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Studies on the Expression of Somatic Crossing over in *Glycine max* L.

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Summary. Variety *T*219 of *Glycine max* L. has spontaneous yellow, dark green and double (yellow-dark green) spots on the leaves of plants of genotype $Y_{11}\,y_{11}$ but no such spots are found on leaves of $Y_{11}\,Y_{11}$ or $y_{11}\,y_{11}$ plants. It was suggested (Vig and Paddock, 1968) that the double spots result from somatic crossing over whereas the two types of single spots primarily originate from chromosomal disturbances.

Cold shocks disproportionately increased the frequency of double spots, but ethyl methanesulfonate (*EMS*) did not do so. However, in most cases of each treatment, the frequency of single spots increased. It is suggested that *EMS* is not very potent in bringing about somatic recombination whereas cold shocks are. Plants from a sample of seeds of variety *L*65–1237 that had been harvested in 1968 at Urbana, Illinois, did not express the spotting phenomenon, but plants from seeds harvested in 1969 at Reno did have spots. Application of mitomycin *C*(*MC*) to the seeds of this variety as well as of *T*219 increased the incidence of double spots manyfold indicating that *MC* can reveal the potential for somatic crossing over in a variety which might not otherwise express it. Soaking dry seeds of *L*65–1237 in aqueous solutions of *MC* for intervals as short as 2 hours was found effective in increasing the frequency of double spots. The role of *MC* in relation to *DNA* synthesis and somatic crossing over is discussed. Application of the chromosome-breaking agent, daunomycin (*DM*) was ineffective in causing double spots.

Introduction

Sometime ago Vig and Paddock (1968) reported evidence for the spontaneous occurrence of somatic crossing over in *Glycine max* L. (Soybean) variety *T*219. It was shown that the incidence of the phenomenon can be increased several fold by the application of mitomycin *C*(*MC*) which also has been used for this purpose in *Saccharomyces* (Holliday, 1964) and *Ustilago* (Morpurgo, 1963). Using *Ustilago* and *Saccharomyces* as experimental material Holliday (1964) could not decide whether the new DNA (the strands being synthesized) or the old *DNA* (already synthesized strands) is involved in the process of crossing over in somatic tissues. According to him "experiments are necessary which would detect recombination in the absence of genetic replication". There are no reports other than the *Glycine* work mentioned above which deal with specific induction of somatic crossing over in a higher plant. The work of Blixt with *Pisum* (Blixt 1965), of Redei (See Redei, 1963; Hirono and Redei, 1965) with *Arabidopsis* and of Brink (see Brink, 1964; Sastri *et al.* 1965, Styles, 1967) with *Zea mays* are all attempts to study somatic mosaicism but not of the nature discussed above.

In the *Glycine max T*219 system one has the advantage of being able to distinguish the heterozygotes from either homozygote by phenotype, the differences being based on a single gene pair $Y_{11} - y_{11}$ governing chlorophyll development. Thus $Y_{11}\,Y_{11}$ plants are dark green in color of stem and leaves, $Y_{11}\,y_{11}$ are light green, and $y_{11}\,y_{11}$ are golden yellow. The two simple leaves of $Y_{11}\,y_{11}$ plants are dotted with three kinds of spots: dark green resembling the color of $Y_{11}\,Y_{11}$ plants, yellow resembling the $y_{11}\,y_{11}$ phenotype and double spots — a dark green spot adjacent to a yellow spot of similar size and shape, the two spots making a mirror image of each other. The first compound leaf has the same three kinds of spots, but in lower frequency and larger size than the spots of the simple leaves. The leaves of homozygotes are devoid of such spots, thus ruling out the possibility of explaining them as simple point mutations. In their studies involving the use of *MC*, Vig and Paddock (1968) found that the frequency of double spots increases disproportionately more than that of either type of single spot. This indicated that *MC* is capable of inducing somatic crossing over affecting the Y_{11} locus and also that in large single spots have a difrent mode of origin than double spots. Subsequently it was shown that several types of chromosomal disturbances may be involved in the formation of single spots (Vig, 1969).

Ethyl methanesulfonate (*EMS*) has been suggested to be chromosomal as well as genic mutagen (Burton and Powell, 1966; Satpathy and Arnason, 1969; Kaul, 1969; Malling and de Serres, 1968) but has not been shown to cause somatic crossing over. Thus if one treats the *T*219 material with *EMS* the frequency of different types of spots should not be comparable with the spectrum seen in case of *MC*. On the other hand, cold shocks which do increase the frequency of somatic segregation in organisms such as *Drosophila* (Brosseau, 1957) should result in a greater increase in frequency of double spots than of single spots. Also

it is not known if prolonged lowered temperatures will cause a disproportionate increase in frequency of double spots or if sudden shocks of low temperatures are more effective in this regard.

Variety *L*65-1237 derives from a cross between *T*219 and Clark-*k* and has individuals which are heterozygous at the Y_{11} locus. But a seed sample obtained from the 1968 crop grown in Urbana, Illinois expressed the spotting phenomenon in 1969 only in exceedingly low frequency. The rare spots seen were usually double.

The present study was carried out with the following points in view: (1) to see the effect on *T*219 of *EMS* as well as prolonged temperatures and cold shocks on the induction of spots and to make qualitative comparisons with the results obtained by using *MC*; (2) to see whether the Y_{11} y_{11} plants of *L*65-1237, which exhibit only rare spots ordinarily, are vulnerable to an increased frequency of spots under the influence of agents such as *MC*, and to make a comparison with *T*219 subjected to similar treatments; (3) to compare *T*219 and *L*65-1237 plants from seeds of similar age raised under similar conditions; (4) to find out if increased frequency of spots can be induced by *MC* treatment administered prior to the onset of new *DNA* synthesis which in legumes is generally not sooner than 20 hours after the seeds are placed in water (see Miksche, 1966; Jakob and Bovey, 1969) and (5) to find out if the new antibiotic daunomycin (*DM*) which is capable of causing chromosomal breakage in leukocytes (Vig *et al.*, 1968) but does not cause somatic recombination (Vig *et al.* 1970) can cause spotting in *L*65-1237.

Materials and Methods

All experiments were conducted using seeds not over one year old. The seeds of variety *T*219 were obtained from the Ohio Biological Supply Company, Columbus, Ohio, as well as from the Carolina Biological Supply Company, Gladstone, Oregon. Variety *T*219 is a derivative from the seed lot of *A*591-1 and has been a standard variety for almost two decades. The seeds of variety *L*65-1237 were obtained through the courtesy of Dr. R. L. Bernard, U.S. Regional Soybean Laborators, Urbana, Illinois. To study objective No. 3 (see introduction) seeds of *T*219 and *L*65-1237 were obtained from crops grown in 1969 at the Agricultural Experiment Station of the University of Nevada, Reno. An additional seed sample from the 1969 crop of *T*219 was procured from Ames, Iowa. Daunomycin was obtained from Farmatilia Research Lab., Italy. Mitomycin C was purchased from Nutritional Biochemicals, Cleveland, Ohio, and *EMS* from Eastman Organic Chemicals, New York. Stock solutions of *EMS* and *DM* at 0.5% and *MC* at 0.005% were prepared and diluted at time of use to desired concentrations with distilled water.

Dry seeds were soaked in excessive quantities of the diluted solutions. Controls were always submerged in distilled water at the same temperature as that of the solution of the chemical and for the same duration of time. Facilities for cold shocks were available in the temperature controlled rooms of the Ohio State University. For cold shocks, experiments were carried out by growing the plants under the following conditions: (i) flats, after sowing the seeds, left in the greenhouse environment as controls, (ii) flats, after sowing the seeds, transferred to a temperature regime of 74 °F during a light period of 15 hours and 64 °F during a dark period of 9 hours at a constant relative humidity of 74% and (iii) flats with seeds or seedlings, subjected to the treatments as in (ii), excepting that these were exposed to 37 °F for a period of middle 6 hours during every 15 hours of light.

The spots on the entire upper surface of the two single leaves and the first compound leaf were counted. No distinction was made for quantitative purposes between large and small spots. Insect injury was distinguishable from the spots, without confusion. To facilitate calculations, each leaflet of the compound leaf was considered equivalent to a simple leaf. Double spots were counted as one.

Experimental Results

EMS treatments: A wide range of combinations of concentrations and times was tried under greenhouse conditions so as to make an analysis of the potentialities of the chemical. The results were unexpectedly disappointing since most of the combinations tried proved highly lethal. Data from all the combinations with LD_{50} or above were discarded.

A reasonable degree of survival and spotting was observed when the chemical was used at concentration of 0.25% for 18 hours in winter of 1966 (Experiment 1), 0.25% for 24 hours during summer of 1966 (Experiment 2) and 0.125% for 24 hours and 0.25% for 24 hours in spring of 1967 (Experiment 3). Table 1 is a summary of the results.

A slight overall decrease in frequency of all three kinds of spots is associated with the treatment in Experiment 1, but this experiment is less reliable than the other two because fewer leaves were scored. In Experiment 2, the *EMS* treatment seems to have made no difference in spot frequencies. In Experiment 3, each *EMS* treatment made a striking increase in frequency of each kind of spot. Also in Experiment 3, the higher concentration of *EMS* was accompanied by a greater increase in frequency of each of the 3 kinds of spots than the increase accompanying the lower concentration. In both Experiments 2 and 3, any given proportion of the frequencies of two types of spots remains more or less comparable across all 5 cases (2 controls and 3 treatments). It is particularly noteworthy that *EMS* treatment did not cause the frequency of double spots to increase disproportionately to the frequencies of dark green, yellow, or total spots.

The most common cause of post-germination mortality was the incapability of the hypocotyl to straighten up after emerging from the soil. In several cases germination was diminished due to inability of the hypocotyl to push out of soil. That is, when presumably ungerminated seeds were dug out there were several which were decaying but partially germinated, or even fully germinated. Several of the seedlings which got a good start did not develop beyond partial expansion of the first two, or simple, leaves.

Table 1. *Spot Frequency and Proportions from EMS treated material*

Date	Treatment	No. of leaves	Spot frequency per sample leaf				Proportions of spots per sample leaf			
			DG	*Yl*	*Db*	*T*	*DG/Db*	*Yl/Db*	*DG/Yl*	*T/Db*
Winter, 1966 (Expt. 1)	Control	40	0.60	0.40	0.10	1.10	6.00	4.00	1.50	11.00
	Ethyl methanesulfonate 0.25%—18 hr	45	0.16	0.36	0.07	0.59	2.29	5.14	0.44	8.43
Summer, 1966 (Expt. 2)	Control	145	0.39	0.17	0.10	0.66	3.90	1.70	2.28	6.60
	Ethyl methanesulfonate 0.25%—24 hr	180	0.31	0.31	0.10	0.72	3.10	3.10	1.00	7.20
Spring, 1967 (Expt. 3)	Control	275	0.15	0.09	0.05	0.29	3.00	1.80	1.67	5.80
	Ethyl methanesulfonate 0.125%—24 hr	140	1.19	0.59	0.27	2.06	4.41	2.19	2.02	7.63
	0.25 %—24 hr	100	3.27	2.30	0.83	6.40	3.94	2.77	1.42	7.71

DG = dark green; *Yl* = yellow; *Db* = double; and *T* = total spots.

Table 2. *Spot frequency and Proportions from the cold treatment experiments*

Experiment:	Treatment	No. of leaves	Spot frequency per sample leaf				Proportion of spots per sample leaf			
			DG	*Yl*	*Db*	*T*	*DG/Db*	*Yl/Db*	*DG/Yl*	*T/Db*
4	Control	115	0.15	0.15	0.02	0.31	7.50	7.50	1.00	15.50
	74°F+64°F cycle	105	0.65	0.08	0.09	0.81	7.22	0.89	8.12	9.00
	74°F/64°F+37°F	110	1.61	0.60	0.79	3.00	2.04	0.76	2.68	3.80
5	Control	125	0.06	0.18	0.02	0.27	3.00	9.00	0.33	13.50
	74°F—64°F cycle	100	0.48	0.13	0.09	0.70	5.33	1.44	3.69	7.78
	74°F/64°F+37°F	120	2.93	0.81	1.38	5.12	2.12	0.59	3.62	3.71
6	Control	225	0.10	0.23	0.05	0.38	2.00	4.60	0.43	7.60
	74°F/64°F cycle	250	0.56	0.27	0.22	1.05	2.55	1.23	2.07	6.73
	74°F/64°F+37°F	210	1.06	0.43	0.50	2.00	2.12	0.86	2.47	4.00

Table 3. *Data on the type and frequency of spots found on the two simple leaves and first compound leaf of $Y_{11}y_{11}$ plants from untreated seed*

Variety and source	No. of leaflets studied	Spots per leaf studied				Proportion of spots			
		DG	*Yl*	*Db*	*T*	*DG/Db*	*Yl/Db*	*DG/Yl*	*T/Db*
*T*219, Ohio	100	0.35	0.23	0.03	0.61	11.67	7.67	1.52	20.33
*T*219, Oregon	50	0.72	1.54	0.88	3.14	0.82	1.74	0.47	3.57
*L*65—1237, Illinois	500	0.002	0.000	0.01	0.01	0.20	—	—	1.20

Table 4. *Data on the type and frequency of spots found on the two simple leaves and first compound leaf of $Y_{11}y_{11}$ plants grown from 1969 seed (untreated)*

Variety and source	No. of leaflets studied	Spots per leaf studied				Proportion of spots			
		DG	*Yl*	*Db*	*T*	*DG/Db*	*Yl/Db*	*DG/Yl*	*T/Db*
*T*219, Reno	139	0.27	0.17	0.17	0.58	1.59	0.82	1.93	3.41
*T*219, Ames	95	0.19	0.22	0.18	0.59	1.05	1.22	0.86	3.30
*L*65—1237, Reno	55	0.47	0.13	0.24	0.84	1.96	0.59	3.61	3.50

Table 5. *Data on the type and frequency of spots on the two simple leaves and first compound leaf on $Y_{11}y_{11}$ plants treated with Mitomycin C*

Variety	Concentration of Mitomycin C (%)	Duration of seed treatment (hr)	No. of leaflets studied	Spots per leaf studied				Proportion of spots			
				DG	*Yl*	*Db*	*T*	*DG/Db*	*DG/Yl*	*Db/Yl*	*T/Db*
*L*65-1237, Illinois	0.000	—	400	0.00	0.00	0.00	0.00	—	—	—	—
	0.0025	12	120	0.77	1.17	1.88	3.82	0.41	0.62	0.66	2.03
	0.0025	24	60	0.62	0.90	3.28	4.80	0.19	0.27	0.69	1.46
*T*219, Ohio	0.0000	—	115	0.29	0.25	0.13	0.67	2.23	1.92	1.16	5.15
	0.0025	24	110	1.41	0.62	0.95	2.97	1.48	0.65	2.27	3.13
	0.0050	24	30	0.87	0.63	0.83	2.33	1.05	0.76	1.38	2.81

Low Temperature treatments: The treatments were (Experiments No. 4, 5 and 6) conducted three times to increase the reliability of the data. Summarized results are given in Table 2. In the three mild low temperature treatments without a 37 °F shock, the frequency of yellow spots was affected least. It decreased only slightly in Experiments 4 and 5, and increased only slightly in Experiment 6. Dark green and double spot frequencies increased, respectively, in all 3 experiments. In each of the 3 treatments with a 37 °F shock, all 3 types of spots had obvious increases in frequency. The increase of double spots was disproportionately greater than that of the other two types and especially as compared to that of total spots, i.e. the ratio of total spots divided by double spots is less than the same ratio in the control. Also, the increase of dark green spots was disproportionately greater than that of yellow spots.

Varietal comparisons and minimum period for MC treatment required for appearance of spots: Untreated seeds of variety *T*219 obtained from Ohio and Oregon, grown to study the frequency of spots in the control population, had all the three kinds of spots on their leaves (Table 4). However, plants raised from untreated seed of comparable age from variety *L*65-1237 from Illinois did not have any spots worth mentioning. These results give the impression that the two varieties have differential potentials for the expression of spots even when grown under identical conditions. To analyse whether this is so or the differences relate to the environments under which the plants which were the source of these seeds were grown, the following experiment was performed. Seeds of *L*65-1237 and *T*219 harvested at Reno and *T*219 harvested at Ames, and all from 1969 crops, were grown in the greenhouse. All three samples had all 3 kinds of spots (Table 4) indicating that the environment in which the mother plant is grown might affect the expression of spotting.

In the next experiment, *MC* at concentrations of 0.0025% in aqueous solutions was applied for 12 and 24 hours to seeds of *L*65-1237 from Illinois to see whether spots can be induced. For comparison, seeds of *T*219 from Ohio were treated with 0.0025% of *MC* for a period of 24 hours. The results given in Table 5 indicate that *MC* is not only capable of increasing the frequency of spots in *T*219 but also induces spots in *L*65-1237. This is of interest in that a variety which does not express the phenomenon under ordinary conditions can be subjected to its initiation and induction, thereby indicating that potential for the phenomenon may be already available.

The frequency of double spots in *L*65-1237 from Illinois treated with 0.0025% of *MC* for either 12 or 24 hours was greater than the frequency of spots of either other type. This is a strong support to the idea that double spots are a result of somatic crossing over. Also, the frequency of yellow and double spots is greater than that in *T*219, Ohio treated equivalently but dark green spots occurred less often. The seeds of *T*219, Ohio, were also treated with twice the concentration of the drug (0.0050%) for 24 hours. The frequency of spots seems lowered as compared to the treatment with 0.0025% of *MC* for 24 hours (Table 5) possibly because of an adverse effect of *MC* on growth and expansion of leaves. Even so there was the usual disproportionate increase in frequency of double spots compared to that of total spots.

Experiments were conducted to study whether induction of double spots requires synthesis of new *DNA* at the time of administration of *MC*. Seeds of *L*65-1237 harvested in Illinois were therefore treated with 0.004% of *MC* for 2 hours and 0.0025% for 4 hours. All 3 kinds of spots were found in both treatments (Table 6).

Also, experiments in this study were performed to see if the new antibiotic mutagen *DM* is capable of causing double spots. The results (Table 7) gave little indication of any parallelism of action between the two drugs. The few spots observed in treatments with *DM* could have resulted from the chromosomal aberrations which *DM* is capable of inducing (Vig,

Table 6. *Data on the type and frequency of spots induced by short treatments of mitomycin C to dry seeds of L65—1237*

Concentration of the drug (%)	Duration of treatment (*hr*)	No. of leaflets studied	Spot frequency per sample leaf				Proportion of spots			
			DG	*Yl*	*Db*	*T*	*DG/Db*	*Yl/Db*	*DG/Yl*	*T/Db*
0.000	—	80	0.00	0.00	0.01	0.01	—	—	—	1.00
0.0025	4	70	0.10	0.23	0.21	0.54	0.48	1.09	0.44	2.57
0.004	2	80	0.29	0.54	0.51	1.34	0.57	1.06	0.54	2.63

Table 7. *Data on the type and frequency of spots on the two simple leaves and first compound leaf of L65—1237 given seed treatment with aqueous daunomycin solution*

Concentration of the drug (%)	Duration of treatment (*hr*)	No. of leaflets studied	Spot frequency per sample leaf				Proportion of spots			
			DG	*Yl*	*Db*	*T*	*DG/Db*	*Yl/Db*	*DG/Yl*	*T/Db*
0.000	—	80	0.00	0.00	0.00	0.00	—	—	—	—
0.01	12	120	0.02	0.01	0.07	0.10	0.29	0.14	2.00	1.44
0.02	12	120	0.01	0.01	0.05	0.07	0.20	0.20	1.00	1.40

et al., 1968), although notice that all 3 kinds of spots did occur, and double spots were the most frequent kind.

Discussion

These studies on the induction of spots in soybean leaves indicate that *EMS* is capable of increasing the frequency of spots in variety *T*219. However, most of the spots induced are single i.e., dark-green or yellow.

The overall picture which emerges from a study of the proportionality of frequencies suggests that *EMS* favors the increase of single spots more than of doubles. These observations are in line with the hypothesis that single spots, at least in part, may result from chromosomal disturbances other than those bringing about somatic crossing over (Vig, 1969) and that *EMS* is capable of causing such disturbances (Kaul, 1969, Burton and Powell, 1966). The results obtained here are clearly different from those obtained using *MC* (Vig and Paddock, 1968) and support our notion that the two chemicals cause spotting by different mechanisms.

Experiments carried out on the induction of mosaicism on the abdomen of *Drosophila melanogaster* (Brosseau, 1957, Stern, 1936; Stern and Rentschler, 1936) support the idea that external agents such as temperature have an influence on the frequency of mosaicism. During our earlier work, an increase in the frequency of spots on the material grown during cooler days was casually observed. It was thus tempting to test the idea if lowering of temperatures during growth of plants would increase the frequency of spots. The plants raised at lower temperatures, especially those given cold temperature shocks at 37 °F for 6 hours daily, had a much higher spot frequency than those plants which were grown in the continuously warm greenhouse. This is in agreement with the findings of Stern and Rentschler (1936) in that they observed more spots on the abdomens of flies cultured at 25 °C than those raised at 30 °C. The proportions of spots, however, do fluctuate in some cases but this is more due to variation in the frequency of the yellow spots than in that of the doubles.

The effect of low temperature in increasing mosaicism may be discussed from several angles. The low temperature may directly influence (disturb) chromosomal processes or the formation or functioning of the spindle. More plausibly it may alter the efficiency of some 'active chemical' in the seeds and thus increase the frequency of the spots.

Varietal Comparisons: It is of interest that the plants from the seeds of variety *L*65-1237 obtained from Illinois had no spots when sown at Reno within a few months of harvesting but the seeds obtained from the plants grown in Reno did express the potential for spotting. It may mean that certain environments cause the production and accumulation of some active chemical(s) responsible for spotting whereas other environments do not. However, the essential potential for such a phenomenon must be available always since the use of *MC* helped induce spots even in *L*65-1237 from Illinois.

DNA Synthesis: Studies made with the embryos of *Vicia faba* (Davidson, 1966; Jakob and Bovey, 1969) and soybean (Miksche, 1966) indicate that onset of synthesis of *DNA* takes about 15—24 hours after placing the seeds in water. Jakob and Bovey (1969) calculated that about 96% of the meristematic cells in the embryos of germinating seeds of *Vicia faba* variety *major* were in G_1 and only about 4% in G_2. A similar general situation was met with in *Allium cepa* by Jensen (1963). However, for *Glycine max* (Miksche, 1966) the relationship between *DNA* synthesis and onset of mitosis seems different i.e., a larger number of embryonic cells is in G_2.

That double spots can be induced by as brief a treatment with *MC* as 2 hours (Table 6) might indicate that at least a major amount of *DNA* synthesis is not associated with the phenomenon. It remains to be seen if some undetected aspect of *DNA* synthesis is required for double spot formation. Esposito and Holliday (1964) pointed out that *DNA* synthesis is associated directly with the process of somatic recombination in *Ustilago maydis* and the inhibition of the synthesis of this nucleic acid by fluorodeoxyuridine stimulates the process. The present results do not agree with this unless *MC* binds to the non-replicating *DNA* but does not become effective until the time of replication (Iyer and Szybalsky, 1963). Whether the mechanism of somatic crossing over in the two organisms is somewhat different or there is differential sensitivity among phases of the cell cycle during treatment is not clear. There also is no precise explanation as to how *MC* will interact with new *DNA* in bringing about double spots. Pontecorvo's (1958) suggestion that "somatic pairing as cytologically detectable at metaphase in a few organisms has something to do with mitotic crossing over is not very helpful" seems very reasonable. On the other hand, it is proposed here that localized pairing of segments of homologous chromosomes in interphase cells is associated with the phenomenon of breakage and reunion of chromosomes. This idea gains support from the works of Chauhan and Abel (1968), Grell (1967) and Maguire (1967) who suggest such pairing in pre-meiotic interphase, as well as from the work of Brown and Cottom (1968) who found such association in pre-meiotic mitosis in several organisms. Such localized pairing and exchanges could be associated with heterochromatic segments of the involved chromosomes. It may also be mentioned that the physical state of the cell is responsible for the degree of induction (or perhaps expression) of the phenomenon of somatic crossing over. Several of our current experiments (Vig, unpublished) show that effectivity of *MC* can increase, to an extent, with an increase in the

water content and metabolic advancement of the seed but the decline starts at almost the same time at which *DNA* synthesis is known to initiate. Experiments are being conducted to see if the spot frequency peaks are associated with *DNA* synthesis or protein synthesis in any way.

The frequency of spots as observed after *DM* treatment and the relationship between dark green versus other types of spots indicate that the action of *DM* is more like that of *EMS* than like that of *MC*. It has been shown that in human leukocytes *DM* does not cause preferential reunion among homologous chromosomes to the extent of qualifying as an agent capable of inducing a high degree of somatic crossing over (Vig *et al.*, 1970).

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References

1. Blixt, S.: Studies of induced mutations in peas. XI. Leaf spotting induced by mutagenic agents. Agri. Hort. Genet. **23**, 172–186 (1965). — 2. Brink, R. A.: Genetic repression of R action in maize. In: Role of chromosomes in development, M. Locke (Ed.), Soc. Stud. Developm. Growth (Symp.) **23**, 183–230 (1964). — 3. Brosseau, G. E.: The environmental modifications of somatic crossing over in *Drosophila melanogaster* with special reference to developmental phase. J. Exp. Zoo. **136**, 567–593 (1957). — 4. Brown, W. V., Cottom, G.: Somatic pairing as a regular preliminary to meiosis. Bull. Torrey Bot. Club **95**, 369–378 (1968). — 5. Burton, G. W., Powell, J. B.: Morphological and cytological response of pearl millet, *Pennisetum typhoides*, to thermal neutrons and *EMS* seed treatments. Crop Sci. **6**, 180–182 (1966). — 6. Chauhan, K. P. S., Abel, W. O.: Evidence for the association of homologous chromosomes during premeiotic stages in *Impatiens* and *Salvia*. Chromosoma **25**, 297–302 (1968). — 7. Davidson, D.: The onset of mitosis and *DNA* synthesis in roots of germinating beans. Amer. J. Bot. **53**, 491–495 (1966). — 8. Esposito, R. E., Holliday, R.: The effect of 5-fluorodeoxyuridine on genetic replication and mitotic crossing over in synchronized cultures of *Ustilago maydis*. Genetics **50**, 1009–1017 (1964). — 9. Grell, R.: Pairing at the chromosomal level. J. Comp. Cell Physiol. **70** (Suppl. 1), 119–146 (1967). — 10. Hirono, Y., Redei, G. P.: Induced premeiotic exchanges of linked markers in the angiosperm *Arabidopsis*. Genetics **51**, 519–526 (1965). — 11. Holliday, R.: The induction of mitotic recombination by mitomycin C in *Ustilago* and *Saccharomyces*. Genetics **50**, 323–335 (1964). — 12. Iyer, V. N., Szybalsky, W.: A molecular mechanism of mitomycin C action: Linkage of complementary *DNA* strands. Proc. Natl. Acad. Sci. (U.S.) **50**, 355–362 (1963). — 13. Jakob, K. M., Bovey, F.: Early nucleic acid and protein synthesis and mitosis in the primary root tips of germinating *Vicia faba*. Exptl. Cell Res. **59**, 118–126 (1969). — 14. Jensen, W. A.: The relation of *DNA* to the early stages of cell development in root tips. Ann. Histochem. **8**, 127–132 (1963). — 15. Kaul, B. L.: Interaction of chromatid breaks induced by nitrosoguanidine in combination with some radiomimetics and *x*-rays. Chromosoma **26**, 469–474 (1969). — 16. Maguire, M. P.: Chromosome pairing in altered constitutions and models of synapsis and crossing over. Genet. Res. **12**, 21–27 (1967). — 17. Malling, H. V., De Serres, F. J.: Identification of genetic alterations induced by ethyl methanesulfonate in *Neurospora crassa*. Mutation Res. **6**, 181–193 (1968). — 18. Morpurgo, G.: Induction of mitotic crossing over in *Aspergillus nidulans* by bifunctional alkylating agents. Genetics **48**, 1259 to 1263 (1963). — 19. Miksche, J. P.: *DNA* synthesis in primary root of *Glycine max* during germination. Can. J. Bot. **44**, 789–794 (1966). — 20. Pontecorvo, G.: Trends in Genetic Analysis. New York: University of Columbia Press 1958. — 21. Redei, G. F.: Somatic instability caused by a cystein sensitive gene in *Arabidopsis*. Science **139**, 767–769 (1963). — 22. Sastri, G. R. K., Cooper, H. B., Brink, R. A.: Paramutations and somatic mosaicism in maize. Genetics **52**, 407–424 (1965). — 23. Satpathy, D., Arnason, T. J.: Post treatment temperature effects on mutation frequency in *EMS*-treated barley. Canad. J. Genet. Cytol. **11**, 522–530 (1969). — 24. Stern, C.: Somatic crossing over and segregation in *Drosophila melanogaster*. Genetics **21**, 525–630 (1936). — 25. Stern, C., Rentschler, V.: The effect of temperature on the frequency of somatic crossing over in *Drosophila melanogaster*. Proc. Natl. Acad. Sci. (US) **22**, 451 (1936). — 26. Styles, E. D.: The metastable nature of paramutable *R* allele in maize. II. Characteristics of alleles differing in geographic origin. Genetics **55**, 399–409 (1967). — 27. Vig, B. K.: Relationship between mitotic events and leaf spotting in *Glycine max*. Can. J. Gen. Cytol. **11**, 147–152 (1969). — 28. Vig, B. K., Kontras, S. B., Paddock, E. F., Samuels, L. D.: Daunomycin induced chromosomal aberrations and the influence of arginine in modifying the effect of the drug. Mutation Res. **7**, 279 to 287 (1968). — 29. Vig, B. K., Paddock, E. F.: Alteration by mitomycin *C* of spot frequencies in soybean leaves. J. Hered. **59**, 225–229 (1968). — 30. Vig, B. K., Samuels, L. D., Kontras, S. B.: Specificity of daunomycin in causing chromosome aberrations in human leukocytes. Chromosoma **29**, 62–73 (1970).

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