RADIATION-INDUCED POLYGENIC MUTATION IN *ARABIDOPSIS THALIANA*

IV. SELECTION FOR NUMBER OF SEED PER POD

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SUMMARY

Selection for high and low number of seed per pod was practised in families derived by selfing single irradiated plants from a homozygous line of *Arabidopsis thaliana* (Heyn.). Such plants were given a single initial dose of 100, 200 or 300 krad of ^{60}Co gamma rays. Selection was also carried out in the control. Selection in the high lines acted principally to reconstitute the control genotype, though this was not entirely successful and most high lines became homozygous for deleterious mutations. "Positive" mutations that increased the number of seeds per pod to above the control level were at best very rare and expressed only in certain environments.

Large selection gains were found in the low lines, though their size was not linearly related to dose. This may have been due to the occurrence of chromosome aberrations such as translocations and inversions.

The results with this character, probably an important component of fitness, contrast with those obtained previously with flowering time, both with respect to the asymmetry of the selection response and its dose dependence. The low frequency of "positive" mutations suggests that radiation-induced quantitative variation may have only limited value for crop plant improvement.

1. INTRODUCTION

THE genetic properties ascribed to radiation-induced mutations in polygenic systems controlling quantitative characters might be expected to vary, like those of their spontaneous or naturally occurring counterparts, according to the influence of such factors as the environment, the breeding system of the irradiated species, the genetic architecture of the particular gene system studied and gene coadaptation within this system (Mather, 1960). Consequently, important variables may include the nature of the character studied, whether an important component of fitness or not and whether previously subject to directional or stabilising selection, and the existence of pleiotropic effects among the mutations. A general understanding of the importance and effect of these factors could be of use in two ways. First, newly induced and therefore relatively unselected mutations offer a means of disrupting integration within gene systems that is in some respects analogous to hybridisation between individuals from isolated populations and consequently provide an alternative method of studying such integration. Second, a general understanding of the effect of these factors would be useful in the appraisal of the potential value of induced quantitative variation in programme of crop plant improvement.

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Previous papers in this series (Lawrence, 1968a, b, 1975a) described experiments with *Arabidopsis thaliana* designed to examine some of the properties of mutations affecting flowering time, a character which may be a relatively minor component of fitness in this predominantly inbreeding species and subject to selection for an intermediate phenotype. By way of contrast, this and the following paper describe experiments in which the properties of mutations influencing the number of seeds per pod are studied, a character which is probably a major component of fitness and subject predominantly to directional selection.

2. MATERIALS AND METHODS

The plants grown in the present experiment were derived from the same Standard inbred line used in the earlier experiments (Lawrence, 1968a, b, 1974). It was highly inbred when first received and was subsequently maintained by the self-pollination of a single plant for nearly 20 generations before the start of the present work. Plants were grown in soil in a glass-house and seed irradiated with ^{60}Co gamma rays at a dose-rate of approximately 400 krad/hour. The cultural technique and irradiation conditions used have been described in detail in earlier papers (Lawrence, 1968a, b).

The experiment was initiated by irradiating different samples of seed from the inbred line with doses of either 0, 100, 200 or 300 krad and self-pollinating four of the resulting X_1 plants, chosen randomly, in each of the four dose groups. Selection for high and low seed numbers was started in X_2 and the 32 lines maintained thereafter by self-pollination of a single plant up to the X_6 generation. Selection was carried out in the X_2 to X_4 generations, inclusive. The average number of seeds per pod in each plant was assessed by collecting the first five pods formed, which were always on the main stem, soaking them in 70 per cent ethanol for at least one day and then counting the number of full seeds through the wall of the intact pod, submerged in water, with the aid of a binocular microscope. Care must be taken with this method, particularly when the number of seeds is high and to ensure an accurate count frequent checks were made by splitting pods and counting isolated seeds. Similarly, frequent intercomparisons were made between the different scorers to ensure a uniform counting procedure. Occasionally one or more of the first five pods was damaged or missing, in which case the next five available were harvested. This is unimportant since it was shown (Lawrence, unpublished) that there is no systematic variation in the numbers of seeds per pod in at least the first 20 pods on plants grown in the conditions used. It was also shown that the average number of seeds per pod in these samples of five pods correlates highly with the total seed yield of the plant.

Selection was carried out in families obtained by sowing 105 seeds, and the highest or lowest yielding plant was selected to continue the line subject to two restrictions; that it possessed a more or less normal phenotype and that it produced at least 150 seeds. Since plants grown in these conditions can produce hundreds of pods, the latter restriction was rarely invoked even in the lines selected for low fertility. Selected plants were transferred to a pot, all stems trimmed back to the rosette, and the plants placed in a growth room under well-isolated conditions to produce self-pollinated seed. Such precautions are essential to avoid accidental out-

crossing, especially in semi-sterile plants, and great care was taken to avoid cross-pollination in this way.

The performance of the 32 selection lines was assessed in the X_6 generation in two independent trials of eight blocks, each line being represented by one plot of 10 plants per block.

3. RESULTS

Table 1 gives the average number of seeds per pod and the variance between plants, averaged over the four replicate lines, in families of the X_2 to X_4 generation inclusive. In the X_2 generation the number of seeds per pod progressively decreased, and the variance progressively increased, with

TABLE 1

Mean ($\bar{x}$) and variance within families (var. x) of number of seeds per pod averaged over the four replicate lines in the X_2 , X_3 , and X_4 generations. n = total number of plants

Dose (krad)	Selection (high or low)	Generation								
		X_2 4/8/66			X_3 18/4/67			X_4 10/8/67		
		$\bar{x}$	var. x	n	$\bar{x}$	var. x	n	$\bar{x}$	var. x	n
0	H	47.47	69.80	328	47.59	32.33	391	53.46	32.88	391
	L				49.58	26.00	370	54.35	39.94	363
100	H	41.62	125.04	297	47.28	55.76	376	51.59	21.09	388
	L				35.85	51.49	379	22.09	81.41	322
200	H	39.69	141.60	252	48.14	46.69	397	50.80	49.96	382
	L				24.01	179.77	350	36.23	130.66	353
300	H	32.86	179.12	271	43.06	60.86	341	53.52	37.95	325
	L				32.47	91.05	358	34.20	67.83	333

increasing dose and this was due to an increasing skewness in the direction of low fertility at higher dose levels. In no instance was the yield of an individual plant or pod higher in the irradiated families than the highest in the control. The greater part of the selection response shown in X_4 was achieved by the X_3 generation, with the exception of the 100 krad low lines and the 300 krad high lines. The 200 krad low lines showed *less* response in X_4 and this was probably due to the segregation and fixation of structural aberrations in chromosomes, discussed later in more detail. The variance in irradiated families selected for high fertility decreased to the control level by X_4 with the exception of the 200 krad high lines, but high variances were observed in the low selection lines. This was partly due to persistent segregation in these families, particularly in two of the 200 krad low lines, but was probably also due to the poorer developmental stability of lines with relatively low fertility. Persistent segregation, in the form of pronounced bimodality in the distribution within families, was observed in two of the 200 krad low lines in both X_3 and particularly X_4 . The two peaks in the distribution were of equal size, and seed was taken from plants located in both the high and the low peak in each of the 200

krad low lines showing bimodality, the resulting lines being maintained until X_6 , when they were grown together with the other lines in the two trials. All plants derived from the high peaks gave seed yields and variances similar to those of the control, but all from the low peaks segregated as before. This situation is consistent with the segregation of chromosome interchanges or inversions in these families and the selection of the infertile heterozygote. Since the interchange or inversion homozygote is unlikely to be as fertile as the heterozygote, its chance fixation would lead to a reduction of the selection response of the kind observed in another 200 krad low line.

Overall, the data from the first three generations of selection (table 1) suggest that the mutations induced in X_1 plants act exclusively to reduce fertility; there is little evidence that mutations increasing the number of

TABLE 2

Average number of seeds per pod in the 32 lines, selected for either low or high fertility, grown in two X^6 trials. Trial 1 (top figure) sown: 2/4/68. Trial 2 (bottom figure) sown: 22/5/68

Replicate line No.	0 krad		100 krad		200 krad		300 krad	
	High	Low	High	Low	High	Low	High	Low
1	56.83	57.88	55.86	7.19	54.92	37.53	59.60	39.92
	43.89	45.80	42.34	6.30	46.52	28.06	43.81	20.67
2	58.76	57.02	58.98	57.60	57.50	24.33	58.71	11.06
	44.59	44.84	43.88	45.38	44.30	28.64	39.46	7.48
3	57.53	56.72	57.40	18.95	58.37	37.06	57.39	28.66
	41.31	43.26	42.63	10.40	43.12	27.27	45.76	12.95
4	56.52	56.34	55.27	11.50	57.94	17.33	58.63	41.63
	44.91	44.71	32.03	3.93	41.66	11.74	44.57	21.08
Trial 1	57.41	56.99	56.88	23.81	57.18	29.06	53.58	30.32
Trial 2	43.67	44.65	40.22	16.50	43.90	23.93	43.40	15.55
Average	50.54	50.81	48.62	20.16	50.54	26.49	50.99	22.93

seeds per pod have been induced. Selection in the high lines seemed therefore merely to discriminate against the induced mutations and reconstitute the control genotype, though some chance fixation of mutations no doubt occurred. There is, however, one curious feature of the results; that is the lines which most nearly approach the control with respect to seed yield are those which received the highest dose. This may mean that mutations increasing the number of seed per pod were in fact induced at a very low frequency but that their action was swamped by chance fixation of mutations with the opposite effect.

Results from the two trials (table 2) and their analysis (table 3) support and extend these conclusions. Combining results from both trials, it can be seen that there was no response to selection in the control, and that the response in irradiated families was predominantly in the direction of low fertility. The dose-response in the low direction was not linear, the greatest response occurring at the lowest dose though the replicate lines

were very heterogeneous. Possibly some mutations which individually lower fertility act synergistically together to produce lethals and are therefore removed from the families receiving higher doses. Overall, there was no significant variation among the high lines, though the yield of the lowest dose group was a little less, and of the highest dose group a little more than the control.

The two trials were, however, markedly heterogeneous. The first trial was grown in a "high yield" environment in which all lines, particularly the low ones, performed better relative to the control than in the second

TABLE 3
Analysis of variance in number of seeds per pod in the two X_6 trials

Item	d.f.	M.S.	V.R.
(1) <i>Lines</i>	31	3883.77	31.10***
High lines	15	61.20	1.38 n.s.
between doses	3	76.42	1.72 n.s.
within doses	12	57.33	1.29 n.s.
Low lines	15	4533.17	27.64***
between doses	3	12641.20	40.05**
within doses	12	2506.16	19.88***
High versus low lines	1	51482.19	68.97 n.s.
(2) <i>Lines × Trials</i>	31	124.87	8.96***
High lines × trials	15	44.33	3.18***
between doses × trials	3	37.32	< 1 n.s.
within doses × trials	12	46.08	3.31
Low lines × trials	15	163.98	11.77***
between doses × trials	3	315.62	2.50 n.s.
within doses × trials	12	126.06	9.05***
High v. low lines × trials	1	746.40	53.58***
(3) <i>Trials</i>	1	19368.20	1390.39***
(4) <i>Blocks</i> —trial 1	7	228.37	13.97***
trial 2	7	159.89	13.88***
(5) <i>Error</i> —trial 1	217	16.35	} 13.93
trial 2	217	11.52	
(6) <i>Total</i>	511	—	—

* $P = 0.05-0.01$; ** $P = 0.01-0.001$; *** $P < 0.001$.

trial, which was grown in a "low yield" environment. It is not known which environmental factors were responsible for this difference. In the first trial the average number of seeds per pod in the 300 krad high lines was $2.2\frac{1}{2}$ per cent greater than in the control, an increase which was significant ($P = 0.05-0.01$), but in the second trial the yield of these lines was less than in the control. The results therefore suggest that the proportion of mutations increasing the number of seeds per pod is at best very small and that they are expressed only in optimal environments.

In addition to the number of seeds per pod, flowering time was also recorded in the two trials with a view to examining the lines for correlated changes in this unselected character. The data are given in table 4 and

TABLE 4

The average number of days between sowing and flowering (opening of the first flower) in the two X_6 trials

Replicate line No.	0 krad		100 krad		200 krad		300 krad	
	High	Low	High	Low	High	Low	High	Low
1	32.21 26.56	31.78 26.23	31.60 25.58	32.10 25.94	31.49 25.65	32.09 25.86	32.56 26.13	33.18 26.68
2	32.11 26.01	32.45 26.10	31.50 25.76	31.38 25.36	31.94 26.35	31.28 25.76	32.69 26.15	37.64 30.65
3	32.10 26.09	32.13 26.21	32.35 26.03	33.69 27.68	32.79 26.53	33.14 27.46	31.84 26.03	34.14 28.83
4	31.86 25.73	31.15 25.85	32.99 26.60	34.84 27.08	32.11 26.63	34.14 27.01	32.14 25.83	33.20 26.98
Trial 1	32.07	31.88	32.11	33.00	32.08	32.66	32.31	34.54
Trial 2	26.10	26.10	25.99	26.51	26.29	26.53	26.03	28.28
Average	29.08	28.99	29.05	29.76	29.18	29.59	29.17	31.41

TABLE 5

Analysis of variance in flowering time in the two X_6 trials

Item	d.f.	M.S.	V.R.
(1) <i>Lines</i>	31	20.52	18.32***
High lines	15	2.15	5.81***
between doses	3	0.27	< 1 n.s.
within doses	12	2.61	7.05***
Low lines	15	34.61	18.71***
between doses	3	68.78	2.64 n.s.
within doses	12	26.07	13.30***
High versus low lines	1	84.82	229.24***
(2) <i>Lines × Trials</i>	31	1.12	3.03***
High lines × trials	15	0.42	1.14 n.s.
between doses × trials	3	0.68	1.84 n.s.
within doses × trials	12	0.36	< 1 n.s.
Low lines × trials	15	1.85	5.00***
between doses × trials	3	1.41	< 1 n.s.
within doses × trials	12	1.96	5.30***
High v. low lines × trials	1	0.49	1.32 n.s.
(3) <i>Trials</i>	1	4766.54	12882.54***
(4) <i>Blocks</i> —trial 1	7	13.74	37.14***
trial 2	7	0.82	2.22*
(5) <i>Error</i> —trial 1	217	0.36	} 0.37
trial 2	217	0.37	
(6) <i>Total</i>	511	—	—

*P = 0.05-0.01; ***P < 0.001.

their analysis in table 5. Random fixation of mutations affecting flowering time in the high lines resulted in only a small, though consistent, change in flowering time in the late direction. It would therefore appear that a more or less equal proportion of these mutations act in the early and late directions as found previously (Lawrence, 1968*a, b*). Infertile lines have a marked tendency to flower later than the control, however, supporting previous observations of such pleiotropic effects. A significant negative correlation exists between the number of seeds per pod and flowering time in the 24 irradiated lines, though the correlation is not significant among the high or low lines separately.

Trial 1	all irradiated lines	$r = -0.623$	$P(22) = 0.001$
	high irradiated lines	$r = -0.200$	$P(10) > 0.10$
	low irradiated lines	$r = -0.515$	$P(10) = 0.10-0.05$
Trial 2	all irradiated lines	$r = -0.643$	$P(22) = 0.001$
	high irradiated lines	$r = -0.538$	$P(10) = 0.10-0.05$
	low irradiated lines	$r = -0.547$	$P(10) = 0.10-0.05$

4. DISCUSSION

The main object of the experiments reported here was to examine the genetic variation in number of seeds per pod induced by ionising radiation, both with respect to the relative amounts of variation that could be utilised by selection in the high and low directions and to the dose dependence of these selection responses. The results obtained with this character, probably a major component of fitness subject to natural selection which is substantially in the upward direction, could then be compared with previous data concerning flowering time, a character which is probably a minor component of fitness subject to natural selection toward some intermediate phenotype.

The two sets of data would appear to contrast both with respect to the symmetry of selection response and to dose dependence. With regard to flowering time, artificial selection in irradiated populations was about equally successful in the early and late directions and the size of the selection response was linearly related to accumulated radiation dose in both directions (Lawrence, 1968*a*). Similar results were also obtained from a series of acute doses (Lawrence, *loc. cit.*), though an asymmetric response was found in a later experiment where the plants were grown in test-tubes under artificial light rather than in soil in a glasshouse (Lawrence, 1975*a*). With regard to fertility, however, the proportion of mutations increasing the number of seeds per pod was at best very small, and moreover they are probably expressed in only a limited range of favourable environments. Similarly, the greatest response to selection, at least in the direction of low fertility, was found in lines receiving the lowest dose, so that the dose-response is clearly non-linear.

While there were some differences in procedure in the two sets of experiments, it nevertheless seems probable that these contrasting results originate in the differences between the properties of the gene systems controlling flowering time and the number of seeds per pod. The great majority of induced mutations can be assumed to reduce fitness, and such a reduction might be manifest in either early or late flowering since the optimum is

probably some intermediate phenotype. With respect to fertility, however, loss of fitness will be associated largely with a reduced number of seeds per pod. The small proportion of mutations which appear to increase the number of seeds per pod may then represent that kind of allele favoured by natural selection—the so-called progressive mutations—or may themselves be thought to lead to a loss of fitness; the physiological resources available to individual pods cannot be expected to sustain an unlimited increase in the number of seeds they contain. Whether such limits were approached in the present experiments remains a moot point, however, since the maximum number of seeds per pod observed far exceeded the average in most plants. In short, the results are compatible with the conclusion that *all* induced mutations, within the limits of detection of the experiments, reduce fitness. Taking the most favourable position, the proportion of mutations increasing fitness must at best be very small. Since these are presumably the kind of mutations required for crop plant improvement, the results suggest that induced variation may have only limited value for this purpose.

The non-linear relation between dose and response to selection for low seed number is unexpected and puzzling. In part it may be due to the occurrence of chromosome aberrations such as translocations and inversions which uniquely affect fertility but not other characters such as flowering time. In the heterozygous condition such aberrations could mask the effect of mutations and prevent their selection. Subsequent fixation of the aberration in the homozygous condition, which seems to have occurred in several instances, would then lead to an increase in fertility. There is evidence (Lawrence, 1975*b*) that one out of two of the 300 krad low lines tested, one of two 200 krad low lines (other than the two heterozygous for aberrations) but none out of two 100 krad low lines tested were homozygous for aberrations. In part, the non-linear dose response may also be due to lethal interactions between mutations which individually lower fertility, leading to their elimination from families in the higher dose groups.

If the evidence pointing to the existence of a small number of mutations which increase the number of seeds per pod is accepted, the response to selection in this direction may in fact be linearly related to dose. Since most mutations decrease fertility, it seems likely that some of them were unavoidably fixed in the homozygous state in the high lines during selection and inbreeding. This perhaps explains why the 100 krad high lines had lower fertility than the control. Similarly, the observation that the 200 and 300 krad high lines had, respectively, equal and slightly greater numbers of seed per pod than the control possibly implies a linear dose response for "positive" mutations, though the selection responses were too small, and heterogeneity between replicate lines too great, for the regression to be statistically significant.

On the face of it, the lack of substantial and consistent response in the direction of high fertility would appear to conflict with the results from several experiments with crop plants, notably those of Gaul *et al.* (1969) with barley. On the basis of results from selection for grain yield in irradiated populations of three varieties of barley, carried out over eight or more generations, Gaul states that "it is relatively easy to select micro-mutants that have yielding potential 10 per cent higher than the mother

variety". It is clear from table 2 that such a statement cannot be made concerning the present experiment. Although it is possible that the difference between the two sets of results arise from unknown factors to do with the species or particular conditions used, certain features of the barley data suggest that the difference may only be apparent. Substantial responses to selection, in the high as well as the low direction, were found in the control populations of all three varieties, indicating considerable genetic heterogeneity in the starting material. Such responses account for about half of the gain in the irradiated populations. Gaul *et al.* (1969) ascribe this to the occurrence of spontaneous mutation. The doses of radiation given to the barley seeds were sufficient to cause extensive partial sterility, and it is therefore possible that outbreeding was encouraged. At the same time the irradiation may have stimulated recombination in portions of the genome normally untouched by this process. As a consequence, response to selection would be expected to be greater in the irradiated than in the control populations. In other words, it is possible that irradiation led to the efficient *utilisation* of pre-existing variation rather than the *induction* of new variation.

It might be objected that the source of the genetic variation is of no consequence in the face of the undoubted gains in crop yield. This is not so, however, for two reasons. From the practical point of view, if the gains arise from spontaneous variation, it will be more efficient to identify and intercross the variants directly, possibly together with irradiation of the hybrid progeny. From the theoretical point of view, conclusions regarding the properties of the "induced" mutations could be misleading, as are predictions concerning the consequences of further rounds of irradiation and selection. Gaul *et al.* (1969) suggest, for example, that their data support the view that induced micro-mutations lack appreciably adverse pleiotropic effects. Spontaneous variants that have been subject to natural selection would of course be just of this type. Similarly, the data are taken to support conclusions drawn from a model proposed by Fisher (1930) in which micro-mutations are supposedly more suitable than macro-mutations for plant breeding since they are more likely to improve fitness. As pointed out previously (Lawrence, 1975a), Fisher's model cannot be used in this context, and the question of the relative merits of micro- and macro-mutation breeding remains open. Finally, Gaul *et al.* (1969) predict that the response to selection in successive rounds of irradiation and selection should be equal to that found in the first, and this is indeed true, at least up to a point, when the variation is actually induced (Lawrence, 1968a, 1975a). However, in experiments such as that of Brock (1966) or Khadr and Frey (1965) where, as shown by selection, the control material was initially heterogeneous, the second round of irradiation and selection can give drastically reduced response because all the spontaneous variation had been exhausted in the first round. It is clear that where relatively small gains are obtained with fairly intense selection, rigorous control of the experimental material, not normally achieved with field crops, must be maintained in order to avoid ambiguous results.

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