MICROGRAFTING: A TOOL FOR THE PLANT PROPAGATOR

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Micrografting is a relatively new technique for the production of grafted plants *in vitro*. It was developed by Murashige *et al.* (4) in the early 1970's to rid *Citrus* cultivars of viruses. This technique can be more effective than thermotherapy, apical meristem culture, or embryogenesis (*in vivo* or *in vitro*) in the elimination of viruses from desired cultivars. Micrografted plants bypass the juvenile phase which does not occur if viruses are eliminated through nucellar embryony in *Citrus*.

Micrografting requires very few materials, but it does require precise manipulation of small tissues and plant organs. Seeds must be available that can be sown aseptically *in vitro* and thus serve as seedling rootstocks. Shoot-tips (0.1 to 0.2 mm in length) are taken from surface disinfested scions and placed on the decapitated seedling under aseptic conditions (Figure 1, a-d). If successful, the grafted plant develops and is then tested for the presence of viruses.

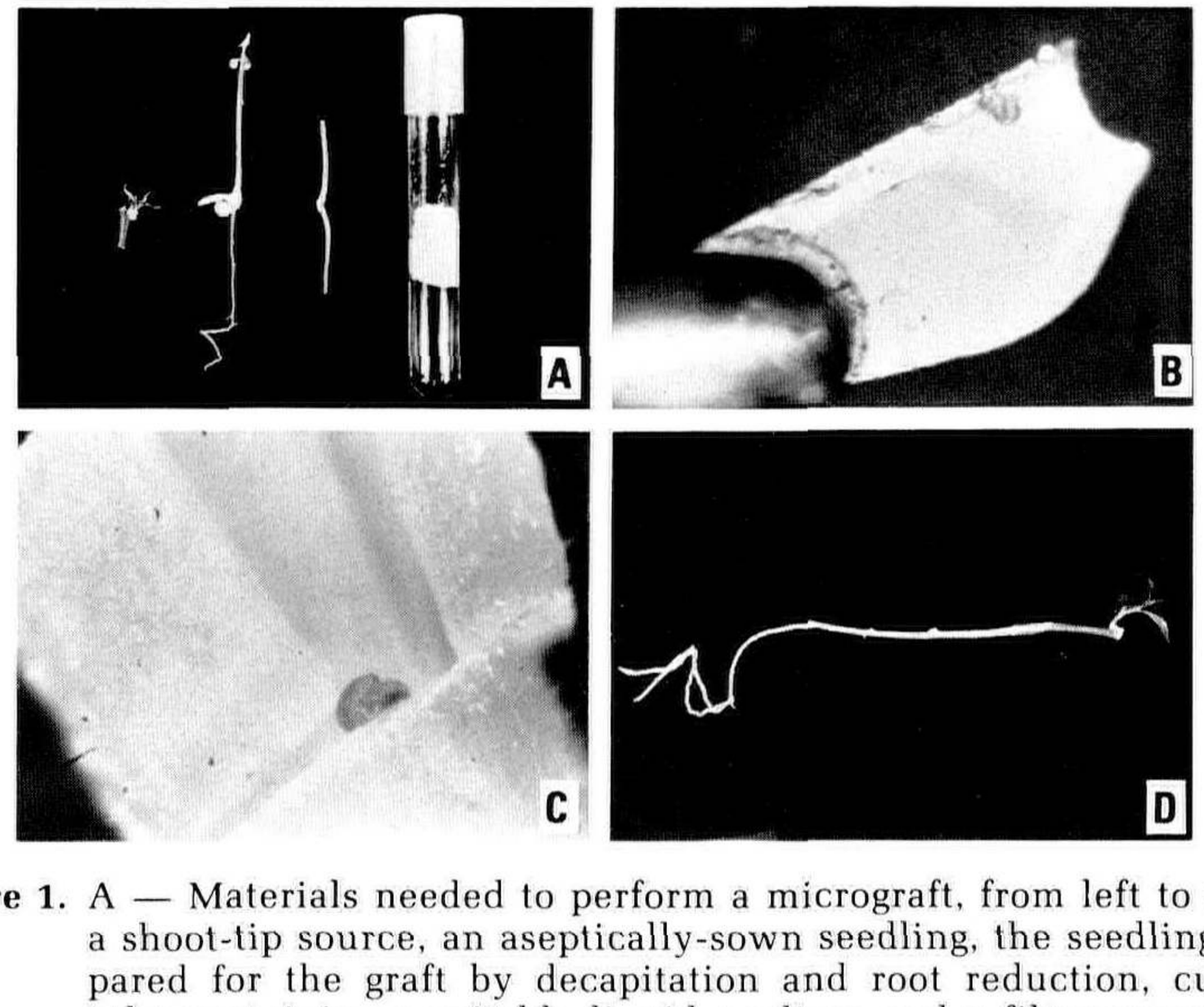

Figure 1. A — Materials needed to perform a micrograft, from left to right: a shoot-tip source, an aseptically-sown seedling, the seedling prepared for the graft by decapitation and root reduction, culture tube containing a suitable liquid medium and a filter paper support; B — A shoot-tip (0.2 mm) on a razor blade fragment; C — Shoot-tip on the cortical surface in an inverted-T incision; D — successful, intact *Citrus* micrograft after ca. 3 weeks in culture; notice root regrowth and shoot emergence from inverted-T incision.

The majority of micrografting work to date has focused on the elimination of viruses from citrus and stone fruits (3,4). However, there are other possible areas of interest to plant propagators and horticulturists that might be investigated using this technique. Cultivar improvement is the subject of extensive effort by horticulturists/geneticists. Whether by genetic or cultural means, improved growth rates, improved nutritional and water use efficiencies, and improved flowering characteristics are goals of many breeding programs. The results of repeated culturing of shoot apices of geranium, *in vitro*, suggests that latent viruses may be present in these plants and, when eliminated or diminished, result in increased plant vigor, water and nutrient use efficiency, and uniformity of flowering (W. Oglevee, personal communication). If latent viruses (non-pathogenic) are present and disrupt normal metabolism, their elimination by micrografting might be feasible and the hypothesis that latent viruses affect cellular metabolism could be tested. Improvement might be defined as the elimination of non-pathogenic viruses responsible for variegation (*Euonymus, Nandina)* or improvement might be measured in physiological terms such as increased photosynthetic rates.

Rejuvenation is an area of extreme interest to plant propagators working with woody perennials that show a concomitant loss of rootability as the plant develops ontogenetically. French workers have found that by repeatedly grafting mature, difficult-to-root, Douglas fir trees, the plants are gradually rejuvenated and by the fifth serial graft the plants root easily (2). Might it be possible to supplant the five serial grafts utilizing fully-developed buds by a single micrograft utilizing a shoot-tip measuring 0.1 to 0.2 mm? The micrografting technique is available to test this hypothesis using difficult-to-root *Eucalyptus spp.* as shoot-tip donors and aseptically sown seeds as rootstocks.

Micrografting is a difficult technique with success rates usually below 50%. This generally is not a limitation in virus elimination since once a single plant has been identified as virus-free it can be propagated by conventional means. However, to be able to perform some of the experimentation outlined above, it clearly would be an advantage to have success rates that approach 100%.

In *Citrus*, several parameters have been studied to increase the success rate. The placement of the apical meristem on the seedling rootstock has been found to be critical (1,5). Placement of the shoot-tip in contact with the vascular ring or on the cortical surface in an inverted-T incision have been shown to be the most successful treatments (Figure 2). It has been recommended to place *Citrus* shoot-tips on the seedling root-

stock in an inverted-T to maximize the possibility of success (1,5).

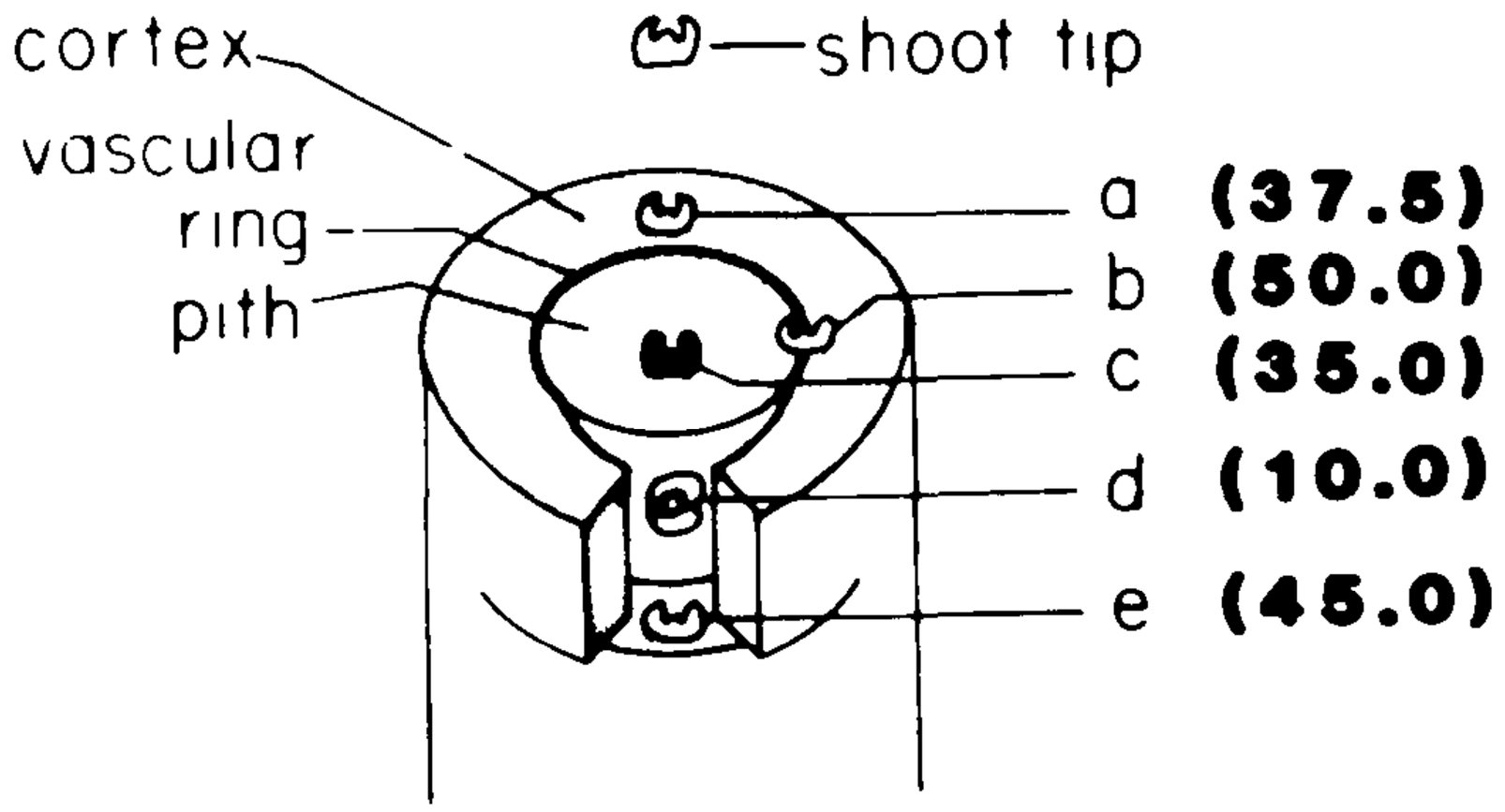

Figure 2. Various locations on the seedling rootstock where the shoot-tip may be placed. Bold numbers in parentheses are the percent successful micrografts when the shoot-tip is placed in that location Drawing and data from Navarro *et al* (5)

The age of the developing seedling rootstock also affects the success rate in *Citrus* (Figure 3). The greatest success was achieved using seedlings two weeks after sowing (5). When younger seedlings were used as rootstocks the micrografted shoot-tips were overwhelmed by callus growth and after 2 weeks the shoot-tips tended to die after being micrografted.

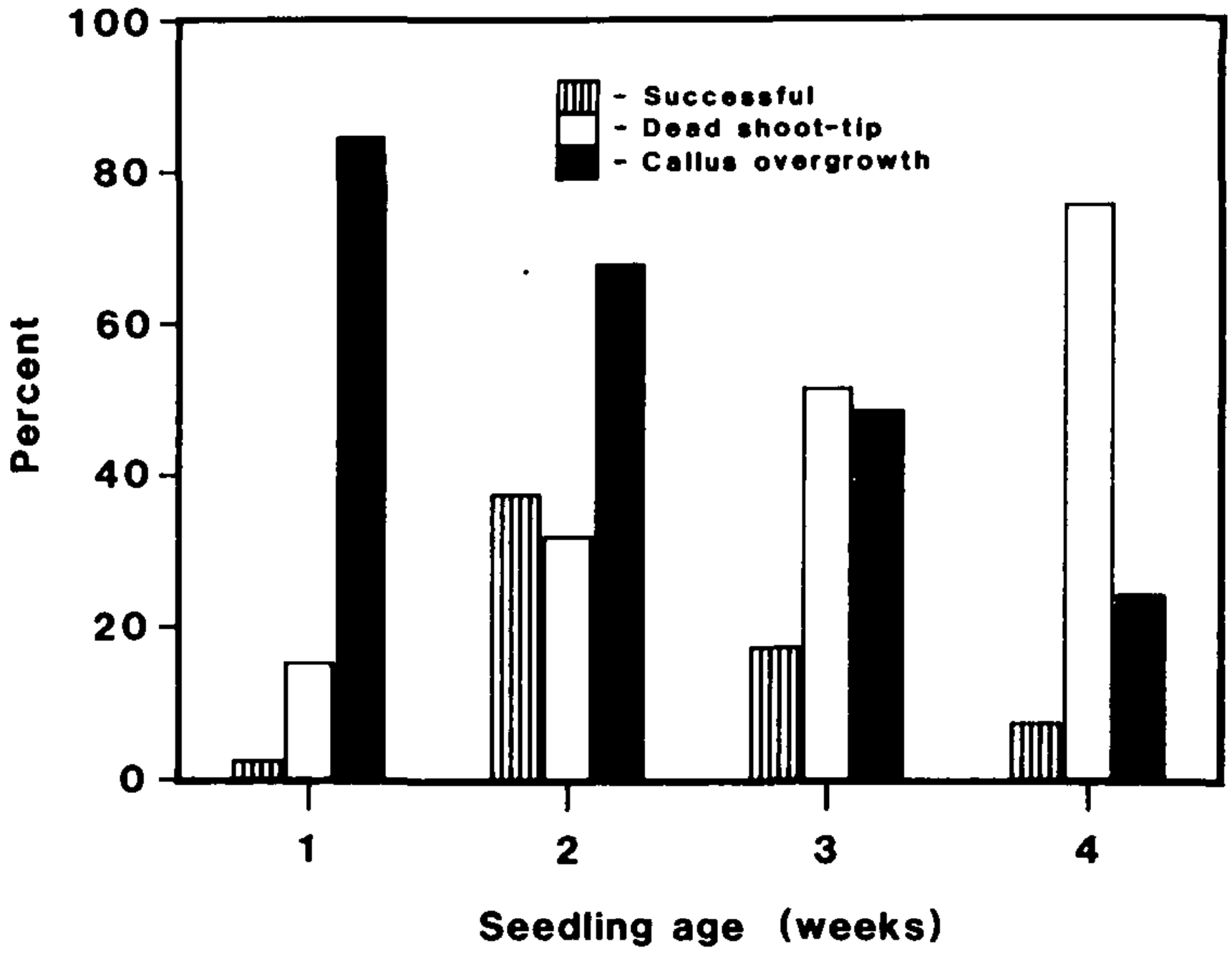

Figure 3. The effect of seedling age on the rate of success from *Citrus* micrografts Data from Navarro *et al.* (5).

There is an inverse relationship between the size of the shoot-tip and the micrografting success rate. Navarro *et al.* (5) found that increasing the shoot-tip size of 'Robertson' navel orange from 0.05 mm to 0.5 mm also increased the success rate from 1.8% to 47.3%. One must keep in mind, however, that larger shoot-tips also are more likely to contain viruses.

There are well-established occurrences of graft incompatabilities in fruit trees and this seems also to occur in micrografting. Edriss and Burger (1) have shown varying micrografting success rates in combinations of 'Troyer' citrange, 'Carrizo' citrange, and 'Sacaton' citrumelo rootstocks and 'Mexican' lime, 'Valencia' orange, and 'Star Ruby' grapefruit (Table 1). Jonard *et al.* have been able to detect early incompatabilities which only appear in the orchard after several years (3).

Table 1. Percent successful grafts among 3 *Citrus* scions and 3 trifoliolate rootstocks. Values are means ± 1 standard deviation.

	Percent successful grafts		
Rootstock	'Mexican' lime	'Valencia' orange	'Star Ruby' grapefruit
'Troyer' citrange	24 4 ± 6.2	17.7 ± 5 5	23 3 ± 5.2
'Carrizo' citrange	63 8 ± 8.1	50 0 ± 6 9	38 3 ± 4 8
'Sacaton' citrumelo	64 4 ± 7 0	44 4 ± 6 2	28.2 ± 4.8

from Edriss and Burger (1)

Pretreatments of the shoot-tip and/or seedling rootstock have been shown to increase the micrografting success rate. Jonard *et al.* (3) treated peach shoot-tips in 0.1 mg zeatin/l and increased the success rate by 300% (Table 2). Edriss and Burger (1) found that a pre-grafting treatment of the seedling trifoliolate rootstocks in 10 mg 2,4-D/l or 1 mg kinetin/l increased success rates by 200% (Table 3).

Table 2. Effects of pre-treatments on the success of in vitro micrografting of peach trees

Treatment of apex with zeatin		Number of grafts,	Number of living	Number of developed plants,	Percent success
mg/l	Hours	N_1	apices,	N_2	N_2/N_1
0		23	11	5	21.7
0 01	48	20	10	10	50
0 01	240	19	16	11	57 9
0 1	48	25	16	16	64
1 0	48	10	3	2	20

from Jonard *et al.* (3)

Each species that is used in micrografting will certainly have its own special requirements for success. The work cited

here is presented only as a reference of parameters that have been studied and have been found to affect the micrografting procedure.

Table 3. The effect of growth regulator pre-treatments on the grafting success of 'Star Ruby' grapefruit onto 3 rootstock cultivars.

Pre-treatment	Conc. (mg/l)	Percent successful grafts 'Troyer'	'Carrizo'	'Sacaton'	Mean ± S.D.
2,4-D	1	50	26.6	66 6	47.8 ± 16 4
2,4-D	10	75 4	73 3	78.5	75 7 ± 2.1
Kinetin	1	66 6	85.0	71 4	74 4 ± 7 8
Kinetin	10	33 3	56.6	33 3	41 1 ± 11 0
2,4-D + Kinetin	1 + 10	44 4	44.4	41 6	43.5 ± 1 3
2,4-D + Kinetin	10 + 1	61.6	55.5	50 0	55.7 ± 4 7
Water (control)	—	23 3	38.5	28 6	30 1 ± 6 3

from Edriss and Burger (1)

LITERATURE CITED

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PROSPECTS FOR GENETIC ENGINEERING IN PLANT PROPAGATION

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The rapidly evolving technology of genetic engineering is opening up exciting new possibilities for plant science and for plant propagation. Although, until now, practical applications of gene splicing techniques have lagged behind fundamental advances, several applications are now ripe for exploitation and it is these that I wish to address.