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Radiation induced developmental instability in *Arabidopsis thaliana**

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Summary. In a study on the nature of induced genetic variations in quantitative characters using the self-pollinated plant *Arabidopsis thaliana*, it was observed that not only quantitative performance but also intra-line variability, or developmental instability, was affected by induced mutations. Emphasis was placed on the latter.

Seeds of *Landsberg* strain were used. Two irradiated (20 kR and 80 kR) populations together with a control population were propagated by self-fertilization for six generations using the "one-parent to one-offspring" scheme. In M_3 and in M_6 generations, plants were taken at random and their M_4 and M_7 progeny lines were grown to study the possible occurrence of polygenic mutations. Both in M_4 and M_7 lines, in addition to an increase in genetic variances, the environmental component of variation was also increased. The mean and within-line standard deviations were found to be uncorrelated in M_7. A selection experiment showed the differences in developmental instability among M_7 lines to be genetic. From these it was concluded that radiation has induced a genetic change causing an increase in intra-line variability, or developmental instability.

Selected higher and lower instability lines were grown under different environmental conditions. It was observed that with constant temperature, the developmental instability increased, but with changing temperature it decreased. Each environment was assigned a value based on the mean performance of all lines in this environment. Phenotypic plasticity of a line was measured by the regression of this line on environmental value. It was found that phenotypic plasticity was not correlated with developmental instability.

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Key words: Radiation induction – Intra-line variability – Environmental component – Developmental instability – Phenotypic plasticity

Introduction

Intragenotypic variation under a certain environmental condition is known as developmental instability. This variation is considered to be due to local fluctuation in environmental conditions outside and/or inside the organism and also to mistakes in the biochemical steps of the developmental process. Several workers (Sakai and Shimamoto 1965; Paxman 1956; Mather 1953; Jinks and Mather 1955) studied intra-organ or inter-plant variabilities to show that developmental instability is genetically controlled. Mather (1953) and Reeve (1960) have further shown that such quantitative variability is susceptible to selection pressure. Intragenotypic quantitative variability as described above may be measured in two ways: (1) intragenotypic difference measured as variation between genetically identical plants grown under the same environmental condition, and (2) intragenotypic difference measured as variability between repeated structures in a single plant. By these two methods the magnitude of developmental instability can be estimated. The present experiment deals with the first type only.

There is another type of phenotypic variation which can be observed by growing individuals of the same genotype in different environments. The variability measured in this way express sensitivity in response to environmental change and is known as phenotypic plasticity.

It is generally assumed that developmental instability is due to accidents in the developmental process and is unrelated to external environmental conditions; or if related, the environmental effects are not discernible (Sakai and Shimamoto 1965). Phenotypic plasticity is considered to occur in response

to change in environment. Both of these types express quantitative variability and are genetically controlled (Bradshaw 1965; Mather 1953; Paxman 1956; Sakai and Shimamoto 1965).

Lack of stability is considered to be non-adaptive (Paxman 1956). Levin (1970), however, proposed that developmental instability may serve as a new way for adaptation to an unfavourable environment through a possible increase in diversity of phenotypic expression by poorly buffered genotypes so as to be selected and recanalised to adapt to a new environment.

The purpose of the present investigation was to determine whether (1) the developmental instability is genetically controlled; (2) the imbalance in development could result from induced mutations; (3) the magnitude of instability is influenced by external conditions; and (4) the phenotypic plasticity is related to developmental instability.

Material and methods

Seeds of a self-pollinating plant species *Arabidopsis thaliana* were obtained from the Induced Mutation Laboratory of the National Institute of Genetics, Misima, Japan. The Landsberg strain was used.

To ensure uniform germination, seeds were subject to a cold-treatment at 10 °C for seven days on moist filter paper before germination. When the data of quantitative characters were recorded, plants were grown at a density of 5 × 5 cm.

Seeds were divided into three lots each containing approximately 2,000 dry seeds; the first lot received 20 kR of gamma radiation and the second 80 kR, each from a ^{137}Cs source at a dose rate of 40 kR/h, while a third was used as an unirradiated control. The plants were grown in a phytotron adjusted at 25 °C, 4,800±500 lux of light and 65±5% relative humidity in sand culture with supplementary nutrition (nitrogen content in the medium was 30 ppm).

Inbred progenies from irradiated and control seeds were propagated by the "one-parent to one-offspring" scheme (Sakai and Suzuki 1964) to avoid the effect of both natural selection and sampling bias. The number of plants should remain the same by this method of propagation, but in practice it decreased considerably on account of incidental loss, seed sterility and failure in germination.

In the M_3 generation, 20 plants from each of the two irradiated populations and 14 plants from the control population were taken at random to develop M_4 progeny lines. Side by side, the "one-parent to one-offspring" scheme of propagation was continued till the M_6 generation to attain higher homozygosity. In M_6, a little more than 100 plants were again taken at random from each of the three populations to raise M_7 progeny lines for the experimental study.

In the M_4 experiment, the irradiated populations each consisted of 20 lines with 20 plants per line. The control population consisted of 14 lines, each with 10 plants. Seven quantitative characters, days to first flowering, plant height, leaf number, leaf length, leaf breadth, rosette diameter and pod number were recorded. Plant height and pod number were recorded 40 days after seed sowing and the other characters for each plant on its first flowering date.

In the M_7 experiment, the control and 20 kR populations consisted of 100 lines each, and the 80 kR population 105 lines. A line consisted of 21 plants, and three quantitative characters, days to first flowering, plant height and rosette diameter at first flowering date, were recorded.

Table 1. Environmental conditions based on different combinations of nitrogen levels and temperature

Designation	Nitrogen (ppm)	Temperature (°C)
E_1	0	25
E_2	40	25
E_3	30	25
E_4	30	20
E_5	30	30
E_6	30	20/30
E_7	30	10/30 approx.

Lines showing higher (H) and lower (L) variabilities were selected from among the M_7 lines, both for days to first flowering and plant height. A progeny test was performed with M_8 progenies and heritabilities estimated on the basis of realized response to selection.

Further the lines selected for higher and lower variability of days to first flowering were grown under five different environmental conditions; the lines selected for variability in plant height were tested under seven different environmental conditions. The environmental conditions used are given in Table 1. E_6 and E_7 refer to changing temperature.

The data of M_4 and M_7 lines were subject to analysis of variance to estimate the genetic and environmental components of variances.

The developmental instability was estimated in terms of within-line standard deviations. The line mean values under different environmental conditions were used as measures of environmental response of the line. Each environment was assigned a value based on the mean performance of all lines in this environment. Then phenotypic plasticity of a line was measured by the regression of its response on the environmental value as done by Finlay and Wilkinson (1963).

Results

1 Observations on the M_4 lines

Analysis of M_4 data showed that irradiation had induced little change in population means except for days to first flowering and plant height in the 20 kR population. However, Table 2 shows that inter-line variances increased significantly. Irradiated populations showed larger intra-population genetic variances than the control in all the characters recorded (Table 3). In four characters, days to first flowering, plant height, leaf number and pod number, the 80 kR population had larger genetic variation than the 20 kR population, but in the other three, the 20 kR population showed larger genetic variances. Probably the high dosage of irradiation (80 kR) had produced more inviable mutations than 20 kR, resulting in a decrease in genetic variability among surviving plants.

Besides the genetic component of variation, the irradiated population showed an increase in within-line variances. Frequency distributions of within-line

Table 2. Mean and mean squares of Anova for seven characters in M_4 lines

Character	Population	Population mean	Between line		Within line	
			df	Mean square	df	Mean square
Days to first flowering	Control	28.25	13	8.95**	126	2.49
	20kR	24.27	19	129.26**	376	6.04
	80kR	29.28	19	252.51**	358	5.88
Plant height (cm)	Control	24.61	13	20.89**	126	7.38
	20kR	27.63	19	90.78**	376	11.97
	80kR	25.78	19	317.82**	358	19.84
Leaf number	Control	9.52	13	1.31	126	1.13
	20kR	9.61	19	21.77**	376	1.79
	80kR	10.97	19	37.33**	358	2.73
Leaf length (mm)	Control	15.97	13	7.85	126	5.75
	20kR	13.53	19	61.86**	376	4.56
	80kR	14.21	19	24.21**	358	6.73
Leaf breadth (mm)	Control	6.37	13	1.56	126	0.87
	20kR	5.39	19	8.30**	376	0.83
	80kR	5.68	19	1.27	358	1.23
Rosette diameter (cm)	Control	3.27	13	52.34*	126	24.36
	20kR	2.92	19	353.71**	376	24.92
	80kR	3.14	19	210.31**	358	35.45
Pod number	Control	25.56	13	232.79**	126	56.99
	20kR	37.02	19	519.63**	376	178.56
	80kR	42.39	19	3641.45**	358	288.78

**, * Significant at 1% and 5% levels respectively

Table 3. Estimates of genetic variances of seven characters in M_4 lines

Character	Control	20kR	80kR
Days to first flowering	0.6959	6.2231	13.0520
Plant height	1.3521	3.9805	15.7701
Leaf number	0.0189	1.0087	1.8514
Leaf length	0.2092	2.8900	0.9126
Leaf breadth	0.0686	0.3817	0.1786
Rosette diameter	2.7979	16.6060	9.2300
Pod number	17.5809	17.2260	177.4313

standard deviations in irradiated populations were wider in range than those in the control population (Table 4). The occurrence of enhanced within-line variations in M_4 lines suggested that the irradiated lines might have had a larger environmental variation, in addition to possible genetic segregation within line.

2 Observations on the M_7 lines

Analysis of M_7 data showed little change in mean values due to irradiation except for days to first flowering in 20 kR. The inter-line variances were significantly larger than those in the control (Table 5). The genetic variances in irradiated populations were always larger than those in the control population (Table 6). However, as observed in M_4, whether the 80 kR population had a larger genetic variance depended on the character. It is also recognized from the table that the irradiated population generally had larger environmental variances than the control. The frequency distribution of within-line standard deviations showed more lines with large standard deviations than the control (Table 7).

Estimates of correlation coefficients between line mean and within-line standard deviation indicated that in the control and 20 kR populations certain characters showed only slight correlation, but in the 80 kR population no significant correlation (Table 8). Thus, it may be considered that the increase in within-line variability in irradiated lines was not affected by any change in line mean. Analysis of variance of the within-line standard deviations showed that the irradiated and control populations differed significantly (Table 9). Correlations were also computed among the within-line standard deviations of different characters. The insignificant correlation coefficients indicated that within-line variability in one character was not at all associated with that in other characters.

From these experimental results with the M_7 lines, it was confirmed that induced genic mutations brought about an increase in within-line variation.

Table 4. Distribution of within-line standard deviations for seven characters in M_4 lines

Character	Population	Within-line standard deviation										No. of lines
		0.6	1.4	2.2	3.0	3.8	4.6	5.4	6.2	7.0	7.8	
	Control		7	6	1							14
Days to flower	20kR		3	11	2	2	2					20
	80kR		3	4	9	4						20
	Control		2	3	6	2	1					14
Plant height	20kR		1	4	5	5	3	1		1		20
(cm)	80kR				4	7	4	1	3			20
	Control		13	1								14
Leaf number	20kR		13	5	2							20
	80kR		10	7	2	1						20
	Control			7	6	1						14
Leaf length	20kR		4	7	7	1	1					20
(mm)	80kR			4	11	5						20
	Control	2	12									14
Leaf breadth	20kR	5	15									20
(mm)	80kR		18	2								20
	Control					6	3	2	1	1	1	14
Rosette diameter	20kR				2	5	4	4	3	1	1	20
(cm)	80kR					1	4	4	5	3	3	20
	Control				1			2	5	2	4	14
Pod number	20kR									1	19	20
	80kR										20	20

Table 5. Means and mean squares of Anova for three characters in M_7 lines

Character	Population	Population mean	Between line		Within line	
			df	Mean square	df	Mean square
	Control	28.53	99	1.51*	1877	1.13
Days to first	20kR	24.02	99	2.99**	1968	1.51
flowering	80kR	20.89	104	3.09*	1996	2.35
	Control	24.00	99	3.03*	1877	1.62
Plant height	20kR	26.82	99	3.07**	1968	1.54
(cm)	80kR	22.33	104	50.19**	1996	2.85
	Control	1.65	99	0.22**	1877	0.12
Rosette diameter	20kR	1.75	99	0.61**	1968	0.23
(cm)	80kR	1.65	104	0.41**	1996	0.22

**, * Significant at 1% and 5% levels respectively

Table 6. Estimates of genetic and environmental component of variances for three characters in M_7 lines

Character	Population	Genetic variance	Environmental variance
	Control	0.0196	1.1285
Days to	20kR	0.0714	1.5093
flower	80kR	0.0370	2.3501
	Control	0.0713	1.6193
Plant	20kR	0.0739	1.5366
height	80kR	2.3661	2.8499
	Control	0.0048	0.1219
Rosette	20kR	0.0180	0.2396
diameter	80kR	0.0097	0.2144

3 Selection experiments

Two selection experiments were carried out to find if the M_7 lines were genetically different from each other with regard to within-line variability. For this purpose the 80 kR population was chosen since it showed no interdependency between mean and standard deviation in any of the three characters. Lines with within-line standard deviations greater than 1.8 and 2.6 were selected as the high variability (H) group for days to first flowering and plant height, respectively. Lines with standard deviations not greater than 1.2 and 1.04 for the two characters were selected as the low variability (L) group. For days to first flowering, 8 H and 8 L lines,

Table 7. Frequency distribution of within-line standard deviation in M_7 lines

Character	Population	Within-line standard deviation								No. of lines
		0.2	0.6	1.0	1.4	1.8	2.2	2.6	3.0	
	Control	5	78	17						100
Days to first	20kR	1	56	38	5					100
flowering	80kR	1	13	52	35	4				105
	Control	2	38	57	3					100
Plant	20kR		45	54	1					100
height	80kR		27	32	28	6	6	3	3	105
	Control	84	16							100
Rosette	20kR	26	74							100
diameter	80kR	27	78							105

Table 8. Estimated correlation coefficients between mean and within-line standard deviation of three characters in M_7 lines

Population	Days to first flowering	Plant height	Rosette diameter
Control	0.476*	0.348*	0.106
20kR	0.201*	0.170	0.205*
80kR	-0.020	0.192	-0.137

* Significant at 5% level

Table 9. Analysis of variance of within-line standard deviations in control and irradiated populations in M_7 lines

Source of variation	df	Mean square		
		Days to first flowering	Plant height	Rosette diameter
Populations	2	5.4921**	4.7478**	0.4963**
C vs R	1	6.8441**	1.8570**	0.9589**
20kR vs 80kR	1	4.1401**	7.6387**	0.0338**
Error	301	0.0540	0.1406	0.0078

C: control, R: Irradiated
** Significant at 1% level

and for plant height 10 H and 11 L lines were selected. Their M_8 progeny lines were grown and within-line standard deviations were calculated.

Results of the selection experiments are shown in Figs. 1 and 2. Realized heritability was computed by the ratio of the difference between H and L groups in M_8 to those in M_7, i.e. the ratio of actual gain to selection differential. A t-test was performed on the difference between H and L groups in M_8 to find if the effect of the selection was significant.

It was found that response to the selection was insignificant ($h^2=0.20$) for the within-line standard deviation in days to first flowering but significant for that in plant height ($h^2=0.34$), confirming that the magnitude of within-line variability was under genetic control.

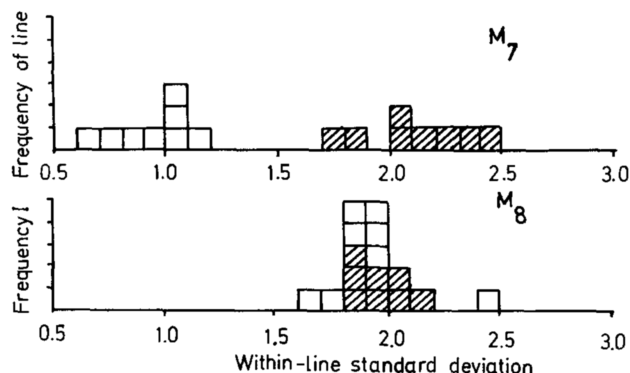

Fig. 1. Frequency distribution of within-line standard deviations of selected H (hatched block) and L (open block) lines in M_7 and their M_8 progeny lines for days to first flowering

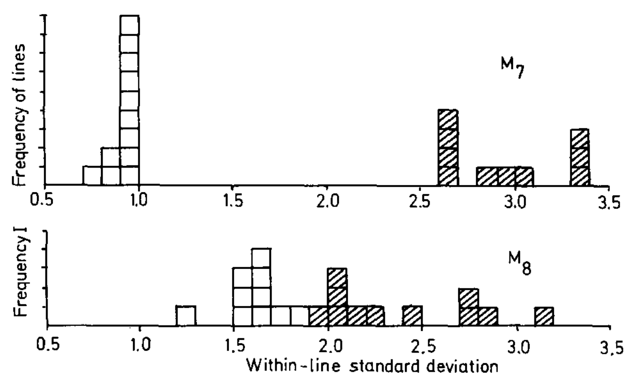

Fig. 2. Frequency distribution of within-line standard deviation of selected H (hatched block) and L (open block) lines in M_7 and their M_8 progeny lines for plant height

4 Responses of selected lines to different environmental conditions

In a separate experiment the H and L line groups were subjected to different environmental conditions to find to what extent the lines respond to those conditions. In this experiment, 8 lines each of higher and lower

Table 10. Analysis of variance of days to first flowering and plant height under different environmental conditions

Source of variation	Days to flower		Plant height	
	df	Mean square	df	Mean square
Environment	4	391.4457**	6	532.6335**
Line	15	1.5318	20	1.1880
H : L group	1	0.2207	1	0.3610
Within group	14	1.6254	19	1.2315
Environment × line	60	1.9013**	120	3.6586**
Env. × group	4	3.0786	6	2.2690
Env. × within group	56	1.8173**	114	3.7317**
Pooled within plot (error)	2488	0.1173[a]	4460	0.0875[a]

[a] Pooled within plot MS/n (n = harmonic mean of number of plants per plot)
** Significant at 1% level

Table 11. Analysis of variance of within-line standard deviations of two characters under different environmental conditions

Source of variation	Days to flower		Plant height	
	df	Mean square	df	Mean square
Between group (H : L)	1	7.6045**	1	10.6751**
Between environment	4	3.0206**	6	42.5004**
Env. × group	4	0.3580	6	1.4717*
Between line within group	14	0.2311	19	0.9575*
Residual[a]	56	0.2642	114	0.500

[a] Environment × line within group variance was taken as error
*, ** Significant at 5% and 1% levels, respectively

variability were use for days to first flowering, while the lines for plant height were the same as used for the selection experiment.

a) Variations in environmental response. In response to changes in environment, the number of days to first flowering and plant height of the lines changed significantly. Environmental response was measured by the change of line means in response to environments. Results of analysis of variance of line mean values of both characters showed that (1) variation due to environments was significant, suggesting that phenotypic expression of characters was variable in response to environmental conditions, and (2) environment × line interaction was significant, suggesting that the mode of environmental response was not the same among lines (Table 10). It may also be noticed that lines selected for higher and lower within-line variability showed little difference in an average performance over the different environments used.

Coefficient of regression of response of environmental value was computed for each line as a measure of phenotypic plasticity. Those for days to first flowering ranged from 0.77 to 1.21 and from 0.84 to 1.19 for H and L groups, respectively, and those for plant height from 0.68 to 1.35 and from 0.55 to 1.28 for H and L groups. The difference between the two groups with regard to the coefficient of regression was not statistically significant.

b) Environmental changes of within-line variability. The magnitude of within-line variability for days to first flowering and plant height differed according to environments. Analysis of variance of within-line standard deviations of the two characters showed that the mean squares due to the difference between H and L groups, environments and environment × group interaction were significant for plant height, while those due to groups and environments were only significant in days to first flowering (Table 11).

Line groups with high and low within-line variability in plant height were examined under seven environmental conditions including two changing temperature conditions. The environment E_6 was obtained in a phytotron where only the temperature fluctuated. The E_7 was obtained in a glass house under natural

conditions. It was observed that under the environment E_6, the magnitude of within-line variability was minimized in comparison to other environmental conditions.

Discussion

On account of high homozygosity of the M_6 parent plants, within-line variability observed in M_7 lines may be considered to be caused by developmental instability. The within-line standard deviation provides a quantitative estimate of developmental instability. There has been evidence showing that developmental instability is genetically controlled, as mentioned in the introduction. The present investigation also proves that developmental instability is governed by genes and can be induced by irradiation. The genetic control of developmental instability was also proved by the selection experiment, and heritability values for developmental instability were estimated.

Bradshaw (1965) considered that lack of stability is a manifestation of the more general phenomenon of plasticity, and stability could be used as synonymous with non-plasticity. This argument raised the question: do developmental instability and phenotypic plasticity have the same basis? Sensitivity in response to environmental change may be considered as phenotypic plasticity, and its quantitative expression may be given by the regression coefficients of response on environmental value. Two line groups selected for high and low within-line variability did not differ with regard to the regression coefficients (1.01 and 0.98 for H and L regarding plant height and 0.99 and 1.01 for H and L regarding days to first flowering). Scatter diagrams also clearly show that there is no difference between the two line groups in distribution of the regression coefficients

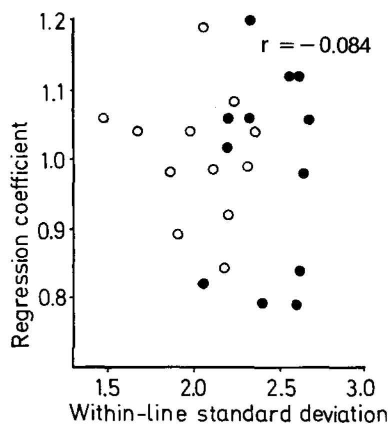

Fig. 3. Relationship between phenotypic plasticity and developmental instability in days to first flowering. (O: H line, O: L line)

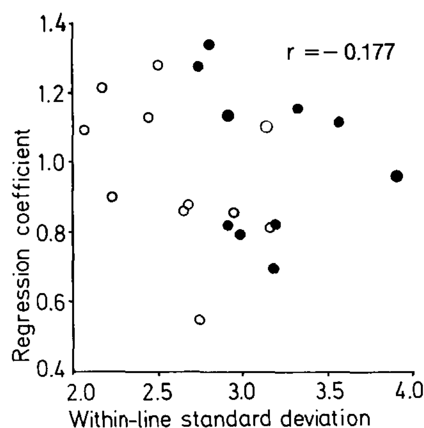

Fig. 4. Relationship between plasticity and developmental instability in plant height. (O: H line, O: L line)

though the two groups are well separated with regard to within-line standard deviation (Figs. 3 and 4). From these results, it may be concluded that developmental instability and phenotypic plasticity are governed by different genetic systems. Perkins and Jinks (1973) pointed out that the sensitivity to macro-environments and to micro-environments within the same treatments were uncorrelated. This might be compared with the relationship between phenotypic plasticity and developmental instability.

The present experiment also showed that under constant temperature conditions developmental instability was strongly expressed, while under changing temperature conditions it was lowered. Went (1953) reported similar phenomena in plants and recognised that fluctuation of day and night temperatures was a necessity for stable expression of characters in plants. Under the changing temperature condition E_7 (10/30 °C), however, it was found that the magnitude of developmental instability was increased. This probably implies that the night temperature of 10 °C was too low for the plants to tolerate.

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