RESEARCH ARTICLE

I. Heat stress in *Triticum*: kinetics of Ca and Mg accumulation

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ABSTRACT

In heat stressed genotypes of *Triticum aestivum* L. (Sever and Golia) and *Triticum turgidum subsp. durum* (Acalou and TE 9306), chosen according to its genetic background diversity, Ca and Mg accumulations were correlated with its photosynthetic performance. It was found that with high temperatures the concentrations of Ca increased in the shoots, whereas the accumulation of Mg augmented only in bread wheat genotypes. Under heat stress, the pattern of electrolytes release in Golia and Acalou remained similar, but the extrusion rates became higher in Sever. During grain filling, the levels of total chlorophylls decreased in the heat stressed genotypes (excepting in Sever). In all the *Triticum* genotypes, stomatal conductance and the net carboxilation rate displayed similar trends. After anthesis, the net carboxilation rates did not vary in Acalou and TE 9306 but changed significantly in the heat stressed bread wheat, being found antagonistic patterns in both genotypes. During grain filling, the mean internal CO_2 concentration only increased significantly in the heat stressed Golia. In Golia and TE 9306, the transpiration rates didn't vary between the control and heat stress treatments, but in Sever and Acalou, significant increases were found. Our data identified synergistic patterns among Ca, Mg, chlorophylls accumulation, and stomatal conductance, whose implications on photosynthesis are discussed.

Key words: *Triticum aestivum* L.; *Triticum turgidum subsp. durum*; net photosynthesis; nutrients uptake and translocation; stomatal conductance.

INTRODUCTION

Optimal mean temperature for the crops growth cycle might vary between 15-18ºC (Chowdhury and Wardlaw, 1978), being 20ºC the optimal value for grain filling (Dupont and Altenbach, 2003). Moreover, the yield performance of wheat genotypes is strongly affected by heat stress (Wardlaw et al., 2002). It was pointed by Wardlaw et al. (1989) that a global reduction in crops production of about 3-4% occurs when the mean temperature increases by 1ºC above optimum. Ciaffi et al. (1996) further concluded that, even short periods of high temperature (35-40ºC), during grain filling, could have a negative effect on yield and quality.

At a physiological level, considering the interactions between crops yield and the mobilization of photoassimilates, it has been reported (Sharkey, 2005) that moderate heat stress (35-45ºC) might implicate photosynthesis inhibition without damaging photosystem II. Yet, when temperatures rise to *ca*. 45ºC (Çjánek et al., 1998; Yamane et al., 1998), the thermal

damage of the photosynthetic oxygen evolving complex (Nash et al.., 1985; Enami et al., 1994) affects the electron transport (Mohanty et al., 2002; Kouřil et al., 2004), increasing Chl *a* fluorescence (Havaux, 1992; Bukhov and Mohanty, 1993). In the heat stressed *Triticum*, a reversible photosynthetic inhibition and an alteration of the chloroplasts structure (namely, grana stacking) has also been reported (Sharkova and Bubolo, 1996). Nevertheless, the heat stress effects after anthesis is complex since integrate wheat responses to periods with moderate high temperatures (25/32ºC) (Wardlaw et al.., 1989) and plant behaviors to short periods with high temperatures (> 32ºC) (Blumenthal et al.., 1991; Stone and Nicolas, 1994).

At the membranes surface of plant cells, Ca bonds the phosphate and carboxylate groups of phospholipids and proteins (Caldwell and Haug 1981; Legge et al., 1982), while Mg strongly interacts with nucleophilic ligands (*i.e.*, phosphoryl groups) through ionic bonding (Günther, 1981). Nevertheless, although both nutrients might regulate cellular pH and cation-anion stability (Marschner, 1995), during plant growth Mg^{2+} uptake can be depressed by Ca^{2+} (Marschner, 1995), unbalancing photosynthetic carboxylations (Günther, 1981; Bergmann, 1992; Schoefs and Bertrand, 1997) and isoprenoids (Jiang and Huang, 2001) accumulation. Accordingly, under heat stress, although Mg^{2+} might stabilize the covalent bonds of Chl, its depression might limit the synthesis of this molecule (Marschner, 1995). Moreover, it has been reported that in this context Ca^{2+} can minimize Chl degradation (Jiang and Huang, 2001) and in the guard cells shield the stomata functioning (Webb et al., 1996; Hare et al., 1998), thus attenuating the heat stress effects through transpiration (Palta, 1996).

The main hypothesis of this study considers that under heat stress Ca and Mg accumulation in bread and durum wheat are linked to the wheat photosynthetic performance. Accordingly, two genotypes of *Triticum aestivum* (Sever from Portugal and Golia from Italy) and of *Triticum turgidum subsp. turgidum* (TE 9306 from Portugal and Acalou from France), having different tolerance to high temperature after anthesis (Maçãs et al., 1999, 2000; Dias et al, 2008, 2009; Dias and Lidon 2009), were used as a test system. An insight in the magnitude of the implications of heat stress on the photosynthetic apparatus is presented and discussed, considering Ca and Mg roots uptake and translocation to the shoots and spikes.

Abbreviations: A - net carbon assimilation rate; Chl - chlorophyll; C_i - internal CO_2 concentration; E - transpiration rate; GF - grain filling; PM - physiological maturity; g_s - stomatal conductance to water.

MATERIALS AND METHODS

***Plant material and growth conditions*:** Bread wheat (*Triticum aestivum* L. genotypes Sever and Golia) and durum wheat (*Triticum turgidum subsp. durum* genotypes TE 9306 and Acalou) grains were washed in distilled water and sterilized by immersion in mercury dichloride solution (1:1000) for 2 min. The grains were next washed five times in deionizer water and placed in an oven at 28ºC for 24 h. Immediately thereafter the seeds were grown in a greenhouse (under natural light, between March and May in Lisbon/Portugal – 38° 42' N; 9° 05' W; photoperiod varying between 12 and 14 h) in 25 x 21 cm pots containing a 1:1 perlite and vermiculite mixture. The experiment was conducted using 136 pots. Half of these pots were putted under heat stress after anthesis. For each genotype 17 replicates were used (with and without heat stress). Ten seeds were grown per pot and two weeks later five were selected, being the others removed. Accordingly, 680 plants were used. In each plant all tillers were removed, keeping only the main culm. Plants were irrigated weakly but alternatively with distillated water or with a standard nutrient solution (in ml/100L, starter/pre-anthesis/post-anthesis, Ca $(NO_3)_2$ 100/100/50; KNO_3 50/200/100; KH_2PO_4 100/100/100; $MgSO_4$ 200/200/100; K_2SiO_3 100/100/0; $Fe(NO_3)_3$ 20/5/5; EDTA 25/5/5; $MnCl_2$ 5/10/5; $ZnSO_4$ 20/10/10; H_3BO_3 10/5/2; $CuSO_4$ 5/5/3; Na_2MoO_4 15/5/5). During the vegetative and reproductive growth, plants were kept under similar environment conditions. At anthesis, the plants were divided in two groups and submitted to control and heat stress conditions, with mean temperatures (day/night) of 25/14ºC and 31/20ºC, respectively, applied throughout the grain filling period. The average of day/night temperatures was calculated as the mean readings of each two hours, during each 24 h period. For the analytical measurements, plants were chosen at random, after all physically damaged or deformed plants were discarded.

***Technical analysis*:** The concentrations of Ca and Mg, were determined in roots, shoots and spikes (at booting-69/70 days after anthesis; grain filling-108/109 and 109/112 days after anthesis as well as at the physiological maturity

– 127/130 days after anthesis, for the genotypes submitted to control and heat stress conditions, respectively). Five randomized plants of each genotype, from each heat treatment, were used for nutrients analysis. Plant samples were washed, the fresh weight was determined in each fraction and, therefore, dry weight was measured after dryness, in an oven for 100 ºC, during 72 h. For Ca and Mg, 1 g of dry material, from each sample, was mineralized through incineration at *ca*. 550 ºC, and followed by nitric acid digestion (Vandecasteele and Block, 1993). A Unicam model 939 absorption unit, equipped with a hollow cathode lamp was used for these metals determinations. The mean concentration values of the nutrients and biomass yields of the roots, shoots and spikes (and grain weight for the grains) were used to determine the related total mean content in the *Triticum* plants. The net uptake was determined adding these values. The mean of the translocation rates were obtained by calculating, on a percentage basis, the ratio between the means of these metal contents in the shoot and their net absorption.

Electrolyte leakage was measured on flag leaf tissues, taken during the grain filling period. Five 0.5 cm^2 sections of 3 flag leaves were sampled for each genotype (control and heat stress treatments) and incubated in deionized water. The electrolytic conductance was determined with a Crison 522 conductimeter, according to Ketchie (1969). Seven conductometric readings were made after 0, 2, 4, 5, 7, 8 and 24 h. For each measurement, the results were expressed, as a rate, in $\mu Scm^{-1} h^{-1}$.

Total Chl of the bread and durum wheat flag leaves were extracted in 100% acetone. Samples were measured spectrophotometrically, using four replicates, according to Lichtenthaler (1987).

Leaf gas-exchange measurements were performed on flag leaves, using an infrared gas analyzer (CIRAS 1, PP Systems, UK) with an external CO_2 concentration of *ca*. 370 ppm and a PPFD of *ca*. 300 $\mu mol\ m^{-2}\ s^{-1}$ (previously found to be saturating under ambient CO_2). Net photosynthesis, stomatal conductance, intercellular CO_2 and transpiration rates were determined (Fig. 1) during the grain filling period (102 – 103 and 103 - 106 days after emergence, for control and heat stress, respectively). These parameters were measured at 4 different times of the day (the mean values of 2 measurements made before noon time was named as am, and the other two, in the afternoon, as pm).

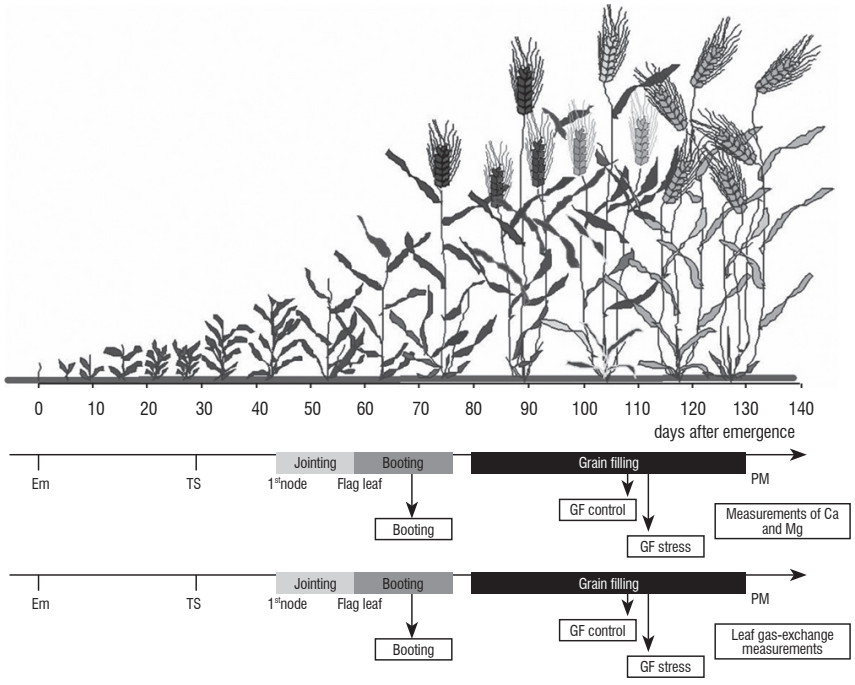

Figure 1. Point in time of Ca, Mg and leaf gas-exchange measurements during the bread and durum wheat phenological development.

***Statistical analysis*:** Statistic analysis were performed with a two-way ANOVA ($p \leq 0.05$), using *STATISTICA*, version 6 (2001), by *StatSoft, Inc*. In tables, different letters in the same column refer to significant differences between genotypes. Between treatments, ns, *, ** and *** refer to: non significant, $P \leq 0.05$, $P \leq 0.01$ and $P \leq 0.001$, respectively.

RESULTS

After anthesis, bread and durum wheat genotypes followed growth conditions similar to those reported by Chinoy (1947) and Wardlaw et al. (1980, 1989), being submitted to a consistent period of moderate high temperatures in the day/night periods.

Among the genotypes, the levels of Ca varied between 2 - 27 mg/g, with the lower values found mostly in the spike (Table 1). In the control treatments, between booting and grain filling, the levels of Ca (Table 1) tend to increase in the shoot, (except in Sever). Between grain filling and maturity a somewhat opposite trend was found, decreasing the contents of Ca in the shoots of Golia and Acalou, while increased in the roots of the durum wheat genotypes (Table 1). At booting the levels of Ca did not show significantly differences in the shoot amongst the genotypes (Table 1).

Table 1. Calcium concentration in different plant parts, on three stages of plant growth (booting, grain filling and maturity), of control and heat stress bread and durum wheat genotypes. Each value is the mean ± S.E. of three replicates. Different letters in the same column refer to significant differences between genotypes. Between treatments, ns, * and ** refer to: non significant, $P \leq 0.05$ and $P \leq 0.01$, respectively.

	Booting	Grain filling		Maturity	
		Control	Heat stress	Control	Heat stress
		Root (mg/g_{dw})			
Golia	9.09±0.06a	8.67±1.09a	6.55±1.74a ns	7.79±0.23a	12.41±2.24a ns
Sever	5.87±0.18b	10.59±3.09a	9.85±1.32a ns	7.66±1.49a	10.92±0.08a ns
Bread wheat	7.48±0.93	9.63±1.45	8.20±1.30 ns	7.72±0.62	11.66±1.01 *
Acalou	12.29±2.98a	7.95±2.23a	11.57±3.67a ns	19.72±1.80a	11.10±3.24a ns
TE 9306	4.14±0.09a	7.51±1.88a	7.86±0.12a ns	16.38±0.81a	13.19±2.19a ns
Durum wheat	8.22±2.65	7.73±1.20	9.71±1.84 ns	18.05±1.26	12.14±1.71 ns
		Shoot (mg/g_{dw})			
Golia	11.55±2.42a	16.90±1.28a	16.02±1.79a ns	8.70±1.08a	12.77±2.80a ns
Sever	13.14±0.91a	9.31±0.32b	27.50±0.51b **	10.36±0.61a	15.91±1.39a ns
Bread wheat	12.35±1.15	13.10±2.26	21.76±3.40 **	9.53±0.70	14.34±1.57 *
Acalou	11.31±1.74a	14.03±2.54a	14.65±0.27a ns	12.57±0.67a	11.26±2.57a ns
TE 9306	12.08±2.86a	13.29±2.71a	16.57±2.87a ns	17.29±3.71a	14.54±0.93a ns
Durum wheat	11.69±1.38	13.66±1.53	15.61±1.30 ns	14.93±2.06	12.90±1.47 ns
		Spike (mg/g_{dw})			
Golia		2.20±0.33a	5.98±0.31a *	4.40±0.46a	3.70±0.81a ns
Sever		3.95±0.31a	10.23±1.77a ns	2.42±0.46a	2.57±0.52a ns
Bread wheat		3.08±0.54	8.11±1.43 **	3.41±0.63	3.14±0.51 ns
Acalou		2.45±0.39a	3.73±0.12a ns	2.63±0.75a	5.81±1.48a ns
TE 9306		9.04±2.66a	11.28±2.24a ns	5.11±0.61a	5.73±1.54a ns
Durum wheat		5.74±2.20	7.51±2.36 ns	3.87±0.82	5.77±0.87 ns

Under heat stress, during grain filling, Ca contents in the spike increased in all the genotypes (significantly in Golia), something that happened also in shoot for Sever . A non-significant increase was also found at maturity, in the roots and shoots of the bread wheat genotypes, while the opposite occurred in the durum genotypes (Table 1). Relatively to the control, the proportion of Ca translocated to the shoot during grain filling was higher in all the wheat genotypes submitted

to heat treatment (Fig. 2). With high temperatures, in Golia, during grain filling, total accumulation and shoot accumulation decreased, 19% and 5%, respectively (Fig. 2). This trend was linked to the decreasing rates in the beginning of the grain filling period (to 74%, in heat stressed plants –Table 2). Yet, the decrease of total accumulation of Ca in the plants did not affect its levels in the spike (which increased significantly), during this growth phase (Table 1), due to an higher translocation of Ca to the shoot (76% to 89% - Fig. 2). Until the beginning of the grain filling, in the heat stressed plants of Sever, the rates of Ca total accumulation showed a 2.6 fold increase. This increase triggered an higher total accumulation of Ca in the shoot (Fig. 2), as well as the significant augmentation of its concentration in the shoot, during grain filling (Table 1).

At booting, the contents of Mg in the control treatment varied (in mg/g) between 2.0 - 2.9 and 2.3 - 3.2 in the roots and shoots, respectively. A similar trend was detected in all the genotypes, among the different growth phases, with the levels of Mg in the roots and shoots decreasing between booting and grain filling, but increasing thereafter until maturity (Table 3). During grain filling this variation became significant in Sever (excepting in the roots) (Table 3). When the plants were submitted, at this stage, to higher temperatures, the concentrations of this metal in the roots decreased, due to an increasing (about 11%) of its translocation to the shoot (Fig. 3), which is related with the levels of Mg in the shoots and spikes under heat stress (Table 3). Indeed, the accumulation in the shoot was higher (*ca*. 60%), although the total accumulation only increased about 40% (Fig. 3).

At maturity, in heat stressed plants of Golia, total and shoot accumulation of Mg increased 36% and 55%, respectively; a similar trend was found for Sever during grain filling, 41% and 60%, respectively (Fig. 3). In both bread wheat genotypes this increase was coupled to a significant augmentation of Mg in the shoots and spikes (Table 3). In the durum wheat genotypes, this trend was not found. Total accumulation of Mg decreased in the heat stressed plants of Acalou during grain filling. The increasing rates of this metal translocated to the shoot (Fig. 3), and the decreasing shoot biomass of heat stressed plants were not sufficient to block the negative effect on the concentration of Mg in the shoots of this genotype (Table 3). Under heat stress, during grain filling, in the genotype TE 9306, total and shoot accumulation of Mg decreased (Fig. 3), triggering a significant reduction of this metal in the spike (Table 3). In this genotype, during this growth phase, the translocation of Mg to the shoot only revealed slight variations (Table 2; Fig. 3). The rate of total accumulation of Mg, under heat stress, remained higher during all the grain growth period in Golia (Table 2). The higher rate of total accumulation in the heat stressed plants (comparatively to the control), between booting and grain filling (Table 2), prompted an increased accumulation in the shoot relatively to the root. In durum wheat genotypes, during grain filling, an opposite trend was found (i.e., the levels of Mg decreased significantly between the treatments) in the shoot of Acalou (at maturity) and in the roots and spikes of TE 9306 (Table 3).

Table 2. Rate of total accumulation of Ca and Mg, in three different periods of growth cycle, for control and heat stressed plants.

	Emergence-Booting	Booting-Grain filling		Grain filling-Maturity	
Genotypes	Control	Control	Heat stress	Control	Heat stress
Ca ($mg_{dw}.plant^{-1}.day^{-1}$)					
Golia	0.28	1.42	1.05	-1.83	-0.82
Sever	0.37	1.06	2.80	-0.70	-6.75
Acalou	0.42	1.04	0.95	-0.38	-0.51
TE 9306	0.43	1.15	1.48	0.30	-1.92
Mg ($mg_{dw}.plant^{-1}.day^{-1}$)					
Golia	0.08	0.22	0.24	0.06	0.52
Sever	0.08	0.22	0.36	0.49	-0.04
Acalou	0.09	0.20	0.20	0.31	0.19
TE 9306	0.09	0.40	0.17	-0.06	0.84

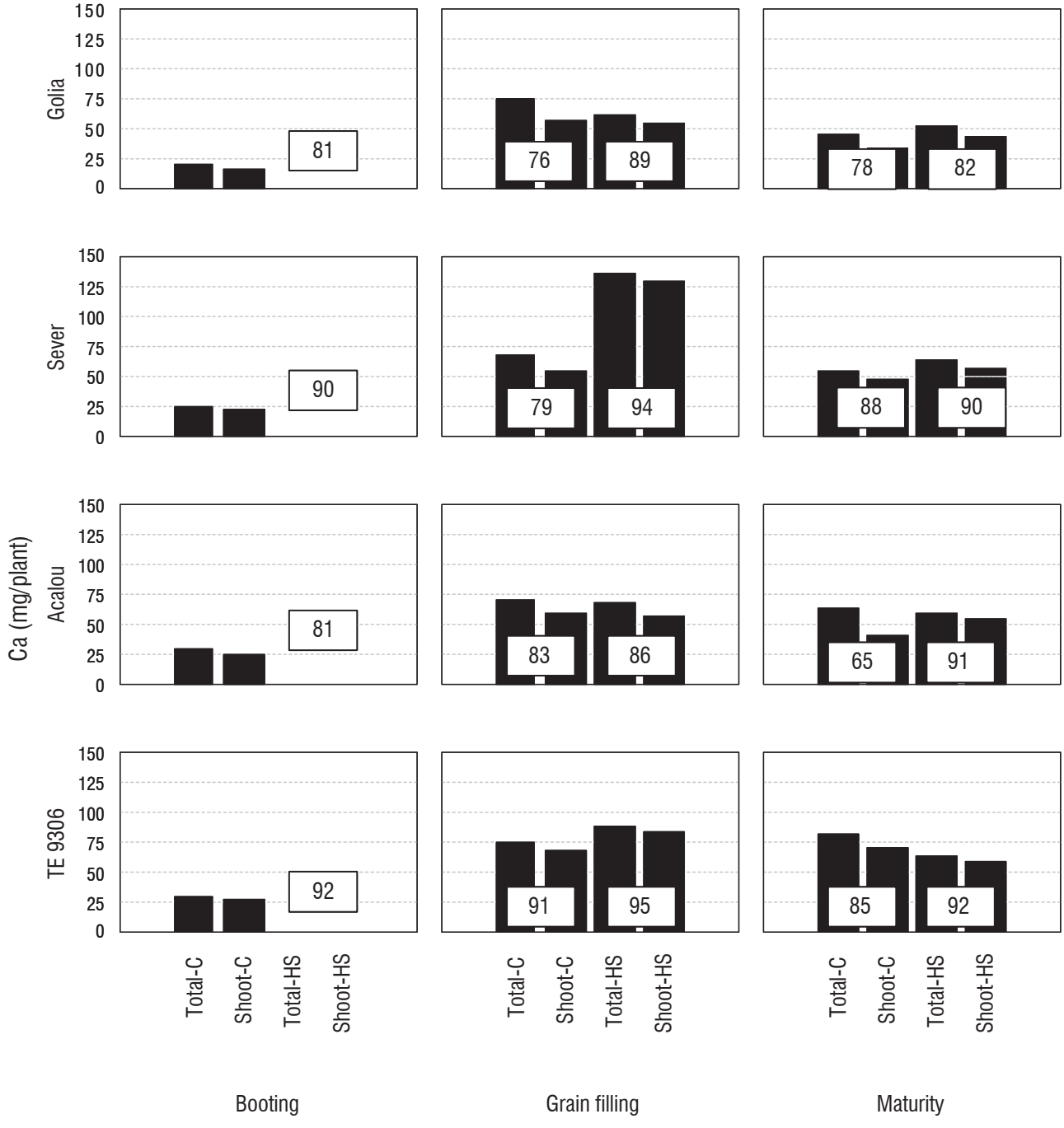

Figure 2. Total plant and shoot accumulation of Ca in bread and durum wheat genotypes in three stages of growth cycle, in control and heat stress treatments. Values inside boxes refer to Ca translocation (%) from roots to shoots. Total C, Shoot C, Total HS and Shoot HS stand for total plant and shoot accumulation in the control treatment, and total plant and shoot accumulation in the heat stress treatment, respectively.

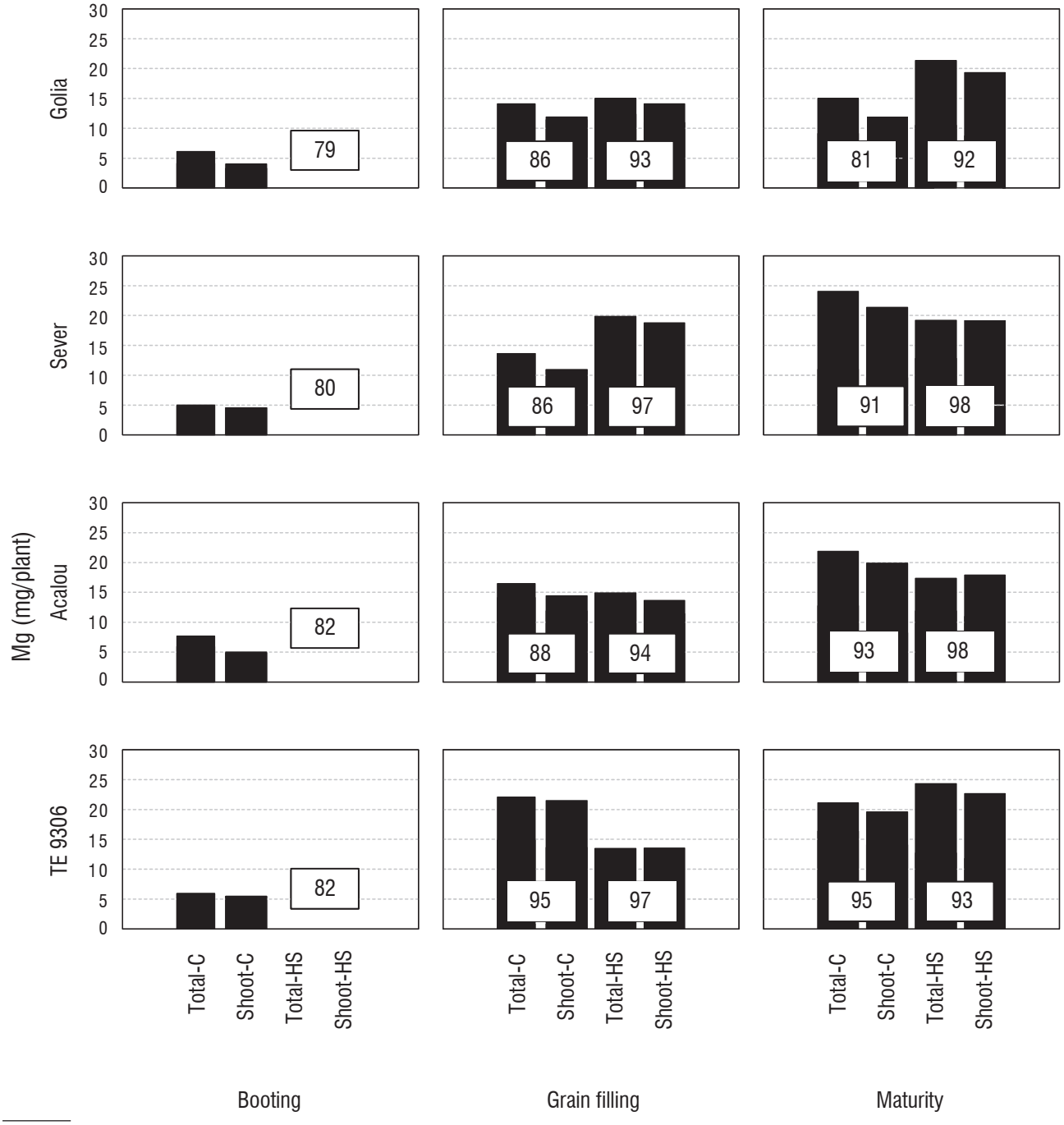

Figure 3. Total plant and shoot accumulation of Mg in bread and durum wheat genotypes in control and heat stress treatments. Values inside boxes refer to Ca translocation (%) from roots to shoots. Total C, Shoot C, Total HS and Shoot HS stand for total plant and shoot accumulation in the control treatment, and total plant and shoot accumulation in the heat stress treatment, respectively.

Table 3. Magnesium concentration in different plant parts, on three stages of plant growth (booting, grain filling and maturity), of control and heat stress bread and durum wheat genotypes. Each value is the mean ± S.E. of three replicates. Different letters in the same column refer to significant differences between genotypes. Between treatments, ns, *, **and *** refer to: non significant, $P \leq 0.05$, $P \leq 0.01$ and $P \leq 0.001$, respectively.

	Booting	Grain filling		Maturity	
		Control	Heat stress	Control	Heat stress
		Root (mg/g_{dw})			
Golia	2.86±0.20a	0.94±0.06a	1.05±0.06a ns	2.34±0.36a	2.11±0.49a ns
Sever	2.48±0.23a	1.50±0.18a	0.59±0.01b *	2.69±0.58a	0.85±0.02a ns
Bread wheat	2.67±0.17	1.22±0.18	0.82±0.13 *	2.51±0.30	1.48±0.42 ns
Acalou	2.46±0.21a	1.11±0.23a	1.04±0.01a ns	1.22±0.05a	0.78±0.24a ns
TE 9306	2.00±0.07a	1.20±0.02a	0.58±0.02b **	1.32±0.22a	4.18±0.75b ns
Durum wheat	2.23±0.16	1.15±0.10	0.81±0.14 *	1.27±0.10	2.48±1.03 *
		Shoot (mg/g_{dw})			
Golia	3.24±0.02a	2.63±0.04a	3.49±0.38a ns	3.64±0.03a	3.97±0.04a *
Sever	2.48±0.17b	1.73±0.03b	3.25±0.03a ***	3.93±0.55a	4.88±0.10b ns
Bread wheat	2.86±0.23	2.18±0.26	3.37±0.17 **	3.79±0.24	4.43±0.27 ns
Acalou	2.44±0.07a	1.80±0.05a	2.02±0.02a ns	4.06±0.39a	1.58±0.32a *
TE 9306	2.25±0.38a	2.50±0.38a	2.65±0.64a ns	3.99±0.48a	3.92±0.49a ns
Durum wheat	2.35±0.16	2.15±0.25	2.33±0.32 ns	4.03±0.25	2.75±0.72 *
		Spike (mg/g_{dw})			
Golia		1.45±0.01a	1.91±0.19a ns	1.25±0.17a	2.50±0.14a *
Sever		1.25±0.01b	2.13±0.08a **	1.63±0.35a	1.06±0.08b ns
Bread wheat		1.35±0.06	2.02±0.11 **	1.44±0.19	1.78±0.42 ns
Acalou		1.67±0.06a	1.60±0.08a ns	1.97±0.37a	2.73±0.66a ns
TE 9306		3.62±0.16b	1.67±0.10a **	1.92±0.03a	2.93±0.64a ns
Durum wheat		2.65±0.57	1.63±0.06 ***	1.95±0.15	2.83±0.38 ns

At grain filling, in all the control and heat stressed genotypes, the rate of electrolyte release (Fig. 4) decreased during the 24 hours of the assay. In TE 9306 this extrusion rate showed minor variations with high temperatures, but the opposite was found for Acalou (Fig. 4). Under heat stress, the pattern of the extrusion rates in Golia and Acalou remained similar, but the opposite was found for Sever, being the electrolyte release higher under heat stress conditions (Fig. 4).

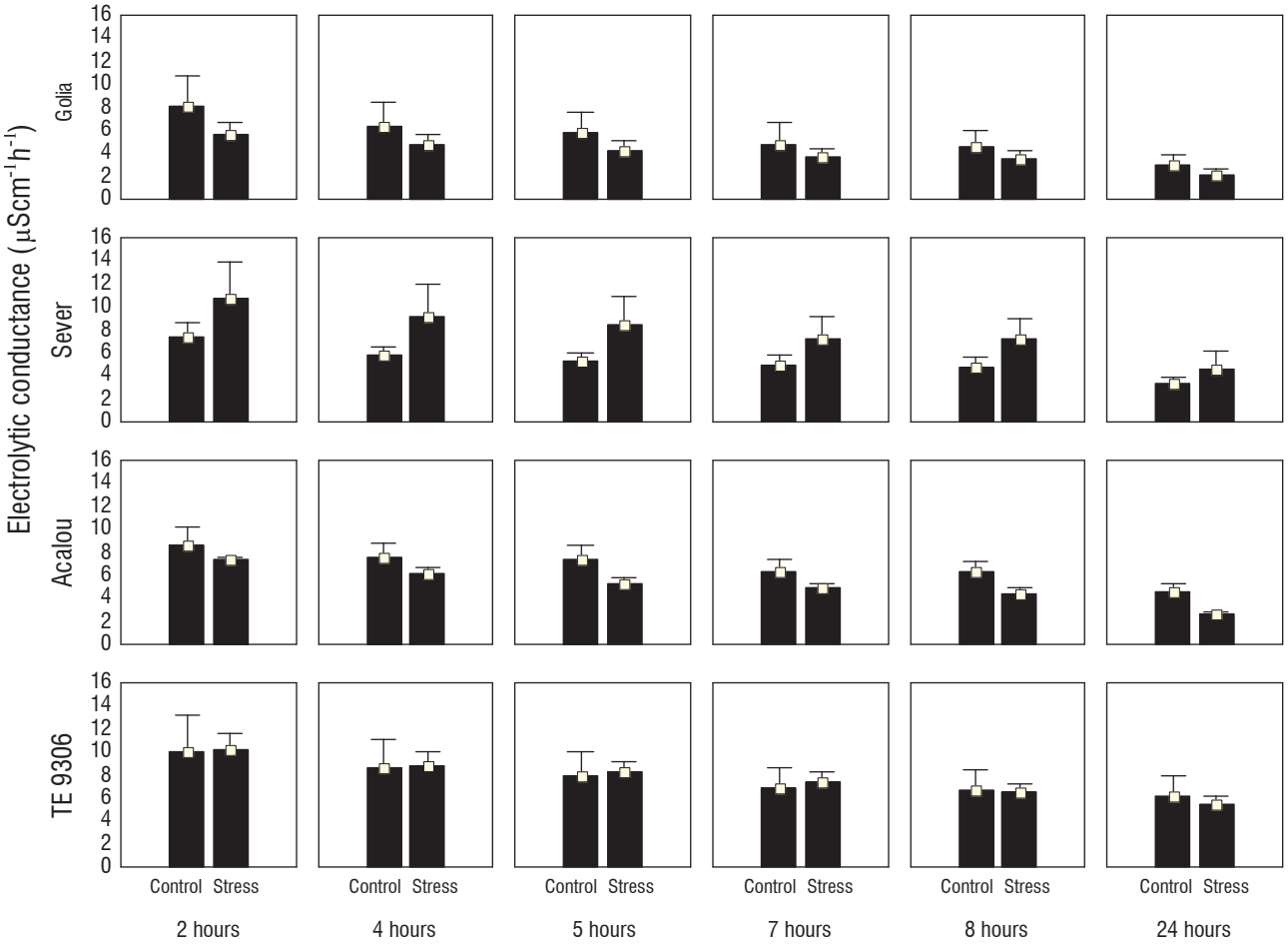

Figure 4. Electrolytic conductance in wheat flag leaves, during grain filling (control and heat stress treatments). Each column represents the mean value of three replicates (each replication is the mean value of 5 discs). Vertical trays stand for SE.

During grain filling, the levels of total Chl tend to decrease in the heat stressed genotypes (except in Sever), significantly only in Acalou (Table 4).

Table 4. Total chlorophyll content in wheat flag leaves, 102-103 and 103-106 days after emergence, for control and heat stress, respectively. Data are mean ± S.E. for n = 4 replicates.

	Total chlorophyll (mg/m^2)	
Genotypes	Control	Heat stress
Golia	784±52	738±44 ns
Sever	752±50	904±15 *
Acalou	798±21	701±28 *
TE 9306	795±11	688±69 ns

After anthesis A changed significantly in the heat stressed bread wheat, being found antagonistic patterns, since in Golia the A values decreased, whereas an increase was found in Sever (Table 5). Something similar was observed with the durum genotypes between the control and stress conditions, although in Acalou and TE 9306, these changes were mostly not significant (Table 5). In all the *Triticum* genotypes g_s and A displayed quite parallel trends (Table 5). The mean of g_S values decreased (although not significantly) in the heat stressed Golia and TE 9306, while in Sever and Acalou opposite patterns were found (Table 5). During grain filling, the heat stressed Golia revealed a significant increase of the mean C_i (Table 5), indicating that CO_2 contents were not a limiting factor despite some decrease in g_s. Moreover, C_i did not significantly changed

in the remaining wheat genotypes, except in Sever where an enhancement was found for pm values (Table 5). In Golia and TE 9306, E didn't vary significantly between the control and heat stress, but in Sever and Acalou, significant increases of 26 and 36%, respectively were found in the morning and of 121% in Sever in the afternoon (Table 5).

Table 5. A, g_s, C_i, E in bread and durum wheat flag leaves, measured on the morning and afternoon in the two temperature treatments, during plants life cycle. Data are mean ± S.E. for n = 12, each replication represents the mean value of 3 readings. Between treatments, ns, *, ** and *** refer to: non significant; $P \leq 0.05$; $P \leq 0.01$ and ***$p \leq 0.001$, respectively.

A (μmol CO_2 $m^{-2}s^{-1}$)						
	am		**pm**		**Daily Mean**	
	Control	Heat stress	Control	Heat stress	Control	Heat stress
Golia	34.5±0.6	22.3±2.8 **	31.5±1.3	21.7±1.8 **	33.0	22.0
Sever	29.6±1.4	30.8±2.8 ns	20.8±0.5	35.4±0.9 ***	26.7	32.4
Acalou	30.3±1.1	31.0±1.2 ns	32.5±0.9	36.2±0.3 **	31.4	33.6
TE 9306	31.4±1.2	29.0±2.7 ns	36.4±0.2	30.8±4.1 ns	33.9	29.9
g_s (mmol H_2O $m^{-2}s^{-1}$)						
	am		**pm**		**Daily Mean**	
	Control	Heat stress	Control	Heat stress	Control	Heat stress
Golia	603±50	484±49 ns	604±33	544±82 ns	604	514
Sever	459±35	595±29 *	309±4	965±75 ***	409	718
Acalou	604±23	585±19 ns	539±55	617±14 ns	572	601
TE 9306	598±44	523±12 ns	752±35	687±19 ns	675	605
C_i (ppm)						
	am		**pm**		**Daily Mean**	
	Control	Heat stress	Control	Heat stress	Control	Heat stress
Golia	254±5	279±5 **	267±3	283±2 **	261	281
Sever	251±4	267±10 ns	246±3	276±5 **	249	270
Acalou	267±4	266±2 ns	255±5	257±2 ns	261	262
TE 9306	263±6	264±7 ns	269±4	276±10 ns	266	270
E (μmol H_2O $m^{-2}s^{-1}$)						
	am		**pm**		**Daily Mean**	
	Control	Heat stress	Control	Heat stress	Control	Heat stress
Golia	8.3±0.7	8.1±0.4 ns	9.6±0.2	9.6±0.5 ns	9.0	8.8
Sever	6.8±0.3	8.6±0.2 ***	7.3±0.1	16.2±0.9 ***	6.9	11.1
Acalou	7.3±0.3	9.9±0.2 ***	10.9±0.8	9.2±0.2 *	9.1	9.5
TE 9306	8.1±0.3	8.4±0.1 ns	10.6±0.5	10.5±0.3 ns	9.3	9.4

DISCUSSION

During grain filling, the significant increase of Ca concentration in bread wheat genotypes (Table 1; Fig. 2) indicated that heat stress increases Ca assimilation from roots to the shoots and spikes. The increasing levels of Ca^{2+} might accumulate in the citosol, alleviating heat injury (Biyaseheva et al., 1993; Palta, 1996; Gong et al., 1998; Jiang and Huang, 2001), increasing cellular survival (Bamberg et al., 1998; Gong et al., 1998) and limiting oxidative damage (Larkindale and Knight, 2002), namely Chl photodestruction, as previously found by Jiang and Huang (2001), working

with heat stressed *Festuca arundinacea* L. and *Poa pratensis* L. Our data also indicated that Ca accumulation was linked to a higher tolerance to heat stress, suggesting that the concentrations of Ca shield the Chl levels in Sever (Table 4). Nevertheless, as increasing levels of this nutrient were not coupled to the maintenance of the selectivity of membrane permeability (Fig. 3), this pattern disagrees with the findings of Cooke et al. (1986) and Coria et al. (1998) that suggested different interspecific metabolisms for Ca in sugar beet and potato. During the life wheat genotypes cycle, Mg uptake was not significantly depressed by Ca and, therefore, an antagonistic interaction between these nutrients accumulations in the shoots could not be found (Table 1, 3). Nevertheless, shoot Mg accumulation displayed a synergistic pattern with Chl accumulation. During grain filling, the levels of Mg in the shoot of heat stressed Sever (Table 3), linked to a higher translocation rate from roots (Fig. 3) supported the observed increase (Table 4) of Chl contents (Bergmann, 1992; Schoefs and Bertrand, 1997). At maturity, the significant decrease of Mg contents in the leaves of Acalou (Table 3) resulted of a decreased rate of its translocation from roots (Fig. 4) and contributed to the inhibition of Chl accumulation (Table 4). Further supporting a different extend of tolerance (Al-khatib and Paulsen, 1989; Maçãs et al., 1999; 2000; Dias et al, 2008, 2009; Dias and Lidon 2009) implicating these nutrients interactions, in the heat stressed durum wheat, following a lower Ca and Mg shoot accumulation at maturity, TE 9306 showed a decreased mean A relatively to Acalou, that paralleled with a diminished Chl content (Table 4, 5). In bread wheat, Sever showed much higher tolerance to high temperatures than Golia, the later showing strong A decreases. Such A sensitivity to heat stress in Golia, agree with the observations in *Pisum sativum* (Haldimann and Feller, 2005) where was suggested an inhibition of photosynthesis due to efficiency losses in the use of photochemical energy and in the acyclic electron transport. Moreover, in Sever and Acalou, the maintenance or increased g_S values might have favored the maintenance of high A values, in a process that is coupled with the levels of Ca in the shoot (Table 1), probably coupling cellular turgescence (Hare et al.., 1998) to an osmotic adjustment modulated by the symplast and apoplast Ca^{2+} levels (Palta, 1996). Furthermore, such increased g_s under heat stress values would explain the higher E values in these genotypes (Table 5), probably allowing a better leaf cooling process that implicated evaporation, as previously reported by Fischer et al. (1998). That further protects the photosynthetic machinery allowing higher A values.

From our data we conclude that independently of the genetic background diversity, in the heat stressed wheat, Ca and Mg accumulation rates are synergistically linked with Chl accumulation. Additionally, Ca is further implicated in gs regulation, which determines the bread and durum wheat photosynthetic performance.

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