EFFECT OF SEED TREATMENTS WITH X-RAY AND PHOSPHORUS 32 ON TOMATO PLANTS OF FIRST, SECOND, AND THIRD GENERATIONS

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Received January 30, 1956

Many observers have reported an increase in variability following exposure of pollen or seeds to the effect of ionizing radiation. In several respects the tomato is favorable material for such studies. It is self-pollinating, and recessive mutants can therefore be uncovered promptly. It is a perennial and can be propagated by cuttings, so that plants of a previous generation can be retained, and two generations can be grown in one year. The normal cytological and genetical behavior is well known. In a recent report (Tomato Genetics Cooperative Report No. 4, 1954) 108 mutant genes of the tomato are listed, and the number is increasing rapidly. Lindstrom (1933), with radium, and MacArthur (1934), with X-rays, succeeded in inducing mutations in this species.

Exposure of pollen to radiation has been much more used than exposure of seeds. The latter method seemed to offer certain advantages in using a radioactive isotope, since, when seeds are soaked in a solution containing P32, the radioactive isotope may enter the nuclei of the embryo before and during germination and the multicellular organism is exposed to decreasing amounts of beta radiation during a period of several weeks.

The studies reported in this paper include a single experiment with X-rays and nine with phosphorus 32 during three generations of mature plants.

EXPERIMENT WITH X-RAYS

Seeds of an inbred line of First Early, a highly fruitful tomato variety, were exposed to X-radiation. Eighty-one seeds were kept on moist filter paper for two days at 80°F before irradiation, and the same number were irradiated in a dry state. Each lot received somewhat less than 10,000r at the rate of 300r per minute. The source of X-rays was a Coolidge tube with tungsten target, operating at 80 KV and 3 milliamperes and without a filter. The results of this experiment were as follows.

After planting, 83 percent of the dry-treated seed and 77 percent of the wet-treated seed emerged. Only one of the 45 seeds planted as a control failed to grow. Germination of the treated seeds, especially of the wet lot, was slower and more irregular than that of the control seeds.

The plants that grew from the control seeds were normal in every way, and most of those from dry-treated seeds appeared to be normal. Most of the 46 plants from wet-treated seeds developed very slowly and many of them were abnormal in meiosis and partially male sterile; 5 were almost totally unfruitful, 5 were only fairly fruitful, and 36 had a full crop by the end of the season.

1 Paper No. 898, University of California Citrus Experiment Station, Riverside, California.
All of the plants which grew from wet-treated seeds were diploid, having 24 chromosomes. Four plants had very few pollen mother cells and these remained in masses during meiosis. In nine plants non-reduction was frequent, and in three of them, there was one unequal pair. Five plants had one tetravalent, one had two trivalents or a hexavalent, one had an inversion bridge and fragment, and two had a trabant-like fragment which was sometimes free. In a few cells in three plants the chromosomes were elongated and formed stickiness bridges at first anaphase.

The seeds that were irradiated and the plants that developed from them were called the R1 generation; the generation from selfing R1 was called R2, and so on. Twenty R1 plants of the “wet” series produced seeds from open pollination. Small R2 populations of from 9 to 18 seeds were planted from each, making a total of 235 seeds, of which 206 germinated.

One R2 family contained 3 plants closely resembling the gene mutant wiry (w), and 30 normal plants. The wiry-like mutant differed from wiry (w) in that the ovary was less apocarpous, and, like the thread-leaved mutant described by Schiefflin (1933), was partially female fertile. A normal plant of this family was selfed and gave in R3 26 wiry-like and 59 normal plants, or approximately the expected monohybrid ratio.

No other previously known mutants occurred in the progenies of the R1 plants. A few apparent mutants occurred in 10 of the 20 R2 families, these mutants including one wholly sterile plant, one having a variegated leaf, several plants having fruit that was somewhat smaller than normal, and some on which the fruit ripened later than that of the control plants.

EXPERIMENTS WITH P32

Dosage, seed germination, and subsequent growth

Seeds of tomato varieties originating from single plants of lines inbred for three to six generations were soaked in solutions containing P32. (This isotope was supplied by the Oak Ridge National Laboratory, Oak Ridge, Tennessee, as phosphate in acid solution, denoted “P-32-P-1 processed, high specific activity”; half life 14.3 days.) In the nine treatments, the initial dose per seed, expressed as microcuries of P32 in solution per number of seeds soaked, ranged from 2.7 to 10.8 microcuries. The temperature during soaking was 70-85°F. Enough distilled water was subsequently added so that some liquid remained in the container at the end of treatment. The non-treated seeds received distilled water only.

The percentage of seed germination was reduced by an initial dose of 5.1 microcuries per seed and 9 days' soaking, but was normal with an initial dose of as much as 10.8 microcuries per seed and 5 days' soaking (table 1, T3, T5, T7, and control 53.61.) It is probable that the longer soaking time interfered with respiration and growth. Germination of treated seeds, even with the lowest dose, was slower and more irregular than in the control. Subsequent growth was much slower, especially with the higher dosage. The mortality both before and after transplanting to the field was also higher than in the control.

The initial dosage per seed used in the nine experiments with P32 is shown in table 1. Reduction of fruitfulness was in general proportionate to dosage. Treat-
TABLE 1

**Effect of \( {P}^{32} \) treatment of tomato seeds on germination and plant survival**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Family</th>
<th>Variety</th>
<th>Date of treatment</th>
<th>Number of seeds treated</th>
<th>Initial dosage ( \mu \text{C} )</th>
<th>Total volume of liquid, ml</th>
<th>Duration of treatment, days</th>
<th>Seed germination percentage</th>
<th>Number of plants In greenhouse</th>
<th>In field</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1</td>
<td>52 C</td>
<td>Riverside</td>
<td>3–1–52</td>
<td>50</td>
<td>271</td>
<td>5.4</td>
<td>2.0</td>
<td>3</td>
<td>3 (100)</td>
<td>36 (94)</td>
</tr>
<tr>
<td>T2</td>
<td>52 B</td>
<td>&quot;</td>
<td>3–1–52</td>
<td>50</td>
<td>136</td>
<td>2.7</td>
<td>2.0</td>
<td>3</td>
<td>3 (100)</td>
<td>34 (94)</td>
</tr>
<tr>
<td>Control</td>
<td>52 A</td>
<td>&quot;</td>
<td>3–1–52</td>
<td>50</td>
<td>0</td>
<td>0</td>
<td>2.0</td>
<td>3</td>
<td>3 (100)</td>
<td>12 (96)</td>
</tr>
<tr>
<td>T3</td>
<td>52.129</td>
<td>&quot;</td>
<td>5–13–52</td>
<td>50</td>
<td>256</td>
<td>5.1</td>
<td>2.0</td>
<td>9</td>
<td>58 (29)</td>
<td>21 (29)</td>
</tr>
<tr>
<td>T4</td>
<td>52.130</td>
<td>&quot;</td>
<td>5–13–52</td>
<td>50</td>
<td>256</td>
<td>5.1</td>
<td>2.0</td>
<td>9</td>
<td>42 (21)</td>
<td>21 (21)</td>
</tr>
<tr>
<td>Control</td>
<td>52.128</td>
<td>&quot;</td>
<td>5–13–52</td>
<td>—</td>
<td>0</td>
<td>0</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>9 (9)</td>
</tr>
<tr>
<td>T5</td>
<td>53.62</td>
<td>Canary</td>
<td>5–4–53</td>
<td>50</td>
<td>540</td>
<td>10.8</td>
<td>1.0</td>
<td>5</td>
<td>98 (45)</td>
<td>33 (45)</td>
</tr>
<tr>
<td>T6</td>
<td>53.63</td>
<td>Export</td>
<td>5–9–53</td>
<td>25</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>9</td>
<td>88 (21)</td>
<td>21 (21)</td>
</tr>
<tr>
<td>Control</td>
<td>53.61</td>
<td>&quot;</td>
<td>5–4–53</td>
<td>50</td>
<td>0</td>
<td>0</td>
<td>1.0</td>
<td>5</td>
<td>92 (23)</td>
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<tr>
<td>T7</td>
<td>54.20</td>
<td>&quot;</td>
<td>3–10–54</td>
<td>70</td>
<td>756</td>
<td>10.8</td>
<td>1.4</td>
<td>5</td>
<td>100 (20)</td>
<td>0 (20)</td>
</tr>
<tr>
<td>Control</td>
<td>54.21</td>
<td>&quot;</td>
<td>3–10–54</td>
<td>70</td>
<td>0</td>
<td>0</td>
<td>1.4</td>
<td>5</td>
<td>95 (4)</td>
<td>—</td>
</tr>
<tr>
<td>T8</td>
<td>54.220</td>
<td>&quot;</td>
<td>9–15–54</td>
<td>50</td>
<td>540</td>
<td>10.8</td>
<td>1.0</td>
<td>3</td>
<td>98 (49)</td>
<td>—</td>
</tr>
<tr>
<td>T9</td>
<td>54.221</td>
<td>&quot;</td>
<td>9–15–54</td>
<td>50</td>
<td>234</td>
<td>4.7</td>
<td>1.0</td>
<td>3</td>
<td>98 (49)</td>
<td>—</td>
</tr>
<tr>
<td>Control</td>
<td>54.219</td>
<td>&quot;</td>
<td>9–15–54</td>
<td>25</td>
<td>0</td>
<td>0</td>
<td>.5</td>
<td>3</td>
<td>96 (4)</td>
<td>—</td>
</tr>
</tbody>
</table>

† Includes 1 cc nutrient solution complete but without phosphorus.
‡ Residual liquid after soaking 50 seeds in 540 microcuries for 5 days.
§ Fifty treated seeds were sacrificed for assay of radioactivity in seed.

ments 1 and 2 were given at the same time and with portions of the same sample of active material, but in T1 the initial dose of \( {P}^{32} \) was twice that in T2. In T2, 44 percent of the R_1 plants were as fruitful as the control and the rest were less fruitful. In T1 only 9 percent were as fruitful as the control and the rest were less fruitful. On the other hand, two lots of seed which received the same initial dose per seed (T5 and T7) but with different preparations and possible dilutions of \( {P}^{32} \), gave very different results. In T7, germination was perfect, but every plant died in the cotyledon stage. The control plants developed normally. In T5, 76 percent of the plants developed beyond the cotyledon stage, and all but six of these survived transplanting to the field. The sample of \( {P}^{32} \) used in T7 gave a heavy precipitate at pH 7, whereas the other sample gave only a light precipitate. In T8 with the same initial dose and only 3 days' soaking, 98 percent of the plants developed well.

In T7, 70 seeds received an initial dose of 756 \( \mu \text{C} \) or 10.8 \( \mu \text{C} \) per seed and the soaking period was 5 days. The average total activity during the 5 day period, therefore, was 675 \( \mu \text{C} \) and the activity per seed was 9.6 \( \mu \text{C} \). If 1 \( \mu \text{C} \) of \( {P}^{32} \) produces 43 roentgens per day, during the 5 day period of treatment each seed received 43 \( \times \) 9.6 \( \times \) 5 = 2060r. After the 5 day treatment in T7 25 of the 70 seeds were removed from the liquid without draining, reduced to ash and counted with a Nuclear Geiger Counter. These seeds were found to contain 27.83 \( \mu \text{C} \). Another lot of 25 seeds was thoroughly washed in distilled water, drained, ashed, and counted. The amount of activity in these was 10.14 \( \mu \text{C} \), so that washing and draining removed 64 percent of the \( {P}^{32} \). The amount present in the washed seeds leads to the conclusion
that of the 595 μC remaining after soaking 70 seeds for 5 days, 28.4 μC or about 4.8 percent was actually in the seeds. Comparison of the dosage in r in the X-ray experiment previously described with T5 or T7 shows that the P$^{32}$ treated seeds received during the 5 days' soaking period about twice that of the X-rayed seeds.

The differences in growth of treated seeds in the same culture is believed to be due to differences in the amount of P$^{32}$ absorbed. Individual seeds after soaking gave different readings with a portable beta radiation monitor. Previous work by Gustafsson et al. (1950) showed that barley kernels soaked in a similar manner varied widely in their intake of P$^{32}$.

Somatic changes in $R_1$ and $R_2$

The nontreated and treated seeds, some of which had germinated, were planted about 2 inches apart in flats in the greenhouse. In some $R_1$ plants the cotyledons were narrower than usual, slightly distorted, and abnormally pale green. Most of these chlorotic seedlings died in the cotyledon stage and all were sterile. In T5, six weeks after planting, one third of the young plants had twin or triple shoots. Apparently some cells of the original apical meristem were injured by the treatment.

Somatic changes were frequent in which only a portion of the plant was involved. The extent of the change varied from a single leaflet to an entire branch on an

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Figure 1.—Transverse section of fresh stem of chimera R$_2$ plant 27G1. Top, normal pubescent sector with chlorophyll in cortex; bottom nearly glabrous green; right upper side, above large trichome, pubescent albino; left lower side glabrous albino sector.
otherwise normal plant. Chlorophyll variegation was by far the most frequent and consisted of normal and rather dull pale green tissue. In T5, seven of the 34 plants which passed the cotyledon stage were variegated and this condition persisted in plants grown from cuttings of five of them. In T8, 27 of the 49 young seedlings were variegated and in all but five of them the variegation was persistent.

Another somatic change in R1 plants was an almost complete loss of pubescence on part of the plant. In one case both the number of loculi in the ovary and the shape of the leaves was changed on different branches of the same plant.

Somatic changes occurred much less frequently in R2 than in R1. Among 750 R2 plants, only 12 were variegated. Seven of these came from 38 not variegated R1 parents and four from 17 variegated R1 parents. Wholly pale green or non-pubescent branches were sterile.

The most remarkable somatic change was found in R2 plant 54.27.G1, from an apparently normal R1 plant. The leaves were very irregularly variegated with green and white, often distorted and nearly white at the edges. Some of the immature fruits were variegated. The stem and leaves contained green and non-green cortical and mesophyll cells. In addition, well-defined almost non-pubescent areas occurred on the stem and leaves (fig. 1), and occasionally this plant produced a weak and sterile non-pubescent pure green shoot with smaller leaves. No normal leaves occurred. This plant was nearly pollen sterile, but produced seeds from pollination with a control plant. The progeny were non-variegated, but the only plant from selfing was variegated. In the same R2 nearly all 11 plants were greyish-green and 8 were partially or wholly male sterile. No somatic changes were found in the controls. R2 families from three variegated parents contained green and albino seedlings in the ratios 4:2, 10:4, and 15:1. No albinos occurred in R2 from non-variegated parents.

Mutations in R2 of T5

Seeds of the R2 generation from selfing 24 plants from P32-treated seeds, and seeds from variant branches of two of these parent plants were planted, making a total of 55 sowings and 750 R2 plants. No seeds germinated from either the normal or variant branches of one R1 plant, and none from another parent. In one family there were five plants resembling in flesh color of fruit the well known r (yellow flesh) mutant. One of these crossed with r+/r gave one yellow-fleshed and one red-fleshed plant. Probably the new mutant is identical with r. A single plant having tangerine flesh occurred in another R2. It was identical in flesh and flower color with the tangerine mutant (l) in the variety Golden Jubilee and was proved by an allelic test to be at the same locus, but it was virescent. An allelic test and chromatographic separation of the ripe fruit pigments in an adsorbent column indicated identity with a virescent tangerine mutant obtained from Dr. F. O. Holmes, Rockefeller Institute, New York. As only 54 different R1 plants were tested and one of them was probably r+r and another l+l, a high mutation rate in the embryonic cells of the treated seeds is suggested. A mutant resembling a (anthocyanless) appeared in R2 from T7, but an allelic test showed that it is not identical with a.

Besides the two which probably are identical with known mutants, several others
occurred which are believed to be gene mutants. Meiosis was normal in all of them. The most striking mutant had bright-yellow foliage and a pale-yellow corolla and stigma. The hypocotyl contained less anthocyan than normal $a^+$ sibs. It may appropriately be called "aurea" ($au$). Only the oldest leaves turn green, either in the field or in the greenhouse. From selfing aurea, 8 aurea seedlings were obtained; the backcrosses ($au^+ \times au$) $\times au$ and ($au \times au^+$) $\times au$ gave, respectively, 5:9 and 6:6 $au:au^+$. Progenies from reciprocal crosses with $l$ (lutescent) or with the virescent tangerine were all green or wild type. Apparently aurea is a new mutant and viable especially in outcrosses despite its deficiency of green color. It seems to be a good seedling character. Aurea occurred in the same R$_2$ of only 15 plants as the $r$ mutant.

Brachytic mutants occurred in five different R$_2$ families from T5. Two brachytics occurred in one R$_2$, and one of them had more loculi in the ovary than the control. Two brachytic mutants from different R$_2$ families were recessive to the control type, but some brachytics did not breed true. One brachytic mutant occurred among the check plants. A multilocular variant in another R$_2$ proved to be a trisomic, rather like triplo-5 (Rick and Barton 1954).

Other mutant types in R$_2$ differed from the control line in the number of flowers in the inflorescence, leaf shape, male sterility, time of maturity of the fruit, fruit size, and in having blue-green leaves. Some of them, especially those having earlier maturity and male sterility, may have useful practical applications. No mutants were found in the control plants.

**Pollen sterility**

Pollen of fully opened flowers was examined in iodine solution (1 gram KI, 0.3 gram I, and 100 ml H$_2$O). Such preparations must be examined immediately. Grains that were of normal size, round, and light yellow were counted as normal. Immature grains near maturity and defective grains which have failed to mature contain starch grains which stain purple; younger grains tend to collapse in iodine. When counts of normal and empty pollen were needed acetocarmine was used. Both methods probably tend to overestimate the proportion of viable grains.

A nominal scale was used for rating pollen quality. This scale was based on the percentage of normal pollen grains found in three or four flowers taken from different

![Figure 2](image.png)

**Figure 2.**—53.62.24 A. Diakinesis, dissimilar homologues unpaired. B. and C. First anaphase C is far more common than B.
parts of the young plant, as follows: 75–100 percent (++), 50 percent (+), 25 percent (±), and 0 percent (0). Pollen was rated before the amount of fruit set was known. A similar scale was used for rating fruitfulness, beginning with the highest degree (+ +), followed by three intermediate degrees (+, ±, and −), and concluding with no fruit (0). It is clear that in many plants pollen sterility was a major cause of reduction in fruitfulness and fertility, and that they are closely related. Plants having very few fruits usually produced no seeds.

In most cases the pollen was the same for the entire plant, but an occasional R1 plant had two distinct types of pollen. In a few plants two types of chromosome structure and behavior could be shown to account for the difference in pollen fertility. The hairy or more normal-appearing part of one plant, for instance, had an unequal pair of chromosomes and no pollen or fruit (fig. 2) whereas the glabrous portion had ± pollen and normal meiosis and bore two seedless fruits.

In the earlier experiments with P³² the variety used set so little fruit that comparisons of pollen and fruit set were difficult. Nevertheless, the records suggest clearly that in these families, poor pollen and unfruitfulness were associated. Also, T2 with 2.7 microcuries per seed caused less damage to pollen fertility and fruitfulness than T1 with 5.4 microcuries. The correspondence between grade of pollen and grade of fruitfulness in individual plants was very good.

In a second series involving the same variety (table 1, T3 and T4), 17 of the 33 plants recorded from treated seeds had poor pollen and were low in fertility. Four of these plants were wholly male sterile, and the other 13 had less than 12 percent good pollen and extremely few seeds. The control plants were ++, both in pollen and in fruitfulness.

For the later experiments a very fruitful variety, Canary Export, was used. The plants were extraordinarily disease free in 1953 and 1954, and the controls were uniformly fruitful. In T5 the heavy initial dose of 10.8 microcuries per seed had a proportionately great effect on pollen fertility. The results are shown in table 2. T5 reduced pollen fertility and fruitfulness more drastically than T6. In T5, 10 plants were wholly male sterile, and even by late August only one plant in the whole population had a full crop. Three of the plants without fruit in T5 may have been female sterile. In T6 there was apparently enough activity in the residual liquid to affect

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Initial dosage μC per seed</th>
<th>Rating</th>
<th>Date recorded</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0</td>
<td>+ +</td>
<td>7/14/53</td>
</tr>
<tr>
<td>T5</td>
<td>10.8</td>
<td>+ +</td>
<td>8/23/53</td>
</tr>
<tr>
<td>T6</td>
<td>Residual from T5</td>
<td>+ +</td>
<td>7/14/53</td>
</tr>
</tbody>
</table>

*Effect of P³² treatments on pollen fertility and fruitfulness of Canary Export variety of tomato*
TABLE 3

Relation between fruitfulness of R1 parents and pollen fertility and fruitfulness of R2 progeny of Canary Export tomato in T5 – 10.8 μC per seed*

<table>
<thead>
<tr>
<th></th>
<th>Pollen fertility</th>
<th>Fruitfulness</th>
<th>Fruitfulness</th>
<th>Fruitfulness</th>
<th>Fruitfulness</th>
<th>Date of rating</th>
</tr>
</thead>
<tbody>
<tr>
<td>R1 plants</td>
<td></td>
<td>++</td>
<td>+</td>
<td>±</td>
<td>–</td>
<td>0</td>
</tr>
<tr>
<td>Pollen fertility</td>
<td>26</td>
<td>36</td>
<td>23</td>
<td>10</td>
<td>5</td>
<td>7/11/54</td>
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<tr>
<td>Fruitfulness</td>
<td>27</td>
<td>43</td>
<td>6</td>
<td>8</td>
<td>14</td>
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<tr>
<td>Fruitfulness</td>
<td>67</td>
<td>12</td>
<td>10</td>
<td>7</td>
<td>6</td>
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<tr>
<td>Control</td>
<td>Fruitfulness</td>
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<td>3</td>
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<td></td>
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<td>0</td>
<td></td>
<td></td>
<td>8/24/54</td>
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<tr>
<td>R2 plants</td>
<td></td>
<td>++</td>
<td>+</td>
<td>±</td>
<td>–</td>
<td>0</td>
</tr>
<tr>
<td>Pollen fertility</td>
<td>6</td>
<td>15</td>
<td>31</td>
<td>16</td>
<td>10</td>
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<td>12</td>
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<tr>
<td>Fruitfulness</td>
<td>28</td>
<td>17</td>
<td>13</td>
<td>6</td>
<td>8</td>
<td>8/30/54</td>
</tr>
</tbody>
</table>

* For description of rating scale see text pages 580 and 581.

pollen fertility materially. Nontreated populations of Canary Export were highly fruitful.

In R2 after T5 none of the seventeen populations were entirely ++ in fruitfulness. Seven populations contained from 1 to 5 plants with no good pollen and no fruit. Six others contained 1 or 2 plants with poor pollen and extremely few fruits. One population from a ++ parent had one sterile mutant and two plants with greatly reduced fruitfulness.

The data in table 3 show that 66 percent of all plants from ++ or + parents, and only 39 percent from ± or – parents, had a full or ++ crop of fruit by August 30. A wide range of fertility grades was found in most families, and attainment of the ++ grade was much slower than in the controls.

Cytological irregularities in R1 and R2

It has not always been possible to find cytological abnormalities to account for unfruitfulness in either R1 or R2. Unfruitful plants with few buds, few p.m.c., green anthers in mature flowers, or abnormal flowers with or without pollen mother cells have been found in R2. The meiotic process may appear to be normal although development of the plant is affected.

P.m.c. of the R1 plants with poor pollen were examined in orcein. The data in table 4 show that translocations occurred more frequently than inversions and that plants with fragments were rare. All R1 plants had 24 chromosomes.

Segregation of the members of an unequal pair of chromosomes in the parent gave rise in R2 to a male sterile plant with 11 normal pairs and one exceptionally small pair (fig. 6) and another plant with an exceptionally large pair. A variegated plant in the same family had 11 normal pairs and a trivalent with two normal-sized and one smaller extra chromosome (fig. 5). Male sterility was apparently due to a homozygous deficiency. One male sterile R1 plant was trisomic (fig. 8).
TABLE 4

The effect of irradiation on chromosomes in pollen mother cells (p.m.c.) and on pollen of tomato plants in R₁

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Initial dose μC per seed</th>
<th>Number of plants</th>
</tr>
</thead>
<tbody>
<tr>
<td>X-ray (wet)</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>T3</td>
<td>5.1</td>
<td></td>
</tr>
<tr>
<td>T4</td>
<td>5.1</td>
<td></td>
</tr>
<tr>
<td>T5</td>
<td>10.8</td>
<td></td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>P.m.c. few and in masses in prophase</th>
<th>Frequent non-reduction</th>
<th>Ring of four</th>
<th>Ring of six or two chains of three</th>
<th>Elongation and stickiness bridges</th>
<th>Inversion bridges with fragments</th>
<th>No good mature pollen</th>
<th>12 II and fragment</th>
<th>Two trivalent like fragments</th>
</tr>
</thead>
<tbody>
<tr>
<td>X-ray (wet)</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>T3</td>
<td>4</td>
<td>6</td>
<td>3</td>
<td>5</td>
<td>1</td>
<td>3</td>
<td>1</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>T4</td>
<td>5</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>—</td>
<td>2</td>
<td>1</td>
<td>—</td>
</tr>
<tr>
<td>T5</td>
<td>6</td>
<td>6</td>
<td>2</td>
<td>6*</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>7</td>
<td>1</td>
</tr>
</tbody>
</table>

* One plant had two rings of four.

In another R₂ family from a parent with a slightly unequal pair of chromosomes, four abnormal plants occurred. Three of these were male sterile. Two of the male sterile plants had few normal buds and few p.m.c. and one plant was minute.

One R₂ plant, 54.27.G1 as already noted, was a mosaic of green, white, pubescent, and non-pubescent tissue (fig. 1). It was nearly male sterile but female fertile. Neither the parent plant nor the sibs in R₂ were variegated, and all sibs had 12 pairs of chromosomes. The mosaic R₃ plant has a small fragment which is often free, and two trivalents in meiosis (fig. 4A and 4B). Variegation may be due to abnormal distribution of the small fragment in the somatic cells. Bridges which occur in p.m.c. of the mosaic plant may be either simple or complex (fig. 4C), their form depending on whether the chromosome with the inversion was in a pair or a trivalent.

In another R₂ family, a variegated mutant had poorly paired chromosomes, frequent non-reduction, and the p.m.c. tended to remain in masses. Another plant in this

![Figure 3](image-url)

**Figure 3.**—54.23. A. Ring from parent of 54.23. (Only one ring was found.) B. Typical diakinesis in 54.23.2. C. Pachytene from 54.23.3 which had the same ring as plant 2. D-G. Two types of rings and pachytene figures from plant 6. Note that pachytenes C and E and rings in A, B, and F are alike.
FIGURE 4.—27G1 A. Two trivalents and a fragment at diakinesis. B. The fragment in a trivalent. C. Bridges at first anaphase.

FIGURE 5.—54.36.6. Trivalent at diakinesis.

FIGURE 6.—54.35.3. The small pair is at the top of the drawing in both A and B.

FIGURE 7.—54.43.12. A. Trivalent at diakinesis. B. Pair and single chromosome at pachytene.

FIGURE 8.—52.130.3. A. Trivalent at first metaphase, involving two very large and one small dividing chromosome. B. Trivalent at pachytene.
family was trisomic, probably triplo-5 (fig. 7). This indicates that failure of normal pairing was also present in the parent.

In three plants out of 15 in another R₂ family, the four microspores of the tetrad failed to separate and no normal pollen developed. This condition is probably caused by a recessive gene.

R₂ from an unfruitful (+−) plant with one ring of four chromosomes consisted of seven plants all of which had the same type of ring of four and unfruitfulness. Another R₂ family from a parent with one ring of four chromosomes consisted of three plants with one ring of four similar to the ring found in the parent (fig. 3A and C), one male sterile plant with two rings of four (fig. 3D–G), and two plants with normal pairing. One of these was very fruitful, the other male sterile. The latter was crossed with a normal. F₁, selfed, gave some plants with one and others with two rings of four.

**DISCUSSION**

An important difference between the X-ray and the P³² treatments was in duration. The seeds were exposed to X-ray for 33 minutes, whereas the P³² acted during the soaking period and until the absorbed fraction had completely disintegrated, a period of about six weeks. Nevertheless, these methods gave very similar types of chromosome changes. Table 4 shows that the proportion of each type of aberration which occurred in meiosis was also very similar after X-ray and P³² treatment. There was much male sterility after both types of treatment and well known mutants were induced.

X-ray treatment of seeds which had been wet for two days was much more effective than treatment of dry seed. This agrees with the results of Sax and Brumfield (1943). It appears that hydrated cells are much more sensitive to irradiation than dormant cells.

Chlorophyll variegation was frequent in R₁ with the P³² treatment and usually involved a small part of the plant. Probably this is due to the large number of cell divisions and advanced stage of development of some cells during the relatively long exposure to P³².

The most common immediate effect of P³² was variegation in green color. When whole branches were chlorotic, they were sterile. Possibly in plants which escaped with little or no apparent plastid injury, delayed cell division or development was a factor. Ehrenberg and Nybom (1954) reported the prevalence in R₁ and R₂ of chlorophyll mutations in barley exposed to ionizing radiations. In tomatoes only a few variegated plants occurred in R₂.

With P³² the changes in chlorophyll and pubescence in R₁ and R₂ may be direct and permanent effects of radiation on the plastids and somatic cells. Similar somatic changes accompanied by sterility have occasionally been reported on nontreated plants. The albino seedlings in R₂ from variegated plants may simply have received injured plastids. Some recessive gene mutations that affect the chlorophyll, also a dominant H and a recessive hl (Butler 1952), that affect pubescence, do not cause sterility.

Many young seedlings in R₁ of T5 had two and a few had more than two phenotypically different parts. Due to death of the chlorotic part or overgrowth of more normal tissues, only 3 mature plants had two distinct phenotypes. In each case,
these two parts differed in pollen fertility. Male sterility was obviously due to segregation of a very unequal pair of chromosomes in one case. It seems probable that cells with large deficiencies or much fragmentation are eliminated at an early stage.

Triploids and male steriles are the most frequent abnormalities found in untreated cultures of tomatoes. Haploids, tetraploids, trisomics and deficiencies occur rarely.

After X-ray and P32 treatment, translocation was by far the commonest chromosomal change observed in meiosis of R1 plants. Both Gottschalk (1951) and Barton (1954) report numerous translocations after X-ray treatment of tomato pollen mother cells or pollen. In our material, segmental interchange occurred between two, three, or four non-homologous chromosomes giving rise, respectively, to one ring of four, two chains of three or a chain of 6, or two rings of four in meiosis.

In R2 the same types of chromosome aberration that were found in R1 recur, and in addition trisomics, deficient trisomics, and homozygous deficiencies are found. One would expect to find occasional trisomics as long as weakness of pairing continues to exist in the line. Deficiencies would recur because plants with an unequal pair may be phenotypically normal. The latter have much nonviable pollen, but some of them can produce a fairly normal late crop of fruit.

In R1 and R2 after T5, the frequent plastid changes in some populations may have caused physiological disturbances which result in such conditions as (a) few p.m.c. that remain in masses, (b) greenish, browning, or separated anthers and (c) few flowers, and thus, might be responsible for some of the reduction in fruitfulness. Even some of the periodical nondisjunction might be due to lack of proper nourishment or to absence of some hormones during a critical time. However, this is certainly not always the cause, since in some plants in both R1 and R2 nondisjunction was clearly due to an obvious size difference in one pair. Probably many such differences were too small to be detected at the later stages of meiosis.

The R2 data suggest an increased gene mutation rate caused by beta radiation of the seeds. In one R1 plant from T5, gene mutations occurred, both to r (yellow flesh) in linkage group II and to aurea (au). The mutations r and t, rarely occur spontaneously. The virescence of the tangerine mutant suggests the possibility that two loci are affected. The tangerine t mutant, first listed by the W. Atlee Burpee Seed Company about 1940, is non-virescent, but another almost equally virescent tangerine type is known. The five yellow fleshed (rr) mutant plants were less fruitful than the ten red-fleshed sibs. The r mutation probably is much older than tangerine and has recurred in the Pearson variety, according to Rick (1947). He estimates (Rick 1945) a normal gametic mutation rate of 20 per 100,000 for genes affecting unfruitfulness. The wiry mutation which appeared in R2 from X-ray treatment seems to occur more frequently, but the allelism of the various wiry mutants is not known. Another probable gene mutant anthocyanless occurred in R2. It is reasonable to assume that small deficiencies that hardly affect chromosome pairing account for some of the variations.

**SUMMARY**

1. Treatment with 10,000r X-rays for 33 minutes, or soaking seeds in P32 solution, caused formation of unequal pairs, segmental interchange, weakened pairing, inver-
Seed Treatment Effects in Tomato

1. Seed treatment effects in meiosis and elongation and stickiness in meiosis of R₁ plants from treated seeds of tomatoes.

2. In R₂ the abnormalities observed in R₁ again occurred, and in addition there were trisomics, deficiency trisomics, homozygous deficient, and duplications.

3. Seedlings in R₁ after P³² treatment often had twin or triple shoots and were variegated or occasionally non-pubescent. Most mature plants had relatively normal growth, but some showed somatic changes and consisted of two or more phenotypically and cytologically different parts.

4. In R₂ from P³² treatment, a small proportion of seedlings and mature plants were variegated, pale grey-green, or albino. One R₁ family consisted of pale-green plants of reduced fertility, and one slow-growing plant which was a complete mosaic of white, green, glabrous, and hairy tissues. Possibly this may be explained by the irregular distribution of a small fragment.

5. From the X-ray treatment, a mutant resembling wiry w was obtained which behaved as a simple recessive.

6. In R₂ from beta radiation of P³², yellow (r) and tangerine (t) flesh color of fruit gene mutants occurred, also aurea, anthocyanless, male sterile, brachytic, and other mutants of which the genetic nature is unknown.

7. Both X-ray and P³² caused much pollen sterility in R₁ and eight R₂ families segregated for complete pollen abortion. Some of the sterility is clearly due to cytological causes; in other cases it seems to be genetic. In most of the other R₂ families fertility was much reduced.

8. An initial dosage of 10.8 microcuries per seed and 5 days' soaking did not prevent germination, but retarded plant growth and drastically affected the plastids, chromosomes, and genes. It probably is close to the lethal dose for tomato seeds.

Acknowledgments

The authors are indebted to DRS. F. M. Turrell, J. W. Cameron, R. K. Soost, and Mr. John Weber for help in measuring and applying the ionizing agents, and to Mr. C. D. McCarty for the chromatography.

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