RESEARCH ARTICLE

IV. Heat stress in *Triticum*: kinetics of Fe and Mn accumulation

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ABSTRACT

The interactions between Fe/Mn accumulation and the photosynthetic light reactions were investigated in heat stressed bread and durum wheat genotypes (*Triticum aestivum* L. and *Triticum turgidum subsp.durum*). Four genotypes were chosen according to its genetic background diversity. Plants were grown in a greenhouse at two different day/night temperatures regimes (control - 25/14ºC and heat stress - 31/20ºC), during the grain filling phase. The contents and uptake/translocation of Fe and Mn were evaluated on booting, grain filling and maturity and correlated with chlorophyll a fluorescence parameters. It was found that, under heat stress, the concentrations of Fe in the culm and leaves diminished in bread wheat, but increased in durum wheat. An opposite trend was found on the contents of Fe in the spike, being this effect higher in Sever. During grain filling, the concentrations of Mn in the heat stressed plants raised significantly in the shoot for all genotypes (excepting Golia). After anthesis, the proportion of Mn translocated also augmented with high temperatures. At maturity, the same trend was found in the translocated proportion of Fe. During grain filling, all the studied fluorescence parameters did not vary significantly except q_P and q_E that decreased in the heat stressed Golia and F_V and F_v/F_m that increases in Acalou. It was concluded that under heat stress, during the grain filling period, Fe and Mn translocation to the shoots accelerates, eventually to overcome the negative effects on several photosynthetic physiological traits.

Key words: heat stress; nutrients; translocation; *Triticum aestivum* L.; *Triticum turgidum subsp. durum*; uptake.

INTRODUCTION

The optimal mean temperature for the crops growth cycle varies between 15-18ºC (Chowdhury and Wardlaw 1978), being 20ºC the optimal value for grain filling (Dupont and Altenbach, 2003; Jenner, 1991; Russell and Wilson, 1994). Moreover, under heat stress, the yield performance of wheat genotypes is strongly affected (Spiertz, 1977; Wardlaw et al., 2002), occurring a global reduction of about 3-4% when the mean temperature increases 1ºC above the optimum value (Wardlaw et al., 1989). During grain filling, heat shocks (about 35-40ºC) also have a negative effect on grain quality and dry weight (Ciaffi et al., 1996). In this context, moderate heat stress (35-45ºC) can inhibit photosynthesis without damaging PS II (Sharkey 2005). Yet, when temperatures rise c.a. 45ºC (Çjánek et al., 1998, Yamane et al., 1998), the thermal damage of the photosynthetic oxygen evolving complex (Enami et al. 1994, Mohanty et al. 2002) affects the electron transport (Bukhov et al., 1999; Kouřil et al., 2004; Mohanty et al., 2002). In the heat stressed *Triticum*, the alteration of chloroplasts structure (namely, grana stacking) has further been reported (Sharkova and Bubolo, 1996), with moderate high temperatures triggering a thylakoid permeability deviation that affects the proton gradient (Bukhov et al., 1999; Schrader 2004), the cyclic photophosphorylation and eventually the b_6/f cytochrome complex (Sharkey 2005).

Considering that plant growth is metabolically driven, the adjustment between the nutrient status and high temperature becomes overexpressed if a feed-back inhibition can develop. In this context, Fe and Mn are essential for plant growth being required appropriate contents at intracellular level, with ratios between them from 1.5 to 2.5 (Bergmann 1992; Alvarez-Tinaut et al., 1980), due to its impact for the photosynthetic electron transfer, reduction of nitrites and sulfates, chlorophyll synthesis and on the nucleic acid metabolism (Boardman, 1975; Nicholas, 1975; Mengel and Kirkby, 1978; Price et al., 1972; Wild and Jones, 1988). However, under stress conditions, Fe and Mn uptake by the roots and the translocation to the shoot shows opposite effects, leading to nutritional unbalancing (Alvarez-Tinaut et al., 1980; Brown and Devine, 1980; Terry, 1979).

Working with 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), integrating different genomic characteristics with different tolerance to high temperature after anthesis (Maçãs et al., 1999; 2000), an insight in the magnitude of the implications of heat stress on the photosynthetic light reactions, considering the Fe and Mn uptake (in the roots) and translocation (to the shoots, spikes and grains), during the consecutives development stages, is discussed being presented an integrative physiological perspective.

Abbreviations: DAE = days after emergence. - F_0 = minimal fluorescence. - F_m = maximal fluorescence. - F_v = variable fluorescence. - F_v/F_m = maximum photochemical efficiency of photosystem II. - GF = grain filling. – NPQ = Stern-Volmer non-photochemical quenching coefficient. - PPFD = photosynthetic photon flux density. - PS II = photosystem II. - q_E = energy-dependent quenching. - q_P and q_{NP} = photochemical and non-photochemical quenchings. - Φe = quantum yield of photosynthetic non-cyclic electron transport.

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 28 ºC 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 hours) 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. During all the experiment the position of the pots was changed weekly, to minimize the effects due to irradiance variations. Plants were irrigated weakly but alternatively with distillated water or with a standard nutrient solution, alternately (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 two different temperature conditions (controlled and heat stress). Under heat stress the plants were submitted to temperatures that rose until 40 ºC. During the grain filling period, control plants grew under regimes with mean temperatures (day/night) of 25/14 ºC and 31/20 ºC (control and heat stress conditions, respectively). The average of day/night temperatures was calculated as the mean readings of each two hours, during each 24 h period.

Nutrients analysis: The concentrations of Fe and Mn were determined in roots, shoots and spikes (at booting-69/70 days after anthesis; grain filling-108/109 and 109/112 days after anthesis, for the genotypes submitted to control and heat stress conditions, respectively) and also in the grain at maturity. 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. From each sample, 1 g $_{dw}$ 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 Fe and Mn, 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 mean content in all *Triticum* roots, shoots and grains. The net uptake was determined adding these values.

Pigments and chlorophyll fluorescence measurements: Total Chl of the bread and durum wheat flag leaves were extracted in 100% acetone and measured spectrophotometrically, using four replicates, according to (Lichtenthaler, 1987). Chl *a* fluorescence parameters were measured (Fig. 2) in three different moments (at booting – 58/59 DAE, at middle GF – between 89 and 92 DAE and at final GF - between 115 and 118 DAE), using a PAM 2000 system (H. Walz, Effeltrich, Germany), on leaf pieces placed inside the LD2/2 O_2 electrode, under CO_2 saturating conditions, at 25 °C, similarly to described in Ramalho et al. (1997; 2002). Briefly, measurements of F_0 and F_v/F_m, were taken from dark-adapted leaves, while photosynthetic steady-state conditions (with a PPFD of 450 μmol m^{-2} s^{-1}) were used to determine q_P, q_{NP} and φ_e (Genty et al., 1989; Krupa et al., 1993; Van Kooten and Snell, 1990). For q_E (Quick and Stitt, 1989) determination, a saturating flash was applied after the recovery of the fast component of the rise observed in F_0' (usually 90–110 s following the removal of actinic illumination). For the saturating flashes, a PPFD of *ca*. 4400 μmol m^{-2} s^{-1}, with duration of 0.8 s, was used.

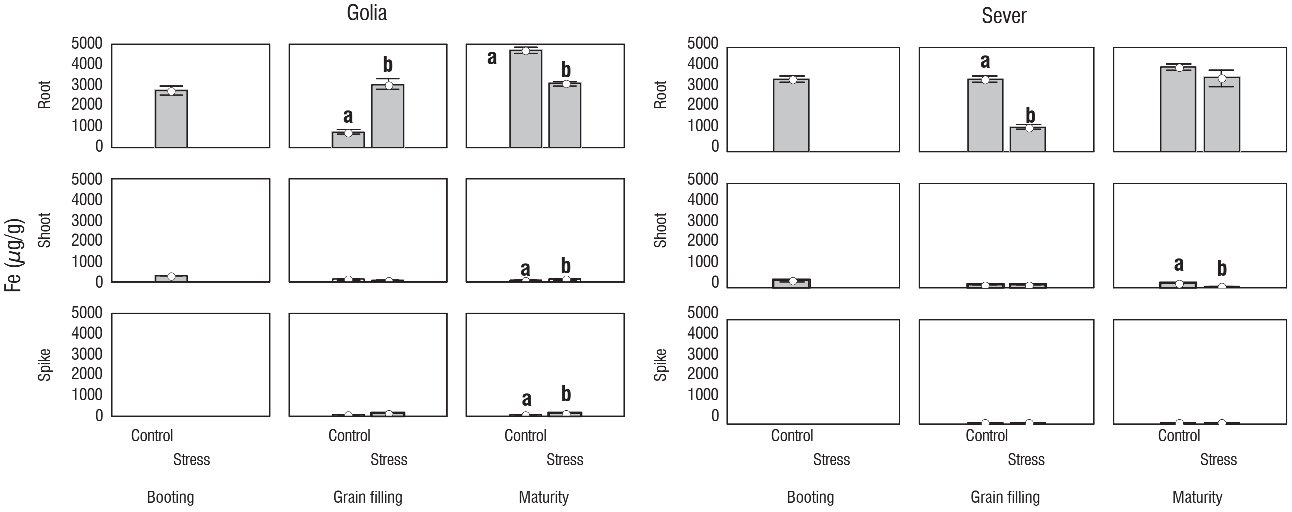

Figure 2. Iron concentration in different parts of the bread wheat plants (left-genotype Golia; right-genotype Sever), for each studied stage of growth cycle (booting, grain filling and maturity) in the two temperature treatments (n=3). Vertical trays stands by SE. Letters a and b stands by significant different means.

Statistic analysis: Statistic analysis were performed with a two-way ANOVA ($p \leq 0.05$), using *STATISTICA*, version 6 (2001), by *StatSoft, Inc*. In figures, each value is the mean ± S.E. of three replicates and letters a and b stands for significant different means. 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 AND DISCUSSION

After anthesis, the studied *Triticum* genotypes faced a consistent period of moderate high temperatures and, additionally, also suffered short periods with mean daily temperatures higher than 32 ºC (Fig.1). These growth conditions prompted characteristic effects of moderate high temperatures (25/32 ºC) after anthesis (Wardlaw et al., 1980; 1989) and specific behaviors (Blumenthal et al. 1991a, b; Randall and Moss, 1990; Stone and

Nicolas, 1994; 1995) associated to heat shocks (> 32 ºC). Accordingly, as found in previous studies by our research group, after anthesis *Triticum aestivum* L. genotype Sever displayed an higher tolerance to heat stress than the genotype Golia (Maçãs et al., 1999; Dias and Lidon, 2009, Dias et al, 2008, 2009) and *Triticum turgidum subsp. durum* genotype TE 9306 tolerance prevailed relatively to the genotype Acalou (Maçãs et al., 2000, Dias and Lidon, 2009; Dias et al 2008, 2009).

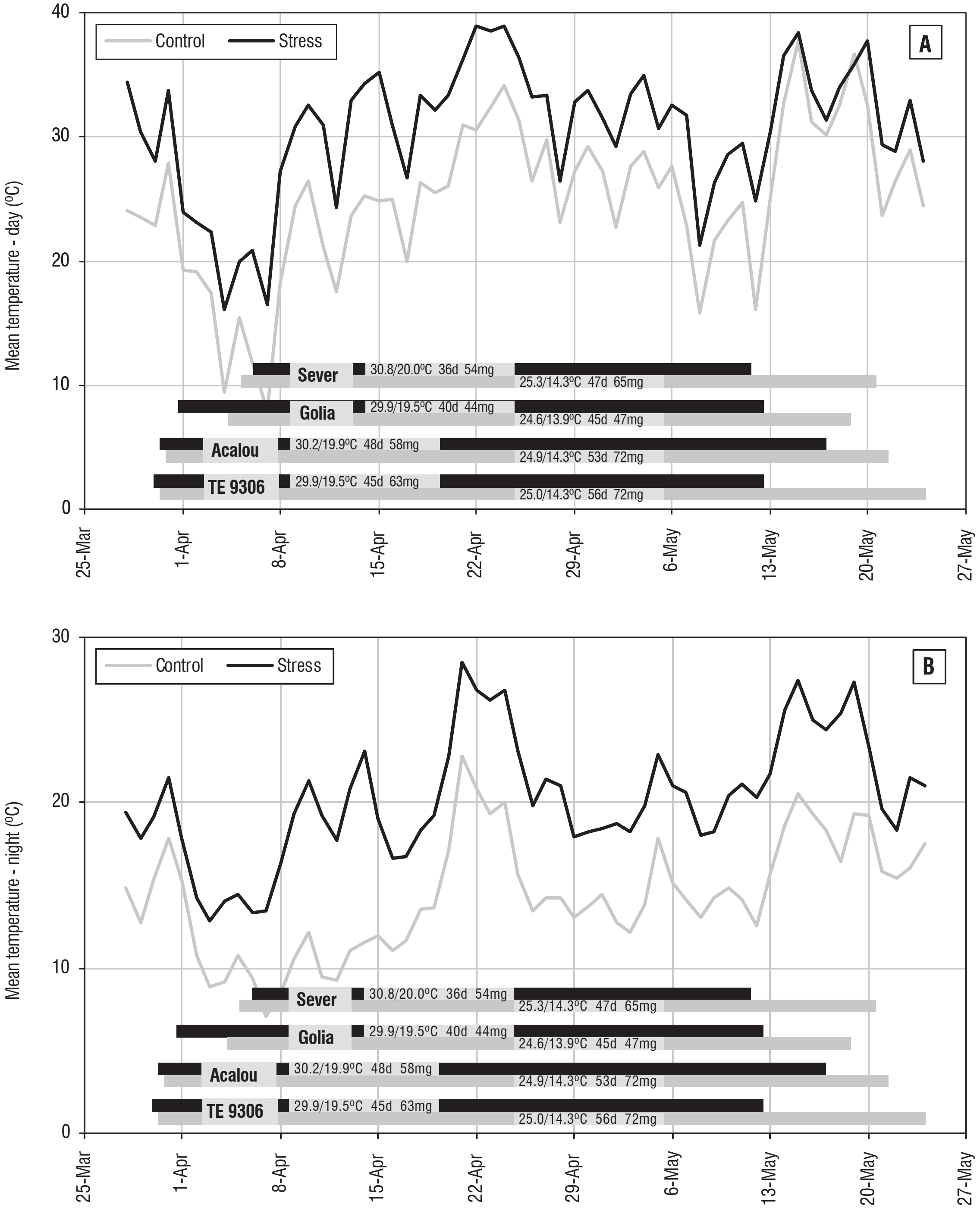

Figure 1. Mean daily temperature during the day (A) and night (B), in the grain filling period, for both treatments. Bars represent the grain filling period for each genotype. Values inside each bar stand by (for each genotype/treatment) from left to right: mean temperature during this period (day/night), duration (days) and grain weight at maturity.

As previously found by Lásztiti (1987) working with triticale, under our defined growth conditions the contents of Fe in the shoot of the *Triticum* genotypes revealed some similarities (Figs. 2, 3 and Table 1). Under heat stress, during grain filling, the concentrations of Fe in the culm and leaves of bread wheat genotypes decrease (Fig. 2), persisting this pattern at maturity in Sever. At this late stage, Fe levels in the control of Sever were significantly higher relatively to Golia in the shoot (Table 1), but decreased significantly (*ca*. 55%) under heat stress conditions (Fig. 2). Moreover, in Golia, the contents of Fe increased significantly (Fig. 2) in the shoot and spike (about 73% and 62%, respectively).

Table 1. Iron concentration (μg/g) 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		
Golia	2780±207a	744±97a	3057±252a *	4700±124a	3084±79a **
Sever	3513±139a	3484±123b	1195±104b **	4057±151a	3531±413a ns
Bread wheat	3147±235	2114±794	2126±549 ns	4378±202	3308±215 **
Acalou	3823±75a	936±46a	1889±11a **	3758±219a	2450±270a ns
TE 9306	2612±123b	1216±175a	2149±108a *	1895±29b	2367±233a ns
Durum wheat	3218±354	1076±109	2019±87 ***	2827±546	2408±148 ns
			Shoot		
Golia	284±7a	108±16a	87±7a ns	66±4a	114±7a *
Sever	350±33a	153±2a	148±11b ns	206±7b	92±15a *
Bread wheat	317±23	130±15	118±19 ns	136±41	103±9 *
Acalou	271±19a	121±17a	144±24a ns	91±3a	126±17a ns
TE 9306	139±1b	121±11a	147±8a ns	61±15a	78±2a ns
Durum wheat	205±39	121±8	146±10 ns	76±11	102±16 ns
			Spike		
Golia		93±16a	127±7a ns	105±8a	170±6a *
Sever		100±4a	109±2a ns	68±5a	64±7b ns
Bread wheat		97±7	118±6 ns	86±11	117±31 **
Acalou		115±7a	81±1a *	39±4a	112±16a *
TE 9306		99±1a	66±11a ns	81±9b	71±7a ns
Durum wheat		107±5	73±6 **	60±13	92±14 *
				Grain	
Golia				65±1a	75±11a ns
Sever				32±2b	63±4a *
Bread wheat				48±10	69±6 *
Acalou				55±12a	71±1a ns
TE 9306				45±3a	50±5a ns
Durum wheat				50±6	61±7 ns

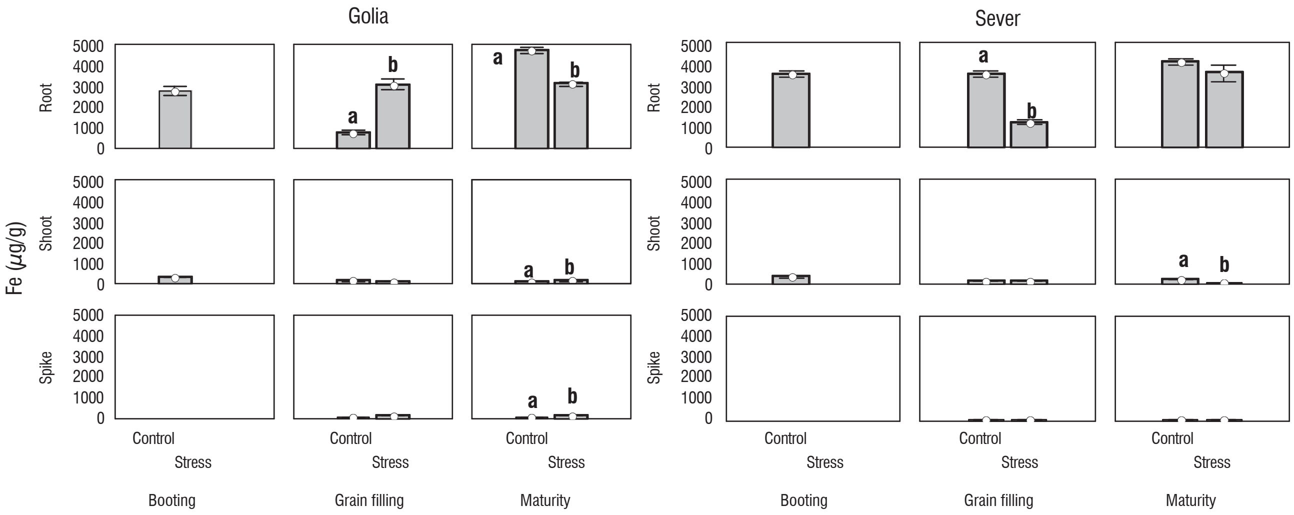

Figure 2. Iron concentration in different parts of the bread wheat plants (left-genotype Golia; right-genotype Sever), for each studied stage of growth cycle (booting, grain filling and maturity) in the two temperature treatments (n=3). Vertical trays stands by SE. Letters a and b stands by significant different means.

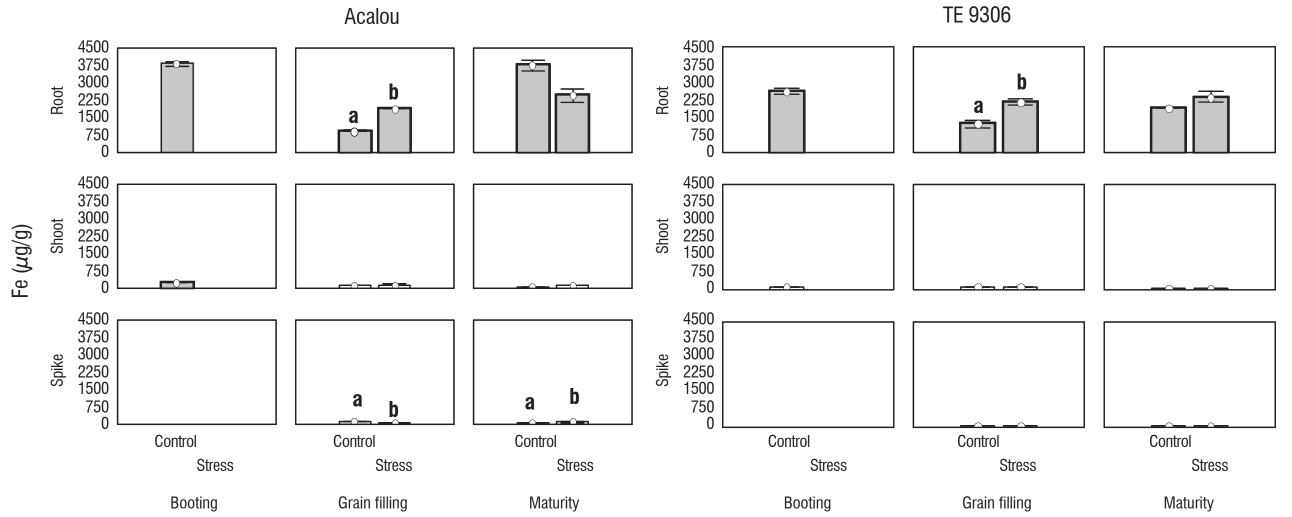

Figure 3. Iron concentration in different parts of the durum wheat plants (left-genotype Acalou; right-genotype TE 9306), for each studied stage of growth cycle (booting, grain filling and maturity) in the two temperature treatments (n=3). Vertical trays stands by SE. Letters a and b stands by significant different means.

Concerning to durum wheat, with high temperature, the Fe levels of shoots displayed a different behavior relatively to bread wheat (Fig. 3 and Table 1): during grain filling Fe increased (although not significantly); in the spike, while at maturity only in Acalou a significant increase was found, persisting thereafter this trend. At maturity, the contents of Fe in the shoot remained similar to those described by other authors for *Triticum* (Davis et al., 1984). The heterogeneous patterns displayed by Fe accumulation in the shoot tissues pointed the occurrence, at a different extent, of a limiting factor linking the intensive growth of these cereals to the heat stress (Kabata-Pendias and Pendias, 1992). Eventually, the availability of citrates chelates, required for Fe transport in the xylem (Kabata-Pendias and Pendias, 1992) changed in heat stressed genotypes, becoming restricted in Sever at maturity, therefore inducing these plant responses.

During grain filling, the contents of Mn, in the roots of Sever, decreased with high temperatures (Fig. 4, Table 2), while the opposite was observed in the durum wheat genotypes (Fig. 5, Table 2). At maturity, such increase was now observed in Golia, while the other genotypes presented heat unaffected contents. These findings gave further evidence that Mn uptake is metabolically controlled (being the high temperature an interacting factor), apparently in a quite similar pathway to that of other divalent cations species such as Mg^{2+} and Ca^{2+}. At booting, the concentrations of Mn in the shoots (Figs. 4, 5 and Table 2), were similar to those described by Bergmann (1992), for bread and durum wheat. During grain filling the high temperatures provoked Mn increases of 28%, 108%, 66% and 33%, in Golia, Sever, Acalou and TE 9306, respectively. At maturity, this trend persisted only in Sever and TE 9306 (Fig. 5), while a decrease was observed in the other 2 genotypes (significant in Golia). Even so, under heat stress, the Mn concentration in Golia was significantly higher than in Sever (Table 2), what would be related to the already significant higher concentration in the control, which largely surpasses values previously described for *Triticum* (Dikeman et al., 1982; Davis et al., 1984).

Table 2. Manganese concentration (µg/g) 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 ≤ 0.05, P ≤ 0.01 and P ≤ 0.001, respectively.

	Booting	Grain filling		Maturity	
		Control	Heat stress	Control	Heat stress
			Root		
Golia	53.76±5.13a	66.71±2.60a	68.58±1.66a ns	105.15±4.18a	134.81±0.48a *
Sever	64.03±3.42a	84.34±2.87b	36.35±0.32b **	86.27±5.76a	85.79±7.77b ns
Bread wheat	58.89±3.89	75.52±5.33	52.46±9.33 ***	95.71±6.18	110.30±14.50 ns
Acalou	67.75±0.23a	29.79±0.41a	33.89±0.23a *	82.34±6.40a	89.79±3.52a ns
TE 9306	51.81±1.63b	36.88±0.23b	52.29±1.07b **	55.13±0.39a	57.58±2.78b ns
Durum wheat	59.78±4.65	33.34±2.05	43.09±5.33 ***	68.74±8.28	73.68±9.48 ns
			Shoot		
Golia	68.52±0.01a	89.52±1.13a	114.94±6.53a ns	130.23±4.40a	91.38±1.93a *
Sever	55.27±0.38b	28.54±0.07b	59.30±0.88b ***	36.60±1.37b	54.20±4.12b ns
Bread wheat	61.89±3.83	59.03±17.61	87.12±16.29 **	83.41±27.09	72.79±10.89 *
Acalou	41.38±3.08a	21.93±0.22a	36.34±0.83a **	56.57±2.98a	26.37±12.65a ns
TE 9306	60.95±3.97a	39.05±3.12b	53.12±0.16b *	39.97±0.31b	69.82±2.96a **
Durum wheat	51.16±6.01	30.49±5.11	44.73±4.86 ***	48.27±4.95	48.09±13.62 ns
			Spike		
Golia		43.71±0.70a	46.65±0.52a ns	39.45 ±0.12a	44.23±1.37a ns
Sever		30.42±0.80b	33.10±2.32b ns	25.65±0.56b	25.76±0.48b ns
Bread wheat		37.06±3.86	39.87±4.03 ns	32.55±3.99	35.00±5.37 *
Acalou		36.38±0.41a	34.08±2.12a ns	32.30±0.52a	35.97±1.18a ns
TE 9306		39.05±0.25b	46.03±0.89b *	66.92±30.30a	44.92±0.49b ns
Durum wheat		37.71±0.80	40.05±3.58 ns	49.61±15.91	40.44±2.64 ns
					Grain
Golia				38.94±2.73a	35.92±1.64a ns
Sever				29.50±0.12a	26.68±0.96b ns
Bread wheat				34.22±2.94	31.30±2.78 ns
Acalou				24.08±3.35a	30.62±0.56a ns
TE 9306				32.56±0.66a	35.58±1.06a ns
Durum wheat				28.32±2.82	33.10±1.51 ns

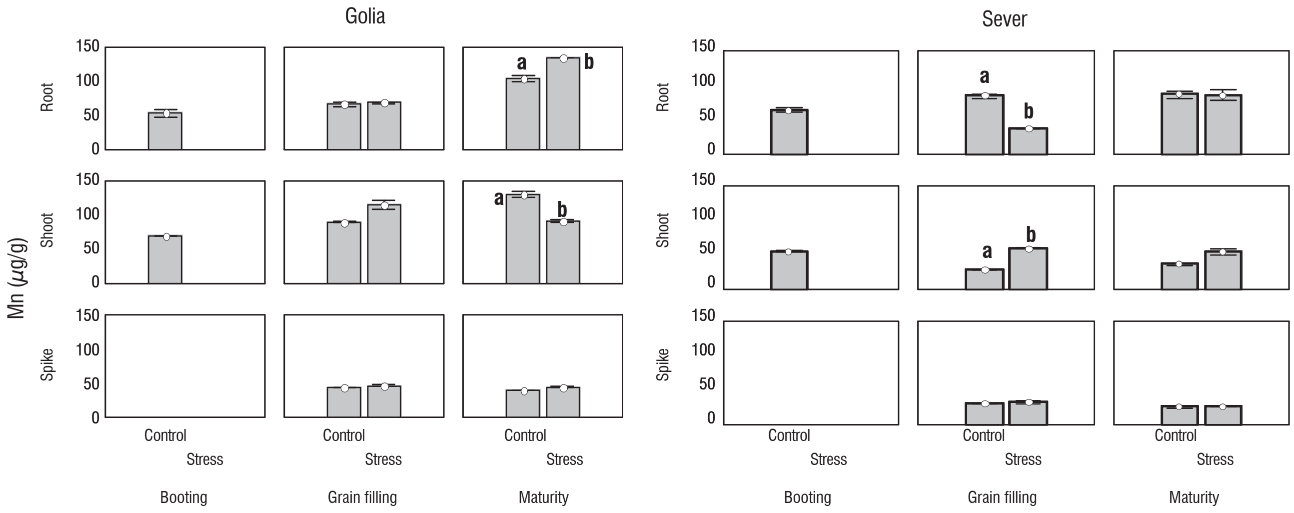

Figure 4. Manganese concentration in different parts of the bread wheat plants (left-genotype Golia; right-genotype Sever), for each studied stage of growth cycle (booting, grain filling and maturity) in the two temperature treatments (n=3). Vertical trays stands by SE. Letters a and b stands by significant different means.

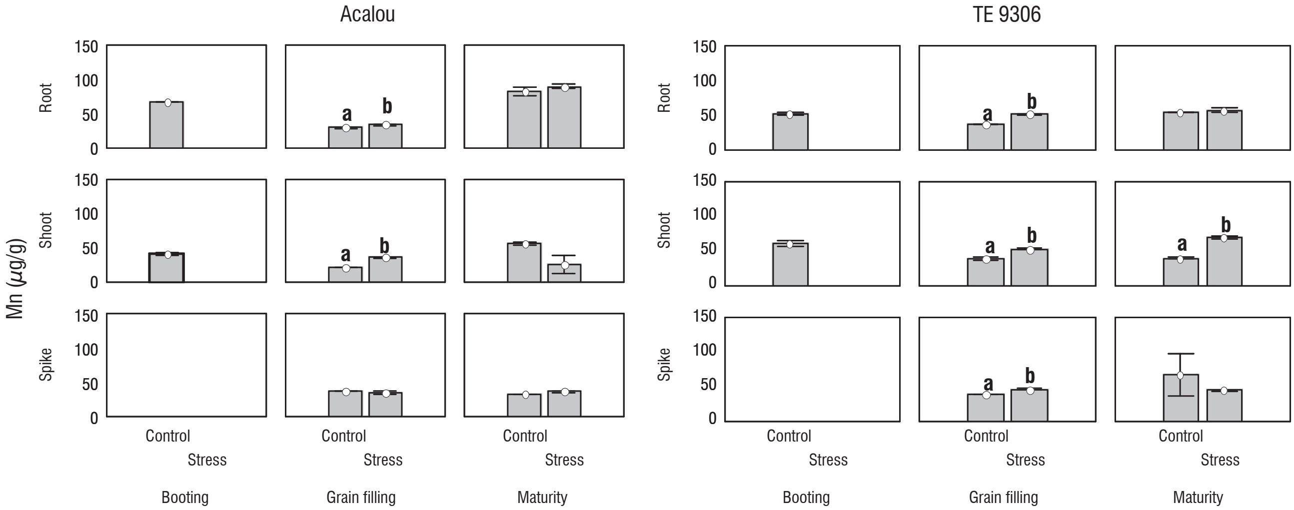

Figure 5. Manganese concentration in different parts of the durum wheat plants (left-genotype Golia; right-genotype Sever), for each studied stage of growth cycle (booting, grain filling and maturity) in the two temperature treatments (n=3). Vertical trays stands by SE. Letters a and b stands by significant different means.

The amount of Fe and Mn in the grain depends on the levels redistributed by the roots during grin development and the amount redistributed to the grain from vegetative tissue via phloem (Garnett and Graham, 2005). In this context, considering the Fe/Mn antagonistic interactions (Alvarez-Tinaut *et al*., 1980, Bergmann, 1992) the heat stress effects on shoot Mn content in Golia (as well as in Sever and Acalou) might be coupled to the changes on Fe contents (what was observed also in the grain filling stage). In non-stress conditions, during grain filling and at maturity, the Fe/Mn ratios in Golia (*ca.* 1.2 and 0.5, respectively) showed only a slight unbalance for Fe. Yet, under stress this limiting factor increased during grain filling, whereas became attenuated at maturity (Fe/Mn ratios of about 0.8 and 1.3, respectively). Concerning to Acalou and TE 9306, the high values of the Fe/Mn ratio (5.5 and 3.1, respectively), during grain filling, in non-stress conditions, probably further pointed an unbalanced nutrient status (implicating a Mn limitation), what seems to be ameliorated under heat stress. At maturity these genotypes further displayed antagonic behaviors (ratios of about 4.8

and 1.1, respectively), concomitantly confirming complex metabolic interactions.

It has long been reported that photosynthesis might vary significantly among wheat genotypes (Al-Khatib and Paulsen, 1990). In this context, under heat stress conditions, the Chl contents (Table 3) at the final grain filling increased significantly in Golia (although the levels of this molecule remained similar to those under control conditions, during booting – data not shown), strongly decreased in TE 9306 and showed non-significant rises in Sever and Acalou. The higher Chl levels in the heat stressed Golia (Table 3) correlated with the related increase of iron contents (Table 1); in Sever and Acalou these variations could not been found, while in the stressed TE 9306 the lower levels of Chl followed a non-significant variation of that nutrient. The correlation between Chl and Fe contents (except in TE 9306), at a physiological level, shield isoprenoid synthesis (Lidon and Henriques, 1992), which further modulate the cyclic photophosphorylation. In this context, under heat stress, F_0 and q_{NP} did not vary significantly, in all the wheat genotypes (Table 4), indicating an higher efficiency of the excitation energy transfer from the associated PS II antennae (thus, implicating Chl structure and organization) in a close association to an efficient utilization of chemical energy in the Calvin Cycle (Yordanov et al., 1999).

Table 3. Total chlorophyll and total carotenoids content in wheat flag leaves during grain filling, in the two temperature treatments. Data are mean ± S.E. for n = 4 replicates. Between treatments, ns and *refer to non significant and P ≤ 0.05, respectively.

	Total chlorophyll (mg/m^2)		Total carotenoids (mg/m^2)	
Genotypes	Control	Heat stress	Control	Heat stress
Golia	537±25	636±15 *	105±5	125±4 *
Sever	433±89	652±4 ns	84±11	117±2 ns
Acalou	559±25	600±85 ns	116±5	117±11ns
TE 9306	726±80	467±27 *	131±7	100±2*

Table 4. Chlorophyll a fluorescence parameters measured in the flag leaves of the bread and durum wheat genotypes, under control and heat stress conditions, in the grain filling phase. Data are mean ± S.E. for n = 4 replicates. Between treatments, ns, *, **refer to non significant, P ≤ 0.05, P ≤ 0.01 respectively.

	Golia		**Sever**		**Acalou**		**TE 9306**	
	Control	**Heat stress**	**Control**	**Heat stress**	**Control**	**Heat stress**	**Control**	**Heat stress**
F_0	33.50±0.89	30.63±0.38*	33.75±0.63	30.38±0.31**	32.63±0.13	31.88±0.83ns	32.13±0.63	31.25±0.83ns
F_v	173.88±2.16	157.00±2.38**	171.13±4.06	159.00±3.02ns	170.63±0.55	158.88±4.83ns	166.50±4.03	151.50±4.66ns
F_v/F_m	0.839±0.002	0.837±0.001ns	0.835±0.001	0.840±0.001*	0.839±0.001	0.833±0.002*	0.838±0.001	0.829±0.002*
q_P	0.725±0.034	0.702±0.017ns	0.717±0.021	0.675±0.027ns	0.774±0.011	0.709±0.024ns	0.723±0.008	0.737±0.024ns
q_{NP}	0.684±0.005	0.608±0.016*	0.703±0.008	0.677±0.024ns	0.630±0.007	0.638±0.017ns	0.670±0.022	0.559±0.028*
NPQ	1.400±0.049	1.043±0.061*	1.457±0.057	1.340±0.120ns	1.117±0.033	1.143±0.062ns	1.297±0.120	0.875±0.101*
q_E	0.469±0.018	0.403±0.012*	0.518±0.002	0.513±0.028ns	0.425±0.014	0.394±0.024ns	0.465±0.023	0.366±0.031ns

Additionally, as previously found in rice grown with high levels of Mn (Lidon et al., 2004), as F_v/F_m showed a strong stability in all genotypes (even if marginal significant changes occurred). Nevertheless, the lower levels of Mn during grain filling, in Golia (Table 2) seems to be linked to q_P and q_E (Table 4), justifying a decreasing efficiency in the conversion of the excitation energy used in the photochemical processes (Haldimann and Feller, 2005) and therefore a decreasing intra-thylakoidal gradient. In this context, the lower Mn levels could become a limitation in the water splitting complex, thus further shortening the proportion of energy absorbed by Chls associated to PS II (Table 2,4). On the other hand, in the other heat stressed genotypes, as Mn level did not decrease significantly, these fluorescence parameters remained unaffected (Table 4), reflecting a maintenance of a efficient functioning of the photosynthetic apparatus.

The total accumulation of Fe in all the genotypes remained sharply higher when compared with other data (Lidon, 2000), a higher proportion of this nutrient accumulated in the roots (Table 5). However, the contents of Fe in the shoot, spike and grain (Table 1) are similar to those reported by other authors (Bergmann, 1992; Davis et al., 1984; Lidon 2000). Under heat stress conditions, the proportion of total Fe accumulation in the grain of all genotypes (Table 5), following the tendency to accumulate in grain observed in Table 1.

Table 5. Iron total plant accumulation and respective allocation in different plant parts, on three stages of plant growth (booting - Boot, grain filling - GF and maturity - Mat) of control and heat stress bread and durum wheat genotypes.

	Golia					Sever				
	Control			Heat stress		Control			Heat stress	
	Boot	GF	Mat	GF	Mat	Boot	GF	Mat	GF	Mat
Total plant accumulation (μg per Plant)	1552	2197	6459	3676	3385	2154	5622	4369	1949	2556
% Maturity	24	34	100	109	100	49	129	100	76	100
% Plant parts	Boot	GF	Mat	GF	Mat	Boot	GF	Mat	GF	Mat
Root	75	72	91	83	71	72	82	76	52	77
Shoot	25	15	2	5	7	28	11	16	23	10
Spike		13	7	12	22		7	8	25	13
Grain			3		7			4		9
	Acalou					TE 9306				
	Control			Heat stress		Control			Heat stress	
	Boot	GF	Mat	GF	Mat	Boot	GF	Mat	GF	Mat
Total plant accumulation (μg per Plant)	2363	2327	4688	2346	1958	1772	1825	2063	1894	1470
% Maturity	50	50	100	120	100	86	88	100	129	100
% Plant parts	Boot	GF	Mat	GF	Mat	Boot	GF	Mat	GF	Mat
Root	76	63	91	66	57	82	62	71	69	65
Shoot	24	18	4	17	14	18	15	7	16	11
Spike		19	4	17	28		24	22	16	23
Grain			5		15			9		13

Between grain filling and maturity stages, under heat stress, the levels of total Mn in the plants were similar (Table 6). These data suggest that, under heat stress, the amount of Mn linked to the photoassimilates mobilized from plant tissues to the grain maintained the levels of this metal (Table 2) in the grain of the durum wheat genotypes (but not in bread wheat). Moreover, with high temperatures, Fe translocation to the shoots increased only at maturity, but did not trigger a higher concentration in all the genotypes, therefore limiting the variations of the light photosynthetic reactions. Among genotypes, during grain filling and at maturity, the proportion of Mn accumulated in the roots tend to be lower under heat stress, further revealing an increased translocated proportion to the shoots (which favored the Hill reactions in the photosynthetic apparatus), during grain filling, independently of total accumulation in the plant.

Table 6. Manganese total plant accumulation and respective allocation in different plant parts, on three stages of plant growth (booting - Boot, grain filling - GF and maturity - Mat) of control and heat stress bread and durum wheat genotypes.

	Golia					Sever				
	Control			Heat stress		Control			Heat stress	
	Boot	GF	Mat	GF	Mat	Boot	GF	Mat	GF	Mat
Total plant accumulation (μg per Plant)	116	548	556	477	490	124	349	326	358	332
% Maturity	21	99	100	97	100	38	107	100	108	100
% Plant parts	Boot	GF	Mat	GF	Mat	Boot	GF	Mat	GF	Mat
Root	19	26	24	14	21	23	32	22	9	14
Shoot	81	49	48	51	39	77	34	38	50	45
Spike		25	29	35	40		34	40	41	41
Grain			24		14			46		31
	Acalou					TE 9306				
	Control			Heat stress		Control			Heat stress	
	Boot	GF	Mat	GF	Mat	Boot	GF	Mat	GF	Mat
Total plant accumulation (μg per Plant)	119	264	390	295	279	168	291	507	346	389
% Maturity	31	68	100	106	100	33	57	100	89	100
% Plant parts	Boot	GF	Mat	GF	Mat	Boot	GF	Mat	GF	Mat
Root	27	18	24	9	15	17	12	8	9	6
Shoot	73	29	31	33	21	83	30	19	31	39
Spike		53	45	57	64		58	73	60	55
Grain			28		44			27		35

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