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SOME X-RAY EFFECTS IN PETUNIA[1]

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Of 945 *Petunia* plants grown from seed arising from x-irradiated sperms and untreated eggs or from untreated sperms and x-irradiated eggs, 907 reached maturity. Forty-three diploid (n = 7) controls were grown simultaneously. The following classes were represented:

1. *Seed from normal eggs fertilized by sperms from pollen grains receiving 13,200 r to 18,000 r at increments of 600 r (nine groups).*—There were three additional groups of seed arising from pollen treated at 20,000, 22,200, and 25,800 r. Plants from these seeds are referred to hereafter by the dosage number as well as plant number.

2. *Seed derived from treated eggs fertilized by untreated sperms.*—In this class ovaries were treated with 2,400 and 3,000 r. Plants arising from these seeds are referred to hereafter as ♀ 2,400 and ♀ 3,000 r as well as by plant number.

3. *Seed from untreated eggs fertilized by sperms from pollen grains treated prior to anthesis when the anthers were turgid.*—Plants from such seeds are referred to as Anther 5,400 or Anther 6,600 as well as by plant number.

Of the 945 plants, 206 showed effects of x-ray treatment; 38 of these died during the following growing season presumably because of such treatment. There was no damage apparent in 739 plants, but an effort was made to determine whether or not these phenotypically normal individuals were affected.

Methods.—Technical factors for irradiation were: target distance 15 cm., filter ½ mm. of aluminum, 120 KV, 10 milliamps, H.V.L. = 1.6 mm. of aluminum. Mature pollen (seed Class 1) was treated by putting the pollen in No. 2 gelatin capsules, fastening the capsules on a stage of Scotch tape and centering them carefully under the target. In treating ovaries (Class 2) the flowers were held in place with strips of Scotch tape on the ring stage. Lead shields protected

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all flower parts except the ovary. In order to treat pollen prior to anthesis (Class 3) the anthers were placed in a capsule, irradiated, and then allowed to ripen and shatter within the capsule. A more detailed account of the method is to be found in a previous report (McQuade, 1952).

Cross-sections were from paraffin-embedded leaves stained with safranin and fast green; meiotic preparations were aceto-carmine smears. In making pollen counts, those grains which did not stain were scored as non-viable.

PLANTS WITH VISIBLE EFFECTS OF X-RAY TREATMENT

Phenotypes.—Among the abnormal phenotypes certain characters appeared rather frequently:

Leaf.—
1. Surface wrinkled
2. Surface with light spots (pl. 28, fig. 1)
3. Color pale to dark green, the paleness often associated with thinness in cross-section
4. Margin lobed (pl. 28, fig. 2)
5. Outline elliptical to suborbicular (pls. 28 and 29)
6. Margin involute (pl. 28, fig. 3); margin revolute (pl. 30, fig. 5)
7. Variable petiole length, the sessile condition being often associated with the spatulate condition

Stem.—
1. Stiff, strongly upright
2. Thin, soft, weak
3. Fusion between a shoot and pedicel
4. Fasciation (pl. 29, fig. 5)

Flower.—
1. Corolla with numerous white spots (pl. 29, fig. 6)
2. Corolla with spots of various colors including white and pink through purple (pl. 28, fig. 1)
3. Distortion of corolla, corolla-tube, calyx, stamens, pistil (pl. 29, fig. 4)
4. Warty protuberances over corolla
5. Failure to flower

Habit.—
1. Miniature
2. Abundant weak leaders (pl. 29, fig. 3)
3. Tall strong habit associated with strong stem
4. Globose, stunted (pl. 29, fig. 2)
5. Recumbent
6. Rosette

Among the teratological conditions observed were two extreme forms. Plate 29, fig. 1, shows flowers in which calyx members have been superseded by petaloid structures one of which is subtending an anther. In another case, parts of the stigmatic rim developed petaloid or sepaloid structures.

The characters listed were combined in a number of ways. For example, plant 52, 18,000 r, had thin, spotted leaves, weak stems, spotted corollas. Plant 25, 15,600 r, had thick, lobed leaves with light spots and spotted corollas.

Leaf Abnormalities.—Since most of the plants with visible x-ray effects showed leaf abnormalities, attention was given to the morphology in these disorders.

1. Plants 23, 20,000 r, 29, 18,000 r, and others (pl. 30, figs. 1, 2, 3) were characterized by light spots on their leaves. These light areas were found to be thin places and were seen first in material sectioned free-hand. When this was studied in microtomed tissue later, the dark areas were found to contain palisade and spongy parenchymas, and the light or thin spots to consist largely of undifferentiated cells.

2. Plant 26, 25,800 r (pl. 30, figs. 5, 7) had leaves so completely revolute as to be circular in cross-section. The curvature was brought about by a strongly developed upper epidermis opposed by a weak lower epidermis. Mesophyll cells were irregular but tended to be round; the columnar definition of a palisade parenchyma was lacking.

3. Plant 40, 20,000 r (pl. 30, fig. 6) possessed a homogeneous mesophyll the cells of which appeared generally spherical if somewhat irregular in outline. These cells were rather densely packed. Only occasionally was there found a columnar cell reminiscent of a palisade parenchyma and then its long axis was apt to be parallel, rather than perpendicular, to the epidermis.

4. Plant 78, 20,000 r (pl. 30, fig. 4) had a palisade parenchyma which could be identified as such but it represented a squat rather than normal cell type.

Cytological Data.—Cytological data were gathered on thirteen plants with visible radiation effects. These plants represented dosage groups 16,200 r, 17,400 r, 20,000 r, 25,800 r, applied to mature pollen, ovaries treated at 2400 r, and unripe anthers treated with 5,400 r. Where controls showed a normal meiosis these obviously affected individuals were characterized by some of the following disorders:

1. The presence of fragments.
2. A chromosome or chromosomes not taking the customary position on the equatorial plate at metaphase I or II.
3. The presence of an extra chromosome at a pole or the absence of a chromosome at a pole following anaphase I.
4. Pollen tetrad abnormalities in the form of micro-grains, in addition to the four nuclei characteristic of this stage, or fewer than four nuclei at conclusion of telophase II. Occasionally large nuclei were found after telophase II.
5. Disorganization of the metaphase I plate to such an extent that a plate might consist of several clumps of bivalent chromosomes. Often these clumps of bivalents were oriented in different dirctions.

The following cytological observations on five plants give a fair picture of the meiotic disorders generally encountered.

1. Plant 23, 20,000 r, was the first plant in which the leaf spots were investigated (pl. 30, fig. 1). Its flowers were miniature and produced little pollen; when enough pollen was finally gathered for a count, it proved to be 92.67 per cent non-viable.

Apparently normal telophase II cells	18
4 nuclei + 1 micro-nucleus	14
2 nuclei only	4
2 nuclei + 1 micro-nucleus	3
1 nucleus only	2
3 nuclei	2
3 nuclei + 1 micro-nucleus	6
3 nuclei + 2 micro-nuclei	1
	50

Additional pollen tetrad material indicated extensive disorder in which one or more nuclei were malformed and some nuclei appeared diffuse.

2. Plant 37, 20,000 r, possessed leaves with light spots and corollas that were spotted. Its pollen was 48.04 per cent non-staining; when selfed it did not set seed.

Diakinesis cells with 7 bivalent chromosomes	8
Diakinesis cells with 7 bivalents + 1 large fragment	10
	18
Apparently normal anaphase I cells	83
Cells with bridges or bridges + fragments	25
Cells with lagging chromosome or fragment	12
	120

Among these anaphase I cells was one in which 7 chromosomes could be counted at one pole, 6 at the other, with a laggard between.

3. Plant 55, 25,800 r, had leaves which were spotted and mildly lobed. Its corollas were spotted, and one pedicel was partially fused to the shoot from which it arose. Its pollen grains were 98.66 per cent non-staining.

Apparently normal metaphase I cells	9
Cells with 1 bivalent off plate	11
Cells with 2 bivalents off plate	3
Cells with 3 bivalents off plate	1
	24

Other cells were found in which bivalents were arranged in several clumps, some improperly oriented.

Apparently normal anaphase I cells	1
Cells with 1 bivalent between poles	1
	2
Apparently normal telophase II cells	6
Cells with no chromatin at one pole	3
	9

4. Plant 76, ♀ 2,400 r, exhibited spotted leaves and corollas.

Apparently normal metaphase II cells		125
a. 7 chromosomes at one plate, other plate undetermined	13	
b. 7 chromosomes at both plates	2	
c. Chromosome numbers undetermined but no laggards or fragments present	110	
Abnormal metaphase II cells		77
a. 1 chromosome or large fragment between plates or at one side	52	
b. 7 chromosomes on one plate, other uncounted; laggard between plates	1	
c. 7 chromosomes on one plate, 6 on other; laggard between plates	3	
d. 7 chromosomes on one plate, 6 on other; no laggard	1	
e. 8 chromosomes on one plate, 6 on other	2	
f. 8 chromosomes on one plate, other uncounted	3	
g. One bridge or remains of bridge	15	
		202

Apparently normal tetrads	91
Tetrad + 1 micro-nucleus	25
Tetrad + 2 micro-nuclei	2
	118

Distribution of chromosomes is therefore seen to be irregular at telophase II. In one cell, for example, there were two groups of 7 chromosomes, one group of 6, one small undetermined group, and 2 chromosomes at separate points not belonging to any particular groups.

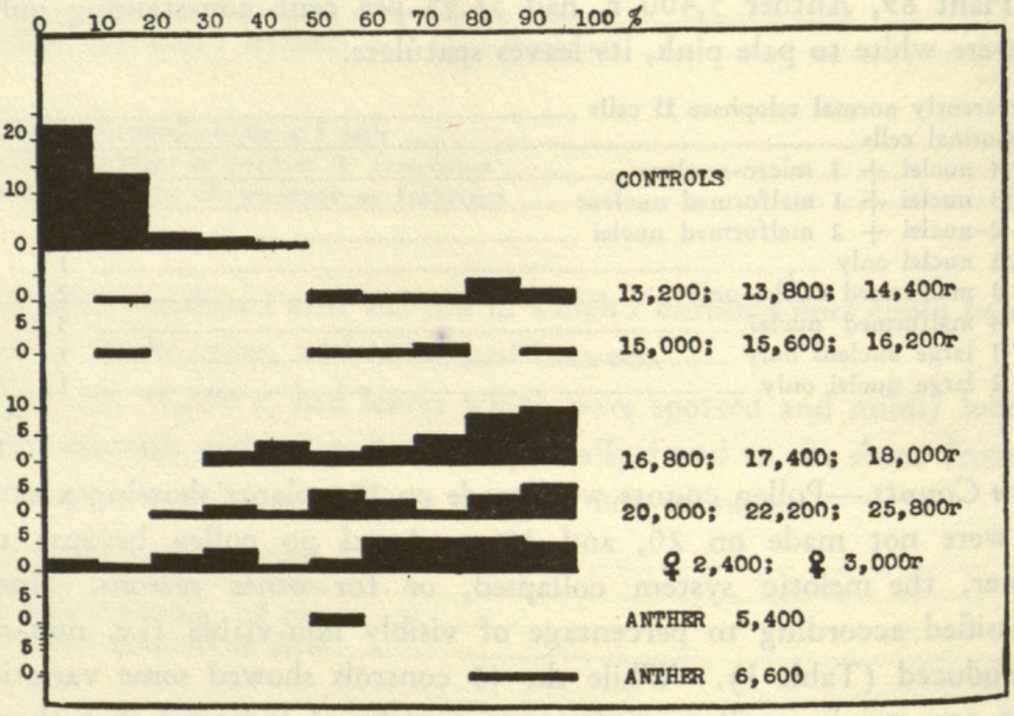
5. Plant 69, Anther 5,400 r, had 56.95 per cent non-staining pollen. Its flowers were white to pale pink, its leaves spatulate.

Apparently normal telophase II cells	64
Abnormal cells	38
4 nuclei + 1 micro-nucleus	1
3 nuclei + 1 malformed nucleus	19
2 nuclei + 2 malformed nuclei	8
3 nuclei only	1
3 malformed nuclei only	2
4 malformed nuclei	5
1 large nucleus only	1
2 large nuclei only	1
	102

Pollen Counts.—Pollen counts were made on 130 plants showing x-ray effects. Counts were not made on 20, and 18 produced no pollen because they did not flower, the meiotic system collapsed, or for other reasons. Individuals were classified according to percentage of visibly non-viable (i.e. non-staining) pollen produced (Table I). While the 43 controls showed some variation with regard to non-staining pollen, a glance at text-fig. 1 indicates that those plants showing x-ray effects tended to have high percentages of non-viable pollen. Since dosage delivered may have varied ± 10 per cent, the class frequencies of non-staining pollen grains were lumped as indicated to produce text-figs. 1 and 2.

TABLE I

Treatment	Number of plants	Plants producing no pollen	Plants in which no counts were made	Classes: per cent non-staining pollen									
				0 –10	10–20	20–30	30–40	40–50	50–60	60–70	70–80	80–90	90–100
Controls													
None	43			23	14	3	2	1					
Plants with visible X-ray effects													
13,200 r	9	1	1		1				1	1		3	1
13,800 r	2										1		1
14,400 r	2								1			1	
15,000 r	3	1	1						1				1
15,600 r	5	1			1				1	1	1		
16,200 r	4	2									1		1
16,800 r	7							1				2	4
17,400 r	16	1	2					2	1	1	2	4	3
18,000 r	21	2	4				2	1	1	2	3	3	3
20,000 r	34	5	6			1	2	1	5	3	1	4	6
22,200 r	1										1		
25,800 r	18	2	3							1	5	2	5
♀ 2,400 r	36	1	3	2	1	3	4	1	2	6	4	5	4
♀ 3,000 r	4										2	1	1
Anther 5,400 r	2								2				
Anther 6,600 r	4	2										1	1
	168	18	20										



Text-fig. 1. Controls and plants showing x-ray effects. Figures at left represent numbers of individuals per class; those at top, per cent of non-staining pollen.

PLANTS SHOWING NO VISIBLE X-RAY EFFECTS

Cytological Data.—Cytological observations were gathered on five plants. Unfortunately no data were gathered in plants where the percentages of non-staining pollen grains were in the high ranges.

1. Plant No. 2, 16,200 r, produced pollen grains of which 47.70 per cent did not stain. Of 27 anaphase I cells studied, none showed disorders. In seven of these cells the distribution of chromosomes was determined to be 7 per pole.

2. Plant No. 3, 16,200 r, had 6.01 per cent non-staining pollen. No disorders were observed in the 49 anaphase I cells studied, and the distribution of chromosomes was determined to be regularly 7 per pole.

3. Plant No. 5, 20,000 r, produced pollen of which 48.0 per cent of the grains were non-staining. Examination of anaphase II cells indicated no disorders, with distribution of chromosomes being 7 per pole.

4. For plant No. 12, 20,000 r, no pollen count was made. Examination of 20 anaphase I cells indicated no abnormalities. In 20 of 23 metaphase II cells, 7 chromosomes ready for division were counted on one plate, while on the other plate the chromosomes could not be counted. In the remaining three cells 7 chromosomes ready for division were counted on both plates. No micro-grains were evident in pollen tetrads.

Pollen Counts.—Table II summarizes the pollen counts for phenotypically normal individuals. Text-fig. 2 indicates that these often had high percentages of non-staining pollen as compared to controls.

TABLE II
PHENOTYPICALLY NORMAL INDIVIDUALS

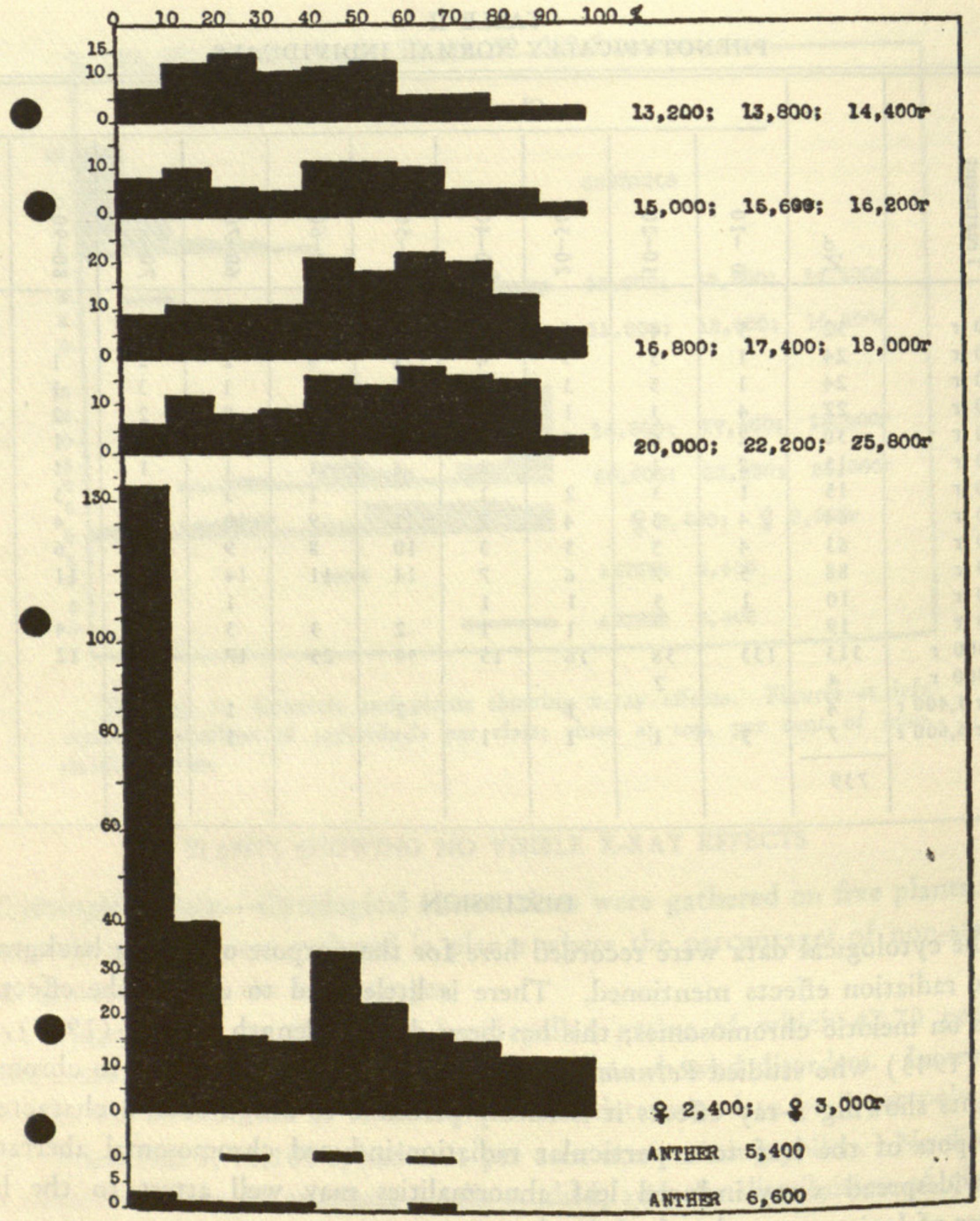
Treatment	No.	Classes: per cent non-staining pollen									
		0–10	10–20	20–30	30–40	40–50	50–60	60–70	70–80	80–90	90–100
13,200 r	32	5	4	6	3	6	6	2			
13,800 r	24	1	3	5	4	1	5	2	2	1	
14,400 r	24	1	5	3	3	4	1	1	3	1	2
15,000 r	27	4	3	1	1	2	4	8	2	2	
15,600 r	30	2	6	5	3	3	6	1	2	1	1
16,200 r	15	2	1		1	6	1	1	1	1	1
16,800 r	15	1	3	2	1		1	3	1	3	
17,400 r	64	4	3	4	7	11	9	10	9	4	3
18,000 r	63	4	5	5	3	10	8	9	10	6	3
20,000 r	88	5	7	6	7	14	11	14	13	11	
22,200 r	10	1	5	1	1			1	1		
25,800 r	19			1	1	2	3	3	2	4	3
♀ 2,400 r	313	133	38	16	15	34	23	17	13	12	12
♀ 3,000 r	4		2						2		
Anther 5,400 r	4			1		2		1			
Anther 6,600 r	7	3	1	1	1			1			
	739										

DISCUSSION

The cytological data were recorded here for the purpose of giving background to the radiation effects mentioned. There is little need to discuss the effects of x-rays on meiotic chromosomes; this has been done at length by Lea (1947), and Rick (1943) who studied *Petunia*. In view of the extensive damage to chromatin in plants showing x-ray effects it is hardly plausible to assign such a character as light spots of the leaf to a particular radiation-induced chromosomal aberration. The widespread x-ray-induced leaf abnormalities may well attest to the large number of loci concerned in leaf development.

It is suggested that in the leaf abnormalities considered here cells often appear undifferentiated rather than differentiated abnormally. In plant No. 40 (pl. 30, fig. 6), the mesophyll cells resemble the undifferentiated cells of tobacco (Avery, 1933) and apple (MacDaniels and Cowart, 1944) near the marginal meristem of the young leaf. In plant No. 78 (pl. 30, fig. 4), the mesophyll reflects somewhat the columnar condition of mesophyll in the young apple leaf during rapid cell division (MacDaniels and Cowart, 1944). In plant No. 26 (pl. 30, figs. 5 and 7), the mesophyll is irregular with some of the upper cells becoming columnar but most of them remaining undifferentiated.

The situation in which a clearly defined epidermis covers an undifferentiated mesophyll (plant 26) is clarified by Avery (1933) who points out that, in tobacco, epidermal cells acquire their characteristic appearance very early but that



Text-fig. 2. Phenotypically normal plants. Figures at left represent numbers of individuals; those at top, per cent of non-staining pollen.

palisade cells do not undergo similar development until the leaf is 4–5 mm. long. Here it seems there has been time enough for some epidermal development before mesophyll development has been halted.

No phenotypic differences were observed between plants grown from seed arising from treated sperms and untreated eggs and plants from seed from untreated sperm and treated eggs.

In general, it appeared that visible radiation effects were associated with a high degree of pollen non-viability although there were some affected plants that had a smaller percentage of non-staining pollen than some with no visible effects. That pollen non-viability could occur independently of visible radiation effects is apparent from text-fig. 2.

It is not suggested that the histograms present a full picture of x-ray effects, since they do not account for the seeds that failed to germinate or individuals that died in the seedling stage. The estimation of pollen non-viability is probably conservative in that pollen grains which stained would not necessarily germinate, or if they germinated might not function properly because of damage of a sort not immediately detectable. For example, plant 37, 20,000 r, with pollen grains of which 48.04 per cent were non-staining did not set seed when selfed; plant 36, 20,000 r, with pollen 64.06 per cent non-staining, set two fruits.

SUMMARY

1. Nine hundred and seven of 945 *Petunia* plants were grown to maturity from seed arising from x-irradiated sperms and untreated eggs or untreated sperms and x-irradiated eggs. Of these, 206 plants showed x-ray effects; 38 died. No effects were apparent in 739 plants.

2. Cytological observations and observations on pollen viability indicated that phenotypic effects of x-ray treatment were usually accompanied by high percentages of pollen non-viability. It was also demonstrated that x-ray treatment often resulted in high percentages of pollen non-viability in plants whose phenotypes were normal.

3. No phenotypic differences were observed in plants in which x-ray effects were brought about through treatment of pollen as opposed to ovaries.

4. The leaf morphology resulting from x-ray treatments described above were considered.

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EXPLANATION OF PLATE 28

1. 20,000 r (Plant 23. See also pl. 30, fig. 1). Light and dark areas on leaf. × about ¼.

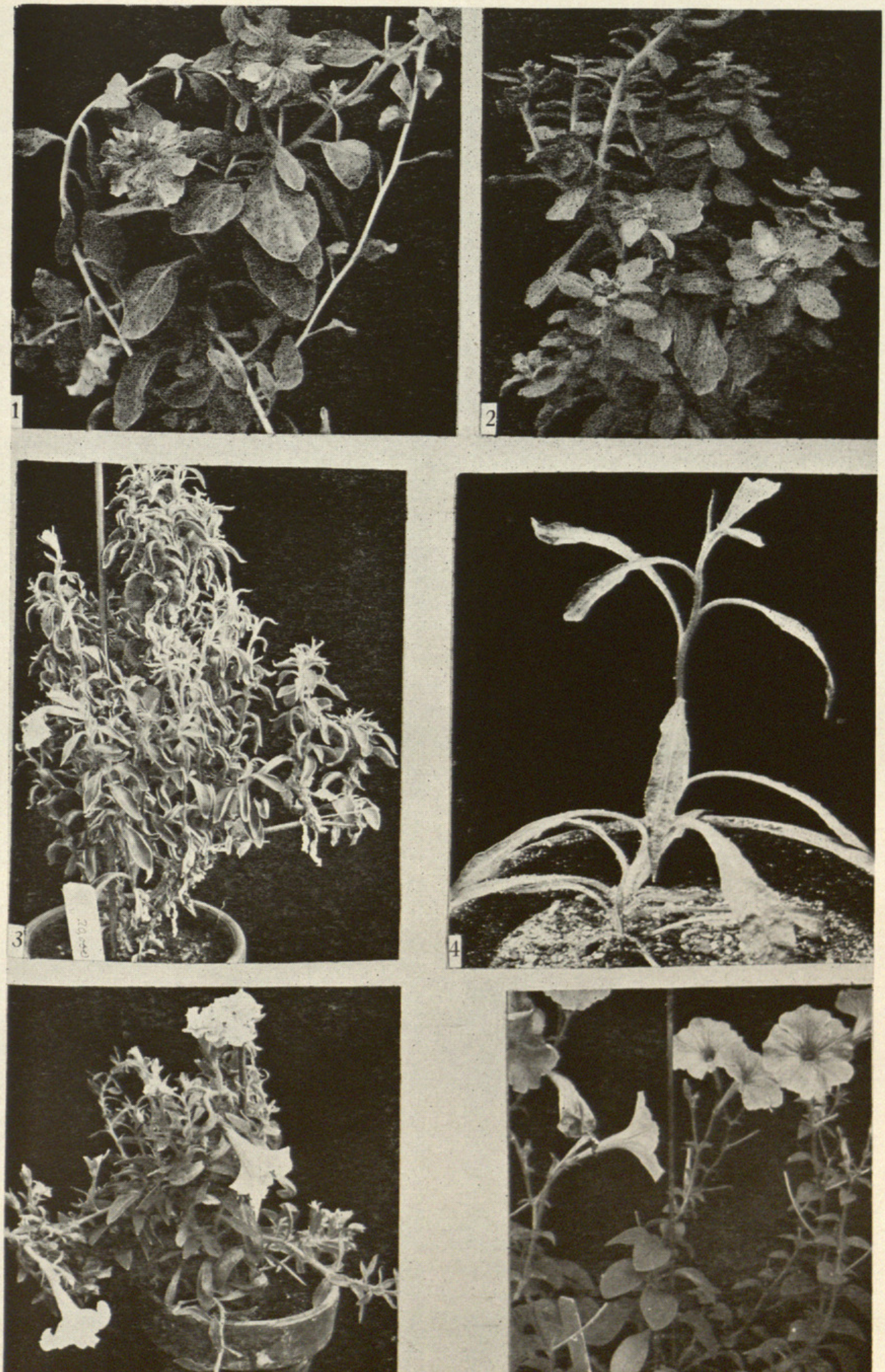
2. 15,600 r (Plant 25). Leaf margins lobed. × about ¼.

3. 20,000 r. Leaf margins involute. × about ¼.

4. 18,000 r. Elliptical leaf. × about ½.

5. 20,000 r. Spatulate leaf. × about ½.

6. Control. × about ¼.



McQUADE—X-RAY EFFECTS IN PETUNIA