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THE ISOLATION AND CYTOGENETICS OF A MONOTELOTRISOMIC SERIES AND ACCESSORY CHROMOSOMES IN BARLEY (HORDEUM VULGARE L.)

by

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To

My Parents

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ABSTRACT

Trisomic lines for each of chromosomes 1, 3, 4, 5, 6 and 7 of barley (Hordeum vulgare, L.) were subjected to mutagens viz., EMS, DES, HA, FUdR and γ-rays with the objective of inducing telocentrics in the extra chromosome. Lethality of DES and EMS on trisomic seeds and sterility caused by HA and γ-rays in meiosis treatments account for failure to recover any telocentrics from these treatments. In contrast, low doses of γ-rays, alone or in combination with FUdR, significantly increased frequencies of telocentrics over the control. All chromosome breaks induced in chromosome 7 were localized at the centromere and the adjacent region of the long arm.

A total of 17 telotrisomics studied represented 11 distinct chromosome arms. Analysis of karyotype, and crosses to translocations and genetic markers, showed these telotrisomics to constitute a series which lacks only short arms of chromosomes 2 and 4 and the long arm of chromosome 7.

The karyotype analysis indicated an arm ratio higher than the standard for chromosome 1 but a lower arm ratio for chromosome 3. Assignment of genetic markers to specific arms of chromosomes 3 and 5 contradicted prior assumptions based on karyotype analysis, indicating that the existing genetic maps of these chromosomes are reversed.

Telotrisomics for long arms resembled their related trisomics whereas those for short arms were virtually indistinguishable from normal diploids. The extra telocentric associated with its homologues in a heteromorphic trivalent in 82% and 77.6% of PMC's at diakinesis and M_I, respectively. Univalent telocentrics divided precociously at A_I, lagged at T_I and T_{II} resulting in a frequency of 26.7% quartets with micronuclei. Comparisons between satellited and non-satellited telocentrics of chromosome 6 suggested that the erratic behavior of the satellited telocentric was due to the presence of the nucleolus and its interference with chiasma formation. Transmission of telocentrics in selfed progenies averaged 31.1% with no obvious transmission through pollen.

Correlations of arm length in relation to chromosome association and transmission were positive and highly significant. Correlation between the frequency of univalents at M_I and quartets with micronuclei (meiotic index) was also positive and highly significant, suggesting that the meiotic index may be used as a convenient measure for chromosome stability. A telocentric shift was detected in the progeny of telotrisomic 2n+2L.

Accessory chromosomes in barley occurred spontaneously among the progeny of trisomics. They are smaller than normal telocentrics with a globular appearance. Their number per cell varied in both somatic and germinal cells. At meiosis, they were positioned at the equatorial plate, divided precociously at A_I and T_I, and lagged and formed micronuclei at the quartet stage. It is assumed that they arose through misdivision from normal chromosomes resulting in loss of their pairing arms.

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GENERAL INTRODUCTION

Chromosome engineering is a very fruitful technique for plant improvement and is extensively used in breeding programs. Interspecific and intergeneric hybridization, induced ploidy and aneuploidy are good examples of that use.

Although a great deal of genetic and cytological work has been conducted with cultivated barley (Hordeum vulgare L. Edmend. Lam.), its diploid nature had limited chromosome engineering in most cases to translocations. Tsuchiya (1960) established the first complete trisomic series in barley. Therefore, it was used to assign the seven linkage groups to the individual chromosomes. However, trisomic analysis, has not been capable of establishing the relationship between the genetic and cytological map with regard to the centromere position. It seems that telocentrics are the only suitable method to achieve such a purpose. A telocentric chromosome is a single chromosome arm with a terminal centromere. It may replace a normal homologue in polyploids (monotelosomics) or may exist in addition to the normal disomic complement (monotelotrisomics). Since deficiencies or deletions even for small segments are generally lethal in diploids, only telotrisomics are tolerated.

Telotrisomics are very useful in the study of genetic activity of each chromosome arm as well as for linkage analysis and for locating the centromere. Since telotrisomics carry only an extra chromosome arm, they are more vigorous and fertile than their related trisomics (Fedak, 1969).

To date, less than half of the fourteen possible telocentrics have been studied in barley. The aim of this investigation was to establish a telotrisomic series in barley and to this end, trisomics were subjected to different mutagens in order to induce telocentrics in the extra chromosome. Using karyotype analysis the isolated telocentrics then were classified into acrocentrics, true telocentrics and accessory chomosomes. True telotrisomics were identified by means of crossing each to appropriate translocation and genetic marker stocks and studying the meiotic behavior of the F_1-progeny. Each identified telotrisomic was studied in detail morphologically and cytologically, in order to establish the relationship between arm length and chromosome behavior, as well as chromosome behavior and stability. In addition, accessory chromosomes were identified and studied cytologically in somatic and sporogenous cells.

The present thesis is divided into the following sections:

Section I: Induction of telocentrics.

Section II: Cytogenetics of telotrisomics.

Section III: Cytogenetics of accessory chromosomes.

SECTION - I

INDUCTION OF TELOCENTRICS

INTRODUCTION

Although trisomics have been utilized extensively in genetic studies of plants, in some species (e.g. Hordeum vulgare L.) certain difficulties have been encountered in their use. Among these characteristics are poor growth of trisomics relative to normal diploids and low fertility and infrequent transmission of the extra chromosome. For these reasons attention has been drawn to the use of telotrisomics which in comparison with trisomics are more vigorous and fertile.

Telotrisomics involve only an additional chromosome arm rather than a whole chromosome and thereby provide an opportunity to map the position of the centromere relative to known genes located on each arm. To-date the position of the centromere on most of the seven linkage groups of barley has not been clearly demonstrated.

Although telotrisomics in barley occur spontaneously, they do so at a frequency too low for practical purposes (Tsuchiya, 1971; Yu, 1968). The observations of some workers (Ramage et al., 1961; Hagberg et al., 1963) that certain induced translocations involved breaks within centromeres suggest that mutagenic treatments may be an effective method to produce telocentrics.

In the following section, the results of four experiments are reported, each designed to measure the effects of various mutagenic agents on the induction of telotrisomics of barley (H. vulgare L.).

REVIEW OF LITERATURE

Telocentric Chromosomes

Misdivision. Darlington (1939) considered that centromers of different chromosomes were all alike in their form and behavior. Lima De Faria (1958), could distinguish four chromomeres in pachytene chromosomes of maize, disposed in a longitudinal line within the centromere region. He suggested that the centromere structure is a tandem reverse.

Darlington (1939) and Sears (1952) outlined detailed studies of univalent misdivision in Fritillaria and wheat, respectively. Their observations indicated that univalents may divide transversely (misdivide) at the centromere either during A_I or their division products at A_{II} to give rise to chromosomes with terminal centromeres (telocentrics). Moreover, a single centromere could misdivide into four functional parts. Brown (1958) believed that misdivision of the centromere depends more upon a change in timing of normal centromere behavior than upon centromere structure. Marks (1957) considered four points of break; three of them within the centromere, giving rise to true telocentrics with a centromere either complete or deficient, while the fourth occurs in the adjacent arm resulting in an acrocentric. However, from the genetic viewpoint, recombination within a very short arm would be seriously affected due to the restriction in the frequency of crossing over.

Occurrence. The first observation of a telocentric was made by Huskins (1934) in wheat. He described a heteromorphic bivalent caused

by the loss of approximately one half of a chromosome. Among the progeny of trisomic-5 in maize, Rhoades (1936) isolated a plant with an extra fragment chromosome and identified it as the short arm of chromosome-5. Moreover, in Datura, many plants with one or two extra telocentrics were found in the progeny of trisomics (Blakeslee and Avery, 1938). Smith (1947) found a compensating telotrisomic in the progeny of an irradiated spike of T. monococcum. He assumed that a break occurred within the centromere and one of the resulting arms subsequently formed an isochromosome.

While telocentrics are common in the progeny of wheat aneuploids, they are very seldom found in the progeny of barley trisomics. Tsuchiya (1960) and Yu (1968) observed that about 24% of microsporocytes (PMC's) with an extra chromosome contained a univalent. In 50% of these cells, the univalent was positioned at the equatorial plate and occassionally it misdivided. Therefore, the actual frequency of telocentrics in the progeny of these trisomics was very low (0.17% Tsuchiya, 1971). For this reason it was necessary to explore some artificial methods such as using mutagens to induce telocentrics in barley.

Mutagensis has been one of the most extensively investigated fields and as a result, literature pertaining to this field of research is voluminous. However, several excellent reviews are available including those of Praaken (1959), Gaul (1964), Wallace (1964), and Auerbach (1967) who presented a detailed survey of the physical properties, biological manifestations, methodology and some of the results obtained with mutagenic

treatment of plant species. In addition Nilan and his co-workers (1963, 1964, 1968) discussed the factors which modify mutagenic effects. The following review deals with only certain specific aspects of induced chromosomal aberrations as they pertain to the present study.

Mutagens

Two main groups of mutagens are recognized, physical radiation and chemicals. Although both groups induce similar effects, their mode of action is quite different.

Radiation. The penetration of radiation into material is accompanied by an energy transfer known as excitation. In addition, ionizing radiation is capable of producing ion pairs when they interact with matter. This ionization, as it is called, as well as excitation may cause a direct change in nucleic acids resulting in mutations.

Ultraviolet light, the only form of non-ionizing radiation, does not have the ability to ionize but transfers its energy by excitation. Due to its low penetration ability, its use in plant mutation work is generally limited to pollen irradiation (Fabergé, 1957; Stienitz-Sears and Sears, 1957). The mutagenic influence of ultraviolet rays is highest at wave lengths that show the highest absorption by nucleic acids (Praaken, 1959). Ultraviolet radiation increases point mutations relative to chromosome breaks, and when they occur they tend to rejoin less easily and therefore are likely to produce deficiencies which are of limited values.

In contrast, the deep penetration of ionizing radiations (x-rays, γ-rays and neutrons) causes chromosome breakage. However, their effi-

ciency is dependent on the energy dissipation and/or ion density along the track of the ionizing particles that are ejected in the tissue. Since x-rays and γ-rays dissipate their energy in biological material by the production of the smaller electrons, fast neutrons by ejection of the larger protons, and thermal neutrons to a great extent by emission of the even larger alfa particles, a successively increased ability to break chromosomes is therefore expected from the use of these three mutagens. This was confirmed by Leroy (1968) who found that neutrons had a greater mutagenic efficiency than γ-rays for the same survival of M_1-plants. In contrast to non-ionizing rays, all breaks induced by ionizing radiations have generally a relatively high rejoining ability which favor chromosome rearrangements such as translocations, inversions, and duplications.

Chemicals. A large variety of chemical mutagens are known. Alkylating agents, the most potent chemical mutagens, produce in addition to point mutations all types of chromosome aberrations. Because their genetical effects are very similar to those of ionizing radiation, the term "radiomimetic agents" is often applied to them. The alkylating agents exert their biological effects by alkylation of nucleophilic sites in DNA, and more specifically, they attack the N-7 atom of guanine which is thought as the most important biological action of these agents (Caspersson et al. 1967).

Another important class of chemical mutagens are the base analogs or inhibitors of DNA synthesis. They induce mainly chromosmoal aberrations

consisting of gaps and open breaks which are due to interruptions in DNA replication in cells completing the synthesis phase. Torsions and tensions produced by chromosome coiling at prophase as well as anaphase movement would result in fragmentation of these chromosomes. The fact that chromosome rearrangements are rare or entirely absent among aberrations induced by some base analogs as 5-fluorodeoxyuridine (FUdR) indicate that rejoining of chromosomal breaks is inhibited. Taylor et al. (1962) reported that FUdR binds irreversibly to the enzyme thymidlate synthetase and inhibits thymidlic acid synthesis which is needed for DNA replication. Thus, the mutagen is not only capable of inducing chromosome breaks, but it should also inhibit the rejoining of breaks induced by irradiation. This was accomplished by using concentrations lower than those needed for the production of chromosomal breaks. Irradiation in the presence of FUdR greatly increased the frequency of free fragments accompanied by a decrease in the frequency of chromosomal bridges (Kihlman, 1962; Taylor et al. 1962; Moutschen-Dahmen et al. 1966).

Chromosome breaking agents exert their effects during different stages of the cell cycle. Most radiomimetic agents have only a delayed effect, i.e. chromosome aberrations induced in early interphase cells (Kihlman, 1963). Ionizing radiation induce aberrations at all stages of the cell cycle, i.e. non-delayed and delayed effects. Base analogs have a non-delayed effect resulting in the immediate appearance of chromosomal gaps and open breaks in addition to a delayed effect.

Although chemicals have similar effects to those induced by ionizing radiations, three major differences between these two mutagens are known to exist. These are:

(1) Treatment with chemical results in a deficiency of chromosome rearrangements relative to gene mutations. This deficiency is not due to a shortage in chromosome breaks but to the inhibition of the process of rejoining (annealing) (Auerbach, 1967).

Froese-Gertzen et al. (1964) found that x-rays caused similar decreases in seedlings growth and spike fertility whereas EMS affected fertility more than seedlings growth. The cytological analysis of the treated material showed that reduction in fertility was directly related to the frequency of chromosome aberrations after x-rays treatment. In contrast, the pronounced decrease in fertility after EMS treatment was accompanied by a very low frequency of chromosome abnormalities. These results confirm those of Sato and Gaul (1967) who pointed out that chromosome abnormalities were not considered to be high enough to account for the extreme sterility induced in barley by EMS. They concluded that small deficiencies may be the main cause of sterility in EMS-treated material.

In an attempt to study the nature of sterility exhibited by irradiated M_1 plants, Ekberg (1969) analyzed 95 lines of barley in which partial sterility had been induced by various mutagenic agents. She found that in radiation treated material translocations and inversions were the most predominate form of chromosomal aberrations (82%), while these types

were in minority (27%) after EMS treatment. The majority in the latter case were lethals which cause abortion of seeds and/or gametes in heterozygous plants.

(2) A tendency exists for chemical-induced injuries to genetic material to remain latent over a period that may extend over many cell cycles, i.e. delayed effect. Since the formation of a chromosome rearrangement requires the simultaneous break of two chromosomes within the same cell, a potential rearrangement is lost if the two breaks open at different stages of the cell cycle. This could be due to a limited injury to DNA after the completion of its synthesis, resulting in chromatid type aberrations.

(3) The occurrence of a nonrandom distribution of chromosome breakages after treatment with chemical mutagens. In contrast to radiation, chromosome breaks induced by chemical mutagens are, in most cases, localized at certain regions known to be heterochromatic (Kihlman, 1963; Caspersson et al. 1969; Natarajan et al. 1969). Treatments of Vicia faba roots with 8-ethoxycaffiene and maleic hydrozide induced chromosome aberrations localized to the secondary constriction and the centromere region of metacentric M-chromosome, respectively (Kihlman, 1963). Caspersson et al. (1967) reported that chromosome breaks induced by quinacrine mustard occurred predominantly in the same well defined regions known in Vicia to be heterochromatic. In barley, Singh et al. (1970) found that about 50% of chromosomal breaks induced by L.S.D. were confined to the centromere region. The probable limitation of chromosome

aberrations to heterochromatin indicate that though the effect of chemicals may be due to alkylation of DNA, not all chromosomal DNA is susceptible (Grant and Heslot, 1966).

It should be kept in mind that localization of chromosome breaks at certain loci is a specific feature for any species treated with certain mutagen. Thus, generalization in only the broadest terms can be made.

MATERIAL AND METHODS

Two trisomic series of barley (H. vulgare L. 2n=15), one a 6-rowed type (OAC-21 x Montcalm) initially produced by Larter (personal communication) and the other a 2-rowed type cv. Betzes, Eslick and Ramage (1969) were used in the present study. Either dry seeds or trisomic seedlings and plants of each line were subjected to various mutagenic agents with the objective of producing individual telocentrics for the extra chromosome. Four experiments were carried out, each of which is discussed individually in the section to follow.

Experiment A

In this experiment, dry seeds of two trisomic stocks (Trisomics 4 and 5) were treated independently with two chemical mutagens, diethyl sulfate (DES) and ethylmethanesulfonate (EMS). Prior to treatment, seeds were presoaked in distilled water for 2 hours and subsequently transferred to freshly prepared 0.2M solutions of the mutagen. Treatment times were 2 and 24 hours at room temperature for DES and EMS respectively. Following treatment, seeds were washed thoroughly in tap water, chilled for one week ($+2^{\circ}C$) and then allowed to germinate on moist blotters. Trisomic plants were identified on the basis of root-tip analyses and were grown to maturity.

Experiments B and C

These experiments were designed to test the effect of hydroxylamine (HA) and γ-rays on meiosis. In experiment B, aqueous solutions of HA

were injected into the upper culm of individual tillers of trisomics 3 and 7 at the premeiotic stage (Sinha, 1967). The treatment was timed so that meiotic cells would be in contact with the solution during their actively dividing state. A concentration of 10 ug/ml of freshly prepared HA solution was used to which 5 ml/100 ml of solution of 0.01% "Tween 20" was added as a wetting agent. For control purposes, a few tillers were injected with distilled water containing "Tween 20" only. The treated heads and the control were tagged, and to prevent outcrossing each spike was bagged at the time of heading. In experiment C, five plants (cv. Betzes) trisomic for each of chromosomes 4, 5 and 6 were irradiated using a Co^{60} source. Two disomic plants served as control, one being cv. Betzes, the other OAC-21. Plants were treated when it was estimated that most of their tillers were initiating meiosis. A total of approximately 10,000 rads were given at a dose rate of 108 rads/hour. Following irradiation, both trisomic and control plants were allowed to reach maturity in the greenhouse.

Experiment D

In this experiment, the effect of low dosages of γ -rays alone and in combination with 5-fluorodeoxyuridine (FUdR) were applied to seedlings trisomic for chromosomes 1, 4 and 7, respectively. For each trisomic, seedlings were divided into three lots of 10 plants each and subjected to γ -irradiation from a Co^{60}-source adjusted to deliver 25 rads/second. The following treatments were applied:

Lot (1) 150 rads (6 sec.)

Lot (2) 500 rads (20 sec.)

Lot (3) 150 rads to seedlings that were pre-soaked in a 10^{-7}M aqueous solution of FUdR for three hours, then washed in tap water for one hour immediately prior to irradiation. Following treatment, the seedlings were planted.

All plant material, both treated and control was grown under a controlled temperature of 18 ± 2^{o}C with a 16 hour photoperiod. On the assumption that induced telotrisomics would exhibit higher fertility than their parental trisomics, those spikes of treated plants which exceeded the trisomic in seed-set were threshed individually, the remaining spikes were bulk harvested. Ten seedlings from each individual spike, as well as a random sample from the bulk, were cytologically analyzed on the basis of root-tip counts. If trisomic segregation occurred in individual samples, the data were pooled for statistical analysis. For Chi-square analyses of data on chromosome frequencies, the transmission rates of control for each chromosome were used to calculate the expected frequencies. Because of the low frequencies of telotrisomics in the control material, an average rate established over all trisomic lines was used to calculate the expected values.

RESULTS AND DISCUSSION

Experiment A

A comparison between the effect of the two chemical mutagens DES and EMS on dry seeds of trisomics 4 and 5, showed that DES caused 52 and 65% lethality respectively compared to 34 and 30% induced by EMS (Table I-1). This lethality may be due in part to the post-treatment storage period. Mikaelsen et al. (1968) found that storing barley seeds treated with DES and EMS increased the frequencies of fragments per cell and after 15 days of storage, the treated seeds failed to germinate. It seemed that trisomic seeds were more sensitive to both mutagens than disomics as revealed by a significant reduction in the frequencies of trisomics in treated populations ($P < .005$ for each trisomic). This could be expected since trisomic seeds are smaller than disomic ones (Tsuchiya, 1960) and small seeds show more physiological damage from mutagenic treatment than do large seeds (Heiner, 1963).

DES treatment resulted in a marked reduction in seed-set (Table I-1) accompanied by an increase in transmission of the extra chromosome (Table I-2). In contrast, EMS treatment did not result in the same degree of reduction in seed-set as did DES. However, it resulted in a slight decrease in transmission of trisomic-4 only. These results agree with the finding that DES tends to induce more acentric fragments (deficiencies) than does EMS (Mikaelsen et al., 1968), which could be compensated for by the presence of an extra chromosome, there-

Table I-1. Survival of Disomics and Trisomics Among M_1-Seedlings from Dormant Seeds Treated With EMS and DES.

Mutagen	No. of treated seeds	Trisomic 4					Trisomic 5				
		Survival %	Chr. number 14 %	Chr. number 15 %	X^2 (1)	Fertility %	Survival %	Chr. number 14 %	Chr. number 15 %	X^2	Fertility %
EMS	150	66.0	95.0	5.0	33.0**	41.2	70.0	83.0	17.0	5.8**	49.3
DES	100	48.0	96.0	4.0	18.0**	28.6	35.0	91.0	9.0	60.2**	35.9
Control (2)	120	95.3	66.0	34.0	-	61.4	93.1	72.0	28.0	-	63.5

(1) X^2 calculated for transmission of the extra chromosome.

(2) Soaked in distilled water.

Table I-2. Chromosome Segregation Among M_2-Seedlings from M_1-Irradiated Trisomics.

Mutagen	Trisomic 4					Trisomic 5				
	No. of Seedlings	Chr. Constitution 14 %	15 %	14+t (1) %	X^2 (2)	No. of Seedlings	Chr. Constitution 14 %	15 %	14+t %	X^2
EMS	180	78.9	21.1	-	9.6**	100	74.0	26.0	-	0.1
DES	124	56.5	43.5	-	6.3**	109	64.3	35.7	-	4.3*
Control (3)	136	67.3	32.2	-		111	71.0	28.1	0.9	

(1) 14+t: 2n + an extra fragment.

(2) X^2 calculated for transmission of the extra chromosome.

(3) Untreated trisomics.

fore favored higher frequencies of trisomics. Results from this experiment did not reveal any telocentrics.

Experiments B and C

Treatment of hydroxylamine and γ-rays at meiosis drastically decreased the size of population of the treated material so that conclusive results were not achieved. It was evident however, that injection of HA into the individual tillers (average of 3 tillers/plant) caused complete sterility of 15 plants of trisomic-3 and a reduced seed-set on 13 plants of trisomic-7 from which 67 seeds were recovered. Of these, 10 seeds (14.9%) germinated giving rise to disomics and trisomics only. Control plants (injected with distilled water and wetting agent) gave slightly lower seed-set than non-treated material (Table I-3).

On the other hand, irradiation with γ-rays during meiosis (Experiment C) severely damaged the plants and all tillers on trisomic plants were killed. Disomics, in contrast, matured and produced near-normal seed-set. Seeds harvested on 3 trisomic-6 plants (secondary growth) showed a parental chromosomal segregation with a decrease in the frequency of trisomics than untreated material (Table I-3).

It seems that some tillers of the control plants were more developed and probably had passed the stage of meiosis before treatment. Hence, radiosensitivity of disomics and trisomics could be attributed to differences in ontogenetic stages of plants at the time of treatment. In general, results from treatments at meiosis were inadequate and did not

Table I-3. The Effect of HA and γ-Rays on Trisomic and Disomic Plants Treated During Meiosis.

Treatment	Trisomic Line	No. of Treated Heads	No. of Seeds	Germination %	Chr. Segregation 14 %	15 %	14+t %	No. of Seedlings Examined
HA (injection)	3	43	0	-	-	-	-	-
	Control	14	79	85.1				
	7	35	67	14.9	80.0	20.0	-	10
	Control	11	187	82.3				
γ-rays (10 Kr)	4	-	-	-				-
	5	-	-	-				-
	6		92 (1)	90	87.8	12.2	-	81
	Control: 6 (2)	-	266	93.6	72.2	27.8		249
	OAC-21	4 (3)	192	94.0	-	-		-
	Betzes	3 (3)	84	86.0	-	-		-

(1) Secondary growth.

(2) Untreated material.

(3) One disomic plant each.

result in the development of telocentrics.

Experiment D

The effect of γ-rays alone or in combination with FUdR on trisomic seedlings of chromosomes 1, 4 and 7 was studied in this experiment. Irradiation at the early seedling stage did not disturb the growing seedlings and all 80 treated seedlings grew to maturity.

Trisomic-1. Plants with an extra chromosome-1 are readily identified by a bushy type appearance. The formation of non-bush tillers may indicate a loss of the extra long arm of chromosome-1. Two treatments (irradiation with 150 and 500r) were carried out on trisomic-1 of which 20 M_1 plants were classified morphologically at maturity into three appropriate classes: (a) one chimeric plant, (b) 4 non-bushy and (c) 15 bushy plants. A cytological analysis of these classes was conducted and the following observations were made:

(a) in the chimeric plant, all early tillers were mutant characterized by normal height and carried normal highly fertile heads. Root tips of M_2-mutant seedlings showed disomic counts, while those of late tillers (non-mutant) were trisomics.

(b) the non-bush class included four plants with near-normal vigor and short heads exhibiting a considerable degree of sterility (average 30%). M_2-seedlings of these plants involved disomics and trisomics but only a trisomic progeny from one plant was raised which segregated into 20% bush and 80% non-bush (parental) plants.

Meiosis in the last mentioned group showed it to carry an isochromo-

some in addition to the normal complement (secondary trisomics). The fact that all tillers of these plants were mutant may indicate that diplontic selection was effective so as to preferentially promote mutant tissue growth and/or the development of isomutants was enhanced since trisomics originate from small, less differentiated seeds (Tsuchiya, 1960; Jacobsen, 1966).

(c) due to the low fertility on the bushy plants, only a few heads were cytologically examined individually and most seeds were checked in bulk. One of these heads yielded in addition to disomics and trisomics, a compensating telotrisomic, i.e., a plant carrying 13 normal chromosomes, a telocentric, and an isochromosome. The compensating telotrisomic, though isolated among disomics and trisomics, seemed to have arisen from a small sector involving few florets or even most of the spike. Similar small mutant sectors have been observed in irradiated barley by other workers (Gelin, 1956; Eriksson, 1965). The bulk seeds of the first treatment (150 rads) yielded only disomics and trisomics (Table I-4) whereas the second treatment (500r) yielded 2 plants each carrying an extra fragment.

Trisomics 4 and 7. Unlike trisomic-1 plants, trisomics for chromosome 4 and 7 were not distinguishable from normals under favorable growing conditions. Subsequently, no selection for non-parental types was practiced. Fifty-seven heads were selected from both trisomics showing high fertility and all were later confirmed as trisomics except for two

Table I-4. Chromosome Constitution of M_2-Progenies from Plants Irradiated at Seedling Stage.

Treatment	Trisomic 1				Trisomic 4				Trisomic 7				Total of Treatments			
		Chr. Constitution				Chr. Constitution				Chr. Constitution						
	Total Seedling	14 %	15 %	14+f %	Total Seedlings	14 %	15 %	14+f %	Total Seedlings	14 %	15 %	14+f %	No. of Seedlings	14 %	15 %	14+f %
150 rads	68	69.1	30.9	-	183	65.1	34.9		118	62.8	34.7	2.5*	369	65.1	34.1	0.8
500 rads	120	66.7	30.8	2.5*	201	79.1	20.4**	0.5	90	72.3	26.6	1.1	411	74	24.8	1.2
150 rads + FUdR	-	-	-	-	207	63.3	37.2	0.5	174	63.9	35.0	1.1	381	51.2	48.0	0.8
Total of lines	188	28.6	30.8	1.6	591	68.9	30.8	0.3	382	66.5	33.0	1.5*	161	68.6	31.5	0.9
Control	105	72.4	26.7	0.9	295	67.1	32.9	-	298	69.3	30.1	0.6				

* Significant at .05 (X^2 calculated for frequency of 14+f).

** Significant at .005 (X^2 calculated for transmission).

heads of trisomic-4 and one head of trisomic-7 which had disomic complements. Nevertheless, from the bulk seeds of trisomic-4, a plant containing an acrocentric and another a true telocentric, were isolated from 500 rads and 150 rads + FUdR, respectively. Also, the six fragments isolated from different treatments of trisomic-7 (Table I-4) were either telo- or acrocentrics for the short arm of chromosome 7. This indicated that all breaks were localized at the centromere or in a region of about one-third of the long arm of chromosome 7.

As shown in Table I-4, the transmission rate of the three studied trisomics was not affected by treatment except for the second dosage (500 rads) applied to trisomic-4 which showed a significantly lower frequency of M_2-trisomics ($P < .005$) than the control. Moreover, the first and second treatments (150 and 500 rads) of trisomics 7 and 1, respectively, resulted in significantly higher ($P < .05$) frequencies of telocentrics than in the control. When the data were pooled and tested within trisomics and treatments, only trisomic-7 responded to treatment and resulted in higher frequencies of telocentrics ($P < .05$). The three treatments were not different from one another, and the concept that FUdR enhances chromosomal breaks was not supported (Kihlman, 1962; Taylor et al. 1962; Moutschen-Dahmen et al. 1966).

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SECTION - II

CYTOGENETICS OF TELOTRISOMICS

INTRODUCTION

Telotrisomics in barley were first isolated by Kerber (personal communication) in the progeny of Herta x Wong triploid hybrid, later by Tsuchiya (1960) in the progeny of an autotriploid plant of cultivar, S.E.16. Other telotrisomics were found among the progenies of 6-rowed OAC-21 x Montcalm trisomics (Yu, 1968). To date, out of the 14 possible telotrisomics, only 7 have been isolated from different trisomic series (Tsuchiya, 1971a, 1972b). Of these, 5 were identified as: (2n+1L), (2n+1S), (2n+2L), (2n+4S) and (2n+5L). The other two lines belong to chromosomes 2 and 3 but have not as yet been assigned to their specific arms (Tsuchiya, 1972b). The morphological and cytological aspects of these telotrisomics have been investigated. Moreover, most of these telotrisomics were used to determine the arm location of several genes (Fedak, 1969; Tsuchiya, 1972b).

In the present study, a total of 11 monotelotrisomics in addition to a compensating telotrisomic were isolated among the progeny of trisomics. Of these, three resulted from mutagen treatment while the remaining occurred spontaneously. By crossing each telotrisomic to translocation markers, the chromosome involved in the telocentric condition was identified. The specific arm existing as a telocentric was determined by using a karyotype analysis and crosses to genetic markers. The identified telotrisomics were studied morphologically and cytologically. In addition, transmission and fertility were investigated. The following section is devoted to a discussion of these aspects.

REVIEW OF LITERATURE

Terminology

Plants with an extra telocentric were termed telosomic trisomics (Burnham, 1962), monotelotrisomics (Kimber and Sears, 1968) and Triplo- followed by an abbreviation to designate the arm involved (Khush and Rick, 1968). Tsuchiya (1972b) recently, used the last terminology to designate barley telotrisomics. For example, telotrisomic for the long arm of chromosome 2 is designated Triplo-2L. In the present investigation however, the designation of Kimber and Sears (1968) will be used throughout.

Identification of telotrisomics

Morphological Identification. The presence of an extra chromosome arm within a diploid complement may manifest itself in a change in the plant's morphology. Such effects may be comparable to those produced by the presence of an extra whole chromosome in the related trisomic. In Nicotiana sylvestris, out of 22 monotelotrisomics studied, only two were similar to the corresponding primary trisomics. Another three were different from both diploids and trisomics, while the rest were more or less similar to diploids (Goodspeed and Avery, 1939). Tomato telotrisomics for the long arms were strikingly similar in gross morphology to the corresponding primary trisomics (Khush and Rick, 1968). Those for short arms were nondescript, being distinguishable from diploids only under optimum conditions. In barley, telotrisomics (2n+1L) and (2n+2L) were described as bush and slender, respectively, like their putative trisomic

parents (Tsuchiya, 1971a). However, telotrisomic (2n+4S) shows some similarity to its related trisomic and (2n+1S) is not distinguishable from its diploid sibs (Fedak, 1969; Tsuchiya, 1972b).

Cytological Identification. Tsuchiya (1961) crossed 2 known translocation testers carrying one chromosome in common to each primary trisomic of barley. In the critical cross, the extra chromosome will share homologies with the chromosomes involved in the quadrivalent and will form a heteromorphic pentavalent while the remaining chromosomes form 5 bivalents. In non-critical crosses the extra chromosome will synapse with its homologues to form a trivalent while the remainder of the genome will associate into one quadrivalent and 4 bivalents. Thus, the observation of pentavalents in PMC's indicates that the extra chromosome is homologous to one of the 2 known chromosomes involved in the interchange. Because of the abundance of translocation testers that involve all seven chromosomes of barley, this technique was succesfully used to identify different trisomics and telotrisomic lines (Tsuchiya, 1966, 1967; Yu, 1968; Fedak, 1969).

Genetic Identification. Since translocation testers are only capable of identifying the extra chromosome involved, genetic markers known for their location are used to assign the specific arm which is present as an extra telocentric (Khush and Rick, 1968; Fedak et al. 1971; Tsuchiya, 1971b). Thus, the telotrisomic is crossed as a female to a homozygous recessive genetic marker and the F_1-monotelotrisomic plants are either selfed or backcrossed to the genetic marker. An F_2-trisomic ratio or deviation

from a BC_1-F_1 1:1 ratio will indicate that the marker is located on the extra telocentric, otherwise a disomic ratio is observed.

Karyotype Analysis. The chromosome complement of barley is one in which all chromosomes are metacentrics and very similar in overall length. Genetic mapping has been used primarily in the chromosomal linkage studies with this species and as a result the relationship between genetic and cytological maps is not yet definitely established. The identification of telotrisomics using genetic markers is therefore controversial. Recently, Tsuchiya (1972a) using a karyotype analysis revealed that a telotrisomic assigned genetically as (2n+5S) by Fedak et al. (1971) actually involves the long arm of chromosome 5 (2n+5L). Subsequently, he reversed the present map of chromosome 5, i.e. those genes that appear on the long arm are in fact, located on the short one and vice-versa.

On the other hand, Bentzer et al. (1971) pointed out sources of technical errors in karyotype analysis. In their work, three pairs of chromosomes with different arm ratios were drawn and measured by each of the authors independently. Then, the same cells were photographed with different magnifications and the arm ratios were calculated in all cases. They concluded that:

(1) the degree of contraction of chromosomes should be uniform and maximal,

(2) at least 10 chromosome sets should be measured,

(3) in the course of an investigation, all measurements and/or drawings should be made by the same person.

Cytological Behavior of Telotrisomics

Although telotrisomics have been detected in many species, few studies have been conducted with them. Fedak (1969) reviewed the expected cytological behavior of telotrisomics during meiosis. The actual behavior of four barley telotrisomics was described by Fedak et al. (1971) who showed that the extra telocentrics were associated in heteromorphic trivalents in 61.1% of the cells at M_I; in the remaining cells they remained as univalents. The predominant trivalent types were ring-rods and tandem-chains. At M_I, the univalent telocentrics were positioned on the equatorial plate in 49.8% of cells, dividing precociously in 12.2%, and lagging in 15.7 and 16.0% at A_I and A_{II}, respectively. As a result, an average of 16.2% of the quartets contained one or more micronuclei and the frequency of stainable pollen averaged 84.0%.

The frequency of chromosome associations in six telotrisomics of tomato (Khush and Rick, 1968) were much lower than in barley telotrisomics, averaging only 44.7%. Their transmission rates through the female were relatively high (36.6%) as compared with 29.7% for barley.

Breeding Behavior

Telotrisomics normally produce n and n + telo gametes. In most cases, both kinds of gametes function on the female side whereas only n gametes function on the male side (Khush and Rick, 1968; Fedak, 1969; Tsuchiya, 1971c). The progeny resulting from selfing such telotrisomics normally consist of telotrisomics and disomics in proportions depending

upon the transmission frequencies of n and n + telo female gametes. However, ditelotetrasomics and the related trisomics are occasionally detected at low frequencies; the first resulting from male transmission while the second occurring when the 2 intact homologues undergo non-disjunction at A_I. On the average, about 29.7% of the selfed progeny of barley monotelotrisomics carry an extra telo while the remainder are diploids.

In Datura, Blakeslee and Avery (1938) found about 44.1% of progenies of selfed telotrisomics carried an extra telocentric. Moreover, ditelotetrasomics and the related primary trisomics occurred at frequencies of 7.7 and 1.0%, respectively. In the progeny of tomato telotrisomics, ditelotetrasomics and the related primary trisomics occurred at a very low frequency. However, the transmission rate of the extra telocentric ranged from 10.- 48.5% with an average of 36.6%.

In a ditelotetrasomic line of barley, the transmission rate of the extra telo through the pollen was much lower (44%) than the expected 97% (Fedak, 1969), indicating that unbalanced gametes (n=7 + telo) are at a selective disadvantage (certation effect). Tsuchiya (1971c) reported an average of 2.19% male transmission of the extra telocentric in four barley telotrisomics. Among 685 seedlings obtained from crossing disomic plants with pollen from telotrisomics, he found 15 telotrisomics, 2 trisomics, 1 haploid and 1 triploid plant, while the remaining were disomics.

Chromosome Mapping

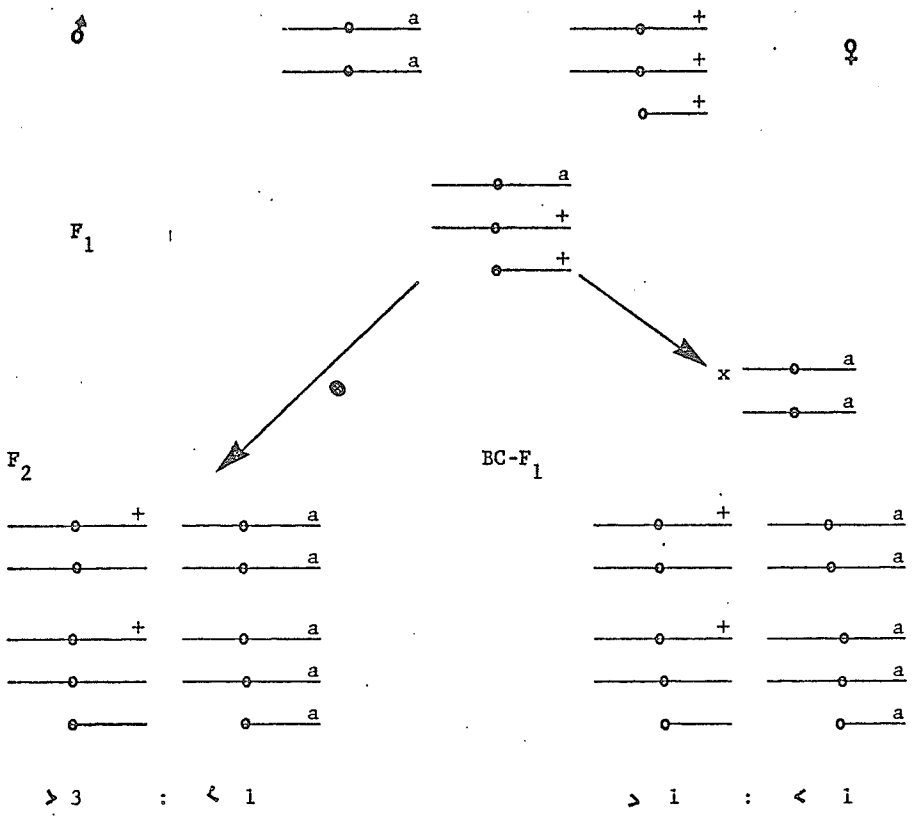
Telotrisomics have been successfully used for locating genes and mapping chromosomes (Rhoades, 1936; Moseman and Smith, 1954; Fedak, 1969; Tsuchiya, 1972b), the usual technique being as follows:

Telotrisomics carrying dominant alleles are crossed as female parents to homozygous recessive stocks and the F_1-telotrisomic plants are allowed to self-pollinate. The F_2-families involving the non-critical arm would segregate as normal disomics (3:1 ratio) while those for the critical arm will exhibit a trisomic ratio for the gene in question (Fig. II-1). Since the telocentric carries the normal allele, all telotrisomic progeny should have the normal phenotype except where crossing over took place. Thus, the proportion of recessive telotrisomics obtained reflects the recombinants and is subsequently used to determine the gene-centromere distance.

Rhoades (1936) used the same approach but instead of selfing the F_1-telo-plants he backcrossed them as female to the recessive stock. The BC progeny, of the disomic parents segregated 1:1 as expected while the trisomic parents deviated from such a ratio depending upon the frequency with which the telo was included in the female gamete.

As another approach (Rhoades, 1940), a telotrisomic carrying the dominant alleles "Bm and A_2" on the telocentric chromosome and recessive alleles on both homologues was used as a male and was crossed to a homo-

A. Critical cross.



B. Non-critical cross.

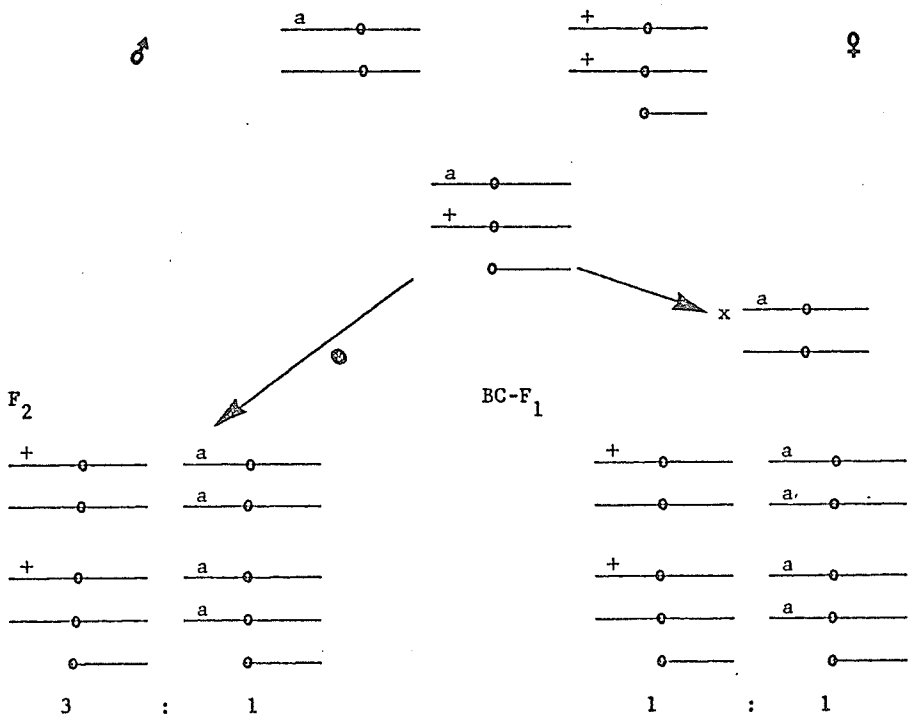


Fig. II-1. Chromosomes mapping by telotrisomics.

zygous recessive female, i.e.,

♀ bm a2 / bm a2 x bm a2 / bm a2 / + + ♂

Gametes: bm a2

1) bm a2
2) + +
3) + a2
4) bm +

bm a2 / bm a2	+ + / bm a2	+ a2 / bm a2	bm + / bm a2

% = bm-centromere % = bm-a2

Since the telocentric was not transmitted through the pollen, all progeny was diploid. Plants with dominant phenotype were the result of crossing over between the telocentric and normal homologues. Thus, the recombination values were calculated directly from frequencies of the progeny carrying the dominant phenotype. The gene with the lower recombination value "Bm" was therefore located closer to the centromere.

Moseman and Smith (1954) used a telocentric and a 3-point test to determine the arm location and linear order of four genes in T. monococcum. Recombination values were calculated from F_2 and F_3 data by either the product or maximum likelihood weighted methods.

Thresholds

Although telocentrics, have been used successfully in cytological and genetical studies, certain problems may accompany their use. For instance in somatic cells, telocentrics tend ocassionally to be lost or to give rise to isochromosomes. Nawashin (1916) expressed the opinion that all centromeres are interstitial and when telocentrics arise through misdivision, they are either converted to isochromosomes or are lost. Marks (1957) however, defended the concept that some species do have naturally occurring and persistent telocentric chromosomes. He suggested that telocentrics are rare because of genetic imbalance of the gametes carrying them brought about by chromosomal deficiencies or duplications. This was supported by Jones and Colden's (1968) observations on the complement of Tradescantia micrantha, in which all chromosomes are true telocentrics. They pointed out that this tetraploid species originated from a diploid bi-armed complement. In wheat, Steinitz-Sears (1966) found that about 75% of telocentrics isolated among the progeny of monosomic-3B were unstable and cannot be recovered at meiosis. She attributed telocentric instability to centromere completeness rather than position. Yet, cytological examinations of pachytene chromosomes in tomato indicated that certain unstable telocentrics revealed a centromere about half the size of the normal one, while stable telocentrics had a centromere of normal size (Khush and Rick, 1968).

Telocentrics may tend to reduce crossing over in the proximal regions (close to the centromere) resulting in shorter distances than estimated

by genetical methods. In cotton, Endrizzi and Kohl (1966) detected a decrease in crossing over at the centromere region of chromosome-6 between a telocentric and its normal homologue. Sears and Briggle (1969) suggested that reduced crossing over at the proximal regions of telocentrics may be accounted for by a distal shift of chiasmata rather than a reduction in their frequency. This phenomenon has the effect of increasing the estimated distances between proximal genes on account of those between distal ones.

Moreover, Revees et al. (1968) suggested that segregation among the diploid progeny of heterozygous telotrisomics could be modified if crossing over takes place between the telocentric and its normal homologue. They concluded that deviation from normal Mendelian ratios, though small, could be detected in populations of normal sizes if certain types of disjunction and failure of pairing of the telocentric occurred at a frequency high enough to be observed.

Sybenga (1965) reported that pairing of the short telocentric (satellited arm) of the satellited chromosome of rye was less efficient than that of the non-satellited one. He pointed out that the presence of the nucleolus in species that initiate pairing at distal ends such as in rye, prevents the formation of a chiasma at the satellite region. On the other hand, the formation of a chiasma between the centromere and the nucleolus will result in a breakdown of the trivalent since no chiasma was formed at the satellite.

MATERIAL AND METHODS

The telotrisomics used in this study were derived from the 7 trisomic lines of barley (H. vulgare, L., 2n=15) initially produced by Larter (personal communication). A total of 30 lines (Table II-5), each carrying an extra fragment, occurred either spontaneously or upon the mutagenic treatments described in Section I, and were classified cytologically on the basis of the fragment morphology into the following categories:

(a) 14 + an acrocentric

(b) 14 + a telocentric (monotelotrisomic)

(c) 14 + 1 or more tiny fragments designated as accessory chromosomes.

Only the last 2 groups were studied and the results are presented in this section and the next to follow.

Monotelotrisomics

The telotrisomic group comprised 17 different lines, each carrying a true telocentric chromosome. Of these, 2 belonged to chromosome 3 and were unstable; 4 were duplicates for telocentrics 1S, 6L, and 7S. In those cases in which more than one telo was obtained for a specific arm, only one of spontaneous occurrence was chosen for study.

Thus, a total of 11 lines each representing a different telocentric are reported in this section. All occurred spontaneously except telocentrics 1L and 4L which were obtained from irradiating trisomics 1 and 4, respectively. Moreover, a compensating telotrisomic (13 normal + an

Table II-5. Source of Telotrisomic (2n + a fragment) Stocks Used in the Present Study.

Chromosome Number	Parental Trisomic	Occurrence	Identity of the Fragment
14 + f	Trisomic 1	Spontaneous	2n + 1S
14 + f	Trisomic 1	♂-rays (500r)	Compensating telotrisomic
14 + f	Compensating telo	Spontaneous	2n + 1L
14 + f	Trisomic 1	♂-rays (500r)	2n + acrocentric
14 + f	Trisomic 1	♂-rays (500r)	A tiny fragment (lost)
14 + f	Trisomic 2	Spontaneous	2n + 2L
14 + f	Trisomic 2	Spontaneous	2n + acrocentric
14 + f	2n + 2L	Spontaneous	2n + acrocentric
14 + f	Trisomic 3	Spontaneous	2n + 3L
14 + f	Trisomic 3	Spontaneous	2n + 3S
14 + f	Trisomic 3	Spontaneous	2n + telo (lost)
14 + f	Trisomic 3	Spontaneous	2n + telo (lost)
14 + f	Trisomic 4	Spontaneous	2n + tiny fragment (Accessory)
14 + f	Trisomic 4	♂-rays (150r)	2n + acrocentric
14 + f	Trisomic 4	♂-rays (150r)	2n + 4L
14 + f	Trisomic 5	Spontaneous	2n + 5S
14 + f	Trisomic 5	Spontaneous	2n + 5L
14 + f	Trisomic 5	Spontaneous	2n + acrocentric
14 + f	Trisomic 6	Spontaneous	2n + 6S
14 + f	Trisomic 6	Spontaneous	2n + 6L
14 + f	Trisomic 6	Spontaneous	2n + telo (lost)
14 + f	Trisomic 6	Spontaneous	2n + tiny fragment (Accessory)
14 + f	Trisomic 7	Spontaneous	2n + 7S
14 + f	Trisomic 7	Spontaneous	2n + acrocentric
14 + f	Trisomic 7	Spontaneous	2n + tiny)
14 + f	Trisomic 7	Spontaneous	2n + tiny) Accessories
14 + f	Trisomic 7	Spontaneous	2n + tiny)
14 + f	Trisomic 7	♂-rays (150r)	2n + acro
14 + f	Trisomic 7	♂-rays (150r)	2n + telo
14 + f	Trisomic 7	♂-rays (500r)	2n + telo
14 + f	Trisomic 7	♂-rays (150r)	2n + acro
14 + f	Trisomic 7	♂-rays (150r)	2n + acro
14 + f	Trisomic 7	♂-rays (150r)	2n + acro

iso + a telocentric) which was obtained among the progeny of an irradiated trisomic 1 seedlings was also studied but the data are presented separately because of its unusual chromosomal constitution.

Telotrisomics were first identified on the basis of the trisomic parent from which each was isolated and in conjunction with the plant morphology of the parental plant in comparison with that of the derived telotrisomic. Each telotrisomic line was then crossed, as a female parent, to 2 homozygous translocations (Table II-6) having in common the translocated chromosome from which the tentatively identified telocentric was derived. Identification was considered positive when pentavalent associations in PMC's of F_1-telotrisomic plants were observed.

To assign the specific arm involved, each telotrisomic was crossed to known linkage markers chosen on the basis of their description and arm location (Table II-6). A Chi-square analysis of F_2-data was made to distinguish between disomic and trisomic ratios. In all cases, the goodness of fit to a trisomic F_2-ratio was the criterion used to identify the arm involved (Tsuchiya, 1967; Fedak, 1969).

Chromosome counts and karyotype analyses were based on root-tip squashes. Seeds were placed on moist blotters in germination boxes and stored at $+2^{o}C$ for 2 to 3 weeks to obtain uniform germination. Root-tips were pretreated at $+2^{o}C$ for 24 hours, fixed in Carnoy's 3:1 and stained with Feulgen. A karyotype analysis was conducted by measuring the relative length of a telocentric as a percent of the total length

Table II-6. Translocation Testers and Genetic Markers Used to Identify Telotrisomics.

Telotrisomic line	Translocation Testers	Genetic Markers (arm location)
1S	$T_1$4a, $T_1$6a	br(S), n(L)
1L	- -	- -
2L	$T_2$3a, $T_2$4a	or(S)
3L	$T_2$3a, $T_3$7b	al(L), an(L), uz(S)
3S	$T_2$3a, $T_3$7b	uz(S)
4L	$T_1$4a, $T_4$5a	-
5S	$T_3$5a, $T_4$5a	trd(S)
5L	Crossed to line 5S	trd(L)
6S	$T_1$6a, $T_2$6a	-
6L	$T_1$6a, $T_2$6a	-
7S	$T_3$7b, $T_5$7b	-

of the haploid complement. Ten cells were measured for each individual karyotype. Relative measurements of telocentrics were compared to the standard karyotype published by Burnham and Hagberg (1956).

Meiotic behavior of the identified telotrisomics was studied thoroughly in PMC's. Spikes were killed and fixed in Carnoy's 6:3:1 for 48 hours, then stored in 70% ethanol. Slide preparations were made using temporary acetocarmine smears. Pollen fertility was determined by staining mature pollen with lactophenol in cotton blue (Johansen, 1940) and scoring the percentage of stainable pollen. The eleven telocentrics were then ranked in descending order of lengths and these data were used for correlation analysis to study the relationship between arm length and chromosome behavior at meiosis. In addition, telocentric chromosome behavior at different stages of meiosis was also investigated.

All plants were grown under controlled temperature of $18 \pm 2^{o}C$ and a 16-hour photoperiod. Morphological data including plant height, number of tillers, heading date and fertility were scored for each line on 10 telotrisomic and disomic plants were grown together on beds in the greenhouse. To complement these data, a brief description of the most conspicuous characters of each telotrisomic was attempted.

RESULTS AND DISCUSSION

The results presented herein comprise a study of twelve telotrisomics. Three of these, a compensating telotrisomic, and telotrisomics 2n+1L and 2n+4L were isolated in the progeny of irradiated trisomics 1 and 4. The compensating telotrisomic was first studied and in its progeny a true telotrisomic 2n+1L was isolated. Hence, the results of eleven true telotrisomics including their identification as well as their morphological and meiotic behavior are presented in the section to follow.

A. The Compensating Telotrisomic

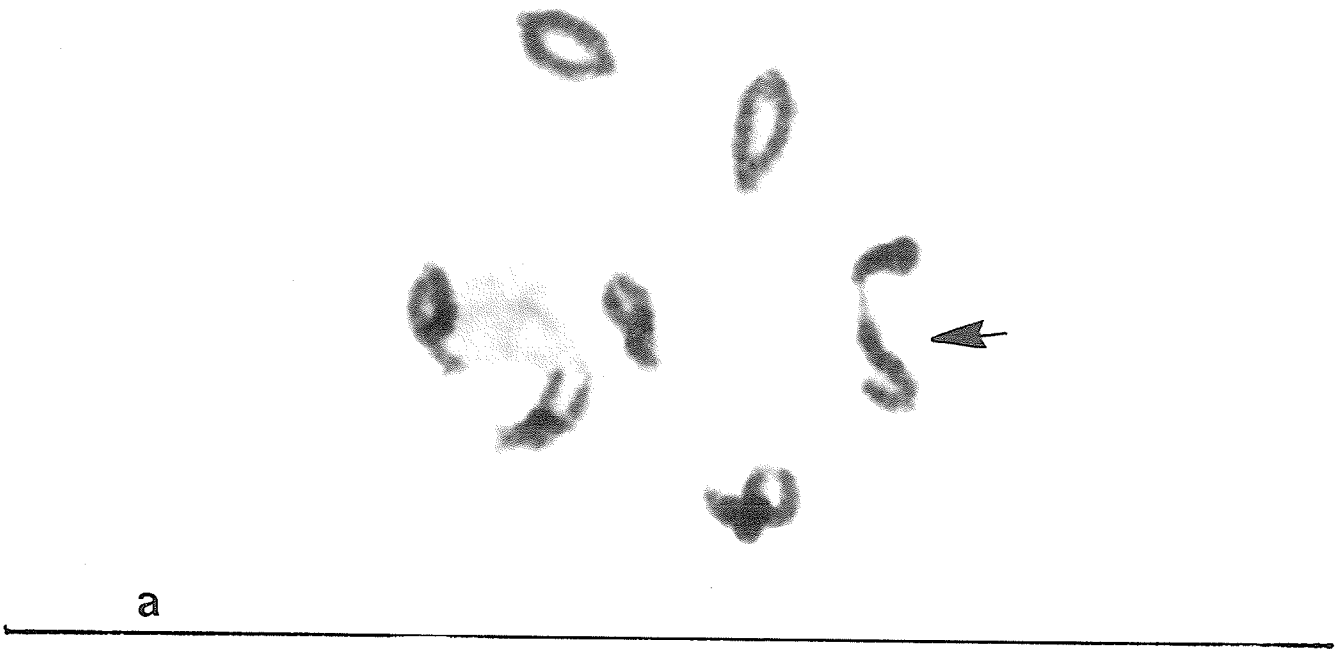
A telotrisomic line isolated in the progeny of an irradiated trisomic-1 plant was similar in stature to normal disomic plants. Spikes were slightly lax and highly fertile and occasionally some spikes showed multiple spikelets. In general, the plants were similar to true telotrisomic 2n+1S.

The meiotic behavior of telotrisomic plants showed 6 bivalents + a heteromorphic trivalent in most cells (Fig. II-2a), otherwise 7 bivalents (including a heteromorphic) in addition to an isochromosome occurred (Fig. II-2b). Although both the telocentric and isochromosome paired with another homologue, they did not pair with one another indicating that their arms were nonhomologous and that they compensated for a missing homologue. In other words, the extra chromosome arm was carried by the isochromosome and did not constitute the telocentric itself. The frequencies of cells of the compensating telotrisomic with various configurations

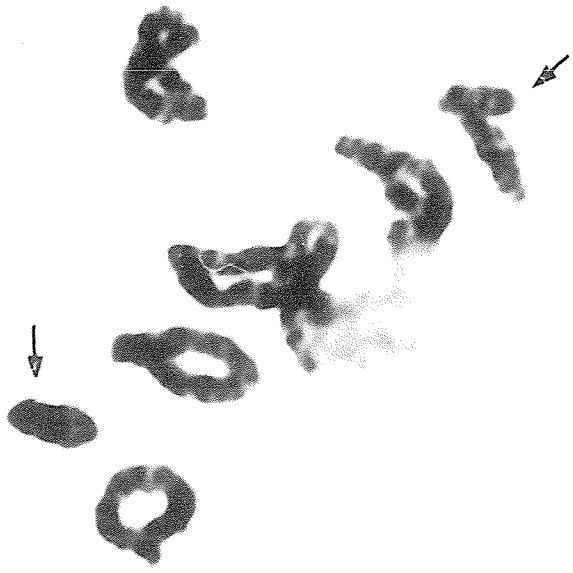
Figure II-2. Chromosome configurations at diakinesis in PMC's of a compensating telotrisomic.

(a) 6" + til"'

(b) 6" + tl" + i'



a



b

at diakinesis and M_I are shown in Table II-7. It was evident that the failure of the isochromosome to associate with its normal homologue was due to inter-arm pairing within the iso, which resulted in the formation of a ring univalent in 20.7% of cells observed at diakinesis. Moreover, when a heteromorphic trivalent occurred, the 2 intact chromosomes associated in distal regions only since proximal regions of the isochromosome paired with themselves. In contrast, the telocentric was seen as a univalent in approximately only 4% of the cells since no competition for pairing existed in its arm.

If random orientation of the trivalent at M_I occurred, a disjunction of 7'-7'+t' and 8'-6'+t' in a ratio of 2:1 should be observed. The actual percentage of cells undergoing 8'-6'+t' disjunction was 8.6% instead of the 33% expected on the basis of random disjunction (Table II-8). Presumably this discrepancy of the non-random orientation and distribution of the trisome occurred at M_I and A_I, respectively.

The frequency of quartets with micronuclei was similar to the frequency of cells with a lagging chromosome at A_I (Table II-8). This indicated that lagging chromosomes at A_I were subsequently excluded. Pollen fertility of the compensating telotrisomic was lower than that of normal disomics and telotrisomics 1S as might be expected since pollen carrying an isochromosome instead of a normal homologue is not viable.

The compensating telotrisomic gave rise to a variety of plants with different chromosome numbers. Meiosis was examined in all 39 aneuploid plants arising from this stock (Table II-9) and the following observations

Table II-7. Chromosome Configuration at Diakinesis and M_I of a Compensating Telotrisomic.

Stage	No. of Cells	Chromosome Configuration %		
		6" + til"'	7" + i'	7" + t'
Diakinesis	135	74.1	20.7	5.2
M_I	661	72.2	23.8	4.1

Table II-8. Chromosome Behavior at A_I, T_I and Tetrad Stage of a Compensating Telotricomic.

Stage	No. of Cells	Without Laggards %	With Laggards %
A_I	221	66.6*	33.4
T_I	528	84.8	15.2
Tetrad	209	66.0	34.0

* These are 8.6% 8'-6'+t' and 58.0% 7'-7'+t'.

Table II-9. Breeding Behavior of Compensating Telotrisomic.

Chromosome No.	No. of Plants	Meiotic Chromosome Constitution	% of Total Population	Notes
14	49	7"	55.7	Disomic
14 + t	2	7" + t'	2.3	True (2n+1L)
14 + t	33	6"+til"'	35.5	Compensating types
13 + 2t	1	6" + i' + t"	1.1	
14 + 2t	1	6" + i" + t"	1.1	
15	2	7" + i'	2.3	Secondary Trisomic
TOTAL	88			

were made:

(1) two plants carried an extra telocentric excluding the isochromosome (true telotrisomics) and were identified by their "bush type" characteristics as being telotrisomic 1L,

(2) two plants were isolated with an extra-isochromosome, i.e., secondary trisomics,

(3) the remaining 35 plants were compensating types, carrying one or more isochromosomes.

The isolation of a true monotelotrisomic 1L indicated that the extra arm in the compensating telotrisomic was the short arm of chromosome 1 while the telo was the long arm. Moreover, the excess of the compensating types in the progeny was expected on the basis of preferential distribution of trisome members at A_I (Table II-8). This distribution increased the frequency of (6'+t'+i') and (n) gametes at the expense of (6'+i') and (7'+t') gametes. Fedak (1969) and Tsuchiya (1972b) found that the addition of an extra short arm of chromosome-1 had no deleterious effects on the gametes carrying it, subsequently it was often transmitted through the pollen. This could be another reason for the excess of compensating types in the progeny reported above. On the other hand, the occurrence of telotrisomics 2n+1L and secondary trisomics (2n+i') in almost equal frequencies was a result of the rare occurrence of a type of disjunction of the trisome in which the normal homologue accompanied either the telo or the isochromosome to the pole.

The compensating telotrisomic described above, with the chromosomal complement of 13'+i'+t' was isolated in the progeny of an irradiated trisomic-1 plant. This genotype required fertilization of a (6'+i'+t') gamete with a normal one (n=7). In diploid barley, it is not possible that such a genotype could arise from 2 gametes, one carrying an iso and the other carrying the telo, or vice versa, because deficiencies could be involved on either side. Darlington (1939) and Sears (1952) observed that three arms of a misdividing univalent moved to one pole giving rise to either a normal chromosome + a telo, or an isochromosome + a telo. In trisomics of diploid species such as barley, the occurrence of a univalent was always accompanied by seven normal bivalents (Yu, 1968), thus the products of misdivision of a univalent are, in fact, additional components of a normal haploid complement of a gamete. This evidence disputes the possibility of spontaneous occurrence of the compensating telotrisomic. Thus it was assumed that irradiation resulted in the break of one of the three members of chromosome-1 within the centromere, the long arm then healed while the short arm formed an isochromosome.

B. The True Monotelotrisomics

The eleven monotelotrisomic lines listed in Table II-6 were identified on the basis of cytological and genetical methods. They were then studied for plant morphology and chromosome behavior at meiosis.

Identification of Telotrisomics

1. Cytological Identification

(a) Crosses to Translocation Testers: Chromosomes involved in the telotrisomic condition were positively identified by crossing each telotrisomic to two translocation testers (Table II-10) having one translocated chromosome in common. At meiosis, pentavalents were observed at diakinesis and M_I of F_1-hybrid combinations involving the nine telotrisomics listed in Table II-10 (Figs. II-3a, 3b). Thus, the telocentric was considered to belong to the translocated chromosome existing in common.

In case of telotrisomics of chromosome 5, the two lines were intercrossed using a ditelotetrasomic derived from line 5a used as a pollen parent. Meiosis of an F_1-plant carrying 14'+2t' (double telotrisomic) showed that both telos paired with another homologue at different arms (Fig. II-3c) indicating that they represent different arms of chromosome 5. This test was not repeated for telotrisomics of chromosomes 1, 3 and 6 because of a lack of ditelotetrasomics in this material.

(b) Karyotype Analysis: The use of karyotype analysis in barley has been limited due to the fact that 4 of the non-satellited chromosomes (i.e. chromosomes 1-4) are submedian with almost similar physical length. Tsuchiya (1960) and Yu (1968) reported that karyotype analysis of trisomic-4 did not give any conclusive evidence as to the shape of the extra chromosome. For these reasons, karyotype analyses using telocentric relative length, i.e. the length of an individual telocentric in per cent

Table II-10. M_I Configurations of F_1-Hybrids Between Telotrisomics and Translocation Testers.

Trisomic Parent	Translocation Tester								
	$T_1$4a	$T_1$6a	$T_2$6a	$T_2$3a	$T_2$4a	$T_3$7b	$T_3$5a	$T_4$5a	$T_5$7b
1a	V	V							
2b				V	V				
3a				V		V			
3d				V		V			
4b	V							V	
5a							V	V	
5b	Crossed to a ditelotrisomic of 5a.								
6a		V	V						
6b		V	V						
7a						V			V

Figure II-3. Cytological identification of telotrisomics.

(a) Diakinesis with $5'' + 1^{tv}$ (critical cross).

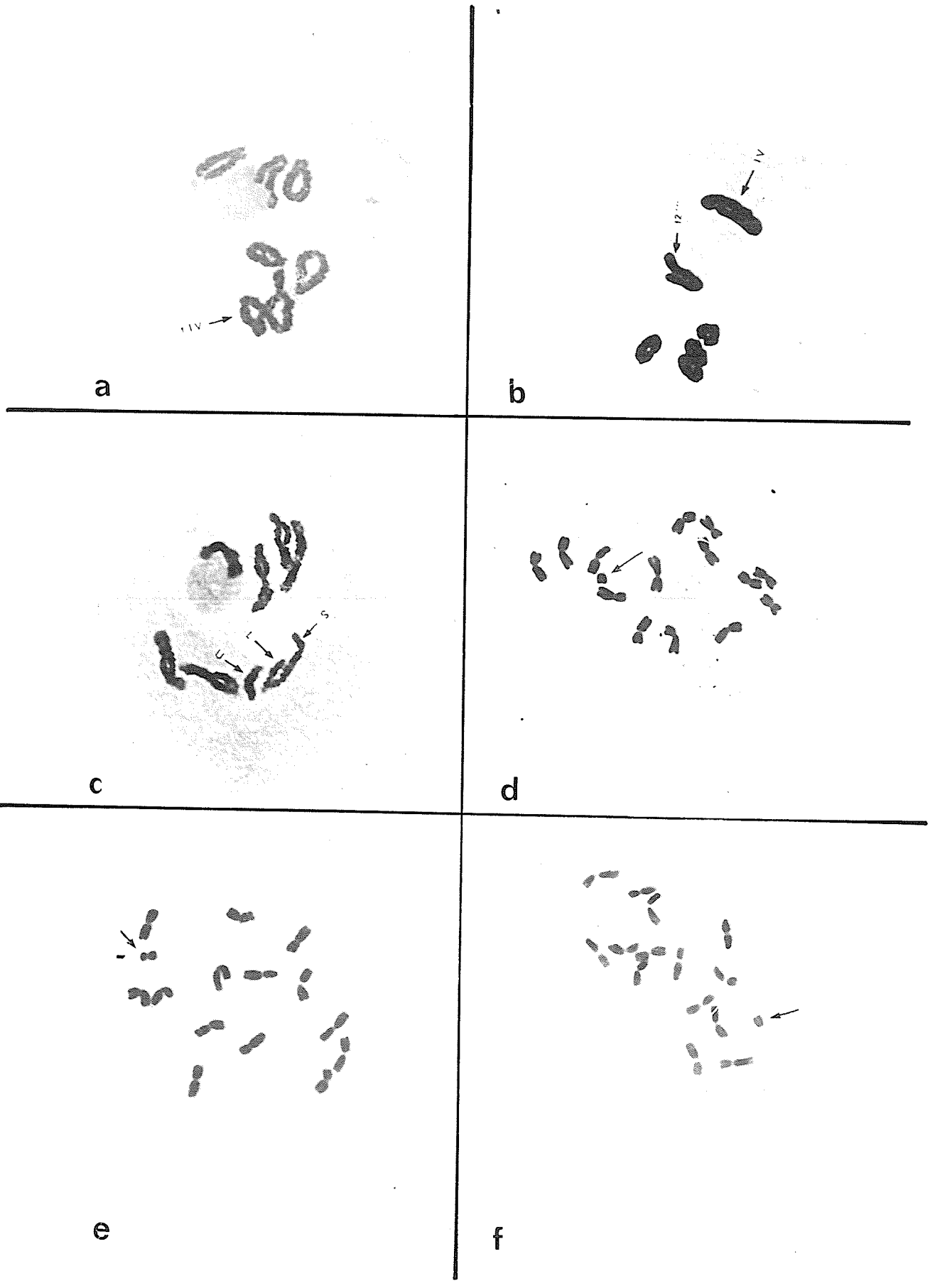
(b) M_I with $4'' + 1^{IV} + t2'''$ (non-critical cross).

(c) Diplotene with $6'' + 2t1''' + 1'$ in a double telotrisomc from a cross between 5a and 5b lines.

(d) Somatic chromosomes of 2n + 5S.

(e) Somatic chromosomes of 2n + 6S.

(f) Somatic chromosomes of 2n + 7S.


a
b
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e
f

of the haploid complement, were used in the present study to relate the length of a telocentric in terms of that of both the short and long arms of a chromosome. Photomicrographs of ten cells of each line were measured and the identity of each telocentric was determined with the aid of comparisons to the standard karyotype established by Burnham and Hagberg in 1956. It should be pointed out that comparisons between the observed relative lengths and those of the standard karyotype indicated that telocentrics 1L, 4L, 5S, 5L, 6S, 6L and 7S were very similar to those of the standard (Table II-11). In contrast, telocentrics 1S, 2L and 3L in the present material were longer than the standard (7.52, 9.02 and 8.29% compared to 6.79, 8.25 and 7.39%, respectively), while the short arm of chromosome-3 was shorter than the standard (6.24 vs. 6.80%). Therefore, the arm ratios of chromosome-1 and 3 in the present material were 0.793 and 0.752 compared to 0.746 and 0.920%, respectively, of Burnham and Hagberg's standard karyotype.

2. Genetic Identification

Monotelotrisomics were crossed to genetic marker stocks in which gene arm relationships were known and F_2-populations from both telotrisomic and disomic F_1-plants were grown. Data for F_2-segregations were scored on the progenies from individial F_1-plants and were then checked separately for X^2-values. If the data were consistent, they were pooled for each cross and an overall X^2-value was calculated. All F_2-populations from disomic F_1-plants showed a good fit for the expected disomic (3:1) ratio (Table II-12).

Table II-11. Relative Length and Arm Ratio of Somatic Chromosomes of Eleven Monotelotrisomics as Compared to the Disomic Standard Karyotype.

Chromosome No.	Telotrisomic relative length*			Standard**		
	Short	Long	Ratio S/L	Short	Long	Ratio S/L
	***					
1	7.52 (a)	9.14 (b)	0.793	6.79	9.09	0.746
2	-	9.02 (b)	-	7.09	8.25	0.859
3	6.24 (a)	8.29 (d)	0.752	6.80	7.39	0.920
4	-	7.82 (b)	-	6.01	7.80	0.771
5	5.17 (a)	7.15 (b)	0.723	5.14	7.04	0.730
6 (Sat.)	6.98 (a)	7.11 (b)		6.80	7.01	0.610
7 (Sat.)	5.92 (a)	-		5.47	9.10	0.410

* Length of individual telocentric/length of the haploid complement (average of 10 cells).

** Calculated from the standard karyotype published by Burnham and Hagberg (1956).

*** Telotrisomic line designation.

Table II-12. X^2 Analysis of F_2-Populations of F_1-Monotelotrisomics and Their Disomic Sibs from Crosses to Genetic Markers.

Linkage Group	Marker Gene (arm location)	No. of Plants			X^{2*}		
		X	x	Total	3:1	5:1	7:1
Telo 1S	Brbr(S)	172	40	212	4.30	0.74	
	Disomics	124	43	167	0.05		
	Nn(L)	214	55	269	3.00		
	Disomics	93	25	118	0.10		
2L	Or or(s)	364	98	462	3.54		
	Disomic	87	37	134	1.50		
3S	Alal(L)	583	121	704	22.90	0.14	
	Disomics	112	30	142	1.14		
	An an(L)	137	14	151	19.90	5.94	1.43
	Disomics	69	17	86	1.30		
	Uz uz(S)	202	70	272	0.07		
	Disomics	159	41	200	2.16		
3L	Uz uz(S)	370	53	423	33.50	5.21	0.00
	Disomics	173	58	231	0.00		
5S	Trd trd(S)	111	39	150	0.08		
	Disomics	206	57	263	1.54		
5L	Trd trd(S)	138	21	159	11.79	1.30	
	Disomics	82	32	114	0.57		

* X^2 values for one degree of freedom at .05 = 3.84 and, .01 = 6.63.

The genes "br" and "n" governing brachytic and naked respectively, are reported to be located on short and long arms of chromosome-1, respectively (Nilan, 1964). The segregating F_2-populations obtained from monotelotrisomic F_1-hybrids (2n+1S x "br" and "n") gave a good fit to a disomic F_2-ratio for "n" ($P = > .05$), and a trisomic ratio for gene "br" ($P = < .05$; Table II-12). This indicated that the extra telocentric was in fact the short arm of chromosome-1. Similarly, a disomic ratio for the gene "or" (for orange seedling) and which is located on the short arm of chromosome-2, indicated that the telotrisomic line carried the long arm of chromosome-2 as the telo. These results confirmed their prior identification using karyotype analysis, thus the cytological maps of chromosomes 1 and 2 correspond with their reported genetic maps.

Three genetic marker stocks carrying the genes "an" for albino seedling and "al" for albina lemma, both on the long arm of chromosome 3, as well as "uz" for uzu on the short arm of chromosome 3, were crossed to telotrisomic 3S. Meanwhile, telotrisomic 3L was crossed to the genetic marker "uz" only. Crosses involving telotrisomic 3S gave trisomic ratios for "an", "al", and disomic for "uz", while telotrisomic 3L showed a trisomic ratio for the gene "uz" (Table II-12). It was concluded on the basis of these ratios that genes "an" and "al" are on the short arm while "uz" is located on the long arm of chromosome-3. Since genes "an" and "al" were previously mapped on the long arm and "uz" on the short arm of chromosome-3 respectively (Robertson, 1971), it is concluded that the reported genetic map is a reversed.

The gene "trd" for "third outer glume" is purported to be located on the short arm of chromosome-5 (Nilan, 1964). The clear-cut ratios observed upon crossing "trd" with both telotrisomics of chromosome-5 (Table II-12) clearly indicated that contrary to previous reports, gene "trd" is located on the long arm of this chromosome. This agrees with Tsuchiya's (1972a) recent suggestion that the gene order on the existing map of chromosome-5 should be reversed.

Telotrisomic lines 1L and 4L were not crossed to genetic markers but were designated as being the long arms of chromosome 1 and 4, by virtue of karyotype analysis and on the basis of their morphological characters in comparison with their corresponding trisomics, "bush" and "robust" respectively. Moreover, because monotelotrisomics 6S and 7S are satellited (Figs. II-3e, 3f) they were designated as being the short arms of chromosomes 6 and 7, respectively. Conversely, the non-satellited arm of chromosome 6 represents the long arm of this chromosome.

Plant Morphology of Derived Telotrisomics

Because the parental trisomics were initially derived from an intercultivar cross (4n OAC-21 x 2n Montcalm) morphological observations were made on both telotrisomics and their disomic sibs for five characters listed in Table II-13. The most significant morphological features of each telotrisomic are described to complement the data in the table.

A description of the morphological characteristics of the telotrisomics observed in this study follows:

Table II-13. Morphological Characteristics of Barley Telotrisomics, Their Disomic Sibs and the Parental Trisomics.

Genotype	Height (ins.)	No. of Tillers	Days to Heading	Florets/ Spike	% Fertility	Fertility of Parental Trisomic in %
2n + 1S	27	3	76	44	81.7	54.2
2n + 1L*	13	10	90	29	53.1	
2n - Disomic	30	5	74	53	92.1	
2n + 2L*	33	3	97	44	56.8	35.9
2n - Disomic	30	5	85	54	93	
2n + 3S	23	3	93	53	73.0	7.6
2n + 3L*	27	4	94	46	45.6	
2n - Disomic	32	7	84	58	94.2	
2n + 4L*	19	4	80	54	82.9	61.4
2n - Disomic	26	8	76	55	94	
2n + 5S	33	6	65	56	76.9	63.5
2n + 5L*	27	6	49	52	64.6	
2n - Disomic	30	8	55	59	93.0	
2n + 6S	32	4	56	33	91.4	45.1
2n + 6L*	27	4	76	49	78.9	
2n - Disomic	30	7	74	60	94.3	
2n + 7S	30	5	60	59	76.8	58.7
2n - Disomic	32	7	59	61	95.6	

* Similar to their trisomic parents (parental types).

Telo 1L: Dwarf plants with high tillering capacity, short narrow dark green leaves, occasionally fused; short reduced heads with long awns; usually, one of the three anthers degenerate; narrow seeds with a naked gap on both sides because of incomplete attachment between palea and lemma; very late maturity.

Telo 1S: Slightly shorter than normal in stature with nearly complete fertility; virtually indistinguishable from disomics.

Telo 2L: Plants taller than disomics; thin culms with long narrow drooping leaves, light-green (yellowish) in color; spikes long and lax with compressed awns (accordion like); often one of the lateral spikelets missing; seeds thin and slender; maturity late.

Telo 3L: Plants similar in stature to disomics; leaves long, greyish green (pale) in color; flag leaves usually very small and drooping with twisted tips; spikes do not emerge completely from the sheath, slightly compact; fertility poor; culms soft at maturity.

Telo 3S: Plants shorter than disomics with long hairy leaves; spikes short and dense with about the normal number of spikelets; fertility high.

Telo 4L: Plants were shorter than normals with thick stems, short, broad, dark green leaves. Spikes had coarse diverged awns and high fertility.

Telo 5S: Slightly taller than disomics with very long spikes; generally, indistinguishable from disomics.

Telo 5L: Plants nearly normal in appearance but all plant parts reduced in size; spikes small showing considerable sterility.

Telo 6S: Tall with very few tillers; spikes short and reduced but almost completely fertile; indistinguishable from normals.

Telo 6L: Plants slightly shorter than normal; leaves, broad, coarse and erect; some spikelets may be missing; seeds wide and plump.

Telo 7S: Plants similar to normal; semi-prostrate tillers; spikes highly fertile with a number of the basal spikelets sterile because of the absence of either male or both male and female sex organs.

Although phenotypic comparisons between different telotrisomics were difficult to make, certain conclusions were reached regarding their identity:

(1) Monotelotrisomics carrying the long arms are quite distinct from each other morphologically and were similar to their trisomic parents.

(2) Four telotrisomics (1S, 5S, 6S and 7S) had no apparent effect on plant morphology and therefore were indistinguishable from normal.

(3) The long arm of chromosome-1 affects plant height and tillering capacity since it caused a remarkable decrease in plant height ($\geq$ 50%) and an increase in tiller number (100%).

(4) Four telotrisomics (1S, 4L, 6L and 7S) has no effect on heading date, another five (1L, 2L, 3L, 3S and 5S) delay heading from 9 to 16 days. In contrast, telotrisomics 5L and 6S headed 6 and 18 days respectively earlier than their disomic sibs.

(5) Monotelotrisomics were generally more vigorous and fertile than their trisomic parents (Table II-13). For instance, the fertility of telotrisomics 3S and 3L were 73.0 and 45.6%, respectively compared to 7.6%

for their parental trisomic.

Meiotic Behavior of Telotrisomics

Chromosome behavior of eleven different monotelotrisomics were studied at different stages of meiosis. For each line, at least 3 plants were analyzed and a description of chromosome behavior at the various meiotic stages follows:

Meiotic First Division.

Diakinesis: PMC's of all monotelotrisomics contained associations of 6"+t2"' (heteromorphic trivalent) or 7"+t' (Figs. II-4a, 4b) with average frequencies of 79.1 and 20.4% respectively for each configuration (Table II-14). In a few cells (0.5%), the telo associated with one homologue in a hetermorphic bivalent while the other homologue behaved as a univalent. Monotelotrisomic (2n+1L) showed the highest frequency of associations (86.3%) while monotelotrisomics (2n+6S) exhibited the lowest (46.6%). The observed types of trivalents were tandem-chain (56%), ring-rod (43.0%), and tri-radial in about 1% of the cells.

Fedak (1969) observed that 42.8% of telotrisomic-1S PMC's contained two or more nucleolei per cell. In the present study, almost all PMC's of nine telotrisomics (non-satellited) including telo-1S, had one and occasionally 2 nucleolei. Moreover, none of these telocentrics was seen attached to a nucleolus. These results indicated that only the satellited telocentrics 6S and 7S had nucleolar organizing activity.

A study of PMC's in which telocentrics 6S and 7S were present revealed information regarding their nucleolar organizing activity. In

Figure II-4. Chromosome configurations of monotelotrisomics at diakinesis and M_I.

(a) 6' + t2"' (diakinesis).

(b) 7" + t' (diakinesis).

(c) 6" + t2"', a heteromorphic trivalent associated with nucleolus (diakinesis of 2n + 6S).

(d) 6" + t2"' (tandem v trivalent at M_I).

(e) 6" + t2"' (triradial trivalent at M_I).

(f) 7" + t' (M_I).

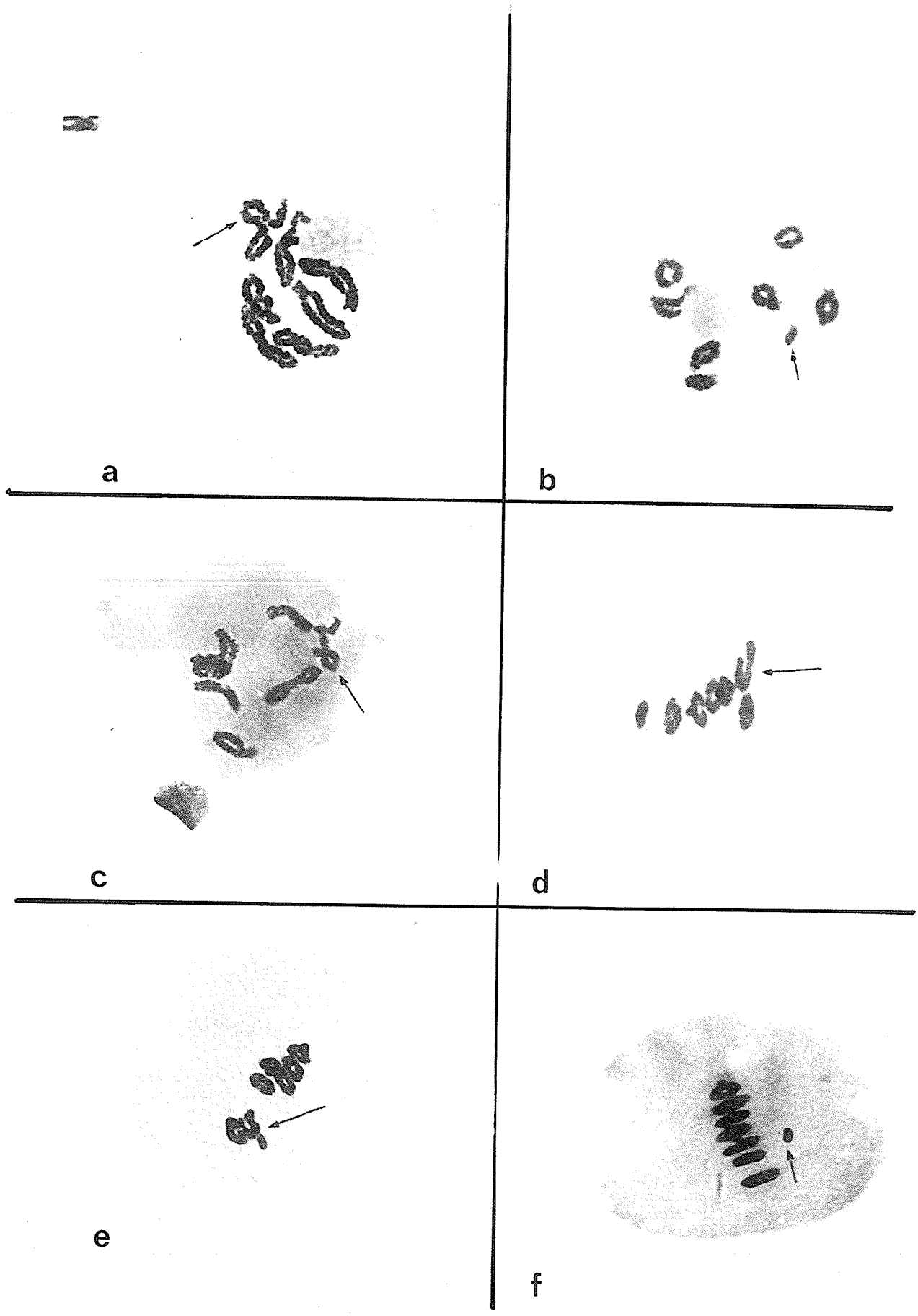

a
b
c
d
e
f

Table II-14. Frequencies of Chromosome Configurations at Diakinesis and M_I in Monotelotrisomics.

Genotype	Diakinesis				M_I			
	No. of Cells	6" + 1"' %	7" + 1' %	6'+t1"+1' %	No. of Cells	6" + 1"' %	7" + 1' %	6"+t1"+1' %
2n + 1S	376	81.9	18.1		1021	78.4	21.6	
2n + 1L	160	86.3	12.1	1.6	574	82.6	17.4	
2n + 2L	182	85.4	13.3	1.3	645	78.6	21.4	
2n + 3S	146	82.2	21.4	0.5	985	74.9	25.9	
2n + 3L	201	78.1	17.2	0.6	719	77.2	22.8	
2n + 4L	179	84.8	14.1	1.5	850	79.3	20.7	
2n + 5S	192	77.6	22.4		745	72.1	27.8	0.03
2n + 5L	225	84.9	15.1		552	80.6	19.4	
2n + 6S	227	46.6	53.4		489	42.7	57.3	
2n + 6L	227	86.3	13.7		627	79.4	20.6	
2n + 7S	143	76.3	23.7		766	73.2	26.8	
TOTAL	2258	Ave. 79.1	20.4	0.5	7973	74.5	25.4	-

telotrisomic 6S, 90.3% of cells contained one nucleolus with an attached telocentric (Fig. II-4c). The remaining cells contained two nucleoli of different sizes with the telocentric always associated with the smaller of the two. On the other hand, telocentric 7S associated with a nucleolus in approximately 30.0% of the observed cells and when a second small nucleolus was formed, the trivalent was seen attached to it. This agrees with previous reports that the nucleolar organizing capacity of telocentric 7S is weaker than that of chromosome 6S (Tsuchiya, 1960; Yu, 1968; Fedak, 1969).

Metaphase I: The frequencies of different types of configurations as observed at M_I are shown in Table II-14. As might be expected, all telotrisomics at M_I exhibited the same trend as in diakinesis in that a preponderance of the PMC's contained (6"+t"') configurations relative to 7"+t' associations. Also, a shift in trivalent types from ring-rod trivalents to tandem ones was evident (Figs. II-4d, 4e).

In cells with (7"+t') the position of the univalent relative to the equatorial plate varied from cell to cell (Fig. II-4f) and appeared to be positioned on a random basis. However, a Chi-square analysis to determine if differences existed among telocentrics, showed a satisfactory fit to a 1:1 ratio for all telocentrics except 6S and 7S (Table II-15). In this case, both telocentric univalents were not distributed at random with telocentric 6S exhibiting greater tendency to be positioned on the equatorial plate.

Anaphase and Telophase I: Observations at A_I disclosed a 7'-7'+t' disjunction in approximately 72.0% of the cells at A_I (Table II-16).

Table II-15. Position of Telocentrics Relative to Equatorial Plate at M_I.

Genotype	No. of Cells With a Univalent	Position of Univalent		P (1:1)
		on plate	off plate	
Unsat. telos.				
2n + 1S	328	198	230	0.10-.05
2n + 1L	90	58	42	0.25-.1
2n + 2L	136	79	57	0.1 -.05
2n + 3S	247	124	123	> 0.9
2n + 3L	164	92	72	0.25-.1
2n + 4L	176	78	98	0.25-.1
2n + 5S	208	114	94	0.25-.1
2n + 5L	107	63	44	0.10-.05
2n + 6L	129	56	73	0.25-.10
Sat. telos.				
2n + 6S	380	175	105	< 0.01
2n + 7S	205	122	83	0.05-.01

Table II-16. Chromosome Behavior at A_I and T_I of Monotelotrisomics.

Genotype	A_I			T_I	
	No. of Cells	Cells With 7-8 Disjunction %	Cells With Lagging Telos %	No. of Cells	With Laggards %
2n + 1S	284	75.4	24.6	608	19.3
2n + 1L	166	75.8	24.2	285	6.7
2n + 2L	159	74.6	25.4	377	14.1
2n + 3S	96	76.0	24.0	572	17.0
2n + 3L	106	85.8	14.2	445	20.9
2n + 4L	95	82.1	17.9	481	12.9
2n + 5S	170	71.2	28.1	173	27.7
2n + 5L	101	72.3	27.7	188	25.5
2n + 6S	127	33.9	66.1*	463	19.4
2n + 6L	120	75.0	25.0	249	15.3
2n + 7S	75	66.7	33.3	356	16.3
TOTAL	1449	Ave. 71.7	28.3	4197	17.7

* 50.0 and 16.1% were in the divided and undivided state, respectively.

Occasionally, a disjunction 8'-6'+t' was observed (Figs. II-5a, 5b) while in the remaining cells, a univalent telo either in the divided or undivided state was positioned on the equatorial plate region. Trivalents seemed to disjoin regularly on a 2-1 basis at A_I, consequently no irregularity occurred in these cells at T_I. In contrast, univalents divided precociously and lagged in most cases at T_I. Telotrisomic 6S showed the highest frequency of cells with a lagging telocentric (66.1%) which divided precociously in 50.0% of the observed A_I cells (Table II-16).

Although the two chromatids of a univalent occasionally showed signs of separation at M_I and A_I, this separation was ordinarily completed too late for the monads to be included in one or both of the telophase nuclei. Thus, at T_I the univalent or its two separated chromatids, were observed lying in the cytoplasm at any position between the equatorial plate and the poles. As shown in Table II-16, the frequencies of cells with laggards at T_I in ten telotrisomics were somewhat higher but comparable to the frequencies of cells with lagging telocentric at A_I. In contrast, telocentric 6S although it divided precociously in 50.0% of A_I cells, only 19.4% of the cells with laggards were observed at T_I. Since 66.1% of A_I cells in this telotrisomic had univalent telocentrics, it is obvious that most univalent telocentrics that divided at A_I and T_I were included in the daughter nuclei while those undivided univalents lagged in the cytoplasm.

Meiotic Second Division. Difficulties encountered in the study of chromosome behavior at second meiotic division involve the short duration

Figure II-5. Chromosome behavior of monotelotrisomics at A_I and quartet stage.

(a) 7'-7'+t' disjunction.

(b) 8'-6'+t' disjunction.

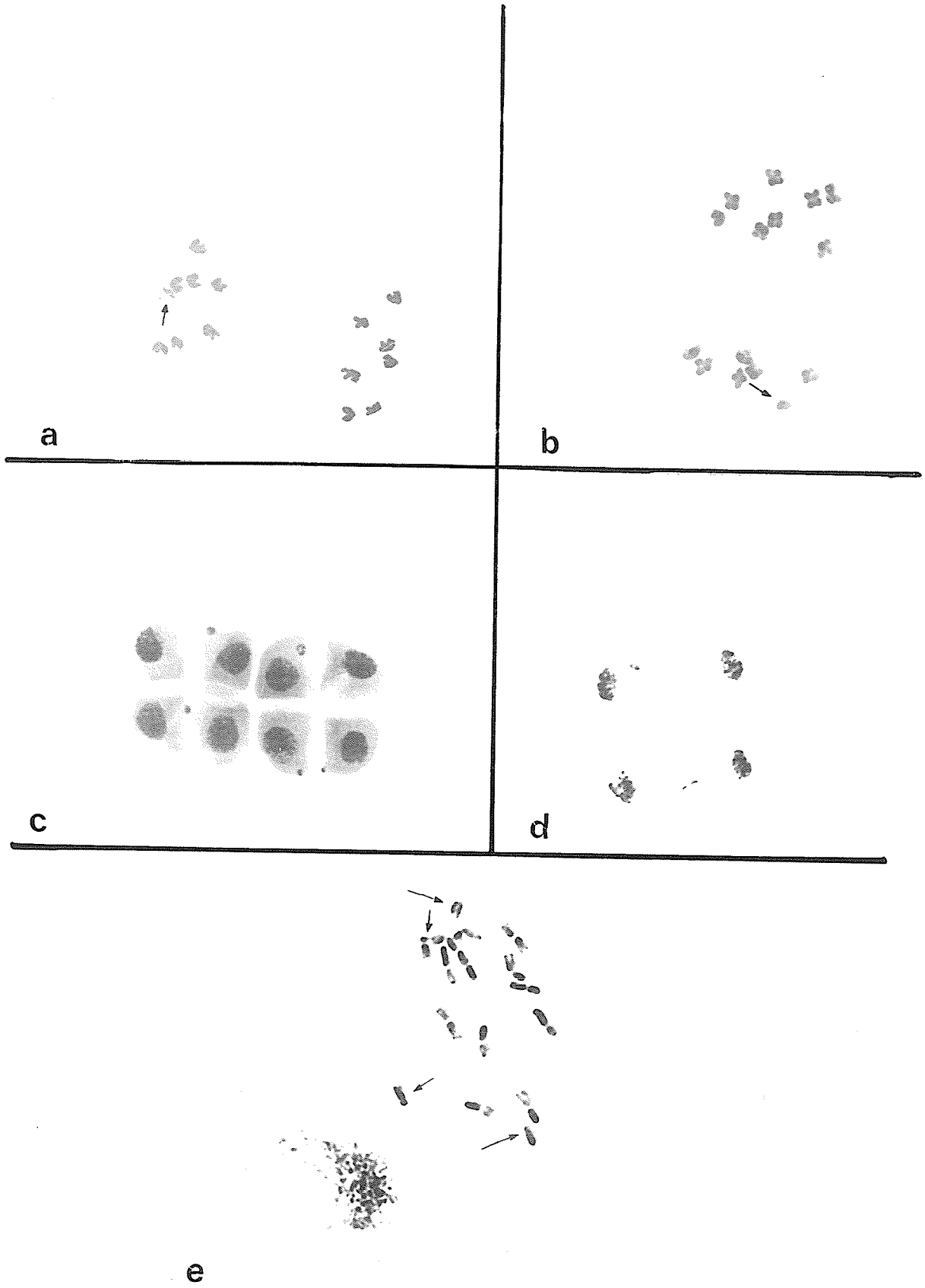
(c) Micronuclei at quartet stage in (2n+6S):

(1) 2 micronuclei of same size

(2) 3 micronuclei of different sizes

(d) Lagging and fragmentation in telotrisomic 2n+6S.

(e) Somatic chromosomes of a plant with 16 chromosomes (12 normal + 2 telo 2L + 2 acro 2S).


a
b
c
d
e

of stages and the added problem of scoring all observations simultaneously on both daughter cells. Lindgren et al. (1969) and Bennet et al. (1971) found that the relative duration of second division of barley was less than 30% of the total meiotic cycle. P_{II} and A_{II} occupied, in equal proportions, about one-third of this time and M_{II} and T_{II} each occupied about one-third of the total period. Such short durations result in small numbers of cells detected at each stage. Another difficulty is that of obtaining satisfactory chromosome spreads in both daughter cells. For these reasons, observations at the second division stages were confined to relatively small number of cells compared to those scored at the first division.

Abnormalities at the second division consisted mainly of non-synchrony of divisions in the two daughter cells coupled with a lagging and fragmentation of the telocentric chromosomes. It was observed that cells with eight dyads (7'+telo), often divided later than the daughter cells with normal complements.

At M_{II}, cells with seven dyads and 7 dyads + 1 or 2 monads were observed. These monads behaved as irregularly as M_I univalents. An average of 16.0% of the cells observed at M_{II} showed that 1 or 2 monads were not aligned at the equatorial plate. However, monads which moved to the equatorial plate were not able to migrate at A_{II} when the dyads disjoined and moved to the poles as in a normal mitosis. Those lagging elements finally moved to the poles but too late to be included in T_{II} nuclei. They formed micronuclei when the second cytokinesis was completed.

In telotrisomic 6S, monads which were aligned at the M_{II} plate were frequently fragmented at A_{II} and T_{II} and resulted in two fragments of different sizes (Fig. II-5d). Since one fragment was approximately twice the length of the other, it was assumed that the secondary constriction was the locus of disarticulation.

Quartet Stage. It has been assumed that the formation of micronuclei indicate the exclusion of univalent chromosomes from daughter nuclei because of their abnormal meiotic behavior. Love (1940) used the frequency of quartets with micronuclei as a relative measure of meiotic stability which he termed "the meiotic index".

Frequencies of quartets with different numbers of micronuclei for eleven telotrisomics are shown in Table II-17. Telotrisomic 1S had the lowest frequency (16.2%) while telocentric 6S because of its aberrent behavior at the previous stages of meiosis exhibited the highest frequency (68.7%). Quartets with more than two micronuclei were very rare in all telotrisomics. An exception was telocentric 6S which because of its fragmentation, it caused a relatively high frequency of quartets with more than two micronuclei (17.2%). When the size of micronuclei was considered for telocentric 6S, most quartets showed the following features:

(a) two micronuclei of the same size, with no fragmentation (Fig. II-5c),

(b) three micronuclei of different sizes, i.e. fragmentation of one chromatid (Fig. II-5c).

Table II-17. Frequencies of Micronuclei in Quartets of Monotelotrisomics.

Genotype	No. of Quartets	% of Total			
		0 m.n.	1 m.n.	2 m.n.	3 or more m.n.
2n + 1S	1661	83.8	10.6	5.6	
2n + 1L	652	77.9	8.4	12.6	1.1
2n + 2L	671	76.0	12.9	11.1	
2n + 3S	927	80.5	13.7	5.8	
2n + 3L	1070	77.9	13.3	8.7	0.2
2n + 4L	948	70.0	7.5	19.4	3.2
2n + 5S	845	77.9	13.3	8.9	
2n + 5L	723	76.6	10.9	11.9	0.6
2n + 6S	1030	31.3	7.5	44.0	17.2
2n + 6L	552	79.0	13.1	7.9	
2n + 7S	716	76.5	13.7	9.8	
TOTAL	9795	Ave. 73.3	11.4	13.3	2.0

(c) four micronuclei, the two members of a pair being of equal size but different from the other pair, i.e. fragmentation of both chromatids.

Some of the abnormal configurations observed at meiosis of telotrisomics but not listed include:

(a) a cell with an asynaptic effect at A_I,

(b) four quartets with most chromosomes forming micronuclei, apparently as a result of asynapsis,

(c) one linear quartet.

Observations at different stages of meiosis in monotelotrisomics were reported by Tsuchiya (1966, 1967) and Fedak (1969). In the present study frequencies of cells with either a trivalent at M_I, a dividing univalent at A_I, laggards at T_I, or quartets with micronuclei were comparable to those reported before considering that a large number of telocentrics, were involved subsequently with greater variation in chromosome behavior. Telotrisomic 6S showed the highest frequency of: (a) cells with univalent telos at diakinesis and M_I (Table II-14); (b) dividing univalents at A_I and T_I (Table II-16) and (c) quartets with micronuclei (Table II-17). In addition it tended to position itself at the M_I-plate and to fragment at T_{II}. Yu (1968) found that among barley trisomics, trisomic-6 had the highest frequencies of abnormalities such as univalents at M_I, laggards at T_I and T_{II}, and quartets with micronuclei. Comparisons between the two telotrisomics of chromosome-6 and their tentative parental trisomic, showed that the short arm amplified the abnormalities

detected in the parental trisomic while the long arm exhibited similar behavior as telotrisomics involving other chromosomes. The presence of the long arm in the trisomic parent, i.e. intact chromosome-6, seemed to attenuate the effect of the short arm. Moreover, chromosome-6 is median with two arms almost similar in length but the shortest arm carries a strong secondary constriction. The fact that the short arm is very active as a nucleolar organizer indicates that the formation of the nucleolus may interfere with the presence of chiasmata thus the ability of the telocentric and its attached homologues to associate as a trivalent configuration. Subsequently, the univalent telocentric detaches from the bivalent prematurely though still attached to the nucleolus. These findings are supported by Sybenga's (1965) observations on the nucleolar organizing chromosome of rye and its derivative telocentrics. He found that the ratio of "crossing over potentials" (long/short) was too large for a submedian chromosome and subsequently attributed it to the nucleolus interfering with chiasma formation on the short arm.

Some other conspicuous features of telocentric 6S were its tendency to be positioned on the equatorial plate, its late division followed by fast movement on the spindle allowing it to be included in the daughter nuclei, and fragmentation at A_{II} and T_{II}. It is suggested that such behavior either results from the presence of the secondary constriction or the occurrence of strong neocentric activity. The latter is more

probable and is considered the cause of fragmentation.

Pollen Viability of Telotrisomics

An estimate of pollen viability, as determined by stainability, was examined in the eleven derived telotrisomics (Table II-18). All lines showed a relatively high frequency of stainable pollen with telos 5S and 7S exhibiting the highest frequency of 96.5% followed by telotrisomic-1S at 95.6%. Telotrisomic 1L had the lowest frequency of 83.2%. Although both telotrisomics 3L and 3S showed similar frequencies (91.3 and 91.4%, respectively) telo-3L had the lowest frequency of seed-set among the eleven telotrisomics (Table II-13). Telotrisomic 6S, notwithstanding its erratic behavior during meiosis, exhibited a high frequency of stainable pollen (95.2%). This is explainable on the basis that the exclusion of the extra telocentric would increase the frequency of normal gametes.

Transmission of Telocentrics

1. Through Egg. Data on transmission through the egg was obtained from crosses to translocation testers and genetic markers using monotelotrisomics as female parents. The transmission of the telo ranged from 20% for telo 2L to 40% for telo 3S. In most cases transmission in selfed progenies were much higher than in crosses (Table II-19).

2. Through Pollen. Out of 260 seeds obtained using monotelotrisomics as pollen only one telotrisomic was recovered, that from telotrisomic 5S. The data were very limited however, and did not allow for conclusions regarding male transmission.

Table II-18. Percentage of Stained Pollen in Monotelotrisomics.

Genotype	No. of Pollen	% of Total Stained
2n	1026*	96.9
2n + 1S	1560	95.6
2n + 1L	1737	83.2
2n + 2L	2052	89.0
2n + 3S	1425	91.4
2n + 3L	1607	91.3
2n + 4L	1569	91.2
2n + 5S	2114	96.5
2n + 5L	1374	93.6
2n + 6S	2255	95.2
2n + 6L	2494	89.7
2n + 7S	1782	96.5
TOTAL	19969	Ave. 92.1

*Average of eleven disomic sibs.

Table II-19. Transmission Frequencies of Telocentrics in the Progenies of Selfed and (2n + telo) x 2n Crosses.

Genotype	Progenies of Selfed Telotrisomics				Progenies of Telotrisomics x 2n Crosses*			
	No. of Seedlings	% 2n	% 2n + telo	% Others**	No. of Seedlings	% 2n	% 2n + t	% Others**
2n + 1S	162	77.7	21.6	0.61	42	79.0	21.0	
2n + 1L	96	60.5	39.5		22	66.0	34.0	
2n + 2L	179	55.4	43.5	1.1	25	76.0	20.0	4.0
2n + 3S	119	69.0	31.0		50	60.0	40.0	
2n + 3L	104	71.2	28.8		21	75.0	25.0	
2n + 4L	103	64.1	35.9		39	72.0	28.0	
2n + 5S	163	65.0	30.7	4.3	28	64.3	35.7	
2n + 5L	107	72.0	28.0		36	61.4	33.0	5.6
2n + 6S	251	83.3	16.7		67	77.2	22.8	
2n + 6L	114	66.7	31.5	1.8	21	70.0	30.0	
2n + 7S	102	64.7	35.3		34	76.8	23.2	
TOTAL	1500	Ave.	31.1		TOTAL 385	Ave.	28.2	

* Translocation testers and genetic markers used as pollen parent.

** Related primary trisomics.

Tsuchiya (1971c) reported an average of 2.19% pollen transmission in four telotrisomics but that such a low frequency was insufficient to account for differences between transmission in crosses and selfed progenies. Ramage (1955) found a delay in the development of eggs carrying an extra chromosome compared to normal ones. This delay may favor certain complements at the time of pollination thereby may result in differences similar to those observed in this study.

3. In Selfed Progenies. With normal meiotic behavior, selfed telotrisomics would be expected to produce equal proportions of n = 7 and n = 7 + telo gametes. Because the extra telocentric will lag and be subsequently eliminated in some cells, the expected frequency of gametes with 7 + telo could be obtained by subtracting the frequency of microcytes with micronuclei from the maximum 50%. The calculated frequency was found to be 38.8% on average which was in good agreement with the observed frequency (Ave. 31.3%). The low transmission of telotrisomics 6S (16.7%) was expected due to its erratic behavior and elimination at meiosis.

4. Primary Trisomics. The related primary trisomics were recovered at low frequencies in selfed progenies and in F_1-hybrids of telotrisomics x translocation and genetic markers (Table II-19). Trisomics arise when an egg with 8-chromosomes is fertilized by a normal 7-chromosome sperm nucleus. The formation of 8-chromosome-eggs result from 8'-6'+t' disjunction at A_I which rarely occurred in telotrisomics (Table II-16). This could explain the rarity of related trisomics in the progeny of telotrisomics.

Correlation Studies of Chromosome Association and Arm Length and Behavior

In the present study, only telocentrics were observed undergoing irregularities at the various stages of meiosis. Also, the terminal position of the centromere in all eleven telocentrics excluded a major variable (centromere position) in the study of chromosome behavior and subsequent chiasma formation. Therefore, the data were used to study chromosome association at earlier stages of meiosis in relation to:

(a) chromosome (arm) length; (b) chromosome lagging and elimination at later stages of meiosis.

Relative measurements of telocentrics in somatic cells were compared to the standard karyotype published by Burnham and Hagberg in 1956 (Table II-11). The eleven telocentrics were then ranked according to their length from the longest to the shortest (Table II-20) and were used for correlation analyses.

Using all eleven telotrisomics, correlations between arm length, and frequency of cells with trivalents at diakinesis and M_I were not significant. The analyses were then repeated on only ten lines excluding telocentric 6S because of its erratic behavior as discussed earlier.

Einset (1943) observed that in corn, trisomics for the short chromosomes exhibited higher frequencies of univalents than those trisomic for the longer ones. This agrees with the present study where positive correlations were found between arm length and chromosome associations (trivalents) at diakinesis and M_I ($r = +.84$ and $+.83$, respectively, Table

Table II-20. A Summary of Chromosome and Breeding Behavior (Percentages) of Eleven Telotrisomics Listed in Order of Long to Short Telocentrics.

Telotrisomic	Trivalents at DK	Trivalents at M_I	Without a Univalent at A_I	Free of Laggards at T_I	Quartets Free Micronuclei	Stainable Pollen	Fertility	Transmission in Self Progenies
1L	86.3	82.6	75.8	93.3	77.9	83.2	53.1	39.5
2L	85.4	78.6	74.6	85.9	76.0	89.0	58.5	43.5
4L	84.8	79.3	82.1	87.1	70.0	91.2	82.9	35.9
3L	78.1	77.2	85.8	79.1	77.9	91.3	45.6	28.8
6L	86.3	79.4	75.0	84.7	79.0	89.7	78.9	31.5
5L	84.9	80.6	72.3	74.5	76.6	93.6	64.6	28.0
3S	82.2	74.9	76.0	83.0	80.5	91.4	73.0	31.0
6S	46.6	42.7	33.9	80.6	31.3	95.2	91.4	16.7
1S	81.9	78.4	75.4	80.7	82.1	95.6	81.7	21.6
7S	76.4	73.2	66.7	83.7	76.5	96.5	76.8	35.3
5S	77.6	72.1	71.2	72.3	77.9	96.5	76.9	30.7
Ave.	82.0	77.6	71.6	82.3	73.3	92.1	71.2	31.1

II-21). This is interpreted to mean that the longer the chromosome, the greater is the chance of it forming chiasmata with its homologues thereby allowing the 3 chromosomes to remain associated.

A negative correlation (r=-0.9 between arm length of telo and frequency of stainable pollen is explained by the fact that short chromosomes are more liable to failure in synapsis and elimination than longer chromosomes, thereby increasing the frequency of normal pollen. Also, the genetic component carried by a telocentric may affect pollen viability. In this case, it is expected that long chromosomes (telos) may involve larger amounts of genetic material vital to gamete development than shorter telocentrics.

Correlations for chromosome behavior at different stages of meiosis were determined on data from eleven telotrisomics, the results of which are presented in Table II-21. The correlation between the frequency of trivalents at M_I and cells with a trivalent at diakinesis was positive and highly significant ($r = +0.97$), as was the correlation between trivalent frequency and the numbers of laggard-free cells at A_I ($r = +0.93$). Interestingly a poor correlation was obtained between trivalent frequency at M_I and laggard-free cells at T_I. A possible explanation of this, in view of the highly positive correlation between M_I and A_I, is if one assumes that univalents at M_I may either migrate to the poles or move to the equatorial plate where they divide at A_I and T_I. The rate of inclusion of univalents and/or their division products in daughter nuclei seems to be dependent more on the time of division and subsequent movement

Table II-21. List of Correlations Carried Out on Eleven Telotrisomics.

Correlation of:	In Relation to	r-value
Arm length	Frequency of cells with trivalents at diakinesis.	+0.84**
	Frequency of cells with trivalents at M_I.	+0.83**
	Frequency of stainable pollen	-0.90**
Frequency of trivalents at M_I	Frequency of trivalents at diakinesis.	+0.97**
	Frequency of cells without a lagging univalent at A_I.	+0.93**
	Frequency of cells free of laggards at T_I.	+0.24
	Frequency of quartets free of micronuclei.	+0.93**
	Transmission rates.	+0.61*
Frequency of stainable pollen	Frequency of trivalents at M_I.	+0.50
	Frequency of quartets free of micronuclei.	+0.21
	Transmission rates.	+0.60

* Significant at .05.

** Significant at .01.

of univalents than on their number. This is supported by the fact that the frequency of cells with laggards at T_I, seemed to be independent of the frequency of cells with a lagging telocentric at A_I. For example telotrisomic 1S exhibited 21.6% of its cells with univalents at M_I compared to 57.3% for telotrisomic 6S. However, the frequency of cells with laggards at T_I were similar for both telocentrics (19.3 and 19.4%, respectively). The positive correlation between chromosome associations (trivalents) at M_I and quartets free of micronuclei indicate that most univalents observed at M_I were excluded from the daughter nuclei at the quartet stage.

Correlations involving pollen viability in relation to frequency of trivalents at M_I, to quartets free of micronuclei, and to transmission rates, were all non-significant (Table II-21). This indicates that there are other non-cytological factors that affect pollen viability.

The positive correlation between trivalents at M_I and transmission in selfed progenies ($r = +0.61$) agree with the concept that long chromosomes have a greater chance to associate with their homologues, consequently they behave regularly and are transmitted more frequently than short chromosomes.

Telocentric Shift

In the progeny of telotricomic (2n+2L), a plant was found which had a chromosome constitution of 13'+2L+ an acrocentric chromosome. All PMC's of this plant contained 6" + a heteromorphic trivalent (the acrocentric and the telo pairing with another homologue). Morphologically,

this plant appeared as a normal disomic with almost complete fertility.

The origin of this plant is speculatory, however in telotrisomic 2n+2L, the telocentric paired with one homologue while the other homologue behaved as a univalent. At anaphase-I, the heteromorphic bivalent disjoined in a normal manner while the univalent misdivided, giving rise to an acrocentric for the short arm. This event would result in a gamete with 6 normal chromosomes, a telocentric (2L) and an acrocentric (2S) which could give rise to a plant with the constitution of the one in question. Cytological observations supports this assumption since chromosomes involved in the telotrisomic condition occasionally failed to pair and behaved as univalents. The progeny from the aforementioned plant consisted of diploids, parentals (13'+2L+ acro) plants with chromosome-2 replaced by 4 chromosomes (12'+2 telo + 2 acro, Fig. II-5e), plants with an extra telocentric (2L) and plants with an extra acrocentric (2S). The last mentioned class, although carrying an extra arm, (acrocentric) genetically speaking had undergone a complete shift from a long arm telo (in parental 2n+2L) to short arm type.

Telocentric shift has not been reported previously in self progenies of telotrisomics. However, Tsuchiya (1971d) observed a number of telotrisomic-1L plants in the progeny of a cross between telotricomic-1S and a disomic mutant. He assumed that the centromere of chromosome-1 frequently misdivided in certain genetic backgrounds. This finding should serve as a warning when using telotrisomics for genetical studies. The use of morphological characters as an aid in the identification of telo-

trisomics, could be of great importance since in most cases the two telocentrics of the same chromosome are not cytologically distinguishable.

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SECTION - III

CYTOGENETICS OF ACCESSORY CHROMOSOMES

INTRODUCTION

During the course of the present study, a number of plants, each carrying one or more fragments were isolated from the progeny of trisomics. These fragment chromosomes showed distinct characteristics from the usual telotrisomics and in general, they could be characterized by the following features:

(1) their morphology was different from that of normal chromosomes,

(2) they varied in number in somatic tissues from cell to cell and also from somatic to germinal cells,

(3) they exhibited abnormal behavior at meiosis such as orientation and late movement at M_I and A_I, respectively,

(4) they had very little effect on plant morphology.

For the purpose of this thesis, these "extra" chromosomes were termed accessory chromosomes and were considered as separate from the usual telotrisomics. Because accessory chromosomes have never before been isolated in barley, a thorough study of their cytological behavior was carried out, the results of which are presented in the following section.

REVIEW OF LITERATURE

Accessory Chromosomes: General

Normal chromosomes are indispensable elements of the hereditary constitution of organisms and changes in chromosome number generally have marked effects on the individual. In contrast, accessory chromosomes are by no means indispensable. They are as a rule deleterious and only occur in some individuals of a population.

The term accessory chromosome has been reserved for those chromosomes that occur in varying numbers over and above the normal complement ("A" chromosomes) of an individual or population and which have a minimal genetic effect on the organism possessing them (Battaglia, 1964). In general, accessory chromosomes constitute a miscellaneous assortment of non-autosomal extra-chromosomes. There are often more than one type in the same species (Battaglia, 1964). Accessory chromosomes had been reported in more than 460 species of 163 genera most of them being outbreeders (Brown and Bertke, 1969). The subject, though controversial, has been reviewed in full detail by Randolf (1928, 1941), Muntzing (1949, 1954), Battaglia (1964), and others (Brown and Bertke, 1969; Ostergren, 1947). Because of the extensive literature on the subject this review will be limited to the most relevant aspects which may help to explain the results obtained in the present study.

Chromosome Number and Morphology

The usual number of accessories in a nucleus is 1 or 2, however higher numbers are known in corn (Randolf, 1941), rye (Muntzing, 1954)

and Crepis sp. (Frost, 1960, 1962). By appropriate crossing, higher and higher numbers have been accumulated in these same species. In terms of size, accessories show a wide range of various sizes, however most are smaller than normal chromosomes as observed in rye (Muntzing, 1954), corn (Randolf, 1941) and in Crepis (Frost, 1960, 1962). In many cases, one type of accessory may give rise to another (Vosa, 1966).

With regard to the position of the centromere, accessory chromosomes may occur as metacentrics, acrocentrics or telocentrics (Battaglia, 1964).

Origin of Accessory Chromosomes

The mechanism of origin of accessory chromosomes is unknown, although it is generally assumed that they are derivatives of autosomes consisting of a normal centromere and residual proximal heterochromatin (Brown and Bertke, 1969). There are at least two general hypotheses regarding the origin of accessory chromosomes.

Ancient Origin. It has been currently suggested that the origin of accessory chromosomes is a primitive trait, probably during speciation (Ostergren, 1947; Muntzing, 1954, 1957). In rye for example, the primitive strains have higher frequencies of accessories than the cultivated ones. Yet, Muntzing (1954) considers the occurrence of accessories in rye as a primitive trait and is of importance in determining the center of origin of cultivated rye.

Chromosome Rearrangements. Regardless of their time of origin, many phenomena which cause chromosome rearrangements were suggested as an underlying mechanism for accessories formation. Battaglia (1964)

cited two events considered to be important:

a) Misdivision: It seems that misdivision of the centromere and successive formation of telocentrics and isochromosomes may be responsible for the occurrence of many existing accessories,

b) Conservation of the Centromere: The loss of the pairing ends of an A-chromosomes may give rise to small chromosomes comprised of the centromere and adjacent heterochromatin. This event could be the result of fragmentation or unequal reciprocal translocations for many successive generations. Jackson (1960) found that the small accessories of Haplopappus gracilis correspond in size and morphology to the centromeres and adjacent chromatin of H. ravenii chromosomes which were lost in the evolution of H. gracilis.

Heterochromatinization

Accessory chromosomes, although they originated from normal chromosomes, seem to have a transition in their chromatin phase resulting in non-homology with their putative A-chromosomes.

Fernandes (cf. Battaglia) suggested that in triploids or interspecific crosses involving genes which control the quantity of active chromatin, some of the extra euchromatic chromosomes may be transformed to heterochromatin and result in heterochromatic accessories. Another possibility is that the lagging euchromatin chromosomes which form micronuclei, persist in the cytoplasm and undergo a degeneration or transformation to heterochromatin. Later, such chromosomes will be included in

the nucleus and be transmitted by fertilization. He also assumed that heterochromatinization, though determined by genes, is an irreversible mechanism.

Cytological Behavior of Accessory Chromosomes

The most unusual characteristic of accessories is their irregular behavior and distribution during cell division. This has given rise to the suggestion that this class of chromosome has a genic system of its own which determines their fate. One such system would appear to involve the determination of numbers of accessories persisting in specific tissues or organs of the species in question. In somatic cells, mechanisms proposed for this purpose involve:

a) Elimination: A phenomenon which results in the elimination of accessories from certain tissues or organs is known to exist in several species, of which Crepis capillaris will serve as an example (Rutishauser and Rothlisberger, 1966). In this species, accessories may be eliminated from the primary and secondary roots as well as from the leaves. In all cases however, they persist in shoots, the organs which pass them to the next generation.

b) Numerical Increases: Frost and Ostergren (1959) and Frost (1960, 1962) observed a special mechanism for the increase of accessories in Crepis conzyaefolia and C. pannonica. Plants with 1, 2, or 3 accessories in somatic tissues always contained twice the number of accessories in their PMC's. This was explained by an endomitotic reduplication restricted

to the accessories. In C. capillaris such a mechanism acts through chromatic non-disjunction after transformation of the shoot apex so that by the time a flower formation, about 80% of the sporangeous tissue contained a doubled number of accessories (Rutishauser and Rothlisberger, 1966). Other tissues of the inflorescence contained either none or at the most, only one accessory. In other species such as rye and corn, the numerical increase (i.e. through chromatid non-disjunction) occurs during first or second pollen mitosis so that the generative nucleus receives twice the number of accessories while the vegetative nucleus receives none (Randolf, 1941; Muntzing, 1946). This phenomenon was termed "post meiotic preferential distribution".

Similar mechanisms for either maintaining or varying the number of accessory chromosomes in plant populations act during the pollen mother cell stage. For example, non-homologous pairing between accessories and normal chromosomes (A chromosomes) was occasionally observed in some species (Li and Jackson, 1961). This type of pairing is described as end-to-end association and was attributed to stickiness rather than homology. Among accessory chromosomes, homologous pairing is quite common. When only one accessory chromosome is present, it behaves as a univalent, two may pair and form a bivalent while three or more accessories are capable of forming multivalents (Sarvella, 1959). In many cases the paired accessories may disassociate before M_I (Bosmark, 1954). Univalent accessories of rye (Bosmark, 1954; Muntzing, 1966), corn (Randolf, 1941; Hakonsson, 1957) and Crepis (Frost, 1960, 1962), though often

divided by late A_I, were included in the daughter telophase nuclei. Thus, at the second division, the divided univalents were unable to move, therefore they lagged and formed micronuclei. In contrast, accessory chromosomes of Anthoxanthum sp. divide only at A_{II} (Battaglia, 1964). Consequently, they gave rise to few if any micronuclei at the tetrad stage.

Cytological analysis of the behavior of accessories in diploid and tetraploid rye revealed that abnormalities in both cases were similar (Sarvella, 1959). Stickiness, bridges, lagging, abnormal orientation of bivalents, restitution nuclei, and contracted M_I-chromosomes were observed in both diploid and tetraploid PMC's. Moreover, in tetraploids, misdivision of autosomes and tripolar spindles were also seen. Chromosome pairing, though it increased in both diploids and tetraploids as the number of accessories increased, was much poorer in tetraploids than in diploids. In addition, univalents were more frequent than expected in an autotetraploid and high multivalent frequencies were also rare.

Phenotypic Effects of Accessory Chromosomes

In general, the presence of a limited number of accessories has no remarkable morphological effects on the carrier plants. However, when present in larger numbers they do affect the phenotype. In corn, the occurrence of accessories above a certain threshold caused decreased vigour and lowered fertility until, at a maximum number of about 30 accessories per plant, the plants were poorly developed and highly sterile (Randolf, 1941). Muntzing (1966a) found an inverse correlation between kernel weight and number of accessories in rye. Conversely, Moss (1966) found that accessories increase kernel weight but delayed germination.

MATERIAL AND METHODS

The chromosomes described herein, were isolated among the progenies of a trisomic series from (OAC-21 x Montcalm) established by Larter (personal communication). They were characterized by small size and irregular behavior in both somatic and germinal cells. The lack of pairing between these chromosomes and the regular complement suggested a lack of homology. Subsequently, they were considered as accessory chromosomes (B-type) and were studied separately from other true telotrisomics.

Several different forms of these chromosomes were observed but only two, one carrying a satellite, the other a non-satellited chromosome, were thoroughly studied in somatic as well as reproductive tissues.

Cytological techniques described in section II (Materials and Methods) were used for the study of mitosis and meiosis.

RESULTS

Mitosis

Chromosome Morphology of Accessories. Although the accessory chromosomes observed in this study showed a wide range of size, they were generally smaller in dimensions than most telocentrics of barley.

a) Satellited Accessories: These chromosomes occurred in the progeny of trisomic-6. Plants with one, two and three accessories were observed. The accessory chromosome of this type was a submedian with a long arm carrying a satellite (Fig. III-6b). One of the three segments comprising this chromosome may be deleted resulting in a small chromosome with two segments only. However, since the deleted accessory associated with the nucleolus at meiotic prophases, it was concluded that the short arm was the missing segment. In somatic prophases these chromosomes seemed to be largely euchromatin. Plants with one or two accessories had the same number in all somatic cells, whereas PMC's from these same plants contained different numbers of accessories from cell to cell.

b) Non-Satellited Accessories: These chromosomes generally were small and globular in appearance (Fig. III-6a). Their length in relation to the normal telocentrics was slightly shorter than the shortest chromosome arm of the barley complement (telo 5S). In somatic prophases they were positively heteropycnotic. Examination of somatic anaphases indicated that depending on the chromosome, the position of the centromere

Figure III-6. Somatic and meiotic accessory chromosomes.

(a) 2n + one acc. chromosomes.

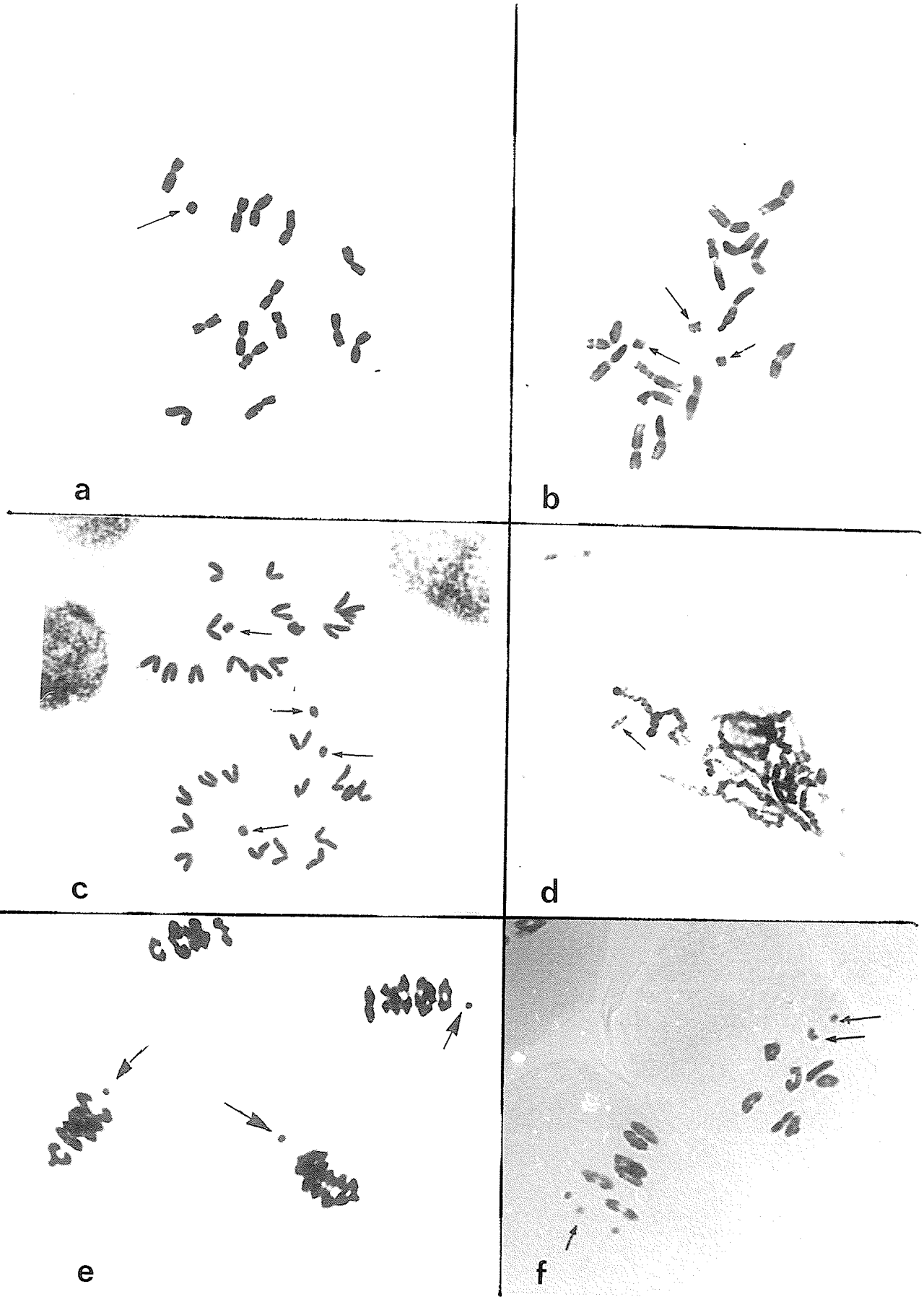
(b) 2n + 3 satellited accessories.

(c) Somatic anaphase (2n + 2 acc.).

(d) Pachytene (2n + one accessory).

(e) M_I, 2n + univalent accessory.

(d) M_I, 2n + 3 accessories (bivalent + univalent and trivalent).


a
b
c
d
e
f

varied from a median to a terminal position (Fig. III-6c).

Root tip cells with zero, one and two accessories were observed in the same plant. It seemed probable that the variation in number as seen in somatic anaphase cells resulted from chromatid non-disjunction - a recognized mechanism for somatic elimination. Thus, one of the daughter cells would receive two accessories while the other received none.

Somatic Elimination. In order to study somatic elimination of accessories, chromosome counts were scored in cells from different roots of the same plant and from different plants (Table III-22). It was clear that in some roots most cells were devoid of accessories while other roots contained at least one accessory per cell. Also within any one root tip, the frequency of cells with two accessories was not equal to those with a correspondingly reduced number as might be expected if both events arise as a result of the same mechanism.

Due to the variation in number from cell to cell, there was no basic number of accessories in these plants. However, a minimum of one accessory per cell was always observed in PMC's.

Meiosis

Plants carrying one, two or three satellited accessories were studied in PMC's. In the case of non-satellited accessories, only one chromosome was present in PMC's at any one time.

Prophase-I: Because of the small size of non-satellited accessories, it was not possible to locate them in most cells whereas it was possible to identify the satellited accessories attached to the nucleolus. In

Table III-22. Chromosome Numbers in Root Tips From Two Lines of Barley Carrying Accessory Chromosomes.

Line	Plant No.	No. of Counted Cells		
		2n + 0	2n + 1 acc	2n + 2 acc
714	5	5	3	1
	5	12	2	-
	5	4	1	-
	9	6	24	3
	7	13	19	4
	7	1	13	1
	63	9	18	2
	63	21	40	7
710	88	13	59	2
	88	17	32	5
	19	-	16	2
		101	227	27

those cells in which it could be followed, a non-satellited accessory was observed as a univalent as early as pachytene (Fig. III-6d). At diplotene and diakinesis, this chromosome appeared as a mass of chromatin lying in the cytoplasm. The satellited accessories were associated with the nucleolus as univalents, bivalents or trivalents depending upon the number existing in PMC's at diplotene and diakinesis. PMC's with three accessories contained a trivalent in 20.8% of the cells, a bivalent + univalent in 63.9%, while the remaining cells (15.3%) had only univalents (Table III-23). In addition, PMC's with two accessories showed a frequency of 83.7% and 16.3% of the cells with a bivalent and two univalents, respectively.

A relatively high frequency of PMC's (24.4%) of a plant carrying three accessories were tetraploid (Table. III-24). Chromosome pairing within these cells showed that about 62.2% contained 14 bivalents while the remaining cells had one or two and occasionally 4 quadrivalents (Fig. III-7a). Also, some cells (3.2%) were partial tetraploids with either 10 bivalents or $6'' + 2^{IV}$ only. Tetraploid cells exhibited a range of 5-7 accessories per cell while in comparison all diploid cells contained only two or three accessories.

Chromosome pairing between accessory chromosomes and those of the normal complement was observed in only a very few cells and appeared to be an end-to-end pairing, probably as a result of stickiness rather than true homology.

Table III-23. Frequencies of Different Configurations of Accessories in Plants With One, Two and Three Accessories.

Stage	Configuration of Accessories	Percent of Cells Observed (No. of Cells) 2n + 1 acc	2n + 2 acc	2n + 3 acc
Diakinesis	Univalents	100 (89)	16.3 (166)	15.3 (219)*
	Bivalents	-	83.7	63.9
	Trivalents	-	-	20.8
M_I	On plate	97.9 (525)	100 (166)	100 (334)
A_I	Dividing	89.8 (76)	12.0 (117)	-
T_I	With laggards	96.3 (189)	15.5 (233)	-
M_{II}	On plate	85.9 (185)	-	-
Tetrad	With micronuclei	75.0 (627)	18.8 (727)	47.6 (412)**

* Two cells contained 2 accessories attached to a normal bivalent.

** Abnormal quartets (irregular shape).

Table III-24. Chromosome Configurations at Diakinesis and M_I in PMC's From a Plant With 2n + 3 acc.

Chromosome Associations	Diakinesis	M_I
Diploids	219	244
Tetraploids	53	90
14" + acc	33	43
1-4 quadrivalent	20	47
Others (partial tetraploids)	7	
No. of cells observed	279	334

Figure III-7. Meiotic behavior of accessory chromosomes (cont.).

(a) M_I in a tetraploid cells.

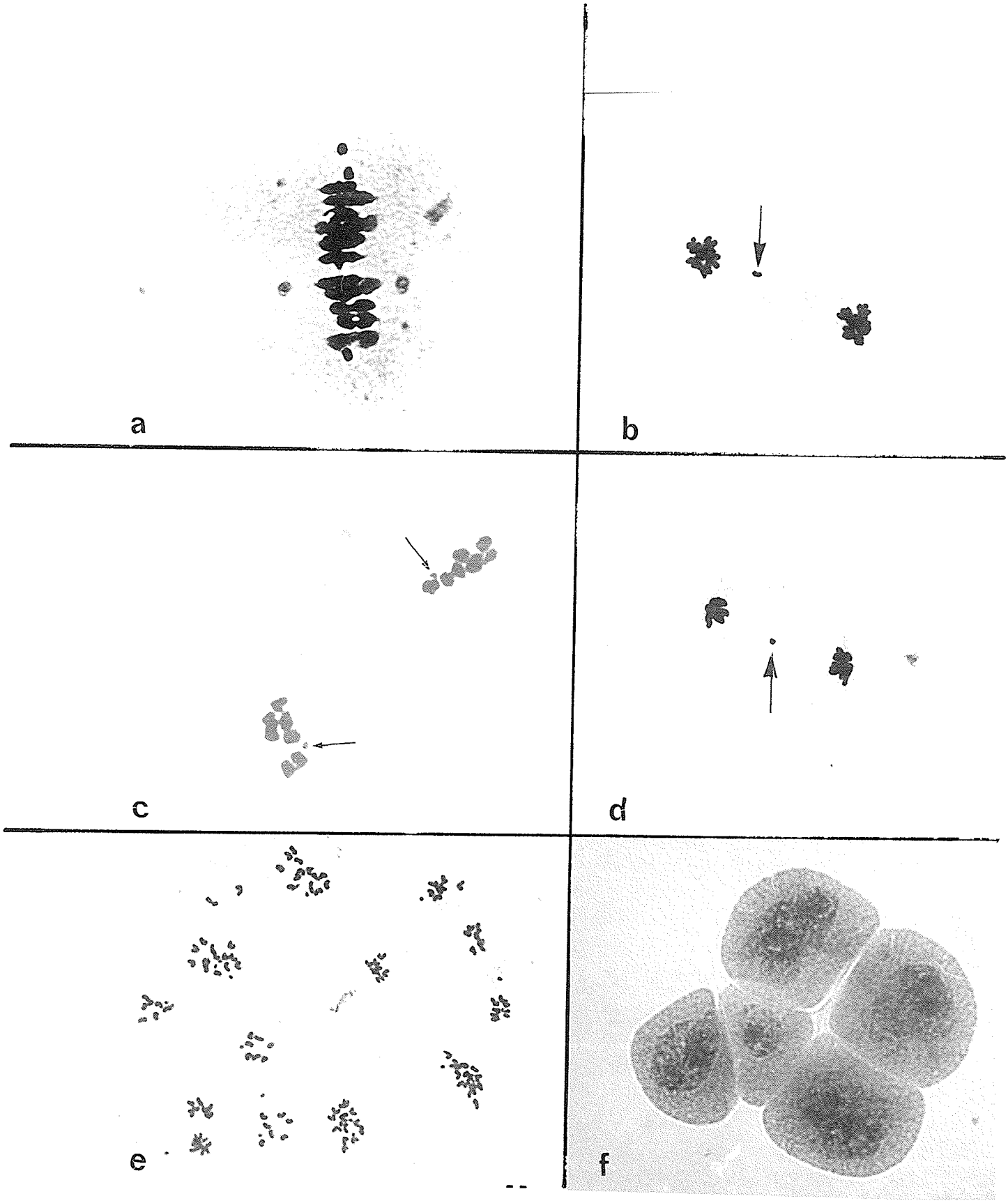
(b) T_I, lagging and dividing univalent accessory.

(c) 7 dyad + one monad accessory at M_{II}.

(d) Lagging accessory at T_{II}.

(e) Equational division at A_I, asynaptic effect.

(f) Accessories forming an extra meiocyte.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Metaphase-I: All accessories were seen positioned peripherally on the equatorial plate at M_I as either univalents, bivalents, or trivalents (Figs. III-6e, 6f). The paired accessories disjoined in a regular manner while univalents divided precociously at late A_I or T_I after all normal chromosomes had migrated to the poles (Fig. III-7b). Despite their late movement, the two monads of each accessory were included in the daughter nuclei in most cells. Subsequently, a very low frequency of cells with laggards (3.7%) was observed at T_I (Table III-23).

In some PMC's, once the M_I-chromosomes disjoined all chromosomes divided equationally resulting in 14 or 28 monads at each pole, in addition to varying numbers of accessories (Fig. III-7e). Apparently, second division was not completed in such cells and only dyads with large nuclei were seen at the quartet stage. Multipolar spindles and non-equal distribution of the chromosomes were also seen in both diploid and tetraploid cells which resulted in the formation of three or four nuclei at T_I. Thus when cytokinesis followed, tetrads of different shapes and sizes were observed.

Second Division: As might be expected, in the second division most daughter cells showed 7 dyads + one or more accessory monads (Fig. III-7c). These monads lagged at A_{II} and formed micronuclei at the quartet stage. As shown in Table III-23, the frequency of quartets with micronuclei ranged from 18.8% for plants with two accessories to 75% for plants with one accessory chromosome, indicating that elimination of univalent

accessories readily occurred in most quartets. In PMC's with three accessories, almost all quartets contained micronuclei. Quartets with abnormal linear or orthodox arrangement of meiocytes occurred in a frequency of 47.6% of the observed quartets (Fig. III-7f).

Transmission of Accessory Chromosomes

The transmission frequencies of accessories were determined in selfed progenies by counting chromosome numbers in somatic metaphase cells. The frequency of plants with one or more accessories ranged from 20.0 to 61.6% in the progeny of plants with one and two accessories, respectively. Only one plant carrying 14' + 3 accessories was recovered. However, somatic elimination may have altered these frequencies since verification by chromosome counts was not made at meiosis.

The effect of accessory chromosomes on fertility was pronounced depending upon the number present. Plants with one accessory had nearly normal seed set (average 89%); while those with two accessories had lowered fertility. The one and only plant with three accessories was completely sterile.

Table III-25 shows a summary of the observed behavior of telocentrics (averages of 11 telotrisomics) in comparison with accessories. It is clear that there was a lack of homology as evidence by the absence of chromosome pairing between univalent accessories and other chromosomes. It was also apparent that accessories were localized at the equatorial region and subsequently divided precociously at A_I. In this respect they

Table III-25. Frequencies of Cells With Different Configurations at Meiosis in Telotrisomics* and Accessory Chromosomes.

Stage	2n + acc. %	2n + telo %
Diakinesis 7" + 1'	100	18.0
M_I (on plate)	97	23.4
A_I (dividing)	89.9	28.4
T_I (with laggards)	96.3	17.7
Tetrad (with micronuclei)	45.0	26.7
Transmission	20.0	30.3

* Average of eleven telotrisomics.

have the same property as univalent accessories observed in other species. Abnormalities at the second division were similar but there occurred a much higher frequency of micronuclei at the quartet stage. Abnormalities in PMC's with three accessories were not recorded in telotrisomics.

DISCUSSION

White (1950) stated that "if enough individuals of enough populations of almost any angiosperm species were examined one might expect to find accessory chromosomes". Thus, the finding of accessories in a species is more or less dependent on the number of individuals examined, however in any given species the number of individuals carrying them is very small. Accessory chromosomes described in this study were isolated from a population of about 7000 plants descended from triploids and their trisomic derivatives. These triploids were derived from an intercultivar cross between OAC-21 (4n) and Montcalm (2n). The fact that cv. OAC-21 is a selection from an old six-rowed variety (Mandscheuri) and was released some sixty years ago, makes it a possible candidate for the origin of accessory chromosomes. According to Muntzing (1954) accessories are very rare in highly uniform European rye varieties but they are more frequent in regions where more primitive strains are cultivated. Subsequently, Moss (1966) maintained that rigorous selection for uniformity and isolation in cultivated rye has decreased the mean number of accessories. A similar situation may have existed in barley.

Powell and Nilan (1968) reported the presence of one or two minute paracentric inversions in the variety OAC-21. Because of competition for pairing among the three elements of a trivalent, an inverted chromosome could be passed on unchanged even after many selfed generations. Acentric fragments produced by these inversions cannot be the origin of

accessories unless they were translocated to another centric fragment resulting from misdivision of extra chromosomes. However, interchange chromosomes though small, are expected to pair with their putative parental chromosomes to form multivalents at low frequencies. Such multivalents were not observed in this material.

Another aspect is the finding that satellited telocentrics are capable of fragmentation at the satellite region. Very short telocentrics lacking the satellite are thus formed. If such small chromosomes involved a change in their structure, e.g., an inversion, it is likely that they will fail to associate with their homologues and subsequently behave as univalents.

The occurrence of centromere misdivision and successive formation of telocentrics may give a clue to the origin of accessories. In barley trisomics as in other species, the extra chromosome may misdivide giving rise to telo- or acrocentrics. An acrocentric with a very short arm may undergo another misdivision within either the centromere or the long arm resulting in the formation of a very short telocentric or metacentric, respectively. These short chromosomes may be exclusively heterochromatin (Kusanagi,1966) and may exhibit its distinct characters such as heteropycnosis and chromatic non-disjunction. Also, since chiasmata are localized at distal regions of barley chromosomes, one is unlikely to detect pairing between these chromosomes even if it should occur.

Accessory chromosomes of barley exhibited characteristics observed

in accessories of other species, viz. somatic elimination, variation in number within plants, and irregular behavior at meiosis.

The constancy of accessories within plants was studied in root-tip mitosis. It was found that there was a small but obvious variation in the number of accessories within root tips of same plant. Such variation has been reported in other species (Muntzing, 1966b; Frost, 1960, 1962).

During meiotic prophases, accessories paired with normal chromosomes in only a very few cells. These associations were probably due to some kind of stickiness rather than homology similar to that observed in several species. However, the lack of homology could be due to drastic changes in chromosome structure such as the presence of an inversion in the chromosome region which gave rise to accessories. The accessory chromosomes localize themselves at the equatorial region which suggest that they are no longer under the control of the normal complement. In PMC's with three accessories, tripolar anaphases and misdivision of normal chromosomes at A_I were observed. In addition, restituted cells and unequal distribution of the chromosomes, indicated a genic action on spindle formation and function. Darlington and Thomas (1941) suggested that in Sorghum a spindle defect leading to polymitosis was partly caused by an excess of heterochromatin. In rye, the action of accessories seemed to delay or prevent spindle formation and appeared to be under genic control and not merely mechanical in nature (Bosmark, 1954). Thus the frequency of abnormal cells increased with increasing numbers of accessories.

Tsuchiya (1960) observed that some PMC's were united to form syncytes. In the present material, about half of the restituted cells contained multivalents which indicated that restitution occurred during late mitotic divisions. In Crepis capillaris, Rutishauser and Rothlisberger (1966) found that an endomitosis restricted to the accessory chromosomes occurred at one late mitotic division in those cells that give rise to the sporangeous tissue. If a similar mechanism acts in barley, it means that accumulation of certain genes carried by accessories had lead to a spontaneous restitution of at least some chromosomes to counter-balance the effect of these genes. This was supported by the observation of some partially tetraploid cells with either 10" or 6" + 2^{IV} only.

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GENERAL DISCUSSION

and

BIBLIOGRAPHY

GENERAL DISCUSSION

The establishment of a complete telotrisomic set (14 telos) involving all seven chromosomes has been the most urgent and important task of barley cytogeneticists and breeders. The aim of the present study was to derive a telotrisomic series in barley. Progenies of selfed trisomics are the most prolific source of telotrisomics in H. vulgare. However, the frequencies of telotrisomics in these progenies are too low for practical purposes (Tsuchiya, 1971a) and in addition the seed-set on some of the parental trisomics is very low. Subsequently, it was necessary to explore some new techniques to accelerate the occurrence of telocentrics.

Ramage et al. (1961) and Hagberg et al. (1963) found that radiation induced translocations involved breaks within the centromeres of some chromosomes. Therefore irradiation as well as other chemical mutations were used in this study to induce telocentrics. On the assumption that gametes carrying chromosome breaks involving the extra-chromosome would be viable, while breaks in other chromosomes would lead to deficiencies and gametic failure, trisomics were chosen for mutagen treatments. Four chemical mutagens, viz., diethyle sulfate (DES), ethyle methanesulfonate (EMS), hydroxylamine (HA), and 5-fluorodeoxyuridine (FUdR) in addition to x-rays were used. The first two chemicals were tested since they are known as the most potent alkylating agents. Results from DES and EMS treatments coincide with the general concept that both mutagens induce gene mutations and when chromosomal aberrations do occur they are mainly deficiencies.

Somers and Hsu (1962) reported that treating Chinese hamster cells with HA induced chromosome breaks at all regions with a high percentage of breaks occurring at the centromere region. Plants injected with HA at meiosis were almost completely sterile and too few progenies from treated plants were obtained for analytical purposes.

Taylor et al. (1962) reported that FUdR suppressed thymidlic acid synthetase thus inhibited chromosomal interchanges induced by ionizing radiation. In this study FUdR was used in combination with x-rays in order to increase the frequency of free fragments. The frequencies of telotrisomics in the progenies of trisomics treated with x-rays alone and in combination with FUdR were not significantly different from one another thus did not support the assumption that FUdR enhances free fragment formation.

Trisomics were treated at different stages of plant development: dormant dry seeds (soaking); during meiosis (injection of HA and irradiation); and at the seedling stage (irradiation). Soaking dry seeds obtained from trisomics in mutagen solutions, though useful to elucidate the sensitivity of the extra chromosome to mutagens, was a tedious method. Trisomic seeds had to first be verified on the basis of root-tip count in populations in which only at 30% of the seedlings are trisomics. On the other hand, mutations observed in root cells do not necessarily involve the shoot cells, therefore the mutation spectrum in M_2-populations may be independent of that observed in the M_1-root cells. Trisomic plants either injected with HA or irrdiated at meiosis were almost com-

pletely sterile. These lethal effects agree with Hermlin's (1970) results on irradiated barley plants. He found that sterility levels of 50% or more were induced by irradiation at meiosis but was not reached even with a ten-fold dose delivered at tillering stage. From similar results from the irradiation of rice plants, Kawai and Inoshita (1965) suggested that treatment at the tillering stage is a relatively effective method for mutation induction.

Another advantage of treatment at early stages of ontogenesis is the ability of young plants to compensate for damaged tillers. Jacobsen (1966) found that up to seven additional prospective mutant sectors were present in barley embryos which may play a part when a large number of the main meristems are killed. In the present study, all the reliable results were obtained from seedling treatments. Two of the eight treatments applied to the seedlings gave higher frequencies of telocentrics than the control. Moreover, among the eleven obtained telocentrics, three (1L, 4L and 7S) were isolated for the first time from these trisomics. The localization of chromosome breaks at a proximal region of the short arm of chromosome-7 may indicate that mutagens are not the appropriate method for the induction of telocentric 7L. Thus, it may be worthwhile to cross trisomic 7 to a desynaptic gene "ds" which eventually will reduce pairing between the extra-chromosome and its homologues which in turn could result in a higher frequency of induced telocentrics. A tertiary trisomic involving an extra chromosome 7 translocated within

the centromere may also serve for the purpose of isolating telocentric 7L.

Differences in response to mutagens reflect the differences in genetic background, i.e., sensitivity of each chromosome to different mutagens. For instance, chromosome-5 was more resistant to the effect of DES and EMS than chromosome-4; trisomic 7 succeeded to set seed although somewhat reduced from that of the normal while trisomic-3 was completely sterile.

Nearly a complete telotrisomic series (eleven out of fourteen) were established in this study. Nine of these were chosen from the spontaneously occurring telotrisomics while the other two occurred in treated material. On the basis of the putative trisomic parent from which each telo was isolated, they were assigned to the seven linkage groups. Translocation testers were then used to verify the chromosome involved. Because translocations are incapable of identifying the particular chromosome arm involved, karyotype analysis and linkage markers were used for this purpose.

Identification of the chromosome arm involved using linkage markers, though time consuming because of the need to calculate F_2-segregation ratios, was employed to compare the genetic map with the cytological one. Despite the fact that barley chromosomes are metacentrics, it was inescapable to identify telocentrics by karyotype analysis in order to establish their cytological identity. These identities were then compared to the existing linkage maps using linkage markers. Because mapping of barley

chromosomes was done by means of genetical methods without the aid of cytological studies, there is no assurance that any of these maps will coincide with the cytological map. In the present study, however, the cytological maps of chromosomes 1 and 2 agreed with the existing genetical maps since genes on short arms of these chromosomes gave trisomic and disomic F_2-ratios when they were crossed to telotrisomics 1S and 2L, respectively.

In the present study, telocentrics 3S and 3L exhibited relative lengths of 6.24 and 8.29% and arm ratio of 0.75 compared to 6.8, 7.39% and 0.93 of the standard karyotype published by Burnham and Hagberg in 1956. The arm ratio observed in this material is very similar to that reported for chromosome-4 in the standard. Also, it should be pointed out that karyotype analysis of trisomic-4 did not give conclusive evidence as to the shape of the extra chromosome (Tsuchiya, 1960, and Yu, 1968). However, it is premature to conclude whether chromosome-3 in the present material carried a structural change or it is in fact the same chromosome previously associated with linkage group 4. Another aspect of chromosome-3 is that contrary to previous reports (Robertson, 1971) its genetic map should be reversed, i.e., those genes which appear on the short arm are in fact on long and vice-versa.

The cytological identification of telotrisomics 5S and 5L using a karyotype analysis did not agree with the genetical assignment. When both telotrisomics were crossed to "trd", a gene located on the short

arm of chromosome-5 (Robertson, 1971), the telotrisomic for the short arm gave a disomic ratio while that for the long exhibited a trisomic ratio. Since telocentric 5S is the shortest of the twelve non-satellited telocentrics, moreover since the difference in length between 5S and 5L is clear cut (arm ratio S/L = .77), it was concluded that the existing linkage map of chromosome 5 is reversed to that previously reported for this chromosome. Recently, Tsuchiya (1972a) arrived at the same conclusion by studying a karyotype analysis of a telotrisomic identified as 5L by Fedak (1969).

Telotrisomic 4L was identified in somatic cells but was not crossed to genetic markers. Tsuchiya (1972b) reported that a telotrisomic 4S (as identified by crosses to genetic markers) exhibited many characteristics similar to the primary trisomic "Robust". From his description it seems that this telocentric is the same chromosome arm identified in this study as 4L. Certain conclusions are not possible since he did not attempt a karyotype analysis as was done in the present study.

The three telotrisomics of chromosomes 6 and 7 were identified on the basis of presence or absence of the satellite in somatic cells. Thus no comparisons between cytological and genetical maps were done on these particular chromosomes.

The phenotypic effects of the presence of an additional single chromosome arm were studied in eleven telotrisomics. It was clear that long arm telotrisomics manifested similar effects, but of lesser magnitude, to their related trisomics. The short arm telotrisomics were

indistinguishable in most cases from normal diploids. However, the undisturbed growth and high fertility compared to trisomics, is a great advantage of telotrisomics over their parental trisomics. This was also achieved in tomato (Khush and Rick, 1968) in which certain telotrisomics resembled their trisomic parents at the same time being highly vigorous and fertile.

Data on meiotic behavior of the present telotrisomics agree with previous reports by Tsuchiya (1969, 1967) and Fedak (1969) on barley. Tomato telocentrics exhibited a much lower frequencies of chromosome association (Ave. 47%) at diakinesis compared to 82% in the present study. Since 23.4% of PMC's contained a univalent at diakinesis, elimination of telocentrics as micronuclei at the tetrad stage occurred as expected in about one fourth of the observed quartets. When the frequency of meiocytes with micronuclei was subtracted from the maximum 50% about 38.8% of the total number of viable gametes were expected to carry the extra telocentric. This is in close agreement with the observed transmisstion frequencies in selfed progenies since the extra telocentric was apparently not transmitted through the pollen.

One of the conspicuous observations is the erratic behavior of telocentric 6S, the main nucleolar organizing chromosome. More than one half of PMC's (63.4%) of this telotrisomic contained a univalent at diakinesis. Subsequently, it misdivided in 50% of cells at A_I and formed micronuclei in 68.7% of cells observed at the quartet stage. The presence of

an inversion on this telocentric may explain its failure in pairing, therefore the aberrent behavior observed. Although Powell and Nilan (1968) reported the presence of one or two minute inversions in cv. OAC-21, there is no evidence that any of these inversions existed in chromosome 6. On the other hand, Fedak (1969) studying a telotrisomic 6S, isolated from trisomic 6 of (Herta x Wong) trisomic series, oberved a similar trend to that reported in the present study. In his material, the frequencies of cells with a univalent at diakinesis, M_I, dividing telo at A_I, cells with laggards at T_I and quartets with micronuclei were 64.9, 78.6, 11.3, 25.4 and 23.3%, respectively, compared to 63.4, 67.3, 50.0, 19.4 and 68.7% in the present material. However, the transmission of the extra telocentric was relatively low (9.9%) in his material compared to 16.7% in the present study, considering that elimination of the extra telocentric as micronuclei occurred in only 23.3% of quartets in his material. It is clear that comparable frequencies were observed in both materials at early stages of meiosis and transmission. Otherwise Fedak's material exhibited a very low frequency of quartets with micronuclei compared to the present material.

Comparisons between the behavior of both telotrisomics of chromosome 6 showed that the non-satellited arm exhibited regular behavior similar to other telocentrics. The abnormal behavior of the short arm may be attributable to the presence of the secondary constriction. Sybenga (1965) found that the ratio (L/S) of "crossing-over potentials" of the two arms of the satellite chromosome of rye was approximately 2.0

which is large for a submedian chromosome. He explained such deviation to a reduced efficiency of pairing in the region of the nucleolus, especially when three nucleoli are present before their fusion. In species in which pairing is initiated at distal ends, such as barley (Kasha and Burnham, 1965), inhibition of pairing at a certain locus will result in the failure of pairing at distal segments. In the case of the satellited telocentric, a chiasma is formed between the centromere and the nucleolus. Consequently, the trivalent will be reduced to a bivalent + a univalent telocentric in the absence of a chiasma at the satellite region. Because of the increasing use of telocentrics in chromosome mapping, it is of interest to study the effect of the secondary constriction on crossing-over since in many cases they carry economically important genes.

Correlation analysis for arm length and behavior at meiosis was in agreement with Einset (1943) findings that long chromosomes have a greater chance of pairing and forming chiasmata so that chromosomes of a trivalent are held intact. Subsequently, they may be transmitted at higher frequencies than shorter chromosomes. The highly positive correlation between univalents at M_I and micronuclei at the tetrad stage suggest that the later stage may be suitable for screening for meiotic stability. Also, the negative correlation between pollen viability and meiotic behavior indicated the importance of the effect of genetic make-up of pollen on its viability.

When using telocentrics for chromosome mapping, attention should be paid to the possible arm shift in telotrisomics. Thus, it seems essential

to use morphological markers on each line to detect such events.

The isolation of accessory chromosomes seems to relate to the vast population examined in this study. There is no doubt that accessories arise from the normal chromosomes regardless of whether their occurrence is an ancient or recent trait. Accessory chromosomes are identified on the basis of specific characters not known for normal chromosomes such as somatic elimination, non-homology and irregular behavior at meiosis. Certain events such as misdivision of the extra-chromosome and the presence of inversions may also have accelerated the differentiation of these chromosomes. The fact that accessories involve a miscellaneous group of chromosomes makes it difficult to compare barley accessories with those in any certain species. However, it was possible to identify and characterize these chromosomes in both somatic and pollen mother cells. However, further investigations are needed to substantiate the present results and to elucidate the role of accessory chromosomes on single plants and populations of barley.

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