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Scientific Note

In vitro development of sugarcane seedlings using ethephon or gibberellin

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Abstract

The use of plant growth regulators is directly related to the success of *in vitro* propagation, which is an advantageous alternative to obtain seedlings on a commercial scale. This study aimed to evaluate the *in vitro* development of 'IAC 95-5000' sugarcane seedlings after the addition of different doses of ethephon (0, 25, 50, 100 and 200 mg L^{-1}) or gibberellic acid (0, 2.5, 5.0, 7.5 and 10.0 mg L^{-1}) to the culture medium. Ethephon increased the number of tillers (up to 231.70%), reduced height of the main tiller (44.66 to 60.47%), and did not affect the shoot´s fresh and dry mass. On the other hand, gibberellin decreased the number of tillers and negatively changed biomass partitioning. It is concluded that the use of ethephon is a potential strategy to enhance *in vitro* production of 'IAC 95-5000' sugarcane seedlings, since it increased the number of usable shoots in subsequent subcultures, and its effects on height reduction can be reversible. However, the use of the tested doses of gibberellic acid is not recommended, because it impaired seedling development of this sugarcane variety.

Keywords: ethylene, gibberellic acid, tissue culture, *Saccharum* spp

Sugarcane is the most efficient bioenergetic crop in tropical and subtropical regions, being also the major source (80%) of sugar in the world (Waclawovsky et al., 2010; Smiullah et al., 2013). Furthermore, it is used for animal feeding, production of acetic acid, butanol and biopolymers, as well as a potential source of cellulose for textile fiber (Garcia et al., 2007; Costa et al., 2013). Due to its wide use and economic relevance, high investments are been applied in sugarcane breeding and seedling production; for this purpose, several biotechnological tools were employed (Snyman et al., 2011; Smiullah et al., 2013; Ramiro et al., 2016). The *in vitro* tissue culture stands out among these tools, since it allows large-scale production of uniform and disease-free sugarcane seedlings (Begum et al., 2011; Snyman et al., 2011; Smiullah et al., 2013).

The success of *in vitro* culture depends on multiple factors, such as plant varieties, type of tissue explant, culture conditions, type and composition of medium, and use of plant growth regulators (Garcia et al., 2007; Begum et al., 2011; Snyman et al., 2011; Smiullah et al., 2013; Maluta et al., 2013; Hajari et al., 2015). There are several studies about the influence of plant growth regulators on '*in vitro*' culture; however,

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ethylene and gibberellin effects on sugarcane development are scarce. Ethylene is a gaseous plant hormone that has been related to the fruit ripening, senescence, flowering, and in several processes involving in the perception and signaling of external stimulus (Tsuchisaka et al., 2009; Davies, 2010; Taiz & Zeiger, 2013). Under *in vitro* conditions, ethylene enhanced the regeneration efficiency, increased the shoots number and height, improved rooting and stimulated the tracheary element differentiation (Prameswara et al., 2009; Pesquet & Tuominem, 2011; Yasmin et al., 2014).

Regarding gibberellins, this plant hormone influences germination, seedling growth, stem elongation, leaf development, bud outgrowth, flower induction, and xylem expansion (Gabriele et al., 2010; Ikezaki et al., 2010; Mauriat et al., 2011; Nadeau et al., 2011; Zhao et al., 2011; Taiz & Zeiger, 2013). Application of gibberellin to the culture media inhibited adventitious root formation (Niu et al., 2013), but promoted shoot development and multiplication in *Lotus corniculatus* (Nikolic et al., 2010) and *Selaginella microphylla* (Jha et al., 2013). This study aimed to evaluate the effects of different doses of ethephon or gibberellic acid on *in vitro* development of sugarcane seedlings.

In vitro pre-established seedlings of sugarcane (*Saccharum spp.* variety IAC 95-5000) were selected and propagated into vials (350 mL) with 50 mL of solid MS medium (Murashige & Skoog, 1962) containing 30 g L^{-1} of sucrose. For seedling development, the flasks were kept for 20 days in a growth chamber at 25 ± 2°C and 16-hour light (30 mM cm^{-2}), 8-hour dark lighting photoperiod.

Seedlings with 4 cm-height and a single stem were selected from the ones that were cultivated in the flasks mentioned above. Then, seedlings were put for the second time in MS medium (Murashige & Skoog, 1962). At this stage, plant growth regulators were added to the medium. Ethephon (2-chloroethylphosphonic acid) at concentrations of 0, 25, 50, 100 and 200 mg L^{-1} and gibberellic acid (GA_3) at concentrations of 0, 2.5, 5.0, 7.5 and 10.0 mg L^{-1} were used. For comparison, the same control (without addition of plant growth regulators) was used as reference for both experiments (ethephon and gibberellin). After this step, the flasks were put back in the growth chamber.

On the 30th day after the addition of plant growth regulators to the culture medium, seedlings were collected to analyze the following parameters: number of tillers; height of the main tiller [measured from the shoot collar to the tip of the highest leaf (cm)]; shoot fresh and dry mass [stem and leaves (mg)]; and roots fresh and dry mass (mg). Furthermore, root: shoot ratio (both for the fresh and dry masses) was also calculated.

The experiment was arranged in a completely randomized design, with 5 treatments and 5 replicates, totaling 25 plots with five seedlings each. Obtained data were submitted to analysis of variance and regression (both at 5% error probability) using the SAS statistic software (SAS Institute, 2011). Significant regression equations (linear, quadratic or cubic) were used to express the variable behavior as a function of increased doses. To fit the statistical assumptions of the variance analysis, data of root: shoot fresh mass ratio (gibberellic acid trial), shoots dry mass (gibberellic acid trial) and height (ethephon trial) were transformed to $\log_{10}x$, $x^{-1.1}$, 1/x (respectively), as recommended by tool "Guided Data Analysis" of the SAS program (SAS Institute, 2011).

As observed in Figure 1, the addition of ethephon to the culture medium significantly increased the number of tillers in sugarcane seedlings (up to 231.70%). According Davies (1995), ethephon is a compound absorbed by plants and converted into ethylene inside cells, leading to the autocatalytic production of endogenous ethylene. The increased ethylene content in plant tissues can promote tillering by inhibiting basipetal transport of auxin; however, it can also decrease cell division (Davies, 1995), which can explain the reduction in main tiller height in seedlings (from 44.66 to 60.47%, Figure 1) (. Interestingly, this hormone can generate the same effect by decreasing the endogenous synthesis of gibberellin, which is a hormone responsible for cell elongation and division (Pearce et al., 1996).

The positive role of ethylene on sugarcane tillering was also reported by Mishra et al. (2014) by using contrasting approaches. These

authors applied both ethylene inhibitors (AVG, AOA and $CoCl_2$) and precursor (ethephon) in the culture media, being observed reductions and increments, respectively, in the number of tiller production. It seems that ethylene plays a key role in the success of *in vitro* culture of several species by improving shoot development. Application of ethylene in the culture media stimulated the tillering in rice (Yasmin et al., 2014) and *Ptilotus spp*. (Prameswara et al., 2009). On the other hand, there were reductions in the percentage of tomato plants with buds, when explants were grown under sub-optimum ethylene concentrations (Trujillo-Moya & Gisbert, 2012).

Ethephon exerted no effects on the total biomass of sugarcane shoots, even after the high increase in the number of tillers, probably due to the concurrent decrease in the height and increase in the thickness of the main tiller (Figure 2). The increased main tiller thickness may be triggered by increasing parenchymatous cell volume (Martins & Castro, 1999) and enhancing tracheary element differentiation (Pesquet & Tuominen, 2011) due to ethylene overproduction in explant tissues. Upon ethephon application, there were decreases in the root dry mass and changes in the root-shoot partitioning (Figure 1). Although ethylene can improve root formation (Prameswara et al., 2009) and development (Trujillo-Moya & Gisbert, 2012), its effects are dose and species-dependent. Upon explant exposure to 25 mg L-1 ethephon, there was little effect on the root dry mass, but a high increase in the number of tillers (Figure 1). However, the use of higher doses of ethephon than the one mentioned above caused negative effects on the root dry mass.

The use of gibberellin also triggered reductions in the root fresh mass of treated seedlings when compared to control (Figure 3), probably by inhibiting the initiation of lateral root primordia (Gou et al., 2010) and/ or by unbalancing the synthesis or transport of endogenous hormones (Nikolic et al., 2010). In rice seedlings, there were reductions in the adventitious root growth after application of gibberellin in the media (Lo et al., 2008).

Gibberellin-treated explants exhibited a more elongated (up to 34.70%) and thinner main tiller than the control explants (Figure 4). According to Davies (1995), gibberellin usually stimulates bud development due to increases in cell division and elongation, providing increments in internode length and, consequently, in plant height.

The stimulatory effect of gibberellin on plant height was also observed in pea explants treated with an anti-gibberellin compound (Ribalta et al., 2014). The internode length was increased by the decreasing concentration of this compound, which did not affected the number of nodes. However, gibberellin diminished the diameter of the main tiller, which also presented an abnormal growth (Figure 4). These results may be triggered by decreases in the radial volume

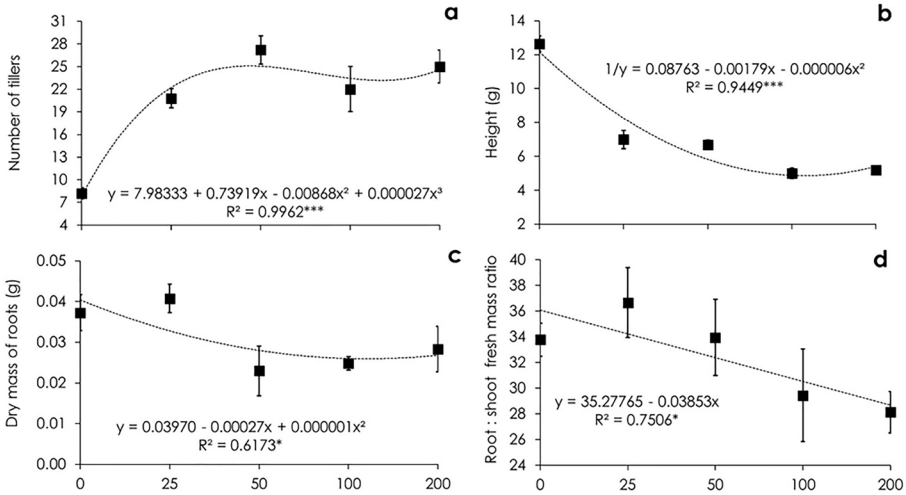

Figure 1. Effects of ethephon (mg L^{-1}) on the number of tillers (a); main tiller height (b); roots dry mass (c) and root: shoot fresh mass ratio (d) of sugarcane seedlings 'IAC 95-5000'. Bars: standard errors. * and ***: significant regression equations at 0.05 and 0.0001 of error probability, respectively.

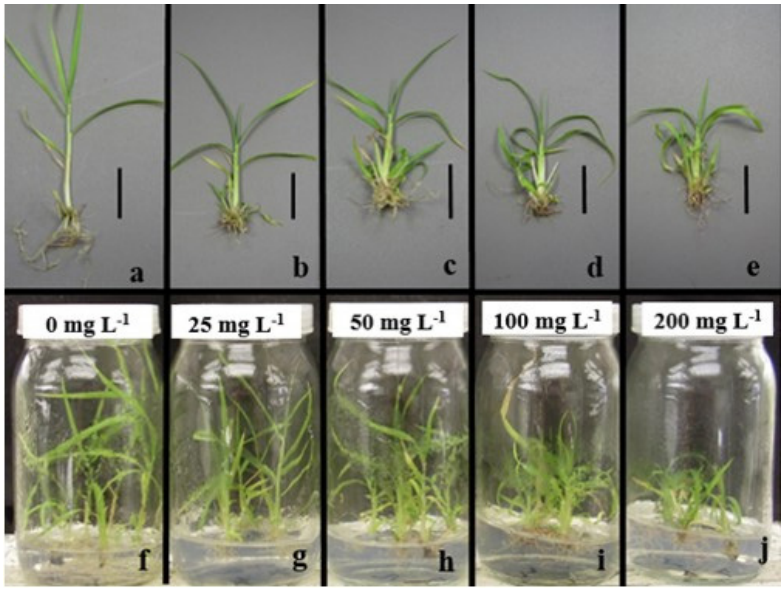

Figure 2. Morphophysiological changes caused by ethephon on 'IAC 95-5000' sugarcane seedlings, at 30 days after addition of this plant regulator to the culture medium. Scale bars: 2 cm.

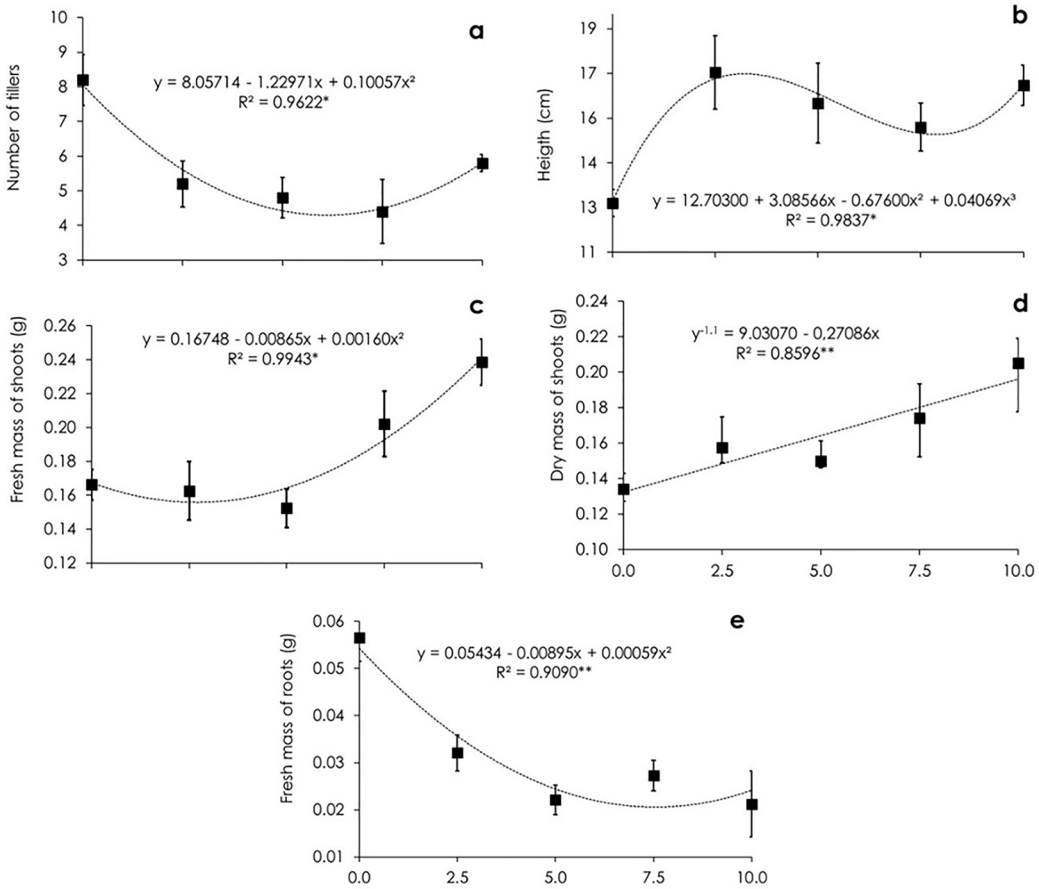

Figure 3. Gibberellic acid (mg L^{-1}) effects on the number of tillers (a); main tiller height (b); shoot fresh (c) and dry (d) mass and roots fresh (e) mass of sugarcane seedlings 'IAC 95-5000'. Bar: standard error. * and **: significant regression equations at 0.05 and 0.001 of error probability, respectively.

of parenchymatous cells, and reductions in the amount of sclerenchyma fibers in sugarcane (Martins & Castro, 1999). Since these fibers are responsible for plant support, the high lodging tendency of explants treated with gibberellic acid may be related to the reductions in the amount of sclerenchyma fibers. Application of gibberellin also inhibited the sugarcane tillering (approximately 48%, Figure 3) and changed the root: shoot ratios (Figure 5), probably by inhibition of the meristem initiation and/or by changing metabolism of several endogenous hormones (Gaspar et al., 1996).

It is concluded that addition of ethephon to the culture medium is a potential strategy to enhance *in vitro* production of 'IAC 95-5000' sugarcane, since it increases the number of usable shoots in subsequent subcultures. However, tested doses of gibberellin negatively affected the seedling development, because it impaired shoot and root growth.

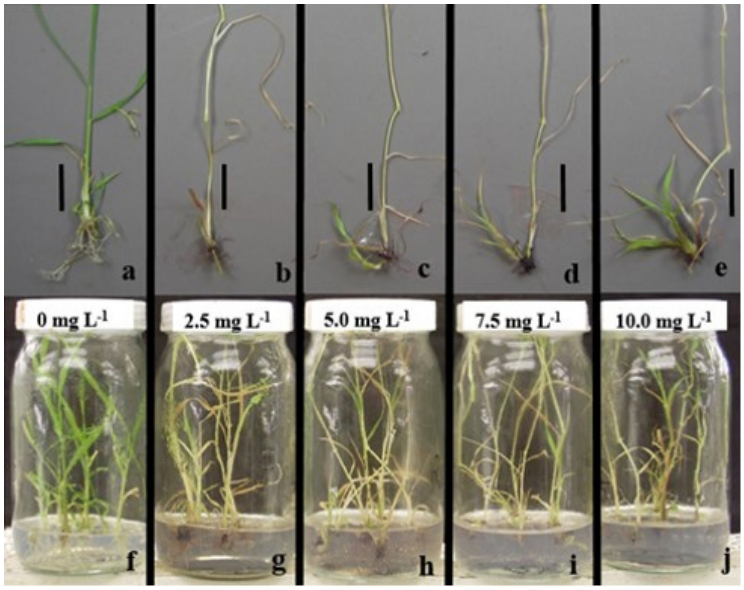

Figure 4.Morphophysiological changes caused by gibberelic acid on 'IAC 95-5000' sugarcane seedlings, at 30 days after addition of this plant growth regulator to the culture medium. Scale bars: 2 cm.

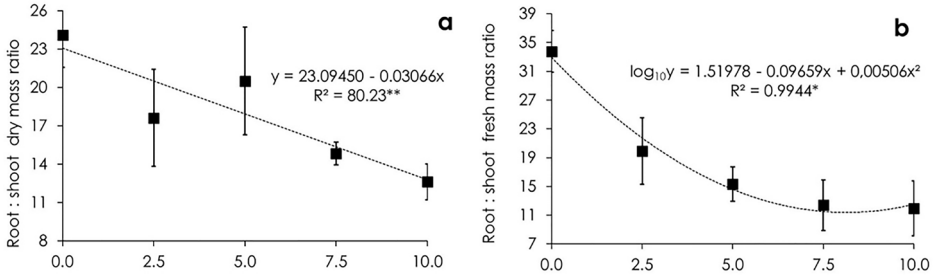

Figure 5. Gibberellic acid (mg L^{-1}) effects on root: shoot ratio based on dry (a) or fresh (b) mass of sugarcane seedlings 'IAC 95-5000'. Bar: standard error. * and **: significant regression equations at 0.05 and 0.001 of error probability, respectively.

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