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 qP and qE that decreased in the heat stressed Golia and Fv and Fv/Fm 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 (Çiçek 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; Kouil 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 \( b_0/f \) 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 nitrates 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 (Maças 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. - \( F_{\infty} \) = maximal fluorescence. - \( F_v \) = variable fluorescence. - \( F_m/F_{\infty} \) = 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. - \( q_f \) and \( q_{NP} \) = photochemical and non-photochemical quenchings. - \( \Phi_E \) = 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 °C 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 distilled 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\(_2\)PO\(_4\) 100/100/100; MgSO\(_4\) 200/200/100; K\(_2\)SiO\(_3\) 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\(_2\)BO\(_3\) 10/5/2; CuSO\(_4\) 5/5/3; Na\(_2\)MoO\(_4\) 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 °C. During the grain filling period, control plants grew under regimes with mean temperatures (day/night) of 25 ± 14 °C and 31/20 °C (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 °C during 72 h. From each sample, 1 g was mineralized through incineration at ca. 550 °C, 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 O2 electrode, under CO2 saturating conditions, at 25 °C, similarly to described in Ramalho et al. (1997; 2002). Briefly, measurements of F0 and Fv/Fm 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 qϕ, qϕ and qϕ (Genty et al., 1989; Krupa et al., 1993; Van Kooten and Snell, 1990). For qϕ (Quick and Stitt, 1989) determination, a saturating flash was applied after the recovery of the fast component of the rise observed in F0′ (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.

**Statistic analysis:** Statistic analysis were performed with a two-way ANOVA (p≤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 ≤ 0.05, P≤ 0.01 and P≤ 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 °C (Fig.1). These growth conditions prompted characteristic effects of moderate high temperatures (25/32 °C) 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 ≤ 0.05, P ≤ 0.01 and P ≤ 0.001, respectively.

|                | Booting                | Grain filling             | Maturity                |
|----------------|------------------------|---------------------------|-------------------------|
|                | Control | Heat stress | 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         |
| TE9306         | 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         |
| TE9306         | 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      | 105±8a | 170±6a | *          |
| Sever          | 100±4a   | 109±2a      | 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 | *          |
| TE9306         | 99±1a    | 66±11a      | 81±9b | 71±7a | ns         |
| Durum wheat    | 107±5    | 73±6 | **         | 60±13 | 92±14 | *          |
| Grain          |         |             |         |             |         |             |
| Golia          | 65±1a    | 75±11a      | 55±12a | 71±1a | ns         |
| Sever          | 32±2b    | 63±4a | *          | 48±10 | 69±6 | *          |
| Bread wheat    | 48±10    | 69±6 | *          |         |         |            |
| Acalou         | 55±12a   | 71±1a | ns         |         |         |            |
| TE9306         | 45±3a    | 50±5a | ns         |         |         |            |
| Durum wheat    | 50±6     | 61±7 | ns         |         |         |            |
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 | 105.15±4.18a |
| Sever          | 64.03±3.42a | 84.34±2.87b   | 36.35±0.32b | 86.27±5.76a  |
| Bread wheat    | 58.89±3.89 | 75.52±5.33    | 52.46±9.33 | 95.71±6.18   |
| Acalou         | 67.75±0.23a | 29.79±0.41a   | 33.89±0.23a | 82.34±6.40a  |
| TE 9306        | 51.81±1.63b | 36.88±0.23b   | 52.29±1.07b | 55.13±0.39a  |
| Durum wheat    | 59.78±4.65 | 33.34±2.05    | 43.09±5.33 | 68.74±4.28   |
| Shoot          |         |               |          |
| Golia          | 68.52±0.01a | 89.52±1.13a   | 114.94±6.53a | 130.23±4.40a |
| Sever          | 55.27±0.38b | 26.54±0.07b   | 59.30±0.88b | 36.60±1.37b  |
| Bread wheat    | 61.89±3.83 | 59.03±17.61   | 87.12±16.29 | 83.41±27.09  |
| Acalou         | 41.38±3.08a | 21.93±0.22a   | 36.34±0.83a | 56.57±2.98a  |
| TE 9306        | 60.95±3.97a | 39.05±3.12b   | 53.12±0.16b | 39.97±0.31b  |
| Durum wheat    | 51.16±6.01 | 30.49±5.11    | 44.73±4.86 | 48.27±4.95   |
| Spike          |         |               |          |
| Golia          | 43.71±0.70a | 46.65±0.52a   | 39.45±0.12a | 44.23±1.37a  |
| Sever          | 30.42±0.80b | 33.10±2.32b   | 25.65±0.56b | 25.76±0.48b  |
| Bread wheat    | 37.06±3.86 | 39.87±4.03    | 32.55±3.99 | 35.00±5.37   |
| Acalou         | 36.38±0.41a | 34.08±2.12a   | 32.30±0.52a | 35.97±1.18a  |
| TE 9306        | 39.05±0.25b | 46.03±0.89b   | 66.92±30.30a | 44.92±0.49b  |
| Durum wheat    | 37.71±0.80 | 40.05±3.58    | 49.61±15.91 | 40.44±2.64   |
| Grain          |         |               |          |
| Golia          | 38.94±2.73a | 35.92±1.64a   | 35.97±1.18a | 35.97±1.18a  |
| Sever          | 29.50±0.12a | 26.68±0.96a   | 25.65±0.56b | 25.76±0.48b  |
| Bread wheat    | 34.22±2.94 | 31.30±2.78    | 32.30±0.52a | 35.97±1.18a  |
| Acalou         | 24.08±3.35a | 30.62±0.56a   | 32.56±0.66a | 35.58±1.06a  |
| TE 9306        | 32.56±0.66a | 35.58±1.06a   | 32.56±0.66a | 35.58±1.06a  |
| Durum wheat    | 28.32±2.82 | 33.10±1.51    | 32.56±0.66a | 35.58±1.06a  |
The amount of Fe and Mn in the grain depends on the levels redistributed by the roots during grain 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-Tinat 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 be 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, F0 and qNP 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, *, ** refer to non significant and P ≤ 0.05, P ≤ 0.01 respectively.

| Genotypes | Total chlorophyll (mg/m²) | Total carotenoids (mg/m²) |
|-----------|---------------------------|---------------------------|
| 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±11 ns                 |
| TE 9306   | 726±80                    | 467±27 *                  | 131±7                     | 100±2*                    |

Additionally, as previously found in rice grown with high levels of Mn (Lidon et al., 2004), as Fv/Fm 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 qP and qE (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 | Golia Sever | Golia Sever | Golia Sever |
|----------------------|------------|------------|------------|------------|
|                      | Control    | Heat stress | Control    | Heat stress |
|                      | Boot       | GF         | Mat        | Boot       | GF         | Mat        | Boot       | GF         | Mat        | Boot       | GF         | Mat        |
| Total plant accumulation (μg per Plant) | 1552       | 2197       | 6459       | 3676       | 3385       | 2154       | 5922       | 4369       | 1949       | 2556       |
| % Maturity           | 24         | 34         | 100        | 109        | 100        | 49         | 129        | 100        | 76         | 100        |
| % Plant parts        | Boot       | GF         | Mat        | Boot       | GF         | Mat        | Boot       | 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         | 12         | 22         | 7          | 8          | 25         | 13         |
| Grain                | 3          | 7          | 4          | 4          | 9          | 4          | 4          | 9          | 4          |

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 | Golia Sever | Golia Sever | Golia Sever |
|----------------------|------------|------------|------------|------------|
|                      | Control    | Heat stress | Control    | Heat stress |
|                      | Boot       | GF         | Mat        | Boot       | GF         | Mat        | Boot       | GF         | Mat        | Boot       | 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        | Boot       | GF         | Mat        | Boot       | GF         | Mat        |
| Root                 | 76         | 63         | 91         | 66         | 57         | 82         | 62         | 71         | 69         | 65         |
| Shoot                | 24         | 18         | 4          | 4          | 17         | 17         | 15         | 15         | 16         | 11         |
| Spike                | 19         | 4          | 17         | 28         | 17         | 28         | 24         | 22         | 16         | 23         |
| Grain                | 5          | 5          | 15         | 9          | 15         | 9          | 15         | 9          | 15         | 9          |

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