

## Methodology for radiation-induced mutations in main lateral buds in *Prunus avium* L. cv. Bing

(Kaedah mutasi teraruh sinaran pada tunas sisi utama *Prunus avium* L. kultivar Bing)

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Key words: acute, fractionated, M1V2 trees, mutation frequency, growth-reduced

### Abstrak

Dahan mata tunas ceri kultivar Bing yang dorman didedahkan pada sinaran gama 4–12 kR secara akut atau berperingkat di dalam udara atau dalam air. Mutan daun dan kerdil dikenal pasti. Antara 2 942 pokok M1V2 (merangkumi semua perlakuan) kekerapan mutan keseluruhannya ialah 7.3% iaitu 0.34% mutan kerdil, 2.3% mutan keseluruhan daun dan 4.7% mutan sebahagian daun. Nisbah tinggi/lilitan batang/bilangan buku untuk semua M1V2 mutan kerdil dan lapan antara M1V3 ialah 2 sisihan piawai kurang daripada pokok bandingan. Kekerapan mutan hasil daripada aruhan di dalam udara tidak nyata bezanya daripada aruhan di dalam air. Aruhan dos berperingkat juga tidak nyata bezanya daripada aruhan akut. Untuk mendapatkan mutan kerdil secara cekap, disyorkan pembiakan mata tunas M1V1 dari bahagian atas.

### Abstract

Dormant scions of sweet cherry cv. Bing were exposed to acute or fractionated doses of 4–12 kR gamma rays, in air or in water. Leaf and growth-reduced mutants of M1V2 trees were identified. Among 2 942 M1V2 trees (including all treatments), the overall mutation frequency was 7.3%, i.e. 0.34% growth-reduced mutants, 2.3% total leaf mutants and 4.7% partial leaf mutants. The height/diameter/number of nodes (H/D/N) ratios of all the M1V2 growth-reduced mutants and eight of the M1V3 were 2 standard deviations below that of control plants. The mutation frequencies for irradiation in air and for fractionated doses were respectively not significantly higher than those for irradiation in water and for acute doses. For efficient recovery of growth-reduced mutants, we recommend propagating only the upper buds of M1V1 shoots of sweet cherry.

### Introduction

Various biological and environmental factors affect radiation-induced mutation frequency and spectrum in plants. Such factors include cultivars (Decourtye 1970; Visser et al. 1971; Lacey and Campbell 1982; Zagaja et al. 1982), developmental

stage of bud primordia (Merricle and Merricle 1961; Sparrow et al. 1961), age and size of buds (Lapins et al. 1969; Lapins 1973; Katagiri and Lapins 1974), number of bud primordia (Pratt 1968), position of buds on irradiated shoot (Lapins and Hough 1970; Lapins 1971; Thompson 1979), dose and

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dose rate (Sparrow et al. 1961; Nishida 1973; Donini 1982), and environmental conditions before, during and after treatment (Sparrow and Woodwell 1962; Conger et al. 1977; Broertjes and van Harten 1978).

Campbell and Lacey (1973) as well as Lacey and Campbell (1977, 1979, 1982) irradiated apple scions in water and suggested that water probably provides a more even dose distribution. Use of chronic and recurrent irradiation techniques has resulted in increased dose tolerance, higher mutation frequency and broader mutation spectrum in vegetatively propagated plants (Fujita and Nakajima 1973; Nakajima 1973; Fujita and Wada 1982). In apple, desirable mutants were obtained mostly from chronic treatment while acute treatment resulted in pollen sterility in most cases (Ikeda and Nishida 1982). On the contrary, Sparrow et al. (1961) reported that chronic exposures or dose fractionation reduced somatic mutations.

Smaller trees are desirable because they can be planted at higher density and orchard operations are facilitated. Lapins (1973, 1975), Donini (1976, 1982) and Thompson (1979) had succeeded in getting radiation-induced compact mutants in sweet cherry while Lapins (1965, 1969), Decourtye (1970), Visser (1973), Visser et al. (1971), Zagaja and Przybyla (1973) as well as Lacey and Campbell (1977, 1979) in apple. The objectives of this study were to manipulate irradiation techniques viz. acute and fractionated gamma irradiation in air and in water to increase mutation frequency and size of mutant sectors in Bing, a major commercial sweet cherry cultivar in western USA as well as to identify bud positions on the primary shoot which yield the highest frequency of whole plant mutants, including growth-reduced mutants, to optimize recovery.

## Materials and methods

Dormant scionwoods of sweet cherry cv. Bing were obtained from a commercial nursery in mid-February 1983 and stored at 0 °C taken out when they were to be irradiated. In mid-April, 26 cm scions, 25 or 50 scions per treatment were placed basal ends in the <sup>60</sup>Co radiation chamber at the Radiation Center, Oregon State University and exposed to acute or fractionated doses of 4–12 kR gamma rays, in air or in water. A mean dose of 671 rad/min (average of 786 rad/min at the perimeter and 557 rad/min at the centreline) was used to determine approximate target exposures in the high flux chamber. Dose fractionation consisted of 1–2.5 kR exposures at 12 h intervals.

Irradiated and control scions were grafted immediately onto Mazzard seedling rootstocks. Buds from vigorous M1V1\* shoots were patch or *T*-budded in sequence from base to apex onto seedling rootstocks, two to a rootstock, in mid-September. The basal 5–6 nodes of the cutback V1 shoot were allowed to grow and produce M1V2\*\* shoots 'in place'.

During the following summer (1984), the budded (B) and cutback (C) V2 trees were examined in mid-July and in mid-August, for partial and total leaf mutants as well as for growth-reduced mutations. Partial leaf mutants are defined as having one to most of the leaves aberrant or partially aberrant, and total leaf mutants are probably periclinal chimeras had all leaves aberrant. Compact growth habit and shorter plants that either stopped growth early or were slower growing are the two types of growth-reduced mutants identified. Compact mutants are noticeably shorter, have a thicker stem for their height and have shorter internodes than normal. Plants approximating 13–16 nodes per 25 cm mid-section of the stem were categorized as semi-compacts, and those with 17 nodes or more as compacts. Trees that were shorter, or stopped growth early were also selected.

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\*hereafter referred to as V1; \*\* hereafter referred to as V2

Table 1. Comparison of height (H), diameter (D) and number of nodes (N) in a 25 cm mid-shoot section for potential growth-reduced mutants in V2 (1984) and V3 (1985) of Bing

Tree	V2 mutants				V3 mutants			
	Ht. (cm)	Diam. (cm)	No.	H/D/N	Ht. (cm)	Diam. (cm)	No.	H/D/N
Control								
Mean	167 (100)*	1.1	11	13.8 (100)	194 (100)	1.4	12	11.5 (100)
Std. dev.	23	0.2	1	1.7	13	0.1	1	1.4
Growth-reduced								
16-58	71 (43)	1.2	12	4.9 (36)	124 (64)	1.2	12	8.6 (75)
31-137	110 (66)	1.1	10	10.0 (72)	58 (30)	0.8	17	4.3 (37)
33-41	112 (67)	0.9	17	7.3 (53)	81 (42)	0.9	13	6.9 (60)
30-18	114 (68)	1.0	14	8.1 (59)	83 (43)	0.8	13	8.0 (70)
23-105	120 (72)	1.1	14	7.8 (57)	99 (51)	1.1	14	6.4 (56)
32-19	134 (80)	1.3	14	7.4 (54)	139 (72)	1.6	11	7.9 (69)
22-133	142 (85)	1.4	17	6.0 (43)	160 (82)	1.3	13	9.5 (83)
24-121	148 (89)	1.1	16	8.4 (61)	163 (84)	1.3	14	9.0 (78)
31-111	154 (92)	1.4	13	8.5 (62)	178 (92)	1.3	12	11.4 (99)
14-22	–	–	–	–	148 (76)	1.2	15	8.2 (71)
44-108	–	–	–	–	80 (41)	0.9	19	4.7 (41)

V2 = budded on Mazzard seedling rootstocks

V3 = budded on Colt rootstocks; data based on 3–16 surviving V3 trees

\*% of control

Plant height (H), diameter at mid-point (D) and the number of nodes (N) in a 25 cm mid-shoot section of potential growth-reduced mutants and of control trees were recorded in early September (1984) and used for determining ratios of growth parameters. Potential growth-reduced mutants were propagated onto seedling rootstocks in mid-September, using 10 buds/selection for verification in M1V3\*. Height, diameter and number of nodes of V3 trees were recorded when trees were dormant. V2 trees identified as having late bud-break on 9 April 1985 were also repropagated for verification in V3. The Chi-square test and Statistical Packages for Social Sciences (SPSSX 1983) were used to analyse the data.

## Results

### *Growth-reduced mutants*

A total of 11 growth-reduced mutants characterized by 43–92% of normal height were identified in the V2. Height, diameter

and number of nodes of the V2 and V3 mutants are presented in *Table 1*. The growth-reduced trait of all mutants except 31-111 (which appeared normal) was also expressed in the V3s as indicated by both their lower average height and H/D/N ratios as compared with controls. The H/D/N ratios of all the V2 mutants and eight of the V3 were 2 standard deviations below that of control plants. Three mutants, 31-137, 23-105 and 44-108, with V3 heights ranging from 30% to 51% and H/D/N ratios 37% to 56% of normal were promising compacts, whereas five mutants, 16-58, 32-19, 22-133, 24-121 and 14-22, with V3 heights ranging from 64% to 84% and H/D/N ratios 69% to 83% of normal were promising semi-compacts. Potential semi-compact mutant 22-133 which was 85% of control height in V2 (and 82% in V3), had a very low H/D/N ratio in V2 (43% of normal) but a considerably higher ratio in V3 (83% of normal). No reversions to normal were

\* hereafter referred to as V3

Table 2. Frequencies of mutations in budded and cutback V2 populations for the various treatments, 1984

Population	No. of V2 trees	Partial leaf mutants		Total leaf mutants		Growth-reduced mutants		All mutants	
		No.	%	No.	%	No.	%	No.	%
Budded	1 667	58	3.5b	53	3.2b	9	0.54	120	7.2a
Cutback	1 275	80	6.3a	15	1.2a	1	0.08	96	7.8a
Total	2 942	138	4.7	68	2.3	10	0.34	216	7.3

Mean values in each column and row with different letters are significantly different at  $p = 0.01$  using Chi-square test

evident in V3 populations that showed growth-reduced habit.

### ***Partial and total leaf mutants***

The number of mutant leaves per partial mutant tree ranged from 1 to 21, with a mean of 6.9 and standard deviation of 5.2.

### ***Mutation frequencies in budded vs. cutback populations***

There were 1 667 budded (B) V2 trees and 1 275 V2 shoots from cutback (C) V1s (Table 2). An average of five (range 1–7) shoots grew from the cutback V1 shoots. Whereas the overall frequency of mutations was similar in C (7.8%) and B (7.2%), the relative proportion of the three types of mutants was significantly different in the two populations. The frequency of total mutants was higher in B (3.2%) than in C (1.2%) as was the frequency of growth-reduced mutants, 0.54% in B vs. 0.08% in C. However, the frequency of partial leaf mutants was higher in C (6.3%) than in B (3.5%).

### ***Effect of irradiation in air and water on mutation frequencies***

Irradiation in air produced higher but not significant frequencies of overall mutations, of partial leaf mutants and of total leaf mutants than that in water. In air, the highest frequency of partial leaf mutants, total leaf mutants, growth-reduced mutants and overall mutations was obtained with acute 3 kR and fractionated 4–5 kR exposures (Table 3). At higher dosages in air, mutation

rates declined. In water, there was not such a clear cut dosage-mutation relationship; the highest frequencies of total leaf mutants were obtained with 6.5 kR, whereas the highest frequencies of partial leaf mutants were seen for lower doses of 4.5 and 5.5 kR. At higher exposures, acute in air (5 and 6 kR) and in water (8.5 kR), and with fractionated dosage in water (10.5 kR), there were very few or no surviving V1 shoots for repropagation (figures not included in Table 3).

### ***Effect of acute vs. fractionation of irradiation dosages on mutation frequencies***

The overall mutation frequency for fractionated dosages was not significantly higher than for acute dosages (8.0% vs. 7.1%, Table 3). Fractionation of dosages induced a higher but not significant frequency of partial leaf mutants than acute doses (5.6% vs. 3.8%), and a similar frequency of total leaf mutants (2.1% vs. 2.7%). Although the numbers were small, there was a twofold increase in growth-reduced mutants with acute dosages (0.49% vs. 0.25%).

### ***Mutation frequency in relation to bud position on the V1 shoot***

The frequency of mutations of Bing V2 plants as related to bud position on V1 shoot is shown in Table 4 and Table 5. Basal buds had 5.9% partial and 1.6% total mutants, whereas upper buds had 3.0% partial and 3.3% total mutants. With upper bud positions, there was a clear cut decreasing

Table 3. Mutation frequencies of partial and total leaf mutants, and growth-reduced mutants (budded and cutback populations combined) from the various irradiation techniques and doses

Dose (kR)	No. of V1 shoots	No. of V2 shoots	Partial leaf mutants		Total leaf mutants		Growth-reduced mutants		All mutants	
			No.	%	No.	%	No.	%	No.	%
<b>In air</b>										
Acute										
3	26	240	10	4.2	12	5.0	2	0.83	24	10.0
4	14	138	4	2.9	4	2.9	0	0	8	5.8
Fractionated										
4	16	200	20	10.0	3	1.5	0	0	23	11.5
5	12	121	12	9.9	3	2.5	1	0.83	16	13.2
6	9	122	5	4.1	1	0.8	0	0	6	4.9
7	2	49	0	0	1	2.0	0	0	1	2.0
Subtotal	79	870	51	5.9	24	2.8	3	0.34	78	9.0a
<b>In water</b>										
Acute										
4.5	23	462	19	4.1	8	1.7	2	0.43	29	6.3
5.5	19	311	12	3.9	7	2.3	1	0.32	20	6.4
6.5	7	62	1	1.6	2	3.2	1	1.60	4	6.5
Fractionated										
4.5	13	257	15	5.8	3	1.2	1	0.39	19	7.4
5.5	13	322	24	7.5	7	2.2	1	0.31	32	9.9
6.5	16	298	7	2.4	9	3.0	0	0	14	4.7
7.5	15	247	8	3.2	7	2.8	1	0.40	16	6.5
Subtotal	106	1959	86	4.4	43	2.2	7	0.36	136	6.9a
<b>Subtotal</b>										
Acute	89	1213	46	3.8a	33	2.7a	6	0.49	85	7.0a
Frac.	96	1616	91	5.6a	34	2.1a	4	0.25	127	7.9a

Mean values in each column and row with the same letter are not significantly different at  $p = 0.01$  using Chi-square test

trend in partials. Total leaf mutants seems to increase at least to bud positions 16–20 and, the highest frequency (3.8%) was at bud positions 31–39. Although the numbers of growth-reduced mutants were small, the highest frequency occurred at bud positions 21–25.

#### ***Distribution of mutant trees in V2 families***

Of the 184 V2 families in the budded population, 101 (55%) had no mutation, 58 (32%) had only one mutant tree and the remaining 25 (14%) families had 2–6 mutant trees per family.

Of the 83 families in which mutations were identified, only six (7%) possibly had the same mutation repeated in two or more

trees. Although low budding success (54%) undoubtedly broke some of the runs, there were no cases where all surviving members of a family had the same mutation. Thus, there were no whole family runs. In families with a single mutant tree, the tree was derived from bud positions 7–33 on the V1 shoots. Growth-reduced mutants were derived from bud positions 6, 8, 15, 20, 21, 25 and 28. In the six families with two similar mutant trees, all originated in the higher bud positions (12–25) on the V1 shoot. In the family with five different types of mutants, the trees originated from bud positions 8, 23, 25, 28 and 29; the first tree was a partial leaf mutant and the latter four were dissimilar total leaf mutants. In three

Table 4. Frequency of mutations in cutback and budded V2 trees as related to bud positions on V1 shoots (all treatments)

Population	V1 bud position	No. of trees	Partial leaf mutants		Total leaf mutants		Growth-reduced mutants		All mutants	
			No.	%	No.	%	No.	%	No.	%
Cutback	2–6	1 275	80	6.3	15	1.2	1	0.08	96	7.5
Budded	7–10	415	20	4.8	12	2.9	1	0.24	33	7.6
	11–15	501	20	4.0	14	2.8	2	0.40	36	7.2
	16–20	387	10	2.6	16	4.1	2	0.52	28	7.2
	21–25	223	6	2.7	7	3.1	3	1.35	16	7.2
	26–30	88	1	1.1	2	2.3	1	1.14	4	4.5
	31–39	53	1	1.9	2	3.8	0	0	3	5.7
Total		2 942	138	4.7	68	2.3	10	0.34	216	7.3

Table 5. Frequency of mutations as related to bud positions grouped according to basal and upper, or post-irradiation origin

Population	V1 bud position	No. of trees	Partial leaf mutants		Total leaf mutants		Growth-reduced mutants		All mutants	
			No.	%	No.	%	No.	%	No.	%
Basal bud	2–10	1 690	100	5.9	27	1.6	2	0.12	129	7.6a
Upper bud	11–39	1 252	38	3.0	41	3.3	8	0.60	87	6.9a

Mean values with the same letters are not significant different at  $p = 0.05$  using Chi-square test

of the 2-plant mutant repeats, the similar mutant trees were derived from adjacent pairs of bud positions 12–17 on the V1 shoot. In the other three repeats, the V2 trees were derived from bud positions 9–25 and were separated by 2–4 nodes on the V1 shoot.

Of the 275 V2 families in the cutback population, only six (2%) out of 275 families with 3–6 V2 shoots had repeats of 2–4 similar partial leaf mutants.

## Discussion

Although we could increase dose tolerance of dormant buds of cherry by irradiation in water, the overall mutation frequency was less than with irradiation in air at lower exposures. In water (fractionated), the overall mutation frequency of Bing in this study was 7.2% compared with 9.3% in air (fractionated). In water (acute), the overall mutation frequency of Napoleon cherry was reported to be 7.5% (Saamin and Thompson 1996), whereas in air (acute), it was 8.8% (Thompson 1979). The trend of lower

mutation rates for irradiation in water lends support to the hypothesis that water provides a more even dose distribution (Lacey 1976; Lacey and Campbell 1979) which buffers the meristematic cells against damage as well as against mutation.

Acute irradiation, especially in air, resulted in 4.2% total leaf mutants, whereas fractionated irradiation resulted in 1.6% total leaf mutants. Acute irradiation in air probably gave a more direct hit and was high enough to result in lethality of many meristematic cells. A mutant cell, being surrounded by fewer non-mutant cells, has a greater chance of surviving intrasomatic competition and becoming expressed in a larger sector and, thus being identified as total leaf mutants. With fractionation, a low frequency of total leaf mutants (1.6%) was accompanied by a relatively high frequency of partial leaf mutants (7.5%). Exposures to 2–2.5 kR in dose fractionation are insufficient to cause substantial meristematic cell elimination. There is probably scattered

cell damage resulting in small sectors which are recovered as partial leaf mutants. Both random and localized radiation damage in shoot apices of Bing cherry has been reported (Saamin and Thompson 1989). High frequency of total leaf mutants (4.2%) with acute exposures is accompanied by a relatively low frequency of partial leaf mutants (3.7%). This is expected because with a larger mutant sector there will be an increasing chance of recovery of total leaf mutants.

The decreasing mutation frequencies at higher exposures could be the result of high lethality of mutant cells or competitive disadvantage of affected cells in growth centres. Using moderate acute exposures of 3–4 kR in air or 4.5–5.5 kR in water, induction of total leaf mutants and growth-reduced mutants was most successful. Higher mutation rates (consisting mainly of partials) occurred in plants derived from basal buds 2–10 in Bing (*Table 4*) and 6–10 as reported in Napoleon (Saamin and Thompson 1996). At higher bud positions, there is a trend for decreasing mutation rates, which was also reported in apple (Lapins and Hough 1970). Others have also found higher mutation rates in those plant parts where axillary buds were already initiated at the time of irradiation (Bauer 1957; Pratt 1967; Lapins et al. 1969; Lapins 1971; Thompson 1979; Donini 1982).

One needs to be able to locate the bud positions on the V1 shoot which yields the highest frequency of total leaf mutants for efficient recovery of stable and useful mutants. In Bing, the frequency of total mutants (total leaf mutants plus growth-reduced mutants) tends to increase with upper bud positions, at least to 21–25, and remains high to the uppermost positions propagated (31–39). The highest frequency of total leaf mutants (0.9%) for Napoleon in previous reports was at bud positions 31–39 (Thompson 1979; Saamin and Thompson 1996), the highest frequency of total leaf mutants (3.2%) was also at the highest bud position. Lapins (1971) also observed in

Bing that the proportion of total mutants was slightly higher from the upper buds than from the basal. Apparently while the shoot is growing, there has been sufficient time for the original mutant cell to divide and form larger sectors which occupy entire lateral bud meristems on V1 shoots. Increased recovery of total leaf mutants was also accompanied by higher frequency of growth-reduced mutants. To be easily identified, growth-reduced mutants need to be total or at least near total mutants. Higher frequency of growth-reduced mutants in Bing was also related to higher frequency of total leaf mutants. Thus, high mutation rates per se may not be the best guide for determining optimum dosage but the desired mutation type should be taken into account.

For efficient recovery of useful mutants, we and also Thompson (1979) recommend propagation of upper buds of V1 shoot of cherry rather than basal buds as recommended by Donini (1982).

In our acute irradiation study and that of Thompson (1979), the frequency of total mutants and the proportion of mutants that are totals are higher in Bing than reported in Napoleon (Saamin and Thompson 1996). Lapins (1971) also obtained a higher frequency of whole shoot mutants in Bing (4.2%) compared with Thompson's (1979) report on Napoleon (2.7%). Differences in stage of development of dormant buds at the time of irradiation, genetic constitution, and perhaps in apical organization and ontogeny of shoots are plausible explanations for the differential response of the two cultivars.

In apple, Campbell and Lacey (1973) as well as Lacey and Campbell (1979, 1982) recovered up to 22% overall mutations for irradiation in water, whereas 13% was the highest we obtained in Bing cherry (*Table 3*). They also obtained runs of compact mutants in the V2 which indicates that all apical initials (at least in the histogenic layers involved with compact growth) in the V1 shoot are mutant and that these mutant initial cells remain in the critical position for mutation to occur

throughout the V1 shoot. This situation also enables preselection of total compact mutants in the V1 in apple, whereas this has not been possible in cherry. Decourtye (1970) recommended propagation of buds 8–12, whereas Lacey and Campbell (1979) recommended slightly higher buds 11–17. Pratt (1968) reported that irradiation damage in the apical meristem of cherry is random, whereas in apple it has been reported to be localized in the promeristem with recovery from the peripheral meristem (Pratt 1967; Lapins and Hough 1970). Thus, V1 shoots in apple must have a stable, recovered apex, whereas in cherry V1 shoots there is much instability. The mode of recovery in cherry has not been reported, and is currently under investigation. In our study and Thompson's (1979), mutant V2 trees were randomly distributed within families. Thompson explained that this could be due to constant shifting of apical cell initials in the V1. The occurrence of predominantly longitudinal mutant sectors in V2 partial leaf mutants suggests that apical cell initials have become more stable in secondary shoots.

The 10 (31-111 excluded) promising growth-reduced mutants in Bing showed great variation in height, internode length, number of nodes, stem diameter and H/D/N ratio (*Table 1*). This suggests that the dwarf trait is not monogenic. Variability lends to versatility in potential value of the growth-reduced mutants. Mutants that are 50–80% of control height may be more suitable for high density planting in orchard, whereas the more compact ones may be useful for home gardens. Compact Stella is about half standard size and Compact Lambert is one quarter the size of Lambert (Lane 1977).

### Conclusion

- The highest frequency of total leaf mutants in Bing was acute 3 kR irradiation in air. Although the buds tolerated higher dosages with water and fractionation, there was a higher frequency of total leaf mutants with acute irradiation in air.

- Irradiation in water led to a slight decrease in overall mutation frequency even with higher dosages, but especially a decreased frequency of totals and increased proportion of partials.
- Higher dosages not only decreased survival but also tended to decrease mutation frequency.
- Fractionation in Bing resulted in slightly higher overall mutation frequency but also higher frequency of partial leaf mutants and lower frequency of total leaf mutants.
- The frequency of compact mutants in Bing (0.34%) was found to be higher than that reported in Napoleon (0.14%) (Saamin and Thompson 1996).
- For efficient recovery of total leaf mutants, we recommend propagating only the upper buds (above position 10) on V1 shoots of sweet cherry. Basal buds have higher overall mutation frequency but lower frequency of total leaf mutants.

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