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EFFECT OF SOFT AND HARD X-RAY IRRADIATED POLLEN ON POD AND SEED DEVELOPMENT FOLLOWING CROSS-POLLINATION IN *PHASEOLUS VULGARIS*

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ABSTRACT

The freshly opened flowers of *Phaseolus vulgaris* cv. Mexican Black were collected and exposed to 0, 45, 90, 135, 180, 225 Gy of hard X-rays (250 kV, 15mA) and 45, 90, 135, 180, 225, 450, 675 and 900 Gy of soft X-rays (30 kV, 4mA). Irradiated pollen was used to pollinate flowers of cv. Concord to compare the biological effects of soft and hard X-rays based on success in pollination, pod and embryo development. There was negative relationship between hard X-ray exposures and percentage of successful pollination and pod set. Soft X-rays increased pod abscission and decreased seed bearing pods only up to 225 Gy. At higher doses of soft X-rays (450–900 Gy), the trend of damage either reversed or remained unaltered, i.e. similar to that of hard X-rays. The effects of both types of X irradiation were different at the same doses. There was substantial increase in the parthenocarpic pods per successful pollination with soft X-rays. Such pods were seedless and smaller in size than those obtained after selfing or crossing with unirradiated pollen. However, there was no difference in the number of ovules present in both types of pods.

Key words: *Phaseolus vulgaris*, soft and hard X-rays, pollen irradiation, parthenocarpy.

Previous work has shown that exposure of pollen to semilethal doses of ionizing radiations leads to pollen killing [1–5], retardation of pollen tube growth [6, 7], changes in its enzyme activity and biochemical constituents [8], and its capacity to fertilize and promote fruit [9, 10] and seed [11–14] development on the female plants. We have studied the deleterious effects of soft and hard X-rays on pollen manifested in seed and pod development in *Phaseolus vulgaris*.

MATERIALS AND METHODS

The parents chosen for this study were cv. Concord and Mexican Black of *Phaseolus vulgaris*. These genotypes differ in a number of major gene loci. Concord has the recessive characters: white seed coat, small seed, green stem, and white

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flower; while Mexican Black has the corresponding dominant characters: black seed coat, large seed, pigmented stem, and violet flower. The genotypes were grown in a glasshouse under 16 h day length at 17±1°C during day and 12±1°C in night.

Freshly opened flowers of cv. Mexican Black were collected at 8 A.M. and divided in several groups. On each date, flowers were exposed to 0, 45, 90, 135, 180 and 225 Gy of hard X-rays and 45, 90, 135, 180, 225, 450, 675 and 900 Gy of soft X-rays. Hard X-ray treatment was given with Marconi X-ray Machine (250 kV, 15 mA; 0.373 kR/min), while Philips Pw 1212 X-ray Fluorescence Spectrometer (30 kV, 4 mA; 3.03 kR/sec) was used as the source of soft X-rays. Pollen irradiated with each dose was used to pollinate the emasculated flowers of cv. Concord.

In each treatment, the effect of irradiated pollen on pod and embryo development was studied. These included percentage of successful pollinations, pods abscised, pods set, small and seedless pods, seed bearing pods, fertilized ovules, aborted embryos, and normal seeds. The unsuccessfully pollinated flowers withered and dropped within 72 h, while swelling of ovary into pod was taken as an index of successful pollination. Some of the developed pods also abscised within 3–14 days after pollination. At maturity, the remaining pods were classified into seedless and seed bearing pods.

RESULTS

Irradiation of pollen grains with increasing doses of hard X-rays led to proportionate reduction in percentage of successful pollination (Table 1). X irradiation increased embryo abortion and pod abscission, and decreased pod set, number of

Table 1. Effects of hard X irradiation of pollen on pod and embryo development in *P. vulgaris*

X-ray dose (Gy)	Pollinations attempted	Pollinations successful (%)	Pod development: abscised	Pod development: formed	Pod development: seedless	Pod development: seed bearing	Embryo development: fertilized ovules	Embryo development: aborted embryos	Embryo development: seed set
0	103	63.10	11 / 0.17	54 / 0.83	4 / 0.06	50 / 0.77	169 / 2.6	6 / 0.09	164 / 2.51
45	153	49.01	69 / 0.92	6 / 0.08	5 / 0.07	1 / 0.01	1 / 0.01	— / —	1 / 0.01
90	138	23.19	30 / 0.94	2 / 0.06	1 / 0.03	1 / 0.03	3 / 0.09	— / —	3 / 0.009
135	145	11.03	14 / 0.88	2 / 0.02	— / —	2 / 0.01	5 / 0.31	— / —	5 / 0.31
180	131	11.45	13 / 0.87	2 / 0.13	1 / 0.06	1 / 0.06	2 / 0.13	— / —	2 / 0.13
225	156	3.84	6 / 1.00	— / —	— / —	— / —	— / —	— / —	— / —

Above the line—total number; below the line—number per successful pollination.

seed bearing pods, and fertilized ovules, computed per successful pollination. There was no effect on the frequency of small and seedless pods. All the developed pods abscised under the influence of the highest dose. Aborted embryos were observed in the crosses made with unirradiated pollen, but not found in those involving irradiated pollen. The seeds obtained in different treatments were F_1 seeds, as could be inferred from the expression of dominant marker genes of the pollen parent.

Successful pollination was lower than control in the treatment with soft X-rays (Table 2). Percentage of successful pollination started declining at 450 Gy dose, and at 900 Gy dose the effect was comparable to that of 135 Gy hard X-rays. Pod abscission increased and pod set decreased per successful pollination only up to 225 Gy dose. However, the trend reversed after 450 Gy. The proportion of seedless pods per successful pollination increased following treatment with 45 to 225 Gy soft X-rays, but decreased after 450 Gy. The ratio of seed bearing pods decreased with increasing dose. Fertilized ovules and seed set per successful pollination had negative relationship with increasing doses of soft X-rays. In contrast with hard X-rays, aborted embryos were obtained in all the soft X-ray treatments under study. However, there was no relationship between radiation dose and frequency of aborted embryos per successful pollination.

Table 2. Effects of soft X irradiation of pollen on pod and embryo development in *P. vulgaris*

X-ray dose (Gy)	Pollinations attempted	Pollinations successful (%)	Pod development: abscised	formed	seedless	seed bearing	Embryo development: fertilized ovules	aborted embryos	seed set
0	103	63.10	11	54	4	50	169	6	163
			0.17	0.83	0.06	0.77	2.60	0.09	2.51
45	171	36.84	24	39	24	15	33	7	26
			0.38	0.62	0.38	0.24	0.52	0.11	0.41
90	149	40.27	16	44	33	11	18	3	15
			0.27	0.73	0.55	0.18	0.30	0.05	0.25
135	157	45.51	16	55	47	8	15	2	13
			0.22	0.78	0.67	0.11	0.21	0.03	0.18
180	140	52.85	11	64	56	8	13	2	11
			0.15	0.87	0.76	0.11	0.18	0.03	0.15
225	163	58.28	7	88	83	5	7	1	6
			0.07	0.93	0.88	0.05	0.07	0.01	0.06
450	138	28.98	28	12	9	3	7	2	5
			0.70	0.30	0.23	0.07	0.17	0.05	0.12
675	158	17.72	22	6	5	1	4	1	3
			0.79	0.21	0.17	0.04	0.14	0.03	0.11
900	119	10.92	11	2	1	1	3	1	2
			0.85	0.15	0.08	0.07	0.23	0.08	0.15

Above the line—total number; below the line—number per successful pollination.

DISCUSSION

The present study compares the biological effects of pollen irradiation with hard (Table 1) and soft (Table 2) X-rays on pollination, pod and seed development. There was negative relationship between the dose of hard X-rays and percentage of successful pollination. However, the reverse was true for the soft X-ray doses up to 225 Gy. Even the lowest dose of hard X-rays had a conspicuous effect on the development of the remaining pods which remained almost at the same level with further increase in dose. Unlike hard X-rays, the trend was linear with the soft X-ray doses ranging from 45 to 225 Gy. At higher doses of soft X-rays (450–900 Gy), the trend either reversed or remained unchanged, and was similar to that of hard X-rays. The effect of the two types of X irradiation was different even at the same dose.

The most pronounced difference between hard and soft irradiation was in high rate of parthenocarpic development of fruits, which were seedless and smaller than the normal pods (Fig. 1). Such pod set was at the rate of 0.06 per successful pollination in the crosses using unirradiated pollen, which increased from 0.38 at 45 Gy to 0.88 at 225 Gy. Such pods did not contain a single aborted or normal seed, and were 5.53 ± 0.46 and 0.34 ± 0.11 cm in length and diameter, respectively, as compared to 8.80 ± 0.91 and 0.89 ± 0.27 cm in the normal pods. However, there was no difference in the number of ovules formed in both types of pods (Fig. 2). Umatsen and Nishiyama [10] observed high percentage of fruit set in tomato following pollen irradiation with hard X-ray doses of 1 to 10 kR. However, the average size and

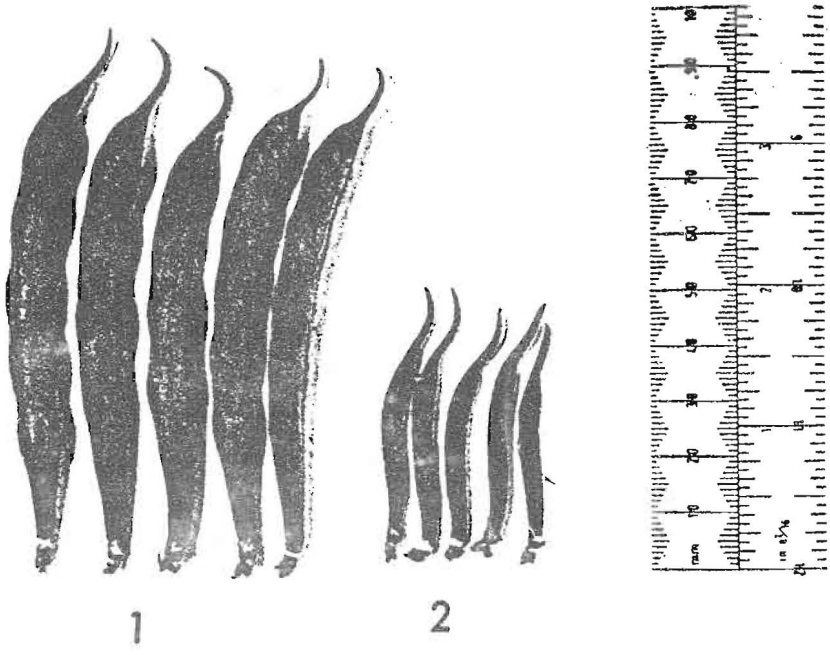

Fig. 1. Control (1), and parthenocarpically developed pods (2).

weight of fruits gradually decreased with increasing dose of X-rays. No seeds formed at 9 kR. Cantan et al. [15] also reported reduction in percentage of normal fruits and number of seeds per fruit in tomato when pollen exposed to 3–5 kR gamma radiation was used. Low level of radiation apparently does not prevent the pollen from effecting fertilization in *Lilium formosanum*, even though seed set percentage decreased [11].

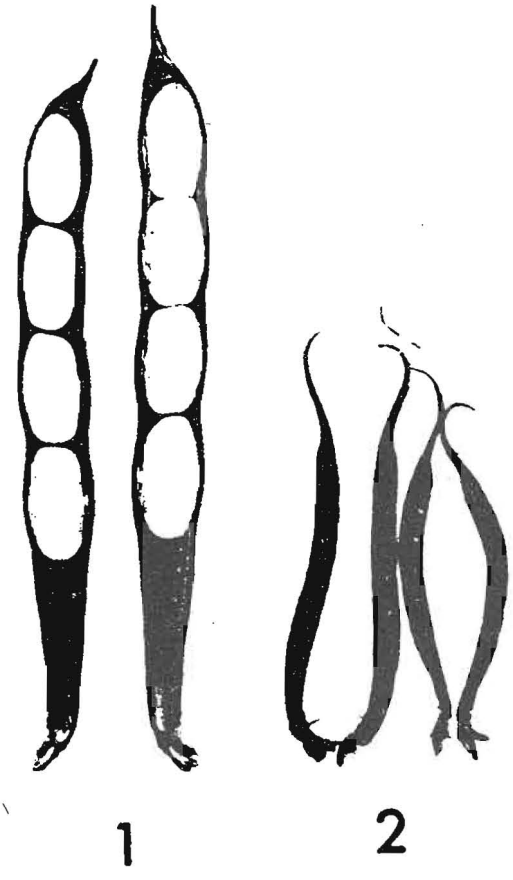

Fig. 2. Well developed seeds in control (1), and absence of seeds in parthenocarpically developed pods (2).

The fertilized pods sometimes abscise or temporarily cease to grow even under conditions of normal growth. Ceasation of growth in such fruits is occasionally induced by adverse environments, such as, high temperature and light [16, 17]. It can also be caused by competition for assimilates with other fruits [18]. As the experiments in this study were conducted under controlled conditions, the possibility

of variation due to environmental effects is ruled out. Parthenocarpic development of pods, similar to those recorded in the present study, has also been observed in the emasculated unpollinated flowers of *Phaseolus vulgaris* (Smartt, unpublished). In order to know the degree of parthenocarpy under natural conditions, 216 and 269 flowers of cv. Concord and Mexican Black, respectively, were emasculated and left unpollinated. In less than 2% cases, small seedless pods, similar to those obtained in the present study, developed. These pods remained green for longer time and matured later than the normal pods. Similar were the characteristics of the seedless smaller pods formed in the crosses made with unirradiated pollen.

Aborted seeds were not obtained in hard X-ray treatments, although these were found in the crosses with soft X-ray irradiated pollen. In maize, UV irradiation of pollen is known to cause embryo abortion [13, 14]. One of the reasons of seed abortion perhaps is fertilization with partly damaged pollen. Kernel abortion is believed to be a result of chromosomal rearrangements in the pollen grain and disruption of the mitotic cycle during embryo and endosperm development after fertilization [1]. The absence of aborted seeds in the treatments with even low doses of hard X-rays suggests that pollen was completely damaged and rendered incapable of fertilization. Nevertheless, even after irradiation with soft and hard X-rays, a few pollen grains escaped damage and were able to effect fertilization, leading to the production of normal seeds. The number of such seeds per successful pollination was higher with soft than with hard X-rays at the same dose.

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