PHOTOSYNTHETIC CHARACTERISTICS OF CAROB TREE (CERATONIA SILIQUA L.) AND CHEMICAL COMPOSITION OF ITS FRUIT ON DIURNAL AND SEASONAL BASIS

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Abstract

The photosynthetic capacity of carob tree (Ceratonia siliqua L.) and the quality indices of the fruits growing under natural conditions, at Athens and Rethymno in Greece, were measured on diurnal and seasonal basis. The highest photosynthesis is observed during May compared to June and October, which is correlated well with the high developmental rates, the optimal temperatures and water availability. C. siliqua growing at Athens site attained higher photosynthesis parameters than trees growing in Rethymno. The decline of CO2 assimilation rate during the hot and dry season was rather a non-stomatal effect, since it is not accompanied by low stomatal conductance. Photoinhibition damage during June with concomitant reduction in electron transport rate in Photosystem 2 and Photosystem 1 may occur. Despite the low soil water and the extremely high air temperatures during the June, carob showed an important capacity to control water loss (A/gs). The total sugar content significantly increases with seasonal changes and reaches its highest value in October when pods are fully ripe, while polyphenols and proteins gradually decrease. The climatic conditions prevailing in the Mediterranean basin do not threaten the survival of C. siliqua.

Introduction

The countries surrounding Mediterranean basin face common developmental challenges in agriculture, the environment, and water (Sakcali et al., 2008). Scientists agree upon on the fact that it is urgent to preserve the natural ecosystems in the Mediterranean, whose resources can contribute significantly to the food shortage in the region. The basin experiences an arid climate and is characterized by strong seasonality, with mild and rainy winters and long warm and dry summers. Plant diversity in the region is exposed to diverse environmental stresses, in particular drought, high evaporative demands, high temperatures during summertime and low temperatures, combined with relatively high light intensity during wintertime and low nutrient contents in the soil (Rubio-Casal et al., 2010). Most climate change scenarios suggest that there will be an increase in aridity in many areas of the globe. Models of global climate predictions indicate that a further 1.1-6.4°C warming is expected for the Mediterranean basin during this century. Therefore, plants are likely to experience increasing high temperature stress. This is attracting greater attention towards the research into plant responses to these stressful conditions (Houghton et al., 2001; Vertovec et al., 2001; Sakcali & Ozturk, 2004). High and low temperature extremes in the Mediterranean may be an important constraining factor for plants, restricting productivity and influencing their distribution (Larcher, 2000). On the other hand, high summer temperatures may provoke a decrease in net photosynthetic rate, mainly due to overheating of leaves (Rubio-Casal et al., 2010). In fact, the photosynthetic behaviour of plants has started attracting much attention lately. The ecophysiological changes related to a temperature increase are closely related with the plant water content (Vardar & Ozturk, 1972; Cornic, 2003; Pietro et al., 2009).

Among the typical representatives of the rich plant diversity in the basin Ceratonia siliqua L. (carob tree) and Capparis spinosa L. occupy the first places as naturally growing multipurpose plant resources together with olive (Vardar et al., 1972; Ozturk & Vardar, 1975; Ozturk et al., 2010; Vekiari et al., 2011; Arefi et al., 2012). This species is of considerable economic importance. The edible pods are used as a cocoa substitute, being rich in sucrose, polyphenols and proteins but poor in fat, they also contain vitamins A and B and several minerals (Calixto & Canellas, 1982; Ozturk et al., 1995; Battle & Tous, 1997). Due to their chemical composition, the carobs are used as food as well as medicine (Ozturk et al., 2010). The polyphenols, because of their strong antioxidant activity, play protective role against oxidative stress thus, they have potential health benefits (Calixto & Canellas, 1982; Arts & Hollman, 2005).

The plant is well adapted to the conditions of the Mediterranean climate (Nunes et al., 1992). The drought resistance strategies in plants with leathery leaves like carob have been studied by water relation parameters measured in leaves and or shoots, like leaf water status, evaporative flux, shoot or leaf vulnerability to xylem cavitation (Salleo & Lo Gullo, 1990; Harvey & Van Den Driessche, 1997; Salleo et al., 2000; Sakcali & Ozturk, 2004). Previous work with 3 to 4-year old carob trees grown in pots has focused on the effects of temperature, light and drought on gas exchange, stomatal behaviour and photosynthetic characteristics (Rhzopoulosou & Nunes, 1981; Nunes & Matos, 1987). These results suggest that the species has a high photosynthetic productivity and is particularly tolerant to low soil water content. The gas exchange characteristics in combination with chemical characteristics of adult trees growing under natural conditions have not been investigated before. The aim of this investigation therefore was to study the impact of season and time of the day on the photosynthetic capacity of the leaves and the quality indices of the pods. The results obtained will serve to make all the previous results more precise and can be used in increasing the potential growth of carob in these environments.
Materials and Methods

Two sites were selected in Greece, the first one being an urban field in Athens and second one a rural field at Rethymno Mountain in Crete, where wild type *Ceratonia siliqua* L. (carob tree) grow widely (Chartzoulakis & Psarras, 2003). All selected trees used were 6-7 years old, 2.0-2.5m tall, healthy and with no infection. Study sites experienced typical Mediterranean climate: irradiance and temperature being high at 22nd June, with practically no rainfall between June and September, whereas wintertime is wet and cool. Air temperature, photosynthetic photon flux density and relative humidity were measured at the same time as the physiological measurements by a portable gas exchange system (Li-6400, LiCor, Lincoln, NE, Table 1).

Table 1. Climatic conditions at the two studied areas (maximum and minimum photosynthetic photon flux density [PPFD], maximum and minimum air temperature [T], relative humidity [RH]), ± se during the measured days of the experiment of 2007 and 2008.

<table>
<thead>
<tr>
<th>Sampling date</th>
<th>Studied area</th>
<th>PPFDmax (μmol m^-2 s^-1)</th>
<th>PPFDmin (μmol m^-2 s^-1)</th>
<th>Tmax (°C)</th>
<th>Tmin (°C)</th>
<th>RH (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2nd May</td>
<td>Athens</td>
<td>1280 ± 93</td>
<td>410 ± 34</td>
<td>24 ± 2</td>
<td>16 ± 3</td>
<td>60 ± 2</td>
</tr>
<tr>
<td></td>
<td>Rethymno</td>
<td>1320 ± 100</td>
<td>350 ± 47</td>
<td>28 ± 3</td>
<td>18 ± 2</td>
<td>71 ± 3</td>
</tr>
<tr>
<td>22nd Jun</td>
<td>Athens</td>
<td>1750 ± 89</td>
<td>690 ± 73</td>
<td>33 ± 4</td>
<td>20 ± 2</td>
<td>52 ± 1</td>
</tr>
<tr>
<td></td>
<td>Rethymno</td>
<td>1920 ± 130</td>
<td>740 ± 85</td>
<td>36 ± 4</td>
<td>22 ± 3</td>
<td>55 ± 3</td>
</tr>
<tr>
<td>24th Oct</td>
<td>Athens</td>
<td>945 ± 56</td>
<td>160 ± 25</td>
<td>21 ± 4</td>
<td>13 ± 1</td>
<td>67 ± 4</td>
</tr>
<tr>
<td></td>
<td>Rethymno</td>
<td>1100 ± 110</td>
<td>250 ± 35</td>
<td>25 ± 3</td>
<td>15 ± 2</td>
<td>74 ± 4</td>
</tr>
</tbody>
</table>

Maximum values measured at 14.00pm and minimum at 7.00am

Gas exchange measurements were made using a LI-6400 analyzer (open system) connected to a 6400-02B leaf chamber (LI-COR Inc., Lincoln, NE, USA) with an integrated 6400-02B LED light source. Net photosynthetic rate (A), stomatal conductance (gs), intrinsic WUE (A/gs) and transpiration rate (E) were determined at a CO₂ concentration of 365μmol mol⁻¹, ambient relative humidity and using standard formulas (Von Caemmerer and Farquhar 1981). Seasonal photosynthetic variations were monitored on May, June and October of 2007 and 2008 from 11:00 to 14:30h (each month corresponding to one season), every 15 days. One leaf per tree of six trees was examined. Diurnal photosynthetic variations were monitored on 2nd May, 22nd June and 24th October of 2007 and 2008, on five adults leaves of different trees from similar positions and orientations in the canopy facing southwards (receiving maximum light during the day) on the trees. Diurnal cycles of gas exchange were carried out at 1-h intervals from early morning to late afternoon under natural conditions.

Chemical composition analysis of carob pods followed the photosynthetic measurements and it was made at the same periods. The total polyphenols were extracted according to the method described by Ayaz et al., (2007) and determined by the Folin - Ciocalteu method with calibration curves for gallic acid. A spectrophotometer UV/VIS was used at a wavelength of 725nm. Data were expressed as mg gallic acid equivalents. Protein concentration (expressed %) was determined by the method of Kjeldahl (Anon., 1984). The sugar content was determined by the method described by Perez et al., (1997).

The statistical analysis was performed using the SPSS 10.0 software (SPSS Inc, Chicago, IL, USA). The results were subjected to a one-way ANOVA, using the Tukey test to check significant differences between means (p<0.05).

Results and Discussion

Gas exchange measurements generally report the sensitivity of plant species to different environmental conditions e.g. air temperature, drought, solar radiation, soil substrate, CO₂ concentration (Moustakas et al., 1997; Issa et al., 2001; Sakcali & Ozturk, 2004). The carob plants growing at Athens or at Rethymno sites revealed the highest gas exchange indices during May compared to June and October. The highest mean seasonal net photosynthesis values were observed in May in the trees growing at Athens (16.3 μmol m⁻² s⁻¹), while a progressive decrease of CO₂ assimilation during June and October for both sites was recorded (Fig. 1A). The peak transpiration rate was also recorded during May, which was 11.9 and 10.0 mmol m⁻² s⁻¹ at Athens and Rethymno, respectively (Fig. 1B). No significant differences in stomatal conductance (gs) among months were observed, which however displayed a progressive decrease from May to October (Fig. 1C). In contrast, the seasonal depression of gs for trees grown at Athens was more evident (Fig. 1C).

Intrinsic water use efficiency (A/gs) was at the annual minimum in October for plants growing on both sites due to a decrease in the photosynthesis rather than stomatal closure (Fig. 1D). The overall seasonal dependence of photosynthesis was primarily correlated with temperature and light intensity as well. Analysis of diurnal data (7:00-18:00 h) on photosynthesis of *C. siliqua* showed that the daily A, gs, E and A/gs values differed significantly between the trees at the two studied sites. The diurnal trends of these parameters revealed higher values in trees growing at Athens than those growing at Rethymno site. Similar findings have been reported by Radoglou & Rafiroyannis (2003) in the broad leaved species planted in Greece.
During a daily spring measurement (2nd May), net CO₂ assimilation rate showed a steady increase during morning, reached a maximum (12.4 and 15.4 μmol m⁻² s⁻¹ for Rethymno and Athens, respectively) at noon and after attaining the peak value it decreased gradually till 18:00 when the sun was setting (Fig. 2A). Stomatal conductance (gs) and transpiration rate (E) on this date followed a similar pattern to the net photosynthesis at both sites. The highest gs value was recorded at midday and it was two times higher at Athens than at Rethymno (0.298 and 0.6 mol m⁻² s⁻¹, respectively, Fig. 2B). The E value at Athens site was 24% higher than that measured at Rethymno site during midday (Fig. 2C). The daily intrinsic water use efficiency pattern (A/gs) at Rethymno resembled those of A, gs, E, but that of Athens was quite different, later displayed the higher value at 13:00h (Fig. 2D).

From March to May an intensive formation of new leaves, rapid extension of branches and expansion of fruits occurs (Nunes et al., 1992). The high growth activity in May is expressed not only by the higher photosynthetic rates, compared to June and October, but also by an increase in the proteins and polyphenols in carob pods. On the other hand, the products formed by photosynthesis may have to be partitioned between the formation of leaves and storage during the October because starch builds up in the leaves (Diamantoglou & Meletiou-Christou, 1980).

During 22nd June, differences were found between measurements at different times of the same day. The highest value of net CO₂ assimilation at Athens was obtained during at 11:00h being 10.9 μmol m⁻² s⁻¹, while at Rethymno site it was recorded at 12:00h being 9.2 μmol m⁻² s⁻¹; thereafter a rather continuous decline was observed (Fig. 3A). Similar A/gs patterns were detected (Fig. 3D). The highest gs and E at Rethymno site was measured at midday 12:00 and 13:00h, whereas at Athens site at 13:00 and 11:00h, respectively (Figs. 3B & 3C).

The low A values recorded in the early morning (7:00h) of the autumn day (24th October), at both Athens and Rethymno site, can be attributed to both low E and low PPFD. Net CO₂ assimilation increased sharply from 0.3 and 0.8 μmol m⁻² s⁻¹ at 7:00h at Rethymno and Athens, respectively, to 6.7 and 8.1 μmol m⁻² s⁻¹ at midday then decreased abruptly to 2.2 and 3.0 μmol m⁻² s⁻¹ at 16:00h, when the sun was setting (Fig. 4A). Stomatal conductance and intrinsic water use efficiency also showed similar trend with net photosynthesis, with higher values at 12:00h (Figs. 4B & 4D). The lowest E was recorded in the early morning (7:00h), followed by an increase to the maximal rates at midday (~ 7.0 mmol m⁻² s⁻¹, at both sites) and then decreasing during the afternoon hours (Fig. 4C).
Fig. 2. Diurnal courses of net CO₂ assimilation rate (A), stomatal conductance (B), transpiration rate (C) and intrinsic water use efficiency (D) of *Ceratonia siliqua* L. growing naturally at two sites Rethymno and Athens measured at 2nd May. Each data point is the mean of five measurements. * shows significant difference at p<0.05 between the studied areas by Tukey test.

Fig. 3. Diurnal courses of net CO₂ assimilation rate (A), stomatal conductance (B), transpiration rate (C) and intrinsic water use efficiency (D) of *Ceratonia siliqua* L. growing naturally at two sites Rethymno and Athens measured at 22nd June. Each data point is the mean of five measurements. * shows significant difference at p<0.05 between the studied areas by Tukey test.
Carob fruit moisture, which is an indicator of the stage of ripeness, was the lowest at October measurements at both sites (Table 2). Moisture on 24th Oct sharply dropped by 88 and 83% at Athens and Rethymno site (p<0.05) as compared to the values on 2nd May. According to Battle & Tous (1997) the carob pod development follows a sigmoid growing curve and could be divided into three stages. In the first stage, during late autumn and winter, the beans show hardly any increase in the weight (fresh and dry), which is due to slow growth. The second stage starts at the beginning of spring when the pod enters an active and fast growth period (April to June). In third stage, the fruit grows slowly, ripens and starts becoming dry after June and during early autumn and changes color from green to brown.

The low moisture value, found in our measurements, coincides with the third growth stage in which the maturity of the pods has been achieved. On the other hand, in spring (2nd May) a fast development and an active metabolism occur, while the pods are small in size and green. Total polyphenols and proteins on a dry basis of carob pods gradually decreased with the ripening, revealing a significant loss on both sites. More precisely, at Athens site in the month of October, proteins and polyphenols showed a loss of 52 and 86% (P<0.05) of their value measured on 2nd May, while at Rethymno site loss was 52 and 90% (p<0.05, Table 2). In contrast, during the course of our measurements the total sugar content significantly increased reaching the highest value in autumn at ripening stage. At both sites, Athens and Rethymno, the sugars doubled their value compared with those of spring (Table 2).

Environmental changes as high temperature and low rainfall lead to high evapo-transpiration losses, increasing the drought stress problems in the plants (Flexas, 2003). The trees in the Mediterranean are expected to grow in a more hot and dry environment (Ozturk et al., 2010). Vitale et al., (2007) observed a strong relationship between stomatal conductance and environmental factors under Mediterranean climatic conditions. They mention that the summer values of stomatal conductance are closely related with the rate of accumulated precipitation, as well as different water-use strategies adopted by plants in response to drought stress during this period. In addition, Awada et al., (2003) recorded maximum photosynthesis for pine tree during June, while other Mediterranean pine tree species showed maximum values during July and August. This interspecific difference could be due to the negative effects of high air temperature. Manes et al., (1997) referred a drop in photosynthesis of Pinus pinea at noon during summertime, together with stomatal closure, coinciding with high air temperature (35°C) and with leaf water potentials similar to those recorded during autumn. In our study, no stomatal closure and no decreased net photosynthesis at noon of June were recorded, suggesting that there is no biochemical pathway limitation of photosynthesis and that carob species can tolerate the high air temperature showing a high intrinsic water use efficiency (A/gs) at midday.
Table 2. Moisture, proteins, total sugars and total polyphenols of carob pods of the two studied areas, during the measured days of the experiment of 2007 and 2008. Mean values of triplicate analyses.

In the same row followed by different letter differ significantly at p<0.05.

<table>
<thead>
<tr>
<th>Parameters (% dry weight)</th>
<th>Studied area</th>
<th>Sampling date</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>22nd May</td>
</tr>
<tr>
<td>Moisture</td>
<td>Athens</td>
<td>78.1&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Rethymno</td>
<td>22.2&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Proteins</td>
<td>Athens</td>
<td>10.6&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Rethymno</td>
<td>13.3&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Total sugars</td>
<td>Athens</td>
<td>18.1&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Rethymno</td>
<td>46.4&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Total polyphenols</td>
<td>Athens</td>
<td>13.6&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Rethymno</td>
<td>24.8&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

The net photosynthesis reached its peak value in May, when water was abundant and temperature was moderately high. These high CO<sub>2</sub> assimilation rates correlate well with the increased carbohydrate demand due to high growth rates of the trees. Thereafter, it got reduced in summer paralleling reduced carboxylation efficiency. As regards our findings related to the seasonal patterns, even though photosynthesis was minimum in June this was not due to stomatal closure. It seems that photoinhibition damage may occur during summertime (June) with concomitant reduction in electron transport rate in Photosystem 2 and Photosystem 1. Thus, the decline of CO<sub>2</sub> assimilation rate during the hot and dry season seems to be rather a non-stomatal effect. C. siliqua is able to maintain intrinsic WUE at severe summer conditions, by adopting a water-balancing strategy during midday. The gas exchange measurements demonstrated that C. siliqua is a species that can be considered as suitable candidate for natural reforestation of warm and arid areas of the east Mediterranean basin. Ozturk et al., (2010) have previously reported that C. siliqua is drought resistant species, and is not affected by environmental aridity. Our results also demonstrate that the extreme climatic conditions occurring in Mediterranean basin do not threaten the survival of this species, as it is happens with pine tree (Rubio-Casal et al., 2010). The capacity of carob tree to access water resources maintains its photosynthetic assimilation and produces positive balance of CO<sub>2</sub> fixation. According to Ozturk et al., (1983), Salleo & Lo Gullo (1989), Vertovee et al., (2001) and Sakcali & Ozturk (2004), C. siliqua is included among the water spending species on the basis of high transpiration and stomatal conductivity values. Indeed, despite the high transpiration and conductivity towards the middle of the day in carob water loss is less during June, which could be attributed to the fact that probably root hydraulic activity replaces this water loss. Thus, our findings reinforce previous aspect, that carob trees possess a high productive potential under long and dry periods of the Mediterranean and are highly suitable both for plantation (ecologically) as well as for agricultural production (economically).

Acknowledgements

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References


Flexas, J. 2003. Unravelling the sequence of photosynthetic responses to progressive drought in C<sub>3</sub> plants: current


