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A Contribution from Europe"**

FACTORS INVOLVED IN MICROPROPAGATION AND SHOOT-TIP GRAFTING OF APPLE (*Malus domestica* Borkh.) AND PEAR (*Pyrus sp.* L.)*

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Introduction

Micrografting is an *in vitro* grafting technique. This procedure involves the placement of a meristem tip or shoots tip explant onto a decapitated rootstock that has been grown aseptically from seed or micropropagated (Hartmann *et al*., 2002). In Iraq, the demand of Iraqi 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. Micrografting has several unique uses including: production of disease-free plants by grafting small meristem tips (Zilka *et al*., 2002), virus indexing by micrografting to susceptible understocks (Zimmerman, 1993), early detection of grafting incompatibility relationships (Jonard, 1986), propagation of novel plants created in tissue cultures that are difficult-to-root (Barros *et al*., 2005) and small micrografted trees are a convenient way to exchange germplasm between countries (Navarro *et al*., 1975).

The *in vitro* grafting of apple shoots has been described in a preliminary report (Lundergan *et al*., 1978) and in more detail (Sparks *et al*., 1977). Micrografting of apple was successful (Huang and Millikan, 1980). Faggioli *et al*. (1997) reported a successful *in vitro* micrografting of *Pyrus communis* shoot tips.

The major aims of the current investigation included: developing a protocol for *in vitro* shoot tip grafting for different cultivars to obtain a source for clean budding material by testing different factors involving in micrografting technique success. Furthermore, developing a skill for new *in vitro* grafting techniques suitable for deciduous fruit trees production of disease-free plants.

Materials and Methods

The present investigation was conducted in Plant Tissue Culture laboratory of the Scientific Research Center at the University of Duhok, Iraq during the period from January, 2008 to May, 2009. Ten grafts were prepared and tested for each factor involved in grafting procedure. Micrografting success was determined after four weeks by the graft uptake, and the subsequent growth of the bud on the microscion. The investigated factors included: the interaction of rootstock and scion sources, the effect of BA on grafting success percentage was tested by treating the decapitated rootstocks and the excised shoot tips with different concentrations of BA (0.0, 0.5, 1.0 and 2.0 mgl^{-1}) for 10 min. prior to micrografting. In a separate experiment, BA was added to the nutrient medium used for culture of grafted plants at 0.0, 0.5, 1.0 and 2.0 mgl^{-1}, different sucrose concentrations were investigated as well by adding 15, 30, and 45 g/l to the nutrient media, To determine the need for aeration in the grafted plants and to compare between the grafting success percentages in both agar solidified medium and liquid medium, the effect of

the physical status of the medium was also tested. Agar (7 g/ l) was used in the case of solid medium and agar was omitted in the case of liquid medium, **a**n agar drop from the solidified culture medium was placed on the cut area of the rootstock to help in avoiding desiccation, which may enhance the union between scion and stock, **t**wo kinds of supporting agents (filter papers and cotton) tested in the case of using liquid medium in order to clarify their effects on micrografting success, and two different methods of micrografting were tested, inverted T-budding and Cleft (Split) grafting to examine their effects on micrografting success percentage.

All experiments were designed as randomized complete block design (RCBD). The comparison between means was carried out according to Duncan's multiple range test ($P < 0.05$) using a computerized program of SAS (SAS, 2001).

Results and Discussion

Figure (1, A) demonstrates that different grafting success percentages were achieved between different apple and pear rootstocks and scions combinations. The highest micrografting success (90%) was obtained while grafting *P. calleryana* pear on *P. calleryana* stocks followed by 80% micrografting success percent in case of the grafting of MM106 apple scions on MM106 apple stocks which were significantly higher than the grafting of heterografting between different cultivars. High significant percentage of successful grafts (80%) was obtained when shoot tips of Anna apple and Aly-Sur pear were obtained from tissue culture grown plantlets, as compared to the lowest percentages of successful grafts (30 and 40%) which were obtained when shoot tips were obtained from flushes in trees grown under field conditions (Figure 1, B).

A significant difference was noticed in apple micrografting success while using *in vitro* micropropagated shoots as rootstocks (60%) when compared with the use of *in vitro* germinated seedlings (48%). The same micrografting success (60%) was achieved for pear micrografting with both kinds of rootstocks (Figure 1, C).

The effect of BA on the ability of graft union to form successfully varied from 30 to 90% between the four treatments. The success rate of the untreated (control) microscions was significantly lower than the other treatments with 40 and 30% for both apple and pear, respectively. Medium supplemented with 2 mgl^{-1} BA produced the highest grafting success percent (90%) for apple, whereas medium supplemented with 1 and 2 mgl^{-1} BA gave the highest grafting success percent (90%) for pear (Figure 1, D). This confirmed the value of using cytokinins to improve grafting success by promoting and inducing callus growth and the formation of graft union between rootstocks and scions. Figure (1, E) shows that dipping rootstocks and scions in different BA concentration solutions before micrografting had no significant influence on micrografting success in both apple and pear. In the four treatments, wetness of microscions and the graft area caused the failure of the graft union to form. Only 10 to 40% graft unions formed successfully.

Micrografting success improved with the increase in sugar levels till 30 g/l in both apple and pear micrografts. The mean values were 50% and 40% at 15 g/l sucrose level which rose up to 60% and 70% at 30 g/l level but grafting success percentage lowered down to 30 and 40% on the media supplemented with 45 g/l sucrose for both apple and pear micrografts, respectively. The mean values indicated that sugar levels influenced the graft success whereas both apple and pear responded almost similarly (Figure 1, F). Figure (1, G) shows that using liquid medium raised successful grafts percentage to 60 and 70% from only 10% in case of solidified media with agar for both apple and pear micrografts, respectively. These results confirmed that using liquid medium was more effective than solid media.

Figure (1, H) declares that micrografts in which an agar drop was added to their grafted area were highly succeeded (70 and 60%) as compared with those grafted without an agar drop (10%) for both apple and pear micrografts respectively. The major cause of micrografting union failure without an agar drop might be due to desiccation (George *et al.*, 2008). 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. Micrografting successes of 50 and 60% and 60 and 70% were obtained while using filter paper and cotton for both apple and pear micrografts, respectively (Figure 1, I).

The comparison between T-budding and cleft grafting (apex shoot-tip) methods on successful grafts of apple and pear is shown in figure (1, J). By inverted-T incision there were 80 and 90% successful micrografts in Anna on MM106 apples and Aly-Sur on *P. calleryana* pears, respectively. Lower success of 70% in both apple and pear was observed when scion was grafted by surface placement using cleft grafting.

The *in vitro* micrografting of apple and pear was successfully achieved. The establishment of a micrografting protocol for clonally propagating true-to-type mature apple and pear genotypes might be an efficient technique overcoming conventional apple and pear propagation problems. *In vitro* grafting of 0.1-0.2 mm-long shoot tips on two weeks old rootstock seedlings or *in vitro* rooted shoots enabled getting successful grafts that could be transplanted to soil with high survival rates. Shoot tip grafting might also have important application of basic research in the field of graft compatibility, physiology of graft union and plant aging. A high mortality rate was found with grafted plants transferred to soil. Successful gradually acclimatized micrografts of both apple and pear reached to 85 and 90%, respectively. Figure (2) shows successful apple micrografting on liquid medium and using different supporting agents.

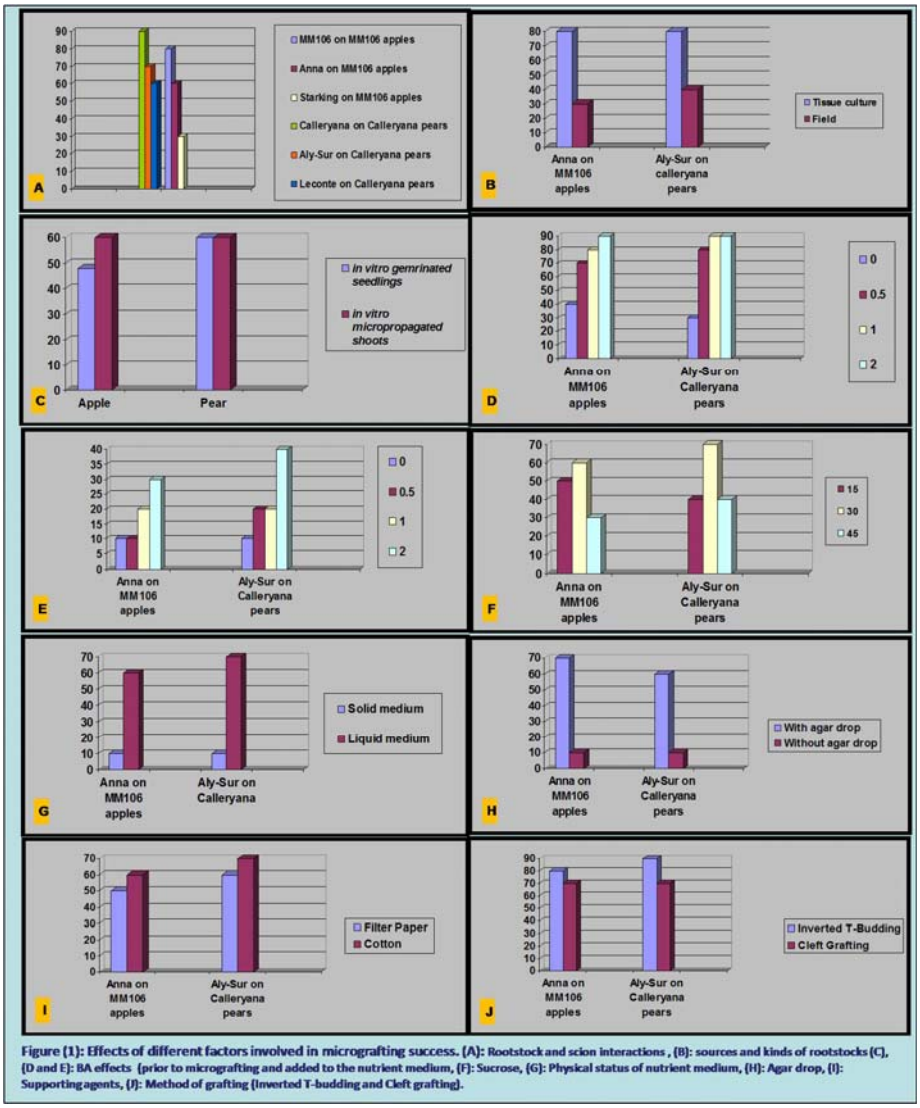

Figure (1): Effects of different factors involved in micrografting success. (A): Rootstock and scion interactions , (B): sources and kinds of rootstocks (C), (D and E): BA effects (prior to micrografting and added to the nutrient medium, (F): Sucrose, (G): Physical status of nutrient medium, (H): Agar drop, (I): Supporting agents, (J): Method of grafting (Inverted T-budding and Cleft grafting).

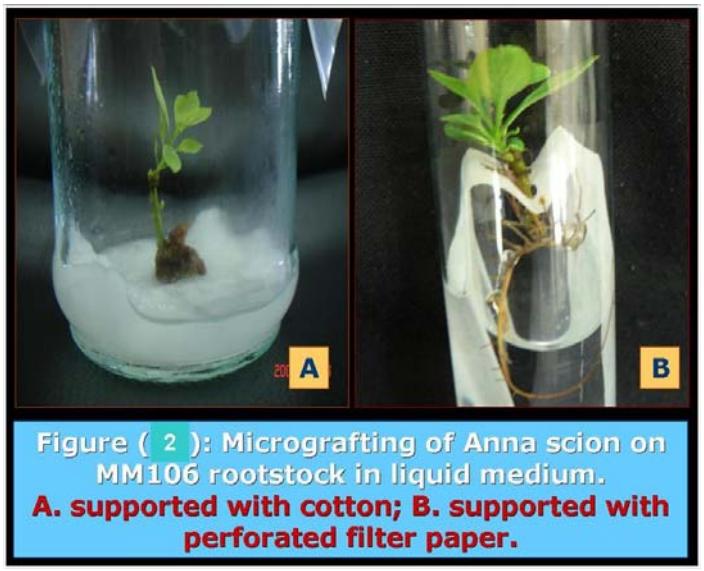

Figure (2): Micrografting of Anna scion on MM106 rootstock in liquid medium.
A. supported with cotton; B. supported with perforated filter paper.

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