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Full Length Research Paper

Effect of indole butyric acid on micrografting of cactus

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Grafting is a common technique to propagate cacti species. *Gymnocalycium mihanovichii* is an ornamental plant and they should be grafted to root stock containing chlorophyll. In this research, exogenous auxin treatments were applied for grafting improvement. *G. mihanovichii* and *Trichocereus spachianus* were used as a scion and root stock, respectively. Indole butyric acid (IBA) was used as an auxin. Plants were treated with four different concentrations of IBA (0, 50, 100 and 150 ppm) and repeated at three different times (3, 9 and 15 days after micrografting). Measured parameters were scion height and diameter, cambial layer diameter, areole numbers, activated areole numbers and successful graft percentage. The histological studies were done on grafted plants with cross section. Auxin of 100 ppm was the most effective treatment to improve measured parameters. Auxin at the optimal concentrations, especially at 100 ppm, resulted in better vascular differentiation, an important process in grafting. Therefore, the optimal concentration of IBA was 100 ppm, especially when it was repeated three times. The obtained results from the present study indicated that IBA at the optimal concentration is an effective treatment, and may lead to increased successful grafts.

Key words: *Gymnocalycium mihanovichii*, *Trichocereus spachianus*, micrografting, hormone, auxin, areole, ornamental plant, vascular differentiation.

INTRODUCTION

Plant grafting is an ancient and a widely used technique (Hartmann et al., 1997) that potentially can combine the advantages of rapid *in vitro* multiplication with the increased productivity (Gebhardt and Goldbach, 1988). It is commonly used to propagate rare ornamental species like cactus species (Estrada-Luna et al., 2002). Elimination of viruses, rejuvenation of mature tissues, year round plant production, make specific genotypic combinations to increase plant productivity and extend ecological limits of a particular plant species to tolerate edaphic conditions are several mentioned advantages of micrografting (Richardson, 1996; Hartmann et al., 1997; Estrada-Luna et al., 2002). Although grafting in commercial propagation has been restricted because of poor success that is attributed to difficulties in the use of unreliable techniques, there are problems with fungal or bacterial contamination and dehydration stress of tissues in the graft union area (Estrada-Luna et al., 2002). Several authors have defined the sequence of events during a compatible graft union formation: Formation of the union, development of a necrotic layer and proliferation of callus bridge at the graft interface prior to the binding of vascular cambium across the callus bridge, differentiation of new vascular cambium, forming a continuous cambial connection between rootstock and scion (Moore, 1984; Hartmann et al., 1997; Estrada-Luna et al., 2002, Hartmann, 2002). Production of new xylem and phloem thus permits the vascular connection between the scion and rootstock (Aloni et al., 2010). Grafting is a common practice to propagate cacti species. Grafted cacti are now regarded as one of the most admired ornamental indoor plants worldwide.

Based on the fact that hormones are involved in rootstock–scion interactions, in many studies various plant growth regulators have been used for grafting improvement. Auxin is one of the most effective hormones on growth, differentiation and development.

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Gymnocalycium mihanovichii is an ornamental plant. It does not have chlorophyll and should be grafted to root-stock containing chlorophyll. However, the preparation of scion of these ornamental plants is difficult. The present study was carried out to investigate the effects of different concentrations of indole butyric acid (IBA) as an auxin on grafted *G. mihanovichii* with *Trichocereus spachianus* as rootstock, and to find the optimal auxin concentration to improve the graft-take percentage of these ornamental plants.

MATERIALS AND METHODS

G. mihanovichii and *T. spachianus* were used as a scion and rootstock, respectively. These plants were prepared from Kasra green house in Semnan, Iran.

Preparation of plants for grafting and treatments

Rootstock with 9 and 2.5 cm height and diameter, respectively were used in controlled conditions. IBA was used as an auxin. Grafted plants were treated with four different concentrations including 0, 50, 100 and 150 ppm at grafting time. In addition to auxin treatment at grafting time, IBA treatment was repeated three different times at 3, 9 and 15 days after grafting. In each auxin concentration, it was repeated once (3 days after grafting), twice (3 and 9 days after grafting) and three times (3, 9 and 15 days after grafting). For example: in 50-1, 50-2 and 50-3 treatment groups, this treatment of auxin were repeated once, twice and three times, respectively at 3, 9 and 15 days after micrografting. We did the same procedure for 100 and 150 ppm. Therefore, plants were treated in ten different treatment groups including control (C), 50-1, 50-2, 50-3, 100-1, 100-2, 100-3, 150-1, 150-2 and 150-3. The grafted plants were maintained in controlled conditions (humidity 45% and 20^{o}C).

Measured parameters

Thirty days after the last treatment, plants were harvested. Measured parameters were scion height and diameter, cambial layer diameter, areole numbers, activated areole numbers and graft take percentage.

Cross section

Histological studies were performed with cross section. Forty-five days after the last auxin treatments, plants were harvested for cross section. Ethanol 80% was used as a fixator. Handy cross sections were done in about 2.5 mm around graft zone.

Statistical procedure

Analysis of variance was performed on all data sets. Duncan's test with probability of 0.05 was used to show significant differences between treatments. All data are presented as mean ± SE.

RESULTS

The obtained results from present research indicated that the effects of exogenous auxin, IBA, on the measured characters were dependent on applied concentrations where treatment of 100 ppm had significantly enhancing effects. Applied auxin treatments in 100-2 and 100-3 group resulted in significantly increased scion diameter (Figure 1, table 1). Application of auxin in 100-1, 100-2 and 100-3 treatment groups also led to significantly increased scion height (Figure 2, table 1). IBA of 100 ppm cause an increased cambial layer diameter in 100-1, 100-2 and 100-3 treatment groups, but the observed increased amounts of it in 50-2, 50-3 and 150-1 were not significant (Figure 3, table 1). IBA of 100 ppm was the only effective treatment on areole numbers. The number of areoles in 100-1, 100-2 and 100-3 treatment groups were significantly higher than untreated control plants with the highest amount found in 100-3 group (Figure 4, table 1).

Furthermore, as a result of application of IBA of 100 ppm, the numbers of activated areoles were enhanced (Figure 5), whereas the number of activated areoles in other treatment groups remained unchanged (Figure 5, table 1). The amounts of the successful graft percentage improved due to applied auxin treatments at suitable concentrations, with the highest amounts of it (Figure 6, table 1) found in treatment groups including 100-1, 100-2, 100-3 and 150-1. Auxin effects on vascular differentiation were completely dependent on applied concentration and treatment times. Auxin of 100 ppm had significantly stimulating effects on the differentiation of vascular system (Figure 7), whereas 150 ppm had enhancing effects only at 150-1, fewer than 100 ppm. In conclusion, the optimal concentration of IBA was 100 ppm, especially when it was repeated three times, 100-3, was most effective.

DISCUSSION

Different factors may have an influence on graft success: inherent system of cellular incompatibility, formation of plasmodesmata, vascular tissue connections, and the presence of plant growth regulators and peroxidases (Usenik et al., 2006). Based on many studies, callus bridge formation between the rootstock and scion and the differentiation of new vascular tissue from callus cells, together with vascular tissue connections are proposed as a crucial events for a successful rootstock–scion interaction (Moore, 1984; Andrews and Marquez, 1993; Wang and Kollmann, 1996; Hartmann et al., 1997; Estrada-Luna et al., 2002; Aloni et al., 2010; Martínez-Ballesta et al., 2010). The scion–rootstock connection is fundamental for optimal growth, water and nutrient uptake and transport (Martínez-Ballesta et al., 2010). The formation of vascular bridges across the grafting zone is a primary need for grafting establishment (Aloni et al., 2010). In grafted plants, the vascular regeneration is complicated processes, which include structural differentiation of the parenchymatous tissue from both sides of the graft union into xylem and phloem tubes (Aloni et al., 2010). Vascular development includes

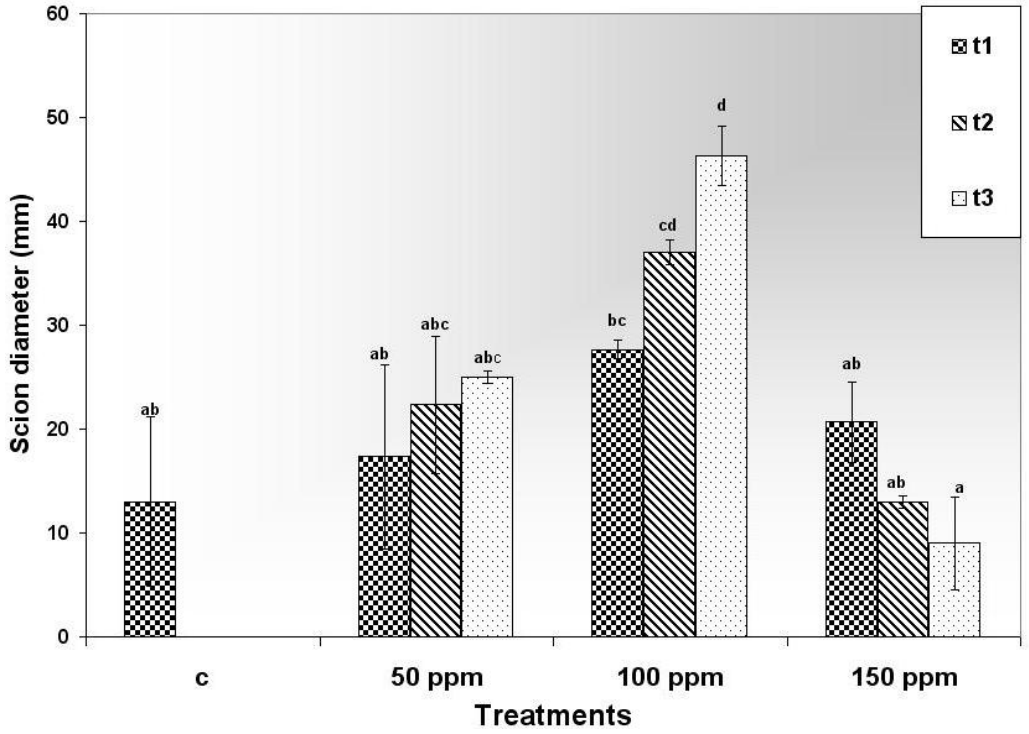


Figure 1. The effect of application of IBA on the scion diameter (X ± SE) in treatment groups. Each applied concentrations of IBA, 50, 100 and 150 ppm, was repeated once (t1), twice (t2) and three times (t3) in ten different treatment groups.

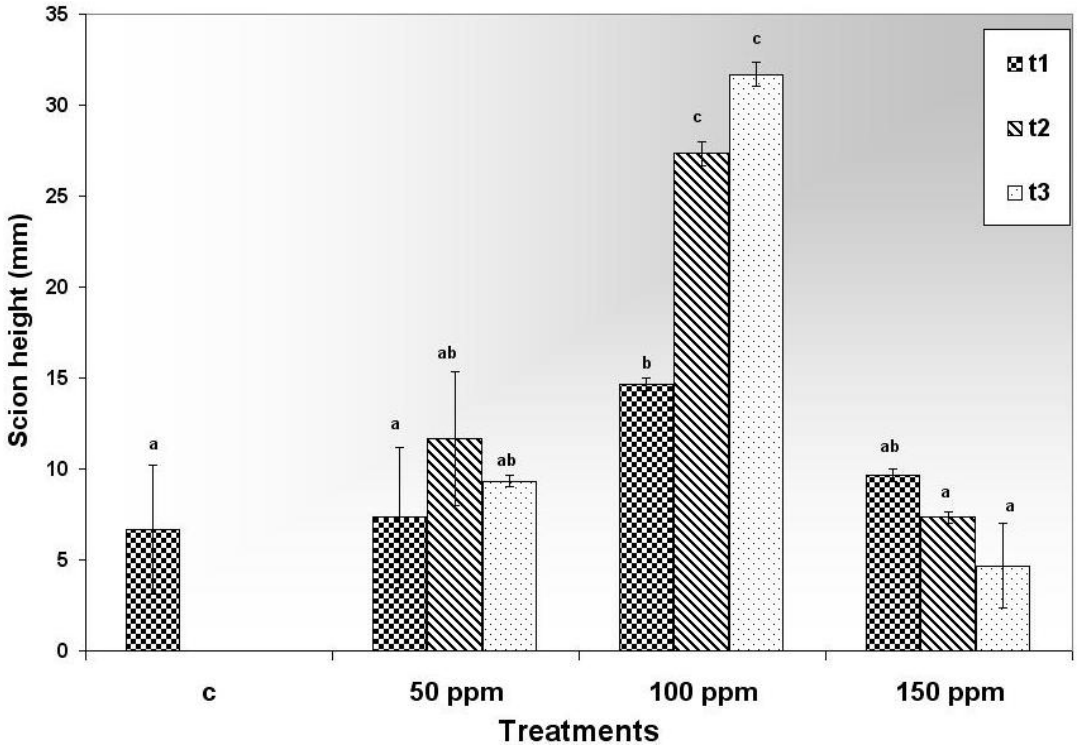


Figure 2. The effect of applied IBA treatment on the scion height (X ± SE) in the treatment groups. Each applied concentrations of IBA, 50, 100 and 150 ppm, was repeated once (t1), twice (t2) and three times (t3) in ten different treatment groups.

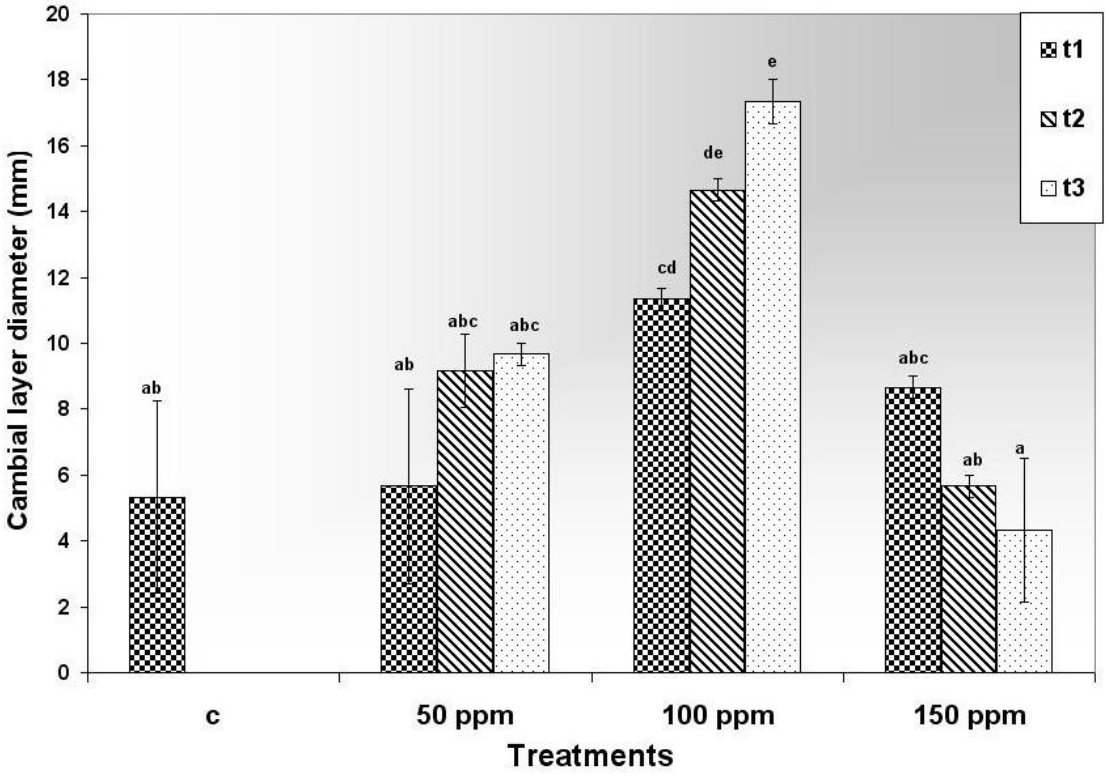


Figure 3. Changes induced in the cambial layer diameter (X ± SE) by the application of IBA. Each applied concentrations of IBA, 50, 100 and 150 ppm, was repeated once (t1), twice (t2) and three times (t3) in ten different treatment groups.

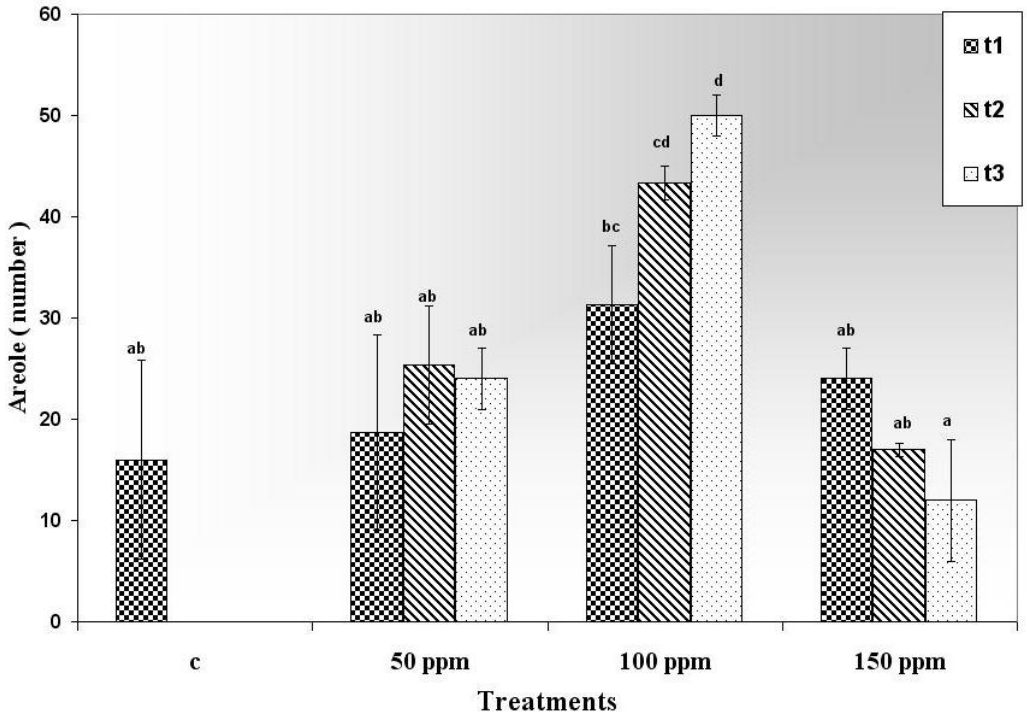


Figure 4. The effect of IBA treatment on the areole numbers (X ± SE) in treatment groups. Each applied concentrations of IBA, 50, 100 and 150 ppm, was repeated once (t1), twice (t2) and three times (t3) in ten different treatment groups.

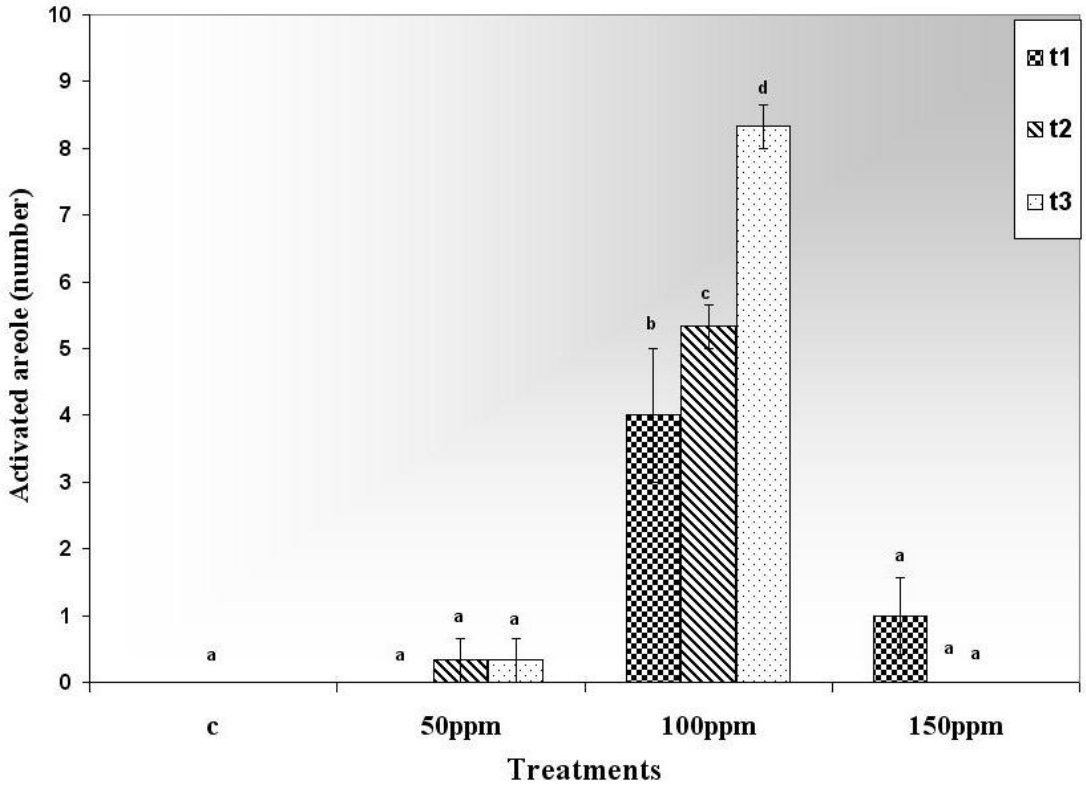


Figure 5. The effect of IBA treatment on activation of the areoles (X ± SE) in treatment groups. Each applied concentrations of IBA, 50, 100 and 150 ppm, was repeated once (t1), twice (t2) and three times (t3) in ten different treatment groups.

formation of the longitudinal pattern of primary vascular strands; formation of the radial pattern of xylem and phloem within vascular strands; differentiation of specialized cell types from xylem and phloem precursors; and cell proliferation and cell differentiation within the vascular cambium (Dengler, 2001). The re-establishment of vascular continuity through the interface zone is the critical event that determines the compatibility between the stock and the scion on the development of graft union formation (Estrada-Luna et al., 2002). The physiological disturbances induced by vascular union discontinuities at the graft union may lead to growth inhibition due to restricted communication between scion and rootstock (Martínez-Ballesta et al., 2010). Physiological studies have clearly demonstrated that the signals for induction of procambial cell formation are derived from apex and that exogenous auxin could replace the function of apex in the induction of procambial cell formation (Aloni 1987; Sachs, 1991; Zheng-Hua, 2002). The morphology of the vascular system is modified during graft union formation (Martínez-Ballesta et al., 2010).

The obtained results from the present research indicated that the effects of exogenous auxin, IBA, on the measured characters were dependent on applied concentrations where treatment of 100 ppm had significantly enhancing effects. The highest amounts of scion height and diameter, cambial layer diameter, areole numbers, the activated areole numbers and successful graft percentage were observed in 100 ppm treatment, especially in 100-3 group. Auxin effects on vascular differentiation and successful grafts were completely dependent on applied concentration and treatment times. Auxin of 100 ppm had significantly enhancing effect on the differentiation of vascular system, whereas 150 ppm had increasing effects only at 150-3, fewer than 100 ppm. It seems that 150 ppm was an excessive concentration and led to desirable results only when the mentioned treatment was used once. Auxin treatment of 50 ppm resulted in suitable results (much fewer than treatment of 100 ppm) when it was repeated three times, 50-3. Therefore, the optimal concentration was 100 ppm, especially when it was repeated three times, 100-3.

The plant hormone auxin regulates numerous developmental processes, and can affect cell division, cell growth, or cell differentiation depending on the content (Wilmoth et al., 2005). It is well documented that IAA has predominant control over many aspects of vascular tissue development, including the induction of primary vascular tissues, the positioning of primary vascular bundles and the activity of vascular cambia (Cooke et al., 2002). The relationships between scion and stock are affected by growth regulators. In grafting, an important substance

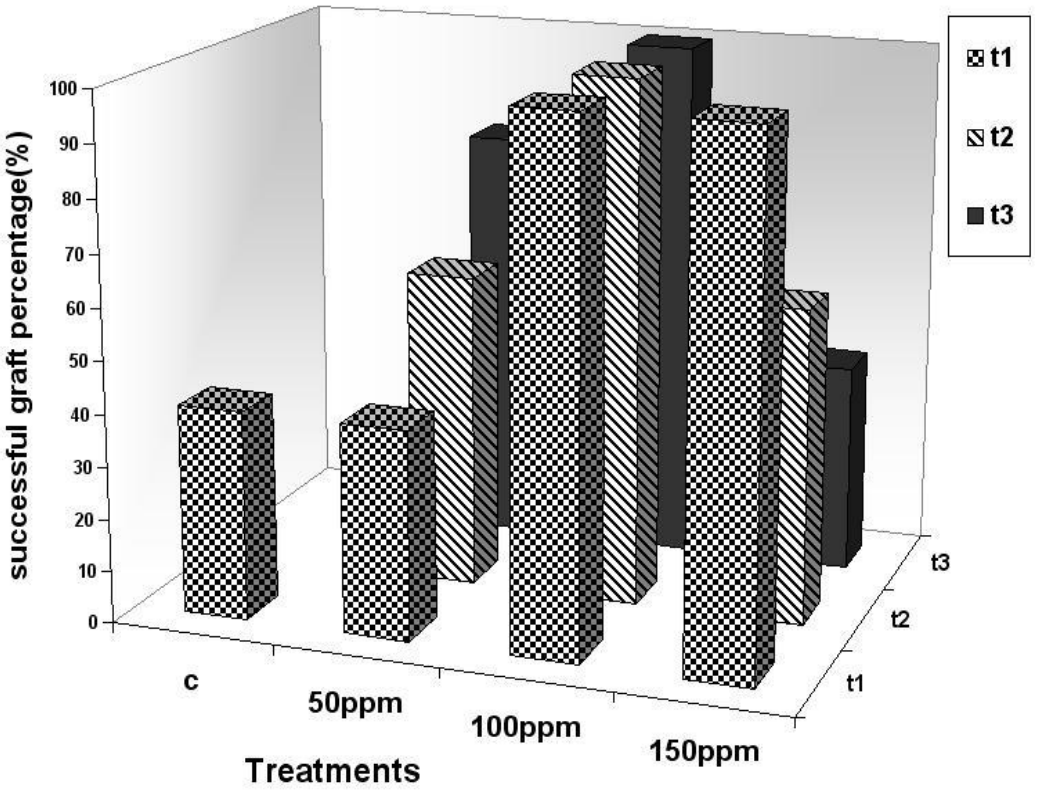


Figure 6. The effect of IBA treatment on successful graft percentage (X ± SE) in treatment groups. Each applied concentrations of IBA, 50, 100 and 150 ppm, Repeated once (t1), twice (t2), three times (t3) in ten different treatment groups.

involved in the development of compatible unions is auxin, which is released from vascular strands of the stock and scion and induces the differentiation of vascular tissues, functioning as morphogenic substances (Moore, 1984; Aloni et al., 1987, 2003; Pina and Eraaea, 2005). Auxin is involved in the development of compatible unions and induces the differentiation of vascular tissues (Usenik et al., 2006). The importance of plant growth regulators for improving the performance of grafted vegetable seedlings have been recognized (Aloni et al., 2010). Vascular system has the considerable adaptive capacities (Sachs, 1989). Auxin induces many developmental effects by regulating gene expression (Wilmoth et al., 2005). In an intact plant, provascular strands differentiate at predictable positions within all major organs, but adaptive responses to wounding or abnormal growth conditions demonstrate considerable flexibility of vascular patterning (Sachs, 1989; Mattsson et al., 1999). If the original connection is interrupted, new vascular strands can be formed even in mature organs (Lyndon, 1990).

Auxin may act as a patterning agent for differentiation of vascular tissues (Zheng-Hua, 2002). Auxin can induce xylem tracheary element differentiation in suspension culture cells of suitable species (Berleth and Mattson, 2000). Auxin translocation from the scion to the rootstock was found to accelerate the formation of a successful graft in cactus (Shimomura and Fujihara, 1977). The differentiation occurs along a narrow line of cells rather than in a field around the source. The signal also mediates oriented differentiation that eventually forms a continuous strand and the response is polar (Mattsson et al., 1999). Mattsson et al., (1999) suggested that auxin transport is required for vascular tissue continuity and the restriction of vascular differentiation to narrow strands. Zheng-Hua (2002) emphasizes that polar auxin flow has important roles in vascular cambium activity. Indole acetic acid (IAA) does not just trigger vascular differentiation per se, but also induces the formation of a continuous vascular strand (Berleth and Mattson, 2000).

In addition to effects on cell differentiation, auxins promote starch hydrolysis and the mobilization of sugars and nutrients to the cutting base (Das et al., 1997). Auxin application can replace leaf primordia in inducing vascular connections in stems and local auxin sources can induce the formation of new vascular strands from parenchymatic cells (Berleth and Mattson, 2000). Vascular regeneration experiments in which hormones were applied exogenously to stem segments indicate that low concentrations of indole acetic acid (IAA) stimulate

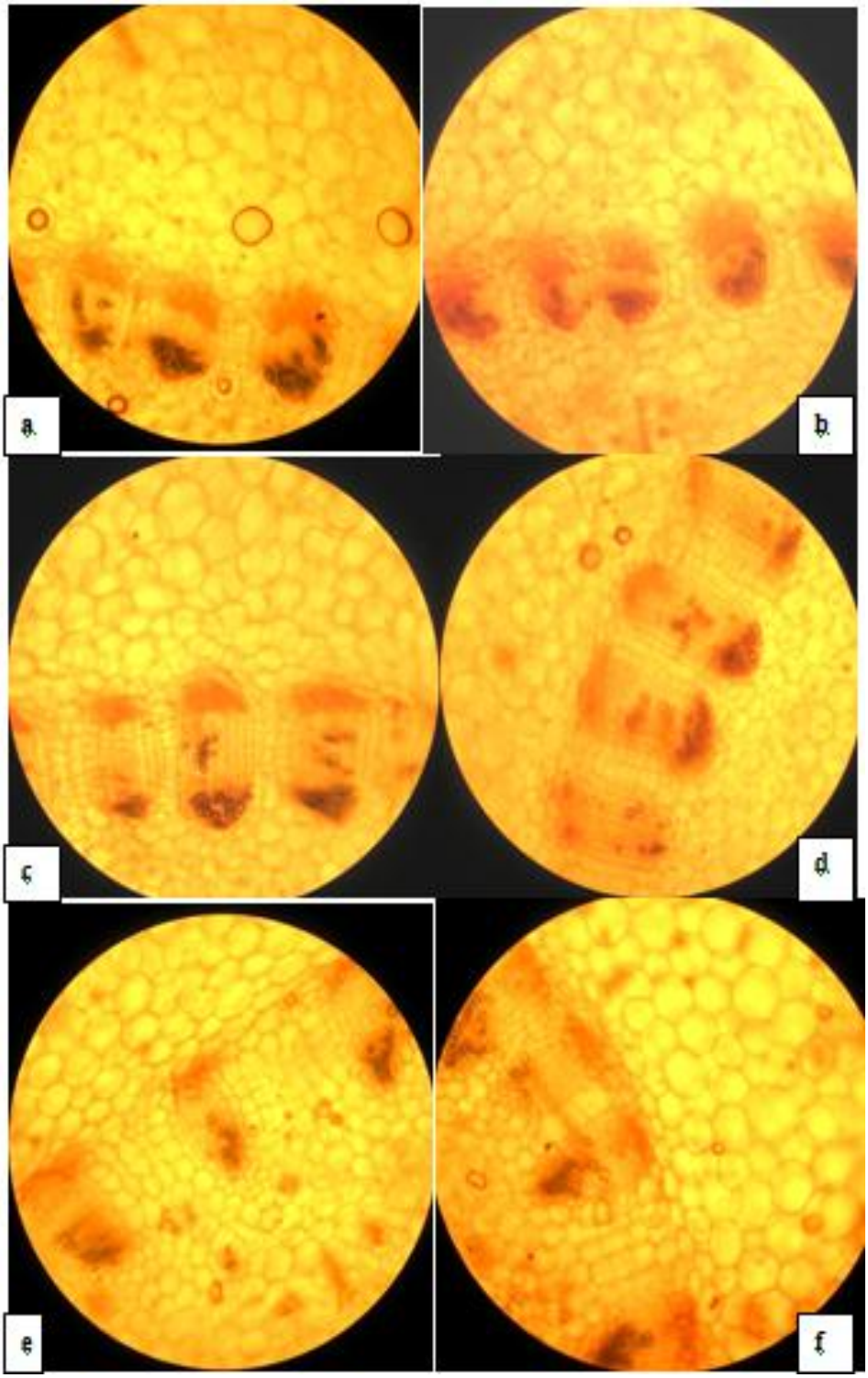
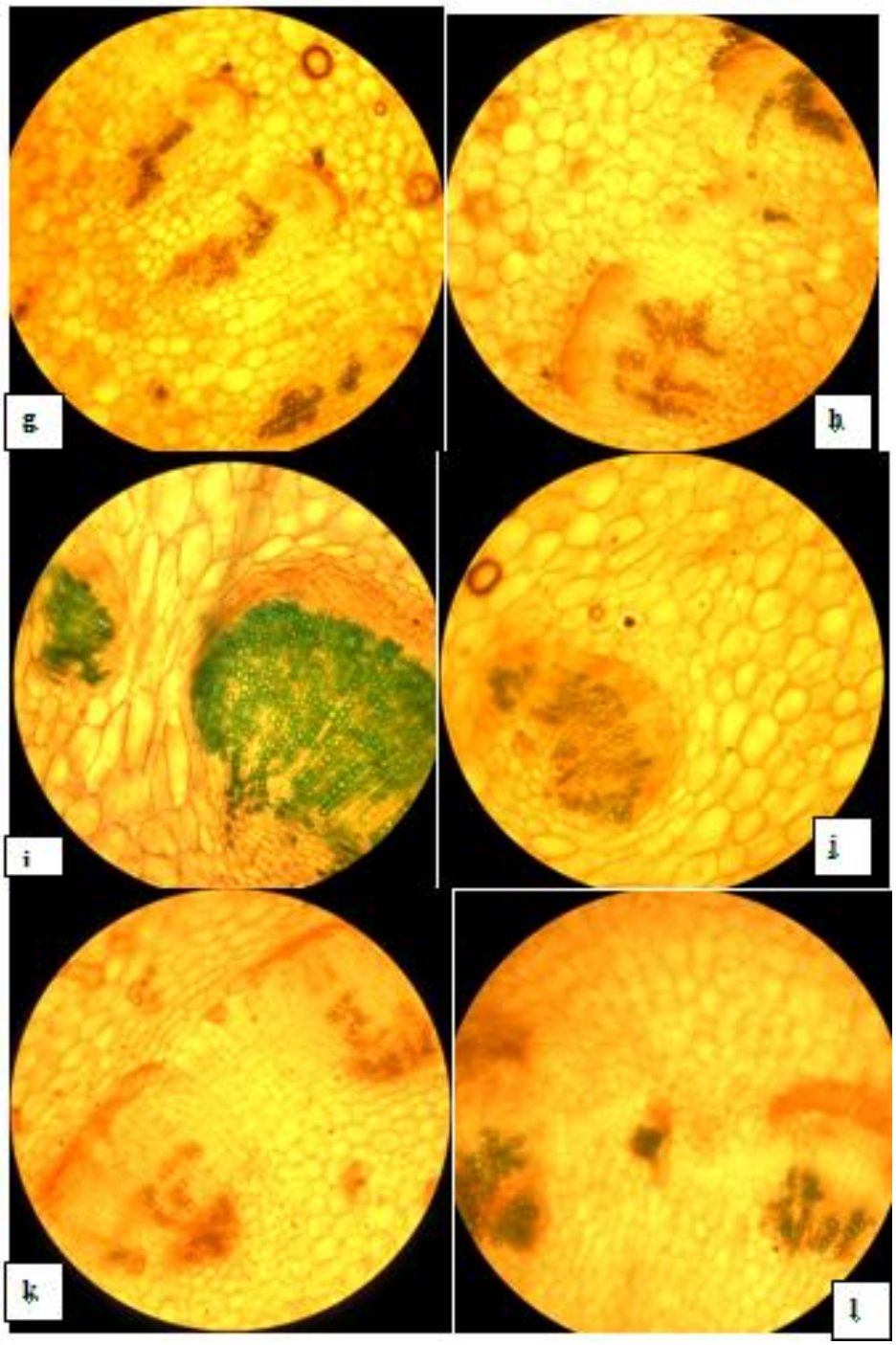


Figure 7. Light microscopic images of cross section of grated plants, 45 days after the last auxin treatments. a, b: Control plant; c, d, e, and f: 50 ppm; g, h, i and j: 100 ppm; k, l, m, n and o: 150 ppm.

phloem differentiation, whereas higher levels induce xylem differentiation (Aloni, 1980, 1987, 1995, 2001, 2010). However, excessive auxin concentrations did not result in respective increases in cutting dry mass (Mesen et al., 1997). Several reports suggest that inhibition by high auxin concentrations may be due to auxin-induced



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ethylene production (Mulkey et al., 1982; Rahman et al., 2001; Aloni et al., 2010). In addition, ethylene may trigger production of reactive oxygen species (Aloni et al., 2010) which may lead to reduced growth and successful graft percentage.

The obtained results from the present research illustrated that activation of the areole takes place under the influence of the auxin, IBA. The most activated areoles were observed in 100-3 treatment group. An activation of axillary meristem has been influenced by auxins (Rubluo et al., 2002). Explants exposed to auxins displayed areole activation, whereas control explants did not (Rubluo et al., 2002). Areole activation through breaking apical dominance is the most efficient way to attain micropropagation in cacti (Rubluo et al., 2002).

Also, in histological part of the presented research, the

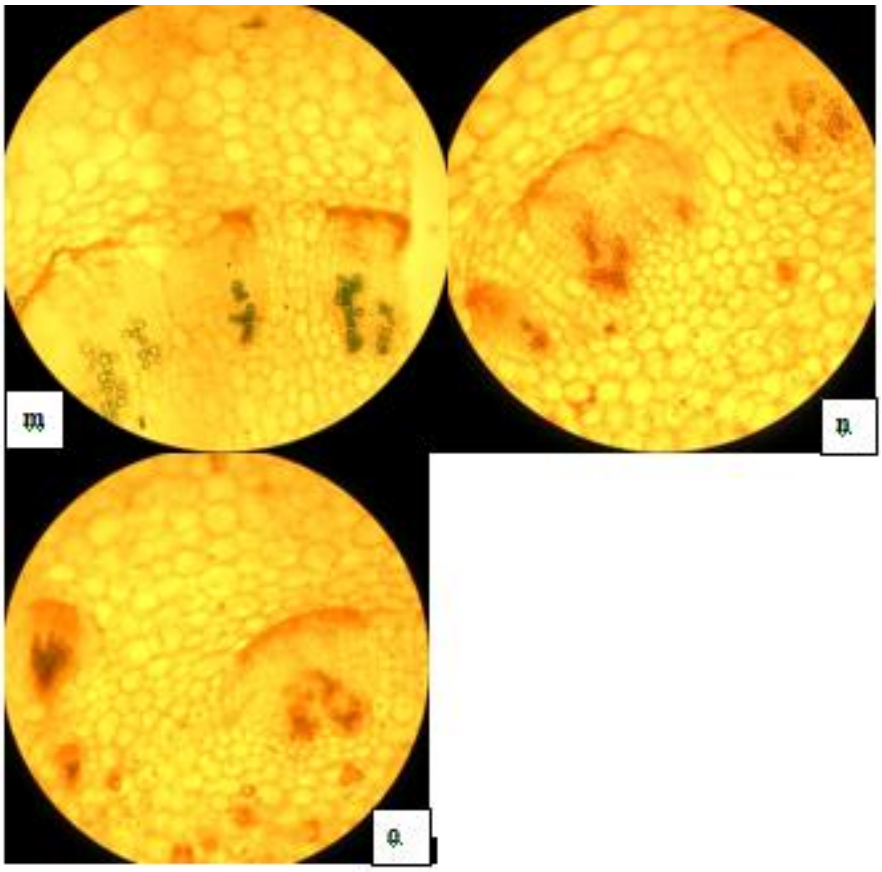

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Table 1. The effect of IBA treatment on some important parameter of the micro grafted plants (X ± SE) in treatment groups including control (C), 50-1, 50-2, 50-3, 100-1, 100-2, 100-3, 150-1, 150-2 and 150-3.

Treatment group*	Scion diameter (mm)	Scion height (mm)	Cambial layer diameter (mm)	Areole (number)	Activated areole (number)	Successful graft percentage (%)
C	13^{ab}	6.667^{a}	5.33^{ab}	16^{ab}	0^{a}	40
50-1	17.33^{ab}	7.33^{a}	5.66^{ab}	18.66^{ab}	0^{a}	40
50-2	22.33^{abc}	11.66^{ab}	9.16^{abc}	25.33^{ab}	0.33^{a}	60
50-3	25^{abc}	9.33^{ab}	9.66^{abc}	24^{ab}	0.33^{a}	80
100-1	27.66^{bc}	14.66^{b}	11.33^{cd}	31.33^{bc}	4^{b}	100
100-2	37^{cd}	27.33^{c}	14.66^{de}	43.33^{cd}	5.33^{c}	100
100-3	46.33^{d}	31.66^{c}	17.33^{e}	50^{d}	8.33^{d}	100
150-1	20.66^{ab}	9.66^{ab}	8.66^{abc}	24^{ab}	1^{a}	100
150-2	13^{ab}	7.33^{a}	5.66^{ab}	17^{ab}	0^{a}	60
150-3	9^{a}	4.66^{a}	4.33^{a}	12^{a}	0^{a}	40

*: The first number in each treatment group indicates the auxin concentration, while the second one relates to repeated times. Data are means of three replicates. Mean values followed by different letters (a, b, c, d, e) are significantly different ($p<0.05$), according to the Duncan's test.

morphological differences of vascular system were found between different treatments as shown in Figure 7. This confirms that local auxin is effective in differentiation of vascular system and it can modified morphology of vascular system where responses are dependent on applied concentrations. The obtained results from the present research suggest that auxins may be involved in morphogenetic responses in Cactaceae and the response is dependent on applied concentrations and probably type of used auxin. Finally, micrografting in

companion with optimal auxin treatment has the strong potential for large scale production of this cactus and might be extended to the propagation of other micrografted cacti species.

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REFERENCES

Aloni B, Cohen R, Karni L, Aktas H, Edelstein M (2010). Hormonal signaling in rootstock-scion interactions. Sci. Hortic. 127: 119-126.

Aloni R (1980). Role of auxin and sucrose in the differentiation of sieve and tracheary elements in plant tissue cultures. Planta, 150: 255-263.

Aloni R (1987). Differentiation of vascular tissues. Ann. Rev. Plant Physiol. 38: 179-204.

Aloni R (1995). The induction of vascular tissues by auxin and cytokinin. In: Davis PJ ed., Plant Hormones: Physiology, Biochemistry and Molecular Biology, Kluwer Academic publishers, Dordrecht, Netherland, pp. 531-546.

Aloni R (2001). Foliar and axial aspects of vascular differentiation-hypotheses and evidence. J. Plant Growth Regul. 20: 22-34.

Andrews PK, Marquez CS (1993). Graft incompatibility. Hortc. Rev. 15: 183-232.

Berleth T, Mattson J, Hardtke CS (2000). Vascular continuity and auxin signals. Trends Plant Sci. 5(9): 387-393.

Cooke TJ, Poli D, Sztein AE, Cohen JD (2002). Evolutionary patterns in auxin action. Plant Mol. Biol. 49: 319-338.

Das P, Basak UC, Das AB (1997). Metabolic changes during rooting in pre-girdled stem cuttings and air-layers of *Heritiera*. Bot. Bull. Acad. Sinica (Taipei) 38: 91-95.

Dengler NG (2001). Regulation of Vascular Development. J. Plant Growth Regul. 20: 1-13.

Estrada-Luna AA, López-Peralta C, Cárdenas-Soriano E (2002). *In vitro* micrografting and the histology of graft union formation of selected species of prickly pear cactus (*Opuntia* spp.). Sci. Hortic.14: 317-327.

Gebhardt K, Goldbach H (1988). Establishment, graft union characteristics and growth of *Prunus* micrografts. Physiol. Plant. 72: 153-159.

Hartmann HT, Kester DE, Davies FT, Geneve RL (2002). Plant propagation. principles and practices, seventh ed. Prentice Hall, Upper Saddle River, NJ, ISBN 0-13-679235-9, p. 849.

Hartmann HT, Kester DE, Davies FT, Geneve RL (1997). Plant propagation. principles and practices, sixth ed. Prentice Hall, Upper Saddle River, NJ, p. 757. ISBN 0-13-206103-1.

Lyndon RF (1990). Plant Development, The Cellular Basis. Boston, MA: Unwin Hyman, pp. 135-165.

Moore R (1984). A model for graft compatibility–incompatibility in higher plants. Am. J. Bot. 71: 752-758.

Martínez-Ballesta MC, Alcaraz-López C, Muries B, Mota-Cadenas C, Carvajal M (2010). Physiological aspects of rootstock-scion interactions. Sci. Hortic. 127: 112-118.

Mattsson J, Sung ZR, Berleth T (1999). Responses of plant vascular systems to auxin transport inhibition. Development, 126: 2979-2991.

Mesen F, Newton AC, Leakey RRB (1997). Vegetative propagation of *Cordia alliodora* (Ruiz & Pavon) Oken: the effects of IBA concentration, propagation medium and cutting origin. Forest Ecol. Manage. 92: 45-54.

Moore R (1984). A model for graft compatibility-incompatibility in higher plants. Am. J. Bot. 71(5): 751-758.

Mulkey TJ, Kuzmanoff KM, Evans ML (1982). Promotion of growth and shift in the auxin dose/response relationship in maize roots treated with the ethylene biosynthesis inhibitors aminoethoxyvinylglycine and cobalt. Plant Sci. Lett. 25: 43-48.

Pina A, Errea P (2005). A review of new advances in mechanism of graft compatibility-incompatibility. Sci. Hortic. 106: 1-11.

Rahman A, Amakawa T, Goto N, Tsurumi S (2001). Auxin is a positive regulator of ethylene-mediated response in the growth of Arabidopsis roots. Plant Cell Physiol. 42: 301-307.

Richardson FVM, Mac Ant Saoir S, Harvey BMR (1996). A study of the graft union in in vitro micrografted apple. Plant Growth Regul. 20: 17-23.

Rubluo A, Marı´n-Herna´ndez T, Duval K, Vargas A, Ma´rquez-Guzma J (2002). Auxin induced morphogenetic responses in long-term in vitro subcultured Mammillaria san-angelensis Sa´nchez-Mejorada (Cactaceae). Sci. Hortic. 95: 341-349.

Sachs T (1991). Cell polarity and tissue patterning in plants. Development, Suppl. 91(1): 83-93.

Sachs T (1989). The development of vascular networks during leaf development. Curr. Top Plant Biochem. Physiol. 8: 168-183.

Shimomura T, Fujihara K (1977). Physiological study of graft union formation in cactus. II. Role of auxin on vascular connection between stock and scion I. Jpn. Soc. Hortic. Sci. 45: 397-406.

Usenik V, KrsˇkB, Vicˇan M, Sˇtampar F (2006). Early detection of graft incompatibility in apricot (*Prunus armeniaca* L.) using phenol analyses. Sci. Hortic. 109: 332-338.

Wang Y, Kollmann R (1996). Vascular differentiation in the graft union of in vitro-grafts with different compatibility, Structural and functional aspects. J. Plant Physiol. 147: 521-533.

Wilmoth JC, Wang S, Tiwari SB, Joshi AD, Hagen G, Guilfoyle TJ, Alonso JM, Ecker JR, Reed JW (2005). NPH4/ARF7 and ARF19 promote leaf expansion and auxin-induced lateral root formation. Plant J. 43: 118-130.

Zheng-Hua YE (2002). Vascular tissue differentiation in plants. Annu. Rev. Plant Biol. 53: 183-202.