradation of xylem-borne ABA was also an important factor (Jia & Zhang, 1999).

Identification of drought-tolerant and drought-sensitive ecotypes

The results of the cluster analysis are shown as a dendrogram in Fig. 7. On the right site (the 'dry twig' of the tree), there were two clusters with relatively low distance between provenances, and the distance between the provenances within the clusters was also very small. One of these consist of two provenances 'bov' and 'kloe', where the beech nuts originated from habitats with relative low rainfall per year (680 and 586 mm yr^{-1}, respectively). In these two provenances, the water potential in roots and shoots and the transpiration rate were less or even unaffected by drought. The concentrations of fructose, ABA and proline in leaf dry matter were also remarkably low and not noticeably increased by drought treatment. This behaviour can be linked to a drought stress-tolerant ecotype.

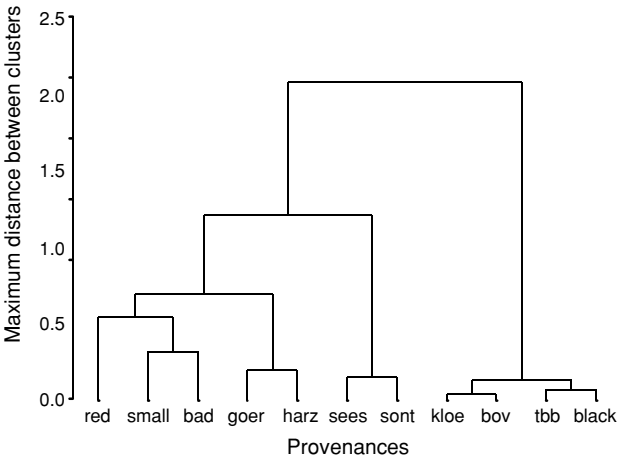

Figure 7 Dendrogram presentation of cluster analysis. The codes of provenances are given in Table 1. The analysis of cluster were performed with the procedure CLUST of SAS release 8.0 (SAS Institute Inc.) using the complete linkage of parameters significantly affected by drought treatment: relative water content in leaves, axes and roots, transpiration rate, predawn water potential in root and shoot, sucrose, proline and abscisic acid concentration in leaf dry matter.

On the other side of the cluster analysis dendrogram the differences between provenances and clusters were markedly greater, indicating a greater heterogeneity of drought sensitivity. However, a homogeneous cluster of two provenances with high annual precipitations between 1150 mm yr^{-1} and 1700 mm yr^{-1} in the habitat was also found here ('sees' and 'sont'). In both provenances, water potential in root and shoot as well as transpiration was strongly decreased and in addition, concentrations of fructose, ABA and proline were high in the controls and significantly increased due to drought.

Three provenances from dry habitats were located within the 'wet twig' of the cluster tree: 'red', 'small', and 'goer'. These provenances showed a relative drought-sensitive behaviour with regard to transpiration and in part to shoot water potential, hexoses, proline and ABA. By contrast, the provenances from wet habitats were always located in the 'wet twig' of the cluster tree. This indicates that there could be an adaptation of beech to dry habitats and creation of drought-tolerant ecotypes. Nevertheless, the classification of the habitats only by annual rainfall may be too crude. Other factors such as evapotranspiration, water capacity of the soil, distribution of rainfall during the vegetation period, relative humidity, availability of ground water, etc., should be taken into account.

General conclusion

Although the overall effect of drought treatment was not as pronounced as expected, and the differences between the provenances were not always significant, the behaviour in response to drought could be partly related to the amount of annual precipitation in the habitat according to a cluster

analysis. All provenances from wet habitats responded like drought-sensitive ecotypes, but not all provenances from dry habitats responded like drought-tolerant ecotypes. Therefore, it is suggested that only beech provenances from dry habitats with qualities of drought-tolerant ecotypes be used for reafforestation. These might be better adjusted to the predicted climate changes.

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