Theoretical and Applied Genetics 41, 145–149 (1971)

Increase Induced by Colchicine in the Incidence of Somatic Crossing over in *Glycine max*

B. K. VIG

Department of Biology, University of Nevada, Reno, Nevada (USA)

Summary. The frequency of somatic crossing over in *Glycine max* has been significantly increased by soaking the dry seeds in aqueous solutions of 0.0025, 0.005 and 0.01% colchicine. This increase was quite consistent for several treatments involving time × concentration interaction as well as in cases where post-treatment with mitomycin *C* was given. Results indicate that colchicine is inefficient in disturbing the arrangements of segments of homologous chromosomes involved in the process of somatic exchanges before the onset of the process of somatic crossing over.

Introduction

In their analysis of somatic association in common wheat (*Triticum aestivum*) Avivi, Feldman and Bushuk (1969) attributed the spatial relationship between homologous chromosomes in the interphase nucleus to the attachment of centromers to annuli of the nuclear membrane at certain predetermined, almost definite regions. They remarked that homologous centromers (chromosomes!) are held together in mitotic interphase by microtubulus which are reoriented remnants of the spindle apparatus. Their studies involved the distribution of distances between two homologous telocentric chromosomes, $3B^L-3B^L$, studied in the root tip cells with or without pretreatment with colchicine. An increase in this distance in colchicine treated material as compared to the water treated roots was taken as indicative of the disruptive action of colchicine on the spindle material or microtubules which held the homologous chromosomes together. These studies, however, give no insight into the precise timing of colchicine action and the data do not indicate whether it is the centromere or other parts of the chromosomes which might be involved in spatial separation of the metaphase chromosomes.

Using cells from the testes of cranefly (*Nephrotoma suturalis*) it has been shown that a large number of microtubules of spindle origin are associated with telophase nuclei. These are regularly spaced and connected to the chromatin (Behnke and Forer, 1966). Sometimes a bridging material between the microtubules and chromosomes is also seen suggesting the role of the former in holding the homologues together. Carlson (1956) indicating the role of microtubules in the interphase nucleus in holding the centrometres to nuclear membrane showed that such relationship could be disturbed by colchicine application. Bajer's work (1968) indicates the incorporation of some spindle material into telophase nuclei but does not suggest a definite role.

Commings (1968) collected information concerning the role of nuclear membrane in regulated positioning of chromosomes and their parts in interphase. He suggested several points along the length of the chromosome which might be attached to the nuclear envelope to facilitate replication of DNA, somatic and meiotic pairing, etc. It was suggested that all the heterochromatic zones are not only in close proximity to the nuclear membrane, but perhaps, also in proximity to corresponding parts of the homologous chromosome (Maguire, 1967). Such association on the part of somatic chromosomes is invoked to explain our results in connection with the phenomenon of somatic crossing over (Serebovsky, 1926; Stern, 1936; Hendrychova-Tomkova, 1964; Grunberg, 1966; Vig and Paddock, 1968, 1970). The suggestion has also been made that somatic crossing over is accomplished through participation of proximal heterochromatin (Grell, 1969). These ideas receive support from the observations that the frequency of somatic crossing over can be increased several fold by using agents like mitomycin *C* (Holliday, 1964; Vig and Paddock, 1968) which are known to attack heterochromatic zones preferentially (Rao and Natarajan, 1967; Shaw and Cohen, 1965).

Colchicine has been reported to bind to nuclear proteins and to microtubules in general (Borisy and Taylor, 1967; Shelanski and Taylor, 1967) and thus disrupt the association between homologous chromosomes (see above). However, the measurements of centromere distance, as done in *Triticum* (Avivi, Feldman and Bushuk, 1969) do not give an insight into the time of action of colchicine since not much is known about how homologous chromosomes are arranged in the interphase nucleus. Dewey and Miller (1969), however, found evidence of colchicine interaction with macromolecules involved in chromosome structure 'only as the cell prepares for division', since cells in G_1 were almost unaffected by the chemical in their experiments on radiation-induced chromosome aberrations.

In view of the above, colchicine may have an influence on the frequency of somatic crossing over

by reducing the frequency of closeness of association of homologous parts of chromosomes in interphase. Such a system should be a very sensitive indicator of the disruption of associations between homologous segments of chromosomes. Also a study using colchicine should indicate if the disruption of microtubules occurs before or after the initiation of somatic crossing over. It was with these objectives that the investigations of the effect of colchicine treatment on somatic crossing over were undertaken.

Material and Methods

Varieties T219 and L65-1237 of *Glycine max* (soybean) have a gene Y_{11} which controls chlorophyll development so that $Y_{11}Y_{11}$ plants have dark green stems and leaves, $Y_{11}y_{11}$ have light green stems and leaves and $y_{11}y_{11}$ plants are golden yellow. The leaves of $Y_{11}y_{11}$ plants from about a year old seed of L65-1237 are spotted with areas of dark green or yellow color (resembling respectively, $Y_{11}Y_{11}$ and $y_{11}y_{11}$ phenotypes), which are separated from each other, as well as twin spots which have both the above components placed next to each other forming mirror images (Fig. 1). No spots were observed on dark green or yellow plants. The frequency of three types of spots (called dark-green, yellow and double or twin) increases many fold with the application of mitomycin *C* indicating that at least a majority of these spots which are located on the two simple leaves and rarely on the first compound leaf originate due to somatic crossing over (Vig and Paddock, 1968; Vig, 1969).

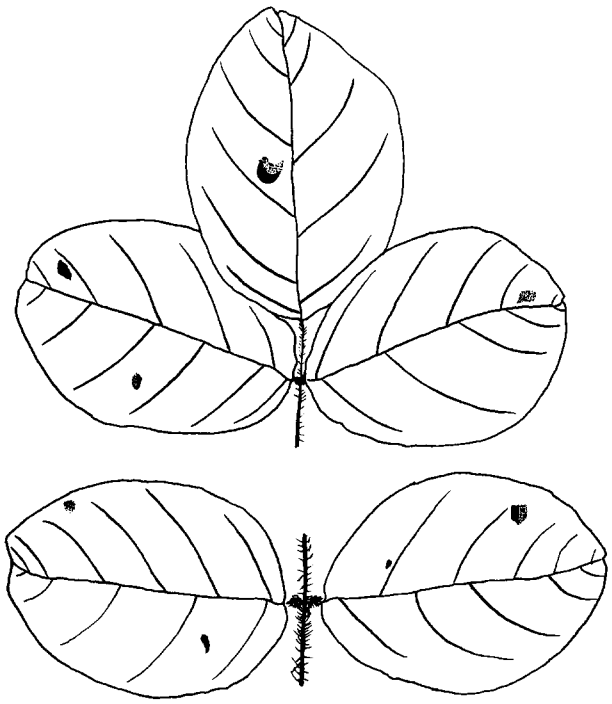

Fig. 1. Diagramatic illustration of the three kinds (dark-green = solid black; yellow = hatched and double) of spots on the two simple (bottom) and first compound (top) leaves of variety L65-1237 of *Glycine max*

Seeds of variety *L*65-1237 were used in this study. Comparisons were made of the material presoaked in water with the one from colchicine treated seeds without any post-treatment; or of the materials pretreated with water or colchicine and then post-treated with Mitomycin *C* with a view to enhance the frequency of the occurrence of the phenomenon of somatic crossing over. In 9 cm petri dishes 60 to 80 seeds were soaked for 4, 8 or 16 hr in 25 cc of distilled water or 25 cc of 0.0025%, 0.005% or 0.01% aqueous colchicine solution (0.01% = 2.5×10^{-3} M colchicine). In experiments using mitomycin *C*, seeds, presoaked in water or colchicine, were soaked for 4 hr in 20 cc of 0.0025% solution of this antibiotic. Seeds were thoroughly washed in running tap water before changing the chemical or before sowing in galvanized iron flats in the greenhouse at 75—85° F and 40—50% humidity.

Spots were counted when seedlings were about 8″ high and had well developed simple leaves and the first compound leaf. Only the upper surface of leaves was used for spot counting (the lower surface has few, if any, spots). To facilitate calculations, every compound leaf was considered equivalent to three simple leaves, thus providing 5 leaf-equivalents per plant.

Results

The pilot experiment was carried out to compare the spot frequency on the leaves from the seeds treated with 0.0025% colchicine or water for 8 to 16 hours. Similar pretreatments were also given with colchicine or water to the seeds treated with 0.0025% mitomycin *C*. Results summarized in Table 1 clearly indicate the higher frequency of spots in treatments where colchicine replaced water. This is not only true of the total frequency of spots but also for the individual types of spots in every case analysed in the three sets of treatments. An increase of 45% or more

Table 1. *Type and frequency of spots in three sets of materials treated with water or colchicine (0.0025%) and with or without post-treatment with Mitomycin C (0.0025%)*

Seed treatment (hr)			Type* and frequency of spots per leaf				
Water or Colchicine	Post-treatment with Mitomycin *C*	No. of leaves	*DG*	*Yl*	*Db*	*T*	*t* (Calculated)
A. 8 hr Water	none	100	0.31	0.22	0.15	0.68	$0.05 > p > 0.1$
8 hr Colchicine	none	115	0.46	0.35	0.40	1.21	
B. 8 hr Water	4 hr	135	0.86	0.68	0.87	2.41	$0.025 > p > 0.05$
8 hr Colchicine	4 hr	115	1.35	0.96	1.20	3.50	
C. 16 hr Water	4 hr	150	1.33	1.17	1.89	4.39	$0.001 > p > 0.005$
16 hr Colchicine	4 hr	125	3.35	1.34	2.05	6.74	

* *DG* = Dark Green; *Yl* = yellow; *Db* = double or twin; and *T* = total spots.

in the frequency of total spots is observed in treatments with colchicine as compared to the ones with water. Statistical analysis (using *t*-test) indicated significant increase of spots in colchicine treated material.

Experiments were repeated using 0.005% colchicine instead of 0.0025% as in the above study. The results given in Table 2 confirm the observations made earlier, i.e. the application of colchicine with or without mitomycin *C* post-treatment increases the incidence of somatic crossing over. The general tendency here again is an increase in frequency of spots of all kinds. In one instance, however, a very small decrease was observed in the frequency of yellow spots in the colchicine treated material (Table 2, B, post-treatment with mitomycin *C*) which reflected on the frequency of total spots per leaf. (It may be mentioned that the yellow spots are the easiest to be overlooked on the light green background especially when all the layers of leaf tissue do not form the genetic mosaic which gives rise to the formation of spots in this material.)

Table 3 gives a summary of the results from another experiment conducted by using 0.01% of colchicine in different combinations of treatment period. The results have only confirmed the foregoing observations; colchicine treatment increases the frequency of spot formation in *Glycine max* var. L65-1237. The general decrease in spot frequency observed in these experiments appears to be due to experimental conditions other than an increase in the concentration of colchicine.

In a few cases a plant or two had exceptionally high frequency of spots on its leaves (e.g. 25—32 spots per leaf were observed in some cases). This resulted in a great increase in variance and eventually lowered the calculated value of t as seen in some cases in Table 2 and 3. However, when these abnormal readings were taken out of the raw data, the value of t indicated 90% confidence in most cases.

Discussion

The increase in the frequency of spots by colchicine is of interest from several points. An important aspect of this study is the indication of non-correlation of the timing of induction of somatic crossing over versus the time of disruptive action of colchicine on the spindle proteins holding the homologous

Table 2. *Types and frequency of spots in three sets of materials treated with water or 0.005% aqueous colchicine for different time periods and with or without post-treatment with Mitomycin C (0.0025%)*

Seed treatment			Type and frequency of spots per leaf				
Water or Colchicine	Post-treatment with Mitomycin *C*	No. of leaves	*DG*	*Yl*	*Db*	*T*	*t* (calculated)
A. 4 hr Water	none	100	0.20	0.20	0.19	0.59	$0.20 > p > 0.40$
4 hr Colchicine	none	125	0.35	0.23	0.21	0.79	
4 hr Water	4 hr	135	0.40	0.41	0.41	1.22	$0.025 > p > 0.05$
4 hr Colchicine	4 hr	105	0.62	0.69	0.69	2.00	
B. 8 hr Water	none	170	0.12	0.14	0.16	0.42	$0.05 > p > 0.10$
8 hr Colchicine	none	180	0.29	0.23	0.24	0.76	
8 hr Water	4 hr	130	0.45	0.64	0.62	1.71	
8 hr Colchicine	4 hr	105	0.52	0.50	0.63	1.65	
C. 16 hr Water	none	150	0.18	0.11	0.14	0.43	$0.05 > p > 0.10$
16 hr Colchine	none	125	0.52	0.38	0.32	1.22	
16 hr Water	4 hr	150	0.51	0.51	0.75	1.77	$0.20 > p > 0.40$
16 hr Colchicine	4 hr	150	0.91	0.75	0.95	2.61	

Table 3. *Types and frequency of spots in three sets of materials treated with water or 0.01% colchicine solution for 4 or 8 hours and with or without post-treatment with Mitomycin C (0.0025%)*

Seed treatment			Type and frequency of spots per leaf				
Water or Colchicine	Post-treatment with Mitomycin *C*	No. of leaves	*DG*	*Yl*	*Db*	*T*	*t* (calculated)
A. 4 hr Water	none	105	0.19	0.17	0.14	0.50	$0.20 > p > 0.40$
4 hr Colchicine	none	170	0.29	0.18	0.25	0.72	
4 hr Water	4 hr	95	0.31	0.31	0.23	0.84	$0.025 > p > 0.05$
4 hr Colchicine	4 hr	110	0.56	0.45	0.50	1.51	
B. 8 hr Water	none	135	0.25	0.22	0.13	0.61	$0.40 > p > 0.50$
8 hr Colchicine	none	120	0.35	0.23	0.13	0.72	
8 hr Water	4 hr	125	0.40	0.47	0.26	1.12	$p > 0.001$
8 hr Colchicine	4 hr	105	0.66	0.66	0.59	1.90	

chromosomes together in relation to the spatial arrangement of corresponding segments of homologous chromosomes. If colchicine can disrupt this arrangement fully or partially before the onset of the process of somatic exchange the frequency of spots in the materials treated with colchicine (with or witout mitomycin *C*) should be lower than the corresponding water-treated materials. The observations indicate (that, at least apparently) not only that colchicine is incapable of disturbing the interphase arrangements of chromosomes in this material but, on the other hand, helps to increase, somehow, the incidence of occurrence of somatic exchange, especially when followed by post-treatment with mitomycin *C*.

Even though colchicine is known to act mostly on the proteinaceous material (MacLeod and Davidson, 1968; Borisy and Taylor, 1967; Shelanski and Taylor, 1967) the results from the present experiments do not elucidate the mode of genetic transfer in *Glycine max* somatic cells and do not indicate that the process of genetic recombination necessarily involves DNA as suggested by some (e.g. Holliday, 1964). In fact the experiments using mitomycin *C* indicate that the synthesis (and perhaps involvement) of genetic material is not required for the accomplishment of somatic exchanges (i.e. breakage-reunion) since the exposure of seeds to mitomycin *C* during 4–8 hours after initiation of soaking in water increases the frequency of spots several-fold. The fact that mitomycin *C* may bind to DNA (Iyer and Szybalsky, 1963) to become effective at a later time does not hold because the effect of mitomycin *C* increases with an increase in the physiological activities of the water soaked seeds and is maximum around 20 hr (Vig, unpublished) when *Glycine max* hardly initiates its DNA synthesis (Miksche, 1966) (a large number of embryonic cells are in G_2). Also, there is evidence that alkylating agents like ethylmethane sulfonate do not increase the frequency of somatic crossing over in *Glycine* (Vig and Paddock, 1970). One might thus speculate concerning the role of mitomycin *C* and colchicine in breaking the weak points of chromosomes to help recombination (also see ref. 1).

Even though there is no apparent reason as to why colchicine increases the frequency of somatic crossing over in *Glycine*, it is not difficult to speculate as to why the present results do not correlate with the observations that colchicine leads to disruption of arrangement between homologues. The observations between distances of chromosomes in wheat were made at metaphase (Avivi, Feldman and Bushuk, 1969), whereas in case of somatic crossing over one observes the results of events which might not be effected by damage to the tubules holding the chromosome and/or centromeres in position. In other words, the chromosomes even in the nucleus with damaged micro-tubules are not disturbed until late interphase or prophase and complementary transfer leading to somatic crossing over might be accomplished before this disturbance. Also, the findings of Dewey and Miller (1969) that colcemid does not affect radiation-induced aberrations in chinese hamster chromosomes until late prophase support the hypothesis that somatic crossing over might be initiated when cells are in early interphase, long before colchicine becomes disruptive to interphase proteins. Secondly, the fibers observed running through chromatin mass of interphase nuclei (Behnke and Forer, 1966) may not be responsible for positioning of homologous parts of the genome involved in somatic exchange. One might also be tempted to agree with the hypothesis of chromosome substitution as a result of doubling followed by somatic reduction similar to the work in *Sorghum* (Sanders and Franzke, 1969). Such an idea has, however, been refuted by some (Dermen, 1964).

Dewey and Miller (1969) successfully recovered chromatid exchanges by irradiating colcemid pretreated cells in mitosis or G_1 when no DNA synthesis is observable. It not only indicates that genetic reunion may not require DNA synthesis but also shows that relative compactness of G_1 chromosomal strands can be affected under some circumstances. Similar results have been obtained by Wolff (1970) where he found urea and glycerol effective in loosening the relational compactness of the strands of G_1 chromosome. These results support the idea that colchicine can increase the incidence of somatic crossing over even when cells are treated in pre-DNA-synthetic phase. Similar argument can be used for mitomycin *C* which has been shown to cause chromatid (and perhaps also subchromatid) type of aberration in human leukocytes treated in G_1 (Nowell, 1964; Kihlman, 1966).

Literature Cited

1. Apparao, K., Gopinath, D. M.: Colchicine induced mutations in trispecific *Nicotiana* hybrid. Canad. J. Genet. Cytol. **10**, 782–787 (1968). — 2. Avivi, L., Feldman, M., Bushuk, W.: The mechanism of somatic association in common wheat, *Triticum aestivum* L. I. Supression of somatic association by colchicine. Genetics **62**, 745–752 (1969). — 3. Bajer, A.: Behavior and fine structure of spindle fibers during mitosis in endosperm. Chromosoma **25**, 249–281 (1968). — 4. Behnke, O., Forer, A.: Intranuclear microtubules. Science **153**, 1536–1537 (1966). — 5. Borisy, G. G., Taylor, E. W.: The mechanism of action of colchicine. Binding of colchicine — ^{3}H to cellular proteins. J. Cell Biol. **34**, 525–533 (1967). — 6. Carlson, J. G.: On the mitotic movements of chromosomes. Science **124**, 203–206 (1956). — 7. Commings, D. E.: The rationale for an ordered arrangement of chromatin in the interphase nucleus. Am. J. Human Genet. **20**, 440–460 (1968). — 8. Dermen, H.: The question of colchicine induced somatic reduction and the production of homozygous diploid mutants in *Sorghum*. Amer. Natur. **98**, 105–108 (1964). — 9. Dewey, W. C., Miller, H. H.: X-ray induction of chromatid exchanges in mitotic and G_1 chinese hamster cells pretreated with colcemid. Exptl. Cell Res. **57**, 63–70 (1969). — 10. Grell, R. F.: Meiotic and somatic pairing. In: "Genetic Organization", edited by E. W. Caspari and A. W. Ravin, pp. 361–492. New York and London: Academic Press 1969. — 11. Grun-

berg, H.: The case of somatic crossing over in the mouse. Genet. Res. **7**, 58–75 (1966). – 12. Hendrychova-Tomkova, J.: Local somatic colony changes in *Salvia splendens*. J. Genet. **59**, 7–13 (1964). – 13. Holliday, R.: The induction of mitotic recombination by mitomycin *C* in *Ustilago* and *Saccharomyces*. Genetics **50**, 323–335 (1964). – 14. 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). – 15. Kihlman, A.: Actions of chemicals on dividing cells. Englewood Cliffs, New Jersey: Prentice-Hall, Inc. 1966. – 16. MacLeod, R. D., Davidson, D.: Changes in mitotic indices in roots of *Vicia faba* L. Exptl. Cell Res. **52**, 541–554 (1968). – 17. Maguire, M. P.: Evidence for homologous pairing of chromosomes prior to meiotic prophase in maize. Chromosoma **21**, 221–231 (1967). – 18. Miksche, J. P.: DNA synthesis in primary root of *Glycine max* during germination. Can. J. Bot. **44**, 789–794 (1966). – 19. Nowell, P. C.: Mitotic inhibition and chromosome damage by mitomycin in human leukocyte cultures. Exptl. Cell Res. **33**, 445–449 (1964). – 20. Rao, R. N., Natarajan, A. T.: Somatic association in relation to chemically induced chromosome aberrations in *Vicia faba*. Genetics **57**, 821–835 (1967). – 21. Sanders, M. R., Franzke, F.: Colchicine-induced complex diploid mutants from tetraploid seedlings. J. Heredity **60**, 137 to 148 (1969). – 22. Serebovsky, A. S.: Somatic segregation in domestic fowl. J. Genet. **16**, 33–42 (1926). – 23. Shaw, M., Cohen, M. M.: Chromosome exchanges in human leukocyte induced by Mitomycin C. Genetics **51**, 181–190 (1965). – 24. Shelanski, M. L., Taylor, E. W.: Isolation of a protein subunit from microtubules. J. Cell Biol. **34**, 549–554 (1967). – 25. Stern, C.: Somatic crossing over and segregation in *Drosophila melanogaster*. Genetics **21**, 525–630 (1936). – 26. Vig, B. K.: Relationship between mitotic events and leaf spotting in *Glycine max*. Can. J. Genet. Cytol. **11**, 147–152 (1969). – 27. Vig, B. K., Paddock, E. F.: Alteration of spot frequencies in leaves of *Glycine max* (L.) Merrill by mitomycin. J. Heredity **59**, 225–229 (1968). – 28. Vig, B. K., Paddock, E. F.: Studies on the expression of somatic crossing over in *Glycine max*. Theor. Appl. Genet. **40**, 316–321 (1970). – 29. Wolff, S.: On the "tertiary" structure of chromosome. Mutation Res. **10**, 405–414 (1970).

Received November 25, 1970

Communicated by H. Stubbe

Dr. B. K. Vig
Department of Biology
University of Nevada
Reno, Nevada 89507 (USA)