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Indole-3-butyric acid and myo-inositol impacts on *in vitro* rooting of the cherry rootstocks CAB-6P and Gisela 6

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Abstract

The present study investigates the effects of indole-3-butyric acid (IBA) alone and in combination with myo-inositol on *in vitro* rooting and biochemical responses in the cherry rootstocks CAB-6P (*Prunus cerasus* L.) and Gisela 6 (*Prunus cerasus* × *Prunus canescens*). For the CAB-6P rootstock, the best results for root number (6.31), fresh mass (FM), dry mass (DM), and rooting percentage (100 %) were obtained on Murashige and Skoog (MS) medium with 2 mg dm^{-3} IBA and maximum root length (30.57 mm) was obtained at 1 mg dm^{-3} IBA. Myo-inositol suppressed the positive effects of IBA on root length. In the Gisela 6 explants, the inclusion of 2 mg dm^{-3} IBA together with 0.5 mg dm^{-3} of myo-inositol in the culture medium significantly enhanced root number (9.91) and root FM and DM. The root length was maximum in the combination of the lowest IBA and myo-inositol concentrations (0.5 mg dm^{-3}). The rooting percentage was the greatest (100 %) with the application of 1 mg dm^{-3} IBA alone. In both explants, the application of IBA alone or in combination with myo-inositol resulted in a lower leaf proline content in comparison with the control (without growth regulators). The maximum leaf chlorophyll content was at 1 mg dm^{-3} IBA in the CAB-6P whereas at 2 mg dm^{-3} IBA and 1 mg dm^{-3} myo-inositol in Gisela 6. Addition of myo-inositol mostly increased sugar content in comparison with control or IBA alone in both rootstocks.

Additional key words: auxin, chlorophyll, growth analysis, proline, *Prunus cerasus*, sugars.

Introduction

CAB-6P is a valuable rootstock as all cherry cultivars grafted on it have less vigor (-30 %), better fruit quality, and higher yield efficiency in comparison to those grafted on sweet cherry seedlings. Gisela 6 tolerates soils of poorer quality than does Gisela 5; in addition, its vigor ranges between that of Gisela 5 and *Prunus avium*.

Cyclic forms of polyols, which include myo-inositol, are widely present in plants (Noiraud *et al*. 2001). The promotory and/or inhibitory effects of myo-inositol have been extensively reported in the literature. Myo-inositol as a component of the Murashige and Skoog (1962; MS) culture medium has been used for many plant species and is considered to be a promoting and even an essential in many types of tissue cultures (Loewus and Dickinson 1982). Myo-inositol is involved in the transport of indole-3-acetic acid (IAA) and controls growth and cellular expansion that is induced by IAA (Loewus and Murthy 2000). In addition, inositol has been implicated in membrane biogenesis, signal transduction, ion channel physiology, and membrane dynamics (Loewus and Murthy 2000). However, myo-inositol combined with auxins inhibited root elongation (Crozier *et al*. 2000) and reduced the rooting percentage (Donnely 1976) whereas myo-inositol alone did not significantly alter chlorophyll content (Teixeira da Silva *et al*. 2006) or rooting (Goforth and Torrey 1977, Jarvis and Booth 1981).

There have been a limited number of studies regarding the effects of various growth regulators and sugar alcohols on cherry rooting *in vitro,* namely cherry rootstocks. Therefore, the purpose of this study was to test the *in vitro* effects of various concentrations of myo-inositol together with indole-3-butyric acid (IBA) on rooting parameters and content of chlorophylls (*a*+*b*), total sugars, and proline in two commercial cherry rootstocks, CAB-6P and Gisela 6.

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Abbreviations: ETR - electron transport rate; IAA - indole-3-acetic acid; IBA - indole-3-butyric acid; LAI - leaf area index; MS medium - Murashige and Skoog medium.

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Materials and methods

The effect of myo-inositol was studied in *in vitro* experiments employing the cherry rootstocks CAB-6P (*P. cerasus* L.) and Gisela 6 (*P. cerasus* × *P. canescens*). Preliminary results indicated that certain combinations of IBA and myo-inositol were not effective (unpublished data). Therefore, to avoid factorial treatments that required a greater number of explants, the following concentrations of IBA + myo-inositol [mg dm^{-3}] were tested: *1*) 0+0 (control), *2*) 0.5+0.5, *3*) 0.5+1, *4*) 0.5+2, *5*) 1+0, *6*) 1+0.5, *7*) 1+1, *8*) 2+0, *9*) 2+0.5, and *10*) 2+1.

Shoot tip explants from previous *in vitro* cultures of 1.5 - 2.5 cm in length were used as explants. The initial material was certified as a virus free. The explants were grown in glass tubes containing 10 cm^3 of MS medium with 30 g dm^{-3} sucrose and 6 g dm^{-3} *Bacto-agar* (pH 5.8). The medium was autoclaved at 121 °C for 20 min. One explant was transferred aseptically to each test tube which was then covered with aluminium foil. The cultures were maintained in a growth room with a 16-h photoperiod, irradiance of 150 μmol m^{-2} s^{-1} provided by cool white fluorescent lamps (36 W, *Philips*, Eindhoven, The Netherlands), and temperature of 22 ± 1 °C. Six weeks after transferring the explants to the rooting medium, the root number per rooted explant, root length, root fresh mass (FM), root dry mass (DM), percentage of rooting, shoot length, shoot FM and DM, and content of chlorophylls (*a*+*b*) in leaves, and sugars and proline in leaves and roots were recorded.

For chlorophyll extraction, 0.1 g of frozen leaves was placed in 25 cm^3 glass test tubes and 15 cm^3 of 96 % (v/v) ethanol was added to each tube. The tubes were incubated in a water bath at temperature of 79.8 °C until there was complete discoloration of the samples (about 4 h). The chlorophyll content was determined according to Wintermans and De Mots (1965).

For proline and sugar extractions, 0.1 g of frozen plant material chopped into small pieces was placed in 25 cm^3 glass test tubes containing 15 cm^3 of 80 % (v/v) ethanol and incubated in a water bath of 60 °C for 30 min (Khan *et al.* 2000). The extract was filtered through a *Whatman No. 1* filter paper and free proline was measured with acid ninhydrin solution (Troll and Lindsley 1955). Total sugars were measured with the anthrone reagent (Plummer 1987).

The experimental layout was completely randomized and the data were analysed with *ANOVA* using the statistical package *SPSS v. 17.0* (*SPSS Inc.*, Chicago, IL, USA). The experiment was repeated twice and each value was the mean of 10 replicates regarding rooting characteristics and biochemical measurements. The reported data are the means of the two experiments. To compare the means, the Duncan's multiple range test and standard error at $P \leq 0.05$ were used.

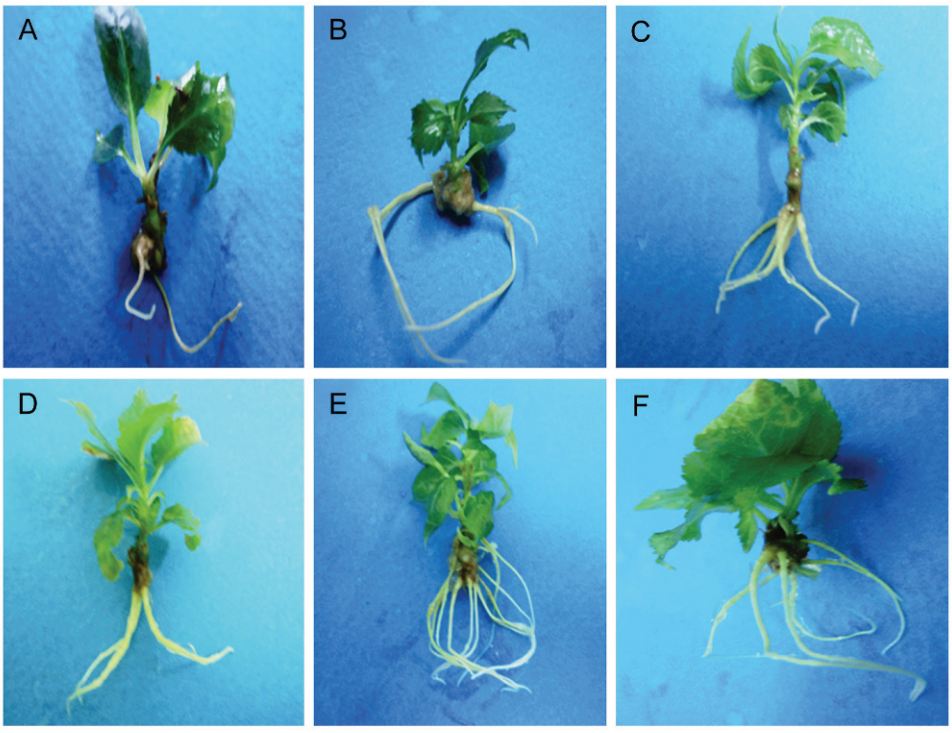

Fig. 1. Effect of IBA alone or in combination with myo-inositol on *in vitro* rooting. *A* - CAB-6P, control; *B* - CAB-6P, 1 mg dm^{-3} IBA; *C* - CAB-6P, 2 mg dm^{-3} IBA; *D* - Gisela 6, control; E - Gisela 6, 2 mg dm^{-3} IBA + 0.5 mg dm^{-3} myo-inositol; *F* - Gisela 6, 0.5 mg dm^{-3} IBA + 0.5 mg dm^{-3} myo-inositol.

Results

For the CAB-6P rootstock, the minimum values of the rooting characteristics were in the control plants, with the exception of root length and rooting percentage (Table 1, Fig. 1*A*). Maximum root length (30.57 mm) was at 1 mg dm^{-3} IBA (Fig. 1*B*). When 2 mg dm^{-3} IBA was incorporated into the culture medium, the number of roots per rooted explant (6.31), root FM and DM, and the rooting percentage (100 %) were significantly higher than those of control plants (Fig. 1*C*). Nevertheless, the maximum number of roots per rooted explant (7.0) was obtained in medium with 0.5 mg dm^{-3} IBA + 1 mg dm^{-3} myo-inositol, however, the rooting percentage in this medium was low (16.67 %) which is half of the control.

For the Gisela 6 rootstock, the control exhibited the minimum values in all the rooting characteristics (Table 1, Fig. 1*D*). The maximum number of roots per rooted explant (9.91) and the root FM and DM were obtained in the medium with 2 mg dm^{-3} IBA + 0.5 mg dm^{-3} myo-inositol (Fig. 1*E*). Compared to the control, the root number was five times greater under this treatment. On the other hand, maximum root length (27.42 mm) was at 0.5 mg dm^{-3} IBA + 0.5 mg dm^{-3} myo-inositol (Fig. 1*F*). The maximum rooting percentage (100 %) was obtained by adding 1 mg dm^{-3} IBA in the culture medium individually, where the rooting percentage was 3.33 times greater than that of the control.

The maximum shoot length of the CAB-6P regenerants was in the control treatment (Table 2) and shoot length decreased after addition of IBA either individually or simultaneously with myo-inositol. The best results for shoot FM and DM were recorded under the combination of the lowest IBA (0.5 mg dm^{-3}) and the highest myo-inositol (2 mg dm^{-3}) concentrations, where both the FM and DM of the shoots were 2 times greater than in the control. On the other hand, for the Gisela 6 rootstock, the maximum length, FM and DM of shoots were when 2 mg dm^{-3} IBA was combined with 0.5 mg dm^{-3} myo-inositol.

Regarding CAB-6P, it was found that addition of IBA irrespective of its concentration significantly increased leaf chlorophyll content expressed on a DM basis (Table 2). In contrast, a combination of IBA and myo-inositol did not significantly alter leaf chlorophyll content expressed on a DM basis and decreased the chlorophyll content expressed on a FM basis. Administering IBA either separately or with myo-inositol did not substantially alter sugar content in the leaves and roots of CAB-6P compared to the control (Table 3). The maximum sugar

Table 1. Effect of IBA alone and in combination with myo-inositol (Myo) on cherry rootstocks CAB-6P and Gisela 6 rooting characteristics. Means ± SE, $n = 10$. Those denoted by the same letter are not significantly different according to Duncan's multiple range test at $P \leq 0.05$. Significant effects at $P \leq 0.05$ (*), 0.01 (**), or 0.001 (***) according to 2-way *ANOVA*.

Rootstock	Treatments [mg dm^{-3}]	Root number [$explant^{-1}$]	Length [mm]	Fresh mass [g]	Dry mass [g]	Rooting [%]
CAB-6P	control	1.00 ± 0.02 a	25.00 ± 0.36 b	0.012 ± 0.001 a	0.001 ± 0.001 a	33.33 c
	0.5 IBA+0.5 Myo	4.00 ± 0.19 b	15.52 ± 0.60 a	0.021 ± 0.001 ab	0.002 ± 0.001 ab	33.33 c
	0.5 IBA+1.0 Myo	7.00 ± 0.15 d	12.51 ± 0.03 a	0.040 ± 0.001 cde	0.004 ± 0.001 cd	16.67 a
	0.5 IBA+2.0 Myo	4.00 ± 0.26 b	15.00 ± 0.53 a	0.030 ± 0.001 bc	0.003 ± 0.001 bc	25.00 b
	1 IBA	2.79 ± 0.32 b	30.57 ± 3.47 c	0.050 ± 0.007 def	0.005 ± 0.001 de	70.00 f
	1 IBA+0.5 Myo	3.38 ± 0.32 b	13.92 ± 0.83 a	0.028 ± 0.003 bc	0.003 ± 0.001 bc	66.67 e
	1 IBA+1.0 Myo	5.43 ± 0.36 c	15.15 ± 0.55 a	0.054 ± 0.002 ef	0.006 ± 0.001 e	53.85 d
	2 IBA	6.31 ± 0.62 cd	21.94 ± 1.68 b	0.080 ± 0.010 g	0.008 ± 0.001 f	100.00 i
	2 IBA+0.5 Myo	5.62 ± 0.78 c	12.53 ± 0.90 a	0.057 ± 0.008 f	0.006 ± 0.001 e	76.92 g
	2 IBA+1.0 Myo	3.86 ± 0.38 b	14.20 ± 1.16 a	0.035 ± 0.004 bcd	0.004 ± 0.001 cd	93.33 h
ANOVA	IBA	***	*	***	***	***
	Myo	***	***	***	***	***
	IBA×Myo	***	**	***	***	***
Gisela 6	Control	2.00 ± 0.12 a	17.43 ± 0.78 b	0.030 ± 0.001 a	0.003 ± 0.001 a	30.00 a
	0.5 IBA+0.5 Myo	4.57 ± 0.49 ab	27.42 ± 2.02 d	0.048 ± 0.003 ab	0.006 ± 0.001 b	70.00 d
	0.5 IBA+1.0 Myo	4.33 ± 0.77 ab	20.41 ± 2.42 bc	0.026 ± 0.003 a	0.003 ± 0.001 a	90.00 f
	0.5 IBA+2.0 Myo	5.00 ± 0.52 bc	20.51 ± 2.10 bc	0.045 ± 0.003 a	0.006 ± 0.001 b	80.00 e
	1 IBA	6.30 ± 0.53 bcd	21.55 ± 1.86 bc	0.067 ± 0.004 b	0.010 ± 0.001 c	100.00 g
	1 IBA+0.5 Myo	7.40 ± 0.20 cde	25.13 ± 0.90 cd	0.090 ± 0.006 c	0.011 ± 0.001 c	55.56 b
	1 IBA+1.0 Myo	8.33 ± 0.47 de	19.70 ± 1.21 b	0.090 ± 0.002 c	0.009 ± 0.001 c	60.00 c
	2 IBA	6.25 ± 0.81 bcd	11.17 ± 1.40 a	0.142 ± 0.011 d	0.015 ± 0.001 d	80.00 e
	2 IBA+0.5 Myo	9.91 ± 1.32 e	17.73 ± 1.91 b	0.200 ± 0.015 e	0.019 ± 0.001 e	91.67 f
	2 IBA+1.0 Myo	8.33 ± 1.44 de	19.17 ± 1.52 b	0.157 ± 0.010 d	0.016 ± 0.001 d	90.00 f
ANOVA	IBA	***	***	***	***	***
	Myo	***	***	***	***	***
	IBA×Myo	**	**	*	ns	***

Table 2. Effect of IBA alone and in combination with myo-inositol on shoot length, fresh mass, dry mass, and chlorophyll content of the cherry rootstocks CAB-6P and Gisela 6. Means ± SE, n = 10. Those denoted by the same letter are not significantly different according to Duncan's multiple range test at $P \leq 0.05$. Significant effects at $P \leq 0.05$ (*), 0.01 (**), or 0.001 (***) according to 2-way *ANOVA*.

Rootstock	Treatments [mg dm^{-3}]	Shoot length [mm]	Fresh mass [g]	Dry mass [g]	Chl *a*+*b* [mg g^{-1}(f.m.)]	Chl *a*+*b* [mg g^{-1}(d.m.)]
CAB-6P	control	24.17 ± 0.43 e	0.099 ± 0.001 ab	0.010 ± 0.001 ab	3.334 ± 0.441 bc	15.117 ± 2.078 a
	0.5 IBA+0.5 Myo	13.75 ± 0.74 bc	0.170 ± 0.011 cd	0.017 ± 0.001 cd	1.459 ± 0.435 a	12.385 ± 3.483 a
	0.5 IBA+1.0 Myo	11.25 ± 0.38 ab	0.139 ± 0.012 bc	0.014 ± 0.001 bc	1.596 ± 0.257 a	12.330 ± 2.043 a
	0.5 IBA+2.0 Myo	10.42 ± 1.32 a	0.215 ± 0.025 e	0.022 ± 0.003 e	1.560 ± 0.337 a	14.927 ± 2.617 a
	1 IBA	16.50 ± 1.21 cd	0.124 ± 0.013 abc	0.013 ± 0.001 abc	4.113 ± 0.637 c	25.321 ± 3.803 b
	1 IBA+0.5 Myo	15.10 ± 0.84 cd	0.144 ± 0.015 c	0.015 ± 0.001 c	2.175 ± 0.361 ab	19.274 ± 3.461ab
	1 IBA+1.0 Myo	16.15 ± 1.32 cd	0.147 ± 0.011 c	0.015 ± 0.001 c	1.586 ± 0.324 a	13.075 ± 1.498 a
	2 IBA	17.31 ± 1.34 d	0.084 ± 0.008 a	0.008 ± 0.001 a	3.213 ± 0.424 bc	26.874 ± 2.744 b
	2 IBA+0.5 Myo	13.85 ± 1.96 bc	0.204 ± 0.014 de	0.020 ± 0.001 de	1.653 ± 0.272 a	14.785 ± 2.877 a
	2 IBA+1.0 Myo	10.33 ± 0.25 a	0.170 ± 0.021 cd	0.017 ± 0.002 cd	1.231± 0.198 a	11.447 ± 0.813 a
ANOVA	IBA	***	ns	ns	ns	*
	Myo	***	***	***	***	***
	IBA×Myo	*	**	**	ns	ns
Gisela 6	Control	19.00 ± 1.03 a	0.264 ± 0.021 b	0.026 ± 0.002 b	1.939 ± 0.210 ab	13.580 ± 0.265 a
	0.5 IBA+0.5 Myo	22.00 ± 0.91 abcd	0.187 ± 0.009 a	0.019 ± 0.001 a	2.672 ± 0.762 abc	20.545 ± 3.916 a
	0.5 IBA+1.0 Myo	22.50 ± 0.69 abcd	0.340 ± 0.021 c	0.034 ± 0.002 c	2.237 ± 0.570 abc	14.396 ± 2.900 a
	0.5 IBA+2.0 Myo	20.00 ± 1.07 ab	0.218 ± 0.010 ab	0.022 ± 0.001 ab	2.402 ± 0.130 abc	18.735 ± 3.875 a
	1 IBA	23.00 ± 1.26 bcd	0.313 ± 0.022 c	0.031 ± 0.002 c	2.535 ± 0.776 abc	18.327 ± 4.494 a
	1 IBA+0.5 Myo	21.67 ± 1.23 abc	0.234 ± 0.008 ab	0.023 ± 0.001 ab	1.813 ± 0.208 ab	19.118 ± 3.303 a
	1 IBA+1.0 Myo	23.00 ± 1.10 bcd	0.251 ± 0.006 b	0.025 ± 0.001 b	4.036 ± 1.275 c	17.826 ± 2.460 a
	2 IBA	21.50 ± 1.38 abc	0.239 ± 0.021 b	0.024 ± 0.002 ab	1.167 ± 0.251 a	16.006 ± 0.774 a
	2 IBA+0.5 Myo	25.42 ± 1.30 d	0.559 ± 0.018 d	0.056 ± 0.002 d	2.415 ± 0.065 abc	22.737 ± 0.769 a
	2 IBA+1.0 Myo	24.00 ± 1.35 cd	0.349 ± 0.021 c	0.035 ± 0.002 c	3.263 ± 0.572 bc	32.633 ± 5.719 b
ANOVA	IBA	*	***	***	ns	ns
	Myo	ns	***	***	ns	ns
	IBA×Myo	ns	***	***	ns	*

content in the leaves was observed at the 2 mg dm^{-3} IBA + 1 mg dm^{-3} myo-inositol. Also the proline accumulation in leaves of CAB-6P was not significantly influenced by IBA concentration. However, the combination of IBA with myo-inositol significantly reduced the proline content in the leaves. Regarding the roots, there was a significant reduction in the proline content when the culture medium was supplemented with IBA individually or with myo-inositol.

For Gisela 6, the leaf chlorophyll content expressed per FM basis was 2 times higher in the medium with 1 mg dm^{-3} IBA + 1 mg dm^{-3} myo-inositol and it was 2.5 times higher in the medium with 2 mg dm^{-3} IBA + 1 mg dm^{-3} myo-inositol when expressed per DM basis (Table 2) in comparison to the control. There were no statistically significant alteration in the sugar content in the leaves of the Gisela 6 rootstock when any amount of myo-inositol is combined with the lowest IBA concentration (0.5 mg dm^{-3}) (Table 3). However, the sugar content in the leaves was reduced at higher IBA concentrations alone or in combination with myo-inositol. The maximum proline content in the leaves and the roots, and the sugar content in the roots were observed in the control treatment. With the exception of 0.5 and 1 mg dm^{-3} IBA combined with 0.5 mg dm^{-3} myo-inositol, all other treatments reduced leaf proline content. Myo-inositol, irrespective of its concentration, in combination with the highest IBA concentration significantly decreased the sugar and proline content in the roots. On the other hand, there was no significant change in the proline content in the roots at 1 mg dm^{-3} IBA alone or in combination with myo-inositol.

Discussion

The results of the present paper indicate that different concentrations of IBA and myo-inositol exerted different effects on the rooting characteristics, shoot growth, and on the various biochemical parameters studied. Moreover, the rootstocks used in the experiment showed different responses. For example, low concentrations of myo-inositol combined with a high IBA concentration increased the root FM and DM of Gisela 6 whereas decreased them in CAB-6P. Our findings regarding the Gisela 6 rootstock are in agreement with those

Table 3. Effect of IBA alone and in combination with myo-inositol on content of sugars and proline in the leaves and roots of cherry rootstocks CAB-6P and Gisela 6. Means ± SE, n = 10. Those denoted by the same letter are not significantly different according to Duncan's multiple range test at $P \leq 0.05$. Significant effects at $P \leq 0.05$ (*), 0.01 (**), or 0.001 (***) according to 2-way *ANOVA*.

Rootstock	Treatments [mg dm^{-3}]	Leaf sugar content [μmol g^{-1}(f.m.)]	Leaf proline content [μmol g^{-1}(f.m.)]	Root sugar content [μmol g^{-1}(f.m.)]	Root proline content [μmol g^{-1}(f.m.)]
CAB-6P	control	36.938 ± 1.415 abc	3.209 ± 0.266 b	36.444 ± 11.675 a	6.543 ± 2.242 b
	0.5 IBA+0.5 Myo	40.796 ± 3.558 bc	1.255 ± 0.124 a	45.826 ± 5.369 a	2.441 ± 0.563 a
	0.5 IBA+1.0 Myo	34.924 ± 3.927 abc	1.103 ± 0.292 a	32.000 ± 3.214 a	2.232 ± 0.524 a
	0.5 IBA+2.0 Myo	31.082 ± 6.892 ab	1.348 ± 0.025 a	46.853 ± 6.587 a	1.962 ± 0.487 a
	1 IBA	36.709 ± 1.730 abc	2.745 ± 0.017 b	32.773 ± 7.027 a	1.492 ± 0.102 a
	1 IBA+0.5 Myo	31.121 ± 1.822 ab	1.041 ± 0.181 a	49.190 ± 4.913 a	1.950 ± 0.291 a
	1 IBA+1.0 Myo	29.005 ± 4.715 a	0.993 ± 0.168 a	44.538 ± 1.326 a	1.577 ± 0.321 a
	2 IBA	40.567 ± 2.591 abc	3.006 ±0.756 b	39.303 ± 4.572 a	1.504 ± 0.083 a
	2 IBA+0.5 Myo	42.487 ± 2.889 bc	1.021 ± 0.146 a	45.068 ± 5.094 a	1.416 ± 0.087 a
	2 IBA+1.0 Myo	45.512 ± 2.993 c	1.418 ± 0.367 a	38.115 ± 4.381 a	1.616 ± 0.099 a
ANOVA	IBA	*	ns	ns	***
	Myo	ns	***	ns	ns
	IBA×Myo	ns	ns	ns	ns
Gisela 6	Control	70.502 ± 5.044 de	3.297 ± 0.100 e	71.357 ± 11.266 d	4.669 ± 1.469 c
	0.5 IBA+0.5 Myo	64.800 ± 5.660 cd	3.060 ± 0.041 de	67.005 ± 0.214 d	2.416 ± 0.067 ab
	0.5 IBA+1.0 Myo	64.453 ± 6.040 cd	2.654 ± 0.105 bc	48.403 ± 2.614 bc	3.094 ± 0.593abc
	0.5 IBA+2.0 Myo	76.969 ± 0.923 e	2.815 ± 0.013 cd	58.903 ± 0.214 cd	2.530 ± 0.067 ab
	1 IBA	56.294 ± 0.998 c	2.555 ± 0.047 abc	62.291 ± 2.594 cd	3.034 ± 0.311abc
	1 IBA+0.5 Myo	61.555 ± 1.586 cd	3.091 ± 0.080 de	49.110 ± 0.065 bc	4.554 ± 0.933 c
	1 IBA+1.0 Myo	40.648 ± 3.777 ab	2.486 ± 0.054 ab	59.333 ± 2.684 cd	4.012 ± 0.252 bc
	2 IBA	45.284 ± 1.546 b	2.830 ± 0.073 cd	36.683 ± 4.827 ab	1.557 ± 0.092 a
	2 IBA+0.5 Myo	32.744 ± 0.769 a	2.448 ± 0.113 ab	32.533 ± 3.445 a	1.736 ± 0.172 a
	2 IBA+1.0 Myo	36.383 ± 2.079 ab	2.294 ± 0.215 a	36.952 ± 7.591 ab	1.507 ± 0.053 a
ANOVA	IBA	***	***	***	***
	Myo	**	***	ns	ns
	IBA×Myo	**	**	*	ns

for the medicinal plant *Hyoscyamus niger*, where 5.55 to 33.3 mM myo-inositol in combination with 0.5 mg dm^{-3} IBA significantly increased root FM and DM (Hong *et al*. 2010). The increase in the root FM can be attributed to an increase in cell volume and/or expansion of new cells. Rapid cell division or expansion requires certain quantities of cell wall biosynthesis precursors. Both IBA and inositol play significant roles in cell wall biosynthesis and signal transduction (Lott *et al*. 1995). The promotory effect of myo-inositol on root mass in the Gisela 6 rootstock indicates that its amount in the MS medium is suboptimal. Similar results were reported after IAA and myo-inositol addition in the medium for carrot tissues cultured *in vitro* (Neumann and Raafat 1973). On the other hand, the inhibitory effects of myo-inositol on CAB-6P are in agreement with those reported by Teixeira da Silva *et al*. (2006) in the *Cymbidium* hybrid. Crozier *et al.* (2000) reported that binding auxins to inositols leads to their inactivation which might explain the inhibitory effect that myo-inositol added to IBA had on the root elongation of CAB-6P explants. In contrast to this result, the addition of 10 or 100 mg dm^{-3} myo-inositol to a culture of *Pteris* doubled the rate of root elongation (Goforth and Torrey 1977). An exception was recorded in the Gisela 6 rootstock, where low myo-inositol concentration promoted root length only in conjunction with the 0.5 and 1 mg dm^{-3} IBA. Therefore, to give maximum response for each rootstock, a certain optimum IBA and myo-inositol concentration or a combination of the two is needed.

In the present study, myo-inositol limited the rooting percentage in both the CAB-6P and Gisela 6 explants. Similar results were reported by Donnelly (1976) in raspberries. However, in other plant species, myo-inositol improved rooting (Jarvis and Booth 1981). In Gisela 6 explants, myo-inositol reinforced the positive effect of IBA (2 mg dm^{-3}) concerning the number of roots. Our data further suggest that myo-inositol enabled to divert the explants from cell division to cell differentiation and root formation. Similarly to the Gisela 6 explants, myo-inositol increased the number of roots in *Haworthia* (Kaul and Sabharwal 1975). A combination of myo-inositol, IBA, and sucrose increased the cambial activity and cell division in roots of *Raphanus* (Torrey and Loomis 1967) and for turnip (Peterson 1973). In contrast, the reduced number of roots per rooted explant in CAB-6P when myo-inositol (1 mg dm^{-3}) was added to the culture medium containing IBA (2 mg dm^{-3}) was due to reduced cell division. According to Biffen and Hanke (1990) myo-inositol has a role in maintaining cell division in soybean callus tissue. In the CAB-6P explants, myo-inositol exogenously applied, possibly increased its

intracellular content in the roots to toxic level, leading to reduced cell division and root number.

In the present study, 0.5 mg dm^{-3} myo-inositol plus 2 mg dm^{-3} IBA gave better results regarding the root number and the root FM and DM per rooted explant in the Gisela 6 rootstock than 2 mg dm^{-3} IBA alone. This enhancement on rooting characteristics can be ascribed to the strong polar basipetal movement of myo-inositol and IBA from shoot apex to roots apex, leading to increased cell division. The same hypothesis has been reported by Kruszewski and Jacobs (1974) for auxins and thiamine in tomato. We did not observe any such synergistic effect in CAB-6P.

The decrease in the rooting parameters of CAB-6P in the presence of IBA and myo-inositol could be explained by a couple of reasons, *e.g*., acceleration of IBA degradation due to the additional application of myo-inositol or inactivation of IBA due to the production of complexes with amino acids, sugars, and inositols (Wiesman *et al.* 1989). The involvement of myo-inositol in IAA metabolism (Epstein *et al*. 1980) and its beneficial effect on the rooting of *in vivo* pea cuttings (Jarvis and Booth 1981) suggest that inositols may help to regulate the formation of adventitious roots.

Our results showed that the increase in myo-inositol concentration inhibits the shoot length of both rootstocks which is in agreement with the results by Sepahvand *et al*. (2012) using the peach rootstock GF-677. However, Al-Sulaiman (2010) found no significant effects of myo-inositol on the shoot length of *Ziziphus spina-christi*.

The increase in shoot FM and DM of both rootstocks by IBA and myo-inositol application can be attributed to an increase in thickness which does not occur uniformly. Apart from the different growth rate among the plant parts, a temporal change in growth also occurs.

It is generally known that the increase in the shoot length of the explant and therefore in leaf number results in a higher leaf area index (LAI) and chlorophyll content, as well as greater photosynthetic rate. It must be noted that although chlorophyll content is not directly related to photosynthetic capacity (Fujiwara *et al*. 1992), it is a good indicator of the status of the photosynthetic apparatus (Seon *et al*. 2000). This is important in the Gisela 6 rootstock, where 1 mg dm^{-3} myo-inositol in combination with the highest IBA concentration increased leaf chlorophyll content, when compared to the effects of IBA (2 mg dm^{-3}) alone. In contrast, there was a negative effect of IBA and myo-inositol on chlorophyll content in CAB-6P. These findings agree with Neumann and Raafat (1973) who observed a decrease in chlorophyll content in carrot tissues *in vitro* due to the addition of IAA and myo-inositol. On the other hand, chlorophyll content in *Cymbidium* was not significantly altered by myo-inositol (Teixeira da Silva *et al*. 2006). Furthermore, in *Orthosiphon stamineus* grown *in vitro*, IBA did not modify the chloroplast ultrastructure (Stoyanova-Koleva *et al*. 2012) whereas Guha and Usha Rao (2012) found that under Mg^{+2} deficiency and in the absence of the naphthalene acetic acid, the ultrastructure of the cortical cells of *Cymbidium* showed progressive disorganization and disintegration of the chloroplast membranes.

Application of IBA has been associated with an increased content of soluble sugars and proline in pea (El-Shraiy and Hegazi 2009), onion (Sing *et al*. 1995), and maize (Amin *et al*. 2006). This is in contrast to our findings where the application of IBA negatively affected the content of proline and sugars in the leaves and roots of the Gisela 6 whereas it had no effect on CAB-6P.

Inositols are involved in the metabolism of sugars as precursors of cell wall components (Loewus and Loewus 1980) and the application of myo-inositol increased its endogenous content in soybean leaves (Kosina *et al.* 2009). In the CAB-6P rootstock, myo-inositol showed no correlation with sugar or proline content in the leaves or roots, irrespective of the IBA concentration. In contrast, in the Gisela 6, there was a decrease in the sugar content in the roots due to myo-inositol (1 mg dm^{-3}) in the presence of 0.5 mg dm^{-3} IBA. Ernstsen and Hansen (1986) found thatthe soluble sugar content in pine cuttings was increased during the adventitious root formation whereas the endogenous content of myo-inositol and inositol derivatives was diminished. In the Gisela 6 rootstock, the external application of myo-inositol possibly increased its endogenous content in the roots, resulting in the decrease of sugar content.

In the present study, myo-inositol decreased the proline content in the leaves of CAB-6P, but in the case of the Gisela 6 this decrease occurred only when myo-inositol was applied together with the highest IBA concentration. The function of proline in stressed plants is often explained by its property as an osmolyte (Saradhi *et al*. 1995) as well as its other positive roles which include the stabilization of proteins (Anjum *et al*. 2000), the scavenging hydroxyl radicals (Smirnoff and Cumbes 1989), and the regulation of the cytosolic pH (Venekamp 1989).

In conclusion, the findings indicate that myo-inositol affects *in vitro* rooting of CAB-6P and Gisela 6 rootstocks. Furthermore, it is clear that it influences leaf chlorophyll content, sugar biosynthesis and metabolism, as well as the proline accumulation in both the leaves and the roots.

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