Foliar application of phytohormones enhances growth of maize and soybean seedlings

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RESUMEN

Las fitohormonas tales como giberelinas, auxinas y citoquininas son compuestos promotores del crecimiento que, adicionados a fertilizantes foliares, podrían contribuir a mejorar el crecimiento y desarrollo de la planta. Este trabajo fue realizado para evaluar los efectos de la aplicación de fitohormonas exógenas, tanto solas como en mezclas, en leguminosas y gramíneas creciendo en cámaras con condiciones controladas de humedad, temperatura y ciclos de luz/oscuridad. La aplicación de fitohormonas ocasionó una considerable mejora en el crecimiento de plantas de soja y maíz. El formulado a base de la mezcla de fitohormonas, cada una en la concentración mínima requerida para lograr un efecto cuantificable, permitió mejorar significativamente las variables de crecimiento consideradas de importancia para aumentar la productividad. Así, la adición de esta mezcla de fitohormonas a productos comerciales utilizados como fertilizantes para aplicaciones foliares podría mejorar el crecimiento y rendimiento en leguminosas y gramíneas.

Palabras clave: fertilizante foliar, maíz, fitohormonas, crecimiento vegetal, soja.

ABSTRACT

Phytohormones such as gibberellins, auxins and cytokinins are plant growth promoting factors which added to foliar fertilizers can modulate plant growth and development of agricultural species. This work was performed to study the effects of exogenously applied phytohormones both alone and in mixtures, on a legume and a cereal growing in chambers with controlled conditions of humidity, temperature and light/dark cycle. It was found that application of phytohormones resulted in a considerable increase in growth of soybean and maize plants. The mixture of phytohormones formulated with the lowest concentration of each required to enhance plant growth, allowed a significant improvement on several growth parameters involved in productivity. Thus, the addition of this mixture to commercial products as foliar fertilizers may render potential improvement of legume and cereal yields.

Keywords: *foliar fertilizer, maize, phytohormones, plant growth, soybean.*

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INTRODUCTION

The central zone of Argentine is a wide plain of fertile soils with a relatively short farming history (Restovich *et al*., 2012). Until the early 1980s, crop production increased through expansion on uncultivated lands, but during the last three decades additional increases were achieved through more intensive use of external inputs, technology and management (Tomei and Upham, 2009). Agricultural intensification advanced via warm-season crops, specially soybean and maize. Nowadays, soybean production accounts for more than 50% of the area cultivated with grains, concentrated in the provinces of Santa Fe, Buenos Aires, Entre Rios and Córdoba and Argentina is ranked as the third country in maize production (FAO, 2016).

The need to feed a growing global population exerts a constant pressure on crop production. One of the techniques available to increase crop production is foliar fertilization, used to complement soil supply or to correct deficiencies during crop development (Chen, 2006). The addition of plant growth regulators ("hormones") to foliar commercial fertilizer has been available for a number of years, and recently there has been a renewed interest. Plant hormones contained in commercial foliar fertilizers are gibberellins, auxins such as indole butyric acid (IBA) and cytokinins such as benzylamino purine (BAP). Gibberellic acid (GA3) is the gibberellin most used to promote cell elongation and seed germination. Several studies have confirmed the GAs role for plant growth and development (Depuydt *et al*., 2016). Auxins and cytokinins are similarly involved in plant development. The effects of each phytohormone may depend on the concentration or activity of another, which is known as a cross signaling (cross-talk) mechanism (Jones *et al*., 2012). Thus, the mixture of different hormones applied as foliar fertilizers may cause detrimental or beneficial effects to the plant. Rukasz and Michalek, (2004) showed that the different plant hormones such as GAs auxins and cytokinins elicited responses such as tilling enhancement and increased developmental rate and in many cases the responses are dependent on the cultivars under study. In addition, the application of phytohormones to foliar fertilizers has the potential to complement such formulations, triggering signals cascades that allow a better availability of these nutrients and their translocation to demanding sites. However, knowledge about the appropriate doses of each compound and/or hormonal mixtures to be applied for optimum growth used in foliar fertilizer is not clear. Indeed, it is also difficult to know how a plant might respond to phytohormones applied used as foliar fertilizer at early vegetative stages, since tissue sensitivity may vary among species and limits between benefit or injury are strictly dose–dependent. Thus, the aim of this study was to help developing a foliar formulation based on the lowest concentration of phytohormones necessary to enhance growth in soybean and maize plants.

MATERIALS AND METHODS

Seeds of *Glycine max* and *Zea mays* were sown in pots (300 cm^3) filled with a sterilized clay-loam substrate (200 °C for 24 h). Six seeds of each species were sown per pot, and pots were placed in chambers with controlled conditions of humidity, temperature and cycle of light/dark (16 h light of 200 µmol m^{-2} sec^{-1} at 28 °C/ 8 h dark at 20 °C, 80% RH). Six pots, three for each species, were used as subsamples for each treatment, irrigated with nutrient solution (Hoagland 25%) throughout the growing season. Each treatment was replicated 4 times in a randomized design.

Two applications were performed by foliar spray to each plant at 15 and 22 days after sowing; physiological growth parameters were measured 72 h after the second application. Two different concentrations of each hormone were applied: GA3 at a concentration of 5 (GA+) and 0.5 mg/l (GA); IBA (indolebutyric acid) also at 5 (IBA+) and 0.5 mg/l (IBA), FAP (furfurilamino purine) at 900 ng/l(FAP+) and 90 ng/l (FAP), and BAP (benzylamino purine) at 900 (BAP+) and 90 ng/l (BAP). Plants were sprayed with distilled water (DW) as a negative control. A commercial product (CP) with a similar composition was used for comparison: it contained 5 mg/l of GA3 and 9 mg/l of FAP and BAP (Stoller, Córdoba, Argentina).

A mixture of all the hormone solutions was obtained by using the lowest concentrations employed in the individual treatments (M), i.e. 0.5 mg/l of GA3 and IBA and 90 ng/l of BAP and FAP. Hormones were obtained from Sigma Chemicals (St. Louis, USA).

Shoot height, root length, first internode length and leaf area were evaluated 72 h after the second application in both species. Roots and shoots from control and inoculated plants were separated and their fresh weight (FW) recorded. Samples were dried in an oven at 70 ºC until constant dry weight (DW) was obtained. FW and DW were expressed as grams of shoot or root per plant (g plant^{-1}). Dry matter content (%) was calculated as DW/FW *100 for root and shoot per each plant.

Content of total chlorophyll in leaves of each species was assessed using a chlorophylometer (Hansatech CL-O1, Hansatech Instruments Ltd, Norfolk, UK) and stomatal conductance with a leaf porometer (Decagon Devices, Pullman, WA, USA) in response to different treatments: hormones at each dosage, negative control (distilled water or DW), positive control (commercial product or CP) and the mixture of hormones (M).

Data were statistically analyzed using InfoStat (InfoStat software version 2016, Universidad Nacional de Córdoba, Córdoba, Argentina). Significant effects of hormonal treatments on plant growth and biomass production were assessed by analyses of variance (ANOVA) as a completely randomized design. Significant differences among treatments were calculated by the use of pair-wise comparisons with Duncan's test ($p<0.05$).

RESULTS

No significant differences were observed on shoot height in maize plants sprayed with the highest concentration of phytohormones in comparison with the negative controls. However, when maize plants were sprayed with a low concentration, the shoot height significantly increased reaching the values of positive controls (Figure 1). The concentrations of each hormone used in this study did not show statistically significant differences in the following parameters: root length, internode first length, and leaf area in comparison with the negative controls (data not shown). Figure 1 also shows that the different treatments with phytohormones were effective on shoot height promotion in soybean plants in comparison with the negative controls.

Regarding soybean, and independently of the GA concentration used, an increase on shoot height was observed as compared to negative controls (distilled water). On the contrary, only the lowest concentration of IBA, FAP and BAP sprayed had a significant increase on the shoot height as compared to negative controls. Soybean root length, internode first length, and leaf area were not modified with

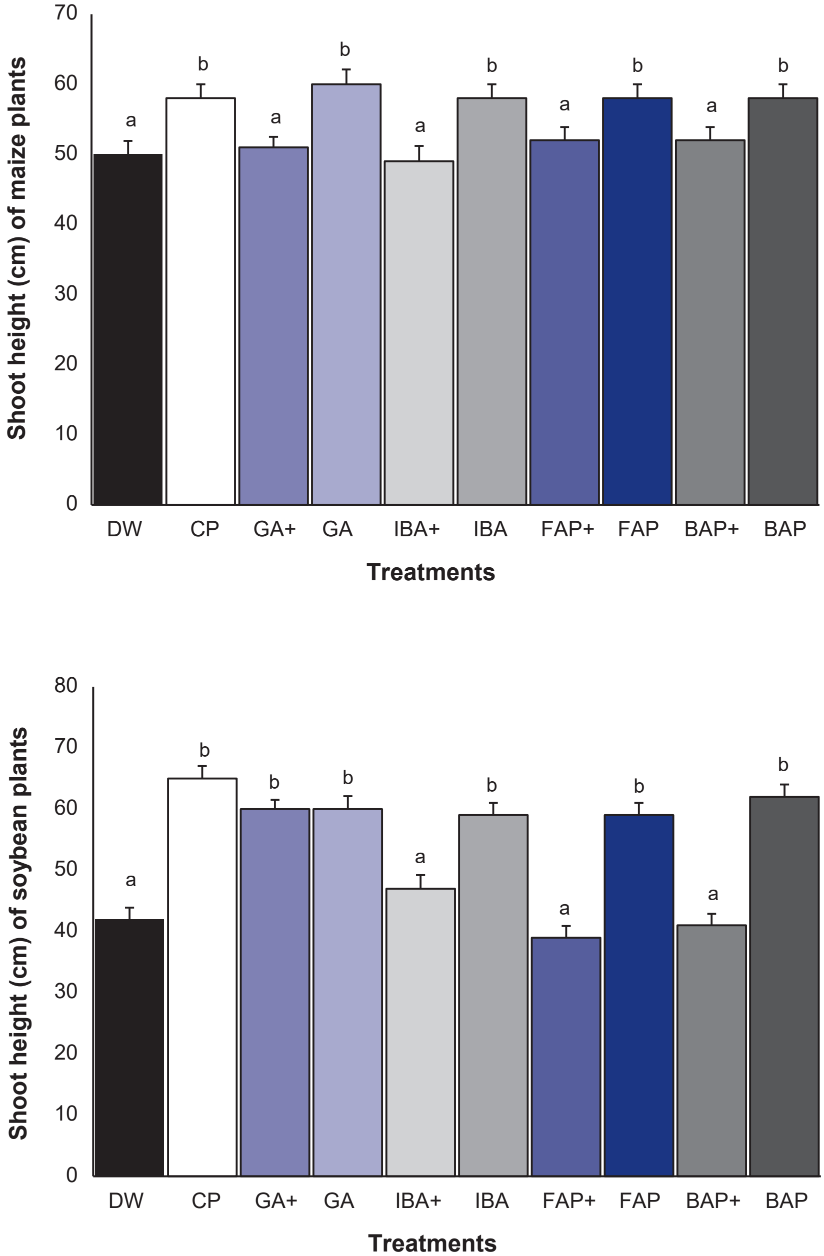

Figure 1. Effect of different phytohormones on shoot height in maize and soybean seedlings. DW (negative control, distilled water), CP (positive control, commercial product), GA+ (GA3: 5 mg/l), GA (GA3: 0.5 mg / l), IBA+ (IBA: 5 mg/l), IBA (IBA: 0.5 mg/l), FAP+ (FAP: 900 ng/l), FAP (FAP: 90 ng /l), BAP+ (BAP: 900 ng/l) and BAP (BAP: 90 ng /l). Data were from four replicated experiments (total n=30 plants per treatment), and represent means± S.E. Different letters above data indicate significant differences within species (P < 0.05).

respecto to neither concentrations of each hormone used in this study (data not shown).

The high GA concentration did not increase chlorophyll content in any of the two species as compared to negative controls, but the lowest concentration increased it, reaching the values obtained with positive controls (Table 1). Remarkably, the BAP and FAP treatments significantly increased the chlorophyll content as compared to positive controls. Stomatal conductance in both maize and soybean plants was not modified by treatments (data not shown).

As shown in Figure 2, the mixture obtained with the lowest concentrations of hormones promoted shoot growth in both species. The values obtained with the mixture exceed those registered with positive controls.

A significant increase in dry matter content in response to the mixture of phytohormones was observed for soybean roots (Figure 3). Instead, shoot dry matter content increased in both species respective to negative controls and soybean plants also showed a significant increment as compared with positive controls. The general aspect of soybean plants treated with the mixture of phytohormones is shown in Figure 4.

DISCUSSION

Phytohormones cause physiological and morphological changes that are dependent on their concentrations, tissue sensitivity and species involved. Results of this study demonstrated that foliar application of phytohormones can enhance growth in soybean and maize. Plant growth regulators can stimulate, inhibit, or otherwise alter plants' physiological processes depending on concentration (Santner and Estelle, 2009). In this study, the foliar application of phytohormones had different effects depending of their concentration, being the lowest concentration which provoked height enhancement. Furthermore, root growth of both, soybean and maize, was not affected by foliar applications of hormones whereas shoot growth was positively affected. The observed increases in shoot growth were paralleled by an increase in dry matter, suggesting a change in tissue anatomy that would make shoots as physically resistant and perhaps more tolerant to both biotic and abiotic stress. BAP and FAP treatments significantly increased the chlorophyll content as compared to positive controls, independently of the concentration sprayed. This response may be associated to the effect of cytokinins on primary metabolism. It has been shown that a useful effect of cytokinins spraying is the delay of leaf yellowing due to a postponement of leaf senescence. Therefore, this phytohormone has potential uses such as to increase crop productivity, to prolong post-harvest storage, and to increase stress tolerance (Hirayama and Shinozaki, 2010; Iqbal and Ashraf, 2013).

The addition of inorganic nutrients to complement the hormone mixture could help to achieve better availability

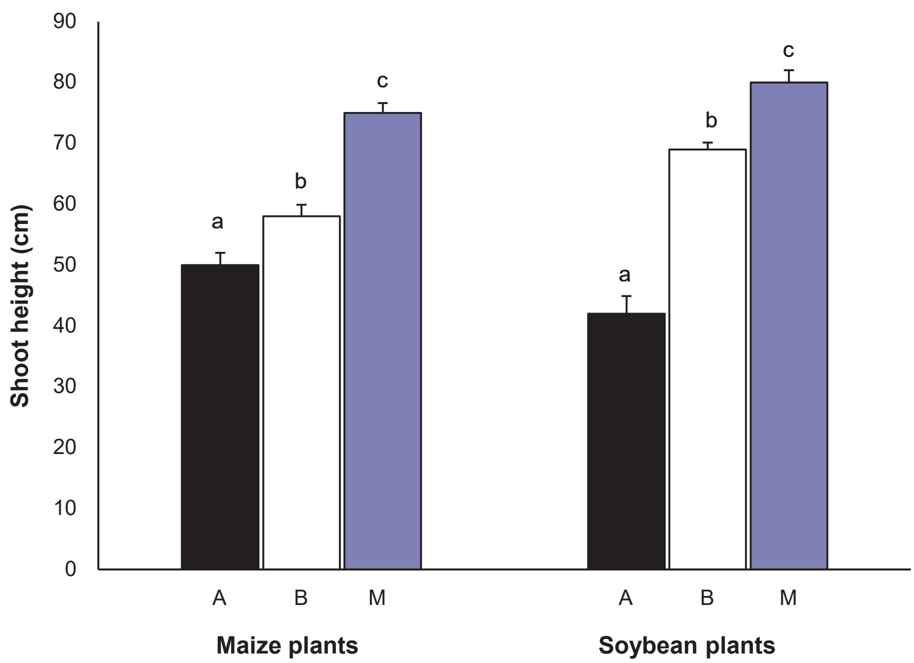

Figure 2. Effect of different treatments on shoot height in maize and soybean plants. WD: negative control (distilled water), CP: positive control (commercial product) and M: mixture of GA3, IBA, BAP and FAP in low concentrations. Bars represent means ± S.E. Different letters above data indicate significant differences within species ($P < 0.05$).

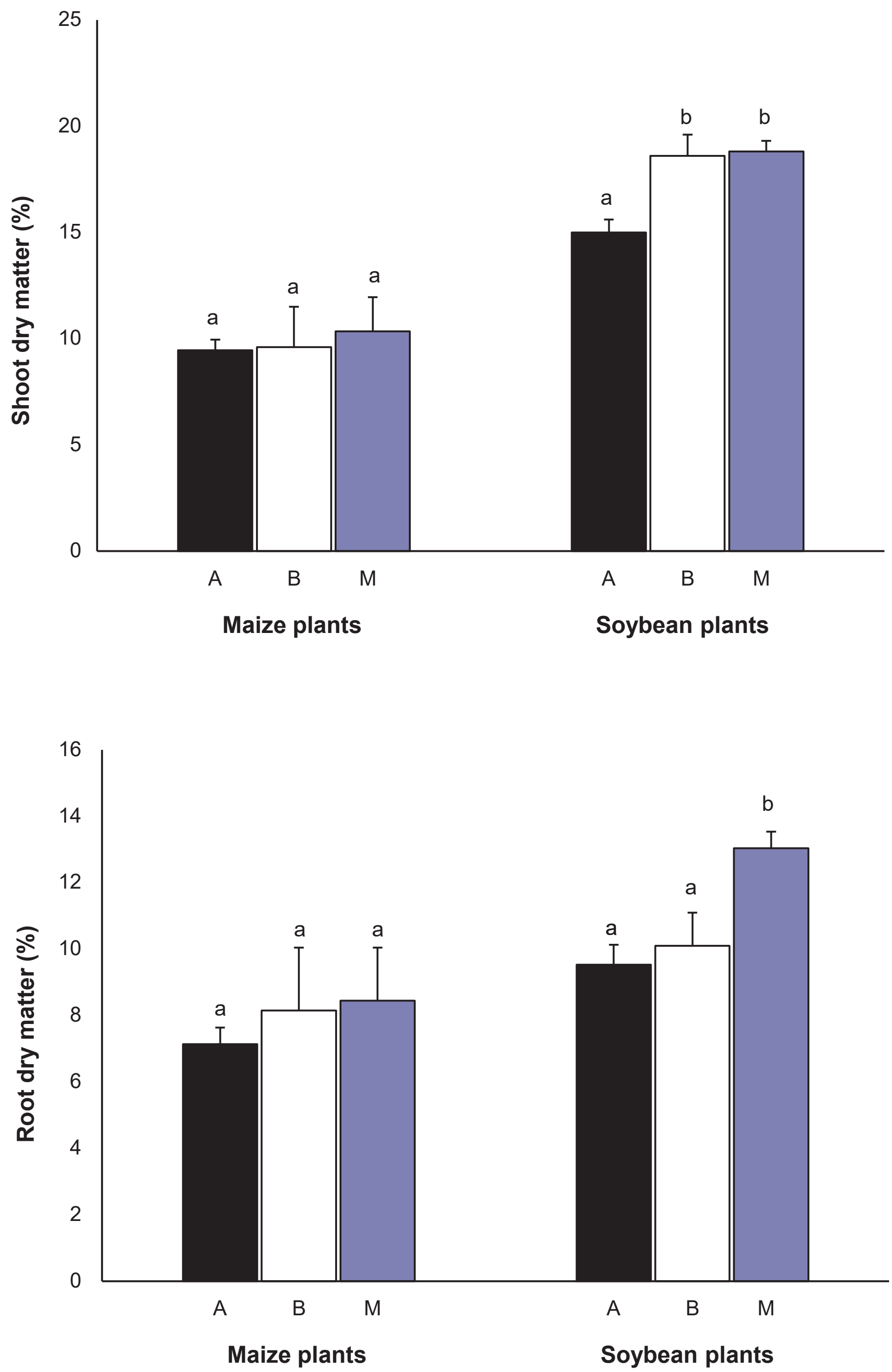

Figure 3. Shoot and root dry matter content in maize and soybean plants. Treatments: DW: negative control (distilled water), CP: positive control (commercial product) and M: mixture of selected phytohormones. Bars represent means ± S.E. Different letters above data indicate significant differences within species ($P < 0.05$).

of nutrients favoring rapid absorption and accelerating its translocation to the sites of increased requirement. Such a fertilizer would improve plant growth by triggering specific physiological processes, increasing plant size and performance (Beaton and Nelson, 2005). Our results suggest that the formulation found to be more effective in this work could be applied to other economically important species to increase productivity. Further studies with various horticultural species should be conducted to test this proposal. Also, the time of application within the plant life cycles, as well as responses of different cultivars are variables to be analyzed.

CONCLUSIONS

The present work was carried out to obtain a formulation based on the lowest concentration of phytohormones necessary to enhance plant growth, to be employed in foliar application. The balanced formulation applied significantly improved physiological parameters of plants that are important to enhance productivity. Thus, it would be expected that this type of fertilization could help ensuring plant establishment under field conditions, and even perhaps an increased productivity in terms of number of seeds, physiological maturity and seed filling. Finally, the use of this formulation for commercial products as foliar fertilizers provides a potential tool for legumes and grasses productivity improvement with a more efficient use of the product itself, saving money and reducing potential environmental impacts thanks to fewer losses.

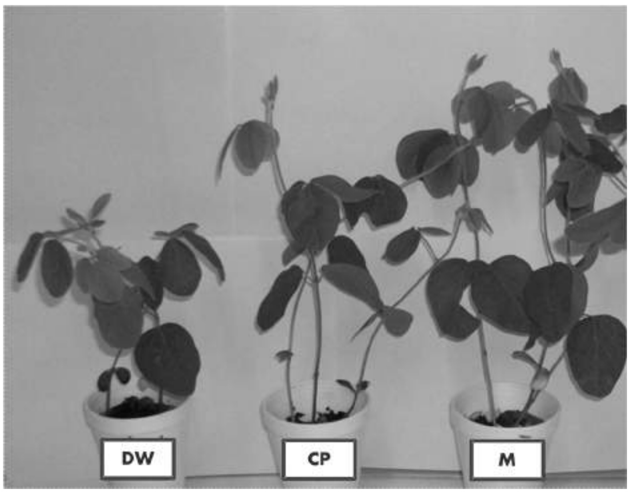

Figure 4. Effect of mixture of selected hormones on growth in soybean plants**.** DW: negative control (distilled water), CP: positive control (commercial product) and M: mixture of low-concentration selected phytohormones.

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Treatments	Total chlorophyll (CU) of maize plants	Total chlorophyll (CU) of soybean plants
DW	6.34 a	9.03 b
CP	8.32 b	9.13 b
GA+	6.09 a	8.7 b
GA	8.9 b	10.2 b
IBA+	11.4 c	10.4 b
IBA	11.5 c	10.5 b
FAP+	11.3 c	11.4 c
FAP	11.5 c	11.9 c
BAP+	11.4 c	10.7 b
BAP	11 c	11.4 c

Table 1. Chlorophyll content in maize and soybean plants.
Different letters above data indicate significant differences within species ($P < 0.05$).