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In vitro Grafting of Pear (*Pyrus* spp.)

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Abstract: The success of *in vitro* grafting of shoot tips of pear (*Pyrus* spp.) has been examined. Shoot tips (meristem plus 2-3 leaf premordia) from *in vitro* propagated Le-Cont cv. shoot were grafted *in vitro* onto decapitated shoots of *Pyrus betulaefolia* as rootstocks. The technique of grafting and the effect of scion size on grafting success was studied. Shoot tips consisted of two different sizes, <0.5 cm long and>0.5 cm but<1cm long were examined. Grafting success was significantly depended on the method of grafting and size of the scion. The highest percentage of successful grafts, axillary shoots development percentage, micrograft length increase and number of new buds formation (83%, 80%, 3.5cm and 4, respectively) were obtained with scion length greater than 0.5cm, while the scion of smaller than 0.5cm was achieved 37% of successful grafting. In conclusion, *in vitro* grafting is an alternative suitable propagation method leading to higher growth potential of grafted populations.

Key words: *In vitro* grafting · Scions · Rootstocks · Pear

INTRODUCTION

Micrografting is an *in vitro* technique. This procedures, involve the placement of a meristem tip or shoot tip explants onto a decapitated rootstock that has been grown aseptically from seed or micropropagated [1]. In Egypt, the demand of Egyptian's farmers for pome fruit rootstocks is usually met by importing them from abroad which costs the government high amounts of money. On the other hand, propagation of such rootstocks by cuttings is rather difficult and characterized by very low rooting success. Thus, production of good deciduous rootstocks is one of the most important objectives in horticulture industry. Grafting and budding of seedling rootstocks is the usual methods used for vegetative propagation [2]. Microshoots that are difficult to root, or do not form roots at all *in vitro* can be micrografted onto rootstocks to obtain rooted plants [3]. Grafting is a common practice to propagate *Pyrus* spp. 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 tissue in the graft union area [4]. Several authors have defined the sequence of events during a compatible graft union formation: Formation of the union, development of necrotic layer and proliferation of callus bridge at the graft interface prior to the binding of vascular cambium across the callus bridge, formation between the rootstock and scion and the differentiation of new vascular cambium, tissue from callus cells, together with vascular tissue connections are proposed as a crucial event for a successful rootstock-scion interaction. [1, 4- 6]. Production of new xylem and phloem thus permits the vascular connection between the scion and rootstock [6]. *In vitro* micrografting has been reported in many plants such as apple and *Pyrus* spp. [7], cashew [3,8,9], pistachio [10-12], *Olea europea* [13], almond [14], *Prunus avium* L. (cherry var.) [15] grapes, [16, 17], citrus [18] and cactus [19].

The aim of this study was establishment of micrografting protocol for clonal propagation of true-to-type mature pear genotype might be an efficient technique for overcoming conventional pear propagation problems such as resolve the seasonal dependence for grafting. This technology has the potential for large scale

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production in a short period of *Pyrus* spp. plants in a short period. Finally, the technique presents several advantages and could offer serious opportunities for rapid mass propagation for healthy plant materials.

MATERIALS AND METHODS

Establishment of the Rootstocks: Stem segments were used as explants; they were sterilized with 20% Clorox (sodium hypochlorite at 1.5%) for 20 min. then washed three times with distilled water. Explants were soaked in filtrated antioxidant solution (100 mg/l ascorbic acid and 150 mg/l citric acid) for starting culture. Sterilized explants were cultured on Murashige and Skoog (MS) [20] solidified medium with 3 g/l with phytagel and containing 2 mg/l N6-benzyl amino purine (BAP). Thereafter, the shoots were transferred to MS medium enriched with 3 mg/l indole-3-butyric acid (IBA) for rooting, [21]. All media were sterilized autoclaving at 121°C and 1.1 kg/cm for 20 min. Cultures were maintained in incubator for 4 weeks under 16/8 photoperiods and 25+1°C. Eight weeks old shoots were selected as rootstocks for *in vitro* grafting operation.

Preparation of Scion: Stem segments from adult trees (Le-Cont cv.) grown in field were sterilized with 3% (v/v) sodium hypochlorite solution containing 5 drops of Tween–20 and rinsed with three changes of sterile deionized water under aseptic conditions in a laminar flow hood. A single stem segment was cultured in each tube containing 15 ml of solid MC Cow's Woody Plant Medium (WPM) [22] medium supplemented with 30 g/l sucrose, 100 mg/l myo- insitol, 3 g/l phytagel. *In vitro* regenerated shoots were micropropagated and subcultured every 3 weeks. After 2 or 3 subcultures, elite shoot tips were used as microscions for *in vitro* grafting.

Grafting Experiments Procedure and Maintenance of *in Vitro* Grafts: Prior to *in vitro* grafting, all leaves except 2-3 of the smallest leaf primordia were removed from the scion explants. For the rootstocks, the leaves were removed from 3-4 cm along for stem and all lateral shoots were removed, leaving a single shoot. The rootstock shoot was decapitated approx. 3 cm above the medium surface. A shoot tip (meristem plus 2-3 leaf primordial) was excised with a different scalpel from the scion donor plants and placed on the decapitated shoot. Since, the diameter of the rootstock was greater than that of the scion; the latter was placed off in the center. In order to prevent cross-contamination between each cut, the scalpel was dipped in a 1.25% (v/v) sodium hypochlorite solution, air dried, dipped in 95% ethanol and flamed. To prevent dehydration of *in vitro* grafted shoot tips, 2 mm^3 cube of solid WPM was placed next to the graft union. Two different sizes of scion were evaluated in each *in vitro* grafted pair: half of the experimental units were performed with scion less than 0.5 cm long and the other half with scions more than 0.5 cm, but less than 1 cm long. Thirty micrografts were done for each scion's size. The number of successful micrografts was recorded 5- 6 weeks after micrografting and the percentage of success with respect to scion size was recorded.

Micrografted plants were cultured into WPM medium containing 2.0 mg/l BAP and 3 mg/l IBA with control group, which did not contain any plant growth regulators (PGRs). Lateral shoots that developed from the rootstock were removed. Approximately 10-15 ml of warm WPM was added to the top of old medium at 20 days intervals. Cultures were incubated at 25°C±1 with a 16h photoperiod provided by cool white fluorescent lambs at 3000 Lux for 6 weeks then, grafted plantlets were exposed to natural conditions in greenhouse.

Acclimatization: Successfully grafted plantlets were removed from the culture vessels and rinsed with tap water to remove remaining phytagel from the root system. Subsequently, they were transplanted into individual commercial plastic pots filled with an autoclaved mix of sand, perlite and peatmoss [1:1:1 (v/v)] covered with transparent bags to maintain a 90±5% relative humidity. During the first 2 weeks the bags were gradually remove to allow air exchange for plant acclimatization.

Statistical Analysis: The *in vitro* experiments were subjected to the completely randomized design. Variance analysis of data was carried out ANOVA for statistical analysis. The differences among means for all treatments were tested for significance at 5% level by using Duncan's multiple range test [23]. Means followed by the same letter are not significantly different.

RESULTS

Grafting success was significantly dependent upon the method of grafting and the size of scion. Of the initial 60 micrografts, all formed white callus at the union graft and 83% of scions more than 0.5 cm established successful grafts (Table 1). Successfully micrografted scions were green; stem elongation was observed 10-12 days after *in vitro* grafting (Fig.1) and production of

Table 1: Success rate and growth of *Pyrus* spp. shoots after 6 weeks micrografting.

			Scion's callus growth			
Size of scion (cm)	Number of micrografts	Successful micrografts (%)	Graft union %	Axillary shoot development %	Micrograft length increment (cm)	Number of new buds
> 0.5 cm	30	83 a	100 a	80 a	3.5 a	4 a
< 0.5 cm	30	37 b	100 a	50 b	1.5 b	2 b

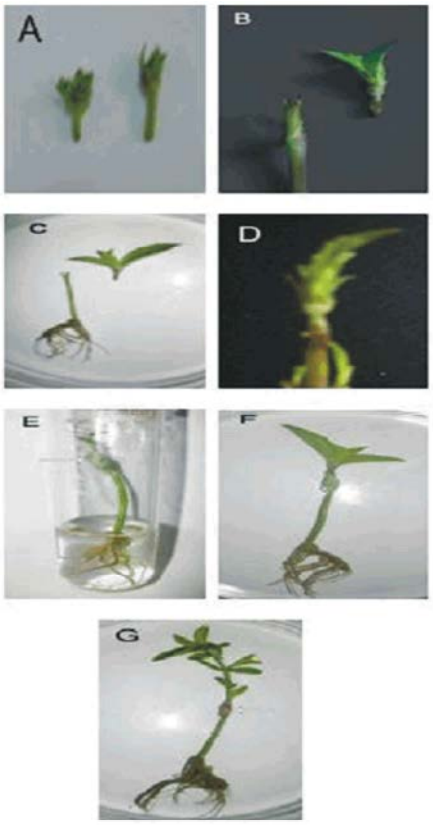

Fig. 1: *In vitro* grafting of pear (*Pyrus* spp.).

A. Size of shoot tips of Le-cont cv. (scion).
B. Preparation of shoot tip (scion) and stem section (rootstock) for *in vitro* grafting.
C. Aspect of shoot tip (scion) and rooted stem section (rootstock).
D. Shoot tip of Le-cont cv. (scion) *in vitro* grafted onto *Pyrus betulaefolia* (rootstock). showing graft union point (see arrow).
E. Callus formation in union point (see arrow).
F. Shoot tip of Le-cont cv. (scion) *in vitro* grafted onto *Pyrus betulaefolia* (rootstock). After 25 days from *in vitro* grafting.
G. Shoot tip of Le-cont cv. (scion) *in vitro* grafted onto *Pyrus betulaefolia* (rootstock). After 45 days from *in vitro* grafting (showing development of new leaves and increase of stem length)

new leaves was observed 2-3 weeks after micrografting. Eighty percent of axillary shoots were developed, while length increment and number of new buds were 3.5 cm and 4, respectively. The scion that less than 0.5 cm long gave 37% successful micrografts. Moreover, 50% (Table1) callus was visible at all graft unions. While, the length increment was 1.5 cm and number of new buds were two buds. Concerning, graft union percentage recorded 100% in both sizes of scion. In this research a swelling in union zone between rootstock and scion was observed. Successfull micrografts were of pear gradually of pear were acclimatized which reached 75% of survival in the greenhouse.

DISCUSSION

Developing a workable *in vitro* grafting model system for pear is of great importance due to the tremendous advantages of this technique as recorded for other species such as peach [24] olive [25], cactus [19], grapevine [17,26], pistachio [11,12, 27], almond and cherry [15]. The *in vitro* grafting of pear was successfully achieved. The establishment of *in vitro* grafting protocol for clonally propagation of true-to-type mature pear genotype might be an efficient technique overcoming conventional pear propagation problems. Murashige *et al*. [28] reported that successful *in vitro* grafting of citrus ranges from 5 to 40% depending on the genotype. Size of the scion and placement on the decapitated rootstock can affect success; Navarro *et al*. [29] observed that the success rates of 14.6% and 34.6% were obtained when the scion consisted of the meristem plus two leaf primordia and the meristem plus four leaf primordial of citrus, respectively. Larger scion sizes are likely to provide better recovery of plants; the possibilities of recovering healthy material are reduced. On the other hand, for the majority of plants this is due to the small size of shoot tip organ making problematic the excision, handling grafting and subsequent maintains of grafted assembly and leading to the drying of shoot tips and low graft integration. Larger size of shoot tip is making handling of grafting easier. However, they contain more phenolic compounds and

hormonal concentrations results in higher polyphenol oxidases and peroxidases activity and hence higher browning and drying of fresh tissue just before and beyond grafting member's integration [30]. Micrograft in which a phytagel drop was added to grafted area was highly successes as compared with those grafted without an phytagel drop for pear micrografts. The major cause of *in vitro* grafting union failure without a phytagel drop might be due to desiccation [31]. Adding an agar drop usually prevents scion drying and makes the transport of different materials possible and holds the graft units together until the fusion took place. Better vascular differentiation, an important process in grafting [9]. The scion rootstock connection is fundamental for optimal growth, water and nutrient uptake and transport [32]. Also, the formation of vascular bridges across the grafting zone is a primary need for grafting establishment [33]. In addition, cortical and pithy parenchyma cells had divided to produce a multi-layer callus, above and below of the necrotic zone were formed to physically join the scion to the rootstock, which caused a slight swelling of the tissues close to the interface [4].

In grafted plants, the vascular regeneration is complicated processes, which include structural differentiation of the parenchmatous tissue from both sides of the graft union into xylem and phloem tubes [33].Vascular development includes formation of longitudinal pattern of primary vascular strands, formation of 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 [34]. Regarding to the re-establishment of vascular especially xylem continuity through the interface zone is the critical event that determines the compatibility between the rootstock and the scion on the development of graft union formation [4]. This is because the restoration of the vascular bundies ensures the flow of substance between the rootstock and scion [5]. Therefore, technique of *in vitro* grafting is a safe and an alternative method for producing genetically uniform, disease free planting material and micropropagation of fruit trees. It is also, important to developed protocols for efficient regeneration of plants from adult trees.

REFERENCES

1. Shartmann, H.T., D.E. Kester, F.T. Davies and R.L. Geneve, 2002. Plant Propagation Principles and Practices. 7th Ed. Prentice Hall. Upper Saddle River. NJ, ISBN., 0-13-679235-9: 849.
2. Yildirm, H., A. Onay, V. Suzever, E. Tilkat and Y. Toktli 2010. Micrografting of almond (*Prumus dulcis* Mill.) cultivars "Ferragnes". Sci. Hort, 125: 361-367.
3. Thimmappaiah Puthra, G.T. and S.R. Anil, 2002. *In vitro* grafting of cashew (*Anacardium occidentale* L.). Sci. Hort, 92: 177-182.
4. Estrade-Luna, A.A., C. Lopez-Peralta and S. Cardenas, 2002. In vitro micrografting and the histology of graft union formation of selected species of prickly pear cactus (*Opuntia* spp.). Sci. Hort, 14: 317-327.
5. Moor, R., 1984. A model for graft compatibility-incompatibility in higher plants. Am. J. Bot., 71(5): 751-758.
6. Aloni, B., R. Cohen, L. Karni, H. Aktas and M. Edelstein, 2010. Hormonal signaling in rootstock-Scion interaction. Sci. Hort, 127: 119-126.
7. Rafail, S. and M.S. Mosleh, 2010. Factors involved in micropropagation and shoot tip grafting of apple (*Malus domestica* Borkh.) and pear (*Pyrus* sp. L.) World Food System A contribution from Europe, pp: 1-4.
8. Ramanayake, S.M.S.D. and T. Kovoor, 1999. *In vitro* micrografting of cashew (*Anacardium occidentale* L.). Sci. Hort, 92(2): 177-182.
9. Mneney, E.E. and S.H. Mantell, 2001. *In vitro* micrografting of cashew. Plant Cell Tissue Organ Culture, 66: 49-58.
10. Abousalim, A. and S.H. Mantell, 1992. Micrografting of pistachio (*Pistacia vera*. cv. "Mateur"). Plant Cell, Tissue and Organ Culture, 29: 231-234.
11. Canan, C., O. Mehmet, T. Hakan, S. Kamil and I. Elman, 2006. In vitro micrografting of pistachio, *Pistacia vera* L. Siirt, on wild pistachio rootstocks. J. Cell and Molecular Biol, 5: 25-31.
12. Onay, A., E. Tilkat, C.I. Skalan and S. Namli, 2006. Micrografting of Pistachio (*Pistacia vera* cv. Siirt). In: Mohan Jain S, Haggman H (Ed) Protocols for Micropropagation of Woody Trees and Fruits: Springer, the Netherland, pp: 289-298.
13. Revilla, M.A., P. Jose, C. Abelardo and R. Roberto, 1996. In vitro reinvigoration of mature olive trees (*Olea europea* L.) through micrografting. In vitro Cell Dev. Biol. Plant., 32: 257-261.
14. Channuntapipat, C., M. Sedgley and G. Collins, 2003. Micropropagation of almond cultivars Non Pareil and Neplus Ultra and the hybrid rootstock Titan x Nemaguard. Sci. Hort., 98: 473-484.

15. Mohammad E. Amiri, 2006. In vitro techniques study the shoot tip grafting of *Prunus avium* L. (cherry) var. Seeyahe Mashad. J. of Food Agric. and Environ., 4(1): 151-154.
16. Tangolar, S.G., K. Ercik and S. Tangolar, 2003. Obtaining plants using *in vitro* micrografting method in some grapevine varieties (*Vitis vinifera* L.). Biotechnology and Biotechnology Eq., 17: 50-55.
17. Mohammad, A.A. and B.H. Mohammad, 2010. *In vitro* micro-grafting of some Iranian grapevine cultivars. Romanian Biotechnological Letters, 15(5): 5576-5580.
18. Ali, A.N., J.J. Muhammad, A. Haider and M. Qasim, 2007. *In vitro* studies on micrografting technique in two cultivars of citrus to produce virus free plants. Pak. J. Bot., 39(5): 1773-1778.
19. Ali, R.L.M., O.A. Zahra and R. Lotfolah, 2012. Effect of indole butyric acid on micrografting of cactus. African J. Biotechnology, 11(24): 6484-6493.
20. Murashige, T. and F. Skoog, 1962. A revised medium for rapid growth of and bioassays with tobacco tissue culture. Physiol. Plant., 15: 473-497.
21. Hassanen, S.A. and F.G. Mahdia, 2012. *In vitro* propagation of pear (*Pyrus betulaefolia*) rootstock. American-Eurasian J. Agric. and Environ. Sci., 12(4): 484-489.
22. Mc Cown, B.H. and G. Lloyd, 1981. Woody Plant Medium (WPM) a mineral nutrient formulation for microculture of woody plant species. Hort. Sci., 16: 453.
23. Duncan, D.B., 1955. Multiple Range and Multiple F. Test. Biometrics, 11: 1-42
24. Navarro, G., M. Llacer, M. Cambra, J.M. Arregui and J. Juarez, 1982. Shoot tip grafting *in vitro* for elimination of viruses in peach plants. (*Prunus persica Batsch*). Acta Hort., 130: 185-192.
25. Farah, F., R. Samaneh, P. Maryam, A. Saideh and H. Mehdi, 2011. Micrografting and micropropagation of olive (*Olea europea* L.) Iranian cultivar; Zard. African J. Plant Science, 5(11): 671-675.
26. Nas, M.N. and P.E. Read, 2003. Simultaneous micrografting, rooting acclimatization of micropropagated American chest nut, grapevine and hybrid hazelnut. Euro. J. Hort. Sci., 68(5): 234-237.
27. Tikat, A., A. Onay and Y.O. Tokatli, 2009. In vitro rooting improvement of adult pistachio, *Pistacia vera* L. 'ATLI'. Acta Hort. (ISHSI), 839: 215-221.
28. Murashige, T., W.P. Bitlers, T.S. Rangan, E.M. Naner, C.N. Roistacher and P.B. Holliday, 1972. A technique of shoot apex grafting and its utilization toward recovering virus free citrus clones. Hort. Science, 7: 8-9.
29. Navarro, L., C.N. Roistacher and T. Murashige, 1975. Improvement of shoot tip grafting *in vitro* for virus-free citrus. J. American Soc. Hort. Sci., 10: 471-479.
30. Jonard, R., 1986. Micropropagation and its Applications to Tree Improvement, In: Y.P.S Bajaj (Eds) Biotechnology in Agriculture and Forestry. Trees I. Springer Verlag, Berlin, 1: 31-48.
31. George, E.F., A.H. Michael and D.K. Greek, 2008. Plant Propagation by Tissue Culture (3rd Edition). The Background, Springer, pp: 1.
32. Martinez-Ballesta, M.C., C. Alcaraz-Lopez, B. Muries, C. Mota-Cadenas and M. Carvajal, 2010. Physiological aspects of rootstock-scion interactions. Sci. Hort, 127: 112-118.
33. Alon, B., R. Cohan, Karni, H. Aktas and M. Edelstein, 2010. Hormonal signaling in rootstock-scion interactions. Sci. Hort, 127: 119-126.
34. Dengler, N.G., 2001. Regulation of vascular development. J. Plant Growth Regulation.