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Effects of Chronic Irradiation Upon a Self-Incompatible Clone of *Lycopersicum peruvianum* *

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Summary. Flovering plants from a self-incompatible clone of *Lycopersicum peruvianum* were exposed during 90 days to different dose-rates of gamma-rays ranging from 2 to 17 rad per hour. Irradiation was continuous, except for a daily interruption of 7 hours.

Whereas irradiation at dose-rates higher than 7.50 rad per hour seriously inhibited bud formation and floral development, chronic exposure at dose-rates ranging from 3 to 7 rad/hour increased the number of seeds per plant. This stimulation is not due to a higher number of seeds per fruit but to a very significant rise in the number of fruits per plant. As irradiation treatment did not significantly increase the number of flowers per plant and did not appear to stimulate pollen tube germination in the styles, it is concluded that the recorded increase in fruit-setting essentially resulted from a radio-induced inhibition of the processes which control floral abscission in the absence of cross-pollination.

Three plants with reduced self-incompatibility and one completely parthenocarpic individual were observed in the M_2 progeny but no evidence was obtained that irradiation could induce a permanent type of self-compatibility in *L. peruvianum*.

Introduction

It has been shown by several authors that irradiation could constitute, in certain cases, a useful tool for breaking down reproductive barriers between species (TANAKA, 1957; SWAMINATHAN and MURTY, 1959; DAVIES and WALL, 1961) and for inducing self-compatibility in cross-pollinated plants. Concerning this last effect, the results available in the literature clearly indicate that radio-induced modifications in self-incompatibility essentially derive from mutations of the incompatibility genes involved (LEWIS, 1949, 1951; LEWIS and CROWE, 1954; PANDEY, 1959; LINSKENS, SCHRAUWEN and VAN DEN DONK, 1960; SHARMA and BOYES, 1961). In some instances, however, irradiation has been observed to induce a temporary breakdown of the incompatibility reaction which can be ascribed to unstable cytological changes in the pollen (LEWIS, 1951; BREWBAKER and NATARAJAN, 1960; BREWBAKER and EMERY, 1960; PANDEY, 1963) or to a direct alteration of the inhibitory substances in incompatible styles (LINSKENS, SCHRAUWEN and VAN DEN DONK, 1960; KUMAR and HECHT 1965).

The main purpose of the present article is to describe a radio-induced increase in seed-set per plant which was observed during chronic irradiation of a self-incompatible clone of *Lycopersicum peruvianum* and which does not appear to be related to any of the phenomena described above but to a direct effect of irradiation upon floral abscission processes.

Material and Methods

Two separate irradiation experiments were performed in the gamma-greenhouse (300 $Cu^{137}Cs$) of the Association during the summer months of 1966 and 1967. In the first experiment, which was primarily designed for inducing genetic mutations for self-compatibility in *L. peruvianum*, 8 plants from a self-incompatible clone (*L. peruvianum* 6006, kindly provided by the Institute of Horticultural Plant Breeding in Wageningen) were irradiated during 90 days at each of the distances corresponding to 5 pre-established dose-rates (1.90, 3.00, 4.70, 7.50 and 17.30 rad per hour). All plants had reached anthesis for the first cluster stage when irradiation began and irradiation was continuous, except for a daily interruption from 10 AM to 5 PM (7 hours). Temperature was maintained at 23 °C during the day and 17 °C at night. Relative humidity (70%) remained constant throughout the experiment whereas light conditions were those of Dutch summer. In order to decrease the considerable amount of flower dropping which occurs naturally in a clonal population of *L. peruvianum* and which might prevent slow-growing pollen tubes to reach the ovules before floral abscission (DAVIES and WALL, 1961), all control and irradiated plants were sprayed weekly with 25 ppm alpha-naphthalene acetic acid. Pollination was ensured by shaking individually, on alternate days, each open floret with an electric vibrator. The main criterium investigated was the number of seeds produced per plant. This is a compound character which can be decomposed, in four different ways, into several elementary factors (Table 1 and Fig 1). Three months after harvest, the seeds obtained were sown and the resulting progeny screened for self-compatibility.

As the results obtained in this first experiment (1966) had shown that irradiation treatment markedly increased the capacity of the plants to set fruits, a second experiment was made in 1967 to test the reproducibility of this result with the same clone and under similar environmental conditions but in the absence of hormone treatment. The criteria observed were the following: total number of flowers produced per plant, number of clusters, number of fruits and number of seeded fruits per plant at the end of the irradiation period (90 days, as in the first experiment). In addition, pollen tube growth in the style at each of the focal distances tested and in reciprocal crosses between irradiated and non-irradiated plants was examined by means of U.V. microscopy using the technique of MARTIN (1959). Observations were based on 15 styles per treatment.

Results and Discussion

1. Effects of Irradiation Upon Fertility and Compatibility Relationships in the Treated Generation

A. Experiment with Hormone Treatment

It can be seen from table 1, which list the average values recorded for each criterium together with their confidence limits and their F value, that irradiation effects, when statistically reliable, were either depressive or stimulating, depending upon the dose-rate and the criterium considered. Figure 1, which sum-

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marizes the data obtained in terms of the difference between control and irradiated means, shows that all depressions were restricted to the highest dose-rate (17.30 rad/hour) whereas all stimulating effects were confined to the 7.50 and 4.70 rad/hour dose-rates, no significant differences being recorded between control and irradiated values at the two lowest rates.

first experiment, it was not possible to know if the increase in fruit-setting resulted from a larger number of flowers per cluster on the irradiated plants or from a radio-induced inhibition of floral abscission. The possibility existed, furthermore, that the weekly spraying with hormones modified radio-sensitivities and interacted with irradiation effects. These aspects

Table 1. *Fertility relationships in a clonal population of self incompatible L. peruvianum exposed to chronic irradiation of gamma-rays (experiment of 1966)*

Average number of — Rad/hour	0.00	1.90	3.00	4.70	7.50	17.30	F calculated	C.L. 95%
Clusters per plant	5.38	5.38	5.63	6.75	6.50	2.50^D	4.08**	2.27
Fructiferous clusters per plant	3.88	2.88	5.13	4.75	3.38	0.88^D	4.19**	1.95
Fruits per plant	25.50	26.00	32.50	63.30^S	57.60^S	4.50	7.15**	24.90
Fruits per cluster(1)	3.84	4.45	6.16	9.52^S	8.83^S	2.05	6.12**	3.57
Fruits per fructiferous cluster(1)	6.73	7.60	9.67	12.21	17.94^S	4.38	8.40**	5.03
Seeded fruits per plant	3.88	2.25	3.13	2.75	8.50^S	0.38^D	7.08**	3.10
Seeded fruits per cluster(1)	0.71	0.48	0.59	0.38	1.36	0.10	4.20**	0.63
Seeded fruits per fructiferous cluster(1)	1.09	0.89	0.78	0.51	2.61^S	0.31^D	11.71**	0.73
Seeds per plant	7.00	7.60	15.60	13.10	21.50^S	0.40	2.38	14.50
Seeds per cluster	1.41	2.06	3.01	1.58	3.60	0.10	2.21	2.58
Seeds per fructiferous cluster(1)	1.78	4.14	3.66	2.53	6.35^S	0.31	2.03	4.44
Seeds per fruit(1)	0.61	0.67	0.48	0.19	0.35	0.04	1.11	0.70
Seeds per seeded fruit(1)	1.55	4.05	4.34	3.37	2.22	1.00	1.58	3.31

F (table) 95% = 2.44 ** = significant at the 99% level S = significantly stimulated
F (table) 99% = 3.49 D = significantly depressed

(1) The value listed for each compound criterium represents the average product of the elementary factors recorded for each plant. Because of plant to plant fluctuations this average product necessarily differs from the product of the averages calculated for each elementary factor.

The depressive effects were statistically significant for four criteria, namely, number of clusters per plant, number of fructiferous clusters, seeded fruits per fructiferous cluster and number of seeded fruits per plant. In all these cases, depression was essentially due to the fact that irradiation at a dose-rate of 17.30 rad/hour prevented cluster formation and subsequent flowering and fruiting processes.

At dose-rates of 7.50 rad/hour and lower, depressive effects were no longer observed and were often replaced by significant increases over control values. The deviations are particularly interesting in the case of seed-set per plant (Table 1) as they appear to indicate a radio-induced increase in self-compatibility which reaches statistical significance at 7.50 rad/hour. However, it can be seen from Figure 1 that this stimulation effect does not result from a rise in seed-set per fruit but from a very significant increase in the number of fruits and of seeded fruits per cluster. Such a stimulatory action of irradiation treatment upon fruit formation appears rather remarkable, especially if one considers the detrimental effects of the treatments upon pollen fertility (Table 2) and if one takes into account the fact that irradiation at similar dose-rates is known to inhibit rather severely the fruit-setting capacity of self-compatible species of *Lycopersicum* (YAMAKAWA and SPARROW, 1965; DE NETTANCOURT and CONTANT, 1966). As the number of flowers per cluster had not been recorded in the

Table 2. *Irradiation effects on pollen stainability*
(Counts were based on 250 grains per flower and 3 flowers per plant. All staining tests were carried out with fast-green in lacto-propionic acid)

Rad/hour	0.00	1.90	3.00	4.70	7.50
% non stainable pollen	4.58	5.83	6.49	11.62	33.65

were investigated in the course of the second irradiation experiment.

B. Experiment without Hormone Treatment

The results obtained in this second experiment are compiled in Table 3 and Table 4. From a comparison of the data in Tables 1 and 3, it can be seen that the irradiation response of the *peruvianum* clone did not vary very much from one year to the other. Due to a possible effect of the hormone treatment or to a more rapid growth-rate in 1966, the general radiotolerance of the plants was greater in the first experiment whereas seed-set per plant and seed-set per fruit were definitely higher in 1967 than in 1966. This increase in self-compatibility can possibly be attributed to a reduction in vigor or to an aging effect (MCGUIRE and RICK, 1954) in the *peruvianum* clone. Yet, the trends in response were the same for all criteria investigated and the absence of hormone treatment in the 1967 experiment did not modify the stimulating effects of irradiation upon the number of fruits and of seeded fruits per plant (Table 3). It is obvious, furthermore, that the observed increases in fruit-set do not result from a larger number of flowers per plant but from an increase in the probability that a flower shall form a fruit. In contrast, the stimulating effect of irradiation upon the number of seeded fruits is solely due to the total increment in the number of fruits and does not represent any radio-induced increase in the probability that a fruit should set seeds. This probability is even significantly reduced at the dose rate of 7.50 rad/hour (Table 3).

It is difficult to explain the stimulating effect of irradiation upon fruit-setting. From Table 4 it can be

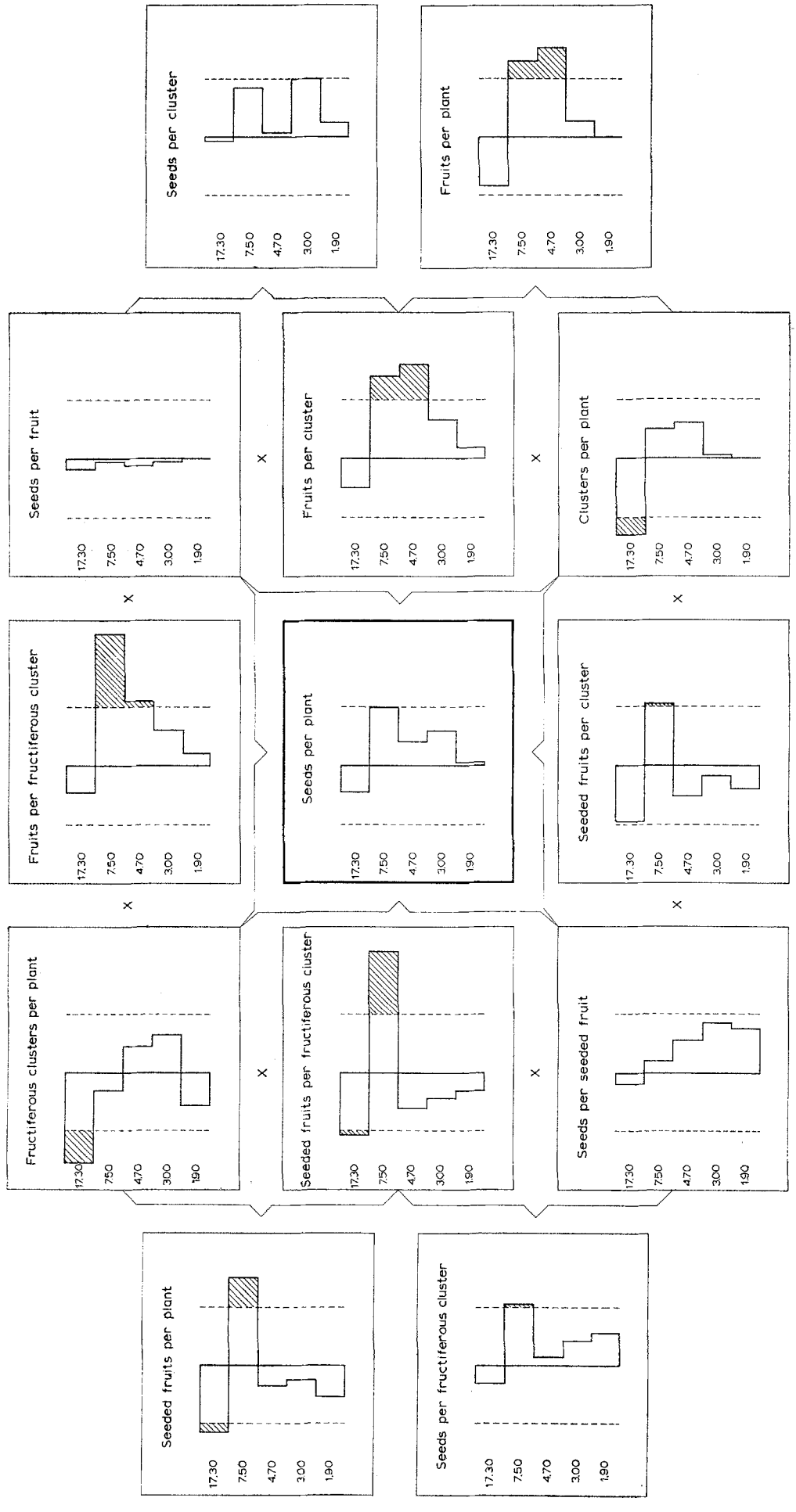

Fig. 1. Deviations from control means and 95% confidence limits (dotted lines) for the 13 criteria examined in the clonal population of *L. peruvianum* after chronic exposure to 17.30, 7.50, 4.70, 3.00 and 1.90 rad/hour. — For each criterium, negative deviations (depression) are on the left of the axis whereas positive differences (stimulation) appear on the right. The X signs and the accolades indicate the contribution of each factor to seed-set per plant

Table 3. *Effects of chronic irradiation upon flower production and fruit formation in a clonal population of L. peruvianum which was not sprayed with alpha-naphthalene acetic acid* (experiment of 1967)

Criterium / Rad/hour	0.00	1.90	3.00	4.70	7.50	*F* calculated	C.L. 95%
Flowers per plant	71.00	84.00	92.50	96.00	98.75	1.31	33.79
Fruits per plant	5.25	20.33	22.00	29.75	51.25^S	5.16**	25.51
Seeded fruits per plant	3.00	11.33^S	11.25^S	14.00^S	4.75	5.39**	6.99
Probability that a flower forms a fruit	6.60	23.50	25.50	31.60	53.20^S	4.45**	27.51
Probability that a fruit sets seeds	59.30	74.40	55.00	57.20	8.90^D	4.44**	41.11

F (table) 95% = 3.11 * = significant at the 95% level S = significantly stimulated
F (table) 99% = 5.03 ** = significant at the 99% level D = significantly depressed

seen that irradiation slightly enhanced pollen tube growth in incompatible styles. The recorded values are, however, very much smaller than those obtained in the case of compatible crosses and do not deviate significantly from the control data. It is therefore probable that the observed enhancement in fruit-setting was not the consequence of an improved pollen tube growth but resulted from a direct action of irradiation upon the physiological processes regulating flower abscission in the absence of cross-pollination.

Table 4. *Average number of pollen tubes having reached at least one third of the style length 24 hours after pollination.* (The dose-rates at which were exposed the pollen donors and the female plants are expressed in rad per hour). $F = 13.16$, 95% confidence limits = 7.7

♀ / ♂	0.00	1.90	3.60	4.70	7.50	CC*
0.00	0.3	0.9	2.1	0.9	0.8	37.14
1.90	0.6	4.8				
3.60	0.4		0.6			
4.70	1.0			1.4		
7.50	1.1				0.5	

* CC = non-irradiated pollen from a *peruvianum* line fully compatible with the clone used in the present study.

2. Analysis of M_2 Population

With the hope of detecting mutant plants with a self-compatible phenotype more than 700 M_2 individuals from the 1966 experiment and from a preliminary experiment carried out in the spring of 1966 were screened for their capacity to set seeds under isolated conditions. All investigations yielded the same results as those reported by DAVIES and WALL (1961) and no evidence was obtained that irradiation treatment could induce a permanent type of self-compatibility similar to the one present in *L. esculentum*. Yet, three M_2 plants were observed to set fruits sporadically and to produce an average of 10 to 30 seeds per seeded fruit. The results were however very irregular, varied from cluster to cluster and appeared to depend very much upon the environmental conditions prevailing at the time of flower opening. As it is known (MARTIN, 1961; RICK 1963) that incompatibility in hybrids of *Lycopersicum* may be governed by more than one gene, it is possible that secondary genes are also operating in *L. peruvianum* and slightly modify the effects of the *S* alleles which MCGUIRE and RICK (1954) have found to control self-incompatibility in this species. If this was the case, one could then ascribe the improvement in self-compatibility which was observed in the 3 M_2 plants to recombination or alteration of modifier genes rather than to induced mutations at the *S* locus. Further selection for self-compatibility shall be extended to the M_3 progenies of these 3 M_2 plants.

Another M_2 individual was found to be completely parthenocarpic with more than 95 % of the flowers setting fruits. This plant, which appears to resemble the parthenocarpic clone used by DAVIES and WALL (1961) in their acute irradiation experiments, is being subjected to further testing in a cross-pollination programme.

Zusammenfassung

Blühende Pflanzen eines selbstunverträglichen Klons von *Lycopersicum peruvianum* wurden 90 Tage hindurch verschiedenen Dosen von Gammastrahlen ausgesetzt (2 bis 17 rad/h). Die Bestrahlungsdauer betrug täglich sieben Stunden.

Während Bestrahlungen in Dosierungen von mehr als 7,5 rad/h die Knospenbildung und Blütenentwicklung stark hemmten, steigerte eine Dauerbestrahlung mit 3 bis 7 rad/h die Samenzahl je Pflanze. Diese Erscheinung beruht nicht auf einer Zunahme der Samenzahl der einzelnen Früchte, sondern auf einer hochsignifikanten Steigerung der Fruchtzahl der einzelnen Pflanzen. Da durch die Behandlung die Blütenzahl nicht signifikant zunimmt und das Einwachsen des Pollens in den Griffel nicht gefördert wird, ist anzunehmen, daß die festgestellte Zunahme des Fruchtansatzes im wesentlichen auf eine strahleninduzierte Hemmung des Blütenverlustes bei Ausbleiben der Fremdbefruchtung zurückzuführen ist.

Drei Pflanzen mit herabgesetzter Selbstunverträglichkeit und ein vollständig parthenokarpes Exemplar wurden in der M_2-Generation angetroffen; es wurde kein Grund für die Annahme gefunden, die Bestrahlung könne eine erbliche Selbstverträglichkeit bei *Lycopersicum peruvianum* hervorrufen.

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