INDUCED PREMEIOTIC EXCHANGE OF LINKED MARKERS IN THE ANGIOSPERM ARABIDOPSIS[1,2,3]

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Received October 5, 1964

IT is well known that X rays produce various types of chromosomal rearrangements and deficiencies which may cause variegation, but induced reassortment of markers on homologous chromatids appears not to have been reported. In higher plants, somatic variegation can rarely be subjected to critical analysis. One major difficulty is the limited number of appropriate somatic markers usable to distinguish the mechanisms causing variegation. Another is that, although it may be possible, by dedifferentiating and redifferentiating somatic cells, to obtain progenies from the critical segments (cf. STEWARD, MAPES, KENT and HOLSTEN 1964), this is not yet simple and practical for classical genetic analyses.

The felicitous material used in this study reduced these barriers, and permitted several somatic sectors of interest to be subjected to genetic analysis (progeny test).

Investigations on this problem would not have been initiated in *Arabidopsis thaliana*, a typical higher plant, had not twin spots appeared in some experiments designed primarily to induce and detect locus-specific mutations and deletions (RÉDEI 1960, unpublished; HIRONO and RÉDEI 1963b). This report demonstrates induced premeiotic exchange of linked markers in an angiosperm, simulating crossing over. The analysis to follow indicates, however, a different mechanism, possibly a translocation between homologous chromatids.

MATERIALS AND METHODS

Three markers in linkage group 4 (RÉDEI and HIRONO 1964) were used (Figure 1). Their linkage relations are: *gi*^{2}—24.8 ± 1.2—*ch*^{1}—7.9 ± 0.4—*pa*. Plants homozygous for *gi*^{2} remain vegetative four to six times longer than the wild type and thus produce giant rosettes (RÉDEI 1962). Plants homozygous for *ch*^{1} are yellow-green owing to the absence of chlorophyll b and a reduction of chlorophyll a content. Tissues homo- or hemizygous for *ch*^{1} may be confirmed by simple and reliable chemical tests (HIRONO and RÉDEI 1963a). Plants homozygous for *pa* are dwarf, and appear dark green owing to thicker leaf tissues. The pigment content and composition of this mutant is not distinguishable from that of the wild type.

Since the mass production of F_1 seed by crossing is generally burdensome in Arabidopsis, F_2 material has been used. The constitution of the F_1 was *gi* + *pa* / + *ch* +. The genotype of F_2

[1] Based on a dissertation submitted by Y. HIRONO in partial fulfillment of the requirements for the degree of Doctor of Philosophy, at the University of Missouri.

[2] This investigation was supported by Public Health Service Research Grant GM 11474 and by National Science Foundation Grant GB 1174.

[3] Contribution from the Missouri Agricultural Experiment Station. Journal Series Number 28219. Approved by the Director.

plants of special interest was ascertained by further progeny test. Only triple heterozygotes were used for further analysis.

In order to induce somatic sectoring, 24-hour presoaked seeds from F_1 plants (i.e. F_2 populations) were subjected to about 13,000 r of X rays (150 kv, 9 ma, 720 r/min). The treated material was cultured in pots in the greenhouse. During the first 3 to 4 weeks, the plants were illuminated for only 8 hours daily in order to facilitate the detection of sectors and produce several shoots with higher seed yield for progeny test (Arabidopsis is a long-day plant).

For the genetic identification of yellow-green sectors, pigments were extracted from a piece of the leaf and examined spectrophotometrically (RÖBBELEN 1957) or by paper chromatography (HIRONO and RÉDEI 1963a). These convenient techniques permit an accurate determination of the pigment composition of sectors even if areas as small as one fourth the surface of an average size rosette leaf are involved. A piece of tissue of 5 mg fresh weight is sufficient for the analysis.

Seeds were harvested separately from each of the shoots which had developed from sectors, and were subjected to progeny analysis.

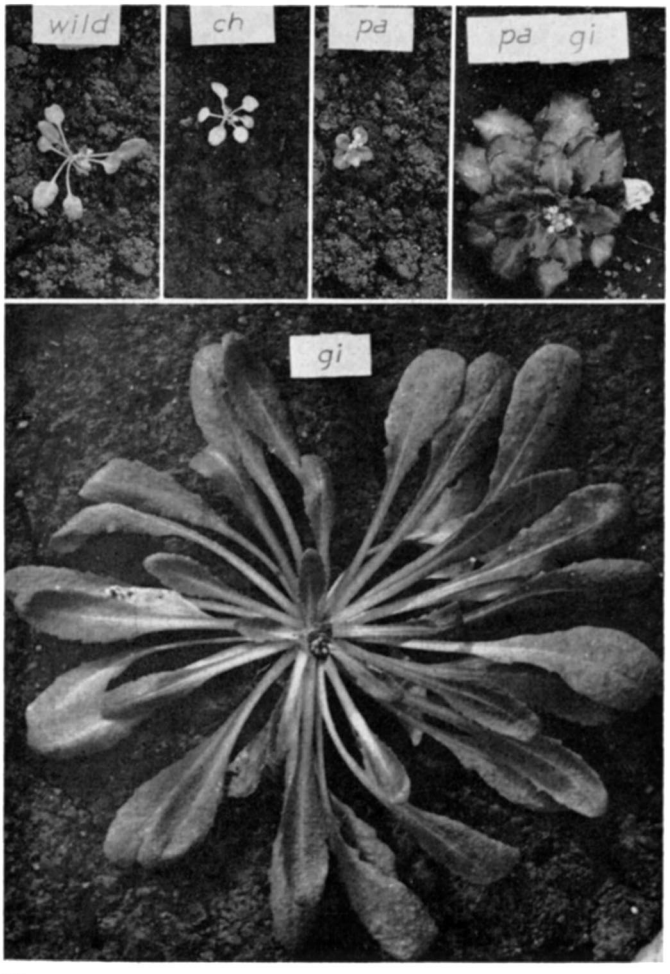

FIGURE 1.—Phenotypes of markers used for the experiments. *ch*[1]: yellow-green, *pa* : dwarf and dark green, *gi*[2]: giant rosette. All plants were cultured under identical conditions and were in the same developmental stage.

Since both sides of the *ch* locus were marked, determination of the mechanism through which *ch* sectors appeared in the triple heterozygotes was feasible. The origin of *pa* sectors can be studied in a similar way although with less facility.

RESULTS

After X-irradiation yellow-green, dark green and very rarely other kinds of sectors (presumably due to the direct physiological effect of the irradiation) appeared on the plants. The *ch* sectors were clearly distinguishable visually from normal green. The number of plants exhibiting larger yellow-green sectors varied from 5 to 10% of the heterozygous *ch* plants. The sectors extended most frequently to half of the leaf, sometimes to less than half, and occasionally to a whole leaf. Rarely even several neighboring leaves developed *ch* phenotype. All the 15 yellow-green sectors chemically analyzed proved to be chlorophyll b deficient and might be either of *ch/o* or of *ch/ch* constitution. All clear yellow sectors obtained by X rays therefore appeared to be *ch* type.

Dark green sectors appeared less frequently than the yellow-green (*ch*) ones, and confirmation of the *pa* phenotype was not possible by chemical means. In about 5% of the plants heterozygous for *pa*, the dark green sectors were tentatively identified as *pa* in phenotype. In a few instances, yellow-green and dark green sectors were adjacent (twin spots). The information available in Arabidopsis does not allow one to determine with certainty whether the occurrence of twin spots is due to mere coincidence of two independent events or to a mechanism which gives rise to reciprocal, complementary products.

Occasionally the yellow-green sectors developed into flowering shoots and a direct genetic analysis of the progenies was feasible. As yet, shoots originating from *pa* sectors could not be identified by phenotype. Seed was harvested separately from different branches of the sectorial plants when possible (Figure 2).

One sectorial plant produced quite remarkable selfed offspring. The seed of the yellow stem developed predominantly (78.3%) into yellow-green (*ch*) plants. The existence of a few non-*ch* seedlings revealed that the yellow shoot of the original plant was a genetic chimera. Progeny tests of these normal green plants have demonstrated that the constitution of the particular seed before irradiation was *gi ch* +/+ + *pa*.

By taking the number of wild phenotype plants in the immediate progeny of the yellow-green stem as a point of departure, it is possible to estimate the total contribution of the green cells to the progeny and to separate them from the totals. After such a separation approximately equal numbers of *gi*$^+$ *ch* and *gi ch* individuals remain (Table 1).

One *ch* strand was apparently tagged as in the original treated seed (*gi ch* in coupling). The other, however, was *gi*$^+$ *ch*, carrying a marker that was originally on the other strand. The *pa* marker, which had been in repulsion to *gi* and *ch*, vanished from the progeny of the yellow cells. The transmission of the exchanged strand was impaired.

Genotypes of 87 *gi*$^+$ *ch* and 60 *gi ch* individuals have been determined by progeny tests (Table 2). In the absence of any abnormality we would expect

FIGURE 2.—Somatic sectoring in Arabidopsis. One rosette leaf half normal green (wild-type), half yellow-green (*ch*). One flowering branch (left) is yellow-green (*ch*) and the other (right) is green (wild-type).

one-third (ca. 29) of the phenotypically gi^+ *ch* individuals to be homozygotes. Only 13 were found, and it is assumed that the strands which survived were mainly those which recovered from the defect through an additional meiotic recombination.

Although in some cases the number of seeds obtained was insufficient for detailed analysis, in five families of *gi ch* +/+ *ch* + genotype the ratio of phenotypes gi^+ to *gi* deviated significantly (P = 5%) from the normal 3:1 ratio. In

TABLE 1

Segregants obtained from the yellow stem

	Phenotype								
	+++	gi ++	+ ch +	++ pa	gi ch +	gi + pa	+ ch pa	gi ch pa	Total
Immediate progeny of the yellow stem									
	40	11	120	16	126	1	0	0	314
	(12.7%)	(3.5%)	(38.2%)	(5.1%)	(40.1%)	(0.3%)	.	.	(100%)
Segregants attributable to an unseen wild sector in the yellow stem									
	40	9	11	22	13	2	0	0	97
Phenotypes attributed to the yellow sector									
	..	..	109	..	113	..	.	.	222

TABLE 2

Genotypes of the yellow plants from the yellow sector

	Phenotype			
	+ *ch* +		*gi ch* +	
Genotype	$\frac{+\,ch\,+}{+\,ch\,+}$	$\frac{gi\,ch\,+}{+\,ch\,+}$	$\frac{gi\,ch\,+}{gi\,ch\,+}$	$\frac{gi\,ch\,pa}{gi\,ch\,+}$
Number of progenies	13	74	59	1

two of these families the ratio was about 2:1. In the remaining three families about 5:1 was observed. These observations demonstrate that the factor which caused the abnormal segregation in the immediate progeny of the sector was transmitted, sometimes recombined, to the following generation. The segregation data indicate that the defect was originally in the gi^+ *ch* strand.

Only one of the 60 *gi ch* individuals analyzed was found to possess the *pa* marker also (Table 2). This may have been derived from a chimeric tissue.

The green branch of the same individual did not appear to be *pa*, yet dwarfs (total of *pa*) comprised 56.7% of its selfed progeny (Table 3). The predominance of the *pa* phenotype cannot be due to random segregation. At first it appeared as if this branch might include the complementary products of a mitotic exchange. Further studies indicated, however, that the case was not so simple. The majority of the wild-type plants produced progenies showing a segregation pattern similar to that of the immediate progeny of the green sector (Table 3). The transmission of some pa^+ strands was abnormal, causing an excess of *pa* phenotypes. There was no evidence that an exchange product complementary to the + *ch* + strand was also included in this green sector, though the recovery of some reciprocal products cannot be ruled out.

A defect has been located on the *gi ch* + strand between the *ch* and *pa* loci.

TABLE 3

Analysis of the first and second generation progenies of the green sector

	Phenotype								
	+++	gi ++	+ ch +	++ pa	gi ch +	gi + pa	+ ch pa	gi ch pa	Total
Segregation of the immediate progeny of the green stem									
Observed	85	23	0	128	3	17	0	1	257
	(33.1%)	(8.9%)		(49.8%)	(1.2%)	(6.6%)		(0.3%)	(100%)
Second generation of the majority of wild phenotype plants from the green stem									
Observed	1031	226	20	1532	33	175	0	5	3023
	(34.1%)	(7.5%)	(0.7%)	(50.7%)	(0.9%)	(5.8%)		(0.2%)	(100%)
Expected theoretically	(41.2%)	(9.1%)	(11.5%)	(22.2%)	(13.3%)	(2.5%)	(0.1%)	(0.2%)	(100%)

The theoretical aspects and the method used for the localization will be published separately (HIRONO in preparation).

Essentially similar recombination phenomena have been observed in several other cases currently being analyzed.

DISCUSSION

Somatic sectoring of irradiated heterozygotes may be due to widely different mechanisms. The available evidence permits a discussion limited to only a few alternative interpretations.

The constitution of the yellow-green sector could involve a long deficiency including both the *ch* and *pa* regions. The simultaneous loss of the ch^+ and *pa* alleles in a plant of *gi ch* +/+ + *pa* constitution would uncover the *ch* marker on the homologous chromosome, producing a yellow sector that would be expected to lack *pa* in its progeny, as observed. At first glance, a deficiency also appeared to satisfy the abnormal segregation ratio observed. If the transmission of the deficiency strand were zero (a reasonable assumption for a deficiency at least 8 map units long), and if there were about 29% recombination between *gi* and the deficiency, a phenotypic ratio of about 1:1 for gi^+:*gi* would be expected. The standard map distance between gi^2 and ch^1 is 24.8 ± 1.2 units. A longer map distance or some transmission of the deficiency chromosome would be required in order to realize a less anomalous ratio. In the second generation of some of the yellow-sector progeny, an abnormal phenotypic ratio of 2 gi^+ : 1 *gi* was observed, indicating a low but definite transmission of the affected strand, even though the immediate progeny had shown a 1 : 1 ratio. The difference between the immediate and second generation progenies is probably not genetic, but rather due to sampling error. Presumably the second generation data indicate the real measure of the abnormality.

In order to get the observed 2:1 phenotypic ratio, about 80% overall transmission of the deficiency is required, if homozygotes are inviable. The progeny-test indicated a much lower transmission. It is very unlikely that a chromosome missing a segment at least 8 map units long, including two known major genes, would be transmitted so well in a diploid.

Some other cases of sectoring were ascribed to deletion of the *ch* locus. In these plants the *pa* marker was recovered, though its transmission was impaired.

Nondisjunction as the cause of the sector is ruled out by the segregation of linked markers in the progeny of the yellow-green sector.

Translocation of the ch^+ *pa* region to a nonhomologous chromosome which subsequently would move to the same pole as the nontranslocated ch^+ *pa* strand could bring about hemizygosity for the *ch* allele and thus cause the appearance of yellow-green sectors (cf. JONES 1937, 1938). The consequence of such a translocation, as far as the yellow-green sector is concerned, would be essentially the same as that of a deficiency, which has already been found inadequate to explain the sector.

A mutation of ch^+ to *ch* and simultaneous deletion of the recessive *pa* allele, or loss of ch^+ and reversion of *pa* to pa^+ might perhaps also explain the observa-

tions. Back mutation of *pa* has not been observed so far, however, and the frequency of forward mutation at the *ch* locus is lower than that of this apparent recombination. Thus the necessary coincidences cannot be expected to occur frequently enough to account for the phenomenon.

It is most plausible that an induced exchange at a four-strand stage in the meristematic cells caused the development of the yellow-green sector. Observations of apparent somatic pairing (STEINITZ-SEARS 1963) in Arabidopsis suggest that homologues may often be close together, increasing the chance of exchange of this type.

The progeny of the yellow-green sector displayed abnormal segregation, apparently due to the presence of a defect on the gi^+ *ch* strand. This indicates that the sector either was not brought about by a normal reciprocal crossover, or that a defect originated separately from the exchange.

The recovery of the expected reciprocal products has not been demonstrated in this experiment. The failure of recovery of both reciprocal products in separate branches is not surprising, inasmuch as embryos were exposed to radiation; subsequent to exposure there is ample opportunity for selection against dwarf tissues. The particular pattern of differentiation of the chimera may also randomly fail to include certain cells in the meristem. It may be that the reciprocal *pa* product was included in the analyzed green sectors, but owing to the complications caused by the chimeric situation it escaped identification.

The recombination event indicates that the centromere is left of the *gi* locus. This information generally can be obtained by genetic means only when tetrad or half-tetrad analysis is feasible.

Spontaneous occurrence of *ch* sectors has not been observed. Recombination has been accompanied by chromosomal defects in all the cases of somatic recombination seen so far in this system. This exchange therefore does not seem to be similar to meiotic crossing over.

The effect of X rays on meiotic crossing over is controversial. Both in Drosophila (BECKER 1957) and Aspergillus (KÄFER 1960, 1963; MORPURGO 1962), ionizing radiation increases the rate of mitotic recombination, but the mechanism responsible for the increase has not been identified.

The rarity or absence of natural mitotic recombination may be primarily due to the general absence of intimate chromosome pairing. There may also exist some other causes. In the present case, it is possible that the X rays damaged mechanisms that normally prevent recombination in mitotic cells, thus creating opportunities for exchanges. The irradiation did not cause hereditary alteration of this type, however, because in later generations recombination has not continued to occur.

It has not been learned what is the relationship between the chromosomal damage and the exchange. It is possible that chromosome breakage is a condition which must be met if somatic recombination is to take place. The exchange may be of a reciprocal chromatid-translocation type, which insofar as some of the end results are concerned is very similar to crossing over.

Though it is widely assumed, tacitly or explicitly, that the mechanism of meiotic crossing over and mitotic recombination is identical (cf. PONTECORVO

1958; PRITCHARD 1963), the experimental basis of this belief is not firmly established. In Drosophila only phenotypic evidence is available, since non-germinal tissues are studied. Progeny tests are feasible in fungi. Unfortunately cytogenetic confirmation of mitotic crossing over is still missing. Chromatid exchange in human tissue cultures has been reported recently (GERMAN 1964). Mitotic crossing over has not been reported to occur in higher organisms with the exception of Drosophila. Distinction between somatic recombination, premeiotic exchange and mitotic crossing over (*parameiosis*, HURST and FOGEL 1964) seems desirable. Because of differences in the analytical methods it is impossible to estimate what percentage of the cases reported as mitotic crossing over could be due to a mechanism similar to that discussed in this paper.

We acknowledge gratefully the valuable discussions with DR. G. Y. KIKUDOME and appreciate the assistance of DR. E. H. COE, JR. and DR. E. L. PINNELL in the preparation of the manuscript.

SUMMARY

Plants of *Arabidopsis thaliana* heterozygous for linkage group 4 markers, when irradiated, developed somatic sectors and occasional "twin spots." In the sexual progenies of the sectors, premeiotic exchange between linked markers has been demonstrated by a test comparable to half-tetrad analysis. Since this recombination is associated with chromosomal aberrations, it appears that a recombination mechanism different from meiotic crossing over is involved.

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