Application of abscisic acid promotes yield in field-cultured soybean by enhancing production of carbohydrates and their allocation in seed

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Abstract. This study investigates the effect of abscisic acid (ABA) and gibberellin (GA₃) applications on physiological and productive parameters in complementary experiments performed with soybean cultured in the field for 3 crop seasons and in the greenhouse for 1 crop season. ABA 300 mg/L was sprayed at the V7 and R2 phenological stages, while GA₃ 300 mg/L was sprayed at R2 and repeated 7 days later. GA₃-treated plants had longer shoots and ABA-treated plants had greater dry weight of aerial parts. Nodule formation was not affected, but both shoot diameter and root density were greater in ABA-treated plants. ABA increased leaf area and chlorophyll content, while GA₃ diminished them. In ABA-treated plants, there was a significant reduction in leaf conductance 24 h after the hormone had been applied, but then the conductance values started to rise and equalled those of the controls 11 days later. Although there were no differences in number of pods, in GA₃-treated plants the number of seeds per pod was lower, and in ABA-treated plants, young pods were bigger. ABA application increased soybean yield by enhancing carbon allocation and partitioning to the seed. Exogenous ABA also improved the seed quality since it did not affect protein levels but enhanced oil concentration, while GA₃ spraying increased oil concentration but diminished seed proteins.

Additional keywords: Glycine max, plant hormones, field crops, seed yield.

Introduction

An appropriate use of time and resources is a crucial factor for obtaining maximal profits in agriculture. One way in which this can be achieved is by growing more than one crop per year. In the agricultural environments of the Argentinean Pampas, a viable option is to rotate wheat with soybean, which has an additional positive effect on the soil carbon balance because it increases the organic matter content (Andrade and Calviño 2004). However, water availability is a major constraint to the implementation of such a strategy. The success of any crop not only depends on the availability of soil water, but also on the plant ability to use it (Andriani 1997). The short-cycle soybean cultivars which are used to rotate with wheat have high yield potential in conditions of sufficient water availability, while long-cycle cultivars are more flexible and stable in yield under limiting conditions (pers. comm.). Therefore, a way to improve yield in short-cycke soybean cultivars is to improve water-use efficiency during maximum irradiance by stomatal control. Theoretically, this benefit may be achieved by inducing a partial closure of stomata in such a way that the intracellular CO₂ concentration suffices for maximal photosynthesis while water losses are minimal (Kang et al. 1998). Abscisic acid (ABA) is a phytohormone known to be responsible for stomatal closure

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under water limitation (Wilkinson and Davies 2002). The signal transduction in guard cells in response to ABA has been well documented (Luan 2002; Pei and Kuchitsu 2005). Therefore, ABA has been reported to play a key role as a water stress alleviator, either in diurnal variations or long-term drought. In Ilex paraguarienses, ABA sprayed on the leaves alleviated diurnal water stress, allowing more growth and dry matter accumulation in shoots and leaves (Sansberro et al. 2004). Similarly, in a previous study, we found that exogenous ABA increased yield in field-cultured wheat by alleviating temporary water stress and favouring assimilate allocation in grains (Travaglia et al. 2007). Recently, it has been shown in Arabidopsis thaliana that the root-to-shoot signal is of a hydraulic nature, but ABA is necessary for the signal to be detected by the aerial part (Christmann et al. 2007). Therefore, exogenous application of the hormone may be a way to improve the plant response to stressful conditions.

Seed yield in soybean is highly dependent on the number of seeds per pod and pods per plant per unit area. Consequently, when field culture conditions are optimal during the plant critical periods, increased pod and seed set can be expected. If radiation levels, water and nutrient availability, and plant health are good, the establishment of seed and pods will depend mainly on the plant photosynthetic capacity and on the plant ability to use assimilates (Kantolic and Slafer 2003). Particularly, the effects of exogenous ABA on plants growing in well watered soils are contradictory. In rice, ABA applied 11-15 days post-flowering increased grain weight (Yang et al. 2003), while in soybean, ABA sprayed during flowering diminished photosynthesis and the number of pods per plant (Liu et al. 2004). Regarding the effect of ABA on overall plant growth, it has been reported that the hormone modifies vegetative growth, producing changes such as increases in the root-to-shoot relationship, stimulation of lateral root growth, and formation of root hairs (Trewavas and Jones 1991). According to Liang and Harris (2005), ABA increases lateral root density in Leguminosae, which may be of benefit in obtaining a greater number of nodules per plant. However, other authors observed that exogenous ABA diminished the number and weight of nodules in soybean (Cho and Harper 1993), and inhibited nodule formation in Pisum sativum (Phillips 1971), Glycine max (Bano and Harper 2002), Trifolium repens, and Lotus japonicus (Suzuki et al. 2004).

Other plant hormones that regulate various biochemical and physiological processes during plant ontogeny are gibberellins (GAs). The most extensively studied response to these hormones in numerous plant species is promotion of shoot elongation, which has been reported for Arabidopsis thaliana and maize mutants deficient in GAs (Hedden and Proebsting 1999). In addition, it has been observed that GAs improve carbohydrate production by stimulating the rapid discharge from leaves, hence facilitating their transport and accumulation in sink organs (Daie 1987). In sorghum, GA₃ has been found to increase total dry matter (Pao and Morgan 1986), promote accumulation of shoot total carbohydrates, and diminish starch content in leaves (Bastián et al. 1999). In field-grown soybean plants, GA3 sprayed on the leaves increased the leaf area index, growth rate, and net assimilation (Sarkar et al. 2002), which was associated with improved vegetative growth and retardation of fruit development (Guiamét et al. 1987). Regarding yield in soybean, it has been shown that applications of GA₃ diminished harvest by 8-11% by reducing seed weight (Mislevy et al. 1989). The effect of GA₃ may, however, vary according to the time of application: when it was applied 3 days before flowering it did not affect the number of flowers, but reduced their viability and hence the pod number; when the application was during flowering, it slightly reduced the number of pods; during post-flowering there were no effects (Birnberg and Brenner 1987). In addition, it has been reported that foliar applications of Uniconazole (an inhibitor of GA synthesis) at the beginning of flowering promoted biomass accumulation and yield by favouring assimilate translocation (Zhang et al. 2007).

Although ABA and GA roles in basic physiology have been extensively studied, information about the involvement of these hormones in the eco-physiology of field crops is limited. Therefore, the aim of this study was to evaluate the effect of ABA and GA_3 applications on physiological and productive parameters in complementary experiments performed with soybean cultured in the field and in the greenhouse.

Materials and methods

Field experiments

A soybean (*Glvcine max* L.) cultivar with a short growth cycle (GM 3.4, DeKalb, Argentina) was sown in the experimental field of the Universidad Nacional de Río Cuarto campus, Río Cuarto, Province of Córdoba, Argentina (33°07'S, 64°14'W), during the 2004–05, 2005–06, and 2006–07 crop seasons. The total rainfall was 611, 466, and 571 mm for the 3 crop seasons, respectively, which provided a good water supply to the crop, both edaphically and atmospheric. The experiment was a complete randomised block design with 6 replications. Each plot was 6 m long with 4 rows 0.7-m apart. Sowing density was 32 seeds/m², and at harvest there were 23 plants/m². Treatments were: (1) control, sprayed with water; (2) ABA 300 mg/L (Lomon Biotech, Beijing, China, 90% purity), sprayed at the V7 phenological stage (7 leaves) and repeated at R2 (flowering); (3) GA₃ 300 mg/L, sprayed at flowering and repeated 7 days later. All the solutions contained 0.1% Triton as emulsificant, and the spraying was done at sunset to avoid ABA photodestruction. Weeds were controlled manually and pests were controlled using the standard treatments performed by farmers. At the R5 phenological stage (beginning of seed filling) the following parameters were recorded: shoot length, weight of aerial part (in fumeventilated oven at 65°C), number of nodes, shoot diameter, leaf area (LA, with a LAI-2000 Plant Canopy Analyzer, LI-COR, USA), and photosynthetic pigments. Conductance and leaf temperature were measured in the 3rd fully expanded leaf (1 leaf per plant, 15 plants per treatment) at 01:00 hours in the control and ABA-treated plants from 24 h to 25 days after the first spraying, by using a portable porometer (LI-COR, USA). For pigment assessment, samples of the 3rd trifoliate leaf were frozen in liquid nitrogen and 50 mg of fresh weight of these samples were homogenised in a mortar with 10 mL of 80% acetone. After 1 h at 4°C the homogenate was centrifuged 5 min at 5000 r.p.m., and aliquots of the supernatant were read in a spectrophotometer at 650 and 665 nm for chlorophyll a and b, respectively, in a total volume to which 5 mL of 1 M NaOH and 15 mL of diethyl ether were added. Carotenoid content was assessed from the ethereal fraction at 450 nm (modified from Mackinney 1938). 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 homogenised 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 r.p.m. Aliquots of 20 µL were taken and added to 500 µL of reactive phenol (0.5% phenol in water), followed by 2.5 mL of sulfuric acid. A 0-100 mg/mL serial dilution of glucose was used to determine the calibration curve. After 10 min at room temperature, the mixture was recorded by spectrophotometer at 488 nm, and the results of the samples were compared with those of the glucose calibration curve (modified from Daniels et al. 1994). Percentage of carbon remobilisation (% CR) was estimated according to Yang et al. (2000): % CR = [(NSCsa -NSCsm)/(NSCsa - 100)], where NSCsa is non-structural soluble carbohydrates in shoots at flowering, and NSCsm is nonstructural soluble carbohydrates in shoots at maturity. Pod

samples were taken at different stages to compare size among treatments. Harvest was manually performed 10 days after the R8 stage, from a surface of 5.52 m^2 (5 m long and 2 rows, 0.5 m on each end and 2 rows were left as borders). From these plants the following variables were assessed: pod number, seed number per pod, seed weight/m, protein content, and oil content (NIR spectroscopy). Data were analysed using ANOVA and tested by l.s.d. with the Fisher α Test at P=0.05 (Program InfoStat/Profesional 1.1, Universidad Nacional de Córdoba, Estadística y Diseño, Facultad de Ciencias Agropecuarias).

Greenhouse experiment

During the 2006–07 crop season, an experiment was performed in the greenhouse in order to corroborate the hormonal effects observed on root growth and nodulation in field conditions. Pregerminated soybean seeds (same cultivar as per the field experiment) were sown in 24-L pots filled with a mixture (1:1) of vermiculite and soil taken from the experimental field used. The plants were subjected to a natural photoperiod and watered periodically at field capacity. Treatments were as in the field experiment: 150 pots were randomly located, and an extra set of pots with plants was used as a border. At stage R5–6 (80 days post-seeding, which is expected to be the stage of maximum nodulation) the total root biomass was measured, along with number of nodules per plant and nodule weight. Nodule viability was assessed by checking the pink colour in transversal cuts.

Results

Under field conditions, there were no differences in average shoot length between the control and ABA-treated plants, but GA₃-treated ones had longer shoots. Conversely, the dry weight of aerial parts was significantly greater in ABA-treated plants than in the controls and GA₃-treated plants (Table 1).

In the greenhouse experiments, although there were no differences among treatments in node number of the main shoot, both shoot diameter and root density (number of lateral roots) were greater in ABA-treated plants than in controls and GA₃-treated plants (data not shown). The greater production of lateral roots is expressed as root dry weight (Table 2). There were no significant differences in number, weight, and viability of nodules in plants treated with the 2 hormones compared with the control, although the nodule biomass in ABA-treated plants (main the GA₃-treated plants (Table 2).

During the 3 crop seasons, ABA increased leaf area (LA) compared with control plants grown in the field for leaves 2 and 3 (from the apex). GA_3 consistently diminished LA, with the lamina narrowing towards the apical part (Fig. 1). Although both the total chlorophyll content and the carotenoid

Table 1. Average length of the main shoot and dry weight (DW) for 3 crop seasons of experiments with field-grown soybean plants at R5 Treatments: control, ABA-, and GA₃-treated plants. Values followed by the same letter are not significantly different at P < 0.05 by the Fisher α test

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	Control	ABA	GA ₃
Length (cm) DW aerial part (g)	45.4a 4.66a	47.0a 7.62b	80.0b 5.68a

content increased in ABA-treated plants, only chlorophyll levels were significantly different (Table 3). Leaves also remained green longer in ABA-treated plants (data not shown). In ABA-treated plants, there was a significant reduction in leaf conductance 24 h after the hormone had been applied, but then the conductance values started to rise and equalled those of the control 11 days later (Fig. 2). Also, ABA-treated plants showed lower average foliar temperature (~1°C). Carbohydrate content in shoots was significantly greater at flowering in the ABA-treated plants, but this difference disappeared at harvest (Fig. 3). Therefore, an increased carbohydrate remobilisation (21%) in the ABAtreated plants from the shoot to the seeds could be estimated. Although there were no significant differences in number of pods, in GA₃-treated plants the number of seeds per pod was significantly lower than in control and ABA-treated ones (Table 4). When the pod size was monitored during plant ontogeny, it was observed that young pods were bigger in ABA-treated plants. Such differences disappeared as pods



Fig. 1. Average leaf area (LA) at different plant levels for 3 crop season of experiments with field-grown soybean plants at R5. Treatments: control, ABA- and GA₃-treated plants. Bars indicate s.e.m. of 30 plants. Columns with the same letter are not significantly different at P < 0.05 by the Fisher α test.

Table 2. Dry weight (DW) of roots and nodule dry weight per plantof soybean plants grown in plastic pots (24 L) for 80 days during the2006-07 crop season

Treatments: control, ABA-, and GA₃-treated plants. Values followed by the same letter are not significantly different at P < 0.05 by the Fisher α test

	Control	ABA	GA ₃
Root DW (g)	1.74a	2.82b	2.46ab
Nodule DW (mg)	17.05ab	20.96b	13.45a

Table 3. Average of total chlorophyll and carotene fresh weight (FW) content for 3 crop seasons of experiments with field-grown soybean plants at R5

Treatments: control, ABA-, and GA₃-treated plants. Values followed by the same letter are not significantly different at P < 0.05 by the Fisher α test

	Control	ABA	GA3
Chlorophyll (µg/g FW)	433.28a	510.25b	407.26a
Carotene (µg/g FW)	0.67ab	0.73b	0.59a



Fig. 2. Average values of leaf conductance (*sg*) in the 3rd fully expanded leaf (1 leaf per plant, 15 plants per treatment) at 01:00 p.m. for 3 crop seasons of experiments with control and ABA-treated field-grown soybean plants from 24 h to 25 days after the first spraying. Bars indicate s.e.m. of the 15 plants.



Fig. 3. Carbohydrate content of shoots at flowering and harvest for 3 crop seasons of experiments with field-grown soybean plants assessed at harvest. Treatments: control, ABA-, and GA₃-treated plants. Bars indicate s.e.m. of 15 plants. Columns with the same letter are not significantly different at P < 0.05 by the Fisher α test.

Table 4. Number of seeds per pod, seed weight per meter, and protein and oil percentage in seed for 3 crop seasons of experiments with field-grown soybean plants assessed at harvest

Treatments: control, ABA-, and GA₃-treated plants. Values followed by the same letter are not significantly different at P < 0.05 by the Fisher α test

	Control	ABA	GA ₃
Seeds/pod	2.03b	2.13b	1.67a
Seed wt (g/m)	92.77a	101.08b	91.88a
Protein (%)	40.35b	39.95b	39.3a
Oil (%)	21.45a	21.88b	21.90b

developed (data not shown). Finally, the seed weight/m increased significantly in ABA-treated plants (Table 4), which in turn increased seed yield. Moreover, protein content was the same but oil concentration was greater in the ABA treatments, while GA_3 spraying also increased oil concentration, but seed protein was substantially lower (Table 4).

Discussion

The results presented here suggest that ABA sprayed on leaves increased total biomass, including seed yield, of soybean plants grown in field conditions. Abscisic acid enhanced the number of lateral roots and therefore density of the root system, which has been found in other species (Trewavas and Jones 1991). More lateral roots carried more nodules, especially compared with GA₃-treated plants. These results are in agreement with those reporting more biomass and more nodules in well irrigated soybean plants (Racca et al. 1980), especially when they were treated with inhibitors of ABA and GA biosynthesis (Zhang et al. 2004). Phillips (1971) reported, however, that by treating soybean plants with an inhibitor of ABA synthesis, the number of nodules per lateral root was increased. Working with hypernodulant soybean mutants, Cho and Harper (1993) pointed out that exogenous ABA lowered the number and dry weight of nodules. It is worth noting that the diverse results reported may be the consequence of different approaches, i.e. assessing nodules per lateral root v. nodules per plant, different varieties, and/or different times, and/or doses.

For the cultivar and the dose used, no restriction of shoot growth was observed as had been previously reported (Sloger and Caldwell 1970), which may be related to enhanced carbon allocation in shoots, as we had previously found in wheat (Travaglia et al. 2007). As expected, GA3 stimulated shoot length. The ABA-treated plants also showed enhanced LA. which may increase light interception (Andrade and Calviño 2004). An opposite effect was observed in GA3-treated plants. It has been extensively reported in the literature that ABA restricts photosynthesis in various plant species grown under controlled conditions (Daie and Campbell 1981; Xu et al. 1995; Gong et al. 1998; Wilkinson and Davies 2002; Reddy et al. 2004; Liu et al. 2005). Conversely, our results showed that increases in dry matter content were correlated with increases in chlorophylls. According to Ivanov et al. (1995), exogenous ABA protected the photosystem II complex of barley plants subjected to high irradiance at 5°C for 7 days. In the present results, even though the plants were not subjected to prolonged water stress (some midday stress may be envisaged because of the unbalance between transpiration and water absorption), the ABA-treated plants showed greater levels of chlorophylls and the leaves persisted for long periods of time. The implication is that ABA protected the photosynthetic apparatus and consequently, more dry matter was produced and accumulated. In contrast, GA₃ did not modify the concentration of photosynthetic pigments, although there are reports of paclobutrazol (inhibitor of GA biosynthesis) treatments enhancing the chlorophyll content in soybean (Sankhla et al. 1985).

The increases in stomatal conductance found in ABA-treated plants 6 days after the hormone was applied were rather unexpected since it has been extensively demonstrated that exogenous ABA promotes stomatal closure (Zhang and Outlaw 2001, and references included therein). However, we had previously found in field-cultured wheat that ABA-treated plants closed their stomata immediately after the application, but then, during maximum irradiance, 'half-opened' stomata predominated (Travaglia 2008). A similar stomatal response has been observed in ABA-treated *Ilex paraguariensis* plants (Sansberro *et al.* 2004). That is, partial closure of stomata might initially allow carbon dioxide entry into photosynthetic cells while water losses are minimal, but in the mid-term it keeps water evaporation more constant in a way that the leaf temperature can be lower than in non-treated plants.

Abscisic acid application increased soybean yield by enhancing carbon allocation and partitioning to the seeds, which is in agreement with results found in rice (Zhang *et al.* 1998; Yang *et al.* 2001, 2004) and our own previous results in wheat (Travaglia *et al.* 2007). Seed quality in soybean is related to protein and oil content. Some authors suggest that increased yield may affect quality, especially oil content (Stark 1924; Yin and Vyn 2005), while others state that protein levels are affected (Shannon *et al.* 1972; Burton 1984; Yin and Vyn 2005). By contrast, our results indicate that ABA not only increased yield but also improved the seed quality since it did not affect protein levels but enhanced oil yield. Gibberellin A₃ increased oil concentration, but diminished seed proteins.

In conclusion, the results obtained in the experiments with soybean grown in field conditions support the idea that ABA enhances yield by a combination of factors. That is, ABA increased the number of lateral roots and therefore density of the root system; improved carbon allocation in shoots; increased dry matter content in correlation with increases in total chlorophyll; kept the stomatal conductance more stable over time, alleviating water stress in plant tissues (edaphic or temporary); and enhanced carbon allocation and partitioning to the seeds, which contained more oil.

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