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Chiasma Formation in the Short Arm of the Nucleolar Chromosome of the Tomato*

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Summary. A translocation heterozygote in tomato (*Lycopersicon esculentum*) is shown to have a cyclical type of interchange between the long arms of chromosomes 1, 2 (nucleolar) and 3. A study of chromosome association in this plant at metaphase I has indicated that in 21% of the cells a ring of six chromosomes is present. Since an open ring hexavalent can occur only if there is chiasma formation in all the translocated segments and in all the short arms of the three chromosomes, it is concluded that there is considerable frequency of chiasma formation in the short arm of the nucleolar chromosome. This conclusion contradicts the previous observations that chiasma formation is either absent or very rare in the entirely dark staining chromatic, sometimes referred to as heterochromatic, short arm of the nucleolar chromosome.

Introduction

When the pachytene chromosomes of tomato are stained with common nuclear stains, the regions adjacent to the centromeres in all chromosomes become stained darkly compared to the regions away from the centromeres. These darkly and lightly staining regions were called "chromatic" and "achromatic" regions by Brown (1949), but in the more recent literature the terms "heterochromatic" and "euchromatic" regions respectively have been used (Khush *et al.*, 1964). Apart from differences in their staining properties, these regions have been reported to possess certain important genetic differences as well. As different from the achromatic regions, the chromatic regions are devoid of genes (or nearly so), and chiasma formation and crossing-over are supposed to occur very rarely if ever.

Cytological evidence for the absence of chiasmata in the chromatic regions was provided by Brown (1949) and Barton (1951). While Brown (1949) observed the absence of chiasmata in the chromatic regions by cytological observations, Barton (1951) confirmed the above observation by studying chromosome configurations in a translocation heterozygote. This had a reciprocal exchange between the entirely chromatic short arm of chromosome 2 (nucleolar) and the chromosome 12. At metaphase I in this plant he observed chains of 4 (63%), 2 pairs (33%) and a trivalent and a univalent (4%) — but never a ring of four. The absence of a ring of four in this case was interpreted by Barton as due to the lack of chiasma formation in the short arm of chromosome 2. But break in this arm may have reduced pairing below the level of detection (in 154 cells).

From the present study on a translocation heterozygote involving the nucleolar and two other chromosomes, it is found that chiasma formation in the short arm of the nucleolar chromosome is not lacking, or it is not as rare as it has been supposed to be.

Material and method

The translocation heterozygote was detected in *Lycopersicon esculentum*, var. Moneymaker in a shoot cutting from a plant raised from seed irradiated with thermal neutrons (R. B. Contant kindly supplied the material). This shoot was rooted in sand and grown to maturity.

For cytological study, young anthers were fixed for 48 hours or more in 3:1 of ethyl alcohol and propionic acid saturated with iron acetate and prepared as aceto-carmine squashes.

Results

Identification of the translocation break points at pachytene

A preliminary examination of pachytene bivalents in well spread pollen mother cells indicated that except chromosomes 1, 2 and 3, all were completely normal, and all the normal ones could be traced individually from one end to the other. The three longest chromosomes 1, 2 and 3 were invariably connected to each other in the achromatic regions of their long arms forming cross-shaped figures that are characteristic of translocation pairing (Fig. 1). All the three short arms as well as the end portions of all the three long arms of these chromosomes were normal. Since non-homologous arms of tomato chromosomes can pair very closely (Ramanna, 1969), it is impossible to localize the exact points of interchange of the chromosomes. However, from the study of 15 configurations, the probable lengths of the translocated segments (*a*, *b* and *c*) were determined (Figs. 2 and 3) but this is only a broad generalization.

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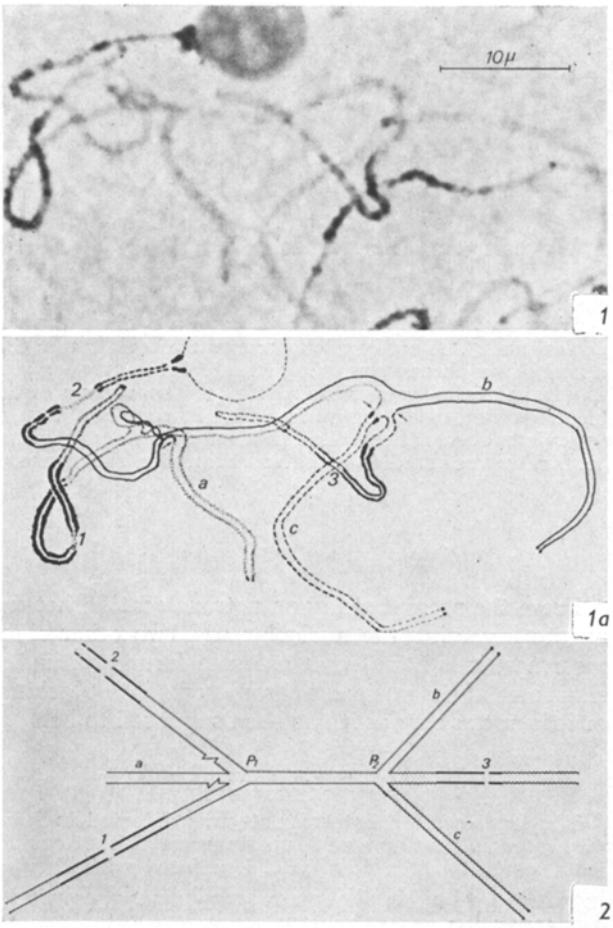


Fig. 1. Pachytene chromosomes 1, 2 and 3 showing translocation pairing
Fig. 1a. Interpretive drawing of Fig. 1. Arrows indicate the approximate points of interchanges
Fig. 2. Diagramatic representation of pachytene pairing of the three translocated chromosomes. The translocated segments of chromosomes 1, 2 and 3 are indicated by letters *a*, *b* and *c* respectively, the centromeres with their distinctive chromatic regions by 1, 2 and 3

The translocated segments can be distinguished from each other. The segment *a*, representing the end part of the long arm of chromosome 1, has a very inconspicuous telomere and this segment is always associated with the point connecting (P1) chromosomes 1 and 2. The segments *b* and *c*, representing the end parts of the long arm of chromosomes 2 and 3 respectively, have telomeres but the segment *c*, unlike *b*, has a group of chromomeres in the achromatic region. The segments *b* and *c* are always connected to the interstitial segment of chromosome 3 (P2). In view of the positions of the translocated segments and the respective chromosome arms (Fig. 2), it may be concluded that this is a cyclical type of translocation mentioned by Burnham (1962). According to this, breaks have occurred in the long arms of chromosomes 1, 2 and 3; a segment of chromosome 1 is translocated to chromosome 2; a segment of chromosome 2 is translocated to chromosome 3; and a segment of chromosome 3 is translocated to chromosome 1 (Fig. 3).

Chromosome association at diplotene

In 76% of the cells a ring of 6, invariably attached to the nucleolus, is present and all other chromosomes are paired normally to form 9 bivalents (Figs. 4, 5 and 7). In 21% of the cells the configurations are chains of 6, also attached to the nucleolus, and in 3% of the cells they could not be distinguished clearly. Because of the nucleolar attachment, the chromosome 2 can always be recognized at this stage. The other two pairs, 1 and 3, in the hexavalent have slight but clearly recognisable differences in size especially in chromatic segments (Figs. 4—7) which helps to identify them. Since the different pairs can be identified at this stage, it is possible to detect whether chiasmata are formed in the translocated segments *a*, *b* and *c* or in the respective interstitial segments or both. When there are chiasmata only in the translocated segments and the three short arms a simple open ring of 6 will result as in Fig. 4. 38% of the rings were of this type. In addition, if there is chiasma formation in the interstitial segments of chromosomes 1, 2 and 3, the configurations illustrated in Figs. 5, 6 and 7 respectively will result. 26% of the rings had interstitial chiasmata in chromosome 1, 22% in chromosome 2 and 8% in chromosome 3. In 6% of the rings there appeared to be 2 interstitial chiasmata.

In any case, from the study of diplotene no conclusion can be made regarding the presence or absence

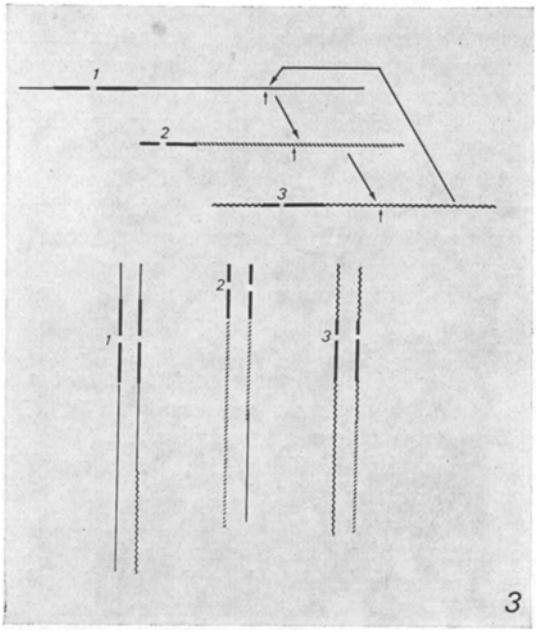


Fig. 3. Diagram showing the cyclical translocation between chromosomes 1, 2 and 3 (small vertical arrows indicate break points)

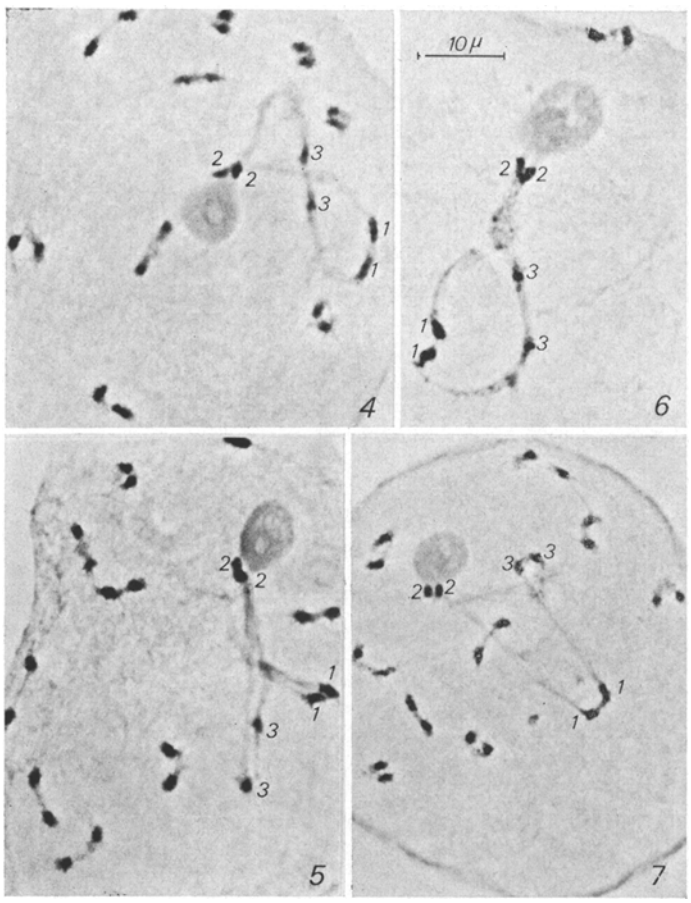
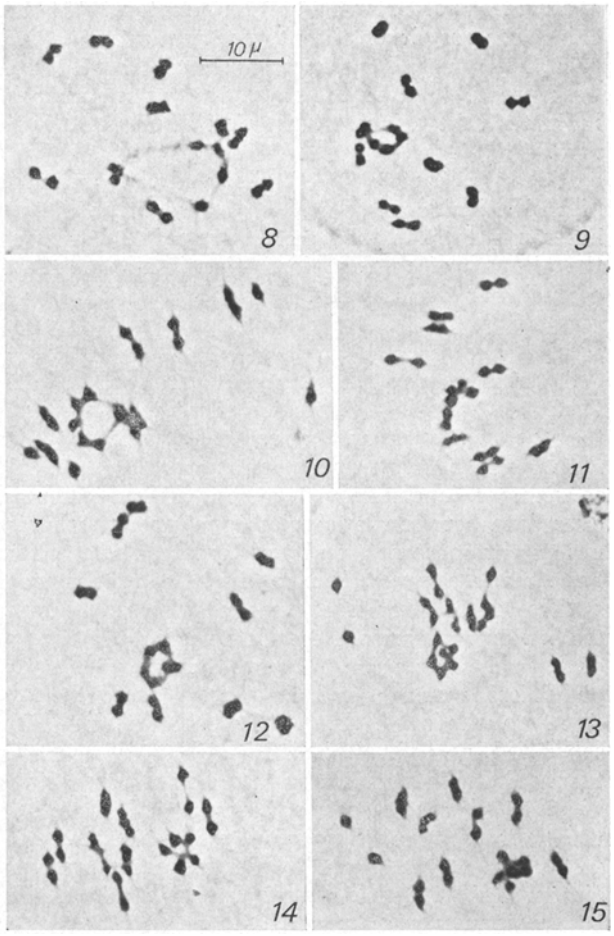


Fig. 4. Diplotene showing a simple ring of 6 attached to nucleolus + 9 bivalents. There is no interstitial chiasmata in this ring
Fig. 5. Diplotene-hexavalent showing interstitial chiasmata in chromosome 1
Fig. 6. Diplotene-hexavalent showing interstitial chiasmata in chromosome 2
Fig. 7. Diplotene-hexavalent showing interstitial chiasmata in chromosome 3



Figs. 8—9. Pro-metaphase I showing rings of 6
Fig. 10. Metaphase I showing a ring of 6
Fig. 11. Metaphase I showing a chain of 6
Figs. 12—14. Metaphase I showing interstitial chiasmata in the rings of 6
Fig. 15. Metaphase I showing complicated chromosome configuration which cannot be categorised

of chiasmata in the short arm of the nucleolar chromosome because of nucleolar attachment.

Chromosome association at metaphase I

At late diakinesis and pro-metaphase I the chromosomes are not yet arranged on the equatorial plate and the centromeres are not subjected to traction forces of the spindle fibers. Except that the nucleolus is absent from the cells, these stages are not very different from diakinesis (Figs. 8 and 9). Therefore, no conclusion can be reached regarding chiasma formation in the short arm of the nucleolar chromosome even at this stage.

As in diakinesis, at mid and late metaphase I, either rings of 6 (Figs. 10, 12, 13 and 14) or chains of 6 (Fig. 11) are present in addition to 9 normal bivalents. 36% of the hexavalents at this stage are chains of 6 and 21% are rings of 6. In the remaining 43% of the cells the configurations were, partly because of interstitial chiasmata, aggregated to such an extent that they could not be interpreted (Fig. 15). Among the 21% with rings, 8% had open rings (Fig. 10) and 13% had rings with interstitial chiasmata (Figs. 12, 13 and 14). Of these 13%, 3% lacked chiasmata in one of the arms and derived their ring shape from interstitial chiasmata. Thus of all the cells 18% had chiasmata in all the arms.

Discussion

If each connection between different chromosomes at metaphase I of meiosis results from the formation of at least one chiasma, the ring of 6 (Fig. 9) has a minimum of 6 chiasmata formed in the three translocated segments and the three short arms. Failure of chiasma formation in any one of them should result in a chain of 6. This is the expected result if the

observations of Brown (1949) and Barton (1951) that chiasmata are either absent or very rare in the short arm of the nucleolar chromosome were to be correct. Presence of 18% of the rings of 6 at metaphase I indicates that chiasma formation in this arm is not rare.

Although Barton (1951) observed the occurrence of a high incidence of chains of 4 in a translocation heterozygote, it is not a conclusive evidence to prove the absence of chiasma formation in the short arm of nucleolar chromosome. This condition may as well result from the failure of chiasma formation of the arms of chromosome 12.

Apart from the cytological evidence, Khush and Rick (1967 and 1968) have provided genetic evidence to support the view that crossing-over in the chromatic (heterochromatic) region is much lower than in achromatic (euchromatic) region. Based on cross-over data between cytologically mapped marker genes on chromosome 4 and 9 of tomato, these authors have made a generalization that "heterochromatin suffers much lower cross-over rates than euchromatin". For comparison, they have cited the example of *ah—wd*, present on 9L and 9S respectively (i. e., on different arms), and *ah—marm*, present on 9L (i. e., on the same arm). The former (*ah—wd*) is separated by a physical distance of 9.4 μ (at pachytene) of heterochromatin but has a cross-over distance of only 3—4 centimorgans (i. e., 0.37 cM/μ), whereas the later (*ah—marm*) is physically separated by a distance of 10.2 μ (at pachytene) but has a cross-over distance of 38 centimorgans (i. e., 3.8 cM/μ). This 10 times lower cross-over rate between *ah—wd*, when compared to *ah—marm* is explained as due to heterochromatin. But the existance of a centromere between *ah — wd*, unlike *ah—marm*, has not been taken into account by these authors. At least in the two examples they have cited, the one mentioned above and *ful-ra* on chromosome 4, it is certainly not proved that the lower rate of crossing-over is not due to the presence of a centromere but due to the presence of heterochromatin alone.

Thus, there seems to be no clear evidence to prove that there is any marked difference between chromatic and achromatic regions with regard to chiasma formation and crossing-over.

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