Orchard Variation in Micropropagated Trees of 'Redspur Delicious' Apple

Richard H. Zimmerman

U.S. Department of Agriculture, Agricultural Research Service, Fruit Laboratory, Beltsville, MD 20705-2350

Additional index words. *Malus ×domestica*, tissue culture

Abstract. Micropropagated trees of 'Redspur Delicious' apple (*Malus ×domestica* Borkh.), planted as small, actively growing trees in May 1982, lacked uniformity in tree size, appearance, and flowering by the spring of 1986. Only four of the 18 trees had a typical spur-type growth habit; these four trees had 80% more spurs per meter of shoot, 8 to 10 times as many flowers the first year of flowering and 9.5-fold higher early fruit yields, but were 40% smaller after 14 years in the orchard and had 25% less cumulative fruit yield than the nonspur types. Shoots from the spur-type trees were recultured in 1988 and the resulting trees planted in an orchard in 1990. These latter trees were uniform in appearance and all had typical spur-type growth, with about 30% more spurs per meter of shoot growth than the spur-type trees from which they were propagated. Micropropagating spur-type apples from previously micropropagated trees that have maintained clonal fidelity may overcome the potential problem of clonal variation in orchard planted micropropagated trees.

Variation among clonally propagated plants is not desirable, since the purpose of clonal propagation is to produce identical copies of the parent plant. An early concern with tissue culture of fruit crops was whether the micropropagated plants maintained clonal fidelity. This determination requires long-term field evaluation. Numerous standard and spur-type apple cultivars have been evaluated in long-term field experiments at Beltsville (Zimmerman and Miller, 1991; Zimmerman and Steffens, 1995, 1996). During these extensive evaluations, micropropagated trees of only one cultivar, 'Redspur Delicious', exhibited tree-to-tree variation, with most trees not retaining the spur-type growth habit. I decided to determine whether new tissue cultures established from the micropropagated trees that retained the spur-type habit would yield uniform spur-type trees. Results from this study are reported here.

Materials and Methods

Trees of 'Redspur Delicious' were micropropagated and field planted as actively growing plants about 40 to 50 cm tall in May 1982 (Zimmerman, 1983). The orchard site and cultural practices used have been described in detail (Zimmerman and Miller, 1991; Zimmerman and Steffens, 1995, 1996). Since differences in growth habit, size, flowering intensity, and yield in the first fruiting year (1986) were evident among the 18 trees of this cultivar, detailed data were collected on 10 shoots per tree. Variables measured were length of

shoot growth in the previous year (1985) and number of lateral branches (>5 cm) and spurs (<5 cm) on the measured shoot.

Shoots from three of the four trees with a distinct spur-type growth habit in this first micropropagation generation (MP₁) were successfully re-established in tissue culture in 1988 and were then micropropagated using the same methods as cited above. In 1990, 25 trees from each of the three MP₁ trees, which then formed the second micropropagation generation (MP₂), were planted in an orchard in a replicated study using a randomized complete block design with single tree plots. In 1994, data on the previous year's shoot growth and number of laterals and spurs, similar to the data on the original trees, were collected on

three to five shoots of each of these MP₂ trees. Each year the number of flower clusters was estimated using a rating scale (see footnote, Table 2), all fruit were harvested to obtain yield, and trunk diameter was measured and then converted to trunk cross-sectional area (TCSA) on all MP₁ and MP₂ trees. Yield efficiency was calculated as kg of cumulative fruit yield per cm² TCSA. Data were analyzed using the General Linear Model Procedure of SAS (SAS Institute, 1987).

Results

Spur-type trees of the MP₁ generation differed from nonspur trees when measured after the 1986 growing season with the spur-types having shorter shoots, more spurs, more total laterals (shoots plus spurs), a higher spur-to-lateral ratio, and fewer lateral shoots >5 cm long (Table 1). At this time, the spur-type trees also had a smaller TCSA, but a higher fruit yield and more flowers (Table 2). After an additional 9 years in the orchard, the spur-type trees were still smaller and had a lower cumulative fruit yield than the nonspur trees, although the yield efficiency of the spur-type trees was higher (Table 2). Differences in growth habit, size, and appearance among the original 18 trees have persisted until now.

The second set (MP₂) of micropropagated spur-type trees began flowering and fruiting one growing season earlier than the MP₁ trees. In 1994, the average flowering rating for all MP₂ trees was 5 (see Table 2 for rating scale) and cumulative fruit yield for 1993-94 was 6 kg per tree; both values were slightly less than the ones recorded for spur-type MP₁ trees in 1986 (Table 2) when they were the same age. The mean TCSA of 32.3 cm² was also slightly less than that of the spur-type MP₁ trees (Table 2). The MP₂ trees were similar among groups (tree 1 = MP₂, tree 2 = MP₂, tree 3 = MP₂) in the vegetative growth characteristics measured at

Table 1. Shoot growth characteristics of original (MP₁) micropropagated 'Redspur Delicious' trees after five growing seasons in an orchard, 1982-86.

Character measured	Spur	Nonspur	F ratio
Shoot length in previous year (1985) (cm)	81	110	19.14***
Laterals per meter of shoot			
Total ²	32.1	21.7	109.6***
Shoots (>5 cm long)	4.4	6.3	8.95**
Spurs (<5 cm long)	27.7	15.4	120.72***
Ratio of spurs : laterals >5 cm	8.7 ¹	3.1 ¹	102.94***

²Spurs plus shoots >5 cm long.

¹Values shown are means of ratios calculated for all branches measured.

, *Significant at $P \leq 0.01$ or 0.001, respectively.

Table 2. Trunk cross-sectional area (TCSA), flowering, and fruiting of original (MP₁) micropropagated 'Redspur Delicious' trees in 1986 and in 1995.

Character measured	Spur	Nonspur	F ratio
Flowering rating ² 1986	5.5	2.6	29.59***
Yield 1986 (kg per tree)	9.5	1.0	67.36***
TCSA ³ 1986 (cm ²)	36.5	49.5	7.01*
TCSA 1995 (cm ²)	129	207	15.83**
Cumulative yield through 1995 (kg per tree)	251	336	10.06**
Yield efficiency (cumulative kg·cm ⁻² TCSA)	2.0	1.7	5.37*

²Rating scale for number of flower clusters per tree: 1 = none; 2 = 1-5; 3 = 6-10; 4 = 11-25; 5 = 26-50; 6 = 51-100; 7 = 101-250; 8 = 251-500; 9 = >500.

³TCSA at 30 cm above the soil line.

*, **, ***Significant at $P \leq 0.05$, 0.01 or 0.001, respectively.

Received for publication 23 Sept. 1996. Accepted for publication 21 Jan. 1997. The cost of publishing this paper was defrayed in part by the payment of page charges. Under postal regulations, this paper therefore must be hereby marked advertisement solely to indicate this fact.

Table 3. Comparison of original micropropagated (MP₁) spur-type trees with set (MP₂) of micropropagated trees obtained from them. Data after five growing seasons in an orchard.^a

Character measured	Tree 1		Tree 2		Tree 3	
	MP ₁	MP ₂	MP ₁	MP ₂	MP ₁	MP ₂
Shoot length previous year (cm)	54 ± 15 ^z	46 ± 12 ^y	85 ± 17 ^z	50 ± 11 ^y	80 ± 16 ^z	48 ± 12 ^y
Laterals per meter of shoot						
Total	33 ± 4	37 ± 8	31 ± 4	38 ± 8	31 ± 2	37 ± 7
Shoots (>5 cm long)	5 ± 3	2 ± 4	4 ± 3	1 ± 2	4 ± 3	2 ± 3
Spurs (<5 cm long)	28 ± 4	35 ± 10	27 ± 4	37 ± 9	27 ± 3	35 ± 9

^aMean ± standard deviation based on 10 shoots from a single tree.

^bMean ± standard deviation based on 22 single tree replicates because of deer browse damage to trees in other replicates. Differences among the three sets of MP₂ trees nonsignificant for all characters measured.

the end of the fifth growing season (1994) (Table 3). In contrast to the MP₁ trees, all the MP₂ trees in this planting were spur-type with a uniform overall appearance. The MP₂ trees tended to have shorter shoots and more spurs than the MP₁ trees from which they were derived (Table 3).

Discussion

All spur-type strains of 'Delicious' apple have originated as mutations, and 'Redspur Delicious' originated as a whole tree sport about 1954 (Fisher and Ketchie, 1989). The variation in growth habit and flowering and fruiting characteristics of micropropagated trees of 'Redspur Delicious' apple reported here is unique among the more than 35 cultivars evaluated in our program, including 10 spur-types.

The chimeral nature of the original mutation for spur-type growth habit means that propagules from the spur-type tree may revert to the original nonspur form. This reversion is obviously undesirable for commercial orchard practices. Faedi and Rosati (1985) reported that some clones of 'Delicious' and 'Golden Delicious' are more prone to reversion after budding or grafting than others and, among the clones tested, those of 'Golden Delicious' were more likely to exhibit reversion than those of 'Delicious'. They also found that buds from the lower one-third of the budstick were more likely to revert. Budwood taken from trees of one clone, in which the source trees did not show reversion until 2 years after budwood was collected, produced more than 90% reverted propagules after 10 years in the field (Faedi and Rosati, 1985). Thus, careful selection of the clone, of budwood source trees, and

of budwood is essential to minimize the probability of reversion.

When a spur-type clone (Perleberg 3) of 'Golden Delicious', which normally has a low frequency of reversion (Faedi and Rosati, 1985), was micropropagated, the spur habit was lost in all trees planted in the field (Rosati and Gaggioli, 1987). They concluded that this loss of spur habit was not a mutation back to the nonspur form, since they used several shoot tips to establish the cultures for micropropagation, and the source tree continued to show normal spur-type growth for 6 years after the shoot tips had been collected. Why tissue culture resulted in the loss of the spur-type growth habit in this case is unknown.

'Redspur Delicious' has some tendency to revert to the nonspur habit in the orchard (S.S. Miller, personal communication). Whether the loss of the spur habit in the 'Redspur Delicious' MP₁ trees reported here is a result of micropropagation or is related to some instability in the original mutation is not yet known. One possibility is that some of the multiple shoot tips used to establish the cultures from which the MP₁ trees were produced were chimeral. Whether the product of chimeral starting tissue or a reversion induced by tissue culture, this type of reversion was not found in nine other spur-type strains of several cultivars that have been micropropagated and grown in the orchard here at Beltsville.

The MP₁ spur-type trees performed as anticipated in comparison with the MP₁ trees that had reverted to a standard growth habit. Fruiting of the spur-type trees began earlier, but ultimately the larger size of the reverted trees resulted in more fruit produced per tree. Nevertheless, the spur-type trees could have pro-

duced higher yields per ha had they been grown at a much closer spacing.

The question remains why no reversion occurred in the MP₂ trees. Perhaps the MP₁ trees that were stable after micropropagation were then a stable source for further tissue culturing. When chimeral origin or reversion to a different form may be a problem, growing out micropropagated trees for several years and repropagating from those showing complete clonal fidelity might be an appropriate method to prevent problems in the orchard.

Literature Cited

- Faedi, W. and P. Rosati. 1985. Reversion in spur clones of Delicious and Golden Delicious apple trees. *Acta Hort.* 159:57-61.
- Fisher, D.V. and D.O. Ketchie. 1989. Survey of literature on red strains of 'Delicious'. Washington State Univ. Ext. Bul. 1515.
- Rosati, P. and D. Gaggioli. 1987. Field performance of micropropagated peach rootstocks and scion cultivars of sour cherry and apple. *Acta Hort.* 212:379-390.
- SAS Institute. 1987. SAS/STAT guide for personal computers, ver. 6. SAS Institute, Cary, N.C.
- Zimmerman, R.H. 1983. Factors affecting in vitro propagation of apple cultivars. *Acta Hort.* 131:171-178.
- Zimmerman, R.H. and S.S. Miller. 1991. Orchard growth and fruiting of micropropagated apple trees. *J. Amer. Soc. Hort. Sci.* 116:780-785.
- Zimmerman, R.H. and G.L. Steffens. 1995. Cultivar, planting density, and plant growth regulator effects on growth and fruiting of tissue-cultured apple trees. *J. Amer. Soc. Hort. Sci.* 120:183-193.
- Zimmerman, R.H. and G.L. Steffens. 1996. Long-term evaluation of micropropagated apple trees: Vegetative growth, cropping, and photosynthesis. *Scientia Hort.* 66:69-76.