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Regulation of the diosgenin expression in *Trigonella foenum-graecum* plants by different plant growth regulators

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Abstract

The effect of indole-3-acetic acid (10^{-4} and 10^{-5} M), gibberellic acid (10^{-4} and 10^{-5} M) and ethephon (50 ppm) as an ethylene releasing compound, on the diosgenin synthesis and/or accumulation process and their effects on the growth of *Trigonella foenum-graecum* plants were investigated. Treatment with 10^{-5} and 10^{-4} M gibberellic acid led to 43 and 19% increases, respectively, of diosgenin in 30-day-old whole plants. These increases might be associated with the action that this growth regulator has in stimulating plant growth and the biosynthetic pathway of this sapogenin. A smaller increase was obtained with the 10^{-5} M indole-3-acetic acid treatment (6%, in 30 day-old plants), probably due to a stimulation of the biosynthetic pathway, alone, since no effect on growth was observed. Treatment with 50 ppm ethephon increased the diosgenin levels observed in the leaves of 15- and 30-day-old plants, growth of the whole plant being substantially reduced at 30 days in comparison with the growth observed in control plants. These figures reflect a 77% increase in diosgenin levels in 15-day-old plants and a decrease of 68% in 30-day-old plants.

Keywords: Diosgenin; Dioscin; Ethephon; Gibberellic acid; Indole-3-acetic acid; Steroidal sapogenin

1. Introduction

It is known that the developmental processes in plants are regulated by the action and balance of the different groups of growth regulators, which may act as promotors or inhibitors of these processes. However, there is little available information concerning the possible involvement of these compounds in the secondary metabolism of plant: terpenes (Coggings, Scora, Lewis, & Knapp, 1969; García Puig et al., 1993; Ortuño et al., 1993; Ortuño, Oncina, Botía, & Del Rio, 1998; Wilson, Shaw, McDonald, Greany, & Yokohama, 1990), phenols (Berhow & Vandercook, 1992; Cho & Harper, 1993; Del Río et al., 1995; García Puig et al., 1995; Hinderer, Peterson, & Seitz, 1984; Shaw et al., 1991) and alkaloids (Cho, Kim, & Pedersen, 1988).

Among these secondary compounds, diosgenin, a steroidal sapogenin belonging to the group of triterpenes, is of great interest in the pharmaceutical industry since it has an estrogenic effect on the mammary gland (Aradhana, Rao, & Kale, 1992), plays an important role in the control of cholesterol metabolism (Cayen & Dvornik, 1979; Holland, Rahman, Morris, Coleman, & Billington, 1993; Marzolo & Nervi, 1989; Roman, Thewles, & Coleman, 1995; Sauvaire, Ribes, Baccou, & Loubatieeres Mariani, 1991), and produces changes in lipoxygenase activity in human erythroleukemia cells (Nappez, Liagre, & Beneytout, 1995). It also produces morphological and biochemical changes in megakaryocyte cells (Beneytout, Nappez, Leboutet, & Malinvaud, 1995).

Several plant material sources have been described for the isolation of diosgenin including *Dioscorea*, *Costus* and *Trigonella* (Cooke, 1970; Dasgupta & Pandey, 1970; Fazli, 1967; Mehta & Staba, 1970; Puri, Jefferies & Hardman, 1976; Taylor et al., 1997), with the last having the advantage of being an annual plant with a short crop cycle (Leung & Foster, 1996). However, very little is known about the process of diosgenin synthesis and its localization in this last plant material (Ortuño et al., 1998).

The possibility of modulation the processes of diosgenin synthesis and/or accumulation by the use of phytohormones has scarcely been described (Ortuño et al., 1998) and then not always is sufficient depth to characterize the nature of the changes produced by hormonal action and to establish the corresponding

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correlations between such changes and the expression of this secondary compound (Jain & Agrawal, 1988).

In the present investigation, we have analyzed the effect of indole-3-acetic acid (IAA), gibberellic acid (GA_3) and the ethepon as an ethylene-releasing compound, on diosgenin production and relate this effect with possible changes in *Trigonella foenum-graecum* plant developmental processes.

2. Materials and methods

2.1. Plant material, hormonal treatments and measurement of growth

Seeds of *Trigonella foenum-graecum* were supplied by Plantaforma, León (Spain). The seeds were soaked for 24 h in water (to obtain control plants) or in aqueous solution of IAA (10^{-4} and 10^{-5} M), GA_3 (10^{-4} and 10^{-5} M) and ethephon (50 ppm). After soaking, the seeds were germinated in sterile peat and maintained in a green-house. Seedlings were harvested 15 and 30 days after germination. To study the growth of control and treated plants, stem and root length, leaf surface and the fresh and dry weight of these organs, together with the corresponding data for the whole plants were analyzed at different times.

2.2. Isolation and measurement of diosgenin

For the isolation of dioscin and its subsequent hydrolysis to diosgenin, we followed the method proposed by Sauvaire and Baccou (1978), which was optimized for our work conditions (Ortuño et al., 1998). The analyses were performed with a Hewlett–Packard liquid chromotograph (model HP 1050) with a diode-array detector (range scanned: 190–500 nm). Reverse phase chromotographic separation was carried out on a μBondapak C_{18} (250 × 4.6 mm i.d.) analysis column. The particle size was 5 μm, and isocratic separation was performed using a mixture of acetonitrile:water (90:10;v/v) at a flow of 1 ml/min at 35°C. Changes in absorbance were recorded in the V/UV diode-array detector at 214 nm. This compound ($R_t = 12.27$ min) was quantified by HPLC in the chromatographic conditions describe above, and the response obtained was compared with the corresponding external standards. The identity of diosgenin was confirmed by reference to its mass spectrum (Hewlett–Packard Mass Spectometer model 5989), as described in our previous paper (Ortuño et al., 1998).

2.3. Chemicals

Diosgenin and gibberellic acid (GA_3) were purchased from Sigma (USA), indole-3-acetic acid (IAA) from Merck (FRG), ethephon [commercial Ethrel, 48% (2-chloroethyl)phosphonic acid] from Etisa (Spain).

3. Results and discussion

3.1. Effect of indole-3-acetic acid

The results concerning the effect of IAA on *Trigonella foenum-graecum* plant growth are depicted in Fig. 1, in which it can be seen that neither of the concentrations used (10^{-4} and 10^{-5} M) had much effect on this parameter. The data obtained at 15 and 30 days for leaf

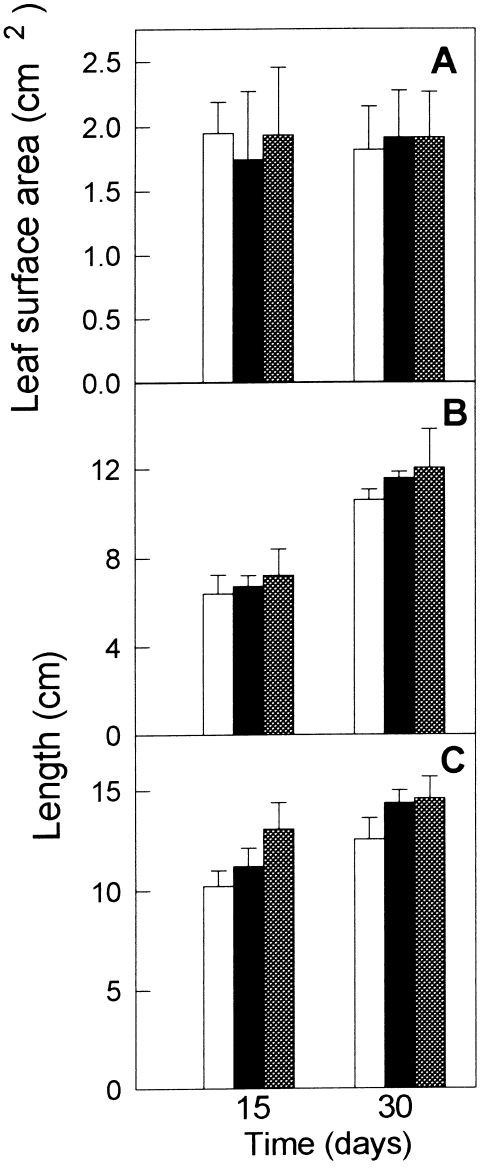

Fig. 1. Effect of IAA on *Trigonella foenum-graecum* plant growth. 15 and 30 days after treatment with 10^{-4} M (■) and 10^{-5} M (▩) IAA, the leaf surface area (A, cm^2), stem (B, cm) and root (C, cm) length were determined in control (□) and treated plants. Data represent the mean values ± SE ($n = 10$).

surface area (Fig. 1A), stem (Fig. 1B) and root (Fig. 1C) length, and the fresh and dry weights of the different organs and whole plant (data not shown) did not differ significantly from those obtained for the control plants.

Fig. 2 shows the results obtained when analysing the effect of IAA on the diosgenin levels observed in the different organs of this plant. As can be seen from measurements made 15 days after treatment with 10^{-5} M IAA, the concentration of diosgenin had risen 55% in leaves and 73% in stems (Fig. 2A and B). At 30 days it was still possible to observe the stimulatory effect of this treatment on leaf diosgenin levels, concentrations being about 6% higher than in the control plants of a corresponding age (see Fig. 2A).

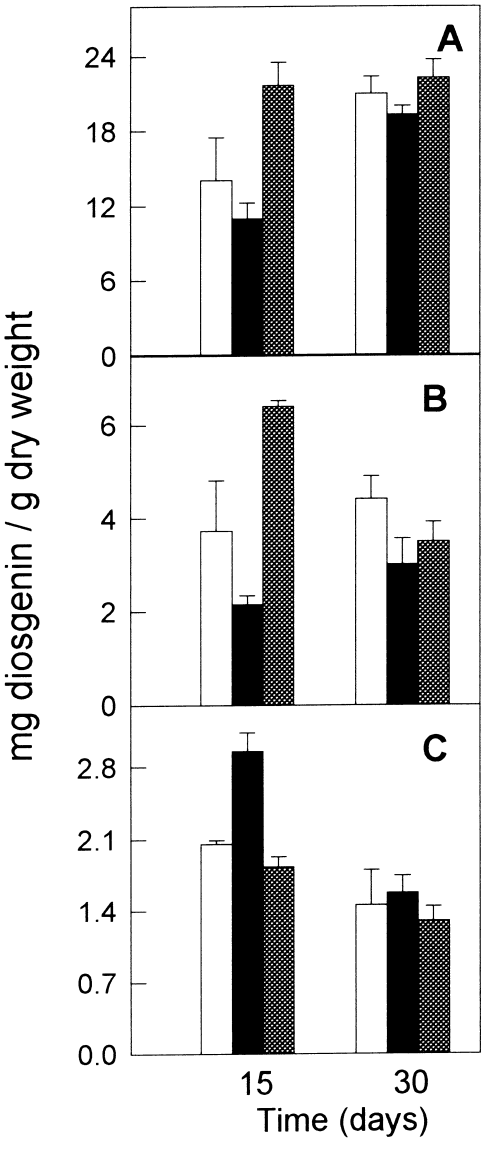

Fig. 2. Effect of IAA on diosgenin levels in plants of *Trigonella foenum-graecum*. Control (□) and treated (10^{-4} M, ■; 10^{-5} M ■) plants were analyzed 15 and 30 days after treatment. The diosgenin levels (mg/g dry weight) were determined in leaf (A), stem (B) and root (C). The experiments were carried out in triplicate and the vertical bars denote ±SE.

At whole plant level, the above-mentioned changes described in the processes of diosgenin synthesis and/or accumulation, caused by the 10^{-5} M treatment, result in an increase in diosgenin levels of 65 and 6% at 15 and 30 days, respectively (Table 1).

When we examine the effect of the 10^{-4} M IAA treatment, an increase of about 44% in the diosgenin levels in the roots of 15 day-old plants is observed (Fig. 2C). This may be put down to a temporary increase of this sapogenin in the root arising from its transport from leaves and stems, as suggested in previous studies (Ortuño et al., 1998), since an analysis of diosgenin levels in the whole plant points to no significant differences between those observed in the control plants at both ages and those observed in the plants treated with 10^{-4} M IAA (Table 1).

The results obtained for treatment with 10^{-5} M IAA support the idea that the stimulatory effect of IAA on the diosgenin synthesis and /or accumulation process in this plant material (Fig. 2, Table 1) may be due to the activation of certain steps of the biosynthetic pathway, since no effect on growth was observed (see Fig. 1).

3.2. Effect of gibberellic acid

Leaf growth in 15-day-old plants treated with GA_3 (10^{-4} M and 10^{-5} M) is similar to that observed in the control plants. However, an increase of around 18% was observed for this parameter in 30-day-old plants after both GA_3 treatments (Fig. 3A).

The greatest increase of growth was observed in the stem at 30 days (Fig. 3B): 24.5 and 40.5% for 10^{-4} M and 10^{-5} M GA_3, respectively. In roots (Fig. 3C), the effect of GA_3 on prolongation was evident at 15 and 30 days for both concentrations (10^{-4} and 10^{-5} M with increases of about 27 and 18%, respectively), although, in this organ the optimal concentration was 10^{-4} M as opposed to the 10^{-5} M which was optimal for the stem. The stimulatory effect of both concentrations on stem and root growth, and leaf area, also brings about an

Table 1
Diosgenin levels in whole plants of *Trigonella foenum-graecum*. The data represent mean values ± SE ($n = 3$) of the diosgenin (mg) in control and treated plants

	Diosgenin (mg/whole plant)	
Treatment	15-days-old	30-days-old
Control	0.26 ± 0.01	1.13 ± 0.2
IAA (10^{-4} M)	0.23 ± 0.02	1.11 ± 0.4
IAA (10^{-5} M)	0.43 ± 0.02	1.26 ± 0.2
GA_3 (10^{-4} M)	0.35 ± 0.03	1.35 ± 0.3
GA_3 (10^{-5} M)	0.35 ± 0.06	1.62 ± 0.4
Ethephon (50 ppm)	0.46 ± 0.01	0.36 ± 0.02

increase in the fresh and dry weight of the whole plant at 30 days (23 and 28%, respectively, data not shown).

As regards the effect of this phytohormone on diosgenin levels in *Trigonella*, the results point to an increase of 153 and 221% in the stem 15 days after treatment with 10^{-4} M and 10^{-5} M GA_3, respectively (Fig. 4B). At 30 days, of stimulatory effect of GA_3 was observed in leaves, with increases of 45% over the control being observed (Fig. 4A).

At whole plant level, diosgenin levels increased by 34% in 15-day-old plants after treatment by both GA_3 concentrations, and by 19 and 43% after treatment with 10^{-4} M and 10^{-5} M, respectively, in 30-day-old plants (Table 1).

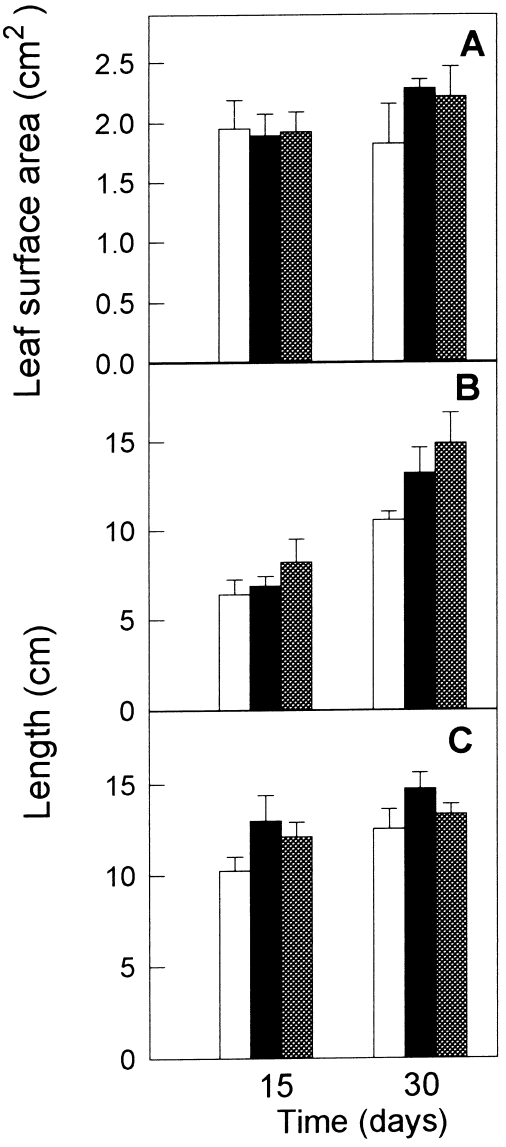

Fig. 3. Effect of GA3 on *Trigonella foenum-graecum* plant growth. 15 and 30 days after treatment with 10^{-4} M (■) and 10^{-5} M (▩) GA_3, the leaf surface area (A, cm^2), stem (B, cm) and root (C, cm) length were determined in control (□) and treated plants. Data represent the mean values ± SE ($n = 10$).

Based on the results obtained, we suggest that the increased levels of this sapogenin after treatment with GA_3 (Fig. 4, Table 1) might in part be due to the stimulatory effect of this phytoregulator on the growth of the plant (see Fig. 3), although we do not discount that GA_3 might also activate the diosgenin biosynthetic pathway. In support of this hypothesis, other authors have suggested that gibberellic acid might affect the activities of some of the enzymes involved in the biosynthetic pathway of other secondary metabolites (Hinderer et al., 1984).

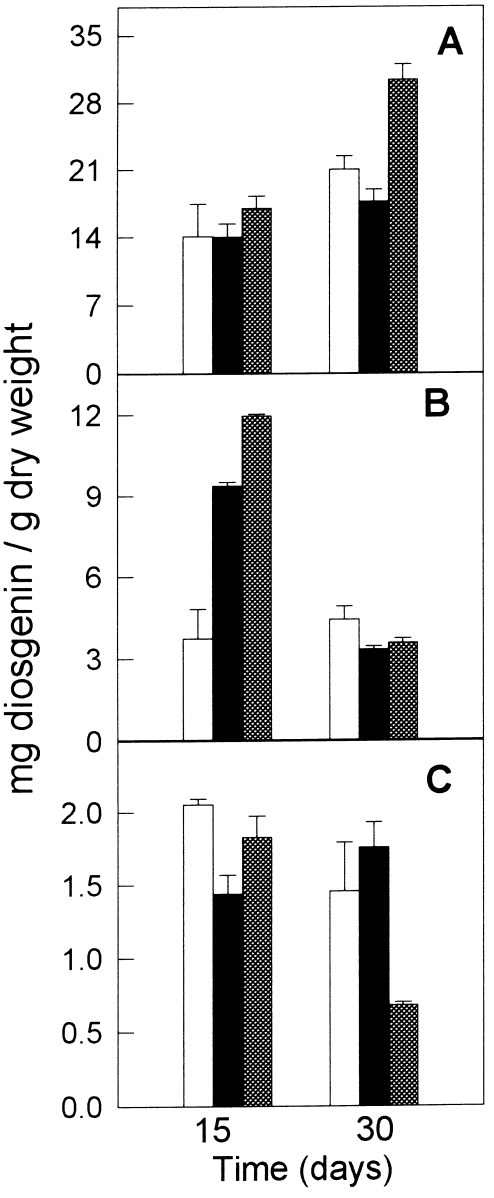

Fig. 4. Effect of GA_3 on diosgenin levels in plants of *Trigonella foenum-graecum*. Control (□) and treated (10^{-4} M, ■; 10^{-5} M ▩) plants were analyzed 15 and 30 days after treatment. The diosgenin levels (mg/g dry weight) were determined in leaf (A), stem (B) and root (C). The experiments were carried out in triplicate and the vertical bars denote ± SE.

The greater stimulatory effect of GA_3 on diosgenin expression in this plant material compared with that observed with IAA is interesting to note. Similar results were obtained by Jain and Agrawal (1988).

3.3. Effect of ethephon

When the growth of *Trigonella foenum-graecum* plants treated with 50 ppm Ethephon are analysed, an inhibitory effect of this hormone on the different organs is evident (Fig. 5). At 15 and 30 days, the leaf surface area and stem and root length are less in treated plants than in the control plants (Fig. 5A–C, respectively). Such effects lead to a reduction in the fresh weight of the whole plant at 15 days of 20% and in the dry weight of 29%. These figures are even more pronounced at 30 days when reductions of about 66 and 78%, respectively, were observed (data not shown).

The results concerning diosgenin levels in *Trigonella foenum-graecum* plants are depicted in Fig. 6. As can be seen, treatment with 50 ppm ethephon led to increased diosgenin levels in leaves of 141% at 15 days and 53% at 30 days, although the concentrations recorded in stems and roots remained below those recorded in the control plants at the same ages.

When diosgenin levels were analysed at the whole plant level, an increase of 77% was observed at 15 days (Table 1). This would mainly arise from stimulation of diosgenin synthesis and/or accumulation in the leaf

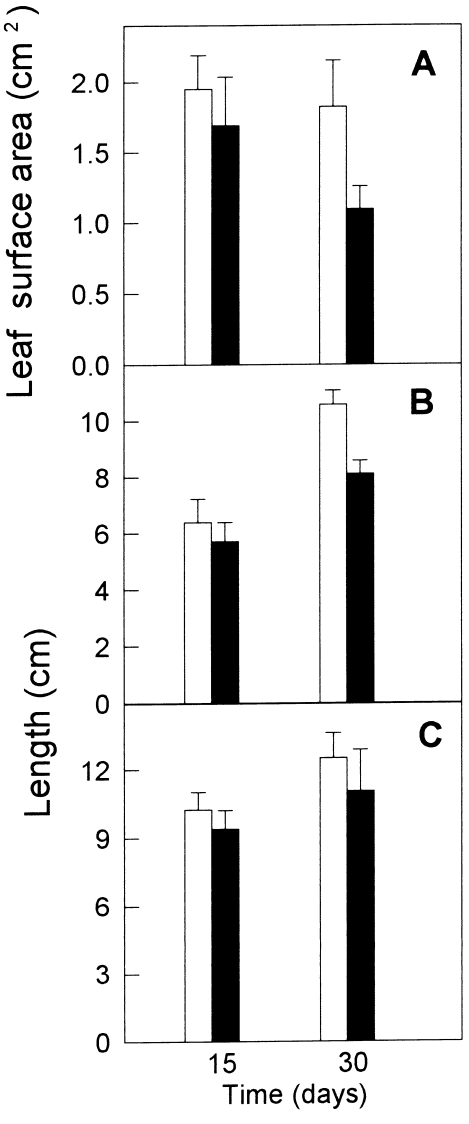

Fig. 5. Effect of ethephon on *Trigonella foenum-graecum* plant growth. 15 and 30 days after treatment with 50 ppm ethephon (■), the leaf surface area (A, cm^2), stem (B, cm) and root (C, cm) length were determined in control (□) and treated plants. Data represent the mean values ± SE ($n = 10$).

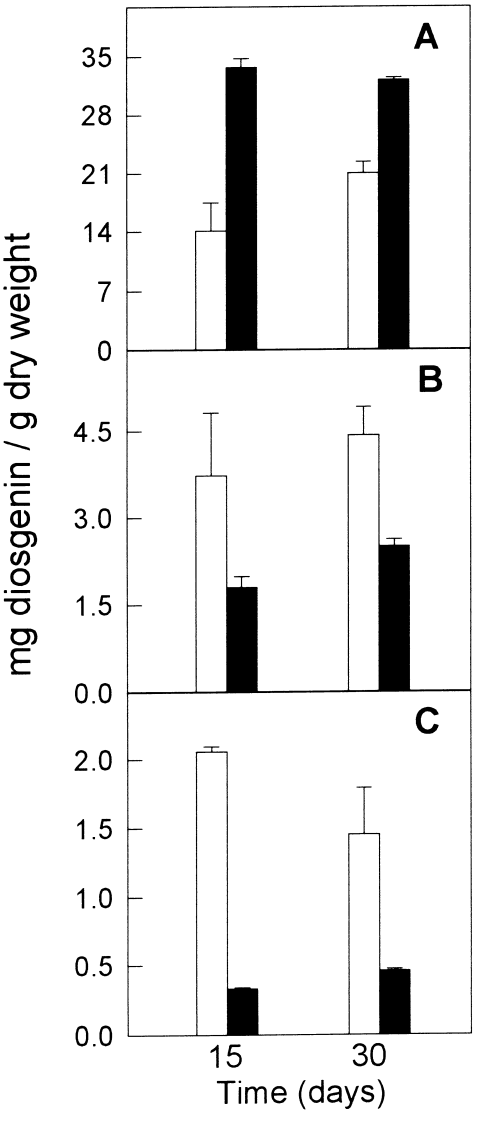

Fig. 6. Effect of ethephon on diosgenin levels in plants of *Trigonella foenum-graecum*. Control (□) and treated (50 ppm, ■) plants were analyzed 15 and 30 days after treatment. The diosgenin levels (mg/g dry weight) were determined in leaf (A), stem (B) and root (C). The experiments were carried out in triplicate and the vertical bars denote ± SE.

although these levels would be slightly reduced by the inhibitory effect that 50 ppm ethephon had on growth at this age. At 30 days, however, diosgenin levels in the whole plants were 68% lower than in the corresponding controls, mainly due to the inhibitory effect on growth that this treatment displayed in plants of this age.

In support of these results other contributions, although few, suggest that this phytoregulator may modulate the expression of other secondary compounds of a terpenic (García Puig et al., 1993; Ortuño et al., 1993), phenolic (García Puig et al., 1995) and alkaloid (Cho et al., 1988) nature.

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