SHORT COMMUNICATION

Exogenous Abscisic Acid Increases Carbohydrate Accumulation and Redistribution to the Grains in Wheat Grown Under Field Conditions of Soil Water Restriction

**Claudia Travaglia · Ana C. Cohen ·
Herminda Reinoso · Carlos Castillo ·
Rubén Bottini**

Abstract This work investigates the effects of abscisic acid (ABA) on physiologic parameters related to yield in wheat (*Triticum aestivum*) grown under field conditions with water restriction ranging between 45.7% and 49.5% of field capacity during anthesis and postanthesis. ABA (300 mg L^{-1}) was sprayed onto the plants at the beginning of shoot lengthening which significantly promoted leaf area and higher concentrations of chlorophylls and carotenoids in flag leaf at anthesis. ABA also increased soluble carbohydrates in shoots at anthesis, which were then re-exported to the grains at maturity. This correlated with a yield increase that was achieved by a higher number and weight of grains per spike, but protein content was not significantly affected.

C. Travaglia · H. Reinoso
Departamento de Ciencias Naturales, Facultad de Ciencias
Exactas, FísicoQuímicas y Naturales, Universidad Nacional de
Río Cuarto, Río Cuarto, Córdoba, Argentina

C. Castillo
Área de Producción de Cereales, Facultad de Agronomía y
Veterinaria, Universidad Nacional de Río Cuarto, Río Cuarto,
Córdoba, Argentina

A. C. Cohen · R. Bottini
Cátedra de Química Orgánica y Biológica, Facultad de Ciencias
Agrarias, Universidad Nacional de Cuyo, Centro Universitario,
Mendoza, Argentina

R. Bottini (✉)
Facultad de Ciencias Agrarias, Almirante Brown 500,
M5528AHB Chacras de Coria, Argentina
e-mail: rbottini@fca.uncu.edu.ar

Introduction

Abscisic acid (ABA) is a plant hormone generally involved in plant responses to stress (Dood and Davies 2005). Accumulated evidence suggests that drought induces increases in xylematic ABA, which acts as the signaling molecule involved in the response of plants to stress (Davies and Zhang 1991). In fact, there is strong evidence that ABA plays an important role in the regulation of stomatal closure in plants under drought, which therefore restrains growth (Mansfield and others 1990). There are reports, however, claiming that ABA can promote dry matter accumulation in sink organs and that the level of the hormone correlates with the growth rate of fruits or seeds (Schussler and others 1984, 1991; Wang and others 1987). The grain-filling rate and grain weight in rice plants was significantly increased by spraying ABA at the initial grain-filling stage (Yang and others 2003b). In *Ilex paraguariensis* (yerba mate) plants, exogenous ABA promoted dry matter accumulation in shoots and leaves by alleviating diurnal water stress (Sansberro and others 2004).

Abscisic acid has also been reported to be involved in transport of assimilates to developing seeds of cereals (Dewdney and McWha 1978, 1979; Tietz and others 1981; Brenner and Cheikh 1995; Yang and others 1999). Yang and others (2000, 2001, 2003a) reported that a mild water stress imposed during grain filling enhances carbon remobilization from vegetative tissues to grains, accelerating the grain-filling rate in rice and wheat. However, the proposal that ABA is involved in the regulation of assimilate partitioning toward developing seeds has remained in dispute (Jones and Brenner 1987; Ober and Setter 1990; Schussler and others 1991; de Bruijn and Vreugdenbill 1992; Sharp and LeNoble 2002). Little is known about whether and how applications of ABA influence the grain-filling process.

The purpose of this study was to examine the effect of ABA on physiologic parameters and yield in wheat plants grown under field conditions of water restriction.

Materials and Methods

The experiment was conducted in the experimental field at the Universidad de Río Cuarto, Río Cuarto, Provincia de Córdoba, Argentina (33° 07′ S, 64° 14′ W) during winter-spring of 2004. Total rainfall received by the crop was of 181 mm. Water deficit was estimated when the soil water content fell below 50%; such a situation was reached at anthesis (last 10 days of September) with a water content of 49.5% of field capacity, which was expected to affect ear growth and grain number, and continued during postanthesis up to 45.7% of field capacity (5–25 days after anthesis). A wheat cultivar currently used by farmers, Condor Relmó, was sown at a density of 250 plants m^{-2} following the technique of zero tillering and with no artificial irrigation. The soil corresponds to the Hapludol type. Before sowing, $(NH_4)_2HPO_4$ and urea were applied at 80 and 140 kg ha^{-1}, respectively. Weeds were controlled with MISIL (methyl metsulphuron + dicamba, Dupont, Argentina) just after plant emergence (3 leaves and 1 tiller).

The experiment consisted of a randomized block design, with six replicates of 10-m^2 plots (2 × 5 m) with rows at 0.175 m. Treatments consisted of foliar sprays of water (control) or a (Lomon Biotech, Beijing, China, 90% purity) 300-mg L^{-1} solution of ABA in an amount of approximately 100 L ha^{-1} at the beginning of shoot enlargement, and repeated at anthesis. Both solutions included 0.1% ethanol (a minimum amount to dissolve the ABA), and 0.1% of Triton X, and spraying was done at dawn to prevent ABA photodestruction. The dose of ABA was chosen after preliminary experiments and according to experience with other species (see Sansberro and others 2004).

Starting from the time of ABA application, leaf area was assessed with a portable leaf area meter (LI-COR model LI-3000). Samples were obtained from anthesis to physiologic maturity. Sampling consisted of both flag leaf and penultimate internode being severed, weighed, and cut into small sections of 2–4 mm. Samples were then frozen in liquid N$_2$ and stored at −20°C until processing. Shoot biomass was determined on a dry weight (DW) basis by placing sample aliquots for 7 days at 65°C in a fan-ventilated oven.

For pigment measurement, 50 mg DW of flag leaf was homogenized in a mortar with 10 ml of 80% acetone. The homogenate was loaded in Eppendorf tubes and after 1 h at 4°C to allow pigment extraction, it was centrifuged (twice) 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 millimeters of 1 M NaOH

and 15 ml of diethyl ether were added to the total volume. Carotene content was assessed from the ethereal fraction by spectrophotometry at 450 nm.

Total water-soluble carbohydrate determination 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 in Eppendorf tubes and centrifuged twice for 10 min at 7500 rpm. Aliquots of 20 μl were taken and added to 500 μl 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^{-1} 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), where % CR = [(NSCsa – NSCsm)/(NSCsa × 100]; NSCsa = nonstructural soluble carbohydrates in shoots at anthesis and NSCsm = nonstructural soluble carbohydrates in shoots at maturity.

At harvest the individual grain and ear dry weights were measured. All the plants (except those from the border) in the plots were harvested at maturity for determination of grain yield. Yield components, that is, the spikes per square meter, kernels per spike, and kernel weight, were determined from 50 harvested plants randomly sampled. Protein content of the grains was assessed by IR spectroscopy as described by Cuniberti (2001). The results were analyzed for variance using the InfoStat statistical analysis software (professional version 1.1, infostat@agro.uncor.edu), and the LSD Fisher α 5% test was used to compare differences among treatments. Different letters mean significant differences with $p \leq 0.05$. Bars represent standard errors of the mean value.

Results and Discussion

Figure 1 shows that flag leaf expansion was higher in the ABA-treated plants. Photosynthetic pigment content was also higher in flag leaves of ABA-treated plants (Figure 2). Although a higher pigment concentration may not warrant more photosynthesis, an increase in dry matter accumulation was observed in shoots of ABA-treated plants at both anthesis (Figure 3, upper) and maturity (Figure 3, lower), therefore suggesting an improved assimilation and partition of carbon. Indeed, total soluble carbohydrate content increased significantly in shoots of wheat plants treated with ABA at anthesis but not at maturity (Figure 4, left). More importantly, those results mean that a higher proportion of total soluble carbohydrates was transported to
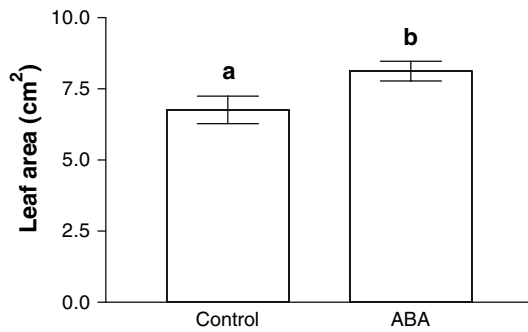
Fig. 1 Leaf area (cm^2) of flag leaves of control and ABA-treated wheat plants at anthesis. Bars show SE of six replicates (each one an average of 4 measurements on each plot). Different letters mean significant differences with $p \leq 0.05$ for the Fisher α test

grains (that is, they were exported to that sink) in the ABA-treated plants (Figure 4, right). The increased carbon partition was finally expressed in a higher weight of 1000 kernels (Figure 5A), and a higher number of kernels per spike (Figure 5B) and yield (Figure 5C) of wheat plants treated with ABA. Importantly, there was no difference in grain protein content when comparing ABA-treated plants with controls (data not shown).

The results demonstrate that ABA applied early in the beginning of shoot enlargement (that is, when plants were
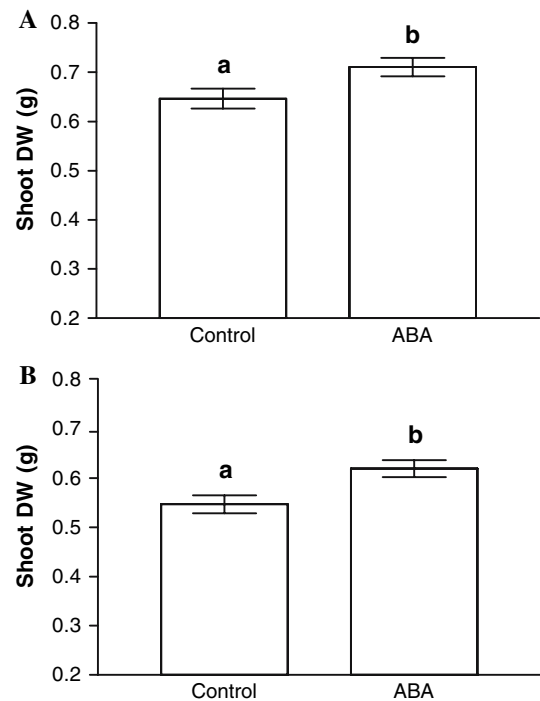

Fig. 2 **A** Chlorophyll *a*, chlorophyll *b*, and total chlorophyll content (μg g DW^{-1}) in flag leaves of control and ABA-treated wheat plants at anthesis. **B** Carotene content (mg DW^{-1}) in flag leaves of control and ABA-treated wheat plants at anthesis. Bars show SE of six replicates (each one an average of 3 plants randomly selected on each plot). Different letters mean significant differences with $p \leq 0.05$ for the Fisher α test
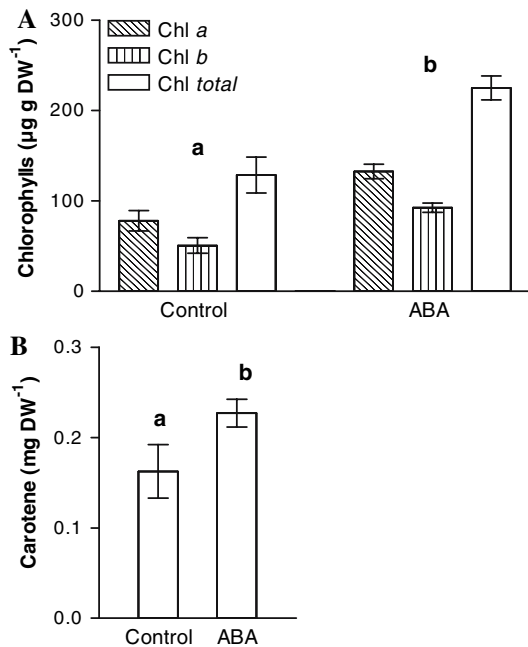

Fig. 3 Shoot dry weight (DW) (g) of wheat plants, treated or not with ABA, assessed at anthesis (**A**) and harvest (**B**). Bars show SE of six replicates (each one an average of 3 plants randomly selected on each plot). Different letters mean significant differences with $p \leq 0.05$ for the Fisher α test

already "turned on" to flowering) and repeated at anthesis significantly promoted grain yield. ABA effects were associated with a higher pigment content that purportedly promoted photosynthetic efficiency, which was reflected in higher dry matter accumulation in shoots. This dry matter increase is associated with accumulation of soluble carbohydrates that are then re-exported to the grains as they develop. The latter are confirmatory of previous reports claiming carbohydrate accumulation in storage organs as stimulated by ABA induced by drought (de Bruijn and Vreugdenbill 1992).

In addition, one must consider that the protective role of ABA over pigments may be related to stimulation of the nonphotochemical quenching imposed to increase the level of xanthophylls. Ivanov (1995) found that barley seedlings treated with ABA had markedly increased (122%) carotene and xanthophylls levels, which play an important role in maintaining the integrity of the photosynthetic membranes under situations of oxidative stress (Havaux 1998; Munné-Bosch and Alegre 2002). In our experiments with wheat, ABA promoted a greater concentration of both chlorophylls and carotenoids, and therefore yellowness was prevented. Consequently, ABA may promote (although indirectly) greater stability of the photosynthetic apparatus, allowing more photosynthesis and thus higher accumulation of dry
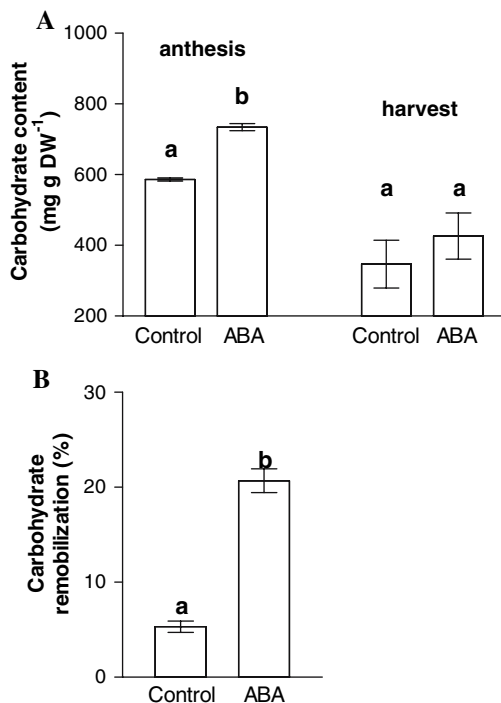
Fig. 4 **A** Total soluble carbohydrate content (mg g DW^{-1}) assessed in shoots of wheat plants treated or not with ABA at anthesis and harvest. **B** An estimate of the total soluble carbohydrate remobilization (in %) in shoots of wheat plants as calculated from data shown in the graph on the left. Bars show SE of six replicates (each one an average of three plants randomly selected on each plot). Different letters mean significant differences with $p \leq 0.05$ for the Fisher α test
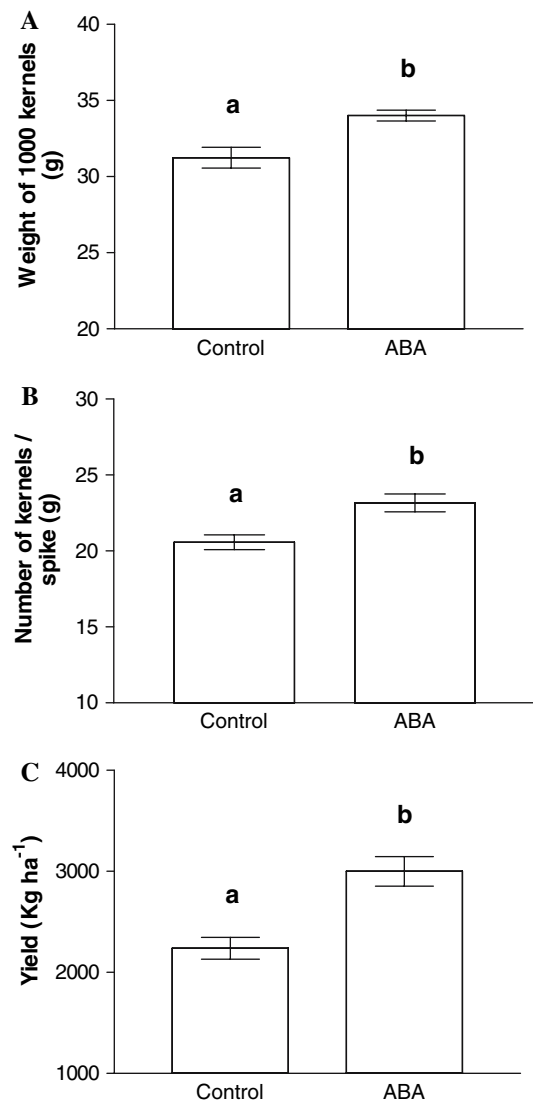
Fig. 5 Weight (g) of 1000 kernels (**A**), number of kernels per spike (**B**), and yield (kg ha^{-1}) (**C**) of wheat plants treated or not with ABA. Bars show SE from 50 plants randomly selected from the six plots. Different letters mean significant differences with $p \leq 0.05$ for the Fisher α test

matter in the harvested products (Thomas and Howarth 2000).

Considering that there is an inverse relationship between grain yield and protein content (Cuniberti 2001), it was logical to expect that exogenous ABA might promote higher yields but lower protein. However, our results did not show significant differences in grain protein levels between ABA-treated and control plants; this is a positive result because ABA may promote higher grain production without affecting quality.

Overall, the results obtained by our study furnish valuable information regarding the possibilities of increasing wheat production by either ABA sprayed onto the leaves, controlled drought treatments, or plant selection associated with higher ABA levels during ontogeny and/or organ location.

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