

# Exogenous ABA Increases Yield in Field-Grown Wheat with Moderate Water Restriction

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**Abstract** Water stress is one of the most important environmental factors that regulate a plant's growth and development. In agronomic practice the effects of water stress are translated into low yield and/or reduced quality. Abscisic acid (ABA) sprays (1 mM) were applied to wheat plants at different phenological stages and the effects on several physiological variables and on yield were evaluated under field conditions at different water regimes. Studies were conducted in the field across three consecutive winter–spring seasons. ABA treatments were applied at the beginning of shoot enlargement and repeated at anthesis. Exogenous ABA increased shoot dry weight and maintained a high concentration of photosynthetic pigments for a longer period of time during grain growth and maturation. Although ABA applications increased stomatal closure immediately after its application, the longer-term effect was to allow for a greater ostiolar opening of the stomatal pore which resulted in increased conductance of

gases and water vapor. ABA also improved the transport of photoassimilates from the leaves and stem to the developing grains, that is, it effectively increased the sink strength of the grains. This correlated with a yield increase without significantly changing the protein quality in the grains. Thus, elevated ABA levels from exogenous application or genetic selection could help improve agricultural production of grains in arid areas where irrigation is not possible.

**Keywords** Abscisic acid · Wheat (*Triticum aestivum* L.) · Water stress · Field crops · Grain yield · Carbohydrates

## Introduction

Water stress is one of the most important environmental factors that regulate a plant's growth and development. In agronomic practice the effects of water stress are translated into low yield and/or reduced crop quality (Israelsen and Hansen 1985). Therefore, reducing the negative effects of water stress is a key factor in increasing yields and quality in rain-fed crops. A large number of past studies have reported on the effects of water stress on a wide range of crops. For example, in wheat (*Triticum aestivum* L.), water stress occurring at or near anthesis (20–30 previous days and 10 subsequent days) was found to affect the number of grains per spike as well as the weight of the grains, causing a reduction in the productivity and quality of the grain crop (Yang and others 2001; Slafer and others 2003).

Plants have evolved mechanisms that allow them to perceive the incoming stresses and rapidly regulate their physiology and metabolism to cope with them (Jiang and Zhang 2003). At the whole-plant level the effect of water stress is usually perceived as a decrease in photosynthesis

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and vegetative shoot growth. At the molecular level, an important negative effect of water stress may be in part a consequence of the oxidative damage to the plant cell produced by osmotic stress, due to an imbalance between production of reactive oxygen species (ROS) and antioxidant defenses (Sharma and Dubey 2005; Hu and others 2006).

The plant hormone abscisic acid (ABA) appears to play a major role in a plant's responses to water stress (Dodd and Davies 2005). Considerable evidence suggests that drought induces an increase in xylem tissue ABA levels, which then act as signaling molecules with regard to the plant's response to the stress (Spollen and others 2000; Gazzarrini and McCourt 2001). For example, there is strong evidence that ABA plays an important role in the regulation of stomatal closure during drought and this stomatal closure appears to reduce shoot growth (Sauter and others 2001; Davies and others 2005) while regulating the balance between water loss (Davies and Zhang 1991) and CO<sub>2</sub> uptake and assimilation (Avigad and Dey 1997). Although it has been widely speculated that ABA may be causally related to growth inhibition (Dodd and Davies 2005), young tissues do have high levels of ABA and mutants deficient in ABA are severely dwarfed because they are unable to control transpiration and thus cannot maintain cellular turgor. In a study by Finkelstein and Rock (2002), one ABA-deficient mutant had normal cell expansion and growth restored after being treated with exogenous ABA. Also, in *Ilex paraguariensis*, ABA sprayed onto the leaves alleviated diurnal water stress and allowed more growth and dry matter accumulation in both stems and leaves (Sansberro and others 2004).

ABA also promoted dry matter accumulation in several organs and its level was strongly correlated with the growth rates of both fruits and seeds (Wang and others 1998). Isogenic lines of wheat containing high levels of endogenous ABA appear to be better at osmoregulation and exhibit better growth and higher yields under water stress (Quarrie and others 1999). This suggests that an appropriate level of ABA will be necessary for plants to grow successfully under stress conditions (Spollen and others 2000).

In a previous study we found that exogenous ABA increased yield in field-grown wheat and that water stress was alleviated temporarily during anthesis and postanthesis stages, with the remobilization of assimilates to the grains being favored (Travaglia and others 2007). An important observation about these findings is that the beneficial effects of ABA manifest themselves only when water stress conditions exist.

Although there is substantial evidence about the effects of ABA on plants, most investigations have been performed under controlled laboratory or greenhouse

conditions. Little is known about the effects of applied ABA in field-grown crops. The purpose of this study was to evaluate the effect of exogenous ABA applied to wheat plants at several different phenological stages on physiological variables and yield under field conditions with different water regimes.

## Materials and Methods

### Site Description

Experiments were conducted in the field at the Universidad de Río Cuarto, Río Cuarto, Provincia de Córdoba, Argentina (33° 07' S, 64° 14' W). The soils of this region correspond to the Hapludol type, with a thick texture, minimal contents of organic matter, and edaphic water, factors that limit the potential for high grain yield. The moisture retention curves of Hapludol soils are characterized by an abrupt change in the slope at approximately 0.3–0.4 MPa, where small decreases in water content cause important increases in the forces of water retention. This is a characteristic phenomenon of these textural classes of soils. Table 1 presents the water constant of each soil horizon and their corresponding depth.

### Climatic Conditions

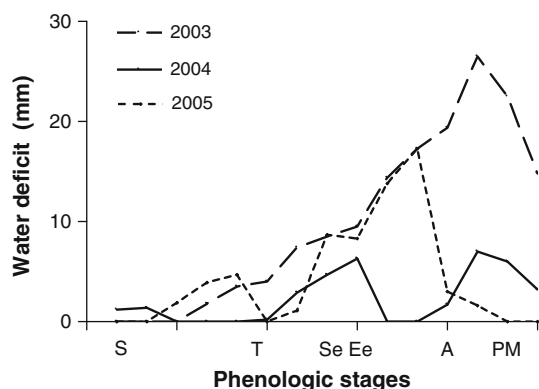
During 2003, the wheat crop developed under intense soil water stress (Fig. 1). At sowing, the soil contained approximately 95 mm of available water in the first meter, which allowed the germination and establishment of the seedling. This value fell sharply, and in the pre-stem elongation stage there was only 24 mm of available water in the soil profile. The total rainfall was 62 mm.

In 2004, during sowing and development of the crop, the climatic conditions were less severe (Fig. 1). Rainfall in July resulted in a good accumulation of water in the soil profile, favoring the emergence and establishment of the wheat seedlings. During plant development, total rainfall

**Table 1** Water constants of the field soil profile

Soil profile	Depth (cm)	FC (%)	PWP (%)	AW (%)
Ap1	00–05	21.83	9.33	12.50
Ap2	05–14	20.23	8.97	11.26
Ad	14–22	19.06	9.15	9.91
Bw1	22–35	19.7	9.14	10.56
Bw2	35–49	18.16	9.09	9.07
BC	49–66	16.77	8.21	8.56
C	66–82	16.52	7.42	9.10

FC field capacity, PWP permanent wilting point, AW available water



**Fig. 1** Water deficit (mm) for each year and during phenologic stages of wheat. Water deficit was estimated when the soil water content fell below 50%. S, sowing; T, tillering; Se, stem elongation; Ee, ear emergence; A, anthesis; PM, physiological maturity. Records kept by the Meteorological Station, UNRC

was 181 mm; nevertheless, there was a moderate drought during the critical anthesis/postanthesis period.

In 2005, after rainfall occurred in March, there were no other rainfall events for several months. This led to a gradual decrease of edaphic water that reached its lowest value (20% of the maximum storage capacity) by mid-October, which was the beginning of the critical anthesis/postanthesis period (Fig. 1). Then the soil water content began to recover due to rainfall, reaching 62% of field capacity at the end of November, a level that represents good soil moisture storage for that time of the year. The total rainfall in 2005 was 201 mm.

### Experimental Procedure

The experiments were conducted during winter–spring of 2003, 2004, and 2005. Treatments consisted of foliar sprays of water (control) or a 300-ppm solution of ABA ( $\pm$ -S-*cis*, *trans* abscisic acid, 90%, Kelinon Agrochemical Co., Beijing, China) in an amount of 1 mM and 100 l ha<sup>-1</sup> at the beginning of shoot enlargement and repeated at anthesis, according to Zadocks' scale (Zadocks and others 1974). Both solutions included 0.1% ethanol (a minimum amount used to dissolve the ABA) and 0.1% of Triton X. Spraying was done at dusk to prevent ABA photodestruction.

The dose of ABA was chosen after preliminary experiments that tested doses from 150 to 350 ppm (Travaglia and others 2007) and according to experience with other species (Sansberro and others 2004). Spraying the dose chosen (300 ppm) effectively yielded about 7.5 mg phytohormone per m<sup>2</sup>.

Each experiment consisted of a randomized block design with six replicates of 10-m<sup>2</sup> plots (2 m × 5 m) with rows spaced 0.175 m apart. The wheat cultivars used were

common for this region (Prointa Gaucho in 2003, Guapo Back in 2004, and Condor Relmo in 2005). For each experiment, (NH<sub>4</sub>)<sub>2</sub>HPO<sub>4</sub> and urea were applied before sowing at 80 and 140 kg ha<sup>-1</sup>, respectively. Seeds were sown at a density of 250 plants m<sup>-2</sup> following the technique of zero tilling and with no artificial irrigation. Weeds were controlled with MISIL (methyl metsulfuron + dicamba, Dupont, Argentina) just after plant emergence (3 leaves and 1 tiller).

### Measurements

Shoot biomass without spikes was determined on a dry weight (DW) basis by placing sample aliquots for 7 days at 65°C in a fan-ventilated oven. Measurements were expressed per unit area (m<sup>2</sup>). Samples were obtained at the postanthesis stage.

For pigment measurement, 50 mg fresh weight (FW) of the flag leaf was homogenized in a mortar with 10 ml of 80% acetone. The homogenate was loaded into Eppendorf tubes, and after 1 h at 4°C to allow pigment extraction, it was centrifuged (twice) for 5 min at 5000 rpm. Aliquots were taken and chlorophyll *a* and *b* levels were measured by spectrophotometry at 650 and 665 nm, respectively. Five milliliters of 1 M NaOH and 15 ml of diethyl ether were added to the total volume. Carotene content was assessed from the ether fraction by spectrophotometry at 450 nm (modified from Mac Kinney 1938). Samples were obtained at the anthesis stage.

Measurements of lipid peroxidation of shoots at anthesis were performed with the thiobarbituric acid (TBA) test, which determines malondialdehyde (MDA) as an end product of lipid peroxidation. MDA was thus used as an indicator of cell membrane stress. Briefly, 200 mg of shoots were homogenized in 2 ml of 0.1% (w/v) trichloroacetic acid (TCA) solution on ice. The homogenate was centrifuged at 14,000 rpm for 10 min, and 0.5 ml of the supernatant was added to 0.5 ml of 0.5% (w/v) TBA in 20% TCA. The mixture was incubated in boiling water for 20 min and the reaction was stopped by incubation in ice. Samples were then centrifuged for 5 min at 14,000 rpm, and the absorbance of the supernatant was measured at 532 nm, subtracting the value for nonspecific absorption at 600 nm (Hodges and others 1999).

Stomatal conductance, transpiration rate (mmol m<sup>-2</sup> s<sup>-1</sup>), and leaf temperature (°C) were measured on flag leaves at 7 h, 7 days, and 21 days after application of ABA by using a portable porometer LI-COR model LI-3000 (LI-COR Biosciences, Lincoln, NE). Concomitantly, a layer of acrylic (synthetic nail amend) was brushed onto the abaxial side of the leaf, allowed to dry for a few seconds, then carefully extracted and mounted for microscope observation to check for stomatal aperture (D'Ambrioglio de

Argüeso 1986). A standard Zeiss model 16 microscope was used to assess the histological preparations, and photomicrographs were taken with a Zeiss Axiophot microscope equipped with image capture and digitization (AxioVision 4.3, with camera AxioCam HRC).

Determination of total water-soluble carbohydrate was based on the phenol–sulfuric acid method. Six samples were processed for each treatment, and 100 mg of shoot was homogenized in a mortar with 1.5 ml of 1% TCA. The homogenate was loaded into Eppendorf tubes and centrifuged twice at 7500 rpm for 10 min. Aliquots of 20  $\mu$ l were taken and added to 500  $\mu$ l of phenol reactive (phenol 0.5% in water), followed by 2.5 ml of sulfuric acid. A serial dilution of glucose from 0 to 100 mg ml<sup>-1</sup> was used to determine the calibration curve. After 10 min at room temperature, the mixture was recorded by spectrophotometry at 488 nm and the results of the samples were compared with those of the glucose calibration curve (modified from Daniels and others 1994). Percentage of carbon remobilization (% CR) was estimated according to Yang and others (2000),

$$\%CR = [(NSCsa - NSCsm)/(NSCsa) \times 100]$$

where NSCsa is nonstructural soluble carbohydrates in shoots at anthesis and NSCsm is nonstructural soluble carbohydrates in shoots at maturity.

At harvest, the yield components and the number and average weight of grains per spike were all measured. All the plants (except those from the border) in 1 m<sup>2</sup> per plot for each treatment were harvested at maturity for determination of grain yield. Protein content of the grains was assessed by IR spectroscopy as described by Cuniberti (2001).

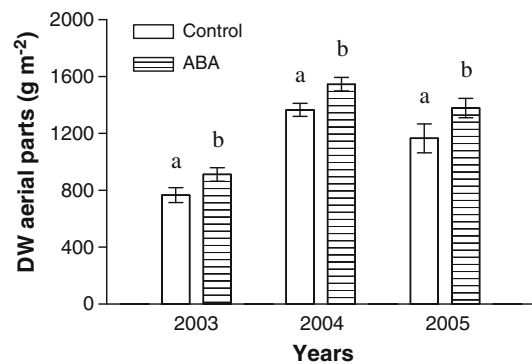
### Statistical Analysis

The results were analyzed for variance using the InfoStat statistical analysis software (professional version 1.1), and the LSD Fisher 5% test was used to compare differences among treatments. In the tables, different letters mean significant differences with  $P \leq 0.05$ . In the figures, bars represent standard errors of the mean value.

## Results

### Biomass Production

In the 3 years of our study, shoot dry weight was always greater in ABA-treated plants relative to control plants (Fig. 2), whereas the dry weight of roots was not affected (data not shown).



**Fig. 2** Dry weight (DW) of aerial parts without spikes ( $\text{g m}^{-2}$ ) of wheat plants in the postanthesis stage, treated with or without ABA. Bars show standard error (SE) of mean value. Different letters mean significant differences in each year, with  $P \leq 0.05$  for the Fisher's test

### Photosynthetic Pigments

In 2003, ABA-treated plants showed significant increases in chlorophyll *a* and total chlorophyll, but not in carotene (Table 2). In 2004, both chlorophyll *a* and *b* and carotene contents increased in ABA-treated plants. In 2005, the carotene content and total chlorophyll levels increased significantly in ABA-treated plants (Table 2). During all 3 years the wheat plant's leaves also remained green longer in ABA-treated plants relative to control plants, for example, 5 days in 2003 and 10 days in each of 2004 and 2005.

### Putative Antioxidant Defense Systems

In 2003, a year with more water stress, MDA levels were greater than during 2004 and 2005 (Table 2, see Controls). In 2003 and 2005, the MDA levels in ABA-treated plants were similar to those in control plants. However, in 2004 the MDA content was significantly decreased in ABA-treated plants (Table 2).

### Stomatal Behavior

In 2003, ABA-treated and control plants showed similar percentages (95%) of stomata closed at noon. Likewise, the values of conductance and transpiration were similar (data not shown). In 2004 and 2005, ABA-treated plants showed 10% more stomata closed than control plants 7 h after hormone application (97 and 87%, respectively). This 10% closure caused a significant reduction in leaf conductance and transpiration rate. Subsequently, the values of these variables started to rise and equaled those of the control plants 7 days later, at which time the leaves of ABA-treated plants exhibited lower temperatures. By 21 days after ABA treatment, both leaf conductance and

**Table 2** Average values of chlorophylls, carotene ( $\mu\text{g g}^{-1}$  FW), and MDA ( $\mu\text{mol g}^{-1}$  FW)

	2003		2004		2005	
	Control	ABA	Control	ABA	Control	ABA
Chl a	166.68a	230.00b	78.04a	132.58b	305.50a	388.13a
Chl b	86.19a	98.90a	50.74a	92.56b	149.99a	196.09a
Chl t	252.87a	328.90b	128.78a	225.14b	455.49a	584.28b
Carotene	0.404a	0.450a	0.163a	0.227b	0.373a	0.450b
MDA	0.0236a	0.0221a	0.0116b	0.0096a	0.0099a	0.0098a

All values are for control versus ABA-treated wheat plants where tissue was harvested at anthesis. Values within a row followed by different letters mean significant differences in each year, with  $P \leq 0.05$  for the Fisher  $\alpha$  test

**Table 3** Average values of leaf conductance ( $\text{sg, mmol m}^{-2} \text{s}^{-1}$ ), transpiration rate ( $\text{mmol m}^{-2} \text{s}^{-1}$ ), and temperature ( $^{\circ}\text{C}$ ) for the flag leaf of wheat plants, treated with or without ABA

	7 h		7 days		21 days	
	Control	ABA	Control	ABA	Control	ABA
Conductance	47.16b	30.57a	24.05a	26.63a	23.23a	32.67b
Transpiration	1.915b	1.222a	1.130a	1.143a	0.449a	0.716b
Temperature	31.66a	31.56a	32.70b	31.66a	27.19a	26.91a

Measurements were made at 7 h, 7 days, and 21 days after application of ABA. Values within a row followed by different letters mean significant differences in each year, with  $P \leq 0.05$  for the Fisher  $\alpha$  test

transpiration rate increased (Table 3). Analysis of the flag leaf's abaxial epidermis showed that the percentages of open and closed stomata were similar in ABA-treated plants and control plants, but stomatal behavior was different. Specifically, in the leaves of ABA-treated plants, a minimum opening of the ostiole was observed for the closed stomata, and the open stomata showed an average pore width of  $3.08 \mu\text{m}$ , whereas in control plants it was  $1.35 \mu\text{m}$  (data not shown).

#### Remobilization of Carbohydrates Reserves from Leaf and Stems to the Grains

During all 3 years of the study ABA-treated plants showed increased mobilization of total soluble carbohydrates to the

**Table 4** Difference between the average mobilization of total stem water-soluble carbohydrates to the grain heads for wheat plants treated with ABA, relative to control plants, in each year analyzed

Year	Carbohydrates mobilization (%)		
	Control	ABA	ABA effect
2003	40.87a	73.86b	+32.99
2004	04.92a	20.75b	+15.83
2005	32.47a	43.18b	+10.71

Tissues were harvested at anthesis and maturity. Values within a row followed by different letters mean significant differences in each year, with  $P \leq 0.05$  for the Fisher  $\alpha$  test

developing grains relative to control plants. The biggest difference was found in 2003, the year of most severe water stress (Table 4).

#### Yield and Some Yield Components

During the 3 years of treatment with ABA, the number of grains produced per spike increased but there was no increase in yield (weight) per hectare. Grain protein, however, was increased by ABA treatment in 2003 (Table 5). In 2004, ABA treatment also increased the weight of grains per spike (Table 5), giving an appreciable (34%) and significant increase in the yield per hectare relative to the control treatment (Fig. 3). In 2005, ABA application again significantly increased the number and weight of grains per spike (Table 5) and the harvest yield (11%) per hectare (Fig. 3).

## Discussion

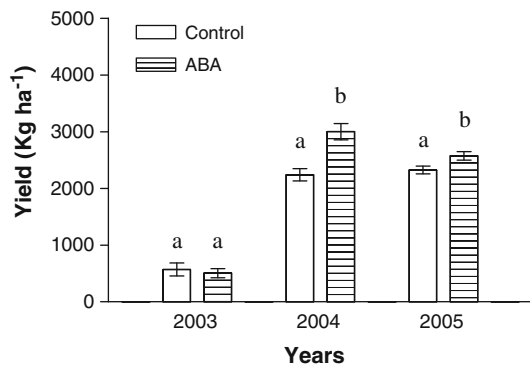
### Biomass Production

In our study, field-grown wheat plants under water stress that had been treated with ABA showed greater shoot biomass accumulation (Fig. 2). In contrast, the dry weight of roots was not affected. This effect of ABA has also been seen in studies carried out under controlled conditions with

**Table 5** Average numbers of kernels per spike, weight (g) per 1000 kernels, and grain protein content for wheat plants treated with or without ABA in each year

	2003		2004		2005	
	Control	ABA	Control	ABA	Control	ABA
No. grain	25a	28b	21a	23b	29a	31b
Weight grain	0.565a	0.610a	0.644a	0.746b	1.023a	1.170b
Proteins	14.4a	15.2b	11.7a	11.5a	11.7a	11.7a

Values within a row followed by different letters mean significant differences in each year, with  $P \leq 0.05$  for the Fisher  $\alpha$  test



**Fig. 3** Yield ( $\text{kg ha}^{-1}$ ) of wheat plants treated with or without ABA in each year. Bars show standard error (SE) of mean value. Different letters mean significant differences in each year, with  $P \leq 0.05$  for the Fisher  $\alpha$  test

different levels of water deficit, for example, in *Arabidopsis* and *Ilex paraguariensis*, where the plants sprayed with ABA had greater growth than those with lower (endogenous) levels of ABA (Finkelstein and Rock 2002; Sansberro and others 2004). In agreement with our previous findings (Travaglia and others 2007), our new results suggest that ABA is an important regulator of both cell and whole-plant water content, likely due to an increased turgor pressure which allows for optimal cellular expansion (Acevedo and others 1971).

#### Photosynthetic Pigments

A more prolonged photosynthetic activity favors a greater accumulation of dry material in products to harvest (Thomas and Howarth 2000). In this respect we note that during all 3 years the leaves of the ABA-treated wheat plants remained green longer than the leaves of control plants, for example, from 5 days longer in 2003 to 10 days longer in 2004 and 2005. Increases in 2003 for ABA-treated plants were due mainly to chlorophyll *a*, whereas for 2004 and 2005 ABA induced significant increases in both chlorophyll *a* and *b* and in carotene (Table 2). Our previous results (Travaglia and others 2007) and the findings reported in this study indicate that application of ABA maintains or increases both of these

chlorophylls for plants growing under water stress, thereby delaying leaf senescence.

Enhanced carotene levels were also observed in 2004 and 2005. Carotenoids could be a contributory factor in maintaining the integrity of the photosynthetic apparatus, especially the chloroplast, as they have been shown to indirectly reduce the formation of reactive oxygen species (ROS) (Foyer and Harbinson 1994). However, there are numerous reports that applied ABA inhibits the photosynthetic process (Reddy and others 2004; Liu and others 2005), although Ivanov and others (1995) have reported that ABA applied to seedlings of barley had a protective effect on the photosystem II (PSII) complex, thereby allowing the plants to avoid the harmful effect of high-intensity light. Ivanov and others (1995) have also reported that the plants with exogenous ABA had increased total carotene concentrations as well as increased xanthophyll cycle activity. The latter is known to play an important role in maintaining the integrity of the photosynthetic membranes under oxidative stress (Munné-Bosch and Alegre 2002).

#### Putative Antioxidant Defense Systems

An increase in levels of MDA is an indicator of greater lipoperoxidation and thus increased stress on the integrity of cell membranes (Heath and Packer 1968). In 2003, the most severe drought stress year, control wheat plants (not treated with ABA) showed appreciably higher MDA levels than in 2004 and 2005 (Table 2). This is consistent with the observation by Sgherri and others (1993) that the intensity of oxidative damage is positively correlated with the intensity of stress. In 2004, the MDA content decreased in ABA-treated plants (Table 2); this may indicate a lower oxidative stress, which is in agreement with previous studies (Foyer and Harbinson 1994).

Other laboratory studies indicate that rye plants pre-treated with ABA showed lower levels of MDA and hydroperoxides and increases in the concentration of polyunsaturated fatty acids (Kurchii 2002). Likewise, increased ABA in maize leaves under water stress was correlated with an increase in the synthesis of antioxidant

molecules (Jiang and Zhang 2002). The supposition that one long-term effect of ABA may be to help protect the plant from oxidative stress thus seems justified.

### Stomatal Behavior

The effect of ABA on stomatal control and signal transduction in guard cells has been widely reported (Davies and others 2005; Pei and Kuchitsu 2005). In 2003, this study showed that ABA-treated and control plants had a similar percentage (95%) of stomata closed at noon. Likewise, values for leaf conductance and transpiration were similar. In 2004 and 2005, our ABA-treated plants had 10% more closed stomata than control plants 7 h after hormone application and this caused a significant reduction in conductance and transpiration rate. However, these variables started to change and within 7 days ABA-treated plants were similar to controls. Also, their leaves showed lower temperatures than control plants. By 21 days after ABA treatment, leaf conductance and transpiration rates were greater than were seen for control plants (Table 3). Thus, under moderate water deficiency (2004 and 2005), ABA treatment had a long-term effect on stomatal behavior, yielding a larger ostiolar opening at 1:00 p.m. (maximal irradiance) than control plants, with the ostiolar opening allowing ABA-treated plants to maintain better stomatal conductance than control plants. This effect could result in a better balance between the water loss by transpiration and the uptake of CO<sub>2</sub>, producing greater photosynthetic rates during the day; for example, when there is a partial closure of stomata, the transpiration rate decreases more than photosynthesis (Montaldi 1995). Stomatal response in cereals may not be as “standard” as it is in dicots. In fact, there are old textbook citations (Sutcliffe 1977) stating that. However, in the present study there is not enough information to further advance the discussion of wheat (or cereal) stomatal physiology except to be speculative. To assess the specific and direct effects of sprayed ABA on wheat stomatal aperture/closure, more frequent time-course measurements should be performed. To measure ABA levels in the “bulk” leaf tissue of the different treatments may not be as informative as expected because the levels on the target cells (occlusive cells) may not be the same as in the whole tissues. In any case, the general effects were noticed on dry matter transport rather than on plant water balance. This confirms previously published results (Travaglia and others 2007).

### Remobilization of Carbohydrate Reserves

In wheat, it is possible that assimilates produced prior to anthesis are deposited in the grains through remobilization from reserves located in the stems. There are reports

(Kerepesi and others 1998; Slafer and others 2003) that show a positive relationship between sugar content and the degree of tolerance to abiotic stress in cereals, suggesting that increased sugar content increases tolerance in plants. In this study we found that during all 3 years ABA-treated plants increased remobilization of total soluble carbohydrates to the grain compared to controls. The greatest difference was found in 2003 under severe water stress (Table 4). In agreement with these results, it has been shown that the application of ABA in postanthesis of rice and wheat plants grown in pots and subjected to moderate water stress during grain filling can increase the remobilization of carbon from the photosynthetic tissue assets to grains, thus accelerating filling rate (Yang and others 2001, 2004). Thus, as suggested in a previous study (Travaglia and others 2007), ABA could be regarded as an essential regulator in the translocation and partitioning of photoassimilates for grain filling in cereals.

### Yield and Some Yield Components

Several yield components, that is, number of grains per unit area and average weight per grain, can be affected by temporary drought stress (Blum and Pnuel 1990). Under this condition the grain-filling stage is reduced to produce a rapid maturation of spikes, which affects their number, the quantity of grains per spike, and the weight per grain (Yang and others 2001). However, we found that during the 3 years under different levels of water restriction, treatment with ABA increased the number of grains per spike. It also increased the weight per grain, except in 2003 when the most severe water stress occurred (Table 5). Therefore, in 2004 and 2005, ABA applications increased the yield per unit area over control (34 and 11%, respectively) (Fig. 3).

Moreover, much of the nutritional value of wheat is determined by the protein content in its grains. Because of this, the main concern has been to develop wheats that are not only more productive but also contain a high percentage of protein in the grain. This is difficult to achieve considering that there is an inverse relationship between yield and protein content (Cuniberti 2001). In our study, the grain protein content in 2003 (severe drought) increased in ABA-treated plants, whereas in the next 2 years there was no difference in grain protein between ABA-treated and control plants (Table 5). As we reported in a previous study (Travaglia and others 2007) and in the present study, ABA treatment in 2004 promoted greater grain production per hectare without affecting grain (protein) quality, an effect also observed in 2005.

Evidence obtained in this study suggests that ABA could help improve agricultural production in rain-fed crops, with consequent economic and environmental benefits because wheat is valuable not only for the grain that it produces but

also for the use of stubble and the increased soil carbon levels. This is a very promising result and should increase interest in the use of grain genotypes with naturally elevated ABA content and in the possible use of applied (s)-ABA to grain crops in arid zones. Although the costs of ABA applications in agriculture may not be economically feasible now, the present results open the possibility for its future use as long as the commercial product is available on the market. Its use will not pose any environmental threat because ABA is a natural compound produced by plants, fungi, and bacteria.

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