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Exogenous auxin improves root morphology and restores growth of grafted cucumber seedlings

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Abstract

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The aim of this study was to investigate the effects of exogenously applied auxin over the rootstock cuttings on root morphology parameters and stand establishment rate of salt-stressed cutting grafted cucumber seedlings. For that purpose, before grafting, the cut ends of the rootstocks were soaked for few seconds into auxin solution (indole-3-acetic acid (IAA) or indole-3- butyric acid (IBA))at different concentrations and afterwards were grafted by the root pruned splice grafting (RPSG) method. Ten days after grafting, the grafted seedlings were transplanted into individual pots where two different levels of salt-stress (0 and 50 mM) were established. Root morphology parameters, as well as dry matter of the root system and the whole plants were successively analysed to randomly selected plants. Exogenous auxins improved root morphology parameters and restored root growth under salinity conditions. The best results were obtained through the application of IBA, which promoted a better rootstock – scion relationship, presumably due to faster development of phloem and xylem tubes, and the promotion of a vigorous root system which increases plant's absorbing capabilities for water and nutrients under adverse soil conditions.

Keywords: root length; root surface area; root volume; relative growth rate; salinity

The use of grafted seedlings has been increasingly popular in the production of many fruit-bearing vegetables such as watermelon, cucumber, oriental melon, muskmelon, tomato, eggplant and red pepper (Lee 2007). Although the main purpose of grafted seedlings is to increase the yield and quality of fruits by combining a disease resistant rootstock with a genetically superior scion (Lee 2007), improved tolerance to environmental stresses such as low soil temperatures, flooding, salinity and high B concentrations has also been shown (Edelstein et al. 2012).

During the entire history of vegetable grafting, a number of grafting methods have been employed including insertion graft, inarching, and the use of inter-stock, approach graft, cleft graft, pin-graft, and side graft (Lee 2007; Yassin, Hussen 2015). Recently, the cutting grafting method (also known as root pruned splice grafting; RPSG), a recent development of common splice grafting method (SG) is used in the production of fruit vegetables, particularly among the Cucurbitaceae and Solanaceae. In case of common splice grafting (SG) method, one cotyledon, along with the visible growing point of the rootstock, is cut with a razor blade following the angle of the leaf petiole. Following that, the hypocotyl of the scion is cut on a 35° to 45° angle on one side only, and the two cut surfaces are matched and held together with a grafting clip (Hassell et al. 2008). Root pruning splice grafting (RPSG) employs the same procedures as SG methods except the rootstock hypocotyl is cut (Fig. 1a) to remove the roots (Davis et al. 2008). Therefore, after grafting, the grafted cuttings must be transplanted into growing medium for rooting (Shibuya et al. 2007).

Different from common grafting methods (SG) when the root system is intact, in the cutting graft-

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ing method (RPSG) new adventitious roots start to develop soon after grafted cuttings are planted into growing substrate (Fig. 1b). Thus, cutting grafted seedlings go over simultaneous processes of meristem development at the grafting point and the initiation and growth of a new adventitious root system at the cut end of rootstock hypocotyl. In a recent published paper, root excised grafted (RPSG) muskmelon seedlings started to show active and rapid root regeneration at 8 days after grafting (DAG) and reached similar root length and surface area as the root-intact plants at 16 DAG (Guan, Zhao 2015). Earlier reports showing that thanks to a prioritized assimilate allocation toward, the newly started roots grew very fast, and by the end of nursery period the dry matter of the root system in RPSG grafted seedlings reached that of intact roots of common splice grafted (SG) seedlings (Lee et al. 2010; Babaj et al. 2014). There are also evidences that RPSG seedlings grew faster than intact SG seedlings immediately after transplanting and could better resist the negative effects of salinity (Balliu et al. 2014).

The anatomical changes that occur during graft union formation include the death of cell layers at the graft interface, cohesion of the scion and rootstock (RS), proliferation of callus cells at the graft interface, and vascular differentiation across the graft interface to establish vascular continuity (Aloni et al. 2008), while adventitious rooting (AR) in vegetative cuttings passes through two main phases: (a) induction, and (b) formation (da Costa et al. 2013). There are several reports showing that both; graft union formation and AR formation are largely dependent on the presence and concentration of plant growth hormones (mostly auxin) (Ye 2002; Overvoorde et al. 2010; Aloni 2013, 2014; Diaz-Sala 2014). Auxin can also act when exogenously supplied, entering the stem via the cut surface of cuttings by inducing cell dedifferentiation, leading to a new root meristem later on (da Costa et al. 2013). Because of that, the use of exogenously supplied auxin is a common practice of vegetative propagation of many ornamental plants, fruits and forest trees.

Although the importance of plant growth regulators for improving the performance of grafted vegetable seedlings has long been recognized, their implementation in the field has not been materialized, mostly because the risks vs. benefits to the grafted crops have not been defined yet (Aloni et al. 2010). Accordingly, information about the possible effects of exogenously applied auxin on the intensity of adventitious root formation and rootstock-scion relationships of cutting grafted vegetable seedlings is very rare. Therefore, the aim of this experiment was to study the effects of different exogenous auxin compounds (indole-3-acetic acid (IAA) and indole-3- butyric acid (IBA)) application on the improvement of rooting and healing rate of cutting grafted (RPSG) cucumber seedlings and of their growth performance under salinity stress conditions immediately after transplanting.

MATERIALS AND METHODS

Plant material and experimental set-up. Graded seeds of cucumber (Ekron F_1) and rootstock; *Cucur-*

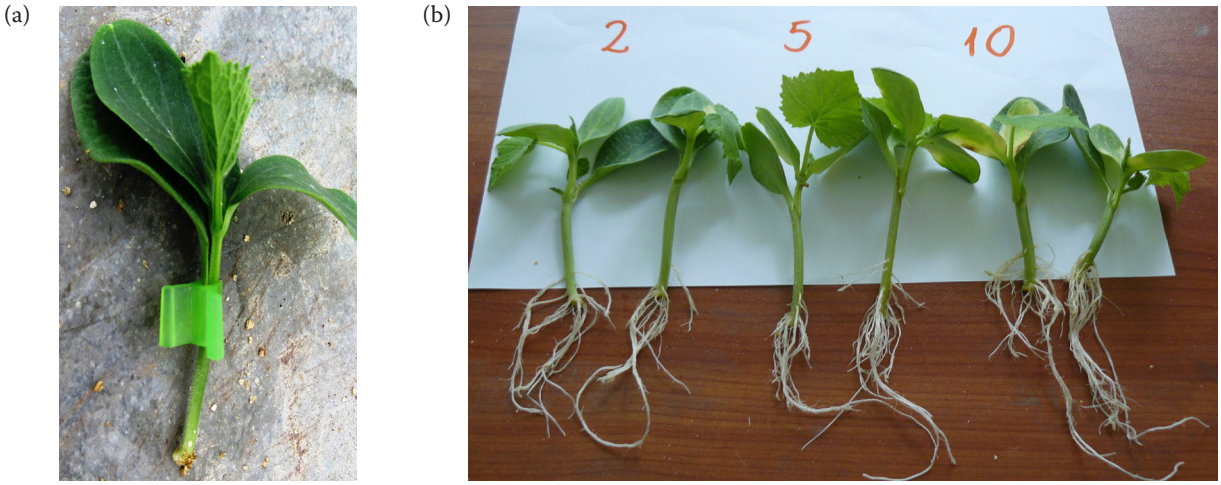

Fig. 1. The grafting moment by RPSG method (a) and adventious root development in IBA 2, 5 and 10 ppm treated grafted cucumber seedlings, 10 days after grafting (b)

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bita maxima × *C. moschata* (cv. 'Nimbus' F_1) were sown in foam trays filled with vermiculite. Fourteen days after sowing, grafting was conducted by the RPSG (graft cutting) method. The cuttings were obtained by excising the rootstock plants at the root neck, and then auxin application was conducted by basal quick deep method (Boyer, Blythe 2013). For that purpose, IAA or IBA were prepared, each at 4 different concentrations (5, 10, 20 and 50 ppm). The cut end of the rootstocks were soaked in the solution of the respective variant for few seconds and after grafting was conducted, grafted cuttings were transplanted in individual cells (60 cm^3 each) of foam containers filled with vermiculite. An equal number of grafted plants were used as control (no auxin application on the cut end of rootstock cuttings). Immediately after grafting, the containers were placed in a growth chamber (KBW 400, Keison Products, Essex, England) maintained at an air temperature of 26°C, a relative humidity (RH) of 100% at three successive days and then gradually decreased to 90%, and a PPFD (Photosynthetic Photon Flux Density) of 100μmol/m^2·s with a photoperiod of 12 hours. White fluorescent lamps were used for illumination.

10 days after grafting, grafted seedlings were individually transplanted into 200 cm^3 plastic pots filled with nutrient-saturated (1 g/l Terraflex T; N, P_2O_5, K_2O 18%, 7%, 25% + TE; ICL Fertilizers, Belgium) vermiculite. Two different levels of salt-stress (0 and 50 mM NaCl) were established by the addition of different amounts of sodium chloride (NaCl) to the nutrient solution. Control seedlings (no auxin application) and IAA and IBA treated seedlings were equally distributed to both salinity treatments according to a full factorial design with the following factors; auxin (IAA; IBA), auxin concentration (0, 5, 10, 20, 50 ppm) and salinity (0, 50 mM NaCl). After transplanting, the plants were equally watered with the respective nutrient solutions until 17 days after grafting, maintaining appropriate substrate humidity in the pots.

Plant sampling and measurements. Five, ten and seventeen days after grafting, 10 plants were randomly selected for each treatment, harvested and then dissected and separated into roots, stems and leaves. The root system was gently washed free of adhering vermiculate particles, and scanned with an Epson Expression/STD 4800 Scanner. Subsequently, the acquired root images were analysed with the WinRHIZO Arabidopsis software (Regent Instruments Inc., Quebec, Canada), and the growth parameters of root system; root length (RL), root projected area (RPA), root surface area (RSA), root volume (RV) and root tips (RT) were measured and recorded. The plant organs were subsequently dried (65°C, 48 h) and dry matter (DM) of roots and shoots of each sample was separately weighed to an accuracy of 0.001 g (TP 303; Denver Instruments GmbH, Göttingen, Germany). Based on the determined dry matter, the relative growth rate (RGR) of the whole plant (RGR_{Plant}) and the root system (RGR_{Root}) between DAG 10 and 17 were calculated for each treatment according to methods described by Hoffmann and Poorter (2002), Hunt et al. (2002) and Hunt (2003). Both parameters were used as indicators to assess the growth rate of grafted seedlings immediately after transplanting under different NaCl treatments.

Statistics. A ten-replicate complete randomized factorial block design was used. Differences in DM, RGR, RL, RPA, RSA, RV and RT were tested by three way ANOVA, using the PC program StatPlus 2009 (AnalystSoft Inc., Walnut, USA). Each significant ANOVA result ($P < 0.05$) was followed by the Tukey-Kramer test at $P < 0.05$ as post-hoc test to compare pair-wise means within and among treatments. Values given throughout the text are means ± SD.

RESULTS AND DISCUSSION

The relationships between scion and stock in grafted seedlings are affected by growth regulators (Pina, Errea 2005). Hormonal signals, auxin in particular, are believed to play an important role in the wound healing and vascular regeneration within the graft union zone (Pina, Errea 2005; Goldschmidt 2014), as well as profoundly influence root morphology, increasing lateral root production and inducing adventitious roots (Aloni et al. 2006; Overvoorde et al. 2010; Woodward, Bartel 2005). The present data as well confirm the influence of exogenous auxin application on adventitious root formation and root architecture in the rootstocks of root pruned splice grafted (RPSG) cucumber seedlings. Five and ten days after grafting, total root length (RL), root projected area (RPA), root surface area (RSA) and root volume (RV) and the number of root tips (RT) of IAA and IBA treated grafted cucumber seedlings were significantly higher than in the control seedlings (Table 1).

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Table 1. Total root length (RL), root projected area (RPA), root surface area (RSA), root average diameter (RAD), root volume (RV), and the number of root tips (RT) of IAA (0, 5, 10, 20, 50 ppm) and IBA (0, 5, 10, 20, 50 ppm) treated cutting grafted cucumber seedlings 5 and 10 days after grafting

Auxin	Concent.	RL (cm)	RPA (cm^2)	RSA (cm^2)	RAD (mm)	RV (cm^3)	RT (No.)
5 days after grafting							
	0	34.7 ± 4.1^{b}	2.89 ± 0.2^{b}	9.1 ± 0.6^{b}	0.87 ± 0.2	0.20 ± 0.01^{b}	154 ± 25^{ab}
	5	48.0 ± 3.8^{a}	3.66 ± 0.5^{a}	11.7 ± 1.3^{a}	0.88 ± 0.2	0.24 ± 0.02^{ab}	145 ± 30^{ab}
IAA	10	52.8 ± 3.7^{a}	3.84 ± 0.4^{a}	12.0 ± 1.4^{a}	0.73 ± 0.1	0.22 ± 0.02^{ab}	172 ± 41^{a}
	20	52.1 ± 5.8^{a}	3.68 ± 0.3^{a}	11.5 ± 1.0^{a}	0.77 ± 0.1	0.22 ± 0.02^{ab}	169 ± 38^{a}
	50	37.8 ± 5.7^{b}	3.01 ± 0.4^{ab}	9.4 ± 0.8^{b}	0.82 ± 0.1	0.19 ± 0.02^{b}	111 ± 14^{b}
	0	35.7 ± 4.4^{b}	2.89 ± 0.2^{b}	9.1 ± 0.6^{b}	0.87 ± 0.2	0.20 ± 0.01^{b}	154 ± 25^{ab}
	5	45.0 ± 5.6^{a}	3.57 ± 0.4^{a}	10.9 ± 1.0^{a}	0.75 ± 0.1	0.21 ± 0.02^{ab}	154 ± 26^{ab}
IBA	10	41.4 ± 3.4^{ab}	3.38 ± 0.3^{a}	10.6 ± 1.2^{ab}	0.91 ± 0.2	0.24 ± 0.02^{ab}	119 ± 15^{ab}
	20	46.2 ± 4.0^{a}	4.12 ± 0.4^{a}	12.9 ± 1.3^{a}	0.93 ± 0.1	0.28 ± 0.04^{a}	158 ± 30^{ab}
	50	29.8 ± 4.7^{b}	2.95 ± 0.6^{b}	9.2 ± 1.3^{b}	0.99 ± 0.1	0.23 ± 0.02^{ab}	118 ± 12^{ab}
Significance							
Auxin type (H)		***	ns	ns	*	***	ns
Concentration (C)		***	***	***	ns	***	***
A × C		***	*	**	*	***	**
10 days after grafting							
	0	124.0 ± 9.7^{c}	6.47 ± 0.7^{b}	20.3 ± 3.3^{b}	0.59 ± 0.14^{a}	0.29 ± 0.03^{c}	531 ± 59b
	5	131.0 ± 18^{bc}	6.07 ± 0.8^{b}	19.1 ± 1.9^{c}	0.46 ± 0.02^{b}	0.22 ± 0.02^{c}	506 ± 57^{b}
IAA	10	154.5 ± 19^{b}	7.29 ± 1.0^{ab}	22.9 ± 3.1^{b}	0.48 ± 0.05^{b}	0.27 ± 0.15^{c}	583 ± 71^{b}
	20	183.4 ± 14^{a}	8.72 ± 0.8^{a}	27.4 ± 2.8^{b}	0.48 ± 0.03^{b}	0.32 ± 0.02^{b}	712 ± 56^{a}
	50	132.0 ± 14b^{c}	5.69 ± 0.8^{b}	17.8 ± 2.0^{c}	0.43 ± 0.05^{b}	0.19 ± 0.04^{d}	548 ± 114^{b}
	0	124.0 ± 9.7^{c}	6.47 ± 0.7^{b}	20.3 ± 3.3^{b}	0.59 ± 0.14^{a}	0.29 ± 0.03^{c}	531 ± 59^{b}
	5	197.4 ± 17^{a}	8.94 ± 1.3^{a}	27.0 ± 3.2^{b}	0.44 ± 0.03^{b}	0.31 ± 0.04^{b}	627 ± 89^{b}
IBA	10	213.9 ± 19^{a}	10.25 ± 1.2^{a}	32.2 ± 3.3^{a}	0.48 ± 0.05^{b}	0.40 ± 0.05^{b}	800 ± 145^{a}
	20	185.8 ± 22^{a}	8.51 ± 1.1^{a}	26.7 ± 2.5^{b}	0.59 ± 0.06^{a}	0.59 ± 0.04^{a}	567 ± 119^{b}
	50	133.2 ± 12^{bc}	7.83 ± 0.8^{ab}	24.6 ± 2.5^{b}	0.60 ± 0.07^{a}	0.37 ± 0.06^{b}	609 ± 94^{b}
Significance							
Auxin type (A)		***	***	***	**	***	**
Concentration (C)		***	***	***	***	***	***
A × C		***	***	***	***	***	***

different letters indicate significant differences within each parameter (Tukey-Kramer test, $P < 0.05$; mean ± SD, $n = 10$); IAA – indole-3-acetic acid; IBA – indole-3- butyric acid; Concentr. – concentration; ns – non significant; *,**,*** – significant at P < 0.05, P < 0.01 and P < 0.001 respectively

The gradual increase of auxin concentration (IAA or IBA) from 5 to 20 ppm was followed by a steady increase of all root morphology parameters in auxin-treated versus the control plants (Table 1). However, similar to earlier reports (Aloni et al. 2006; Tanimoto, 2005) the further increase of auxin concentration to 50 ppm has dropped the root morphology parameters down to the level of control plants (no auxin application). The results fit well with the classical view of concentration dependency of auxin action on plant growth and the regulatory function of auxin in root growth.

As it is previously reported (Tanimoto 2005), in contrast to the accelerating effect on stem elongation, in relatively high concentration auxin strongly decelerates root elongation in a wide range of concentrations. Though the inhibition mechanism of root growth in grafted cucumber seedlings by

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such low levels of auxin (50 ppm) is not sufficiently explained, it is ascribed at least partially, to the growth inhibitory effect of ethylene produced by auxin action (Woodward, Bartel 2005; Aloni et al. 2011; Aloni 2013) or increased production of reactive oxygen species (ROS) such as superoxide radicals, hydrogen peroxide and hydroxyl radicals (Aloni et al. 2010). Meantime, the significantly higher number of root tips in auxin-treated plants (Table 1) indicates that the longest total root length of auxin-treated seedlings versus control was a consequence of a more branched root system, rather than longer individual roots. This finding fits well with the common presumption that auxin influences root morphology by inhibiting root elongation and increasing lateral root production (Overvoorde et al. 2010; da Costa et al. 2013; Kazan 2013).

As it has been largely reported (Copeman et al. 1996; Cuartero et al. 2006; Huang et al. 2009; Edelstein et al. 2011; Porcel et al. 2012) plants growth decreases with increasing salinity that severely inhibits root development (Estañ et al. 2005; Huang et al. 2009; Aloni et al. 2011) and greatly reduces plant stand establishment (Evelin et al. 2009). That was also fully confirmed by our results. One week after transplanting (DAG 10 to DAG 17) root length, root projected and root surface area, root volume, and total number of root tips in salt-stressed plants (50 mM NaCl), though not always significantly, were smaller than the respective values of non-salt-stressed plants (Table 2). The average root diameter was the only parameter that was not affected by the raise of salinity (Table 2).

Though the involvement of hormonal effects in the responses of grafted seedlings to abiotic stresses is not fully clear (Aloni et al. 2011), it seems that the use of plant hormones before grafting also alleviates negative effects of salinity over transplanted seedlings (DAG 10 to DAG17). After being exposed for a week (DAG 10 to DAG 17) to saline conditions, auxin-treated seedling (5, 10 and 20 ppm IBA) resulted with significantly higher values of root morphology parameters than the control plants (Table 2). Essentially, the enhanced salt tolerance of grafted vegetables has been often associated with specific characteristics of root system, such as root length and surface area, which play an active role in ions and water uptake (Himmelbauer et al. 2004; Colla et al. 2010). Considering these earlier findings, there is a speculation that the better growth performance (faster RGR, higher DM) of IBA treated grafted cucumber seedlings exposed to salinity stress (Table 3) might be attributed, at least to some extent, to their more abundant root system. Indeed, IBA applications (5, 10 and 20 ppm) have restored RL, RPA, RSA, RV, RT and RV to the level of control plants under non-saline conditions, or even higher than that (Table 3). Yet, no significant differences were found between IAA treated seedlings and control plants, neither in non-saline or saline conditions (Table 3). The differences between IBA and IAA treated plants were especially large regarding total root surface area and root volume. One week after transplanting (DAG 17) the respective average values of IBA seedlings were almost twice higher than IAA treated seedlings (Table 2).

The successful application of the grafting technique depends on the compatibility of the graft union in terms of fast formation of the vascular connection between the root stock and the scion and fast renewal of root and canopy growth (Aloni et al. 2008). In grafted plants, vascular elements are regenerated by complex processes which include structural differentiation of parenchymatous tissue into xylem and phloem tubes (Fernández-García et al. 2004; Aloni et al. 2008). The process was reported to be initiated 4–7 DAG, and fully developed by 11 DAG in melon (Aloni et al. 2008) and between 4 and 8 DAG and fully developed after 15 DAG in tomato (Fernández-Garcíaet al. 2004). Insufficient connection of vascular bundles between the scion and the rootstock decreases the water flow and consequently the scion growth decreased, too (Martínez-Ballesta et al. 2010). Considering that, the relative growth rate of plants (RGR_{Plant}) in a certain period could be considered as an integral indicator of grafting success and of the intensity of relationships between rootstock and scion. Therefore, the significantly higher values of RGR_{Plant} and RGR_{Root} (DAG 5 to DAG 17) in IBA treated seedlings (Table 3) indicated the faster development of their vascular bundles versus control and IAA treated plants. That led to a more intensive rootstock-scion communication, which subsequently resulted in significantly higher plant (DM_{Plant}) and root (DM_{Root}) dry matter in IBA treated seedlings by DAG 17 (Table 3).

The highest DM_{Root} and DM_{Plant} values were obtained at 10 and 20 ppm IBA variants (Table 4). Slight, but not significant differences were also

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Table 2. Total root length (RL), root projected area (RPA), root surface area (RSA), root average diameter (RAD), root volume (RV), and the number of root tips (RT) of IAA (0, 5, 10, 20, 50 ppm) and IBA (0, 5, 10, 20, 50 ppm) treated cutting grafted cucumber seedlings one week after transplanting (DAG 17) into pots with two different levels of soil salinity (0 and 50mM NaCl)

Auxin	Sal.	Concent.	RL (cm)	RPA (cm2)	RSA (cm^2)	RAD (mm)	RV (cm^3)	RT (No.)	RF (No.)
IAA	0	0	260 ± 30^{b}	10.5 ± 1.50^{c}	35.5 ± 3.3^{c}	0.41 ± 0.03^{c}	0.39 ± 0.07^{e}	935 ± 33^{b}	2,412 ± 358
		5	217 ± 28^{c}	8.57 ± 1.73^{cd}	26.9 ± 5.9^{cd}	0.39 ± 0.05^{d}	0.27 ± 0.05^{g}	724 ± 136^{bc}	1,742 ± 411
		10	306 ± 9^{a}	10.7 ± 0.62^{c}	38.1 ± 4.1^{c}	0.36 ± 0.04^{d}	0.34 ± 0.04^{f}	1,173 ± 152^{a}	2,978 ± 556
		20	224 ± 20^{c}	7.75 ± 0.86^{d}	24.4 ± 2.7^{d}	0.35 ± 0.04^{d}	0.21 ± 0.02^{f}	876 ± 93^{b}	1,775 ± 167
		50	218 ± 21^{c}	7.79 ± 0.80^{d}	24.5 ± 2.5^{d}	0.36 ± 0.05^{d}	0.22 ± 0.02^{f}	823 ± 152^{b}	1,712 ± 241
	50	0	180 ± 18^{c}	6.83 ± 0.76^{d}	21.6 ± 1.7^{d}	0.38 ± 0.06^{d}	0.21 ± 0.04^{f}	692 ± 101^{c}	1,516 ± 270
		5	209 ± 21^{c}	7.40 ± 1.03^{d}	23.2 ± 1.8^{d}	0.36 ± 0.02^{d}	0.21 ± 0.03^{f}	915 ± 28^{b}	1,714 ± 148
		10	226 ± 24^{c}	7.18 ± 0.88^{d}	22.4 ± 1.9^{d}	0.32 ± 0.03e	0.18 ± 0.02^{h}	783 ± 44^{bc}	1,768 ± 257
		20	202 ± 21^{c}	7.66 ± 1.27^{d}	24.0 ± 3.9^{d}	0.38 ± 0.04^{d}	0.23 ± 0.05^{g}	749 ± 100^{bc}	1,708 ± 248
		50	186 ± 24^{c}	6.56 ± 1.05^{d}	20.6 ± 2.5^{d}	0.36 ± 0.03^{d}	0.18 ± 0.03^{h}	800 ± 92^{bc}	1,473 ± 229
IBA	0	0	260 ± 30^{b}	10.50 ± 1.50^{c}	35.5 ± 3.3^{c}	0.41 ± 0.03^{c}	0.39 ± 0.07^{e}	935 ± 33^{b}	2,412 ± 358
		5	300 ± 30^{ab}	11.92 ± 1.31^{bc}	37.5 ± 5.0^{bc}	0.40 ± 0.05^{c}	0.38 ± 0.03^{e}	961 ± 78^{ab}	2,621 ± 398
		10	337 ± 27^{a}	14.54 ± 1.45^{b}	45.7 ± 4.5^{b}	0.43 ± 0.02^{c}	0.49 ± 0.06^{c}	1,102 ± 122^{a}	2,866 ± 247
		20	344 ± 23^{a}	17.62 ± 1.23^{a}	55.3 ± 4.6^{a}	0.51 ± 0.04^{a}	0.71 ± 0.07^{a}	1,138 ± 87^{a}	3,313 ± 317
		50	292 ± 34^{ab}	14.18 ± 1.91^{b}	44.5 ± 5.4^{b}	0.48 ± 0.07^{a}	0.55 ± 0.09^{b}	1,111 ± 83^{a}	2,650 ± 445
	50	0	180 ± 18^{c}	6.83 ± 0.76^{d}	21.6 ± 1.7^{d}	0.38 ± 0.06^{d}	0.21 ± 0.04^{g}	692 ± 101^{c}	1,516 ± 270
		5	263 ± 29^{b}	10.58 ± 1.34^{c}	33.2 ± 4.3^{c}	0.40 ± 0.02^{c}	0.33 ± 0.05^{f}	853 ± 72^{b}	2,044 ± 216
		10	246 ± 23^{b}	11.43 ± 1.47^{bc}	35.9 ± 3.7^{c}	0.46 ± 0.06^{b}	0.42 ± 0.07^{d}	932 ± 101^{b}	2,145 ± 257
		20	248 ± 23^{b}	13.36 ± 1.45^{b}	42.0 ± 4.4^{b}	0.54 ± 0.07^{a}	0.57 ± 0.10^{b}	850 ± 128^{b}	2,382 ± 220
		50	209 ± 23^{c}	11.99 ± 0.70^{bc}	35.8 ± 4.1^{c}	0.54 ± 0.06^{a}	0.47 ± 0.03^{c}	834 ± 77^{b}	2,131 ± 170
Significance									
Auxin type (A)			***	***	***	***	***	***	***
Hormone concentration (C)			***	***	***	***	***	***	***
Salinity (S)			***	***	***	ns	***	***	***
A × S × C			ns	*	***	ns	**	***	**

different letters indicate significant differences within each parameter (Tukey-Kramer test, $P < 0.05$; mean± SD, $n = 10$); DAG – days after grafting; Concent – concentration; Sal. – salinity; ns – non significant; *,**,*** – significant at $P < 0.05$, $P < 0.01$ and $P < 0.001$, respectively

found between control and IAA treated variants. The raise of salinity to 50 mM NaCl significantly reduced the dry weight of roots (DM_{Root}) and of the entire plant (DM_{Plant}) in control plants, but contrary to that, no significant reduction of root and plant dry matter was recorded in IAA and IBA treated seedlings. Interestingly, in 20 ppm IBA variant the dry matter of roots and the whole plant under salinity conditions (50 mM) was restored even at a higher level than that of control plants (no auxin application) under normal condition (Table 3).

As significant differences between auxin-treated and control seedlings were found since 5DAG, till there, no differences were found between IAA and IBA seedlings. Interestingly, gradually enlarging and statistically significant advantages of IBA versus IAA seedlings were recorded 10 and 17 day after grafting. That could be explained by the differences existing between IAA and IBA mode of action. While IAA has an immediate root promoting effect and is rapidly oxidized by the plant tissue (Štefančič et al. 2005), IBA acts as a storage form which is gradually converted to IAA and thus contributes to the maintenance of IAA gradients that are required for root development in a longer time span (Fusconi 2014; Woodward, Bartel2005). Hence, higher stabil-

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Table 3. DM_{Root}, DM_{Plant} and DM_{Root}: DM_{Plant} ratio of IAA (0, 5, 10, 20, 50 ppm) and IBA (0, 5, 10, 20, 50 ppm) treated cutting grafted cucumber seedlings, 10 and 17 days after grafting, and RGR_{Root} and RGR_{Plant} from 5 to 17 day after grafting (DAG), under two different levels of substrate salinity (0 and 50mM NaCl)

Auxin	Sal.	Concent.	10 DAG		17 DAG		5 DAG to 17 DAG	
			DM_{Root}	DM_{Plant}	DM_{Root}	DM_{Plant}	RGR_{Root}	RGR_{Plant}
IAA	0	0	0.018 ± 0.002	0.191 ± 0.03	0.027 ± 0.01^{b}	0.395 ± 0.04^{b}	0.238 ± 0.01^{c}	0.050 ± 0.01^{b}
		5	0.015 ± 0.005	0.213 ± 0.03	0.022 ± 0.01^{b}	0.375 ± 0.04^{b}	0.239 ± 0.01^{c}	0.039 ± 0.01^{bc}
		10	0.016 ± 0.011	0.200 ± 0.05	0.017 ± 0.00^{bc}	0.344 ± 0.05^{b}	0.237 ± 0.01^{c}	0.038 ± 0.01^{bc}
		20	0.020 ± 0.004	0.226 ± 0.02	0.019 ± 0.00^{b}	0.337 ± 0.03^{b}	0.244 ± 0.01^{bc}	0.042 ± 0.01^{b}
		50	0.012 ± 0.004	0.195 ± 0.02	0.023 ± 0.01^{b}	0.309 ± 0.04^{b}	0.249 ± 0.01^{bc}	0.032 ± 0.01^{c}
	50	0			0.013 ± 0.00^{c}	0.262 ± 0.08^{c}	0.212 ± 0.01^{d}	0.031 ± 0.01^{c}
		5			0.017 ± 0.00^{bc}	0.292 ± 0.07^{bc}	0.220 ± 0.02^{c}	0.028 ± 0.01^{c}
		10			0.016 ± 0.01^{bc}	0.330 ± 0.03^{b}	0.234 ± 0.01^{bc}	0.035 ± 0.01^{bc}
		20			0.017 ± 0.01^{bc}	0.344 ± 0.04^{b}	0.246 ± 0.01^{bc}	0.044 ± 0.01^{b}
		50			0.010 ± 0.00^{c}	0.274 ± 0.03^{c}	0.240 ± 0.01^{bc}	0.025 ± 0.00^{d}
IBA	0	0	0.018 ± 0.002	0.191 ± 0.03	0.027 ± 0.01^{b}	0.395 ± 0.04^{b}	0.238 ± 0.01^{c}	0.050 ± 0.01^{b}
		5	0.019 ± 0.005	0.194 ± 0.04	0.029 ± 0.01^{b}	0.424 ± 0.05^{ab}	0.255 ± 0.01^{b}	0.060 ± 0.01^{ab}
		10	0.025 ± 0.007	0.225 ± 0.03	0.036 ± 0.01^{ab}	0.441 ± 0.03^{ab}	0.269 ± 0.01^{a}	0.065 ± 0.01^{a}
		20	0.024 ± 0.011	0.213 ± 0.06	0.046 ± 0.01^{a}	0.498 ± 0.06^{a}	0.260 ± 0.01^{a}	0.067 ± 0.01^{a}
		50	0.025 ± 0.009	0.223 ± 0.02	0.035 ± 0.01^{ab}	0.487 ± 0.06^{a}	0.264 ± 0.01^{a}	0.067 ± 0.01^{a}
	50	0			0.013 ± 0.00^{c}	0.262 ± 0.08^{c}	0.210 ± 0.01^{d}	0.032 ± 0.01^{c}
		5			0.027 ± 0.01^{b}	0.399 ± 0.03^{b}	0.251 ± 0.01^{b}	0.056 ± 0.01^{b}
		10			0.029 ± 0.01^{b}	0.377 ± 0.07^{b}	0.257 ± 0.01^{b}	0.053 ± 0.01^{b}
		20			0.035 ± 0.01^{ab}	0.483 ± 0.07^{ab}	0.257 ± 0.01^{b}	0.065 ± 0.01^{a}
		50			0.027 ± 0.01^{bc}	0.375 ± 0.06^{b}	0.245 ± 0.01^{bc}	0.048 ± 0.01^{b}
Significance								
Auxin type (A)			***	ns	***	***	***	***
Hormone concentration (C)			ns	ns	*	***	***	***
A × C			ns	ns	**	***	***	***
Salinity (S)					***	***	***	***
A × S × C					ns	ns	ns	ns

different letters indicate significant differences within each parameter Tukey-Kramer test, $P < 0.05$; mean ± SD, $n = 10$; ns – non significant; Concent – concentration; Sal. – salinity; DM – dry matter; RGR – relative growth rate; IAA – indole-3-acetic-acid; IBA-indole-3-butyric-acid

ity of IBA than IAA seems to be the reason of the superior capability of indol-3-butyric acid (IBA)of promoting adventitious root formation in cutting grafted cucumber seedlings.

In conclusion, it should be said that the use of low concentration auxin compounds (10–20 ppm) before grafting could improve the growth parameters of grafted cucumber seedlings and their performance after transplanting in saline environments. The best results were obtained through the application of IBA, which promotes better rootstock – scion relationship, presumably due to faster development of phloem and xylem tubes, and a more vigorous root system by which plants are more capable of absorbing water and nutrients under adverse soil conditions.

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