In vivo Micrografts in Almond and Their Application in Breeding Programs

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SUMMARY. The techniques of micrografting provide useful tools for the early propagation of breeding lines in variety improvement programs. In this study, the success of various in vivo micrografts of buds of 'Nonpareil' almond (*Prunus dulcis*) seedlings have been examined. Variables tested include type of microscion, the rootstock genotype, and the growth stage of the rootstock. Microscions tested included small [3 mm (0.12 inches)] microwedges from either unsprouted or recently sprouted buds. Approach micrografts were also evaluated. Three-month-old woody rootstocks tested included 'Nemaguard' and 'Nemared' peach (*P. persica*) rootstocks, and the 'Hansen' (peach x almond) hybrid rootstocks. 'Nemared' rootstocks were also grafted after 1 month of growth when the tissue was still herbaceous. Micrografts onto woody rootstocks and using recently sprouted buds showed the best results. With very young or weak seedlings, where the recovery of microscions was difficult, the use of approach micrografts onto herbaceous rootstock proved a useful technique.

Grafting has been widely used over the centuries for the asexual propagation of fruit trees. Micrografts, developed in the 1970s, consist of the grafting of millimeter-size vegetative buds usu-

ally under aseptic conditions. Initially, this technique was used for virus elimination in fruit trees (Navarro et al., 1975; 1982; Deogratias et al., 1986; Gebhart and Goldbach, 1988). It has since become widely used in fruit tree micropropagation. More recently, it has been employed for the early assessment of rootstock-scion incompatibility (Jonard et al., 1990), the commercial multiplication of new germplasm (Hammerschlag and Scorza, 1991; Monteuiis, 1995), virus detection (Tanne et al., 1993), and the evaluation of virus resistance (Martínez-Gómez and Dicenta, 2000).

Very little information is available concerning the effectiveness of different micrograft techniques for almond. Initial surveys in almond showed a success rate between 15% and 80% depending on microscion origin, mode of grafting and condition of the rootstock (Ghorbel et al., 1998). Efficient micrografting offers several advantages to tree crop breeding programs, including the early propagation of new genotypes and the invigoration of weak seedlings.

The objective of this study was to examine the success of *in vivo* micrografts of 'Nonpareil' almond seedling buds under different conditions. Variables examined in this study included the rootstock genotype, the type of microscion used, and the growth stage of the rootstock.

Materials and methods

PLANT MATERIAL. Buds from 6-week-old 'Nonpareil' almond seedlings [with stems of about 5 mm (0.19 inches) in diameter] were used for *in vivo* micrografts. For approach micrografts, younger 3-week-old 'Nonpareil' seedlings [at about 3 mm (0.12 inches) in diameter] were assayed. Rootstocks evaluated included 'Hansen' hybrid, and 'Nemaguard' and 'Nemared' peach rootstocks, which were 3 months old with stem cambium becoming woody. One-month-old 'Nemared' peach rootstocks still at the herbaceous stage were also tested. Rootstocks were growing in 5-L (1.35-gal) pots in the greenhouse at a temperature of 25 to 30 °C (77 to 86 °F) and a photoperiod of 16 h.

MICROGRAFT TECHNIQUE. Micrografts were performed in aseptic *in vivo* conditions, with all grafting tools being previously sterilized with alcohol. Microscions tested included small

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Fig. 1. Details of microscions at about 5 mm (0.2 inches) stem length showing unsprouted (A), and recently sprouted (B) buds.

[3 to 5 mm (0.12 to 0.19 inches)] microwedges from both unsprouted and recently sprouted buds (Fig. 1), and approach micrografts. ‘Nemared’ rootstocks were grafted either after 3 months of growth when the tissue had become woody [at about 8 mm (0.31 inches) stem diameter], or after about 1 month of growth when the tissue was still herbaceous [at about 3 mm in diameter]. Microscions were inserted and covered with parafilm (Aglis, Medel Co., Tokyo). Two weeks after micrografting the rootstocks were pruned above the micrografts and the parafilm on the sprouted bud treatments was removed.

STATISTICAL ANALYSIS. Each treatment was replicated three times, with 10 trees per replication. The percentage of both bud survival and micrografts showing continued growth was recorded. Subsequent growth of the micrografts (height and diameter) was examined at 1, 2, and 3 months after initial pruning. An analysis of variance was computed and means were separated by LSD test at $P < 0.05$ (SAS Institute, 1989).

Results and discussion

SUCCESS OF MICROGRAFTS. Significant differences were observed in the percentage of both bud survival (takes) as well as micrograft bud growth among the different microscions, the different rootstock and the different stage of rootstock assayed (Table 1). The failure of micrografts when using sprouted buds was usually due to either dehydration and/or fungal infection. Bud dehydration was the major cause of failure when using unsprouted bud or approach micrografts. Of the unsprouted buds that did not grow,

67% died primarily due to dehydration while the remaining 33% remained dormant. The survival sprouted buds was higher on woody rootstocks as compared to the herbaceous rootstocks. For unsprouted buds these differences were not observed. Successful takes for approach micrografts on herbaceous ‘Nemared’ rootstocks were higher (averaging 50%) as compared to woody rootstocks (30%). In general, micrografts using recently sprouted buds performed better (69% showing continued bud growth) than micrografts with unsprouted buds (54%). Success as high as 85% was observed for micrografts using recently sprouted buds on ‘Hansen’ rootstock (Table 1). Smaller, though often significant differences were observed among the rootstocks used. The average (sprouted and unsprouted) for continued growth using woody rootstocks was 67.5% as compared to 40.0% for the herbaceous stage. The improved

performance of woody rootstock for micrografts contrasts with the results of approach micrograft, where herbaceous rootstocks showed better performance over woody (50% versus 30%) (Table 1). Under our conditions, approach micrografts performed less well than the other micropropagation techniques. Approach micrografts were still useful, however, for very young or weak seedlings, and where the recovery of microscions is difficult.

Our results are similar to those of an earlier study by Ghorbel et al. (1998) using in vitro conditions, where success of up to 80% with microscions of about 4 mm (0.16 inches) was reported. Similarly, success of micrografts using recently sprouted buds under in vitro conditions was reported to be from 60 and 80% for peach (Martínez-Gómez and Dicenta, 2000). In a comparison of micrografting success under in vivo and in vitro conditions, Monteuiis (1995) obtained similar results in mango (*Acacia mangium*) (about 50%), while Ozambak and Schmidt (1991) showed a better performance for in vivo micrografting in cherry (*Prunus avium*) (57% in vitro versus 71% in vivo).

GROWTH OF MICROGRAFTS.

Micrograft growth differences were not significant among the different rootstocks used (Table 2). However, significant differences were observed for the type of microscion used (sprouted or unsprouted). Consistent, though only occasionally significant, growth differences were also observed for the growth stage (woody or herbaceous) of the ‘Nemared’ rootstock used. Over all rootstocks and stages,

Table 1. Percentage of bud survival and of micrografts showing subsequent bud growth for the different treatments examined.

Rootstock			Success of micrografts	
Genotype	Stage	Microscion	Bud survival ²	Bud growth ²
Hansen	Woody	Unsprouted bud	90 a	65 bc
		Sprouted bud	85 ab	85 a
Nemaguard	Woody	Unsprouted bud	75 bc	60 cd
		Sprouted bud	65 c	65 bc
Nemared	Woody	Unsprouted bud	80ab	60 cd
		Sprouted bud	75 bc	75ab
		Approach	30 e	30 e
Nemared	Herbaceous	Unsprouted bud	75 bc	30 e
		Sprouted bud	50 d	50 d
LSD		Approach	50 d	50 d
			14.0	12.5

²Grouping within columns by LSD test at $P < 0.05$.

Table 2. Height and diameter of shoots from the different micrografting treatments examined.

			1 month		2 months		3 months	
Rootstock			Ht	Diam	Ht	Diam	Ht	Diam
Genotype	Stage	Microscion	(cm) ^{ys}	(mm) ^{ys}	(cm) ^{ys}	(mm) ^{ys}	(cm) ^{ys}	(mm) ^{ys}
Hansen	Woody	Unsprouted bud	5.2 bc	1.8 b	13.6 b	2.6 b	39.8 b	3.6 c
		Sprouted bud	14.0 a	2.6 a	24.8 a	4.6 a	63.9 a	6.2 a
Nemaguard	Woody	Unsprouted bud	5.0 bc	1.5 b	14.0 b	2.6 b	36.0 b	3.2 c
		Sprouted bud	9.9 b	2.2a	18.6ab	3.2ab	56.0a	5.5 a
Nemared	Woody	Unsprouted bud	5.6 b	1.6 b	12.6 b	2.8 b	39.0 b	4.1 bc
		Sprouted bud	10.9ab	2.3a	22.0a	4.0a	60.5a	6.6a
		Approach	7.0 b	1.2 b	12.2 b	2.4 b	38.0 b	4.2 bc
Nemared	Herbaceous	Unsprouted bud	3.0 c	1.0 c	8.8 bc	1.8 bc	29.6 bc	3.2 c
		Sprouted bud	7.6 b	1.8ab	16.6ab	2.6 b	55.0a	4.6 bc
		Approach	5.2 b	1.3 b	10.6 b	2.1 b	31.0 bc	4.0 bc
LSD			3.9	0.5	6.2	0.8	14.5	1.3

^zGrouping within columns by LSD test at $P < 0.05$.

^y1 cm = 0.39 inches; 1 mm = 0.039 inches.

micrografts using sprouted buds grew better than micrografts with unsprouted bud or approach micrografts. Three months after micrografting, the shoots from sprouted buds showed an average height of 58.6 cm (22.82 inches) and diameter of 5.7 mm (0.22 inches) for combined treatments, as compared to 36.1 cm (14.01 inches) and 3.5 mm (0.14 inches) respectively for unsprouted buds, and 34.5 cm (13.45 inches) in height with a diameter of 4.1 mm (0.16 inches) for approach micrografts. Early growth vigor may be desirable as Gebhart and Goldbach (1988) had previously found evidence for a positive correlation between the initial growth of micrografts and the later vigor of the plants in the field.

We have found the use of micrografts to be particularly valuable in the breeding program to rapidly propagate important genotypes, to invigorate weak material, and to eliminate virus contamination (see also Navarro et al., 1975; 1982; Deogratias et al., 1986; Gebhart and Goldbach, 1988). Use of in vivo rather than in vitro micrografts also avoids tissue culture transplant shock when transplanting from the sterile in vitro conditions. For commercial propagation, the micrografting of unsprouted buds, while less effective, is less tedious and hence more appropriate for routine work. While more difficult, the use of sprouted buds is justified when a high rate of success is desirable, owing either to the value of the scion or a limited availability of bud wood. We are presently employing this technique to recover aneuploids and haploids of almond which occur at low frequen-

cies in sexual embryos with twin seeds (multiple embryos within the same seedcoat). Often, such aneuploid material show very weak growth due to their particular genetic condition and their poorly developed state within twin seeds (see also Hesse, 1971; Gulcan, 1975). Over 80% of the aneuploids identified by this program have been salvaged from eventual seedling collapse by micrografting within the first 3 weeks of growth.

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