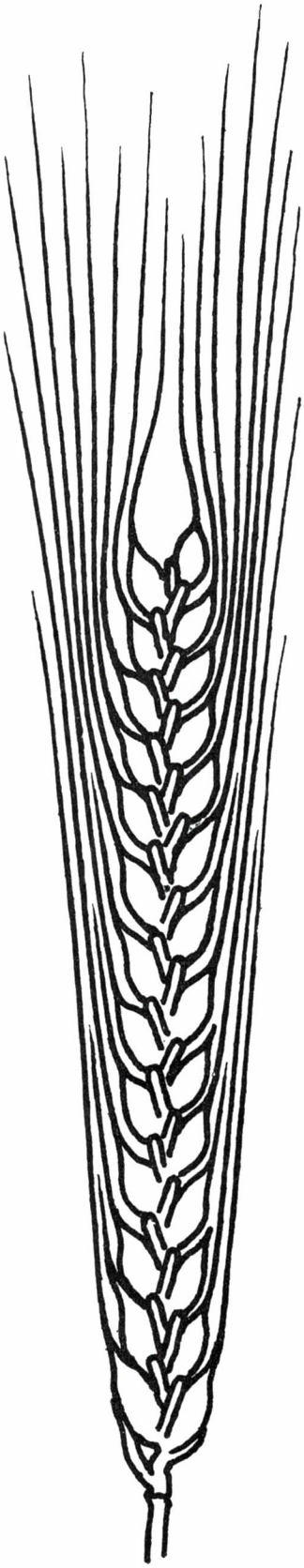
To Dr. M. Feldman
with the best compliments of
T. Tsuchiya



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Chromosome aberrations and their use in genetics and breeding in barley – trisomics and aneuploids

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INTRODUCTION

Aneuploids have been used extensively in the study of genetics and plant breeding. Monosomics and nullisomics have been used mostly in polyploid plants; trisomics and tetrasomics to a lesser extent. In diploid plants, including barley, it is impossible or very difficult to produce monosomics and nullisomics. Trisomics have been the main aneuploids used in studies of genetics and breeding of such diploid plants as *Datura*, tomato, maize, barley, and others (see Burnham 1962).

Trisomics are especially useful in assigning genes and linkage groups to particular chromosomes. They have been used in the establishment of linkage groups in *Datura*, maize, tomato, and spinach (cf. Burnham 1962).

In barley the trisomics were not used for linkage studies and other cytogenetic investigations until recently because the trisomics were only rarely obtained spontaneously (Kattermann 1939, Smith 1941) and not produced artificially (cf. Smith 1951, Tsuchiya 1958a, c).

McLennan (1947, unpublished) and McLennan and Burnham (1948) obtained some trisomic plants among the progeny of a partially asynaptic strain with long chromosomes (cf. Burnham 1946). Six simple and two double trisomic plants were produced in progenies of a hypotriploid plant ($2n = 20$) of a cultivated two-rowed variety (Tsuchiya 1949, 1950, 1952a). A considerable number of trisomics and aneuploids have been produced among the progenies of autotriploids (Kerber 1954, 1958, unpublished; Larter and Enns 1962, unpublished; Tsuchiya 1954, 1958c, 1960a, c, d, 1961, 1963a, b, unpublished), and interchange heterozygotes (Burnham et al. 1954; Hagberg 1954; Nishimura 1961, Ramage 1955, 1960; Ramage and Day 1960; Tsuchiya 1960b).

Complete sets of the seven primary trisomics have been established in 3 varieties; a cultivated six-rowed variety, Mars (Ramage 1955, 1960), a wild two-rowed variety (Tsuchiya 1954, 1958c, 1960a, c, d) and a cultivated two-rowed variety, Shin Ebisu No. 16 (Tsuchiya 1963a, b, unpublished). Series of trisomic types have been obtained among progenies of autotriploid Gateway (cultivated 6-rowed) and triploid hybrids (Herta × Wong) (Kerber 1954, 1958, unpublished), autotriploid Montcalm (cultivated

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6-rowed) (Larter and Enns 1960, 1962, unpublished), and autotriploid two-rowed variety (Ising 1961, unpublished).

The morphological and cytological aspects of these trisomic plants have been investigated. Linkage studies have also been carried out in certain of the trisomic series (Kerber 1958, unpublished; Ramage 1955, 1960; Takahashi and Hayashi 1962; Takahashi et al. 1962; Tsuchiya 1956, 1959a, 1960a; Tsuchiya et al. 1960).

The present writer has produced two complete sets of primary trisomics, one in a wild and the other in a cultivated two-rowed barley variety. The set in the cultivated variety has not been studied thoroughly, but the wild series has been investigated in considerable detail. This paper will deal mainly with the trisomics and aneuploids obtained from the wild variety.

MATERIALS AND METHODS

The trisomic plants used in this study were obtained from the progeny of autotriploid plants of a wild two-rowed barley variety, *Hordeum spontaneum* C. KOCH v. *transcaspicum* VAV. The triploid plants used in this study were obtained from the cross 4x × 2x and from progenies of triploids and trisomics.

Simple trisomics and other aneuploids were isolated by somatic chromosome counts. The simple trisomics were roughly grouped into 7 different types mainly by their gross morphological features.

Analysis of karyotypes and meiotic chromosome behaviors were made in most of the trisomics in the first generation and some in the second and later generations to test the nature of trisomics; primary, secondary, or tertiary trisomics or other aberrations.

Most of the first-generation trisomics, and many of the trisomics in the later generations, were crossed with linkage testers carrying several gene markers.

The detailed description of materials used and the experimental methods will be given in each paragraph. Cytological materials were fixed in acetic alcohol (1:3) and stained by a modified acetocarmine squash method (Tsuchiya 1957, cf. Rattenbury 1956).

ORIGIN AND OCCURRENCE OF TRISOMICS

Trisomic plants and other aneuploids were obtained exclusively from autotriploids; directly from the progenies of autotriploids and indirectly through other aneuploids, for example, double and triple trisomics, etc.

The seed fertility and germination percentages are relatively high in the barley triploids but differ in different cross combinations and also in different years. By selfing from triploids and from reciprocal crosses of triploids with diploids an abundance of seeds was obtained (table 1). A total of 865 plants were obtained from 1,273 seeds (table 1).

Chromosome numbers in the progeny of autotriploid plants are shown in table 2. The chromosome number ranged from 2n = 14 to 26. No plants with a chromosome number of 22, 23 or 24 were found.

The variation in chromosome number was most extreme in the progeny of selfed autotriploids. Simple trisomics were most frequent followed by the diploid and 16-chromosome plants. The frequency of 17- and 18-chromosome plants was low. Triploid

TABLE 1. *Fertility and germination of seeds from autotriploids in a wild barley variety*

Cross combinations	Number of		Fertility	Number of seeds		
	florets	seeds		sown	germinated	(%)
3x × selfing	2432	465	19.12	452	190	(42.03)
3x × 2x	540	138	25.56	136	84	(61.76)
2x × 3x	938	686	73.13	685	591	(86.28)
total	3910	1289	—	1273	865	—

and hypotriploid plants (2n = 21 and 19) and hypotetraploids (2n = 25 and 26) were also obtained but with a very low frequency.

The variation in chromosome number was less in progenies from crosses of 2x × 3x (table 2). The most frequent class was diploid (79 percent) with simple trisomics amounting to 13.5 percent. No 17- and 18-chromosome plants were found. Surprisingly, triploid and near triploid plants having 2n = 21-, 20- and 19-chromosomes were obtained.

Most of the aneuploid plants obtained were viable and vigorous to some extent. Among the aneuploids the simple trisomics were the most vigorous, and some were comparable to normal diploids. However, the vigor and the growth habit are extremely different in plants having chromosome numbers from 2n = 16 to 20: some are very vigorous and fertile while others are weak and highly sterile. Differences in vigor and fertility were observed in different trisomics as has been reported earlier (Tsuchiya 1954, 1958c,

TABLE 2. *Chromosome numbers in the progenies of autotriploid plants of a wild two-rowed barley*, Hordeum spontaneum *var.* transcaspicum

Chromosome number (2n)	3x selfing		3x × 2x		2x × 3x		Total number
	no.	%	no.	%	no.	%	
14	37	19.47	21	25.00	467	79.02	525
14 + 1f	2	1.05	0	0	21	3.55	23
15	92	48.42	50	59.52	80	13.54	222
15 + 1f	3	1.58	0	0	3	0.51	6
16	36	18.94	8	9.52	3	0.51	47
17	6	3.16	1	1.19	0	0	7
18	3	1.58	0	0	0	0	3
19	1	0.53	0	0	2	0.34	3
20	0	0	0	0	1	0.17	1
21	2	1.05	0	0	2	0.34	4
22	0	0	0	0	0	0	0
23	0	0	0	0	0	0	0
24	0	0	0	0	0	0	0
25	1	0.53	0	0	0	0	1
26	1	0.53	0	0	0	0	1
not identified	6	3.16	4	4.76	12	2.30	22
total	190	—	84	—	591	—	865

1960a, cf. Kerber 1954, 1958; Ramage 1955, 1960). This wild barley material tolerates an extra chromosome rather well. Unpublished data on the simple trisomics and other aneuploids in a cultivated two-rowed variety (Shin Ebisu No. 16) have shown a comparable tolerance of extra chromosomes (Tsuchiya 1963a, unpublished).

The simple trisomic plants were classified into 7 morphological groups and named as follows: Bush, Slender, Pale, Robust, Pseudonormal, Purple and Semierect.

Table 3 shows the frequency of the different types of primary trisomics in the progenies of autotriploids: 3x selfing, 3x × 2x and 2x × 3x. The frequency of the various types varies with the source. 3x selfing and 3x × 2x are similar with the exceptions of Bush and Pale; Bush is lowest in 3x selfing and highest in 3x × 2x, and Pale is highest in 3x selfing and lower in 3x × 2x.

TABLE 3. *The frequency of seven types of primary simple trisomics in the progenies of autotriploids of* H. spontaneum *var.* transcaspicum

Type of trisomic	3x selfing		3x × 2x		2x × 3x		Total number
	no.	%	no.	%	no.	%	
Bush	8	8.69	15	30.00	0	0	23
Slender	6	6.52	8	16.00	6	7.50	20
Pale	26	28.26	6	12.00	0	0	32
Robust	11	11.96	4	8.00	2	2.50	17
Pseudonormal	8	8.69	4	8.00	3	3.75	15
Purple	13	14.13	6	12.00	33	41.25	52
Semierect	15	16.30	6	12.00	21	26.25	42
not identified	5	5.43	1	2.00	15	18.75	21
total	92	—	50	—	80	—	222

In contrast, the situation is quite different in the progenies from 2x × 3x: No trisomics for chromosome 1 (Bush) and 3 (Pale) were obtained from this cross, though they occurred most frequently from 3x × 2x (Bush) and 3x selfing (Pale) (table 3). The frequency of the trisomic for chromosome 2 (Slender) from 2x × 3x is almost comparable to that in 3x selfing and is lower than that in 3x × 2x. Trisomics for chromosomes 4 and 5 were obtained with the lowest frequency. In the 2x × 3x progenies the trisomics for chromosome 6 (Purple) and 7 (Semierect) occurred with an extremely high frequency; about 41 percent of Purple and 26 percent of Semierect. The causes of the differences in the frequency of different types from different cross combinations of triploids and diploids will be discussed elsewhere.

CHARACTERISTICS OF THE PRIMARY TRISOMICS

The simple trisomics and other chromosomal types were first isolated by the somatic chromosome counts of the roottips taken from the germinating seeds. By close observations throughout the growing period from seedling to maturity the simple trisomics

TABLE 4. *Diagnostic morphological features of the seven primary simple trisomics in barley as*

Type of trisomic	Growth habit	Leaf characters [1])
Bush	Bushy; dwarf with many tillers.	Short, narrow, dark green colour; frequent occurrence of fused leaves. Index 20.0.
Slender	Bushy with many tillers; slender in appearance.	Very long, thin, narrow and hanging down at almost vertical position; dark green. Index largest, 25.3.
Pale	Nearly normal with slightly oblique stems; seemingly weak.	Extremely twisted leaf tips with pale color; thin and drooping at almost vertica position; very small flag leaf. Index 17.7.
Robust	Robust and vigorous.	Long, wide, thick and dark green with very wavy margins. Index 13.3.
Pseudonormal	Closely resembles normal diploids.	Inversely twisted small leaves. Index 16.6.
Purple	Robust and coarse, semi-prostrate.	Thick, wide, long, coarse and dark green; dark purple color in leaf sheaths. Index 14.0.
Semierect	Semi erect with short and straight leaves.	Straight, short, wide and coarse leaves with coarse texture. Index smallest, 12.8.
Diploid	Prostrate or spreading type.	Relatively long with slightly waved margins. Index 16.4

[1]) Leaf index (length/width ratio) was calculated from a measurement of the leaf preceding the flag leaf.

TABLE 5. *Fertility and germination percentage of seven primary simple trisomics*

Type of trisomic	Pollen-fertility	Seed fertility				Germination percentage		
		selfing	open	(2x+1)×2x	2x×(2x+1)	selfing	(2x+1)×2x	2x×(2x+1)
	%	%	%	%	%	%	%	%
Bush	97.2	68.0	72.1	82.5	91.4	90.3	85.3	84.4
Slender	94.7	65.1	73.2	71.1	93.7	76.8	76.5	81.8
Pale	72.3	88.3	88.5	81.3	97.9	81.4	85.5	73.4
Robust	92.6	80.5	90.3	87.3	96.1	95.0	89.3	85.7
Pseudonormal	93.6	90.5	91.0	76.6	94.7	86.4	86.4	72.0
Purple	96.5	82.8	89.2	82.0	97.2	90.8	89.9	61.5
Semierect	96.6	86.2	81.1	81.7	91.7	83.9	86.3	71.3
average	*91.9*	*80.2*	*83.6*	*80.4*	*95.2*	*86.4*	*85.6*	*75.2*
diploid	99.2	91.3	97.2	—	—	93.2	—	—

xpressed under field condition

Other outstanding features	Ramage's trisomic type
hort ears with relatively long awns; multiple or compound spikelets in 6% of spikelet triplets; degeneration of one or two anthers in 34% of orets; very long empty glumes; rachilla frequently changes to awn-like ody.	b
lenderness in all the plant parts; short awn; very short stomatal guard ells; small seeds. Relatively low seed fertility.	f
)ense ears with short and weak awns; short empty glumes; tiny rachilla; maciated seeds. Low pollen fertility.	c
ong ears with long and coarse awns; thin and soft glumes. Emaciated eeds.	e
lants nearly normal appearance but somewhat smaller than diploids. mall ears with short and thin awns. Empty glumes and rachilla are short. eeds small.	a
horter ears with relatively long and coarse awns; extremely long empty lumes; rachilla axis is long but rachilla hairs very short. Seeds shorter nd wider.	g
hort and lax ears with short and coarse awns; large seeds; very large mpty glumes; rachilla axis and rachilla hairs are the longest of all seven ypes.	d

were grouped into 7 different morphological types. The following characters were used as criteria in identifying trisomic types:

1. Seedling characters: leaf shape and color, and abnormalities
2. Growth habit at the early growing stage: root system and tillering; erect, semierect and procumbent
3. Plant height, and length and thickness of culm of adult plants
4. Leaves: length, width, index, color, texture, and abnormalities
5. Spikes: length, width, number of spikelets per spike, density (length of rachis internodes), abnormalities in spike, spikelet triplets, florets and other floral organs, texture of lemma and palea
6. Awns: length, width, thickness and abnormalities
7. Kernels: length, width, thickness, and abnormalities
8. Empty glumes: length and texture
9. Rachilla: length of axis, length and density of rachilla hairs
10. Time of heading and maturity
11. Brittleness of rachis: tightness in Pale
12. General aspects of trisomic plants, especially in group planting of each type

Measurements of some plant organs were made and microscopical observations were made on other characters mentioned above (Tsuchiya 1958c).

Simple trisomics are distinctive in their morphological characters, and also in pollen and seed fertility. Detailed descriptions of the trisomics have been reported (Tsuchiya 1956, 1958c, 1960a). Characteristics which are helpful in identification of the 7 trisomic types are shown in table 4. The typical seedling characters, leaves of seedlings, growing plants, spikes, and seeds of trisomics and of the diploid are illustrated in the figs. 1 to 5. Each of the primary simple trisomic types differs from the normal diploid and also from the others in a large number of characters. Some differences are minute and inconspicuous but others are gross and striking; some are qualitative and others quantitative. Each trisomic type represents a unique combination of a number of

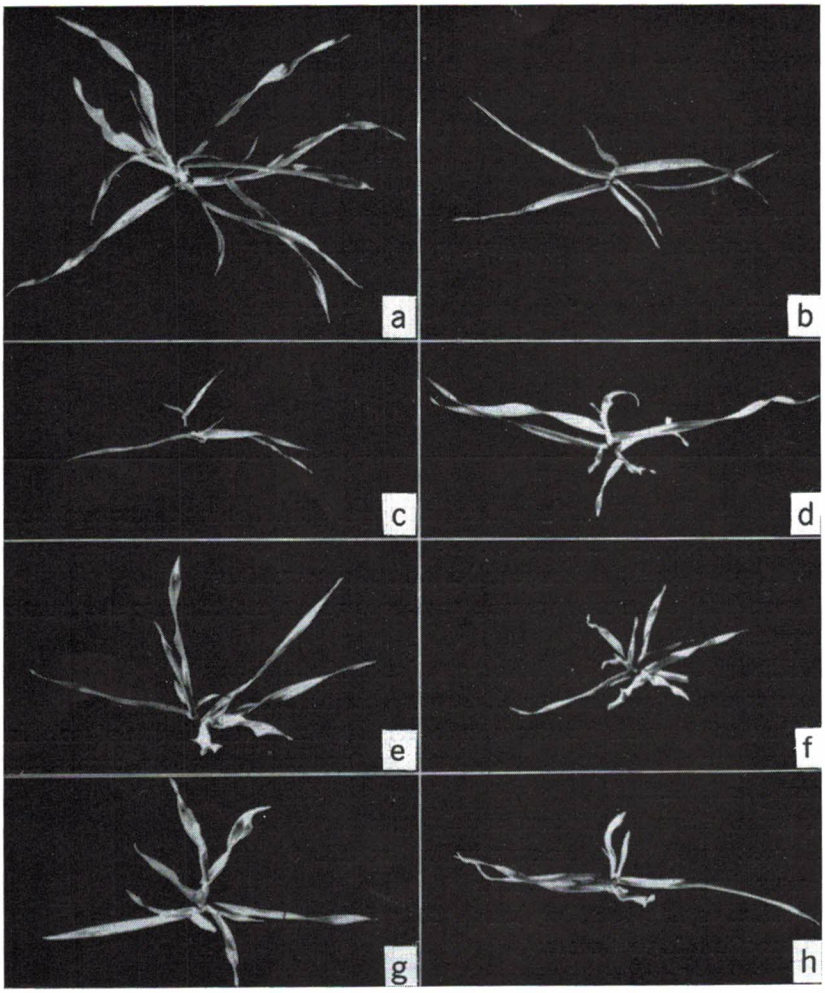

FIG. 1. Seedlings of diploid (a) and triomsics (b–h) viewed from above

a. diploid, b. Bush, c. Slender, d. Pale, e. Robust, f. Pseudonormal, g. Purple, h. Semierect

characters (table 4 and figs. 1-5). Thus, each of the seven trisomic types is easily distinguished from normal diploids and from the other trisomic at various stages from seedling to adult plants, especially at the tillering stage. The independence of the seven primary types has been morphologically established from the above descriptions and figures. (cf. Tsuchiya 1954, 1956, 1958c, 1960a).

The seed fertility of the trisomic types was relatively high in comparison with other barley trisomics previously reported by several authors (cf. Tsuchiya 1958c, 1960a).

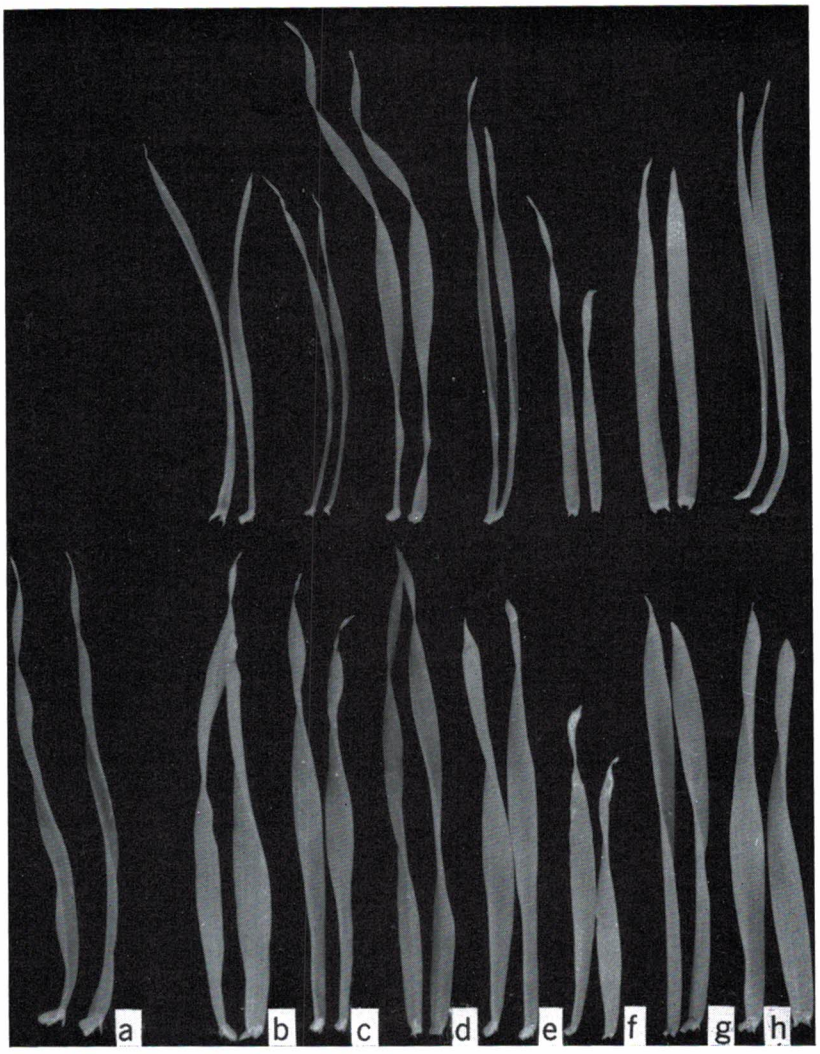

FIG. 2. Leaves of trisomic (upper row) and diploid (lower row) seedlings at early stage. See for explanation fig. 1
Upper row: Trisomics from trisomic parents
Lower row: Diploids from diploid and trisomic parents

It is also noteworthy here that completely fertile spikes were occasionally observed in most of the trisomic types.

Seed fertility and germination percentage are given in table 5. Seed fertility was lowest in Slender (69.8%), and next in Bush (74.2%). The remaining 5 types were similar in fertility. The causes of the low fertility in Slender are unknown but the reduced seed fertility in Bush may be partly ascribed to the frequent occurrence of multiple spikelets which were mostly sterile (Tsuchiya 1958c, 1960a).

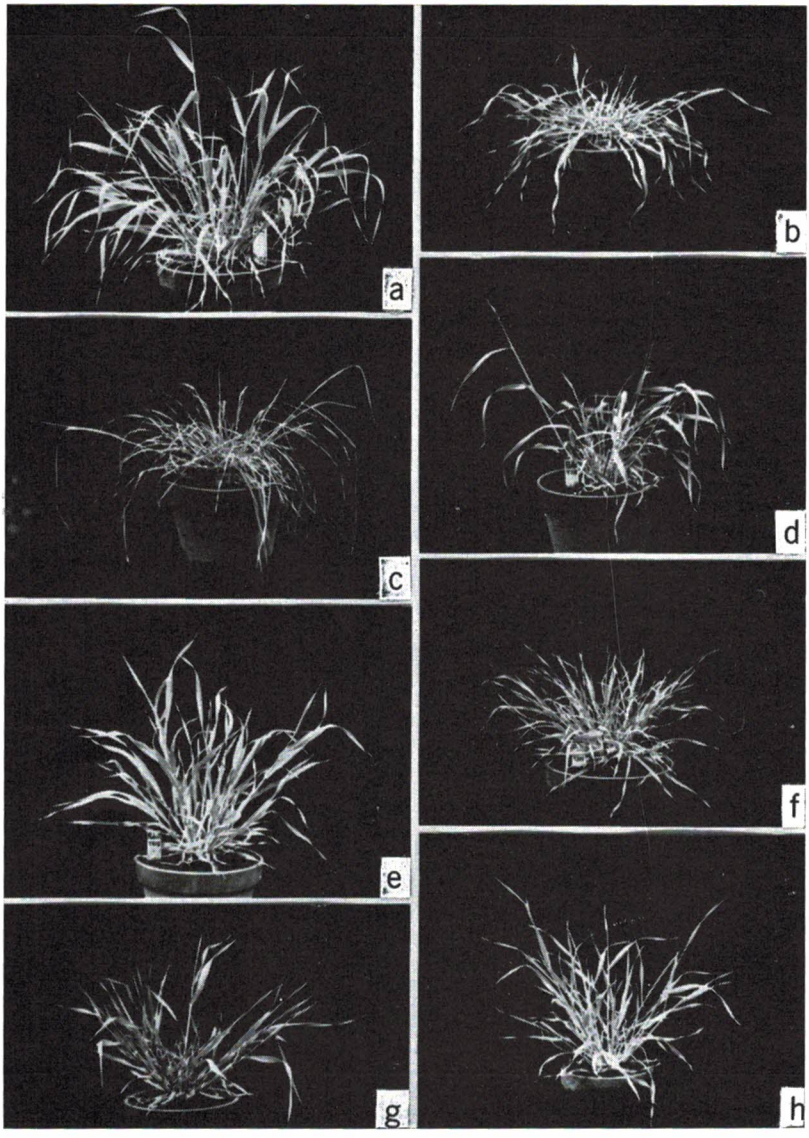

FIG. 3. Plants of diploid and trisomics at the stage preceding the heading (× 0.5). See for explanation fig. 1

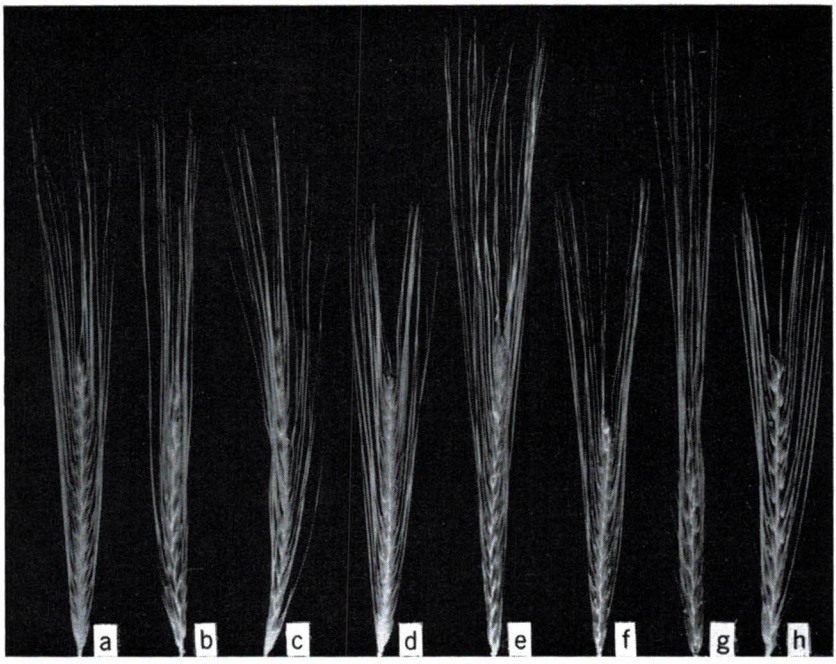

FIG. 4. Spikes of diploid and trisomics ($\times$ 0.44). See for explanation fig. 1

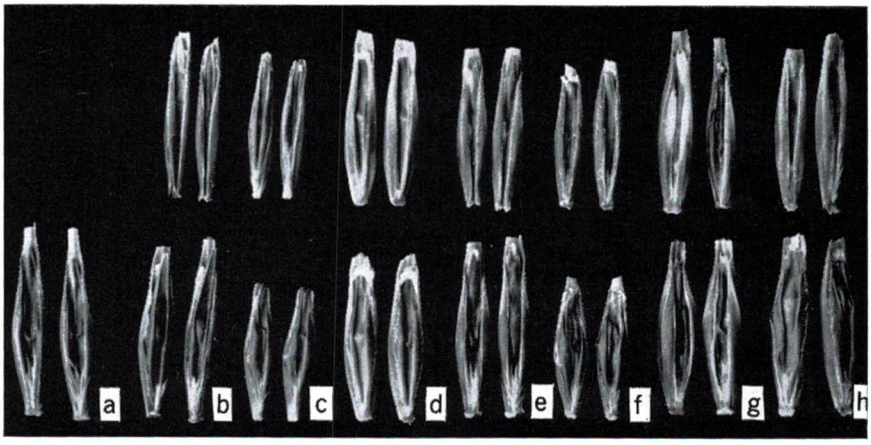

FIG. 5. Comparison of seed size of trisomics (upper row) and diploids (lower row) ($\times$ 2.4).
See for explanation fig. 1
Upper row: The seeds from which trisomics emerged
Lower row: The seeds from which diploids emerged
These seeds are produced by parental diploid and trisomics

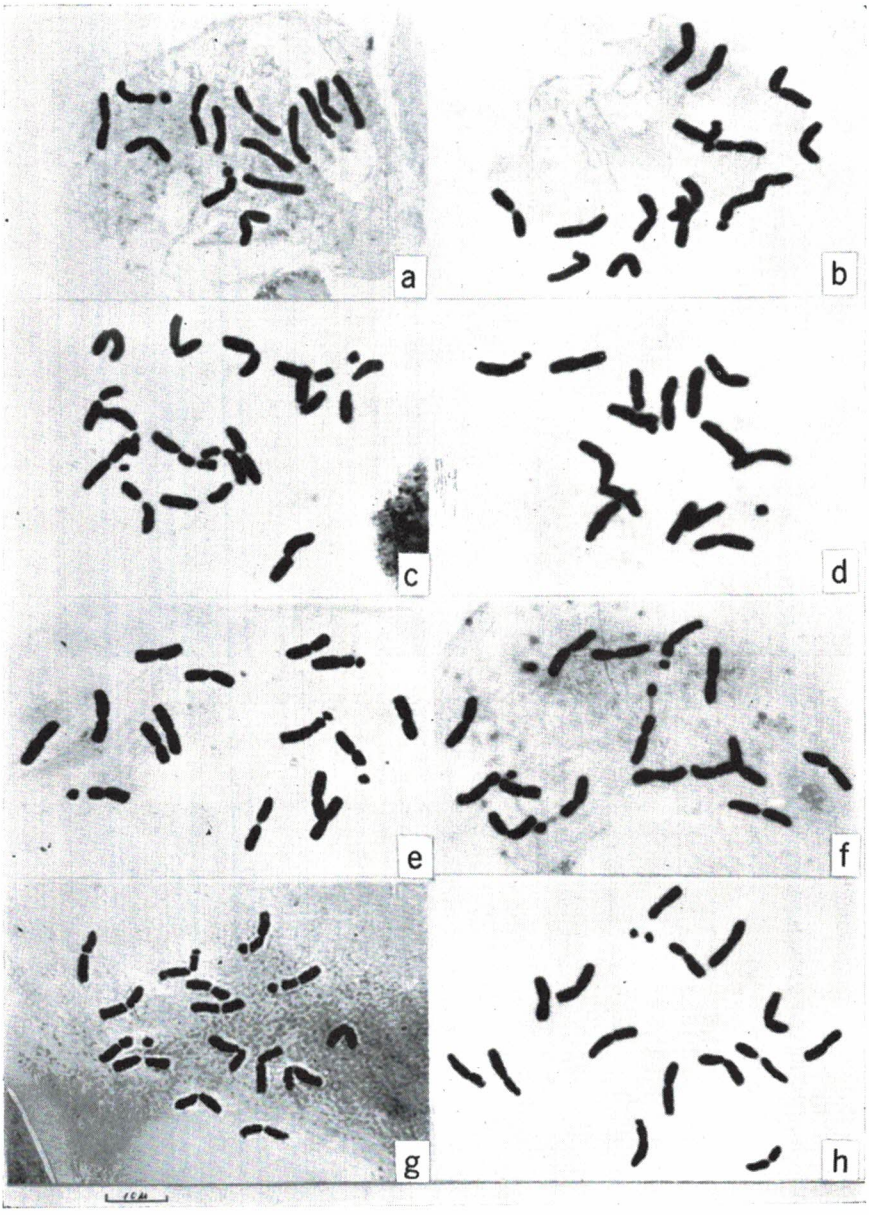

FIG. 6. Somatic metaphase chromosomes in root tip squashes of diploid (2n = 14) and trisomics (2n = 15) of barley

a. diploid, b. Bush, c. Slender, d. Pale, e. Robust, f. Pseudonormal, g. Purple, showing three chromosome 6 with larger satellite, h. Semierect, showing three chromosome 7 with smaller satellite

As shown in table 5, the germination percentages were high enough to maintain the trisomic stocks by selfing and by $2x + 1 \times 2x$ crosses. The germination of $2x \times 2x + 1$ was also good (table 5).

CYTOLOGICAL IDENTIFICATION OF THE TRISOMIC TYPES

Karyotype analysis was not carried out thoroughly for the largest 4 chromosomes. Only the two SAT-chromosomes (6 and 7) and the smallest non-SAT-chromosome (5) were studied by karyotype analysis. Purple is trisomic for chromosome 6, Semierect for chromosome 7, and Pseudonormal for chromosome 5. Bush, Slender, Pale and Robust are trisomic for chromosomes 1-4.

The largest 4 chromosomes, as well as chromosomes 5, 6, and 7 were analyzed by the use of Burnham's interchange testers. The seven trisomic types were crossed as female with the interchange testers and meiosis of the trisomic F_1 hybrids were analyzed.

If the extra chromosome of a trisomic type is homologous with one of the interchanged chromosomes, the F_1 trisomic hybrids would show a configuration of $1_V + 5_{II}$ (primary trisomic interchange heterozygote), while if the extra chromosome is not-homologous with the interchanged chromosomes the configuration would be $I_{IV} + 1_{III} + 4_{II}$ (fig. 7).

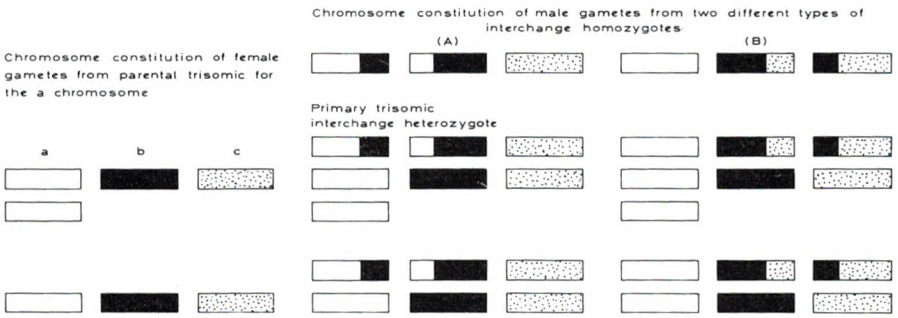

FIG. 7. Diagrammatic representation of the identification procedure for the extra chromosome of trisomics using the translocation testers. a, b, and c represent 3 different chromosomes of barley complement. (A) Interchange between a and b, (B) Interchange between b and c

Twenty-seven of the possible 42 cross combinations, using 6 interchange testers, have been analyzed. The results are given in table 6. The extra chromosomes of Bush, Slender, Pale, and Robust are chromosomes 1, 2, 3, and 4, respectively. The results confirmed that the extra chromosomes of Pseudonormal, Purple and Semierect are chromosomes 5, 6, and 7.

Thus the interrelationships of trisomics and chromosomes as well as linkage groups have been established as shown in table 10 (cf. Kramer and Swomley-Blander 1961, Tsuchiya 1961).

TABLE 6. *Chromosome configurations in trisomic F_1 hybrids between seven trisomics and 6 interchange testers.*

Trisomic type	Burnham's interchange tester						Extra chromosome determined
	a – b	b – d	c – d	c – e	e – f	f – g	
Bush	V[1]	V	—[2]	IV + III[3]	IV + III	—	b
Slender	IV + III	—	—	IV + III	V	—	f
Pale	IV + III	IV + III	V	V	IV + III	IV + III	c
Robust	IV + III	IV + III	IV + III	—	—	—	e
Pseudonormal	V	IV + III	IV + III	—	IV + III	—	a
Purple	—	—	—	—	—	V	g
Semierect	IV + III	V	V	IV + III	IV + III	—	d

[1] V: $1_V + 5_{II}$
[2] —: Not yet studied
[3] IV + III: $1_{IV} + 1_{III} + 4_{II}$

Chromosome configurations and the types of trivalents at diakinesis and MI were studied in detail as shown in table 7 (Tsuchiya 1960a). The frequency of different chromosome configurations and the different types of trivalents vary with different trisomic types. This situation suggests that the differences may be due to frequency and terminalization of chiasmata, although the chiasmata have not been analyzed.
The types of trivalents are mostly chains, ring-and-rod (frying-pan), Y-shape and triple-arc, indicating that all the trisomics obtained from autotriploids were primary types. The results of studies on the nucleolus-chromosome relationships will be given later (cf. table 13).

GENETICAL IDENTIFICATION OF TRISOMICS AND CYTOGENETIC ESTABLISHMENT OF LINKAGE GROUPS IN BARLEY

The inheritance studies in trisomics using marker genes provide the data for the genetical identification of trisomic types and also serve to establish linkage groups. Back cross and selfing methods have been compared by Rick and Barton (1954) in tomato with the conclusion that the latter method has several advantages and also higher efficiency than the former.
The trisomics in the wild variety have shown sufficiently high fertility in all seven primary types to be usable (table 5). This high fertility has facilitated the genetic studies of trisomics, especially using the selfing method.
The trisomic types were crossed as female with diploid linkage testers having one or more marker genes. The trisomic F_1 plants were selfed to give F_2 progenies in which segregating ratios were examined. The procedures are as follows: first of all, the F_2 plants derived from a cross combination were pooled and tested for a 3:1 ratio. If a segregating ratio deviated significantly from a 3:1 then the population was tested against a trisomic ratio.

TABLE 7. *Chromosome associations and types of trivalents at diakinesis (DK) and metaphase-I (MI) in seven trisomic types (in per cent)*

Type of trisomic	Stage	Chromosome associations				Types of trivalents				No.triv. obsvd.
		$_{1}III + _{6}II$	$_{7}II + _{1}I$	others	no.spor. obsvd.	chain	frying pan	Y-type	triple-arc	
Bush	DK	82.9	17.1	—	140	27.6	60.3	0	12.1	116
	MI	70.7	28.7	0.7	150	67.3	31.8	0.9	0	107
Slender	DK	89.3	10.7	—	420	53.1	43.2	1.1	2.7	375
	MI	78.6	20.0	1.4	1280	69.2	29.8	0.4	0.6	1007
Pale	DK	94.3	5.7	—	265	40.8	56.0	0	1.0	250
	MI	76.0	23.8	0.2	2060	62.4	36.1	0.9	0.6	1569
Robust	DK	92.9	7.1	—	42	46.2	48.7	0	5.1	39
	MI	77.7	21.9	0.4	480	64.9	33.0	1.1	1.1	373
Pseudonormal	DK	79.5	20.5	—	200	49.7	47.1	0.6	2.5	159
	MI	63.1	36.5	0.4	260	72.0	25.6	1.8	0.6	164
Purple	DK	89.0	11.0	—	100	41.6	53.9	0	4.5	89
	MI	77.7	22.3	0.1	1200	60.2	37.8	0.5	0.1	933
Semierect	DK	93.9	6.1	—	262	20.3	70.7	0.8	8.1	246
	MI	71.7	28.2	0.2	660	47.1	46.7	5.7	0.4	473
average	*DK*	*89.1*	*10.9*	—	*1429 (total)*	*40.6*	*54.1*	*0.4*	*4.9*	*1273 (total)*
	MI	*75.9*	*23.7*	*0.5*	*6090 (total)*	*62.5*	*35.5*	*1.3*	*0.02*	*4626 (total)*

TABLE 8. *Summary of F_2 data from those crosses in barley showing trisomic ratios; all from duplex trisomic plants; VVv, BBb, etc.*[1]

Name of trisomic	Chromo-some	Gene pair	Observed numbers in 2n		Observed numbers in 2n + 1		Total			% rec.	% 2n+1
			dom.	rec.	dom.	rec.	dom.	rec.	total		
Bush	1	*Nn*	281	24	89	0	370	24	394	6.1	22.6
,,	1	*Br br*	280	25	90	0	370	25	395	6.3	22.8
,,	1	*Fc fc*	258	16	138	0	396	16	412	3.9	33.5
Slender	2	*Vv*	235	29	61	0	296	29	325	8.9	18.8
Pale	3	*Uz uz*	357	17	136	0	493	17	510	3.3	26.7
Robust	4	*Kk*	53	11	24	0	77	11	88	12.5	27.3
,,	4	*Bl bl*	201	25	128	0	329	25	354	7.1	36.2
Pseudonormal	5	*Bb*	76	3	39	0	115	3	118	2.5	33.1
Purple	6	[2]									
Semierect	7	*Ss*	100	9	45	2	145	11	156	7.1	30.1
total (avg. %)			*1841*	*159*	*750*	*2*	*2591*	*161*		*6.4*	*27.9*

[1]) Rearranged by Dr. C. R. Burnham (1962) from the data presented in Tsuchiya (1959a) and Tsuchiya et al. (1960)

[2]) Only disomic ratios were observed with markers for the linkage groups then known

Expected trisomic ratios have been investigated by several workers (Blakeslee and Farnham 1923, Lesley 1937, Rhoades 1933, and others). Expected trisomic ratios used in this study were based on maximum equational segregation (cf. Burnham 1962, Tsuchiya 1960a).

The tests of segregations of 10 allele pairs for the seven trisomic types were performed in 66 of the possible 70 cross combinations. Fifty-two out of 66 populations proved to fit the disomic expectations. Four more populations were not analyzed in detail because the number of plants in the populations was too small to be tested for the segregating ratios.

In the remaining 10 populations the segregating ratios deviated significantly from disomic expectations. These were then tested against trisomic ratios after dividing the populations into diploid and trisomic portions (table 8). The 10 populations contained no homozygous recessive trisomics with the exception of Semierect tested against *Ss*, in which two homozygous recessive trisomics *(sss)* were obtained among 47 trisomics in F_2 from an F_1 duplex trisomic *(SSs)*.

TABLE 9. *Summary of tests against disomic and/or trisomic ratio of ten marker genes involved in respective linkage groups of barley*

Type of trisomic	Marker genes in respective linkage groups tested against trisomic ratio	disomic ratio									
		I	II	III	IV	IV	V	V	VI	VII	VII
Bush	*n* (III)										
,,	*br* (VII)	v	B	x	–	bl	s	r	uz	x	x
,,	*f* (VII)										
Slender	*v* (I)	x	B	n	K	bl	s	r	uz	–	f_c
Pale	*uz* (VI)	v	B	n	K	bl	s	r	x	br	f_c
Robust	*K* (IV)	v	B	n	x[1]	x	–	r	uz	br	f_c
,,	*bl* (IV)										
Pseudonormal	*B* (II)	v	x	–	K	–	s	–	–	–	f_c
Purple	– –	v	B	n	K	bl	s	r	uz	br	f_c
Semierect	*s* (V)	v	B	n	–	–	x	x	uz	br	f_c
,,	*r* (V)										
diploid (control)	– –	v	B	n	K	–	s	r	uz	br	f_c

[1] F_3 data, all others F_2 data. x Trisomic ratio. – Test not yet completed

The segregation of all 10 gene pairs fitted the expected trisomic ratio in the diploid and trisomic portions, indicating that each marker gene is carried on the extra chromosome of the respective trisomic type except for Purple, the trisomic for chromosome 6 (tables 8 and 9). It is noteworthy here that the trisomic for chromosome 1 (Bush) showed trisomic segregations for characters previously thought to be in separate linkage groups, III *(Nn)* and VII *(Brbr)*. This result confirmed a conclusion arrived at earlier from linkage tests with interchange testers by Kramer et al. (1954).

From the results of genetic studies, all trisomic types except for Purple were genetically identified and the relationships between trisomics and genetic linkage groups were established (tables 9 and 10). For the Purple trisomic for chromosome 6 the tests for genetic markers have not been completed though F_1 hybrids have been obtained. However, the independence of Purple from the other 6 types has been clearly established by cytological, genetical and morphological investigations of trisomics (Tsuchiya 1956, 1958c, 1959a, 1960a, 1961). The same conclusion has been drawn from cytogenetic studies of interchange lines (cf. Kramer and Swomley-Blander 1961, Kramer et al. 1954, Ramage and Suneson 1958, Ramage et al. 1961).
The relationships between trisomics, new linkage groups and chromosomes, previous linkage groups, translocation intercross designations and type gene pairs are thus, established as shown in table 10 (Kramer and Swomley-Blander 1961, Tsuchiya 1961).

TABLE 10. *Interrelationships between trisomics, chromosomes, and key marker genes in the respective linkage groups of barley*

Trisomic type	New linkage group and chromosome numbers	Key marker gene pair	Previous designations	
			chromosome	linkage group
Bush	1	*Nn*	b	III, VII
Slender	2	*Vv*	f	I
Pale	3	*Uzuz*	c	VI
Robust	4	*Kk*	e	IV
Pseudonormal	5	*Bb*	a	II
Purple	6	*Oo*	g	new
Semierect	7	*Rr*	d	V

RATES OF TRANSMISSION

The rates of transmission were studied in selfing and reciprocal crosses of trisomics with diploids; the results are shown in table 11. The trisomic plants were recovered only from selfed trisomics and $2x + 1 \times 2x$; the average being 27.5 percent for the former and 22.7 percent for the latter. No trisomic plants have been obtained so far from the cross $2x \times 2x + 1$ though the number tested is small. All the seven trisomic types have produced exactly the same types in their progenies as the parental types with only a few exceptions. Only one secondary trisomic has been found (in an F_1 hybrid line of Slender $\times$ Colsess V). A pericentric inversion of chromosome 6 was found in a pedigree of Purple (Tsuchiya 1960a, 1962b, c, 1963c).
Unrelated types have never been obtained in the progenies of the 7 types, though such off-type plants as haploids and triploids have been obtained rarely (Tsuchiya 1960a, 1962a).
Generally speaking, the rate of transmission of the primary trisomics is sufficiently high to maintain the stocks. The high fertility and germination percentage (table 5) and the high transmission rates (table 11) have facilitated the maintenance of the trisomics and tests for the segregating ratios in F_2 or later generations by selfing.

The transmission rate is related to the size of seeds, especially their width and thickness as has been previously reported (Tsuchiya 1960a; cf. Ramage 1955, 1960, Ramage and Day 1961). The size difference of diploid- and trisomic-producing seeds is shown in fig. 5.

TABLE 11. *Rates of transmission in the barley trisomics*

Type of trisomic	Frequency in progenies from								
	(2x + 1) selfed			(2x + 1) × 2x			2x × (2x + 1)		
	total plants identified	2x + 1		total plants identified	2x + 1		total plants identified	2x + 1	
		number	%		number	%		number	%
Bush	2084	597	28.65	249	62	24.90	54	0	0
Slender	927	181	19.53	160	44	27.50	129	0	0
Pale	1793	448	24.99	357	97	27.17	175	0	0
Robust	622	210	33.76	141	16	11.34	102	0	0
Pseudonormal	250	73	29.20	51	5	9.80	118	0	0
Purple	1031	329	31.91	161	28	17.39	65	0	0
Semierect	816	234	28.68	102	25	24.51	97	0	0

USEFULNESS OF TRISOMICS IN GENETICS AND BREEDING

Linkage studies – Assigning genes to the chromosomes

The usefulness of trisomics in assigning genes and/or linkage groups to a particular chromosome may be illustrated by the example given below. Takahashi et al. (1957) found that a linkage group consisting of 4 genes *(yh-sh-Hs, Hn)* [1]) could not be associated with any of the 7 linkage groups (I-VII) by the conventional gene analytical method. Using Tsuchiya's trisomic series in the wild variety, Takahashi and Hayashi (1962) and Takahashi et al. (1962) were able to assign the floating linkage group to chromosome and linkage group 4 (former group IV) (table 12). Takahashi et al. (1962) have also examined several other genes against the trisomics with the results shown in table 12.

Cytological studies, especially the nucleolus chromosome relationship

Trisomics have some advantages in the study of various cytological problems related to the existence of an extra chromosome. Among others it is especially suitable to the study of nucleolus-chromosome relationships and also nucleolus organizing capacity of each chromosome as has been made in maize (Lin 1955).

[1]) *yh* – yellow head; *sh* – spring habit of growth; *Hs* – hairyness of the leaf sheaths at the base; *Hn* – lemma hairs on the nerves and completely linked with *Hs* (Takahashi et al. 1957).

The extra chromosomes in barley had been expected to affect the nucleolus-organizing capacity in the trisomic condition, especially in the trisomics for satellited chromosomes. The seven trisomic types have provided suitable material for the study of this subject. The number of nucleoli at late prophase are shown in table 13.

TABLE 12. *Summary of trisomic analysis for 5 genes* [1])

Chromosome / Trisomic type	1 Bush	2 Slender	3 Pale	4 Robust	5 Pseudo-normal	6 Purple	7 Semi-erect
yellow head *(yh)*	[2])	[2])	[2])	link.	[2])	[2])	[2])
hairy sheath *(Hs)*	[2])	–	[2])	link.	–	–	[2])
permanent wave *(pw)*	[2])	–	link.	[2])	[2])	–	–
fragile stem *(fs_2)*	[2])	–	[2])	[2])	link.	–	–
subjacent hood *(sk)*	[2])	–	[2])	[2])	[2])	–	[2])

[1]) From Takahashi et al. (1962)
[2]) Segregated in a 3:1 ratio in both disomic and trisomic groups

TABLE 13. *Frequency of sporocytes with varying number of nucleolus at late prophase of meiosis in trisomic plants (in percent).*

Type of trisomic	Number of nucleolus per sporocyte				Number of sporocytes observed
	1	2	3	4	
Bush	97.9	2.1	0	0	140
Slender	98.3	1.7	0	0	420
Pale	98.1	1.9	0	0	265
Robust	100.0	0	0	0	42
Pseudonormal	98.5	1.5	0	0	200
Purple	88.0	10.0	1.0	1.0	100
Semierect	93.9	5.7	0.4	0	262
average	*96.9*	*2.9*	*0.1*	*0.1*	*1429 (total)*
diploid	99.2	0.8	0	0	509

Usually only one nucleolus was observed in most sporocytes although two or more nucleoli were observed with varying frequencies in the different trisomic types (table 13).

In many sporocytes of 5 trisomic types for chromosomes 1 – 5 one or rarely two bivalents are associated with the nucleolus or nucleoli at late diplotene and diakinesis. In Purple, the trisomic for chromosome 6, all but a few sporocytes showed the trivalent associated with the nucleolus or nucleoli; only rarely a bivalent and a univalent attached to the nucleolus (nucleoli). The trisomic for chromosome 7 (Semierect) showed a considerable number of sporocytes in which the trivalent or univalent together with one or two bivalents were attached to the nucleolus or nucleoli.

From the results mentioned above it has become clear that chromosomes 6 and 7 have the strongest nucleolus organizing capacity, and the former is stronger than the latter. Also the other five non-satellited chromosomes may have a different degree of nucleolus organizing capacity, though it seems to be very weak.

Effects of extra chromosome

Morphological effects

The characteristics of the extra chromosomes in the homozygous trisomics have been described in detail (tables 4 and 5; cf. Tsuchiya 1958c, 1959a, 1960a). The effects of extra chromosomes in some hybrid combinations are described below.

The most striking is the effect on the expression of the hooded character. There are three different types of hooded barley; normal hood *(K)*, elevated hood *(K^e)*, and subjacent hood *(sk)* (Takahashi 1955, Takahashi et al. 1953). The genes *K* and *K^e* belong to the linkage group 4 (former IV) together with the recessive awned allele *(k)*, but *sk* has not yet been associated with any of the linkage groups (table 12; cf. Takahashi et al. 1953, 1962).

In the study of trisomic inheritance the seven trisomic types were crossed with a linkage tester, Colsess V which had a marker gene *K* for normal hood. The F_1 hybrids and F_2 trisomic segregants showed marked differences in the expression of the hooded character as shown in fig. 8. These results suggest the existence of a modifier or polygene system which controls the hooded character expression.

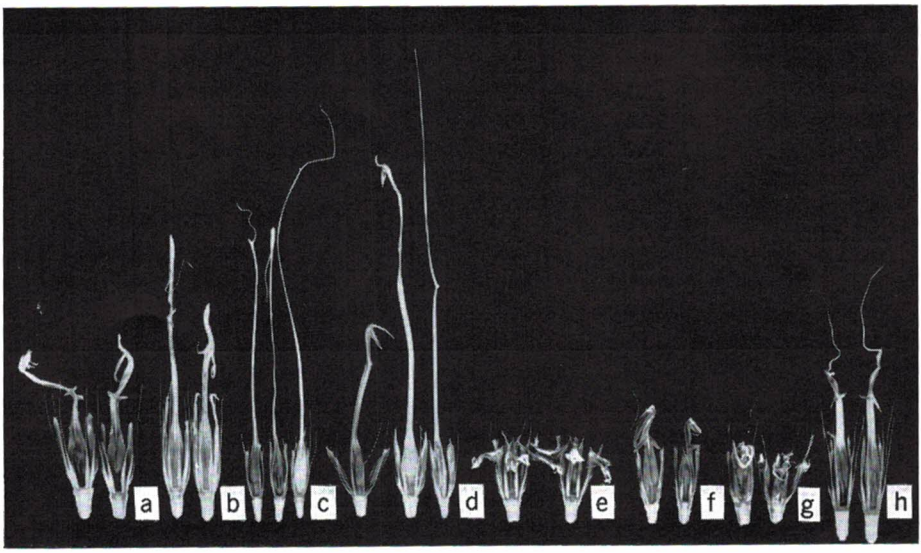

FIG. 8. Hooded character expression in trisomics and diploid
a. normal hood in diploid (*Kk*); b, c and d. elevated hood in Bush (*Kk*), Slender (*Kk*), and Pale (*Kk*), respectively; e. subjacent-like hood in Robust (*Kkk*); f and g. subjacent-like hood in Pseudonormal (*Kk*) and Purple (*Kk*), respectively; h. almost normal hood in Semierect (*Kk*)

Next is the expression of the six-rowed character in trisomic hybrids. Usually the F_1 hybrids between two- and six-rowed varieties show an intermediate phenotype. The F_1 hybrids, both diploid and trisomic, between trisomics *(H. spontaneum* v. *transcaspicum)* and some six-rowed linkage testers *(H. vulgare)*, showed the intermediate row character (having a considerable development of lateral florets). However, the degree of lateral development was different from trisomic type to type. In Pale (trisomic for chromosome 3) some of the lateral florets set seeds; fertility of lateral florets of trisomic F_1 hybrids between Pale and Colsess V, and Pale × Fragile stem was 3.84 percent and 10.17 percent, respectively. The fertility of central florets was 69.2 and 81.9 percent, respectively.

In contrast the lateral florets were completely sterile in the other six trisomic types.

These results suggest that the trisomic plants may be useful for the analysis of modifier or polygene systems which control the character expression of some major genes.

Cytological effects

Generally speaking, mitotic and meiotic processes are almost normal in most of the trisomic plants. All the cells divided normally at mitosis with only a few exceptions which showed a few tetraploid cells (mixoploidy) (Tsuchiya unpublished).

At meiosis most of the cells showed $1_{III} + 6_{II}$ and $7_{II} + 1_I$, and only rarely $6_{II} + 3_I$. The meiotic behavior of chromosomes are, in general, almost the same as those reported in other species (cf. Tsuchiya 1959a, 1960a). Two trisomic types, however, showed specific abnormalities in meiosis.

In Slender (trisomic for chromosome 2), about 1 percent of the sporocytes showed complete asynapsis having 15 univalents at diakinesis and MI. This effect was observed in all Slender trisomics. The double trisomic plants having specific slender characteristics also showed the same asynaptic phenomenon (16 univalents) as the simple trisomic Slender. Thus the asynaptic behavior may be ascribed to the specific effect of chromosome 2 (Tsuchiya 1959c, 1960a). The asynaptic phenomenon was not recorded in Slender of Wase (Early) Golden Melon (Tsuchiya 1952a). The cause of this difference is not known.

Next is the syncyte formation in Pale (trisomic for chromosome 3). Various types of syncytes (polyploid cells) consisting of 2–7 nuclei were observed in some plants of Pale, though Pale plants did not always show syncyte formation (Tsuchiya 1959c, 1960a). In contrast to the asynaptic effect of chromosome 2 in Slender, the effect of chromosome 3 on syncyte formation in Pale does not seem to be absolute, showing different degrees of penetration because some Pale plants did not show the phenomenon. It may be worthy to mention here that, however, a double trisomic plant having Pale characteristics also showed extreme syncytes in meiosis (Tsuchiya unpublished)

FUTURE PROBLEMS IN THE TRISOMIC STUDIES

Comparison of trisomic series from different sources

Rick and Notani (1961) have reported differences in tolerance of aneuploidy and in character expression in trisomics between primitive and highly selected horticultural varieties of tomato.

In barley two complete series of trisomics were previously reported by Ramage (1955, 1960) and Tsuchiya (1954, 1958c, 1959a, 1960a). From a comparison of the results obtained by Ramage (l.c.) and Tsuchiya (l.c.) it has become clear that the characteristics of the trisomic types are expressed more distinctly in the wild variety than in the cultivated one. The greatest difference was the much lower fertility in the series in the cultivated variety (cf. Burnham 1962, Tsuchiya 1958c, 1960a).

In his previous paper the present writer stated that the extreme differences between the two series could be attributed to the differences of:

1. the original variety from which the trisomics were established, namely, cultivated 6-rowed variety, Mars, by Ramage (l.c.) and wild 2-rowed variety, *H. spontaneum* v. *transcaspicum*, by Tsuchiya (l.c.)
2. the methods by which the trisomic series were produced, namely, translocation heterozygotes by Ramage and autotriploids by Tsuchiya.

At any rate, it would be highly desirable to compare different trisomic series and other aneuploids in various combinations as given below in order to find the causes of differences in the tolerance of aneuploidy, in character expression of trisomics and especially in seed fertility.

1. Autotriploid vs. reciprocal translocation (RT) in the same variety.
2. Wild vs. cultivated varieties for the same source; autotriploid and interchange.
3. Two-rowed vs. six-rowed varieties from the same source.
4. Different varieties within six-rowed or two-rowed barley.

In addition to the two complete sets of primary simple trisomics just mentioned, three other almost complete series have been recently induced by Ising (1961, unpublished) from triploid of two-rowed variety and by Kerber (1954, 1958, unpublished), and Larter and Enns (1962, unpublished) from triploids of six-rowed cultivars. Further, a new series of seven primary trisomics has been established in a cultivated two-rowed variety, Shin Ebisu No. 16 (Tsuchiya 1963a, b, unpublished).

Thus, at present, we have six complete or almost complete sets of primary simple trisomics of which only one set has been derived from interchange heterozygotes of a six-rowed variety, Mars (Ramage 1955, 1960), and the other five sets from autotriploids and triploid hybrids.

As mentioned above the trisomic series have not been established from different sources in the same variety. But trisomic sets have been established in five different varieties from autotriploids, so that it will be possible to make comparisons between wild and cultivated two-rowed varieties, between two-rowed and six-rowed cultivars, and between autotriploid and interchange heterozygote in six-rowed cultivated varieties. This year the present writer has made a preliminary comparison of some characters including fertility between two trisomic series from autotriploids of wild and cultivated two-rowed varieties.

Generally speaking the external morphology of the new trisomics of the cultivated variety, Shin Ebisu No. 16, is very similar to the previous set from the wild variety and it was easy to distinguish the trisomics from diploid sibs and also from each other. The fertility is rather different in the two series. The fertility of the new series is very low in selfing with only one exception (Purple) which shows almost complete seed fertility in selfing, $2x + 1 \times 2x$, and $2x \times 2x + 1$. However, the fertility has been raised greatly by artificial self pollination and also by reciprocal crosses with diploids.

The trisomics from autotriploid plants of cultivated six-rowed varieties seem to be

different from those from two-rowed varieties. From the comparison of all the complete series of trisomics so far obtained the causes of differences between these series will be disclosed.

Linkage studies

Production and use of balanced tertiary trisomics

The procedure of the production and the use of the balanced tertiary trisomics has been thoroughly described by Ramage (1963) in this Symposium.

Assigning new genes to the particular chromosome

Our linkage studies in barley are an example of the most advanced researches in this field of plant cytogenetics like in maize, tomato, and *Pisum*, and the linkage maps have been thought to be well established. However, as has been learned from the study of a floating linkage group (Takahashi et al. 1957, 1962) and from the fact that there are many non-designated genes, some barley chromosomes still have many regions which are as yet unmarked with any known gene. Consequently, a number of genes have been shown to be difficult or impossible to assign to the linkage groups by the conventional method of gene analysis (cf. Takahashi et al. 1957, 1962; Robertson 1963, Woodward 1957). Further it has been assumed that the barley chromosomes in different varieties differ as to their content of duplications (cf. Hagberg 1959, 1960), inversions (cf. Powell and Nilan 1963) or other structural aberrations (cf. Sharma and Sharma 1959). The trisomic method is applicable to linkage studies irrespective of such structural changes of chromosomes as mentioned above. At any rate, further extensive studies of linkages in barley are required to establish more complete linkage maps in which a sufficient number of genes are located on the chromosomes and also to reach a better understanding of the structure of barley chromosomes.

There is a vast number of spontaneous and artificially induced mutations in many cultivated barley varieties (cf. Hagberg 1963; Gustafsson 1947; Scholz 1957; Scholz and Lehmann 1962; Stubbe and Bandlow 1947; and others). There are also about 300 viable mutations induced by X- and γ-irradiations of some two-rowed cultivated varieties grown in Japan (Tsuchiya 1962d).

The trisomic series have also been established in 6 varieties, including wild and cultivated; two- and six-rowed; and also triploid- and interchange derivatives. Thus it becomes easier to assign newly obtained genes to a particular chromosome by means of trisomic inheritance.

Comparison of mutability between chromosomes of barley

In the mutation research in barley we have obtained a vast number of chlorophyll deficient mutations (cf. Gustafsson 1940, 1947, Holm 1954; others) which are mostly lethal and difficult to assign to the correct linkage groups by the conventional gene analytical method. In fact only a few chlorophyll-deficient lethal genes have been assigned to the particular chromosomes or linkage groups in barley (cf. Holm 1963; Robertson 1963; Robertson et al. 1941, 1947, 1955; Takahashi 1956a, b).

The trisomics provide a method to assign new mutant genes directly to a particular chromosome. The trisomics have some advantages for this purpose. First of all the

smaller seeds produced by trisomic plants have shown a much higher frequency of trisomics than the larger seeds (Ramage 1955, 1960; Ramage and Day 1961; Tsychiya 1960a). Thus it is easy to treat the selected trisomic seeds by X-or γ-rays or chemical mutagens.

The procedure is as follows:

1. Select the smaller seeds
2. Treat the seeds by radiations or chemical mutagens and grow the X_1 plants
3. From each trisomic X_1 plant collect the seeds from each spike separately
4. Examine X_2 spike progenies to observe the segregating ratios of mutated genes (trisomic or disomic). Here we should be careful to check for disturbances of X_2 segregations of mutated genes by chromosomal aberrations or other factors
5. Thus we should repeat the test for segregations in the X_3 generation. Seeds collected from trisomic plants in X_2 are tested again in X_3

By this procedure new mutant genes would be assigned directly to a particular chromosome provided the mutation occurred on the extra chromosome of a trisomic. Of course, the mutant genes occurring on disomic chromosomes cannot be assigned directly by this method.

It would be possible, further, to compare the mutation rates by this method between the seven chromosomes in the barley complement.

This method, however, has some disadvantages in connection with such chromosomal aberrations as reciprocal translocations, deficiencies and others that may be induced by the irradiation. To avoid the simultaneous occurrence of chromosome aberrations which will disturb the usual trisomic segregating ratios (Moh and Smith 1951; Gaul 1960; and others), such chemical mutagens as diethyl sulfate and ethyl methane sulfonate, would be recommended because these chemicals produce a high frequency of chlorophyll and viable mutations and a very low frequency of chromosomal aberrations (cf. Nilan et al. 1963).

Uses of trisomics as a tool for evolutionary studies

Trisomics are useful in the experimental study of evolution. Herewith is reported an instance observed during the course of cytogenetic studies of trisomics in barley.

A pericentric inversion accompanied by a deletion has been observed in the 4th generation of a pedigree of a Purple trisomic for chromosome 6 (53–3–1) (Tsuchiya 1960a, 1962b, c, 1963c). The changed chromosome was about 18 percent shorter than the original chromosome 6 and had a dicentric-like appearance on the somatic metaphase plate. Due to the trisomic condition, no deleterious effects of the inversion-deficiency chromosome have been observed on viability and fertility; the new trisomics having the inverted chromosome are vigorous and highly fertile. The meiotic chromosome behavior from late prophase to quartets is almost like that of the original, primary Purple trisomic (Tsuchiya 1962b, 1963c, unpublished).

Tetrasomic plants having two inverted chromosomes in addition to the normal 14 chromosome complement were obtained in the siblings and the progeny of this new Purple trisomic (Tsuchiya 1962c, unpublished). Further, some other chromosomal aberrants having 15-, 16- and 17-chromosomes were also obtained in a pedigree of the new Purple trisomic.

In all 16-chromosome plants, configurations of $I_{IV} + 6_{II}$, $1_{III} + 6_{II} + 1_I$, 8_{II} were observed at MI. The most interesting is the configuration observed in plant No. 60-602-1 (1961) which has the karyotype of $2n = 16 = 14 + \dot{1} + \dot{1}$. In this plant all the MI sporocytes showed the configurations of $1_{III} + 6_{II} + 1_I$ and $7_{II} + 2_I$ with a few exceptions, indicating that at least one of the two extra chromosomes was not able to associate with the normal complement. From the study of mitotic and meiotic chromosomes it was ascertained that the unpaired chromosome had a median centromere and was shorter than any of the normal barley chromosomes. The meiotic behavior of this chromosome is very similar to the B chromosomes observed in other plant species. The plant having a chromosome constitution of $2n = 16 = 14 + \dot{1} + 1$ was also obtained (60-602-3 and its progeny in 1962/'63) from which many plants having $2n = 16 = 14 + 1 + 1$ were obtained in the next generation (Tsuchiya unpublished). It is expected that barley plants having new karyotypes or a new basic number, $n = 8$, will be created from such 16-chromosome aberrants.

DISCUSSION AND CONCLUSION

The establishment of possible types of primary trisomics is desirable for the study of trisomics. The establishment of a complete set of trisomics has been achieved in a limited number of plant species. All or almost all of the expected primary trisomics have been obtained in *Datura*, tomato, maize, *Nicotiana sylvestris*, spinach, *Antirrhinum*, and some others (cf. Burnham 1962).

As the possible sources of simple trisomics the following ~~six~~ seven are usually used (cf. Burnham 1962, Tsuchiya 1960a):

1. Normal diploids (spontaneous occurrence)
2. Asynaptic plants (uneven distribution of chromosomes at meiosis)
3. Progeny of irradiated plants (interchange heterozygotes and other chromosomal variants)
4. Simple trisomics (one trisomic types produce occasionally some unrelated types)
5. Double-, triple-trisomics and other polysomic plants (two, three, or more different types will be produced)
6. Triploids
7. Tetraploids (corn)

The best source is among the progeny of autotriploids as has been shown in many plant species (cf. Burnham 1962, Tsuchiya 1960a).

In barley the primary trisomics have been obtained from all the sources but 4 and 7 mentioned above. However, triploids had not been used in barley as the source of trisomics until 1950 because of the difficulty in inducing triploids by the cross 4x × 2x or reciprocal cross. The present writer has succeeded in producing considerable numbers of autotriploid plants from the cross 4x × 2x and also in the progenies of autotriploids and trisomics (cf. Tsuchiya 1958a, 1960a, c, d).

The autotriploids in barley showed relatively high seed fertility in selfing and reciprocal crosses with diploids and also good germination rates of the seeds (table 1). From these autotriploids a number of simple trisomics have been obtained (table 2; cf. Tsuchiya 1960a, c, d). The following is a comparison of the frequency of occurrence of simple trisomics from various sources (table 14).

As shown in table 14 the frequency of simple trisomics is quite high in the progenies of autotriploids; especially in the progenies of selfing and 3x × 2x. Even in the progenies of 2x × 3x the frequency of simple trisomics is still higher than any of the other sources.

Some double and triple trisomics, and also other aneuploids having 18-, 19- and 20-chromosomes, were relatively vigorous and fertile. They have been shown to be a good source of simple trisomics in barley (Tsuchiya unpublished).

TABLE 14. *Frequency of simple trisomics from various sources in barley*

Source	Total plants examined	Frequency of 2x + 1		Reference
		number	%	
spontaneous [1])	202	3	1.5	Smith 1941
spontaneous, partially sterile [2])	10	3	30.0	Kattermann 1939
partially asynaptic plants [3])	1000	14+	>1.4	McLennan 1947, unpublished McLennan and Burnham 1948
interchange heterozygotes	12[4])	4	33.4	Burnham et al. 1954
,,	289	7	2.4	Hagberg 1954
,,	6699	117	1.7	Ramage 1955, 1960
,,	5386	154	2.9	Ramage and Day 1960
,,	1629	92	5.7	Ramage and Day 1960
,,	78	2	2.6	Tsuchiya 1960
autotriploid self	25	12	48.0	Kerber 1958
triploid hybrid self	50	26	52.0	Kerber 1954, 1958
autotriploid self	190	92	48.4	Tsuchiya 1954, 1958c, 1960a, c, d, 1963a, unpublished
autotriploid × 2x	84	50	59.5	
2x × autotriploid	591	80	13.5	
autotriploid self	60	29	48.3	Tsuchiya 1963a, unpublished
autotriploid × 2x	24	8	33.3	
2x × autotriploid	25	1	4.0	
autotriploid? self	15	5	33.3	Ising 1961
autotriploid self	–	–	20–25	Larter and Enns 1962, unpublished

[1]) Probably interchange heterozygotes (cf. Ramage 1955, 1960)

[2]) Probably trisomic plants (cf. Tsuchiya 1958d)

[3]) Burnham's 'long chromosome' (Burnham 1946, McLennan 1947)

[4]) Only off-type plants having deviating characters.

If an autotriploid plant is once produced, then triploids and near triploids will be derived from the progenies of the triploids. Triploid plants were also obtained from the progeny of some trisomic plants (Tsuchiya 1960a, unpublished). They will, further, serve as a source of trisomics and aneuploids (table 2, Tsuchiya unpublished). Thus the triploids have proved to be the best source of trisomics in barley (Tsuchiya 1952a, 1954, 1958c, 1960a, c, d, unpublished; cf. Ising 1961, unpublished; Kerber 1954, 1958, unpublished; Larter and Enns 1962, unpublished).

The trisomic plants from interchange heterozygotes and homozygotes of long chromosomes (partial asynapsis) of a cultivated six-rowed variety, Mars, showed relatively low seed fertility and less conspicuous differences in external morphology in some trisomic types (McLennan 1947, unpublished; McLennan and Burnham 1948, Ramage 1955, 1960). Also trisomics from autotriploids of cultivated six-rowed varieties, Gateway (Kerber 1958, unpublished) and Montcalm (Larter and Enns 1962, unpublished), have shown a less distinctive morphology. Two trisomic series in wild and cultivated two-rowed barley (Tsuchiya 1963a, b, unpublished) showed distinctive morphological traits by which the trisomic types were easily distinguished from normal diploids and also from each other.
The primary simple trisomics so far obtained in barley including complete and incomplete sets seem to be divided into 3 distinct groups regarding the fertility.

1. The trisomics from RT heterozygotes of a cultivated 6-rowed variety, Mars, showed the lowest average fertility (8.2%) ranging from 0.0 to 36.0 percent (Ramage 1955, 1960).
2. The trisomics from autotriploids of wild 2-rowed barley, *H. spontaneum* v. *transcaspicum*, showed the highest fertility; the average of 7 types was 80.2 percent, ranging from 65.0 to 92.0 (Tsuchiya 1958c, 1960a).
3. All of the others from autotriploids and triploid hybrids of cultivated 6- and 2-rowed varieties showed a wide variation from 0.0 to about 95.0 percent, the average being 40–50 percent. The seed fertility was, however, raised greatly by the artificial self pollination in all seven types of Shin Ebisu No. 16 (Tsuchiya unpublished), and reciprocal crosses with diploid plants in Wase (Early) Golden Melon (Tsuchiya 1952a) and Shin Ebisu No. 16 (Tsuchiya unpublished). In these cases, the low fertility would be, at least partly, ascribed to some mechanical difficulties in pollen shedding. The true causes of the differences in fertility observed in different trisomic series are, however, not explained completely.

The trisomics may be used for various phases of cytogenetic studies as has been reviewed and discussed by Burnham (1962) in his extensive *Discussions in cytogenetics*. Burnham (l.c.) presented 13 items of special use of aneuploids; most of the items given are concerned with trisomics. Among others 3 were concerned with the secondary trisomics and one with tertiary trisomics.
As to the tertiary trisomics, Ramage (1963) has reviewed and discussed them intensively at this Symposium.

In barley the telocentric and isochromosomes seem to be very rare or poorly viable. In the course of investigations of trisomics only one secondary trisomic plant was observed (in an F_1 population of Slender $\times$ Colsess V) although more than several thousand trisomics and aneuploids have been investigated during the past decade (Tsuchiya unpublished). The scarcity of secondary trisomics and telocentrics may be ascribed to the meiotic behavior of chromosomes in barley; namely, misdivision was only rarely observed in meiosis of autotriploids, trisomics and other aneuploids of barley. In this connection no further attention will be paid to such chromosomes.
The present paper is mostly concerned with the primary simple trisomics in barley with a short consideration of a pericentric inversion accompanied by a deletion.

The primary trisomics are very useful in the study of linkages. In order to assign the genes to particular chromosomes the following five methods have been used:

1. Conventional gene analytical method
2. Translocation analysis
3. Trisomic analysis
4. Deficiency analysis
5. Others (fragments, and others)

The methods 2 to 5 are concerned with cytogenetic procedures. The last 2 methods have not been used in barley.

Translocation analysis has been carried out extensively by many researchers and has brought about much progress in linkage studies of barley (cf. Burnham and Hagberg 1956; Hagberg 1958, 1959, 1960, 1963; Ramage 1963; Ramage et al. 1961; and others). Trisomics were used at first for linkage analysis in barley by Ramage (1955, 1960) followed by Tsuchiya (1956, 1959a, 1960a), Tsuchiya et al. (1960), Kerber (1958, unpublished) and played a part in establishing the linkage groups cytogenetically. The trisomic analysis also confirmed the result obtained by Kramer et al. (1954) (Tsuchiya 1956, 1959a, 1960a, 1961). The trisomic method was used to assign a floating linkage group (Takahashi et al. 1957) to chromosome 4 (Robust) (Takahashi and Hayashi 1962, Takahashi et al. 1962). Thus the trisomic method is now being used to assign new genes to particular chromosomes of barley.

If the marker and new genes to be analyzed are located far from one another on a chromosome it would be very difficult or impossible to detect linkage between them by the conventional method of gene analysis. In fact, we have a large number of genes which have rigidly resisted detection of their linkages by the conventional genetic method. As has been described above the trisomics are useful for the study of linkages or to assign the genes to particular chromosomes, because each gene can be associated with the extra chromosome by the trisomic method.

However, the trisomic method also has some disadvantages in linkage studies. If the trisomic plants have low seed fertility (which was observed in some trisomics of cultivated varieties) the F_2 method would be difficult to use. The backcross method is less effective and extremely laborious in barley (cf. Rick and Barton 1954). Thus, it is desirable that the trisomic sets have sufficiently high fertility and vigor to give many seeds by selfing. Fortunately the trisomic sets in the wild variety have very high seed fertility with selfing and crossing with diploids. Most of the other sets showed various degrees of sterility.

The trisomic set should be produced in a variety having many dominant character because the study of trisomic inheritance is facilitated if F_1 hybrids are *AAa*. Thus the wild species having many dominant alleles is suitable as a source of a trisomic set. However, the wild species of barley, *Hordeum spontaneum*, has some undesirable features, i.e., brittleness of rachis, winter habit of growth and a long dormancy of the seeds which result in uneven germination. Thus, it appears desirable to establish sets from other varieties. Efforts to establish such sets are being continued. Most of the trisomic series established in six-rowed varieties are not complete; Kerber's series include some hybrid derivatives and Larter's series have not been completed. The new trisomic series in a cultivated two-rowed variety, Shin Ebisu No. 16, have been identified mainly by the morphological similarities to the previous set in the wild variety.

Cytological and genetical identifications are still not complete. One or two generations will be necessary to complete the cytological and genetical identification of the trisomics in Shin Ebisu No. 16.

The other problem in the study of trisomic inheritance is concerned with the spontaneous structural changes that take place in the course of breeding. Various kinds of structural changes would be expected to take place and to be perpetuated due to the trisomic condition as was found in *Datura* (Blakeslee 1927; cf. Blakeslee and Avery 1938). An example of a spontaneous structural change in barley is an inversion in chromosome 6.

A pericentric inversion accompanied by a deletion in chromosome 6 was obtained in a pedigree of Purple (Tsuchiya 1962b, 1963c). Because of the trisomic condition such a severe structural change has shown no deleterious effects on the viability and fertility which were almost comparable to the normal diploid and a little better than the primary trisomic Purple. The primary and new Purple trisomic type would be difficult to distinguish from a study of meiotic chromosome behavior and external morphology because the differences between the two types are only slightly expressed. The new trisomic type, having the inversion-deficiency can be distinguished by a somatic chromosome study; the changed chromosome 6 shows a dicentric-like appearance (Tsuchiya 1962b, 1963c).

This same type of change could occur in one of the non-satellited chromosomes. If the two or three breakage points (one in one arm and the others in the other arm) occurred at almost the same distance from the centromere and if the deleted segment is so short, the derived chromosome would be almost the same shape as the original chromosome and the trisomic plants having this new extra chromosome could not be distinguished from the primary type. A simple minute deletion could occur which would not give any change in chromosome morphology. Such changes of chromosomes also would not give any detectable changes in external morphology of the trisomic plants because of the trisomic condition; the newly formed trisomic plants could not be distinguished from the corresponding primary trisomics either morphologically or cytologically.

The spontaneous occurrence of such kinds of aberrations cannot be ruled out from the finding of the new Purple trisomic type having an inversion-deficiency.

Some other cytological aberrations have been obtained in the progenies of trisomics and aneuploids as well as triploids (Tsuchiya 1960a). In a progeny of Pseudonormal (trisomic for chromosome 5) several plants showing almost typical characteristics of Pseudonormal have exhibited a small fragment instead of an extra normal chromosome 5 (Tsuchiya unpublished). Plants having various types of fragments are rather common in the progeny of autotriploids (table 2) and trisomics (Tsuchiya 1960a, Ising 1961, unpublished).

If trisomics having such aberrations, and also plants with fragments, are used for genetic studies, the genes located on the deleted or missing segments would show a disomic segregation instead of trisomic inheritance.

Based on findings and speculations mentioned above, it is recommended that at least several plants in a certain trisomic type should be used for genetic studies. Of course, karyotype analysis and the study of meiosis for parental and F_1 (or F_2) plants, are necessary procedures in trisomic analysis of linkages.

The usefulness of trisomics in the study of nucleolus-chromosome relationships and morphological and cytological effects of extra chromosomes has been revealed from

the examples presented in this paper. However, the data so far obtained are still far from sufficient to explain all of the problems. A thorough consideration will be made after sufficient data will be obtained.

As has been shown in this paper the cytogenetic studies of trisomics in barley have advanced during the last decade. Further, by the establishment of 6 series of primary trisomics from different sources it has become possible to make comparisons of the effects of extra chromosomes upon the cytogenetic behaviors between different sets.

From close examination of the results so far obtained in trisomics of barley it has become clear that the trisomics are useful for other purposes than those discussed above. The comparison of mutability between chromosomes of the barley complement and the experimental study of evolution are promising; the latter has been suggested by Burnham (1962) but the former has not been considered so far.

In conclusion, we still have many projects in the study of trisomics of barley to be worked out, though a considerable amount of information has been accumulated in the last decade.

SUMMARY

The seven possible types of primary simple trisomics and other aneuploids were produced from autotriploid plants of a wild two-rowed variety of barley, *Hordeum spontaneum* C. KOCH v. *transcaspicum* VAV. Data are presented on the occurrence of trisomics and aneuploids, and morphological, cytological and genetical identifications of primary trisomic types. The usefulness of trisomics in genetics and plant breeding is considered with some examples of their use. Future problems to be studied and some recommendations in the study of trisomics are presented. The results of observational and experimental studies are summarized as follows:

1. Trisomics and other aneuploids were frequently obtained in the progenies of autotriploid plants. The most frequent was the simple trisomic ($2n = 15$) (48.4% in selfing and 59.5% in the $3x \times 2x$). The simple trisomics amounted to about 14 percent in the cross $2x \times 3x$, and the diploids amounted to about 80 per cent. A number of similar types of trisomics were obtained in the progenies of double and triple trisomics which were siblings of the simple trisomics in the progenies of triploids. From these results the writer arrived at the conclusion that the triploids are the best source of trisomics in barley.

2. The simple trisomics were classified into seven morphological types, mainly by their distinctive characteristics, and named as follows: Bush, Slender, Pale, Robust, Pseudonormal, Purple, and Semierect. The seven trisomic types are readily distinguished from normal diploids and from each other by their distinctive traits which are expressed in the whole growing period; from the very early seedling stage to maturity, in almost all of the plant organs. Identification of trisomics from their diploid sibs can be made from the size of seeds in some trisomic types.

3. The trisomic plants were very vigorous and had high pollen fertility; the average of the seven types was 91.9 per cent ranging from 92.6 to 97.2 per cent with an exception of Pale (72.3%).

The seed fertility was also relatively high in all of the seven trisomic types; the average

of the seven types was 80.2 per cent in selfing, 80.4 in 2x + 1 × 2x, and 95.2 in 2x × 2x + 1.

The germination percentage was high; the averages of the seven types in selfing and 2x + 1 × 2x were 86.4 and 85.6 percent, respectively. The germination of the seeds from 2x × 2x + 1 was slightly lower than those from the selfing and 2x + 1 × 2x, showing an average of 75.2 percent.

4. The seven trisomic types were cytologically identified by the karyotype analysis and the study of meiotic configurations of F_1 hybrids between trisomics and Burnham's interchange testers.

5. The chromosome behavior in meiosis of the seven trisomic types was, in general, similar and resembled the trisomics in the other plant species. The chromosome configurations at diakinesis and MI were $I_{III} + 6_{II}$ and $7_{II} + 1_I$ in most of the sporocytes and the types of trivalents were long-rod, V- and Y-shape, frying-pan and triple-arc which are specific to the primary trisomics. The frequency of various chromosome configurations and the types of trivalents differ at different stages of meiosis, and in different types of trisomics. These differences are helpful in testing the independence of different trisomic types. The asynaptic phenomenon observed in Slender and the syncyte formation in Pale are also helpful in identifying these two types.

6. Nucleolus-chromosome relationships were investigated in detail in all seven types with the following results: The nucleolus organizing capacity of chromosome 6 is the strongest of all seven chromosomes, showing the highest number of nucleoli (four) per sporocyte and the highest frequency of sporocytes with 2 to 4 nucleoli (12%) in Purple. The next is chromosome 7 which is the extra chromosome of Semierect, in which the trivalent is attached to the nucleolus, along with the bivalent of chromosome 6. In the remaining 5 trisomic types most of the sporocytes had only one nucleolus associated with one or two bivalents; two nucleoli were observed in a sporocyte in only a few cases and very rarely the trivalent was associated with one nucleolus or two nucleoli.

7. The results of genetic studies of these trisomic types have provided evidence of independence of the seven trisomic types and also six linkage groups. Further definite evidence was obtained for locating two previous linkage groups, III*(Nn)* and VII *(Brbr, Fc_1fc_2)*, on the extra chromosome of Bush (chromosome 1), confirming the results obtained by Kramer et al. (1954) from the genetic studies of interchange testers. In the trisomic for chromosome 6 (Purple) all gene markers tested, which belonged to the previous linkage groups, I–VII, showed disomic ratios thereby indicating the independence of Purple.

8. Rates of transmission of the extra chromosomes are high through the ovules in all the seven types, but they vary from type to type. The average of seven types was 27.5 percent ranging from 19.5 to 33.8 percent in selfing; the average was 22.7 percent with a range from 9.8 to 27.5 percent in 2x + 1 × 2x. No transmission was observed through the pollen. Trisomic plants occurred more frequently from the smaller seeds than from the larger.

9. The effects of extra chromosomes are expressed in many morphological characters and in some cytological behaviors; some are strongly and some are slightly expressed;

some are qualitative and others quantitative; some are expressed fairly well at the very early seedling stage and others at heading time or throughout the whole period of growth.

10. High seed fertility, high germination capacity, high rates of transmission, vigorous growth, and fairly well expressed effects of extra chromosomes, have made a successful performance of genetic experiments possible.

11. The usefulness of trisomics in various phases of genetics and breeding has been illustrated.

Future problems to be studied in trisomics are presented. Some recommendations have been made for the study of trisomics, especially for genetic investigations in relation to the occurrence of a pericentric inversion accompanied by a deletion.

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