ANALYSIS OF INDUCED QUANTITATIVE VARIATION II. THE RECOMBINANT INBRED LINES

N. R. INGRAM and J. L. JINKS

Department of Genetics, University of Birmingham, P.O. Box 363,
Birmingham B15 2TT, U.K.

Received 11.ix.81.

SUMMARY

On average, irradiation of F_2 seed of the cross of varieties 1 and 5 of *Nicotiana rustica* increases the additive genetical variation among inbred families in the F_6 by about 60 per cent. This agrees with the increase predicted from a hierarchical analysis of F_3 and F_4 families but it is greater than that predicted from an F_2 triple test cross analysis. This is as expected (Virk, Pooni, Ingram and Jinks, 1981). Both predictions, however, overestimate the increase observed in final height but underestimate those for leaf width and flowering time. The underestimates but not the overestimates can be accounted for if irradiation has increased the rate of recombination in the lineages derived from the irradiated F_2 seed.

The increased genetical variation following irradiation is accompanied by a shift in the distribution of F_6 family means towards the lower end; the range of family means is extended and more families have means which are lower than that of the lower scoring parent of the cross. But final height is again unusual in showing a corresponding increase in the range at the upper end of the distribution and more families with means which are higher than that of the taller parent of the cross.

1. INTRODUCTION

VIRK, Pooni, Ingram and Jinks (1981) estimated, by triple test cross and hierarchical inbreeding analyses, the increase in additive genetical variation induced in the F_2 of a cross between pure breeding varieties 1 and 5 of *Nicotiana rustica* by gamma radiation. These estimates have now been used to predict the properties of the recombinant inbred lines that can be derived from the irradiated and non-irradiated F_2 plants. The properties of the actual inbred lines have been assessed at the F_6 and RF_6 generations to compare with these predictions.

2. MATERIALS AND METHOD

Two randomly chosen plants were selfed from each of the 50 F_4 and RF_4 families in the 1979 experiment of Virk *et al.* (1981), and their progenies raised during the following winter. Two randomly chosen plants from each of these F_5 and RF_5 families were selfed resulting in a maximum of 200 F_6 and RF_6 families, that is, 8 F_6 or RF_6 families from each of the 25 control and irradiated F_2 seeds. Because of accidental losses the actual number of families in the F_6 and RF_6 fell short of this maximum by 12 and 11, respectively, but none of the original F_2 plants was represented by less than four of the possible eight families. These missing families have subsequently been regenerated but too late to be included in the current assessments.

The available 88 F_6 and 189 RF_6 families were grown in a completely randomised experiment during the summer of 1980. As additional controls 80 F_{15} families derived by single seed descent from an F_2 of the same cross of varieties 1 and 5 (Perkins and Jinks, 1973), the parental varieties themselves and their F_1 cross were also included in this experiment. Eight individually randomised plants were raised from each of the 460 families. The experiment was scored for leaf width (LW), flowering time (FT), height at flowering time (HFT) and final height (FH) as in the previous experiment.

The variance of the means of the 80 F_{15} families is a direct estimate of D , the additive genetical component of variation. For the F_6 and RF_6 families the variance of their means is $\frac{15}{16}D + \frac{15}{1024}H$. We can ignore the trivial contribution of the dominance component, H and treat this as an estimate of $\frac{15}{16}D$ from which we can obtain D .

3. RESULTS

Estimates of D and E_w , the environmental component of variation, from the F_{15} , F_6 and RF_6 for each of the four characters are given in table 1. The estimates of D from the 80 F_{15} families and the 88 F_6 families

TABLE 1

Estimates of the additive genetical and environmental components of variation for the F_6 , RF_6 , and F_{15} recombinant inbred lines

		F_6	F_{15}	RF_6
LW	D	10.9 $\pm$ 1.12	11.5 $\pm$ 1.79	17.1 $\pm$ 1.76
	E_w	7.1 $\pm$ 1.55	8.3 $\pm$ 2.26	7.1 $\pm$ 1.54
FT	D	39.4 $\pm$ 4.04	38.4 $\pm$ 6.00	57.9 $\pm$ 6.00
	E_w	17.4 $\pm$ 1.77	16.6 $\pm$ 2.59	15.4 $\pm$ 1.59
HFT	D	271.0 $\pm$ 27.80	275.2 $\pm$ 42.98	420.2 $\pm$ 43.22
	E_w	146.7 $\pm$ 15.05	143.0 $\pm$ 22.33	145.9 $\pm$ 15.00
FH	D	222.1 $\pm$ 22.79	192.3 $\pm$ 30.03	379.1 $\pm$ 38.89
	E_w	174.9 $\pm$ 17.90	153.8 $\pm$ 24.03	179.9 $\pm$ 18.51

are, as expected, very close for all characters the largest difference (FH) having a probability of only 40 to 50 per cent. In contrast the estimates from the 189 RF_6 families derived from the irradiated F_2 are all significantly greater than the corresponding estimates from the control F_6 . This increase in variance among RF_6 families ranges from 47 per cent for FT to 71 per cent for FH (table 2). The percentage increases in this variance that were expected from the triple test cross and hierarchical analyses of the F_2 and RF_2 (tables 3 and 5, Virk *et al.*, 1981) along with those observed in the F_6 and RF_6 (table 1) are summarised in table 2.

All three sources correctly predict the largest increase in variation following irradiation for FH but they all overestimate its magnitude. At the other extreme all three sources of prediction underestimate the increase in variation for LW and FT. For the fourth character HFT the predictions from the hierarchical analyses are good but the triple test cross again gives an underestimate. Over all four characters, therefore, the triple test cross gives predictions as good as, or better than the hierarchical analyses for two characters, FT and FH, but much worse for the other two, LW and

TABLE 2

Comparison of the percentage increase in the variance of recombinant inbred lines expected following irradiation of the F₂ with that observed

Source Character	Triple test cross	Expected		Observed RF ₆ /F ₆
		Hierarchical inbreeding* (1)	(2)	
LW	1	20	21	57
FT	26	23	17	47
HFT	9	76	55	55
FH	106	135	112	71

* (1) is based on the estimate of D . (2) is based on the estimates of D_1 and D_2 and the relative contributions that they make to the total additive genetic variation of recombinant inbred lines.

HFT. Reasons for these differences have been discussed in our earlier paper (Virk *et al.*, 1981) and will be returned to later (section 4).

The value of the increased variation in the RF₆ cannot be assessed without reference to the mean of the distribution relative to that of the F₆ control. The corresponding means for the four characters are, therefore, given in table 3. With the exception of flowering time the mean of the RF₆ is lower than that of the F₆, that is, on average the RF₆ plants are smaller and later flowering but for no character is the difference between the means significant.

The expected means of the F₆ and RF₆ are $m + \frac{1}{32}[h]$. If we ignore the trivial contribution of $\frac{1}{32}[h]$ we can estimate the F₆ and RF₆ means from the hierarchical inbreeding experiment as $2\bar{F}_4 - \bar{F}_3$ and $2\bar{RF}_4 - \bar{RF}_3$, respectively. The differences between these estimates for the four characters are given in table 4. They agree with the differences observed for LW, HFT and FH but not for FT but all the expected differences are like those observed, in being non-significant. We neither expect nor observe, therefore, any significant directional changes as a result of mutation.

Not even an approximate estimate of m can be obtained from the families of the F₂ or RF₂ triple test crosses. We can, however, estimate $m + \frac{1}{2}[h]$ from both which, if we assume $\frac{1}{2}[h]$ is approximately the same for the control and irradiated material, allows us to estimate the difference between the two m 's. These differences are given in table 4. Again all are negative, showing a fall in the expected means of the RF₆ relative to those of the F₆, but two of them, FT and HFT, are significant.

There are two further ways in which we can examine the outcome in the F₆ and RF₆; one is to compare the number of families whose means

TABLE 3

The means of the F₆ and RF₆ recombinant inbred lines

Character	F ₆	RF ₆	Difference
LW	17.01 ± 0.75	16.07 ± 0.85	-0.94 ± 1.13
FT	47.14 ± 1.25	47.23 ± 1.39	+0.09 ± 1.87
HFT	76.05 ± 3.30	74.18 ± 4.06	-1.87 ± 5.23
FH	131.64 ± 2.84	129.37 ± 3.89	-2.27 ± 4.82

TABLE 4

Differences in the expected means of the F_6 and RF_6 predicted from the hierarchical inbreeding and triple test cross experiments

Character	Hierarchical	Triple test cross
LW	-0.21 ± 0.99	-0.25 ± 0.23
FT	-0.82 ± 2.35	-1.38 ± 0.44
HFT	-4.32 ± 6.08	-2.52 ± 1.12
FH	-3.84 ± 5.99	-1.62 ± 1.07

fall outside of the parental range of varieties 1 and 5 (table 5); the other is to compare the ranges of family means (table 6). The frequencies in table 5 are not particularly informative for LW and FT because all of the F_6 and RF_6 families fall outside of the parental range. The only difference, therefore, is a small shift in the distribution of the RF_6 families towards the lower end of the range ($<\bar{P}_2$) but for neither character is this significant. For HFT and FH, however, there is a significant increase in the frequency of RF_6 relative to F_6 families falling outside of the parental range. For HFT this is accompanied by a shift of the distribution towards the lower end of the range but for FH the increase occurs equally at both ends of the distribution. For all characters, therefore, there is an increase in the frequency of families at the lower end of the distribution ($<\bar{P}_2$) following irradiation of the F_2 , but for only one character FH is there a corresponding increase at the upper end of the distribution ($>\bar{P}_1$).

The ranges of F_6 and RF_6 family means in table 6 give a similar picture. The range is significantly extended at the lower end of the distribution in

TABLE 5

The frequency of F_6 and RF_6 families whose means fall outside of the parental range of varieties 1 and 5

Character		$<\bar{P}_2$	$>\bar{P}_1$	Total outside parental range
LW	F_6	124	64	88
	RF_6	133	56	189
FT	F_6	52	136	88
	RF_6	61	128	189
HFT	F_6	52	103	155
	RF_6	79	91	170
FH	F_6	63	50	113
	RF_6	75	62	137

TABLE 6

The range of family means in the F_6 and RF_6 generations

Character		Maximum	Minimum
LW	F_6	25.81 ± 0.48	6.25 ± 0.58
	RF_6	25.50 ± 0.91	4.17 ± 0.33
FT	F_6	61.88 ± 1.46	36.00 ± 0.33
	RF_6	62.62 ± 2.02	34.25 ± 0.31
HFT	F_6	120.57 ± 3.55	45.12 ± 3.58
	RF_6	117.06 ± 2.18	37.17 ± 2.08
FH	F_6	163.62 ± 2.74	78.00 ± 4.17
	RF_6	167.63 ± 2.33	65.37 ± 3.82

the RF_6 for all four characters. It is extended at the upper end for FT and FH and contracted for LW and HFT but in no case is the difference between the F_6 and RF_6 significant.

4. CONCLUSIONS

On average over the four characters studied, irradiation of F_2 seed has increased the genetical variation among inbred families in the F_6 by about 60 per cent (table 2). This agrees well with the average predicted increase from the hierarchical analysis although within this average the increase for one character (FH) was overestimated and for two others (LW and FT) underestimated. The average increase predicted from the triple test cross analysis was lower than the 60 per cent observed in the F_6 but again within this average the increase for FH was overestimated and those for the remaining three characters underestimated, the predictions for LW and HFT being particularly low (table 2).

That the triple test cross analysis gives the poorer predictions comes as no surprise since the predictor, the estimate of D , is known to be biased by any dominance arising from mutation at loci at which the testers do not differ (Virk *et al.*, 1981). These triple test cross and hierarchical design predictors are however consistent in overestimating the increase for one character and underestimating it for two others and this suggests a common cause. One such possibility is an interaction between the genotypes and seasonal difference between 1979, when the triple test cross and hierarchical experiments were grown, and 1980, when the F_6 and RF_6 families were assessed.

A second possibility, that would account for the underestimates for LW, FT and HFT, but not the overestimates for FH, is the repulsion linkages which were detected in the hierarchical experiment (Virk *et al.*, 1981). The linkages themselves could not explain the underestimates but if the irradiation had led to an increase in the rate of recombination in the lineages derived from the RF_2 it could account for the unpredictably high increase in the genetical variation in the RF_6 relative to that in the F_6 .

In general the increased genetical variation in the RF_6 has led to a higher frequency of inbred families with lower than average means and the occurrence of extreme families with means that are lower than any that can be found in the F_6 (tables 5 and 6). The only exception is again final height where there is a similar increase in frequency but a smaller increase in range at the upper end of the distribution. On the whole, the predictions from the triple test cross and hierarchical inbreeding analyses are in agreement with these observed directional shifts in the means of the distributions, although few of the predicted changes are significant (table 4).

Acknowledgments.—We acknowledge receipt of an SRC studentship to N.R.I.

5. REFERENCES

- PERKINS, J. M., AND JINKS, J. L. 1973. The assessment and specificity of environmental and genotype-environmental components of variability. *Heredity*, 30, 111–126.
 VIRK, D. S., POONI, G. S., INGRAM, N. R., AND JINKS, J. L. 1981. Analysis of induced quantitative variation. I. Hierarchical and triple test cross analysis. *Heredity* (in the press).