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Modification of Reproductive Biology, and Fruit Characteristics of Citrus by Bud Irrigation

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למחקר החקלאות
בית-דגן

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Title

MODIFICATION OF REPRODUCTIVE BIOLOGY AND FRUIT CHARACTERISTICS OF CITRUS
BY BUD IRRADIATION

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MODIFICATION OF REPRODUCTIVE BIOLOGY AND FRUIT CHARACTERISTIC OF CITRUS BY BUD IRRADIATION

C. ABSTRACT

Budwood of seven easy peeling cultivars, and of one selection, as well as of 'Chandler pummelo' was irradiated in Israel with gamma rays to induce and observe mutations in mV_1 and mV_2 . A new method in citrus involving wedge grafting budwood with 3-5 buds and base sheltered from irradiation, gave significantly higher survival, enabling also use of higher dose rates. Irradiation of three orange and two easy peeling cultivars was performed in Florida. Irradiation of buds immersed in water decreased bud mortality of all cultivars tested in comparison with buds irradiated in air. Troyer citrange was used in Israel as a rootstock for irradiated buds and scions, while in Florida Carrizo citrange as a rootstock proved superior to Swingle citrumelo and Rangpur x Troyer hybrid.

Cytogenetic studies in both countries showed that in seedless clones derived from irradiated seed and budwood, seedlessness was always associated with decreased male fertility. In several cases it could be demonstrated that infertility is caused by structural rearrangements of chromosomes. A fairly good correlation was also established between highly reduced seed number in 'Murcott' selections and pollen stainability.

A fair number of consistently low seeded selections of 'Murcott' were made. Several of these also showed variation as to fruit composition and time of ripening. In 'Yafit' and 'Orah' mV_1 shoots bearing low seeded fruit were also observed.

On the basis of work performed in Israel, selection in mV_2 in the field seemed the more preferable approach. Similar oriented work was performed in Florida, mainly in the greenhouse with mV_1 , as field work suffered from the 1989 freeze.

The horticultural significance of the results has been discussed.

D. OBJECTIVES OF THE ORIGINAL RESEARCH

The objective of the study was to assess and evaluate the effect of bud irradiation as a tool for modifying fruit characteristics, with special regard to seedlessness, fruit components and color. Further aims were to improve efficiency of the selection procedure for desirable mutants induced by irradiation, as well as an effort to correlate reduction in seed number with cytological findings on sterility.

In previous studies, on the effect of irradiation in inducing mutants in citrus, performed on a limited scale in Israel and in Florida, seedless mutants of 'Minneola' tangelo, 'Eureka' and 'Villafranca' lemon were obtained in Israel (Spiegel-Roy and Vardi, 1989; Spiegel-Roy et al., 1985, 1990), while seedless grapefruit and orange (Hearn, 1984, 1986) resulted from the work in Florida. These mutants were used for cytogenetic studies in both laboratories.

In the present study further genotypes, on a larger scale, were treated by gamma rays. Significant differences were found concerning radiosensitivity between cultivars, in method of treatment (air compared to water immersion of budwood during irradiation), as well as in feasibility of selection performed at the first vegetative stage (mV_1) compared to the second vegetative stage (mV_2).

E. DESCRIPTION AND DISCUSSION OF RESEARCH RESULTS (ISRAEL)

a. Irradiation procedure, radiosensitivity and plant establishment

During the first two years of the research budwood of several easy peeling seeded cultivars: 'Murcott', 'Nova', 'Mikhal', 'Ellendale' and our selections 'Yafit', 'Shani', 'Orah' 19/81 as well as 'Chandler pummelo' (see table 1) were irradiated by gamma rays. Based on previous experience, 3-4.5 kR doses have been applied at the rate of 50 kR per hour at the Nahal Soreq Center by R. Padova. Though with high doses a higher mutations rate can be expected, these gave rise (in many cases) to a high percentage of bud mortality. In order to achieve a higher rate of survival of buds we carried out an additional experiment with a different method, somewhat similar to that used with deciduous fruit trees. Budwood with 3-5 buds has been shielded at its base with lead during irradiation and subsequently wedge-grafted on rootstocks (Sour orange, and Troyer citrange). Applying this system, close to 90% survival was obtained with all

varieties used by us and this method has been therefore largely adopted in further work. It also hastened development and contributed to shortening the lag, generally found with irradiated buds, to start growth. In view of this and because of the use of clonal (non-juvenile) material for mutation breeding, grafted plants from irradiated budwood with certain cultivars e.g. 'Murcott' were found to flower about one year after being set out in the field. 'Mikhal' mandarin proved highly radiosensitive compared to other easy peeling cultivars, while our selection 'Orah' (♀ 'Temple' $\times$ ♂ 'Dancy') proved more tolerant than other material. With 'Murcott', using the wedge-grafting procedure, we were able to obtain a good bud take and start of growth, even after applying 5kR.

Close to 1200 plants of easy peeling varieties from irradiated material (mV_1 and mV_2) have been established in the field during April-June 1989. Further 2000 plants of easy peeling varieties from irradiated material (mV_1 and mV_2) have been established in the field, using the same method, during April-June 1990. About 100 plants of Chandler pummelo from irradiated material were included in plantings of 1989 and 1990.

b. Observations on existing irradiated material

In previous work performed by us, two mutants of seedless lemon and a seedless mutant of 'Minneola' tangelo have been selected, out of mV_2 plants derived from irradiated buds. Under conditions prevailing on the Israeli coast, average seed content per fruit of 'Eureka' lemon, 'Villafranca' lemon and 'Minneola' tangelo were found to be 10.5, 22 and 11.8 respectively. 'Eureka' lemon budwood was irradiated by 6.0 kR and 600 mV_2 plants were planted, while 'Villafranca' lemon and 'Minneola' tangelo were irradiated by 5.0 kR. From the latter two cultivars 120 mV_2 plants and 80 mV_2 plants, respectively, were planted. The three selections showed practically seedless fruits even in the presence of ample foreign pollen, probably as a result of very highly reduced ovule fertility and reduction of pollen stainability (10-20% compared to 80-90% in the check), combined with the parthenocarpic tendency. No measurable decrease in fruit size, in the seedless mutants, has been noted and all of them bore good crops.

c. Observations and preliminary selection in irradiated material

i. 'Murcott' - Most of the 1000 mV_2 irradiated plants of Murcott (doses 3.5 kR and 4.5 kR) planted in the field in Bet-Dagan in July

1987 (at a 4 x 1 m distance with Sour orange as a roostock) flowered during the spring of 1988. An abnormally large number of indehiscent anthers has been noted. This could be attributed either to the every young stage at which flowering occurred (10 months after planting) or to the effect of irradiation. While initial fruit-set seemed satisfactory with most plants, a count made toward the end of July 1988 showed that only 35% of the plants were left with fruit. Excessive fruit drop was probably highly influenced by a heat wave during the third week of May (up to 43°C for several days). The first survey for seed content was done during March 1989. Eight mV_2 plants bearing fruit with up to 5 seeds have been selected for further observations. Most of the fruits that seemed to have resulted from off season flowering did not differ markedly in seed content from fruit set obtained during the normal spring flowering period.

Further observations on mV_1 and mV_2 'Murcott' plants, as well as on 20 check plants from unirradiated budwood, established in the field at the same time as plants from irradiation, were made in 1989 and 1990. While in 1989 only about 400 plants were surveyed, in 1990 over 950 trees that set and maintained fruits were evaluated for seed content of the fruit. During screening we established as the maximum limit 7 seeds per fruit. The average seeds number with unirradiated material is 25. The eight preselections made in March 1989, gave similar seed counts in 1990 and 1991. Additional preselections, based mainly on seed content were made in 1990 and 1991. While most of these preselections showed a markedly reduced seed content compared to the unirradiated check, some of the additional preselections made in 1990 showed a higher seed content (up to 11-12 seeds per fruit) and were eliminated from further observation and propagation. Altogether 20 low seeded preselections were made. These trees as well as the check trees and preselected trees bearing fruits with intermediate seed number (11-12) served as material for determination of pollen stainability, refractometer readings of T.S.S, acid content, and fruit weight (Table 2).

Considerable attention was given to horticultural characteristics. Our observations concerning tree size and vigour showed often higher vigour and more erect growth with plants having a highly reduced seed number compared to the unirradiated check, often also in comparsion with trees from irradiation with the normal (high)

seed number. In a few cases, trees with somewhat compact characteristics were located, regardless of seed number.

Several of the preliminary selections had fruit with a higher TSS content and also somewhat higher TSS/acid ratio (Table 2). As to number of fruits, nearly all preselections set at least some fruit in the year after the 'on' year, but generally a tendency for biennial bearing with fewer fruits during "off year" was observed. It should be noted that 'Murcott' cultivar alternates heavily under most orchard conditions. However, as we were unable to pick the fruit in time from trees under observation, this might have increased the tendency for biennial bearing. While the larger part of the preselections bore fruit of satisfactory fruit size, and in some cases larger fruit than the control, we noted also preselections with a lower than average fruit size.

In 1991 re-propagation of the best selections was made by grafting, on Troyer citrange, 6-12 trees per selection. These plants will be planted at Bet Dagan in 1992.

ii. Additional irradiated plant material

About 1400 plants of different easy peeling varieties from irradiated material (mV_1 and mV_2) have been established in the field during 1988-1990. These included established mandarin cultivars such as: 'Mikhal', 'Ellendale', 'Nova' as well as new selections from our breeding program: 'Yafit' (φ 'Clementine' x σ 'Wilking'), 'Shani' (φ 'Wilking' x σ 'Mikhal'), 'Orah' (φ Temple x σ 'Dancy'), 19/81 (φ 'Wilking' x σ 'Orlando') and 'Chandler pummelo'. Of the varieties mentioned above (see table 1) 'Mikhal', 'Ellendale', 'Nova', 'Shani' and also 'Chandler pummelo' have the ability to set seedless fruit or fruit with only few seeds in the orchard, usually in the absence of pollenizers. This may be also quite pronounced in young trees with yet sparse flowering, even in the presence of potential pollenizers. This situation made selection for seedless mutants, in the above mentioned varieties, more problematic and at least several seasons are required in order to complete the selection. However, examining pollen stainability and possibly also viability could help and hasten selection procedure. Selection, in the above mentioned population, was started in the spring of 1990 and continued on a larger scale in 1991.

In 'Mikhal' and 'Shani' so far, no shoots in mV_1 , nor individuals in mV_2 could be selected deviating significantly from the unirradiated check material. In 'Ellendale' only one individual with 0-1 seeds was found meriting further observation. In 'Nova' also, only one such individual was observed, with only 2-6% pollen stained by acetocarmine. 'Chandler' pummelo started to fruit only in 1991. All plants are of the mV_1 vegetative generation. Selection was done on the basis of individual shoots. One shoot bearing earlier maturing fruit, seedless, deeper in color was selected. Pollen stainability will be checked in the spring of 1992.

The three other genotypes observed: 'Yafit', 'Orah' and 19/81, are self compatible and tend to set copious fruit set with many seeds without foreign pollen. In 'Yafit' one mV_1 shoot was selected in 1990; it bore 5-7 seeds per fruit, compared to 14-30 in the check. Pollen stainability, tested in 1991 reached 30% compared to over 90% in the check. In 'Orah' several mV_1 shoots were selected, among them one bore larger fruit with 4-7 seeds compared to 12-20 in the check; pollen stainability was 20% compared to close to 90% in the check. Both selections were repropagated on Troyer rootstock in 1991. No selection was made with 19/81, as all the material bore fruit with a large number of seeds.

d. Comparison of selection feasibility in mV_1 and mV_2

We were able to compare, with several varieties (e.g. 'Murcott', 'Orah') the feasibility of selection in mV_1 compared to selection, on a larger number of trees, in mV_2 . When individual buds, from irradiation, are propagated as mV_1 , one more year in the nursery, is required to establish mV_2 plants. (We propagated 6-8 mV_2 plants from each mV_1 established in the nursery). Accordingly, somewhat earlier selection in mV_1 than mV_2 can be accomplished. However, this requires sampling by individual shoots. Tagging and labeling in mV_1 is more cumbersome, the number of fruits per shoot varies and may be inadequate for sampling, and furthermore, production per shoot may alternate more pronouncedly than on whole trees. Thus in spite of expectations to terminate selection earlier, no substantial gain in time (years) has been observed by us and additional work was involved. Based on the experience gained we tend to prefer producing mV_2 plants. Another possibility is to produce mV_1 plants by wedge-grafting allowing them to form plants with adequate branching in the nursery.

Such plants can be then considered and treated as mV_2 plants in the orchard. This system, giving also a high percentage of take after irradiation has been employed by us in the new plantings. First fruiting will occur in 1992. In both cases (mV_2 plants and mV_1 plants from wedge-grafting) fruit set on trees will be more adequate and samples of fruit will be taken from whole trees.

e. Experiments on observation of seedless mutants in the screenhouse

'Yafit', 'Orah' and 'Murcott' mV_2 plants have been also grown in 10 liter containers in a screenhouse, in order to hasten start of fruiting. All the three varieties are highly self compatible. Container size proved inadequate to contain the plants for the whole cycle of selection, while larger containers would necessitate copious space if a sizeable vegetative progeny is to be raised. In addition, flowering after one season as observed, was far from being general. Still, some selections with 'Yafit' were made after one year. Because of tree size we were unable to perform repeated observations and we had to transfer the plants to the field for further assessment. In conclusion, no appreciable gain in time has been observed with the three varieties mentioned, although additional labor and expense were involved.

DESCRIPTION AND DISCUSSION OF RESEARCH RESULTS- (U.S.A.)

1. Irradiation and plant establishment

a. Budwood of 'Valencia' sweet orange and 'Murcott' tangor were exposed to 0, 3, 5 or 7 kR (gamma rays from a ^{60}Co source) in air or in water and budded onto Carrizo citrange rootstock. 'Murcott' appeared more radiosensitive than 'Valencia' at all dose levels administered. Bud survival was increased by exposure in water compared with air at 7 kR ('Valencia' and 'Murcott') and 5kR ('Murcott' only).

Half of the plants produced from this experiment were planted in the field and the others were grown in 11.8 liter containers in a heated greenhouse. Field-grown plants were killed back to protective soil banks following exposure to a minimum temperature of -9°C on December 24 and 25, 1989. Most of these trees recovered, but none of them flowered until spring of 1991. Bloom was light and scattered among the 'Valencia' plants; nearly all 'Murcott' plants bloomed and set fruit. This first crop of field-grown fruit will be harvested and

evaluated this season for seed number ('Murcott') and soluble solids/acidity and color ('Valencia').

b. Budwood of 'Hamlin' sweet orange was exposed to 5kR in air or in water and budded onto Carrizo citrange, Swingle citrumelo, or Rangpur x Troyer hybrids (RxT). All untreated buds survived on all rootstocks, and all except one bud on Swingle sprouted and grew. After 6 months, none of the irradiated buds on Swingle had sprouted (from air or water treatment) and only 2.2 and 5.5% on RxT and Carrizo respectively (Table A). RxT was tested to compare its tendency toward precocity with Carrizo, and Swingle was used because of its contribution to field cold hardiness. The poor response of irradiated buds on these two rootstocks has confirmed the value of Carrizo for clonal propagation and precocity. As previous results have indicated, bud survival is enhanced by irradiation in water, as opposed to air. All of these 'Hamlin' orange plants have been planted in the field for subsequent evaluation of juice quality characteristics, particularly juice color.

c. Additional irradiated plant material includes 'Valencia' and 'Midsweet' oranges, 'Murcott' tangor, and 'Fairchild' mandarin. As before, lower fruit acidity (thus earlier maturity) from 'Valencia' and seedlessness in 'Murcott' were the primary objectives sought. 'Midsweet' is a new mid-season sweet orange cultivar in Florida that has significant economic potential. 'Midsweet' fruit have an average of 12.6 seeds per fruit, however, so this cultivar was included in the project to select productive, commercially seedless clones with obvious advantage for either fresh fruit or processing markets. 'Fairchild' is an early-maturing, high quality mandarin hybrid of limited commercial importance, partly because it is very seedy. This cultivar was included in the project to select seedless clones and to study the effect of seedlessness on fruit set, fruit size, parthenocarpy, and yield in a normally seedy mandarin type.

Over 1500 buds of each of the above named cultivars were exposed to 4kR ('Murcott' and 'Fairchild') or 5 kR ('Valencia' and 'Midsweet') through air or water. Although experience has demonstrated enhanced bud survivability following exposure through water, it has not yet been possible to determine whether mutation frequencies are likewise affected. All of these buds have been propagated onto Volkamer lemon rootstocks in 1991 and will be field planted in early 1992.

Preliminary evaluation for specific characteristics is anticipated in 1993-1994. It should be possible to identify improvements and to determine mutation frequencies from these groups (larger than previously created) following 1-3 years observation in the field.

2. Observation of existing irradiated material

Preliminary evaluations of container-grown 'Valencia' and 'Murcott' plants for juice quality and seed number were completed. Trees of both cultivars flowered remontantly in the greenhouse environment. This behavior made fruit evaluation a somewhat difficult exercise. Fruit pedicels were carefully painted with different colors to identify fruit by bloom date, and fruit were harvested at various times by color code. Environmental effects associated with fruit production on containerized plants in the greenhouse likely obscured the effects of mutagenic treatment. For example, seed number of 'Murcott' control fruit ranged from 2 to 14 and 0 to 15 in the first two harvests; although several irradiated 'Murcott' clones produced fruit with few or no seeds, it cannot be concluded with certainty that any irradiated clones were seedless following these first observations of but a few fruits.

The problem of evaluating multiple crops of fruit from 'Valencia' for juice characteristics was more critical, perhaps, than seed number of 'Murcott'. Juice quality was quite variable; even among fruit harvested from some individual control trees, acidity ranged from 0.72 to 1.51% and 0.86 to 1.90%, for example. Again, the great variation observed among fruit from individual control trees cast doubt on the values observed among the irradiated 'Valencia' clones. Although growing trees in the greenhouse insured their survival and resulted in earlier flowering and fruiting than field-grown counterparts, the imposition of environmental effects of critical traits and the small sample size made meaningful, valid selection impossible.

The greenhouse-grown trees were planted in the field in early March 1991, and they continued to behave atypically there. Off-season flowering occurred among 47 and 82% of 'Valencia' and 'Murcott', respectively, and none of the transplanted trees had normal season fruit (Table A). Although only 22% of the field grown 'Valencia' plants flowered in 1991, all of these bloomed during the normal season; 68% of the field-grown 'Murcott' plants likewise had normal bloom. None of the field-grown plants bloomed out of season. So,

although plants survival and early fruiting were improved in the greenhouse environment, the problems associated with remontant flowering and atypical fruit development indicated that field-grown material should be more useful for selection of clones exhibiting the desired characteristics.

All of the clones produced following irradiation, and appropriate control plants have now been (or will soon be) planted in the field. Although funding for this project has been terminated, the plant material produced will be maintained and evaluated for the desired objectives over the next few years. Potentially desirable mutant types will be propagated and planted in appropriately designed advanced field trials to determine stability of trait expression and to evaluate horticultural performance in comparison with the parental clones.

3 Studies of male fertility and meiosis

a. Seedless 'Pineapple' orange clones from irradiated seeds. Various measures of male fertility (including acetocarmine staining and in vitro germination of pollen, pollen grain number per anther, and frequency of tetrads and other abnormal sporads) and meiosis (chromosome pairing relationships, metaphase I and anaphase I behavior) were studied to determine mechanisms underlying seedlessness in 'Pineapple' sweet orange clones developed and selected by C.J. Hearn (USDA-Orlabdo) following seed irradiation. Pollen germination and acetocarmine staining tests were performed in 1989 and 1990 (Table C). Staining percentage ranged from 11.9 to 41.0% in the seedless clones and was 85.1% in the normal control. Germination was 37.2% in the control but ranged from 0.8 to 9.1% among the seedless mutants. Stainability and germination were not correlated with each other, nor with the relative level of seedlessness. The results of pollen viability tests in 1990 were essentially the same as those of 1989. The number of pollen grains per anther was determined; the seedy control had the lowest number per anther, while the most seedless clones produced the greatest number per anther.

Citrus chromosomes are small and, as a consequence, meiotic analysis is difficult compared with other well studied plants such as maize. Nonetheless, the perseverance and experience, it was possible to observe meiosis and to describe chromosome pairing and behavior with confidence. Observations of prophase I (Table D) revealed nearly

complete bivalent pairing in the control with some univalency (7.1% PMCs). Two clones (1-8, 10-60) revealed quadrivalent formations in nearly 20% of PMCs, suggesting reciprocal translocations; univalency was increased also in these two selections. Another clone (10-98) had elevated levels of univalency (53.6% PMCs) up to 6I. No pairing anomalies were observed in a fourth seedless clone (10-85) at prophase I, but 12.7% of PMCs observed at anaphase I had bridges that suggest the existence of a paracentric inversion (Table E). Associated with the inversion was a decrease in the frequency of normal quartet sporads and an increase in the frequency of pentads (Table F). The increased univalency clone 10-98 likewise was associated with decreased frequency of normal quartets, but the frequency of both pentads and hexads dramatically increased. In summary, two of the four seedless clones may harbor reciprocal translocations, one may harbor a paracentric inversion, and the basis of reduced fertility of the other was increased univalency. It is speculated that the later case is chromosomal in nature and not genic because, at most, only three pairs were unable to pair normally. This partially univalent clone had the lowest pollen germination rate and mean seed number of the four studied.

b. Seedless 'Foster' grapefruit clones from bud irradiation. Two seedless clones of 'Foster' grapefruit were compared with the parental clone in terms of male fertility and some meiotic analysis. These clones were selected from irradiated, mature bud-propagated trees by C.J. Hearn (USDA-Orlando). As with the seedless 'Pineapple' selections, pollen staining and in vitro germination percentages of the seedless 'Foster' clones were drastically reduced (Table G); the mean number of pollen grains per anther was increased in one and decreased in the other seedless clone (Table G). Although observations of prophase were few in number, the percentages of PMCs exhibiting normal bivalent pairing were decreased in both mutants (Table H); PMCs with a pair of univalents or quadrivalents were especially increased in clone 9-20. Bridges were observed at anaphase I (26.1%) and II (13.9%) in 9-26 PMCs (Tables I, J). The observations suggest that an inversion in 9-26, and univalency and (perhaps) a translocation in 9-20 are responsible for reduced fertility. In contrast with the seedless 'Pineapple' clones, the decreases in normal tetrad frequency were less in the 'Foster' selection (Table K).

c. Naturally occurring seedless mutants. Studies identical to those described above were carried out with several seedless cultivars and selections. The clones studied and the associated mechanisms of seedlessness suggested are listed and summarized in Table L, along with the rationale used to reach these conclusions. All (except 'Valencia' orange and Mexican lime) possess what might best be described as "chromosome structural hybridity" leading to increased frequencies of univalent pairs; this, in turn, may result in chromosomally unbalanced gametes. It is noteworthy that our observations of Mexican lime meiosis were more indicative of translocation heterozygosity, rather than the inversion described by Iwamasa. We did observe bridge-like structures at anaphase, but we interpreted these to be chromosome chains which are sometimes found with translocation, and not typical anaphase bridges.

Table 1. Number of trees planted during 1987-1990 from irradiated budwood of 7 easy-peeling cultivars, one selection and a pummelo genotype.

Cultivar	Year of planting			
	1987	1988	1989	1990
'Murcott'	1000	500	500	400
'Mikhal' mandarin	-	50	50	100
'Ellendale	-	70	50	35
'Nova'	-	200	100	-
'Yafit'	-	20	70	80
(♀ Clementine x ♂ Wilking)				
'Shani'	-	50	150	-
(♀ Wilking x ♂ Mikhal)				
'Orah'	-	100	200	-
(♀ Temple x ♂ Dancy)				
19/81	-	90	-	-
(♀ Wilking x ♂ Orlando)				
'Chandler pummelo'	-	30	30	70

Table 2. Fruit size, number of seeds per fruit, TSS and acidity and pollen stainability in 17 mV₂ selections from irradiated budwood of 'Murcott', compared to unirradiated check.

Selection	No. of Fruits per tree	No. of fruits tested	Average fruit size (g)	Average No. of seeds per fruit	TSS	Acid %	TSS/acid	Pollen stainability (%)
1	50	10	111	5.3 (2-12)	17.2	0.99	17.4	12.5
2	30	10	108	4.0	16.2	1.44	13.7	10.0
3	30	8	94	2.3 (1-5)	17.1	1.28	13.4	12.5
4	100	10	89	4.3 (3-7)	17.9	1.12	15.9	27.5
5	60	5	79	3.0 (2-4)	19.1	1.44	13.3	21.0
6	50	5	127	3.2 (10-6)	16.2	1.38	11.7	11.5
7	20	10	109	5.5 (4-8)	13.2	1.31	10.1	40.0
8	10	5	82	2.8 (2-3)	20.1	1.63	12.3	6.0
9	50	5	74	3.6 (1-5)	15.7	1.37	13.7	32.0
10	50	10	102	3.1 (1-5)	16.5	0.93	17.7	43.0
11	30	5	86	3.0 (1-5)	16.5	0.89	18.5	30.0
12	50	5	78	2.2 (1-4)	17.2	0.99	17.4	23.5
13	50	10	95	2.9 (1-7)	16.4	0.93	17.6	32.0
14	100	10	107	4.1 (2-6)	15.3	1.15	13.3	12.5
15	50	7	122	3.9 (1-6)	15.9	0.86	18.5	24.5
16	70	10	92	4.0 (2-9)	17.4	0.99	17.5	27.5
17	30	10	95	2.3 (1-5)	19.7	1.31	15.0	5.0
unirradiated check	100	10	97	23 (15-25)	14.5	1.18	12.3	86.0

Table A. 'Hamlin' bud germination (%) following 5 kR treatment in air or water, and propagation on three different rootstocks. Data were collected 6 months after propagation.

	Control	Air	Water
RxT	100 n=8	2.2 n=46	47.8 n=44
Carrizo	100 n=6	5.5 n=18	62.5 n=32
Swingle	83.3 n=6	0 n=22	0 n=21

Table B. Percentage of trees from container-grown greenhouse plants (g) or field planted (f) with fruit from either normal bloom, off-season bloom (mature and/or young fruit and flowers), or without fruit in July 1991.

	Mature fruit	Normal fruit	Young fruit/bloom	No fruit
Valencia (g)	33	0	47	29
Valencia (f)	0	22	0	78
Murcott (g)	18	0	82	32
Murcott (f)	0	68	0	32

Table C. Numbers of seeds per fruit and pollen grains per anther, and percentages of acetocarmine stained and in vitro-germinated pollen grains of 'Pineapple' sweet orange and selected radiation-induced mutants.

Selection	Seeds per fruit (1989)	Acetocarmine stained pollen (1989)		Acetocarmine stained pollen (1990)		Germination percentage (1990)	Pollen grains per anther (1990)
		n ^z	% ^y	n ^z	% ^x		
Pineapple	19.4	1136	75.2 a	1198	85.1 ± 5.5	37.2	12190
10-8	2.7	5463	35.0 b	1254	41.0 ± 6.4	3.4	15440
10-60	0.9	7951	18.6 c	1951	14.9 ± 4.5	1.3	22170
10-85	3.0	2098	17.1 c	1429	16.6 ± 3.2	9.1	14190
10-98	0.7	5089	9.6 c	1347	11.9 ± 4.3	0.8	17500

^zTotal number of pollen grains observed.

^yMean percentage of total number of pollen grains that were stained. Means separated using Waller-Duncan K-ratio t-test with mean square for trees (selection) as error term.

^xPercentage of total number of pollen grains that were stained and standard deviations.

Table D. Meiotic chromosome pairing relationships of 'Pineapple' sweet orange and selected radiation-induced mutants.

Selection ²	PMCs observed	9II		8II + 2I'		7II + 1IV		7II + 4I		6II + 6I		5II + 2IV	
		No.	(%)	No.	(%)	No.	(%)	No.	(%)	No.	(%)	No.	(%)
Pineapple (19.4)	42	39	(92.9)	3	(7.1)	--	--	--	--	--	--	--	--
10-8 (2.7)	58	37	(63.8)	6	(10.3)	12	(20.7)	3	(5.2)	--	--	--	--
10-60 (0.9)	41	24	(58.5)	4	(9.8)	8	(19.5)	3	(7.3)	1	(2.4)	1	(2.4)
10-85 (3.0)	46	45	(97.8)	1	(2.2)	--	--	--	--	--	--	--	--
10-98 (0.7)	125	58	(46.4)	32	(25.6)	--	--	27	(21.6)	8	(6.3)	--	--

¹Numbers in parentheses are mean number of seeds per fruit in 1989.

²Student's t-test revealed no differences among 'Pineapple', 10-8, 10-60, and 10-85; however, 10-98 was significantly different from the other selections for frequency of 8II + 2I PMCs ($t = 2.54$, $\alpha = 0.01$).

Table E. Observations of metaphase I and anaphase I from 'Pineapple' sweet orange and selected radiation-induced mutants.

Selection	Metaphase I			Anaphase I		
	PMCs observed	Precocious division (%) ^z	PMCs observed	Normal (%)	Bridges (%) ^y	Fragments/ laggards (%) ^x
Pineapple	186	2.2	176	90.9	1.1	8.0
10-8	144	5.1	113	85.8	0.9	13.3
10-60	307	3.5	93	89.2	0.0	10.8
10-85	115	4.3	165	52.1	12.7	35.2
10-98	165	38.8	272	54.8	0.0	45.2

^xThis refers to cells in which one or more chromosome pairs were found to be separated and in polar positions while the other pairs were at the metaphase plate.

^yPercentage of the total number of cells observed with one or more bridges, with or without associated fragments.

^zPercentage of the total number of cells observed with one or more fragments and/or lagging chromosomes.

Table F. Percentages of normal sporad cells and abnormalities from 'Pineapple' sweet orange and selected radiation-induced mutants.

Selection	n ²	Tetrad	Pentad	Hexad	Others ³
Pineapple	1284	89.7	6.3	3.8	0.2
10-8	1379	88.4	7.7	3.3	0.6
10-60	1905	75.7	16.9	7.1	0.3
10-85	1716	59.4	35.6	4.5	0.6
10-98	1224	53.7	27.8	15.9	2.6

²Total number observed.

³Others include 1-3 abortive, heptads, and octads.

Table G. Numbers of seeds per fruit and pollen grains per anther, and percentages of acetocarmine stained and in vitro-germinated pollen grains of 'Foster' grapefruit and selected radiation-induced mutants.

Selection	Seeds per fruit (1989)	Acetocarmine stained pollen % (1990)	Germination % (1990)	Pollen grains per anther (1990)
Foster	50.8	90.77	52.25	25,500
9-20	4.5	5.24	2.33	30,880
9-26	1.6	2.45	0.48	18,580

Table H. Meiotic chromosome pairing relationships of 'Foster' grapefruit and selected radiation-induced mutants.

Selection ²	PMCs observed	9II		8II + 2I		7II + 1IV		7II + 4I	
		No.	(%)	No.	(%)	No.	(%)	No.	(%)
Foster (50.8)	80	70	(87.5)	7	(8.8)	3	(3.8)	--	
9-20 (4.5)	34	16	(47.1)	11	(32.4)	6	(17.7)	1	(2.9)
9-26 (1.6)	38	30	(79.0)	4	(10.6)	4	(10.6)	--	

²Numbers in parentheses are mean number of seeds per fruit in 1989.

Table I. Observations of metaphase I and anaphase I from 'Foster' grapefruit and selected radiation-induced mutants.

Selection	Metaphase I			Anaphase I		
	PMCs observed	Precocious division (%) ²	PMCs observed	Normal (%)	Bridges (%) ¹	Fragments/laggards (%) ³
Foster	50	0	42	95.2	2.3	2.3
9-20	40	2.5	257	67.3	0	32.7
9-26	141	2.8	69	73.9	26.1	8.7

¹This refers to cells in which one or more chromosome pairs were found to be separated and in polar positions while the other pairs were at the metaphase plate.

²Percentage of the total number of cells observed with one or more bridges, with or without associated fragments.

³Percentage of the total number of cells observed with one or more fragments and/or lagging chromosomes.

Table J. Observations of metaphase II and anaphase II from 'Foster' grapefruit and selected radiation-induced mutants.

Selection	Metaphase II		Anaphase II		
	PMCs observed	Extra fragments (%) ^z	PMCs observed	Normal (%)	Bridges (%) ^y Fragments/ laggards (%) ^x
Foster	40	2.5	25	100.0	0 0
9-20	175	33.7	26	92.3	0 7.7
9-26	125	12.8	65	86.1	13.9 4.6

^zThis refers to fragments or extra chromosomes observed that likely resulted from chromosomes lagging at AI.

^yPercentage of total PMCs observed with at least one bridge found in one cell.

^xPercentage of the total number of cells observed with one or more fragments and/or lagging chromosomes.

Table K. Percentages of normal sporad cells and abnormalities from 'Foster' grapefruit and selected radiation-induced mutants.

Selection	n [*]	Tetrad	Pentad	Hexad	Others ^y
Foster	447	97.1	2.2	0.7	0
9-20	487	85.2	11.1	2.1	1.6
9-26	384	94.8	4.7	0	0.5

^{*}Total number observed.

^yOthers include 1-3 abortive, heptads, and octads.

Table L. Naturally occurring seedless Citrus selections and evidence for underlying mechanisms suspected.

Selection	Mechanism(s) of Infertility	Evidence
Hamlin orange	Chromosome structural hybridity	High frequency of PMCs with 2-6 univalents
Valencia orange	Translocation heterozygote	High frequency of PMCs with quadrivalent formations
Marsh grapefruit	Chromosome structural hybridity	High frequency of PMCs with 2-6 univalents
Mexican lime	Translocation heterozygote	High frequency of PMCs with quadrivalent formations
Satsuma selection	Chromosome structural hybridity; pollen sterility gene (?)	High frequency of PMCs with univalents; low number of pollen grains per anther
Lee x Nova hybrid	Multiple spindles; chromosome structural hybridity; asynapsis (or desynapsis) gene (?)	PMCs with 2-12 univalents (96.8%)

F. DESCRIPTION OF COOPERATION

Dr. Vardi visited during October 1987 Lake Alfred to discuss and coordinate with Dr. F.G. Gmitter the detailed research plan for the first year. A decision was also taken concerning division of work. Inter alia, it was decided that Dr. Gmitter will deal, within the framework of his work, with cytogenetic studies on male fertility and meiosis as well as carrying out the comparison between performance of material after irradiation in water compared to air. In Bet Dagan work was to be carried out with larger populations from irradiated material, with different varieties, including a comparison of testing and performance of mV_1 vs. mV_2 (including as well as studies on type of material for irradiation and correlation between reduction in seed number with pollen stainability).

During the project period mutual consultations and exchange of information was maintained.

G. EVALUATION OF THE RESEARCH ACHIEVEMENTS WITH RESPECT TO THE AIMS OF THE ORIGINAL RESEARCH PROPOSAL (ISRAEL)

a. Sterility studies

Seedless Israeli 'Villafranca' lemon, seedless 'Eureka' lemon, seedless 'Minneola' tangelo were obtained, in our laboratory, by irradiation. These mutants were examined for pollen sterility and female sterility (seed content). Seed content was very low (0-1) in all years examined, while pollen stainability ranged between 10% to 20% compared to 80-90% in the seeded check.

Similar studies were performed with low seeded selections of 'Murcott' derived from mV_2 irradiated plants, compared to 'normal' seeded 'Murcott'. Average seed number in 17 selections examined ranged from 2.3-5.5 per fruit compared to 23.0 in the unirradiated control. Pollen stainability ranged from 5-43% compared to 86% in the control. A fairly good correlation was thus established between seed number and pollen stainability. Because of the heavy involvement with further objectives of the the research no microscopic work on megaspore and embryo sac development was carried out. Seed content, a measure of female fertility, was widely examined.

b. Screening existing populations

A comparatively large population (1000 plants) of mV_2 'Murcott', has been planted about 5 months before starting the project. We were able to make the first evaluation already in February-March 1988, 4 months after the project was initiated. Moreover, we have been able to screen the same population for 2 more years. Results, as to seed number, as well as other characteristics were fairly consistent from year to year. We were able to make preliminary selections based not only on substantially lower seed number and on pollen stainability, but also on fruit composition (TSS, acids) and time of ripening. Several selections have been repropagated in 1991 and will be planted in 1992 for further observations.

In addition to the work performed on existing populations, new populations of irradiated material (mV_1 and mV_2) were established during the period of the research (fall 1987 - fall 1991) both on 'Murcott' cv. and on 7 additional easy peeling genotypes. 'Chandler' pummelo' was also included. In all populations were were able to observe so far at least one crop. Performance of these genotypes as to seed number and other characteristics have been described in the present report.

c. Comparing irradiation efficiency

Rather extensive information has been gained on the relative performance and problems involved in growing and selecting in the mV_1 vegetative generation as compared to growth and selection in the mV_2 generation. In addition a new method has been evolved in propagating irradiated citrus material by wedge grafting, involving also protection of the base of the scion. This method resulted in a much higher percentage of bud take after irradiation, enabling to raise nearly twofold the radiation dose. Growing and selecting in mV_2 proved more efficient and less laborious than growth and selection in mV_1 .

d. Speeding up screening by use of container grown plants

Growing irradiated budded plants in containers was performed with three varieties. Container size (10 liter) was inadequate for conducting repeated observations on fruit characteristics for three years. Selections were made with only one variety and all plants had to be set out in the orchard for further observations. No appreciable

gain in time with this system can be thus expected, compared to growing out of mV₂ plants in the field.

The results of the research so far have proven of considerable interest concerning methodology of mutation breeding in citrus, with particular relevance to seedlessness. We have also gained information as to possible approaches to the problem with different genotypes and types of pollination system (self pollinating, incompatible). The main effect on reduction of ovule fertility, as evidenced by significantly lower seed number was clearly demonstrated, and a similar effect was found in reduction of pollen fertility, as assessed by pollen stainability. The relatively short period of the research did not allow full assessment and exploitation of other horticulturally significant changes induced. However, with the early cropping of clonal 'Murcott' some interesting variants as to fruit composition were disclosed. Full assessment and appreciation of the induced changes will have to await yield and other records from selected mutants. Its preliminary results will be indeed confirmed by material propagated on a larger scale, this may turn out as a significant contribution to the citrus industry in both countries.

G. EVALUATION OF THE RESEARCH ACHIEVEMENTS WITH RESPECT TO THE ORIGINAL PROPOSAL OBJECTIVES (U.S.A.)

a. Irradiation and plant establishment

Experiments were conducted to develop efficient methods for bud irradiation, plant establishment, and preliminary screening for desired phenotypic changes. Irradiating buds immersed in water decreased bud mortality of all cultivars tested in comparison with buds irradiated in air. It has not been possible within the time frame of this grant to ascertain whether mutation frequencies are equivalent for air or water immersed buds, but this question will likely be answered as evaluation and selections are made among the irradiated cultivar families now growing in the field.

Carrizo citrange has been shown to be an excellent rootstock choice for irradiated bud survival and precocity. 'Valencia' and 'Murcott' progeny flowered and set fruit within 2 years, and some irradiated 'Hamlin' progeny flowered within 9 months post-treatment. Other rootstocks tested because of their positive influence on scion precocity (Rangpur x Troyer hybrid) or cold tolerance and precocity

(Swingle citrumelo) have proven undesirable because of their poor performance regarding bud survival.

b. Development of rapid selection

An experiment was performed to compare the length of time needed to evaluate and make preliminary selections from irradiated progeny grown in containers in the greenhouse or identical progeny grown in the field. Greenhouse-grown plants in containers flowered and set fruit in 2 years or less following treatment. When compared with equivalent field-grown progeny in early 1990, it appeared that the greenhouse selection scheme was the most rapid and efficient method because all of the field plants, were severely damaged by the December 1989 freeze. However, additional time and observations have led this investigator to conclude that, despite some obvious advantages associated with the greenhouse approach, the most effective, efficient way to grow progeny and make preliminary selections from mV_1 plants is to grow them in the field. Remontant flowering and fruit set in the greenhouse environment is very difficult to control and greatly complicated record keeping and meaningful data collection. At best, a maximum of 350 irradiated progeny could be maintained in the greenhouse of growth, flowering, and first fruit evaluation; obviously, space limitations in turn limit the number of irradiated progeny that can be created and evaluated in the greenhouse. Great variation for fruit characteristics, even among fruit from the same bloom on individual control trees, made it impossible to have confidence that specific mutations possibly identified among irradiated progeny were, in fact, genetic mutations rather than greenhouse environment. Furthermore, container-grown plants continued to flower remontantly for one year after being planted in the field, further delaying meaningful evaluation and selection of budlines with the desired phenotypic modifications. Despite the risk of calamity associated with disastrous freeze events, growing mV_1 progeny in the field provides the advantages of being able to screen more and/or larger families as well as resulting in more typical growth, flowering and fruit development and thereby can facilitate meaningful selections of improved budlines.

c. Studies of mechanisms underlying seedlessness

The extensive studies of aspects of male fertility and meiosis in seedless clones produced by irradiation of seed or budwood, as well as

of naturally occurring seedless Citrus selections were the first to address and compare the mechanisms underlying sterility in these two groups.

Seedlessness in all of the seedless clones derived from irradiated seed or budwood was always associated with decreased male fertility. The observed infertility results from structural rearrangements of chromosomes and does not appear to be genic in nature. The naturally occurring seedless Citrus selections studied, likewise, appear to have a chromosomal basis for infertility and seedlessness, with two possible exceptions. The 'Satsuma' mandarin clone and the 'Lee' x 'Nova' hybrid, although characterized by increased univalency, may involve genic factors, as well. For example, it is possible that there exists a gene in the 'Satsuma' that controls pollen grain development post-meiosis (similar to the pollen sterility gene in 'Washington' navel orange), based on an observed high frequency of normal tetrads (> 92%) but very low number of pollen grains per anther (≈ 4800). The 'Lee' x 'Nova' hybrid exhibited multiple spindles and high levels of univalency the possibility of genic control of spindle formation or desynapsis (or asynapsis) cannot be excluded at this time, based on our observations. However, it is clear that the basic mechanisms underlying seedlessness in the irradiated clones are chromosomal and not genic in nature. Therefore, the induction of seedlessness via mutagenesis ought to be possible for most seedy Citrus types at a higher frequency than radio-induction of other useful characteristics where individual genes must be affected.

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