EXTRACTION AND STUDY OF THE AABB TETRAPLOID COMPONENT OF THREE VARIETIES OF *TRITICUM AESTIVUM* L. EM. THELL. AND CHROMOSOME SEGREGATION IN HYBRIDS WITH THE CORRESPONDING PARENTAL AABBDD HEXAPLOID

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Pantouses John Kaltsikes

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ABSTRACT

Using the method described by Kerber (1964) the AABB component was extracted from three varieties of common wheat, Triticum aestivum L. em. Thell. (aestivum group), viz. Prelude, Rescue and Thatcher. All extracted AABB tetraploids exhibited relative to their corresponding AABBDD hexaploid: finer stems and leaves; reduced vigour, height and fertility; delayed maturity, similar stem solidness and smaller and denser spikes. The spike of Thatcher AABB resembled the spike of Triticum compactum Host. There was no resemblance between the extracted tetraploid AABB of any variety and the presently grown durum wheat varieties. Meiosis of all extracted tetraploids was normal.

Bread-making quality of the extracted AABB tetraploids was very poor, similar to durum wheat, with the exception of Prelude AABB which exhibited bread-making quality similar to its AABBDD counterpart. This high quality was attributable, at least in part, to a translocation of a segment, identified as part of the long arm of chromosome 1D, to a chromosome of either the A or B genomes. In addition however, there was cytological evidence to indicate that a second such segmental interchange also may have occured. Attempts are being made to transfer the high bread-making quality of Prelude AABB into a high yielding durum wheat variety.

Disc gel electrophoresis of the endosperm proteins did not reveal any differences in the electrophoretic patterns for the gliadins of the extracted AABB tetraploids and their AABBDD hexaploid counterparts. Prelude and Rescue however, were similar to Stewart 63, a durum wheat variety, whereas Thatcher exhibited a different and more complex electrophoretic

pattern.

Extracted AABB tetraploids were crossed to their corresponding AABBDD hexaploids. Meiosis and chromosome segregation was examined in the resulting pentaploid ($2\underline{n}=5\underline{x}=35$) hybrids. Differences were found in the rate of univalent elimination with Rescue exhibiting the highest and Thatcher the lowest rate. Reciprocal crosses between Thatcher and Rescue and their corresponding AABB extracted tetraploids revealed differences in univalent elimination which were attributed to factors affecting the development of certain zygotic combinations.

Several plants with 15^{II} were isolated in each of the three varieties. These D genome addition lines were of very poor vigour and fertility although they exhibited normal meiotic behaviour.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF TABLES (Continued)

LIST OF PLATES

LIST OF FIGURES

INTRODUCTION

It is generally accepted today that the common wheat of agriculture, Triticum aestivum L. em. Thell. ($2n=6x=42$), genomically AABBDD, originated from the combination of emmer wheat, Triticum turgidum L. var. dicoccoides (Korn), in litt. in Schweinf. Bowden ($2n=4x=28$), genomically AABB, with Triticum tauschii (Coss.) Schmal. ($2n=2x=14$), genomically DD, (Morris and Sears, 1967). The inclusion of the D genome brought about an improvement in bread-making quality of the new species which is lacking in the present-day durum wheat varieties of T. turgidum L. var. durum which originated from the ancestral emmer wheat.

Aneuploid and other analyses of the D genome have confirmed the role of its chromosomes regarding bread-making quality (Kuspira and Unrau, 1957; Welsh and Hehn, 1964; Avila and Favret, 1966; Boyd and Lee, 1967) but at the same time indicated that genes on chromosomes of the A and B genomes are important in determining the quality characteristics of common wheat varieties (Morris et al., 1966); 1968; Welsh et al., 1968). Attempts have been made to produce the seven possible D addition lines carrying fourteen pairs of chromosomes from the A and B genomes plus one pair from the D genome by crossing tetraploid with hexaploid wheat (Matsumura, 1952; Yamashita, 1947) or by crossing the tetrasomics of Chinese Spring, a common wheat variety, to tetraploid wheat (Joppa, 1967). These lines, when produced, could be used in the chromosome by chromosome study of the characteristics which differentiate between tetraploid and hexaploid wheats. Nevertheless, these lines, on account of the mode of their production, are not homozygous and therefore cannot be used in genetic studies without extensive backcrossing.

In view of the above remarks it would be of interest if the AABB component of cultivated common wheat varieties could be extracted (Kerber, 1964). This component then could: (a) be studied to ascertain the quality attributes of the A and B genomes; (b) be used in crosses with the corresponding AABBDD thereby providing a means of producing homozygous D addition lines. If an AABB component of any variety having high bread-making quality could be obtained, it could then be used for the production of high-quality durum wheat in a backcross programme with established durum varieties. The production of such a wheat would be of great interest because (a) durum wheats, on the average, outyield hexaploid wheat varieties, despite a lack of concentrated breeding effort (Shebeski, 1959); (b) they carry important disease resistance genes which lose some of their potential when transferred to hexaploid wheat.

Differences observed in chromosome elimination in the pentaploid hybrids of tetraploid by hexaploid wheat have been attributed to a variety of reasons (Kihara and Matsumura, 1942; Thompson and Cameron, 1928; Lilienfield, 1951; Love, 1940; Joppa, 1967). It was not possible hitherto to ascertain whether the univalents themselves or the background genotype control univalent elimination in these hybrids. The extracted AABB components of hexaploid wheat varieties offer the opportunity of bringing into the same genetic background univalents from different varieties by intercrossing them with the AABBDD components of other varieties.

The purpose of this study was: (a) to extract and study the AABB component from three common wheat varieties grown in Western Canada; (b) to use these components for the production of (1) the seven possible D genome addition lines and, (2) a high quality durum wheat; (c) to study

the chromosome segregation in hybrids between the extracted AABB's and their corresponding AABBDD's; and (d) to examine the causes of dissimilar chromosome segregations in these hybrids.

LITERATURE REVIEW

Extracted AABB tetraploids

Kerber (1964) was the first to extract the AABB genome of two common wheat varieties, namely Selkirk and Canthatch. The plants obtained were of poor vigour and low fertility and did not morphologically resemble the presently grown durum wheat varieties. Their bread-making quality characteristics, however, were similar to that of durum wheat (Kerber, 1968). Boyd and Lee (1967) examined the seed storage proteins of the extracted tetraploid of Canthatch by starch gel electrophoresis. No differences were found in patterns of the tetraploid and the corresponding hexaploid wheats.

When the extracted tetraploids were crossed to various varieties of Triticum tauschii (Coss.) Schmal., they gave rise to synthetic hexaploids, the bread-making quality of which was better than that of durum wheats but lower than that of the varieties from which the AABB component was extracted.

Nishiyama and Maruyama (1965) crossed two common wheat varieties to the variety vesticum of Triticum turgidum var. polonicum and then backcrossed or self-pollinated the F_1 for several generations. In the progeny of one of the varieties they were able to obtain stable tetraploid lines which did not resemble the original tetraploid parent. One of the tetraploid lines obtained was abnormally poor and showed necrosis. An attempt to extract the AABB component from the other variety was not successful.

Chromosome segregation in pentaploid wheat hybrids

Kihara (1921, 1924) investigated chromosome numbers in the F_2 and subsequent generations of the cross T. aestivum cultivar polonicum. He separated the F_2 progeny into three classes. In those plants with 14^{II} + $(1\text{-}7)^{I}$, later generations usually reverted to 14^{II}. The progeny of F_2 plants exhibiting more than 14^{II} usually reverted to 21^{II}. The intermediate group was highly sterile and usually disappeared from the population. Kihara (1924) also noted a greater frequency of parental chromosome combinations than would be expected on the random distribution of univalents. Kihara and his associates carried on an extensive study of the pentaploid hybrids, the results of which can be summarized as follows (Kihara and Matsumura, 1942):

1. Univalents tend to go the poles in groups at AII.
2. Univalents tend to be lost.
3. Eggs and especially pollen with intermediate chromosome numbers tend to be inviable.

Furthermore, Kihara and Matsumara (1942) presented the following formula for the calculation of univalent elimination in the male gametes.

$$\text{Elimination } (\%) = \frac{\Sigma fx}{0.5 \times Nx \times n} \times 100$$

where

x = number of micronuclei per sporocyte

Σf = frequency

Σfx = total number of micronuclei

$N = \Sigma f$ = total number of sporocytes scored

n = number of univalents present in the hybrid

Jenkins and Thompson (1930) examined the chromosome numbers of a small sample from the cross T. aestivum by T. turgidum cultivar emmer and obtained results similar to those of Kihara. Thompson and Cameron (1928) determined the frequency with which univalents were passed through the male and female gametes by backcrossing the F_1 pentaploid hybrids between Marquis and Chinese Spring by spring emmer and Iumillo to each of their respective parents. The univalents tended to be either lost or transmitted as a group with the result that gametes with intermediate chromosome numbers were fewer than expected on the basis of random distribution. There were differences between the crosses in the frequencies with which univalents were transmitted. Thompson (1934) examined the causes for the deficiency of plants with intermediate chromosome numbers in species crosses in wheat. He concluded that it was due mainly to the failure of pollen germination and endospermic effects which caused abortion and abnormal development of the embryo. Moreover, he found that it was primarily the female gametes that were affected by virtue of the fact that chromosome unbalance is confounded by the polyploid nature of the endosperm.

Love (1940) crossed Iumillo to Marquis, Hope and RL-729. The chromosome numbers in F_5, F_6 and F_7 tended to approach the parental combinations but there were several viable intermediate combinations. Watkins (1927) observed and analyzed univalent elimination in a 37- chromosome plant and presented formulae for determining the frequency of their inclusion in the gametes after second meiotic division although excluded from products of first division.

Joppa (1967) crossed Thatcher to three durum varieties, namely Wells, Lakota and Langdon. In the F_2, plants with fourteen bivalents plus zero to seven univalents were observed in frequencies greater than expected.

A number of plants with fifteen bivalents plus one to four univalents were also observed. The durum variety used in the cross did not affect the frequencies of various chromosome combinations in F_2 plants. He also determined the frequencies with which various numbersof univalents were passed through the female gametes of the pentaploid hybrid produced from a cross between the hexaploid variety Ceres and Wells. By assuming that univalents were passed through male gametes with the same frequency as through the female, he calculated expected zygotic frequencies and found that certain classes were deviating significantly from the expected. Kerber (1968) crossed the extracted AABB components of Canthatch, Marquis and Selkirk to their respective AABBDD components. The frequencies with which univalents were passed through the male and female gametes (Marquis) or male gametes (Selkirk and Canthatch) of the pentaploid hybrids were observed as well as the various somatic chromosome combinations in the F_2. No differences were found between the varieties examined.

Production of D genome addition lines

Yamashita (1947) produced some plants with fifteen bivalents by crossing T. aestivum cultivar spelta to T. turgidum cultivar polonicum and backcrossing the pentaploid hybrid to the tetraploid. The lines obtained were of short stature, produced few tillers, and were mostly sterile although certain lines produced a few seeds. Their meiotic behaviour was abnormal and in most cases no plants with fifteen bivalents were recovered in their progeny.

Matsumura (1952) reported on a 30- chromosome plant having twenty-eight chromosomes from T. turgidum cultivar polonicum and a single pair of D genome chromosomes from T. aestivum cultivar spelta. This plant was

relatively stable cytologically.

Joppa (1967) produced D genome addition lines by crossing the tetrasomics of Chinese Spring to the durum varieties Wells and Lakota and then self-pollinating the F_1. Of the eight plants obtained, five were male sterile and three were partially male fertile. Two of these plants carried 4D as the extra pair and the remaining plant carried 5D. Meiosis and the morphological characteristics of these addition lines were also studied. Jones (1967) obtained D addition lines in the cross of hexaploid to tetraploid wheat and in a synthetic T. turgidum cultivar durum X T. tauschii which was then crossed to the durum parent. He was unable to maintain the plants because of their sterility and reversion to the tetraploid level.

MATERIALS AND METHODS

The varieties used in this study were Prelude, Rescue and Thatcher, all three belonging to the spring type common wheat Triticum aestivum L. em. Thell. (aestivum group). Rescue and Thatcher are awnless whereas Rescue is awned and is the earliest of the three in maturity. Prelude and Thatcher are hollow stemmed whereas Rescue is solid.

Extraction of AABB tetraploids

The method described by Kerber (1964) was used for the extraction of the AABB tetraploids. The variety Stewart 63 of Triticum turgidum L. var. durum was used for the initial cross following which there were three, four and five backcrosses made for Prelude, Rescue and Thatcher, respectively. The seed of the extracted tetraploids was increased for three generations before being tested for quality.

Cytological studies

All material for cytological studies was grown in the green-house where the average temperature was 21°C. At least ten plants from each line were examined, unless otherwise indicated. For a study of mitotic chromosome numbers, root-tips were collected in ice-water and were placed at 0°C for twenty-four hours following which they were transferred into a solution of three parts ethyl alcohol and one part glacial acetic acid. They were hydrolyzed in 1N HCl at 60°C for seven minutes, stained in Feulgen and counterstained in 0.5% acetocarmine. For meiotic studies, inflorescences were collected in Carnoy's solution and stained in 1.5% acetocarmine. Pollen fertility was ascertained by scoring those pollen grains that stained in

0.5% acetocarmine solution. To ensure maximum viability of pollen, collections were made while the stigmata were still receptive.

In an attempt to identify the chromosome(s) involved in the translocations observed in Prelude AABB, crosses between this component and members of the ditelosomic series of Chinese Spring were made. The ditelosomic involved in trivalent configurations or heteromorphic bivalents in MI of meiosis of the resulting hybrid was interpreted to identify the chromosomes involved in the translocation.

Plant characteristics and statistical analysis

Height measurements of field grown plants were taken to the nearest centimeter and maturity notes were recorded as the stage when the plots were ready for harvest. Tiller number per plant was obtained from greenhouse grown material. Thousand-kernel weight was the average weight of four 1000-kernel sub-samples.

Data were compared by analysis of variance using a completely random design. The values for rod bivalents, univalents, multivalents and micronuclei per sporocyte were transformed according to the formula $\sqrt{X + 0.5}$ (Steel and Torrie, 1960). Means were compared by Lsd except for the fertility data where means were compared by the Student-Newman-Keuls test (Steel and Torrie, 1960). All means are reported in the original form. The means for height were tested by a t-test. In all cases data were tested for homogeneity before pooling. Contingency tables were used for the comparison of data from different authorities.

Bread-making quality of the extracted tetraploids and their hexaploid counterparts

Flour was milled from each variety on a Buhler experimental mill. Except where stated otherwise, the methods used for evaluating the bread-making quality of the flour were those of the American Association of Cereal Chemists (1962). Other methods used are as given in footnotes to Table V.

Extraction and fractionation of endosperm gliadins

The major soluble fraction of bread wheat endosperm proteins is the prolamin called gliadin. This fraction is defined as the protein that is soluble in 70% aqueous ethanol solution and comprises about 40% of all the proteins in the endosperm of bread wheats. Accordingly this protein fraction plays a major role in the bread-making quality of the flour.

Gliadin was extracted from 5g of flour of each variety by the following procedure. Water and salt-soluble proteins were first removed by extracting twice with 30 ml portions of 5% NaCl solution and once with 30 ml of water. The gliadin was then extracted from the residue with the two 30 ml portions of 70% aqueous ethanol solution. The ethanol extracts were combined and freeze-dried.

The similarity of the gliadins from the extracted tetraploids and their corresponding hexaploid counterparts was examined by disc electrophoresis. The procedure used was essentially the same as described by Davis (1964) except that the concentration of polyacrylamide in the gel was 7%. The gliadin was dissolved to give a 1% solution in 2M dimethylformamide and 0.1 M acetic acid solution. Fifty µl of the protein solution was applied to the column and the electrophoresis was run at pH 3.8. After electrophoresis, the gel was stained in 0.5% amido black dissolved in 7.5%

acetic acid solution, and destained electrophoretically in the same acetic acid solution. The protein bands were viewed with fluorescent underlighting and were handcopied for comparison. Photographs of the gels are not generally satisfactory since they do not show up the faint bands.

Chromosome segregation in the pentaploid hybrids

Pentaploid hybrids were produced by crossing each of the three hexaploid varieties as female, with the corresponding tetraploid (AABB) derivative extracted from it. All material studied was grown in the greenhouse. Progenies of the various F_1 plants were tested for homogeneity before pooling and differences between mean-chromosome numbers were tested for significance using the t-test, except in the case of the reciprocal hybrids of Rescue and Thatcher, where analysis of variance was used.

Production of D genome addition lines

Attempts were made to produce these lines by:

(a) self-pollinating pentaploid hybrids and selecting in their progeny those plants with fifteen chromosome pairs; and

(b) by backcrossing the pentaploid hybrids to their respective AABBDD counterpart and selecting for plants of $15^{II} + 6^{I}$ constitution. These plants were then self-pollinated and the progeny was cytologically analyzed for plants with fifteen bivalents.

RESULTS

Extracted AABB tetraploids

(1) Morphological characteristics

Relative to their corresponding hexaploids, the extracted tetraploids exhibited:

1. Reduced vigour, finer stems and leaves, smaller and denser spikes, with the exception of Thatcher AABB in which the spike resembled that of *Triticum compactum* Host. (Plate 1, Figs. 1-2).
2. Almost identical seed colour but reduced seed size.
3. 1000 kernel weight reduced by about one-half.
4. Same disarticulation, non-fragile rachis and free threshing kernels.
5. Same stem solidness.
6. Delayed maturity (by about one week).
7. No resemblance to the presently grown durum wheat varieties.
8. Shorter stature by approximately 20% (Table I).

The awns of tetraploid Prelude were darker in colour relative to hexaploid Prelude.

The fertility of hexaploid Rescue was significantly different ($P=0.01$) from the other two hexaploid varieties and was comparable to that of the extracted tetraploids (Table II). The means and standard errors for number of tillers per plant for tetraploid Prelude, Rescue, Thatcher and Stewart 63, respectively were 7.93 ± 0.76, 5.62 ± 0.53, 5.30 ± 0.57 and 6.41 ± 0.34. For the hexaploids, mean tiller number per plant was 5.42 ± 0.53, 7.21 ± 0.21, and 7.86 ± 0.43 for Prelude, Rescue and Thatcher, respectively. Thus, tillering was affected differentially in the three extracted tetraploids. Tetraploid Prelude had a significantly greater number of tillers

PLATE 1. Morphological characteristics of spikes and seeds of extracted AABB tetraploid wheats, their hexaploid AABBDD counterparts and hybrids between extracted tetraploids.

Fig. 1 - Hexaploid Rescue, Prelude and Thatcher.

Fig. 2 - Tetraploid (AABB) Rescue, Prelude and Thatcher.

Fig. 3 - Tetraploid Prelude, hybrid of tetraploid Prelude X tetraploid Thatcher, tetraploid Thatcher.

Fig. 4 - Tetraploid Thatcher, hybrid of tetraploid Thatcher X tetraploid Prelude, tetraploid Prelude.

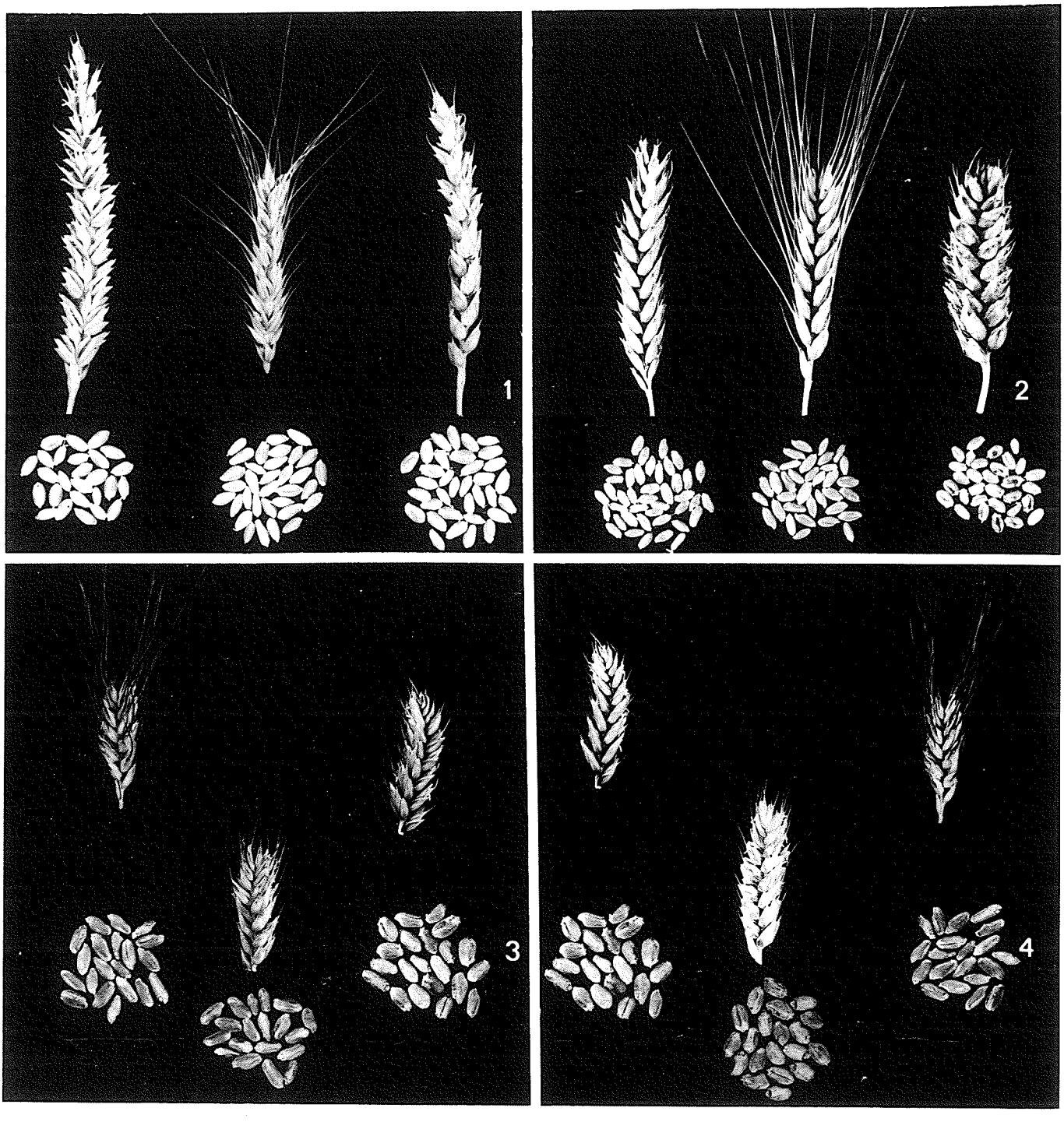
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TABLE I

HEIGHT OF EXTRACTED TETRAPLOID WHEATS AND THEIR HEXAPLOID COUNTERPARTS

Variety	Mean (cm)	St. error (cm)	t-values	Mean in % of hexaploid counterpart
Prelude AABB	76.58	3.32	8.45**	81.32
Prelude	94.16	13.57		
Rescue AABB	85.62	4.41	11.41**	80.01
Rescue	107.00	10.92		
Thatcher AABB	75.20	4.04	12.59**	76.73
Thatcher	98.00	10.10		
Stewart 63	121.50	8.23		

than hexaploid Prelude, whereas both tetraploid Thatcher and Rescue had significantly fewer tillers per plant than their corresponding hexaploids (P=0.01 and 0.05). Comparing the extracted tetraploids to the durum variety Stewart 63, only tetraploid Prelude had significantly (P=0.01) more tillers per plant.

The hybrid between tetraploid Prelude and Thatcher resembled, in both spike and seed characteristics, the female parent (Plate 1, Figs. 3-4).

(2) Meiotic characteristics

There were no significant differences among the extracted tetraploids in any of the meiotic stages which were scored, with the exception of the significantly greater number of bivalents in tetraploid Rescue (Table III). The extracted tetraploids exhibited a more regular meiosis

TABLE II

FERTILITY OF EXTRACTED TETRAPLOID WHEATS AND THEIR HEXAPLOID COUNTERPARTS

Rank	Variety	Mean (seeds) per spikelet)	St. error	Mean in % of the hexaploid counterpart
1.	Thatcher	2.24	0.008	
2.	Prelude	2.21	0.008	
3.	Thatcher AABB	1.87	0.008	83.48
4.	Rescue	1.82	0.008	
5.	Prelude AABB	1.59	0.008	71.94
6.	Rescue AABB	1.16	0.008	63.73

than their hexaploid counterparts, although the differences were not statistically significant. Asynchronous separation of some of the bivalents was observed and univalents, when present, divided at AI. Secondary pairing was seen only rarely. Pollen fertility ranged from 85% to 99% within each extracted tetraploid and no significant differences were observed between the tetraploids. Mean pollen fertility was 94.14%, 94.27% and 94.18% for tetraploid Prelude, Rescue and Thatcher, respectively. In hybrids among themselves and with a durum variety (Durum 60) the extracted tetraploids exhibited a more or less regular meiotic behaviour (Table IV) indicating that there was no extensive contamination by the D genome. Pollen fertility was within the usual range for green-house grown material.

TABLE III

MEIOTIC CHARACTERISTICS OF EXTRACTED TETRAPLOID WHEATS AND THEIR HEXAPLOID COUNTERPARTS

Variety	No. of cells examined	Bivalents			Univalents	No. of quartets scored	Micronuclei per quartet
		Ring	Rod	Total			
Prelude AABB	84	13.48 ± 0.02*	0.48 ± 0.02	13.97 ± 0.41	0.02 ± 0.02	171	0.02 ± 0.01
		(11-14)**	(1-3)	(13-14)	(0-2)		(1-2)
Prelude	94	-	-	20.89 ± 0.05	0.06 ± 0.03	182	0.04. ± 0.03
				(18-21)	(1-2)		(0-5)
Rescue AABB	20	12.90 ± 0.16	1.05 ± 0.16	13.95 ± 0.04	0.10 ± 0.02	153	0.01 ± 0.00
		(12-14)	(0-2)	(13-14)	(0-2)		(0-1)
Rescue	106	-	-	20.88 ± 0.04	0.05 ± 0.02	202	0.06 ± 0.04
				(19-21)	(0-1)		(0-4)
Thatcher AABB	64	13.37 ± 0.10	0.59 ± 0.10	13.96 ± 0.02	0.06 ± 0.04	207	0.01 ± 0.01
		(10-14)	(1-4)	(13-14)	(0-2)		(0-2)
Thatcher	109	-	-	20.90 ± 0.03	0.15 ± 0.05	89	0.04 ± 0.03
				(19-21)	(1-2)		(0-3)

* mean ± s.e. per cell
** range

TABLE IV

MEIOTIC CHARACTERISTICS AND POLLEN FERTILITY OF HYBRIDS BETWEEN EXTRACTED AABB TETRAPLOID WHEATS THEMSELVES AND WITH A DURUM WHEAT (DURUM 60) VARIETY

Hybrid	No. of cells examined	Bivalents			Univalents	Pollen fertility
		Ring	Rod	Total		
Prelude AABB X Durum 60	57	11.85 ± 0.17* (10-14)**	1.66 ± 0.16 (0-5)	13.52 ± 0.07 (12-14)	0.84 ± 0.13 (0-2)	94.14
Durum 60 X Prelude AABB	36	12.22 ± 0.17 (11-14)	1.16 ± 0.14 (0-3)	13.38 ± 0.09 (12-14)	1.22 ± 0.19 (0-2)	94.18
Prelude AABB X Thatcher AABB	154	13.22 ± 0.08 (9-14)	0.65 ± 0.02 (1-5)	13.87 ± 0.07 (11-14)	0.07 ± 0.04 (0-6)	91.37
Thatcher AABB X Prelude AABB	20	13.37 ± 0.10 (10-14)	0.59 ± 0.10 (1-4)	13.96 ± 0.02 (13-14)	0.06 ± 0.04 (0-2)	87.88

* mean + s.e. per cell
** range

(3) Chromosome(s) involved in the translocation(s) in Prelude AABB

Only the crosses to the ditelocentric for the long arm of chromosome 1D($1D^L$) gave trivalent configurations in which the telocentric was involved. Of the five plants examined from this cross, three exhibited such trivalents. From a total of ninety cells scored, the means and standard errors of total bivalents, univalents and trivalents per cell were 13.17 $\pm$ 0.09, 6.35 $\pm$ 0.09 and 0.73 $\pm$ 0.08, respectively. The bivalents were further classified as either ring or rod bivalents with means and standard errors of 11.33 $\pm$ 0.19 and 1.84 $\pm$ 0.20, respectively. There were eight cells in which the telocentric was present as a univalent and twenty cells in which it was seen in a heteromorphic bivalent. In two of the five plants studied, the telocentric was present in the univalent condition indicating that no translocation was present. Obviously, the Prelude AABB population was still segregating for the translocation(s) at the time the study was made.

(4) Bread-making characteristics

Results of bread-making tests indicated that all extracted tetraploids had a higher flour protein content and lower flour yield than their hexaploid counterparts (Table V). An increase of all but one of the parameters shown in Table V indicates improved quality. Flour ash content which depends on ash content of the wheat grain and the milling efficiency, is usually inversely related to bread-making quality. It is generally accepted that in the farinograph test, a flour of good quality develops into a dough of optimum consistency within 5 to 7 minutes of continuous mixing and maintains most of its maximum consistency for a relatively long mixing time. As shown by the dough mixing curves (Fig. 1) two of the

TABLE V

BREAD-MAKING QUALITY CHARACTERISTICS OF EXTRACTED AABB TETRAPLOID WHEATS AND THEIR AABBDD HEXAPLOID COUNTERPARTS

Variety	Prelude		Rescue		Thatcher		Stewart
	AABB	AABBDD	AABB	AABBDD	AABB	AABBDD	63
CHARACTERISTIC							
WHEAT							
Bushel weight, lb.	64	67	63	67	59	66	68
1000 kernel weight, g.	21.5	30.7	17.5	34.9	22.3	31.9	56.2
Moisture, %	11.9	11.5	13.0	11.3	11.3	11.3	12.8
Protein, % (13.5% m.b.[1])	14.7	13.0	15.3	11.4	13.2	13.2	11.8
Flour yield, %	68.2	75.1	58.6	74.6	54.8	74.1	66.1
FLOUR							
Ash, % (14% m.b.)	0.78	0.48	0.94	0.50	0.93	0.45	0.66
Colour, units	5.1	0.4	5.7	0.1	5.6	0.3	0.5
Protein, % (14% m.b.)	13.8	12.3	13.8	10.8	12.2	12.1	10.8
Wet gluten content, %	38.1	37.2	39.8	32.0	35.8	35.6	32.8
Moisture of wet gluten, %	65.2	60.0	62.7	59.6	62.9	59.9	72.7

TABLE V (Continued)

Variety	Prelude		Rescue		Thatcher		Stewart
	AABB	AABBDD	AABB	AABBDD	AABB	AABBDD	63
FLOUR (Continued)							
Protein of gluten, % (d.b.[2])	83.0	76.7	82.1	77.5	84.8	76.6	82.1
Sedimentation value[3]	33.0	54.0	18.0	65.5	17.8	67.0	19.0
Diastatic activity, mg. maltose	523	214	914	356	650	303	459
Amylograph viscosity, B.U.	240	635	30	155	90	420	270
α-amylase activity[4]	1.40	0.51	7.20	1.44	4.10	0.50	0.64
Predicted amylograph viscosity[5], B.U.	400	650	50-100	400	200	700	600
Starch damage, %	63	25	94	34	84	39	62
BREAD							
Baking absorption[6], %	60.4	57.1	60.1	58.7	60.2	62.8	56.5
Loaf volume, c.c./100 g. flour[7]	755	730	435	705	470	785	400
FARINOGRAPH							
Absorption, %	73.4	62.1	82.7	62.7	80.2	67.8	68.5

TABLE V (Continued)

Variety	Prelude		Rescue		Thatcher		Stewart
	AABB	AABBDD	AABB	AABBDD	AABB	AABBDD	63
FARINOGRAPH (Continued)							
Development time, min.	3.0	5.0	2.6	3.5	2.5	4.0	1.8
M.T.I., B.U.	60	50	165	75	100	50	85

[1] m.b. = moisture basis

[2] d.b. = dry basis

[3] A.A. C.C. method No. 56.61

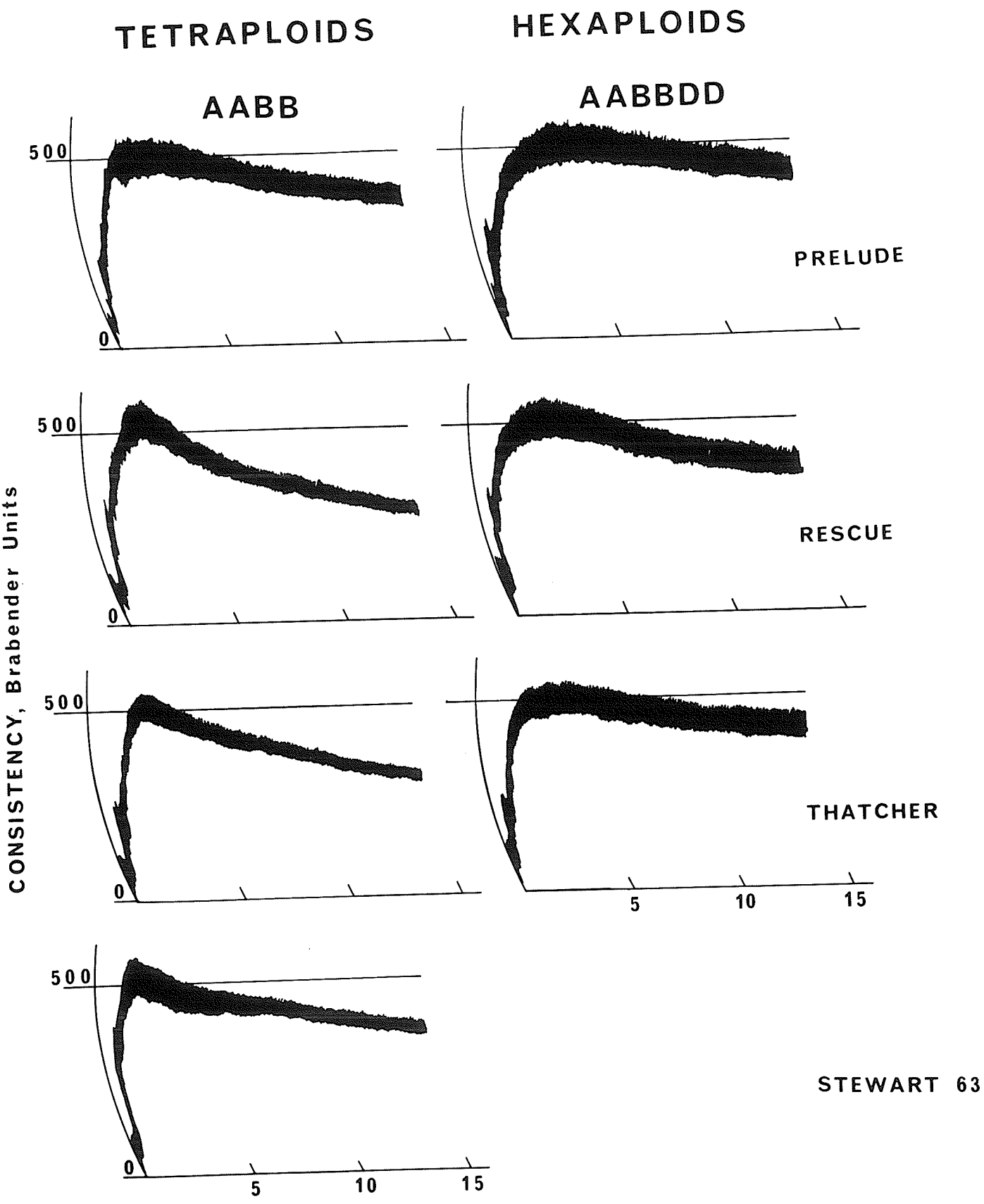
[4] Viscometric method of Tipples (1968)

[5] Predicted from α-amylase activity

[6] Adjusted for proper dough handling at panning.

[7] Remix baking test (Irvine and McMullan, 1960)

FIGURE 1. Redrawn farinograph curves of extracted AABB tetraploid wheats, their AABBDD hexaploid counterparts, and a durum wheat variety (Stewart 63).


TETRAPLOIDS
HEXAPLOIDS
AABB
AABBDD
500
0
PRELUDE
RESCUE
THATCHER
STEWART 63
5
10
15
CONSISTENCY, Brabender Units
MIXING TIME, min.

tetraploids (Thatcher and Rescue) were somewhat weaker than the durum variety Stewart 63 whereas tetraploid Prelude was slightly stronger. In general, however, the farinography curves indicated that the extracted tetraploids were weaker than their hexaploid counterparts. With one exception, the data in Table V show that the bread-making quality of extracted tetraploids was relatively poor, not too unlike that of standard durum varieties (Plate 2, Figs. 1-7). The one exception was tetraploid Prelude which showed superior bread-making quality and was comparable to that of hexaploid wheats.

(5) Disc electrophoresis of the gliadins

Electrophoretic patterns for the gliadins of the extracted tetraploids and their corresponding hexaploids were essentially the same (Plate 3, Figs. 1-7, Fig. 2). The main difference between the extracted tetraploids and their corresponding hexaploids was in the amount of gliadin in that the tetraploid contained a greater amount of this protein than did the hexaploid. Another interesting feature was the marked difference in electrophoretic patterns between the variety Thatcher and the two varieties Rescue and Prelude. The pattern for Thatcher was considerably more complex than were those for either Prelude or Rescue which in turn were similar to the pattern obtained from Stewart 63, a durum wheat variety.

Chromosome segregation in the pentaploid hybrids

(1) F_2 generation

The results of the meiotic studies of the three pentaploid hybrids are summarized in Table VI. Figures 1-6 of Plate 4 show representative cells of the pentaploid hybrids at MI and AI. The hybrid between Prelude

PLATE 2. Representative loaves of extracted AABB tetraploid wheats, their AABBDD hexaploid counterparts, and a durum wheat variety (Stewart 63).

Fig. 1 - Prelude AABB

Fig. 2 - Prelude AABBDD

Fig. 3 - Rescue AABB

Fig. 4 - Rescue AABBDD

Fig. 5 - Thatcher AABB

Fig. 6 - Thatcher AABBDD

Fig. 7 - Stewart 63 (AABB)

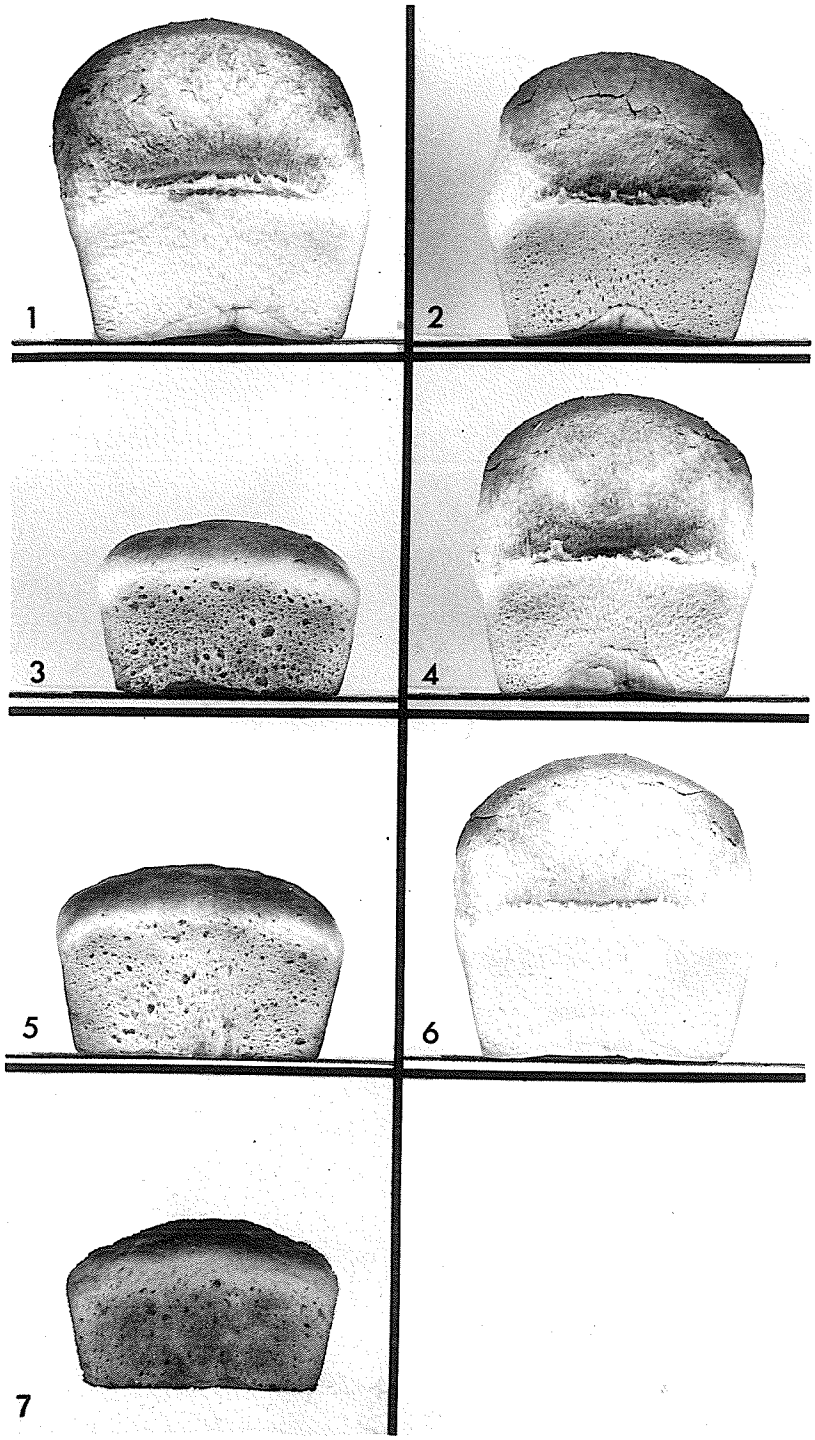

1
2
3
4
5
6
7

PLATE 3. Gliadin electrophoretic patterns of extracted AABB tetraploid wheats, their hexaploid AABBDD counterparts and durum wheat variety (Stewart 63).

Fig. 1 - Prelude AABB

Fig. 2 - Prelude AABBDD

Fig. 3 - Rescue AABB

Fig. 4 - Rescue AABBDD

Fig. 5 - Thatcher AABB

Fig. 6 - Thatcher AABBDD

Fig. 7 - Stewart 63 (AABB)

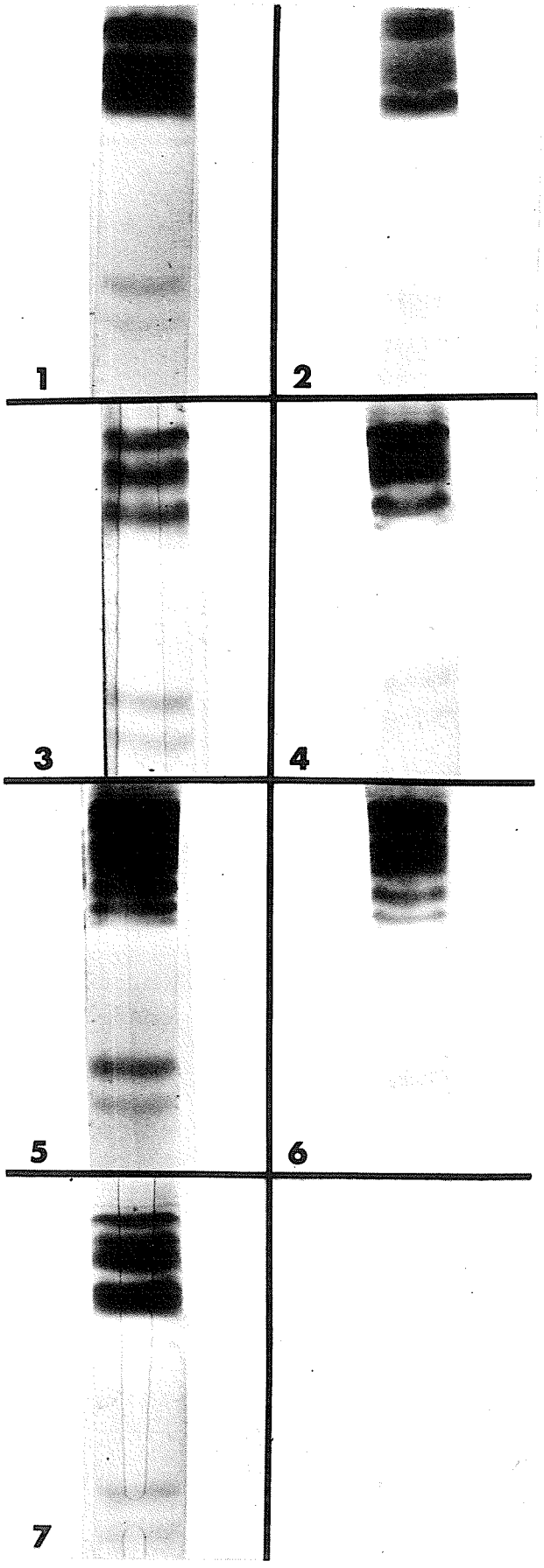

1
2
3
4
5
6
7

FIGURE 2. Diagrammatic presentation of the gliadin electrophoretic patterns of the extracted AABB tetraploid wheats, their hexaploid counterparts and a durum wheat variety (Stewart 63).

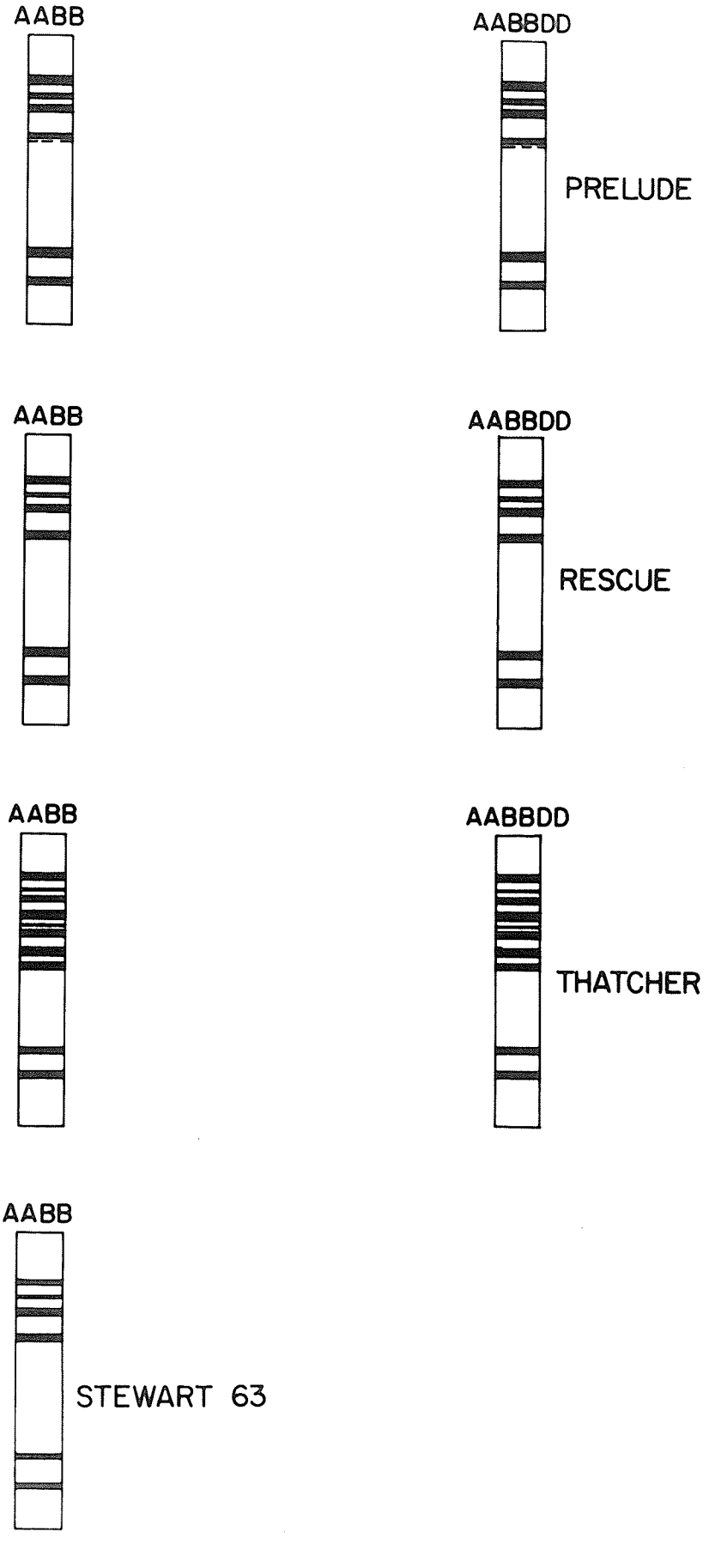

AABB
AABBDD
PRELUDE
AABB
AABBDD
RESCUE
AABB
AABBDD
THATCHER
AABB
STEWART 63

TABLE VI

MEIOTIC CHARACTERISTICS OF PENTAPLOID HYBRIDS OBTAINED FROM CROSSES BETWEEN HEXAPLOID (AABBDD) PARENT WHEATS AND THEIR EXTRACTED (AABB) TETRAPLOID DERIVATIVES

Hybrid	No. of cells examined	Meiotic Stage				
		First metaphase			First anaphase	
		Bivalents	Univalents	Trivalents	Chromosomes dividing late	No. of cells examined
Prelude X Prelude AABB	169	12.61 ± 0.08 (9-15)	5.75 ± 0.10 (3-9)	1.21 ± 0.06 (0-3)	not scored	-
Rescue X Rescue AABB	80	13.95 ± 0.05 (13-14)	7.10 ± 0.01 (7-9)	-	5.79 ± 0.26 (0-7)	34
Thatcher X Thatcher AABB	55	13.98 ± 0.04 (13-16)	6.87 ± 0.19 (3-7)	0.05 ± 0.03 (0-1)	4.95 ± 0.29 (0-7)	42

* mean per cell ± st. error

** range

PLATE 4. Meiosis of pentaploid ($AABB_D$)wheat hybrids obtained from crosses between hexaploid (AABBDD) wheats and their extracted tetraploid (AABB) derivatives.

Fig. 1 - MI of Prelude AABBD

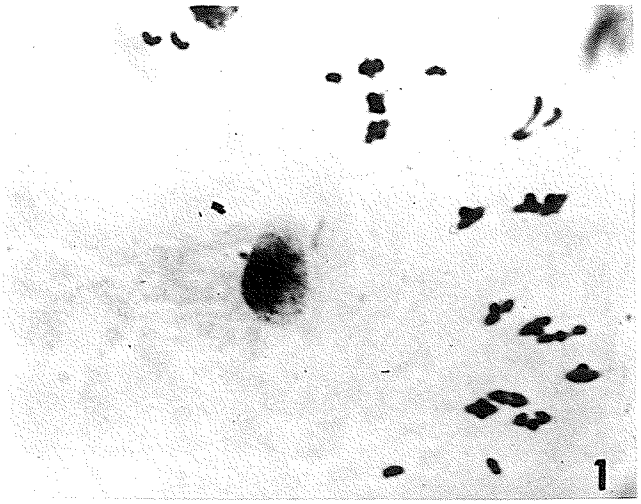
Fig. 2 - AI of Prelude AABBD

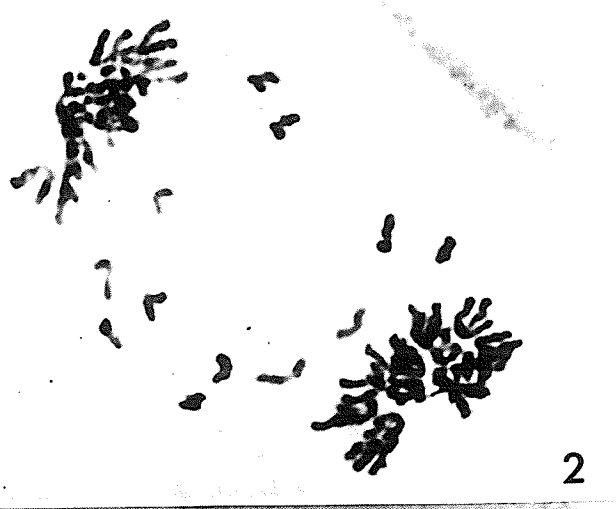
Fig. 3 - MI of Rescue AABBD

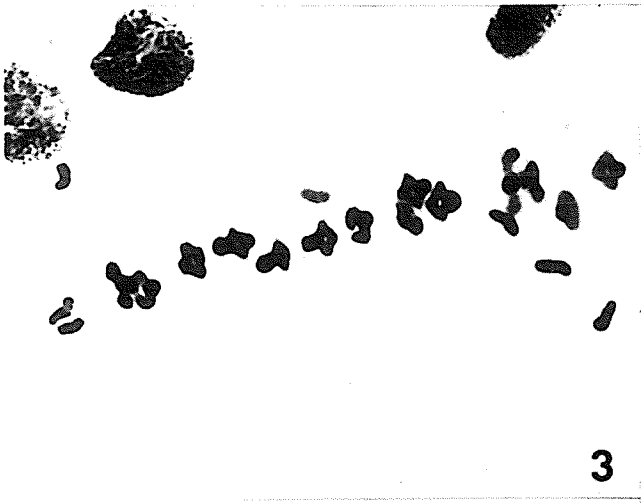
Fig. 4 - AI of Rescue AABBD

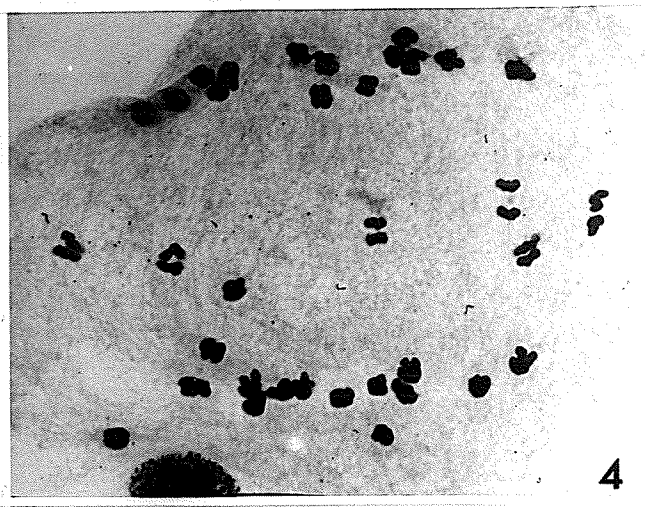
Fig. 5 - MI of Thatcher AABBD

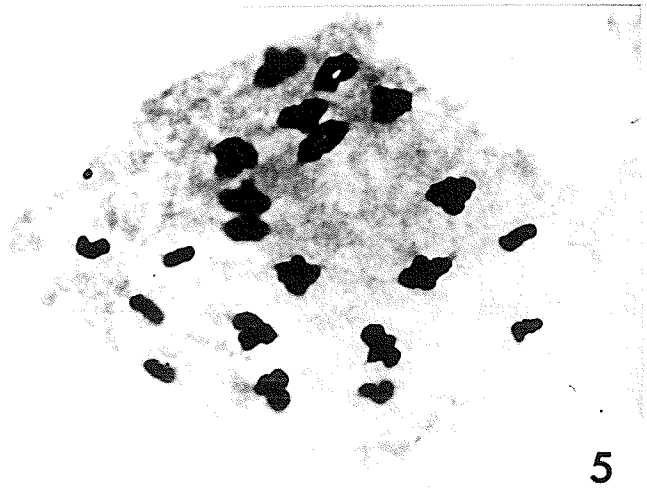
Fig. 6 - AI of Thatcher AABBD

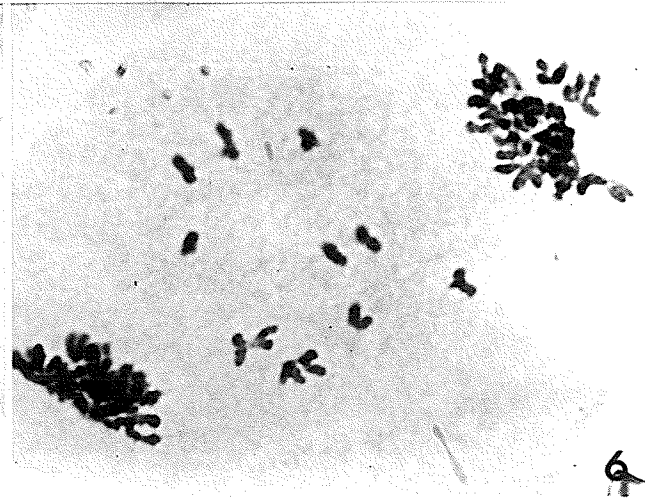

1


2


3


4


5


6

and its extracted tetraploid exhibited one trivalent in most of the cells at MI. However, there were cells in which two or even three trivalents were present, indicating that more than one translocation had occurred. Prelude and Thatcher were also crossed to Stewart 63 and the hybrids were analyzed cytologically at meiosis. The frequency of trivalents in the hybrid Stewart 63 X Thatcher was much less than it was in the case of Prelude X Stewart 63, viz. 0.10 ± 0.01 versus 0.40 ± 0.08 trivalents per cell, respectively. At AI of meiosis the fourteen bivalents of the hybrids segregated normally. The behaviour of the univalents however, was unpredictable in that they either proceeded poleward undivided, or divided late and subsequently were excluded from daughter nuclei, or failed to divide and were lost.

The number of the univalents per cell dividing late at AI of pentaploid hybrids was scored. The means and standard errors were 4.95 ± 0.29 and 5.79 ± 0.21 univalents for pentaploid Thatcher and Rescue, respectively. The means were not significantly different from one another indicating that chromosome elimination took place either late in meiosis or that zygotes with intermediate chromosome numbers were eliminated.

As shown in Table VII, pentaploid hybrids exhibited significant differences in the mean number of micronuclei per sporocyte ($P=0.01$). It is of interest to note that the Thatcher pentaploid which had the highest mean chromosome number in F_2 (Table VIII) had the lowest mean number of micronuclei per sporocyte.

The frequency with which gametes containing 14 to 21 chromosomes were formed in the hybrids can be calculated as follows: Let p be the probability that a given gamete will contain any univalent and 1-p the

TABLE VII

FREQUENCY OF MICROSPORES WITH 0 TO 4 MICRONUCLEI, MEAN NUMBER OF MICRONUCLEI PER SPOROCYTE AND UNIVALENT ELIMINATION IN PENTAPLOID HYBRIDS DERIVED FROM HEXAPLOID (AABBDD) WHEATS X THEIR EXTRACTED TETRAPLOID (AABB) DERIVATIVES

Hybrid	Number of micronuclei per sporocyte						Mean ± s.e. per sporocyte	Univalent elimination
	0	1	2	3	4	Sum		
Prelude X Prelude AABB	461	300	39	20	4	824	0.55 ± 0.02	15.74%
Rescue X Rescue AABB	467	273	50	20	10	820	0.72 ± 0.02	16.48%
Thatcher X Thatcher AABB	574	80	10	6	6	676	0.21 ± 0.02	6.00%

probability that it will not. Furthermore let n equal the number of univalents present in the hybrid. The frequency of gametes carrying zero to n univalents is given by the terms of the expansion of the binomial $(p+q)^n$, where q=1-p. In the present case, n equals seven for Thatcher and Rescue. If it is assumed that no loss of univalents took place (p=0.5) and that univalents were distributed at random in both micro- and megasporogenesis, the expected frequency of gametes with a particular chromosome number could be determined by expanding the binomial $(0.5 + 0.5)^7$. The frequencies of gametes with 14 to 21 chromosomes thus obtained were placed across the top and along the side of a two-way table resulting in a table with 64 cells. The frequency with which a particular zygotic number occurred is the sum of the products of the frequencies of those gametes involved in the combination. This frequency was multiplied by the total shown in each of the rows of Table VIII in order to obtain the expected frequencies. In order for a valid chi-square to be calculated, certain classes were combined so that expected values of two or more were obtained. These tests indicated that in both Thatcher and Rescue, the observed frequencies did not fit the expected ones on the basis of random univalent elimination with no chromosomal loss. This was interpreted to mean that univalent loss did in fact occur.

The formula given by Kihara and Matsumura (1942) was used to calculate univalent elimination from male gametes (Table VIII). Again assuming that the same univalent elimination took place during both micro- and megasporogenesis, the expected frequencies were calculated and tested for fit to the observed values. Deviation of observed frequencies from expected however, was significant. Next it was assumed that not all male gametes

TABLE VIII

FREQUENCY OF VARIOUS SOMATIC CHROMOSOME NUMBERS IN THE F_2 GENERATION OF HYBRIDS BETWEEN HEXAPLOID (AABBDD) WHEATS AND THEIR EXTRACTED TETRAPLOID (AABB) DERIVATIVES

Somatic chromosome number	Hybrid		
	Prelude X Prelude AABB	Rescue X Rescue AABB	Thatcher X Thatcher AABB
28	44	184	5
29	45	80	6
30	32	53	12
31	19	49	13
32	19	16	16
33	16	22	15
34	11	18	33
35	18	25	33
36	7	17	27
37	2	5	18
38	4	8	24
39	2	2	13
40	3	3	6
41	3	1	3
42	5	26	2
TOTAL	230	509	226
Mean chromosome number $\pm$ s.e.	31.45 $\pm$ 0.22	30.91 $\pm$ 0.16	34.80 $\pm$ 0.20

Chi-square = 382.11, 28 d.f. ($P < 0.005$)

are equally functional and furthermore, that independent assortment occurs with no loss of chromosomal material. Once more the observed frequencies did not fit the expected ones.

The next step was to test the hypothesis that a considerable loss of univalents took place but those that remained were distributed at random. By assuming that the observed values for the 28 chromosome class in both Rescue and Thatcherwere correct, the value of p should equal the 14th root of 184/509 for Rescue and 5/226 for Thatcher (Table VIII). In the manner described above, when the expected frequencies were obtained and tested against the observed ones, a poor fit was obtained. Therefore, it was concluded that either the univalents retained were not distributed at random and could not assort independently, or that all classes of gametes were formed according to expectations but they did not function with the same frequency. It became necessary therefore to find the frequency with which the various classes of gametes function in both the male and the female. For this purpose the pentaploid hybrids were crossed to their respective hexaploids and the frequencies observed are presented in Table IX. Using these frequencies, the expected zygotic frequencies were calculated in the manner described above and the results are shown in Table X. Chi-square values indicated a satisfactory fit, except in the case of Rescue.

Within each of the three hybrids, the gametes functioning on both the male and the female side were compared in an RXC table. Chi-square values obtained were 6.47, 1.84 and 8.16 for Prelude, Rescue and Thatcher, respectively. All values were non-significant, indicating that the same proportion of male or female gametes carrying a specific chromosome number functioned on both the male and the female side. Separate analyses of male and

TABLE IX

FREQUENCIES OF VARIOUS CLASSES OF FUNCTIONAL GAMETES AS DETERMINED FROM RECIPROCAL CROSSES BETWEEN PENTAPLOID HYBRIDS (AABBDD X EXTRACTED AABB) AND THE PARENTAL HEXAPLOIDS

Chromosome number	Prelude		Rescue		Thatcher	
	male	female	male	female	male	female
14	35	19	135	119	12	25
15	12	10	35	38	15	28
16	9	2	15	12	21	23
17	2	4	5	7	33	29
18	4	3	7	6	19	21
19	3	4	6	7	23	20
20	2	3	5	6	20	22
21	8	6	39	41	17	14
TOTAL	75	51	247	236	160	182

TABLE X

FREQUENCIES OF SOMATIC CHROMOSOME NUMBERS IN THE F_2 PROGENIES OF PENTAPLOID HYBRIDS AS COMPARED TO EXPECTED ON THE BASIS OF FREQUENCIES OF FUNCTIONAL GAMETES DETERMINED FROM RECIPROCAL CROSSES BETWEEN PENTAPLOID HYBRIDS AND THE PARENTAL HEXAPLOIDS

Chromosome Number	HYBRID					
	Prelude X Prelude AABB		Rescue X Rescue AABB		Thatcher X Thatcher AABB	
	Number of Plants		Number of Plants		Number of Plants	
	Observed	Calculated	Observed	Calculated	Observed	Calculated
28	44	42.22	184	140.62	5	2.33
29	45	36.69	80	81.16	6	5.52
30	32	22.92	53	41.34	12	9.47
31	19	18.53	49	22.09	13	16.34
32	19	10.95	16	19.72	16	19.94
33	16	17.81	22	20.08	15	23.51
34	11	15.21	18	18.22	33	27.49
35	18	30.26	25	94.59	33	28.53
36	7	12.96	17	27.82	27	25.87
37	2	6.40	5	10.77	18	22.15
38	4	4.35	8	5.17	24	17.48
39	2	2.75	2	5.17	13	11.87
40	3	3.55	3	4.79	6	8.55
41	3	2.28	1	3.83	3	5.07
42	5	3.04	26	13.96	2	1.84
TOTAL	230	229.92	509	509.33	226	225.96
chi-square value	25.36	14 d.f.	129.71	14 d.f.	15.11	14 d.f.

female gametic frequencies resulted in a chi-square value of 168.65 and 114.04 for male and female gametes, respectively. Both values were highly significant, indicating that the proportion of the total number of male or female gametes which carried a specific chromosome number differed from variety to variety.

Because the seeds of the F_2 exhibited some shrivelling, they were separated into two classes, shrivelled and non-shrivelled. Embryos of shrivelled seeds were poorly developed and were consequently cultured on a nutrient medium (Norstog and Smith, 1963). Chromosome numbers of the resulting plants were determined and compared by a t-test with those resulting from full seeds. No difference in mean chromosome numbers were observed.

(2) Univalent elimination in reciprocal hybrids

In order to investigate further the possible causes for the differences in the rate of univalent elimination between Thatcher and Rescue (Table VII), the following crosses were made: Rescue (AABBDD) X Thatcher (AABB) reciprocally, code numbered hybrid 1 and 2, respectively; Thatcher (AABBDD) X Rescue (AABB) reciprocally, code numbered hybrid 3 and 4, respectively. Thus univalent chromosomes present in the F_1 hybrids 1 and 2 were contributed by Rescue, and in hybrids 3 and 4 they were contributed by Thatcher. It is significant to note that a common genetic background existed in all hybrids.

Hybrid 3 had a significantly higher mean number of ring bivalents per cell relative to the other three hybrids (Table XI). Hybrids 1 and 2 did not differ significantly in rod bivalents per cell but were significantly different from hybrids 3 and 4. No differences were observed between the hybrids in total number of bivalents or trivalents per cell. Hybrids 1, 2

TABLE XI

MEIOTIC CHARACTERISTICS OF RECIPROCAL HYBRIDS BETWEEN THE EXTRACTED TETRAPLOID DERIVATIVES (AABB) OF THATCHER AND RESCUE AND THEIR HEXAPLOID COUNTERPARTS

Meiotic Stage	Hybrid 1 Rescue X Thatcher AABB	2 Thatcher AABB X Rescue	3 Thatcher X Rescue AABB	4 Rescue AABB X Thatcher
MI				
Ring bivalents	10.48 ± 0.21*(6-13)**	10.52 ± 0.22 (6-14)	11.27 ± 0.22 (9-14)	10.66 ± 0.19 (7-13)
Rod bivalents	1.98 ± 0.15 (0-6)	2.04 ± 0.16 (0-5)	1.33 ± 0.15 (0-4)	1.52 ± 0.15 (0-4)
Total bivalents	12.46 ± 0.19 (10-14)	12.56 ± 0.16 (10-14)	12.61 ± 0.15 (11-14)	12.18 ± 0.15 (9-14)
Univalents	7.64 ± 0.15 (7-11)	7.18 ± 0.14 (4-11)	7.06 ± 0.10 (6-9)	7.02 ± 0.09 (5-9)
Trivalents	0.00 ± 0.00 (0-1)	0.18 ± 0.05 (0-1)	0.21 ± 0.06 (0-1)	0.14 ± 0.04 (0-1)
Quadrivalents	0.52 ± 0.00 (0-1)	0.50 ± 0.07 (0-1)	0.47 ± 0.07 (0-1)	0.76 ± 0.06 (0-1)
Chromosomes				
- lagging at AI	5.36 ± 0.24 (0-8)	5.56 ± 0.22 (0-9)	4.96 ± 0.17 (1-8)	5.84 ± 0.21 (3-8)
- excluded from TI	1.21 ± 0.07 (0-5)	1.06 ± 0.10 (0-3)	1.00 ± 0.12 (0-3)	1.13 ± 0.14 (0-3)
- lagging at AII	4.21 ± 0.28 (3-7)	3.50 ± 0.21 (1-8)	3.19 ± 0.19 (0-7)	3.50 ± 0.21 (1-6)
Micronuclei per sporocyte	0.33 ± 0.02 (0-3)	0.55 ± 0.02 (0-3)	0.41 ± 0.02 (0-3)	0.67 ± 0.07 (0-3)

* mean ± s.e. per cell
** range

and 3 contained significantly fewer quadrivalents per cell relative to hybrid 4. Hybrids 2, 3 and 4 were significantly different from hybrid 1 in mean univalent number per cell while hybrid 3 differed significantly from hybrid 4 in the number of chromosomes lagging at AI. No significant differences were found in the number of chromosomes excluded from the nucleus at TI. All hybrids were significantly different from one another in the number of chromosomes lagging at AII with hybrid 3 exhibiting the fewest laggards. A significant difference existed between all hybrids on the number of micronuclei per sporocyte with the exception of hybrids 2 and 3.

The analyses of chromosome numbers in the F_2 population (Table XII) indicated that the use of the hexaploid as female (hybrids 1 and 3) resulted in a significantly greater number of univalents retained in the progeny of hybrid 1 (univalents contributed by Rescue) than in hybrid 3 (univalents contributed by Thatcher). However, when the hexaploids were used as male, the differences no longer existed. See Appendix 1 for an example of an analysis of variance table.

In the reciprocal hybrids, namely hybrids 1 and 2 and 3 and 4, respectively, the parent which contributed the univalents did not have any effect on their subsequent retention or elimination.

Production of D addition lines

(1) Selfing pentaploid hybrids

From the thirty-chromosome plants which were obtained by selfing the pentaploid hybrids of the three varieties involved (Table VIII), none were found to have fifteen bivalents in MI. All plants examined had fourteen bivalents and two univalents and upon selfing produced only 2.93% 0.00% and 11.66% plants with 30 chromosomes for Prelude, Rescue and Thatcher, respectively. Again, with the exception of one plant in Thatcher, these

TABLE XII

FREQUENCY OF VARIOUS SOMATIC CHROMOSOME NUMBERS IN THE F_2 GENERATION OF HYBRIDS BETWEEN HEXAPLOID (AABBDD) WHEATS AND THEIR EXTRACTED TETRAPLOID (AABB) DERIVATIVES

Chromosome Number	Hybrid			
	Rescue X Thatcher AABB	Thatcher AABB X Rescue	Thatcher X Rescue AABB	Rescue AABB X Thatcher
28	19	15	11	8
29	17	9	13	10
30	27	21	17	16
31	35	18	21	21
32	30	21	12	12
33	29	29	14	20
34	38	26	14	23
35	32	22	12	14
36	30	22	11	11
37	19	26	7	7
38	20	9	6	12
39	11	5	4	5
40	9	5	-	-
41	4	1	-	-
42	-	7	-	2
TOTAL	320	236	142	161
Mean $\pm$ s.e.	33.57 $\pm$ 0.17	33.80 $\pm$ 0.20	32.58 $\pm$ 0.26	33.23 $\pm$ 0.25

plants had fourteen bivalents and two univalents.

(2) Selfing plants of $15^{II} + 6^{I}$ constitution

Only Rescue and Thatcher were used in this study and the distribution of the somatic chromosome number of the resulting progeny is given in Table XIII.

Twenty-five meiotic cells from each of three plants of Rescue and Thatcher were scored at metaphase. A total of 78.67% and 65.72% of the cells from Rescue and Thatcher respectively, had fifteen bivalents while the remainder exhibited fourteen bivalents plus two univalents. The univalents divided at AI, while pollen stainability was 48.63% and 56.35% for Rescue and Thatcher respectively. Upon examination of the flowers, the anthers were found to be non-dehiscent. Attempts to induce seed set by manually rupturing the anthers failed.

The fifteen bivalent plants were of very poor vigour and were semi-erect in growth habit. They differed morphologically from one another within each variety indicating that more than one addition line was obtained. The lack of seed set, however, caused the abandonment of this project.

TABLE XIII

DISTRIBUTION OF SOMATIC CHROMOSOME NUMBERS IN PROGENY RESULTING FROM SELF-POLLINATION OF PLANTS OF $15^{II} + 6^{I}$ CONSTITUTION

Somatic chromosome number	Variety	
	Rescue (percentage)	Thatcher (percentage)
28	0.60	-
29	-	-
30	4.84	2.06
31	7.27	2.06
32	5.45	3.09
33	4.24	4.12
34	8.48	14.43
35	13.33	14.43
36	13.33	22.68
37	11.51	14.43
38	7.87	10.30
39	10.90	7.21
40	9.09	4.12
41	3.03	1.03
42	-	-
TOTAL	165	97

DISCUSSION

Extracted AABB tetraploids

(1) Morphological characteristics

It is to be expected that the removal of a complete genome from a well established species could affect many morphological characteristics. Kerber (1964) also observed spikes resembling Triticum compactum Host. in his extracted tetraploids. A change in seed colour was expected in the tetraploids since one of the genes for seed colour is located on 3D (Sears, 1944). Seed colour of the extracted tetraploids however, was similar to that of the hexaploid counterparts; a result that may be due to a modifying effect of environment. Fertility and vigour of extracted tetraploids produced in this study was superior to those reported by Kerber (1964) and could be due to the fact that fewer backcrosses were made for the extraction of the present material than were made in Kerber's investigation.

(2) Meiotic characteristics

The removal of the D genome did not affect pairing relationships in the resulting AABB tetraploids. It was reported that the diploid-like meiotic behaviour of hexaploid wheat is due to the balance between the action of the gene on the long arm of 5B on one hand, and gene(s) on the short arm of this chromosome in addition to chromosomes 5A and 5D on the other (Feldman, 1966). No hypothesis can be advanced as to the reason why the removal of the 5D genes did not affect pairing, despite the fact that plants nullisomic for 5D were asynaptic (Feldman, 1966). It could be that the effect of the gene(s) on 5D is counterbalanced by genes on other D genome chromosomes and when the whole D genome is removed, no change in pairing relationships is manifested.

With regard to hybrids among extracted tetraploids themselves as compared to hybrids with a durum wheat variety, it is to be noted that the latter exhibited more univalents per cell. This could be explained on the thesis that a cross to a durum variety is more or less an intervarietal cross whereas crosses among extracted tetraploids are intravarietal in the sense that the same durum variety was used for their extraction.

(3) Chromosome(s) involved in the translocation(s) of Prelude AABB

The configurations observed in the meiosis of the hybrid between Prelude AABB and Chinese Spring ditelosomic for $1D^{L}$ indicated that one of the chromosomes involved in one of the translocations was 1D, specifically part of the long arm of 1D. This segment was translocated terminally.

The existence however of plants of Prelude AABB that were free of the translocation, coupled with the evidence from the pentaploid hybrid of Prelude X Prelude AABB (Table VII) that two different translocations might be present in the material under study, brought about the temporary abandonment of further attempts to cytologically identify the translocations involved. This became necessary since it was difficult, if not impossible, to distinguish between the chromosomes involved reciprocally in either of the two translocations. The project will be resumed after individual plant progenies have been grown and examined for the presence of translocations.

(4) Bread-making characteristics

Although the sedimentation value for tetraploid Prelude (Table V), indicated that the quality of its protein for bread might be slightly inferior to that of its hexaploid counterpart, the additional protein content

more than made up for this deficiency. In the actual baking test therefore, the tetraploid yielded a higher loaf volume. The high water absorption of the tetraploids was probably due to the extensive starch damage produced in milling the extremely hard durum-like kernels. However, this factor was not reflected by a proportional increase in the baking absorption.

The data on quality (Table V) present additional evidence on the importance of the D genome in the inheritance of baking quality. The somewhat higher quality of tetraploid Prelude is attributable to two factors: (i) high protein content and, (ii) additional quality due to - (a) the translocation of part of the long arm of 1D to one of the chromosomes of the A or B genomes and, (b) perhaps, to another as yet unidentified segment of another D genome chromosome. Chromosome 1D has been shown to control flour quality characteristics (Welsh and Hehn, 1964) but there have been reports (Morris et al., 1966; Schmidt et al., 1966; Welsh et al., 1968) implicating chromosomes from the A or B genomes as controlling flour quality characteristics. It would appear that flour quality inheritance is complex and differences might exist from variety to variety. Regarding tetraploid Prelude it should be borne in mind that the seed tested was in all probability a mixture of translocation homozygotes and heterozygotes together with seeds free of any translocations. For this reason the possibility that its high bread-making quality is due to genes on chromosomes from the A or B genomes cannot be ruled out, despite the fact that there were only three backcrosses made to hexaploid Prelude.

In order to clarify the situation, individual plant progenies must be grown, analyzed for the presence of translocation and then tested for

bread-making quality. If the lines carrying the translocation do indeed have high bread-making quality and the lines free of translocation do not, then the high quality would be due to the translocation. If all lines have high quality, then the conclusion would be that it is due to genes on chromosomes of the A or B genomes. Regardless of the underlying factors responsible for the high quality of Prelude AABB, it should be possible to incorporate this quality into an established high yielding durum wheat for the production of a high yielding, high-quality durum variety.

(5) Disc electrophoresis of the gliadins

The fact that there was more gliadin in the extracted tetraploids relative to the corresponding hexaploids and essentially the same electrophoretic pattern in both, suggests that differences in bread-making quality between tetraploid and hexaploid wheats could be partly due to quantitative factors. The highly complex pattern for Thatcher may be qualitatively related to its high bread-making quality in relation to the other two varieties. Furthermore, it would appear that the removal of the D genome does not qualitatively affect the gliadin proteins. These results are similar to those obtained by Boyd and Lee (1967) working with the extracted tetraploid of Canthatch. Moreover, Eastin et al. (1967) could not associate differences between gliadin patterns with flour quality characteristics. It seems likely therefore, that the loci in the D genome controlling protein synthesis are duplicated in the A and B genomes and that no clear-cut association can be made between electrophoretic patterns of gliadin and flour quality. It must be emphasized however, that these conclusions are based on results of a single experiment and must, therefore, be confirmed by future experiments.

Chromosome segregation in the pentaploid hybrids

(1) F_2 generation

In the progeny of the pentaploid hybrids, there were more plants with twenty-eight chromosomes than expected on the basis of random univalent segregation. This is in agreement with the results of other workers, although they were using tetraploid wheat species rather than extracted AABB tetraploids (Kihara, 1924; Jenkins and Thompson, 1930). More recently, Joppa (1967) working with Thatcher X durum crosses also observed an excess of twenty-eight chromosome plants. The present data from Thatcher when compared with those of Joppa were found to be homogeneous (Chi-square = 25.49, with 36 d.f.) indicating that for Thatcher at least, the particular AABB used in the cross did not affect chromosome segregation. When these same data, however, were compared with those of Kerber (1968), they were found to be heterogeneous (Chi-square = 472.88 with 52 d.f.) indicating that not all extracted AABB tetraploids affect chromosome segregation in the same way. Similar deviations from the expected patterns of chromosome segregation have been reported by other workers and have been attributed to differential functioning of male and female gametes (Thompson and Cameron, 1928; Kihara and Matsumura, 1942).

Another point that merits attention is that the hybrid between Prelude and Stewart 63 had more trivalents per cell than the hybrid between Thatcher and Stewart 63. This would suggest that the translocation to Prelude AABB must have taken place during the extraction process itself. Mello-Sampayo (1968) observed trivalents at MI of hybrids between Chinese Spring ditelocentrics for D genome chromosomes and Ld222, a durum wheat variety, yet it is known that a translocation is not involved. He attributed

this behaviour to homoeologous pairing and it would seem that the same explanation could apply to the trivalents observed in the present material. If this is true, then the pentaploid wheat hybrids could be used as a bridge for the transfer of desirable characters to or from the tetraploid wheats.

The higher mean F_2 chromosome number of Thatcher relative to Rescue was correlated with a lower frequency of micronuclei per sporocyte (Table VII). The fact that no differences were found in mean chromosome numbers of plants originating from either full or shrivelled seeds indicated that certain zygotic combinations, which are rare in the progeny, die at an early stage.

(2) Univalent elimination in the reciprocal hybrids

It seems that no clear-cut association can be made between the differences in cytological behaviour that were observed in the various meiotic stages examined and the final outcome in terms of mean chromosome numbers (Tables XI and XII). The differences observed indicate that the background genotype or the univalents themselves have little or no effect on univalent elimination. Other factors, i.e. lack of compensation for missing chromosomes which in turn hinders further development, might have a decisive effect on the fate of certain zygotic combinations.

Production of D genome addition lines

(1) Self-pollinating pentaploid hybrids

The lack of plants with fifteen bivalents is apparently due to the small size of the sample. If it is assumed that a univalent has the same probability of either being included in a gamete or eliminated, and

furthermore if it is assumed that this probability is the same in both micro- and megasporogenesis, then the probability that gametes carrying identical D genome chromosomes will unite to form a zygote is $(1/2)^{14}$. It is evident that with the sample sizes obtained (Table VIII) there was not a reasonable chance of obtaining addition lines.

(2) Self-pollinating plants of $15^{II} + 6^{I}$ constitution

The lack of vigour exhibited by the fifteen bivalent plants was unexpected in view of the fact that plants with fourteen bivalents and two univalents were both vigourous and fertile. However, it seems that the inclusion of a pair of homologous D chromosomes is somehow more detrimental to vigour than two non-homologous chromosomes. Joppa (1967) also found some of the D addition lines to be male sterile. It is unlikely that failure to set seed was due to the inability of anthers to dehisce since manually breaking the anther with subsequent pollination of normal appearing stigmata did not result in seed development. Moreover, pollen stainability was found to be reasonably good although it is recognized that pollen stainability may not always be a true indication of pollen viability. It would seem that the zygote, if it was formed at all was arrested in its development at a very early stage.

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APPENDIX 1

ANALYSIS OF VARIANCE TABLE FOR A COMPLETELY RANDOM DESIGN

The data pertain to the number of chromosomes left outside the nucleus at the end of first telophase in the reciprocal hybrids of Table XI, and were transformed according to the formula $\sqrt{x + 1/2}$ prior to analysis.

Source of variation	Degrees of freedom	Sum of squares	Mean square	F value
Among hybrids	3	0.4478	0.1493	1.19
Within hybrids	370	46.5750	0.1259	
TOTAL	373	47.0228		

$F_{3,370} = 1.19$ was smaller than the theoretical $F_{3,370} = 2.60$ at the 5 percent level. It was concluded therefore that no significant differences were present among the hybrids.