Comparison of the Developmental Response of Diploid and Tetraploid *Phalaris* Following Irradiation of the Dry Seed

V. Germination, survival and length of root and shoot¹

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Radiation and radiomimetic substances have been proved to be a powerful tool in creating variability in plants. Enormous amount of work has already been done in cereals and some legumes, the chief being barley and wheat. The present material i. e. *Phalaris canariensis* and *P. minor* has been selected for the present studies because of its favourable size of chromosomes and number. In the present paper the influence of filtered and unfiltered X-rays on germination, survival and length of root and shoot has been discussed.

Material and methods

Dry dormant seeds of *Phalaris canariensis* Linn. ($2n=12$) and *Phalaris minor* Retz ($4n+2=28$) were irradiated at the M. R. C. Unit, Hammersmith Hospital, London. A detailed description of method of irradiation has already been described by Prasad and Godward (1965, 1969a).

Experimental result and discussion

1. *Germination*: The effects of low and high doses of soft and hard X-rays on the percentage of seed germinating was estimated for the two species of *Phalaris* and are summarised in Table 1.

The Table 1 shows that in the M_1 generation, *P. canariensis* 10 Krad of both types of X-rays had little effect on the percentage of seeds germinating; some reduction was effected by 20 Krad and 30 Krad.

In the *P. minor*, a much greater effect was produced with general reduction in germination at all doses.

The progeny seeds (M_2) of such plants of irradiated M_1 seeds as survived to give offspring, showed the same percentage as the control with the exception of *P. minor* M_1 given 30 Krad where only 40% of M_2 seeds germinated. In *P. canariensis*, no M_1 plants given this dose survived to produce M_2 seeds. This expected difference between the tetraploid (*P. minor*) and diploid (*P. canariensis*) will be referred to again at the time of discussing survival.

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Discussion: In the literature a direct relationship between increased dose and reduced germination is generally reported (Gustafsson 1944, Sparrow and Gunckel 1956, Fujii and Matsumura 1958, Matsumura and Fujii 1958, Fujii and Matsumura 1959 a and b, Matsumura and Nezu 1961, Saric *et al.* 1961, and Bremer-Reinders 1962). However Fujii and Matsumura (1958) using ^{60}Co reported that seeds of certain genera (*Triticum vulgare*, *Zea mays*, *Fagopyrum sagittatum*) showed increased percentages of germination at all doses although death of the seedlings followed at high doses. The relationship between germination and survival of seedlings is of interest; though germination percentage may not be reduced with high doses, survival may be reduced or negligible (Fujii and Matsumura 1958, Singh 1961, Micke 1961,

Table 1. Percentage germination of *Phalaris canariensis* and *P. minor* following irradiation of dry seeds (M_1)

Species	Treatment	Soft X-rays Number of seeds irradi- ated	% of seed germination	Treatment	Hard X-rays Number of seeds irradi- ated	% of germinated
<i>P. canariensis</i> (2n)	Control	200	90	Control	200	63.5
	10 Krad	200	98	10 Krad	800	85.2
	30 Krad	200	66.0* (% of con- trol 66.6)	20 Krad	800	53.0 (% of con- trol 83.4)
<i>P. minor</i> (4n+2)	Control	200	50.0	control	300	50.5
	10 Krad	200	24.7	10 Krad	1000	18.6
	30 Krad	200	17.7	20 Krad	1000	17.9

* All M_1 died at the seedling stage.

M_2 generation (Progeny of M_1 generation above, not further irradiated)

Species	Treatment soft X-rays	Number of seeds for germination	Tested % of seeds germinated
<i>P. canariensis</i> (2n)	Control	200	95.0
	10 Krad	4429	97.3
	30 Krad	no seed produced	no seeds produced
<i>P. minor</i> (4n+2)	Control	300	70.0
	10 Krad	19,708	72.8
	30 Krad	500	40.0

etc.) or there may be no survival (Fujii and Matsumura 1958 in *Allium fistulosum* L., *Brassica Rapa* L. var. *nippo-oleifera*). Our result on *P. canariensis* i. e. high percentage of germination with no survival at maturity, may be compared with these. For example, in *P. canariensis*, our figure of germination 66%, survival 0% at 30 Krad may be compared with Fujii and Matsumura's 1958 observation in *Allium fistulosum* var. *aizu* of germination 58%, survival 0% at 20 Krad. Bremer-Reinders (1962) worked on *P. canariensis* using the diploid species and a colchicine-produced teraplod. His results therefore should be comparable with those found in the present work on *P. canariensis* and the approximate tetraploid *P. minor*. These are as follows:¹ In the tetraploid control shows only a low % germination both in M_1 and M_2 as compared with the diploid. The percentages of germination in the tetraploid

at different doses are confusing compared with those of the diploid. This surprising result is not dissimilar to that of Bremer-Reinder (1962) in the artificial tetraploid. However, the survival of seedlings was in all cases greater in the tetraploid as would be expected.

So far as percentage germination of progeny seeds obtained from M_1 is concerned, our finding is similar to that of Amer and Hakeem (1964) in *Lupinus termis* var. *Fayoum 45* (^{60}Co 4 Krad, 7 Krad, 12 Krad, 16 Krad, 20 Krad and 25 Krad), i. e., there is no difference in viability between seeds of the control and the progeny of the irradiated population. The striking exception is the reduced viability of M_2 of *P. minor* given 30 Krad (Table 1, 40% as against the control 70%). Here it must be recalled that the diploid M_1 at 30 Krad was unable to produce any seeds at all. Presumably the effect of tetraploidy is well shown here, in that chromosome damage which could be lethal in the diploid could be carried through to the M_2 seed without causing complete inviability.

2. Survival

Result: Survival of seedlings from germination to maturity was recorded using different doses of hard and soft X-rays in the two species of *Phalaris* and results are shown in Tables 2 and 3.

Table 2. Percentage survival in *Phalaris canariensis* (2n) from seedling to maturity (M_1) with hard and soft X-rays

Days after irradiation	Soft X-rays			Days after irradiation	Hard X-rays		
	Control	10 Krad	30 Krad		Control	10 Krad	20 Krad
10	100	100.0	0.0	15	100	100.0	100.0
20	100	90.5	0.0	30	100	92.4	60.4
30	100	88.0	0.0	45	100	84.5	55.9
40	100	66.0	0.0	60	100	81.4	54.6
50	100	66.0	0.0	75	100	81.4	54.6
60	100	66.0	0.0	90	100	81.4	54.6
70	100	66.0	0.0	105	100	81.4	54.6
Maturity	100	66.0	0.0	Maturity	100	81.4	54.6

Table 3. Percentage survival in *P. minor* (4n+2) from seedling to maturity (M_1) with hard and soft X-rays.

Days after irradiation	Soft X-rays			Hard X-rays		
	Control	10 Krad	30 Krad	Control	10 Krad	20 Krad
15	100	100	100.0	100	100.0	100.0
30	100	100	23.5	100	100.0	100.0
45	100	100	4.4	100	80.5	53.1
60	100	100	2.8	100	80.5	53.1
75	100	100	2.8	100	80.5	53.1
90	100	100	2.8	100	80.5	53.1
105	100	100	2.8	100	80.5	53.1
Maturity	100	100	2.8	100	80.5	53.1

*M*₁ generation:

Survival in P. canariensis: Table 2 shows that though the death of seedling is maximum immediately after germination, it continues up to 40–45 days at 10 Krad (soft and hard) and nearly two months at 20 Krad (hard). After this no further premature death was seen. At 30 Krad (soft) even the first leaf failed to emerge and all seedlings died within ten days after germination.

Survival in P. minor: Percentage survival data (Table 3) show no death of seedling at 10 Krad (soft) from seedling to maturity and until 30 days at 10 Krad and 20 Krad (hard). Gradual death of seedlings was observed up to 60 days at 30 Krad and between 30–45 days at 10 Krad and 20 Krad (hard). At 30 Krad in some cases the first leaf failed to emerge and the seedling died within a short period after germination whereas the remaining seedlings died at the first leaf stage when the length of the leaves varied from 0.3–0.9 cm. All seedlings died within two months from the date of irradiation and before flowering.

The doses used are not quite comparable, since the highest doses were 30 Krad of soft X-rays and 20 Krad of hard X-rays. There is no real difference in survival between the two species after hard X-rays at the two doses 10 Krad and 20 Krad, the first resulting in 80% survival to maturity, and the second 53–54%. Using soft X-rays however, the difference between the species is marked, showing a greater survival in *P. minor* ($4n+2$) and greater sensitivity in *P. canariensis* ($2n$) at both doses, 10 Krad and 30 Krad. The interesting point is the complete elimination of all seedlings of *P. canariensis* ($2n$) at 30 Krad whereas there was a low percentage of survival (2.8%) at this dose in the tetraploid *P. minor* ($4n+2$). The lower dose was 10 Krad of both soft and hard X-rays but it is difficult to draw a conclusion regarding the irradiation effects of this dose on the two species. In both the species the percentage survival seems to indicate that this is an inverse function of dose.

*M*₂ generation: The *M*₂ seeds i. e. the progeny of *M*₁ plants of Table 2 and 3 germinating with no death of seedlings either at seedling stage or during the growth period to maturity. The germination percentage was the same as the control in both species (Table 1).

Discussion: Bremer-Reinders (1962) irradiated the diploid *P. canariensis* and its colchicine-derived tetraploid, and found, in agreement with the results presented here, a greater sensitivity in the diploid as compared with the tetraploid at high doses. It is seen (Table 2 and 3) that death of seedlings continues over a long period which varies with the dose of the radiation. This can easily be explained by assuming the progressive elimination of meristematic cells. We have seen while examining the shoot apical meristem that the cells with multiple bridges are eliminated earlier than the cells with one or two bridges during the growth in agreement with Singh (1961). These cells ultimately fail to divide and finally degenerate. The higher the dose the longer is the period of elimination, and so death of seedlings continues for a longer period.

3. Length of root and shoot

Result: The mean length of root and shoot measured in both the species at 24 hours intervals up to seven days after germination is shown in Figs. 1–4, and ex-

pressed as a percentage of that of the control in Tables 4–5. The relative radiosensitivities of root and shoot shown as the ratio of mean length of root mean length of shoot per day is presented in Table 6.

a) *Effect on the root*: The growth of the root is reduced with increasing dose in both the species (Figs. 1–2). The reduction is greater in *P. canariensis* than in *P. minor*. By comparison with the diploid *P. canariensis*, the tetraploid *P. minor* shows very little variability in root length either in the control or in the irradiated seedlings. At 30 Krad there is almost no growth of the root in either species and none after the third day.

Table 4. Mean length of root in cm expressed as percentage of control

Days after germination	<i>P. canariensis</i>		<i>P. minor</i>	
	Dose		Dose	
	10 Krad	30 Krad	10 Krad	30 Krad
1	64.2	28.5	72.2	27.7
2	52.3	19.0	72.0	24.0
3	50.0	17.8	68.9	20.6
4	45.4	15.1	66.6	20.0
5	47.0	14.7	64.5	19.3
6	50.0	13.8	62.5	18.7
7	48.6	13.5	60.6	18.1

b) *Effect on the shoot*: Fig. 3 gives an indication of initial stimulation of growth in *P. canariensis* at 10 Krad. The greater length of the shoot in the irradiated seedlings in the first two days would appear to be just significant, judging by the only partly overlapping standard deviations. Whereas the length of the irradiated shoot expressed as a percentage of the control is shown (Table 5), the stimulatory effect on *P. canariensis* appears marked during the early part of the growth of the seedlings.

No such effect has been seen in roots of either of the species, or in the shoot *P. minor* (Table 4 and 5, Fig. 4).

There is greater variation in shoot length as growth proceeds in *P. canariensis* (2n) than in *P. minor* (4n+2) both in the controls and after 10 Krad (Figs. 3-4).

Table 5. Mean length of shoot in cm measured with reference to the tip of the first leaf expressed as a percentage of control

Days after germination	Plant			
	<i>P. canariensis</i>		<i>P. minor</i>	
	Dose		Dose	
	10 Krad	30 Krad	10 Krad	30 Krad
1	200.0	no emergence of first leaf	75.0	15.0
2	116.6	—	64.2	17.8
3	104.3	—	82.5	15.0
4	87.8	—	97.7	15.8
5	86.3	—	97.9	16.3
6	91.4	—	96.2	14.8
7	96.3	—	93.3	15.0

At 30 Krad no significant growth was seen in *P. minor* (4n+2) and in *P. canariensis* (2n), even the first leaf failed to emerge and all seedlings died within ten days of germination.

c) *Comparison of radiosensitivity of root and shoot:* The growth depression after 10 Krad expressed as the ratio of mean length of root/mean length of shoot has been tabulated (Table 6). It shows that the root in both the species is more sensitive

to radiation than the shoot and *P. minor* ($4n+2$) is more radioresistant than *P. canariensis*.

Table 6. Ratio of mean length of root/mean length of shoot per day

Days after germination	Plant			
	<i>P. canariensis</i>		<i>P. minor</i>	
	Dose		Dose	
	Control	10 Krad	Control	10 Krad
1	3.50	1.12	0.90	0.86
2	1.76	0.78	0.89	1.00
3	1.10	0.58	0.73	0.61
4	0.80	0.42	0.68	0.47
5	0.77	0.42	0.63	0.42
6	0.76	0.42	0.45	0.39
7	0.69	0.36	0.55	0.36

Discussion: Reduction in growth with increasing doses has been reported by many investigators both in root and shoot at seedling stage, one (Bremer-Reinders, 1962) having worked on *P. canariensis*: but only in the present experiment has the initial stimulation of growth in the shoot of *P. canariensis* ($2n$) at 10 Krad been seen. The controversial topic of stimulation induced by radiation has been discussed in detail by Shull and Mitchell (1933), Wort (1941), Sax (1955) and Kaindall and Linser (1961). Recent reports on seed irradiation followed by stimulation include:—seedling height in wheat (Saric *et al.* 1961); root and coleoptile of maize (Stein and Steffensen 1959), and increased phosphorylation in the corn root-tip (Cherry *et al.* 1961). A possible cause of stimulation brought about by radiation would be the damage to the growth regulating system of the cells, whereby an inhibitor could be removed and increased growth would result. Such an effect would be expected to be more obvious in the diploid; the tetraploid due to the presence of reduplicated genomes being more radioresistant, and this is what is found in the present case.

So far as variation during root and shoot growth is concerned *P. canariensis* ($2n$) is more variable than *P. minor* ($4n+2$) whether irradiated or not. This also is to be expected of a diploid as compared with a related polyploid.

It is difficult to suggest why the root is more sensitive to radiation than the shoot as shown by the present observations (Table 6) and also found by Scarascia *et al.* (1961).

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