CHROMOSOME STUDIES IN ARABIDOPSIS THALIANA¹

LOTTI M. STEINITZ-SEARS

University of Missouri, Columbia, Missouri

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THE advantages of using *Arabidopsis*, a small crucifer, for studies of mutation and other genetic problems were pointed out by G. P. RÉDEI (1962). His establishment of five linkage groups (RÉDEI, private communication) and the occurrence of certain aberrant mutants made a study of *Arabidopsis* chromosomes desirable. The following is a report on the cytology of normal *Arabidopsis thaliana* (L.) Heynh. and the five primary trisomics which have been obtained.

As early as 1907 LAIBACH gave the chromosome number of *A. thaliana* (*Stenophragma Thalianum*) correctly as $2n=10$. His determination was based on meiosis and on prochromosomes (chromocenters) of the nondividing nucleus. LAIBACH worked with a variety of plants and was mostly concerned with proving the individuality and continuity of chromosomes, still a controversial question at that time. WINGE (1925) published a camera lucida drawing of a second meiotic metaphase showing the two plates of five very contracted chromosomes. At this stage, depending on the view, the chromosomes are almost spherical or dumbbell shaped, and no individual identification is possible. JARETZKY (1928) confirmed the presence of five pairs of chromosomes from a second meiotic metaphase plate. MANTON (1932) determined somatic chromosome numbers of *A. pumila* ($2n=32$) and *A. suecica* ($2n=26$ to 28). She states "The results of other investigators with *A. thaliana* ($n=5$) are exceedingly anomalous and, in spite of the number of records, should be confirmed by a somatic count." TITOVA (1935) from somatic counts gave the number as $2n=6$. Her drawing suggests that this was some other species. It seems very unlikely that *A. thaliana* could occur with three pairs of chromosomes. On the other hand, close somatic association might lead to misinterpretation; but in her drawings the two satellited chromosomes lie far apart and this would make it unlikely, according to the author's experience, that the chromosomes showed somatic pairing.

The past major difficulties of cytological determinations and the absence in the literature of photomicrographs of the chromosomes of *Arabidopsis* can be attributed to their small size both in meiosis and mitosis. The anthers within a flower are not well synchronized, and one anther often contains no more than half a dozen cells at a useful stage of meiosis. Although mitoses are frequent, the chromosomes are tiny and may be obscured by free floating prochromosomes from broken cells. It is possible to utilize the prominent prochromosomes for somatic counts, as has indeed been done by RÖBBELEN (1957), since these

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heterochromatic regions agree in number with the number of chromosomes present.

All the material used in this study was grown by G. RÉDEI at the University of Missouri, who also found the polyploid lines, made the crosses involved and selected the trisomics. His help made this study possible and his enthusiastic interest is gratefully acknowledged. The author also wishes to thank E. R. SEARS for confirmation of microscopic determinations and for constructive criticism.

METHODS

To obtain well-stained cytological preparations of *Arabidopsis* it is essential to secure good fixation by evacuating during the initial ten minutes. The best fixation has been obtained with 6 parts methanol:3 parts chloroform:2 parts propionic acid, a mixture used by PIENAAR (1955). The material is transferred to fresh fixative after evacuation and can be used after 48 hours. If refrigerated, satisfactory preparations can be obtained without further transfer for up to two

FIGURE 1.—*Arabidopsis*, grown under short day conditions. (a) normal wild type, $2n = 10$; (b-f) the five primary trisomics.

TABLE 1
Characteristics of trisomics grown under short day conditions

Figure	Color	Cotyledon	Rosette leaves	Petiole	Vigor	No. of offspring		No. analysed in F_4	
						Total	Trisomic	Meiosis	Mitosis
1b	dark	somewhat round	round with sharp shoulders in compact rosette	wide	good	15	: 2	2	2
1c	light	narrow	elongated with smooth margin	normal	poor	18	: 5	4	4
1d*	normal	narrow	narrow with cut margin	curved	fair	30	: 6	2	2
1e	normal	narrow	small	long	weak, slow growth	26	: 2	0†	1
1f	normal	normal	narrow with serrated margin	normal	fair	56	: 2	1	1

* Elongated anthers.
† Seen only as 2n in F_3 .

months. For longer preservation 70 percent ethyl alcohol may be used. For staining, whole inflorescences are placed in aceto-carmin or acetic orcein in a 60°C oven for six hours or overnight. Acetic orcein has the advantage, for most purposes, of not staining the nucleolus or cytoplasm. Orcein does stain pro-chromosomes however, and it is impossible to avoid them completely when taking photomicrographs, since they float throughout the preparations from broken cells. These small round bodies are seen somewhat out of focus in several of the illustrations (e.g. Figure 4a). They do not interfere with observations, as they are easily distinguished from mitotic or meiotic chromosomes in the actual preparations. The squash may be made directly in the stain or in 45 percent acetic acid.

A dissecting microscope is used for freeing the anthers from as much of the other floral tissue as possible. This rather tedious procedure is essential if good results are to be obtained. Buds of diploid *Arabidopsis* are only 0.2 to 0.4 mm long at the time when the anthers, 0.08 to 0.12 mm long, contain microspores in meiosis. Lines with polyploid chromosome numbers have somewhat larger buds and anthers. After the six anthers have been taken out of the bud and all extraneous material has been removed, a coverslip is dropped onto the unbroken anthers. If in meiosis, healthy anthers will usually be broken in the process, and the microspores will float out. With poorly fertile anthers some additional pressure and gentle heating may be required to obtain satisfactory preparations. Slides can be made permanent by any of the usual methods.

OBSERVATIONS

A. thaliana has five pairs of chromosomes. The nucleolar chromosome is the longest (3.7μ at TI) and frequently, but not always, forms a rod-shaped bivalent at MI. It is clearly heterobrachial and usually forms at least one chiasma in the long, satellite-bearing arm (Figure 3b,c). Three pairs are intermediate in size, one with a median, the other two with sub-median kinetochores. The fifth pair is considerably smaller (1.1 to 1.4μ at TI), and its kinetochore appears to be sub-median. This smallest pair almost always occurs as a ring bivalent. Univalent chromosomes, e.g. in trisomic plants (Figure 4d,e), become greatly contracted and dotlike at MI, making it impossible to identify them cytologically. In favorable cells chromosomes involved in a trivalent may be identified at MI or TI.

From genetic and morphological considerations RÉDEI suspected polyploidy in some lines obtained from X-rayed material, and upon analysis some were found to be tetraploid, others hexaploid (Figure 2c,f). From crosses of $4n \times 2n$, a triploid F_1 was obtained. The triploid was fertile and set selfed seed. F_2 's to F_1 's were grown, and those that differed morphologically from their euploid parents were marked and analysed cytologically. The five trisomic types (Figure 1) were obtained, as well as other more complex aneuploids. Table 1 lists the distinguishing characteristics of the plants, and preliminary data on trisomic transmission, and gives reference to the illustrations of each. That these

FIGURE 2.—Somatic cells. (a, b) Non-dividing nuclei with prochromosomes $2n = 10$, and $2n = 11$ respectively. Arrows in b point to two (lower) and three (upper) fused prochromosomes. (c-f) Mitotic metaphases. c, tetraploid; d, diploid; e, trisomic; f, hexaploid ($6n \pm 2$). $5000\times$.

five types are the five different primary trisomics must still be confirmed by genetic and cytological work, but their strikingly different phenotypes make this highly probable. No definite identifications have been made of the trisomics with respect to particular chromosomes as recognized cytologically. Preliminary observations suggest that the characteristically serrate-leaf type (Figure 1f) may be trisomic for the smallest chromosome. The very weak trisomic (Figure 1e) probably has the long, satellited chromosome in triplicate. The round-leaf, narrow-leaf, and elongate types (Figure 1b, 1c and 1d respectively) are trisomic for one of the medium length chromosomes.

Meiosis in *Arabidopsis* is quite orthodox, but some trivalents and quadrivalents are easily mistaken for bivalents (Figure 3e,f). To avoid errors, analysis of chromosome constitution must be based on both MI and TI (Figure 4). Univalents in trisomic plants lie off the plate, (Figure 4e,d) divide at TI, and are usually included in the TI nuclei. Reciprocal translocations in X-rayed material are easily identified as rings and chains and no difficulty is anticipated in determining inversion bridges in *Arabidopsis*. LANGRIDGE (1958) working with such material found in X_2 “. . . abnormal plants with reduced fertility . . . When examined cytologically these are found to be due to deletions and rearrangements.” Of 24 mutants found, he classed 11 as lethals or semilethals, six as chromosomal and seven as morphological. Only two of these were checked cytologically and these two were found to contain a translocation and a possible deletion, respectively (LANGRIDGE, private communication).

Excellent mitoses were often found in premeiotic sporogenous tissue, anther walls, and petal fragments. In the present paper all mitoses shown (Figure 2)

FIGURE 3.—a-c, normal diploid. a, mitotic metaphase; b, c, meiotic metaphase and anaphase, respectively, with arrow pointing to the satellited chromosome. d, MI in the round-leaved trisomic; e, f, quadrivalents at MI and AI. a, ca. $4160\times$. b-f, ca. $3330\times$.

are from such cells, and not from root tips. The chromosomes at mitotic metaphase are small (0.8 to 2.1μ in length) and often their position strikingly suggests (Figure 3a) somatic pairing. However, parallel positioning of two small, not very different chromosomes could simulate somatic pairing. Detailed objective measurement and comparison with other than floral tissue is needed to decide the issue.

Prochromosomes are prominent in all nuclei of non-dividing cells in *Arabidopsis*. Those of the satellited chromosome almost always occupy a position near the nucleolus, while the others usually lie at the nuclear periphery (Figure 2a). When in close proximity prochromosomes may fuse (Figure 2b), and care must be exercised in using them for numerical chromosome determinations.

FIGURE 4.—a and d, MI in the narrow-leaf trisomic (cf. Figure 1c) with $4H1^{III}$ and $5H1^I$, respectively. b and e, MI in the round-leafed trisomic (cf. Figure 1b) with $4H1^{III}$ and $5H1^I$, respectively. c, TI in the narrow-leaf trisomic with six chromosomes near the upper, and five at the lower pole. $5000\times$.

SUMMARY

Arabidopsis thaliana has five pairs of two-armed chromosomes. Although the chromosomes are small, they differ sufficiently in size (1.1μ to 3.7μ in length at TI) and arm ratio that, with the possible exception of two, they can be distinguished from each other. The satellite bearing chromosome is the longest and forms a rod-bivalent in 75 percent of MI plates. In non-dividing nuclei the prominent prochromosomes can be used for determination of chromosome number. From a cross of $4n \times 2n$ the five primary trisomics have been obtained. These differ from diploid and from each other in morphological characters. All are sufficiently vigorous and fertile to be maintained and to be utilized for genetic work.

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