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## Heat and water stressed field-grown soybean: A multivariate study on the relationship between physiological-biochemical traits and yield

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## ABSTRACT

Heat stress (HS) combined with water stress (WS) negatively impact soybean [*Glycine max* (L.) Merr.] production. The aims of this research were to quantify the combined effect of HS and WS during grain filling (GF) on grain yield, and to identify physiological-biochemical traits strongly associated to crop performance and yield determination processes under these stressful field environments. Two soybean cultivars were exposed during GF to ambient temperature or to HS (> 32 °C for 6 h per d) during 21 d; and to field capacity or to WS (20% of available soil water content) during 35 d. Yield, grain number and weight (GN and GW) were significantly reduced under HS combined with WS. In irrigated HS yield and GN were also reduced, albeit final GW was similar to that of control, highlighting compensation mechanisms. Chlorophyll fluorescence analysis indicated that detrimental effects of HS and WS on photosynthesis were via structural capacity aspects but also through damage to photosystem II. The HS × WS decreased the availability of assimilate to grains, but also impaired the sink-grain metabolization of the remobilized products from leaves. A multivariate analysis highlighted strong correlations between quantum efficiency of photosystem II photochemistry, chlorophyll content (SPAD value), and the antioxidant state of leaves, which were all positively correlated with yield. Canopy temperature throughout grain filling ( $CT_{GF}$ ) was negatively associated with GN and GW, the latter also positively associated with SPAD. Explaining these physiological traits 81, 50, and 82% of the total variability of yield, GN and GW, respectively. To our knowledge this is the first report that integrating different scales of study, demonstrates the potential of physiological-biochemical parameters to explain yield variations in field-grown soybean under HS and WS conditions.

## 1. Introduction

Argentina contributes approximately to 18% of world soybean (*Glycine max* L. Merrill) grain, being the third largest producer, and the main exporter of soybean meal and oil worldwide (USDA, 2017). In Argentina, the soybean-growing area covers a wide agro-ecological region highly variable in terms of climatic conditions (i.e. temperature, rainfall and solar radiation) (Carrera et al., 2009). The crop is grown

under rainfed conditions, where precipitations and therefore, soil water availability are one of the main important limiting factors for growth and yield production (Chowdhury et al., 2016). Often low water availability is accompanied by high temperatures, resulting in heat stress and water deficit combination, increasing threat of climatological extremes to crop production thus compromising food security.

There is ample evidence supporting that heat stress episodes and/or water stress during grain filling (GF) decrease soybean yield mainly

**Abbreviations:**  $CT_{GF}$ , canopy temperature throughout grain filling; FRAP, ferric reducing ability of plasma; Fv/Fm, quantum yield of photosystem II; GF, grain filling; GN, grain number; GPr, grain protein content; GW, grain weight; HD, heat difference; HS, heat stress; HST, heat-stressful temperatures; MDA, malondialdehyde; NHS, non-heat-stress; NWS, non-water-stress; PCA, principal component analysis; <sup>a</sup>PSII, quantum efficiency of photosystem II photochemistry; RH, relative humidity; ROS, reactive oxygen species; SPAD, chlorophyll-meter measurements; TLPr, total leaf protein content; TLSS, total leaf soluble sugars; Tm, mean air temperature; Tmn, minimum air temperature; Tmx, maximum air temperature; To, optimum temperature; VPD, vapor pressure deficit; WS, water stress

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through decreases in grain weight (GW) and to a lesser extent in grain number (GN) (Brevedan and Egli, 2003; Dornbos and Mullen, 1991; Rotundo and Westgate, 2010). Multiple physiological, biochemical and molecular alterations could underlie yield responses to these abiotic stresses. For instance, under heat stress and/or water stress conditions plants activate a complex mechanism of responses involving stomatal regulation. In general, stomata close rapidly under drought conditions; and even when under elevated temperatures stomata have been shown to open wide, this response has been observed for warm but also moist environments (Reynolds-Henne et al., 2010). Overall, both drought and heat stress decrease photosynthesis, due to not only an increase of internal resistance to CO<sub>2</sub> diffusion (stomata closure), but also an increase of leaf and canopy temperature, thus favoring RuBisCo oxygenase activity and photorespiration in C3 plants (Prasad et al., 2008; Miyashita et al., 2005; Rollins et al., 2013). Several works observed a negative correlation between canopy temperature and yield under heat stress (Mason and Singh, 2014), water stress (Bahar et al., 2008; Harris et al., 1984) and heat stress combined with water stress (Rollins et al., 2013), most of them in wheat (*Triticum aestivum* L.). Reactive oxygen species (ROS) are by-products of normal aerobic metabolisms, and their generation markedly increase under different environmental stress conditions (Apel and Hirt, 2004). Stomatal closure induced under drought and heat stress decrease the CO<sub>2</sub> availability, unbalancing the light and carbon reaction of photosynthesis and consequently generating an excess of excitation energy condition, where the photosynthetic electron carriers are over reduced and the probability of ROS generation by energy or electron transfer to oxygen are increased (Foyer and Shigeoka, 2011). Together with non-photochemical quenching of chlorophylls, and photorespiration; ROS generation could be assumed as an energy dissipating mechanism, as long as, their generation rates are compensated by ROS scavenging capacity (Tambussi et al., 2004). ROS are highly reactive molecules that provoke deleterious oxidative processes in all kind of cellular macromolecules, inducing senescence symptoms and cell death (Mittler, 2002). To cope with the oxidative stress induced by ROS, plants have a sophisticated antioxidant defense system, composed by enzymes and non-enzymatic reduced compounds (Gill and Tuteja, 2010; Mittler, 2002). If ROS production exceed prolonged overtime the antioxidant system, during late reproductive stages, the reduction in photosynthesis and the increase of oxidative stress could result in significant losses of soybean yield (Siebers et al., 2015).

Photosynthesis is a main target of oxidative processes. The photosynthetic performance of crop canopies under stress conditions can be estimated indirectly through chlorophyll fluorescence techniques (Maxwell and Johnson, 2000). Indeed, maximum quantum yield of photosystem II (Fv/Fm) and quantum efficiency of photosystem II photochemistry (<sup>3</sup>PSII) are robust indicators of the photosynthetic machinery integrity and functional performance, respectively (Bian and Jiang, 2009; Inamullah and Isoda, 2005; Maxwell and Johnson, 2000). It has been documented significant diminutions of Fv/Fm and <sup>3</sup>PSII under heat stress (Lu and Zhang, 1999), water stress (Inamullah and Isoda, 2005; Souza et al., 2004) and the combination of both stresses (Jiang and Huang, 2001) in different crops including soybean. Reductions of the photosynthesis efficiency during GF lead to a decrease in the synthesis of sucrose and starch. If these stressful conditions are prolonged in time, the assimilate source available to the grain is reduced, therefore decreasing not only GW and yield (Brevedan and Egli, 2003) but also grain quality (Carrera et al., 2015; Rotundo and Westgate, 2009).

Soybean grains are highly nitrogen demanding due to their high protein concentrations. Nitrogen redistribution from leaves (mainly from chloroplast protein) is an important source of N for grain protein (GPr) biosynthesis; indeed this contribution is estimated to vary between 50 and 100% (Masclaux et al., 2001). The recycling and remobilization of N from leaves to grains during the filling period are processes associated with senescence, which main macroscopic

symptom is the decrease in chlorophyll content. Stress conditions such as heat and drought during soybean GF accelerate senescence and remobilization of N from leaves, involving a loss of photosynthetic capacity, and thus shortening grain fill duration resulting in deleterious effect on GW (Egli and Bruening, 2007; Gregersen et al., 2013; Masclaux-Daubresse et al., 2010) and composition (Carrera et al., 2015; Rotundo and Westgate, 2009) as previously mentioned.

Although great research efforts have been made towards understanding the effects of heat and water stress on soybean productivity, little attention has been given to the combined effect of both abiotic stresses. Comprehensive research integrating variables measured at different scales including physiological, biochemical, and yield traits evaluated in field-grown crops is still unavailable. This knowledge, however, could contribute to identify physiological and biochemical traits tightly involved in heat and water stress tolerance, useful for soybean genotype selection with improved performance. Gaining insight in the interactive effects of these two stresses on soybean productivity is decisive, since about 90% of the world's soybean production occurs under rainfed conditions, characterized by high temperature and low or erratic rainfall (Thuzar et al., 2010). Moreover, these aspects are particularly important in the context of climate change where rising temperatures and drought are prognosticated to increase by the Intergovernmental Panel on Climate Change (IPCC, 2013). The objectives of this study were: i) to quantify the combined effect of heat and water stress during GF on field-grown soybean yield and its components, GN and GW, and ii) to identify physiological-biochemical traits which could robustly depict the crop performance and yield determining processes under these stressful conditions.

## 2. Materials and methods

### 2.1. Plant culture and experimental design

The experiment was conducted during 2012–2013 growing season at the research station of the National Institute of Agricultural Technology (INTA), located in Manfredi (31°49'S, 63°46'W, Córdoba, Argentina). Two soybean genotypes from Syngenta Company SPS4 × 4 RR and SPS4 × 99 RR (of maturity group IV and indeterminate growth habit) were sown on November, 16th. These genotypes were selected because they are widely used by Argentine soybean farmers and present similar length of the reproductive phase (R1: beginning flowering to R8: full maturity according to Fehr and Caviness (1977)). Inoculated seeds were hand-planted in four rows 0.52 m apart and four meters long using a stand density of 25 plants m<sup>-2</sup>. Chemical (nitrate nitrogen, phosphorus, and pH) and physical (percentage of organic matter, bulk density and texture) analyses of soil samples from the experimental site did not indicate any physical or nutritional constraint on crop development. Crops were grown following cultural practices recommended by INTA's National Soybean Network, which include control of diseases, insects and weeds to avoid yield reduction and/or grain chemical quality alterations.

Average daily maximum, mean, and minimum local temperature data were obtained from INTA Manfredi Meteorological Station. Considering these data, we calculated new variables summarizing temperature conditions during the GF period (from beginning seed, R5 until physiological maturity, R7; Fehr and Caviness, (1977)). Such variables were: average daily maximum, minimum and mean air temperature during grain fill. The experimental design was a split-split plot with two replications, resulting in a three factorial arrangement: water level (the main plot), genotype (sub-plot), and temperature level (sub-sub plot).

Water levels were: i) non-water-stressed (NWS) plots, where soil water was kept near field capacity during the entire growing season using a drip irrigation system, and ii) water-stressed (WS) plots with approximately 20% of soil water available content during 35 d from R5.5 (seeds 6 mm long) phenological stage, to ensure cotyledon cell

division was complete (Rotundo and Westgate, 2010) up to physiological maturity. Rainfalls contribution was excluded by means of two independent mobile rainout shelters (8 m long, 5 m wide, with a maximum height of 6 m; covered with transparent polyethylene 100 μm film). Soil water content was measured on each plot at 20 cm intervals from 0 to 200 cm depth gravimetrically four times in control (non-heat-stressed and non-water-stressed) and in water-stressed plots: before the onset of the stresses (at R3 phenological stage), on the onset of the stresses (at R5.5 phenological stage), at the end of the heat stress treatment (21 d from R5.5 phenological stage), and at full maturity (phenological stage R8). In those plots involving heat stress treatments (irrigated heat-stressed and heat-stressed combined with water-stressed plots) soil water content was measured two times: at the end of the heat stress treatment and at full maturity. The soil is silty loam Typic Haplustoll (USDA Soil Taxonomy). Soil samples were collected from the field and carried out to the laboratory, where they were weighted, and placed in an oven at 105 °C during 48 h, until constant weight. Soil available water content was calculated according to Ritchie (1981) as the ratio between actual crop available soil water and maximum available soil water (Eq. (1)):

$$SAW = \left( \frac{\Theta_a - \Theta_{ll}}{\Theta_{dul} - \Theta_{ll}} \right) \times 100 \quad (1)$$

where  $\Theta_a$  is the volumetric water content at any time,  $\Theta_{dul}$  is the field drained upper limit, and  $\Theta_{ll}$  represents the lower limit obtained from laboratory determinations at  $-1.5$  MPa water potential.

Temperature levels generated two types of plots: i) non-heat-stressed (NHS) plots (ambient temperature) and ii) heat-stressed (HS) plots, with episodes exceeding 32 °C during 6 h (from 10 am to 04 pm) during 21 d from R5.5. Temperature increases were achieved using polyethylene film (100 μm thickness) shelters (4 m long, 2 m wide and 2 m high) opened at the bottom (0.60 m above ground level) to allow adequate gas exchange (Molino, 2011). Air temperature and relative humidity (RH) of each shelter were recorded every 30 min throughout the treatment period by means of button-type digital loggers (DS1923L-F5, resolution: 0.5 °C, 1-button data loggers, Digi-Key Co. Ltd., USA). These sensors were positioned in the center of each shelter at the top of the canopy (at 1.2 m from the ground level). The degree of heat stress was quantified as the difference in mean temperatures between control and heated plots. This variable was defined as heat-stressful temperatures (HST, °C h<sup>-1</sup>) and computed as in Eq. (2):

$$HST = \sum_{i=1}^N (T_x > T_o) / (h > T_o) \quad (2)$$

where  $N$  is the duration of treatment period (in h),  $T_x$  is the average air temperature registered every 30 min (°C),  $T_o$  is optimum temperature (32 °C),  $T_x > T_o$  is the cumulative temperature above  $T_o$ , and  $h > T_o$  is the number of hours with temperatures above  $T_o$  (Neiff et al., 2016). The  $T_o$  was set always at 32 °C because several studies have shown that above this temperature not only grain growth rate but also grain fill duration were reduced in soybean (Egli and Wardlaw, 1980; Gibson and Mullen, 1996). The average vapour pressure deficit (VPD, kPa) during the daily hours of heating treatment was computed as the difference between saturation ( $e_s$ ) and actual vapour pressure ( $e_a$ ) based on the methodology developed by Allen et al. (1998) (Eqs. (3)–(5)):

$$VPD = e_s - e_a \quad (3)$$

$$e_s = \frac{0.6108 \cdot \text{Exp} [(17.27 \cdot T_{mx}) / (T_{mx} + 237.3)] + 0.6108 \cdot \text{Exp} [(17.27 \cdot T_{mn}) / (T_{mn} + 237.3)]}{2} \quad (4)$$

$$e_a = \frac{(T_{mx}) \cdot (RH_{mn}/100) + (T_{mn}) \cdot (RH_{mx}/100)}{2} \quad (5)$$

Where  $T_{mx}$  is the average daily maximum temperature (°C),  $T_{mn}$  is the average daily minimum temperature (°C),  $RH_{mn}$  is the minimum

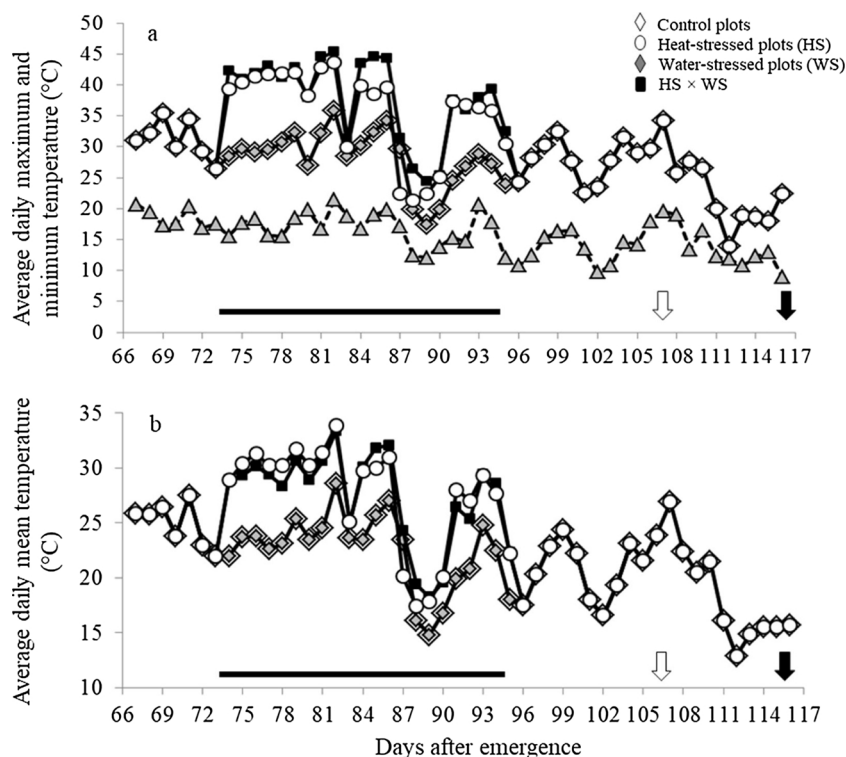
relative humidity (%),  $RH_{mx}$  is the maximum relative humidity (%) considering the daily duration in hours of heating treatment.

## 2.2. Measurement of physiological-biochemical traits during grain filling

All physiological and biochemical determinations were performed on an attached leaflet of the third trifoliolate leaf from the main stem apex of plants from central rows at the 9th d after the end of heat stress imposition (30 d after the plot reached R5.5). The only exception was the canopy temperature measurement which was measured also during the heat stress treatment.

Quantum efficiency of photosystem II photochemistry ( $\Phi_{PSII} = (F_m' - F_t) / F_m'$ ) and maximum quantum yield ( $F_v / F_m = (F_m - F_o) / F_m$ ) in dark-adapted leaves (30 min) were measured from the upper surface using a pulse amplitude modulated fluorometer (FMS2, Hansatech Instruments, Pentney King's Lynn, UK). Measurements were performed on six plants per plot, at 11 and 14 h (when the least change in solar radiation intensity occurred) on cloud less days and winds less than 5 km h<sup>-1</sup>. The variables  $F_m'$  and  $F_t$  represent the maximal fluorescence yield of an illuminated leaf and the steady state fluorescence yield, respectively. Whereas,  $F_o$  and  $F_m$  are the minimal and maximal fluorescence yields of dark-adapted leaves, respectively. Canopy temperature throughout grain filling ( $CT_{GF}$ ) during and after the heat stress treatment was measured using a handheld infrared thermometer (Testo 845, Barcelona, España) maintaining an angle less than 45 ° to the horizontal plane and covering different areas of the plot in order to integrate many leaves. In each experimental unit, five readings were taken during late morning to early afternoon (~12 and 15 h) every five to seven days depending on climatic conditions (readings were made only on clear, sunny days with minimal winds). Although crops attained maximum cover during GF, special care was taken to avoid temperature interference from the soil surface. Chlorophyll-meter measurements (SPAD-502; Minolta Camera Co., Osaka, Japan) were taken in six plants per plot, and were used as an indicator of the leaf chlorophyll status in the leaves, thus indirect estimating leaves senescence.

For biochemical parameters determinations leaflet of tagged trifoliolate leaves were collected, immediately frozen in liquid nitrogen after harvesting, and stored at  $-40$  °C until determination assays. Three leaves discs of 1.3 cm of diameter (3.98 cm<sup>2</sup>) were obtained using a round punch hole tool. The ferric reducing ability of plasma (FRAP), and malondialdehyde (MDA), as well as the total leaf soluble sugars (TLSS), total leaf protein content (TLPr) and leaf starch, were measured on three plants per plot. The FRAP content was determined from 150 mg of leaf grounded into fine powder in liquid nitrogen using a mortar and pestle. We added 1500 μl of 80% v/v cold ethanol and vigorously mixed. The homogenate was centrifuged at 19500 × *g* for 10 min at 4 °C. The supernatant obtained was used for the determination according to the protocol of Benzie and Strain (1996). The FRAP value was calculated using Trolox (Sigma-Aldrich, St. Louis, USA) as the standard reference. Lipid peroxidation was estimated by determining leaf MDA content as described by Heath and Packer (1968) using the thiobarbituric acid method with the following modifications. The homogenate obtained from 150 mg of leaf powder vigorously mixed with 1500 μl of 80% v/v ethanol was heated at 80 °C for 20 min and centrifuged at 19,500 × *g* for 10 min. Equal aliquots of supernatant and 20% trichloroacetic acid containing 5% thiobarbituric acid were mixed and heated at 90 °C for 30 min and then cooled quickly on ice bath. The homogenate was centrifuged at 10,000 × *g* for 5 min and the absorbance of the supernatant was measured at 532 nm. Values were corrected for unspecific turbidity by subtracting the absorbance at 600 nm. The MDA content was calculated by using extinction coefficient of 155 mM<sup>-1</sup> cm<sup>-1</sup>. The TLSS were determined as described by Guan and Janes (1991), using the same supernatant as for the determination of MDA content. Leaf starch content and TLPr were quantified using the pellet obtained from the extraction of the MDA content. The pellet was



**Fig. 1.** Average daily maximum (full line), minimum (grey triangle and dotted line) (a) and mean (b) air temperature during soybean grain fill of control (non-heat-stressed and non-water-stressed), heat-stressed, water-stressed, and heat-stressed combined with water-stressed plots. Black line = duration of heat treatment; white arrow = end of seed fill of water-stressed plots, and heat-stressed combined with water-stressed plots; black arrow = end of seed fill of irrigated heat-stressed plots and control (non-heat-stressed and non-water-stressed) plots.

oven dried at 60 °C for 24 h and then partitioned equally for total protein and total starch determinations. The protocol followed for TLPr was the one described by [Lowry et al. \(1951\)](#) with some modifications. The pellet was re-suspended shaking it in water containing 0.1% v/v tween 20 (Sigma-Aldrich, St. Louis, USA) and heated at 100 °C for 5 min and then centrifuged at 19,500  $\times$  g for 10 min at room temperature. The supernatant was used for determination. The TLPr value was calculated using bovine serum albumin (Sigma-Aldrich, St. Louis, USA) as the standard reference. Leaf starch content was determined as described by [Guan and Janes \(1991\)](#) using glucose (Sigma-Aldrich, St. Louis, USA) as the standard reference.

### 2.3. Yield, grain number, grain weight and grain protein content

At physiological maturity, plants in a 1.13 to 2.08 m<sup>2</sup> area were hand harvested in the two central rows of each plot. Grains were threshed, weighted, and counted. Yield (g m<sup>-2</sup>) and GW (mg grain<sup>-1</sup>) were expressed on a dry moisture basis. Protein concentration (expressed as percentage of dry sample) was determined by near infrared reflectance using an Infratec 1241 (Foss Tecator; Höganäs, Sweden) and following the method recommended by AACC ([AACC, 2000](#)). Protein content (expressed on a dry matter basis) is referred to total mass of the component (mg grain<sup>-1</sup>) and concentration was calculated per unit GW (g 0.1 kg<sup>-1</sup>).

### 2.4. Statistical analyses

In order to explore associations between yield, GN and GW and physiological-biochemical traits a principal component analysis (PCA) was performed ([Johnson and Wichern, 2002](#)). Results of this analysis are visualized through biplot graphs ([Gabriel, 1971](#)) constructed from the first and second principal components (PC 1 and PC 2) derived from the PCA. This technique of reducing the dimension implies a consequent help in the interpretation of the data since it allows to: i) analyze the whole data set instead of individual elements and take into account several factors simultaneously (i.e. the different treatments conditions), and ii) identify associations between observations, between

variables and between variables and observations ([Di Rienzo et al., 2010](#)). The data obtained from the field trials were modeled according to a trifactorial experiment following a split-split plot design with two replications. The level of significance was 5%. The comparison of means was performed using DGC test ([Di Rienzo et al., 2002](#)). To adjust the statistical model, we used a mixed model approach. For this, we used the lme function of the nlme library of R version 2.2.11 ([R Development Core Team, 2010](#)), using the interface implemented in InfoStat ([Di Rienzo et al., 2010](#)). For yield, the statistical model included a variance function to consider that the residual variances increased with the mean response value. Simple and multiple linear regressions were fitted to model yield, GN and GW as functions of physiological-biochemical variables. A stepwise variable selection procedure was run to identify significant variables (at the 0.05 probability level) explaining crop productivity. Model selection was based on Mallows' Cp statistic, the residue analysis, and the determination coefficient ( $R^2$ ) ([Draper and Smith, 1966](#)). All calculations were done with the statistical software InfoStat (InfoStat, <http://www.infostat.com.ar>)

## 3. Results and discussion

### 3.1. Growing conditions during grain filling

Average daily mean, maximum and minimum air temperature during soybean grain fill of NHS and NWS (here after named control plots), HS, WS and heat-stressed combined with water-stressed (here after named HS  $\times$  WS) plots are shown in [Fig. 1a](#) and [b](#). The time elapsed between the onset and removal of the HS treatment was 21 d, during which the average daily maximum air temperature of HS and HS  $\times$  WS were 7.7 °C and 9.9 °C, respectively above the control ([Fig. 1a](#)). Also, the average daily mean air temperature of HS and HS  $\times$  WS was 5.0 °C above the control during the HS treatment ([Fig. 1b](#)). When considering the six-hours daily heating, the average maximum air temperature was 3.9 °C (HS) and 6.2 °C (HS  $\times$  WS) higher than control plots ([Table 1](#)), resulting in higher average mean air temperature of both HS and HS  $\times$  WS in comparison to control (3.2 and

**Table 1**

Average maximum (T<sub>mx</sub>), mean (T<sub>m</sub>) and minimum (T<sub>mn</sub>) air temperature and relative air humidity (RH) during heating. The heat difference (HD; i.e. difference between the mean temperature of non-heat-stressed plots and heat-stressed plots), heat-stressful temperatures (HST, °C h<sup>-1</sup>), and vapor pressure deficit (VPD) were calculated. Fraction of time (h) with exposure to temperatures above 32 °C (h > T<sub>o</sub>) also was calculated during the heating period (6 h around midday during 21 d). All measurements were recorded every 30 min in the center of each shelter at the top of the canopy (at 1.2 m from the ground level).

Meteorological variable	Non-heat-stressed plots	Heat-stressed plots	HS × WS plots <sup>a</sup>
T <sub>mx</sub>	31.6	35.5	37.8
HD <sub>Tmx</sub>	–	3.9	6.2
T <sub>m</sub>	28.1	31.3	33.1
HD <sub>Tm</sub>	–	3.2	5.0
T <sub>mn</sub>	24.6	26.3	27.4
HD <sub>Tm</sub>	–	1.7	2.8
h			
h > T <sub>o</sub>	41.0	70.0	80.6
°C h <sup>-1</sup>			
HST	5.6	10.7	13.1
HD <sub>HST</sub>	–	5.1	7.5
%			
RH	67.2	54.6	45.7
kPa			
VPD	1.6	2.3	3.5

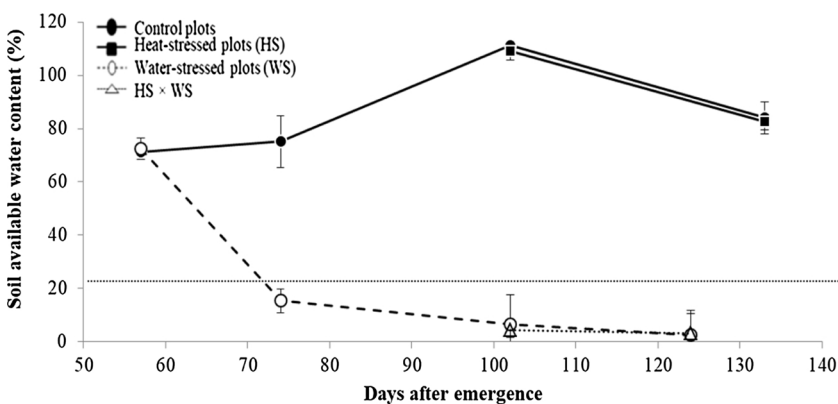
<sup>a</sup> HS × WS plots = heat-stressed × water-stressed plots.

5.0 °C, respectively, Table 1). As a result the intensity of HS (quantified as HST) was slightly higher for HS × WS plots than for HS plots (13.1 and 10.7 °C h<sup>-1</sup>, respectively) (Table 1). On the other hand, artificial heating did not affect RH inside the polyethylene shelters (Table 1), thus the higher VPD observed in heated plots (regardless of the water stress) could be entirely attributed to the higher temperatures achieved during the heating period compared to control plots.

At the onset of treatments (R5.5 phenological stage) soil available water content (%) measured up to 2 m depth in non-water-stressed (irrigated) plots was similar (i.e. 75 ± 10%) for both NHS and HS, and this condition was maintained throughout the stress period ending with 84.0 ± 6.01% and 82.6 ± 3.02%, respectively. In water-stressed treatments, soil available water content was about 16–20% at R5.5 and decreased gradually until 2.58 ± 7.82% in water-stressed plots and 3.02 ± 8.79% in HS × WS plots at physiological maturity, thus resulting in a severe terminal water stress (Fig. 2).

### 3.2. Independent and combined effects of heat and water stress on yield, grain number and grain weight

Yield, GN and GW were not influenced by genotype (P > 0.05), but were significantly affected by individual stresses (HS and WS) and their



**Fig. 2.** Evolution of soil available water content (percent of field capacity) measured up to 2 m depth gravimetrically four times in control (non-heat-stressed and non-water-stressed) and in water-stressed plots: before the onset of the stresses (at R3 phenological stage), on the onset of the stresses (at R5.5 phenological stage), at the end of the heat stress treatment (21 d from R5.5 phenological stage), and at full maturity (phenological stage R8); and two times in irrigated heat-stressed and heat-stressed combined with water-stressed plots: at the end of the heat stress treatment and at full maturity.

**Table 2**

Significance of different water availability (non-water-stressed and water-stressed plots), levels of temperature (non-heat-stressed and heat-stressed plots) and genotype (SPS4 × 4 RR and SPS4 × 99 RR) and their interaction on yield, grain number and grain weight.

Source	Yield	Grain number	Grain weight
Water availability (WA)	*	ns	*
Temperature levels (TL)	*	*	ns
Genotype (Gen)	ns	ns	ns
WA × TL	*	*	*
WA × Gen	ns	ns	ns
TL × Gen	ns	ns	ns
WA × TL × Gen	ns	ns	ns

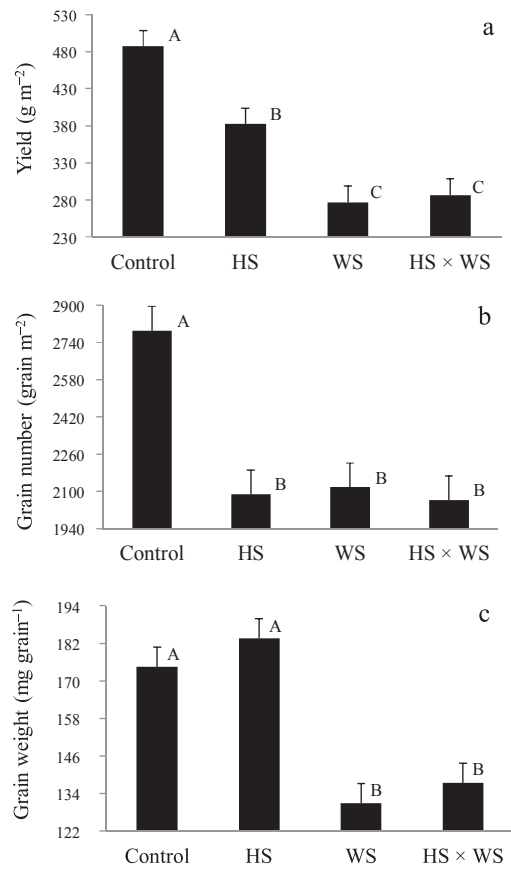
ns, non-significant.

\* Significant at P ≤ 0.05.

interaction, being the only exception the non-significant HS effect on GW (Table 2). For further analyses the genotype data were pooled. The episodes of HS resulted in significant yield reductions in irrigated plots (21%), mainly due to GN diminutions around 25% (Fig. 3a and b). Interestingly, in this treatment GW slightly increased (184 ± 1 mg grain<sup>-1</sup>) in comparison to controls (175 mg grain<sup>-1</sup> ± 7 mg grain<sup>-1</sup>) although this difference was not significant (Fig. 3c). Increases in GW can be expected as a compensatory effect due to the GN reduction particularly if the assimilate source functioning can recover after stress (Andriani et al., 1991). Previous studies by Dornbos and Mullen (1991) showed a 26% yield reduction in greenhouse-grown plants under HS conditions compared to control plants which were explained by drops in the two yield components (~13%). In that study, duration of HS treatments comprised the whole filling period which could explain the decreased in GW, in contrast with our findings. Present knowledge on soybean seed development has established that potential seed weight can be modified until late seed filling which extends the capacity of soybean seeds to modify their weight in response to an improvement in environmental conditions, thus conferring great plasticity to the crop (Borrás et al., 2004).

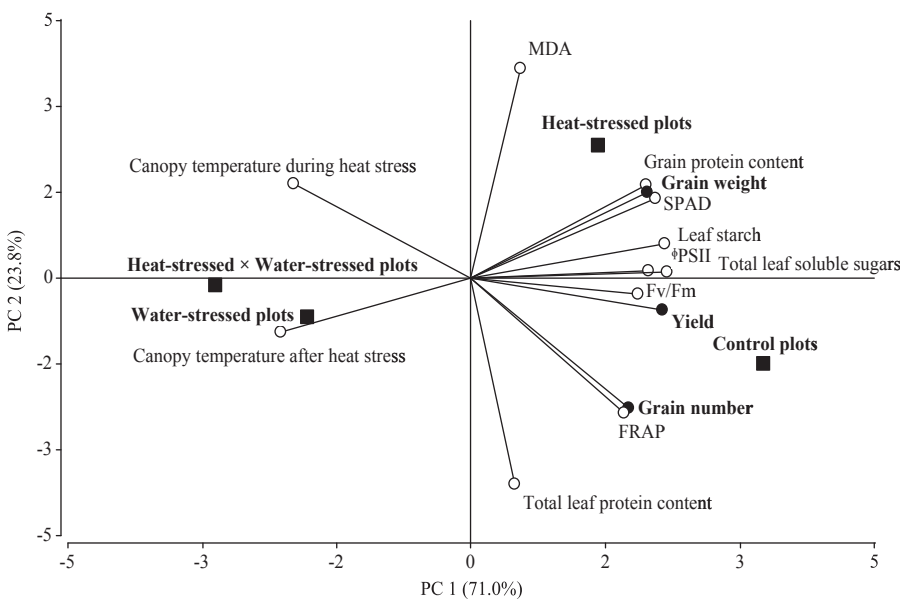
Under WS, yield decreased 43% due to both lower GN (24%) and GW (25%) compared to controls (Fig. 3a–c). Similar yield, as well as GN and GW reductions were observed by Dornbos and Mullen (1991) when plants were grown under severe drought conditions (50% of pot water volume). In rainfed field experiments where irrigation treatments were applied during seed filling Rotundo and Westgate (2010) showed yield differences close to 16%, mainly associated with GW decreases (21%) in non-irrigated plots since GN did not exhibit significant diminutions (<2%). Since in that study, control plots were irrigated after the onset of seed filling, the crop might be subjected to certain level of water stress during the critical period of pod set, reflected in the low yield exhibited by the controls (2195 kg ha<sup>-1</sup>). Thus, the control plots yielding below potential (due to lower GN), would exacerbated more the differences in GW than in GN between control and WS conditions.

When episodes of HS and WS occurred concomitantly yield decreased 41% in comparison to the control, with GN and GW decreasing



**Fig. 3.** Yield (a), grain number (b) and grain weight (c) for soybean crops grown under four water and temperature treatments: control (non-heat-stressed and non-water-stressed), heat-stressed (HS), water-stressed (WS), and heat-stressed × water-stressed (HS × WS) plots, expressed as mean and standard error. Different letters indicate significant differences among means ( $P \leq 0.05$ ).

26 and 21%, respectively. It is documented that the simultaneous occurrence of both heat stress and drought may cause additive or multiplicative (synergism) effect on wheat (Prasad et al., 2011), barley (Rollins et al., 2013) and rapeseed yield (Triboi-Blondel and Renard, 1999), being the impact of the combined stresses considerable larger than the single stress effects. Also in soybean, Dornbos and Mullen



**Fig. 4.** Biplot from the first and second principal components (PC 1 and PC 2) of principal components analysis (PCA) showing relationships between physiological-biochemical variables (white circles) and yield, grain number and grain weight (black squares) of soybean crops grown under different water and stress treatments (black squares): control (non-heat-stressed and non-water-stressed), heat-stressed, water-stressed, and heat-stressed × water-stressed plots. MDA, malondialdehyde; SPAD, leaf chlorophyll meter; <sup>†</sup>PSII, quantum efficiency of photosystem II photochemistry; Fv/Fm, maximum quantum yield of photosystem II; FRAP, ferric reducing ability of plasma. Physiological-biochemical traits were measured at the 9th d after the end of heat stress imposition (30 d after plots reached R5.5). The only exception was the canopy temperature measurement which was measured also during the heat stress treatment.

(1991) reported additive effect of HS × WS, being yield reductions approximately equal to the sum of the percentage reductions of the individual stresses (~65%). In the present work, the hypothesized additive or multiplicative effect due to the interaction of both stresses was not observed. Instead, the magnitude of the reduction was lower than the sum of the individual effects, suggesting that WS was dominant (generated the greatest specific response) possibly because duration of the water stress was larger (whole seed filling) than the heat stress in our study. In the aforementioned studies analyzing additive or multiplicative effects of heat stress and drought on yield, authors applied a same duration for both stresses treatments.

As observed from results yield drops across treatments were due to similar decreases in both yield components, except for irrigated HS plots where yield decreased was only accounted for by GN diminution. Although HS and WS treatments were imposed during seed filling, GN loss highlights the great complexity of soybean yield generation characterized by a large overlapping of reproductive phases which was also accentuated by the indeterminate growth habit of the genotypes used in this study. Earlier studies have shown that the end of the critical period for pod and seed number determination is close to the beginning of growth stage R6 (Board and Tan, 1995; Egli, 1997). Moreover, results from our study in line with others, which also studied HS and/or WS late in the reproductive phase (after growth stage R5) (Bredvan and Egli, 2003; De Souza et al., 1997; Dornbos and Mullen, 1991) highlight that important GN diminutions can be expected with late stress, thus compromising the reproductive plasticity of the crop. One possible explanation could be that although the majority of pods are already fixed around growth stage R5.5, severe stress conditions limiting the photoassimilate translocation to the seeds could result in GN loss.

**3.3. Associations between physiological-biochemical traits under independent or combined heat and water stress**

The first two principal components (PC 1 and PC 2) of the principal components analyses explained 94.8% of total variability in the data (Fig. 4). Correlations between physiological-biochemical traits were explored by the angles between trait's vectors. The cosine of the angle between two trait vectors approximates the association among the traits; acute and obtuse angles indicate positive and negative correlation, respectively; whereas right angles denote no correlation between traits. The PC1 of the biplot (Fig. 4) revealed that <sup>†</sup>PSII, Fv/Fm, leaf starch, TLSS, and FRAP were positively associated, being these vectors oriented towards irrigated control plots. Grain protein and SPAD were

**Table 3**

Mean and standard error for physiological-biochemical variables depicting physiological status of soybean crops grown under four water and heat stress treatments during grain filling: control (non-heat-stressed and non-water-stressed), heat-stressed (HS), water-stressed (WS), and heat-stressed × water-stressed (HS × WS) plots. Variables were measured at the 9th d after the end of heat stress imposition (30 d after the plot reached R5.5). The only exception was the canopy temperature measurement which was measured also during the heat stress treatment. Different letters indicate significant differences among means ( $P \leq 0.05$ ) according to DGC comparison test.  $\Psi$ PSII, quantum efficiency of photosystem II photochemistry; Fv/Fm, maximum quantum yield of photosystem II; SPAD, leaf chlorophyll meter;  $CT_{GF}$ , canopy temperature throughout grain filling; FRAP, ferric reducing ability of plasma; MDA, malondialdehyde.

Variable	Control plots	HS plots	WS plots	HS × WS plots
$\Psi$ PSII	0.31 ± 0.02 <sup>A</sup>	0.30 ± 0.02 <sup>A</sup>	0.29 ± 0.02 <sup>A</sup>	0.26 ± 0.02 <sup>B</sup>
Fv/Fm	0.77 ± 0.02 <sup>A</sup>	0.74 ± 0.02 <sup>A</sup>	0.72 ± 0.02 <sup>A</sup>	0.62 ± 0.03 <sup>B</sup>
Leaf starch (g m <sup>-2</sup> )	5.57 ± 0.73 <sup>A</sup>	5.27 ± 0.71 <sup>A</sup>	3.83 ± 0.65 <sup>B</sup>	3.82 ± 0.26 <sup>B</sup>
Total leaf soluble sugars (g m <sup>-2</sup> )	1.01 ± 0.11 <sup>A</sup>	0.91 ± 0.22 <sup>A</sup>	0.79 ± 0.11 <sup>B</sup>	0.72 ± 0.10 <sup>B</sup>
SPAD	35.48 ± 0.89 <sup>A</sup>	36.84 ± 1.09 <sup>A</sup>	22.38 ± 2.11 <sup>B</sup>	20.68 ± 1.86 <sup>B</sup>
$CT_{GF}$ during HS (°C)	23.58 ± 0.33 <sup>A</sup>	27.94 ± 0.53 <sup>B</sup>	28.89 ± 0.53 <sup>B</sup>	29.73 ± 0.27 <sup>B</sup>
$CT_{GF}$ after HS (°C)	23.57 ± 0.27 <sup>A</sup>	24.05 ± 0.60 <sup>A</sup>	29.49 ± 0.53 <sup>B</sup>	29.60 ± 0.77 <sup>B</sup>
FRAP (mmoles m <sup>-2</sup> )	4.74 ± 0.33 <sup>A</sup>	3.47 ± 0.34 <sup>B</sup>	3.63 ± 0.35 <sup>B</sup>	3.21 ± 0.34 <sup>B</sup>
MDA (moles m <sup>-2</sup> )	3.91 ± 0.28 <sup>A</sup>	5.57 ± 0.41 <sup>B</sup>	3.77 ± 0.37 <sup>A</sup>	4.21 ± 0.44 <sup>A</sup>
Total leaf protein (mg m <sup>-2</sup> )	0.73 ± 0.09 <sup>A</sup>	0.43 ± 0.04 <sup>C</sup>	0.58 ± 0.05 <sup>B</sup>	0.59 ± 0.05 <sup>B</sup>
Grain protein (mg grain <sup>-1</sup> )	65.66 ± 2.11 <sup>A</sup>	68.01 ± 0.78 <sup>A</sup>	48.51 ± 0.83 <sup>B</sup>	51.78 ± 2.82 <sup>B</sup>

positively associated. The vectors of these variables together with the MDA vector were oriented towards irrigated HS plots (Fig. 4). In the opposite site of the plane determined by the two PC, towards WS plots alone, or combined with HS, the  $CT_{GF}$  vectors (both during and after HS treatment) were negatively associated with  $\Psi$ PSII, Fv/Fm, leaf starch, TLSS, FRAP, SPAD and GPr. On PC 2 TLPr was negatively associated with MDA (Fig. 4). The average and standard error for each physiological-biochemical trait ( $\Psi$ PSII, Fv/Fm, leaf starch, TLSS,  $CT_{GF}$ , FRAP, MDA, TLPr, SPAD, GPr) are presented in Table 3.

The  $\Psi$ PSII estimates the proportion of light absorbed by chlorophyll associated with photosystem II (PSII) that is used in photochemistry, thus it is used as a photosynthesis indicator (Maxwell and Johnson, 2000). The average  $\Psi$ PSII value was maximum (0.31) in plants grown under control conditions and was significantly reduced (16%) by HS × WS treatment (Table 3). The  $\Psi$ PSII magnitude is close to findings by Caldwell et al. (1994) and Jumrani et al. (2017) under field conditions; although lower  $\Psi$ PSII values (0.18) were observed in control soybean plants growing in greenhouse (Inamullah and Isoda, 2005). The other fluorescence parameter measured was Fv/Fm, which reflects the potential quantum efficiency of PSII, widely used as a sensitive indicator of damage in the photosynthetic apparatus (Maxwell and Johnson, 2000). A significant diminution of Fv/Fm was observed in HS × WS plots (20%) compared to the maximum values (0.77) in plants grown under control conditions (Table 3). The maximum value observed in this study was in the expected range of variation for most plant species including soybean (Maxwell and Johnson, 2000). There was a general trend for  $\Psi$ PSII and Fv/Fm to decrease in plants exposed to individual stresses, but it was marginal and not significant (Table 3). Analyzing intact leaves of several plant species (*Lycopersicon esculentum* Mill., *Solanum tuberosum* L., *Solanum nigrum* L.) exposed to heat and drought separately or in combination, it has been proposed that PSII is relatively more tolerant to drought stress than to heat stress (Havaux, 1992). In agreement, later studies in different species found no significant effects of drought on  $\Psi$ PSII and Fv/Fm (Bian and Jiang, 2009; Lu and Zhang, 1999; Rollins et al., 2013). The greater sensitiveness of PSII to heat than to water stress was the basis to establish that it is one of the most thermolabile components of the photosynthetic electron transport (Havaux, 1996). Interestingly, it has been demonstrated that the thermal inactivation of PSII is reversible with substantial recovery often only being observed after several days of HS treatments finalization (Bilger et al., 1987; Karim et al., 1999; Seemann et al., 1984). Thus, the not significant effect of HS on  $\Psi$ PSII and Fv/Fm observed in the present work could be pointing out to recovery processes of the mentioned parameters, considering that measurements were taken 9 d after HS imposition. Additionally, a feature of our study is that HS was imposed for only 6 h per day (from 10 am to 04 pm) which could have

allowed for PSII recovery after the stress release. Only plants from plots exposed to the combined effect of heat and continuous drought stress seemed to be unable to recover after the heat stress was released, thus exacerbating the effects of high temperature in comparison to previous studies (Rollins et al., 2013; Xu and Zhou, 2006).

Starch (the temporary carbon reserve in leaves) and sucrose (the transport form of carbohydrate) are the main primary products of photosynthesis (Huber et al., 1984). Thus, they could be used as indicators of the activity of the source (i.e. canopy photosynthetic performance). Significant diminutions of starch and TLSS (which includes sucrose) were observed under water stress both alone (31% and 22%, respectively), or in combination with episodes of HS (31% and 28%, respectively) (Table 3). These results indicate detrimental effect of WS on the rate of photosynthesis in line with results from similar studies (Breedan and Egli, 2003; Huber et al., 1984) that demonstrated carbon assimilation reductions and the immediate fate of the fixed carbon (i.e. leaf carbohydrates levels). The maintenance of high levels of soluble sugars in leaves of plants under WS has been proposed as an osmoregulation mechanism (Chaves et al., 2002). However, in contrast with previous reports showing maintenance or increase of leaves soluble sugars under WS (Porcel and Ruiz-Lozano, 2004; Souza et al., 2004), soluble sugars in our work significantly decreased together with starch (Table 3, Fig. 4). It is probable that the severe and continue WS detrimental effects on carbon assimilation had impaired sucrose production in absolute terms as suggested by Quick et al. (1992). Noticeable, under WS conditions the diminution in carbon assimilation indicated by the significant decreased in leaf starch content was not accompanied by detrimental effects on  $\Psi$ PSII and Fv/Fm ( $P > 0.05$ , Table 3). Possibly, photorespiration processes known to increase during drought, played a key role in the protection of the photosynthetic machinery against excess excitation energy (Wingler et al., 1999).

It is well documented that water stress usually leads to reduction of CO<sub>2</sub> assimilation due to stomatal closure to a great extent (Lu and Zhang, 1999; Quick et al., 1992; Souza et al., 2004), while the canopy transpirational cooling can also be affected with a consequent rise in leaves temperature (Sharkey, 2005) well above the surrounding air temperature. Indeed,  $CT_{GF}$  measurements 9 d after the end of the heat stress (coincidentally with the moment of measurement of the discussed physiological-biochemical traits) showed that regardless of the HS treatment, leaves from water stressed plants were significantly warmer than irrigated ones (Table 3, Fig. 4). Moreover,  $CT_{GF}$  of water stressed plants was 3 °C above the air temperature (29.6 °C vs 26.5 °C) which reveals the canopy cooling inability. In contrast, heated plants of irrigated plots exhibited similar transpirational cooling to control after the HS was removed (similar  $CT_{GF}$  values) indicating the recovery ability of the well-watered plants (Table 3). Significant decreases of leaf

carbohydrates levels together with significant diminution of fluorescence parameters in response to combined HS and WS stress are indicative of photoinhibition associated with structural damage to PSII. It is apparent that under these severe conditions decreases in carbon assimilation may be responding to stomatal closure but also to a non-stomatal component, namely metabolic limitations. During HS treatment in water stressed plants stomatal closure was fostered not only by a progressively reduction of soil water availability but also by high atmospheric VPD (Table 1), which can reduce stomatal conductance in soybean (Bunce, 1984). The inability of the crop to recover after HS removal due to the severe drought conditions (Fig. 2) was clearly depicted by the high canopy temperature in plants from these plots (Table 3).

Many studies have reported that biochemical limitations for carbon assimilation are quantitatively important during severe drought (Grassi and Magnani, 2005) and heat stress (Salvucci and Crafts-Brandner, 2004). For instance, as temperature increases photorespiration rises owing to increases mainly in the relative solubility of  $O_2$  compared to  $CO_2$  (Jordan and Ogren, 1984). Likewise, RuBisCo activase, which plays an essential role on RuBisCo catalytic capacity (Salvucci and Ogren, 1996; Spreitzer and Salvucci, 2002), is particularly sensitive to temperature increases (Crafts-Brandner et al., 1997), being less effective in keeping RuBisCo catalytically competent (Crafts-Brandner and Salvucci, 2002; Morales et al., 2003). It would be interesting to quantify the proportion of carbon assimilation that is decreased due to stomatal closure and due to a non-stomatal component (metabolic limitations). These are important avenues for gaining insights into the mechanisms involved in the response of the primary metabolism to the combined effect of HS and WS.

We additionally analyzed the cellular redox state through FRAP. An important part of the deleterious effects of drought and HS are given by the disruption of cellular redox state, determined by the ROS production and the scavenging rates leading to oxidative stress (Gill and Tuteja, 2010; Mittler, 2002). In the disruption of the leaf redox state, photorespiration has a major impact being one of the fastest ROS producing system in photosynthetic cells under many conditions including high temperatures or water deficits (Foyer et al., 2009; Noctor et al., 2002). In this context, FRAP is a useful cellular redox state marker that could be assumed as the total antioxidant power (Benzie and Strain, 1996). As is observed in the biplot the vector of this trait was oriented towards irrigated controls (Fig. 4), indicating the low level of oxidative processes in the photosynthetic source of assimilates under non-stressful conditions. In coincidence, Table 3 showed that FRAP values were highest in control plots and significantly decreased under HS or WS, evidencing oxidative stress. There was a marginal tendency of higher consume of non-enzymatic antioxidant species in HS  $\times$  WS plots, as indicated by the lowest value of FRAP under this condition compared to the others, but it was not statistically different to values observed under single stresses. On the other hand, double bonds in unsaturated fatty acids are highly sensitive to ROS oxidative effects, leading to lipid peroxidation of plant membranes (Foyer et al., 1994). Peroxides of polyunsaturated fatty acids generate cytotoxic products, being in many cases MDA the most abundant individual aldehydic lipid breakdown product (Esterbauer and Cheeseman, 1990). An increase in MDA values indicates higher membrane lipid peroxidation processes. In the biplot the trait vector of this variable was oriented towards HS plots; indeed episodes of HS increased MDA 43% relative to the control (Table 3), indicating that high temperature resulted in high peroxidation of cell membranes. Similar percentage of increase in MDA content was observed by Jiang and Huang (2001) analyzing two turf-grasses exposed to high temperatures treatments ( $33^\circ C$ ) in controlled environments. In HS  $\times$  WS plots MDA content also increased although this increase was not statistically different from control plots (Table 3). These results are in sharp contrast with those reported by Jiang and Huang (2001) and Xu and Zhou (2006) who observed 7.6-fold and 12.7-fold increase in MDA content, respectively when plants were exposed to

the combined effect of heat stress and drought respect to plants from control treatments, concluding that lipid peroxidation (MDA) was more pronounced under the combined stresses rather than individual stresses alone. It has been demonstrated that the thiobarbituric acid reactive species test for the analysis of MDA is highly sensitive, but especially under severe stress conditions the reaction lacks accuracy and specificity (Davey et al., 2005), because MDA is an intermediate metabolite that can cross react with other compounds producing interferences with spectrophotometric measurements (Kosugi and Kikugawa, 1989). This phenomenon could possibly explain the non-significant increased tendency of MDA content observed in WS plots, even when the oxidative stress was evidence by the significant decrease of FRAP under HS or WS alone or combined (Table 3).

Protein degradation such as RuBisCo during leaf senescence is strongly enhanced by environmental stress (Jiang et al., 1999), and this degradation has been proposed as an index of redox state (Pacifci and Davies, 1990). In addition to the significant higher MDA content, a 42% decrease in TLPr was observed in HS plots compared to control ones (Table 3). Moreover, there was a strong negative association between both variables (Fig. 4) confirmed by fitted regression model which indicated that significant explanation ( $P = 0.01$ ;  $R^2$  adjusted = 0.17) was obtained for TLPr from MDA (data not shown). Possibly disclosing biochemical processes related to damage in proteins (i.e. inhibition of RuBisCo activity) which might be implicated in damage derived from heat stress as demonstrated by Yamauchi et al. (2008) through *in vitro* experiments. It has been estimated that RuBisCo constitutes up to 60% of the total soluble protein in many C3 plants (Friedrich and Huffaker, 1980), and accounts for about 90% of the protein degraded during the early stages of senescence. Thus, the significant decreased in soluble proteins observed in HS plots could be related essentially to degradation of RuBisCo.

On the other hand, under prolonged stress conditions such those generated by the water-stress, the TLPr decreased  $\sim$ 20% along with a decreased in SPAD values. Indeed, the SPAD values in WS and HS  $\times$  WS plots were 37% and 42%, respectively lower than those observed in control plots (Table 3). These results are in agreement with the general chlorophyll drops that occur when soybean plants are subjected to continuous water stress from early seed filling (De Souza et al., 1997; Inamullah and Isoda, 2005). In other species, prolonged water deficit combined with heat stress also decreased chlorophyll content enhancing leaf senescence (Jiang and Huang, 2001; Xu and Zhou, 2006). Declines in leaf chlorophyll, protein and consequently photosynthesis, commonly occurring during leaf senescence begin early in seed filling and can be accelerated under abiotic stresses (Brevedan and Egli, 2003; Egli and Crafts-Brandner, 1996). Senescing leaves are source organs of both carbon and nitrogen to the developing seed. Soybean seed is characterized by its high protein content, where redistribution of N can be an important source for protein biosynthesis, contributing between 50 and 100% to final GPr (Masclaux et al., 2001). In the present study, it was observed that the decrease in TLPr in irrigated HS plots mirrored the significant increase in GPr, exhibiting the grains harvested in these plots similar protein content to that from control ones (Table 3), reflecting remobilization activity from leaves to seeds. Contrasting results emerge when analyzing WS plots alone or combined with HS. Although significant decreases of both TLPr and chlorophyll content (SPAD value) were detected in these plots, GPr decreased close to 25% in comparison to control plots (Table 3), suggesting that grain metabolization of the remobilized N from leaves was impaired in drought conditions. Although GW and GPr are mostly determined by the availability of assimilates (source strength), sink-grain strength also can play an important role (Burstin et al., 2007), through mechanisms associated with both its physical (size) and physiological (activity) capabilities (Herbers and Sonnewald, 1998). For instance, it has been demonstrated that abiotic stressful conditions (heat and/or drought) during seed filling have profound effect on grain metabolism, decreasing sink-grain strength (i.e. the intrinsic capacity of the grain to accumulate storage



compounds) for assimilate utilization of remobilized products of stored reserves from leaves (Triboi and Triboi-Blondel, 2002). Since it has been shown that under normal growing conditions soybean GW experience a large degree of co-limitation by the source and the sink (Borrás et al., 2004), it would be of great interest to study the physiological drivers underpinning grain protein accumulation under drought conditions. Particular attention should be given to analyze if protein accumulation is sink (involving for example differences in protein synthetic capacity through enzymes activities) or source-regulated (comprising N supply from the vegetative organs) or if sink and source co-limitations could occur. Nowadays, we are investigating the involvement of autophagy, as a protein degradation mechanism and nitrogen recycling, and its relationship with cellular redox changes and oxidative stress during the GF period under control and stress conditions.

### 3.4. Correlations between yield, grain number, grain weight and physiological-biochemical traits

The PC1 of the biplot (Fig. 4) revealed that yield and GN vectors were oriented towards irrigated control plots, being positively associated with  $\phi$ PSII, Fv/Fm, leaf starch, TLSS, and FRAP. Grain weight vector more oriented towards irrigated HS plots, was positively associated with GPr and SPAD (Fig. 4). Yield components, i.e. GN and GW were negatively associated with  $CT_{GF}$  during and after HS, respectively. To establish potential associations between yield components and physiological-biochemical traits we fitted regression models including  $\phi$ PSII, Fv/Fm, leaf starch, TLSS,  $CT_{GF}$  during HS,  $CT_{GF}$  after HS, FRAP, MDA, TLPr, SPAD, GPr.

The best fitted regression for yield was a multiple one that included  $\phi$ PSII, SPAD, and FRAP, explaining this model 81% of its total variability (Table 4). The robust association of these variables and yield pointed out to the obvious fact that yield was basically a function of photosynthetic assimilates towards the end of the cycle, when the crop is strongly limited by the source (Borrás et al., 2004). A large body of evidence indicates a close relation between leaf chlorophyll concentration, leaf N content, photosynthesis and crop yield (Betzelberger et al., 2010; Cartelat et al., 2005; Le Bail et al., 2005; Ramesh et al., 2002). Nevertheless, to our knowledge this is the first study that demonstrates the potential of physiological variables, namely  $\phi$ PSII, SPAD and FRAP, to explain yield variations in soybean grown under episodes of HS in combination with WS in field conditions.

Linear regressions showed that the  $CT_{GF}$  during HS treatment was the best predictor for GN, explaining 50% of the total variability of this yield component (Table 4). Across treatments the fitted model indicated that the response of GN to  $CT_{GF}$  was linear and negative, with a slope of  $-87.49 \text{ GN } ^\circ\text{C}^{-1}$ , indicating  $\sim 90 \text{ GN m}^{-2}$  decrease per degree increase in canopy temperature (Table 4). The negative association between  $CT_{GF}$  and GN is widely supported by several studies conducted both under normal (Takai et al., 2010) or abiotic stressful conditions

including drought, heat and their combination (Badaruddin et al., 1999; Gutierrez et al., 2010; Neiff et al., 2015). It is noteworthy that most of these works studied cereals (mainly wheat, maize and rice) as crop models. Some supporting evidence of the negative association between  $CT_{GF}$  and soybean yield was provided by Harris et al. (1984), who evaluating 20 genotypes observed  $822 \text{ kg ha}^{-1}$  more grain yield under irrigated than under dry land treatments which exhibited warmer canopies than irrigated ones. However, results were limited to only yield with no data about its components (i.e. GN and GW).

Canopy temperature strongly depends on stomatal conductance, and positive associations have been noted between stomatal conductance and grain yield, and between both variables and photosynthetic activity (Fischer et al., 1981; Morgan et al., 1993; Takai et al., 2010). Thus, we hypothesize that the decrease in GN in irrigated plots exposed to episodes of HS could be a result of reduction of  $\text{CO}_2$  assimilation due to stomatal closure to a great extent (as suggested by the high canopy temperature values). Which in turn could be promoted by the high VPD owing to the higher temperatures achieved during heating period compared to control plots (Table 1). On the other hand, under a lack of sufficient soil water availability (Fig. 2) to transpire in plants from water stressed plots an exacerbation of stomatal closure is expected (Medina and Gilbert, 2016), thus limiting canopies transpirational cooling, with the consequent rise in leaf temperature (Table 3).

Regression equation for GW showed linearly significant contribution of  $CT_{GF}$  and SPAD both measured after HS (P-value, Table 4), explaining these variables 82% of GW variations across treatments. Noticeable,  $CT_{GF}$  as well as SPAD showed similar contribution in the fitted model (Mallows' CP, Table 4). The regression coefficients linearly relating  $CT_{GF}$  and SPAD with GW indicated increasing GW with decreasing canopy temperatures and increasing SPAD readings. There is supporting evidence on the negative association between  $CT_{GF}$  and GW, for instance Amani et al. (1996) and Olivares-Villegas et al. (2007) found strong and significant ( $P < 0.0001$ ) negative association between  $CT_{GF}$  and wheat GW under heat and drought, respectively. However, to our knowledge this is the first report to demonstrate the strong correlation of  $CT_{GF}$  and GW in heat and water stressed soybean under field environmental conditions. On the other hand, the positive association between SPAD readings during GF and GW is expected since chlorophyll meter measurements is known to be a good predictor of yield at later stages in grain crops including soybean (*Zea mays* L.: Bullock and Anderson, 1998; *Oryza sativa* L.: Ramesh et al., 2002; *Triticum aestivum* L.: Le Bail et al., 2005; *Glycine max* L.: Betzelberger et al., 2010).

## 4. Conclusions

In summary, heat stress combined with water deficit conditions reduced yield and its components (GN and GW). Yield and GN were also reduced in irrigated HS treatments; however, GW was similar to that exhibited by the control. This highlights the capacity of soybean crops to

**Table 4**

Regression equations for soybean yield ( $\text{g m}^{-2}$ ), grain number ( $\text{grain m}^{-2}$ ) and grain weight ( $\text{mg grain}^{-1}$ ) on physiological-biochemical variables during grain filling across the four treatments: control (non-heat-stressed and non-water-stressed), heat-stressed, water-stressed, and heat-stressed  $\times$  water-stressed plots. FRAP, ferric reducing ability of plasma;  $\phi$ PSII, quantum efficiency of photosystem II photochemistry; SPAD, leaf chlorophyll meter;  $CT_{GF}$ , canopy temperature throughout grain filling during and after heat-stress.

Dependent variable	Explanatory variable	Regression coefficient	Standard error	P value	Mallows' Cp	Adjusted R <sup>2</sup>
Yield	Constant	-196.32	96.05	0.0803		0.81
	FRAP	47.72	12.26	0.0060	16.38	
	$\phi$ PSII	776.15	268.81	0.0234	10.42	
	SPAD	5.20	1.66	0.0166	11.69	
Grain number	Constant	4730.85	625.87	< 0.0001		0.50
	$CT_{GF}$ during HS	-87.49	22.41	0.0018	15.23	
Grain weight	Constant	233.69	56.09	0.0013		0.82
	$CT_{GF}$ after HS	-4.47	1.63	0.0181	8.98	
	SPAD	1.38	0.54	0.0260	8.03	

capitalize improved environmental conditions late during reproductive stage through GW modifications in response to compensation mechanisms. Interestingly, even when the air temperature and VPD were highest in the combined stress treatment, yield was no lower than plots with WS alone. By means of chlorophyll fluorescence parameters it was possible to determine that the interaction of both type of stresses affected photosynthesis not only via structural capacity aspects but also by damaging PSII sites. Our findings demonstrated an altered sink-grain capacity for assimilate utilization of remobilized products of stored reserves from leaves, indicating that other factors rather than the availability of assimilate could be involved when HS is combined with WS. Principal component analysis complemented with multiple regression models highlighted many of the included physiological and biochemical parameters (describing the primary metabolism and redox state) late in the reproductive stage as powerful explanatory variables of yield and its components variations, suggesting that they might be useful for cultivar screening. In particular, the outstanding predictor capacity of SPAD and  $CT_{GF}$  are promising traits. Since SPAD and  $CT_{GF}$  measurements are quick, not laborious, not destructive and low cost these predictors could be used as potential selection markers for screening of tolerant soybean genotypes to heat and water stress in field conditions. Further studies are necessary involving more genotypes and years to validate the potential power of the mentioned selection markers for heat and water stressed soybean.

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## References

- AACC, 2000. Approved Methods of the AACC, 10th ed. Am. Assoc. of Cereal Chemists, St. Paul, MN.
- Allen, R.G., Pereira, L.S., Raes, D., Smith, M., 1998. Crop Evapotranspiration-Guidelines for Computing Crop Water Requirements-FAO Irrigation and Drainage Paper 56. FAO, Rome 300 D05109.
- Amani, I., Fischer, R., Reynolds, M., 1996. Canopy temperature depression association with yield of irrigated spring wheat cultivars in a hot climate. *J. Agron. Crop. Sci.* 176, 119–129.
- Andriani, J., Andrade, F., Suero, E., Dardanelli, J., 1991. Water deficits during reproductive growth of soybeans. Their effects on dry matter accumulation, seed yield and its components. *Agronomie* 11, 737–746.
- Apel, K., Hirt, H., 2004. Reactive oxygen species: metabolism, oxidative stress, and signal transduction. *Annu. Rev. Plant Biol.* 55, 373–399.
- Badaruddin, M., Reynolds, M.P., Ageeb, O.A., 1999. Wheat management in warm environments. *Agron. J.* 6, 975–983.
- Bahar, B., Yildirim, M., Barutcular, C., Ibrahim, G., 2008. Effect of canopy temperature depression on grain yield and yield components in bread and durum wheat. *Not. Bot. Hort. Agrobot. Cluj* 36, 34.
- Benzie, I.F., Strain, J.J., 1996. The ferric reducing ability of plasma (FRAP) as a measure of antioxidant power: the FRAP assay. *Anal. Biochem.* 239, 70–76.
- Betzberger, A.M., Gillespie, K.M., Mcgrath, J.M., Koester, R.P., Nelson, R.L., Ainsworth, E.A., 2010. Effects of chronic elevated ozone concentration on antioxidant capacity, photosynthesis and seed yield of 10 soybean cultivars. *Plant Cell Environ.* 33, 1569–1581.
- Bian, S., Jiang, Y., 2009. Reactive oxygen species, antioxidant enzyme activities and gene expression patterns in leaves and roots of *Kentucky bluegrass* in response to drought stress and recovery. *Sci. Hortic.* 120, 264–270.
- Bilger, W., Schreiber, U., Lange, O.L., 1987. Chlorophyll fluorescence as an indicator of heat induced limitation of photosynthesis in *Arbutus unedo* L. In: Tenhunen, J.D., Catarino, F.M., Lange, O.L., Oechel, W.C. (Eds.), *Plant Response to Stress*. Springer, Berlin, Heidelberg, Germany, pp. 391–399.
- Board, J., Tan, Q., 1995. Assimilatory capacity effects on soybean yield components and pod number. *Crop Sci.* 35, 846–851.
- Borrás, L., Slafer, G.A., Otegui, M.E., 2004. Seed dry weight response to source-sink manipulations in wheat, maize and soybean: a quantitative reappraisal. *Field Crops Res.* 86, 131–146.
- Brevedan, R., Egli, D., 2003. Short periods of water stress during seed filling, leaf senescence, and yield of soybean. *Crop Sci.* 43, 2083–2088.
- Bullock, D., Anderson, D., 1998. Evaluation of the Minolta SPAD-502 chlorophyll meter for nitrogen management in corn. *J. Plant Nutr.* 21, 741–755.
- Bunce, J.A., 1984. Effects of humidity on photosynthesis. *J. Exp. Bot.* 35, 1245–1251.
- Burstin, J., Marget, P., Huart, M., Moessner, A., Mangin, B., Duchene, C., Desprez, B., Munier-Jolain, N., Duc, G., 2007. Developmental genes have pleiotropic effects on plant morphology and source capacity, eventually impacting on seed protein content and productivity in pea. *Plant Physiol.* 144, 768–781.
- Caldwell, M., Flint, S., Searles, P., 1994. Spectral balance and UV-B sensitivity of soybean: a field experiment. *Plant Cell Environ.* 17, 267–276.
- Carrera, C., Martínez, M.J., Dardanelli, J., Balzarini, M., 2009. Water deficit effect on the relationship between temperature during the seed fill period and soybean seed oil and protein concentrations. *Crop Sci.* 49, 990–998.
- Carrera, C., Carrão Pazizzi, M., Gontijo Mandarino, J., Leite, R., Ergo, V., Andrade, F., Parola, R., Lascano, R., Vega, C., 2015. Relationship between soybean industrial-nutritional quality and the assimilate source under heat and water stress during seed filling. In: *Proceedings of VII Congreso Brasileiro de Soja-Mercosul* 2015. Florianópolis, Brazil. 22–25 June 2015. Empresa Brasileira de Pesquisa Agropecuária, Florianópolis, Brazil. pp. 99–103.
- Cartelat, A., Cerovic, Z., Goulas, Y., Meyer, S., Lelarge, C., Prioul, J.L., Barbottin, A., Jeuffroy, M.-H., Gate, P., Agati, G., 2005. Optically assessed contents of leaf polyphenolics and chlorophyll as indicators of nitrogen deficiency in wheat (*Triticum aestivum* L.). *Field Crops Res.* 91, 35–49.
- Chaves, M.M., Pereira, J.S., Maroco, J., Rodrigues, M.L., Ricardo, C.P.P., Osório, M.L., Carvalho, I., Faria, T., Pinheiro, C., 2002. How plants cope with water stress in the field? *Photosynth. Growth Ann. Bot.* 89, 907–916.
- Chowdhury, Z.Z., Karim, M.Z., Ashraf, M.A., Khalid, K., 2016. Influence of carbonization temperature on physicochemical properties of biochar derived from slow pyrolysis of durian wood (*Durio zibethinus*) sawdust. *BioResources* 11, 3356–3372.
- Crafts-Brandner, S.J., Salvucci, M.E., 2002. Sensitivity of photosynthesis in a C4 plant, maize, to heat stress. *Plant Physiol.* 129, 1773–1780.
- Crafts-Brandner, S.J., Van de Loo, F.J., Salvucci, M.E., 1997. The two forms of ribulose-1,5-bisphosphate carboxylase/oxygenase activase differ in sensitivity to elevated temperature. *Plant Physiol.* 114, 439–444.
- Davey, M., Stals, E., Panis, B., Keulemans, J., Swennen, R., 2005. High-throughput determination of malondialdehyde in plant tissues. *Anal. Biochem.* 347, 201–207.
- De Souza, P., Egli, D.B., Bruening, W.P., 1997. Water stress during seed filling and leaf senescence in soybean. *Agron. J.* 89, 807–812.
- Di Rienzo, J.A., Guzmán, A.W., Casanoves, F., 2002. A multiple-comparisons method based on the distribution of the root node distance of a binary tree. *J. Agric. Biol. Environ. Stat.* 7, 129–142.
- Di Rienzo, J., Casanoves, F., Balzarini, M., Gonzalez, L., Tablada, M., Robledo, C., 2010. InfoStat: Stastical Software. Grupo InfoStat, FCA, Universidad Nacional de Córdoba, Córdoba, Argentina.
- Dornbos, D., Mullen, R., 1991. Influence of stress during soybean seed fill on seed weight, germination, and seedling growth rate. *Can. J. Plant Sci.* 71, 373–383.
- Draper, N.R., Smith, H., 1966. Selecting the best regression equation. In: *Draper, N.R., Smith, H. (Eds.), Applied Regression Analysis*, third ed. John Wiley and Sons Inc., Hoboken, NJ, USA, pp. 327–368.
- Egli, D., Bruening, W., 2007. Accumulation of nitrogen and dry matter by soybean seeds with genetic differences in protein concentration. *Crop Sci.* 47, 359–366.
- Egli, D., Crafts-Brandner, S., 1996. Soybean. In: *Zamski, E., Shaffer, A. (Eds.), Photoassimilate Distribution in Plants and Crops: Source-sink Relationships*. Marcel Dekker Inc, New York, USA, pp. 595–624.
- Egli, D., Wardlaw, I., 1980. Temperature response of seed growth characteristics of soybeans. *Agron. J.* 72, 560–564.
- Egli, D., 1997. Cultivar maturity and response of soybean to shade stress during seed filling. *Field Crops Res.* 52, 1–8.
- Esterbauer, H., Cheeseman, K.H., 1990. Determination of aldehydic lipid peroxidation products: malonaldehyde and 4-hydroxynonenal. *Methods Enzymol.* 186, 407–421.
- Fehr, W.R., Caviness, C.E., 1977. Stages of soybean development. *Spec. Rep. 80*, Iowa State University, Ames, Iowa.
- Fischer, R., Bidingir, F., Syme, J., Wall, P., 1981. Leaf photosynthesis, leaf permeability, crop growth, and yield of short spring wheat genotypes under irrigation. *Crop Sci.* 21, 367–373.
- Foyer, C.H., Shigeoka, S., 2011. Understanding oxidative stress and antioxidant functions to enhance photosynthesis. *Plant Physiol.* 155, 93–100.
- Foyer, C.H., Lelandais, M., Kunert, K.J., 1994. Photooxidative stress in plants. *Physiol. Plant.* 92, 696–717.
- Foyer, C.H., Bloom, A.J., Queval, G., Noctor, G., 2009. Photorespiratory metabolism: genes, mutants, energetics, and redox signaling. *Annu. Rev. Plant Biol.* 60, 455–484.
- Friedrich, J.W., Huffaker, R.C., 1980. Photosynthesis, leaf resistances, and ribulose-1,5-bisphosphate carboxylase degradation in senescing barley leaves. *Plant Physiol.* 65, 1103–1107.
- Gabriel, K.R., 1971. The biplot graphic display of matrices with application to principal component analysis. *Biometrika* 58, 453–467.
- Gibson, L., Mullen, R., 1996. Soybean seed quality reductions by high day and night temperature. *Crop Sci.* 36, 1615–1619.
- Gill, S.S., Tuteja, N., 2010. Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. *Plant Physiol. Biochem.* 48, 909–930.
- Grassi, G., Magnani, F., 2005. Stomatal, mesophyll conductance and biochemical limitations to photosynthesis as affected by drought and leaf ontogeny in ash and oak trees. *Plant Cell Environ.* 28, 834–849.
- Gregersen, P.L., Culetic, A., Boschian, L., Krupinska, K., 2013. Plant senescence and crop

- productivity. *Plant Mol. Biol.* 82, 603–622.
- Guan, H.P., Janes, H.W., 1991. Light regulation of sink metabolism in tomato fruit: I. Growth and sugar accumulation. *Plant Physiol.* 96, 916–921.
- Gutiérrez, M., Reynolds, M.P., Klatt, A.R., 2010. Association of water spectral indices with plant and soil water relations in contrasting wheat genotypes. *J. Exp. Bot.* 61, 3291–3303.
- Harris, D., Schapaugh, W., Kanemasu, E., 1984. Genetic diversity in soybeans for leaf canopy temperature and the association of leaf canopy temperature and yield. *Crop Sci.* 24, 839–842.
- Havaux, M., 1992. Stress tolerance of photosystem II in vivo. *Plant Physiol.* 100, 424–432.
- Havaux, M., 1996. Short-term responses of photosystem I to heat stress. *Photosynth. Res.* 47, 85–97.
- Heath, R.L., Packer, L., 1968. Photoperoxidation in isolated chloroplasts: I: kinetics and stoichiometry of fatty acid peroxidation. *Arch. Biochem. Biophys.* 125, 189–198.
- Herbers, K., Sonnwald, U., 1998. Molecular determinants of sink strength. *Curr. Opin. Plant Biol.* 1, 207–216.
- Huber, S., Rogers, H., Israel, D., 1984. Effects of CO<sub>2</sub> enrichment on photosynthesis and photosynthate partitioning in soybean (*Glycine max*) leaves. *Physiol. Plant.* 62, 95–101.
- IPCC, 2013. Intergovernmental Panel on Climate Change Website. IPCC Accessed 12 Aug. 2017. <http://www.ipcc.ch>.
- Inamullah, I., Isoda, A., 2005. Adaptive responses of soybean and cotton to water stress II. Changes in CO<sub>2</sub> assimilation rate, chlorophyll fluorescence and photochemical reflectance index in relation to leaf temperature. *Plant Prod. Sci.* 8, 131–138.
- Jiang, Y., Huang, B., 2001. Drought and heat stress injury to two cool-season turfgrasses in relation to antioxidant metabolism and lipid peroxidation. *Crop Sci.* 41, 436–442.
- Jiang, C.Z., Ishihara, K., Satoh, K., Katoh, S., 1999. Loss of the photosynthetic capacity and proteins in senescing leaves at top positions of two cultivars of rice in relation to the source capacities of the leaves for carbon and nitrogen. *Plant Cell Physiol.* 40, 496–503.
- Johnson, R.A., Wichern, D.W., 2002. Principal components. In: Hall, P. (Ed.), *Applied Multivariate Statistical Analysis*, fifth ed. Ed. New Jersey, New Jersey, USA, pp. 430–480.
- Jordan, D.B., Ogren, W.L., 1984. The CO<sub>2</sub>/O<sub>2</sub> specificity of ribulose 1,5-bisphosphate carboxylase/oxygenase. *Planta* 161, 308–313.
- Jumrani, K., Bhatia, V.S., Pandey, G.P., 2017. Impact of elevated temperatures on specific leaf weight, stomatal density, photosynthesis and chlorophyll fluorescence in soybean. *Photosynth. Res.* 131, 333–350.
- Karim, M., Fracheboud, Y., Stamp, P., 1999. Photosynthetic activity of developing leaves of *Zea mays* is less affected by heat stress than that of developed leaves. *Physiol. Plant.* 105, 685–693.
- Kosugi, H., Kikugawa, K., 1989. Potential thiobarbituric acid-reactive substances in peroxidized lipids. *Free Radic. Biol. Med.* 7, 205–208.
- Le Bail, M., Jeuffroy, M.H., Bouchard, C., Barbotin, A., 2005. Is it possible to forecast the grain quality and yield of different varieties of winter wheat from Minolta SPAD meter measurements? *Eur. J. Agron.* 23, 379–391.
- Lowry, O.H., Rosebrough, N.J., Farr, A.L., Randall, R.J., 1951. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* 193, 265–275.
- Lu, C., Zhang, J., 1999. Effects of water stress on photosystem II photochemistry and its thermostability in wheat plants. *J. Exp. Bot.* 50, 1199–1206.
- Masclaux, C., Quilleré, I., Gallais, A., Hirel, B., 2001. The challenge of remobilisation in plant nitrogen economy. A survey of physio-agronomic and molecular approaches. *Ann. Appl. Biol.* 138, 69–81.
- Masclaux-Daubresse, C., Daniel-Vedele, F., Dechorgnat, J., Chardon, F., Gaufichon, L., Suzuki, A., 2010. Nitrogen uptake, assimilation and remobilization in plants: challenges for sustainable and productive agriculture. *Ann. Bot.* 105, 1141–1157.
- Mason, R.E., Singh, R.P., 2014. Considerations when deploying canopy temperature to select high yielding wheat breeding lines under drought and heat stress. *Agronomy* 4, 191–201.
- Maxwell, K., Johnson, G.N., 2000. Chlorophyll fluorescence—a practical guide. *J. Exp. Bot.* 51, 659–668.
- Medina, V., Gilbert, M.E., 2016. Physiological trade-offs of stomatal closure under high evaporative gradients in field grown soybean. *Funct. Plant Biol.* 43, 40–51.
- Mittler, R., 2002. Oxidative stress, antioxidants and stress tolerance. *Trends Plant Sci.* 7, 405–410.
- Miyashita, K., Tanakamaru, S., Maitani, T., Kimura, K., 2005. Recovery responses of photosynthesis, transpiration, and stomatal conductance in kidney bean following drought stress. *Environ. Exp. Bot.* 53, 205–214.
- Molino, J., 2011. Estrés térmico por alta temperatura en soja (*Glycine max* (L.) Merr.): análisis de la dinámica de producción y fijación de vainas y su efecto sobre la determinación del rendimiento MSc. dissertation. Universidad de Buenos Aires, Buenos Aires, Argentina.
- Morales, D., Rodríguez, P., Dell'Amico, J., Nicolas, E., Torrecillas, A., Sanchez-Blanco, M., 2003. High-temperature preconditioning and thermal shock imposition affects water relations, gas exchange and root hydraulic conductivity in tomato. *Biol. Plant.* 47, 203.
- Morgan, J.A., Le Cain, D.R., Mc Caig, T.N., Quick, J.S., 1993. Gas exchange, carbon isotope discrimination, and productivity in winter wheat. *Crop Sci.* 33, 178–186.
- Neiff, N., Dhliwayo, T., Suarez, E.A., Burgueno, J., Trachsel, S., 2015. Using an airborne platform to measure canopy temperature and NDVI under heat stress in maize. *J. Crop Improv.* 29, 669–690.
- Neiff, N., Trachsel, S., Valentini, O.R., Balbi, C.N., Andrade, F.H., 2016. High temperatures around flowering in maize: effects on photosynthesis and grain yield in three genotypes. *Crop Sci.* 56, 2702–2712.
- Noctor, G., Veljovic-Jovanovic, S., Driscoll, S., Novitskaya, L., Foyer, C.H., 2002. Drought and oxidative load in the leaves of C3 plants: a predominant role for photorespiration? *Ann. Bot.* 89, 841–850.
- Olivares-Villegas, J.J., Reynolds, M.P., McDonald, G.K., 2007. Drought-adaptive attributes in the Seri/Babax hexaploid wheat population. *Funct. Plant Biol.* 34, 189–203.
- Pacifici, R.E., Davies, K.J., 1990. Protein degradation as an index of oxidative stress. *Methods Enzymol.* 186, 485–502.
- Pinheiro, J., Bates, D., DebRoy, S., Sarkar, D., 2011. R Development Core Team. nlme: linear and nonlinear mixed effects models. R package version 3.1-97. R Foundation for Statistical Computing, Vienna, Austria.
- Porcel, R., Ruiz-Lozano, J.M., 2004. Arbuscular mycorrhizal influence on leaf water potential, solute accumulation, and oxidative stress in soybean plants subjected to drought stress. *J. Exp. Bot.* 55, 1743–1750.
- Prasad, P.V.V., Staggenborg, S.A., Ristic, Z., 2008. Impacts of drought and/or heat stress on physiological, developmental, growth, and yield processes of crop plants. In: Ahuja, L., Reddy, V., Saseendran, S., Yu, Q. (Eds.), *Response of Crops to Limited Water: Understanding and Modeling Water Stress Effects on Plant Growth Processes*, pp. 301–355. Advances in Agricultural Systems Modeling Series 1; ASA-CSSA: Madison, WI, USA.
- Prasad, P., Pisipati, S., Momčilović, I., Ristic, Z., 2011. Independent and combined effects of high temperature and drought stress during grain filling on plant yield and chloroplast ef-tu expression in spring wheat. *J. Agron. Crop Sci.* 197, 430–441.
- Quick, W., Chaves, M., Wendler, R., David, M., Rodrigues, M., Passaharinho, J., Pereira, J., Adcock, M., Leegood, R., Stitt, M., 1992. The effect of water stress on photosynthetic carbon metabolism in four species grown under field conditions. *Plant Cell Environ.* 15, 25–35.
- Ramesh, K., Chandrasekaran, B., Balasubramanian, T., Bangarusamy, U., Sivasamy, R., Sankaran, N., 2002. Chlorophyll dynamics in rice (*Oryza sativa*) before and after flowering based on SPAD (chlorophyll) meter monitoring and its relation with grain yield. *J. Agron. Crop Sci.* 188, 102–105.
- Reynolds-Henne, C., Langenegger, A., Mani, J., Schenk, N., Zumsteg, A., Feller, U., 2010. Interactions between temperature, drought and stomatal opening in legumes. *Environ. Exp. Bot.* 68, 37–43.
- Ritchie, J., 1981. Water dynamics in the soil-plant-atmosphere system. *Plant Soil* 58, 81–96.
- Rollins, J., Habte, E., Templer, S., Colby, T., Schmidt, J., Von Korff, M., 2013. Leaf proteome alterations in the context of physiological and morphological responses to drought and heat stress in barley (*Hordeum vulgare* L.). *J. Exp. Bot.* 64, 3201–3212.
- Rotundo, J.L., Westgate, M.E., 2009. Meta-analysis of environmental effects on soybean seed composition. *Field Crops Res.* 110, 147–156.
- Rotundo, J.L., Westgate, M.E., 2010. Rate and duration of seed component accumulation in water-stressed soybean. *Crop Sci.* 50, 676–684.
- Salvucci, M.E., Crafts-Brandner, S.J., 2004. Inhibition of photosynthesis by heat stress: the activation state of Rubisco as a limiting factor in photosynthesis. *Physiol. Plant.* 120, 179–186.
- Salvucci, M.E., Ogren, W.L., 1996. The mechanism of Rubisco activase: insights from studies of the properties and structure of the enzyme. *Photosynth. Res.* 47, 1–11.
- Seemans, J.R., Badger, M.R., Berry, J.A., 1984. Variations in the specific activity of ribulose-1,5-bisphosphate carboxylase between species utilizing differing photosynthetic pathways. *Plant Physiol.* 74, 791–794.
- Sharkey, T.D., 2005. Effects of moderate heat stress on photosynthesis: importance of thylakoid reactions, rubisco deactivation, reactive oxygen species, and thermotolerance provided by isoprene. *Plant Cell Environ.* 28, 269–277.
- Siebers, M.H., Yendrek, C.R., Drag, D., Locke, A.M., Rios Acosta, L., Leakey, A.D., Ainsworth, E.A., Bernacchi, C.J., Ort, D.R., 2015. Heat waves imposed during early pod development in soybean (*Glycine max*) cause significant yield loss despite a rapid recovery from oxidative stress. *Global Change Biol.* 21, 3114–3125.
- Souza, R., Machado, E., Silva, J., Lagôa, A., Silveira, J., 2004. Photosynthetic gas exchange, chlorophyll fluorescence and some associated metabolic changes in cowpea (*Vigna unguiculata*) during water stress and recovery. *Environ. Exp. Bot.* 51, 45–56.
- Spreitzer, R.J., Salvucci, M.E., 2002. Rubisco: structure, regulatory interactions, and possibilities for a better enzyme. *Annu. Rev. Plant Biol.* 53.
- Takai, T., Yano, M., Yamamoto, T., 2010. Canopy temperature on clear and cloudy days can be used to estimate varietal differences in stomatal conductance in rice. *Field Crops Res.* 115, 165–170.
- Tambussi, E., Bartoli, C., Guiamet, J., Beltrano, J., Araus, J., 2004. Oxidative stress and photodamage at low temperatures in soybean (*Glycine max* L. Merr.) leaves. *Plant Sci.* 167, 19–26.
- Thuzar, M., Puteh, A., Abdullah, N., Lassim, M.M., Jusoff, K., 2010. The effects of temperature stress on the quality and yield of soya bean [*Glycine max* (L.) Merrill.]. *J. Agric. Sci.* 2, 172–179.
- Triboi, E., Triboi-Blondel, A.M., 2002. Productivity and grain or seed composition: a new approach to an old problem. *Eur. J. Agron.* 16, 163–186.
- Triboi-Blondel, A.M., Renard, M., 1999. Effects of temperature and water stress on fatty acid composition of rapeseed oil. New horizons for an old crop. In: Wratten, N., Salisbury, P.A. (Eds.), *Proceedings of the 10th International Rapeseed Congress*. Canberra, Australia. pp. 26–29.
- USDA (United States Department of Agriculture), 2017. World Agricultural Supply and Demand Estimation. WASDE-566. ISSN: 1554-9089. 40pp. <http://www.usda.gov/oce/commodity/wasde/latest.pdf> (Accessed 07.07.17).
- Wingler, A., Quick, W., Bungard, R., Bailey, K., Lea, P., Leegood, R., 1999. The role of photorespiration during drought stress: an analysis utilizing barley mutants with reduced activities of photorespiratory enzymes. *Plant Cell Environ.* 22, 361–373.
- Xu, Z.Z., Zhou, G.S., 2006. Combined effects of water stress and high temperature on photosynthesis, nitrogen metabolism and lipid peroxidation of a perennial grass *Leymus chinensis*. *Planta* 224, 1080–1090.
- Yamauchi, Y., Furutera, A., Seki, K., Toyoda, Y., Tanaka, K., Sugimoto, Y., 2008. Malondialdehyde generated from peroxidized linolenic acid causes protein modification in heat-stressed plants. *Plant Physiol. Biochem.* 46, 786–793.