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Gamma-ray Induced Meiotic Chromosome Stickiness in Tomato

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Summary. In gamma-ray treated populations of tomato, one of the plants showed stickiness of chromosomes at meiosis. At diakinesis, some of the bivalents tended to fall apart as univalents, and at about the same time or a little later, the bivalents and/or univalents congregated into groups. At metaphase I, the number of such groups varied from 1 to 14 and the groups were of various sizes; the masses of chromatin in which the individuality of chromosomes seemed to be completely lost were spherical, oval or irregular in outline and freely dispersed in the cytoplasm. Some of the masses were so small that they might possibly represent fragments of chromosomes. As the stage passed to anaphase I, the masses dissolved into individual chromosomes, even though stickiness was still persistent but less intense. Laggards in varying numbers were also found. At completion of meiosis, in some proportion of telophase II cells, persistent laggards were found. Pollen fertility and seed set were low. The selfed M_2 progeny of the sticky plant contained a few yellow seedlings which died at the cotyledon stage. Cytological examination of meiosis of some of the individuals of this progeny revealed stickiness again in a majority of plants. Sticky as well as normal plants in M_2 were selfed and the M_3 generation raised. In progenies of both kinds, yellow lethals were found in proportions that gave a good fit with three green to one yellow seedlings in many cases. The occurrence of a sticky plant in M_1 and many such plants in M_2 was assumed to be due to a dominant mutation induced by gamma irradiation. This, besides causing sticky meiosis, also produced recessive yellow lethal mutations.

Key words: Tomato - Meiosis - Sticky Chromosomes - Gamma-Rays - Chlorophyll Deficiency

Introduction

Variations in the meiotic process are known to be governed by environmental as well as genic factors. Since such variation is of considerable significance to the biological world, a large body of evidence concerning its control has accumulated during recent times. Genotypic control of meiosis, in the form of mutations affecting - and causing breakdown of - specific stages, is perhaps the best known. The phase of meiosis most frequently affected seems to be synapsis by spontaneous or induced recessive mutant genes such as desynaptic, asynaptic, sticky etc. and by such environmental factors as temperature, moisture content of soil, X-rays, chemicals etc. In the tomato, apart from the synaptic and male sterile genes, the only gene-governed meiotic abnormality known is precocious centromere division, leading to the formation of aneuploid and polyploid gametes (Clayberg 1959). In the present investigation on irradiated populations, one of the plants exhibiting abnormal meiosis was located, and its aberrant meiosis and the consequential effects on the progenies were studied.

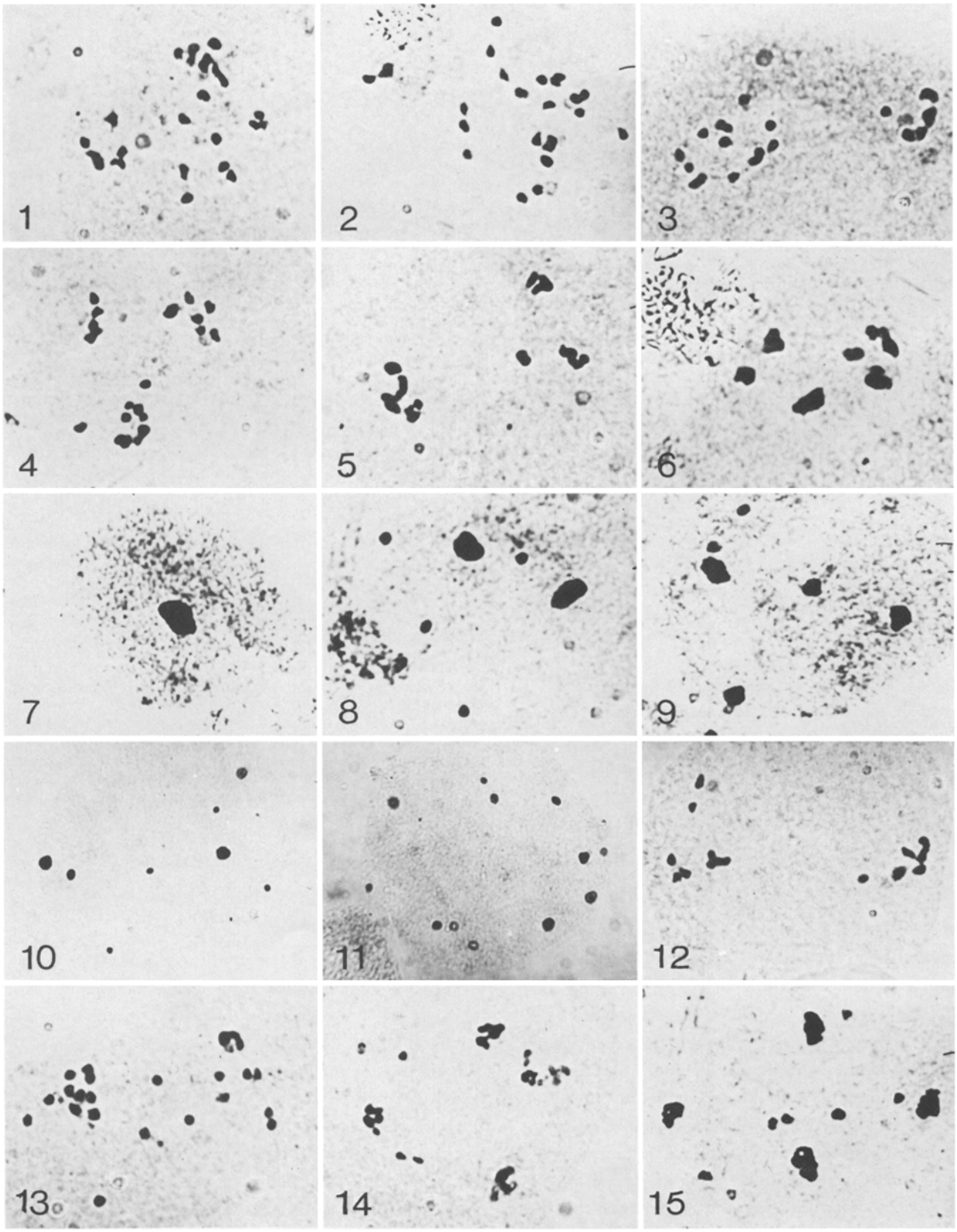
Materials and Methods

Seeds of var. 'Marglobe' were subjected to 20 Kr, 30 Kr and 40 Kr gamma-rays, sown in germinator trays within a week of irradiation and later planted into the field. Cytological studies were made on flower buds fixed in a freshly prepared solution of propionic acid and absolute alcohol (1:3) for 24 hours and stored in 70 % alcohol under refrigeration. Squashes were made in 2 % propionic carmine and destained to the desired extent with 45 % acetic acid.

Results

During the cytological check of the gamma-ray treated populations, one plant from 30 Kr treatment in M_1 generation showed stickiness of chromosomes during meiosis. This plant did not differ significantly from the rest of the treated or control plants in morphological features like height of plant, shape and size of leaf and of floral parts etc. The fruits, however, were smaller than in control plants. The plant showed reduced fertility.

A study of meiosis in the abnormal plant showed that pachytene pairing was perfectly normal, and al-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so at early diakinesis the bivalent organization was normal except that the rod bivalents were more frequently encountered than in control plants. In the comparable mid-diakinesis phase, 2 to 6 univalents per cell were also found in the sticky plant in some cells (Table 1). The average chiasma frequency in the abnormal plant worked out at 13.08 ± 0.3146 per cell and 1.09 ± 0.0281 per bivalent, in contrast to 15.50 ± 0.1828 and 1.29 ± 0.0178 in the control plant, these differences being significant at 1% level. In later phases of diakinesis, some of the bivalents showed a tendency to fall apart into univalents. The number of such univalents observed per cell varied between 0 to 18 (Figs.1 and 2). At about the same time, or a little later, the univalent chromosomes and/or bivalents exhibited a tendency to congregate together into groups. The number of such groups during the transition phase from diakinesis to metaphase I ranged from 1 to 6 with varying numbers of univalents (Figs.3 to 6). The typical orientation of bivalents on the plate at metaphase I was never encountered. The chromatin clumps formed by the chromosomes were of different sizes and number varying from 1 to 14 per cell (Table 2) and were freely dispersed throughout the cytoplasm (Figs.7 to 11). They appeared as dense masses of chromatin with a circular, oval or irregular outline and the identity of individual chromosomes or bivalents in a mass was completely lost (Figs.7 to 11). In certain cases, the size of the chromatin bodies observed was too small to represent masses formed by univalent chromosomes and, therefore, it is believed that fragments of chromosomes, though rare, also occurred. In the process of transformation from metaphase I to anaphase I, the several transition phases studied indicate that the clumps gradually resolved into individual chromosomes. By the time anaphase I was fully set in, it appeared more or less normal although in some cells

Table 1. Chromosome associations at mid-diakinesis in sticky and normal plants of tomato

Bivalents		Univalents	No. of cells	
Ring	Rod		Sticky plant (3-23-22)	Control
-	12	-	1	-
1	11	-	-	6
2	10	-	2	9
3	9	-	4	6
4	8	-	-	18
5	7	-	-	15
-	11	2	1	-
1	10	2	2	-
2	9	2	6	-
3	8	2	3	-
4	7	2	2	-
1	9	4	2	-
-	10	4	1	-
1	8	6	1	-

Table 2. Frequency of cells with large and small sticky chromatin clumps at metaphase I in tomato

		Large clumps				
		1	2	3	4	5
Small clumps	0	4	34	1	36	-
	1	2	15	1	16	-
	2	1	29	5	16	3
	3	4	22	3	7	-
	4	3	7	4	3	-
	5	1	11	5	4	1
	6	1	6	-	3	-
	7	-	5	1	1	1
	8	-	1	-	1	-
	9	-	3	-	-	-
	10	-	1	-	-	-
	11	-	-	1	-	-

chromosome masses of variable number were still present towards either pole making the count of chromosomes at each pole rather difficult (Fig.12). However, the stickiness was much less intense, compared with metaphase I, and the ends of at least some individual chromosomes in a mass could always be

Figs.1-15.
1. Late diakinesis: 6 II and 12 I
2. Late diakinesis: 3 II and 18 I
3 to 6. Transition stages from diakinesis to metaphase I showing the processes of congregation of chromosomes into various groups
7 to 11. Metaphase I: Sticky chromatin masses of variable number, size and shape:
7. One group
8. 2 large and 4 small groups
9. 4 large and 2 small groups
10. 3 large and 5 small groups
11. 4 large and 8 small groups
12. Anaphase I: Persistent stickiness
13. Anaphase I: 4 laggards
14. Telophase II: 2 laggards, one of them dividing
15. Telophase II: 6 persistent laggards
(Magnification: Figs.10 and 11 (X 1500), rest (X 2500))

Table 3. Segregation for green and yellow seedlings in M_3 generation raised from selfed seed of M_2 plants in tomato

Plant No. M_2 generation	Meiosis	M_3 Seedling progeny		χ^2 value (3:1 ratio)	p
		Green	yellow lethal		
23 - 4	Normal	36	20	3.4286	0.20 - 0.05
23 - 7	Normal	63	10	4.9727	0.05 - 0.01
23 - 11	Sticky	38	4	5.3651	0.05 - 0.01
23 - 17	Sticky	27	7	0.3529	0.80 - 0.50
23 - 21	Sticky	1	0	-	-
23 - 22	Sticky	2	1	-	-
23 - 27	Normal	57	15	0.7667	0.50 - 0.20
23 - 28	Normal	48	11	1.1271	0.50 - 0.20
23 - 29	Normal	48	16	0.0000	-

discerned at this stage. Among the cells where the chromosomes could be clearly counted, distributions varying from 12:12 to 14:10 were found in 72% of cells examined; laggards ranging from 1 to 4 (Fig. 13) and occasionally fragments were observed in the rest. The pre-metaphase I association of chromosomes into sticky clumps was more or less random (judging from the variations in number of clumps) in that those bivalents and/or univalents which were nearer to each other spatially within the cell congregated into groups (Figs. 4 and 5) and subsequently dispersed in the cytoplasm. Thus when the individuality of the chromosomes is partly or fully reestablished by dissolution of the sticky masses at anaphase I with the chromosomes moving to the poles (Fig. 13), it is likely that, even though a 12:12 distribution may be accomplished, the gametes resulting from such a distribution might not always represent a typical haploid complement of chromosomes and could be duplication-deficiency types. After the completion of anaphase I, no further meiotic irregularities were found in the second division except those already encountered, such as persistent laggards, fragments etc. (Figs. 14 and 15). The rest of the meiosis was completed and pollen quartets were formed, though micronuclei resulting from persistent laggards were found in some cases. 54.69% of the pollen was shrivelled and unstained and on selfing, though fruits were formed, the number of seeds in each was 25 or less, compared with about 95% pollen fertility and 120 seeds per fruit in the control plants.

The selfed progenies of the sticky chromosome plant were raised in M_2 generation; 4 seedlings out of 72 were yellow (one of them variegated) and died at the cotyledon stage. The remaining 52 green plants were planted into the field. 20 of them died after planting. A cytological check could be made of only 14 plants of the 32 available. 8 showed sticky meiosis, one plant was desynaptic and the remaining 5 were normal. Among the 8 sticky plants, 2 did not show as much stickiness as the parent (showing partial stickiness) but the meiotic phenomenon was more or less the same in all the sticky plants. Like their parent, the sticky plants were highly pollen sterile and 3 of them did not set any seed on self pollination, whereas the normals in the same progeny were fertile. Seed set was also completely lacking in the desynaptic. The somewhat fertile sticky plants and the fertile normals were selfed and their seedling progenies were raised as M_3 generation. The seeds from one of the sticky plants did not germinate. In the progenies of both sticky and normal plants some yellow lethals were produced (Table 3).

Discussion

Beadle (1932) reported the first case of recessive gene governed meiotic chromosome stickiness in maize. Similar stickiness was found in maize after X-irradiation also (Beadle 1937). Subsequently stickiness was observed in inbred lines of *Alopecurus myosuroides* (Johnsson 1944) and *Picea abies* (Andersson 1947). In both cases stickiness was shown to be genotypically controlled. However, Eriksson (1968) in *Larix* and Andersson (1947) in *Picea* found that

stickiness could be induced by external agents like cold shock treatment as well. In *Rosa* the stickiness could be the result of interspecific hybridization and the interaction of genotype and environment (Klásterská and Natarajan 1975).

Since the stickiness observed in the present study occurred in M_1 generation after gamma irradiation and since the selfed progeny of the sticky plant showed segregation for both sticky and normal plants, it is probable that stickiness is governed by a dominant mutation induced by irradiation. As the high sterility was found only in the sticky plants in M_1 and M_2 generations, and the normal segregates of the M_2 generation (excepting the desynaptic) were quite fertile, it is evident that the sterility in the former was due to chromosome stickiness. Since pachytene pairing was normal in sticky plants, the occurrence of many rod bivalents and some univalent chromosomes at diakinesis suggests that there was general reduction in chiasma formation or slipping off of some chiasmata, which accounts for the significant difference in average number of chiasmata per cell and per bivalent between the sticky and normal plants. The occurrence of a desynaptic plant and yellow lethal seedlings in the selfed progeny of the sticky plant in M_2 generation, the appearance of yellow lethals in the selfed progeny of sticky as well as normal plants in M_3 generation, and their absence in any of the other irradiated populations and their progenies, suggest that their origin is in some way connected with stickiness in the parent. The genetic segregations for yellow lethals and green seedlings in at least some of the progenies seem to suggest that the lethals were probably homozygous recessives. The deviations from expectation of a 3:1 ratio of green and yellow lethals in M_2 progeny and in two of the progenies in M_3, however, remain unexplained. The above facts imply that the apparent dominant sticky mutation has manifold effects not only on meiosis but also on the progenies following sticky meiosis.

Endosperm colour variegation and striped seedlings in the progenies of sticky plant were found in maize also, but these were shown to be the consequence of deficiencies of entire chromosomes or segments of chromosomes carrying the respective genes in the concerned tissues (Beadle 1937). In the present investigation, a study of mitosis in root tip cells from selfed seeds of the sticky plant did not give any indication of chromosome elimination and all the progenies of sticky plants examined had 2n = 24 chromosomes; furthermore the yellow lethals appeared not only in the progenies of the sticky plants but also in those of normal segregates. All these facts offer presumptive evidence that yellow lethals represent recessive mutations.

In maize, translocation heterozygotes occurred in the progenies with a greater frequency than in controls (Beadle 1932). Such translocations were not found in the present study but it should be pointed out that the progeny examined was too small to enable recovery of any translocation heterozygotes. Similarly the irregular distribution of chromosomes at anaphase I should result in the occurrence of aneuploids in the progenies. However, no such aneuploids were located.

In sticky plants of maize, abnormality in meiosis starts right from the pachytene stage and continues through anaphase I and a number of chromatin bridges were found at anaphase I. But in tomato, although the stage most affected was metaphase I, the effects of the sticky gene were initiated at diakinesis and persisted into anaphase I, though with less intensity, and no chromosomal bridges were encountered. Thus the effects of the apparently dominant sticky gene in tomato are somewhat similar, though not identical, with the manifestations of the recessive sticky gene in maize. As there were variations in the extent of chromosome stickiness in the sticky plants of the M_2 generation in the present study, it is probable that there are some modifiers also controlling the expression of the sticky gene.

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