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Radiation-induced mutations from accessory buds of sweet cherry, *Prunus avium* L. cv 'Bing'

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Abstract Dormant scions of 'Bing' were exposed to 1–2.5 krad of gamma radiation in order to induce useful mutations. The main buds were excised and the scions grafted to allow the growth of accessory buds into primary (V₁) shoots. The frequency and types of mutations on secondary (V₂) populations are described. In a population of 3324 V₂ shoots, the overall mutation frequency was 6.4%: 4.2% partial, 1.6% total and 0.3% growth-reduced mutants were identified. The experiment was repeated using 3 krad- and 4 krad-fractionated doses in water. Differences in mutation frequency at 3 krad and 4 krad were not significant. Of 2562 surviving V₂ shoots derived from the irradiation of accessory buds of both standard and V₁ shoots, the overall mutation frequency was 3.3%: 1.7% were partial-leaf mutants, 1.0% were total-leaf mutants, and 0.54% were growth-reduced mutants. For maximum mutation rate with adequate survival we suggest acute irradiation of accessory buds in air at dosages approximating LD₅₀ (2.75–3 krad). A larger mutant sector was present in V₁ shoots derived from accessory buds than those from main buds as revealed by the higher number of total mutant repeats in the families.

Key words Accessory buds · Mutation · Growth-reduced · Mutant sector · Gamma radiation

Introduction

Irradiation of a multicellular and histogenically layered plant meristem (Pratt 1963, 1967; Tilney-Bassett 1963) commonly results in mericlinal or periclinal chimeras, and it can take several years and special techniques to identify and isolate the mutant tissue (Lacey and Campbell 1977, 1982; Visser 1973). Adventitious buds have been used to circumvent this problem of chimerism, and in some vegetatively propagated ornamentals this has led to the induction of a very high proportion of total mutants (Broertjes 1966, 1982; Broertjes and Van Harten 1978; Van Harten 1982; Doorenbos and Karper 1975). However, this technique is not applicable for many deciduous fruit trees due to the inability to force adventitious bud growth.

In sweet cherry, *Prunus avium* L., recovery of total-plant mutants in secondary (V₂) trees following irradiation of the main buds is low, about 2–4% (Lapins 1971; Thompson 1979). In contrast, in apple, the frequency of total mutants in the V₂ can be as high as 15% (Lapins 1965). Furthermore, in apple, preselection for compact mutants has been successful in the primary (V₁) generation (Visser et al. 1971; Lacey and Campbell 1979), which indicates that the entire apex in the V₁ shoot is mutant. In contrast, in cherry, mutations occur sporadically on the V₁ shoot, and preselection for compact mutants is not possible (Thompson 1979).

Accessory buds of cherry are in various stages of development and have fewer meristematic cells than the main lateral buds (Katagiri and Lapins 1974). They can be readily induced to produce shoots by removing apical dominance of the main buds, which is achieved by removing these buds (Saamin and Thompson 1996a). Irradiation of accessory buds, in comparison to that of the main buds, resulted in larger mutant sectors in V₁ and the recovery of a higher proportion of total mutants in V₂ (Lapins 1971). In pear, a higher

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frequency of mutations was also observed upon irradiation of accessory buds (Nishida 1973).

The irradiation experiment on accessory buds of 'Bing' cherry presented here had three main objectives, (1) to compare the effects of irradiation dosages in air or in water on mutation frequency; (2) to study the distribution of mutations in relation to bud positions on V_1 shoots; and (3) to increase the size of mutant sectors and thereby improve the efficiency of isolation of total mutants, particularly of the growth-reduced type.

Materials and methods

In 1983, dormant scionwood of 'Bing' was obtained from a commercial nursery in mid-February and stored at 0°C until irradiation on April 16th. The basal 13-cm section of each 1-year shoot was cut and placed basal-down in the low-flux ^{60}Co irradiation chamber. Either 25 or 50 scions per treatment were exposed to 1–2.5 krad acute or fractionated (12 h intervals) gamma rays (dose rate of 145 krad/min) in air or in water. Following irradiation, all main buds were excised, leaving the accessory buds intact. Lanolin was applied onto the cut surfaces, and the scions were grafted onto Mazzard seedlings in the nursery. In mid-September, buds from vigorous V_1 shoots were patch or T-budded, 2 to a rootstock. Because the basal five to six buds on the cutback V_1 shoot were too congested to bud individually, they were allowed to grow and produce V_2 shoots "in place". Leaf-and growth-reduced mutants of budded (B) and cutback (C) V_2 trees were identified in July 1984. Plant height, diameter at mid-point and the number of nodes in the 25-cm mid-shoot section of all V_2 plants were recorded in early September. Growth-reduced mutants were then budded onto seedling rootstock, ten budding per selection for verification in V_3 (tertiary population) the following summer. Height, diameter and number of nodes of the V_3 trees were recorded when the trees were dormant.

For accessory bud irradiation in 1984, dormant scionwood of 'Bing' was obtained from a commercial nursery in late January. V_1 shoots from the previous year's irradiation experiment that were derived from both irradiated main and accessory buds of 'Bing' were also collected in late February for re-irradiation. All scions were held at 0°C until irradiation on April 20th. Fifty scions per treatment were used for 3-krad or 4-krad fractionated doses in water. Dose fractionation consisted of 1- to 1.5-krad exposures at 12-h intervals to cushion irradiation damage at increasing cumulative dosages and to allow possible cellular and chromosomal repair. All main buds

were excised, and both treated and control scions were then grafted in the field. Because of the delayed growth of V_1 shoots from accessory buds, the scions were too immature to bud in the fall. Thus, they were grafted in early April the next year using 2- to 4-bud scions. The three basal buds were too congested and not propagatable. Growth and leaf mutations on V_2 trees were observed in July–August. Plant height, diameter and number of nodes of all V_2 trees were recorded in early September. Each potential growth-reduced mutant was propagated onto 15 seedling rootstock (five buddings per selection, replicated 3 times) for verification in V_3 . Measurements were made as before on V_3 trees in mid-July.

The chi-square test and Statistical Packages for Social Sciences (SPSSX 1983) were used to compare the various treatments.

Results

As the average frequencies of mutations in V_2 's for acute (1.2%) and fractionated (1.9%) dosages were not significantly different in both the (C) and (B) populations, data for these two treatments were combined.

Among the combined 3324 cutback and budded V_2 shoots, the overall mutation frequency was 6.4%: 4.2% were partial-leaf mutants, 1.6% were total-leaf mutants, and 0.3% were growth-reduced mutants (Table 1). Two types of growth-reduced mutants were identified: those with a compact growth habit (a noticeably shorter tree, thicker stem diameter for its height and shorter internodes than normal) and shorter, non-compact plants that simply grew more slowly and/or stopped growth early. Compared to exposures in water, exposures in air had markedly higher frequencies of overall mutations (9.0% vs. 3.6%), partial-leaf mutants (5.7% vs. 3.1%) and total-plant mutants (2.7% vs. 0.5%). Irradiation in water at higher dosages (3 and 4 krad) in 1984 did not result in a mutation frequency higher than that following irradiation at lower dosages, (1–1.25 krad) in 1983. The spectrum of morphological leaf aberrations in partial- and total-leaf mutants was similar to that observed in main bud irradiation (Saamin and Thompson 1996b).

Table 1 Effect of air versus water on mutation frequency of V shoots derived from accessory buds of 'Bing' irradiated in 1983 and 1984

Irradiation method dose (krad)	Number of V trees	Partial mutants (%)	Total plant mutants (%)	Growth reduced mutants (%)	All mutants (%)
1983					
In air (1–1.5)	1569	5.7a	2.7 ^a	0.6 ^a	9.0a
In water (1–2.5)	1755	3.1a	0.5 ^a	0 ^a	3.6b
Total	3324	4.2	1.6	0.3	–
Control (0)	177	0	0	0	0
1984					
In water (3 & 4)	2562	1.7	1.0	0.6	3.3
Control (0)	72	0	0	0	0

a, b: Significantly different within column between rows at $P = 0.01$ using chi-square test: ^a numbers were too small for chi-square test

The overall mutation frequency in V_2 trees was 3.0% for all treatments in both years (Table 2). The frequency of overall mutations (partial-leaf mutants and total-plant mutants) was 4.4% in plants derived from the basal bud positions (2–10), whereas it was only 2.2% from buds 11 to 45. To determine the frequency of mutations in relation to bud position on V_1 shoots, we grouped the V_2 trees into 5–15 bud groups. There was no difference in the relative proportion of partial-leaf mutants and total-plant mutants in the populations derived from upper and lower positions. From buds 11–45, 31% of all the mutants identified were total-plant mutants, whereas from buds 2–10, 28% were total-plant mutants. With a few exceptions, with increasing bud position on the V_1 shoots the frequency of mutants decreased. Although the highest frequency of total-plant mutants (including growth-reduced) was at bud positions 2–10, there was no decrease from the 11th to the 45th position.

Among 3307 V_2 trees, 5 were initially identified as potentially growth reduced with heights 52–75% and H/D/N ratios 45–85% that of the control. As the V_3 heights ranged from 26–50% of the control and the H/D/N ratios were 20–47% of the control V_3 trees were even more compact than their V_2 counterparts (Table 3). Selection 58-16 appears to be the most promising because both the V_2 and

V_3 generations were 50% of the control height and about 45% H/D/N.

Irradiation of accessory buds of 'Bing', 1984

Since differences in mutation frequencies at 3-krad and 4-krad fractionated dosages in water or in air were not significant, the two populations were combined (Table 1). Of the 2562 surviving V_2 shoots derived from the irradiation of accessory buds of V_1 shoots, the overall mutation frequency was 3.3%: 1.7% were partial-leaf mutants; 1.0% were total-leaf mutants and 0.6% were growth-reduced mutants. The 37 V_2 growth-reduced mutants selected in 1984 had heights 36–98% and H/D/N ratios 31–99% that of the control. The height of the V_3 's were 34–95% of that of the control, and 7 mutants were less than 50% shorter than the control. In the V_3 , the growth-reduced mutants also showed marked variation in other traits, such as branching habit, erectness of growth, leaf color, thickness and size.

The size of mutant sector isolated is indicated by the number of mutant trees in each V_2 family. In 1 (0.45%) of 222 budded V_2 families (i.e. V_2 plants derived from a single V_1 shoot), all 12 of the V_2 plants were similarly growth reduced. Twenty-eight (12.6%) of the families

Table 2 The frequency of mutations in 'Bing' V_2 shoots derived from irradiated accessory buds as related to bud position on V shoots, 1983 and 1984 data combined

V bud position	Number of V trees	Number of partial mutants (%) ^a	Number of total plant mutants (%) ^a	Number of growth-reduced (%) ^a	Number of all mutants (%) ^a
2–10	2181	62 (2.8)	27 (1.2)	6 (0.3)	95 (4.4)
11–15	1218	19 (1.6)	8 (0.7)	3 (0.2)	30 (2.5)
16–21	1108	13 (1.2)	7 (0.6)	4 (0.4)	24 (2.2)
22–30	1015	8 (0.8)	7 (0.7)	5 (0.5)	20 (2.0)
31–45	550	5 (0.9)	4 (0.7)	2 (0.4)	11 (2.0)
Total	6072	107 (1.8)	53 (0.9)	20 (0.3)	180 (3.0)

^a Percentage indicated in parentheses

Table 3 Comparison of height, diameter, number of nodes at 25-cm midsection stem of V potential growth-reduced 'Bing' mutants selected in 1984 and their V generation

Tree	V height (cm)	Diameter (mm)	Number of nodes	H/D/N ^a	V height (cm)	Diameter (mm)	Number of nodes	H/D/N ^a
<i>Control (OkR)</i>								
Mean	170 (100) ^b	1.1	11	14.4 (100) ^b	194 (100) ^b	1.4	12	11.5 (100) ^b
SD	22	0.1	1	1.1	13	0.1	1	1.4
<i>Growth-reduced</i>								
55–18	128 (75) ^b	1.0	11	12.2 (85) ^b	57 (29)	0.9	21	3.0 (26) ^b
55–21	110 (65)	0.9	14	9.1 (63)	51 (26)	0.7	20	3.6 (31)
56–70	115 (68)	1.1	9	11.9 (83)	58 (30)	1.0	25	2.3 (20)
58–16	88 (52)	0.8	17	6.5 (45)	91 (50)	1.0	17	5.4 (47)
42–52	–	–	–	–	78 (40)	0.9	22	3.9 (34)

^a Height/diameter × no. of nodes

^b Percentage relative to control

had 2 or more total-leaf mutant members, whereas 10 (4.5%) families had only 1 total-leaf mutant plant. Eleven (5.0%) of the families had 2 or more partial-leaf mutant plants and 25 (11.3%) families had 1 partial-leaf mutant. Bud survival ranged from 50–90% depending on the vigor of the scions and the dosage. Bud survival may have affected the frequency of mutants per family.

Discussion

Katagiri and Lapins (1974) demonstrated that accessory buds have a lower LD₅₀ than main buds. This higher radiosensitivity is due to their less developed meristems. A larger cell population, greater apical size during recovery and faster rate of growth increase the radiotolerance of irradiated apices (Langenauer et al. 1973). As a consequence of the necessity of lower irradiation dosages, the mutation rate was lower in V₂'s derived from accessory buds (2.7%) in this study than in those derived from main buds of 'Bing' (7.3%) irradiated at dosages between 3 and 7.5 krad (Saamin and Thompson 1996b).

Although water and dose fractionation increase the tolerance of meristematic tissues, as indicated by the 50–60% survival of V₁ shoots following 4-krad irradiation of accessory buds (Saamin and Thompson, 1996a), these treatments also buffer against mutations. Sparrow et al. (1961) reported that the yield of somatic mutations was reduced in many species by dose fractionation of acute exposures. As there is evidence of a very sharp decline in the radiotolerance of accessory buds at dosages above LD₅₀ (Saamin and Thompson 1996a), we suggest acute irradiation in air at LD_{≤50} (2.75–3 krad). This may increase the mutation rates somewhat higher than those we obtained at LD₂₅. It is suggested that, with the higher dosages, much larger numbers of scions need to be irradiated in order to obtain sufficient V₁.

Not all accessory bud sites are at the same stages of development. The vigor of the scions needs to be considered. The lowest third of scions have been found to give the best survival (Saamin and Thompson 1996a) so this section was used for this experiment. The key to success is to irradiate young meristems with the fewest cells in order to recover larger mutant sectors and yet have the accessory buds mature enough to grow when the main buds are removed. Also, in order to stimulate the development of more vigorous V₁ shoots from accessory buds, we advise grafting the irradiated scions onto potted rootstocks in a greenhouse. This would provide more mature buds suitable for September budding.

Whereas there were 33% leaf aberrations at the basal bud positions on the V₁, the frequency of mutations in V₂'s derived from these basal buds was 4.5%. Thus, only 14% of the aberrant leaves in V₁ were associated with mutations in their corresponding axillary buds.

The frequency of all mutants that were total, including growth-reduced mutants, was about the same in V₂'s from the budded populations of accessory shoots (49%) as in the budded population of V₂'s from main buds (52%) (Saamin and Thompson 1996b). Lapins (1971) reported a similar proportion of total mutants (53%) from the irradiation of accessory buds but a lower proportion (35%) from main buds. This discrepancy can be explained by the fact that Lapins propagated only buds 6–15 on the V₁ shoots derived from main buds, whereas we propagated all usable buds, up to position 45, and found that the frequency of total mutants did not decrease and in fact slightly increased with the higher buds.

The size of the mutant sector in V₁ can be inferred from the number and distribution of repeats, or runs, of the same mutation within V₂ families. The proportion of V₂ families derived from accessory buds which had runs of total-leaf mutants (13.3%) was much higher than the proportion in families derived from main buds (1.8%) (Saamin and Thompson 1996b). In one case, all the V₂ plants in a family were growth-reduced mutants, which indicated that they originated from either a periclinal chimera or a totally mutant shoot apex. Also, the proportion of families with 2 or more total mutants was double that of families with single partial-leaf mutants. This further strengthened the hypothesis that a larger mutant sector size was present in V₁ shoots derived from accessory buds than in those from main buds. One important advantage of accessory buds is that identification and verification of total-leaf mutants and growth-reduced mutants was much easier when they occurred in runs than when they occurred as single plants.

Repropagation from a larger mutant sector would increase the chance of separating out non-chimeral tissue and thus enhancing the stability of mutants. The experiment had to be discontinued at the early stages of the V₃ generation, thus the stability and fertility of the mutants were not recorded.

In summary, we conclude that: (1) acute irradiation of accessory buds in air at dosages approximating LD₅₀ (2.75–3 krad) may increase mutation rate with adequate survival; (2) a large mutant sector can be induced in the meristem by the irradiation of dormant accessory buds rather than main buds; (3) to recover growth-reduced mutants all buds on the V₁ shoots derived from irradiated accessory buds can be propagated.

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