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Genetic fine-structure of the *GA-1* locus in the higher plant *Arabidopsis thaliana* (L.) Heynh

BY M. KOORNNEEF, J. VAN EDEN, C. J. HANHART AND A. M. M. DE JONGH

Department of Genetics, Agricultural University, Generaal Foulkesweg 53 6703 BM Wageningen, The Netherlands

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SUMMARY

Non-germinating gibberellin (GA) responsive mutants are a powerful tool to study genetic fine structure in higher plants. Nine alleles (EMS- and fast neutron-induced) of the *ga-1* locus of *Arabidopsis thaliana* were tested in a complete half-diallel. No wild type 'recombinants' were found in the selfed progeny of 9 homoallelic combinations (in total 3×10^5 plants); in the progenies from the 36 selfed hetero allelics the wild type frequency ranged from zero to $6{\cdot}6 \times 10^{-4}$. These frequencies allowed the construction of an internally consistent map for five different sites representing eight alleles. The ninth allele covered three sites and thus behaved like an intragenic deletion. The estimate of the total genetic length of the *ga-1* locus was 0·07 cM. The order of the sites was also clearly reflected by the association with proximal outside markers. On the assumption that wild type gametes predominantly arise from reciprocal events, it was shown that a cross-over within the *ga-1* locus leads to positive interference in the adjacent region.

The results are discussed with respect to the mutagen used, the frequencies found in other plant and Drosophila genes, and the possible occurrence of gene conversion.

1. INTRODUCTION

Studies on the genetic fine structure of genes have given useful information about their physical structure, the nature of crossing-over and the nature of particular mutations.

Among eukaryotes, fungi with their high 'resolving power' (high numbers of spores and the availability of 'self-detection' systems) have proved ideal tools for this type of study by means of complementation, recombination and gene conversion analysis (for reviews see Catcheside, 1977, and Fincham, Day & Radford, 1979).

Studies in Drosophila led to qualitatively comparable results, which suggests a common molecular mechanism of meiotic recombination (Hilliker & Chovnick, 1981). Fine structure analyses in higher plants are rare due to the difficulties in

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handling the enormous numbers of plants required. For this reason the most extensive studies are with pollen-grain markers where very large numbers can be easily scored: waxy (*wx*) in maize (Nelson, 1958, 1962, 1968, 1975; Amano, 1968), *glx* in barley (Rosichan *et al.* 1979; Nilan, Kleinhofs & Warner, 1981) and *Adh 1* in maize (Freeling, 1976, 1978). Seedling characters have been studied in maize (*gl-1*) by Salamini & Lorenzoni, 1970, and in barley (*cer-cqu* region) by Wettstein-Knowles and Søgaard, 1980. These genes control wax biosynthesis and deposition. Jörgensen & Jensen (1979) studied the mildew resistance gene *ml-o* in seedlings of barley.

For the analysis of intragenic recombination the recessive non-germinating gibberellin (GA) responsive mutants (gene symbol *ga*) isolated in *Arabidopsis thaliana* (Koornneef & van der Veen, 1980) and tomato (Koornneef *et al.* 1981) seem particularly suitable. For germination these mutants require gibberellin and without further addition of GA they develop into typical dwarfs, but with GA sprays at weekly intervals, they develop into the wild type phenotype or nearly so.

Depending on the allele, the environmental circumstances during seed development and the germination conditions, varying degrees of germination occur without GA ('leakiness' of some mutant alleles). However, subsequently such germinators invariably develop into dwarfs.

The suitability of the *ga*-system for intragenic analysis, among other things its high resolving power, derives from the following aspects:

(1) Recombinants can be identified with certainty as these are 'self-detecting' as germinating seedlings, which are much more vigorous than the mostly rare spontaneous germinators of the *ga*-mutants. In cases of doubt, the dwarf versus non-dwarf contrast is a definite criterion for mutant versus wild type.

(2) Several types of outside markers are available to study joint segregation.

(3) As only few seedlings emerge, sowing can be done closely spaced in Petri dishes and on artificial media (like filter paper, agar, perlite, etc.).

Special advantages of *Arabidopsis* for this type of research are:

(1) It is self-fertilizing under greenhouse conditions.

(2) 1000 up to 5000 of the small seeds go into a 9 cm Petri dish.

(3) Also mutants may produce as many as 5000 seeds per plant.

(4) The short generation interval (2 months for the early ecotypes used) and the small plant size allow the rapid production of large quantities of seeds in climate chambers.

(5) A high number of independently induced non-germinating mutant alleles obtained with ethylmethanesulphonate (EMS), X-rays and fast neutrons (FN) are available at three different loci (Koornneef & van der Veen, 1980). All mutants are induced in the same genetic background (ecotype: Landsberg '*erecta*').

Two preliminary experiments, included in this report as the 1st and 2nd experiment, had indicated the substantial occurrence of wild type plants in the progeny of heteroallelic *ga-1* plants (Koornneef, 1979), in contrast to experiments with *ga-3* mutants (Koornneef & Janssen, unpublished), which yielded only very

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few recombinants. Therefore, in the present study, nine independently induced mutants at the *ga-1* locus were analysed in a complete half-diallel crossing-scheme. They included both EMS and FN induced mutants and one germinating *ga-1* dwarf (a clearly leaky allele); otherwise they were chosen at random.

2. MATERIAL AND METHODS

(i) *Mutant alleles*

The *ga-1* mutants used are listed in Table 1 with respect to mutagen and 'spontaneous germination' (i.e. without addition of GA).

Table 1. Ga-1 *mutants used for fine structure analysis, mutagen used and germination* (%) (*without adding GA*)

Mutant allele	Mutagen*	Germination (%)
NG4	EMS	0
NG5	EMS	1
A428	EMS	15
Bo27	EMS	1
d69	EMS	0
d352	EMS	59
6·59	FN (69 Gy)	0
29·9	FN (47 Gy)	0
31·89	FN (67 Gy)	0

*Ethylmethanesulphonate (EMS): 10 mM, 24 h, 24 °C, in the dark.
Fast neutrons (FN): In Gy (Gray) dose as indicated. 1 Gy = 100 rad.

(ii) *Conditions of culture*

The seeds were sown in 9 cm Petri dishes, either equally spaced (25 seeds/dish) or scattered (250–5000 seeds/dish), on perlite saturated with a standard mineral solution, composed as described by Oostindiër-Braaksma & Feenstra (1973). To break seed dormancy the dishes were kept at 2–4 °C for 4–6 days. Germination was at approx. 24 °C under continuous illumination by fluorescent light tubes (Philips TL 57) at approx. 8 W . m^{-2}. Eight days after incubation at 24 °C the seedlings were scored and when necessary transplanted into soil. To obtain F_1 seeds parental mutant lines were grown and crossed in an air-conditioned greenhouse. For the emasculation technique see Feenstra (1965). To exclude as much as possible unwanted selfing, in the third large experiment parental lines were used, which carried an extra recessive marker. Available for this were lines 6·59 with *ms* (male sterility) and NG5 with f_{ca} (late flowering) and *ap-2* (apetala without petals), and the recessivity of non-germination to the germination of d352. F_1 seeds were sown as described. After a week at 24 °C, checking for wild-type contaminants and scoring of 'spontaneous germination' was done, after which they received GA_{4+7} (mixture of gibberellin GA_4 and GA_7) up to a final concentration of 10 μM in the medium to induce complete germination. A week later the F_1 seedlings were transplanted into soil in an isolated climate chamber (standardized conditions;

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unwanted cross-fertilization excluded). Here temperature was 23 °C, relative humidity approx. 80% and continuous light was by TL 33 fluorescent tubes (12–17 W . m^{-2}) supplemented with incandescent bulbs (4–5 W . m^{-2}). Two weeks after transplanting (dwarf phenotypes clearly visible) GA_{4+7} (100 μM) was sprayed at weekly intervals, in total 2 or 3 times. Harvested F_2 seeds were stored at room temperature for at least 2 months.

(iii) *Testing for wild type recombinants*

Seeds of selfed parental lines and F_2 populations derived from hetero-allelic crosses were sown under the conditions described above. These conditions permit also 100% germination of wild type and of *ga-1* mutants (the latter only when 10 μM GA_{4+7} is included in the medium). The seeds were scattered into Petri dishes at numbers ranging from approx. 250 (F_2's involving the 'germinating allele' d352) up to approx. 5000 (F_2's without 'spontaneous germination'). Counting of the seeds was on the basis of seed weight, determined separately in each experiment for each parental line and each F_2 population. The weight of 1000 seeds is mostly 20–25 mg. Wild-type seedlings are easily recognized, because upon germination they are much more vigorous than the *ga-1* mutants, of which in the case of some 'leaky' alleles a certain proportion of the seeds will germinate (Plate 1). All presumed wild type seedlings (including cases of doubt) were transplanted into soil to check their non-dwarf phenotype. In Expt 3 all wild type recombinants were also progeny tested to check for the expected segregation of *ga-1* mutants: wild type contaminants are not expected to be heterozygous for the *ga-1* locus.

Since the intragenic recombinant gametes are rare and only one half of these are (dominant) wild types, the proportion of wild types found in F_2 populations is a direct estimate of the recombinant fraction (r). See:

$$\frac{r}{2}\,(\text{maternal})+\frac{r}{2}\,(\text{paternal})-\frac{r^2}{4}\simeq r.$$

For calculating the 95% confidence limits a Poisson distribution is assumed.

(iv) *The association of outside markers with intragenic recombinants*

To distinguish between cross-overs and gene conversions, closely linked outside markers are required at both sides of the locus studied. For *ga-1* the situation is not ideal, as this locus is at the end of the chromosome 4 map and the nearest markers are rather distant.

Line NG5 ($ga\text{-}1^1/ga\text{-}1^1$) was provided with the proximal outside markers f_{ca} (late flowering) and *ap-2* (apetala, reduced petals), *f* and *ap* for short. The map positions (in cM) are: *ga-1* – 27·2 – *f* – 30·0 – *ap* (Koornneef, de Bruine & Goetssch, 1980). NG5 was then crossed with the other eight lines carrying *ga-1* alleles. This yielded F_1's $ga\text{-}1^1 . f . ap/ga\text{-}1^{\times} . F . Ap$.

In F_2 the wild-type recombinants (with respect to the *ga-1* locus) arise from one recombinant gamete (*Ga-1*) and one non-recombinant gamete (*ga-1*). Both gametes

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can further carry *F.Ap*, *F.ap*, *f.Ap* or *f.ap*. So recombinants of $4\times4 = 16$ different genotypes (always combinations of *Ga-1* and *ga-1* gametes) may occur. Of these types only *Ga.1.f.ap/ga-1.f.ap* can be directly identified. To determine the other genotypes F_3 progeny testing is required. To distinguish the coupling and repulsion diheterozygotes 40 plants were raised per F_3 line, and when necessary another 40 plants.

With the homozygotes gamete assessment is straightforward: e.g. *F.ap/F.ap* necessarily has orginated from one *Ga-1.F.ap* and one *ga-1.F.ap* gamete. With the four monoheterozygotes and the two diheterozygotes, the linkage phase must be taken into account: e.g. *F.Ap/f.ap* arose from *ga-1.F.Ap* and *Ga-1.f.ap*, when the *ap* and *f* locus are in repulsion with *ga-1*. This can be applied to *F/f* heterozygotes but not to genotypes which are only heterozygous *Ap/ap*, since *ap* segregates almost independently from *ga-1*. In other words, complete gamete assessment cannot be done for *F.Ap/F.ap* and *f.Ap/f.ap*. To save work, none of the *f/f* phenotypes were progeny-tested which implies that *f.Ap/f.ap* also could not be distinguished from *f.Ap/f.Ap*. So in total $4\times4-5 = 11$ gamete combinations were completely assessed, the other five being assessed only for the loci *ga-1* and *f*. From these data (Table 5) the frequencies of the different types of recombinant *Ga-1* gametes with respect to *f* and *ap* are then estimated by a maximum-likelihood procedure (in view of the incomplete assessment). The figures also allow an estimate of the recombination fraction between *f* and *ap* in gametes that did not originate from intragenic recombination at the *ga-1* locus. Estimates of 'ordinary' recombination fractions were calculated by the method of maximum likelihood from F_2 segregation data derived from the cross *ga-1*^{1}*.f.ap/ga-1*^{1}*.f.ap* × wild type.

3. RESULTS

(i) *Intragenic complementation*

The germination percentages of both F_1's and F_2's of all 36 heteroallelic combinations did not exceed the percentages of the higher parent. So there is no indication of even partial intragenic complementation. The same holds for 23 other independently induced *ga-1* alleles as far as mutually tested.

(ii) *Frequencies of wild-type recombinants*

Table 2 presents the frequencies of wild-type *ga-1* alleles found in F_2's of all 36 heteroallelic combinations of the nine *ga-1* mutants. In Table 2 the results of the two preliminary experiments (Koornneef, 1979) and of the large third experiment have been pooled. Only the results from NG4 × 29·9 in Expt 2 were omitted as they were strikingly at variance with all other results, probably as a result of selfing admixture. In all other cases no significant differences were found between the different experiments.

Not a single recombinant was detected among the homoallelic combinations (3×10^5 seeds tested in total, indicating a spontaneous reversion frequency $< 10^{-5}$

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Table 2. *Frequencies of wild type recombinants* ($\times 10^{-4}$) *in selfed progenies of hetero allelic crosses of* ga-1 *mutants, the 95% confidence intervals (between parentheses) and the approx. number of* F_2 *seeds tested*

Allele	NG5	NG4	d69	A428	d352	6·59	Bo27	31·89
29·9	1·6 (0·8–2·8)	4·9 (3·2–7·1)	6·6 (4·5–9·5)	5·6 (4·6–6·8)	5·6 (3·7–8·0)	5·1 (3·8–6·7)	6·1 (4·9–7·5)	3·4 (2·0–4·5)
	75300	55300	45300	178400	50300	100300	156600	87300
	NG5	4·9 (3·6–6·3)	4·1 (3·0–5·4)	5·0 (3·5–5·8)	4·5 (3·0–6·5)	5·2 (3·9–6·9)	5·5 (4·1–7·4)	3·3 (3·2–4·7)
		111000	125300	149100	62300	97300	83000	85300
		NG4	0·0 (0·0–0·5)	1·9 (1·2–2·8)	0·6 (0·1–1·7)	0·5 (0·1–1·7)	2·5 (1·6–3·7)	0·0 (0·0–0·3)
			65300	130900	50300	43300	99800	92900
			d69	2·0 (1·2–3·2)	1·6 (0·7–3·1)	0·5 (0·1–1·3)	1·2 (0·6–2·2)	0·0 (0·0–0·4)
				85300	50300	80300	85300	80300
				A428	0·0 (0·0–0·9)	0·0 (0·0–0·2)	0·6 (0·2–1·1)	0·0 (0·0–0·2)
					34300	127100	126600	162300
					d352	0·0 (0·0–0·6)	0·6 (0·1–1·7)	0·0 (0·0–0·6)
						46600	50300	50300
						6·59	0·1 (0·0–0·6)	0·0 (0·0–0·3)
							89300	109900
							Bo27	0·0 (0·0–0·3)
								101800

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($P < 0{\cdot}05$)). In the majority of the heteroallelic combinations the frequencies of wild type recombinants range from 1×10^{-5} up to $6{\cdot}6 \times 10^{-4}$. In analogy to similar results obtained by previous authors in other organisms, it is concluded that these elevated frequencies reflect intragenic recombination which may include gene conversion.

Table 3. *Summarized recombinational matrix (frequencies* $\times 10^{-4}$*, between parentheses 95 % confidence intervals) compiled by adding together the figures of alleles that do not show recombinants among each other (31·89 not included)*

Allele	NG5	NG4 d69	A428 d352 6·59	Bo27
29·9	1·6 (0·8–2·8)	5·7 (4·3–7·3)	5·4 (4·7–6·3)	6·1 (4·9–7·5)
NG5	—	4·4 (3·6–5·4)	4·8 (4·0–5·2)	5·5 (4·1–7·4)
NG4, d69	—	—	1·3 (1·0–1·7)	1·9 (1·3–2·6)
A428, d352, 6·59	—	—	—	0·4 (0·2–0·7)

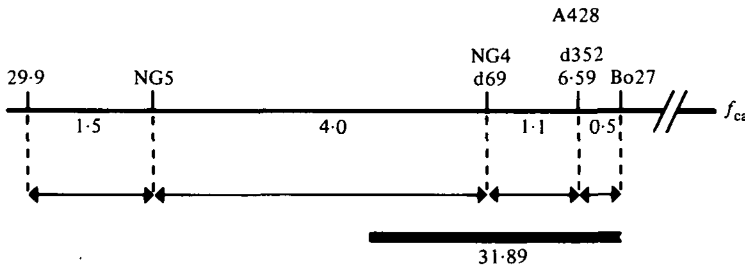

Fig. 1. Genetic fine structure map of the *ga-1* locus. Distances: cM $\times 10^{-2}$.

Table 3 gives the results upon pooling alleles that did not show recombination among each other. These are (*a*) NG4 and d69 and (*b*) A428, d352 and 6·59. The results with 31·89 are not included as they clearly stand apart (see below). It appears possible to construct an internally consistent map (Fig. 1) from the data of Table 3. The distances in Fig. 1 were calculated from the direct estimate of a particular segment and the estimates of its bordering segments, e.g. with an allele order

$$\text{A} \overset{1}{—} \text{B} \overset{2}{—} \text{C} \overset{3}{—} \text{D}, \quad r_1 = (r^{AB} + r^{AC} - r^{BC})/2$$

and

$$r_2 = (r^{BC} + r^{AC} - r^{AB} + r^{BD} - r^{CD})/3.$$

When using this procedure the sum of the intervals becomes $7{\cdot}1 \times 10^{-4} = 0{\cdot}071$ cM. From the results obtained allele 31·89 can be interpreted as an intragenic deletion which covers half of the present *ga-1* map (Fig. 1). The data give no indication for the occurrence of map expansion.

(iii) *Distribution of outside markers*

As *ga-1* is located close to the end of chromosome 4 no distal markers are yet available. The linkage relations of *ga-1* with the two proximal markers *f* and *ap*

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have been derived from the F_2 (*ga-1*^{1}.*f*.*ap*/*ga-1*^{1}.*f*.*ap*) × wild type as shown in Table 4. The estimates of recombination fractions agree with previously published results (Koornneef *et al.* 1980).

Table 4. *Linkage analysis of the F_2 from* (ga-1^1/ga-1^1, f/f, ap/ap) × *wild type*

Plant phenotype	F_2 frequencies	Gamete genotypes	Estimated frequencies
Ga-1/. F/. Ap/.	516	*ga-1 f ap* / *Ga-1 F Ap*	0·481 ± 0·020
ga-1/ga-1 F/. Ap/.	91		
Ga-1/. f/f Ap/.	61		
Ga-1/. F/. ap/ap	99	*ga-1 F Ap* / *Ga-1 f ap*	0·234 ± 0·017
ga-1/ga-1 f/f Ap/.	63		
ga-1/ga-1 F/. ap/ap	17		
Ga-1/. f/f ap/ap	79	*ga-1 f Ap* / *Ga-1 F ap*	0·215 ± 0·017
ga-1/ga-1 f/f ap/ap	50		
	986	*ga-1 F ap* / *Ga-1 f Ap*	0·070 ± 0·012

Estimates of recombination fractions: *ga-1 – f*: 0·304 ± 0·018; *ga-1 – ap*: 0·449 ± 0·022; *f – ap*: 0·284 ± 0·018.

The frequencies of *Ga-1* recombinants with respect to both *f* and *ap*, isolated from F_2's of NG5 × other *ga-1* mutants are presented in Table 5. No significant differences were found for the distribution of the different gametes between the different F_2's. Heterogeneity $\chi^2_{84} = 82{\cdot}6$ ($0{\cdot}4 < P < 0{\cdot}6$). Therefore, the crosses were pooled (see totals in Table 5) to estimate the marker distribution over the *Ga-1* recombinants (Table 6). However, the data obtained with 29·9 were not included as this allele is located at the other side of NG5 (see Fig. 1). From the predominance of the recessive *f* allele with the *Ga-1* recombinant and on the basis of the intragenic map, the order of the alleles with respect to *f* and *ap* is most likely to be 29·9 – NG5 – other *ga-1* alleles – *f* – *ap*.

Assuming that only reciprocal cross-overs give rise to *Ga-1* alleles (i.e. no conversions), it appears that a cross-over within the *ga-1* locus leads to a significant decrease of cross-overs in the adjacent *ga-1* to *f* region (positive interference), but does not affect recombination between *f* and *ap* (see Table 6; 2nd and 4th column). In addition the recombination fraction between *f* and *ap* which can be estimated from the non-recombinant gametes ($r = 0{\cdot}201 \pm 0{\cdot}032$) is in good agreement with that from the recombinant gametes ($r = 0{\cdot}215 \pm 0{\cdot}017$ in Table 4).

Conversely, the position of 29·9, distal to NG5, is expected to lead to a proportional excess of *F* as outside marker. However, the number of plants (12) is too small to confirm this.

4. DISCUSSION

An internally consistent map could be constructed from the frequencies of wild types (intragenic recombinants) occurring in the selfed progenies of plants heteroallelic for mutations at the *ga-1* locus. The deviating results from crosses with allele 31·89 can be readily explained by the hypothesis that 31·89 is an intragenic

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Table 5. *Genotypes of F_2* Ga-1 *recombinant plants derived from crossing* ga-1[1]/ga-1[1] f/f ap/ap *with other* ga-1 *alleles*

Gamete genotype: Recomb. (*Ga-1*)	Non-recomb. (*ga-1*)	Parent line (*ga-1* allele tested with NG5): 29·9	NG4	d69	A428	d352	6·59	Bo27	31·89	Total	Total (29·9 excluded)
F Ap	*F Ap*	2	1	2	1	3	0	2	2	13	11
F Ap	*f ap*	1	4	2	1	1	3	0	0	12	11
F Ap	*f Ap*	0	0	0	0	0	0	0	1	1	1
f Ap	*F Ap*	0	3	5	3	1	3	4	3	22	22
f Ap	*F ap*	0	2	0	0	2	1	1	1	7	7
F ap	*f ap*	0	2	2	1	0	0	2	0	7	7
F ap	*F ap*	0	1	0	0	0	0	0	0	1	1
F ap	*f Ap*	1	1	0	1	0	0	0	1	4	3
f ap	*F Ap*	0	16	13	4	7	11	14	8	73	73
f ap	*f ap*	2	7	11	10	4	8	9	2	53	51
f ap	*F ap*	1	3	2	3	3	2	2	2	18	17
F $^{Ap}_{ap}$	*F* $^{ap}_{Ap}$*	1	2	2	0	0	2	2	2	11	10
f ·	*f* ·†	4	3	10	3	4	7	6	6	43	39
		12	45	49	27	25	37	42	28	265	253

* Single heterozygous *Ap/ap* could not be completely assessed (see text).
† Homozygous *f/f* were not tested (see text), so only *f/f, ap/ap* was completely assessed.

Table 6. *Outside marker distribution of* Ga-1 *recombinants from the pooled F_2's from crosses* ga-1[1]/ga-1[1], f/f, ap/ap × ga-1[1]/ga-1[1], F/F, Ap/Ap

(The F_2 from NG5 × 29·9 was excluded (see text).)

Marker association of *Ga-1* recombinants	Frequency of occurrence	Gamete originates after crossing-over between*	Frequencies expected on the basis of random crossing-over†
f ap	0·614 ± 0·037	No crossing-over	0·481 ± 0·020
F Ap	0·101 ± 0·020	*ga-1/f*	0·234 ± 0·017
f ap	0·212 ± 0·031	*f/ap*	0·215 ± 0·017
F Ap	0·073 ± 0·017	*ga-1/f* and *f/ap*	0·070 ± 0·012

* No gene conversion is assumed.
† From Table 4.

deletion. It may be significant in this respect that 31·89 hardly shows any germination (Table 1), which indicates non-leakiness. It is in no way a general rule that intragenic recombinant frequencies are additive like we found for the *ga-1* locus (see, for example, Carlson, 1959; Fincham *et al.* 1979). In higher plants this lack of additivity was conspicuous for the *Adh1* locus in maize (Freeling, 1976, 1978) and was also noted by Nelson (1968) for *wx* in maize, which made it impossible to construct a map on the basis of intragenic recombinant frequencies.

The frequencies of wild type recombinants among gametes found for the *ga-1* locus in *Arabidopsis* (up to $r/2 = 3{\cdot}3 \times 10^{-4}$) are comparable to those found for other

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well studied plant genes (over $\mathbf{r}/2 = 10 \times 10^{-4}$ for *wx* in maize (Nelson, 1968), up to 20×10^{-4} for *glx* in barley (Nilan *et al.* 1981), up to $6{\cdot}6 \times 10^{-4}$ for *Adh*1 (Freeling, 1978) and up to $5{\cdot}8 \times 10^{-4}$ for *gl-1* (Salamini & Lorenzoni, 1970)). The size of the plant genes including *ga-1* corresponds to the large Drosophila loci like *lz* (Green & Green, 1956), *r* (Carlson, 1971), *dp* (Grace, 1980). This suggests that plant genes in general are 'large'. However, it should not be overlooked that one of the reasons for studying these particular loci was the availability of a number of different alleles, which implies that preferentially loci were chosen with relatively high mutation frequencies. In this connection it is interesting that the *ga-3* locus has a significantly lower mutation frequency than *ga-1* and also shows lower frequencies of intragenic recombinants (Koornneef & Janssen, unpublished). A relation between the genetic size of a gene and its induced mutation frequencies has also been suggested by Chovnick, Ballantyne & Holm (1971) who compared the *ma-l* and *ry* locus of Drosophila.

Amano (1968) found, for his 3 fast-neutron induced *wx* mutants in maize, no wild-type pollen grains from the heteroallelic plants and, moreover, a reduced transmission of the affected chromosome. This was in contrast to his 9 EMS-induced *wx* mutants, 7 of which were able to recombine with the same *wx* tester allele, and suggests that FN may preferentially induce gross chromosomal damage like large deletions. There is an apparent discrepancy with our results, where 2 FN-induced and all 6 EMS-induced alleles show recombination, whilst a third FN-induced allele shows only reduced recombination. This discrepancy may be explained by a difference in mutant selection procedure. Our mutants were selected in M_2 lines (the progenies from selfed mutagen-treated M_1 plants), so that gross chromosomal aberrations have been sieved out due to their low transmissibility through the pollen (certation). In contrast, Amano's *wx* mutants were all identified upon pollination of M_1 plants with pollen from (non-treated) homozygous recessive testers. Therefore, the conclusion seems warranted that FN induces more gross genetic damage than EMS does, but that mutations that pass a certation sieve (i.e. those selected in M_2) are in general not of that type.

The isolation of leaky alleles induced by both EMS and FN at the *ga-1* locus (germinating dwarfs) (Koornneef & van der Veen, 1980; Koornneef, Dellaert & van der Veen, 1982*a*) also points to the recovery of FN-induced alleles with minor genetic damage.

As outside markers were not available at both sides of the *ga-1* locus (*f* and *ap* are both proximal), it was *a priori* impossible to exclude conversion (not followed by a reciprocal event) as a source of wild-type gametes. Let x and y be *ga-1* mutants and f the outside marker in the order x–y–f. Then the heterozygote

$$\frac{x \quad f}{\overline{y \quad F}}$$

may produce *Ga-1* .*f* gametes by (1) reciprocal crossing-over between x and y, (2) conversion of x, and (3) conversion of y followed by crossing-over between *ga-1*

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and *f*. It may produce *Ga-1*.*F* gametes by (1) conversion of *y*, (2) conversion of *x* followed by crossing-over between *ga-1* and *f*. For the order *y–x–f* this relationship is interchanged. It follows that an excess of *f* alleles (or *F* alleles) among *Ga-1* recombinants only allows a conclusion about the order of *x* and *y* when reciprocal events are more frequent than conversion events. When the conversion events are in excess and when in addition conversion of the proximal site is far more frequent than at the distal site, the order inferred on the assumption of only reciprocal events may be erroneous.

On the other hand, no indication was obtained for the occurrence of map expansion. This phenomenon is explained by the relatively frequent occurrence of co-conversion of closely 'linked' sites (Holliday, 1964). Its absence is an indication that recombinants with parental flanking markers (conversions) are relatively rare among the randomly sampled (wild type) recombinant gametes. Moreover, the *ga-1* gene is relatively large and so are the intervals between the sites. With larger intervals these conversions are expected to be relatively infrequent, as the recombination event has a greater probability of being detected as a crossover than as a pure conversion event (Chovnick *et al.* 1971; Hilliker & Chovnick, 1981). However, with some large loci both in *Drosophila* (*r* locus, Carlson, 1971) and in maize (*wx*, Nelson, 1968, 1975; *gl-1*, Salamini & Lorenzoni, 1970) there seem to be exceptions to this rule.

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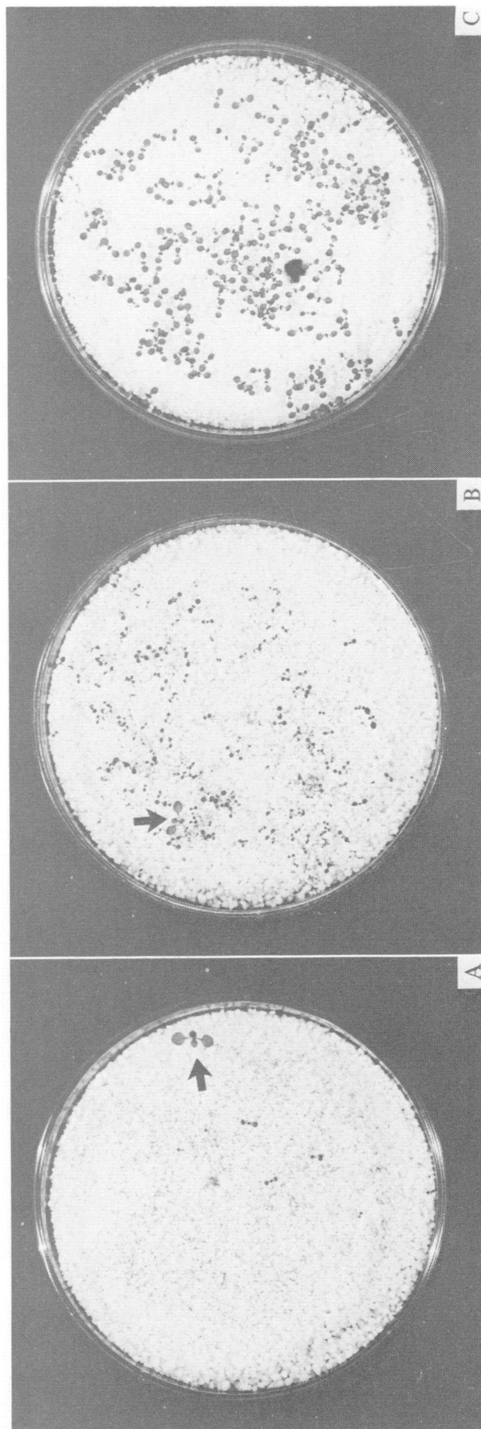

Petri dishes with F_2 progenies of 6·59 × NG4 (A), 6·59 × NG5 (B) and d352 selfed. (C) Arrows indicate wild type seedlings.

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