THE BEHAVIOR AND UTILITY OF SOME MONOTELOTRISOMICS IN HORDEUM

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ABSTRACT

The detection of a number of monotelotrisomics in intervarietal crosses and trisomic progenies of barley initiated the present study. By means of crosses to translocation stocks and established linkage markers, they were identified as 14 + 1S, 14 + 2L, 14 + f^3, 14 + 4L, 14 + 5S and 14 + 6S. Two additional aneuploids detected in the progeny of 14 + 1S; ditelotetrasomic 14 + 2 - 1S and Trisomic Bush, were included in the studies.

A study of plant morphology of five existing monotelotrisomics, revealed that the addition of an extra telocentric produced morphological effects of the same type but less pronounced than did the addition of a whole chromosome as in trisomics.

Meiotic studies showed that the extra telocentrics were associated in heteromorphic trivalents in 63.5 percent of MI cells examined, otherwise they remained as univalents. Ring-rod and tandem chain were the predominant trivalents formed. On the average, univalent telocentrics were orientated on the equatorial plate in 49.8 percent of cells, divided equationally in 12.2 percent and showed TI and TII abnormalities in 9.5 and 20.3 percent of cells examined. Micronuclei were observed in 15.5 percent of the quartets. Monotelotrisomics were transmitted to the progeny at an average frequency of 28.3 percent.

Ditelotetrasomic 14 + 2 - 1S morphologically was highly sterile and later in maturity than diploid sibs. The selfed progeny revealed 7.1 percent diploids, 45.7 percent monotelotrisomics and 47.2 percent ditelos indicating a high rate of transmission. The extra telo was transmitted through 44.0 percent of male gametes. At diakinesis the

extra telocentrics were synapsed with one another or with normal homologues in 98.4 percent of cells examined.

Of eight chromosome-1-associated genes tested with 14 + 1S; $\underline{gs}_3$, $\underline{yv}_2$, sb and ea were found to be located on the short arm of that chromosome. When it was discovered that the frequency of homozygous recessive segregates in monotelotrisomic F_2 progenies was inversely proportional to the gene-centromere distance, the genes were arranged linearly along the short arm relative to two established genes fc and br.

The centromere of chromosome-1 was found to be located between markers $\underline{Bl}_2$ and ert-d. Two new mutant genes, revoluted lead and Parkland spot, for which the symbols rl and ps have been proposed, were found to be inherited as simple recessives and located on the short arm of chromosome 5, along with a new dominant semi-dwarf gene.

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INTRODUCTION

Common barley (Hordeum vulgare L. Emend.Lam), is probably the most extensively studied of all crop plants, with the exception of corn. It has certain characteristics that make it desireable for genetic and cytological studies, including a diploid genetic constitution, low chromosome number, large chromosomes and floral parts and a self-pollinating reproductive habit. The introduction of refined cytological techniques by Tjio and Levan (1950) and the establishment of the standard karyotype by Burnham and Hagberg (1956) have greatly facilitated cytological studies in this crop. In addition, it was finally shown by Tsuchiya (1959a) by means of trisomic analysis, that the seven known linkage groups correspond to the seven pairs of chromosomes. The genetic analysis of this crop however is by no means complete. The centromere positions on the chromosomes have not been located with certainty, and large numbers of associated genes await mapping (Nilan,(1964).

Being a diploid, the amount of chromosome engineering that this crop will tolerate is limited. Tetraploids have been produced in barley by Reinbergs (1964) and a sixteen chromosome plant by Tsuchiya (1969). A number of workers including Ramage (1955), Kerber (1958), Tsuchiya (1959, 1967), Yu (1968) and Eslick and Ramage (1969) have identified and studied trisomics. Trisomics are individuals with a chromosome number of 2n=15, in which one chromosome is present in triplicate.

Reports on monotelotrisomics, (plants with a chromosome number of 2n=14+telocentric) are infrequent and often incomplete. The monotelotrisomics that have been reported, described merely as fragments,

have not been identified nor studied genetically in detail. Such aneuploids, when identified, would be useful in associating genes with their respective chromosome arms by virtue of trisomic ratios, in locating centromere positions and determining gene-centromere distances.

The occurrence of a number of monotelotrisomics in progenies of trisomics made possible the initiation of this study. The extra telocentrics were identified by crosses to translocation stocks and appropriate linkage markers. The meiotic behavior of the extra telocentrics was studied along with their effects on plant morphology. The identified monotelotrisomics were used to associate mutant genes with the proper chromosome arms, to locate the position of one centromere, to locate genes for B amylase activity and to determine if the phenomenon of somatic association of homologous chromosomes was operative in barley.

CHAPTER II

REVIEW OF LITERATURE

A telocentric chromosome is composed of a single chromosome arm with a terminal centromere. Telocentrics may represent component parts of several different genotypes or chromosome constitutions; four of which are outlined below.

(1) Entire complement telocentric.

Jones and Calden (1968) report that the entire complement of Tradescantia micrantha, a tetraploid with a chromosome number of $2n = 24$, is composed of telocentric chromosomes. It probably evolved from an ancestral diploid by centromere breakage in acrocentric chromosomes and loss of inert or deleterious arms, followed by polyploidization.

(2) Monotelosomics

Are plants with one chromosome represented by a telocentric mis-division product for one complete chromosome arm, symbolized by $20'' + t'$. (Kimber and Sears, 1968). Such a loss of chromatin can only be tolerated by polyploids such as wheat, (Sears), oats, (McGinnis et al 1963), and cotton, (Endrizzi and Kohel, 1966). Thus, for each monosomic there are two possible monotelosomics. Sears (1946) reports that monoiso and monotelosomics exist for each of the 21 wheat chromosomes, but in some cases only one of the two possible types has been established. Ditelo-somic genotypes have also been reported by Sears (1946) in wheat and by Dubuc (1968) in oats.

(3) Compensating telocentric

Smith (1947) reported on a plant of Triticum monococcum whose configuration at meiosis was six bivalents, one normal univalent, one

isochromosome and one telocentric. The latter two units were compensating for a normal whole chromosome. A similar situation has been reported by Southern (1969) in Myrmeleoteltix.

(4) Monotelotrisomic

Are plants with one chromosome represented by a trivalent consisting of two complete chromosomes and one telocentric chromosome. In barley this would be symbolized as 7" + t' or 6" + t 2" ' (Kimber and Sears, 1968). They were termed telosomic trisomics by Burnham (1962) followed by an abbreviation to telotrisomics by Khush and Rick (1968), however where applicable the terminology and symbols of Kimber and Sears (1968) will be used throughout this presentation. Monotelotrisomics have been reported in Zea mays (Rhoades, 1936, 1940), Datura stramonium (Blakeslee and Avery, 1938), Nicotiana sylvestris (Goodspeed and Avery, 1939), Triticum monococcum (Smith, 1947; Moseman and Smith, 1954), Secale cereale (Kamonoi and Jenkins, 1962), Hordeum vulgare (Tsuchiya, 1959, 1967; Yu, 1968), Lotus pedunculatus (Chen and Grant, 1968), and Lycopersicon esculentum (Khush and Rick, 1968).

The first observation of telocentric chromosomes was made by Huskins (1928). He observed a heteromorphic bivalent at metaphase I in wheat and described the phenomenon as a cytological abnormality caused by the loss of approximately one-half of a chromosome. Huskins and Spier (1934) carried out further studies on heteromorphic bivalents in wheat. Love (1938) associated a phenotypic effect with the loss of one arm of a chromosome. Heterozygous white-chaff wheat plants displayed 20" + t1" at metaphase I (MI), with one chromosome of the hetermorphic bivalent having a terminal centromere caused by the loss of the short arm.

Homozygous white-chaff plants were homozygous for the deficiency. In subsequent reports, Love (1940, 1941, 1943) described the same material in which misdivision of the centromeres was considered. Following the reports by Upcott (1937), Koller (1938) and Darlington (1939, 1940) that univalents may misdivide, it became obvious that isochromosomes and telocentrics arise through the misdivision of centromeres.

Darlington (1939) and Sears (1952) outlined detailed studies of univalent misdivisions. They generally agree on the type of misdivisions that can occur and the following descriptions represent the centromere breakages that were observed in wheat univalents at anaphase I (AI) by Sears (1952).

(a) One normal chromatid passed to one pole while the two arms of the other passed separately to the other pole or remained acentric on the metaphase plate. This instance would appear to be a combination of a normal longitudinal cleavage followed by a transverse misdivision of the centromere of one chromatid.

(b) Two identical arms went to one pole while the other two arms either went to the other pole or one or both remained acentric on the plate. This is the typical "misdivision" i.e. a transverse rupture of the centromere. It was the most frequent type of misdivision observed and was responsible for the majority of the isochromosomes formed at AI

(c) In the third category, three arms went to one pole while the fourth either went to the other pole or remained acentric on the plate.

The above observations indicate that a centromere is capable of sub-dividing into four functional parts. Marks (1957), Lima de Faria (1958) and Strid (1968) constructed models of the centromere structure

illustrating the possible breakpoints and indicated that, following breakage, this number of functional units may be formed.

Telocentrics may also be produced at A II from some of the A I products. For example, the isochromosomes produced in (b), may divide transversally at the centromere to produce two telocentrics each. Similarly, normal chromatid products of A I may misdivide at A II in the same way to also produce two telocentrics. A true telocentric chromosome, therefore, consists of a single chromosome arm with a terminal centromere. Breaks of this type through the centromere of barley chromosomes have been reported (Ramage et al, 1961).

Telocentric chromosomes have been reported in a number of crops and their sources are as varied as the means used to identify them. In oats, (McGinnis et al, 1963) a short arm telocentric for chromosome 14 arose in the progeny of an intervarietal cross and was identified by karyotype analysis and comparisons with a standard idiogram. The absence of the long arm was associated with a chlorophyll deficiency.

In cotton (2n=4x=52), a telocentric, haplo 17L, was formed through the misdivision of monosomic haplo 17 and was found to be deficient for the long arm (White and Endrizzi, 1965). An additional four telocentrics in cotton were reported by Endrizzi and Kohel (1966). Both telocentrics for chromosome 6, and one for 16, arose through misdivision of known monosomics. Their identity was verified by phenotypic resemblance to known monosomics and supported by genetic studies using known linkage markers. The same authors reported that telo 15 arose in the progeny of a cross involving a trisomic and translocation 4-15. In a hybrid containing this telo and translocation 4-15, a MI

configuration of $24''+1^{tv}$ chain was observed with the telo located terminally in the chain. Identity of the specific arm involved was accomplished by using known linkage markers.

A monotelotrisomic of Triticum monococcum was found in the progeny of a plant with thirteen normal chromosomes, one telocentric and one isochromosome (Smith, 1947). The trisomic ratios observed for group D when the monotelotrisomic was crossed to six of the seven known linkage groups, served to identify the telo.

Individual chromosomes are distinct at the pachytene stage of meiosis in corn, and a telocentric for the short arm of chromosome 5 was identified in this manner (Rhoades, 1936). In the same way, the size of the centromere of the telocentric was found equal to a normal one.

Six monotelotrisomics were produced in the tomato (Khush and Rick, 1968). Five of these occurred spontaneously in progenies of tertiary monosomics and trisomics and one arose in the progeny of a cross involving a tertiary trisomic and disomic. Three of the six, 4L, 3L and 8L, were not truly telocentric but had subterminal centromeres. Pachytene analysis revealed the presence of heterochromatic chromomeres as remnants of the missing arms. In a true sense these telocentrics were actually tertiary trisomics but it was considered that the distally located heterochromatin was inert; thus giving functional telocentrics. It was also shown by pachytene analysis that not all telocentrics had centromeres of equal size. The centromeres of 7L and 10L were of normal size while that of 3S was about one-half normal size. The latter telocentric was unstable and became eliminated in somatic tissue, probably as a result of an inadequate centromere.

Monotelotrisomics were detected at low frequencies in progenies of primary trisomics of Lotus (Chen and Grant, 1968), Secale (Kamonai and Jenkins, 1962), and Hordeum (Tsuchiya, 1967 and Yu, 1968), but no effort was made to identify them nor study their meiotic behavior. Plants with one to three supernumerary fragments were isolated in the progenies of barley primary trisomics(Frost and Ising, 1964). These fragments were characterized by subterminal centromere positions, and therefore were not true telocentrics nor complete trisomics. The transmission rates, and meiotic behavior of the fragments were studied but it was unfortunate that they were not identified nor could their relative chromatin content be accurately assessed.

The telocentric may become associated with several types of chromosome configurations at meiosis. The three possible heteromorphic trivalents that may be produced by the synapsis of 2 homologues plus a homologous telocentric are shown in Figure 1 (page 9). These configurations were obtained by extrapolating from the possible configurations produced when 3 homologues synapse (Darlington, 1965).

The tandem-V and tandem chain are the simplest trivalent configurations, each requiring only one chiasma per arm. A triradial configuration also requires a minimum of 2 chiasmata both in the same arm. A ring-rod structure is the most complex, requiring a total of 3 chiasmata.

In cases where no chiasmata are formed at diplotene between the telocentric and normal chromosome, the telocentric remains unpaired at metaphase leaving the two normal homologues associated in a bivalent.

The telocentrics of tomato monotelotrisomics were found either as univalents or paired with normal homologues at diakinesis, giving

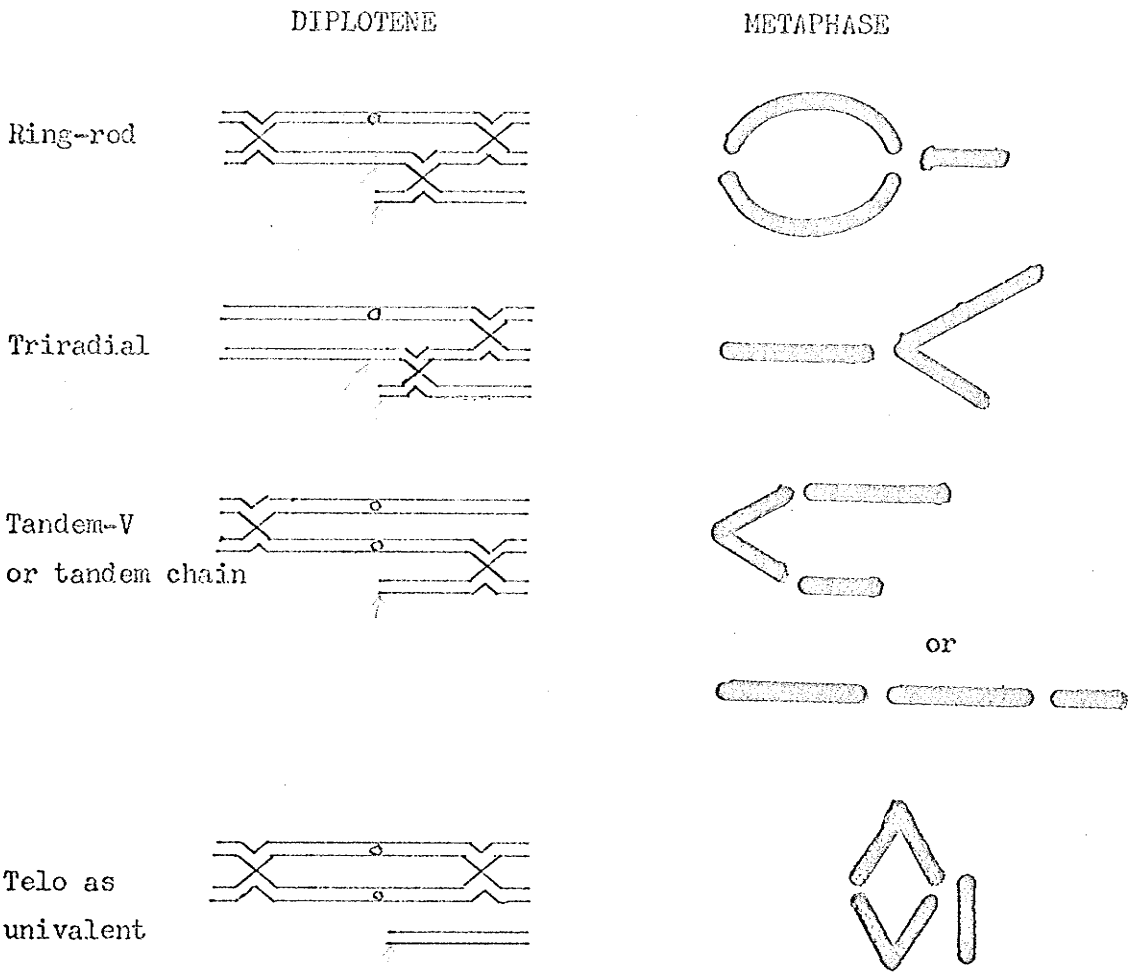


FIGURE 1 METAPHASE I HETEROMORPHIC TRIVALENT CONFIGURATIONS PRODUCED BY THE SYNAPSIS OF TWO NORMAL HOMOLOGUES PLUS A HOMOLOGOUS TELOCENTRIC AT DIPLOTENE

configurations of 12" + t' or 11" + t2" ' at frequencies of 55 and 45 percent, respectively (Khush and Rick, 1968). Only rarely were three univalents observed. The pairing was always non-randmon with normal homologues perferentially pairing with each other, leaving the telo as the univalent. The longer the telocentric arm, the more frequently it became involved in trivalents. The segregation of the three members at anaphase was likewise non-random, but preferentially 2 : 1 with the telo always accompanying a normal member of the pair. Tomato monotelotrisomics were found to be transmitted through the pistillate parent at an average frequency of 36.5 percent; primary trisomics for the corresponding telos were only rarely recovered.

The monotelotrisomic for the short arm of chromosome 5 of corn (Rhoades, 1936) showed much the same type of meiotic behavior as reported for tomato telos, with non-random pairing at meiotic prophase and non-random disjunction at anaphase. Synapsis of homologous segments was initiated at the ends, so that pairing of three chromosomes at one spot was never observed. The frequency of cells with trivalents at metaphase in corn was only 35 percent. The behavior of the telocentric at anaphase was similar to that reported by Sears (1952) for telos of wheat, in that if a telo was paired with another telo or with a normal homologue it behaved normally. If unpaired it could be expected to behave abnormally. First of all, it may or may not become orientated on the equatorial plate. If not, it usually remained in the cytoplasm during the formation of the daughter nuclei and consequently became excluded. When orientated on the plate, it usually migrated to one of the poles, but at a slower pace than normal chromosomes, and again was

subject to exclusion. The univalent telo would occasionally divide at anaphase I, but only after the normal chromosomes had reached the poles. The only type of misdivision possible for the telo was one in which an isochromosome was formed. Sears (1952) reported that monotelosomes of wheat would form isochromosomes at TII with a frequency of 13.3 percent.

The additional fragments observed in barley (Frost and Ising, 1964) exhibited a wide range of meiotic behavior. Meiotic configurations were scored in fifty cells which contained 2n=14+2 fragments. In 58 percent of these cells the two extra fragments were unpaired, in 26 percent they were paired with each other, and in the remaining 16 percent they synapsed with normal chromosomes to form quadrivalents. Non-orientation of univalents on metaphase plantes was observed, with some division at anaphase and many laggards at telophase. Plants with one fragment showed a lower transmission rate on the male than female side, while a transmission rate of .5 was observed on both sides in plants with two fragments. The individual fragments were found to be transmitted at different frequencies.

The trisomics of the Shin Ebisu 16 variety of barley (Tsuchiya, 1967) exhibited an average frequency of 89.9 percent cells with trivalents at diakinesis and 10.1 percent with univalents. The trivalent configurations; ring-rod, chain and triradial- triple-arc; were formed at average frequencies of 54.1 percent, 40.6 percent and 5.3 percent, respectively. 22.7 percent egg transmission of the extra chromosome was observed with virtually no pollen transmission.

Telosomic genotypes are useful for determining the arm location of genes and their distances from the centromere. Monotelosomics were

used to locate gene positions on chromosomes of cotton by Endrizzi and Kohel (1966) using the following method. Monotelosomics carrying dominant alleles were crossed to homozygous recessive diploids. In this material aneuploids were morphologically distinguishable from diploids. F_1 aneuploid hybrids were reciprocally test-crossed to homozygous recessive diploids. The test-cross was used to determine the arm location of a gene. Genes located on the arm present in duplicate segregated in normal disomic ratios, whereas genes on the hemizygous arm segregated as in the case of monosomics.

The reciprocal test cross: $\frac{a \quad o}{a \quad o} \times \frac{a \quad o}{A \quad o}$

gave only disomic progeny; the telocentric is not transmitted through the pollen in cotton hence n-1 gametes did not function. Therefore any dominant allele known to be located on the telo (as determined by the test-cross described above) that was transmitted to the progeny must have been shifted to the normal chromosome as a result of recombination. The frequency of transmission of the dominant allele *via* the normal chromosome was indicative of the amount of recombination and hence the gene-centromere distance.

Driscoll (1966) used ditelos from both arms of chromosome XIII of wheat to determine the arm location of *Iw*, a dominant wax inhibitor gene known to exist on chromosome XIII. Homozygous recessives females ditelosomic for both arms were pollinated by homozygous dominant diploids and the resulting F_1 heterozygous aneuploids were allowed to self-pollinate. F_2 progenies invólving the non-critical arm showed no segregation. The locus therefore was located on the arm that showed

segregation for waxiness in the F_2. Gene-centromere distances were estimated by determining the frequency of aneuploids among the recessive F_2 segregates and applying the Maximum Likelihood formula to the data.

Sears and Loegering (1968) used wheat ditelosomics to map stem rust resistance genes Sr9 and Sr16. The two genes were known to be located on chromosome 2B but their map location was required. Both ditelos 2BS and 2BL were crossed to the homozygous resistant line. F_1 aneuploids were test-crossed as female to the homozygous recessive stock of the same variety; i.e.

$$\frac{r \quad o}{R \quad o} \times \frac{r \quad o}{r \quad o}$$

Susceptible offspring were examined cytologically. Any susceptible individuals carrying two whole chromosomes were indicative of a cross-over between the telo and normal, with the relative frequency of cross-overs reflecting the map distance. Formulas were provided for the determination of the actual map distance. The same effective method was used (Sears and Briggle, 1969) to locate genes for mildew resistance that were located more than 50 units from the centromere.

A monotelotrisomic for the short arm of chromosome 5 of maize was used by Rhoades (1936) to locate the position of the A_2a_2 locus on that arm. A monotelotrisomic homozygous for A_2 was pollinated by an a_2a_2 source. F_1 telotrisomic heterozygotes were test-crossed with a_2 pollen. Disomic parents gave 1 : 1 B.C. ratios as expected while telotrisomics showed deviant ratios in the order of $5A_2 : 3a_2$. The magnitude of the deviation depended on the frequency with which the telocentric

chromosome carrying the dominant allele was included in the female gametes.

Another criterion of the same test that may be used to locate genes on the short arm is the presence in the B.C. progeny of homozygous recessive monotelotrisomic individuals, which would be the result of chromatid crossing-over. This latter test would require smaller progenies and would be of a more critical nature (Rhoades, 1936).

A slightly more efficient technique was used (Rhoades, 1940) to locate the Bm/bm locus relative to A_2/a_2 on the short arm of chromosome 5. The monotelotrisomic plants carrying recessive bm and a_2 alleles on the two normal homologues with Bm and A_2 on the telocentrics, were crossed reciprocally to homozygous recessive diploids. When hyperploid plants were used as pollen parents, diploid progeny plants with dominant phenotype were the result of recombination between the telocentric and normal chromosome, so that the dominant allele from the telo was exchanged for the recessive on the normal homologue. The observed frequency of A_2/a_2 and Bm/bm in the B.C. progeny was 1.74 percent and 0.25 percent respectively, indicating that the latter was located closer to the centromere on that arm. In other words, the frequency of recombination was directly proportional to the gene-centromere distance.

Monotelotrisomics were used to determine the map locations of several genes in the tomato (Khush and Rick, 1968a). The crosses were made between dominant monotelotrisomics as female parents and recessive mutant stocks. F_1 hybrids with a recessive allele on one normal chromosome and dominant alleles on the other homologue and the telo were allowed to produce selfed seed. If a gene was not located on a

particular telocentric arm, both disomic and trisomic progenies segregated in 3 : 1 ratios for the locus in question; otherwise a critical trisomic ratio was observed. For the purposes of such tests it was desirable to separate disomic from monotelotrisomic progeny plants and determine the segregation in each fraction, however the authors point out that even if monotelotrisomic progeny plants cannot be distinguished from diploids, the ratios for the bulk F_2 progeny could still be evaluated but with less discrimination.

It was also stated in the same paper that all monotelotrisomic F_2 plants will be of dominant phenotype. Obviously, the effects of recombination between telocentrics and normal chromosomes weren't considered since it is possible to obtain in such an F_2, monotelotrisomic plants that are homozygous recessive as a result of chromatid crossing-over as illustrated by Burnham (1962).

Reeves et al (1968), reconsidered the above conclusions about trisomic ratios, indicating that crossing-over between normal chromosomes and telos will increase the proportion of dominants at the expense of recessives. The degree of reduction of the mutant category will depend on a number of factors, one of which is the distance between the locus and centromere.

The addition to, or the deletion of, whole chromosomes or portions thereof from the normal genome will often manifest itself in the morphology of the plant. The first report of a distinct phenotype associated with a telocentric condition was that of a white-chaff mutant in wheat (Love, 1938). It was caused by the absence of one arm, i.e. a ditelosomic condition.

In Nicotiana sylvestris, a total of twenty monotelotrisomic plants was studied by Goodspeed and Avery (1939). All but three differed from both diploids and corresponding trisomics from which they arose. Only two plants resembled primary trisomics and one a double monosomic.

The telotrisomic for group D of T. monococcum (Smith, 1947) was shorter than the diploid, with fewer leaves, spikes and culms and had lower fertility. The telotrisomic for the short arm of chromosome 5 of corn (Rhoades, 1936), was intermediate in phenotype between the diploid and trisomic 5, the latter exhibiting thick, broad leaves with blunt tips, stubby tassel and reduced plant height.

Long arm monotelotrisomics of the tomato (Khush and Rick, 1968), resembled the corresponding trisomics in gross morphology, with short arm telos being distinct from diploids only under optimum growth conditions. The two arms of chromosome 3 each produced a distinct phenotype when present in triplicate. The internodes, leaves, flowers and fruit of trisomic 3 were longer and narrower than those of the diploid. In contrast monotelotrisomic 3L had slender but not elongate organs, while 3S had elongate but not slender parts. It follows that each arm conferred a specific feature on plant morphology.

A considerable amount of information exists in the literature on the morphological characteristics associated with a trisomic condition in barley. These effects will be compared to those produced by the presence of single arms in triplicate. In some cases the effects are somewhat comparable. Smith (1941), and McLennan (1947), were among the first to isolate a few barley trisomics. These plants, though weak and sterile, had no obvious morphological features that could be used to

distinguish them from diploids.

Ramage (1955) isolated a number of trisomics and described the morphology of each. Where specific phenotypic features were expressed, they were confined to a weaker, shorter growing condition, with a high degree of sterility.

Tsuchiya (1954) derived all seven trisomic types from H. spontaneum and separated them into seven groups based on plant morphology. Each was significantly different from every other one and from disomic sibs. The seven phenotypes were described using the designations Bush, Slender, Pale, Robust, Pseudo-normal, Pale and Semi-erect, corresponding to chromosomes 1 to 7 in that order. These same standards were later used successfully to identify the seven trisomics of a cultivated variety, Shin Ebisu, No. 16 (Tsuchiya, 1967) and of a six-row cultivated variety (Yu, 1968).

Frost and Ising (1964), studied the effect on plant morphology created by the addition of extra chromosome fragments. They found that the addition of one to three fragments had a retarding effect on vegetative development in the seedling stage. On older plants, the extra fragments had no significant effect on straw length and number of culms; the only two characters measured.

CHAPTER III

MATERIALS AND METHODS

The material containing the telocentrics used in this study was derived from several sources, including the progenies of intervarietal crosses and progenies of trisomics. These sources and their respective authorities are listed in Table I. The trisomic "Bush" used in these studies was obtained in the progeny of monotelotrisomic 1. It was studied for two reasons: first, as a comparison to the behavior of monotelotrisomics and secondly, the series of trisomics isolated by Kerber lacks Bush, consequently this individual has never been studied in that series. The chromosome fromwhich the telocentric was derived was identified by means of crosses to homozygous translocation testers listed in Table II, and subsequent analysis of chromosome pairing at MI in the hybrid. Prior to translocation testing, the telocentrics were putatively identified by either the trisomic they were derived from, their plant morphology, or chromosome morphology at somatic metaphase. On this basis, only a limited number of translocation testers were used and the identity of the telo proved to be the expected.

The criteria of the translocation test were as follows (Tsuchiya, 1961): male gametes from the translocation tester carrying two interchanged chromosomes will synapse with homologous sections of normal chromosomes provided by the female gamete, resulting in a quadrivalent configuration at MI of meiosis in the hybrid. In a critical cross the extra telocentric will share homologies with the chromosomes involved in the quadrivalent, will synapse with them to form a heteromorphic pentavalent (V), while the remaining chromosomes form 5". In a

non-critical cross the telo will synapse with its two homologues forming a heteromorphic trivalent (t2"'), leaving the remainder of the genome associated in 1^{iv} + 4".

Positive identification of the telocentric was achieved when pentavalents were found in two different hybrids involving two different translocation tests that had one translocated chromosome in common, e.g. pentavalents in Tl3a and Tl7a positively identified the telocentric for chromosome 1. As the technique outlined will only identify the telocentric with a specific chromosome, the specific arm involved was identified by means of crosses to known linkage markers, with the use of Chi-square analysis of F_2 data to distinguish between disomic and trisomic ratios. The mutants used for the identification were selected on the basis of their location and description as outlined by Nilan (1964). Following their identification the monotelotrisomics were used first of all to determine the arm location of a number of mutant genes previously shown to be associated with chromosome 1 (Walker et al, 1963). Monotelotrisomic 1L+1S was used to determine the position of the centromere on the linkage map of chromosome 1, using markers located in that area of the chromosome (Nilan, 1964), and finally the arm locationsof a number of previously undescribed mutants were determined.

In all cases, crosses were made between monotelotrisomics as the female parents, receiving pollen from homozygous diploids, recessive for marker genes. F_1 monotelotrisomic hybrids were selected cytologically and allowed to self to produce F_2 seed. For every monotelotrisomic progeny grown, the seed from a corresponding disomic F_1 hybrid was grown out as a check population. Individual F_2 plants were scored for each

segregating character in question and the ratios were determined for the bulk families, i.e. it was not possible in most cases to distinguish morphologically between disomic and aneuploid segregates. Tests for association were made by means of Chi-square analysis of F_2 populations for fits to disomic and trisomic ratios. In all cases a trisomic F_2 ratio was the criterion used to assign a gene to a specific chromosome arm. The majority of F_1 hybrids were in duplex condition, thus making it relatively convenient to distinguish between disomic and trisomic ratios in the F_2. Where 25 percent recessive segregates are expected in simple disomic ratios, the corresponding value for trisomic ratios was in the order of 10 percent. However a few of the F_1 hybrids were in simplex condition in which case 35.6 percent recessives was expected in the F_2. In order to determine the mode of inheritance in these cases, it was necessary to separate disomic from monotelotrisomic segregates by means of chromosome counts and determine the ratios in each group separately.

For studies of somatic metaphase preparations, and for chromosome counts, seeds were germinated on moist blotters in germination boxes, root-tips were collected, processed and stained with Feulgen (Ostergren and Heneen, 1962). Early trials showed that telotrisomic seeds had a lower germination capacity than normal diploids. To overcome this, such seeds were left in germination boxes at +2°C for periods of three to four weeks at which time all seeds germinated equally well.

Material for meiotic studies was grown in either the field or in a growth-chamber at a temperature of 20°C and a 16-hour day. Spikes were collected in the boot stage, killed and fixed in Carnoy's 6 : 3 : 1

(ethanol : chloroform : acetic acid) at room temperature for four to five days, then transferred to 70 percent ethanol and stored under refrigeration for periods of up to several months. Meiotic chromosome behavior was studied in temporary aceto-carmine preparations. Only clearly discernible cells were scored. Suitable preparations were made semi-permanent and stored for photographic purposes. Dilute aceto-carmine was used to determine the proportion of stainable or good pollen for each genotype.

A brief morphological description of each aneuploid was attempted. Because of the diversity of the parentage, each genotype was compared to its disomic sib. Data on monotelotrisomics 1, 3, 5 and 6 were obtained from field-grown material, while the corresponding data for 2, 4 and ditelotetrasomic 14 + 2 - 1S were obtained from plants grown in a growth chamber. At least five plants were used for scoring each of the characters considered. The terminology and symbols proposed by Kimber and Sears (1968) for Triticinae aneuploids will be utilized in this report where applicable.

TABLE I

SOURCE VARIETIES OF TELOTRISOMIC STOCKS

Telotrisomic Type	Source Variety (ies)	Authority
14 + 1S	Herta X Wong	Kerber
14 + 2L	Shin Ebisu 16 (Trisomic)	Tsuchiya
14 + f^3	O A C 21 x Montcalm	Yu
14 + 4L	Shin Ebisu 16 (Trisomic)	Tsuchiya
14 + 5S	O A C 21 x Montcalm	Yu
14 + 6S	Gateway (Trisomic)	Kerber

CHAPTER IV

EXPERIMENTAL RESULTS

1. Identification of Monotelotrisomics

(a) Cytological identification by means of translocation testers.

Barley, with seven pairs of chromosomes, is capable of having fourteen different telocentrics. The karyotype shows that all chromosomes are metacentric and with the exception of two satellited arms, the other twelve arms cannot be distinguished cytologically. Individual chromosomes are not distinct at pachytene, as they are in corn, thus limiting the usefulness of this stage for identification. Consequently, all telotrisomics were putatively identified on the basis of their origin, plant morphology or chromosome morphology, then crossed to specific translocation testers. The translocation stocks used and the results of MI chromosome pairing analysis in the F_1 hybrid are shown in Table II.

A critical cross was identified when a $1^{\overline{tv}}$ + 5" were seen at diakinesis or metaphase I (Figure 2a). Occasionally in such hybrids $1^{\overline{iv}}$ + 5" + t' were seen (Figure 2c). To obtain positive identification of the telocentric, two such critical crosses were required, with the common translocated chromosome identifying the telocentric. In the case of a non-critical cross, a MI configuration of $1^{\overline{iv}}$ + t2"' + 4" was observed (Figure 2b).

It was expected that the heteromorphic quadrivalent would frequently fail to form since the homologous sections involved were quite short. That is, the telo represented only one-half of a chromosome while some of the translocated segments were likewise quite short.

However, difficulty in finding a pentavalent was encountered only for the telocentric involving chromosome 6. Although this telo synapsis with normal chromosomes at a much lower frequency than other telos (Table VII), several cells were found that contained a heteromorphic pentavalent. In addition, the pentavalent was found to be associated with the nucleolus at diakinesis stages. Since telo 6 has a secondary construction, i.e. nucleolar organizing capacity, (Figs. 12 b, c, d, e) this observation is further proof of its identity.

The frequency of cells containing the pentavalent was estimated in hybrids from the cross 14 + telo 1 x T17a. Of 25 cells examined, 44 percent showed $1^{tv} + 5''$, 48 percent $1^{iv} + 5'' + t'$ and 8 percent $6'' + t2'''$.

These results and those presented in table II identify the chromosome from which the telo was derived but not the specific arm involved.

(b) Genetic identification using linkage markers

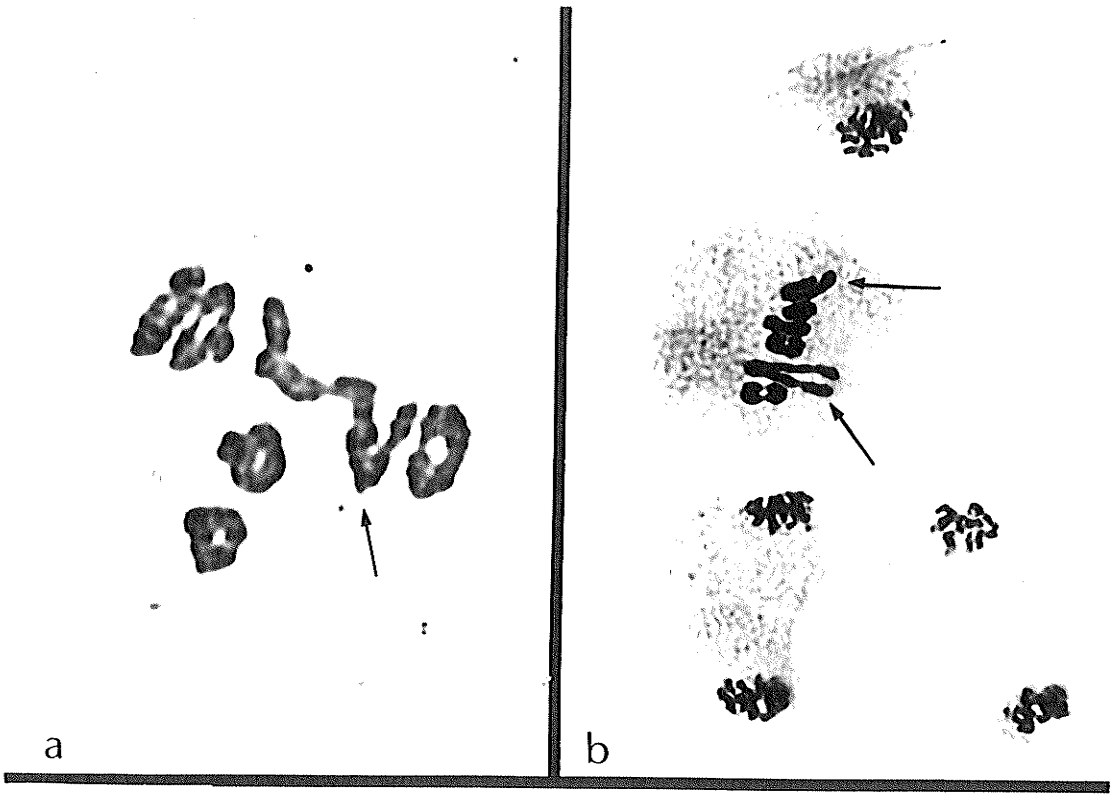
To determine conclusively the identity of each telocentric, the monotelotrisomics were crossed to known linkage markers located on specific chromosome arms. The linkage markers used for this purpose (Table III) were selected according to their location on the chromosomes and descriptions provided by Nilan (1964). F_2 populations for markers Nn, and Br,br were grown in the field with the families from other markers being grown in greenhouse beds. For all linkage markers (Table IV) disomic check F_2 progenies, though not shown, were grown. In each case a Chi-square analysis showed a good fit to an expected disomic ration of 3 : 1 at each of the loci, indicating simple inheritance.

FIGURE 2 - MI CHROMOSOME CONFIGURATIONS IN MONOTELOTRISOMIC F_1 HYBRIDS BETWEEN MONOTELOTRISOMICS AND TRANSLOCATION TESTERS

(a) Critical cross - $1^{\overline{tv}}$ + 5"

(b) Non-critical cross - $1^{\overline{iv}}$ + t2"' + 4"

(c) Critical cross - $1^{\overline{iv}}$ + 5" + t'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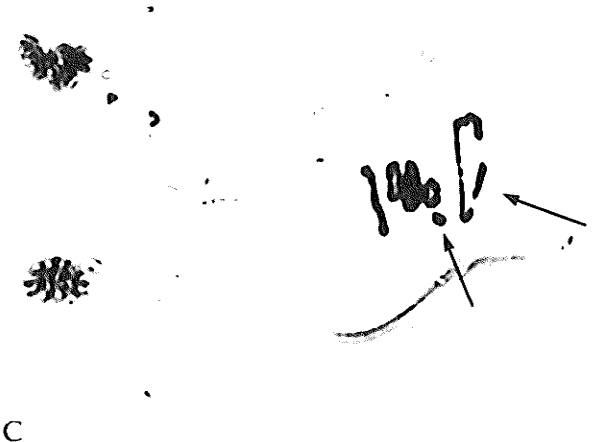
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TABLE II

CHROMOSOME CONFIGURATIONS AT M1 IN MONOTELOTRISOMIC F_1 HYBRIDS BETWEEN TELOTRISOMICS AND TRANSLOCATION STOCKS

Telotrisomic Type	Telo 1	Telo 2	Telo 3	Telo 4	Telo 5	Telo 6
Translocation Tester						
$T_1$3a	V					
$T_1$7a	V					
$T_3$7b	IV + III					
$T_2$3c		V				
$T_2$7b		V				
$T_1$3c			V			
$T_3$7b			V			
$T_4$6a				V		
$T_4$7b				V		
$T_1$6c				IV + III		
$T_2$5a					V	
$T_5$7b					V	
$T_1$6a						V
$T_3$6c						V

Genes n and br were introduced from the same pollen parent and segregated in the same progeny. The segregating progeny obtained from monotelotrisomic-1 crossed as the female parent with the two markers showed a good fit (Table IV) to a disomic ratio for the n locus but an unsatisfactory fit for br because of a deficiency of recessives, hence a trisomic ratio, for this gene. Since br is known to be located on the short arm of chromosome 1, the presence of this arm in triplicate is indicated. In contrast, the progeny from a trisomic (2n=15) which appeared among the F_1 hybrids from this cross showed trisomic F_2 ratios at both n and br loci, indicating that the extra chromosome was a homologue of chromosome 1.

The or gene, located on the short arm of chromosome 2, showed a disomic segregation ratio in crosses to monotelotrisomic-2. This indicates that the telo of chromosome 2 must represent the long arm. The Chi-square data for the F_2 population from telo 2 x or should be interpreted with caution. The population was extremely small; it was obtained from one highly sterile monotelotrisomic F_1 hybrid which produced only nineteen seeds on thirteen spikes. Ocular micrometer measurements were made of all telocentrics to determine their relative lengths in comparison with the lengths shown in the standard karyotype of Burnham and Hagberg (1956). This comparison indicated that telo 2 represents the long arm.

No linkage marker data were obtained for the chromosome 3 fragment. Since it was not a true telocentric, no further identity was required for the purpose of these studies.

A disomic F_2 ratio was obtained for the segregation of K vs. k. Since it is located on the short arm, this result indicates that

TABLE III

LINKAGE GROUPS AND MARKER GENES USED TO IDENTIFY TELOCENTRIC CHROMOSOMES

Linkage Group	Character	Arm Location	Gene Symbol	Genotype of Monotelotrisomics
1	Normal vs. brachytic	Short	Br, br	Br,Br
1	Covered vs. naked caryopsis	Long	N, n	N,N
2	Green vs. orange seedling	Short	Or,or	Or,Or
4	Hooded vs. awned	Short	K,k	k,k
5	Normal vs. 3rd outer glume	Short	Trd,trd	Trd,Trd
5	Normal vs. albino seedling	Short	At,at	At,At
6	Normal vs. orange lemma	Centromere	O,o	O,O
6	Normal vs. uniculm	Centromere	Uc_2,uc_2	Uc_2,Uc_2

monotelotrisomic 4 represents the long arm. In this cross the source of the K gene was dominant giving a simplex F_1 heterozygote, from which a trisomic ratio is distinguished from a disomic one with difficulty. To resolve this ambiguity, 60 F_2 seeds were separated into disomic and monotelotrisomic classes by somatic chromosome counts. Disomic ratios were obtained in both portions (Table V). These results support the conclusion derived from the bulk F_2.

In tests involving telotrisomic 5, a significant deviation from disomic expectation was shown for Trd vs. trd (Table IV). This gene is known to be located on the short arm, indicating that the telo was derived from this same arm. A trisomic ratio was likewise observed for the at gene which is also reported to be located on the short arm.

Disomic segregation patterns were observed at both uc_2 and o loci. They are both known to be located in the centromere region of chromosome 6 (Nilan, 1964). In the current studies a frequency of one percent recombination between the two loci was observed, which is in agreement with Shands (1963), who found them to be very closely linked. The results thus far indicate that telo 6, being satellited, represents the short arm of chromosome 6 and loci uc_2 and o, by virtue of their disomic segregation patterns must be located on the long arm side of the centromere.

On the basis of Chi-square analysis of F_2 populations (Table IV) and karyotype analysis the following barley monotelotrisomics have been positively identified: 14+1S, 14+2L, 14+4L, 14+5S, and 14+6S. The symbols used are the same as those adapted for tomato telotrisomics, (Khush and Rick, 1968a), where the number refers to the chromosome from which the telo was derived and the large case S or L refers to the short

TABLE IV

X^2 ANALYSIS OF F_2 POPULATIONS FROM CROSSES BETWEEN MONOTELOTRISOMIC STOCKS AND LINKAGE MARKERS FOR 3:1 DISOMIC RATIOS

Linkage Group	Marker Gene	Frequency		X^2 3 : 1	P	% Recessive
		X	x			
1	Br,br	1116	82	217.19	< .01	6.8
1	N,n	826	257	.93	.70-.80	23.7
2	Or,or	17	2	2.12	.10-.25	11.5
4	K,k	45	13	.21	.10-.25	22.4
5	Trd,trd	175	20	22.61	< .01	10.3
5	At,at	372	54	34.50	< .01	12.7
6	O,o	273	101	.80	.25-.50	27.0
6	Uc_2,uc_2	275	99	.43	.50-.17	26.5
Identity of BUSH						
1	Br,br	84	3	21.55	< .01	3.4
1	N,n	82	5	17.20	< .01	5.7

or long arm respectively. The ditelotetrasomic, derived from 14+1S, will be referred to henceforth as 14+2-1S and the trisomic derived from the same source as "Bush", the trisomic for chromosome 1.

TABLE V

X^2 ANALYSIS OF F_2 POPULATIONS FROM MONOTELOTRISOMIC 4 x Kk FOR 3 : 1 DISOMIC SEGREGATION

Fraction	Frequency X	Frequency x	X^2 3:1	P	% Recessive
Disomic	29	12	.42	.50 - .75	29.3
Monotelotrisomic	13	6	.44	.50 - .75	31.6

2. Morphological Description of Aneuploid Forms

A considerable amount of detailed morphological data were obtained on each telotrisomic and are in Table VI. The details that will be presented in this outline represent the most significant morphological features of each genotype and are meant to complement the data in the table. The different telotrisomics were quite distinct from each other as determined by the parental background and genotype. The morphological comparisons in each case were made with the corresponding disomic sib.

(a) Monotelotrisomic 14 + 1S (Fig. 3a-B, 3b, 5A, D)

Monotelotrisomic 14 + 1S was derived from an intervarietal cross between Herta and Wong and retained some features from both parents.

For example, the erect, awnletted, six-rowed spike is similar to that of Wong and the mixed blue and yellow aleurone kernels is a characteristic of Herta. The telotrisomics had fewer tillers and were slightly less leafy than the diploids, but neither of these differences was great enough to permit visual differentiation with any degree of certainty. The fertility of the aneuploid was only slightly lower than that of the diploid, making it desirable for genetic studies. Root and coleoptile lengths were measured on one-week-old seedlings of diploids and compared by means of a paired t test to those of monotelotrisomics. The test indicated no significant differences.

(b) Trisomic BUSH (Fig. 3a-D, 5B)

The morphological features were similar to those of the Bush trisomics previously described (Tsuchiya, 1967; Yu, 1968). It was included in this study because the series of trisomics, isolated by Kerber, lacks a representative for chromosome 1. It was lighter in color than diploids because of a waxy bloom covering. It tillered profusely for a prolonged period producing many short, thin culms. From the seedling stage to near maturity it was characterized by numerous, long, narrow, somewhat folded leaves. The florets subtended long awns that were rough in texture and caryopses were incompletely enclosed by lemma and palea.

(c) Ditelotetrasomic 14 + 2 - 1S (Fig. 3a-C, 5C)

Ditelos were medium green in color and medium waxy with an intense red color at the base of the stem, possibly a dosage effect of the Rs (Red stem) gene. Some plants had a very unthrifty appearance from seedling stage to maturity beginning with a prostrate type of vegetative growth. Culms were thin and kinked, bearing few, short, wide leaves.

Spikes were short with awns of medium length, not unlike the diploid. Fertility was low because of the many florets than contained only hyaline, vestigial anthers. The seeds that were produced were larger than those of the diploid.

(d) Monotelotrisomic 14 + 2L (Fig. 3c, 5E, F)

This monotelotrisomic differed from the diploid sibs more than any of the others studied. It closely resembled, Slender, the trisomic for chromosome 2. The two-rowed, long awned spike is characteristic of Shin Ebisu 16, the variety from which it was derived. Monotelotrisomics were lighter in color and shorter growing than the diploids. The very long, narrow, drooping leaves were evident from the early seedling stage to maturity. Many of the leaves would senesce prematurely at the juvenile stage. Numerous blind tillers were produced. This genotype was much later in heading than the diploid, with the spikes most often emerging through the sheath of the flag leaf rather than pushing through the top in the usual manner. This action may have caused a spiral that was observed in the neck of the culm. At the rachis nodes on the spike, instead of the usual spikelets, miniature spikes, each with several spikelets, were often formed, giving the spike a busy appearance (Figure 5F). The fertility of these spikes was low, the seeds produced were small and not fully enclosed by the lemma and palea.

(e) 14 + acrocentric fragment - 3 (Fig. 4a, 5G, H)

These monotelotrisomics were shorter than diploids, somewhat later in heading and maturity, with fewer tillers.

(f) Monotelotrisomic 14 + 4L (Fig. 4b, 5I, J)

Monotelotrisomic 14+4L was derived from the two-row, long awned

variety Shin Ebisu 16. Compared with the diploid sibs, these plants had thick, waxy stems with less waxy leaves that were darker in color. The flag leaf of this aneuploid was comparatively small. A considerable amount of red pigment was observed at the base of the stem and in the auricles. A pronounced spiral was noted at the base of the awns and the neck at the base of the spike. Spikes on mature plants often remained enclosed by the sheath of the flag leaf rather than protruding through the top in the usual manner. An explanation for the phenomenon can be put forth. It was noticed that the top internode on the monotelotrisomics measured 4.6 inches in average length while the same section on diploids measured 7.6 inches. The culms of diploids were composed of two other internodes, measuring 5.0 and 3.1 inches in length on the average. The respective lengths of these internodes in monotelotrisomics were 5.5, 4.7 and a fourth internode at the base measuring 2.5 inches in average length. The features tended to give monotelotrisomics a stouter appearance than that of diploid sibs, but this was of lesser magnitude than the "Robust" characteristics of trisomic 4.

(g) Monotelotrisomic 14 + 5S (Figure 4c, 5KL)

14 + 5S was derived from a six-row long-awned background. Monotelotrisomics were darker in color than corresponding diploids and produced 50 percent more tillers. The culms of tillers were shorter and thinner than those on diploid sibs and had more numerous but narrower leaves. Telotrisomic seeds were smaller in size than diploid seeds.

(h) Monotelotrisomic 14 + 6S (Figure 4d, 5MN)

This monotelotrisomic was derived from a six-row, long awned stock. The plants differed markedly from diploid sibs being darker in color,

TABLE VI

MORPHOLOGICAL CHARACTERISTICS OF BARLEY ANEUPLOIDS AND DISOMIC SIBS

Genotype	Leaf Width mm	Leaf Length mm	L/W Ratio in Leaf	Spike Length mm	Rachis Length mm	Rachis i'node Length mm	Florets/ Ear	% Fertility	Days to Head	Height Ins.	No. of Tillers
14+1S	10.3	151.4	14.7	101.6	59.2	6.0	38.0	52.5	42.4	27.8	19.2
14+2-1S	7.2	79.5	11.1	91.4	50.1	6.0	23.5	11.9	52.7	19.8	14.7
BUSH	6.6	108.2	16.4	139.7	37.7	4.4	12.5	41.5	53.1	18.3	51.6
2n CK	5.7	125.2	22.1	109.2	78.6	5.3	45.0	92.4	40.5	27.3	24.3
14+2L	5.5	146.8	26.7	127.0	45.4	2.5	44.5	10.2	64.2	20.0	12.0
2n CK	8.0	122.7	15.3	160.0	35.0	3.3	18.2	67.9	44.6	25.0	8.0
$14+f_3$	13.5	137.2	10.2	177.8	68.2	4.9	46.0	15.2	58.5	26.4	21.4
2n CK	14.7	131.3	9.0	190.5	73.7	3.3	57.0	33.3	51.0	34.0	44.3
14+4L	9.1	125.0	13.7	142.2	57.7	3.0	27.6	37.6	47.5	26.0	10.0
2n CK	7.3	102.4	14.0	165.1	63.3	4.4	24.4	86.0	44.6	26.5	15.0
14+5S	9.5	118.1	12.4	139.7	65.4	4.1	50.0	62.0	46.2	26.8	39.6
2n CK	11.5	146.1	12.7	177.8	75.2	4.4	53.0	90.6	38.4	35.0	20.0
14+6S	19.0	225.6	11.9	165.1	80.1	4.0	56.0	33.9	56.9	21.2	7.3
2n CK	11.0	165.1	15.1	152.4	70.8	3.3	53.0	66.0	52.1	30.4	29.3

FIGURE 3 - PLANT MORPHOLOGY OF MONOTELOTRISOMICS AND DISOMIC SIBS

(a) A. Disomic Check

B. 1$_4$ + 1S

C. 1$_4$ + 2 - 1S

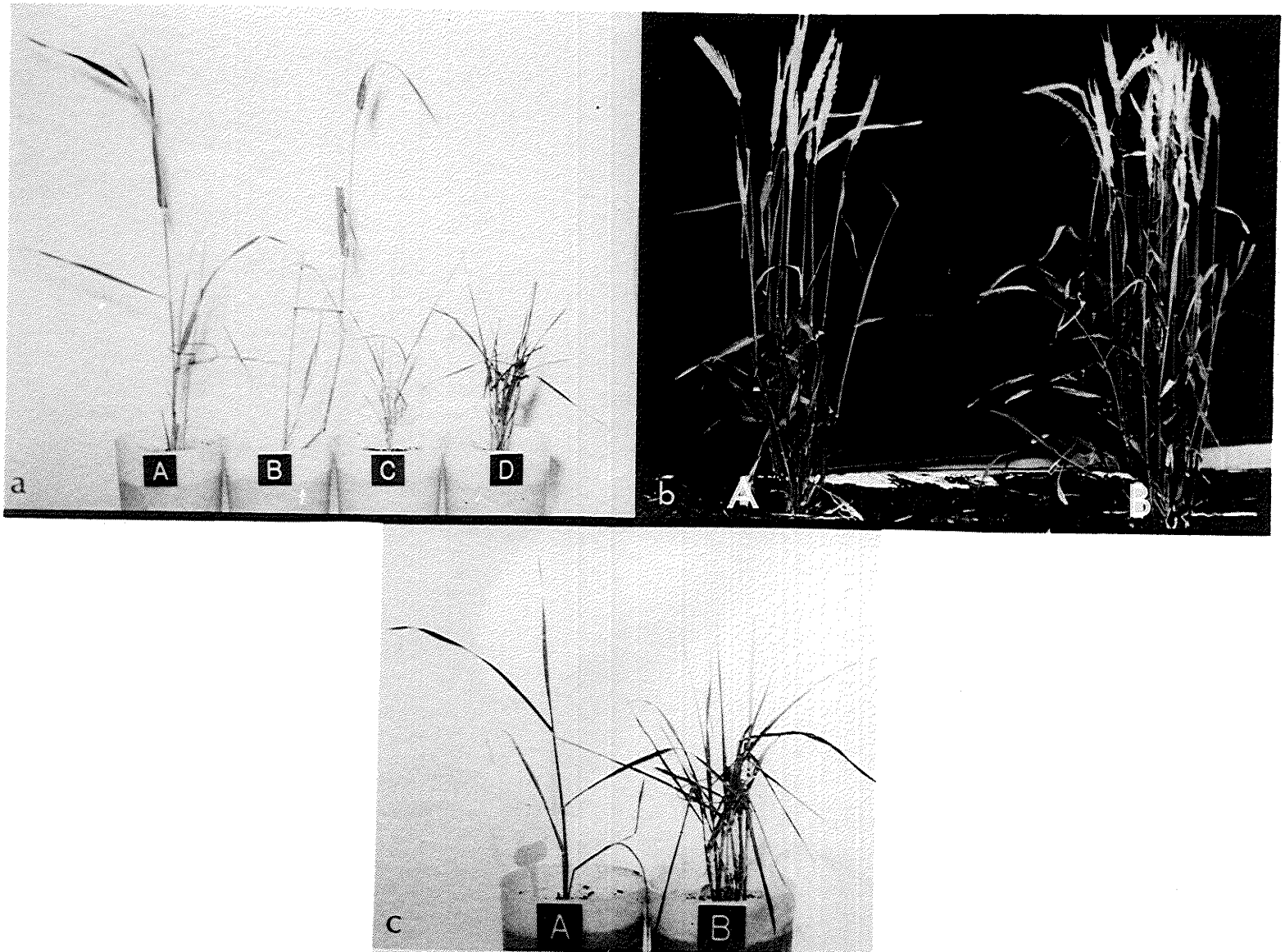
D. Trisomic BUSH

(b) A. 1$_4$ + 1S

B. 2n check

(c) A. 2n check

B. 1$_4$ + 2L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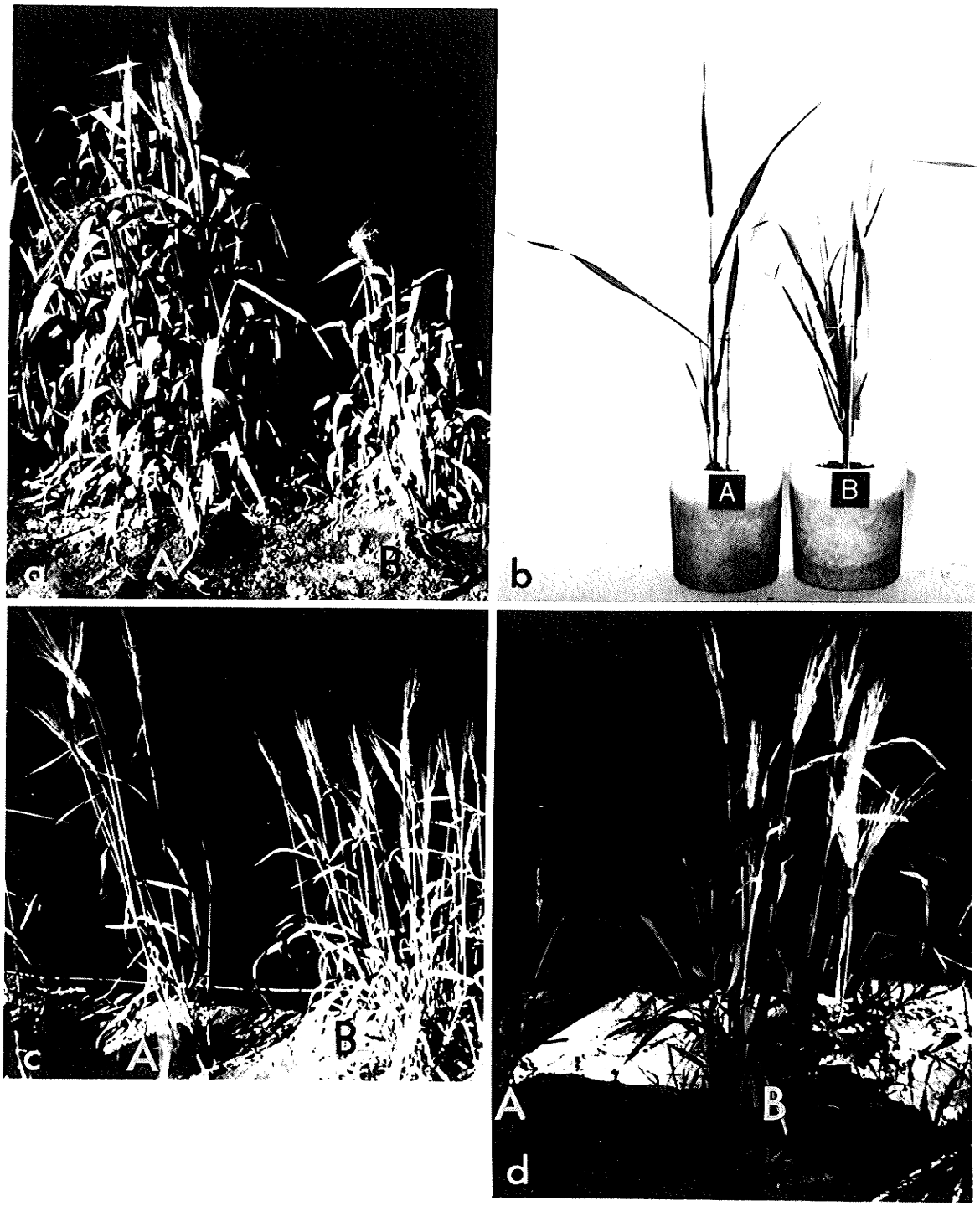
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FIGURE 4 - PLANT MORPHOLOGY OF MONOTELOTRISOMICS AND DISOMIC SIBS

(a) A. 2n check

B. $14 + f^3$

(b) A. 2n check

B. 14 + 4L

(c) A. 2n check

B. 14 + 5S

(d) A. 14 + 6S

B. 2n check

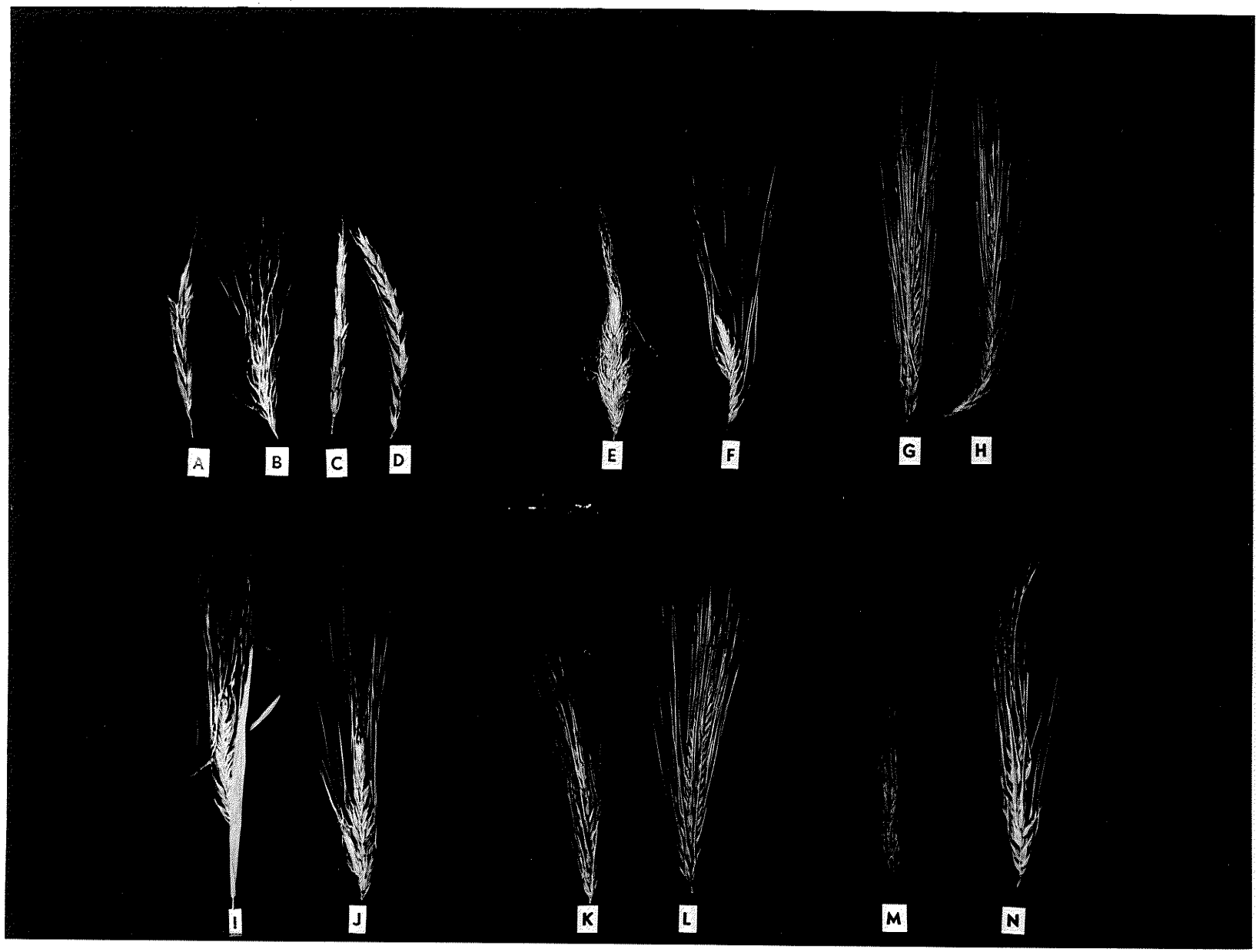
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FIGURE 5 - SPIKE CHARACTERISTICS OF ANEUPLOIDS AND DISOMIC SIBS (2nCK)

(A) 14 + 1S

(B) Trisomic BUSH

(C) 14 + 2 - 1S

(D) 2nCk

(E) 14 + 2L

(F) 2nCK

(G) 14 + f^3

(H) 2nCK

(I) 14 + 4L

(J) 2nCK

(K) 14 + 5S

(L) 2nCK

(M) 14 + 6S

(N) 2nCK

shorter, and with fewer tillers that bore fewer but larger leaves. A slight spiral was noted in the neck and awns. The awns were equal in length to those of the diploids but coarser and more divergent. The nodes showed a purple coloration like that observed in trisomic "Purple". Aneuploid seeds were slightly plumper than diploid seeds.

3. Mitosis in Monotelotrisomics

In Figure 6 (a - g) are shown the somatic metaphase preparations of aneuploids 14 + 1S, 14 + 2 - 1S, 14 + 2L, 14 + f^3, 14 + 4L, 14 + 5S and 14 + 6S, respectively. The extra fragment of chromosome 3 is actually acrocentric, (Fig. 6,d), while the telo of 14 + 6S shows the secondary construction with the large satellite (Fig. 6, g). That the latter extra telocentric has nucleolus organizing capacity is verified by its association with the nucleolus at diakinesis and late diplotene (Fig. 12b, c, d, e). The preparations for the remaining aneuploids (Fig. 6a,c,e,f) indicate that the supernumerary fragments are truly telocentric. Cells of the ditelo 14 + 2 -1S each contain two identical telocentrics (Fig. 6b).

4. Meiosis in Monotelotrisomics

Chromosome behavior at various stages of meiosis was studied in five telotrisomics, one ditelotetrasomic and the trisomic BUSH. The following data represent observations made on several plants of each type. In diploid sporocytes, 7" were always observed at diakinesis and metaphase with the bivalents forming ring configurations in nearly 100 percent of the cases. There were no chromosomal abnormalities in subsequent stages.

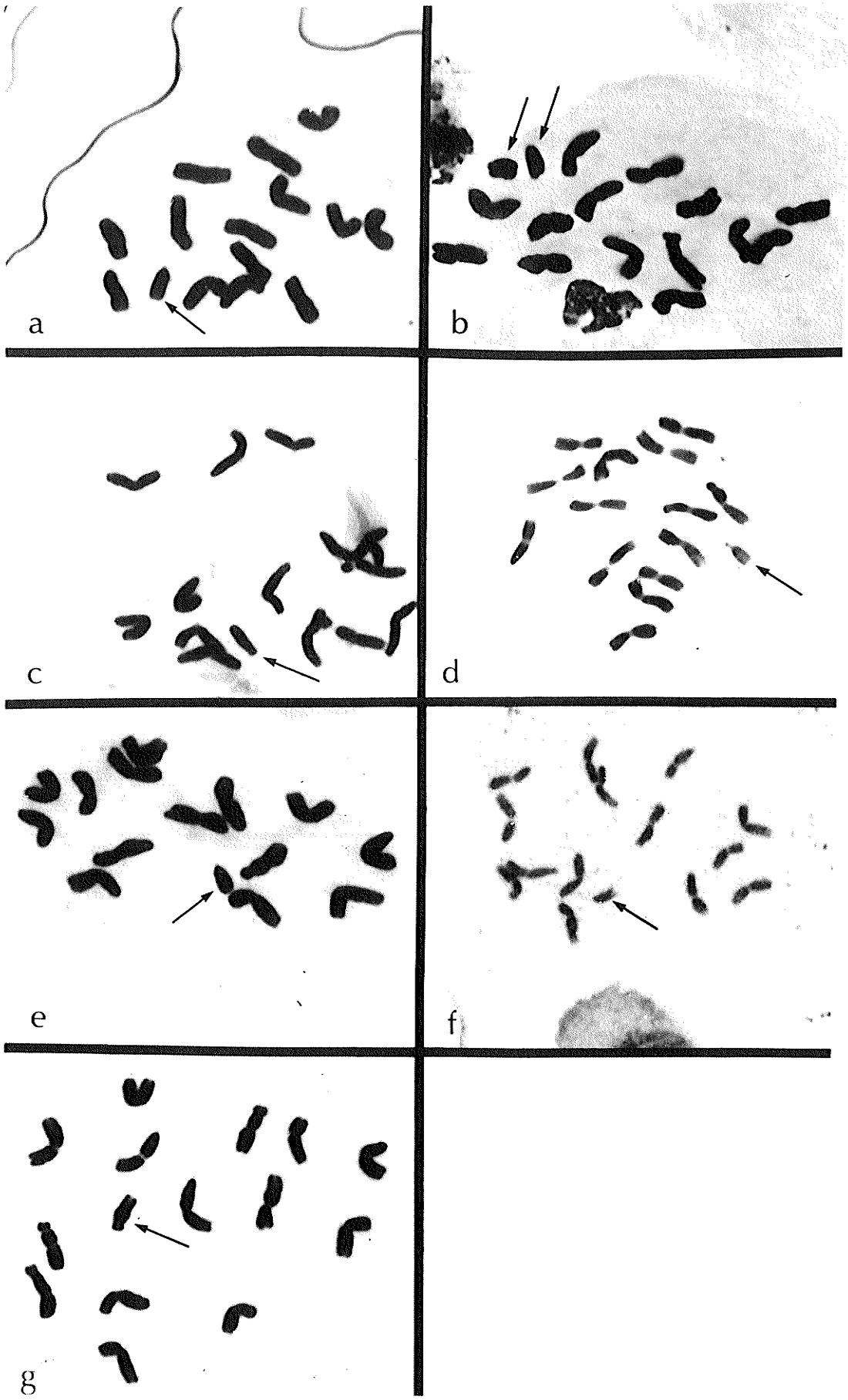
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FIGURE 6 - SOMATIC METAPHASE PREPARATIONS OF BARLEY TELOSOMICS

(a) 14 + 1S

(b) 14 + 2 - 1S

(c) 14 + 2L

(d) 14 + f^3

(e) 14 + 4L

(f) 14 + 5S

(g) 14 + 6S

(a) Diakinesis

At diakinesis of first metaphase the chromosomes commonly formed configurations of either 6" + t2"' or 7" + t' (Fig. 7d) at average frequencies of 73.4 and 26.6 percent (Table VII). All monotelotrisomics with the exception of 14 + 6S, showed essentially the same pattern with 14 + 2L showing the highest frequency of trivalents at 88.5 percent. Telotrisomic 14 + 6S departed significantly from the other frequencies by forming trivalents in only 35.1 percent of the cases. This figure lowered the average for the entire group.

Two types of heteromorphic trivalents were predominantly formed. These were tandem chain - tandem V (Fig. 7c, e) and ring-rod (Figure 7a, f) at average frequencies of 34.5 and 62.6 percent respectively. Even in 14 + 6S, in which trivalents were formed at low frequencies, the ring-rod type was predominant. This appears unusual since it is the more complex trivalent, requiring three chiasmata, whereas the chain type requires only two. The extra chromosome of Trisomic Bush formed two additional types of trivalents at low frequencies. These were triradial and triple arc types.

(b) Metaphase

As the stage of meiosis advanced through diakinesis into metaphase there was a decrease in the frequency of 6"+ t2"' configurations by about 10 percent with a corresponding increase in the frequency of 7" + t'. At the same time the frequency of chain trivalents increased by about 15 percent at the expense of the ring-rod, probably as a result of chiasma terminalization. Telotrisomic 14 + 6S had the highest frequency of ring-rod trivalents with 14 + 4L having the lowest (Table VIII).

TABLE VII

FREQUENCY (%) OF CHROMOSOME ASSOCIATIONS AND TYPES OF TRIVALENTS AT DIAKINESIS IN MONOTELOTRISOMICS, AND TRISOMIC BUSH

	Chromosome Associations				Types of Trivalents			
Genotype	6II+1III	7II + 1I	Other	No. of Cells	Chain	Ring-rod	Other	No. of Trivalents Observed
14+1S	76.4	20.7	2.9	140	44.1	52.3	3.6	111
BUSH (2n=15)	85.9	13.4	.7	142	31.5	61.3	7.2	124
14 + 2L	88.5	11.5	-	279	37.5	62.5	-	247
14 + 4L	67.5	28.8	3.7	191	38.2	56.2	5.6	136
14 + 5S	79.6	19.2	1.2	172	40.0	58.6	1.4	140
14 + 6S	35.1	64.9	-	185	15.4	84.6	-	65
Average of Telos	69.6	29.0	1.4	Total 1109	Average 35.0	70.1	219	Total 1823

TABLE VIII

FREQUENCY (%) OF CHROMOSOME ASSOCIATIONS AND TYPES OF TRIVALENTS AT METAPHASE I IN MONOTELOTRISOMICS, AND TRISOMIC BUSH

	CHROMOSOME ASSOCIATIONS				TYPES OF TRIVALENTS			
Genotype	6II + 1III	7II + 1I	Other	No. of Cells	Chain	Ring-rod	Other	No. Trivalents Observed
14 + 1S	70.6	29.4	-	214	50.0	50.0	0	137
BUSH (2n=15)	75.7	24.3	-	366	46.9	40.4	12.7	277
14 + 2L	79.4	20.0	.6	738	62.2	37.0	.8	563
14 + 4L	64.6	34.9	1.5	370	65.3	33.9	.8	242
14 + 5S	69.5	29.4	1.1	367	54.1	45.1	.8	255
14 + 6S	21.3	78.6	-	225	27.1	72.9	-	48
Average	60.7	38.5	.8	Total 2280	Average 51.7	45.8	2.5	Total 1522

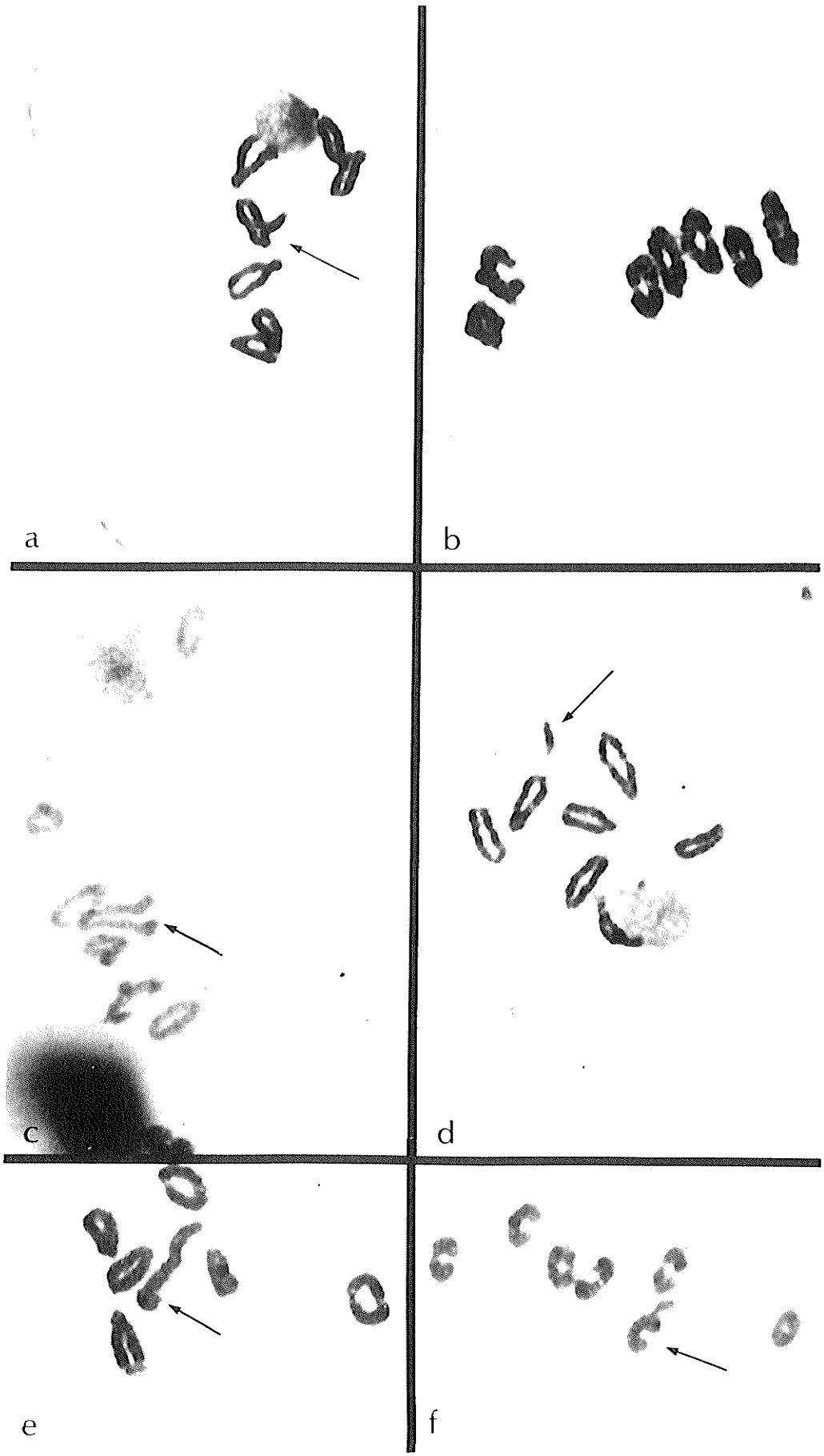
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FIGURE 7 - CHROMOSOME CONFIGURATIONS OF BARLEY MONOTELOTRISOMICS AND TRISOMIC BUSH AT DIAKINESIS AND METAPHASE I.

(a) 6" + t2"' ring-rod trivalent - diakinesis
two bivalents associated with nucleolus

(b) 7" - disomic check - metaphase

(c) 6" + 1"' - tandem-V trivalent metaphase--trisomic BUSH

(d) 7" + t' - telo univalent - diakinesis
two nucleoli per cell

(e) 6" + t2"' - tandem chain trivalent - diakinesis

(f) 6" + t2"' - ring-rod trivalent - metaphase

With reference to the shapes of bivalents, in 50 percent of the sporocytes they were all in the form of rings, in the remainder of the cells one rod bivalent occurred among the ring bivalents. Disomic sibs consistently showed 7" (Fig. 7b) at metaphase.

Some of the unusual configurations observed at diakinesis and metaphase, but not listed in Tables VII and VIII were:

1) In P.M.C.'s of 14 + 1S, two cells each with 6" + 1"' (triradial) were seen at diakinesis.
2) 14 + 2L had one cell with 6" + t1" + 1'.
3) Monotelotrisomic 14 + 4L had one cell of each of:
   a) 5" + t2"' + (1' + 1').
   b) A 6" + 1"' (triradial)
   c) 6" + t1" + (1' + 1')
4) 14 + 5S had one cell with 6" + t2"' (triradial), a cell with only 7", possibly an indication of somatic elimination, and a trisomic cell with 6" + 1"'.

(c) Meiosis in Diltelotetrasomic 14 + 2 - 1S

The two telocentrics of the ditelo 14 + 2 -1S showed a high degree of pairing both with one another and with homologous arms of normal chromosomes 1 (Table IX). At diakinesis the configuration occurring in the highest frequency, was that in which the two telos paired with the two normals to form heteromorphic quadrivalents. This arrangement was observed in 61.3 percent of the cells. Approximately three-quarters of the total quadrivalents observed were in the form of chains, (Fig. 8c), the remaining one-quarter were in the form of ring-rods (Fig. 8d) and triradial types (Fig. 8a). The chain quadrivalents are the simplest

TABLE IX

FREQUENCIES OF COMBINATIONS OF VARIOUS CHROMOSOME ASSOCIATIONS AT DIAKINESIS AND METAPHASE I OF DITELOTETRASOMIC 14 + 2 - 1S

Combination of Associations	Percent of Total Cells	
	Diakinesis	Metaphase
6" + 1 (t"1")	61.3	51.1
6" + t.2" + t'	1.3	9.5
7" + t"	37.1	38.1
7" + (t' + t')	.3	1.3
Total cells observed	318	559
Shape of Quadrivalent		
Chain	74.0	71.4
Ring-rod and triradial	26.0	28.6
Total quadrivalents	196	286

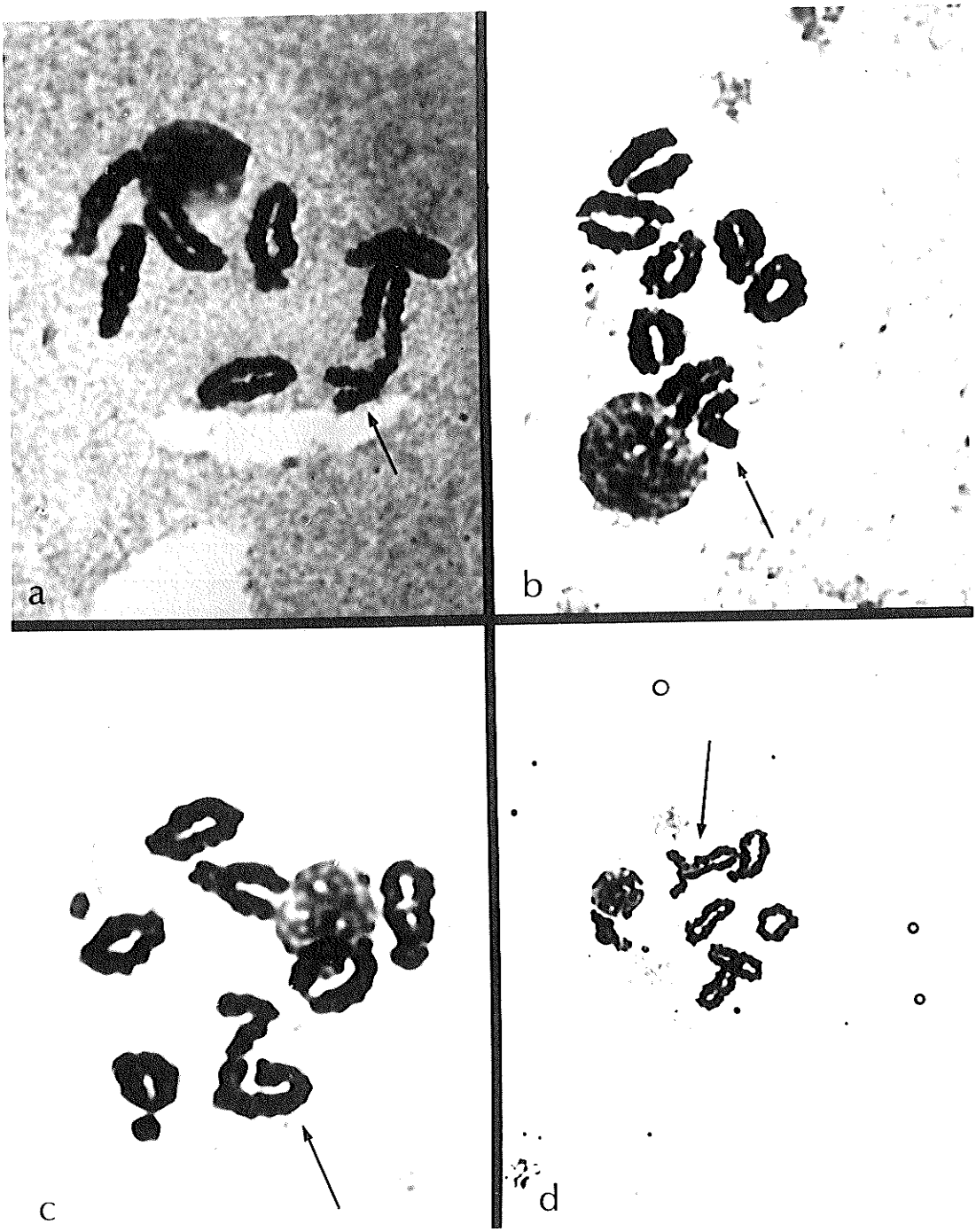
FIGURE 8 - DIAKINESIS CHROMOSOME CONFIGURATIONS OF DITELOTETRASOMIC 14 + 2 - 1S

(a) 6" + 1(t"1") - triradial type quadrivalent

(b) 7" + t"

(c) 6" + 1(t"1") - chain quadrivalent

(d) 6" + 1(t"1") - ring-rod quadrivalent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type, requiring only three chiasmata to hold them intact, while the other forms require four. The next most frequent group was that in which the two telos alone synapsed to form a bivalent (Fig. 8b) at a frequency of 37.1 percent. Least frequent were two categories; one in which one telo was unpaired, and the smallest group in which both telos were unpaired.

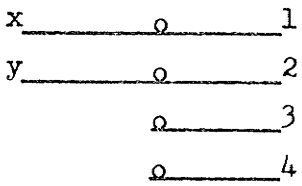
At metaphase, the same combinations were observed in the same relative frequencies as in diakinesis. However, the actual values had changed, probably as a result of chiasma terminalization. For example, the quadrivalent frequency decreased by about 10 percent and there was a corresponding increase in cells with a trivalent plus a univalent telo, which must become detached during terminalization. The proportion of chain to ring-rod quadrivalents was not noticeably altered between diakinesis and metaphase. The frequency of unpaired telos at metaphase was one percent greater than at diakinesis.

Morrison and Rajhathy (1960a, 1960b), and John and Henderson (1962), among others, have shown that two-thirds of the chromosomes in an autotetraploid cell will become associated into quadrivalents at first metaphase, while the remaining one-third form bivalents. A Chi-square test was performed on the data from diakinesis pairing observed in the ditelotetrasomic to determine the goodness of fit to a 2 : 1 ratio of quadrivalents to bivalents. A Chi-square value of 3.3 at 1 degree of freedom indicated no significant deviation from the expected.

In summary, it may be said that chromosome pairing in the ditelotetrasomic occurs in the same pattern as observed in autotetraploids.

To obtain the 2 : 1 ratio, pairing of the four homologous arms of

the ditelotetrasomic must take place in the following way (Sved, 1966):



Pairing between x and y arms is initiated at the ends (Kasha and Burnham, 1965), is complete and results in formation of chiasmata 100 percent of the time. Meanwhile pairing in the short arm also begins at the ends, and the four arms may synapse in three combinations as follows:

1) 1 and 2 plus 3 + 4
2) 1 and 3 plus 2 + 4
3) 1 and 4 plus 2 + 3

Combination 1 will result in the formation of two bivalents while 2 and 3 will both result in quadrivalents, hence the 2 : 1 ratio discussed above.

(d) Univalent behavior at metaphase I

Metaphase I cells were examined to determine if the monotelocentrics as univalents, or as bivalents in the case of the ditelo, would become positioned on the equatorial plate with the normal bivalents. The frequencies of "on plate" and "off plate" locations are presented in Table X. The position of the telo in the cell varied from an "on plate" location (Fig. 9b) to a polar one (Fig. 9a), with all positions in between. Some of the "on plate" univalents were located at the extreme periphery of the plate (Fig. 9c), apparently no longer under the influence of the meiotic spindle, and would likely be eliminated from telophase nuclei because of their immobility. The positions were grouped into two categories only, "on plate" and "off plate", to determine if differences existed between individual telos and whether positioning affected the

FIGURE 9 - POSITION OF UNIVALENT TELOCENTRIC RELATIVE TO EQUATORIAL PLATE

(a) "off plate" - polar location.

(b) "on plate" location., note tandem-V trivalent

(c) at periphery of equatorial plate.

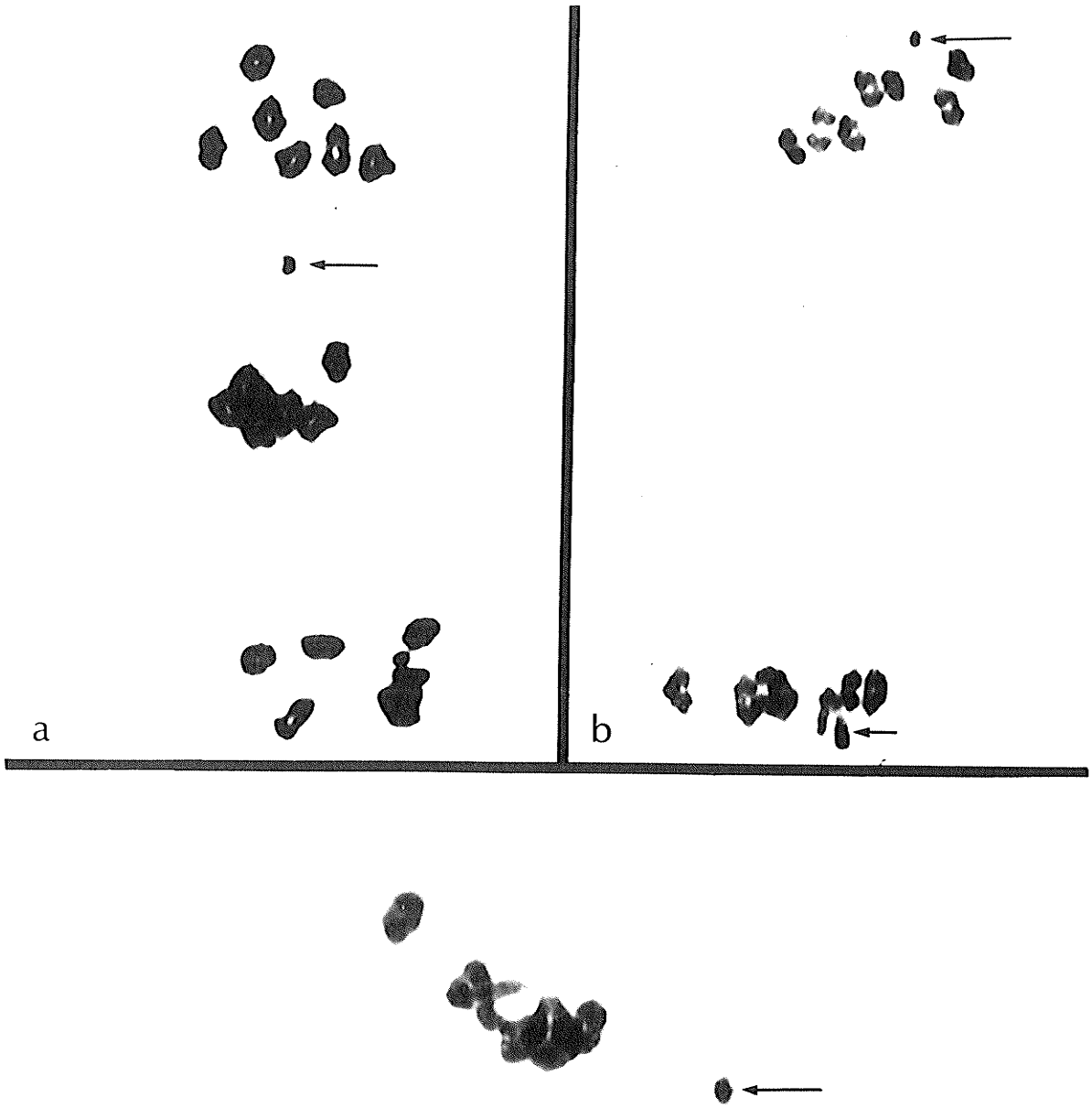

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TABLE X

POSITION OF UNIVALENT AND BIVALENT TELOCENTRICS
RELATIVE TO EQUATORIAL PLATE

Aneuploid Type	Total Cells Observed	Position of telos in %	
		On Plate	Off Plate
14 + 1S	98	64.0	36.0
14 + 2 - 1S	101	95.1	4.9
14 + 2L	95	50.5	49.5
14 + 4L	99	65.6	34.4
14 + 5S	72	48.6	51.4
14 + 6S	114	20.2	79.8
	Ave. of telos	49.8	50.2

TABLE XI

CHROMOSOME BEHAVIOR OF MONOTELOTRISOMICS
AND TRISOMIC BUSH AT AI AND TI

Aneuploid Type	Anaphase I		Telophase I	
	Number Cells Observed	Cells with Dividing Univalents in %	Number Cells Observed	% of Total Cells with Laggards
14 + 1S	71	16.9	214	17.5
Trisomic BUSH	85	9.3	186	11.3
14 + 2L	66	10.6	283	7.8
14 + 4L	132	15.1	356	16.6
14 + 5S	89	10.1	266	11.3
14 + 6S	122	11.3	146	25.4
Total	690	Ave. of Telos 12.2	Total 1537	Ave. of Telos 15.7

frequency of division at AI and subsequent lagging and exclusion at TII. The "on plate" univalents were not examined for orientated or non-orientated positions. Though not specifically scored, the majority of the telo bivalents on the plate assumed orientated positions, i.e. with centromeres aligned with polar regions.

In 95.1 percent of the cells examined, the telo bivalent was in an "on plate" position. In contrast, the telo univalents were located on the plate at a much lower frequency, varying from a high of 65.6 percent for 14 + 4L to a low of 20.2 for 14 + 6S with an average of 49.8 percent for the five monotelotrisomics. The length of the telocentric didn't appear to influence its frequency of positioning on the plate. The shortest arm, 14 + 5S was positioned on the plate in 48.6 percent of cells examined, while the longest 14 + 2L assumed the same position at a frequency of 50.5 percent, while the 14 + 6S univalent, which is intermediate in length between the two, was found in the same position in only 20.2 percent of the cells examined. Perhaps the positioning of the telo affects its rate of division at the subsequent anaphase stage.

(e) Anaphase I and Telophase I

Since continuous chromosome movements cannot be followed directly, their configurations are observed at distinct stages and the intervening steps are obtained by extrapolation. The distribution of chromosomes in daughter cells at anaphase I was observed, i.e. whether 7 : 7 telo dyad or 7+monad : 7+monad distribution, to determine whether the unpaired telocentric had divided at anaphase I.

The telo bivalent in 14 + 2 - 1S divided reductionally in 91.2 percent of the cells observed and in the remaining cells either moved

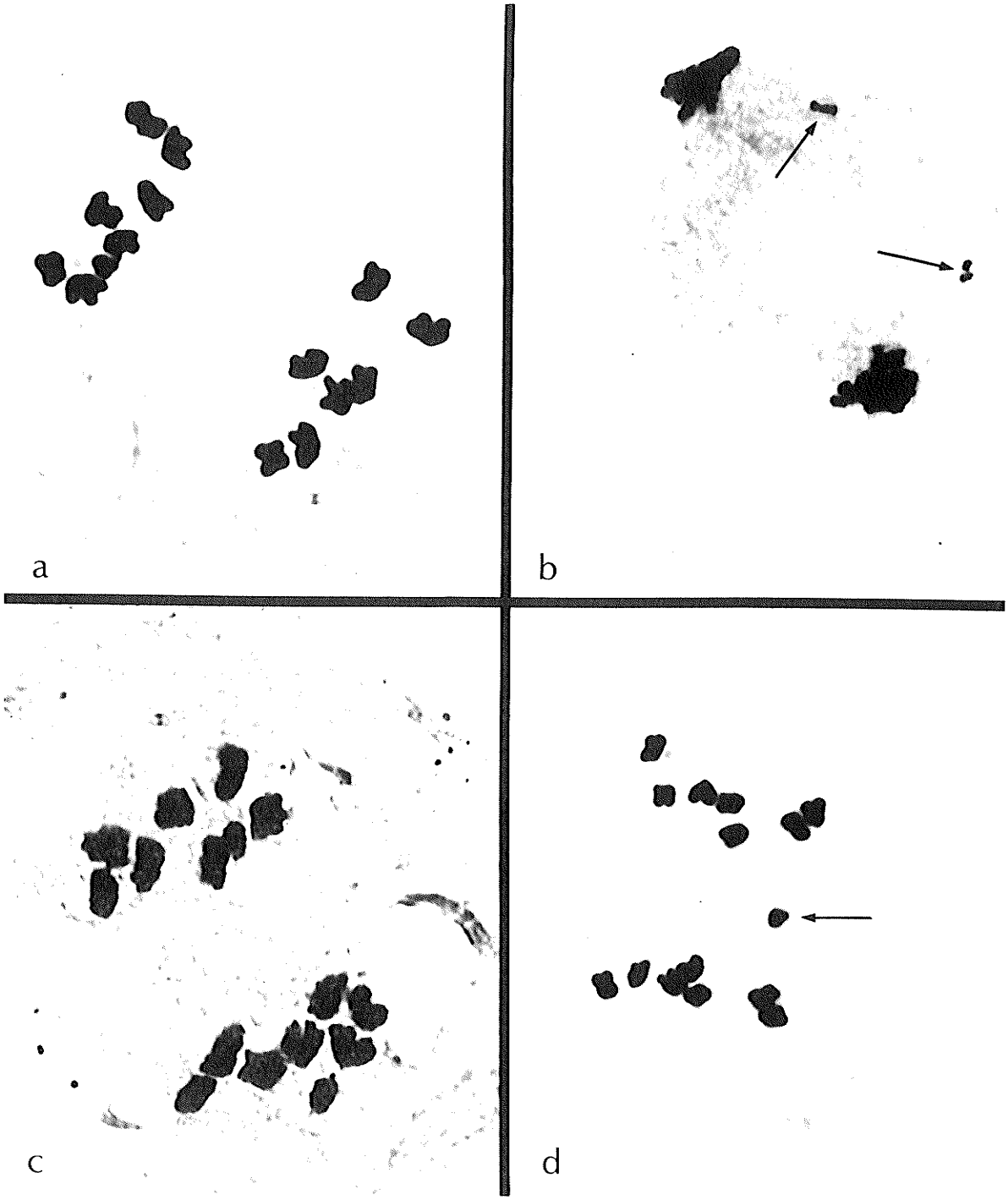
FIGURE 10 - CHROMOSOME BEHAVIOR AT AI AND TI OF MONOTELOTRISOMICS

(a) 7 : 7 + telo dyad disjunction at AI.

(b) lagging and fragmentation of telos at TI.

(c) 7 + monad : 7 + monad disjunction at AI.

(d) lagging dyad at AI.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poleward undivided or remained on the equatorial plate. The high rate of AI reductional division is expected in accordance with a high degree of pairing of the two telos followed by a high frequency of orientation on the metaphase plate (Table X).

In contrast, division of the univalent telos at AI would be equational and therefore precocious. The unpaired telos showed this type of behavior in an average of 12.2 percent of the cells (Table XI), (Fig. 10c). Telotrisomic 1L + 1S showed the highest rate of AI division at 16.9 percent and 1L + 5S the lowest at 10.1 percent. Trisomic Bush showed a lower frequency of AI division than the monotelotrisomics. In the majority of the cells, therefore, the telo as a dyad moved to one of the poles along with seven normal dyads while the other seven moved to the other pole (Fig. 10a). Occasionally the telo dyad was observed lagging on the equatorial plate (Fig. 10d). The univalents that divided at this stage presumably were those that had become orientated on the plate.

The frequency of laggards at telophase was determined. The univalents that divided usually did so after the normal bivalent chromosomes had completed their division and consequently lagged. Occasionally fragmentation of the lagging chromosomes was observed (Fig. 10b) indicating a certain amount of elimination at this stage. The univalents located at the periphery of the equatorial plate, infrequently remained in this position during anaphase and telophase and likely were excluded from telophase nuclei. However, it is likely that some of the laggards were the result of univalents moving onto the plate after the normal bivalents had divided, and dividing late. Again at telophase 1, the

ditelo had the lowest frequency of laggards at 6.9 percent and monotelotrisomic 14 + 6S had the highest frequency at 25.4 followed by 14 + 4L at 16.6 percent. Monotelotrisomic 14 + 2L showed the lowest frequency for the telos at 7.8 percent.

(f) Anaphase II abnormalities

Abnormalities at AII consisted mainly of laggards with occasional fragmentation (Fig. 11a, b). The ditelo 14 + 2 - 1S, approximating a normal chromosome behavior throughout meiosis, again showed a low frequency of abnormalities at AII, at a frequency of 5.5 percent (Table XII). Again the highest frequency of abnormalities, 28.7 percent, was shown by 14 + 6S. This telo showed the highest frequency of abnormalities throughout meiosis, beginning with a high frequency of univalents per

TABLE XII

CHROMOSOME BEHAVIOR AT AII OF MONOTELOTRISOMICS, TRISOMIC BUSH AND DITELOTETRASOMIC 14 + 2 - 1S

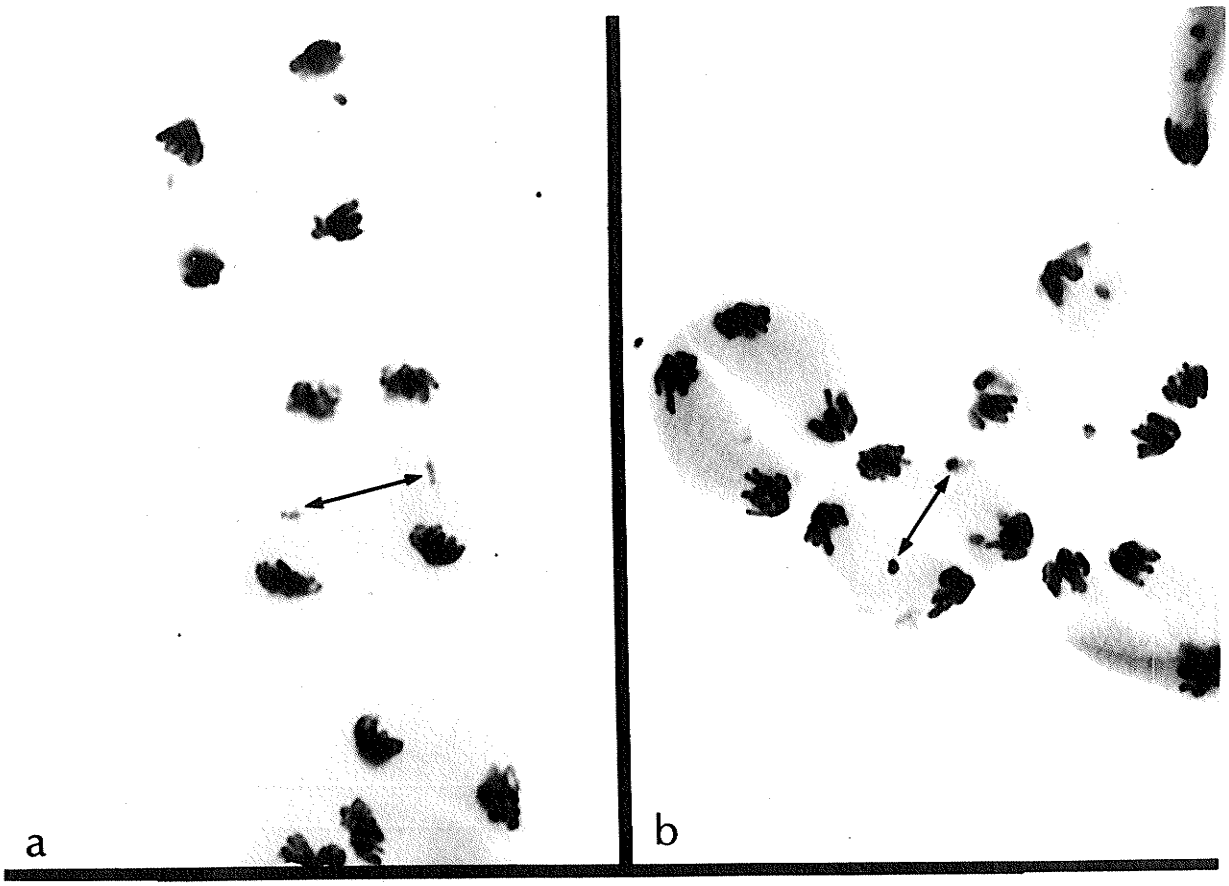
Aneuploid Type	Total Number Cells Observed	Cells with Chromosomal Abnormalities (%)
14 + 1S	138	16.2
14 + 2 - 1S	180	5.5
Trisomic BUSH	146	18.4
14 + 2L	287	11.4
14 + 4L	171	12.7
14 + 5S	328	15.4
14 + 6S	146	28.7
Total	1396	Average of Telos 16.9

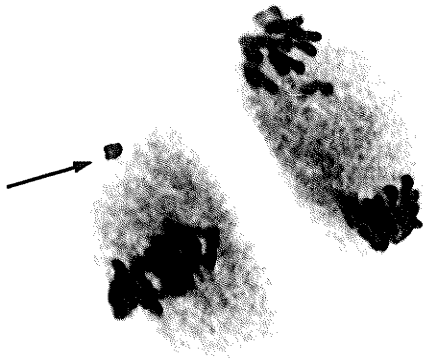
FIGURE 11 - CHROMOSOME ABNORMALITIES AT A II AND T II IN MONOTELOTRISOMICS

(a) Lagging and fragmentation of telo monads in both daughter cells.

(b) Lagging and fragmentation in both daughter cells.

(c) Non-synchronized disjunction in two daughter cells at A II. Daughter cell containing univalent in polar position divides later than its balanced counterpart.





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cell at diakinesis and metaphase, the highest frequency of "off plate" locations at MI and a high frequency of laggards at TI. Whereas the AII abnormalities for the majority of the aneuploids consisted of laggards and some fragmentation; in the case of 14 + 6S there was a high frequency of cells in which the telo had assumed a polar position at MII, while the normal chromosomes were on their respective metaphase plates (Fig. 11c). In the latter figure, the unbalanced daughter cell with the extra univalent in a polar position divided later than the balanced daughter cell.

(g) Nucleoli per cell

On the average, 91.3 percent of the cells examined contained one nucleolus (Fig. 12d, e), while 7.7 percent showed two (Table XIII, Fig. 12b, c). This would suggest one pair of chromosomes with strong nucleolar organizing capacity, and a second pair with a similar but weaker function. The former must be chromosome 6, with the large secondary constriction, and the latter chromosome 7. In the case of field-grown monotelotrisomic 14 + 1S and trisomic Bush, up to three nucleoli per cell were seen at times (Fig. 12a). These consisted of one large nucleolus and two smaller ones; often the third was of miniature size. These observations support those of Tsuchiya (1959) who suggested that the extra chromosome of Bush had a weak nucleolar-organizing capacity. The standard karyotype of Burnham and Hagberg (1956) suggests a minute secondary constriction in the long arm of chromosome 1. Perhaps it is responsible for the extra activity. If so, it is difficult to explain why an extra dose of the short arm should have similar increased activity. Also it is not unreasonable to assume that the minute nucleolus observed

at late diplotene (Fig. 12a) will fuse with one of the others to give two functional nucleoli at diakinesis.

(h) Nucleolus-chromosome relationships

Following the scoring of numbers of nucleoli per cell, observations were made on numbers of bivalents and/or univalents associated with each nucleolus. The results are presented in Table XIV. The average for the group indicated that, in 32.8 percent of cells, one bivalent was associated with the nucleolus, with two bivalents per nucleolus in the remainder. This observation supports the data of Table XIII in which it was shown that there are two major nucleolar-organizing chromosomes.

Cells of telotrisomic 14 + 6S showed a variety of nucleolar-chromosome relationships. In cells with only one nucleolus, the following chromosome arrangements were observed associated with it:

(a) two bivalents plus one univalent (Fig. 12e)

(b) one bivalent plus one trivalent (Fig. 12d)

(c) one bivalent plus one univalent

(d) one bivalent

Where two nucleoli were present, the following patterns were seen:

(a) one bivalent at each

(b) two bivalents at one and one univalent at the other (Fig. 12c)

(c) one bivalent plus univalent at one, with one bivalent at the other (Fig. 12b)

In the case of 14 + 1S, in which up to three nucleoli were seen per cell, only rarely was a second bivalent associated with one of the smaller nucleoli.

TABLE XIII

FREQUENCY (%) OF CELLS WITH VARYING NUMBERS OF NUCLEOLI AT LATE PROPHASE OF MEIOSIS IN MONOTELOTRISOMICS, TRISOMIC BUSH AND DITELO 14 + 2 - 1S

Source	No. of nucleoli per cell				No. Cells Observed
	1	2	3		
14+1S G.C.	99.1	.9	-		107
14+1S field	58.1	34.8	7.2		279
Trisomic BUSH	96.0	3.0	1.0		125
14+2-1S	96.1	3.9	-		152
14+2L	96.5	3.5	-		171
14+4L	98.1	1.9	-		106
14+5S	96.8	3.2	-		364
14+6S	89.8	10.2	-		89
Average of Telos	89.7	9.3	1.0	Total	1393

FIGURE 12 - NUCLEOLUS-CHROMOSOME RELATIONSHIPS IN MONOTELOTRISOMICS

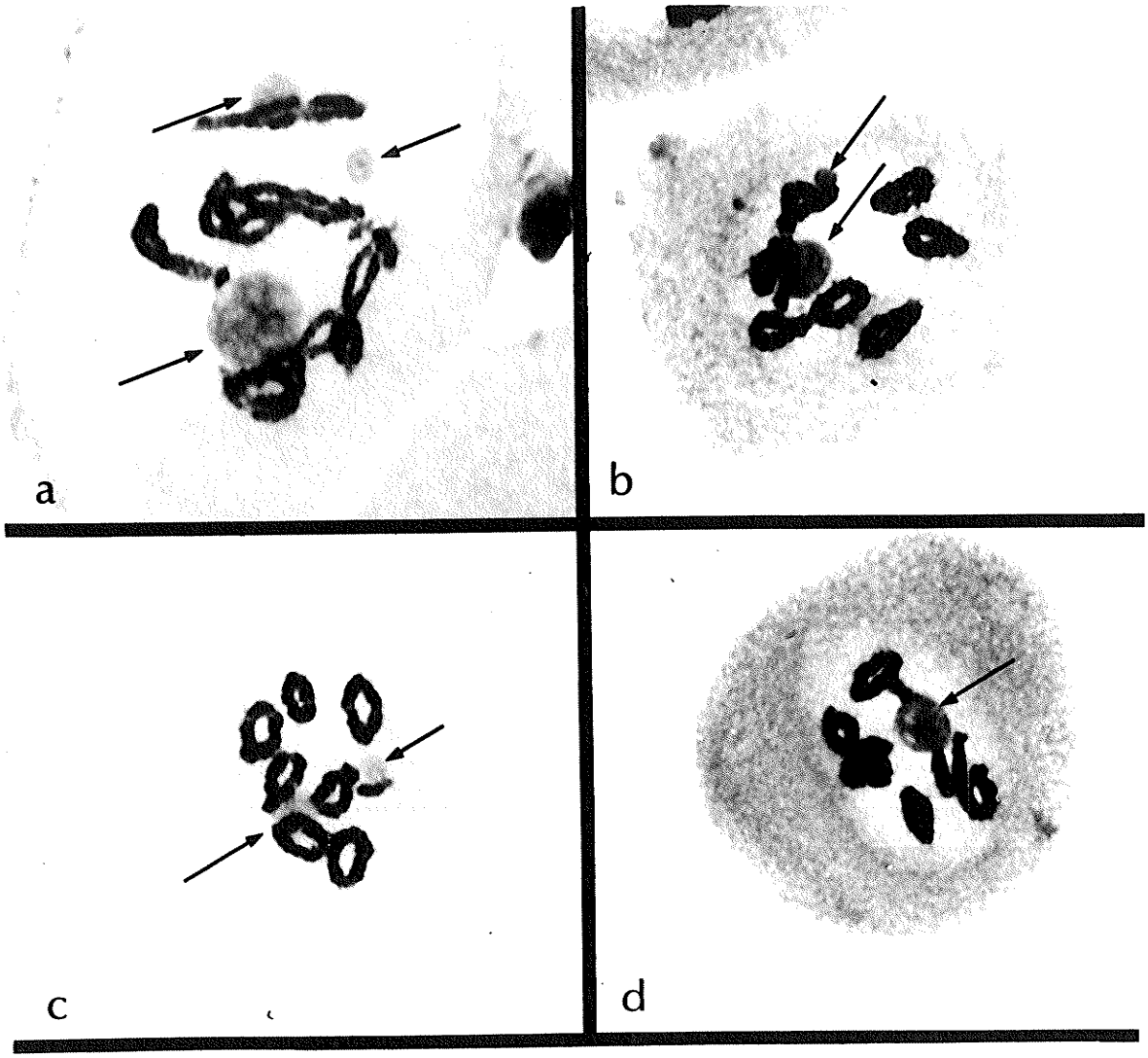
(a) One large, two small nucleoli at late diplotene of 14 + 1S.

(b) One bivalent plus univalent associated with large and one bivalent associated with small nucleolus of 14 + 6S.

(c) Two bivalents at large and one univalent at small nucleolus of 14 + 6S.

(d) One trivalent (ring-rod) plus a bivalent associated with single nucleolus of 14 + 6S.

(e) Two bivalents plus a univalent per single nucleolus of 14 + 6S.



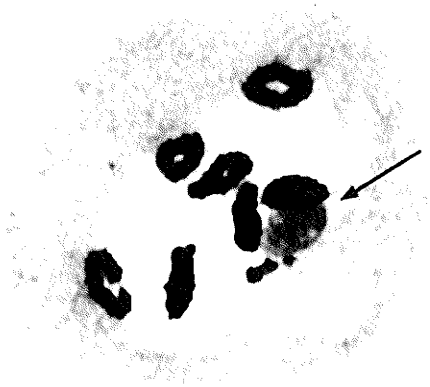


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TABLE XIV

PROPORTION OF CELLS WITH VARYING NUMBERS OF BIVALENTS PER NUCLEOLUS IN MONOTELOTRISOMICS, TRISOMIC BUSH AND DITELO 14 + 2 - 1S

Genotype	bivalents/nucleolus (% of cells) 1	2	No. Cells Observed
14+1S	68.1	31.9	285
14+2-1S	37.5	62.5	40
Trisomic BUSH	21.6	78.4	120
14+2L	23.2	76.8	156
14+4L	16.5	83.5	103
14+5S	51.7	48.3	129
14+6S	42.0	58.0	78
Ave. of Telos	40.3	59.7	911

(i) Micronuclei per quartet

Frequency of micronuclei per quartet was determined for each of the aneuploids and the information summarized in Table XV. It was assumed that micronuclei indicate the exclusion of extra unpaired chromosomes from daughter nuclei because of their abnormal meiotic behavior, such as lagging and precocious division at AI and AII. Of the quartets that exhibited micronuclei, those containing one (Fig. 13b) were more frequent than those containing two (Fig. 13b, c). Telotrisomic 14 + 6S showed the highest frequency of quartets with micronuclei at 23.3 percent, which may be expected as a consequence of its erratic behavior during the preceeding meiotic stages. Such unbalanced meiotic behavior will likely be reflected in the rate of transmission of the extra telocentric.

Whether the micronuclei observed were the result of lagging at anaphase I or anaphase II might be determined by the position of the micronuclei in the quartet. When two micronuclei were present per quartet an alternate arrangement (Fig. 13d), generally should indicate an anaphase I division and exclusion, while an adjacent arrangement (Fig. 13c) should be the result of AII abnormalities, though not absolutely. Scoring of micronuclei positions for a number of telocentrics revealed equal numbers of adjacent and alternate arrangements, indicating that exclusion was caused by both anaphase I and anaphase II divisions of the extra telo.

Ditelo 14 + 2 - 1S showed the lowest frequency of micronuclei in accordance with its regular meiotic behavior up to that point.

TABLE XV

FREQUENCY OF MICRONUCLEI PER QUARTET IN MONOTELOTRISOMICS, TRISOMIC BUSH AND DITELOTRISOMICS 14 + 2 - 1S

Aneuploid Type	Total Quartets Observed		Quartets with micronuclei in %		
			0	1	2
14 + 1S	247		82.6	13.4	4.0
14 + 2 - 1S	358		93.9	5.0	1.1
Trisomic BUSH	89		82.0	12.4	5.6
14 + 2L	162		90.2	6.8	3.0
14 + 4L	443		81.8	11.0	4.2
14 + 5S	422		80.6	10.4	9.0
14 + 6S	273		79.7	15.1	8.2
Total	1994	Average of Telos	83.0	13.4	3.6

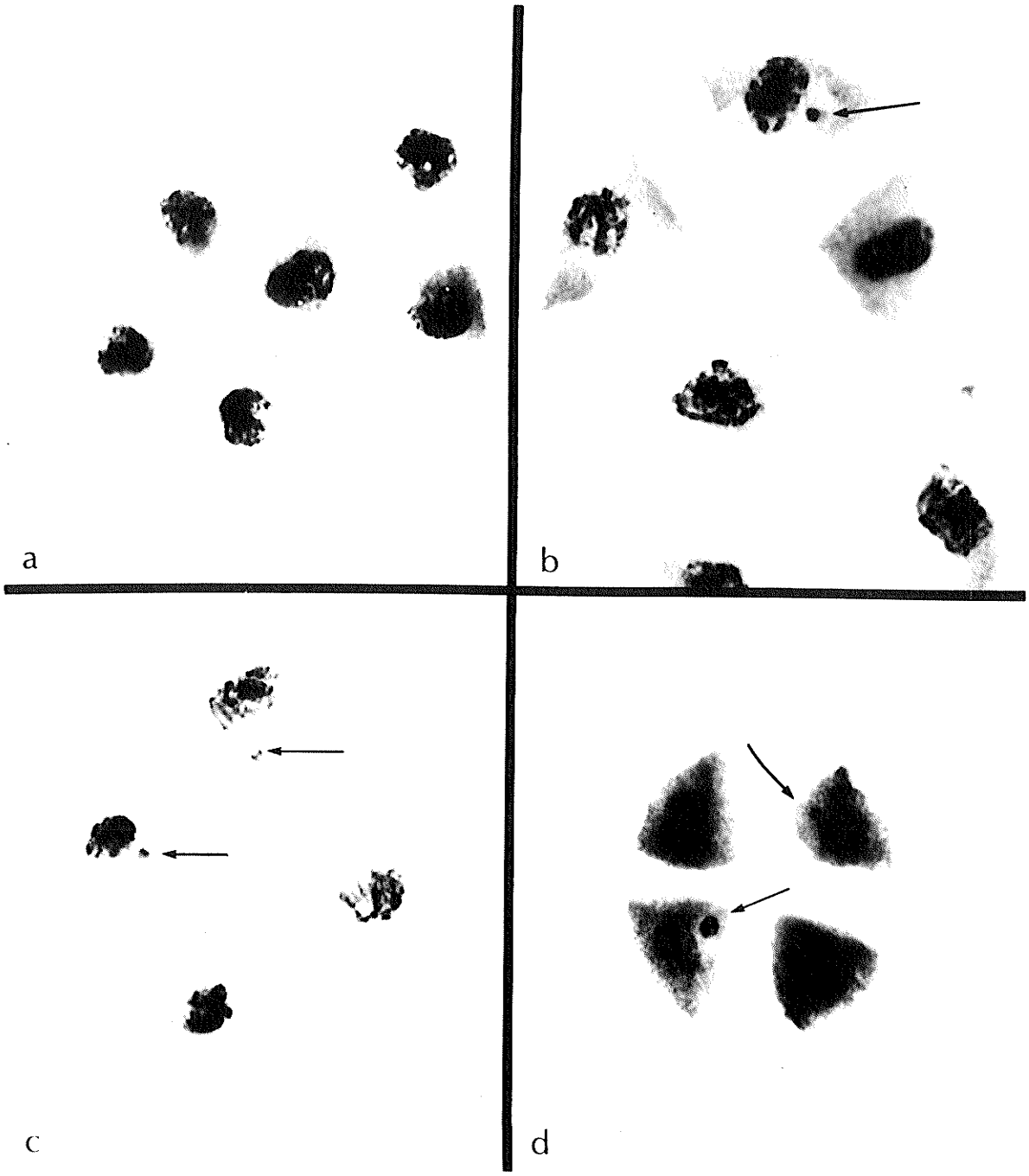
FIGURE 13 - MICRONUCLEI IN QUARTETS OF MONOTELOTRISOMICS

(a) No micronuclei.

(b) One micronucleus.

(c) Two micronuclei - adjacent arrangement.

(d) Two micronuclei - alternate arrangement.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5. Reproductive Properties of Aneuploids

(a) Transmission frequencies of extra chromosomes

The determination of transmission frequencies was made in all cases by counting chromosome numbers in somatic metaphase cells. Scoring was done in this manner since euploid and aneuploid plants were not sufficiently distinct to permit visual identification.

The frequency of transmission of the extra chromosome through selfed aneuploids ranged from a high of 35.5 percent for the acrocentric fragment of chromosome 3 to a low of 9.9 percent for 14 + 6S with an average of 28.3 percent for the group (Table XVI). The small sample size scored for 14 + 6S may not be sufficient for a reliable estimate.

Some of the unusual genotypes recovered in the progenies of selfed monotelotrisomics were as follows:

(a) Five ditelos and two primary trisomics in 14 + 1S,

(b) One triploid in 14 + 4L,

(c) Two primary trisomics in 14 + 5S,

(d) A ring chromosome in somatic cells of 14 + 6S.

There appeared to be no correlation between the lengths of the telocentrics and relative frequencies of their transmission. The following represents the ranking of the telos according to their length from the longest to shortest in relation to transmission frequencies shown in brackets:

14+2L (32.1), 14+4L (29.6), 14+6S (9.9), 14+1S (33.6), 14+5S (26.0)

The relative lengths were derived from a combination of ocular micrometer measurements on somatic cells and comparison with the standard barley karyotype of Burnham et al, (1956). Perhaps the relative magnitude of

TABLE XVI

TRANSMISSION RATES OF THE EXTRA CHROMOSOMES IN PROGENIES OF SELFED MONOTELOTRISOMICS AND 2n = 14 + telo X 2n crosses

Aneuploid	Total Seeds Examined		Progeny of selfed telotrisomics in %[1]		
			2n = 14	2n = 14 + telo	Other
14 + 1S	485		66.7	31.9	1.4
Trisomic BUSH	156		79.5	20.5	-
14 + 2L	143		67.9	32.1	-
14 + f[3]	197		64.5	35.5	-
14 + 4L	209		70.3	29.7	1.0
14 + 5S	258		74.0	26.0	.8
14 + 6S	121		80.1	9.9	.8
Total	1569	Averages	71.1	28.3	.6
			Progenies of 2n + telo X 2n[2] crosses		
14 + 1S	580		68.2	30.2	1.6
14 + 2L	30		76.7	23.3	-
14 + f[3]	72		70.8	29.2	-
14 + 4L	50		72.0	28.0	-
14 + 5S	219		77.2	22.8	1.4
14 + 6S	80		85.0	15.0	-
Total	1031	Averages	72.4	27.1	.5

[1] Selfed progenies included those from parental aneuploids and F_1 hybrids.

[2] The 2n pollen parents were linkage markers and translocation stocks.

deleterrious effects of the individual telos was more of a contributing factor to their transmission rate than was their relative length.

The frequency of monotelotrisomics in the progeny of monotelotrisomic by disomic crosses represents the transmission of the extra telo exclusively through the egg cell. The frequency ranges from a high of 30.2 percent for 14 + 1S to a low of 15.0 percent for 14 + 6S with an overall average frequency of 27.1 (Table XVI). This figure is slightly lower than that observed in selfed aneuploids. The latter may represent a certain very low frequency of pollen transmission also indicated by the occasional recovery of ditelos at a low frequency. However, the results from pollen transmission for 14 + 1S (Table XVII) indicate that the rate must be very low. The small sample sizes for 14 + 2L and 14 + 4L may not have given a true estimate of transmission in these two aneuploids.

Some of the unusual aneuploids observed in the progenies of 2n + 1 x 2n crosses were as follows: seven primary trisomics, one triploid and one 16 chromosome plant from 14 + 1S and three primary trisomics in the progeny of 14 + 5S.

No direct estimates were obtained of the influence of seed size on telo transmission as reported by Ramage (1955) and Tsuchiya (1959), but a general observation was that the smaller seeds, which germinated slower, were the ones bearing the telo. Central and lateral kernels were studied separately but no differences were found between these two groups in frequency of transmission of the telo.

(b) Pollen transmission of extra telocentrics

To determine the rate of transmission of the extra chromosome

through the pollen, the two aneuploids involving chromosome 1 (Table XVII) were used as male parents in crosses to normal diploids. Chromosome numbers were counted in somatic cells of the hybrid progeny to determine transmission rates.

Theoretically, balanced (n= 7) and unbalanced (n= 7 + telo) gametes should be produced at equal frequencies by each monotelotrisomic and all gametes produced by the ditelo should carry 7+ telo, assuming perfect meiotic behavior. Subtracting from these maxima the frequency of gametes carrying micronuclei (Table XV), the monotelotrisomic is still expected to contribute unbalanced gametes at a 36.0 percent frequency, while that expected from the ditelo would be 97.0 percent.

As shown in Table XVII, there was no transmission of the extra chromosome through the pollen of the monotelotrisomic, indicating the inability of unbalanced gametes to compete with the normal. In the case of the ditelo, 44.0 percent of unbalanced gametes were able to function.

Since 97.0 percent of the male gametes produced by the ditelo are unbalanced, and only 44.0 percent of them function, indicates that unbalanced gametes are at a disadvantage. Also it must be considered that the size of the sample studied was small (Table XVII).

(c) Transmission in selfed ditelotetrasomics

No crosses were made using diploid pollen on ditelos as female to determine the actual rate of transmission of the telos through the egg. Having established the transmission rate through the pollen (Table XVII), examination of chromosome numbers of selfed progenies, should give some indication of the rate of transmission through the egg cell. Assuming a level of meiotic abnormalities at megasporogenesis

TABLE XVII

FREQUENCY OF TRANSMISSION OF THE EXTRA CHROMOSOME THROUGH THE POLLEN OF MONOTELOTRISOMIC 14 + 1S AND DITELOTETRASOMIC 14 + 2 - 1S

Pollen Parent	Total Seeds	Chromosome Number			% Transmission of Extra Chromosome
		14	14 + telo	14 + 2 telo	
14 + 1S	54	54	-	-	0.0
14 + 2 - 1S	52	30	22	-	44.0

TABLE XVIII

TRANSMISSION OF THE EXTRA TELOCENTRICS THROUGH SELFED PROGENIES OF DITELOTETRASOMIC 14 + 2 - 1S

Total Seedlings Examined	Chromosome Number			% Transmission of Extra Chromosome
	14	14 + telo	14 + 2 telo	
No. 70	5	32	33	
Percent	7.1	45.7	47.2	92.9

similar to that observed at microsporogenesis that give rise to micronuclei, the eggs would be expected to produce 97 percent unbalanced and 3 percent balanced gametes. Combining this frequency for the egg with that observed in the pollen (Table XVII), a selfed ditelo should produce 1.8 percent diploids, 55.9 telotrisomics and 41.9 percent ditelos as shown in Figure 14. The proportions of the three genotypes that were actually observed are shown in Table XVIII. The total number of seedlings examined was small, but there were more diploids and ditelos formed than expected but fewer monotelotrisomics; the discrepancies, however, were not great. Unlike the transmission through the pollen, the transmission of the extra telo through the egg is close to the expected frequency.

(d) Primary trisomics in monotelotrisomic progenies

Primary trisomic plants were recovered from three different sources during the course of these studies (Table XIX):

(a) progenies of selfed telotrisomics,

(b) F_1 hybrids from 2n = 14 + telo x 2n crosses,

(c) segregating F_2 populations from telotrisomic X linkage marker crosses.

The frequencies with which trisomics occurred in these three populations were 0.59 percent, 1.3 percent and 2.2 percent, respectively (Table XIX). The trisomics occurring in the first two groups were detected by somatic chromosome counts. Trisomics in F_2 populations involving 14 + 1S were detected morphologically by their Bush phenotype. Their identity in the latter case was not verified cytologically.

Undoubtedly trisomics also occurred in F_2 progenies from crosses

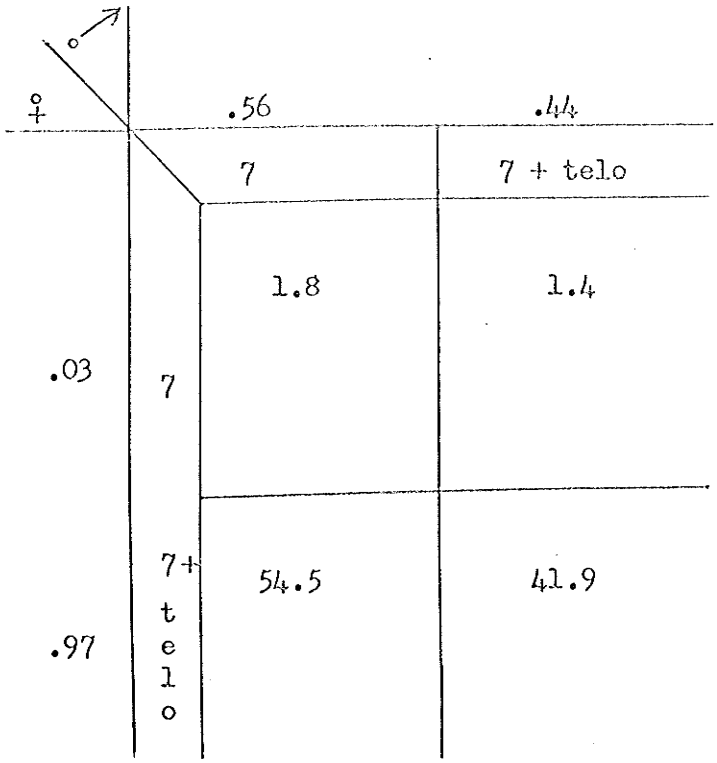


FIGURE 14 EXPECTED FREQUENCY OF ANEUPLOIDS IN DITELO 14+2-1S CONSIDERING OBSERVED RATE OF POLLEN TRANSMISSION OF TELO AND EXPECTED EGG TRANSMISSION

between monotelotrisomic 14 + 5S and linkage markers, but the Pseudo-normal phenotype of trisomic 5 was not distinct enough to permit visual selection.

It is assumed that such trisomics result from the fertilization of an eight chromosome egg by a 7 chromosome sperm nucleus. The eight chromosome egg is formed by a 2 normal : 1 telo disjunction from a trivalent instead of the usual 1 normal + 1 telo : 1 normal type of disjunction.

It has been reported by Chen and Grant (1968) in Lotus pedunculatus that "trisomic shifts" had occurred, i.e. progeny were not trisomic for the same chromosome as the parent. Of the numerous reports on barley trisomics, none describe similar events in that species. In the same vein, the question might be asked, are the primary trisomics that were derived from monotelotrisomics actually trisomic for the same chromosome as the telo? The following evidence will indicate that the individuals examined were the same.

An F_1 trisomic hybrid plant was obtained from the cross 14 + 1S Br,N x br,n with br located on the short n on the long arm of chromosome 1 as indicated in Table III. Of 87 F_2 plants grown from this individual, five were homozygous recessive n,n and three homozygous recessive br,br, i.e. both showed trisomic ratios at their respective loci (Table IV). These data indicate that the trisomic represented chromosome 1 and is evidence in support of its Bush phenotype.

Monotelotrisomic and trisomic F_1 hybrids were obtained from the cross between 14 + 5S x SV103. F_2 populations grown from both of these hybrids showed trisomic segregation at the SV103 locus (Table XXV). If

TABLE XIX

FREQUENCY OF OCCURRENCE OF TRISOMIC PLANTS
IN PROGENIES OF MONOTELOTRISOMICS

	Source	Total Examined	Number of Trisomics	% of Trisomics
1)	selfed monotelotrisomics			
	14+1S	485	2	.41
	14+5S	258	2	.77
				$\bar{x}$ = .59%
2)	14+1S x 2n	580	7	1.2
	14+5S x 2n	219	3	1.4
				$\bar{x}$ = 1.3%
3)	F_2 populations			
	14+1S x mutants	516	13	2.5
	14+1S x br	410	10	2.4
	14+1S x la	502	8	1.6
				$\bar{x}$ = 2.2%

TABLE XX

PERCENTAGE OF GOOD POLLEN IN BARLEY ANEUPLOIDS AND THEIR DISOMIC SIBS

Genotype	Number of Pollen Grains			% Good Pollen
	Empty and Degenerated	Good	Total	
14 + 1 S	67	1010	1077	93.8
2n Check	28	1000	1028	97.2
Trisomic BUSH	157	518	675	76.7
14 + 2 - 1 S	798	1462	2260	64.6
14 + 2 L	120	1031	1151	89.5
2n Check	82	1027	1109	92.6
14 + f^3	376	1000	1376	72.7
2n Check	97	1022	1119	91.3
14 + 4 L	32	1011	1043	96.9
2n Check	29	1010	1039	97.2
14 + 5 S	137	906	1043	86.9
2n Check	75	1009	1084	93.1
14 + 6 S	100	1012	1112	91.0
2n Check	37	1009	1046	96.5
Average of Aneuploids				84.0
Average of Diploid Checks				94.7

FIGURE 15 - POLLEN GRAINS STAINED WITH DILUTE ACETOCARMINE

(a) 2n check

(b) 14 + 1S

(c) Trisomic Bush

(d) 14 + 2 - 1S

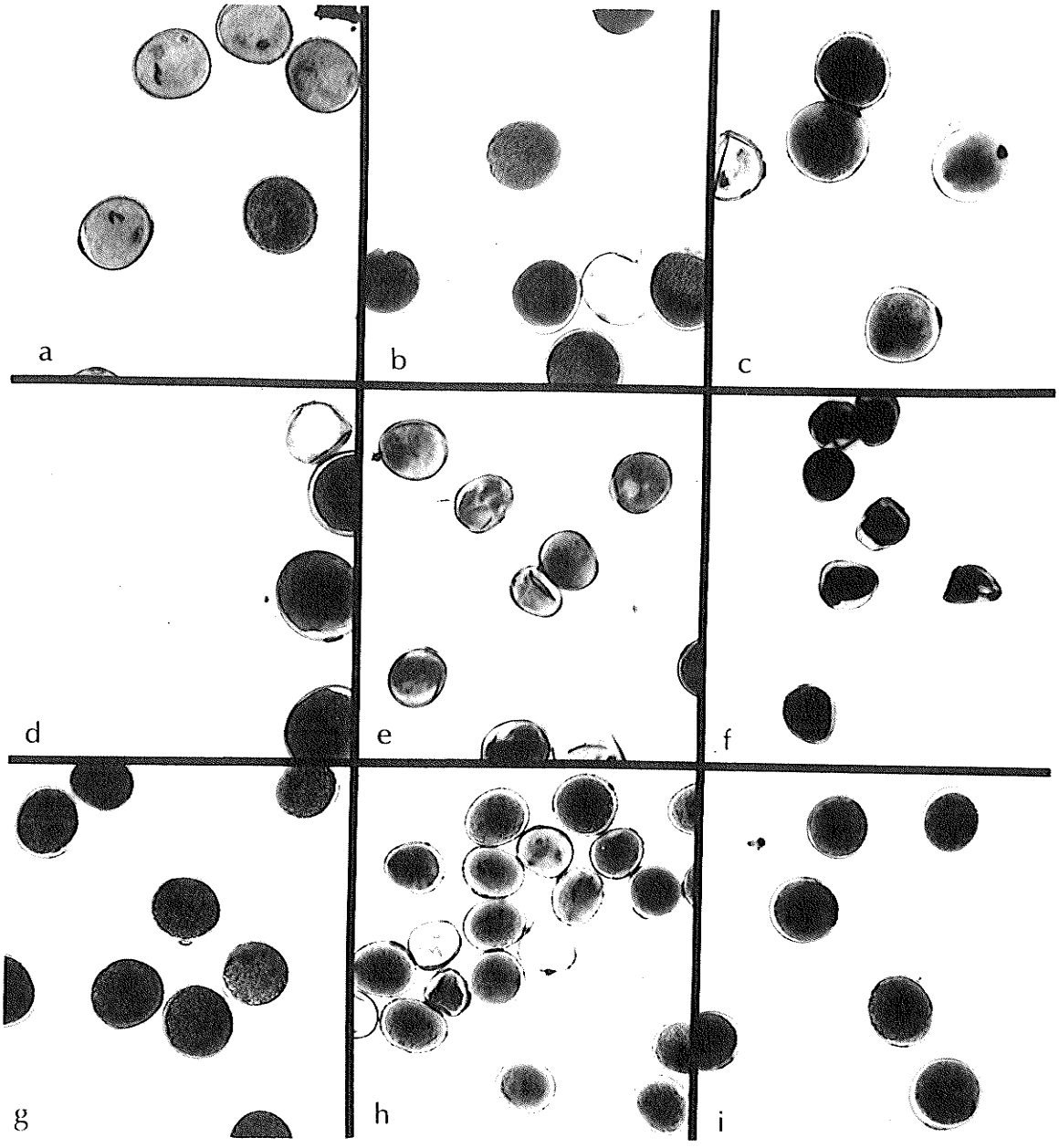
(e) 14 + 2L

(f) 14 + f^3

(g) 14 + 4L

(h) 14 + 5S

(i) 14 + 6S


a
b
c
d
e
f
g
h
i

a trisomic had occurred, the mutant gene in the trisomic population would not have segregated in a trisomic ratio.

(e) Pollen viability

Pollen viability was studied on all monotelotrisomics, the ditelo-tetrasomic and trisomic Bush (Table XX). The plants that were sampled were grown in a growth-chamber under controlled conditions as mentioned previously. Pollen grains, full of cytoplasm, that stained well with aceto-carmine were scored as good pollen, compared to empty or degenerated grains that did not stain (Figure 14a - i). The percent good pollen varied from a high of 96.6 percent for 14 + 4L to 64.6 percent for 14 + 2 - 1S with an average of 84.0 for all aneuploids. Even those monotelotrisomics that set very little seed such as 14 + 2L and 14 + 6S (Table VI) showed a high percentage of good pollen at 89.5 and 91.0 percent, respectively.

The pollen of disomic sibs corresponding to each aneuploid was scored in the same manner. The values ranged from a low of 91.3 for the f^3 disomic check to 97.2 percent for 14 + 1S check. No studies were made of the relationships between pollen grain size and genotype.

6. Genetic Studies

(a) Determination of arm-location of genes associated with chromosome 1

A number of mutant genes reported to be associated with established markers on chromosome 1 (Walker et al, 1963) were crossed to monotelotrisomic 14 + 1S, initially to determine their arm location. These markers, their symbols, and the corresponding genotype of 14 + 1S are listed in Table XXI. The F_2 populations from crosses involving each mutant gene were grown and scored in the field in the summer of 1968. All mutant characters

were clearly expressed with the exception of sb (subnodal bract) which showed variable expressivity as described by Walker et al (1963). The bracts were randomly arranged and were absent on some spikes of mutant plants. The observed F_2 segregations for each marker gene were tested by means of a Chi-square goodness-of-fit test for deviations from a 3 : 1 ratio. Five of the 11 markers listed in Table XXI, la, l_2, lb_3, n and yv showed a good fit to a 3 : 1 ratio (Table XXII). This precludes their location on the short arm of chromosome 1. They must be located either on the long arm or elsewhere in the genome. If located on the long arm, their distances from the centromere on that arm could be determined by the use of monotelotrisomic 1$\frac{1}{4}$ + 1L, Trisomic Bush, or they could be mapped by the conventional three point test.

Table XXII shows the Chi-square analysis for mutant genes br, ea, fc, gs_3, sb and yv_2 that showed a significant deviation from a 3 : 1 ratio for segregation at their individual loci. The high Chi-square values obtained for these genes and the low proportion of recessives in each F_2 (10.7 to 6.8 percent) led to the conclusion that these were trisomic ratios. It was difficult to apply theoretical trisomic ratios to bulk F_2 populations such as these. Theoretical ratios of this sort have been published (Burnham, 1962) and summarized (Tsuchiya, 1959). These ratios are dependant, however, on a number of factors, some of which are: ability to distinguish aneuploid from diploid segregates; the degree of pairing between the extra chromosome and normals; the amount of recombination between these chromosomes; frequency of transmission of the extra chromosome, etc. Estimates were made of some of these parameters in an effort to arrive at a possible trisomic ratio.

TABLE XXI

MARKER GENES ASSOCIATED WITH CHROMOSOME 1, THAT WERE CROSSED TO 14+1S TO DETERMINE ARM LOCATION

Gene Symbol	Character	Genotype of 14+1S
Br, br	Normal vs. brachytic	Br,Br
Ea, ea	Early vs. late heading	Ea,Ea
Fc, fc	Normal vs. chlorina seedling	Fc,Fc
Gs_3, gs_3	Normal vs. glossy sheath	Gs_3,Gs_3
L_2, l_2	Lax vs. compact spike	L_2,L_2
La, la	Lax vs. compact spike	La,La
Lb_3, lb_3	Normal vs. long weak basal i'node	Lb_3, Lb_3
N, n	Covered vs. naked	N,N
Sb, sb	Normal vs. subnodal bract	Sb,Sb
Yv, yv	Green vs. yellow viable	Yv,Yv
Yv_2, yv_2	Green vs. yellow viable $_2$	Yv_2,Yv_2

TABLE XXII

X^2 ANALYSIS OF F_2 POPULATIONS OF CROSSES BETWEEN MONOTELOTRISOMIC 14 + 1S AND MUTANT GENES ASSOCIATED WITH CHROMOSOME 1

Marker	Segregation Classes X	x	Chi-square 3 : 1	P	Percent Recessive
La, la	390	112	1.93	.25 - .10	22.3
L_2, l_2	240	65	2.21	.25 - .10	21.3
Lb_3, lb_3	415	165	3.68	.10 - .05	28.4
N, n	826	257	.93	.50 - .25	23.7
Yv, yv	540	203	2.12	.25 - .10	27.3
Br, br	1116	82	210.61	< .01	6.8
Ea, ea	1032	116	135.84	< .01	10.1
Fc, fc	1387	140	204.12	< .01	9.2
Gs_3, gs_3	520	51	78.63	< .01	8.9
Sb, sb	193	23	23.73	< .01	10.7
Yv_2, yv_2	590	48	104.93	< .01	7.5

As shown in Table VIII, the telo of 14 + 1S enters into trivalent configurations with normal chromosomes in 70.6 percent of metaphase cells. This indicates a high frequency of chiasma formation and it might be assumed that chiasmata are physical manifestations of recombination.

An estimate was made of the amount of chromatid crossing-over occurring in the F_2 population involving the fc gene. A population of 250 F_2 plants was grown in the greenhouse, of which 30 showed a homozygous recessive fcfc, chlorina phenotype. A cytological examination of these 30 individuals revealed that three were monotelotrisomic. According to Burnham (1962), monotelotrisomic recessive homozygotes arising from duplex monotelotrisomics are the result of chromatid crossing over. The frequency of such crossing over in this case would be 10 percent. Putting together a 10 percent frequency of chromatid crossing over coupled with a 30 percent frequency of telo transmission, a trisomic F_2 ratio of 11 : 1 was expected, but it was found that not all F_2 ratios would fit this expectation. In fact a single ratio was not found that would fit all F_2 segregations.

There are a number of variables affecting trisomic ratios of genes that tend to affect individual genes differently. The telocentric in the case of monotelotrisomics was transmitted at a much lower frequency that expected on a random basis. There was no pollen transmission and only 30 percent egg transmission, though there was a potential of 50 percent transmission through each type of gamete. The telo carried the dominant allele in a duplex F_1 heterozygote, and even with no recombination, there would be a lower than expected ratio of dominant : recessive in the F_2, because of loss of the dominant allete with the telocentric.

With the occurrence of recombination the recessive allele would be placed on the telo in exchange for the dominant, then lost with the telo, resulting in a shift of dominant : recessive to a higher ratio. The greater the distance between the locus and centromere, the greater the incidence of recombination, hence a lower proportion of homozygous recessive individuals in the F_2.

It was found that linkage markers fc and br gave 9.2 and 6.8 percent recessive individuals in their respective F_2 populations, (Table XXII). Since br is known to be located distal to fc on chromosome 1 (Nilan, 1964), the relative proportion of recessives obtained in the F_2 is in agreement with the postulates outlined above. Going one step farther, the other markers with trisomic ratios (Table XXII) were placed at relative sites on the linkage map of the short arm of chromosome 1 based on proportion of homozygous recessives obtained in each F_2. They were located relative to the known positions of fc and br (Fig. 16).

(b) Locating the centromere position on chromosome 1

The position of the centromere on the linkage map on chromosome 1 has been reported to be located in the area rs bl_2 n by Robertson (1964), and ac_2 rs bl_2 ert-d n by Nilan (1963). When mapping of translocation breakpoints on chromosome 1,(Ramage and Burnham, 1963),found the centromere to be located in the region of n. Unfortunately only the markers ac_2 and n were considered in that study, the intervening ones shown above were not.

Using monotelotrisomic 14 + 1S and the markers listed in Table XXIII the feasibility of locating the centromere position on this chromosome was tested. By crossing each marker to 14 + 1S and observing the

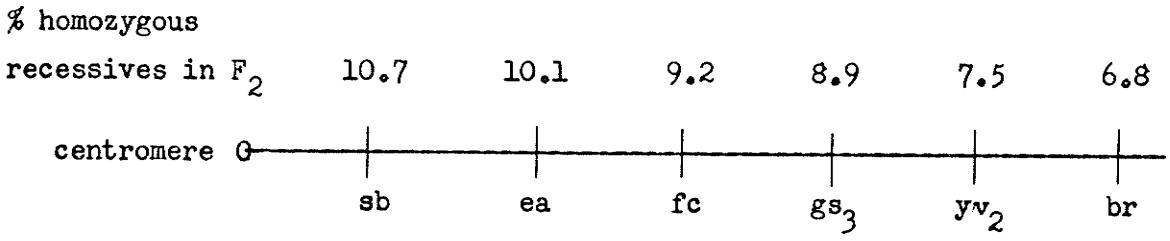


FIGURE 16 ARRANGEMENT OF ASSOCIATED GENES ON SHORT ARM OF CHROMOSOME 1 RELATIVE TO PREVIOUSLY LOCATED GENES fc AND br

ratio for each locus in the F_2, the centromere position should be found located in the interval between the two genes where a trisomic ratio changes to a disomic.

Disomic inheritance for the markers ert-d and n (Table XXIII), indicated by a good fit to a 3 : 1 ratio, confirmed their location on the long arm of chromosome 1.

The determination of inheritance patterns at the Rs and Bl_2 loci was more complex than for the two previously reported markers, n and ert-d. The 14 + 1S parental material was red-stemmed. To determine if this was conditioned by the same Rs gene as the one listed on the linkage map of chromosome 1, it was crossed to a known Rs stock and a test of allelism carried out. A total of 72 disomic and 122 monotelotrisomic F_2 progeny plants were grown. There were no green segregates in these populations, indicating that the red stem in 14 + 1S was conditioned by the Rs gene.

A cross was made between 14 + 1S and a green stem (rs) pollen parent for determination of arm location of Rs. Chi-square analysis of the F_2 data showed a significant deviation from a 3 : 1 ratio (Table XXIII). The high Chi-square value combined with the small proportion of recessive segregates indicated a trisomic ratio, hence a short arm location.

The expression of the red coloration in segregating progenies appeared to be light sensitive. When F_2 plantings were made in rows perpendicular to the long axis of a greenhouse bench, it was impossible to classify accurately individual segregates and arrive at clear-cut ratios. There was an obvious light differential between the edge and centre of the bench, resulting in poor expression of the red color. It

TABLE XXIII

X^2 ANALYSIS OF F_2 POPULATIONS OF CROSSES BETWEEN 14+1S AND MARKERS USED IN LOCATING CENTROMERE POSITION ON CHROMOSOME 1

Marker Gene	Frequency X	Frequency x	X^2 3 : 1	P	Percent Recessive
$\underline{Bl_2}$, $\underline{bl_2}$	515	286	48.96	<.01	35.7
Ert-d, ert-d	94	27	.47	.50 - .25	22.3
N, n	826	257	.93	.50 - .25	23.7
Rs, rs	248	43	16.23	<.01	14.7

was only when plantings were made in single or double rows, parallel to the long axis of the bench and individual plants were more evenly illuminated, that segregates could be classified with any degree of accuracy and clear-cut ratios determined.

The proportion of homozygous recessives in the F_2 population from the cross 14 + 1S (Rs) x rs was 14.8 percent. This is a higher frequency of recessives than reported for any other monotelotrisomic F_2 (Table XXII). With the Rs locus being located in the centromere region of chromosome 1, then the Rs allele located on the telo in the F_1 heterozygote would undergo a small amount of recombination with the rs allele on the normal chromosome. The rs allele therefore would be transmitted to the progeny at a high frequency while located on the normal chromosome, resulting in a high frequency of recessives in the F_2. In the case of a locus located farther from the centromere, a higher frequency of recombination would shift the recessive allele to the telo, with which it would be lost, altering the segregation in the F_2 as a consequence. This result provides further evidence for the postulate formulated earlier about the relationship of recombination to gene-centromere distances.

Chi-square analysis of F_2 segregation at the Bl_2 locus likewise showed a significant deviation from a disomic ratio (Table XXIII). The proportion of recessives in this F_2 population was 35.7 percent, which is different from observed values for other populations shown in Table XXII. In this case, the cross involved 14 + 1S (bl_2,bl_2,bl_2) x Bl_2,Bl_2, resulting in a simplex F_1 heterozygote rather than duplex as in the other cases. The proportion of recessives expected in the F_2 obtained from a simplex F_1 heterozygote is 35.6 percent when diploids and telotrisomics are not

separated.

An 11:7 ratio was used by Kerber (1958) to explain the trisomic segregation observed in the progeny of a simplex heterozygote. The ratio assumes non-transmission of n + 1 pollen, 25 percent transmission of n + 1 female gametes, factors which are not at variance with the situation in the current studies. The transmission rate of the telo 14 + 1S through the egg was slightly higher at 30 percent. No estimate of chromosome segregation was obtained, though it is assumed to be the predominant type because of the proximity of the $\underline{Bl}_2$ locus to the centromere. The Chi-square analysis of goodness-of-fit of observed data to an 11 : 7 ratio gave a value of 3.42 with a P. value of .10 - .05. These results indicate trisomic segregation at the $\underline{Bl}_2$ locus.

This leads to the conclusion that the centromere position on chromosome 1 is located between $\underline{Bl}_2$ and ert-d since Rs and $\underline{Bl}_2$ showed trisomic segregation while ert-d and n proved to be disomic.

(c) Inheritance and arm location of new mutant genes.

The Table XXIV, are listed six mutant stocks that have not been reported previously, nor have they been associated with any chromosome of the complement. These stocks were supplied by Dr. T. Tsuchiya. One additional mutant, Parkland spot, found to be associated with chromosome 5 (S. A. Wells, personal communication) was supplied by Dr. Wells under that name.

Five of these markers, SV53, SV103, Km114, 118 and 174 when crossed to 14 + 1S showed disomic segregation in both disomic and monotelotrisomic progenies indicating that they were inherited as simple recessives and that they were not located on the short arm of chromosome 1.

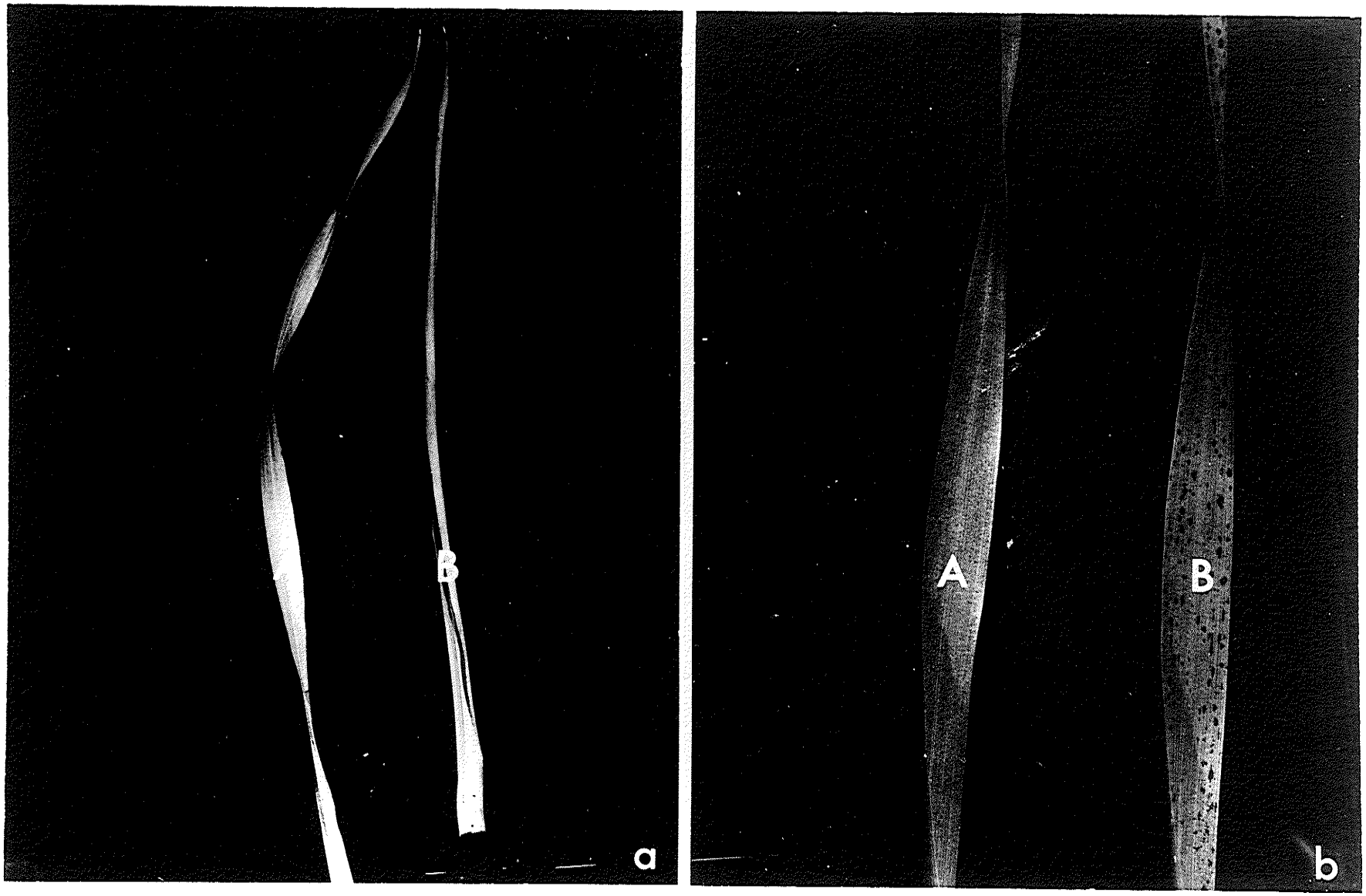
FIGURE 17 - LEAF CHARACTERISTICS OF MUTANTS REVOLUTED LEAF (rl.) AND PARKLAND SPOT (ps).

(a) A - 2n check.

B - Revoluted leaf (rl)-note counter-clockwise spiral.

(b) A - 2n check.

B - Parkland spot (ps)-note leaf spotting.


B
a
A
B
b

Km183 was inherited as a simple dominant. The F_1 hybrid exhibited a semi-dwarf growth habit, i.e. 15 inches in height, when diploid checks of 14 + 1S grew 24 inches tall.

In crosses to 14 + 5S; SV53, 114, 118 and 174 again showed disomic ratios in both disomic and monotelotrisomic progeny indicating that they were not located on the short arm of chromosome 5 either. Chi-square analysis of F_2 monotelotrisomic populations from Parkland spot and SV103 (Table XXV) showed significant deviations from disomic ratios. The high Chi-square values and low frequencies of homozygous recessives indicated that trisomic segregation occurred at these two loci. F_2 disomic check populations showed a good fit to 3 : 1 disomic ratios. This is evidence that mutant genes SV103 and Parkland spot are inherited as simple recessives and are located on the short arm of chromosome 5.

The mutant SV103 is characterized by the tips of juvenile leaves becoming rolled into a tube (Fig. 17a), through a counterclockwise spiral. The character is most prominent at about the three-leaf stage and persists through to near maturity. Segregating populations may be classified with ease at any of these times. A suggested symbol for this mutant is rl, representing the revoluted leaf phenotype.

Parkland spot is characterized by the appearance of small, brown leaf-spot-like specks that appear near the tips of young leaves, beginning at about the three-leaf stage (Fig. 17b). The symbol ps is suggested for this mutant; representing Parkland spot.

The two mutants just described should be useful for genetic studies. They are clearly expressed at an early stage of plant growth, permitting early classification of segregating populations. Mutant plants

TABLE XXIV

NEW MUTANT GENES THAT WERE CROSSED TO MONOTELOTRISOMICS 14+1S AND 14+5S IN ORDER TO DETERMINE THEIR ARM LOCATION

Identity of Mutant	Character
SU 53	glossy sheath
SV 103	revoluted leaf
Km 114	awnless
Km 118	spiral neck
Km 174	erectoides
Km 183	semi-dwarf
Parkland spot	brown flecks on upper half of leaf

TABLE XXV

X^2 ANALYSIS OF F_2 POPULATIONS FROM CROSSES BETWEEN 14 + 5S AND NEW MUTANTS

Mutant Gene	Frequency X	Frequency x	X^2 3 : 1	P	Percent Recessive
14 + 5S x Pspot 2n Check	140	41	.67	.50 - .25	22.7
Monotelotrisomic Progeny	606	100	44.31	<.01	14.2
14 + 5S x SV103 2n Check	50	12	1.05	.50 - .25	19.3
Monotelotrisomic Progeny	330	40	39.74	<.01	10.8
Trisomic Progeny	271	37	27.78	<.01	12.0

are not depressed in growth; are as thrifty as normal sibs, and as fertile if grown to maturity.

Kml83, the semi-dwarf when crossed to 14 + 5S, gave an F_2 ratio of 71 : 15 for dominant : recessive at that locus in the disomic progeny. The calculated Chi-square value for a 3 : 1 ratio amounted to 2.63 which gave a P. value of .10 - .25, indicating a good fit to the expected disomic ratio. The segregation in the monotelotrisomic progeny at the same locus was 131 : 91. The F_1 heterozygote from this cross was dwarfed, indicating a dominant gene, therefore a simplex constitution in the monotelotrisomic. A Chi-square value for goodness-of-fit to an 11 : 7 ratio gave a value of .41 with a probability of .50 to .75. These data indicate that Kml83 is inherited as a simple dominant with the locus located on the short arm of chromosome 5.

(d) Linkage map of short arm of chromosome 5.

Marker genes at and trd were used to identify the short-arm telocentric of chromosome 5. Although only small F_2 populations were grown, it was found that they produced 10.3 and 12.7 percent recessives in their respective F_2 monotelotrisomic progenies. In comparison, ps and rl in their respective F_2 progenies (Table XXV) produced 14.2 and 12.0 percent recessives. On the assumption that the frequency of homozygous recessives in the F_2 is inversely proportional to gene-centromere distance, the four genes can be located at relative positions on the short arm of chromosome 5. The positions of at and trd, determined by classical genetic analysis (Nilan, 1964), are in agreement with positions based on the frequency of homozygous recessives in monotelotrisomic progenies for these two genes (Fig. 18). On the same basis, the genes ps and rl,

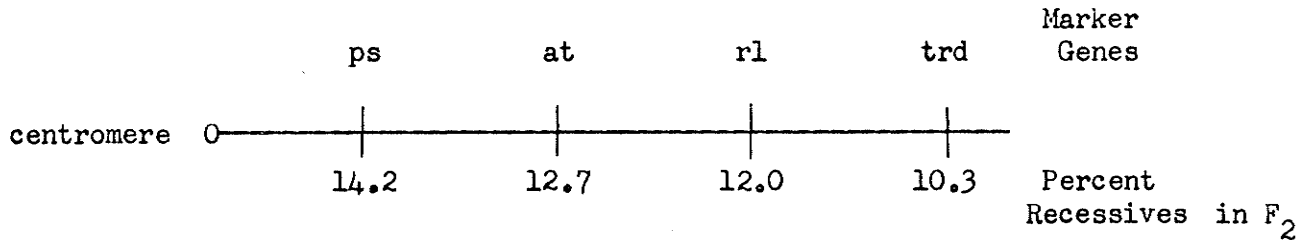


FIGURE 18 - RELATIVE POSITIONS OF FOUR MARKERS ON SHORT ARM OF CHROMOSOME 5, BASED ON PERCENT HOMOZYGOUS RECESSIVE IN F_2 MONOTELOTRISOMIC PROGENY

can be assigned to map positions relative to at and trd as shown in Figure 18.

The position of the centromere on chromosome 5 has not been determined. From available data this position may be determined by approximation. Referring back to the genetic data obtained for chromosome 1; in F_2 populations from crosses with 14 + 1S, the Rs locus, known to be located in the centromere region of chromosome 1, showed 14.7 percent recessives. In crosses with 14 + 5S, 14.2 percent of the F_2 monotelotrisomic progeny were recessive for the ps phenotype. On this premise it can be concluded that the centromere on chromosome 5 is close to the ps locus and distal to the other markers studied on chromosome 5 (Fig. 18). This method of positioning the centromere, though indirect, may give some indication of its location, which at present is not known.

CHAPTER V

DISCUSSION

Telocentric chromosomes in barley have been isolated from several sources. They have arisen spontaneously and in intervarietal crosses but the most prolific source has been trisomics in which they arise through misdivision of unpaired univalents. The reports of Tsuchiya (1967) and Yu (1968) indicate that telocentrics have been detected in the past but never identified nor studied. With the demonstration in this report of their utility, every effort should be made to retain and identify any new ones discovered. Only five monotelotrisomics of the possible 14 required for a complete series have been described in this report. At the time of the preparation of the manuscript (August, 1969) at least two other monotelotrisomics were known to exist, namely 14 + 1S (Tsuchiya, 1967 and unpublished data) and one containing the non-satellited arm of chromosome 6, presumably 14 + 6S (Metcalfe, 1968). The observations by Ramage, et al (1961) and Hagberg, et al (1963) that some induced translocations involved breakage within the centromere of one of the chromosomes, suggest that irradiation of trisomic seeds may induce additional monotelotrisomics.

Because barley chromosomes are structurally metacentric, cytological identification of telocentrics at somatic metaphase is virtually impossible. The use of translocation testers provides irrefutable identification of chromosomes from which telocentrics were derived but not of the specific arms. The identity of the arms utilizing linkage marker data, is delayed through the necessity of producing an F_2 population; however, the recombination values obtained therefrom can be used as points of

reference for future genetic studies.

Plant morphology was affected by the addition of a telocentric to the regular chromosome complementthrough manifestations of a similar type but of lesser magnitude than those produced by the addition of the corresponding whole chromosome as in trisomics. The one exception to this generalization was 14 + 2L. This characteristic lack of morphological distinction eliminates this as a means of separating diploids from aneuploids in segregating generations. On the other hand, the high vigor and fertility of monotelotrisomics is ample compensation for the lack of morphological distinction. The completion of the series of monotelotrisomics will permit the study of contributions to plant morphology of each chromosome arm in triple dose. Plants with the long arm of chromosome 1 in triplicate resembled those trisomic for this chromosome while 14 + 1S was not unlike the diploid. In this respect the designation of short arm is appropriate. Genetically, however, the linkage map of the short arm is more extensive than for the long arm (Nilan, 1964); containing a greater number of located genes. The phenotypic effects lead to the assumption that the existing linkage map may not reflect the true genic content of the long arm nor the importance of the gene functions contained therein. From existing information it may be premature to conclude that in barley, as in the tomato (Khush, et al, 1968), long arm monotelotrisomics resemble trisomics in gross morphology while the short arm counterparts are only rarely distinct from diploids. The results obtained with 14 + 1L and 14 + 1S indicate that this may be the case.

Generally, the frequency of synapsis of the extra telocentric with two normal chromosomes at diakinesis was slightly lower than the

synapsis of the three homologues of trisomics. Trivalent configurations were observed in 73.4 percent of monotelotrisomic and in 89.1 percent of trisomic cells. The higher frequency of trivalents in the latter aneuploid may be the result of a greater number or different arrangement of the hypothetical pairing initiation points called zygomeres (Sybenga, 1968). The behavior of the extra telocentrics in subsequent meiotic stages was similar to that of additional whole chromosomes in trisomics as reported by Yu (1968), resulting in similar transmission frequencies of the extra chromosome for both groups of aneuploids.

One characteristic shared by both series of aneuploids was the abnormal meiotic behavior of additional members of chromosome 6. In 64.9 percent of 14 + 6S cells univalents were observed at diakinesis, while the average for five monotelotrisomics was 26.6 percent. A similar pattern was observed at diakinesis in the Trisomic Purple (Tsuchiya, 1967; Yu, 1968). A high rate of synapsis is expected of telo 6 because of its nucleolar-organizer function. In this capacity the three homologous short arms, through association with the nucleolus, would be in close proximity; a prerequisite for complete synapsis. It is not unreasonable to assume however, that the telocentric may associate with a second nucleolus, precluding its association in a trivalent configuration. When synapsed the telo was most frequently involved in ring-rod trivalents; the more complex type requiring three chiasmata for their formation, whereas chains require only two. The extra chromosome of trisomic 6 displayed a similar low frequency of pairing and likewise ring-rods were the most frequent trivalents formed. No explanation can be offered at present for the abnormal behavior of these two genotypes other than to suggest that

chromosome 6 carries factors that influence chromosome pairing.

The telo of 14 + 6S was only infrequently orientated on the equatorial plate. The majority of univalents were located at the periphery of the plate and in polar regions, presumeably beyond the influence of the meiotic spindle and in a position conducive to exclusion from telophase nuclei. A high frequency of laggards, observed at T11, became excluded from daughter nuclei and formed micronuclei in the quartets. If such meiotic behavior is characteristic of megasporogenesis then it becomes obvious why the transmission rate of 14 + 6S was only 9.9 percent while the average for other monotelotrisomics was 26.4 percent. Ovules with an unbalanced chromosome number may abort to further decrease the transmission frequency of the extra telo.

It was shown that unbalanced male gametes were excluded when in competition with balanced gametes in the case of 14 + 1S, while only one-half of the expected number of unbalanced male gametes of the ditelo 14 + 2 - 1S were involved in fertilization. This may be partially explained by the observation that chromosomes in daughter cells with a balanced chromosome number completed their A II division more rapidly than did their counterparts in the unbalanced daughter cell. A similar phenomenon was observed at A II of trisomics (Yu, 1968) and subsequently at pollen mitosis in which eight-chromosome pollen grains completed mitosis much later than normal microspores. Accordingly, it is believed that the unbalanced gametes would be immature at the time of anthesis and would fail to compete with mature, balanced gametes in germination and pollen tube growth.

Synapsis of homologous arms of ditelo 14 + 2 - 1S is similar to

that observed in autotetraploids (Morrison and Rajhathy, 1960a). Presumeably the process of somatic or premeiotic association is responsible for positioning the four homologous arms in close proximity to each other at the nuclear membrane to which they subsequently become attached through the medium of the centromere and or telomeres (Feldman, 1966; Sved, 1966). The latter concept is somewhat speculative, since nuclear membrane association of barley chromosomes has never been reported. Such an attachment has been reported for some plant species (Pusa, 1965) and other organisms (Moens, 1969). Kumar (1966) by irradiating dry barley seeds, was able to induce a high frequency of dicentrics and lower frequencies of ring configurations among the chromosomes. Dicentrics were formed by breakage and rejoining of chromosomes in closely-associated centromere regions, with rings resulting from similar processes at chromosome ends. Prerequisites for this these phenomena are polarity of homologous centromeres with free-floating chromosome ends, implying an association of homologous centromeres. Conversely, Kasha and Burnham (1965) report that synapsis of homologous chromosomes is initiated at or near the ends, implying as association in regions distal to the centromere. In this case a premeiotic association of some description is mandatory to permit pairing initiation at chromosome ends without the production of interlocking bivalents. Presumeably these latter two functions that of premeiotic association followed by pairing initiation at chromosome ends are responsible for the high degree of pairing observed in the ditelo.

Sears (1952) as a result of studies with wheat telos, indicated that a chromosome even a telocentric displayed a more normal meiotic behavior when in a paired condition. When a comparison was made of the

frequency of abnormalities at various meiotic stages in monotelotrisomics and ditelos (Table XXVI) it was obvious that the same concept applied to barley telocentrics.

TABLE XXVI

FREQUENCIES (%) OF ABNORMALITIES AT MEIOTIC STAGES IN MONOTELOTRISOMICS AND DITELO 14 + 2 - 1S

Frequency of cells with:	Ave. of 5 Monotelotrisomics	Ditelo
Univalents at diakinesis	29.0	1.6
t' orientated on equatorial plate	49.8	95.1
t' division at A1	12.8 (equational)	91.2 (reductional)
T1 laggards	15.7	6.9
T11 abnormalities	16.9	5.5
Micronuclei per quartet	17.0	6.6
Transmission frequency in selfed progenies	28.3	92.9

In every comparison made the monotelo showed a higher frequency of abnormalities than observed in the ditelo. The transmission frequency of the extra telo as univalent was comparatively lower than the transmission observed through the ditelo. The frequency of aneuploids in the selfed progeny of a ditelo at 92.9 percent appears relatively high, however it is a total of mono and ditelos observed at frequencies of 45.7 and 47.2 percent respectively. With perfect meiotic behavior all progeny should have been ditelos. These results indicate indeed, as

suggested by Stebbins (1951) that the increase in the basic chromosome number of a diploid with subsequent meiotic stability is a rare event.

Monotelotrisomics have certain advantages for linkage mapping studies that may not be especially manifest in barley because of the existing extensive linkage maps. They may be used to determine the location of a gene on a chromosome arm bearing no mapped genes, a situation which precludes the use of conventional three-point tests. In addition a monotelotrisomic test, if properly executed, can be used to determine gene-centromere distances on chromosome arms having no known markers.

In the present study a number of mutants were crossed to monotelotrisomics, bulk F_2 populations were grown and by virtue of trisomic ratios the mutants were assigned to specific chromosome arms. This was the simplest type of test, allowing F_1 plants to set selfed-seed from which large F_2 populations were grown. A more efficient technique though more laborious, involves backcrossing the F_1 plant so that chromatid crossing-over can be detected directly in the progeny by scoring the frequency of homozygous recessive monotelotrisomics. Appropriate formulas can be used on such data (Reeves, et al, 1968) to determine gene-centromere distances. This test-cross method will place physical limitation on the size of the progeny that can be produced, but smaller populations are adequate, since the precision of the test is greater than that of a bulk F_2.

An inversely proportional relationship was found between the frequency of homozygous recessives in an F_2 monotelotrisomic progeny and the distance between the locus and centromere thus implying a direct proportion between gene-centromere distance and recombination. On this

basis chromosome-1-associated genes were arranged along the short arm of the chromosome relative to two previously mapped genes. This procedure was carried out despite the statements made by Hagberg and Person (1963), who stated: "The distal localization of chiasmata in barley is not definitely proved but there are many indications that this is the case. This means that chromosomal material is inherited to some extent in seven big blocks where exchange seldom occurs." This statement implies that recombination, if and when it occurs, is confined to the distal regions of the chromosome. The observation reported here of interstitial chiasmata at late diplotene in bivalents possessing two terminal chiasmata is accepted as evidence opposed to the generalization that chiasmata are almost exclusively terminal in barley. In addition the observation of 14.7 and 6.8 percent homozygous recessives in F_2 progenies for genes Rs and br located proximal and distal from the centromere respectively, indicates that the proportionality between gene-centromere distance and recombination is operative in barley in agreement with well documented reports from other crop plants.

However, using telos in trisomic conditions as opposed to the use of mono or ditelos of wheat creates complexities that must be considered before actual gene-centromere distances can be determined accurately. The frequency of trivalent formation as well as the loss of univalents must be considered in the calculations. It is also required that aneuploids be distinct from euploids in segregating generations (Sears, 1966).

The mono or ditelo method will detect cross-overs between a critical gene and a centromere with a probability of 50 percent. In

trisomics or monotelotrisomics on the other hand (taking a hypothetical example) where 72 percent trivalent formation is observed and 50 percent of univalents are lost the probability of a gamete being the critical a a type following crossing-over between the "a" locus and centromere of an AAa individual drops to two percent in monotelotrisomic and five percent in the diploid portion. In order that probabilities of the trisomic method approach those of the mono or ditelo method, following a cross-over, both cross-over chromosomes must pass to one pole at A I and the two chromatids carrying recessive alleles must also preferentially segregate to the same pole at A II.

That telocentric chromosomes may be unstable and become eliminated in somatic tissue has been the subject of a number of reports (Rhoades, 1938, 1940; Sears, 1952; Steinitz-Sears, 1966; Gupta, 1968; and Khush, et al, 1968). Somatic elimination of the extra telocentric has been ob-observed in 14 + 1L individuals through chimera formation (Tsuchiya, 1968). These monotelotrisomics are characteristically Bush in phenotype, i.e. dwarfed compared to diploid sibs, so that diploid tillers can be detected by their taller growth. Chimeras of this type have been observed for the telo IX of wheat (Sears, 1952). There is only a limited amount of evidence for somatic elimination in the remaining monotelotrisomics. Because of morphological similarity between diploid and aneuploid tillers in 14 + 1S and 14 + 5S chimeras could not be detected. In somatic root-tip cells of 14 + 5S, normal diploid cells were observed and spikes from this aneuploid and 14 + 1S were harvested in which no monotelotrisomic seeds were found when 30 percent were expected. Occasionally seeds known to be monotelotrisomic produced entire plants bearing

only diploid seeds. The most common reason offered for this elimination is an incomplete centromere. Telocentrics owe their existence to the fact that partial centromeres are able to function; however, the function may be less than perfect. In barley the unsuitability of the pachytene stage makes it difficult to analyze centromere structures and establish this as the primary reason for somatic elimination of telos.

It has been reported (Endrizzi and Kohel, 1966) that intrachromosomal compensation for recombination has been detected in at least one heteromorphic bivalent of cotton. The absence of one arm was shown to increase the amount of recombination in the existing arm. Does the absence of the long arm in heteromorphic trivalents of monotelotrisomic hybrids increase recombination in the short arm, thus decreasing the frequency of homozygous recessive segregates in the F_2 compared to the situation in trisomics where three whole chromosomes constitute the trivalent? There were 10.8 percent homozygous recessives in the monotelotrisomic F_2 progeny from 14 + 5S x SV103 and 12.0 percent in the trisomic progeny (Table XXV). The lower frequency of recessives in the former is indicative of a higher incidence of recombination in accordance with the previous model and may be taken as evidence for compensation except that a Chi-square value of .033 indicated that the two values were not significantly different. However with existing monotelotrisomics as tools, the concept may be more adequately tested using several markers located at varying distances from the centromere.

The monotelotrisomics identified in the present investigation and others of the same type that should be isolated in the future have several applications. They offer a higher degree of precision than

trisomics in genetic studies and investigations of gene dosage effects on biochemical traits. With existing monotelotrisomics the centromere positions on chromosomes 2, 4, 5 and 6 may now be mapped by techniques previously described. Pollen transmission of the extra telocentrics was observed for the first time in barley and will provide greater versatility for cytogenetic studies than has been available previously.

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