

## Plasticity of leaf anatomy, chemistry and water economy of irrigated sugar beets grown under Mediterranean conditions

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### Abstract

In a three-year (2004-2006), field experiment, we aimed to study changes in leaf physiological traits (leaf water potential- $\Psi_{\text{leaf}}$ , leaf water content-LWC, carbon isotope discrimination- $\Delta$ , specific leaf area-SLA, chlorophyll content as assessed by SPAD and modified SPAD-MSPAD) and elemental composition (K, Na, Ca, Mg, K/Na, Ca/Mg, total leaf salinity-TLS) of sugar beets (cv. Rizor) grown under Mediterranean, irrigated conditions. Each year, soil moisture content (SMC) and leaf parameters were determined 11 times from early June to the end of October. Growing seasons differed as regards water inputs with 2004 being the driest and 2006 being the wettest. Leaf physiology and chemistry showed plasticity to water availability and significant differences were found between years (except for Ca), sampling time and their interaction.  $\Psi_{\text{leaf}}$ , LWC,  $\Delta$ , SLA, K/Na and Ca/Mg were positively affected by SMC while the adverse was evident for chlorophyll content (SPAD and MSPAD), Na, Ca, Mg and TLS. As a Chenopodiaceae, sugar beet used leaf succulence, lowering SLA, to cope with water shortage and maintain LWC ca 90%. Low SLA was related with low  $\Psi_{\text{leaf}}$  and  $\Delta$  values. Succulent leaves were characterized by increased leaf salinity and chlorophyll accumulation, which was ascribed to increased Mg concentrations. Leaf salinity and mainly Na had a negative impact on  $\Delta$ . Antagonistic effects of K on Na or Mg and synergistic effects of Na on Ca and Mg were recorded.

**Keywords:** *Beta vulgaris* L; Carbon isotope discrimination; Drought; Photosynthesis; Specific leaf area; Water use efficiency

### Abbreviations

ANOVA: analysis of variance; asl: above sea level;  $C_i/C_a$ : the ratio of intracellular to ambient CO<sub>2</sub> concentration; CEC: cation exchange capacity; CF-IRMS: continuous flow-isotope ratio mass spectrometer;  $\delta^{13}\text{C}$ : carbon isotope ratio;  $\Delta$ : carbon isotope discrimination; g: stomatal conductance; LA: leaf area; LSD: least significant difference; LWC: leaf water content;  $\Psi_{\text{leaf}}$ : leaf water potential; MSPAD: modified SPAD (SPAD/SLA)

ratio); RGR: relative growth rate; SLA: specific leaf area; SMC: volumetric soil moisture content; SPAD: Soil Plant Analytical Development; PDB: Pee Dee Belemnite; TDR: time domain reflectometry; TLS: total leaf salinity; WUE: water use efficiency

## Introduction

Sugar beet (*Beta vulgaris* L.) is a drought and salinity tolerant species (Francois and Maas, 1994) tracing its origin back to the indigenous in Mediterranean, *Beta maritima* (Winner, 1993). However, soil water availability is the most limiting factor of sugar beet productivity in Mediterranean climates (Morillo-Velarde and Ober, 2006) and the provided irrigation makes these areas prone to salinization due to the semi-arid climate (Subbarao et al., 2003; Navarro et al., 2007). Under these growing conditions, plants face a combination of stresses (drought, osmotic stress, salinity), which is incorrectly characterized as water stress (Chaves et al., 2002; Munns, 2002). To cope with stress, plants produce and accumulate a wide range of organic compounds such as sugars (glucose, sucrose), proline, glycinebetaine and amino acids, which have an osmoprotective role (Gzik, 1996; Ghoulam et al., 2002; Mäck and Hoffmann, 2006; Monreal et al., 2007). Biosynthesis of these compounds is energy consumptive, retards growth and is triggered by the accumulation of specific monovalent (K, Na) and divalent (Ca, Mg) ions (Niazi et al., 2004). The biophysical, biochemical and osmotic role of these ions is very important for plant survival under stressful conditions (Subbarao et al., 2003).

Till now, many works have studied the effects of increased levels of salinity on the ionic composition (K, Na, Ca, Mg) of both halophytes and glycophytes (Martínez-Ballesta et al., 2004; Vicente et al., 2004; Koyro et al., 2006). However, the study of seasonal changes of ions, interactions between them and their effects on plant physiology would be interesting (Arndt et al., 2004). Especially for Na, increased concentrations in leaves were detected not only under saline conditions but also under drought in low salinity soils (Martínez et al., 2003). This was also found in sugar beet, a semi-halophytic member of Chenopodiaceae, grown on clayey soils under the Mediterranean conditions of central Greece. Increased leaf Na levels were related with negative effects on plant photo-assimilatory machinery and water relations (Tsialtas and Maslaris, 2006). Antagonistic or synergistic effects between K, Na, Ca and Mg have already been reported (Pujos and Morard, 1997; Maggio et al., 2000; Munns et al., 2002; Bayuelo-Jiménez et al., 2003) and it would be rather informative to relate ions with the seasonal physiological adaptations of plants to progressive water limitation under Mediterranean conditions (Balaguer et al., 2002).

Studying leaf water economy, many parameters are used to assess plant water status,  $\Psi_{\text{leaf}}$  being the most common (Flexas et al., 2004). Leaf water content (LWC) is an easily determined parameter, which under water limited conditions, is indicative of photosynthetic and water economy behavior (Leidi et al., 1999). A contemporary and powerful tool used to study not only water relationships but also plant ecophysiology as a total, is carbon isotope discrimination ( $\Delta$ , a measure of the  $^{13}\text{C}/^{12}\text{C}$  ratio in plant tissues compared to the air), which is related with stomata functioning in  $\text{C}_3$  species (Brugnoli et al., 1998).  $\Delta$  is commonly used as an indirect assessment of the long-term water use efficiency (WUE, the ratio of the biomass produced to the water consumed to produce it) in  $\text{C}_3$  species (Farquhar et al.,

1989), sugar beet included (Bloch et al., 2006; Monti et al., 2006). Specific leaf area (SLA), a physiological parameter affected by water availability (Rinaldi, 2003), is considered as the best predictor of resource use strategy (Vandramini et al., 2002) and is related with leaf morphology, leaf life span and relative growth rate (RGR) (Castro-Diez et al., 2000; Wright and Westoby, 2001). Contradictory results have been yielded by researches on how stress (drought, salinity) affects leaf chlorophyll, which is the compound responsible for photosynthesis (Subbarao et al., 2001; Balaguer et al., 2002; Niazi et al., 2004; Koyro, 2006).

The aim of this work was to study the seasonal adaptation of sugar beet physiological traits ( $\Psi_{\text{leaf}}$ ,  $\Delta$ , SLA, LWC, chlorophyll content) in relation with changes in concentration of elements (K, Na, Ca, Mg) important for leaf water economy. Relationships between physiological traits or elemental composition and soil water availability were also studied.

## Materials and Methods

### *Experimental site and set up*

In a three-year (2004-2006), field experiment, aiming to study the yield formation during the growing season, sugar beet cv. Rizor (SESVANDERHAVE NV/SA, Tienen, Belgium) was arranged in a Randomized Complete Block design with six replications, in a typical field of eastern Thessaly (39° 33' N, 22° 27' E, 98 m asl). Seeds were mechanically drilled (20 cm on the row and 50 cm between rows) in six-row plots, 8 m long. The seeding was conducted on 18 March (2005) or 23-24 March (2004-2006, respectively) and the preceding crop was cotton (2004) or winter wheat (2005 and 2006). Table 1 presents the soil characteristics before the establishment and Table 2 shows the mean monthly temperature and the water input (rainfall + irrigation) during the growing season. Adequate fertilization was applied as basal (110 kg N ha<sup>-1</sup>, 90 kg P ha<sup>-1</sup>, 265 kg K ha<sup>-1</sup>) and top-dressing (40 kg N ha<sup>-1</sup>). During the growing season, full protection was taken against cercospora leaf spot, powdery mildew, weeds and insects by chemical sprayings.

### *Physiological trait determinations and element measurement*

In each growing season, physiological traits were determined 11 times, in two-week intervals, from early June to the end of October. Leaf chlorophyll content was indirectly assessed, at midday (11.00 h to 13.00 h), using SPAD-502 (Minolta Co Ltd, Osaka, Japan) on 10 full-expanded leaves. Six upper, healthy, full-expanded and full-sunlit leaves were randomly collected, put in a portable refrigerator and immediately transferred to Crop Physiology lab of Larissa factory, Hellenic Sugar Industry SA.  $\Psi_{\text{leaf}}$  was determined using WP 4 system (Decagon Devices, Pullman, USA) on two leaves per plot. Three leaves were used for leaf area (LA) determinations using WinDias image analysis system (Delta-T Devices Ltd, Cambridge, UK) and after drying at 75 °C for 48 h, SLA was estimated as the ratio of LA to dry weight. LWC was estimated by comparing fresh and dry weights. Dried leaves were ground to fine powder using microhammer cutter mill (Glen Creston Ltd, Stanmore, Middlesex, UK). 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 on line sample preparation. Carbon isotope ratio  $\delta^{13}\text{C}$  (‰) in samples was calculated as:

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

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

$$\Delta (\text{‰}) = (\delta_a - \delta_p) / (1 + \delta_p / 1000),$$

where  $\delta_a$  and  $\delta_p$  are  $\delta^{13}\text{C}$  of the air and ground leaf sample, respectively.  $\delta_a$  is ca-8 ‰.

A subsample (0.5-1 g) was burnt in a muffle furnace at 500 °C for at least 4 h and the residue was dissolved in 2 M HCl. Monovalent ions (K and Na) were determined on a flame photometer (Jenway PFP 7, Gransmore Green, Felsted, England) and divalents (Ca and Mg) were measured on an atomic absorption unit (Perkin-Elmer 403, Wellesley, MA, USA).

Table 1. Soil characteristics before the establishment of the experiments. Each value is the mean of six replications. Means in the same column labeled with the same letter did not differ significantly at  $P < 0.05$ . CEC: cation exchange capacity.

Depth (0-30 cm)	Sand	Silt	Clay	pH (1:1)	Total	Organic	CEC (cmol kg <sup>-1</sup> )	Total N (g kg <sup>-1</sup> )	NO <sub>3</sub> -N	P-Olsen	Exch-K	Exch-Na
					CaCO <sub>3</sub>	matter						
					(g kg <sup>-1</sup> )		(mg kg <sup>-1</sup> )					
2004	268 <sup>b</sup>	218 <sup>c</sup>	515 <sup>a</sup>	8.2 <sup>a</sup>	12.0 <sup>b</sup>	14.6 <sup>b</sup>	40.9 <sup>a</sup>	1.33 <sup>ab</sup>	8.3 <sup>b</sup>	14.8 <sup>b</sup>	365 <sup>a</sup>	272 <sup>a</sup>
2005	237 <sup>b</sup>	337 <sup>a</sup>	427 <sup>b</sup>	7.8 <sup>b</sup>	13.5 <sup>b</sup>	13.2 <sup>c</sup>	39.1 <sup>a</sup>	1.25 <sup>b</sup>	8.6 <sup>b</sup>	19.6 <sup>a</sup>	288 <sup>c</sup>	147 <sup>b</sup>
2006	363 <sup>a</sup>	260 <sup>b</sup>	377 <sup>c</sup>	8.3 <sup>a</sup>	81.3 <sup>a</sup>	17.2 <sup>a</sup>	32.9 <sup>b</sup>	1.39 <sup>a</sup>	10.4 <sup>a</sup>	7.1 <sup>c</sup>	331 <sup>b</sup>	271 <sup>a</sup>

Table 2. Monthly mean temperature and monthly rainfall during the growing season. Irrigation water and total water input (rainfall + irrigation) are given.

Month	Temperature (°C)			Rainfall (mm)		
	2004	2005	2006	2004	2005	2006
March	9.5	9.3	9.8	31.9	64.2	34.1
April	13.4	13.7	14.2	43.5	5.7	35.4
May	17.0	20.1	19.1	57.1	16.4	1.9
June	23.4	23.5	23.8	93.2	3.6	15.3
July	26.0	26.9	25.0	4.6	11.3	34.3
August	25.4	25.8	26.8	2.6	24.4	10.4
September	21.0	21.8	20.7	20.1	53.4	108.3
October	17.5	15.4	16.2	37.2	10.2	106.1
	19.2	19.6	19.5	290.2	189.2	345.8
		Irrigation (mm)		260	509	647
		Total (mm)		550.2	698.2	992.8

*Annual water inputs and soil water availability*

Soil moisture content (SMC) at 0-75 cm was monitored in each sampling date using four time domain reflectometry (TDR) sensors (Moisture Point, ESI Environmental Sensors Inc, Victoria, Canada) and results are presented in Figure 1. Sensors were installed a week before the beginning of measurements at randomly selected plots.

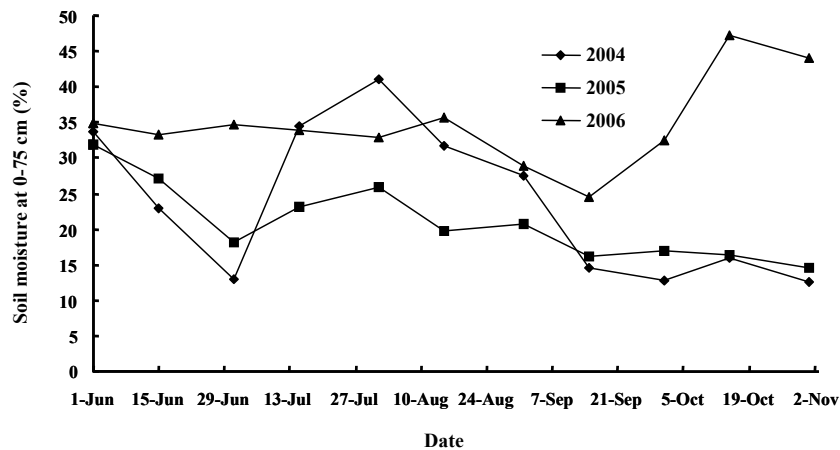


Figure 1. Changes of volumetric soil moisture content (SMC, %) during the growing season.

*Calculations and statistics*

MSPAD values were estimated as the ratio of SPAD to SLA in order to normalize SPAD measurements between years and samplings (Sexton and Carroll, 2002). Also, the K/Na and Ca/Mg ratios were estimated and TLS was calculated as the sum of the elemental concentrations (K, Na, Ca, and Mg).

The data were subjected to ANOVA as a Randomized Complete Block design combined over years with samplings as main factor. Mean values were compared with least significant difference (LSD) test at  $P < 0.05$ . Statistical analysis was carried out with MSTAT-C package (version 1.41, Crop and Soil Sciences Department, Michigan State University, USA).

**Results**

*Year water inputs and soil water availability*

From 2004 to 2006, water inputs (rainfall + irrigation) were increased progressively (Table 1). Water input in 2004 was only 55% of the respective in 2006. This was caused by the combined increase of rainfall and irrigation occurred in 2006. In 2006, SMC remained constant till mid-August, declined till mid-September and finally, increased to the highest

levels of the season due to the autumn rainfall (Figure 1). In 2004 and 2005, SMC was gradually decreased till the end of June, then irrigation or rainfall restored (2005) or increased (2004) its levels and then a gradual depletion toward the end of season was evident (Figure 1).

#### *Seasonal changes of physiological traits and elemental composition*

The main factors (year and sampling) and their interaction affected significantly the traits measured with the exception of Ca for which no year effect was found.

$\Psi_{\text{leaf}}$  was highest in 2006 (-2.40 MPa) but no significant differences were found between 2004 and 2005 (-3.17 and -2.98 MPa, respectively). Seasonal trends in 2004 and 2005 were similar, showing fluctuations between samplings, while in 2005 small changes with time were evident (Figure 2). LWC was lowest in 2004 ( $0.866 \text{ g g}^{-1}$ ), highest in 2006 ( $0.895 \text{ g g}^{-1}$ ) and moderate in 2005 ( $0.876 \text{ g g}^{-1}$ ). A gradual decline with time was found for LWC in all years but restoration took place toward the end of season. This was not true only in the last sampling conducted in 2005 (Figure 2). Seasonal mean  $\Delta$  was analogous to LWC (19.56, 20.04 and 21.73%, respectively). In 2004 and 2005,  $\Delta$  decreased till the beginning of September, then increased (2005) or decreased (2004), but in the last two samplings no significant differences were recorded. In 2006,  $\Delta$  followed a completely different pattern.  $\Delta$  values were abruptly increased between early August to early September, declined to previous levels on mid-September and then increased again till the end of season (Figure 2).

The highest SLA was found in 2006, the lowest in 2004 and moderate in 2005 ( $174.6 \text{ cm}^2 \text{ g}^{-1}$ ,  $124.3 \text{ cm}^2 \text{ g}^{-1}$ ,  $150.7 \text{ cm}^2 \text{ g}^{-1}$ , respectively). Till the beginning of September, a gradual decline with time was evident and thereafter, SLA increased toward the end of season. This trend was more pronounced in 2004 and 2006 (Figure 3).

No significant differences were found for SPAD in 2004 and 2005 (41.48 and 42.15, respectively) while decreased values were recorded in 2006 (39.32). Seasonal patterns of SPAD were similar in 2004 and 2006 showing a maximum at the end of June, then gradually decreased and finally increased toward the end of season. In 2005, SPAD did not show significant fluctuations till the beginning of October and then increased steadily (Figure 3). MSPAD was significantly differentiated between years (0.348, 0.304 and 0.237, respectively) but seasonal patterns were quite different between years (Figure 3).

With the exception of Ca, leaf elemental content was affected by years, sampling and their interaction but seasonal patterns of elements were quite different (Figure 4). The same was also evident for Ca/Mg (Figure 5). The Ca/Mg ratio was highest in 2006 (1.51) and lowest in 2004 (1.15) but not differing significantly with 2005 (1.24). The K/Na ratio increased significantly between 2004 and 2006 (0.36, 0.50, 0.67, respectively) following the analogous increases of SMC. No significant fluctuations between samplings were recorded in 2004 and 2005 while an increase of K/Na with the progress of time, especially at the end of season, was evident in 2006 (Figure 5). TLS followed an inverse trend in relation with SMC. The highest value was found in the dry 2004 ( $97.89 \text{ g kg}^{-1}$ ), the lowest in the wet 2006 ( $83.33 \text{ g kg}^{-1}$ ) and moderate in 2005 ( $93.68 \text{ g kg}^{-1}$ ). After the end of June, TLS did not change significantly till the end of season in 2004 and 2005 (Figure 5). In 2006, a gradual decline was evident from mid July onward.

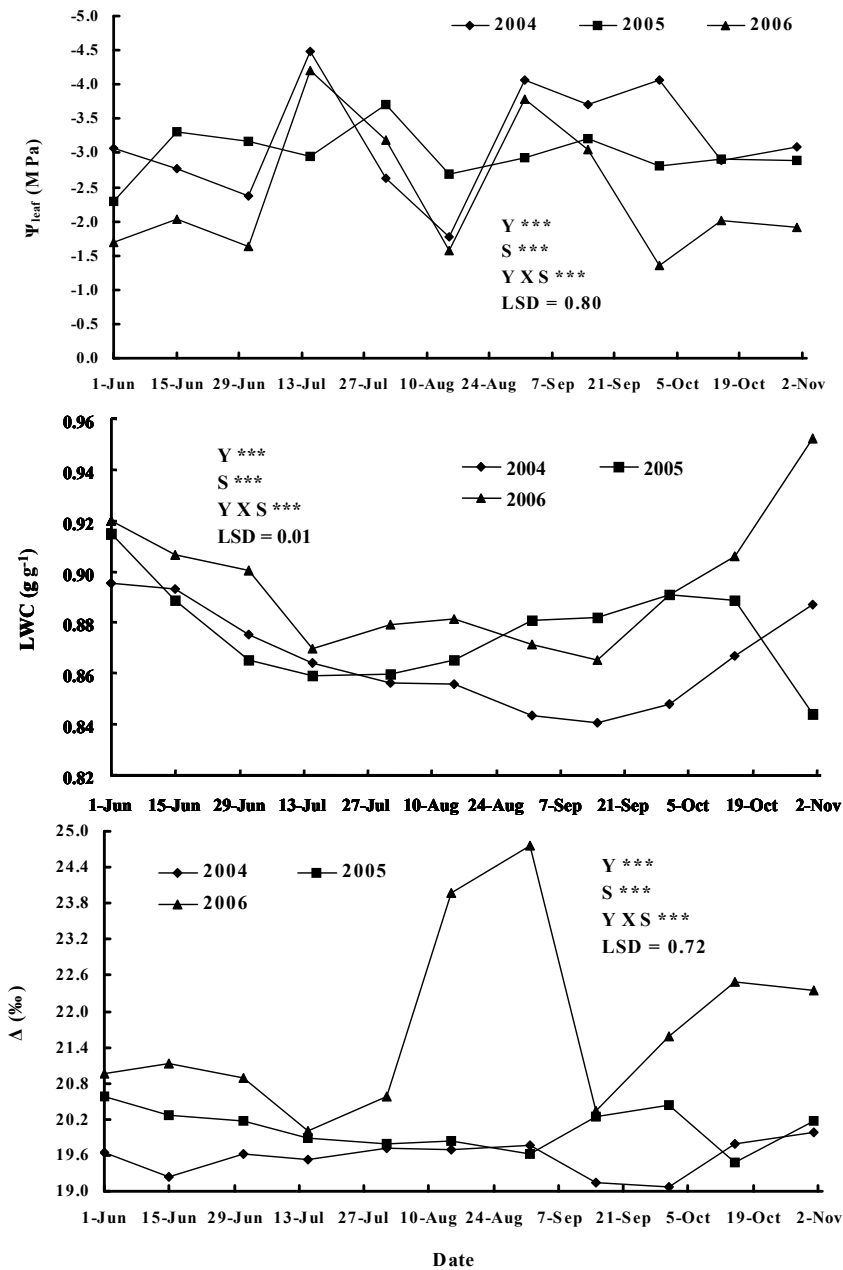


Figure 2. Seasonal patterns of  $\Psi_{leaf}$ , LWC and  $\Delta$  during the growing season. Y: year, S: sampling, \*\*\*:  $P < 0.001$ .

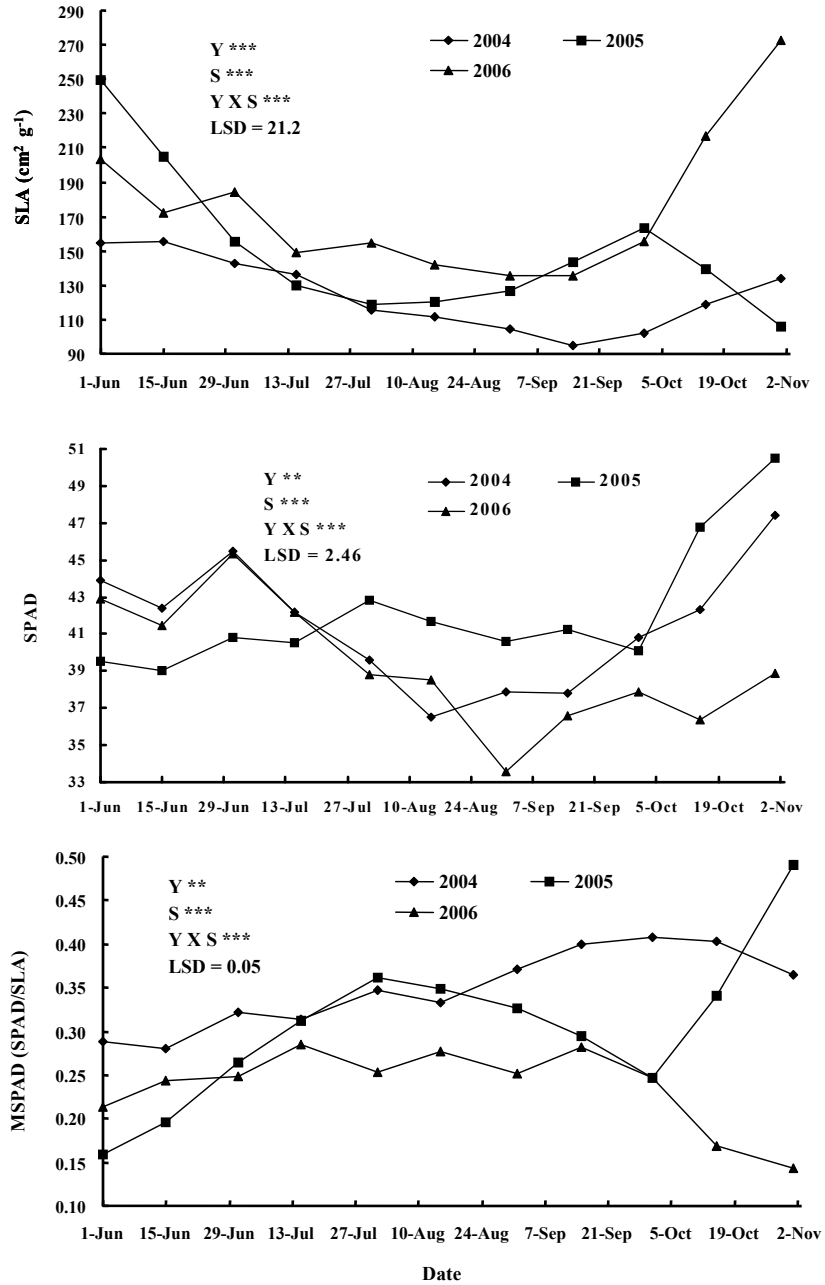


Figure 3. Seasonal patterns of SLA, SPAD and MSPAD during the growing season. Y: year, S: sampling, \*\*:  $P < 0.01$ , \*\*\*:  $P < 0.001$ .



*SMC effects on physiological traits and element content*

With the exception of K, leaf physiological traits and element composition were positively ( $\Psi_{leaf}$ , LWC,  $\Delta$ , SLA, K/Na, Ca/Mg) or negatively (SPAD, MSPAD, Na, Ca, Mg, TLS) affected by SMC (Table 3). SLA, MSPAD and Mg concentration were strongly affected ( $r = 0.55, -0.64, -0.62, P<0.001$ , respectively). LWC,  $\Delta$ , SPAD and TLS were moderately affected by changes in water availability ( $P<0.01$ ) while weak effects of SMC on  $\Psi_{leaf}$  and element concentrations or ratios were evident (Table 3).

Table 3. Correlation coefficients and significance level of the relationships between soil moisture content (SMC, %) and leaf physiological traits or element concentrations.

$\Psi_{leaf}$	LWC	$\Delta$	SLA	SPAD	MSPAD	K	Na	Ca	Mg	K/Na	Ca/Mg	TLS
0.37*	0.48**	0.50**	0.55***	-0.48**	-0.64***	ns	-0.37*	-0.35*	-0.62***	0.42*	0.39*	-0.53**

n = 33, ns: not significant, \*:  $P<0.05$ , \*\*:  $P<0.01$  and \*\*\*:  $P<0.001$ .

*Relationships between physiological traits and elemental composition*

Table 4 presents the correlation coefficients and significance level of the relationships between leaf physiological and chemical traits determined.

$\Psi_{leaf}$  was more related with LWC ( $r = 0.58, P<0.01$ ) than with any other trait or element determined. Also, a positive relationship was found between  $\Psi_{leaf}$  and  $\Delta$  or SLA ( $r = 0.38, P<0.05$ , and  $r = 0.47, P<0.01$ , respectively) and a negative one between  $\Psi_{leaf}$  and MSPAD ( $r = -0.43, P<0.05$ ).

LWC showed a strong, positive correlation with SLA ( $r = 0.91, P<0.001$ ) and negative correlations with MSPAD and Mg ( $r = -0.81$  and  $r = -0.57, P<0.001$ , respectively). A weaker relationship ( $r = -0.45, P<0.01$ ) was found between LWC and TLS while loose correlations were evident between LWC and  $\Delta$  ( $r = 0.41, P<0.05$ ), Ca ( $r = -0.44, P<0.05$ ) or K/Na ( $r = 0.42, P<0.05$ ).

$\Delta$  was strongly and negatively affected by increased leaf Na concentration and TLS ( $r = -0.58, P<0.001$ ). Also,  $\Delta$  was negatively related with increased Mg concentration ( $r = -0.45, P<0.01$ ), MSPAD and SPAD values ( $r = -0.53, r = -0.45, P<0.01$ , respectively). High  $\Delta$  values were related with increased K/Na and Ca/Mg ratios ( $r = 0.52, r = 0.49, P<0.01$ , respectively).

Low SLA was related mainly with high Mg concentration ( $r = -0.71, P<0.001$ ) and secondly with high Ca ( $r = -0.55, P<0.001$ ). A strong, negative relationship between SLA and TLS was also evident ( $r = -0.60, P<0.001$ ) while a loose correlation between SLA and Na concentration was found ( $r = -0.42, P<0.05$ ). Increased SLA promoted selective K accumulation in leaves over accumulation of Na (K/Na,  $r = 0.51, P<0.01$ ).

High leaf chlorophyll content (as assess by MSPAD) was related with increased accumulation of Mg ( $r = 0.81, P<0.001$ ), Ca ( $r = 0.53, P<0.01$ ), Na ( $r = 0.47, P<0.01$ ) and high TLS ( $r = 0.61, P<0.001$ ). High MSPAD was negatively affected K/Na and Ca/Mg ratios ( $r = 0.55, P<0.001$  and  $r = -0.38, P<0.05$ , respectively) and thus low leaf K concentration ( $r = -0.38, P<0.05$ ).

Leaf K concentration was inversely related with Na ( $r = -0.59$ ,  $P < 0.001$ ) and Mg ( $r = -0.44$ ,  $P < 0.01$ ). On contrary, Na and Ca had a positive effect on Mg concentration ( $r = 0.52$ ,  $P < 0.01$  and  $r = 0.68$ ,  $P < 0.001$ , respectively). Potassium promoted selective accumulation of K and Ca in leaves since it was positively related with K/Na ( $r = 0.83$ ,  $P < 0.001$ ) and Ca/Mg ( $r = 0.48$ ,  $P < 0.01$ ). The adverse was evident for Na and Mg. A strong, positive correlation was found between K/Na and Ca/Mg ratios ( $r = 0.65$ ,  $P < 0.001$ ). Accumulation of Na and Mg in leaves had a positive effect ( $r = 0.87$  and  $r = 0.71$ ,  $P < 0.001$ , respectively), Ca had a small effect ( $r = 0.41$ ,  $P < 0.05$ ) and K had no effect on TLS. K/Na and Ca/Mg ratios were negatively related with TLS ( $r = -0.68$ ,  $P < 0.001$  and  $r = -0.44$ ,  $P < 0.01$ , respectively).

## Discussion

Under the semi-arid, Mediterranean conditions of central Greece, sugar beets depend on instable and uncertain water inputs for their growth. Water availability fluctuates within season and between seasons but often this is not reflected on yield due to physiological adaptations of crop to water resources variability (Mäck and Hoffmann, 2006). Study of the in season physiological adaptations to changing water availability could provide useful information on plant mechanisms employed to cope with water shortage (Arndt et al., 2004; Gao et al., 2006).

Physiological traits and element concentrations showed high plasticity to temporal changes of water availability. Sugar beets were adapted to low SMC by decreasing LWC, SLA and  $\Psi_{\text{leaf}}$  and increasing leaf chlorophyll content and salinity. As a member of Chenopodiaceae, sugar beet use leaf succulence as a mechanism to maintain LWC ca 90% and to avoid dehydration (Vendramini et al., 2002). Dependence of SLA on water availability is already known (Rinaldi, 2003) but our findings indicated that SLA responded to short-term changes of SMC. Succulent leaves (low SLA) are characterized by conservative resource-use strategy (Vendramini et al., 2002), low photosynthesis and consequently low RGR (Jensen et al., 2000; Nautiyal et al., 2002). Decreased  $\text{CO}_2$  assimilation in low SLA leaves is ascribed to low  $\Psi_{\text{leaf}}$  and thus to closed stomata (Katerji et al., 1997; Niu et al., 2005). Conservative water use under water-limited conditions was confirmed by low  $\Delta$  values.  $\Delta$  is a reliable tool for studying stomata functioning under field conditions, is affected by both  $g_s$  and  $C_i/C_a$  changes (Brugnoli et al., 1998) and is a good indicator of WUE in sugar beets (Bloch et al., 2006; Monti et al., 2006). Due to its dependence on SMC,  $\Delta$  is a useful monitor of water availability at spatial, temporal and community level (Gao et al., 2006; Chen et al., 2007; Ma et al., 2007). Our results revealed that  $\Delta$  is also a sensitive indicator of SMC at short-term scale. A negative effect of salinity on  $\Delta$  is already reported (Qian et al., 2004; Shahhen and Hood-Novotny, 2005) and it could be ascribed to increased leaf salinity and especially on detrimental effects of Na accumulation in leaves (Tsialtas and Maslaris, 2006).

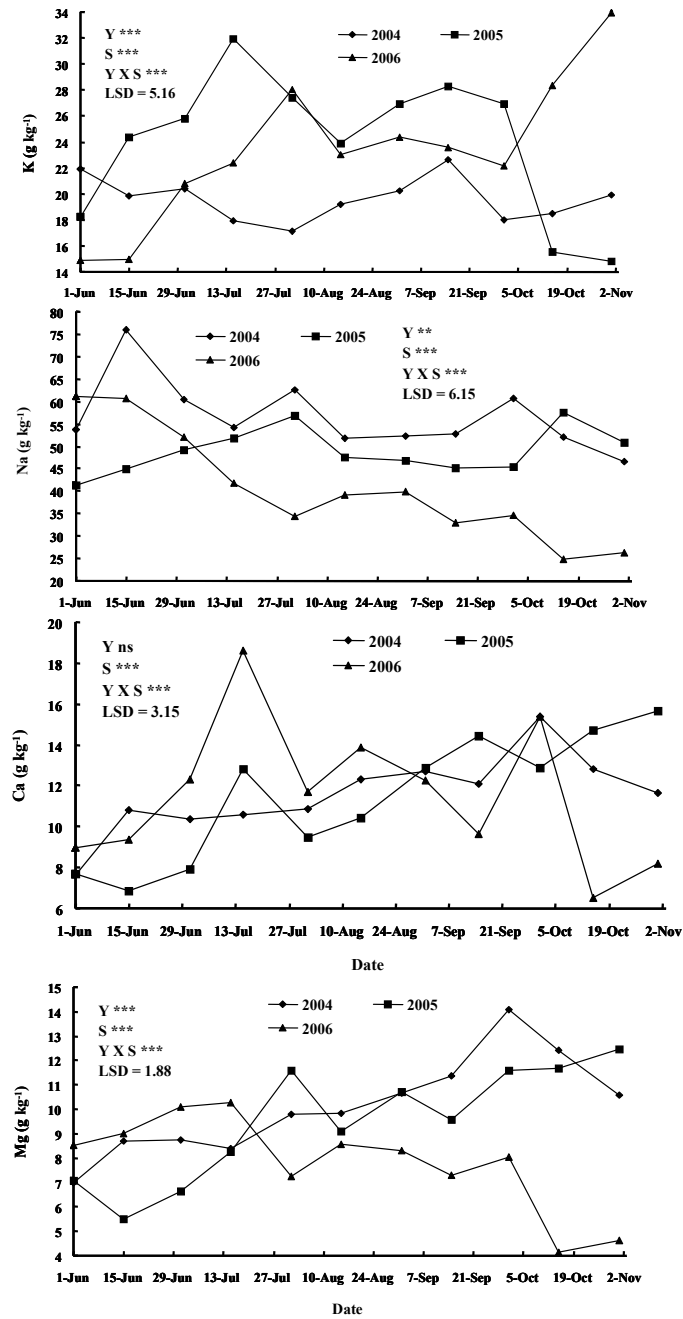


Figure 4. Seasonal changes of element (K, Na, Ca, Mg) concentration during the growing season. Y: year, S: sampling, ns: not significant; \*\*:  $P < 0.01$ , \*\*\*:  $P < 0.001$ .

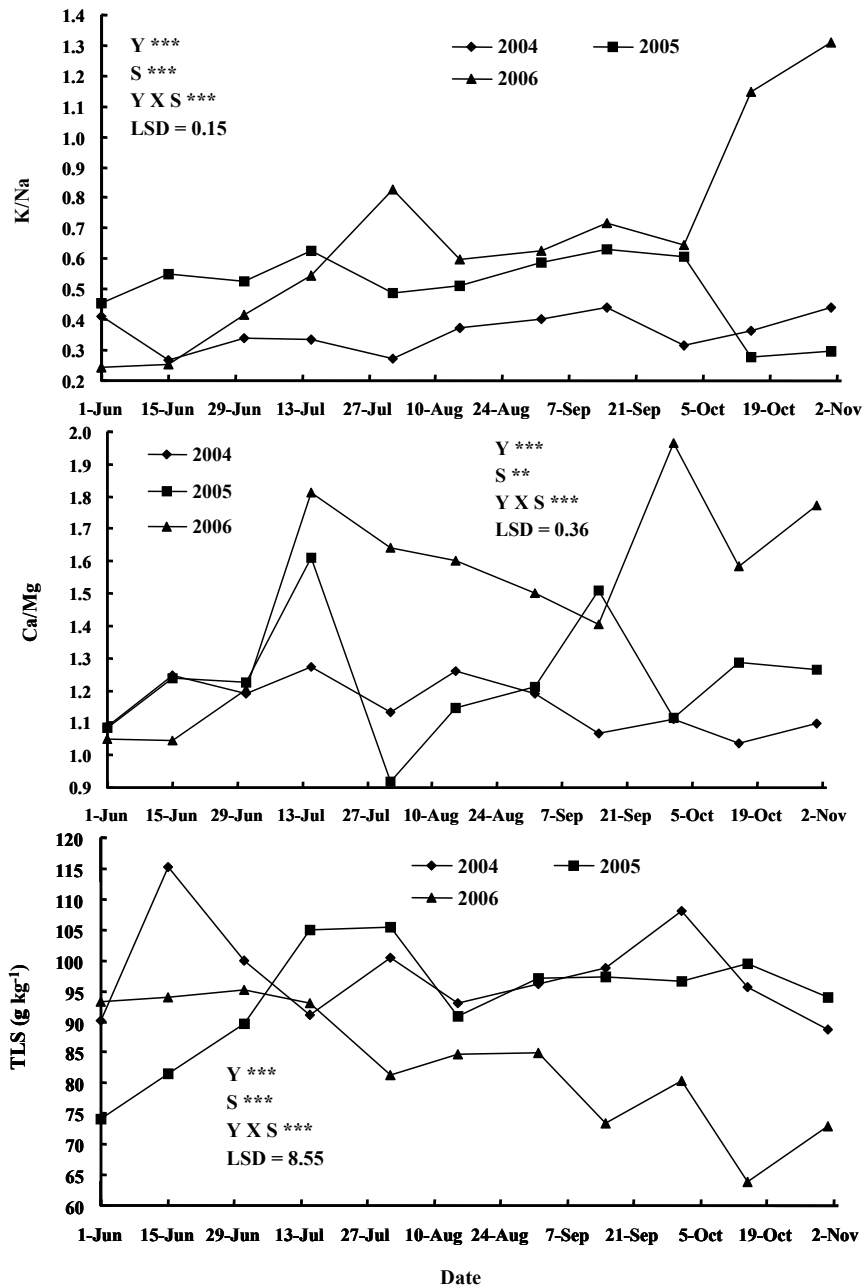


Figure 5. Seasonal changes of elemental ratios (K/Na, Ca/Mg) and TLS during the growing season. Y: year, S: sampling, \*\*:  $P < 0.01$ , \*\*\*:  $P < 0.001$ .

Table 4. Coefficients and significance level of the correlations between the leaf traits determined.

	LWC	$\Delta$	SLA	SPAD	MSPAD	K	Na	Ca	Mg	K/Na	Ca/Mg	TLS
$\Psi_{leaf}$	0.58**	0.38*	0.47**	ns	-0.43*	ns	ns	ns	ns	ns	ns	ns
LWC		0.41*	0.91***	ns	-0.81***	ns	ns	-0.44*	-0.57***	0.42*	ns	-0.45**
$\Delta$			0.41*	-0.45**	-0.53**	ns	-0.58***	ns	-0.45**	0.52**	0.49**	-0.58***
SLA				ns	-0.89***	ns	-0.42*	-0.55***	-0.71***	0.51**	ns	-0.60***
SPAD					0.43*	-0.42*	0.47**	ns	0.42*	-0.49**	-0.35*	0.42*
MSPAD						-0.38*	0.47**	0.53**	0.81***	-0.55***	-0.38*	0.61***
K							-0.59***	ns	-0.44**	0.83***	0.48**	ns
Na								ns	0.52**	-0.86***	-0.65***	0.87***
Ca									0.68***	ns	ns	0.41*
Mg										-0.62****	-0.44*	0.71***
K/Na											0.65***	-0.68***
Ca/Mg												-0.44**

ns: not significant, \*:  $P < 0.05$ , \*\*:  $P < 0.01$ , \*\*\*:  $P < 0.001$ .

Water shortage under semi-arid conditions causes a combination of drought, salinity and osmotic stress (Chaves et al., 2002; Munns, 2002) and this is confirmed by the increased levels of leaf salinity in plants grown under non-saline conditions (Martinez et al., 2003; Tsialtas and Maslaris, 2006). Under stressful conditions, chlorophyll degradation is a common reaction in order the flow of the electrons through photosystems to be reduced and thus the risk of photoinhibition to be restricted (Koyro, 2006). In our work, water shortage and the increased leaf salinity promoted chlorophyll accumulation in leaves (Niazi et al., 2004; García-Valenzuela et al., 2005) as a consequence of the high levels of Mg, an element occupying a central position in chlorophyll (Hermans et al., 2004).

Leaf dehydration and succulence were related with increased concentrations of monovalent (K, Na) and divalent (Ca, Mg) cations, which contribute to osmoregulation and trigger the production of organic osmotica such as sugars, glycinebetaine and amino acids (Koyro, 2006; Gao et al., 2006). Potassium is considered to play a key role in plant water economy affecting guard cell function (Subbarao et al., 2003). Although Tsialtas and Maslaris (2006) reported that K could positively affect  $\Delta$  in sugar beets grown under low soil Na concentrations, this was not confirmed in our work. In the semi-halophytic sugar beet, Na could successfully substitute for K in its osmotic role (Marschner et al., 1981; Robinson et al., 1997; Subbarao et al., 2000; Subbarao et al., 2003) and promote leaf succulence contributing to plant adaptation to drought (Vicente et al., 2004; Hessini et al., 2005). Antagonistic effects between K and Na are common in *Beta* species (Koyro, 2000; Subbarao et al., 2001; Tsialtas and Maslaris, 2006) but analogous effects were also found between K and Mg in our work. Although antagonistic or no effects of Na on Ca and Mg were reported (Koyro, 2000; Munns et al., 2002; Vicente et al., 2004), Na accumulation had synergistic effects on Ca and Mg accumulation in leaves increasing leaf salinity with negative effects on leaf physiology (Tsialtas and Maslaris, 2006; Subbarao et al., 2004).

In conclusion, sugar beets showed plastic response of the leaf physiological traits and elemental contents to water availability. Under water shortage, leaves became succulent lowering SLA, LWC,  $\Delta$  and  $\Psi_{leaf}$  but increasing elemental contents, TLS and chlorophyll content. Low SLA is related with decreased CO<sub>2</sub> assimilation rates and thus low RGR as an effect of stomata closure ( $\Psi_{leaf}$ ). Increased leaf salinity (mainly Na) affected negatively  $\Delta$  but promoted chlorophyll accumulation due to increased Mg concentration. Antagonistic (K on Na or Mg) and synergistic (Na on Ca or Mg) effects between elements were evident.

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