DERIVATION AND STUDY OF PRIMARY TRISOMICS OF COMMON BARLEY, HORDEUM VULGARE L.

A Thesis

Submitted to

the Faculty of Graduate Studies and Research

The University of Manitoba

In Partial Fulfillment

of the Requirements for the Degree

Doctor of Philosophy

---

by

Chao-ping Riley Yu

December 1968

ACKNOWLEDGMENTS

The author wishes to express his gratitude to Dr. E. N. Larter, Rosner research Professor, Department of Plant Science, for his initiation of the project, suggestions and encouragement throughout the course of the investigation and in the preparation of the thesis. The helpful advice and constructive criticisms of Dr. T. Tsuchiya, Department of Plant Science and Dr. W. O. S. Meredith, Grain Research Laboratory are also gratefully acknowledged. The author also wishes to thank his wife Lina for helping with the preparation of the manuscript.

Sincere thanks are also extended to the National Research Council of Canada for financial support.

ABSTRACT

DERIVATION AND STUDY OF PRIMARY TRISOMICS OF COMMON BARLEY (HORDEUM VULGARE L.)

Chao-ping Riley Yu

A triploid derivative from the six-rowed barley species (Hordeum vulgare L.) was produced experimentally with exogenous auxin techniques developed by Larter and Enns (1960). Cytological analyses of progeny from the triploid revealed a high frequency of trisomic plants (40%). Based on their phenotypic appearance, karyomorphological comparisons, and cytogenetic studies with translocation testers, a complete set of primary trisomics was established.

The meiotic chromosome behaviour was generally similar for all seven identified trisomics. In most metaphase I cells the extra chromosome was associated with its homologues in the form of a trivalent, however, about one-fifth of the extra chromosomes remained as univalents and divided at a subsequent stage. The primary trisomic for chromosome 6 exhibited the highest variation in microsporogenesis and was characterized by its secondary constriction region and dominant nucleolar organizing ability.

Pollen mitosis study revealed that the first and second mitotic divisions of 8-chromosome gametes were delayed

relative to those of euhaploid pollen. The average frequency of trisomic plants recovered from selfed trisomics was 26%, and 22% from crosses with disomic male plants.

Individual chromosomes in the triplicate condition were found to have no significant effect either on the amino acid composition or on the total protein content of the seed. The results from quality analyses indicated that chromosomes 1, 2 and 4 were associated with total beta-amylase activity while chromosome 7 carried a gene governing free beta-amylase reaction. There was also evidence of a strong maternal effect on the amylolytic enzyme level in the barley kernel.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER I

INTRODUCTION

Blakeslee and his colleagues (1919) in their early work with the trisomic of Jimson weed (Datura stramonium Thorn A.) were among the first to draw attention to the influence of individual chromosomes in determining the genetical characteristics of plant species. More recently, this line of research has taken on a new emphasis with the classical work of Sears (1954) in the development of an aneuploid series in common wheat (Triticum aestivum L.). In this work each of the twenty-one pairs of homologous chromosomes was characterized as to its influence on the gross morphological development of the plant. Subsequently, the individual arms of each chromosome have been similarly characterized.

From this point interest has turned to studies of the chromosomal effects at the biochemical level. However, because of the polyploid nature of wheat, the interpretation of genetic systems controlling certain properties is complicated by the duplicating effect of genes present on homoeologous chromosomes. Therefore in the analysis of chromosome function at the biochemical level, a diploid organism is to be preferred. In the present study the six-rowed barley species (Hordeum vulgare L.) was selected for two main

reasons:

1. Six-rowed barley is an important crop plant of western Canada, where these studies were conducted.

2. There is already available much cytological and genetic information on this crop thus expediting further work with this species.

The research project as described herein was initiated with the following objectives in view:

1. to establish and identify a primary trisomic series in a six-rowed barley species,

2. to study the cytological and cytogenetical behaviour of the trisomics so established,

3. to investigate the influence of each of the seven linkage groups of barley on the malting and feeding value of this species.

CHAPTER II

REVIEW OF LITERATURE

1. Types of Trisomics and their Uses:

Aneuploidy is a general term referring to organisms whose somatic chromosome number is not a multiple of the basic number of the genome or genomes involved. Organisms which have an extra chromosome in addition to their normal somatic complement, i.e. $2n + 1$, are called trisomics. Since first described in Datura stramonium by Belling and Blakeslee (1926) the occurrence of trisomics in several plant species as well as in man, has led to their classification into four types based on the structure of the extra chromosome.

(a) Primary Type:

In primary trisomics the extra chromosome is homologous with members of one of the chromosome pairs. The trivalent that is often observed at meiosis of this class of trisomic, frequently appears as either an open V-shaped trivalent or a rod-shaped configuration (Belling and Blakeslee, 1924).

Primary trisomics may be used to assign chromosomes and genes to their respective linkage groups. This approach is made possible by the fact that the extra chromosome modifies the expected genetic ratio for segregating alleles located on chromosomes involved in the trisomic (Takahashi

and Hayashi, 1966a, 1966b). Primary trisomics are also useful in determining the relative distance of genes from the centromere as measured by the frequency of double reduction, particularly when the trisomic phenotypes can be distinguished from their disomic forms (Burnham, 1962). In addition, they can be employed to study the effects of extra chromosomes on plant morphology, cytology, qualitative and quantitative characteristics, as well as biochemical reactions. Certain cytological abnormalities might also be analyzed by primary trisomics. Observations at diakinesis or metaphase in crosses of trisomics and abnormal stocks give some indication of particular chromosomal anomalies involved.

(b) Secondary Type:

Secondary trisomics have an extra chromosome which is an isochromosome, i.e. the two arms of the additional chromosome are homologous with one another as well as with one arm of one of the chromosome pairs of the diploid complement. A misdivision of the centromere of the univalent is responsible for the origin of a secondary trisomic from primary trisomics (Darlington, 1938) and may be used for linkage group determination as well as for assigning genetic factors to a specific arm of a particular chromosome.

(c) Tertiary Type:

In tertiary trisomics, the extra chromosome is made up

of a segment of each of two different non-homologous chromosomes, the result of a segmental interchange. During meiosis, the extra chromosome is often included in a chain of five chromosomes.

In addition to the possible application of tertiary trisomics in genetic linkage studies, their use has also been proposed in hybrid barley production (Ramage, 1965). For this purpose, balanced tertiary trisomics are established in such a way that the dominant allele of a marker gene is carried on the extra chromosome, and the recessive allele is carried on the two normal chromosomes that constitute the diploid complement. Ramage suggested that balanced tertiary trisomics with genetic recessive male sterile genes as the markers could constitute the female parents in the commercial production of hybrid seed.

The first successful hybrid barley variety, Hembar, was produced by this approach, and was announced recently by the U. S. Department of Agriculture and the University of Arizona. Hembar yielded 15 to 35 percent more than Arivat, the most widely grown variety in Arizona, also the male parent of the hybrid.

(d) Compensating Type:

Compensating trisomics are $2\underline{n} + 1$ forms in which the absence of an individual chromosome is compensated for by the presence of two rearranged chromosomes. These two

chromosomes may be composed of either two translocated chromosomes or an iso- and an interchanged chromosome. The first one to be recognized was called Nubbin (N6) in Datura and appeared among the offspring of an irradiated plant (Blakeslee, 1927a, 1927b). The compensating types have proved to be particularly useful as a means of locating a new gene in a particular chromosome (Avery, 1959b). When heterozygous, a new gene with its locus in the compensated chromosome will breed true for the gene among its disomic offspring, except through the occurrence of crossing-over.

2. Occurrence and Origin of Trisomics in Plants:

(a) Early work in Datura:

The first trisomic type, although not then recognized as such, appeared in 1915 among cultures of Datura stramonium growing in the botanical garden at Starrs, Connecticut. The plant was called Globe and was considered as an atypical "mutant" because of its globose rather than normally elongated pods, and the peculiar manner in which the Globe characteristics were inherited. The first published account of the Globe "mutant" morphology is found in the 1916 Annual Report of the Station for Experimental Evolution at Cold Spring Harbor. Two years later, the results of extensive breeding experiments with "Globe" plants involved in reciprocal crosses were reported (Blakeslee and Avery, 1916; Avery et al., 1959). By 1919, a considerable number of other

similar types had been discovered and described. In 1930, when a system of numbering the ends of chromosomes was adopted, the types were designated by the chromosome number and the original mutant names were abandoned (Blakeslee and Cleland, 1930). However, the true character of the "mutants" in question was not established until Belling's investigation in 1920 showed the chromosome number of trisomic plants to be $2n = 24 + 1$ instead of the 24 found in normal plants (Blakeslee *et al.*, 1920). At the same time, it was pointed out that if each of these "mutants" was due to the presence of an additional chromosome representing each of the twelve linkage groups in *Datura*, it should be possible by breeding tests to associate any mutant gene with a particular chromosome (Blakeslee, 1923). By means of extra whole chromosomes or segments thereof, it has been possible for the Blakeslee School to secure a wide range of variations in *Datura* including twelve complete primary trisomics as well as many other known types (Avery *et al.*, 1959).

(b) Trisomics in Other Plants:

After the initial work in *Datura*, trisomics were found in *Nicotiana tabacum* and *N. sylvestris* (Clausen and Goodspeed, 1924; Goodspeed and Avery, 1939), *Lycopersicum esculentum* (Lesley, 1926, 1928, 1932; Rick and Barton, 1954), *Matthiola incana* (Forst, 1927, 1931), *Zea mays* (McClintock, 1929, 1931), *Crepis capillaris* and *C. tectorum* (Hollingshead, 1930; Babcock and Navaschin, 1930), *Antirrhinum majus*

(Propach, 1935), Secale cereale (Tagaki, 1935), Oenothera lamarckiana and O. brevistylis (Emerson, 1936; Catcheside, 1954), Petunia hybrida (Levan, 1937; Heseman, 1964), Pisum sativum (Sutton, 1939), Triticum vulgare (Sears, 1939), Medicago spp. (Ledingham, 1940; Kasha and McLennan, 1967), Populus tremula (Johnsson, 1942), Lolium perenne (Myers, 1944), Gossypium hirsutum (Menzel, 1952), Sorghum vulgare (Price and Ross, 1955, 1957), Clarkia unguiculate (Vasek, 1956), Collinsia heterophylla and C. tinctoria (Dhilon and Garber, 1960; Rai and Garber, 1960; Garber, 1964; Chomchalow and Barber, 1964), Spinacia oleracea (Ellis and Janick, 1960), Ricinus communis (Jakob, 1963), Oryza sativa (Sen, 1965; Hu, 1968), Beta vulgaris (Kaltisikes and Evans, 1967) and Lotus peduncula (Chen and Grant, 1968).

(c) Source of Various Types of Trisomics:

Primary trisomics may be obtained in the following ways:

(i) In otherwise normal diploids due to occasional asynapsis, desynapsis or non-disjunction, n + 1 gametes may be formed which if fertilized by normal n gametes would result in primary trisomics. Belling and Blakeslee (1924) found 0.4 per cent of n + 1 pollen grains in the pollen mother cell (PMC) analysis of normal diploid Datura.

(ii) Certain homozygous recessive genetic factors affecting the meiotic pairing and causing relatively high

frequency of univalents give a low percentage of trisomic progeny. Enns and Larter (1960) found a trisomic for chromosome 7 in the progeny of a radiation-induced desynaptic mutant in Hordeum vulgare var. Husky.

(iii) Crossing a tertiary trisomic with a normal diploid will give primaries among the resulting progeny (Ramage, 1955).

(iv) An autotetraploid plant occasionally gives rise to trisomics among its offspring. Randolph and Fischer (1939) reported the occasional trisomic appearing among the offspring of autotetraploid maize plants.

(v) Established tetrasomics when crossed to diploid stocks provide a reliable source of trisomics for any particular chromosome. Blakeslee and Avery (1938) found in Datura that 70.5% of progeny from crosses between diploids with tetrasomics, 63.9% from reciprocal crosses, and 49.9% from a selfed tetrasomic, were trisomic.

(vi) One of the best sources of primary trisomics is found in the progeny of a triploid. In Datura, from a total of 1,252 field-grown offspring resulting from crosses of triploids with diploids, 677 plants (54%) were trisomics (Avery et al., 1959).

Secondary trisomics appeared spontaneously in the offspring of primary trisomics as a result of misdivision of the extra chromosome at the region of the centromere

(Darlington, 1938). A telocentric chromosome is the immediate product of such a misdivision which upon replication produces an isochromosome with two genetically identical arms.

Tertiary trisomics occur regularly, possibly exclusively, among the progeny of interchange heterozygotes. A 3:1 disjunction from a reciprocal translocation produces $\underline{n}$ + 1 gametes which are usually functional through the female. Union of $\underline{n}$ + 1 gametes with normal $\underline{n}$ gametes, results in the formation of tertiary trisomic and primary trisomic interchange heterozygotes (Ramage, 1960).

(d) Methods of Producing Triploids:

Since triploids are the most prolific source of trisomics, methods of producing triploids are worthy of investigation.

Triploids occur occasionally among the progeny of diploids as a result of the spontaneous formation of unreduced ($2\underline{n}$) functional gametes, which unite with normal reduced gametes to form triploid ($3\underline{n}$) individuals. Two such cases were observed to occur in corn (Rhoades, 1933, 1936).

Triploids also occur regularly in the progeny of tetraploid x diploid crosses. The success with which triploids are obtained by intercrossing autotetraploids with diploids depends on the species and direction in which the cross is made. The first triploid Datura was obtained from a cross between a tetraploid and a diploid (Belling and

Blakeslee, 1922). Results obtained in maize by Randolph (1935) indicated that crosses between tetraploid and diploid individuals were more successful if the tetraploid was used as the female parent. In most species, such as barley for example, this cross is difficult to make. Tsuchiya (1953, 1958a) succeeded in obtaining hypotriploid and triploid seeds from crosses between tetraploids and diploids of a number of varieties of two-rowed barley. In six-rowed barley varieties, however, the difficulties of intercrossing individuals with different levels of ploidy were not overcome until the recent development of embryo culture techniques. Larter and Enns (1960) showed that application of exogenous gibberellic acid enhanced triploid embryo development in vivo enabling the triploid embryo to germinate in vitro. By using these techniques, they succeeded in obtaining autotriploid barley both in the six-rowed variety O.A.C. 21 and the two-rowed variety Herta.

Tsuchiya (1967) reported that 48.3% of the progeny from selfed autotriploids and 33.2% of the progeny from crosses between autotriploids and diploids were trisomics. Therefore, at the present time the most efficient method of producing triploids in barley is by intercrossing lines of different levels of ploidy in conjunction with auxin application.

3. Trisomics in Barley:

(a) Derivation:

As shown in Table I, Kattermann (1939) was probably the first one to report trisomics in barley. Three trisomics occurred spontaneously in the progeny of semi-sterile strains of Hordeum distichum. Smith (1941) described three trisomic plants which also arose spontaneously in Hordeum vulgare. He attributed their origin to 8:6 disjunction of the chromosomes at anaphase I of a normal diploid. In studying the descendents of one of Smith's trisomics, Ramage (1955) concluded that they belonged to the tertiary type.

Barley trisomics reported by McLennan (1947; McLennan and Burnham, 1948) were obtained from the progeny of an X-ray-induced mutant "long chromosome" which showed a partial asynapsis at first metaphase (Burnham, 1946).

The first complete set of primary trisomics in barley was established by Tsuchiya (1958b) in a brittle-eared form of a wild two-rowed variety of Hordeum spontaneum. Twenty-four trisomic plants were obtained from an auto-triploid and were classified into seven independent types based initially on the study of external morphology (Tsuchiya, 1954) and later confirmed by cytogenetical analyses (Tsuchiya, 1959a, 1959b, 1961; Tsuchiya et al., 1960). In a two-rowed cultivated variety of Hordeum distichum, Tsuchiya also found many trisomics from the progeny of either autotriploids or hypo-autoploids. Thirty-

TABLE I

TRISOMICS PRODUCED IN GENUS *HORDEUM*

Origin	Type	Species	Variety	Authority
Spontaneous Semi-Sterile	Primary	*H. distichum*	var. ?	Kattermann, 1939
Spontaneous Reciprocal Translocations	Tertiary	*H. vulgare*	var. ?	Smith, 1941
Asynaptic Partially	Primary	*H. vulgare*	var. Mars	McLennan, 1947
Desynaptic	Primary	*H. vulgare*	var. Husky	Larter, 1962 unpublished
Interchange Heterozygotes	Tertiary	*H. vulgare*	var. Mars	Burnham *et al.*, 1954
" "	Tertiary & Primary	*H. vulgare*	var. Mars	Ramage, 1955
" "	Tertiary	*H. distichum*	var. Gull	Hagberg, 1954
Autotriploid	Primary	*H. distichum*	var. Shin Ebisu 16 var. Wase Golden Melon	Tsuchiya, 1950 1952, 1963
"	Primary	*H. vulgare*	var. Gateway	Kerber, 1958 unpublished
"	Primary	*H. vulgare*	var. OAC 21	Larter, 1961 unpublished
"	Primary	*H. distichum*	var. Abed Kenia	Derenne, 1967
Triploid Hybrid	Primary	*H. vulgare*	var. Herta x Wong	Kerber, 1954, 1958
"	Primary	*H. vulgare*	var. OAC 21 x Montcalm	Larter, 1962 unpublished
?	Primary	*H. distichum*	var. Betzes	Ramage, 1968 unpublished

seven primary trisomics were grouped morphologically into seven different classes and six of these classes were genetically and cytologically identified (Tsuchiya, 1950, 1967). Recently Derenne (1967) also reported trisomics in the progeny of an autotriploid of a two-rowed cultivated variety.

In a six-rowed cultivated barley species (Hordeum vulgare) Kerber (1954, 1958) established four trisomics for chromosomes 1, 2, 3 and 7 from two triploid hybrids and another four trisomics for chromosomes 4, 5, 6 and 7 from an autotriploid origin. The trisomic for chromosome 1, however, was not a primary type, but rather a telotrisomic which had only one arm of chromosome 1.

Hagberg (1954) and Burnham et al. (1954) found tertiary trisomics in the progenies of x-ray-induced translocation heterozygotes of six-rowed varieties of barley.

Ramage (1955, 1960) was the first to report on the morphological and cytogenetical identification of barley trisomics. From a set of interchanges involving seven chromosomes of the variety Mars, he isolated tertiary and primary trisomics. The morphological expression of the trisomic types was found to be genetically associated with specific chromosomes.

(b) Morphological identifications:

Smith (1941) described three trisomic barley plants obtained by him as vigorous, producing several tillers, and

about two-thirds the height of normal plants. In contrast, barley trisomics obtained by McLennan and Burnham (1948) were, with few exceptions, dwarf and weak.

Tsuchiya (1954) identified a total of twenty-four trisomics from autotriploid progenies of a two-rowed wild type barley and subsequently classifed them into seven groups. In accordance with their morphological characteristics, these seven groups were designated as: Bush; Slender; Pale; Robust; Pseudo-normal; Purple and Semi-erect. Tsuchiya found the same classification could be applied to a trisomic series later established in a two-rowed cultivated barley (var. Shin Ebisu No. 16). The seven trisomic types of both species were readily distinguished from normal diploids and from each other throughout the whole of the growing period. Moreover, the fertility of the cultivated trisomic material was higher than that of the wild species (Tsuchiya, 1954, 1964, 1967).

On the basis of morphological differences, Ramage (1955) was able to select off-type plants from a large number of progeny of chromosomal interchange stocks. Upon cytological examination, 4 to 35% of these plants were found to be trisomic depending upon the chromosome involved. From the selfed progenies of these trisomics and also from hybrid progenies produced by crossing the unknown trisomic with genetic linkage markers, the constitution of the extra

chromosome was determined in each trisomic. The chromosome for which each was trisomic was identified by comparing the morphological characteristics of trisomics from two or more interchanges which have one chromosome in common, and from F_2 analyses of progenies from crosses of trisomics with appropriate genetic marker testers. Thus, trisomics with similar morphological characteristics obtained from the progenies of different interchanges involving the same chromosome were described by Ramage as follows:

Chromosome 1 (b)[1]: narrow dark-green leaves.

Chromosome 2 or 4 (fore): (i) long narrow leaves which droop or hang in an almost vertical position; (ii) dwarf plants with short wide leaves.

Chromosome 3 or 7 (c or d): (i) dwarf plants with slender stems, short, narrow leaves, and complete sterility when selfed; (ii) normal in appearance and not distinguishable from normal diploids.

Chromosome 5 (a): dwarf, weak and highly sterile with no other readily identifiable morphological characteristics.

Chromosome 6 (g): weak plants with very few tillers, high sterility.

Ramage observed that the smaller seeds produced on a trisomic plant were more often trisomic than the larger seeds.

---

[1]Small letters in parentheses () show the old designations of chromosomes.

This was confirmed by Tsuchiya (1967).

Kerber (1958) was also able to identify by their morphological characteristics four primary trisomic types from the variety, Gateway. A large variation in fertility was found in his trisomic lines.

(c) Genetic and Cytological Studies:

Ramage (1955) crossed each group of trisomic plants with genetic stocks carrying marker genes on both chromosomes involved in the interchange from which they were derived. The results were not complete due to a disease epidemic, but the observed ratios in many cases were obviously not disomic.

Kerber (1958) also showed that one of his Gateway plants was trisomic for linkage group II.

Similar genetic tests were carried out in the two-rowed trisomic series of both wild (Tsuchiya, 1959a; Tsuchiya et al., 1960) and cultivated species (Tsuchiya, 1967) of barley. Morphologically distinct trisomic groups showed trisomic ratios in their F_2 progenies from the crosses between each of the trisomic groups and their critical diploid linkage testers. The two trisomic series were also crossed with seven interchange testers. A configuration of 1 V + 5 II indicated that the extra chromosome in the trisomic was one of those involved in the interchange. Seven trisomics of the wild species and trisomics for

chromosome 3, 4, 5, 6 and 7 of the cultivated variety were identified by this method (Tsuchiya, 1961, 1967). Since the karyotypes of species H. spontaneum and H. distichum are similar (Morrison, 1959), three of the seven trisomic types were identified on the basis of karyotype analyses (Tsuchiya, 1961). These included chromosome 5 which is the shortest of the complement, and the two satellited chromosomes 6 and 7; the largest satellite being associated with 6. The association of the nucleolus organizing capacity with chromosome 6 provided additional evidence for the cytological identification of this chromosome.

In no instance was the extra chromosome found to be transmitted through the pollen. The frequency of trisomics among progenies of a selfed trisomic plant of H. spontaneum varied from a low of 19.53% (Slender) to 33.76% (Robust) with an average of 28.10%. For the cultivated species H. vulgare, the range extended from 12.50% (Bush) to 36.70% (Robust) with an average of 29.10% (Tsuchiya, 1963, 1967).

In the four morphologically distinct primary trisomic types in Gateway as found by Kerber (1958), the trisomic condition was transmitted to 21-26% of the selfed progeny. A transmission rate of the extra chromosome through the male gametes of five unidentified hybrid trisomics averaged 0.4% in a total population of 237 plants.

Kattermann (1939) made detailed meiotic studies on

three trisomic plants which he found in the progeny of a semi-sterile line of two-rowed barley. He scored a frequency of 65% of 6II + 1 III and 35% of 7 II + 1 I at MI. In some PMCs, the extra chromosome was split at metaphase I and lagged at first and second anaphase.

Smith (1941) reported the meiotic behavior of trisomics obtained from reciprocal translocation stocks. In contrast to Kattermann's findings however, he observed a lower frequency of 6 II + 1 III configurations at MI (22%) than 7 II + 1 I associations (78%). The meiotic behavior of trisomics in both the wild and the cultivated species of two-rowed barley were studied in detail by Tsuchiya (1960, 1967) whose findings were in agreement with those of Kattermann.

CHAPTER III

MATERIALS AND METHODS

Primary trisomics described in this study were obtained from the selfed progeny of a triploid plant which in turn was derived from controlled crosses between an autotetraploid O.A.C. 21 and a diploid variety Montcalm of six-rowed barley (Hordeum vulgare L.). Difficulty in the initiation of triploid embryos from such crosses was overcome with leaf-treatment of the seed-bearing parent with gibberellic acid (G.A.) for a 12- to 14-day period. The effect of the G.A. was to stimulate embryo differentiation to a point where embryo culturing could be successfully carried out in vitro (Larter and Enns, 1960). The triploid seedlings once established on artificial culture were transplanted to potted soil to continue their development. Although percentage of seed-set on triploid plants was very low, the problem of seed recovery was alleviated somewhat by virtue of the fact these plants were extremely vigorous which permitted continuous harvesting of newly formed tillers.

The analyses of progeny from the selfed triploid plants were based upon root-tip squashes stained with Feulgen (Ostergren and Henee, 1962). Seed was germinated

on moist blotters and root tips were collected for staining preparation. During the two- to three-day period which was required to prepare and analyze root tip preparations, the germinated seeds were held at a temperature of about 2° C thereby retarding their growth until chromosome counts were determined. Trisomic seedlings were then transplanted to pots.

Meiotic analyses of PMCs and pollen mitoses in this study were carried out using temporary aceto-carmine preparations after fixation in Carnoy's solution. Materials used for meiotic studies were grown in a growth cabinet with fixed temperature of 16 ± 2° C. Mature pollen grains were stained with aqueous KI solution for the purpose of pollen viability studies.

All 15-chromosome plants previously verified by root-tip counts were grouped into classes based upon specific morphological characteristics, viz. plant height, tiller number, stem diameter, leaf shape, plant color and growth habit. Each trisomic was identified with respect to the extra chromosome by means of reciprocal translocation stocks which were used as male parents in crosses with individual trisomic plants. Pentavalent association in the PMCs of F_1 trisomics during MI was used as a criterion for positive cytological identification.

Once established, a complete set of primary trisomics was used for quality analyses. Seed from each of the seven

trisomic types was first germinated and chromosome counts of each seedling were established. Four trisomic seedlings (2n = 15) along with a disomic sib (2n = 14) for each trisomic class were transplanted to plastic pots in a growth cabinet. Since the seeds produced from these plants were used for chemical analyses, it was essential to standardize growing conditions as much as possible. Accordingly, a 16 hour photoperiod and a light intensity of 1800 f.c. was maintained throughout the growth period. Temperature was kept constant at 16 ± 2° C. The soil moisture regime of each pot was controlled by weighing pots every two to three days and adding sufficient distilled water to maintain the moisture level between permanent wilting point and field capacity.

Since approximately one-fourth of the seeds produced on a trisomic plant are expected to carry the extra chromosome, it was necessary to first establish the chromosome constitution of the seeds used for chemical analyses. This was accomplished by dissecting and germinating embryos from such seeds and determining their chromosome number on the basis of root-tip squashes. From these determinations, endosperm tissue from both 14- and 15-chromosome "seeds", as well as from seed of the disomic sib check plants, was grouped according to the chromosome involved and ground separately in a mullite mortar. Based on these endosperm materials, the following quality analyses were made:

(1) total crude protein content in duplicate using a standard Micro-Kjeldahl method;

(2) amino acid composition by chromatographing the 6N HCl hydrolysate on an ion-exchange column using a Technicon Auto-Analyzer;

(3) Beta-amylase determination using the dinitrosalicylic acid procedure of Bendelow (1963).

Because of the limited size of each sample, the following modifications were made on Bendelow's method. Twenty mg. of ground endosperms were extracted with 10 ml. of 0.5% NaCl solution overnight for free beta-amylase determination and with 1% papain solution for total beta-amylase analysis. The extracts were diluted appropriately to provide a proper reading range on a spectrophotometer. Duplicate extractions of amylases were made on different samples of grists and duplicate diastases were made for each extraction. Each recorded sample value, therefore, represented the mean of four determinations.

CHAPTER IV

EXPERIMENTAL RESULTS

1. Derivation of Primary Trisomics in Six-rowed Barley

(a) Description of Triploid (2n = 21) Plants:

In general, triploid plants were similar in their morphological characteristics to their diploid sibs. They exhibited however, a very vigorous growth habit after a rather slow initial seedling stage and produced many tillers during a longer-than-normal growth period.

The meiotic chromosome behavior of the triploid plants was highly variable with a high frequency of trivalent and univalent formation (Figs. 1, a-f). The most commonly observed chromosome configuration was 6 III + 1 II + I (29.1%) and 5 III + 2 II + 2 I (26.7%) (Table II). Fertility of triploids was very low (6%) under greenhouse conditions, but germination of the seeds was reasonably good, averaging approximately 76.8%.

(b) Progeny of Triploids:

Cytological analyses of root-tips of progeny from the selfed triploids revealed a surprisingly high frequency of primary trisomics (Table III). Of a total of 156 seedlings cytologically analyzed, 30% were disomics (2n = 14), more

FIGURE 1 - MEIOTIC CHROMOSOME BEHAVIOUR OF TRIPLOID BARLEY

(a) 6 III + 1 II + 1 I at MI

(b) 4 III + 4 II + 1 I at MI

(c) 4 III + 3 II + 3 I at MI

(d) 11 - 10 disjunction at AI

(e) AI with lagging dyads

(f) AI with lagging daughter univalents

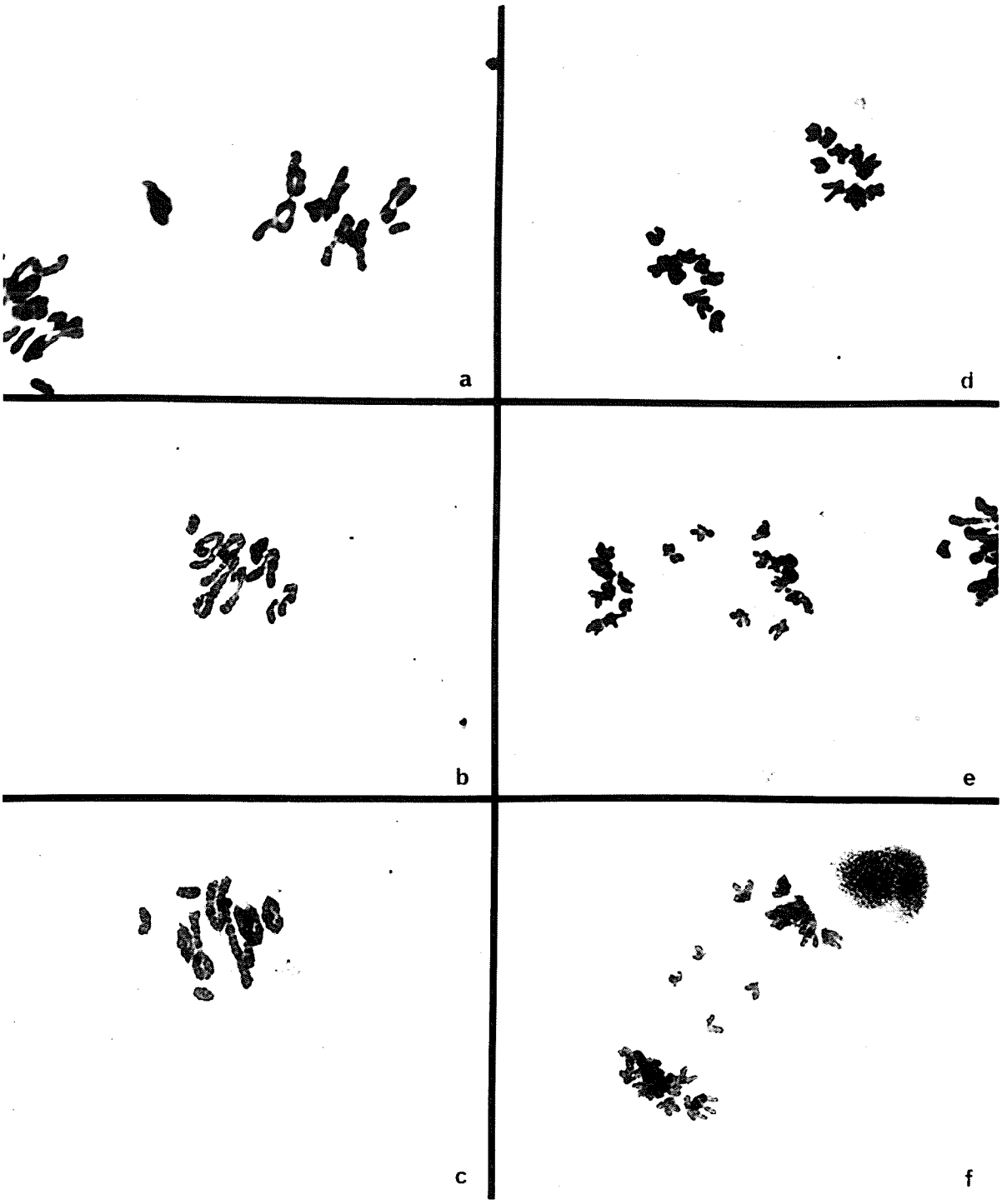

a
d
b
e
c
f

TABLE II

FREQUENCY OF CHROMOSOME CONFIGURATIONS OBSERVED AT FIRST MEIOTIC METAPHASE IN TRIPLOID (2n = 21) BARLEY

Chromosome configuration	Frequency of cells observed	% of total cells observed
7 III	16	18.6
6 III + 1 II + 1 I	25	29.1
5 III + 2 II + 2 I	23	26.7
4 III + 3 II + 3 I	14	16.3
3 III + 4 II + 4 I	6	7.0
2 III + 5 II + 5 I	1	1.2
1 III + 6 II + 6 I	1	1.2
7 II + 7 I	0	0.0
Total	86	100.0

TABLE III

CHROMOSOME CONSTITUTION AND GROWTH CHARACTERISTICS OF PROGENY FROM SELFED TRIPLOID (2n = 21) BARLEY

Chromosome Constitution (2n)	No. of seeds	% of total seeds	Growth Characteristics
14	48	30.77	Normal
14 + telo	1	0.64	Reduced vigor, semi-dwarf, partially sterile
15	63	40.39	" " "
16	26	16.67	Semi-dwarf, late, numerous tillers, highly sterile
17	3	1.92	Dwarf and weak, late, completely sterile
18	3	1.92	Weak seedling, died prematurely
19	1	0.64	" "
20	1	0.64	" "
21	2	1.28	Weak seedling, numerous tillers, late, highly sterile
22	1	0.64	" "
23	1	0.64	" "
24	0	0.00	
25	1	0.64	" "
26	2	1.28	Weak growth, completely sterile
27	2	1.28	Vigorous, partially sterile
28	1	0.64	Normal growth, stocky plant, partially sterile
Total Germinable Seeds	156	100.00	

than 40% were trisomics (2n = 15), 28% possessed a chromosome number greater than 15, and one plant was telotrisomic. The growth characteristics of these plants varied greatly as described in Table III.

(c) Trisomic Lines:

Of the total of 63 trisomic seeds initially identified by root-tip counts, most were successfully reared to the adult stage. These were allowed to self-pollinate in order to perpetuate the seed stocks and to decrease heterozygosity of each trisomic line. From the progenies of self-pollinated trisomic plants, selection for vigor and fertility was practiced. Some lines were lost due to very weak growth and extremely poor fertility. However, a total of 32 lines were saved and selected for further identification.

2. Identification of Individual Trisomic Lines

(a) Morphological Classifications:

A comparison of trisomics derived from the present program with those established by Tsuchiya in two-rowed barley species (1964, 1967), indicated that all the seven possible trisomic types were represented. Since the trisomics of this study were derived from six-rowed varieties, some deviation of morphological expression from that of Tsuchiya's two-rowed material was expected. However, the distinguishing morphological characteristics as

described by Tsuchiya for each of his trisomic types were also expressed in the present series. Therefore it was possible to descriptively characterize each of the newly derived trisomics using the same morphological characteristics as those employed by Tsuchiya. These included such plant morphological features as height of plant, number of tillers, growth period, length and width of leaves, fertility, size of stem, leaf shape, plant color, and growth habit. Accordingly, it was possible to distinguish each of the seven trisomic groups on the basis of the following plant characteristics:

Group I: Numerous tillers; short, dark-green narrow leaves.

Group II: Thin culm; drooping long leaves, poor fertility.

Group III: Normal appearing; slightly pale, very poor fertility.

Group IV: Thick stems; plant stocky.

Group V: Smaller plant; normal appearing, partial sterility.

Group VI: Coarse and erected leaves; purple-colored nodes.

Group VII: Normal appearing; partial sterility.

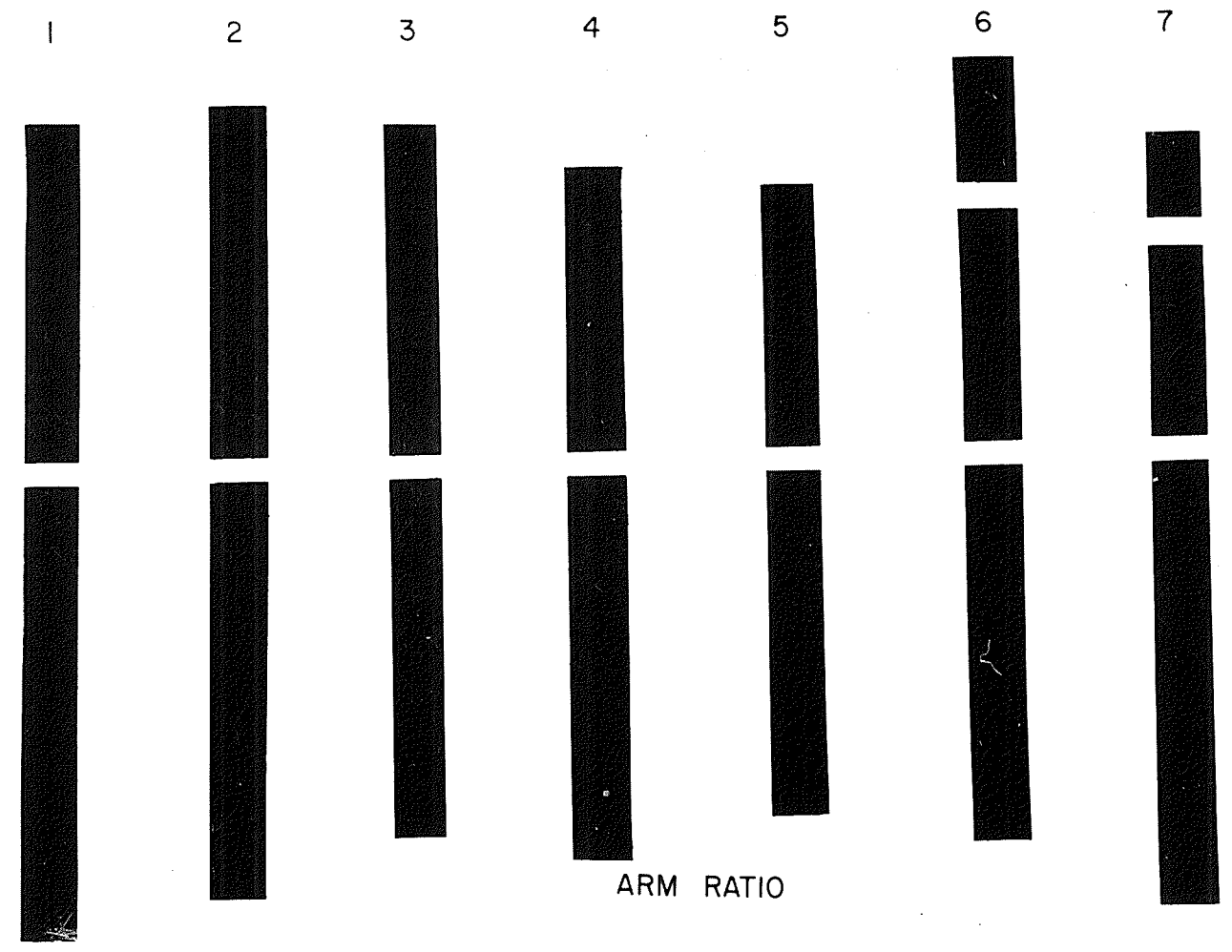
(b) Karyotype analyses:

A karyotype of the somatic chromosomes of barley

based chiefly on data from Tjio and Hagberg (1951) and Burnham and Hagberg (1956), was adopted as a standard model in this study (Fig. 2, Table IV). Photomicrographs and camera lucida drawings of the chromosomes of each trisomic type were studied and measured and subsequently, comparisons were made between idiograms constructed from both trisomic and normal complements. The total chromosome length, centromere position and secondary constrictions were used as major karyomorphological markers to identify the trisome of each trisomic individual.

In general, the chromosome complement of barley is one in which all chromosomes are similar in overall physical length. Within the non-satellited group (chromosomes 1 - 5), however, chromosome 5 is the shortest and plants trisomic for this chromosome were recognized accordingly (Table IV). The ratio of arm lengths (S/L) of each of the trisomes was measured and compared with the standards. The longest non-satellited chromosome with the smallest S/L arm ratio could be distinguished from other chromosomes and was identified as chromosome 1. The average arm ratio for trisome 1 as obtained by measuring chromosomes from 10 mitotic cells was 0.745 ± 0.045 and was very close to 0.746 as shown in the standard idiogram. Greater deviations from standard arm ratios were found for chromosome 2, 3 and 4. Furthermore, perhaps due to the effect of pretreatment or to uneven pressure applied at the time of slide preparation, an

FIGURE 2 - STANDARD IDIOGRAM OF BARLEY SOMATIC CHROMOSOMES



(After Burnham and Hagberg 1956)

TABLE IV

RELATIVE LENGTH AND ARM RATIO OF ROOT-TIP CHROMOSOMES OF DISOMIC BARLEY (STANDARD) AS COMPARED WITH THOSE OF TRISOMICS

Chromosome number	Standard (after Burnham and Hagberg, 1956)					Trisomics	
	Relative total length	Satellite	Arm Length Short	Arm Length Long	Arm Ratio (S/L)	Arm Ratio (S/L)	S. D.
1	136.9	-	58.5	78.4	0.746	0.745	0.043
2	132.2	-	61.1	71.1	0.859	0.870	0.061
3	122.3	-	58.6	63.7	0.920	0.880	0.062
4	119.0	-	51.8	67.2	0.771	0.807	0.047
5	105.0	-	44.3	60.7	0.730	0.713	0.040
6	100.0	20.7	37.9	62.1	0.610	0.630	0.031
7	110.7	14.9	32.2	78.5	0.410	0.460	0.033

disproportionate expansion or contraction of chromosomes was frequently observed. All these factors made it more difficult to accurately identify the other non-satellited chromosomes (2, 3, 4) on the basis of chromosome length and centromere position.

Trisomics for chromosomes 6 or 7 were easily identified on the basis of difference in satellite size. Chromosome 6 carries the larger satellite of the two (Fig. 3).

(c) Cytological Identifications:

In order to substantiate identification based on karyotype analyses, as well as to distinguish between trisomics for the non-satellited chromosomes 2, 3 and 4, crosses were made between trisomics representing each of the seven morphological groups and standard translocation stocks. The results of the meiotic analyses of the resulting F_1 plants are shown in Table V.

Trisomic F_1 plants in which the "extra" chromosome was in common with one of the two chromosomes involved in the interchange (critical cross) exhibited a metaphase configuration of either 1 V + 5 II (Fig. 4, a-f) or 1 IV + 5 II + 1 I (Fig. 5, e-f). For the non-critical crosses, the trisomic F_1 hybrids showed an MI configuration of 1 IV + 1 III + 4 II (Fig. 5, a-b) or 1 IV + 5 II + 1 I (Fig. 5, c-d). It is to be noted that in both critical and non-critical crosses, cells showing a configuration of

FIGURE 3 - CHROMOSOME MORPHOLOGY OF BARLEY TRISOMIC IV

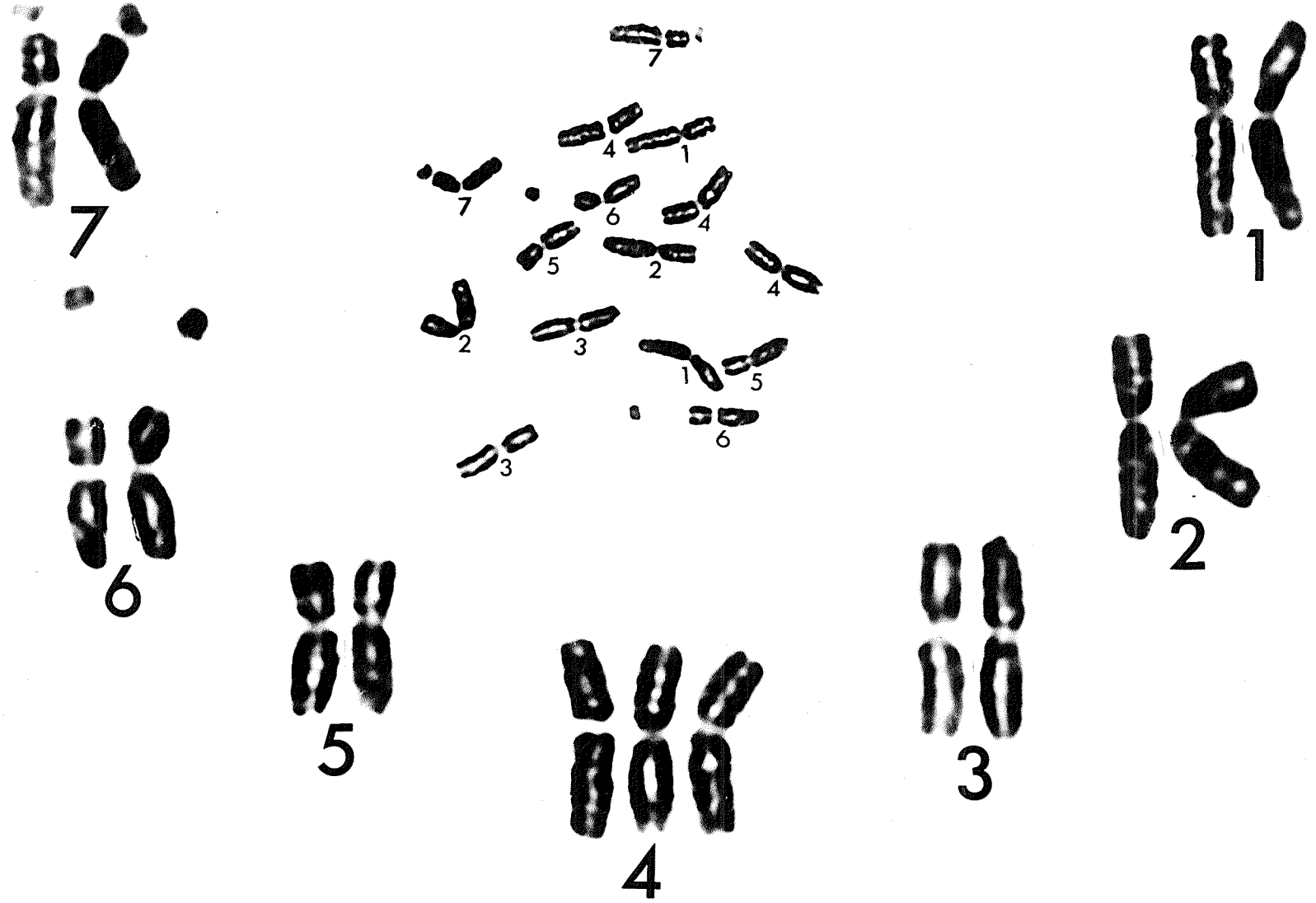

7
6
5
4
3
2
1

TABLE V

MEIOTIC CHROMOSOME CONFIGURATIONS IN TRISOMIC F_1 HYBRIDS FROM CROSSES BETWEEN TRISOMIC LINES AND TRANSLOCATION TESTERS

	Trisomic groups	I	II	III	IV		V			VI		VII
	Line designation	A-29	A-71	A-73	A-2,	A-24	A-3,	A-38,	A-74	A-10,	A-51	A-77
TRANSLOCATION TESTERS	T_{1-2a}	V	V									
	T_{1-3c}	V		V		IV+III			IV+III			
	T_{1-4a}				V							IV+III
	T_{1-5a}						V	V		IV+III	IV+III	
	T_{1-5c}	V						V	V			
	T_{1-6c}									IV+I		
	T_{2-3d}	IV+III										
	T_{2-4a}		V			V						
	T_{2-7b}										IV+III	V
	T_{3-4b}			V	V		IV+III					
	T_{3-7a}											V
	T_{4-5a}		IV+III				V			IV+III		
	T_{5-6a}						V			V		
	T_{5-7b}			IV+III	IV+III	IV+III		V				
	T_{6-7b}					IV+III			IV+III			

FIGURE 4 - CHROMOSOME CONFIGURATION AT MI OF TRISOMIC F_1 PLANTS FROM THE CRITICAL CROSS BETWEEN A TRISOMIC AND TRANSLOCATION STOCKS, SHOWING 1 V + 5 II

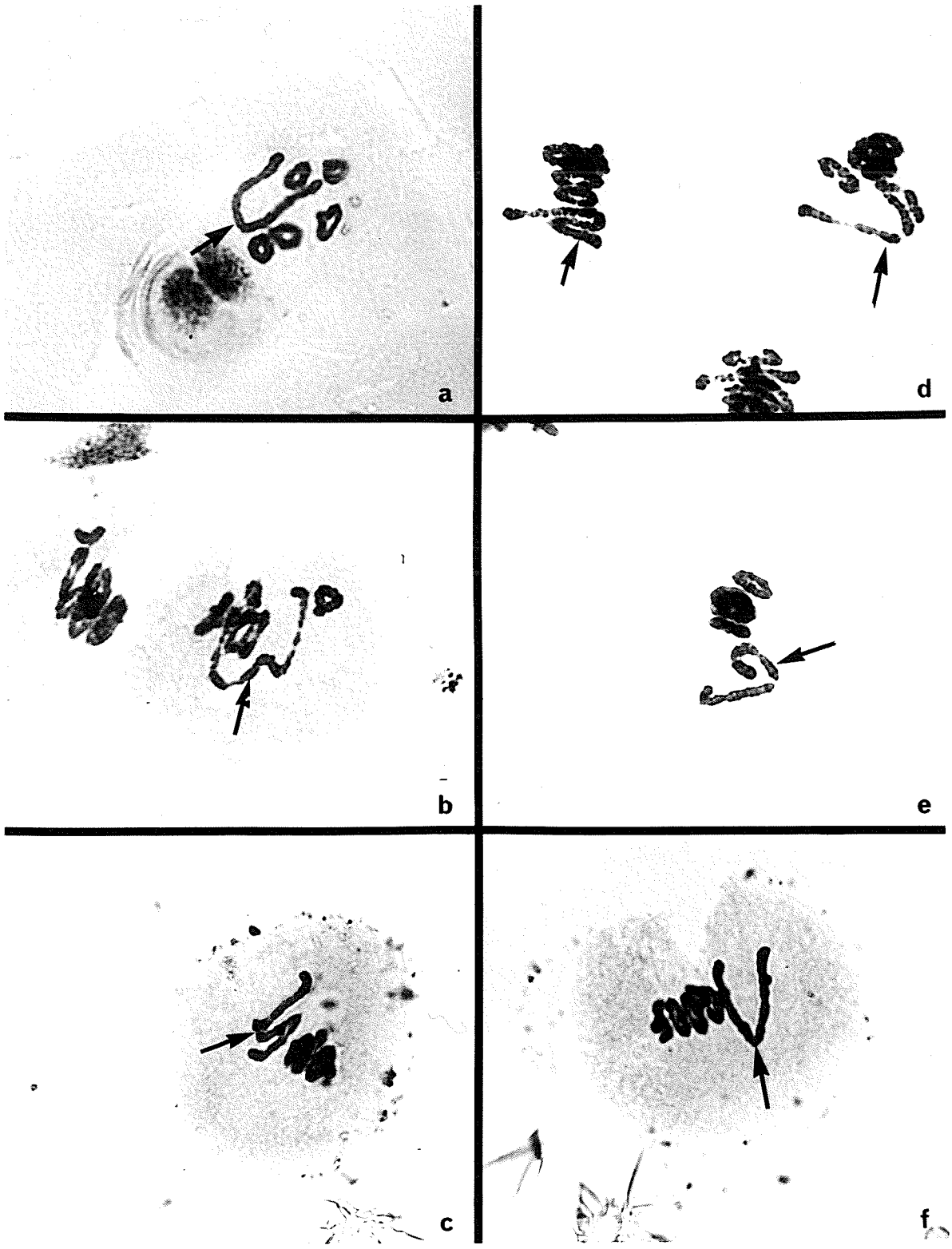
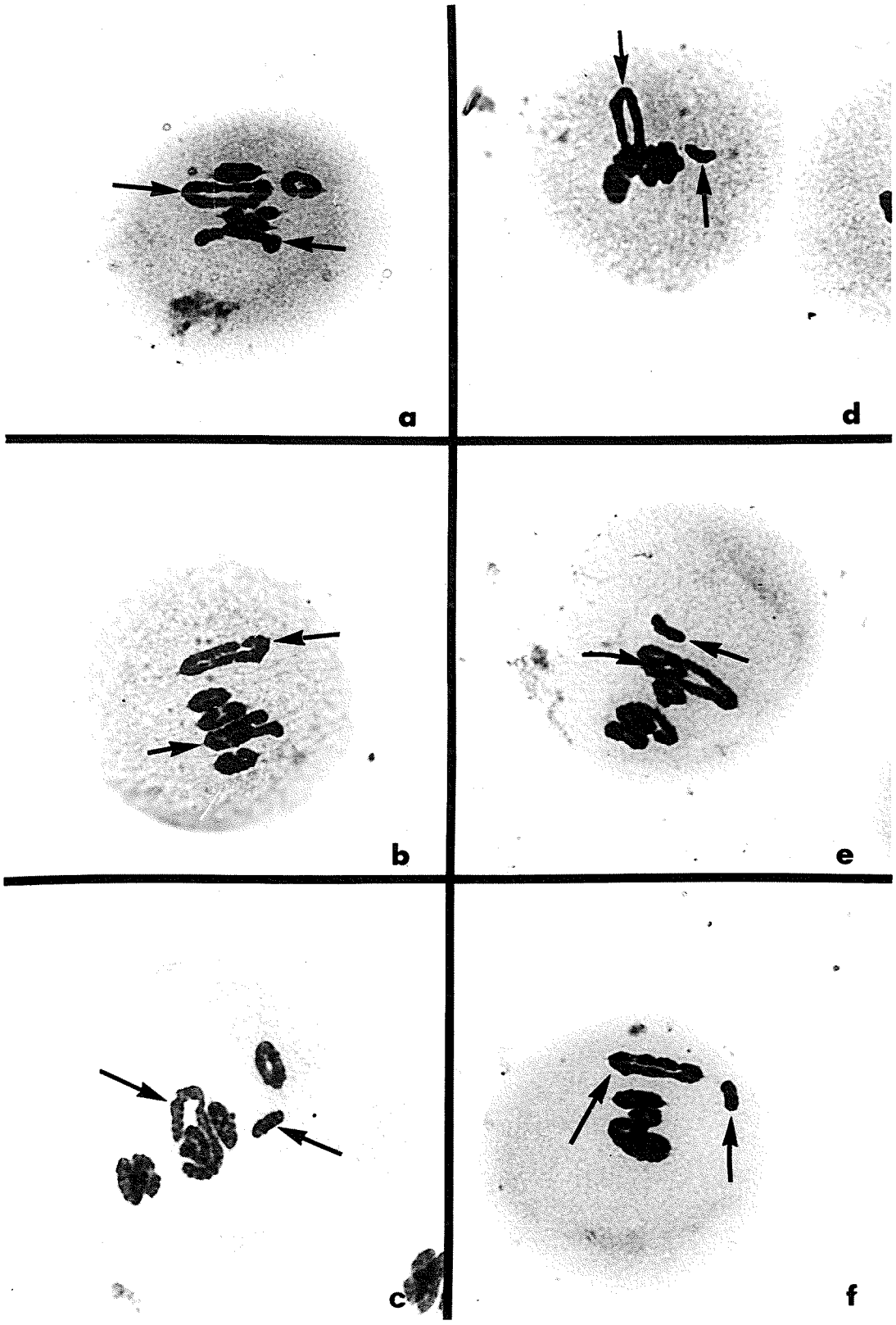

a
d
b
e
c
f

FIGURE 5 - CHROMOSOME CONFIGURATIONS AT MI OF TRISOMIC F_1 PLANTS FROM CROSSES BETWEEN TRISOMICS AND TRANSLOCATION STOCKS

(a-b) 1 IV + 1 III + 4 II from non-critical crosses

(c-d) 1 IV + 5 II + 1 I from non-critical crosses

(e-f) 1 IV + 5 II + 1 I from critical crosses


a
d
b
e
c
f

1 IV + 5 II + 1 I occurred. However, the occurrence of cells with pentavalent configurations was regarded as positive evidence for homology between the trisome and interchanged chromosomes. Among the critical crosses, three failed to show a pentavalent association at metaphase I of F_1 trisomics. Line A-74 which was originally suspected in Group III exhibited a metaphase configuration of 1 IV + 1 III + 4 II or 1 II + 5 II + 1 I in the hybrid. However, a pentavalent was formed when paternal translocation stocks involving chromosome 5 (T_{1-5c}) were used. This indicated that it was previously misclassified and should belong to trisomic Group V (chromosome 5) instead of III. Similarly Line A-10 initially classified as trisomic for chromosome 6, also gave rise to some inconsistencies in chromosome configurations when crossed to translocation stocks involving chromosome 6. Although pentavalents were not formed in crosses with either stocks T_{1-5a} or T_{1-6c} for example, they did occur in hybrids from crosses with T_{5-6a}. The results of karyotype analyses and nucleolar organizing capacity studies (cf. Results, section 2(b) and 4(c)) provided additional evidence that Line A-10 was trisomic for chromosome 6. All other critical crosses gave positive results as shown in Table V.

3. Morphological Characteristics of the Trisomics:

The primary trisomics obtained from the progeny of

triploids differed from one another and from diploid sibs in many morphological characteristics. A preliminary grouping was made on the basis of these morphological differences. Specific differences among the seven cytologically identified trisomic types and between these and the disomic sibs were as follows:

(a) Trisomic I (Figs. 6, a; 8, a and 8, b). Trisomics for chromosome 1 were readily identified by their narrow, dark-green leaves; dwarf growth habit and high tillering capacity (thus the name "Bush"); occasionally, onion-like fused leaves; with spikes usually emerging from the side of the flag leaf. One of the three anthers in a spikelet was often degenerated. The seeds usually were narrow with a naked gap on both sides of the kernel because of incomplete attachment between lemma and palea.

(b) Trisomic II (Figs. 6, b; 8, a and 8, b). Trisomics for chromosome 2 were characterized and readily recognized by their long, narrow leaves which drooped in an almost vertical position; their large auricles and ligules; thin culms; seeds with a naked gap on both sides due to incomplete connection between lemma and palea; slender kernel, and awns compressed "accordion" style.

(c) Trisomic III (Figs. 6, c; 8, a and 8, b). Plants trisomic for chromosome 3 were short with slender culms; leaves characteristically light green in color; flag leaf usually very small and drooping with twisted tip; culm soft

FIGURE 6 - PLANT MORPHOLOGY OF TRISOMICS AND DISOMIC SIBS (CK)

(a) Trisomic I

(b) Trisomic II

(c) Trisomic III

(d) Trisomic IV

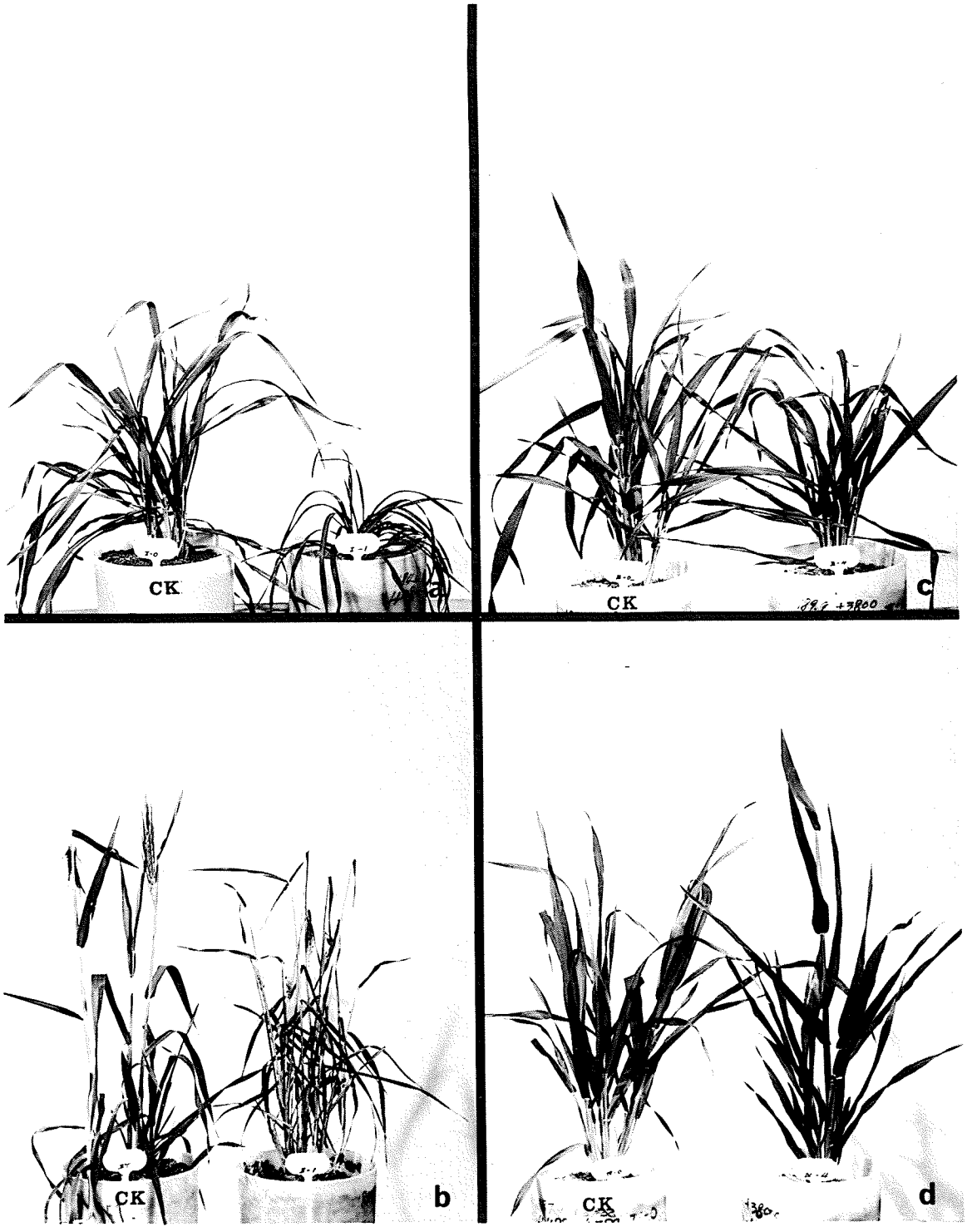

CK
a
CK
c
CK
b
CK
d

FIGURE 7 - PLANT MORPHOLOGY OF TRISOMICS AND DISOMIC SIBS (CK)

(a) Trisomic V

(b) Trisomic VI

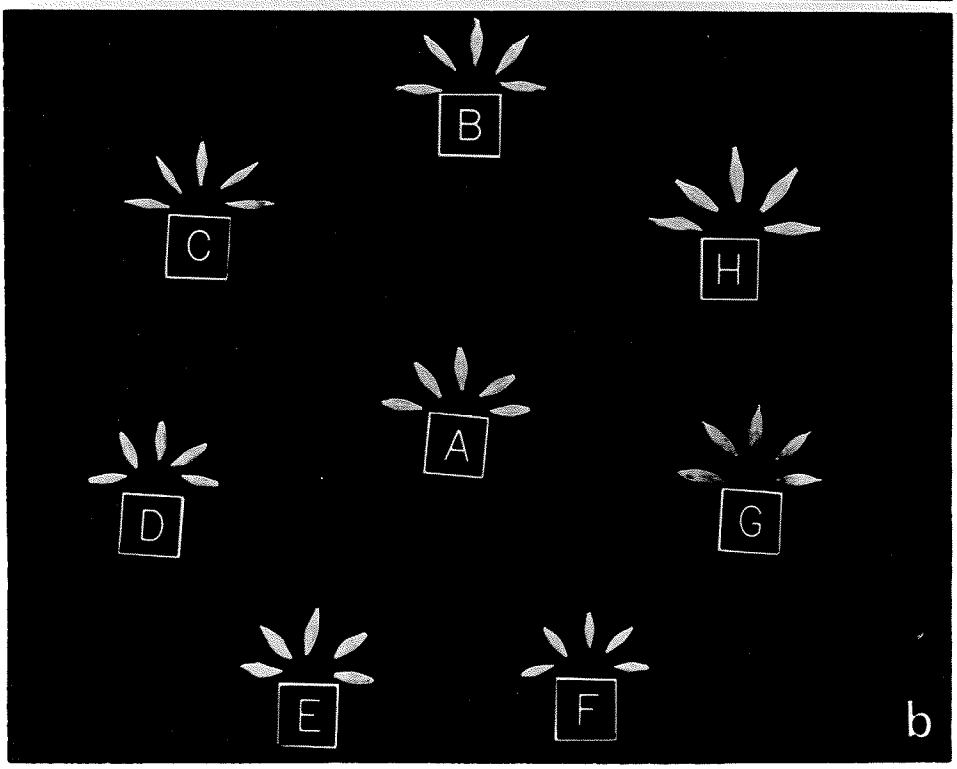
(c) Trisomic VII


CK
a
CK
b


CK
c

FIGURE 8 - COMPARISON OF SPIKE (a) AND KERNEL (b)
MORPHOLOGY OF TRISOMICS WITH DISOMIC SIBS
A: disomic check
B - H: trisomic I - VII


A
B
C
D
E
F
G
H
a


B
C
H
A
D
G
E
F
b

at maturity; basal rachis internodes somewhat curved; spikes relatively dense with very poor fertility.

(d) Trisomic IV (Figs. 6, d; 8, a and 8, b). The trisomics for chromosome 4 were characterized and readily identified by their wide leaves and thick stem; leaves very broad relative to length and dark-green in color. These identifiable characters were particularly pronounced in the flag leaf. The awns diverged giving the spike a ragged appearance. Florets and seeds were small in size in contrast to the robust leaves and stems.

(e) Trisomic V (Figs. 7, a; 8, a and 8, b). All parts of the plants trisomic for chromosome 5 were reduced in size relative to other trisomics but lacked other distinct identifiable morphological characteristics. These plants had smaller leaves with rolled margins and small lax spikes with convergent awns.

(f) Trisomic VI (Figs. 7, b; 8, a and 8, b). The trisomics for chromosome 6 were characterized by their readily identifiable oblique and twisted collars. The plants generally were very tall with few tillers; leaves broad, coarse, dark green and erect. The necks were often moderately kinked. The base of some of the awns were twisted in a kinky form; a light purple color usually could be seen at the base of mature seeds; kernels were wide and plump.

(g) Trisomic VII (Figs. 7, c; 8, a and 8, b). The

trisomics for chromosome 7 usually did not have distinct morphological characteristics which distinguished them from their disomic counterparts. During tillering and early growth stage, these plants exhibited a semi-prostrate habit particularly under field conditions; short culms, small ears and succulent leaves. Spikes were lax and produced a relatively large kernel. A marked gap between the palea and lemma usually occurred.

4. Cytological Studies of Trisomics

(a) Meiotic Studies:

Observations were made on chromosome behaviour in microsporocytes (PMCs) at diakinesis and subsequent meiotic stages of each identified trisomic. Materials subjected to meiotic studies were grown in a growth cabinet with controlled temperature (16 ± 2° C) and data from 2-4 plants of each trisomic were combined for analyses. The results of meiotic behaviour of trisomics are presented in Tables VI, VII, VIII, IX, X and XI, and a general description of each stage follows.

Diakinesis. At diakinesis (DK), pollen mother cells of all seven trisomic types contained either 6 II + 1 III or 7 II + 1 I, the former being more prevalent. All the types of trivalent configurations possible from normal pairing of three homologous chromosomes occurred and were observed in the following descending order of frequency:

ring-rod; tandem-V or tandem chain; triple-arc; and triradial. Detailed frequencies of the trivalent types were scored at metaphase I (Table VI).

Metaphase I. The frequencies of trivalent, bivalent and univalent configurations as observed in PMCs of the seven trisomics at metaphase I (MI) are shown in Table VI. The frequency of 6 II + 1 III combinations decreased in favor of 7 II + 1 I associations as the stage of cell division advanced from diakinesis to MI. The frequencies of the various types of trivalent configurations also changed from diakinesis to MI in that ring-rod (Fig. 9-a) and other configuration (Fig. 9-b & c) frequencies declined in favor of tandem trivalents (Fig. 9-d & e).

Trisomic I had the highest frequency of cells with 6 II + 1 III (81.38%) and trisomic V showed the lowest frequency (70.25%). A tandem trivalent configuration was most prevalent in six trisomic types with the exception of trisomic VI, in which a ring-rod trivalent was most common.

The position of the univalent in relation to the equatorial plate varied from cell to cell at MI and to establish if a difference existed between trisomes in their position, three trisomic types were studied, viz. trisomics I, III and IV. Univalents which were oriented at the equatorial plate with the seven normal bivalents were scored as "on-plate" univalents (Fig. 10, a & b) while those which

TABLE VI

FREQUENCIES OF CHROMOSOME ASSOCIATIONS AND TRIVALENT CONFIGURATIONS AT METAPHASE I IN SEVEN PRIMARY TRISOMIC TYPES

	Chromosome associations			Trivalent types				
Trisomic types	6 II + 1 III %	7 II + 1 I %	Total no. of cells observed	Tandem-V or -chain %	Ring-rod %	Tri-radial %	Triple-arc %	Total no. of trivalent observed
I	81.38	18.62	290	52.94	46.32	0.37	0.37	272
II	81.00	19.00	200	53.95	44.74	1.32	1.32	76
III	75.90	24.00	330	71.49	27.19	0.44	0.44	228
IV	76.91	23.09	550	68.02	30.18	0.90	0.90	222
V	73.10	26.90	548	56.88	42.66	0.46	0.00	218
VI	70.25	29.75	316	35.46	63.05	0.49	0.99	203
VII	79.03	20.97	186	58.02	40.74	0.00	1.23	81

FIGURE 9 - TYPES OF TRIVALENT CONFIGURATIONS

(a) ring-rod

(b) triradial

(c) triple-arc

(d) tandem-V

(e) tandem-chain

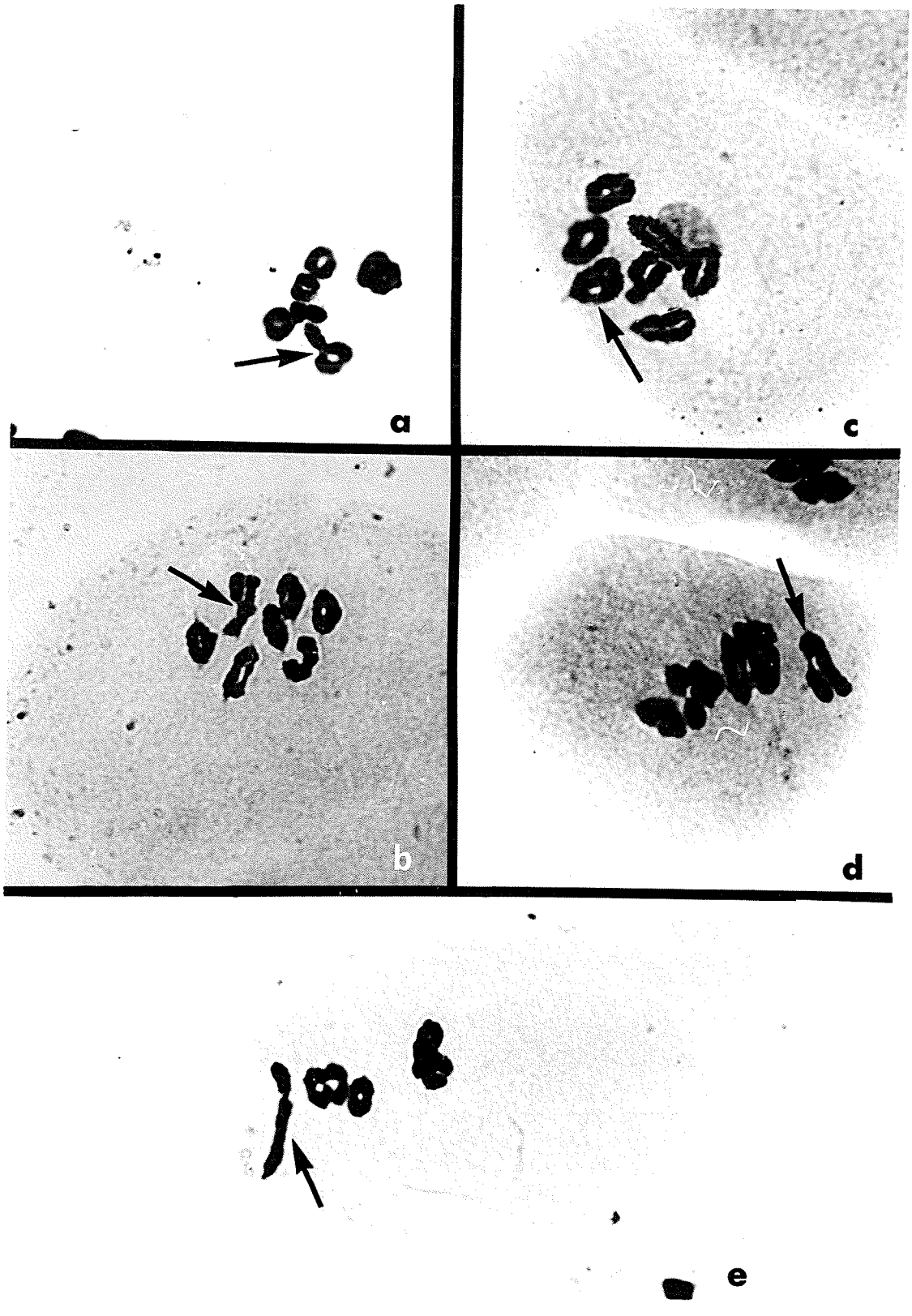
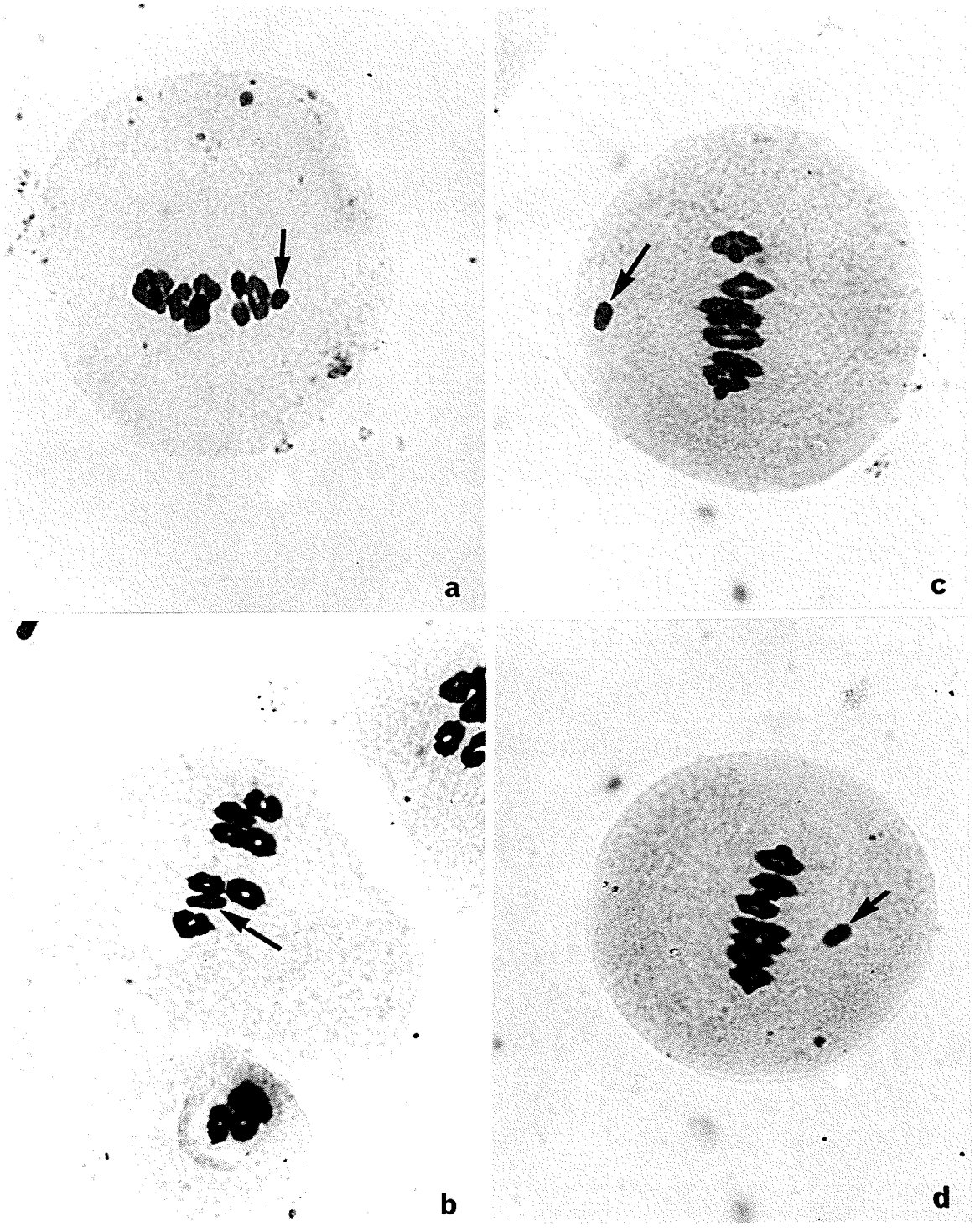

a
c
b
d
e

FIGURE 10 - POSITION OF UNIVALENT IN RELATION TO EQUATORIAL PLATE

(a, b) on-plate

(c, d) off-plate


a
b
c
d

were observed to lie in an area between the equatorial plate and the polar regions were scored as "off-plate" univalents (Fig. 10, c & d) (Table VII). The results indicated that there was no significant difference in the univalent position at MI among the three trisomic types studied. The frequencies of "on-plate" univalents were very similar to the "off-plate" frequencies.

TABLE VII

POSITION OF MI UNIVALENT IN RELATION TO THE EQUATORIAL PLATE AS STUDIED IN THREE PRIMARY TRISOMICS

Trisomic types	Total number of cells observed containing univalents	Univalents in % of total cells observed	
		On-plate	Off-plate
I	81	44.44	55.56
III	57	42.11	57.89
IV	128	57.81	42.19

Anaphase I and Telophase I. Studies of anaphase I (AI) and early telophase I (TI) revealed that the majority of the two daughter nuclei arising from trisomic mother cells contained seven or eight dyads respectively (Fig. 11, a). Presumably therefore, the univalent moved undivided to one or the other of

FIGURE 11 - CHROMOSOME BEHAVIOUR AT AI AND TI

(a) 7 - 8 disjunction at AI

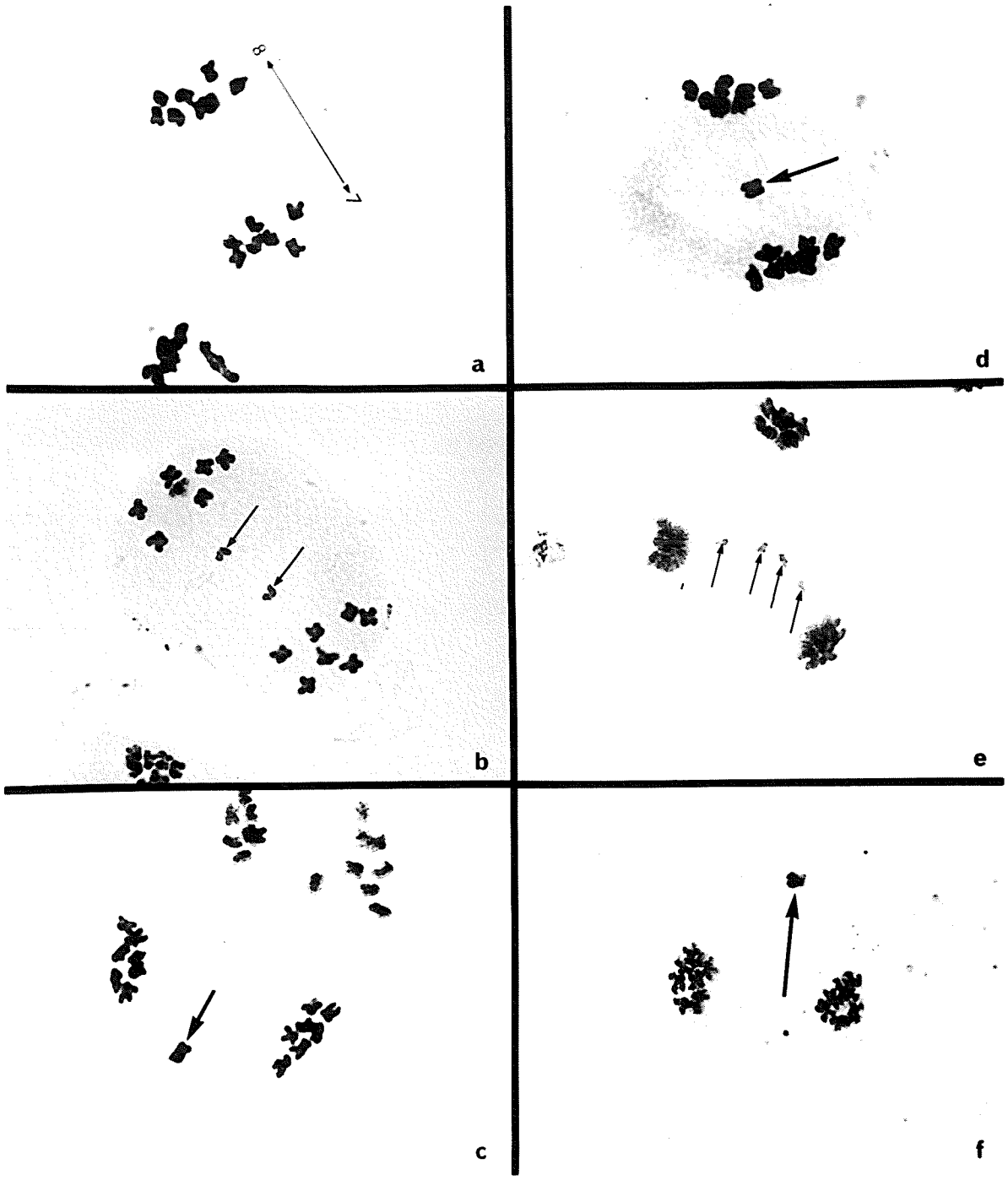
(b) lagging monads in AI

(c) lagging dyad lying at periphery of cell at AI

(d) lagging dyad at AI

(e) fragmentation at TI

(f) lagging and excluded dyad at TI


a
b
c
d
e
f

the two poles along with the other seven dyads of the normal complement. The remaining seven dyads moved to the opposite pole. About one fifth (19.07%) of the PMCs at anaphase I showed a dividing univalent (Fig. 11, b), ranging from 30.85% in trisomic VI to 15.20% in trisomic V (Table VIII). Since all seven barley chromosomes are metacentric (or nearly so), and also since their satellites can not be recognized in meiotic preparations, it was very difficult to distinguish an isochromosome from a normal chromosome at anaphase I. Consequently, there was no accurate way to unequivocally score the univalent as having divided transverseely at AI (i.e. two isochromosome products). It appeared however, that only a very small proportion of divided univalents was caused by misdivision.

At anaphase, dividing univalents usually migrated to the poles at a slower rate than the daughter chromosomes (dyads) of the seven bivalents. Occasionally, the divided univalent was noted to lie on the extreme periphery of the plate apparently beyond the influence of the spindle mechanism (Fig. 11, c). Fragmentation of the lagging chromosomes was also observed infrequently (Fig. 11, e). The laggards scored at telophase I were those which presumably would be finally excluded in the daughter nuclei, and included either one dyad, or one to two monads (Fig. 11, f). In Table VIII it is seen that the proportion of

TABLE VIII

CHROMOSOME BEHAVIOUR AT ANAPHASE I AND TELOPHASE I OF SEVEN PRIMARY TRISOMIC TYPES

Trisomic Types	Anaphase I		Telophase I	
	Total no. of cells observed	% of total cells with div. univalent	Total no. of cells observed	% of total cells with laggards
I	194	22.16	162	6.17
II	137	23.36	102	5.88
III	141	17.02	109	6.42
IV	123	18.70	181	7.73
V	250	15.20	178	9.55
VI	188	30.85	96	11.46
VII	152	15.79	98	9.18

cells with laggards for each of the seven trisomic types varied from 11.46% to 5.88%. Trisomic VI which showed the highest proportion of cells with a divided univalent had also the highest frequency of cells with laggards.

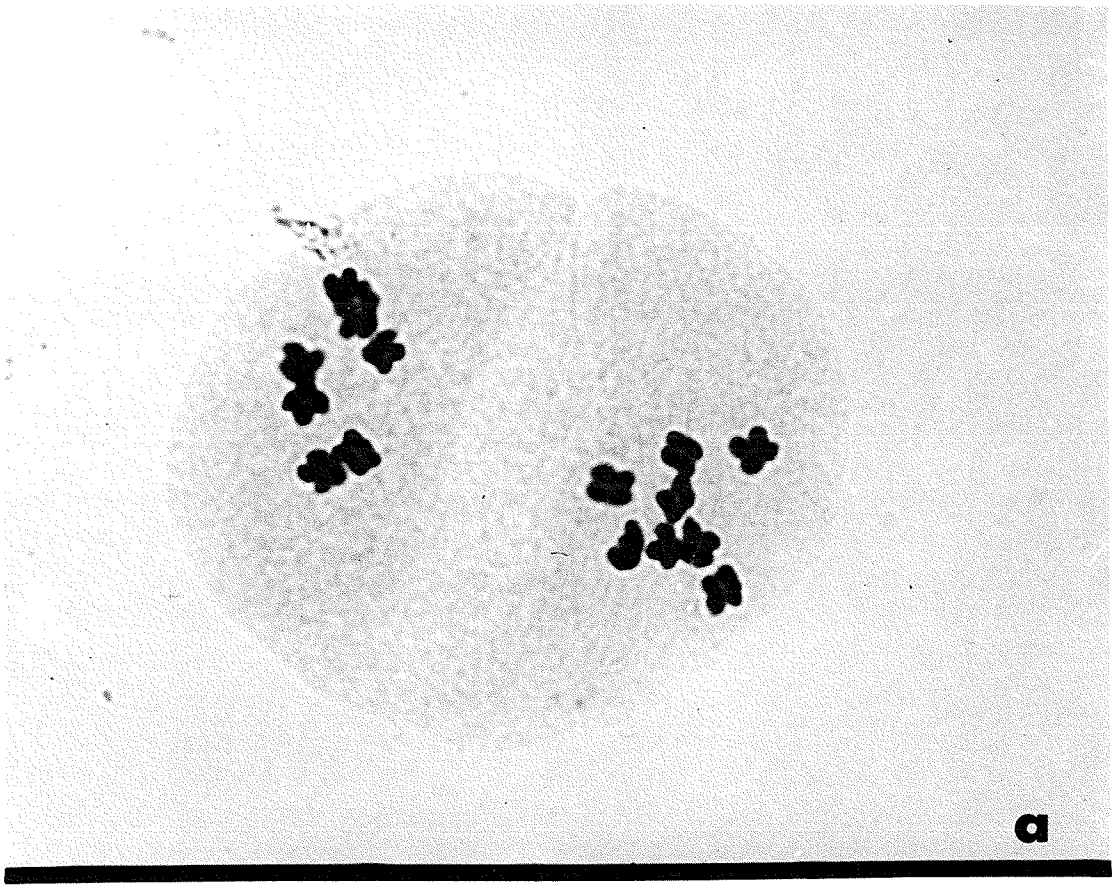
Second division. Because of the short duration of the second meiotic division, also due to the difficulty encountered in obtaining satisfactory chromosome spreads during this stage, studies of chromosome behaviour at meiotic mitosis were limited to a relatively small number of cells from each trisomic class. At metaphase II (MII), most paired daughter cells showed a 7 and 8 distribution of dyads on the plates (Fig. 12, a). Occasionally, a 7-7 alignment was observed in conjunction with lagging chromosomal material which presumably represented first division products of the extra trisome. About 20% of the observed daughter cells contained 7 dyads and one monad. These monads behaved as MI univalents, in that they were observed to be randomly distributed about the equatorial plate in association with the 7 dyads (Fig. 12, b). In a few cells, two monads with 7 dyads were observed.

The frequencies of cells with different chromosomal constitutions observed at MII in each of the seven trisomic types are presented in Table IX. The proportion of daughter cells with 7 dyads was found almost equal to those with 8 dyads. The frequency of daughter cells with 7 dyads

FIGURE 12 - CHROMOSOME BEHAVIOUR AT MII

(a) 7 - 8 distribution

(b) 7 dyads + 1 monad distribution


a

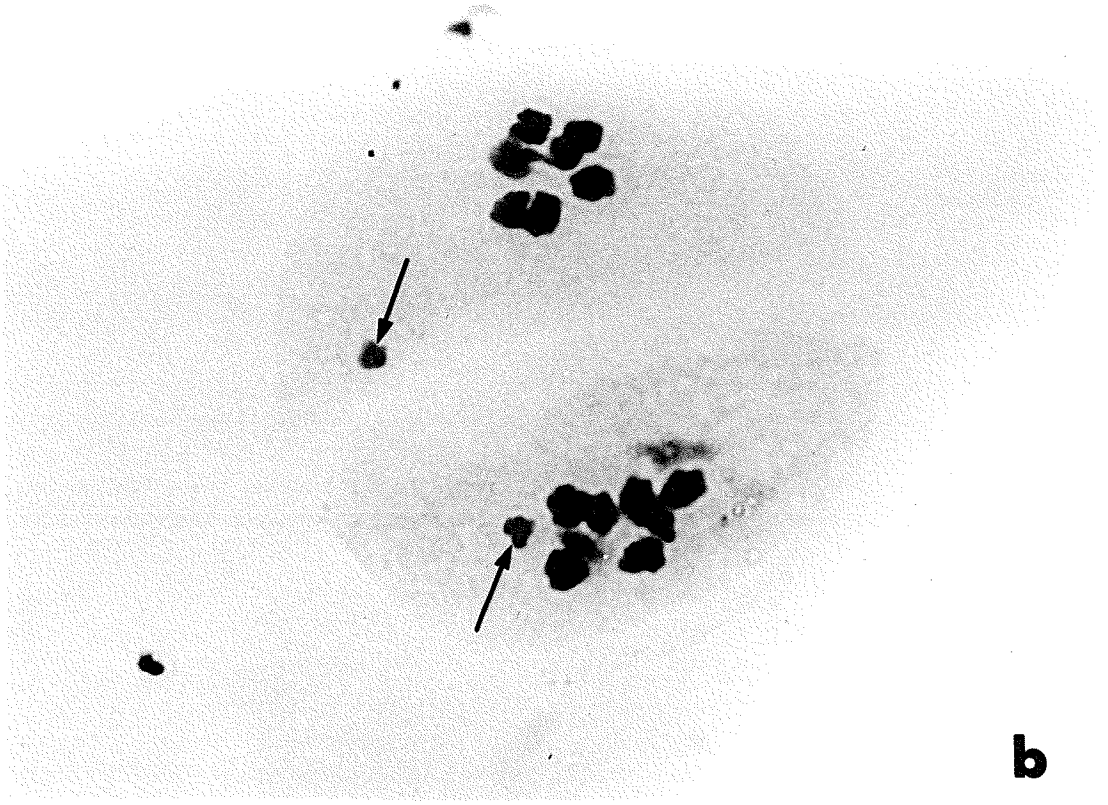

b

TABLE IX

METAPHASE II CHROMOSOME BEHAVIOUR OF SEVEN PRIMARY TRISOMIC TYPES

Trisomic types	Total no. of daughter cells observed	% of total cells observed			
		7 dyads	7 dyads + 1 monad	7 dyads + 2 monads	8 dyads
I	126	41.27	22.22	0.00	36.51
II	152	38.16	22.36	1.32	38.16
III	175	41.71	20.00	1.71	36.57
IV	255	40.78	19.61	1.18	38.43
V	234	40.17	18.38	1.28	40.17
VI	189	38.10	27.51	1.05	33.33
VII	139	42.17	14.39	1.44	41.01

plus one monad at MII ranged from 14.39% in trisomic VII to 27.51% in trisomic VI, averaging 26.63%. Less than 2% of the daughter cells showed 7 dyads and 2 monads.

In anaphase II (AII), dyads which were aligned on the equatorial plate disjoined and moved to the poles as in a normal mitotic division (Fig. 13, a). Cells scored with abnormalities at AII (Table X) included those which exhibited chromosome lagging and fragmentation (Fig. 13, b, c and e). Occasionally, a lagging monad was observed to have undergone misdivision in the centromere region (Fig. 13, f). In addition, a few paired daughter cells showed an unsynchronized separation of dyads at AII. It appeared that daughter cells with 8 dyads initiated chromosomal disjunction later than those containing the normal complement of 7 dyads (Fig. 13, d). The percentages of cells with abnormalities at anaphase II in seven trisomic types varied from 17.30% to 28.57% with average 20.71% (Table X). The highest frequency of abnormality at AII was found in trisomic VI (28.57%) while the lowest occurred in trisomic III (17.30%).

In telophase II (TII), the lagging elements either remained in the cytoplasm or finally moved to the poles. It appeared that the excluded laggards generally exhibited delayed despiralization relative to chromosomes included in the two polar nuclei. As a result, most lagging chromosomal elements were still relatively condensed at anaphase II

FIGURE 13 - CHROMOSOME BEHAVIOUR AT AII AND TII

(a) $\frac{7 - 7}{8 - 8}$ disjunction at AII

(b) $\frac{7 - 7}{7 - 7}$ disjunction at AII with lagging monads in both daughter cells

(c) $\frac{7 - 8}{7 - 7}$ disjunction at AII with single lagging monad

(d) nonsynchronized disjunction of chromosomes between two daughter cells at AII. Cell with extra chromosome tends to divide later than normal 7-chromosome cell

(e) fragmentation at TII

(f) misdivision of the extra monad at AII; 7 + telo ——— 7 + telo disjunction

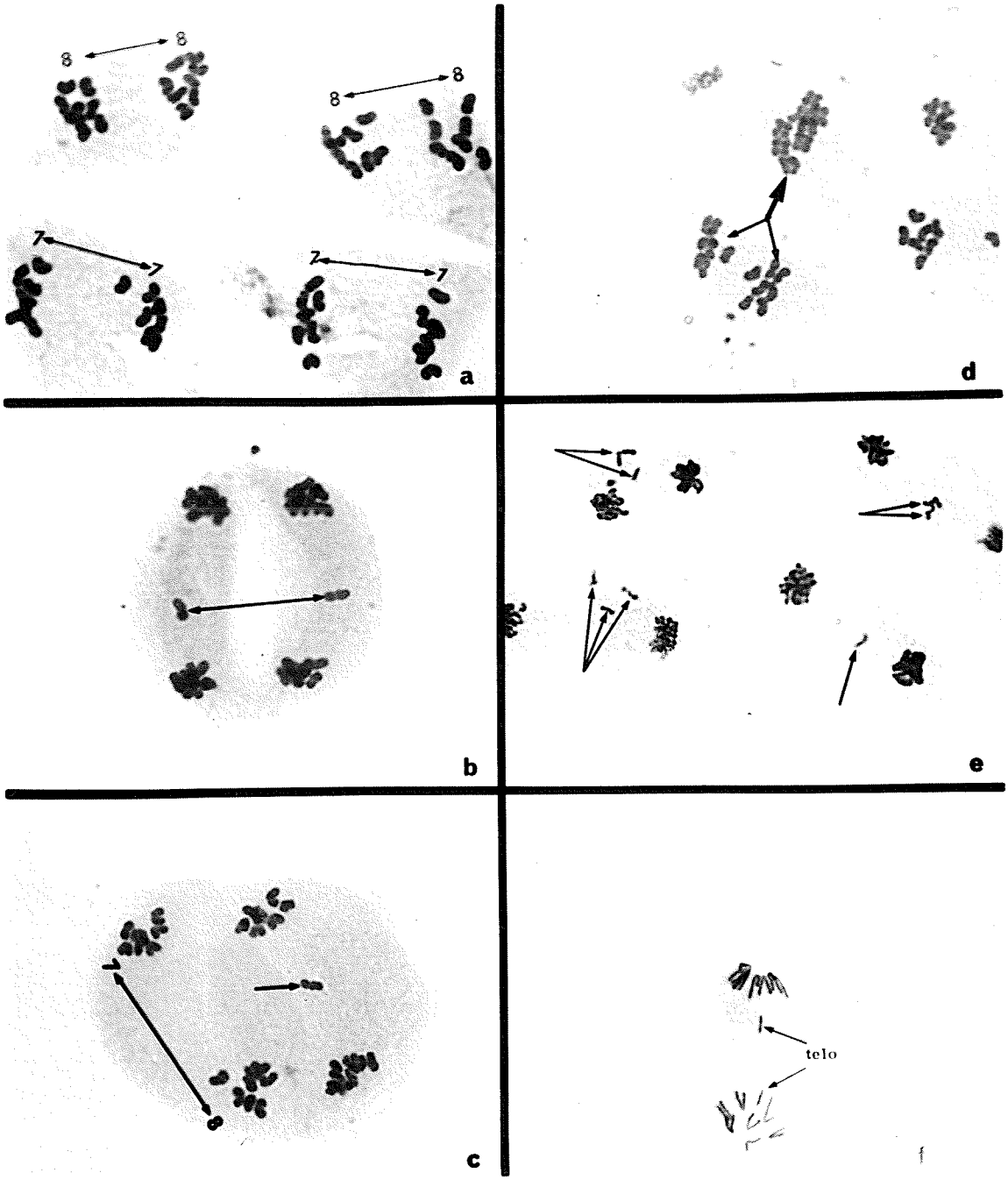

8
8
8
8
7
7
7
7
a
d
b
e
c
telo
f

TABLE X

ANAPHASE II CHROMOSOME BEHAVIOUR OF SEVEN PRIMARY TRISOMIC TYPES

Trisomic types	Total number of daughter cells observed	% of total cells with chromosomal abnormalities*
I	101	21.78
II	84	21.42
III	52	17.30
IV	54	17.30
V	205	19.51
VI	84	28.57
VII	149	18.12

* including chromosome lagging, fragmentation and misdivision.

when the daughter cells were already entering late TII.

The number of micronuclei in quartets was considered as a relative index of the number of excluded chromosomes per cell. Accordingly, the frequencies of quartets with 0, 1, 2 and 3 or more micronuclei per microspore were scored and are presented in Table XI (Fig. 14). Trisomic VI had the highest frequency (24.74%) of the quartets with micronuclei while trisomic V had the lowest (14.10%). Trisomic VI also exhibited the highest frequency of, (a) cells with univalent at MI (Table VI); (b) dividing univalents at AI (Table VIII); (c) laggards at TI (Table VIII); (d) 7 dyads and 1 monad at MII (Table IX); and (e) abnormalities at AII (Table X). These data indicate that most micronuclei in the quartet originated from the univalent at first metaphase. Moreover, if most micronuclei were formed by these univalents dividing at AI and subsequently lagging at TI, then theoretically one-half of the quartets containing two micronuclei should have them located in alternate microspores (Fig. 14, d). However, if most micronuclei were formed from a univalent which divided and lagged at AII, the resulting two micronuclei would appear in adjacent microspores (Fig. 14, c). Among the 75 quartets which had two micronuclei, 36.00% had micronuclei in alternative microspores and 64.00% showed an adjacent relationship. It appeared therefore, that there existed about an equal chance

TABLE XI

FREQUENCIES OF MICRONUCLEI IN QUARTETS OF THE SEVEN PRIMARY TRISOMIC TYPES

Trisomic Types	Total quartets observed	% of total quartets			
		Number of micronuclei per quartet			
		0	1	2	3 or more
I	114	78.95	8.77	10.53	1.75
II	164	79.27	10.98	6.71	3.05
III	108	84.26	6.48	7.41	1.85
IV	130	82.31	11.54	3.85	2.31
V	156	85.90	7.67	4.49	1.92
VI	190	75.26	11.05	9.47	4.21
VII	157	84.21	6.58	5.26	3.95

FIGURE 14 - NUMBER OF MICRONUCLEI (MN) IN QUARTETS

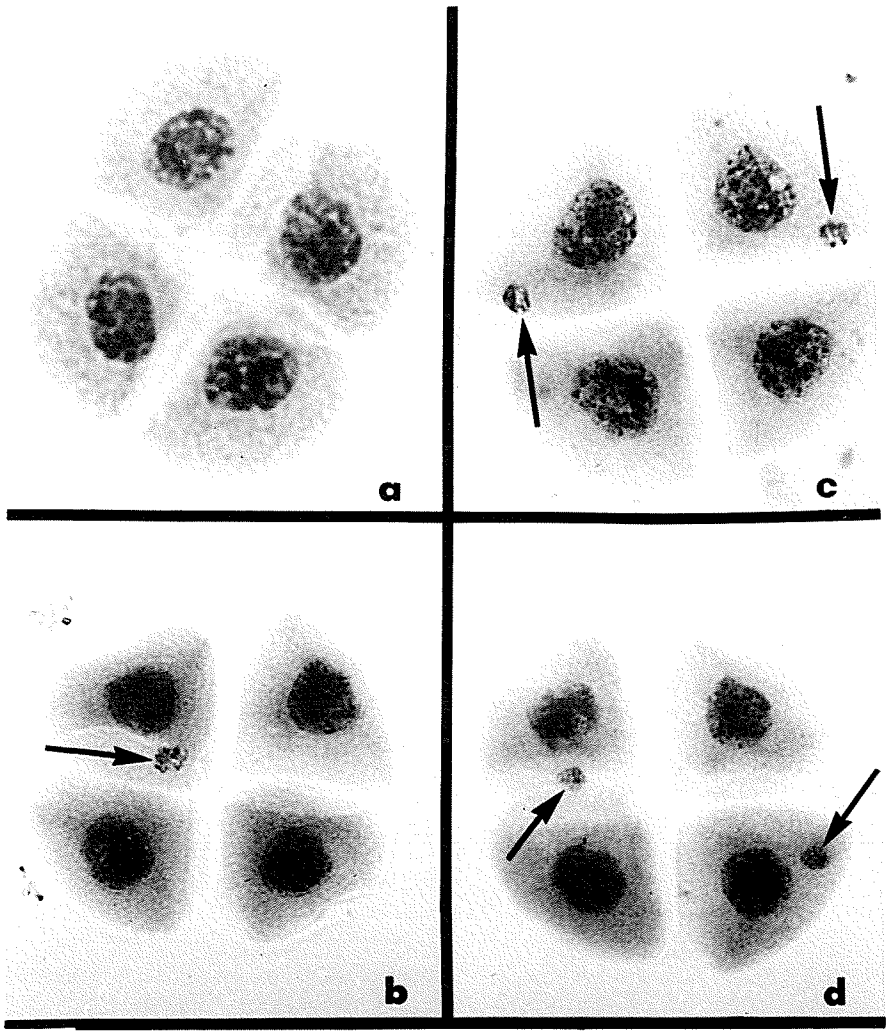
(a) non MN

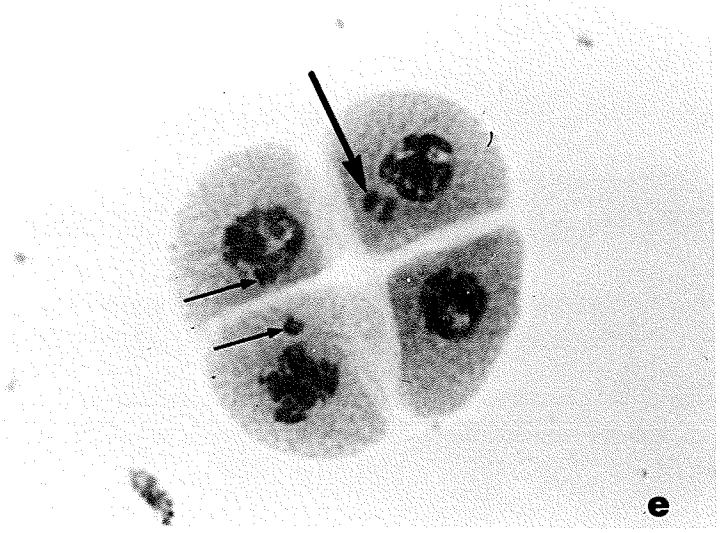
(b) 1 MN

(c) 2 MN (adjacent)

(d) 2 MN (alternate)

(e) 3 MN


a
c
b
d


e

for the univalent chromosome of a trisomic plant to divide at either the first or second meiotic division, thereby resulting in micronuclei formation as described. Only very rarely were both micronuclei observed in the one microspore.

(b) Nucleolar Organizer:

A study of the nucleolus-chromosome relationship was made on the sporocytes of trisomic VI at diakinesis. In most cases, a trivalent or a univalent plus a bivalent was observed attached to the nucleolus (Fig. 15, a). Occasionally, two nucleoli of different sizes appeared in one cell undergoing diakinesis (Fig. 15, b). In a few cases one bivalent would occur, attached to the same nucleolus which associated with the trivalent (Fig. 15, c). This indicated that there was another chromosome, in addition to No. 6, which had nucleolar organizing capacities. Previous reports of an additional nucleolar organizer in barley have been controversial (Tometorp, 1939; Burnham et al., 1954; Tsuchiya, 1960). For this reason a study was made of the sporocytes of trisomic 7 at diakinesis in order to obtain additional information. In 21.5% of 77 sporocytes examined, the trivalent was closely associated with the nucleolus which in turn was attached to a bivalent. These results indicated the existence of an additional nucleolar organizer. However, the nucleolar organizing capacity of chromosome 7 was definitely weaker than that of chromosome 6.

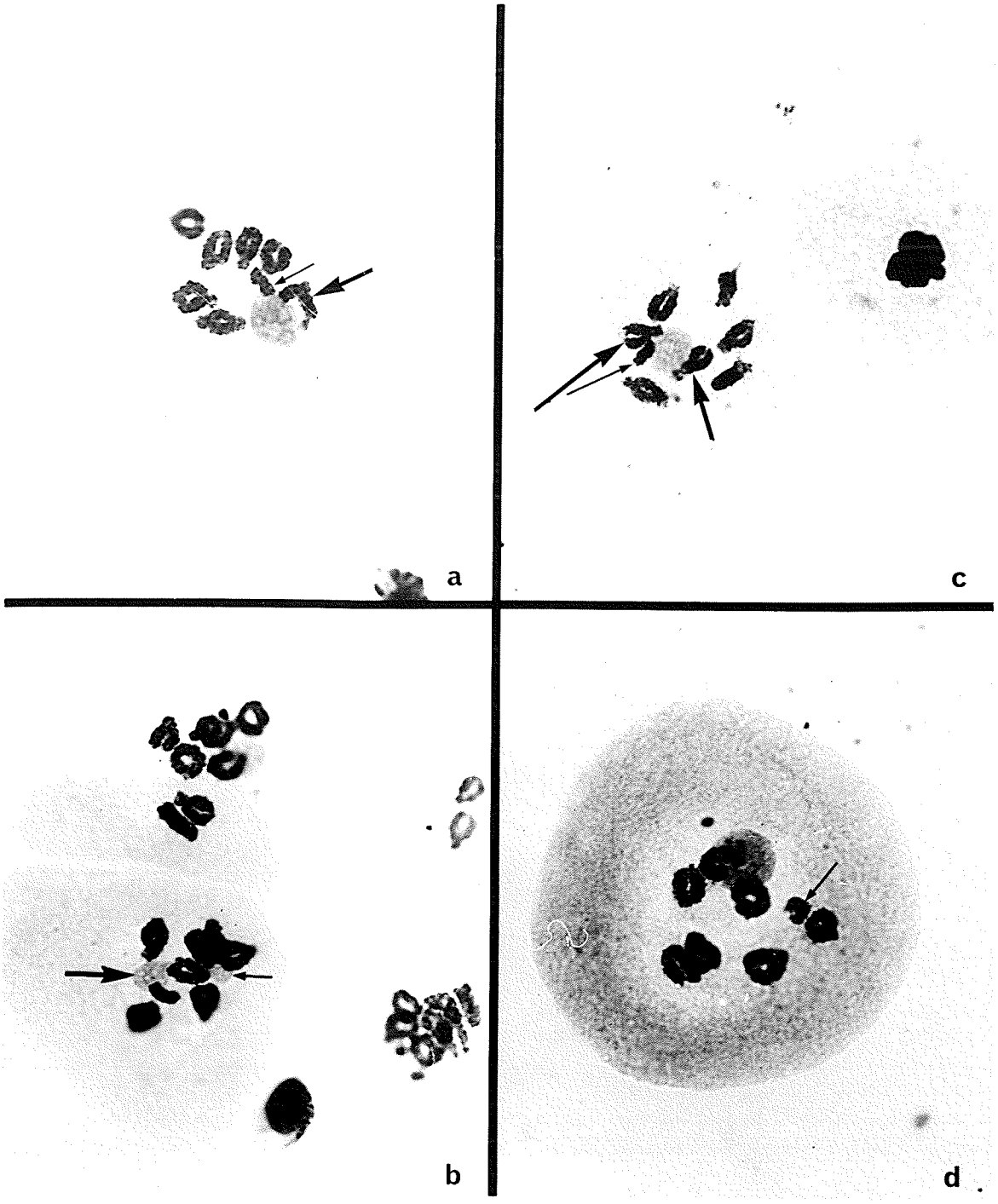
FIGURE 15 - THE BEHAVIOUR OF NUCLEOLAR CHROMOSOMES AT DIAKINESIS

(a) 1 II and 1 I associated with nucleolus of trisomic VI

(b) 1 II and 1 I associated with a larger nucleolus, and 1 II associated with a small nucleolus of trisomic VI

(c) 1 III and 1 II associated with nucleolus of trisomic VI

(d) no association of a non satellited trisome with the nucleolus (trisome 4)


a
c
b
d

(c) Pollen Mitosis:

A study of pollen mitosis was carried out in order to determine the chromosome complement of the male gametophyte of trisomic plants. Unfortunately, the results of this investigation were not completely satisfactory because of the following cytological limitations:

(i) Either the anabolic products of the cell (e.g. starch and protein) or the cellulose wall absorbed the stain and prevented clear definition of the nuclear contents of the pollen grain.

(ii) The pollen grains did not undergo synchronous division at either the first or second mitotic cycle. The critical stage of the first gametophytic division occurred at a time when the flag leaf had completely emerged, although there was a gradation in maturity from central to lateral florets of the spike. Fixation of the material at mid-day gave satisfactory results and prolonged heating of the squash preparation was found to partially overcome the staining problem. A saturated solution of mono-bromonaphthalene was applied before fixation in order to contract and to arrest the metaphase chromosomes.

From the results of limited studies of pollen mitosis in two trisomic lines (trisomic for chromosome 4 and 5), it was evident that the division cycle proceeded in two distinct "waves"--the first composed of euhaploid nuclei containing 7 chromosomes, the second consisting of aneuploid carrying

an additional chromosome (n = 8). At first pollen mitotic division, while the aneuploid nuclei were at metaphase, euhaploid nuclei had already divided and in each cell two daughter nuclei were clearly evident (generative and tube nuclei) (Fig. 16). The second mitosis of the 8 chromosome cells was not observed until close to anthesis. Obviously at the time of pollination, preferential fertilization would occur favoring the earlier maturing gametes from the nuclei with a normal chromosomal complement (n = 7).

5. Reproductive Properties of Trisomics

(a) Fertility and Germination

Trisomics of barley reported by the previous workers (Kattermann, 1939; Smith, 1941; Tsuchiya, 1950, 1959b, 1967; Ramage, 1955; Kerber, 1958) showed various degrees of sterility. The trisomics of the present study generally exhibited reasonably good fertility and germinability which enabled the easy maintenance of most trisomic lines through selfing.

Pollen viability. The materials subjected to pollen viability studies were grown in a growth cabinet with a controlled temperature and moisture regime. The level of pollen viability of the seven primary trisomics was surprisingly high when compared with the fertility (seed set) of each trisomic type. The highest percentage of good pollen was found in trisomic VII of 86.64% which was closely

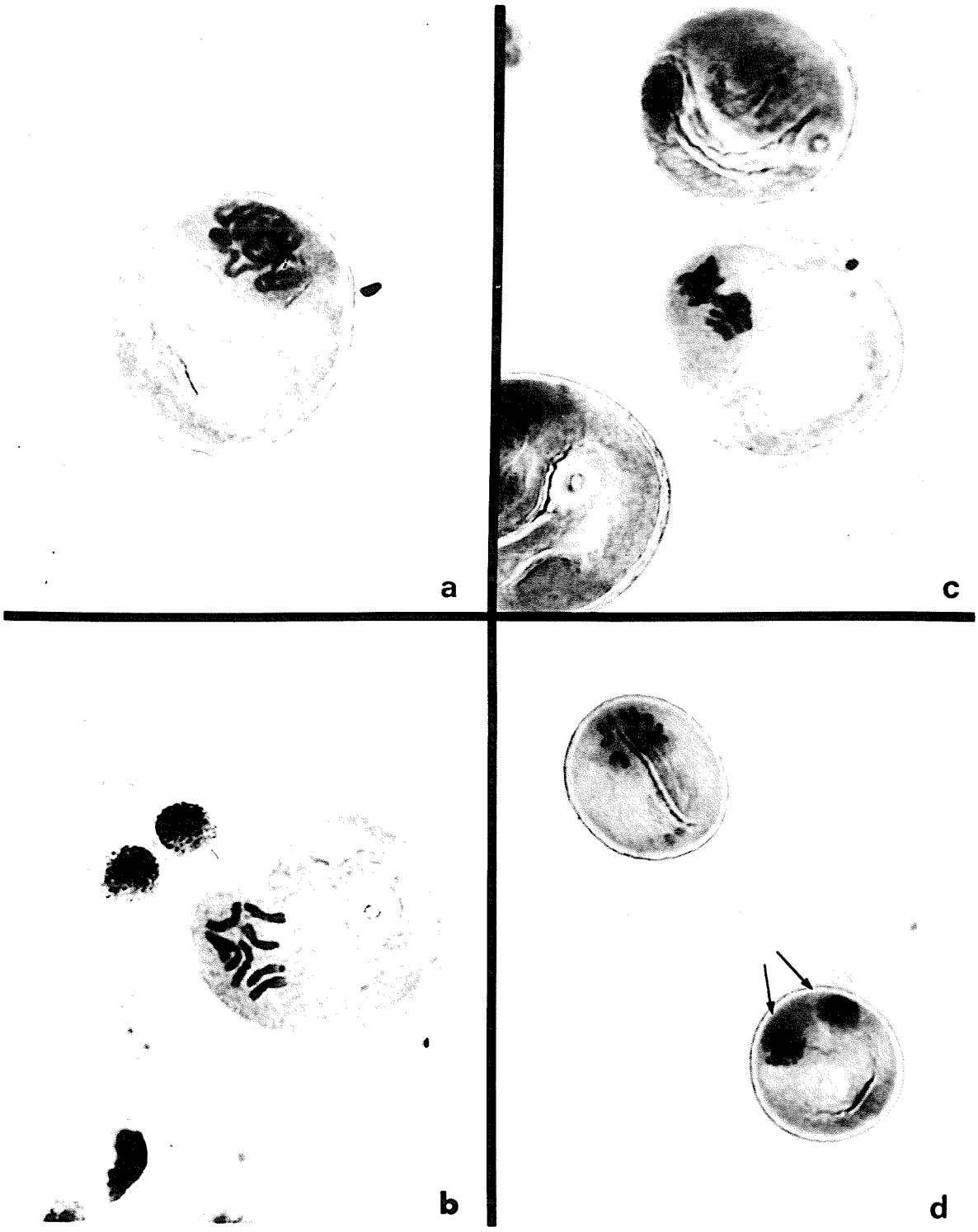
FIGURE 16 - POLLEN MITOSIS OF A BARLEY TRISOMIC

(a) prophase

(b) metaphase

(c) anaphase

(d) metaphase of an 8-chromosome gametophyte adjacent to a presumed euhaploid pollen grain with 2 nuclei.


a
c
b
d

followed by trisomic IV with 86.62%, while trisomic I exhibited the lowest pollen viability of 61.89% (Fig. 17) (Table XII). Interestingly, trisomic III which produced the lowest per cent seed-set among the seven trisomics (7.64%), exhibited a high percentage pollen viability (83.71%).

Floral fertility. It had been noticed that the seed-setting ability of trisomic plants was greatly affected by environment. It was impossible for example, to obtain seed set on plants of trisomic I and III under field conditions. Similarly, the per cent seed-set on trisomics II, V and VI was greatly reduced when grown in the field. Cool, moist conditions were conducive to satisfactory seed-set on plants of all seven trisomic types. Progenies from selfed trisomic plants were used in the study of seed fertility and were grown in a growth cabinet at relatively low temperatures (16 ± 2° C) and controlled moisture levels.

As shown in Table XIII, the highest seed-set was found on trisomic V (63.89%) and the lowest was observed on trisomic III (7.64%). Hand pollination was necessary to produce sufficient seed development on trisomic III in order to ensure its propagation.

Germination rate. Germination rates were studied on seed from selfed trisomics representing each of the seven groups. Seeds after harvesting were stored for a period of at least four weeks before germination. In general,

FIGURE 17 - POLLEN GRAINS STAINED WITH KI_2 SOLUTION

(a) trisomic I (lowest viability)

(b) trisomic II

(c) trisomic III

(d) trisomic IV

(e) trisomic V

(f) trisomic VI

(g) trisomic VII (highest viability)

(h) disomic check

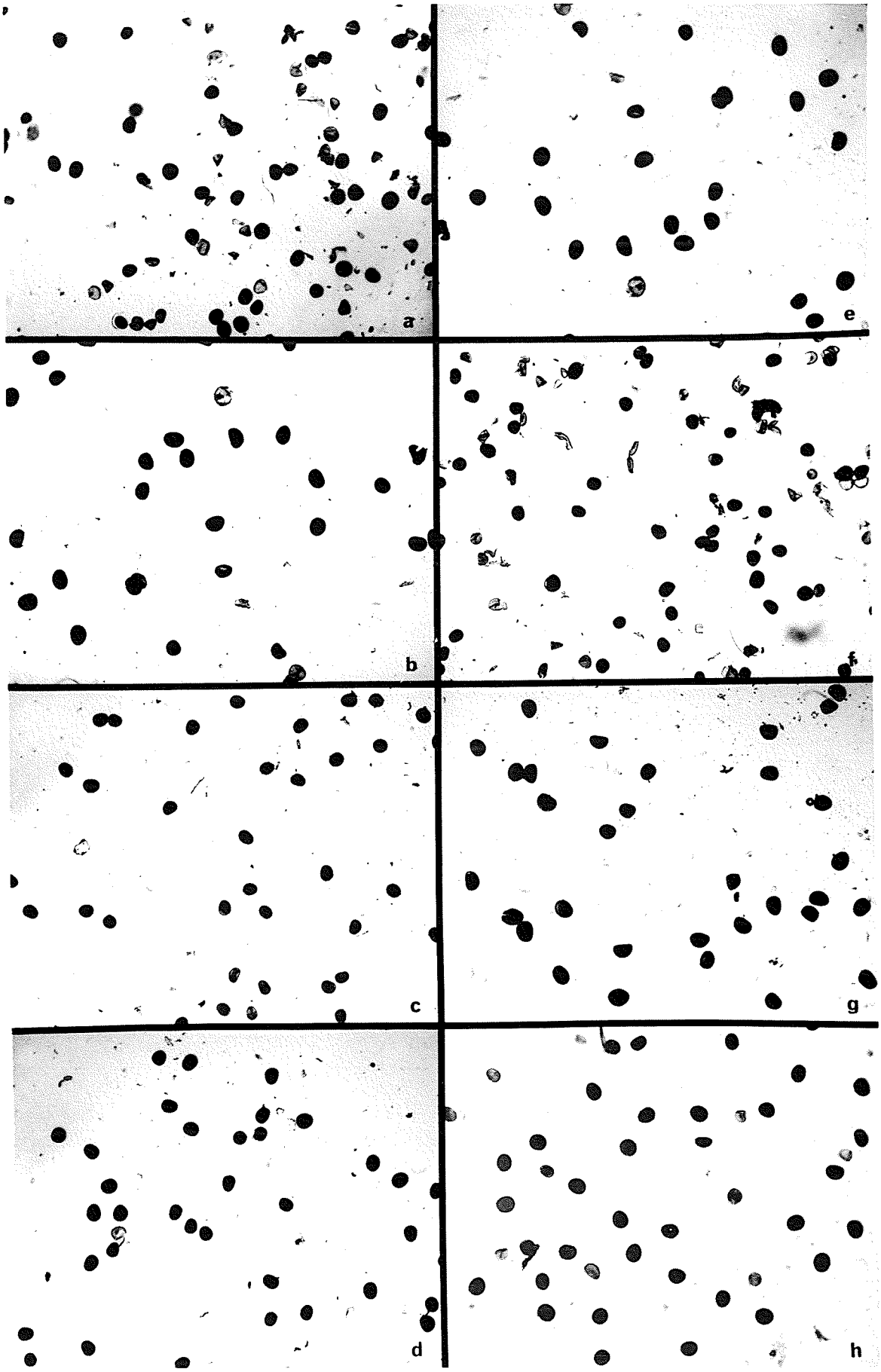

a
e
b
f
c
g
d
h

TABLE XII

AVERAGE POLLEN VIABILITY OF
SEVEN PRIMARY TRISOMIC TYPES

Trisomic Types	Number of pollen grains			Good pollen (%)
	Total	Good	Degenerated	
I	795	492	303	61.89
II	596	468	128	78.52
III	1068	894	174	83.71
IV	852	738	114	86.62
V	1010	797	213	78.91
VI	728	504	224	69.23
VII	891	772	119	86.64
Disomic	1580	1428	152	90.38

TABLE XIII

AVERAGE SELF FERTILITY AND SEED GERMINATION RATE OF SEVEN PRIMARY TRISOMIC TYPES

Trisomic Types	Number of Florets	Number of Seeds	Fertility (%)	Germination (%)
I	120	65	54.17	84.84
II	78	28	35.90	80.49
III	144	11	7.64	88.17
IV	405	249	61.48	92.04
V	576	386	63.89	89.19
VI	144	65	45.14	90.20
VII	143	94	58.74	85.14

trisomic seeds had a tendency to germinate later than disomic seeds harvested from the same plant. It was found however, that a pretreatment of 10 to 14 days at relatively low temperature (2° C), was necessary to induce a rapid and uniform germination rate of seeds of trisomic constitution.

As shown in Table XIII, following pretreatment the germination rates of seeds from the selfed trisomic progenies of all seven types ranged from a high of 92.04% (trisomic IV) to a low of 80.49% (trisomic II).

(b) Transmission of the Extra Chromosome in Trisomics

Data on the frequencies of recovery of trisomic, disomic, telotrisomic, and triploid individuals among the selfed progenies of each of the seven primary trisomic types were accumulated during the course of this study (Table XIV). The telotrisomes were either true telocentrics or acrocentrics. The recovery of a triploid from the progeny of trisomics was actually very rare in that only one $2n = 21$ seedling was recorded from more than 3000 cytologically examined seeds. The frequency of recovery of trisomics from the progeny of selfed trisomics, ranged from 16.18% to 33.19% depending upon the parental trisomic, and averaged 26.09% as shown in Table XIV. The single triploid was recovered from among 336 selfed progeny of trisomic I.

Transmission via the egg. The progenies from crosses between trisomics and reciprocal translocation homozygotes

TABLE XIV

TRANSMISSION RATE OF THE EXTRA CHROMOSOME IN SEVEN PRIMARY TRISOMIC TYPES

Trisomic Types	Total number of seeds studied	Chromosome number 2n=14	2n=15	others[1]
		Progeny of selfed trisomics(%)		
I	336	72.62	25.00	2.38
II	122	69.67	28.69	1.64
III	445	83.15	16.18	0.67
IV	476	65.97	33.19	0.84
V	644	72.52	27.02	0.47
VI	291	77.58	24.05	1.03
VII	264	68.94	30.68	0.38
		Progeny of (2n+1) x 2n [2]		
I	60	83.33	15.00	1.67
II	40	77.50	22.50	0.00
III	74	78.38	20.27	1.35
IV	132	68.94	29.55	1.52
V	165	80.00	20.00	0.00
VI	93	83.87	16.13	0.00
VII	55	80.00	20.00	0.00

[1]Others include telotrisomics and triploid

[2]Reciprocal translocation stocks used as pollen plants

(2n = 14) were used to study the transmission of the extra chromosome through the egg. The results of the frequencies of trisomics among the progenies from (2n + 1) x (2n) crosses are shown in Table XIV. The egg transmission rates varied from a low of 15.00% in trisomic I to a high of 29.55% in trisomic IV with an average of 21.61% transmission among all seven different trisomic types.

Transmission via the pollen. Only very limited data were obtained on the frequency of transmission of the extra chromosome through the pollen. From a total of 58 seeds produced from crosses between disomics used as female x various trisomic types as male, no trisomics were recovered. It is recognized that these data are not adequate to enable one to draw specific conclusions. However supplementary information pertaining to male transmission frequencies was available from a comparison of the number of recovered trisomics from selfed vs. crossed trisomics in which the male was disomic (Table XIV). The selfed progenies generally contained a higher frequency of trisomics than progenies produced from crosses involving disomic male parents (26.09% as compared with 21.16% respectively). This strongly suggests that in general the extra chromosome was transmitted at a very low frequency through the pollen. In trisomic III, frequency of trisomics among the selfed progeny (16.18%) was even lower than that among the progeny from trisomics crossed with disomic male plants (20.27%).

The low transmission rate of selfed trisomic III plants must be affected by its own pollen source. The extremely low seed fertility observed in trisomic III also supports this conclusion.

6. Quality Studies of the Trisomics:

The established primary trisomics derived and identified in this study were used as basic tools to study the genetical basis of some components of malting and feeding quality. Analyses were made of total crude protein content, amino acid composition and total and free beta-amylase activity of seed samples. As indicated earlier, only endosperm tissue was used for chemical analyses; the embryo of each seed was excised for purposes of establishing its chromosome constitution.

(a) Protein content:

On the basis of dry matter, only small differences were observed in the total crude protein content of the endosperm among the seven disomic checks. Comparisons between each disomic check and its trisomic counterpart however, revealed differences of a far greater magnitude (Table XV). The presence or absence of an extra chromosome 3 had little effect on protein content. On the other hand, both chromosome 1 and 2 increased protein content markedly. With the possible exception of trisomic II, protein content of endosperm tissue harvested from individual

TABLE XV

CRUDE PROTEIN CONTENT OF SEED ENDOSPERM TISSUE AS A PER CENT OF DRY MATTER IN SEVEN BARLEY TRISOMICS

Chromosome constitutions	Maternal plant seed embryo tissue		Disomic (2n=14) 2n=14	Trisomic (2n=15) 2n=14	Trisomic (2n=15) 2n=15
% ± S. D. of protein	TRISOMIC TYPES	I	10.12±0.56	15.00±0.72	16.32±0.71
		II	10.02±0.45	13.00±0.69	16.61*
		III	9.59±0.46	11.74±0.68	11.60±0.36
		IV	10.21±0.41	12.80±0.50	14.68±0.08
		V	9.98±0.49	12.89±0.65	14.31±0.72
		VI	9.57±0.76	11.93±0.48	13.41±0.33
		VII	9.90±0.14	11.53±0.02	13.68±0.67

*Single trial

trisomic plants did not vary more than two per cent regardless of whether the seed was trisomic or disomic (Table XV). However, since trisomic seed was generally smaller than disomic seed, and contained correspondingly less carbohydrate in relation to the total yield it was necessary to convert protein level to a single kernel basis. Expressing protein percentage in this way, resulted in differences in protein content between trisomics and disomics being greatly reduced (Table XVI). Nevertheless, the possible influence of chromosome 2 was still evident from the converted data since a relatively wide deviation in protein level (2.64%) occurred between endosperm from trisomic seeds as compared with diploid tissue from kernels on the same trisomic parent plant.

(b) Amino Acid Composition:

The amino acid composition of seed (endosperm) from seven different barley trisomic types was analyzed and the results are shown in Table XVII. Along with each trisomic type, seeds from each of their disomic sib plants were used as control material.

Among the eighteen different amino acids analyzed, lysine was given particular attention because of its importance in feeding rations. Results indicated that with exception of chromosome 4, which showed a positive effect, all trisomic seeds exhibited a reduced lysine content

TABLE XVI

CRUDE PROTEIN CONTENT OF SEED ENDOSPERM TISSUE AS A PER CENT OF SINGLE KERNEL WEIGHT IN SEVEN BARLEY TRISOMICS

Chromosome constitution	Maternal plant seed embryo tissue		Disomic (2n=14) 2n=14	Trisomic (2n=15) 2n=14	Trisomic (2n=15) 2n=15
% of protein per kernel	TRISOMIC TYPES	I	19.08	20.78	19.26
		II	19.24	20.41	17.17
		III	17.98	19.31	18.16
		IV	20.16	21.57	21.51
		V	19.25	19.33	18.33
		VI	18.18	20.95	21.15
		VII	18.17	21.79	20.99

TABLE XVII

AMINO ACID COMPOSITION OF SEED ENDOSPERM AS A PER CENT OF PROTEIN IN SEVEN BARLEY TRISOMICS

Amino Acid	Chromosome constitution		Trisomic types						
	Maternal plant	Seed embryo	I	II	III	IV	V	VI	VII
Alanine	Disomic	2n=14	3.70	4.08	3.98	3.76	4.18	3.97	4.31
	Trisomic	2n=14	3.67	3.31	3.68	3.34	3.06	3.44	3.48
		2n=15	3.95	3.46	3.68	3.49	3.19	3.69	3.70
Arginine	Disomic	2n=14	3.70	5.41	4.52	3.96	4.76	4.90	5.40
	Trisomic	2n=14	4.11	4.01	4.62	4.28	4.55	4.03	4.26
		2n=15	4.57	4.00	4.72	4.91	3.98	4.46	4.72
Aspartic acid	Disomic	2n=14	4.91	4.96	5.09	4.94	5.20	4.95	5.37
	Trisomic	2n=14	4.22	3.81	4.60	4.36	3.45	4.28	4.22
		2n=15	4.53	4.11	4.61	4.49	3.87	4.83	4.52
Cysteine	Disomic	2n=14	2.48	3.38	2.99	2.34	2.66	2.96	2.74
	Trisomic	2n=14	3.22	4.07	3.13	3.15	1.89	2.59	3.46
		2n=15	2.45	4.16	2.80	3.62	2.10	1.48	3.96
Glutamic acid	Disomic	2n=14	26.89	23.80	25.36	24.30	24.80	24.75	23.30
	Trisomic	2n=14	26.00	26.90	25.60	26.67	22.44	27.91	26.00
		2n=15	25.60	26.80	25.70	25.10	31.87	27.94	24.90
Glycine	Disomic	2n=14	3.67	4.25	4.23	3.89	4.24	4.03	4.31
	Trisomic	2n=14	3.56	3.51	3.86	3.46	3.69	3.46	3.66
		2n=15	3.91	3.44	3.95	3.11	3.35	3.75	3.90
Histidine	Disomic	2n=14	1.97	2.56	2.19	2.30	2.52	2.45	2.71
	Trisomic	2n=14	2.52	2.37	2.25	1.98	2.56	1.96	2.23
		2n=15	2.63	2.09	2.59	1.98	1.77	2.39	2.25
Isoleucine	Disomic	2n=14	3.76	3.70	3.71	3.82	3.79	3.75	3.76
	Trisomic	2n=14	3.71	3.67	3.66	3.53	3.51	3.57	3.57
		2n=15	3.77	3.66	3.68	3.15	3.35	3.67	3.75
Leucine	Disomic	2n=14	7.15	7.34	7.53	7.09	7.51	7.20	7.27
	Trisomic	2n=14	7.04	6.94	6.97	6.80	6.98	6.91	7.06
		2n=15	7.50	6.46	7.25	6.81	6.69	7.30	7.01

TABLE XVII (continued)

Amino Acid	Chromosome constitution: Maternal plant	Seed embryo	Trisomic types: I	II	III	IV	V	VI	VII
Lysine	Disomic	2n=14	3.06	3.52	3.49	3.11	3.57	3.91	3.31
	Trisomic	2n=14	2.66	2.69	2.74	2.92	3.05	3.44	2.47
		2n=15	3.00	2.84	2.69	3.40	2.71	3.08	2.75
Methionine	Disomic	2n=14	1.44	2.28	1.31	1.38	1.69	2.21	2.49
	Trisomic	2n=14	1.88	2.56	2.05	1.27	1.32	1.66	2.18
		2n=15	2.15	2.75	2.30	1.91	1.27	0.58	2.17
NH_3	Disomic	2n=14	5.28	4.96	4.87	7.02	5.09	4.90	5.31
	Trisomic	2n=14	5.63	5.12	5.12	5.78	5.37	5.57	4.85
		2n=15	4.83	6.46	4.53	5.45	5.52	5.07	4.70
Phenyl-alanine	Disomic	2n=14	6.03	4.76	4.59	5.17	4.65	4.71	4.98
	Trisomic	2n=14	5.31	5.84	4.99	4.95	4.71	5.31	4.89
		2n=15	5.50	5.24	5.23	5.08	4.75	5.25	4.94
Proline	Disomic	2n=14	12.49	9.81	12.06	14.27	10.40	10.40	9.81
	Trisomic	2n=14	12.00	11.90	12.10	14.34	11.51	14.21	13.50
		2n=15	11.00	11.70	11.70	13.90	12.79	12.34	12.30
Serine	Disomic	2n=14	3.31	3.78	3.60	3.43	3.85	3.82	3.83
	Trisomic	2n=14	3.48	3.32	3.76	3.49	2.89	2.50	3.55
		2n=15	3.62	3.33	3.81	3.26	3.17	3.55	3.59
Threonine	Disomic	2n=14	3.15	3.31	3.20	2.92	3.61	3.35	3.41
	Trisomic	2n=14	3.03	2.89	3.24	3.17	2.34	2.17	3.23
		2n=15	3.16	2.82	3.23	3.00	2.71	3.10	3.24
Tyrosine	Disomic	2n=14	1.73	2.84	1.61	1.01	2.04	2.33	2.12
	Trisomic	2n=14	2.39	2.40	2.37	1.65	1.98	2.28	2.21
		2n=15	2.43	2.00	2.39	2.42	2.04	2.43	2.38
Valine	Disomic	2n=14	5.24	5.46	5.74	5.51	5.47	5.40	5.60
	Trisomic	2n=14	5.29	4.68	5.30	4.84	4.71	4.76	5.17
		2n=15	5.37	5.07	5.15	4.96	4.85	5.11	5.22

relative to their diploid counterpart. The results also showed that an extra dose of any of the seven different chromosomes decreased the aspartic acid content in barley seed endosperm. Similarly, glutamic acid content was decreased by all chromosomes except chromosome 1. Chromosomes 2 and 6 showed some positive association with phenylalanine while 1 and 3 appeared to have some effect in increasing histidine content. Arginine was increased when either chromosome 1 or 4 was present in triplicate and ammonia showed an increase with an extra dose of either chromosome 2 or 5. There existed a positive relationship between the amino acid content of disomic and trisomic seeds from the same trisomic plant which indicated a genetic influence of the maternal plant on amino acid content of the kernel.

(c) Total beta-amylase:

The total beta-amylase (saccharifying) activity of seven trisomic seeds (endosperm) was determined as a per cent of air-dried matter (Table XVIII). In view of the variation in size between the trisomic and disomic seeds, all data were expressed on the basis of a single kernel (Table XIX). In general, total beta-amylase activity of seeds from trisomic plants was higher than that from seeds of disomic sib plants. When disomic and trisomic seeds from only trisomic plants were compared and expressed as a percentage of each disomic check, results indicated that the presence of

TABLE XVIII

TOTAL BETA-AMYLASE SACCHARIFYING ACTIVITY OF SEED ENDOSPERM AS A PER CENT OF AIR-DRIED MATTER IN SEVEN BARLEY TRISOMICS

Trisomic types	% ± S. D. of maltose formed per 5 min.		
	Disomic plant (2n=14)	Trisomic plant (2n=15)	
	Disomic embryo 2n=14	Disomic embryo 2n=14	Trisomic embryo 2n=15
I	19.16±0.84	52.33±0.51	53.41±0.23
II	18.13±0.23	41.45±0.01	53.36±0.19
III	17.52±0.33	26.54±0.14	21.82±0.23
IV	18.60±0.47	24.86±0.23	39.67±0.19
V	17.90±0.37	29.25±0.79	31.12±0.29
VI	18.69±0.37	24.90±0.19	28.32±0.33
VII	17.62±0.51	24.62±1.12	28.18±0.14
	% of disomic check		
I	100	273.1	278.8
II	100	228.6	294.3
III	100	151.5	124.5
IV	100	133.7	213.3
V	100	165.5	173.9
VI	100	133.3	151.5
VII	100	139.8	133.3

TABLE XIX

TOTAL BETA-AMYLASE SACCHARIFYING ACTIVITY OF SEED ENDOSPERM AS A PER CENT OF SINGLE KERNEL WEIGHT IN SEVEN BARLEY TRISOMICS

	% ± S. D. of maltose formed per 5 min.		
	Disomic plant (2n=14)	Trisomic plant (2n=15)	
Trisomic types	Disomic embryo 2n=14	Disomic embryo 2n=14	Trisomic embryo 2n=15
I	36.12	72.43	63.03
II	34.81	65.09	57.10
III	32.85	43.36	34.16
IV	36.73	41.87	57.90
V	34.53	43.88	39.97
VI	35.51	43.83	43.04
VII	32.62	45.14	43.27
	% of disomic check		
I	100	200.7	174.6
II	100	187.0	164.0
III	100	132.0	104.0
IV	100	114.0	157.6
V	100	127.1	115.8
VI	100	123.4	121.2
VII	100	139.6	133.8

either chromosome 1 or 2 in the trisomic condition markedly increased total beta-amylase content. Interestingly, disomic seeds from trisomic 4 also showed a considerable increase whereas trisomic seeds from the same parental trisomic line exhibited only a moderate increase in amylase activity in comparison with its diploid sib.

(b) Free beta-amylase:

The chromosomal effect on the free beta-amylase activity appeared to be more variable than on total beta-amylase (Table XX, XXI). When an extra chromosome of 1, 2, 4 or 7 was present, a marked increase in free beta-amylase activity was found to occur. Seeds with disomic embryos from trisomic VII plants contained very high free beta-amylase activity but when an extra chromosome 7 was present in the embryo, the activity dropped sharply (Table XXI).

The total beta-amylase activity was considered to include bound as well as free beta-amylase. The amount of free beta-amylase as a per cent of the total is shown in Table XXII. These results clearly indicated that the marked increase of free beta-amylase was mainly associated with chromosome 7. The high value recorded for free beta-amylase in diploid seed from plants trisomic for chromosome 7 was reduced close to that of the diploid sib level when seed bearing only trisomic embryos was compared.

TABLE XX

FREE BETA-AMYLASE SACCHARIFYING ACTIVITY OF SEED ENDOSPERM AS A PER CENT OF AIR-DRIED MATTER IN SEVEN BARLEY TRISOMICS

Trisomic types	% ± S. D. of maltose formed per 5 min.		
	Disomic plant (2n=14)	Trisomic plant (2n=15)	
	Disomic embryo 2n=14	Disomic embryo 2n=14	Trisomic embryo 2n=15
I	7.59±0.17	18.65±0.41	25.13±0.23
II	7.60±0.08	17.61±0.30	26.42±0.47
III	6.93±0.08	12.12±0.08	11.92±0.06
IV	6.67±0.05	14.57±0.09	21.03±0.19
V	7.23±0.13	13.91±0.50	15.03±0.20
VI	6.90±0.06	11.87±0.33	12.21±0.33
VII	7.57±0.03	17.26±0.33	15.54±0.46
	% of disomic check		
I	100	245.7	331.1
II	100	231.7	347.6
III	100	174.9	172.0
IV	100	218.4	315.3
V	100	192.4	207.9
VI	100	172.0	177.0
VII	100	228.0	205.3

TABLE XXI

FREE BETA-AMYLASE SACCHARIFYING ACTIVITY OF SEED ENDOSPERM AS A PER CENT OF SINGLE KERNEL WEIGHT IN SEVEN BARLEY TRISOMICS

Trisomic types	% ± S. D. of maltose formed per 5 min.		
	Disomic plant (2n=14)	Trisomic plant (2n=15)	
	Disomic embryo 2n=14	Disomic embryo 2n=14	Trisomic embryo 2n=15
I	14.30	25.83	29.65
II	14.60	27.64	28.28
III	12.99	19.94	18.65
IV	13.17	24.55	30.82
V	13.96	20.86	19.32
VI	13.12	20.89	18.57
VII	13.90	32.62	23.62
	% of disomic check		
I	100	180.6	207.3
II	100	189.3	193.7
III	100	153.5	143.6
IV	100	186.4	234.0
V	100	149.4	138.4
VI	100	159.2	141.5
VII	100	234.7	169.9

TABLE XXII

FREE BETA-AMYLASE SACCHARIFYING ACTIVITY OF SEED ENDOSPERM AS A PER CENT OF TOTAL BETA-AMYLASE CONTENT IN SEVEN BARLEY TRISOMICS

Chromosome constitution	Maternal plant		Disomic (2n=14)	Trisomic (2n=15)	
	Seed embryo tissue		2n=14	2n=14	2n=15
% of total beta-amylase content	TRISOMIC TYPES	I	39.6	35.5	47.0
		II	41.9	42.5	49.0
		III	39.5	46.0	54.6
		IV	35.8	58.6	53.2
		V	40.4	47.5	48.3
		VI	36.9	47.7	43.1
		VII	43.0	72.3	54.6

CHAPTER V

DISCUSSION

Derivation of trisomics

Although progenies from selfed triploids provide an excellent source of trisomics in Hordeum vulgare, the initial production of triploid parental stock has been difficult to achieve. Because of the high degree of incompatibility that exists between tetraploids and diploids of H. vulgare, crosses between these two strains until recently, have proved unsuccessful. Larter and Enns (1960) were the first to overcome this barrier with the use of exogenous auxin. In the present study, a total of 40.39% of the progenies from triploids produced by their method were trisomic, which included all seven possible primary types. The search for spontaneously derived triploids of barley (Muntzing, 1938; Kerber, 1958) obviously can not compare with the frequency with which they can be produced experimentally.

Identification of trisomics

On the basis of the morphological descriptions of trisomics as presented by Tsuchiya (1954, 1964), Ramage (1955), and Kerber (1958), it was possible to distinguish most of the seven trisomic types from one another and also

from their disomic sibs. The four trisomics, I, II, IV and VI were readily recognizable on the basis of plant morphology. Trisomics III, V, and VII however, were not easily distinguishable, consequently it was necessary to establish their identity using karyotype analyses and established translocation marker stocks. Some limitation in the use of karyotype analysis was encountered because of the similarity of arm ratios of unidentified trisomes. Chromosomes 2, 3 and 4 fell into this category. The results of these analyses also indicated that the arm ratio of chromosome 7 of either varieties OAC 21 or Montcalm (0.46±0.022) was much higher than that of variety Mars (0.41), the standard as established by Burnham and Hagberg (1956). Variations in karyotype based on varietal differences can occur and for this reason care must be taken in establishing chromosome identity using karyotype analysis alone.

Linkage markers as well as translocation stocks were employed by previous workers for the identification of barley trisomics (Ramage, 1955; Kerber, 1958; Tsuchiya, 1959a, 1960, 1961, 1967). The use of translocation stocks offers certain advantages over linkage markers. For example, the occurrence of pentavalents at MI of the F_1 hybrid from crosses between the unknown trisomic and translocation stock provide irrefutable evidence for trisomic identification. In contrast, with the use of gene markers the final analyses

of linkage data have to await the results from the F_2 generation. Furthermore, in examining the theoretical ratios in the F_2, consideration must be given to: (1), the extent of chromatid crossing over; (2), the genotype of the trisomic; and (3), the transmission frequency of the trisomic. Since growing numbers of identified translocations exist in barley stocks throughout the world, their use in identifying trisomics is obviously more efficient than that of conventional genetic analysis using linkage testers.

Cytological behaviour of trisomics

The meiotic chromosome behaviour of the seven different trisomic types examined in this study was generally similar. Trisomic VI exhibited the highest frequency of univalents (29.75%) in MI cells and also gave the highest number of AI cells with dividing univalents (30.85%). At AII, trisomic VI again exhibited the highest frequency of abnormality (28.57%) which was expressed in the highest percentage of quartet micronuclei observed among the seven trisomics (27.74%). The reason for the instability of the extra chromosome in trisomic VI is unknown however, cytological studies revealed some interesting aspects of trisome 6. At MI, trivalent associations were predominately tandem-shaped in all trisomic types except VI in which a ring-rod trivalent configuration was more common. A similar observation involving VI was also made by Tsuchiya (1959a, 1967).

A tandem trisomic configuration occurs as a result of the formation of two chiasmata, each involving opposite arms of a different pair of the three trisomes. Ring-rod trivalent configurations are the result of three chiasmata; two in opposite arms of one pair of homologues, the third involving a different pair of homologues of the same trivalent association. The occurrence of a high frequency of ring-rod trivalents of chromosome 6 indicated that the formation of two chiasmata in adjacent arms of the three homologues was not suppressed to the same extent as in other trivalent chromosomes of barley. Chromosome 6 has also been recognized as a major nucleolar organizer. Theoretically therefore, because of their relationship with the nucleolus during early prophase, the three homologues of trisomic VI should be more closely associated with one another during this period than any other two homologues of the chromosomal complement. As pointed out by Sybenga (1966, 1968) in several respects (localization, interaction, activation, and their variation) the nucleolar organizer appear to be a considerable extent analogous to the hypothetical unit of chromosome pairing initiation which he called zygomere. Furthermore, a recent study of telotrisomics of the long arm of chromosome 6, revealed that 85% of the PMCs at MI contain univalents (Fedak, personal communication). This also strongly suggests that the short arm of chromosome 6, which carries a secondary constriction, has some effect on chromosome pairing and

exchange during prophase.

A comparison of the behaviour of the extra chromosome of trisomics with univalents of monosomics of polyploid cereals is of interest. According to the findings of Sanchez-Monge and Mackey (1948) and Sears (1952) in wheat, also McGinnis and Taylor (1961) in oats, most univalents divide at AI. In comparison, only about one fifth of the trisomes of barley divided at first division which suggests that the extra chromosome is much more stable in a trisomic than in a monosomic condition. Homologous pairing apparently enhances the stabilization of an otherwise univalent chromosome at first meiotic division. The results of the present studies also indicated that most of the divided trisomes at first division were those which were in a form of univalents rather than trivalents.

The division of the univalent at first anaphase also causes abnormalities in chromosomal behaviour at subsequent stages of meiosis, viz. lagging and exclusion of the extra chromosome from the microspore. Theoretically, the exclusion or loss of the extra chromosome through meiosis is detectable by micronuclei counts at the quartet stage. If a lagging chromosome does not occur at AI or AII of a trisomic, no micronuclei should be observed in the quartets; therefore, 50% of the gametes will carry an extra chromosome while the remaining 50% will be euhaploid. One micronucleus per quartet is indicative that a laggard at anaphase did occur;

consequently 25% of the gametes will have 8 chromosomes. The occurrence of two or more micronuclei would suggest that the extra chromosome was excluded from all microspores and would not be transmitted to the progeny.

Reproductive properties of trisomics

The results of present studies (Table XI) indicated that about 40% of the male gametes contained 8 chromosomes. The results from transmission studies revealed however, that the extra chromosome was carried through the pollen at a very low frequency (cf. results section 5-6 and Table XIV). Similar results were noted by Kerber (1958). From pollen mitosis studies, it was clear that the extra chromosome was included in male gametes as evidenced by the occurrence of 8 chromosome pollen. Mitotic divisions of such cells were delayed relative to normal cells (n = 7) both in first and second mitosis. Competition between these two classes of gametes would favor the earlier-developing, normal pollen and would operate to the exclusion of the extra chromosome through the male. Megasporogenesis of the trisomics was not studied. Nevertheless it is reasonable to assume that the proportion of 8 chromosome female gametes would approximate that found from microspore analysis. The results of present transmission studies showed however, that the highest transmission rate of the extra chromosome through the egg was only about 30%. A selection against 8 chromosome female

gametes had obviously occurred. In considering the low fertility and germination rate of trisomics relative to normal diploid plants, selection likely occurred at the zygote stage as well as during germination.

Quality studies of trisomics

As mentioned previoulsy, all trisomic lines used in the present studies originated from a cross between autotetraploid barley var. OAC 21 and diploid barley var. Montcalm. Although it is possible that genetic differences may have existed within trisomic lines as a result of parental diversity, such difference should be minimal because: (1), each line had undergone several generations of self pollination prior to their final evaluation; (2), the two parental varieties are known to have similar patterns for malting quality (Meredith, 1946, 1965) and therefore may be considered to have similar genetic make-up for this particular character; (3), in the analysis of each trisomic line, comparisons were made between trisomics and their disomic sibs.

Since the chromosome number of the progeny from trisomic plants was determined by root-tip counts of germinated embryos, only the endosperm portion of a seed was used in the quality analyses. Since the protein-rich germ portion is only a small fraction of the whole grain, the data based on the endosperm analyses alone are quite representative of the whole kernel (Rose and Anderson, 1937). By the same

token, the use of endosperm tissue for amino acid analyses should also be valid since in the seed they are bound in the form of protein (Holme, 1966). Similarly, beta-amylase is produced and stored mainly in the endosperm of a dormant barley seed (Verbeek-Wyndale and Coulier, 1961; Meredith, 1966), therefore an analysis based on endosperm tissue would be indicative of the enzyme content of the seed as a whole.

Total protein content is a quantitative character and is greatly affected by environment. It is generally considered that these characters are controlled by polygenes which because of their numbers, are difficult to assign to specific chromosomes. The results from present studies indicated that with the exception of trisome III there was no distinct difference in total protein content among trisomics. Trisomic III was significantly lower in protein content than all other trisomic lines. This may be an indirect result of the inhibiting effect that an extra dosage of chromosome 3 was found to have on chlorophyll production in that all trisomic III plants were pale green in color. The photosynthetic efficiency of such plants would be impaired and would curtail the metabolic processes involved in protein synthesis. More fruitful results would be obtained if a study was conducted on the effect of individual chromosomes as each controls synthesis of a particular species of protein rather than of overall total

protein.

Beta-amylase is basically a protein in nature, and can be assumed to be associated with relatively few genes. Plant breeders have been successful in incorporating into their breeding materials the necessary genetic determiners governing a satisfactory amyblytic level for industrial purposes. However, a rather limited number of studies of its inheritance have been reported in the literature. The fact that amylase activity is under genetic control in the barley plant is revealed indirectly by the inherent inter-varietal differences that occur for this character (Sallans and Anderson, 1938; Sisler and Banasik, 1951; Necas, 1960). Several studies have been concerned with genetic associations between such components and certain agronomic characteristics. A significant positive correlation between saccharifying activity and protein content has been reported by a number of workers (Anderson et al., 1938; DenHortog and Lambert, 1953; Hsi and Lambert, 1954; Rasmusson and Glass, 1965; Metcalfe et al., 1967). These findings are in good agreement with the present results. As indicated earlier, when the differences in seed size between trisomic and disomic were considered, the high protein content in trisomic seeds was found to be related to relatively low levels of carbohydrate. The high concentration of amylolytic enzymes in trisomic seed possibly interferes with the accumulation of starch or carbohydrate during the latter stages of seed development,

resulting in under-sized kernels generally associated with the trisomic condition.

Based on the results of present quality analyses, trisomic I, II and IV showed a markedly higher level of total beta-amylase than either their respective disomic sibs or other trisomic types. Bendelow (1964; Baker et al., 1968) using the backcross method, demonstrated that the level of free beta-amylase activity was dependent on one incompletely dominant gene. From the findings of the present study, this gene is very likely located on chromosome 7. On the other hand, recent results from electrophoric zymograms have shown unequivocally that barley can produce more than two protein species with beta-amylase activity (Grabar and Daussant, 1964; Waldschmidt-Leitz et al., 1964; Frydenberg and Nielsen, 1965). It is reasonable to assume, therefore, that there are at least three different genes each responsible for one of the three structurally different total beta-amylases. It is concluded from the present results that these three genes are located on chromosome 1, 2 and 4.

The results of present quality analyses also showed that in the progeny of a selfed trisomic, similar patterns of grain protein content and amylase activity were expressed regardless of whether the individual was trisomic or disomic. This is strongly indicative of the influence of the maternal parent on quality characteristics of barley, a phenomenon previously reported for many other plant species (Pawlowski,

1964; Fowler, 1965; Singh and Hadley, 1968).

Based on work with wheat (Jennings et al., 1963a, 1963b, 1963c, 1963d; Buttrose, 1963a, 1963b) DNA content of the endosperm increased with time following fertilization, reaching a peak around the twentieth day. The nucleate activity in the endosperm had completely ceased at this point and rapid cell expansion and heavy deposition of proteins and carbohydrates followed. It appeared that the early development of a kernel was primarily controlled by the genetic information from the maternal plant. At a later stage during embryo development and when the metabolic processes in the maternal plant began to decline, the embryo per se began to assume a major role in kernel development. Therefore the development and growth of a whole seed would appear to be controlled by the interaction of genetic factors between maternal and embryonic tissue. Since a trisomic plant produces two classes of seeds (trisomic and disomic) and each class has a different chromosome constitution in the embryo ($2n=14$ and 15) as well as in the endosperm ($3n=21$ and 23), a difference in enzyme level between these two classes of seeds is expected. The actual data from the present amylase studies showed such a difference to exist both in the total beta-amylase activity associated with chromosome 4 and in the free beta-amylase level associated with chromosome 7. This finding provided experimental

evidence that the quality characteristics of a barley kernel are the products of genic interaction between tissues of the maternal plant and those of the embryo.

BIBLIOGRAPHY

Anderson, J. A. and C. A. Ayre. 1938. Varietial differences in barleys and malts. I. Nitrogen distribution among protein fractions of barley. Can. J. Research (C) 16: 377-390.

Avery, A. G., S. Satina and J. Rietsema. 1959. Blakeslee: The genus Datura. The Ronald Press Company, New York, N. Y.

Babcock, E. B. and M. C. Navaschin. 1930. The genus Crepis. Bibliogr. Genetica 6:1-90.

Baker, R. J., V. M. Bendelow and K. W. Buchannon. 1968. Early generation inheritance of malting quality characters in a barley cross. Crop Sci. 8:446-448.

Belling, J. and A. F. Blakeslee. 1924. The configurations and size of the chromosomes in the trivalent of 25-chromosome Daturas. Proc. Nat. Acad. Sci. 10:116-120.

_____ and _____. 1926. On the attachment of non-homologous chromosome at the reduction division in certain 25-chromosome Daturas. Proc. Nat. Acad. Sci. 12:7-11.

Bendelow, V. M. 1963. Modified procedure for the determination of diastatic activity and alpha-amylase activity. J. Inst. Brewing 69:467-472.

_____. 1964. Inheritance of free beta-amylase in barley. Can. J. Plant Sci. 44:550-554.

Blakeslee, A. F. 1927a. The chromosomal constitution of Nubbin, a compound (2n + 1) type in Datura. Proc. Nat. Acad. Sci. 13:79-85.

_____. 1927b. Nubbin, a compound chromosomal type in Datura. Annals N. Y. Acad. Sci. 30:1-29.

_____ and B. T. Avery, Jr. 1919. Mutations in the Jimson weed. J. Heredity 10:111-120.

_____, J. Belling and M. E. Farnham. 1920. Chromosomal duplication and Mendelian phenomena in Datura mutants. Science 52:388-390.

_____ and M. E. Farnham. 1923. Trisomic inheritance in the Poinsettia mutant of Datura. Am. Nat. 57:481-495.

_____ and R. E. Cleland. 1930. Circle formation in Datura and Oenothera. Proc. Nat. Acad. Sci. 16:177-183.

_____ and A. G. Avery. 1938. Fifteen-year breeding record of 2n + 1 type in Datura stramonium. Cooperation in Research, Carnegie Inst. Wash. Publ. 501:315-351.

Burnham, C. R. 1946. A gene for "long" chromosomes in barley (abst.). Genetics 31:212-213.

_____. 1962. Discussions in Cytogenetics. Burgess Publ. Company, Minneapolis, Minnesota.

_____, F. H. White and R. Livers. 1954. Chromosomal interchanges in barley. Cytologia 19:191-202.

_____ and A. Hagberg. Cytogenetic notes on chromosomal interchanges in barley. Hereditas 42:467-482.

Buttrose, M. S. 1963a. Ultrastructure of the developing wheat endosperm. Aust. J. Biol. Sci. 16:305-317.

_____. 1963b. Ultrastructure of the developing aleurone cells of wheat grain. Aust. J. Biol. Sci. 16:768-774.

Catcheside, D. G. 1954. The genetics of brevistylis in Oenothera. Heredity 8:125-137.

Chen, C. C. and W. F. Grant. 1968. Morphological and cytological identification of the primary trisomics of Lotus pedunculatus. Can. J. Genet. Cytol. 10:161-179.

Chomchalow, N. and E. D. Garber. 1964. The genus Collinsia. XXVI. Polyploidy and trisomy in C. tinctoria. Can. J. Genet. Cytol. 6:488-499.

Clausen, R. E. and T. H. Goodspeed. 1924. Inheritance in Nicotiana tabacum. IV. The trisomic character "Enlarged". Genetics 9:181-197.

Darlington, C. D. 1938. Misdivision and the genetics of the centromere. J. Genet. 37:341-364.

DenHortog, G. T. and J. W. Lambert. 1953. The relationships between certain agronomic and malting quality characters in barley. Agron. J. 45:209-212.

Derenne, P. 1967. Triploide et trisomie chez l'orge cultivée. I. L'état triploide. Bulletin des Recherches Agronomiques de Gembloux 2:242-259.

Dhilon, T. S. and E. D. Garber. 1960. The genus Collinsia. X. Aneuploidy in C. heterophylla. Bot. Gaz. 121:125-133.

Ellis, J. R. and J. Janick. 1960. The chromosome of Spinacia oleracea. Am. J. Bot. 47:210-214.

Emerson, S. H. 1936. The trisomic derivatives of Oenothera Lamarckiana. Genetics 21:200-224.

Enns, H. and E. N. Larter. 1960. Note on the inheritance of DS; a gene governing meiotic chromosome behaviour in barley. Can. J. of Plant Sci. 40:570-571.

Frost, H. B. 1927. Chromosome mutant types in stocks. I. Characters due to extra chromsomes. J. Heredity 18: 475-486.

_____. 1931. Trisomic inheritance of doubleness, complicated by lethals, in Matthiola incana. Proc. Nat. Acad. Sci. 17:499-509.

Fowler, D. B. 1965. Lipid and morphological changes in developing seed of Brassica napus L. Uni. of Saskatchewan, M. Sc. Thesis.

Frydenberg, O. and G. Nielsen. 1965. Amylase isozymes in germinating barley seeds. Hereditas 54:123-139.

Garber, E. D. 1964. The genus Collinsia. XXII. Trisomy in C. heterophylla. Bot. Gaz. 125:46-50.

Goodspeed, T. H. and P. Avery. 1939. Trisomic and other types in Nicotiana sylvestris. J. Genetics 38:381-458.

Grabar, P. and J. Daussant. 1964. Study of barley and malt amylases by immunochemical methods. Cereal Chem. 41: 523-532.

Hagberg, A. 1954. Karyomorphological studies on barley. Hereditas 40:263-264.

Hesman, C. U. 1964. Gytogenetische Untersuchungen an Trisomen von Petunia hybrida. Z. Pflanzenzüch 51:1-11.

Hollingshead, L. 1930. Cytological investigations of hybrids and hybrid deriviatives of Crepis capillaris and Crepis tectorum. Uni. Calif. Publ. Agr. Sci. 6:55-94.

Holme, J. 1966. A review of wheat flour proteins, and their functional properties. Cereal Chem. 36:321-340.

Hsi, C. H. and J. W. Lambert. 1954. Inter- and intra-annual relationships of some agronomic and malting quality characters in barley. Agron. J. 46:470-474.

Hu, C. H. 1968. Establishment of a complete set of trisomics in rice. Proc. XII International Congress Genetics 1:177.

Jakob, K. M. 1963. A trisomic male castor bean plant. J. Heredity 54:292-296.

Jennings, A. C. and R. K. Morton. 1963a. Changes in carbohydrate, protein, and non-protein nitrogenous of developing wheat grain. Aust. J. Biol. Sci. 16:318-331.

_____ and _____. 1963b. Changes in nucleic acids and other phosphorus-containing compound of developing wheat grain. Aust. J. Biol. Sci. 16:332-341.

_____, _____ and B. A. Palk. 1963c. Cytological studies of protein bodies of developing wheat endosperm. Aust. J. Biol. Sci. 16:366-374.

_____ and _____. 1963d. Amino acids and protein synthesis in developing wheat endosperm. Aust. J. Biol. Sci. 16:384-394.

Johnsson, H. 1942. Cytological studies of triploid progenies of Populus tremula L. Hereditas 28:306-312.

Kaltsikes, P. J. and L. E. Evans. 1967. Production and identification of trisomic types in Beta vulgaris. Can. J. Genet. Cytol. 9:691-699.

Kasha, K. J. and H. A. McLennan. 1967. Trisomics in diploid alfalfa. I. Production, fertility and transmission. Chromosoma (Berl.) 21:232-242.

Katterman, G. 1939. Sterilitats Studien bei Hordeum distichum. Zeits Ind. Abs. Ver. 77:63-103.

Kerber, E. R. 1954. Trisomics in barley. Science 120: 808-809.

_____. 1958. A study of autotriploids and trisomics of common barley, Hordeum vulgare L. Uni. of Alberta, Ph. D. Thesis.

Larter, E. N. and H. Enns. 1960. The influence of gibberellic acid on the development of hybrid barley ovules in vivo. Can. J. Genet. Cytol. 2:435-441.

Ledingham, G. F. 1940. Cytological and developmental studies of hybrids between Medicago sativa and a diploid form of M. falcata. Genetics 25:1-15.

Levan, A. 1937. Chromosome numbers in Petunia. Hereditas (Lund.) 23:99-112.

Lesley, J. W. 1926. The genetics of Lycopersicum esculentum Mill. I. The trisomic inheritance of "Dwarf". Genetics 11:352-354.

_____. 1928. A cytological and genetic study of progenies of triploid tomatoes. Genetics 13:1-43.

_____. 1932. Trisomic types of the tomato and their relations to the genes. Genetics 17:545-559.

McClintock, B. 1929. A cytological and genetical study of triploid maize. Genetics 14:180-222.

_____ and H. E. Hill. 1931. The cytological identification of the chromosome associated with the R-G linkage group in Zea mays. Genetics. 16:175-190.

McGinnis, R. C. and D. K. Taylor. 1961. The association of a gene for chlorophyll production with a specific chromosome in Avena sativa. Can. J. Genet. Cytol. 3:436-443.

McLennan, H. A. 1947. Cytogenetic studies of a strain of barley with long chromosomes. Uni. of Minnesota, M. Sc. Thesis.

_____ and Burnham, C. R. 1948. Cytogenetic studies of "long chromsome" barley. Abst. Paper Presented at Am. Soc. Agron. Meet., Fort Collins, Colorado, August 24-27, 1948.

Menzel, M. Y. and M. S. Brown. 1952. Viable dificiency duplication from a translocation in Grosspium hirsutum. Genetics 37:678-692.

Meredith, W. O. S. 1946. Malting quality of Canadian barleys. V. Summary of seven years tests on Montcalm, a new smooth awned variety. Sci. Agr. 26:560-565.

_____. 1965. Combined action of alpha- and beta-amylases in saccharification. Am. Soc. Brew. Chem. Proc. 1965:5-10.

_____. 1966. Distribution of protein and free and bond amylase in cereal species. Am. Soc. Brew. Chem. Proc. 1966:32-38.

Metcalfe, D. R., S. B. Helgason and E. W. Hougen. 1967. A genetic study of malting quality in barley varieties of different ancestral background. Can. J. Genet. Cytol. 9:554-564.

Morrison, J. W. 1959. Cytogenetic studies in the genus Hordeum. I. Chromosome morphology. Can. J. Bot. 37:527-538.

Muntzing, A. 1938. Note on heteroploid twin plant from eleven genera. Hereditas 24:487-491.

Myers, W. M. 1944. Cytological studies of a triploid perennial ryegrass and its progeny. J. Heredity 35:17-23.

Necas, J. 1960. The heritability of the amylolytic activity in barley. Biol. Plantarum 2:1-18.

Ostergren, G. and W. K. Heneen. 1962. A squash technique for chromosome morphological studies. Hereditas 48:332-341.

Pawlowski, S. H. 1964. Seed genotype and oil percentage relationship between seeds of a sunflower. Can. J. Genet. Cytol. 6:293-397.

Price, E. M. and W. M. Ross. 1955. The occurrence of trisomics and other aneuploids in a cross of triploid x diploid Sorghum vulgare. Agron. J. 47:591-592.

_____ and _____. 1957. Cytological study of a triploid x diploid cross of Sorghum vulgare Pers. Agron. J. 49: 237-240.

Propach, H. 1935. Studien über heteroploide Formen von Antrrhinum majus L. II. Die Reifeteilungen in den Pollen mutterzellen der Trisomen anaemica, fusca, purpurea, rotunda, canada. Planta 23:349-357.

Rai, K. S. and E. D. Garber. 1960. The genus Collinsia. XI. Trisomic inheritance in C. heterophylla. Bot. Gaz. 122:109-117.

Ramage, R. T. 1955. The trisomics in barley. Uni. of Minnesota, Ph. D. Thesis.

_____. 1960. Trisomics from interchange heterozygotes in barley. Agron. J. 52:156-159.

_____. 1965. Balanced tertiary trisomic for use in hybrid seed production. Crop Sci. 5:177-178.

Randolph, L. F. 1935. Cytogenetics of tetraploid maize. J. Agr. Research 50:591-605.

_____ and H. E. Fischer. 1939. The occurrence of parthenogenetic diploids in tetraploid maize. Proc. Nat. Acad. Sci. Wash. 25:161-164.

Rasmusson, D. C. and R. L. Glass. 1965. Effectiveness of early generation selection for four quality characters in barley. Crop Sci. 5:389-391.

Rhoades, M. M. 1936. Note on the origin of triploidy in maize. J. Genetics 33:355-357.

_____. 1933. An experimental and theoretical study of chromatid crossing over. Genetics 18:535-555.

Rick, C. M. and D. W. Barton. 1954. Cytological and genetical identification of the primary trisomics of the tomato. Genetics 39:640-666.

Sallans, H. R. and J. A. Anderson. 1938. Varietal differences in barleys and malts. II. Saccharifying activities of barleys and malts and the correlation between them. Can. J. Research (C) 16:405-416.

Sanchez-Monge, E. and J. Mackey. 1948. On the origin of subcompactoids in Triticum vulgare. Hereditas (Lund.) 34:321-337.

Sears, E. R. 1939. Monosomes, trisomics and segmental interchanges from a haploid of Triticum vulgare. Genetics 24:509-523.

_____. 1952. Misdivision of univalent in common wheat. Chromosoma 4:535-550.

_____. 1954. The aneuploids of common wheat. Missouri Agr. Exp. Stat. Research Bull. 572:1-59.

Sen, S. K. 1965. Cytogenetics of trisomics in rice. Cytologia 30:229-238.

Singh, B. B. and H. H. Hadley. 1968. Maternal control of oil synthesis in soybeans, Glycine max (L.) Merr. Crop Sci. 8:622-625.

Sisler, W. W. and O. J. Banasik. 1951. Quality selection in early generation barley hybrids. Agron. J. 43:616.

Smith, L. 1941. An inversion, a reciprocal translocation, trisomics and tetraploids in barley. J. Agr. Research 63:741-750.

Sutton, E. 1939. Trisomics in Pisum sativum derived from an interchange heterozygote. J. Genetics 38:459-476.

Sybenga, J. 1966. The zygomere as hypothetical unit of chromosome pairing initiation. Genetica 37:186-198.

Sybenga, J. 1968. The analysis of systems of chromosome pairing. Proc. XII International Congress Genetics 1: 174.

Tagaki, F. 1935. Karyogenetical studies on rye. I. A trisomic plant. Cytologia 6:496-501.

Takahashi, R. and J. Hayashi. 1966a. Inheritance and linkage studies in barley. II. Assignment of several new mutant genes to their respective linkage groups by the trisomic method of analysis. Borichte des Ohara Instituts für Landwirtschaftliche biologie 8:185-198.

_____ and _____. 1966b. Inheritance and linkage studies in barley. II. Linkage studies of the gene for fragile stem-2 and orientation of linkage map on barley chromosome 5. Berichte des Ohara Instituts fur Landwirtschaftliche Biologie 7:185-198.

Tjio, J. H. and A. Hagberg. 1951. Cytological studies on some X-ray mutants of barley. Ann. Aula Dei 2:149-167.

Tometorp, G. 1939. Cytological studies on haploid Hordeum distichum. Hereditas 25:241-254.

Tsuchiya, T. 1950. Some notes on the progeny of a hypo-triploid barley. Jap. J. Genetics 25:230.

_____. 1953. Some notes on autotriploid barley. Jap. J. Genetics 28:189.

_____. 1954. Trisomics in barley. Jap. J. Genetics 29: 179.

_____. 1958a. Studies on cross compatibility of diploid, triploid and tetraploid barleys. I. Seed development in reciprocal crosses between diploids and induced autotetraploids. Bot. Mag. Tokyo 71:48-52.

_____. 1958b. Studies on trisomics in barley. I. Origin and the characteristics of primary simple trisomics in Hordeum spontaneum C. Koch. Seiken Ziho 9:69-86. (English resume).

_____. 1959a. Genetic studies in trisomic barley. I. Relationships between trisomics and genetic linkage groups of barley. Jap. J. Bot. 17:14-28.

_____. 1959b. Studies on trisomics in barley. Kyoto Uni., Dr. Agr. Thesis.

_____. 1960. Cytogenetic studies of trisomics in barley. Jap. J. Bot. 17:177-213.

_____. 1961. Studies on the trisomics in barley. II. Cytological identification of the extra chromosomes in crosses with Burnham's translocation testers. Jap. J. Genetics 36:444-451.

_____. 1963. Trisomics in a cultivated two-rowed barley variety. Barley Newsletter 6:99-100.

_____. 1964. Chromosome aberrations and their use in genetics and breeding in barley--trisomics and aneuploids. Barley Genetics I (Proc. First International Barley Genet. Symposium):116-150.

_____. 1967. The establishment of a trisomic series in a two-rowed cultivated variety of barley. Can. J. Genet. Cytol. 9:667-682.

_____, J. Hayashi and R. Takahashi. 1960. Genetic studies in trisomic barley. II. Further studies between trisomics and the genetic linkage groups. Jap. J. Genetics 35:153-160.

Vasek, F. C. 1956. Induced aneuploid in Clarkia unguiculata. Am. J. Bot. 43:366-371.

Verbeekwyndale, R and V. Coulier. 1961. Beta-amylase in Barley embryo and endosperm. European Brew. Conv., Proc. Congress. 72:9.

Waldschmidt-Leitz, E., L. Grafinger and M. Westphal. 1964. Zur Struktur der Beta-amylase aus Gerste und Malz. Zeit. f. Phys. Chem. 399:36-41.