Drought adaptation ecophysiological mechanisms of two annual legumes on semi-arid Mediterranean grassland

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The drought adaptation of two annual legumes, Medicago minima (L.) Bartal and Onobrychis aequidentata (Sibth and Sm) D’Urv., was studied at the H$_2$O and CO$_2$ efficient use level over the drought period investigating whether, besides the already known drought adaptation mechanism, annual legumes are dealing with drought by controlling stomatal and mesophyll conductance. O. aequidentata maintained higher leaf water potential and leaf hydraulic conductance than M. minima and consequently exhibited better water balance. The data revealed that these two legumes exhibit a completely different photosynthetic behavior. The photosynthetic rate in M. minima is mainly depended on stomatal conductance while in O. aequidentata seems to be depended both on stomatal and mesophyll conductance. O. aequidentata seems to be more efficient having significantly lower values of the ratio internal CO$_2$ concentration to stomatal conductance at the same value of leaf water potential compared to M. minima. This verifies the higher values of mesophyll conductance and consequently the higher values of carboxylation efficiency. Each one of the two species has different traits to cope with the adverse environmental conditions. The differentiation of drought adaptation mechanisms seems to be a major key for co-existence of several plants of the same life form in Mediterranean grasslands.

Key words: Photosynthesis, transpiration, stomatal conductance, mesophyll conductance, carboxylation efficiency, Medicago minima, Onobrychis aequidentata.

INTRODUCTION

Arid and semi-arid Mediterranean grasslands are very rich in annual species (Koukoura, 2007). Their existence is usually associated with ruderal environments undergoing human disturbance and long summer drought (Jackson and Roy, 1986). Among annual species, several genera of legumes such as Medicago, Trifolium and Onobrychis predominate in the Mediterranean region (Tutin et al., 1968; Mrfat-Vukelic et al., 2000; Koukoura, 2001) and present high genetic diversity (Acar et al., 2001; Fresnillo Fedorenko, 2001). Legumes in general are desirable for introduction to natural pastures due to their ability to produce high quality forage production and to increase nitrogen fixation (Pérez-Corona et al., 1998).

Drought conditions are considered to be the most important environmental factor of plant survival in semi-arid Mediterranean grasslands. In arid areas, annual plants combine a relatively short life cycle with a high growth rate during the wet season to avoid drought, adjusting sink/source allocation by increasing root growth and by decreasing leaf area (Rivero et al., 2007).

Traditionally annual plant species have been considered to be stress avoiders by escaping unfavourable season in the form of dormant seeds. However, plant response to stress is a continuum from stress avoidance to stress tolerance, the latter being more expressed in slowly growing perennial plants (Gurevitch et al., 2002;
Chaves et al., 2003). The maintenance of favourable internal water status and plant functions at low leaf water potential is the main physiological process that contributes to the maintenance of high production under drought periods (Blum, 1996).

In field conditions, there is a positive relationship between water use and dry matter production (Philipp et al., 2007). Water use efficiency (WUE) is a very important index of the relationship between water consumption and biomass production (Wang et al., 2007) relating two physiological parameters: the photosynthetic rate (A) and the transpiration rate (E) (Bacon, 2004). The photosynthetic rate, which is responsible for dry matter production, is regulated by stomatal (gs) and mesophyll conductance (gm). The transpiration rate is predominantly controlled by gs at a given vapour pressure difference. These two parameters (gs, gm) are very important as far as improvements in WUE are concerned (Udayakumar et al., 1998). Due to difficulties to determine gm, Sheshshayee et al. (1996) proposed the ratio internal CO2 concentration to stomatal conductance (Ci/gs) to be used as a good reflection of the mesophyll efficiency for carboxylation. So, carboxylation efficiency (CE) seems to be directly related to gm but at the same time might be indirectly related to gs (Guo et al., 2006).

It is well known that annual legumes such as Medicago minima (L.) Bartal and Onobrychis aequidentata (Sibth and Sm) D’Urv., frequently present in arid and semi-arid grasslands and become dominant species under conditions of aridity or overgrazing (Merou and Papanastasis, 2000; Mrfat-Vukelic et al., 2000; Fresnillo Fedorenko, 2001). These species exhibit an efficient water use to confront drought periods by completing their life cycle, before severe soil and plant leaf water deficit is established. This drought mechanism is realized using two kinds of adaptations: (a) rapid phenological development and (b) developmental plasticity (Gurevich et al., 2002). However, whether the above annual species, beside the drought avoidance mechanism have the ability of H2O and CO2 efficient use controlling the gs and gm is not known.

This paper aimed firstly to investigate whether the two annual legumes M. minima and O. aequidentata differ at the H2O and CO2 efficient use level over the drought period and secondly, whether besides the already known drought adaptation mechanism, annual legumes also exhibit physiological mechanisms, dealing with drought by controlling gs and gm.

MATERIALS AND METHODS

Study area and climate

The research was conducted in 1994 at low elevation Mediterranean grassland in Northern Greece. The experimental area (40°58′N, 28°01′E, altitude 170 m a.s.l), located 25 km north-east from Thessaloniki, was mainly consisting of forage species and few patches of shrubs such as Pyrus amygdaliformis Vilm, Crataegus oxycantha and Jasminum fruticans L.

The climate of the area is characterized as Mediterranean semi-arid. The mean annual air temperature ranged from 4.4°C (January) to 24.7°C (August) and the average annual precipitation was
409 mm (1983 - 1993). Changes in microclimatic parameters photosynthetic photon flux density (PPFD) and vapor pressure deficit (VPD) were obtained during the period of measurements (Figure 1). The values of PPFD and VPD given are averages of five measurements that have been taken on the date and time that plant physiological parameters were measured (see below).

Physiological measurements

Leaf water potential (Ψ), relative water content (RWC), net photosynthetic rate (A), transpiration rate (E) and stomatal conductance (gs) were measured in the two annual legumes M. minima and O. aequidentata. These wild annual species were chosen because they are important, high quality forage species during spring and early summer in the low elevation Mediterranean grasslands. Details on the morphological characteristics of the above two legumes are given by Tutin et al. (1968). Seasonal measurements were obtained on clear sunny days around solar noon (12.00 - 14.00 h) at approximately 15 days intervals. Also, the diurnal pattern of leaf water potential and transpiration rate were measured on three phenological stages (early, vegetative, reproductive) and were taken on a single day every 3 h from 06.00 - 17.30 h local time. All measurements were obtained on mature and intact fully expanded upper leaves. For each parameter, the values presented are averages of five plants. Approximately 10 days after fruit maturation the aerial parts of M. minima and O. aequidentata had been completing the biological life cycle.

Leaf water potential, in both species, was measured using the pressure chamber technique (Koide et al., 1991). The RWC was determined on 4 mm discs from leaves similar in age and orientation, and from the same plant to those used for the Ψ determination. Leaf discs were transferred to the laboratory and their fresh weight (FW) was determined in a saturated atmosphere. Then, leaf discs were floated in distilled water at 4°C for 12 h until reaching a constant weight. Leaf discs were weighed and this weight was taken as saturated weight (SW). The same samples were dried at 70°C for 48 h and then reweighed. The new weight was taken as dry weight (DW). The RWC was calculated by the following formula: RWC = [(FW – DW) / (SW - DW)]*100 (Iannucci et al., 2002).

Furthermore, at the same time A, E, gs and internal CO₂ concentration (Ci) were measured with the portable infrared gas analyser system LI-6200 (LI-COR Lincoln, NE). We also calculated (a) the apparent carboxylation efficiency (CE) by using the formula: CE = A/Ci (Flexas et al., 2001), (b) the ratio Ci/gs to obtain the mesophyll efficiency (Sheshshayee et al., 1996), and (c) the leaf hydraulic conductance (Ks) according to Hubbard et al. (2001).

Statistical analysis

To determine differences in the ecophysiological responses of the two species (first factor) during the growing season (second factor) we performed a two way analysis of variance (ANOVA) on all parameters studied (Steel and Torrie, 1980). Regression analysis was used to determine the relationship between Ci/gs and Ψ for both species; t-test was applied to compare two means.

Moreover, correlation analysis was used to determine the relationship between A, gs and Ci/gs. All analyses were performed using the SPSS (16) statistical package.

RESULTS

Photosynthetic Photon Flux Density and VPD during the experimental period ranged from approximately 654 (first day of the measurements) to 1738 μmol.m⁻².s⁻¹ (last day of the measurements) and from 1.2 to 3.65 kPa respectively (Figure 1).

The two way ANOVA reveals significant differences (F ≥ 37.23, df = 1, all P ≤ 0.0001) between the two species for all tested parameters (that is, A, E, gs, Ψ, Ci/gs, and CE). Also, the time of the season significantly affected (F ≥ 57.01, df = 3, all P ≤ 0.0001) all parameters measured. The interaction between time of the season and species was significant for all parameters above (F ≥ 13.06, df = 3, all P ≤ 0.0001), indicating differential physiological response of the two species throughout the season.

Ψ showed a declining trend (gradual at the beginning and steeper at the end) during the season in both species (Figure 2). However, O. aequidentata expressed significantly higher values of Ψ as well as higher Ks (Figure 3) compared with M. minima (F = 7.4 df = 1, P ≤ 0.01). Only at the end of the growing season (last day of the measurements) Ψ values were similar for the two species (t = 1.16, df = 8, P > 0.05) at approximately -1.5 MPa. At the same low value of Ψ, O. aequidentata exhibited a higher RWC compared to M. minima (Figure 4).

Patterns of E in relation to Ψ (Figure 5a) were similar for the two species until -1.0 MPa (t ≤ 2.1, df = 8, P > 0.05). Then, E of O. aequidentata showed an increasing tendency while it remained relatively stable in M. minima (Figure 5a). At the end of the growing season, O. aequidentata exhibited significantly higher E than M. minima (t = 4.85, df = 8, P < 0.05). The pattern of gs and A in relation to Ψ was similar only in M. minima (Figure 5b, c). As the growing season proceed and Ψ apparently decreased gs of M. minima and O. aequidentata increased in relation to Ψ until -1.0 MPa. Stomatal conductance and photosynthetic rate were both higher in O. aequidentata (Figure 5b, c) throughout the season. There is a positive correlation (r = 0.911, P < 0.001) between A and gs for M. minima and a negative correlation (r = -0.541, P < 0.01) between A and Ci/gs for O. aequidentata.

O. aequidentata at the same Ψ showed lower Ci/gs ratio than M. minima (Figure 6) and higher CE than M. minima up to -1.0 MPa (Figure 7).

DISCUSSION

In order to answer the basic questions of our study, we should focus on the following three basic parameters: the Ψ that expresses the internal water status, the gs that controls the E and the A and the gs that controls the carboxylation efficiency. The relative water content may reflect the balance between water supply to leaf tissue and transpiration rate through its relationship to cell volume (Garcia et al., 2002). The observed higher seasonal values of Ψ (Figure 2) and the higher RWC at the same Ψ in O. aequidentata (Figure 4) could be probably attributed to the higher hydraulic conductance, since E
was higher in *O. aequidentata* compared to *M. minima* (Figure 5a). Indeed, *O. aequidentata* presented higher leaf hydraulic conductance over the growing season compared with that of *M. minima* (Figure 3). The lower values of E in *M. minima* induced by lower values of $g_s$ (Figure 5a, b) can be attributed to the fact that *M. minima* has the ability to decrease $g_s$ at high VPD (Figure 1, 5b). The increase of transpiration rate following increase in VPD and gradual decrease (and its relatively stabilization) at higher VPD is known as feed forward response (Mott and Parkhurst, 1991; Medrano et al., 2002).

The favourable internal water balance of *O. aequidentata* compared to that of *M. minima* (Figures 2, 4) probably implies a higher photosynthetic capacity (Chaves, 1991) in *O. aequidentata*. In fact, the results (Figure 5c) suggest that this is valid. Two factors probably cause the significant differences in photosynthetic rate between the two annual species: $g_s$ and/or $g_m$ well expressed by $C_i/g_s$ or CE (Sheshshayee et al., 1996) (Figures 6, 7).

In most cases, stomatal conductance follows a pattern
similar to that of photosynthesis (Quick et al., 1992). This is only the case in *M. minima* (Figure 5b, c, \( r = 0.911 \)), and it is in agreement with Cornic (2000) who suggests that under field conditions the stomatal closure plays by far the main role in the decline in leaf photosynthesis and that the photosynthetic machinery remains intact thereby allowing the leaf to respond rapidly to changes in environmental water status. Cornic (2000) apparently considers the stomatal conductance as the main regulator of the photosynthetic machinery. The fact that in *O. aequidentata*, the \( A \) and \( g_s \) pattern in relation to \( \Psi \) was not comparable beyond -1.0 MPa (Figures 5 b, c) suggests that under 15\% water deficit (Figure 4) the decrease of photosynthetic rate does not keep up with \( g_s \) and consequently the role of \( g_s \) seems to become less important. The observed difference in \( g_s \) (Figure 5b) might explain the relatively higher values of the photosynthetic rate in *O. aequidentata* at high \( \Psi \). On the other hand, the higher photosynthetic rate of *O. aequidentata* may be attributed to higher mesophyll efficiency (Loreto et al., 1992; De Lucia et al., 2003), considering that \( g_m \) limits the photosynthetic rate at the same magnitude as \( g_s \) (Warren et al., 2003).

Thus, the two legumes exhibit a completely different photosynthetic behavior. The photosynthetic rate in *M. minima* is mainly depended on \( g_s \). This parameter in *O. aequidentata* seems to depend on both \( g_s \) and \( g_m \). From the productive point of view *O. aequidentata* seems to be more efficient (Sheshshayee et al., 1996; Karatassiou, 1999; Bindumadhava et al., 2007) having significantly lower values of \( C_i/g_s \) at the same value of \( \Psi \) compared to *M. minima* (Figure 6), a fact that verifies the higher values of \( g_m \) and consequently the higher values of \( CE \) (Figure 7). The significant negative correlation between \( A \) and \( C_i/g_s \) suggests that the \( A \) in *O. aequidentata* is controlled by mesophyll efficiency (Krishna Prasad et al., 1996). It is well known that the plants are divided in photosynthesis stomatal and non stomatal conductance related (Flexas and Medrano, 2002). Udayakumar et al. (1998) takes it one step ahead considering that there are plants depended on stomatal conductance (\( g_s \)- dependent type) and other plants depended on mesophyll conductance of \( CO_2 \) (\( g_m \)- dependent type). According to this classification *O. aequidentata* seems to belong to the second group.

Consequently, the two annual legumes exhibited different behaviors under the same semi-arid environmental conditions. Our data could not support the existence of physiological mechanisms in *M. minima* to deal with drought conditions. Nevertheless, our data suggest that *O. aequidentata* involves, besides the drought avoidance, physiological mechanisms by controlling \( g_s \) and \( g_m \) that enables it to withstand more efficiently the drought period. Therefore, some wild annual legumes obviously involve in their ecophysiological process, not only, the known phenological mechanism but also the physiological function of \( g_m \) and \( g_s \) controlling \( E \) (water balance), \( A \) and...
CE (biomass production), a drought adaptation mechanism which is involved to ecophysiological process of perennial plants. Each one of the two species has different traits to cope with the adverse environmental conditions. The differentiation of drought adaptation mechanisms seems to be a major key for co-existence of a number of plants from the same life form in Mediterranean grasslands.

Figure 5. Relationship between leaf water potential (Ψ) and (a) transpiration rate (E), (b) stomatal conductance (gs), and (c) photosynthetic rate (A) for *O. aequidentata* (empty symbols) and *M. minima* (filled symbols). Values are means ± SE of five replicates per species.
Figure 6. Relationship between mesophyll efficiency ($C_{i}/g_s$) and leaf water potential ($\Psi$) for *O. aequidentata* (empty symbols) and *M. minima* (filled symbols). Values present means ± SE of five replicates per species.

Figure 7. Relationship between apparent carboxylation efficiency (CE) and leaf water potential ($\Psi$) for *O. aequidentata* (empty symbols) and *M. minima* (filled symbols). Values present means ± SE of five replicates per species.