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Effect of Sitosterol on Root Formation of Cotton Cuttings

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ABSTRACT

A pot experiments were carried out during 2003 and 2004 summer seasons in the greenhouse of the National Research Center, Dokki, Giza, Egypt to determine the effect of sitosterol on root formation of cotton cuttings, growth, yield and its components as well as biochemical constituents of two cotton cultivars. Cuttings of two Egyptian cotton (*Gossypium barbadense* L.) cultivars Giza 85 and Giza 89 were taken from the previous growing season during the activity stage of buds containing two nodes. Cotton cuttings were soaked in sitosterol concentrations (100, 150 and 200 ppm) for 1 h at laboratory conditions as well as control treatment (distilled water). Cotton cuttings were grown in pots N. 30 filled with loamy soil at March 27 and 31 of 2003 and 2004 seasons, respectively. The results indicated that in both cotton cultivars root length was significantly and gradually increased with increasing sitosterol from zero to 150 or 200 ppm. Data also showed that sitosterol application significantly increased boll weight and seed cotton yield per plant with increasing sitosterol up to 200 ppm. Application of 200 ppm sitosterol in Giza 85 produced the highest values in all yield traits in comparison with untreated plants or with plants received 100 or 150 ppm sitosterol. On contrary, in Giza 89 application of 200 ppm sitosterol decreased number of bolls per plant, number of seeds per boll and seed weight per boll in comparison with control plants or with low sitosterol concentrations. Total indoles in stem of Giza 85 was increased by using 100 or 150 ppm sitosterol, while total soluble sugars was increased in roots of Giza 85 at high concentration in both cotton cultivars. Application of 200 ppm sitosterol increased total carbohydrates in root, stem and leaves of Giza 85 while in Giza 89 an opposite trend was noticed by using the highest sitosterol concentration. In general, it could be concluded that application of sitosterol to cotton cuttings lead to encourage the root formation and enhancing the growth and yield of cotton plants.

Key words: Sitosterol, root formation, cotton cuttings, cotton yield, phenol compounds, indoles

INTRODUCTION

Brassinosteroid (BR) is an important phytohormone known to regulate many of the same aspects of plant growth and development, including cell division and expansion, vascular differentiation, root growth, and senescence. Sitosterol is the major compound of steroids that are phytosterol (Deliu *et al*., 1992). Steroids promoted root growth, root weight, root/shoot ratio and rooting ability of rice and maize plants as well as brassinosteroid application (Rufu, 1992; Fujii and Saka, 2001; Abdel-Wahed, 2001). Additionally, rice lamina joint inclination increased with brassinosteroid concentration and exposure period. Etiolated lamina was more sensitive to brassinosteroid than green lamina. Sitosterol had stimulatory effect on vascular differentiation, thickness of the upper epidermal, mesophyl tissue layer, growth and yield as well as chemical composition of wheat (Abdel-Wahed *et al*., 2000). In addition, brassinosteroids are involved in many morphological processes in rice including the elongation and unrolling of leaves and skotomorphogensis (Mori *et al*., 2002). Müssig *et al*. (2003) pointed that low concentrations of EBL and 24-epicastasterone (ECS) raises root length. Regenerated plants have been obtained from explants such as hypocotyl, cotyledon, root, anther and from various cotton species (Özyiğit *et al*., 2007). Somatic embryogenesis and plant regeneration systems have been established from cotton tissue, protoplasts and ovules (Ikram-ul-Haq and Zafar, 2004). However, both processes have many disadvantages, which effects to obtain fertile plants from explants. Some of those are; genotype dependent regeneration, poor rooting, a prolonged culture period, browning that caused death of explants (Kumria *et al*., 2003; Ouma *et al*., 2004; Özyiğit *et al*., 2007).Therefore, brassinosteroids could be generating erect leaf of rice plant (Morinaka *et al*., 2006). Brassinolide doubled the mean root length and the number of mitoses over that of controls at low concentration (Howell *et al*., 2007). Özyiğit and Gözükirmizi (2008) indicated that regenerated plants were all fertile, they did not show obvious changes in overall morphology, and short time in culture did not affect their normal developments. All the plants that regenerated from both genotypes tested exhibited normal phenotype and they were similar.

While some studies have claimed that steroids promote root growth other studies have inhibitory effect. However none of these studies have sought to examine sitosterol effect on formation roots on cuttings to obtain quantities data on the actual characters of cotton plant. The purpose of the present study was to determine the effect of sitosterol on root formation of cotton cuttings, vegetative growth and yield as well as chemical constituents to save cotton seeds for industrial processes.

MATERIALS AND METHODS

The experiments were conducted to study sitosterol effect on root formation, vegetative growth, yield and some chemical constituents of cotton plants. Cuttings of two Egyptian cotton (*Gossypium barbadense* L.) cultivars Giza 85 and 89 were taken from the previous growing season during the activity stage of buds containing two nodes. Cotton cuttings were soaked in sitosterol (Stigmasta-5 en-3B-ol):(24R-24-ethylcholest-5-en-3B-l) concentrations (100, 150 and 200 ppm) for one hour at laboratory conditions as well as control treatment (distilled water). Cotton cuttings were grown in pots N. 30 filled with loamy soil at March 27 and 31 of 2003 and 2004 seasons, respectively. Each pot was contained 10 cuttings, thinned to two after its activity and kept to grow till harvest at green house of National Research Centre, Dokki, Giza, Egypt. The experiments were arranged as a randomized complete design with three replications. Calcium super phosphate fertilizer (15.5% P_2O_5) was added before sowing at a rate of 6 g pot^{-1}, nitrogen fertilizer at a rate of 12 g pot^{-1} as ammonium nitrate (33.5% N) was added at two doses (30 and 45 days after sowing).

Growth and yield measurements: The following growth characters were determined: root length (cm), root, stem and leaves as fresh and dry weight (g). At harvest time, seed cotton yield (g plant^{-1}), number of bolls/plant, lint weight/boll (g), seed weight/boll (g), number of open bolls/plant, average boll weight (g) and number of seeds/boll were also determined.

Biochemical constituents: Total and soluble sugars according to Dubois *et al*. (1956), free amino acids according to Plumer (1978), total phenols (Daniel and George, 1972) and total indoles (Glickmann and Dessaux, 1995) were determined in different dried plant organs.

Statistical analysis: Data were carried out for analysis of variance as described by Snedecor and Cochran (1980). L.S.D. at 5% level of probability was used to compare between means.

RESULTS

Effect of sitosterol on vegetative growth characters of cotton cultivars: Table 1 indicated that root length was significantly and gradually increased with increasing sitosterol from 0 to 150 or 200 ppm, while root fresh and dry weight were significantly decreased with increasing sitosterol up 200 ppm. The same trend was observed with stem fresh and dry weight, but the differences between the treatments were not significant. However, application of 200 ppm sitosterol significantly increased the fresh weight of cotton leaves in comparison to the treatments received 100 or 150 ppm sitosterols while the dry weight of leaves was gradually decreased up to 200 ppm sitosterol. The highest values of root length (66.83 cm) at 200 ppm sitosterol, while the lowest root fresh and dry weight , stem fresh and dry weight and leaves fresh and dry weight were 9.54, 2.92, 25.52,10.48, 23.33 and 6.95 g, respectively at the same sitosterol application. Table 2 indicated that Giza 85 and Giza 89 significantly varied in their growth characters (root fresh weight, root, stem and leaves dry weight/plant) by using different concentration of sitosterol. However, in both Giza 85 and 89, root length was significantly and gradually increased with increasing sitosterol from zero to 150 or 200 ppm, whereas, application of 100 ppm decreased root length of Giza 85 and

Table 1: Effect of sitosterol concentration on vegetative growth characters of cotton plants

		Root weight (g)		Stem weight (g)		Leaves weight (g)	
Sitosterol (ppm)	Root length (cm)	Fresh	Dry	Fresh	Dry	Fresh	Dry
00	41.33	14.70	6.67	30.05	15.07	27.30	8.38
100	38.00	12.71	6.33	29.16	13.20	18.05	7.85
150	49.33	11.68	4.80	26.09	11.38	18.23	7.43
200	66.83	9.54	2.92	25.52	10.48	23.33	6.95
LSD 0.05	6.79	2.11	1.03	NS	NS	4.40	0.95

Table 2: Effect of interaction between cotton cultivars and sitosterol concentrations on vegetative characters

			Root weight (g)		Stem weight (g)		Leaves weight (g)	
Cultivars	Sitosterol (ppm)	Root length (cm)	Fresh	Dry	Fresh	Dry	Fresh	Dry
Giza 85	0.0	53.00	18.77	8.13	31.93	17.07	36.43	11.77
	100	47.67	15.90	10.10	31.16	15.47	20.10	10.30
	150	65.67	15.17	7.10	30.46	17.67	14.67	8.03
	200	81.00	10.17	3.30	24.10	10.00	18.03	7.67
Mean		61.83	15.00	7.18	29.41	15.05	22.31	9.47
Giza 89	0.0	29.67	10.63	5.20	28.17	13.08	18.17	5.00
	100	28.33	8.93	2.68	27.17	10.93	16.00	5.40
	150	33.00	8.20	2.50	21.60	5.10	21.80	6.83
	200	52.67	8.90	2.53	26.93	10.93	28.63	6.13
Mean		35.00	9.17	3.16	25.97	10.01	21.15	6.13
LSD 0.05	V	NS	1.49	0.73	NS	3.76	NS	0.67
	V x S	6.33	2.98	1.45	NS	NS	6.23	1.34

Table 3: Effect of sitosterol on cotton yield and its component

Sitosterol (ppm)	No. of bolls/plant	Average boll weight (g)	Seed weight/boll (g)	Lint weight/boll (g)	No. of seeds/boll	Cotton seed yield/plant (g)
0.0	4.17	1.57	0.96	0.59	12.50	3.00
100	4.17	1.75	1.21	0.65	13.50	4.53
150	4.33	2.00	1.35	0.58	15.00	6.05
200	5.33	2.27	1.16	0.60	14.00	6.37
LSD 0.05	NS	0.22	0.22	NS	NS	0.80

Table 4: Effect of interaction between cotton cultivars and sitosterol concentrations on cotton yield and its components

Cultivars	Sitosterol (ppm)	No. of bolls/plant	Average boll weight (g)	Seed weight/boll (g)	Lint weight/boll (g)	No. of seeds/boll	Cotton seed yield/plant (g)
Giza 85	0.0	3.00	1.79	0.89	0.64	12.67	3.50
	100	4.00	1.95	1.27	0.71	15.00	5.70
	150	4.33	2.25	1.43	0.71	17.67	7.20
	200	6.67	2.55	1.56	0.80	18.34	7.40
Mean		4.50	2.14	1.29	0.72	16.17	5.95
Giza 89	0.0	5.33	1.35	1.03	0.54	11.33	4.10
	100	4.33	1.55	1.15	0.50	12.00	3.37
	150	4.33	1.74	1.25	0.65	12.33	4.90
	200	4.00	1.98	0.76	0.40	9.67	5.33
Mean		4.50	1.66	1.05	0.55	11.33	4.43
LSD 0.05	V	NS	0.15	0.15	0.09	1.80	0.57
	V x S	2.45	NS	0.31	0.18	NS	1.41

Giza 89 in comparison to control or the two other treatments. The highest root length (81.0 cm) was observed with Giza 85 treated with 200 ppm sitosterol, while the lowest (28.33 cm) with Giza 89 treated with 100 ppm sitosterol. On the other hand, application of 150 ppm sitosterol in Giza 89 produced lower root and stem fresh and dry weight 8.20, 2.50, 21.60 and 5.10 g , respectively, while application of 100 ppm sitosterol with Giza 85 resulted in the lowest leaves fresh weight (14.67 g). On contrary application of 200 ppm sitosterol increased the fresh and dry weights of leaves in Giza 89.

Effect of sitosterol on yield and its components of some cotton cultivars: Table 3 showed that sitosterol application significantly increased boll weight and seed cotton yield per plant with increasing sitosterol up to 200 ppm, however seed weight per boll was only significantly increased up to 150 ppm. On the other hand, number of bolls per plant, lint weight per boll and number of seeds per boll were not significantly affected by sitosterol application. Data presented in Table 4 illustrated the interaction between cotton cultivars and sitosterol concentrations, it is obvious that number of bolls, seed and lint weight per boll as well as seed cotton yield per plant were significantly affected by the interaction between sitosterol application and cotton cultivars, while average boll weight and number of seeds per boll were not significantly affected. Data also indicated that Giza 85 and Giza 89 were significantly differed in all yield traits, except number of bolls per plant it was insignificant. Application of 200 ppm sitosterol in Giza 85 produced the highest values in all yield traits in comparison with untreated plants or with plants received 100 or 150 ppm sitosterol. On contrary, in Giza 89 application of 200 ppm sitosterol decreased number of bolls per plant , number of seeds per boll and seed weight per boll in comparison with control plants or with low sitosterol concentrations (Table 4). In general, Giza 85 seems to be more responsible to sitosterol application than Giza 89.

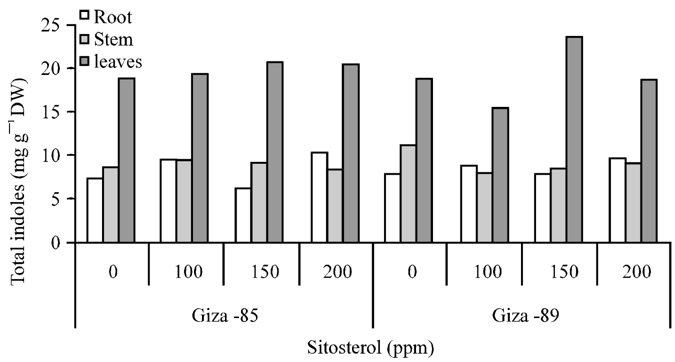

Fig. 1: Effect of sitosterol on total indoles in root, stem and leaves of two cotton cultivars

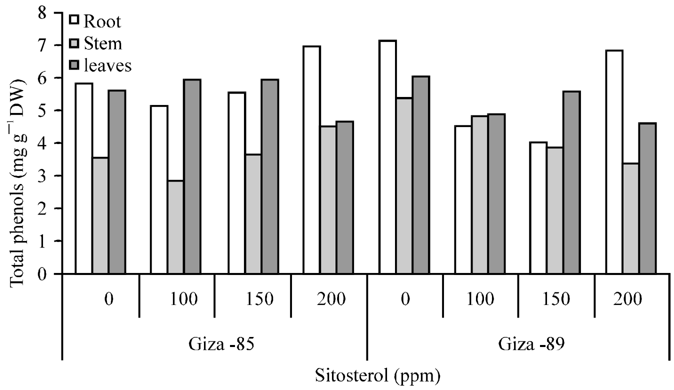

Fig. 2: Effect of sitosterol on total phenols of root, stem and leaves of two cotton cultivars

Effect of sitosterol on biochemical constituents of cotton plants: Data presented in (Fig. 1) illustrated that application of 200 ppm sitosterol increased the total indoles in roots of both cotton cultivars, while it was increased only in Giza 85 cultivar at high sitosterol concentration (200 ppm). In Giza 89 cultivar, application of high concentration of sitosterol increased the total indoles of roots in comparison to control plants. On the other hand, the total indoles in stem of Giza 85 was increased only by using 100 or 150 ppm sitosterol while in Giza 89 the highest total indoles in stem was obtained with untreated plants. However, in both cultivars the total indoles of leaves was increased by using 150 ppm sitosterol in comparison with other sitosterol treatments or with control plants (Fig. 1). Total phenols in root, stem and leaves were also affected by using sitosterol in both cotton cultivars. Fig. 2 illustrated that application of 200 ppm sitosterol increased the total phenols in roots of Giza 85. The same trend was noticed in Giza 89. However, the total phenols in stem of Giza 85 were increased at high sitosterol concentration, while in contrary it was decreased under the same condition in Giza 89. On the other hand, increased the sitosterol concentration up to 200 ppm resulted in a gradually decreased leaves total phenols in both cotton cultivars (Fig. 2).

Data presented in (Fig. 3) indicated that total free amino acids in roots of Giza 85 were increased by using 200 ppm sitosterol and also by using 150 ppm in Giza 89. On contrary in Giza 85 increased sitosterol up to 200 ppm decreased the total free amino acids in stem while it was also decreased at 100 or 200 ppm in Giza 89 in comparison to the other treatments. In contrast,

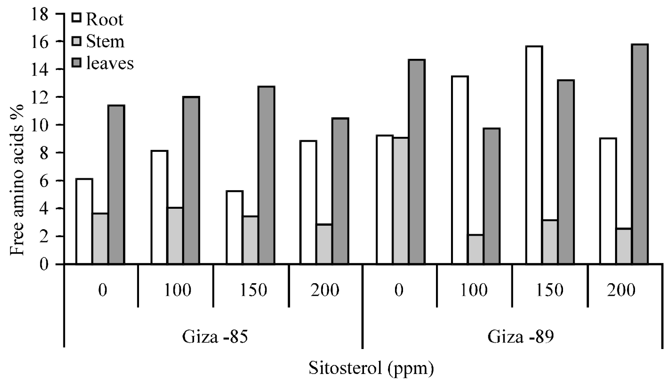

Fig. 3: Effect of sitosterol on total free amino acids in root, stem and leaves of two cotton cultivars

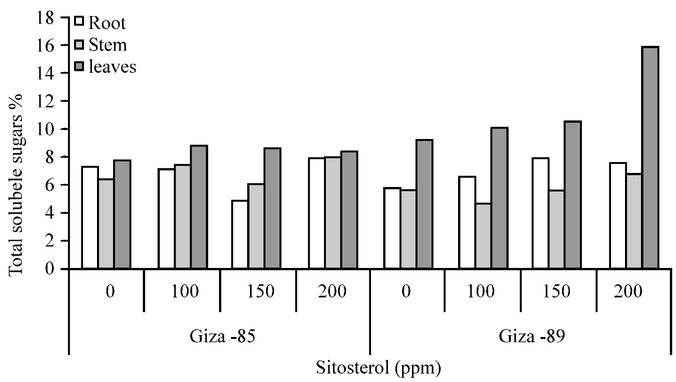

Fig. 4: Effect of sitosterol on total soluble sugars in root, stem and leaves of two cotton cultivars

application of high concentration of sitosterol (200 ppm) decreased the total free amino acids in stem of Giza 85, while it was increased by using the same sitosterol concentration in leaves of Giza 89.

Total soluble sugars was also influenced by using sitosterol concentration, it was increased in roots and stem of Giza 85 at high concentration, while in Giza 89 were increased in both stem and leaves when the plants treated with 150 or 200 ppm of sitosterol (Fig. 4). In this regards, total soluble sugars was increased in stem of both cotton cultivars with increasing sitosterol up 200 ppm. Figure 4 also illustrated that the total soluble sugars in leaves of Giza 85 was increased by using 100 or 150 ppm, whereas in Giza 89 applied of 200 ppm increased the total soluble sugars in comparison to the other treatments. The highest values of total soluble sugars (7.97 and 7.90%) were obtained with root and stem of Giza 85 while leaves of Giza 89 produced the highest values (15.87%).

Figure 5 indicated that application of 200 ppm sitosterol increased the total carbohydrates in root, stem of Giza 85 in comparison to untreated or to other sitosterol concentration, whereas in leaves tend to an opposite trend , it was increased with high concentration. On the other hand, in Giza 89 the total carbohydrates was also decreased in root at 200 ppm or in stem at 150 ppm while in leaves was increased at 150 ppm in comparison to other treatment in the same cultivar.

Concerning, the total carbohydrates in seeds, data in (Fig. 6) indicated that application of 100 or 200 ppm sitosterol increased the total carbohydrates in seeds in Giza 85 cultivar, while in Giza 89 was increased by using 150 or 200 ppm.

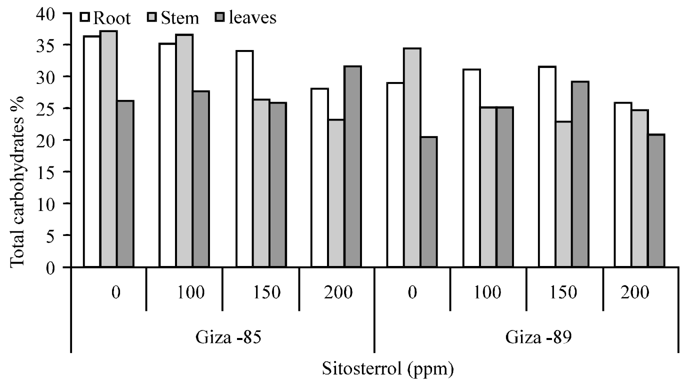

Fig. 5: Effect of sitosterol on total carbohydrates in root, stem and leaves of two cotton cultivars

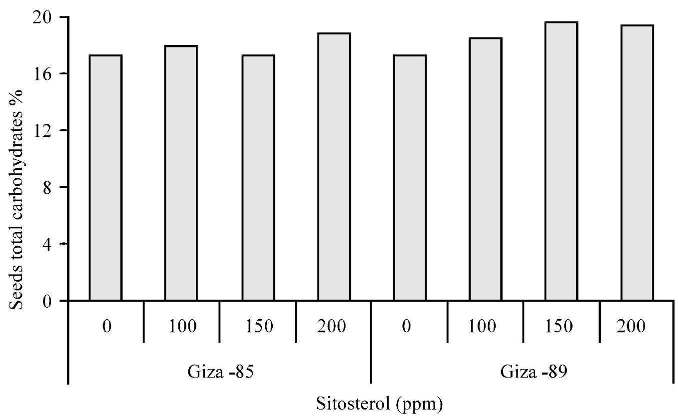

Fig. 6: Effect of sitosterol on total carbohydrates in seeds of two cotton cultivars

DISCUSSION

Applying high sitosterol concentration 200 ppm resulted in the highest root length of both cotton cultivars, while in contrary, the fresh and dry weight root, stem and leaves in Giza 85 were gradually decreased with increasing sitosterol up to 200 ppm and up to 150 ppm in root and stem of Giza 89. This effect might be due to genotypes ability expression on growth characters of cotton plant. The varied stimulatory growth of cotton plant was return to their gene expression sensitivity (Kim *et al*., 2007). Rashad *et al.* (2009) indicated that application of β-sitosterol led to a significant increase shoot dry weight and shoot/root ratio by using 40 ppm β-sitosterol. Also, Mekki *et al.* (2010) on canola pointed that dry weight of canola cultivars was increased by applying stigmasterol up to 400 ppm. Root length was increased by sitosterol application (200 ppm) while, sitosterol concentration had inhibitory effect on plant growth characters compared with untreated plants. This might be return to sitosterol stimulatory effect on formation skotomother cell, cell division and expansion for increasing root characters and boll fertility of cotton plants that led to increasing cotton yield. Whereas, stimulatory effect of brassinosteroids on root growth increased at low concentration and inhibited primary root growth at higher concentration (Kim *et al*., 2007). In this trend, epibrassinolide doubled the root length and number of mitosis of onion plants at low concentration (Howell *et al*., 2007). Similar effect to brassinostroids, both sitosterol and stigmasterol and a typical sterols involved in the regulatory function of plant development and gene expression.

Sterols are essential for normal plant growth and development as brassinostroids (He *et al.*, 2003). Previous studies confirmed the present obtained positive response to the foliar application of brassinostroid on different species (Ahmed and Shalaby, 1994; Kurapov *et al.*, 1996). This means that sterols play a role in plant development including cell expansion, vascular differentiation; etiolating and reproductive development (Clouse and Sasse, 1998; Abdel-Wahed *et al.*, 2000). Moreover, the favorable effect on growth could be attributed to the stimulated action of brassinostroid on phytohormones such as auxins and cytokinins, which turn induced cell elongation and division (Gregory and Mandava, 1982; Bao *et al.*, 2004). Also it was found that brassinostroid cause pronounced elongation of hypocotyls, epicotyls of monocots (Sasse, 1999). Brassinostreoid promoted cell wall formation and resulted in hyperpolarization of cell membranes and accelerated growth cycle (Clouse and Sasse, 1998). Similar results are in agreement with those obtained by Abdel-Wahed (2001), who found that plants treated with steroidal sex hormones or precursors influenced plant development and increased rate of root, fresh and dry weight. Additionally, cell divisions root and shoot growth, embryo growth, flowering, pollen tube growth and callus proliferation (Janeczko and Skoczowski, 2005).

The obtained data also indicated that applying different concentrations of sitosterol to cotton plants cuttings resulted in a gradually and significantly increase of bolls weight, seed weight per boll and seed yield per plant. The increase of these yield traits mainly attributed to foliar application of sitosterol might be due to sitosterol enhanced photosynthetic apparatus, growth parameters cell division and enzymatic activity (Kalinich *et al.*, 1985; Wang, 1997; Clouse and Sasse, 1998). These results are also supported by Abdel-Wahed *et al.* (2003) and Kim *et al.* (2007). Data also indicated that Giza 85 and Giza 89 were significantly differed in all yield traits, except number of bolls per plant were insignificant. Application of 200 ppm sitosterol in Giza 85 produced the highest values in all yield traits in comparison with untreated plants or with plants received 100 or 150 ppm sitosterol. On contrary, in Giza 89 application of 200 ppm sitosterol decreased number of bolls per plant , number of seeds per boll and seed weight per boll in comparison with control plants or with low sitosterol concentrations (Table 4). This effect might be due to its activity as well as brassinosteroids on stimulating variety of physiological responses including changes in enzymatic activities, membrane potential, DNA, RNA and protein synthesis, photosynthetic activity and changes in the balance of other endogenous phytohormones (Mandava, 1988; Szekeres and Konez, 1998). These results are in agreement with those obtained by Abdel-Wahed (2001) on maize (*Zea mays* L.) Abdel-Wahed *et al.* (2003) on rice (*Oryza sativa* L.) Kim *et al.* (2007) on onion (*Allium cepa* L.)

In this concern, applying of sitosterol to cotton cutting resulted in an increase of total indoles and phenols of root, stem and leaves in both cotton cultivars. However, in Giza 89 cultivar, application of high concentration of sitosterol decreased the total indoles of roots in comparison to control plants. On the other hand, the total indoles in stem of Giza 85 were increased only by using 100 or 150 ppm sitosterol. This effect might be due to the effect of brassinosteroids on stimulating variety of physiological responses including changes in enzymatic activities, photosynthetic activity and changes in the balance of other endogenous phytohormones (Mandava, 1988; Szekeres and Konez, 1998). Total phenols in root, stem and leaves were also affected by using sitosterol in both cotton cultivars. Application of 200 ppm sitosterol increased the total phenols in roots of Giza 85 and Giza 89. However, the total phenols in stem of Giza 85 were increased at high sitosterol concentration, while in contrary it was decreased under the same condition in Giza 89. This effect might also be due genotypes sensitivity to sitosterol application and related developmental stage

(Abdel-Wahed *et al.,* 2003) on rice. Data presented in Fig. 3 indicated that total free amino acids in roots of Giza 85 were increased by using 200 ppm sitosterol and also by using 150 or 200 ppm in Giza 89. On contrary, in Giza 85 increased sitosterol up to 200 ppm decreased the total free amino acids in stem and also decreased at 100 or 200 ppm in Giza 89 in comparison to the other treatments. Abdel-Wahed (2008) on soybean (*Glycine max*) and Rashad *et al.* (2009) on Marigold (*Calendula officiinalis* L.) found that using 40 or 80 ppm β-sitosterol improved the biochemical constituents of the plants such as indoles, free amino acids and phenolic compounds in the leaves.

Total soluble sugars, total carbohydrates in different cotton plant organs were also influenced by applying different concentration of sitosterol. Total soluble sugars were increased in root and stem of Giza 85 cultivar with increasing sitosterol up 200 ppm. In Giza 85 leaves it was increased by using 100 or 150 ppm, whereas in Giza 89 applied of 200 ppm increased the total soluble sugars in comparison to the other treatments (Fig. 4). In this regards, application of 200 ppm sitosterol decreased the total carbohydrates in root, stem, leaves and seeds of Giza 85 cultivar, while in leaves it was increased. On the other hand, in Giza 89 total carbohydrates was also decreased in roots at high concentration , while it was increased in leaves at 150 ppm (Figs 5 and 6). These results are in agreement with those obtained by Abdel-Wahed (2001) on maize (*Zea mays* L.); Abdel-Wahed *et al.* (2003) on rice (*Oryza sativa* L.), Kim *et al.* (2007) on onion (*Allium cepa* L.).

CONCLUSION

This study has demonstrated the use of synthetic phytohormone to increase vegetative growth and enhancing root formation of cotton cutting as a new method for cultivation cotton crop. Results from the study revealed that application of different concentration of sitosterol could be enhanced and increased the growth and some chemical constituents of cotton.

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