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Field Performance of ‘Olympus’ Strawberry Subclones

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Abstract. **The field performance of micropropagated and runner-propagated subclones of ‘Olympus’ strawberry *(Fragaria × ananassa* Duch.) was compared. The yield of micropropagated plants was not greater than that of runner-propagated plants. There was significant variability among micropropagated subclones, with the highest yielding subclone having 68% higher yield than the lowest yielding subclone in each of the first 2 years. However, after runner propagation for 4 years, selected subclones showed no differences in yield. Differences among subclones of ‘Olympus’ were not stable and were most likely transient responses to the micropropagation environment. The apparent superiority of the subclones was not genetic.**

Micropropagation is used in commercial propagation of strawberries and in breeding programs to produce many plants rapidly. If virus-free plants are used to initiate the cultures, it offers the advantage of producing disease-free plants. Micropropagated strawberry plants can be stored under refrigeration (Mullin and Schlegel, 1976), making it a valuable technique for the storage of germplasm.

Although there are advantages for the use of micropropagation, there are concerns about genetic changes resulting from this process. Genetic variability of plants regenerated from cells in culture has been demonstrated for many species (Evans et al., 1984). Discrete morphological variants have been observed in micropropagated strawberry plants (Scott and Zanzi, 1980; Sansavini and Gheradi, 1979; Swartz et al., 1981). Leaf. variegation consisting of a narrow white streak in the leaf blades (Scott and Zanzi, 1980; Sansavini and Gheradi, 1979; Swartz et al., 1981) or a chlorosis of the leaves (Scott and Zanzi, 1980; Swartz et al., 1981) has been noted. Additionally, Swartz et al. (1981) found growth changes among micropropagated plants including dwarfs, compact trusses, lack of runner production, and female sterility.

In addition to these distinct morphological variants, morphological and physiological changes as a consequence of propagation have been noted (Cameron and Hancock, 1986; Cameron et al., 1985, 1989; Swartz et al., 1981). Generally, micropropagated plants had greater vigor, runner production, and yields than runner-propagated plants. However, not all cultivars exhibited a yield increase (Cameron et al., 1985). Cameron et al. (1989) noted higher rates of CO_2 assimilation and stomatal conductance in micropropagated ‘Earliglow’ compared to conventionally propagated ‘Earliglow’.

In addition to differences between micropropagated plants and runner-propagated plants, Swartz et al. (1981) also found differences in yield among four micropropagated subclones of ‘Earliglow’. This result suggested that genetic improvement could be made through selection of superior meristem-tip lines (Swartz et al., 1981).

The objective of this study was to determine if micropropagated subclones of ‘Olympus’ differed from runner-propagated subclones or varied among micropropagated subclones and if this variation could be useful in genetic improvement of strawberries. If clonal variation was identified, the stability of this variation would be tested by repropagation and replanting in the field. Commercial propagators had observed differences in runnering capabilities of ‘Olympus’ plants after micropropagation. For this reason, ‘Olympus’ was selected for use in this study.

1984 planting. In 1983, micropropagated subclones of ‘Olympus’ strawberry were propagated from eight source plants from nuclear plant stocks. All source plants indexed negative for virus infection by leaf grafting onto *F. vesca* ‘Alpine’ and ‘UC-5’. Cultures were initiated on 2 and 19 Aug. 1983. Each explant was maintained as a separate subclone. The subclones went through three transfers on full strength MS salts and vitamins (Murashige and Skoog, 1962) with 3% sucrose, 0.2% Gelrite, and 0.5 mg benzyladenine (BA)/liter. Concentrations (mg·liter^{-1}) were: BA, 0.8 on the fourth transfer, 0.5 BA with 0.1 indoleacetic acid (IAA) for the fifth transfer, and 0.7 BA, 0,1 IAA, and 0.1 gibberellic acid (GA,) for the sixth transfer. The seventh transfer was to a rooting medium consisting of half-strength MS salts, 600 mg activated charcoal/liter, and 2 mg IAA/liter. From initiation of cultures to transfer to rooting media required 14 to 16 weeks. A total of 43 micropropagated subclones were obtained from the eight source plants, with four to six micropropagated subclones obtained from each source plant. Plants were not cold stored before planting.

Runner-propagated plants from each source plant were produced in a screenhouse. Only four of the eight source plants produced a sufficient number of runner plants to be included in the field planting.

In June 1984, 47 subclones (43 micropropagated subclones from eight source plants and one runner plant subclone from each of four source plants) were planted at Washington State Univ., Puyallup, in four replications of 10 plants each. Plants were set 61 cm apart in rows on 107 cm centers and allowed to form matted rows.

Plots were hand-harvested and total fruit

Table 1. Field performance of micropropagated and runner-propagated subclones of ‘Olympus’ planted 1984 at Puyallup, Wash.

Harvest components	Subclones: Micropropagated[z]	Subclones: Runner propagated	Significance: Propagation method	Significance: Among micropropagated subclones
1985				
Yield (kg·ha^{-1})	33,800 (23,600–39,500)	32,400 (31,400–33,200)	NS	**
Fruit wt (g)	12.4 (11.1–13.8)	11.9 (11.7–12.1)	*	NS
Fruit rot (%)	12.2 (7.2–16.6)	12.9 (11.6–14.7)	NS	*
Fruit firmness (N)	1.7 (1.4–1.9)	1.7 (1.6–1.9)	NS	NS
Midpoint of harvest (June)	25 (22–26)	26 (25–26)	*	*
1986				
Yield (kg·ha^{-1})	25,200 (17,600–29,500)	27,900 (25,900–32,400)	**	**
Fruit wt (g)	8.7 (7.7–9.8)	9.0 (8.8–9.1)	NS	NS
Fruit rot (%)	3.6 (2.4–4.4)	4.0 (3.3–4.3)	NS	NS
Fruit firmness (N)	1.9 (1.6–2.2)	1.8 (1.7–1.8)	*	NS
Midpoint of harvest (June)	18 (16–20)	18 (18–19)	NS	NS

[z]Means of four replicates of 43 micropropagated subclones and range of subclone means.
[y]Means of four replicates of four runner-propagated subclones and range of subclone means.
[NS,*,**]Nonsignificant or significant at P = 0.05 or 0.01, respectively.

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Table 2. Yield components of micropropagated and runner-propagated 'Olympus' subclones.[z,y]

Subclones	Yield (kg·ha⁻¹, thousands)	Fruit wt (g)	Crowns/plant	Trusses/plant	Fruiting sites/plant	Fruit/plant	Fruiting sites/truss	Fruit/truss	Trusses/crown
				Micropropagated					
3D	27.1 b	9.3 a	23.0 a	42.4 a	293 a	258 a	6.9 a	6.1 a	1.9 a
3F	22.9 bc	8.8 a	19.8 a	37.5 a	258 a	228 ab	6.9 a	6.1 a	1.9 a
3H3	23.3 bc	7.7 a	21.4 a	37.6 a	230 a	204 a–c	6.2 a	5.5 a	1.8 a
4J2	19.2 cd	8.4 a	22.9 a	42.3 a	272 a	228 ab	6.7 a	5.7 a	1.9 a
5F	17.6 d	8.6 a	16.1 a	29.1 a	181 a	138 c	6.2 a	4.8 a	1.9 a
6D	25.4 b	8.7 a	19.8 a	35.0 a	245 a	214 a–c	7.0 a	6.1 a	1.8 a
6H2	26.0 b	8.3 a	20.5 a	40.9 a	255 a	229 ab	6.3 a	5.7 a	2.0 a
6J1	27.4 b	9.2 a	16.8 a	32.6 a	171 a	148 bc	5.4 a	4.6 a	2.0 a
7B	24.2 bc	8.3 a	16.7 a	32.8 a	258 a	222 a–c	7.9 a	6.8 a	2.0 a
7E	25.3 b	8.6 a	20.5 a	43.5 a	259 a	219 a–c	6.4 a	5.5 a	2.2 a
Mean	23.8**	8.6	19.7	37.4	242*	209*	6.6*	5.7	1.9
				Runner-propagated					
3	26.3 b	9.1 a	19.3 a	40.8 a	285 a	255 a	8.0 a	7.0 a	2.1 a
4	25.6 b	9.1 a	28.2 a	50.2 a	326 a	277 a	6.9 a	5.9 a	1.8 a
5	32.4 a	8.9 a	21.1 a	41.6 a	300 a	268 a	7.5 a	6.6 a	2.0 a
6	25.6 b	8.8 a	18.3 a	35.0 a	249 a	206 a–c	7.5 a	6.4 a	2.3 a
Mean	27.5	9.0	21.7	41.9	290	251	7.5	6.5	2.1

[z]Measurements made June 1986 on plants established in 1984.
[y]Means based on four replication of two plants for each subclone. Mean separation, within columns, by Duncan's multiple range test, $P = 0.05$.
*,**Indicate differences between micropropagated and runner-propagated subclones at $P = 0.05$ and 0.01, respectively.

Table 3. Yield of four micropropagated subclones of 'Olympus' in 3 years at Puyallup, Wash.

Subclone	Yield (kg·ha⁻¹, thousands)[z] 1985[y]	1986[y]	1989[x]
3D	39.5 a	27.1 a	15.2 a
7E	38.8 a	25.3 a	15.6 a
3H	24.8 b	24.0 a	13.7 a
5F	23.6 b	17.6 b	15.8 a

[z]Means based on four replications. Mean separation, within columns, by Duncan's multiple range test, $P = 0.05$.
[y]Planting established in 1984.
[x]Planting established in 1988.

weight and the weight of 25 fruit per plot were recorded weekly for all 47 subclones in 1985 and 1986. Firmness (the force required for a 4-mm-diameter cylinder to penetrate the shoulder of a fruit to the depth of 9 mm) was measured using a Hunter Spring Mechanical Force Gauge (Series L; Ametek, Hatfield, Pa.) for five fruit per plot at each harvest. Seasonal weighted averages were calculated for fruit weight and firmness. Data were analyzed as a randomized complete block and variation partitioned into propagation method and variation among micropropagated subclones.

Yield component analysis. Subclones with the greatest and smallest yield and fruit size in 1985 were selected for yield component study in 1986. Yield components were measured for the four runner plant subclones and 10 of the 43 micropropagated subclones. Two plants from-each plot were dug 6 through 10 June 1986. The number of crowns, trusses, fruit, and fruiting sites (sum of fruit number and flower number) was determined for each plant. The number of trusses per crown, fruits per truss, and fruiting sites per truss was calculated. Plot yield and fruit weight were obtained from weekly harvests. Yield was calculated for an eight-plant plot rather than the 10-plant plots for the other subclones. The mean of the measurements on the two plants for each plot and the plot yield were used in analysis of variance and correlations were computed between each component and yield.

1988 planting. Representative plants of each of the micropropagated subclones planted in the field in 1984 were maintained by runner propagation in a screenhouse. In 1987, the two micropropagated subclones with the highest (3D and 7E) and the lowest (3H and SF) yields in 1985 were runner-propagated for field planting. Runner plants of these four micropropagated subclones were planted at Washington State Univ., Puyallup, in May 1988. The cultural system was the same as for the first planting, except each plot consisted of five plants. Plots were hand-harvested in 1989 as in 1985. Data were analyzed as a randomized complete block.

Growth was uniform and very vigorous in all plots. Few runner plants were produced, but most plants developed multiple crowns. Sparse runnering and multiple crowns are common characteristics of 'Olympus' plants (Barritt and Schwartze, 1974). One variegated plant was observed in one plot for each of two micropropagated subclones in the 1984 planting. The overall incidence of variegation in the 1984 planting was 0.12%. No other variant plants were observed.

1984 *planting.* The average yield of the micropropagated subclones and the runner-plant subclones did not differ in 1985 (Table 1). In 1986, the yield of micropropagated subclones was significantly less than for runner-plant subclones. In both years, there were significant differences among micropropagated subclones for yield.

Yield component analysis of 10 micropropagated subclones and the four runner subclones revealed significant differences between runner and micropropagated subclones for yield, number of fruit per plant, fruiting sites per plant (number of fruit and flowers), and fruiting sites per truss, but not for fruit weight, number of crowns, trusses, trusses per crown, or fruit per truss (Table 2). Only yield and number of fruit per plant varied significantly among subclones. Only number of fruit per plant was significantly correlated with yield *(r* = 0.30, *P* = 0.028).

The yields of the micropropagated subclone plants were compared by source plant using single-degree-of-freedom contrasts and there were no differences among source plants. These analyses indicate that the among-subclone variability for yield was on an individual subclone basis and was not associated with the source plant. The highest-yielding subclone in 1985 (3D) and the next-to-lowest-yielding subclone (3H) were both derived from the same source plant.

There was considerable variation in the size of the original explants, ranging from that used for meristem culture (0.25 mm) to that used for micropropagation (1.25 mm). Twelve explants were noted as being large (0.75 to 1.25 mm long) and 11 explants were small (0.25 to 0.50 mm long). The average yield in 1985 was 33,800 $kg \cdot ha^{-1}$ (CV = 7.0%) for subclones derived from large explants and 34,300 $kg \cdot ha^{-1}$ (CV = 9.7%) for subclones from small explants. Although there was variability in the size of the original explant it does not appear to have contributed to the variability among micropropagated subclones.

In addition to the variability in yield, there was variability in other harvest characteristics (Table 1). However, the results were not consistent from year to year. There were differences between micropropagated subclones and runner-plant subclones in 1985 (but not in 1986) for fruit weight and midpoint of harvest; for fruit firmness in 1986 (but not in 1985); and no differences in either year for preharvest fruit rot. There was significant variability among micropropagated subclones in 1985 for preharvest fruit rot and average fruit weight, but not in 1986.

1988 planting. In 1989, there were no differences among the four subclones planted in 1988 for yield, fruit firmness, or fruit weight. Subclone yield in 1989 is given in Table 3 along with their 1985 and 1986 yields from the 1984 planting. In 1989, there were differences among subclones for fruit rot and midpoint of harvest (data not shown). There were no differences among these subclones for fruit rot or midpoint of harvest in the 1984 planting.

In 1985 and 1986 the mean performance of runner-propagated and micropropagated subclones of 'Olympus' was very similar. The greatest differences were among micropropagated subclones, and this was not associated with micropropagation source plants or explant size. By 1989, after additional runner propagation, there were no differences in yield among micropropagated subclones. This indicates that the differences were probably transient responses to the micropropagation environment and not genetic changes.

Previously, strawberry cultivars were identified that responded differentially to micropropagation. Variation was found among subclones of micropropagated 'Earliglow' but not among subclones of micropropagated 'Guardian' (Swartz et al., 1981). Apparently, 'Olympus' has an intermediate response to micropropagation. There was no difference in yield between micropropagated and runner-propagated plants in the 1st year, but there was increased variability among micropropagated subclones.

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