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Seasonal trends and relationships of light, temperature and leaf physiological traits of sugar beets (*Beta vulgaris* L.) grown under semi-arid, Mediterranean conditions

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Abstract

The seasonal changes of leaf physiological traits and its relationship with abiotic factors (photosynthetic active radiation-*PAR*, leaf temperature-T_{leaf}, air temperature-T_{air}, T_{leaf} - T_{air}- ΔT) were studied on sugar beets grown under the semi-arid conditions of central Greece. Sugar beet (*Beta vulgaris* L.) cv *Rizor* was established in a Randomised Complete Block design experiment for two years (2004-2005). From early June till the end of October, 11 determinations of leaf physiological traits (intracellular to ambient CO_2 concentration-c_i, transpiration rate-*E*, stomatal conductance-g_s, net photosynthesis-*A*, instantaneous water use efficiency-*A*/*E*, photosynthetic water use efficiency-A/g_s, chlorophyll content-*SPAD*, carbon isotope discrimination- Δ, *N* concentration and specific leaf area-*SLA*) took place. Temperatutes (T_{leaf} and T_{air}) and ΔT were higher in 2004 when water budget (*WB*, precipitation + irrigation – evapotranspiration) was more negative. Also, seasonal mean of carbon isotope discrimination (Δ) and net photosynthesis (*A*) were lower in 2004 to compare to 2005. A gradual decline with the progress of the growing season was found for *A*, Δ, g_s, *E*, *SLA* and leaf *N* concentration while the adverse trend was evident for water use efficiencies (*WUE*, A/*E* and A/g_s). *PAR* was positively related with g_s and *E* and negatively with *A*/*E*. Temperatures (T_{air} and T_{leaf}) had a positive effect on *E* and a negative one on *A*/*E*. High temperatures (35-37 ºC) degraded leaf chlorophyll but temperatures higher than 37 ºC tended to increase chlorophyll content. ΔT (transpirational cooling) was negatively related with g_s, *E*, and *A* and positively with *A*/*E* and A/g_s. Both *E* and *A* were regulated by g_s which was kept higher or slightly lower than 0.10 mol m^{-2} s^{-1}, a limit indicative of severe drought in C_3 species. Positive relationships were found between *A*, leaf *N* concentration and *SLA*. *SLA* is an indicator of resource-use strategy and can provide useful information about abiotic factor effects on sugar beet physiology and yield. Δ was negatively related with A/g_s showing that it can be an indirect assessment of *WUE* at leaf level.

***Keywords*:** Carbon isotope discrimination; Drought; Photosynthesis; Stomatal conductance; Transpiration

Abbreviations

A: net photosynthesis; *A*/*E*: instantaneous water use efficiency; A/g_s: photosynthetic water use efficiency; *ANOVA*: analysis of variance; c_i: intracellular to ambient CO_2 concentration; c_i/c_a: the ratio of intracellular to ambient CO_2 concentration; *CF-IRMS*: continuous flow-isotope ratio mass spectrometer; *cv*: cultivar; $\Delta^{13}C$: carbon isotope ratio; Δ: carbon isotope discrimination; ΔT: T_{leaf}-T_{air}; *DAS*: days after seeding; *E*: transpiration rate; g_s: stomatal conductance; *LA*: leaf area; *LSD*: least significant difference: *SLA*: specific leaf area; *SPAD*: Soil Plant Analytical Development; *PAR*: photosynthetic active radiation; *PDB*: Pee Dee Belemnite; *RGR*: relative growth rate; T_{air}: air temperature; T_{leaf}: leaf temperature; *TE*: transpiration efficiency; *WB*: water budget; *WUE*: water use efficiency

Introduction

Water availability is the major constraint of sugar beet productivity in the Mediterranean basin (Morillo-Velarde and Ober, 2006). Although sugar beet is considered as a drought tolerant species (Francois and Maas, 1994), supplemental irrigation of ca 550 mm is necessary in order crop profitability to be secured in central Greece (Analogides, 1993). Sugar beet yield is not reduced by water deficits lower than 30% of the demanded water (Winter, 1980). Thus, crop remains profitable under controlled deficit irrigation (Fabeiro et al., 2003). Analogous yield compensation was recently reported under the rain-fed conditions of Germany (Mäck and Hoffmann, 2006).

Although water shortage effects on agronomic performance (root and sugar yield, root internal quality) have already been studied thoroughly (Choluj et al., 2004; Bloch and Hoffmann, 2005), there is not much work on leaf physiology of sugar beets grown under water deficit conditions especially in the field. In Mediterranean environments, what is defined as water stress is actually a combination of drought, salt, temperature and light stresses (Chaves et al., 2002; Munns, 2002). Already, negative effects of increased leaf salinity on sugar beet physiology have been reported (Tsialtas and Maslaris, 2006). Also, Qi and Jaggard (2006) showed that irrigation can not alleviate the negative impact of high temperatures on sugar beet productivity. D'Abrosio et al. (2006) defined 25 ºC as an optimum and higher temperatures decrease photosynthetic rate and increase photorespiration of sugar beets. As a consequence, sucrose production in leaves and accumulation in root is reduced when air temperature deviates above 25 ºC (Kenter et al., 2006). Global warming is expected to expand semi-arid sugar beet growing areas in central and northern Europe (Jones et al., 2003). Adaptations of plant physiology under semi-arid conditions have already been reported (Balaguer et al., 2002; Medrano et al., 2002) but sugar beet leaf physiology and adaptation to such conditions have not been studied. We are aware of only one work conducted in northern Europe which studied seasonal variations of photosynthetic capacity and leaf development in field grown sugar beets (Vandendriessche et al., 1990).

Recently, stable carbon isotopes were employed in sugar beet research. Carbon isotope discrimination (Δ, a measure of the $^{13}C/^{12}C$ ratio in plant tissues compared to air) is a useful tool for the indirect assessment of plant ecophysiology in the field (Dawson et al., 2002). Since Δ is related with the ratio of intracellular to ambient CO_2 concentration (c_i/c_a) and water use efficiency (*WUE*, the ratio of biomass produced to the water consumed to produce it) in C_3 species (Farquhar et al., 1989), it can provide an integrative study of the environmental factors on stomata functioning (Brugnoli et al., 1998). Δ is a good indicator of *WUE* at leaf (Monti et al., 2006 a) or biomass (Bloch et al., 2006) level and varies between growth stages and sugar beet organs (Monti et al., 2006 b). However, no genotypic differences of Δ were found between sugar beet cultivars screened for increased *WUE* (Tsialtas and Karadimos, 2003; Rytter, 2005; Bloch et al., 2006).

In a two-year, field work conducted on sugar beets grown under the Mediterranean conditions of central Greece, we aimed to study a) the seasonal changes of abiotic factors (photosynthetic active radiation-*PAR*, air temperature-T_{air}, leaf temperature-T_{leaf}) and leaf physiological traits (intracellular to ambient CO_2 concentration-c_i, transpiration rate-*E*, stomatal conductance-g_s, net photosynthesis-*A*, instantaneous water use efficiency-*A*/*E*, photosynthetic water use efficiency-A/g_s, chlorophyll content-*SPAD*, carbon isotope discrimination- Δ, *N* concentration and specific leaf area-*SLA*), b) to establish relationships between abiotic factors and physiological traits and c) to explore any relationship between physiological traits, which can provide useful information about sugar beet adaptation and productivity under semi-arid conditions.

Materials and methods

Site and experiment set up

A Randomized Complete Block design experiment with six replications was established for two years (2004-2005) on a typical field of eastern Thessaly (39° 33′ N, 22° 27′ E, 98 m above sea level). Soil was heavy (sand 27 %, silt 22 %, clay 51 %), with pH 8.2, low organic matter content (1.46 %) and low residual N (8.2 mg NO_3-N kg^{-1}). The experiment aimed mainly to monitor seasonal changes of sugar beet yield and quality. Cultivar *Rizor* (SES EUROPE NV/SA, Tienen, Belgium) was mechanically sown (on 23 March 2004 and 18 March 2005) at 20 cm on the row and 50 cm between rows, in six-rowed plots, 10 m long. Adequate fertilization was applied as basal (110 kg N ha^{-1}, 200 kg P_2O_5 ha^{-1} and 320 kg K_2O ha^{-1}) and top-dressing (40 kg N ha^{-1}). During the growing season, full protection was taken against cercospora leaf spot, powdery mildew, weeds and insects by chemical sprayings. Sprinkler irrigation was applied six times from June to September providing 260 mm and 509 mm of water in 2004 and 2005, respectively. The nearest station of State Meteorological Service provided meteorological data. The monthly water budget (*WB*, precipitation + irrigation - evapotranspiration) is presented in Table 1.

Gas exchange measurements and determinations

Beginning in early June, ca 70 days after seeding (*DAS*) and every two weeks till the end of October, gas exchange measurements (*A*, c_i, *E*, g_s) were conducted by the LC*i* portable photosynthesis system (ADC BioScientific Ltd, Hoddesdon, UK). Instantaneous water use efficiency and photosynthetic water use efficiency were calculated as ratios of *A* to *E* or g_s (*A*/*E* and A/g_s), respectively. Five intact, fully expanded and full sun-lit leaves were measured per plot. On parallel, the same system monitored photosynthetic active radiation (*PAR*), air and leaf temperatures (T_{air}, T_{leaf}). ΔT was calculated as T_{leaf}-T_{air}. Leaf chlorophyll content was indirectly assessed using the *SPAD*-502 (Minolta Co Ltd, Osaka, Japan) on 10 leaves per plot. All measurements were conducted at midday (11.00h to 13.00h).

Table 1. Monthly and total water budget (*WB*) during the growing season. *WB* is the algebraic sum of precipitation, irrigation and evapotranspiration in mm.

Month	Monthly water budget (mm)	
	2004	2005
March	-10.88	0.96
April	-14.10	-82.50
May	-41.48	-36.36
June	-5.40	-58.20
July	-41.40	5.58
August	-75.50	-44.86
September	-77.10	-8.20
October	-22.32	3.14
Mean	-37.98	-31.94
Total	-288.18	-220.44

At each sampling occasion, six intact, fully expanded and full sun-lit leaves were collected per plot, put on an iced chest and transferred to the Physiology Laboratory of Larissa factory, Hellenic Sugar Industry SA. Leaf area (*LA*) was determined using the *WinDias* image analysis system (Delta-T Devices Ltd, Cambridge, UK) in three leaves per plot. After leaf drying at 75°C for 48 h, specific leaf area (*SLA*) was estimated as the ratio of *LA* to leaf dry weight. Dried leaves were ground to fine powder using a microhammer cutter mill (Glen Creston Ltd, Stanmore, and Middlesex, UK). Total leaf *N* concentration was measured on a Kjeltec 2300 Analyzer Unit (Foss Tecator, Höganäs, Sweden). Carbon isotope determinations were conducted at Scottish Crop Research Institute on an isotope mass spectrometer (*CF-IRMS*, Europa Tracer Mass, Crewe, UK) coupled to an elemental analyser (Roboprep) for the on-line sample preparation. Carbon isotope ratio $\Delta^{13}C$ (‰) in samples was calculated as:

$[(R_{\text{sample}}-R_{\text{standard}})/R_{\text{standard}}) \times 1000]$,

where R_{sample} and R_{standard} are the $^{13}C/^{12}C$ ratio in the plant tissue and the standard, respectively. The universally accepted standard of Pee Dee Belemnite (*PDB*) limestone was used. Carbon isotope discrimination (Δ) was calculated as:

Δ (‰)= $(\Delta_a - \Delta_p)/(1+ \Delta_p/1000)$,

where Δ_a and Δ_p are $\Delta^{13}C$ of the air and ground leaf sample, respectively. Δ_a is ca -8 ‰.

Statistical analysis

Data were subjected to analysis of variance (*ANOVA*) as a Randomized Complete Block design with years and samplings as main factors. Mean values were compared with the least significant difference (*LSD*) test at $P<0.05$. Statistical analysis was carried out with the M-STAT statistical package (MSTAT-C, version 1.41, Crop and Soil Sciences Department, Michigan State University, USA).

Results

Seasonal pattern of abiotic factors

The *WB* was more negative in 2004 compared to 2005 (Table 1). In 2004, a high *WB* deficit was recorded from July to September while in 2005, water deficit was more intensive at the first growth stages (Table 1). Irradiance (*PAR*) and temperatures (T_{air}, T_{leaf}) were affected by samplings and the years×samplings interaction (Table 2). Maximum *PAR* was recorded earlier than maximum temperatures (120 *DAS* and 134 *DAS*, respectively). After achievement of maxima, abiotic factors showed a decreasing trend toward the end of the growing season (Figure 1). *PAR* was positively related with both T_{air} and T_{leaf} (r^2= 0.57 and r^2=0.59, P<0.001, respectively). ΔT was higher in 2004 compared to 2005 (0.35ºC and 0.20 ºC, respectively) and was affected by samplings and the years×samplings interaction (Table 2). However, in both years, ΔT increased from 134 *DAS* onward (Figure 1).

Seasonal changes of photosynthetic traits

Years had no significant effect on c_{i} and g_{s} (Table 2). Mean *A* was higher in 2005 (15.74 μmol m^{-2} s^{-1}) compared to 2004 (13.76 μmol m^{-2} s^{-1}) but the adverse was evident for A/g_{s} (65.53 and 56.22 μmol mol^{-1}, respectively). This finding could be attributed to the abrupt decline of *A* after 166 *DAS* in 2004 and only at the last sampling, *A* increased to levels higher than 10 μmol m^{-2} s^{-1} (Figure 2). All the traits were affected by samplings and years × samplings interaction (Table 2). *E* and g_{s} were higher early in the season and then declined gradually with the progress of time. The adverse trend was found for A/g_{s} and A/E. No clear seasonal pattern was found for c_{i} (Figure 2).

Seasonal changes of non-photosynthetic traits

Years had no significant effect on *SPAD* (Table 2). Except from Δ, which was not affected by sampling time, the other traits were significantly affected by both samplings and years × samplings interaction (Table 2). In 2004, *SPAD* declined till 150 *DAS* and then increased strictly. A rather different trend was found in 2005 when *SPAD* did not show significant changes till 198 *DAS* and then increased toward the end of the season (Figure 3). Seasonal patterns of *SLA* and leaf *N* concentration were rather similar in both years showing a gradual decline with time and only small increases toward the end of the season. Δ showed different patterns between years from 166 *DAS* onward; in 2004, an abrupt decline till 198 *DAS* was evident while the adverse was found in 2005. In 2004, seasonal pattern of Δ resembled the respective pattern of *A* (Figure 3).

Relationships between abiotic factors and physiological traits

A strong, exponential relationship was found between *PAR* and *E* (r^2= 0.62, P<0.001) while *PAR* and g_{s} were curvilinearly related (Figure 4). Both leaf *N* concentration and *A* were positively related with *PAR* but the relationships were weak ($y=14.87e^{0.0006x}$, r^2=0.34

and y=0.0098x+0.219, r^2=0.33, P<0.01, respectively). As a result of *PAR* effects on *E* and *A*, *A*/*E* decreased significantly with increasing *PAR* (Figure 4).

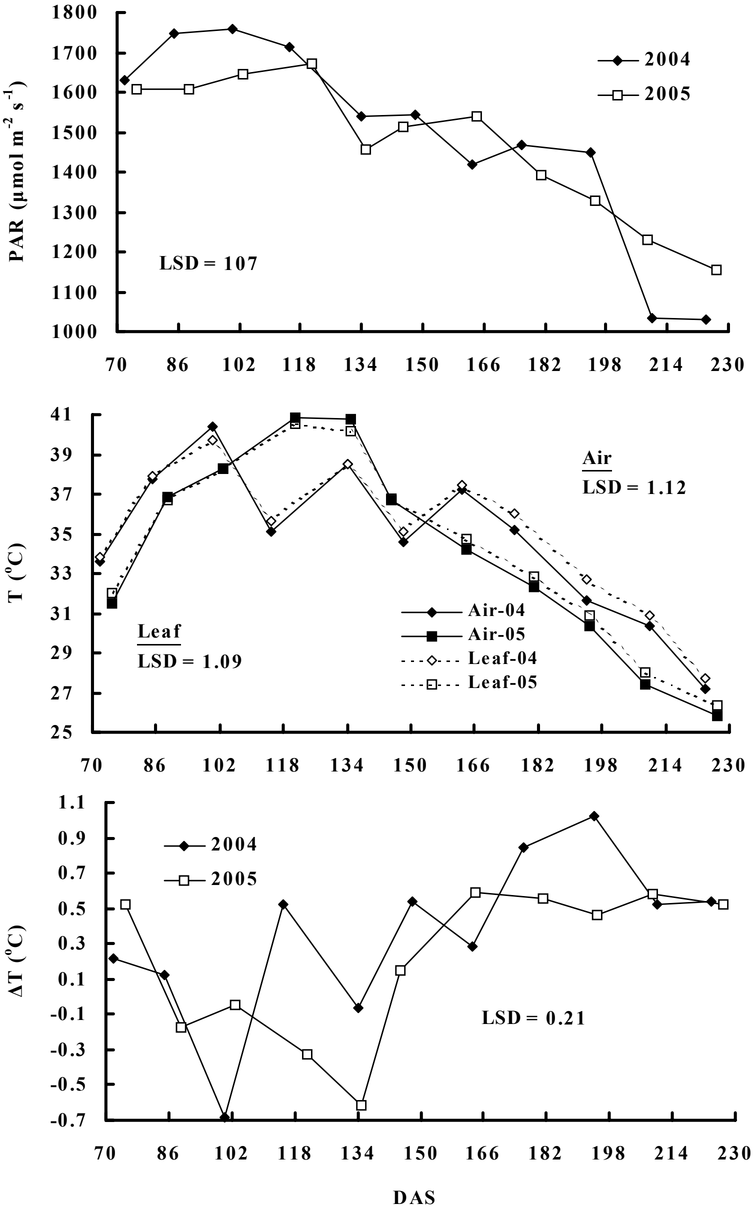

Figure 1. Seasonal trends of *PAR*, temperatures (T_{leaf}, T_{air}) and ΔT. Each point is the mean of six values.

Table 2. Analysis of variance (*ANOVA*) for the abiotic factors and physiological traits determined. Where *PAR*: photosynthetic active radiation (μmol m^{-2} s^{-1}), T_{air} and T_{leaf}: air and leaf temperature, respectively (°C), ΔT: T_{leaf} - T_{air} (°C), c_i: intracellular to ambient CO_2 concentration (μmol mol^{-1}), *E*: transpiration rate (mmol m^{-2} s^{-1}), g_s: stomatal conductance (mol m^{-2} s^{-1}), *A*: net photosynthesis (μmol m^{-2} s^{-1}), *A*/*E*: instantaneous water use efficiency (μmol $mmol^{-1}$), A/g_s: photosynthetic water use efficiency (μmol mol^{-1}), Δ: carbon isotope discrimination (‰), *N*: leaf N concentration (mg g^{-1}), *SLA*: specific leaf area (cm^2 g^{-1}), df: degrees of freedom, CV: coefficient of variation.

		Abiotic factors				Leaf physiological traits									
Source of variation	df	*PAR*	T_{air}	T_{leaf}	ΔT	c_i	*E*	g_s	*A*	*A*/*E*	A/g_s	Δ	*SPAD*	*N*	*SLA*
Blocks	5	***	***	***	*	ns	***	ns	**	ns	*	ns	*	ns	ns
Years (Y)	1	ns	***	***	***	ns	***	ns	***	*	***	***	ns	***	***
Samplings (S)	10	***	***	***	***	***	***	***	***	***	***	ns	***	***	***
Y × S	10	***	***	***	***	***	***	***	***	***	***	**	***	***	***
CV (%)		6.33	2.83	2.75	66.26	10.60	12.60	30.78	11.36	13.11	21.67	2.86	6.38	9.08	15.15

ns: Not significant, *: $P<0.05$, **: $P<0.01$, ***: $P<0.001$

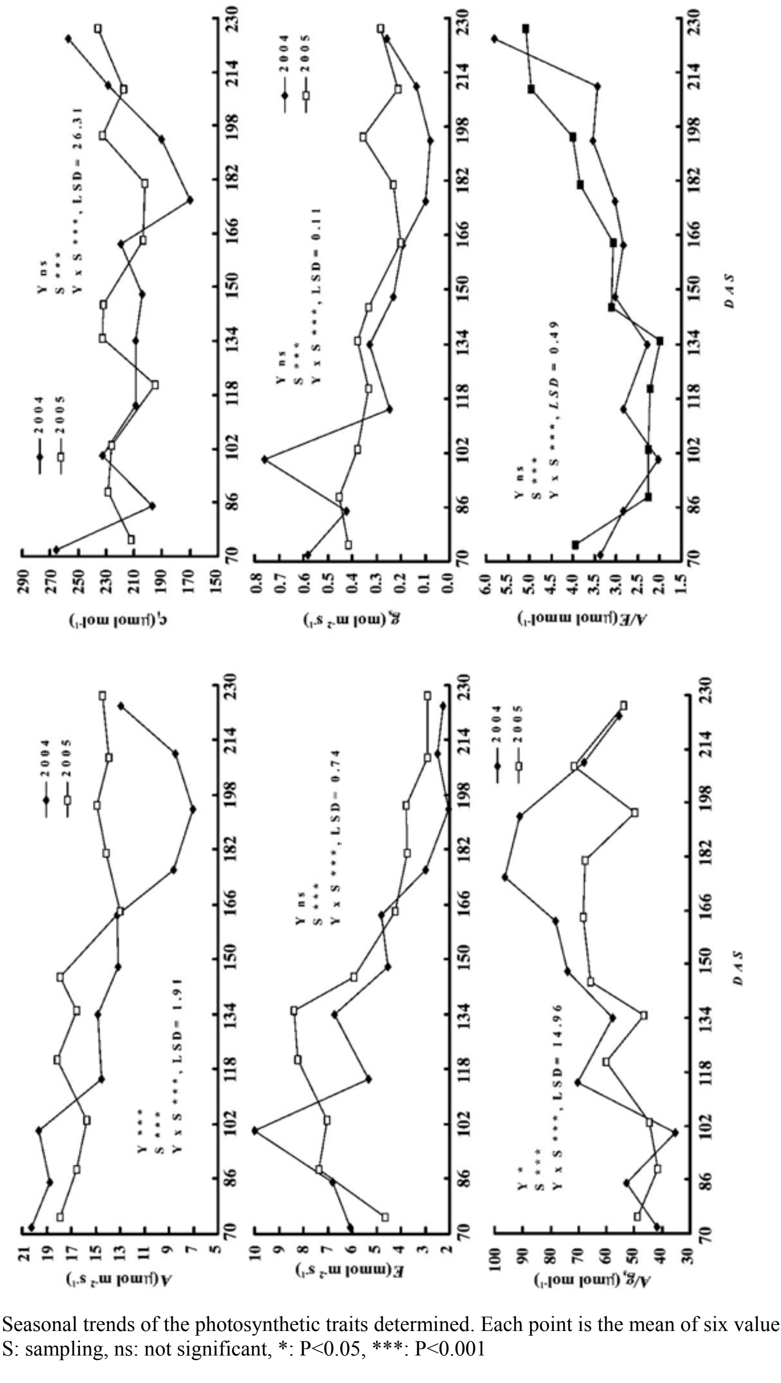

Figure 2. Seasonal trends of the photosynthetic traits determined. Each point is the mean of six values. Y: years, S: sampling, ns: not significant, *: P<0.05, ***: P<0.001

Temperatures (T_{air}, T_{leaf}) had a positive effect on E and a negative one on A/E (Figure 5). A significant quadratic relationship was found between temperatures and *SPAD*. Temperatures up to 35-37 ºC decreased *SPAD* but higher temperatures increased leaf chlorophyll content (Figure 5). A strong, negative correlation was found between ΔT and E and weaker, negative functions related ΔT with g_s and A (Figure 6). *WUE* expressed as A/E or A/g_s was positively related with ΔT (Figure 6). Leaf N concentration was weakly related with ΔT ($y= -10.568x^2 - 5.6103x + 39.683$, $r^2= 0.33$, $P<0.05$).

Relationships between photosynthetic parameters and physiological traits

Stomatal conductance regulated both E and A and strong, quadratic functions were found between the traits. A highly significant, quadratic relationship between leaf N concentration and A was evident (Figure 7). Leaf N was positively related with *SLA*. A similar but weaker relationship was found between *SLA* and A. *SLA* and A/g_s were curvilinearly related (Figure 8). Also, A/gs was significantly related with c_i, leaf N and Δ (Figure 9), with A/g_s-N relationship to be the strongest one ($r^2= 0.71$, $P<0.001$). Δ and A/gs were negatively related but the r^2 and the significance level were low. A weak, logarithmic function was the best-fitted to the A/g_s-c_i relationship (Figure 9).

Discussion

Under Mediterranean conditions, irrigation makes sugar beet crop profitable but yield is practically not reduced by deficits up to 30% of the demanded water (Winter, 1980). However, supplemental irrigation can not alleviate the negative effects of high temperatures on sugar beet crop (Qi and Jaggard, 2006). In semi-arid environments, plants have to cope with a combination of stresses (water, salt, light, temperature), which are falsely considered as drought stress (Chaves et al., 2002; Munns, 2002). Thus, it is interesting to study leaf physiological adaptations of sugar beets under Mediterranean conditions and to relate them with possible effects on yield.

Sugar beets were grown under more water deficit conditions in 2004. This was also confirmed by the lower seasonal Δ values and the higher ΔT found in 2004 compared to 2005. Δ is a reliable indicator of soil water availability (Wang, 2003; Tsialtas and Maslaris, 2006) and in our work, it was sensitive even to short-time scale changes of *WB* since it decreased abruptly after 166 *DAS* in 2004 when *WB* became strongly negative. An analogous to Δ trend was found for A showing that CO_2 assimilation was restricted by the soil water depletion. In temperate environments, A was maximized during July and August and the level of photosynthesis was determinant of sugar beet yield (Vandendriessche et al., 1990). In our work, maximum A was found early in the growing season and was up to 20 $\mu mol\ m^{-2}\ s^{-1}\cdot$ 20 % lower than that reported for northern Europe (Vandendriessche et al., 1990). Alike temperate environments, yield was determined by photosynthesis levels (data not shown).

ΔT, an indicator of leaf ability to regulate temperature *via* transpirational cooling (Dunham, 1993; Kumar and Singh, 1998), was affected by the *WB* deficit and increased toward the end of season. High ΔT values were the result of closed stomata (low g_s) and related with

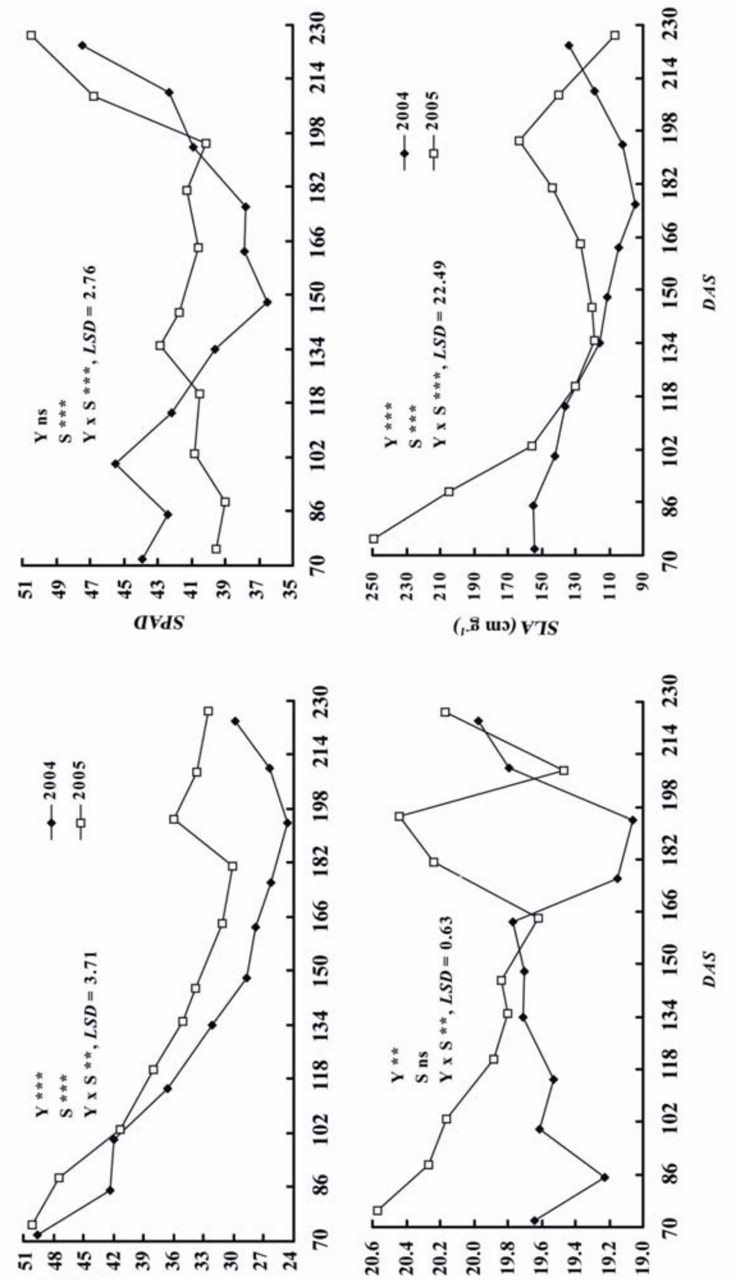

Figure 3. Seasonal trends of the non-photosynthetic leaf traits determined. Each point is the mean of six replications. Y: years, S: sampling, ns: not significant, *: P<0.05, **: P<0.01, ***: P<0.001

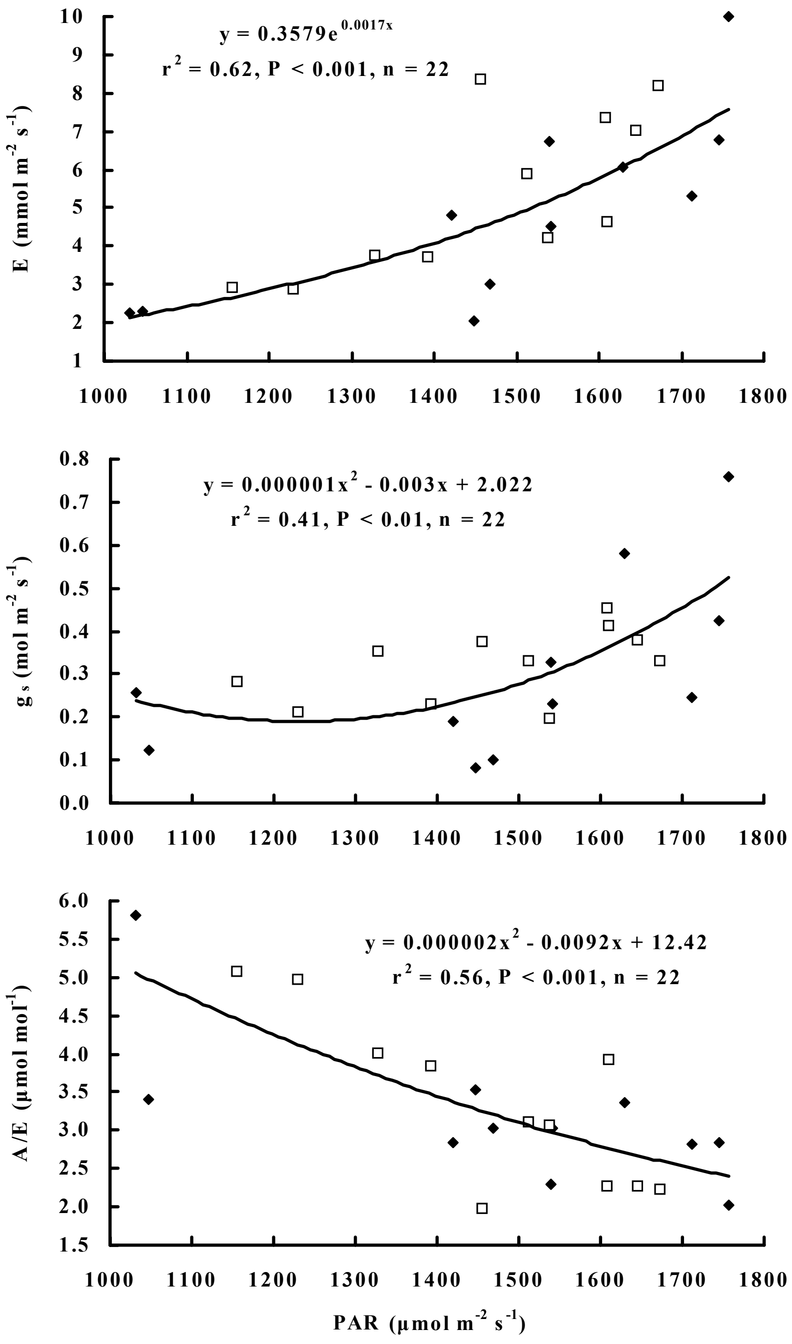

Figure 4. Best-fitted curves of the significant relationships between *PAR* and the physiological traits.

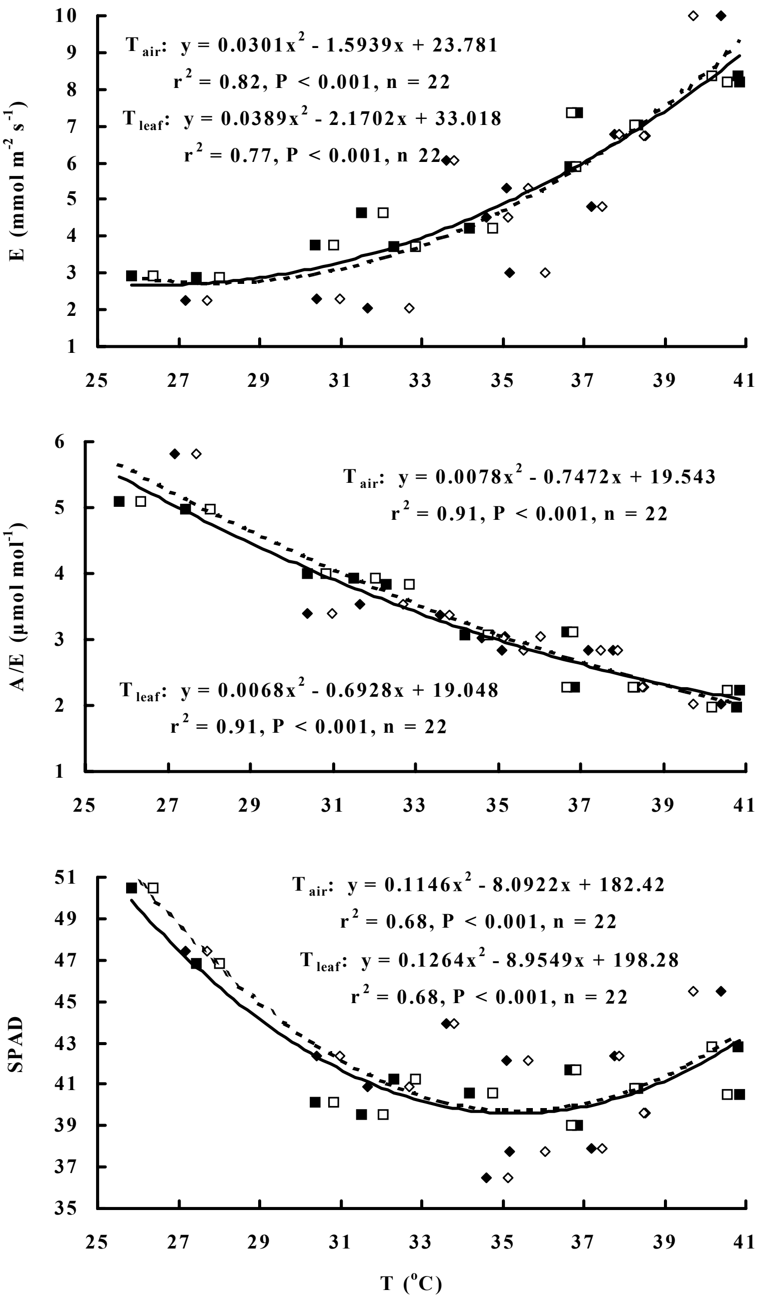

Figure 5. Best-fitted curves of the significant relationships between temperatures (T_{air}, T_{leaf}) and the physiological traits. Open symbols were used for T_{leaf} and filled ones for T_{air}. Diamonds were used for 2004 data and cubes for 2005 data.

low E and A. Consequently, WUE expressed as A/E or A/g_s was positively related with ΔT confirming previous reports that water use is more efficient under stressful conditions (Tsialtas et al., 2001; Wang et al., 2005).

High *PAR* increased stomata functioning (Matsumoto et al. 2005) and was related with high E and low A/E (Yin et al., 2006). Temperatures (T_{air}, T_{leaf}) had analogous but stronger effects on E and A/E. Under Mediterranean conditions, temperature is a main determinant of photosynthesis and its related parameters (Leidi et al., 1999). Optimum temperature for both sugar beet photosynthesis and growth has been defined at 25 ºC (D' Ambrosio et al., 2006; Kenter et al., 2006). Temperatures above the optimum (30-35 ºC) suppress photosynthesis due to the dissipation of the excess excitation energy to other sinks different from CO_2 assimilation (D' Ambrosio et al., 2006). This explains the gradual decline of A with the progress of the growing season and the lower levels of photosynthesis found in our work compared to temperate climates. As a result, sugar beet growth and yield is limited under Mediterranean conditions.

Although there are reports of leaf chlorophyll stability under water stress conditions (Vomáčka and Pospíšilová, 2003), *SPAD*, an indirect assessment of leaf chlorophyll and N contents (Wiesler et al., 2002), decreased with increasing temperature up to 35-37 ºC. Chlorophyll degradation under stressful conditions (drought, salinity) is a plant adaptation in order to reduce the flow of electrons through photosystems and to restrict the risk of photoinhibition (Koyro, 2006). The alleviation of stresses can restore leaf chlorophyll content (Balaguer et al., 2002). In our work, temperatures higher than 37 ºC showed to increase leaf chlorophyll content probably as a reaction of sugar beets to the stressful conditions in accordance with reports on water or osmotic stressed plants (García-Valenzuela et al., 2005; Tsialtas and Maslaris, 2008).

Stomatal conductance (g_s) was proposed as an integrative indicator of water stress, which a plant had experienced (Flexas and Medrano, 2002; Medrano et al., 2002). In 2004, g_s was significantly declined from 166 to 214 *DAS* due to the high *WB* deficit occurred. In both seasons, based on g_s decline with the progress of time, a gradual subjection of sugar beets to water stress was evident. However, only in 2004 g_s reached or dropped below 0.10 mol m^{-2} s^{-1}, which is considered as the lower limit of moderate drought stress in C_3 species (Flexas and Medrano, 2002). The strong regulation of A by g_s was in agreement with reports on water-stressed sugar beets grown under controlled conditions (Monti et al., 2006 a). In sugar beet, stomatal limitations of photosynthesis prevail to non-stomatal ones since photosynthesis is restored after restoration of water supply (Vomáčka and Pospíšilová, 2003). Under semi-arid conditions, sugar beet yield is determined by stomata functioning *via* the regulation of E and consequently, of the water used for the crop growth (Dunham, 1993). Leaf N concentration was positively related with A in accordance with the well-documented positive relationships between A, leaf N and *SLA* reported for many species and different growing conditions (Poorter and Evans, 1998; Gulías et al., 2003).

SLA is considered as the best predictor of resource-use strategy (Vendramini et al., 2002) and is affected by temperature and water status (Rinaldi et al., 2006). In our work, *SLA* decreased with the progress of the growing season as a result of progressive adaptation of sugar beets to the stressful conditions (Nautiyal et al., 2002; Yang et al., 2005). Low *SLA* is related with thicker leaves, longer leaf life span and lower relative growth rate (*RGR*) (Castro-Díez et al., 2000; Wright and Westoby, 2001) and thus, is an indication of plant

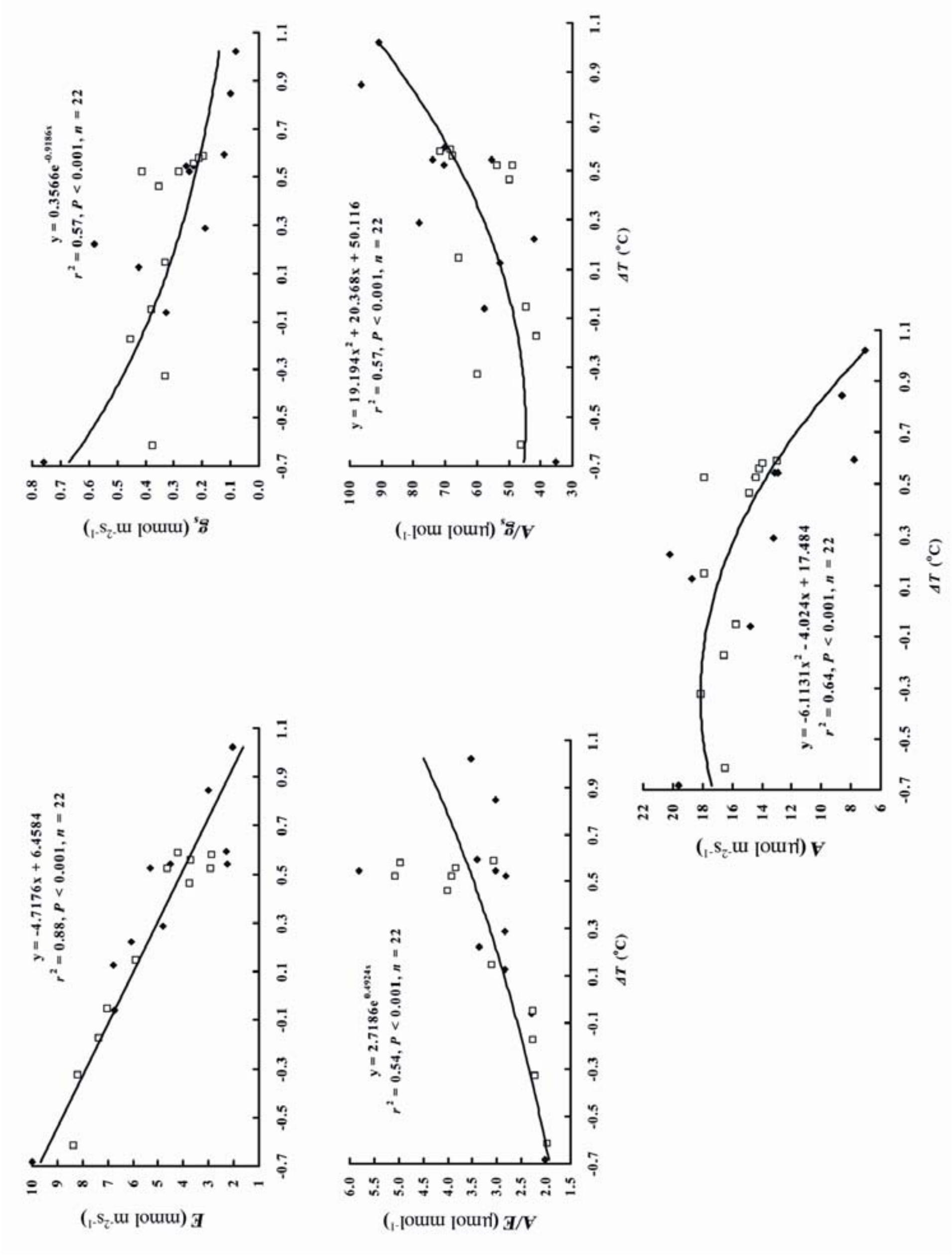

Figure 6. Best-fitted curves of the significant relationships between ΔT and the physiological traits.

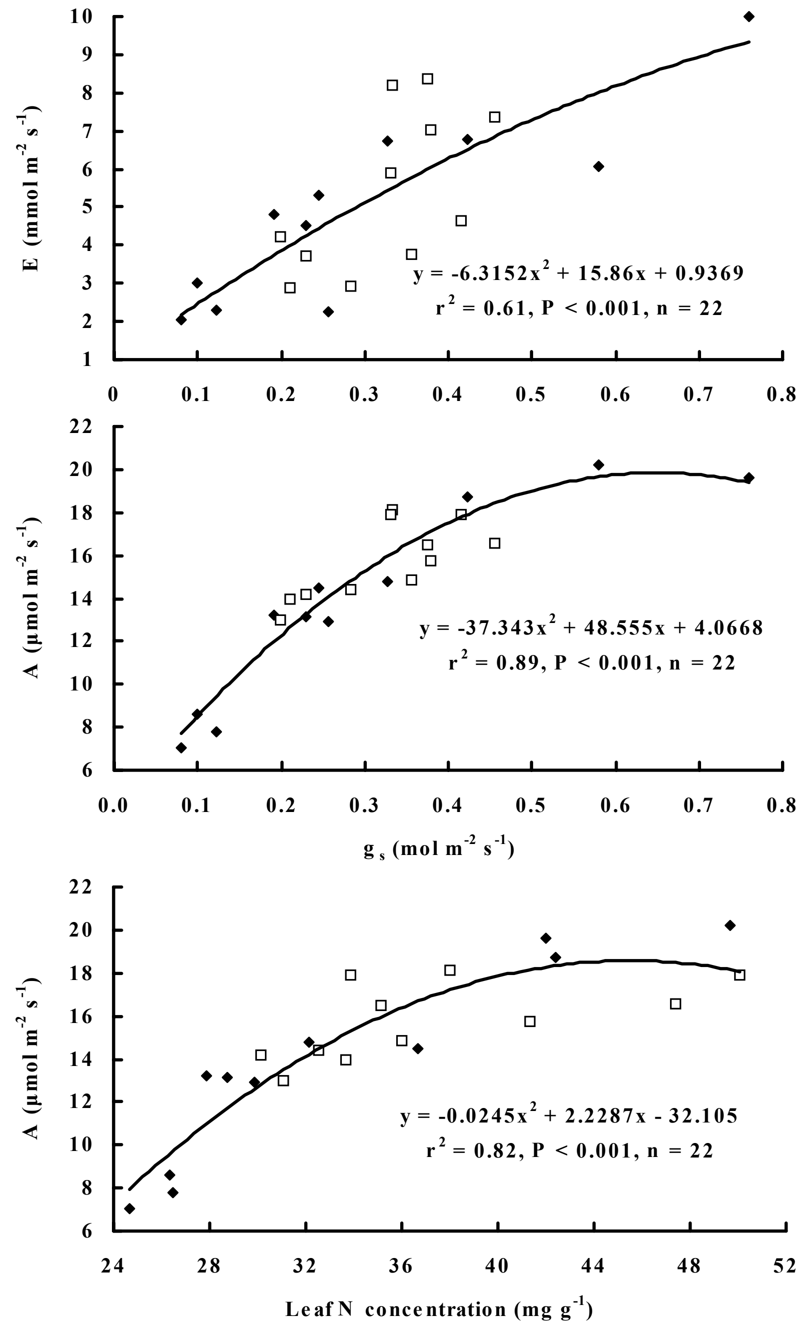

Figure 7. Significant relationships between g_s, E, A and leaf N concentration.

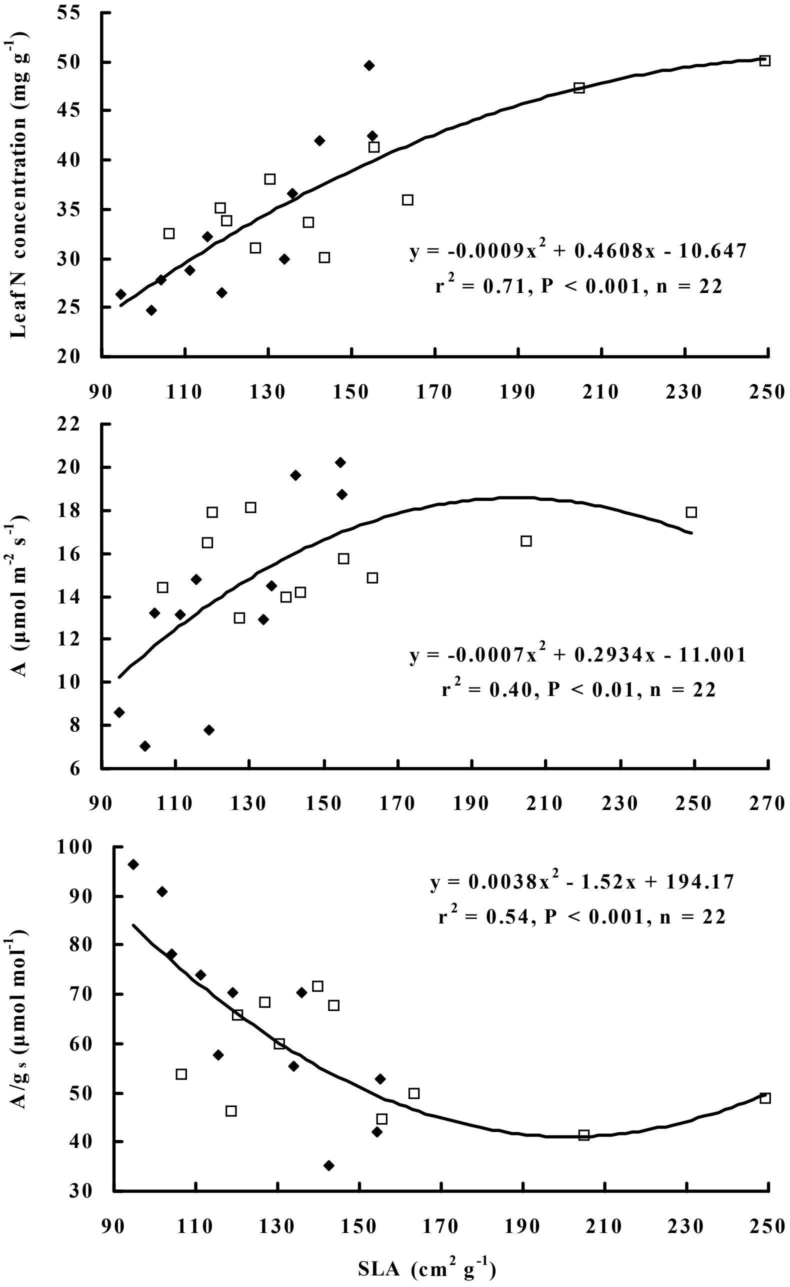

Figure 8. Significant relationships between *SLA*, *A* and leaf *N* concentration.

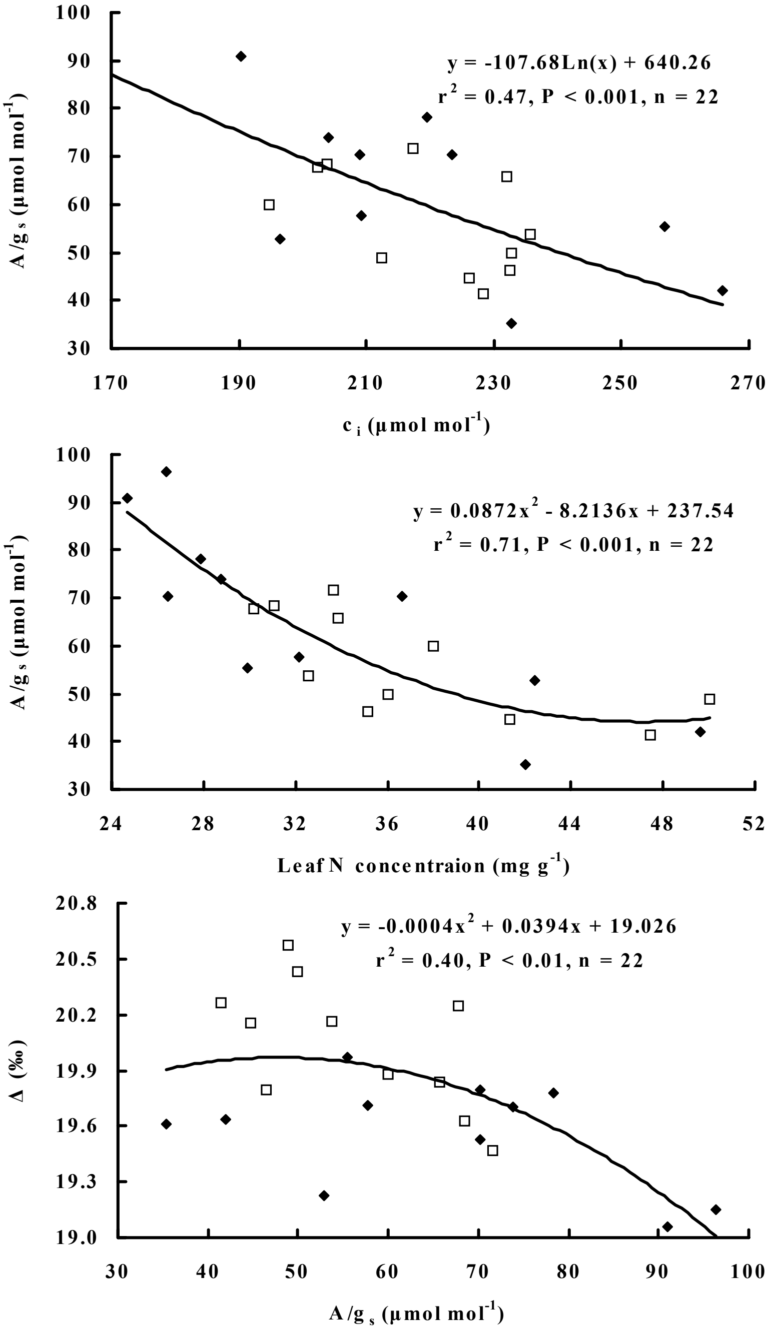

Figure 9. Best-fitted curves of the c_i-A/g_s, leaf N concentration-A/g_s and A/g_s- Δ relationships.

persistence under stressful conditions (Lambers and Poorter, 1992). On the other hand, high *SLA* is related with high leaf expansion rates, root growth and consequently yield (Jensen et al., 2000). Our results are in agreement with the hypothesis that *A* could not be improved without increases of *SLA* due to limitations imposed to photosynthesis by *N* limitations (Gulías et al., 2003). The trade-off of a high *SLA* is a low *WUE* expressed as A/g_s. High A/g_s was the result of increased carboxylation efficiency since a negative relationship between A/g_s and c_i was found (Earl, 2002; Chen et al., 2005). High leaf *N* concentrations had a negative effect on A/g_s in accordance with previous reports (Tsialtas et al., 2001; Tsialtas and Maslaris, 2006). Usually, high leaf *N* concentration is related with high *WUE* i.e. low Δ (Sparks and Ehleringer, 1997) due to reductions in c_i/c_a ratio of the mesophyll caused by the higher photosynthetic capacity of *N*-rich leaves (Williams and Ehleringer, 2000). In our case, the negative A/g_s-N relationship seems to be the impact of soil water availability on *WUE* since in semi-arid environments like Mediterranean, *N* uptake is a function of soil water pulses (Gebauer and Ehleringer, 2000). Δ, a long-term assessment of *WUE*, was negatively related with A/g_s confirming previous findings on sugar beets (Monti et al., 2006 a).

Concluding, sugar beets were grown under more stressful conditions (higher *WB* deficit and Δ*T*) in 2004 and consequently, Δ and *A* were lower in 2004 compared to 2005. Temperatures affected significantly *E* (positively), *A*/*E* and *SPAD* (negatively). Δ*T* (transpirational cooling) was negatively related with *E*, g_s and *A*. Stomata functioning (g_s) regulated *A*. *SLA*, *A* and leaf *N* concentration were positively related. Δ seemed to be a good indicator of leaf *WUE* expressed as A/g_s.

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