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Anatomical and eco-physiological changes in leaves of couch-grass (*Elymus repens* L.), a temperate loess grassland species, after 7 years growth under elevated CO_2 concentration

A.I. ENGLONER[*,**], D. KOVÁCS[*], J. BALOGH[**], and Z. TUBA[*,**,***]

Department of Botany and Plant Physiology, Faculty of Agricultural and Environmental Sciences, Szent István University, H-2103, Hungary[*]
Departmental Research Group of Hungarian Academy of Sciences, Faculty of Agricultural and Environmental Sciences, Szent István University, H-2103, Hungary[**]

Abstract

Leaf anatomy and eco-physiology of *Elymus repens*, a temperate loess grassland species, were determined after seven years of exposure to 700 $\mu mol(CO_2)\ mol^{-1}$ (EC). EC treatment resulted in significant reduction of stomatal density on both surfaces of couch-grass leaves. Thickness of leaves and that of the sclerenchyma tissues between the vessels and the adaxial surfaces, the area of vascular bundle, and the volumes of phloem and tracheary increased at EC while abaxial epidermis and the sclerenchyma layer between the vessel and the abaxial surface were thicker at ambient CO_2 concentration (AC). Stomatal conductance and transpiration rates were lower in EC, while net CO_2 assimilation rate considerably increased at EC exposure. Contents of soluble sugars and starch were higher in EC-treated couch-grass leaves than in plants grown at AC.

Additional key words: mesophyll; net photosynthetic rate; phloem; sclerenchyma; stomatal conductance; stomatal density; tracheary; transpiration rate; water use efficiency.

Introduction

Future increases in atmospheric CO_2 concentration (Houghton *et al.* 1990) will have a direct effect on the productivity of plants through changes in the functioning of the photosynthetic apparatus (Long 1991). As a consequence of increased photosynthesis at elevated CO_2, dry matter production of terrestrial C_3 plants is expected to increase (Cure and Acock 1986). However, in plants which do not have enough sink capacity the increased saccharide content can lead to the downward regulation of photosynthesis as it is frequently reported (Jarvis 1993). Both the increased photosynthetic production and saccharide accumulation could have anatomical consequences. Compared to the large number of papers studying the effects of EC, plant anatomy is far less investigated. However, due to the central role of leaves in photosynthesis, the knowledge, for example, of adaptations in leaf anatomy to changing atmospheric CO_2 concentration is a crucial issue.

The increase of leaf thickness at EC is frequently observed (Kelly *et al.* 1991, Pearson *et al.* 1995, Zhu *et al.* 1997, Bandara *et al.* 1998, Atkin *et al.* 1999, Pritchard *et al.* 1999, Tipping and Murray 1999, Rengifo *et al.* 2002), which is generally explained as the result of larger cell size rather than increased cell division (Radoglou and Jarvis 1990, Pritchard *et al.* 1999). Thicker adaxial and abaxial epidermises were observed in poplar clones grown at higher atmospheric CO_2 concentration (Radoglou and Jarvis 1990), while Thomas and Harvey (1983) described no significant differences in epidermal thickness. In the mesophyll, increased areas of both palisade and spongy layers and vascular tissues are reported in plants growing at elevated CO_2 (Madsen 1973, Radoglou and Jarvis

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[***]Author for correspondence. Fax: (+36)28410804, e-mail: tuba@fau.gau.hu

Abbreviations: AC – present-day CO_2 concentration; EC – elevated CO_2 concentration; E – transpiration rate; g_s – stomatal conductance; OTC – open top chamber; P_N – net CO_2 assimilation rate; PCA – principal component analysis; WUE – water use efficiency.

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1990, Pritchard *et al.* 1999).

However, according to Thomas and Harvey (1983), the enhanced leaf thickness in soybean was the result of increased cell division, *i.e.* an extra layer of palisade cells. Both increasing and decreasing phloem area and intercellular spaces in plants from CO_2 enriched environment have been reported (Radoglou and Jarvis 1990, Pritchard *et al.* 1999) while Byrd and Brown (1989) observed no significant anatomical differences.

Stomatal aperture and stomatal conductance (g_s) are also often reduced in long-term high CO_2 treated plants (Rogers *et al.* 1983), which in turn results in lower rates of transpiration (E). However, data are contradictory whether the decreased g_s is a consequence of the decreased stomata number. Elevated CO_2 did not affect the stomatal density of *Geranium* spp. (Kelly *et al.* 1991), *Rumex obtusifolius* (Pearson *et al.* 1995), and other species (Thomas and Harvey 1983, Bettarini *et al.* 1998), whereas several plants have shown a decline in stomatal density due to elevated CO_2 (Madsen 1973, North *et al.* 1995, Bettarini *et al.* 1998). Tipping and Murray (1999) reported both increased and decreased stomatal frequency in the *Panicum* genus.

One of the most widely reported effects of elevated CO_2 is the increase of water use efficiency, WUE (Rogers *et al.* 1983, Morison and Gifford 1984, Jarvis 1993, Amthor 1995, Beerling and Woodward 1995, Pearson and Brooks 1995, Šantrůček and Sage 1996; for review see Saralabai *et al.* 1997), which can be of particular importance for plants in arid and semi-arid conditions (Sionit *et al.* 1981), like in the Carpathian basin.

As the anatomical responses are frequently contradictory and the data on the long-term responses of natural vegetation including perennial herbaceous grasslands species to elevated atmospheric CO_2 concentrations are particularly sparse (Breymeyer *et al.* 1996), further examination is highly required. In this paper we report the leaf anatomical and eco-physiological responses to 700 μmol(CO_2) mol^{-1} (EC) in a widespread C_3 species *Elymus repens* from a xeric temperate loess grassland after 7 years of exposure. The possible interrelationships between the ecophysiological and anatomical changes are also presented.

Materials and methods

Couch-grass [*Elymus repens* (L.) Gould.] is a perennial weed with large stolons, long leaf blades which may become rolled, and short ligules but well developed auricles. Spikelets are placed with their broader side to the axis of the spike, glumes and lemmas are short-awned or sharply pointed. The investigated species is a dominant member of the temperate loess grassland (steppe) vegetation (*Salvio-Festucetum rupicolae pannonicum* Zólyomi) (Zólyomi and Fekete 1994). The climate is a temperate continental with hot dry summers; mean annual precipitation 500 mm or less; annual mean temperature of 11 ºC; and large annual amplitude of temperature changes (22 ºC).

Grassland monoliths with their original soils in 40 cm depths were transplanted into Open Top Chambers (OTC's) (Tuba *et al.* 1994, 1996) in the Global Climate Change and Plants Experimental Ecological Research Station (Department of Botany and Plant Physiology, Faculty of Agricultural and Environmental Sciences, Szent István University, Gödöllő, 28 km east of Budapest, Hungary). EC was maintained for seven years at 700 μmol mol^{-1} and the ambient CO_2 concentration (AC) at 350 μmol mol^{-1}.

For counting stomatal density, replicas were taken from the middle part of adaxial and abaxial epidermises of leaves of same age and size of 10-10 control and treated couch-grass plants, respectively. 10 measurements per replica were carried out. For mesophyll investigations, 5-5 leaves of different plants were selected and 10 cross sections were taken from the middle part of each leaf. The investigated tissue features of computerised (Demeter *et al.* 1995) sections are as follows (Fig. 1): the thickness of leaves at the vessels and between them, the thickness of abaxial epidermis, and that of the sclerenchyma tissues between the vessels and the adaxial and abaxial surfaces, respectively; the area of the vascular bundle; and the volumes of the phloem and the tracheary.

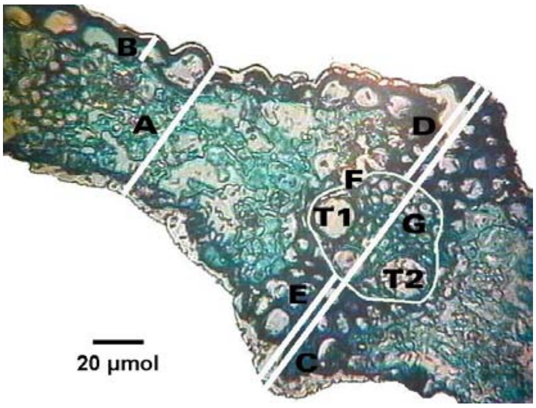

Fig. 1. The investigated tissue features in cross sections of couch-grass leaves. A: leaf thickness between the vessels; B: thickness of abaxial epidermis; C: leaf thickness at the vessels; D: thickness of the sclerenchyma tissue between the vessels and the abaxial epidermis; E: thickness of the sclerenchyma tissue between the vessels and the adaxial epidermis; F: area of the vascular bundle; G: area of the phloem; H: average area of the two trachearies [H = (T1 + T2)/2].

Leaf gas-exchange measurements, namely g_s, net CO_2 assimilation rate (P_N), and E were carried out *in situ*, in the OTS's in the morning hours in seven to nine replicates. The measurements and calculations were carried out as described in Tuba *et al.* (1994, 1996). Water use efficiency (WUE) is given as ratio of P_N to E. Saccharide (starch and soluble sugars) content was measured as described by Tuba *et al.* (1994) as a modified method from Dubois *et al.* (1956) and McCready *et al.* (1950).

Data were evaluated by *t*-tests and standardised principal components analysis (PCA) using the *SYN-TAX* computer program package (Podani 1994, 1997). PCA is an ordination technique, which does not assume *a priori* groups of observations, therefore it is useful to obtain a summary of the correlation structure of variables and objects based on a pooled data set. Its results are illustrated by biplot-like scattergrams in which axes are the principal components, the original variables are shown as vectors, and groups of objects may be represented by convex polygons. The length and directionality of vectors provide insight into the correlation structure of variables and principal components.

Results and discussion

At both CO_2 levels, adaxial epidermis of couch-grass had much more stomata (165 and 68 per mm^2, respectively) than the abaxial one (36 and 23 per mm^2, respectively) (Fig. 2). EC resulted in significant reduction of stomatal density on both surfaces of leaves. This decrease, however, was greater on the upper epidermis than on the lower one (60 and 40 %, respectively).

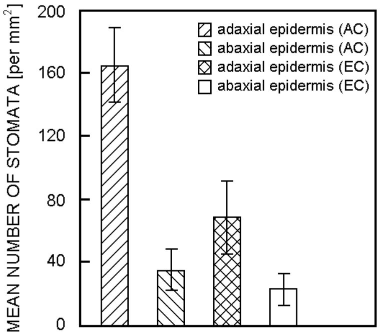

Fig. 2. Mean number of stomata on the adaxial and abaxial sides of *Elymus repens* leaves grown at ambient (AC) and elevated (EC) CO_2 concentrations. *Bars* represent standard deviation.

Table 1. Average thicknes [µm] and area [μm^2] of the investigated tissue features in *Elymus repens* grown at ambient (AC) and elevated (EC) CO_2 concentrations. Tissue features represented by letters A-H are detailed in Fig. 1. Mean ± SD; $^*p<0.05$, $^{**}p<0.01$, n.s. = not significant.

Tissue feature	AC	EC	
A	76.56±15.52	81.08±21.25	ns
B	16.04±5.89	11.00±2.14	**
C	153.33±18.87	166.73±13.57	**
D	46.35±9.19	42.50±5.34	*
E	42.44±6.43	47.03±8.51	**
F	3098.35±643.84	4069.35±421.67	**
G	733.40±214.03	980.35±186.37	**
H	347.53±66.32	368.13±45.02	*

Average thicknesses and areas of the investigated mesophyll tissues are presented in Table 1. Except for the leaf thickness between the vessels (A), all features significantly differed at the two CO_2 concentrations. Abaxial epidermis (B) and the sclerenchyma layer between the vessel and the abaxial surface (D) was thicker in leaves grown at AC, while the other five parameters were larger at EC.

Based on the investigated histological features, the separation of couch-grass leaves exposed to AC and EC were evaluated by standardised principal components analysis (Fig. 3). In spite of some overlap between the polygons, the biplot-like scattergram discloses clear separation of the two groups of leaves. Most vectors representing the PCA variables (*i.e.* the anatomical characters) show high positive correlation with the polygon of treated leaves. The leaf thickness at the vessels (shown by C in the scattergram), the sclerenchyma layer between the vessel and the adaxial epidermis (E), the area of the vascular bundle (F), the volumes of the phloem (G), and those of the tracheary elements (H) were larger in couch-grass plants grown at EC. Variables A, B, and D tended towards a certain group of control leaves (in right bottom of the polygon 1) indicating that these parameters, namely the thickness of leaf between the vessels, that of the abaxial epidermis, and the sclerenchyma layer between the vessel and the abaxial epidermis could reach larger extent in control leaves.

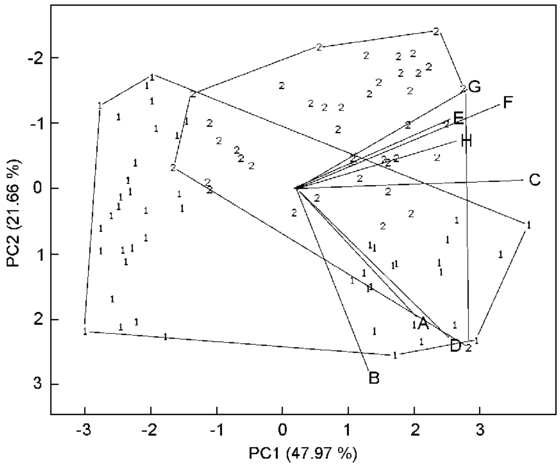

Fig. 3. PCA ordination of the investigated tissue features of *Elymus repens* leaves. Objects enclosed by polygons are: couch-grass leaves grown at (1) ambient or (2) elevated CO_2 concentrations. Tissue features represented as vectors A-H are detailed in Fig. 1. For explanation, see the 'Materials and methods' section.

Considering the physiological responses (Table 2), g_s and E values were lower at EC, while P_N considerably increased. The WUE values, therefore, were higher for plants grown at EC. Both the concentration of soluble sugars and the concentration of starch were considerably higher in plants grown at EC.

Table 2. Stomatal conductance, g_s [mol m^{-2} s^{-1}], net CO_2 assimilation rate, P_N [μmol(CO_2) m^{-2} s^{-1}], transpiration rate, E [μmol(H_2O) m^{-2} s^{-1}], water use efficiency (WUE = P_N/E), and soluble sugar and starch contents [g kg^{-1}] in *Elymus repens* grown at ambient (AC) and elevated (EC) CO_2. Means ± SD.

	AC	EC
g_s	0.05±0.02	0.04±0.04
P_N	5.82±2.08	17.09±9.19
E	1.34±0.54	1.10±0.83
WUE	5.68±4.56	25.25±25.93
Soluble sugars	130.28±1.62	224.57±11.77
Starch	120.99±7.32	181.73±22.66

The decreased E and g_s in *E. repens* grown at EC should be related to the decreased stomata density. Such reduction of stomatal density was reported in other high CO_2 plants (*e.g.* Madsen 1973, North *et al.* 1995, Bettarini *et al.* 1998). Although this decrease in *E. repens* occurred on both surfaces of leaves, it was greater on the adaxial epidermis. Decreasing g_s and E were also observed in EC plants indicating a downward stomatal acclimation (Jarvis 1993, Tuba *et al.* 1996) of couch-grass. This type of acclimation to EC in g_s has been found in other plant species also (DeLucia *et al.* 1985, Jarvis 1993). However, the rates of decrease in g_s and E observed in this species are less pronounced as it was reported in many cases (Jarvis 1993) especially for grasses (Nagy *et al.* 1997, and relevant references cited in it). Therefore the increase in WUE in the present study was primarily due to an increase in P_N and partially to a decrease in E.

Increased leaf thickness of couch-grass at EC (in accordance with Kelly *et al.* 1991, Pearson *et al.* 1995, Atkin *et al.* 1999, Bandara *et al.* 1998, Tipping and Murray 1999, Zhu *et al.* 1997) can be in our case related to the increased amount of accumulated soluble sugars and starch, specifically in mesophyll and vascular tissues. Decrease in thickness of abaxial epidermis and sclerenchyma layer between the vessel and the abaxial epidermis in EC plants, however, requires further investigation. It is also needed to quantify what proportion of the photosynthates is translocated to the roots under EC.

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