STUDIES ON TRANSMISSION OF ALIEN SUBSTITUTION GAMETES

INVOLVING FOUR RYE CHROMOSOMES IN WINTER WHEAT

A Thesis

Presented to

the Faculty of Graduate Studies and Research

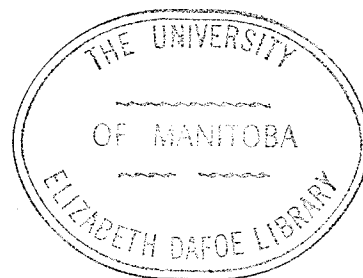University of Manitoba

In Partial Fulfillment

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by

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ABSTRACT

An attempt was made to study the transmission of four different

chromosomes of rye var. Dakold in the form of alien substitution and

alien addition gametes. The four rye chromosomes used in the present

investigation were I, IV, V and VI, designated after Bhattacharyya and

Jenkins (3). Only these four rye chromosomes were used due to the ease

with which they can be morphologically distinguished from the wheat

chromosomes at mitosis. Of the 21 'Kharkov' wheat monosomics, seven

belonging to the D genome were used, firstly in order to have complete

representation of the seven homoeologous groups and secondly because

the D genome is believed to have a primitive arrangement (40). Rye var.

Dakold and winter wheat var. Kharkov were used, because these are being

used in wheat-rye substitution programs underway in the Department of

Plant Science, University of Manitoba.

In order to obtain suitable material for transmission studies,

seven Kharkov monosomics were pollinated by each of the four rye addi-

tion lines (21"W+1"R). Each of these 28 combinations gave essentially

two types of F_1 plants, namely: 42W+1R and 41W+1R plants. Only the

latter were of interest, since they could form four types of gametes,

(1) 20W, (2) 20W+1R, (3) 21W, and (4) 21W+1R. These plants which

formed 20"W+1'W+1'R at metaphase I were used in reciprocal crosses

with normal wheat plants, forming 21"W. The constitution of the gametes

from wheat plant being known, the transmission rates of different

gametes from F_1 plants were then calculated.

An attempt was also made to present a theory in order to derive theoretical ratios of the transmission rates of different gametes. The practical results were then compared with the theoretical ratios. The theory was essentially based on evidence that a rye univalent was lost more often than a wheat univalent and showed that the gametes deficient for rye chromosome were as frequent as 86.4 per cent, while gametes deficient for wheat chromosome were known to be 75 per cent (48).

The theoretical ratios derived were as follows:

1. Female transmission 19(20W): 3(20W+1R): 6(21W): 1(21W+1R)
2. Male transmission
    (a) Compensating effect 12(20W+1R): 25(21W): 4(21W+1R)
    (b) Non-compensating effect 25(21W): 4(21W+1R)

Theory concerning F_2 progenies from F_1 (20"W+1'W+1'R) plants was also presented, but similar ratios were not derived.

The practical results were in accordance with theory. Rye chromosome I showed compensation for chromosomes 1D and 3D of wheat; chromosome IV compensated for 6D; chromosome V and VI did not exhibit any compensating effect whatsoever. It was concluded that chromosome I of Secale cereale var. Dakold was involved in an interchange and thus had segments from two ancestral chromosomes. Chromosome IV was believed to belong to homoeologous group 6 and was compared with the nucleolar organizing chromosome II from rye var. King II. Chromosome V was believed to be a result of a complex interchange or mutations, thus losing all the compensating effect. Complete absence of compensatory effect in chromosome VI (corresponds to rye chromosome I rye var. King II) was attributed to mistaken identification, because there is strong evidence that

this chromosome belongs to homoeologous group 5.

The transmission rates of male nullisomic gametes and non-compen-sating male substitution gametes were also calculated and were 4.4 per cent and .74 per cent respectively.

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INTRODUCTION

It has long been recognized that rye (<u>Secale cereale</u> L.)
possesses certain agronomic traits, which if added to the genetic
background of some of our cereal crops, would greatly improve the
agricultural worth of these species. The incorporation into wheat,
for example, of the high degree of winter hardiness from rye would
result in tremendous economic gains in the field of cereal production
by making possible large-scale winter wheat production on the northern
Great Plains area of North America. Attempts have been made in the
past, therefore, for the transfer of such valuable genetic material
from rye to wheat.

Although hybridization between these two species is success-
ful, lack of chromosome pairing prevents genetic recombination. To
circumvent this barrier to free exchange of genetic material in wheat-
rye hybrids, several attempts have been made to produce amphiploids,
thereby incorporating the complete genomes of both species. Similarly,
the addition of a pair of homologous rye chromosomes to the existing
wheat complement (addition lines), and the substitution of a pair of
homologous rye chromosomes for those of wheat (substitution lines) has
been attempted. These methods have met with only limited success to
date in the improvement of the existing wheat species.

Considerable chromosomal imbalance is introduced in wheat by
the addition or substitution of whole rye genome. Similarly commercial
use of wheat-rye addition lines is also limited due to cytological in-
stability, partial sterility and to certain adverse affects that the

inclusion of genetic material from rye has on wheat. Reports on the development of wheat rye substitution lines are also not encouraging to date. To be successful, such lines must integrate the substituted chromosome completely within the chromosome complex of wheat, and at the same time compensate for the missing wheat chromosome, while providing the additional necessary genetic function for which it was selected.

In order to assess the practical value and limitations of the substitution program, it is essential to have a knowledge of the precise relationship between wheat and rye chromosomes. Riley and Kimber (42) emphasized that substitution can be accomplished only when the alien chromosome is homoeologous with the wheat chromosome for which it is substituted. The work from this laboratory also provided evidence that a rye chromosome is not always transmitted with the same frequency, when it replaces different wheat chromosomes.

The present work was undertaken, therefore, to determine the transmission frequencies of each of four rye chromosomes substituting for different chromosomes of the D genome of wheat. Such a study of transmission rates could supply information as to which rye chromosome would successfully substitute and compensate for one or more particular wheat chromosomes, and would thereby afford a better opportunity for success of future substitution programs involving these two species.

1. <u>Transfer of alien genetic variation to wheat</u>:-

    (a) Amphiploids

    Many attempts have been made to transfer characters to wheat from
closely related genera. One of the common methods used to achieve this
objective has been the production of amphiploids. The first interspeci-
fic hybrid between wheat and rye was produced by Wilson in 1876, but was
sterile. A fertile allopolyploid was produced by Rimpau in 1891.
Vigorous attempts have been made to make wheat rye amphiploid, popularly
known as 'Triticale' a success. Muntzing (25, 26) and Sanchez Monge
(46, 47) have reviewed the results and concluded that none of the Triti-
cale (hexaploid, octaploid, decaploid) have suitable agronomic qualities.

    (b) Addition lines

    Other methods used for the transfer of characters to wheat from re-
lated genera have been the production of alien addition or alien substi-
tution lines and the induction of translocations by ionizing radiations.
The name "alien addition races" was proposed by Clausen (Gerstel, 1945)
to signify races having a normal complement of wheat chromosomes, to
which a pair of homologous chromosomes has been added from a closely re-
lated genus. In alien substitution lines, similarly, a pair of wheat
chromosomes is replaced by a pair of chromosomes from a non-wheat parent.
Riley and Kimber (42) used the term "alien" to designate those species
which when crossed with <u>Triticum aestivum</u> form hybrids of low fertility.

    O'Mara (28) proposed a scheme in order to add a pair of rye chromosomes
to wheat. He crossed a wheat-rye amphiploid with wheat and allowed the

derivatives to self for one or more generations. Following this method,
he obtained a few monosomic additions and three disomic additions of in-
dividual rye chromosomes to the wheat variety Chinese Spring (28, 32).
Subsequently, Chapman and Riley (4) and Riley and Chapman (39) obtained
the disomic addition of the individual rye chromosomes of the variety
King II to the common wheat variety Holdfast. Evans and Jenkins (7) re-
ported the additions of all the seven rye chromosomes individually from
the rye var. Dakold to wheat var. Kharkov. As described by Bhattacharyya
and Jenkins (3), the seven chromosomes of Dakold rye could be morphologi-
cally identified.

Additions of individual pairs of chromosomes to the wheat genome from
genera other than rye have also been tried. Sears (49) reported the suc-
cessful addition of a pair of chromosomes of Aegilops umbellulata to wheat.
Hyde (13) using a "bridging" technique, added chromosomes from Haynaldia
villosa to wheat. He obtained five of the seven possible disomic addi-
tions and six of the seven possible monosomic additions. Gerstel (9) made
similar additions between Nicotiana species and Sadanaga (45) obtained
additions of rye chromosomes to Triticum durum. Muchizuki (24) obtained
six of the seven possible additions from Agropyron elongatum (2n=14) to
Triticum durum var. Stewart.

Because of their genetic instability and partial sterility, the addi-
tion lines reviewed above were of limited agronomic value. The produc-
tion of alien substitution lines, therefore, was considered to be the
next possible avenue of approach and to date several reports of the suc-
cessful substitution of a pair of alien chromosomes in wheat are known.

(c) Substitution lines

Bakshi and Schlehuber (1) reported replacing chromosome 3D of
Triticum aestivum var. Pawnee with one from Agropyron elongatum (2n=70)

carrying resistance to leaf rust. They also mentioned that a comparison of substitution lines with corresponding nullisomics had shown that this Agropyron chromosome provided a high degree of compensation for chromosome 3D. Similarly, Knott (18) demonstrated that lines of wheat carrying a blue endosperm derived from Agropyron elongatum were alien substitution lines.

The development of wheat-rye substitution lines was first reported by O'Mara (29). A rye chromosome, which he designated as I was substituted for chromosome 5A(IX) of wheat, a substitution which occurred in the progeny of a slightly asynaptic addition line. In comparison with the addition line, the substitution line exhibited restored male fertility, increased female fertility, improved meiotic stability and plant vigor. It was apparent, therefore, that rye chromosome I of O'Mara's material compensated favorably for wheat chromosome 5A(IX).

Using O'Mara's material, Smith (52) noted that alien addition male gametes (21W+1R) were at a competitive disadvantage with euploid (21W) gametes. This observation led him to conclude that the genetic material of the rye chromosome was of no advantage in a gamete containing a normal complement of wheat chromosomes. He also argued that an alien substitution gamete was successful, because it had the normal chromosome number.

Knott (19) pointed out that it is important to determine whether an alien chromosome can substitute equally well for each of the wheat chromosomes. It might be expected that substitution would depend on the genetic relationship between substituting alien chromosome and the replaced wheat chromosome. Johnson (15) demonstrated that Agropyron alien substitution gametes having 21 chromosomes behaved much like nullisomic gametes. Exceptions occurred in cases where alien chromosome replaced the genetically related chromosome of homoeologous group 6, for

which the alien chromosome compensated. He compared this situation with the compensating nullisomic-tetrasomics series in wheat described by Sears (51).

Riley (37) found that chromosome II of Secale cereale could substitute for all three homoeologous chromosomes of group 6 of wheat. He, therefore, suggested an evolutionary relationship between the rye and corresponding wheat chromosomes. He further concluded that it would be reasonable to suppose that every chromosome of diploid donor species should be expected to substitute for only one chromosome in every genome of a recipient polyploid species. Johnson (15) also suggested that there may be a relationship between rye chromosome II and an Agropyron chromosome, because both substituted for chromosomes of homoeologous group 6 of T.aestivum.

Jenkins (14) reported the substitution of chromosome I (designated after Bhattacharyya and Jenkins, 1960) of Dakold rye for fourteen chromosomes of wheat, including chromosomes of all seven homoeologous groups. He also described examples in which other rye chromosomes substituted for wheat chromosomes with no apparent relationship to the homoeologous groups. Weinhues (57) similarly suggested that a chromosome of Agropyron intermedium could act as a substitute for ten different wheat chromosomes representing all seven homoeologous groups.

As described by Ray (34), disomic substitutions may be obtained by selfing plants with a chromosome formula of: (a) 20"W+1'W+1'R, (b) 20"W+1'R, (c) 20"W+1'W+1"R. The third method was found to be the most promising due to the abundance of both male and female gametes carrying the substitution. The limitation of the first two methods in the production of substitution lines is a result of the inability of the alien substitution male gametes to function.

Ray (34), while studying the morphology of the different substitution lines showed that when chromosome VI was substituted in turn for chromosomes 5A(IX), 3B(III), 1D(XVII) and 5D(XVIII) of Kharkov, near normal plants were obtained. Plants deviated considerably from normal when rye chromosome V was substituted for any of Kharkov chromosomes.

Riley and Kimber (42) pointed out that the concept of dependence of substitution on homoeology may face complications based on the following factors:

1) Firstly, reciprocal translocations could give rise to structural conditions, such that partial compensation by one alien chromosome for two wheat groups is possible. This situation is, in fact, anticipated in rye substitutions because the chromosome condition of annual rye is structurally different from the basic arrangement of perennial rye.

2) Secondly, mutation at several sites on the alien chromosome may alter its function in such a way as to make it unable to compensate for the missing wheat chromosome.

3) Thirdly, since wheat is a polyploid with much duplication, a chromosome may have become so altered in function that it is not vital for the survival of the organism. Such a wheat chromosome may be substituted by more than one chromosome from an alien species.

By virtue of any one of the above factors, homoeology between alien chromosomes and the homoeologous groups of wheat could break down.

2. Morphology of rye chromosomes:-

The present study involved only four of the seven chromosomes of rye, namely I, IV, V and VI. The selection of only these four was based on the ease with which they could be morphologically identified. The following review will relate to these four chromosomes only.

Bhattacharyya and Jenkins (3) described the karyotype of rye var. Dakold and pointed out that rye chromosomes were quite large in size in comparison to the other members of the _Triticinae_. Average length of Dakold rye chromosomes varied from 9.13 μ to 11.34 μ. On the basis of morphology, five types were suggested.

Chromosome I was classed as Type A, which was characterized by a median or nearly median primary constriction. Of the three chromosomes I, II, III belonging to this type, chromosome I was the largest (11.34 μ) and nearly metacentric with an average arm index of 1.10.

Chromosome IV was described as the single member of Type B. It was long (10.57 μ) with three constrictions. The primary constriction was submedian and the long arm had a secondary constriction at the sub-median position. The short arm carried a short terminal satellite and the arm index was 1.74.

Chromosome V, a solitary member of the Type C, was long (10.39 μ) with a submedian primary constriction and a secondary constriction in a submedian position in the long arm. Average arm index was 1.50. It differed from chromosome IV in having no satellite and in having a short arm, shorter than the corresponding arm of IV.

Chromosome IV was classed as Type D and was comparatively short (9.74 μ) with a submedian primary constriction and a very short terminal satellite on the short arm. The long arm was almost double the length of the short arm and the arm index was 1.82. Chromosome VI differed from IV in being smaller and in having a smaller satellite. The length of the short arm was also less than that of chromosome IV.

Levan (20) described three satellited chromosomes S_1, S_2, S_3 which should correspond to VII, VI and IV, respectively of Bhattacharyya and Jenkin's material.

In Levan's material, two pairs which carried no constriction may correspond to I and II. Similarly, Riley and Chapman (39) observed three pairs with secondary constrictions. Bhattacharyya and Jenkins (3) acknowledged the lack of conformity between their results and those of Riley and Chapman, but believed that chromosomes II and IV of Riley and Chapman's material may correspond to VII and IV in Dakold rye, while chromosome I carrying the hairy neck gene (marker) corresponds to chromosome VI.

Riley and Chapman (39) and Riley (36) produced five addition lines and studied the morphology of rye chromosomes in monosomic addition lines at anaphase I of meiosis. The morphology of four rye chromosomes studied by Riley and Chapman is described as follows:

Chromosome I - carried one short and one extremely long arm with a secondary constriction midway on the long arm. Riley and Chapman's (39) results regarding the morphology of chromosome I differed from those of O'Mara (32), who suggested that chromosome I had a median centromere.

Chromosome II - One arm was more than twice the length of the other. The short arm had a pronounced secondary constriction terminated by a large satellite.

Chromosome III - Both arms were more or less of equal langth.

Chromosome IV - Arms were of unequal lengths and the shorter one carried a small terminal satellite. Chromosome IV had a longer shorter arm relative to the long arm, when compared to II. The satellite in IV was also smaller than in II.

The full set of seven addition lines using wheat var. Holdfast and rye var. King II has now been completed (43).

3. <u>Chromosomes of the D genome of common wheat</u>:-

Sears (48) described in detail the aneuploids of common wheat and all 21 monosomics have been described from the standpoint of plant

morphology. He also studied the morphology of the 21 wheat chromosomes at metaphase I and telephase II of the meiosis. The following table summarizes the morphology of the seven chromosomes of the D genome, as studied by Sears (48):

| Wheat Chromosome | Total Length (u) | | Arm Ratio |
	M I	T II	T II
1D (XVII)	5.02	5.55	1.82:1
2D (XX)	5.58	8.18	1.23:1
3D (XVI)	5.86	7.45	1.37:1
4D (XV)	4.90	6.85	1.80:1
5D (XVIII)	4.83	5.77	1.82:1
6D (XIX)	4.22	5.90	1.11:1
7D (XXI)	6.16	9.06	1.17:1

Riley and Chapman (40) found that seven bivalents and no multivalents were normally formed in the hybrid between wheat var. Chinese Spring and _Aegilops squarrosa_. It was, therefore, concluded that the D genome in the var. Chinese Spring has a primitive arrangement.

4. Behaviour of univalents

Since a study of univalent behaviour was an integral part of the program reported herein, a review of literature of this subject is deemed necessary.

Considerable knowledge of the behaviour of univalents comes from the study of haploids, polyhaploids and interspecific hybrids in the sub-tribe _Triticinae_ (38). In addition, valuable information has come from the extensive work on aneuploid wheats by Sears (48), on barley by Tsuchiya (53), on maize by McClintock and Hill (21), on tobacco by Clausen and Cameron (5), and on oats by McGinnis (22). Certain

conclusions regarding univalent behaviour can be derived from these studies:

1)   Univalents are distributed to the poles at random.

11)   Univalents may pass to either pole without dividing at first division of meiosis and may subsequently divide normally at second division.

111)   There is tendency for the univalents to either lag at the first division or divide at first division and then lag at the second division. Usually the lagging chromosomes are not included in the nuclei resulting from meiosis, but appear as micronuclei in the quartet of spores.

IV)   Univalents which pass to one pole without dividing probably divide at the second division.

V)   Occasionally the univalents may divide at first division of meiosis and may again misdivide at the second division.

V1)   In some case, most or all of the univalents have a tendency to pass to one pole.

Hacker (10) working on oats devised a technique for the calculation of the frequency of functioning deficient and euploid male and female gametes produced by monosomics, knowing the proportions of nullisomic, monosomic and euploid plants obtained in selfed progenies. The following equation was derived:

$$2E_{20} \doteq (N+1-U) \pm \sqrt{(U-1-N)^2 - 4N}$$

Where N = proportion of nullisomics obtained in progeny of a selfed monosomic.

U = proportion of euploids in the progeny of a selfed monosomic.

E_{20} = frequency of nullisomic eggs.

This equation gives two alternative values for E_{20}: one applying to nullisomic eggs, the other to nullisomic pollen. This technique is

quite useful to calculate the frequency of transmission of a single existing univalent through both male and female.

5. <u>Variability of rye chromosome morphology</u>:

Rye chromosomes, in the presence of wheat chromosomes, are known to undergo an alteration in their morphology. Bhattacharyya, Evans and Jenkins (2), for example, pointed out that the morphology of rye chromosomes V and VII changed in the presence of the wheat complement. The total length of wheat chromosome V remained constant, but the average arm index changed from 1.50 in the parental species to 1.29 in the disomic addition line. These workers concluded from their observations that this change in the relative length of the two arms was possibly due to the effect of the new combination of wheat and rye chromosomes. Chromosome VII did not show any change in length and arm index, but the secondary constriction in the short arm became almost inconspicuous in the addition line and appeared to have shifted toward the centromere producing a long terminal satellite.

Ray (34) observed that rye chromosomes V and VI both assumed a somewhat abnormal morphological appearance when substituted into wheat. Chromosome V showed a very short terminal satellite in the short arm and VI exhibited a submedian secondary constriction in the long arm which was very pronounced at metaphase I. It was also observed that a very short terminal satellite in chromosome VI was less densely stained than the main body of the chromosome and sometimes appeared as a fine faintly-stained thread-like body.

6. <u>Behaviour of rye chromosome VI</u>:-

There is a considerable volume of literature relative to the

behaviour of chromosome VI (previously designated by O'Mara as chromosome I.). This chromosome was selected by various workers, because of its effect (hairy neck) on the wheat plant.

O'Mara (30) and Kattermann (16) found two chromosomes each capable of producing the hairy-neck character in the recipient parent. O'Mara (30, 32) pointed out that one had a median constriction, in which the two chromosome arms (isobrachial) were connected by a small isthmus or thread. The other chromosome had a submedian constriction which seemed identical to the first in the degree to which it constricted the chromosome (heterobrachial). O'Mara (32) believed that the latter was derived from the former by the loss of a part of the chromosome, or alternatively, the median chromosome was derived from submedian type by the loss through misdivision of the short arm and duplication of the long arm. O'Mara (30, 32) and Riley and Chapman (39) concluded that the very strong constriction in this chromosome was not the kinetochore constriction but was a secondary constriction of a very pronounced type. The two types which produced the same phenotype were: (1) the normal rye chromosome, which had a subterminal kinetochore and a very pronounced median and secondary constriction; and (2) the long arm of this chromosome, which carried the same constriction in a submedian position and a terminal kinetochore on the short arm.

O'Mara (30) and Riley and Chapman (39) found that the gene for pubescence was located in the long arm of rye chromosome I. Sears (50) subsequently pointed out that the gene for hairy neck must be near the centromere region. Besides hairy neck, the long arm of chromosome I carried genes governing internode length, as found in wheat rye addition lines. The short arm had little or no detectable effect. The long arm, when present twice, on the other hand had all the obvious

effects of two whole chromosomes, (O'Mara 30, 33).

O'Mara (28) showed that when monosomic additions, disomic additions and amphiploids were compared, amphiploids exhibited the least pubescence. Nakajima (27) studied the F_1 plants from crosses of Triticum macha with four species of Secale(cereale, vavilovii, africanum and montanum) and found that they failed to exhibit the hairy neck character. Such observations indicate that the expression of hairy neck in wheat plants is not simply inherited.

O'Mara (30, 32) obtained the five different types of additions arising from the misdivision of rye chromosome VI. They were: (1) the normal univalent; (2) the telocentric chromosome representing the long arm; (3) the isochromosome representing the duplication of the long arm; (4) the telocentric chromosome representing the short arm, and (5) the isochromosome representing duplication of the short arm. Since rye chromosomes were often present as univalents even when present as two homologues, stable additions of rye chromosome to the wheat parent would seem to be difficult to accomplish (32). O'Mara (31) also studied the effect of chromosome substitution on gametic competition. He crossed common wheat with plants having chromosome formula 20"W + 1'W + 1'R (obtained by crossing 20"W + 1"R with common wheat). Excluding products of possible misdivision, the male gametes produced from these individuals were of four types: (1) nullisomic gametes, 20W; (2) alien substitution gametes, 20W + 1R; (3) normal gametes, 21W; (4) alien addition gametes, 21W + 1R. If pollen from these plants is placed on the stigma of normal wheat plants, the resultant progeny should indicate the relative success of the different classes of gametes in competition with one another. The percentage of successful gametes was 60 per cent for

normal gametes, 26 per cent for alien substitution gametes, 8 per cent for addition gametes, and 6 per cent for nullisomic gametes. The data, therefore, indicated that normal gametes did not have an extreme competitive advantage and the substitution gametes affected fertilization once for every 2.3 unions of normal gametes. Smith (52) studied the male and female transmission of these gametes. His results for male transmission were similar to those of O'Mara. The transmission of female gametes was 54 per cent for nullisomic gametes, 19 per cent for normal gametes, 21 per cent for substitution gametes, and 4 per cent for addition gametes.

Materials and Methods

Plant Material:-

Plant material for the present investigation was supplied by the Plant Science Department, University of Manitoba, and included the winter wheat var. Kharkov MC 22 and winter rye var. Dakold 23. Abbreviations "K", "W", and "R" were used and refer to wheat var. Kharkov, wheat chromosomes and rye chromosomes respectively.

The establishment of (1) a monosomic series in Kharkov 22, and (2) disomic addition lines of wheat (Kharkov) and rye (Dakold) was already completed as department projects prior to the initiation of the present study. Seven lines of Kharkov monosomic for each of the seven chromosomes of the D genome, also four rye addition lines disomic for chromosomes I, IV, V and VI (designated after Bhattacharyya and Jenkins) were already available at the commencement of this investigation.

Monosomic Substitutions:-

F_1 plants with the chromosome constitution 20"W+1'W+1'R (20 wheat bivalents + 1 wheat univalent + 1 rye univalent) were obtained using wheat monosomics and rye addition lines in the following way:

(a) Plants with 41 chromosomes were selected by mitotic chromosome counts from root tips of plants obtained in the progeny of the seven different lines of the D genome.

(b) Sample checking was done at meiosis to confirm the monosomic condition of the 41 chromosome plants.

(c) From the progeny of selfed disomic addition individuals, plants with 42W+2R were selected for all the four different rye chromosomes.

(d) Seven different monosomics were pollinated separately by each of the four rye addition lines in order to prepare 28 different F_1 lines. Monosomics were always used as the female parent and the rye addition line as the male parent. The 28 different F_1 lines thus obtained were used in reciprocal crosses with Kharkov in the following manner:

(1) From the seeds obtained from crosses between monosomics and rye addition lines, plants with 41W+1R were selected on the basis of mitotic counts.

(2) Mitotic chromosome counts were made on the germinating seeds of the Kharkov parent in order to ensure that only euploid plants (42W) were used in crosses. The occasional aneuploid was discarded.

(3) Meiosis was studied in F_1 plants with 41W+1R to ascertain the constitution 20"W+1'W+1'R, (i.e., with two univalents).

(4) 41W+1R plants of the above constitution were crossed reciprocally with Kharkov.

(5) The seeds obtained from reciprocal crosses were germinated and the chromosome constitution for each seed determined on the basis of mitotic counts. The chromosomes supplied by Kharkov being known, the male and female transmission frequencies of rye chromosomes were then calculated.

Identification of rye chromosomes:-

The four different rye chromosomes I, IV, V and VI could be identified at the mitotic metaphase on the basis of their morphology.

At meiosis, due to its larger size, the rye univalent always could be differentiated from the wheat univalent. The detailed morphology of the four rye chromosomes has been described in the section of literature review.

Cytological techniques:-

All the plants grown were cytologically examined to establish the chromosome constitution of each plant. To facilitate the handling of material while making crosses, the labels of the pots carried the information pertaining to the plant number as well as the chromosome constitution.

Before collecting root tips for cytological examination, the seeds were placed on moist blotters at a temperature of 0°-2^oc. for about a week in order to overcome dormancy. After a week, they were allowed to remain at room temperature for about 24-36 hours, after which time root tips were collected in tap water and pretreated at 0°-2^oc. for 24 hours. After pretreatment, the root tips were fixed in acetic alcohol (1:3) as outlined by Tsunewaki and Jenkins (54) for a minimum of 24 hours. Fixed root tips were hydrolyzed for 6-8 minutes in N HCl at 60^oc, stained in Feulgen solution for 15 minutes, and squashed in 0.5% solution of acetocarmine.

Meiosis was studied by fixing the spikes in Carnoy's fluid (6:3:1) and staining the pollen mother cells in 0.5% solution of acetocarmine.

Results and Discussion

1. Production of F_1:-

Seven lines of Kharkov wheat representing the seven monosomics in the D genome and four rye addition lines were crossed to obtain the 28 possible F_1 combinations. The results of the progenies of the selfed and crossed material are presented below.

(a) Progeny of selfed monosomics:-

Table I shows the distribution of disomics, monosomics and nulli-somics in the progeny of selfed wheat monosomics. Chi-square tests shows that such a distribution is independent of the wheat chromosome involved (P = .70-.50). The summed data were in good agreement with the expected ratio of 24:73:3 (P = .30-.20).

Table I   -   Distribution of Progenies from selfed wheat monosomics.

Wheat Chromosome Monosomic	Chromosome Number			Total
	42	41	40	
1D	3	20	1	24
2D	3	18	1	22
3D	7	12	-	19
4D	4	16	-	20
5D	4	19	1	24
6D	5	20	-	25
7D	4	20	1	25
Total	30	125	4	159

(b) Progeny of selfed rye addition lines:-

The distribution of disomic lines and other aneuploid types in the progeny of selfed rye addition lines is presented in Table II. A chi-square test for independence showed that all addition lines were not equally stable (P = <.001). A significant chi-square value was obtained and could be attributed to the rye addition line for chromosome VI, which was known to be unstable.

Table II - Distribution of Progenies from addition lines (21"W+1"R).

Addition line	Chromosome Number		Total
	42W+2R	Others	
RA 1	27	1	28
RA IV	10	-	10
RA V	29	1	30
RA VI	31	20	51
Total	97	22	119

(c) Monosomic x rye addition lines:-

The results obtained from crosses of wheat monosomics and rye addition lines for rye chromosome IV, V and VI are presented in Tables III, IV and V respectively. The results pertaining to rye chromosome I are not presented as some of these lines were available when the present program was initiated. These data showed that 41W+1R and 42W+1R were obtained in the ratio of 3:1 as expected. A low frequency of 41W and 42W types occurred as a result of the loss of the rye chromosome or due to occasional selfing of the wheat monosomic parent. The addition line involving chromosome VI of rye was relatively unstable and, as a result, the frequency of 41W and 42W types occurring in its progeny was very high.

Table III - Distribution of Progenies from (20"W+1'W)♀ x (21"W+1"R)♂, where rye chromosome IV is involved.

Wheat Chromosome	Chromosome Number			
Monosomic	41W+1R	42W+1R	Others	Total
1D	4	2+1*	1	8
2D	12	5	4	21
3D	27	10	1	38
4D	21	3	-	24
5D	17	7	1	25
6D	24	5	1	30
7D	4	1	-	5
Total	109	34	8	151

*43W

Table IV - Distribution of Progenies from (20"W+1'W)♀ x (21"W+1"R)♂, where rye chromosome V is involved.

Wheat Chromosome	Chromosome Number			
Monosomic	41W+1R	42W+1R	Others	Total
1D	16	6	2	24
2D	11	5	2	18
3D	8	4	2	14
4D	10	4	-	14
5D	10	3	1	14
6D	12	2	-	14
7D	15	3	1	19
Total	82	27	8	117

Table V - Distribution of Progenies from $(20''W+1'W)\female$ x $(21''W+1''R)\male$, where rye chromosome VI is involved.

Wheat Chromosome Monosomic	Chromosome Number			Total
	41W+1R	42W+1R	Others	
1D	10	5	7	22
2D	11	4+1*	6+2**	23
3D	9	2	2	13
4D	7	4	7+1***+1**	20
5D	11	-	11	22
6D	6	2	5	13
7D	9	3	8	20
Total	63	21	49	133

*43W+1R
**40W+1R+telo
***41W+telo

2. Cytology of F_1 lines:-

(a) Mitosis

In the 28 combinations involving the four rye addition lines and the seven wheat monosomics, two classes of plants with respect to their chromosome constitution were obtained, viz: those of 42W+1R and those of 41W+1R. The rye chromosome was identified on the basis of its characteristic morphology. Fig. 1 and Fig. 2 show typical root-tip cells with the constitution of 2n = 42W+1R and 2n = 41W+1R respectively. Plants with 2n = 42W+1R were not studied in detail since only the substitution lines were of direct value in the present investigation.

Figures 1-2.   Mitotic metaphase of plants from 20"W+1'W+1'R(♀) x
21"W+1"R(♂)   (Arrow indicates a rye chromosome).

Fig. 1.   Metaphase showing 42W+1R(I). (X850).

Fig. 2.   Metaphase showing 41W+1R(3DVI). (X850).

(b) <u>Meiosis</u>

Several plants which proved to be of the constitution 41W+1R at mitosis were studied at meiosis and were found to exhibit a configuration predominantly of 20"+1'W+1'R (Fig.3). At metaphase I, the two univalents were usually found outside the equatorial plate. As most commonly observed at early anaphase I, the bivalents were regularly disjoined, with 20 chromosomes moving to each pole and the two univalents lagging at the plate (Fig.4). Usually the two univalents divided at anaphase I in a regular manner (Fig.5) and were either included in the nuclei at telophase I or were excluded as micronuclei in the cytoplasm (Fig.6). In addition, the following variations in chromosome behaviour were observed: (1) non-division of one of the two lagging univalents, a condition which possibly is more common with wheat univalents (Fig.7); (2) One or the other, or both, univalents misdivided, giving rise to telocentrics or isochromosomes (Fig.8).

In a small percentage of cells at metaphase I (5 per cent approximately), 19"W+3'W+1'R were observed due to asynapsis or desynapsis between two homologous wheat chromosomes (Fig.9). Such a situation could lead to the presence of four lagging univalents observed at anaphase I (Fig.10).

At anaphase II, laggards were not infrequent and consisted mainly of single chromosomes. At that stage it was possible to identify the laggards on the basis of relative size difference between wheat and rye chromosomes, also by the morphology of the rye chromosome itself (Fig.11). The results presented in Table VI are based on analysis of Pollen Mother Cells at late anaphase II. It was apparent that the lagging chromosomes were not included in the pollen nuclei, but formed micronuclei at the quartet stage (Fig.12). The results in Table VI did not include the doubtful cases where lagging chromosomes could not be identified with
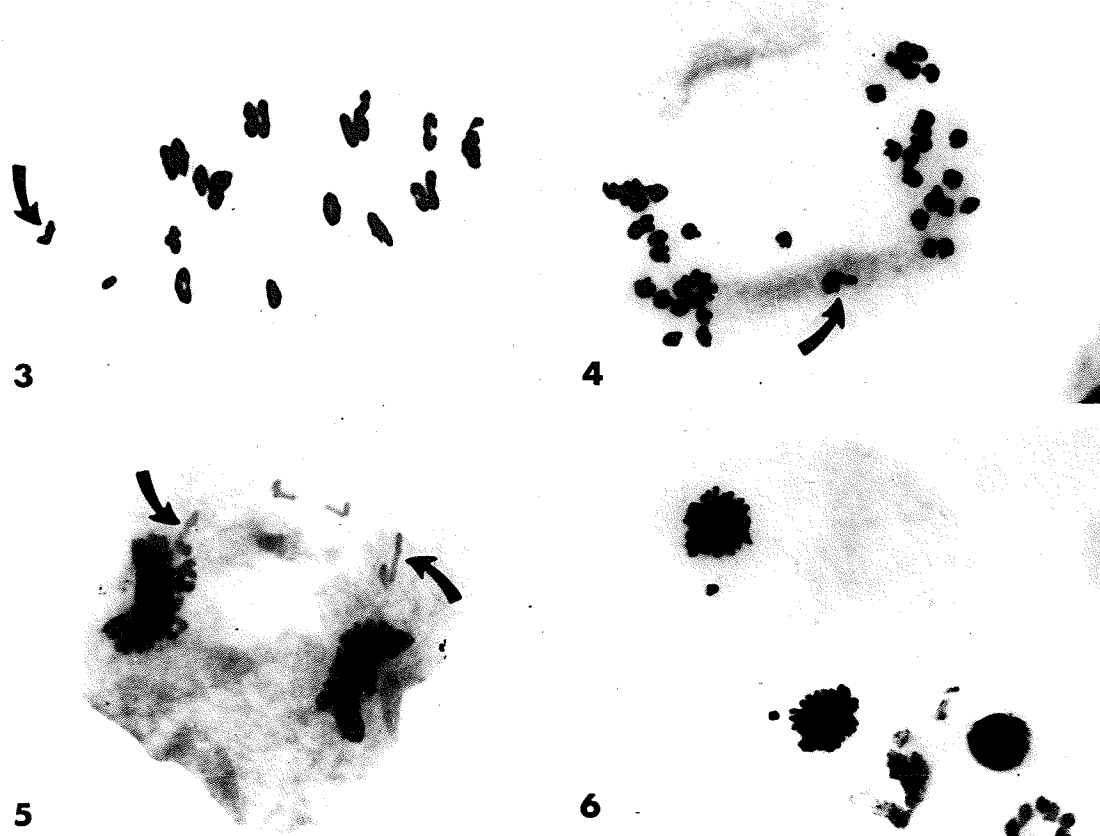
Figures 3-6.   Meiosis in a 41W+1R plant.   (Arrow indicates a rye chromo-
                some).

Fig. 3.   Metaphase I showing 20"W+1'W+1'R (6DVI). (X600).

Fig. 4.   Anaphase I showing 20 chromosomes on each pole and 2
            lagging univalents (6DIV) (X600).

Fig. 5.   Late anaphase I where both univalents have already
            divided (6DIV) (X600).

Fig. 6.   Lagging chromosomes fail to be included and form micro-
            nuclei (6DIV) (X600).
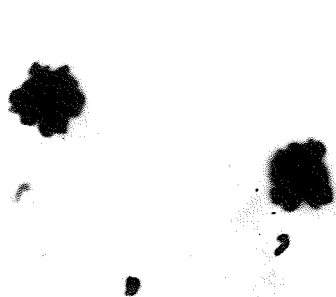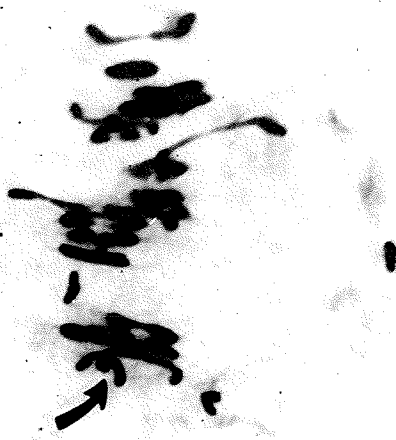
Figures 7-10.   Meiosis in a 41W+1R plant (contd.) (Arrow indicates
                 a rye chromosome.

   Fig.   7.   At anaphase I only one univalent divided; wheat uni-
                 valent undivided (6DIV) (X600).

   Fig.   8.   Anaphase I showing misdivision of univalents (6DIV)
                 (X600).

   Fig.   9.   Metaphase I showing 19"W+3'W+1'R (6DIV) (X600).

   Fig.   10.   Anaphase I showing 19 chromosomes on each pole and
                 four lagging univalents (6DIV) (X550).

Table VI - Distribution of lagging chromosomes at anaphase II and telophase II in (20"W+1'W+1'R) plants.

Lagging Chromosomes \ Line Involved	2D IV	3D IV	4D IV	5D IV	7D IV
0	25	12	7	2	6
1W	5	5	3	-	3
2W	3	3	-	1	2
1R	10	9	3	5	1
2R	8	5	3	1	5
1W+1R	4	7	2	2	3
1W+2R	5	2	5	1	5
2W+1R	1	2	4	1	2
2W+2R	14	6	3	2	2
Total quartest	75	51	30	15	29
Total laggards	36W+55R	36W+44R	24W+31R	11W+16R	23W+30R

certainty. Therefore, the actual relative frequency of quartets exhibiting no laggards was lower than obtained in the present study.

The results in Table VI, pertaining to rye chromosome IV, indicated that this rye univalent was excluded more often than was the chromosome from wheat. This may not be true for the other three rye chromosomes involved in this study, namely I, V and VI; however, similar studies on these chromosomes were not conducted. From other information available on the behaviour of rye univalents, however (see section 3, p.31), it was reasonably clear that the four rye univalents in question probably behave in a similar manner. It was also apparent from these results that the majority of gametes were deficient for both the wheat and rye chromosomes.

Chromosome mosaics like those reported by Watanabe (55,56) in the wheat var. Shirhada were also observed. In a plant from the line 7D (VI) of the chromosome constitution 20"1+1'W+1'R, about 10 cells each with 22 chromosomes exhibited a bivalent frequency ranging from three to six and univalent frequency of 10-16 (Fig.13). These cells were smaller in size than the regular pollen mother cells in this line (Fig.14).
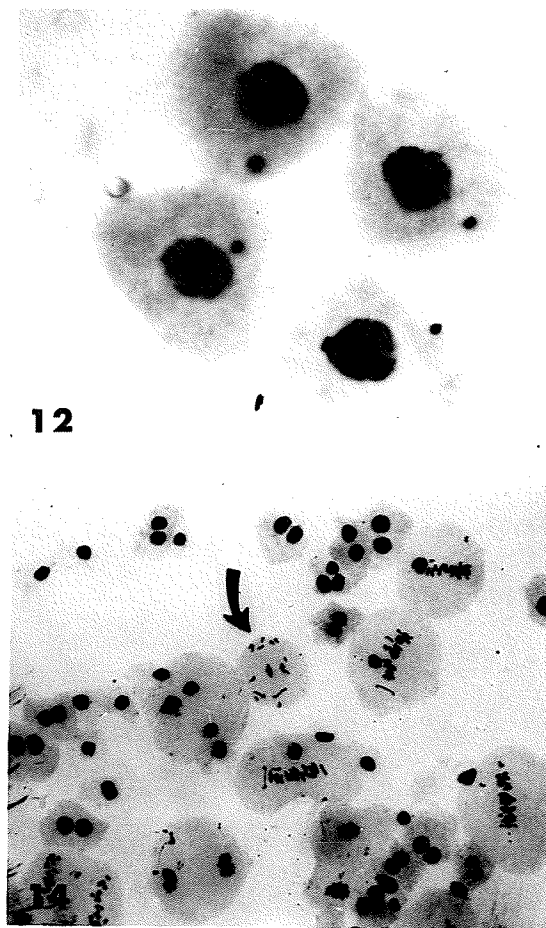
Figures 11-14. Meiosis in a 41W+1R plant (contd.)

  Fig. 11. Anaphase II showing lagging of two rye chromosomes
              (indicated by arrows) and one wheat chromosome (6DIV)
              (X600).

  Fig. 12. Quartet stage showing four micronucleii (6DIV) (X600)

  Fig. 13. An abnormal Pollen Mother Cell showing three bivalents
              and sixteen univalents at metaphase I (7DVI) (X600)

  Fig. 14. Pollen Mother Cells showing the relative size of the
              abnormal cell shown in Fig. 13. (Arrow indicates the
              abnormal cell). (X140)

3. Transmission of rye chromosomes from the F_1 (20"W+1'W+1'R)

In order to obtain theoretical ratios with which the actual re-
sults obtained in this study were compared, the following section on

transmission frequencies has been divided into two subsections, namely;

(A) Theory, and (B) Practical results.

A. Theory

The F_1 material used for transmission studies had the configura-
tion, 20"W+1'W+1'R. Therefore at metaphase I, there were always two

univalents, one representing the wheat chromosome and the other the rye

chromosome. Assuming the rye univalent to behave in a manner identical

to that of wheat, it can be expected to be lost in 75 per cent of the

gametes and would be distributed to the gametes in the following manner:

OWOR	OW1R	1WOR	1W1R
9	3	3	1

In such a situation, if male nullisomic gametes (OWOR) do not

function, and the other three types of gametes function without a certa-

tion effect, the transmission through the male and female would be as

follows:

Female	OWOR	OW1R	1WOR	1W1R
	9	3	3	1
Male	OWOR	OW1R	1WOR	1W1R
	0	3	3	1

Assuming that alien substitution gametes (OW1R) also do not func-

tion through the male, as would be the case if the rye chromosome did

not compensate for the missing wheat chromosome, the transmission of male

gametes would be:

OWOR	OW1R	1WOR	1W1R
0	0	3	1

The fact that an alien chromosome may not always be distributed to the gametes in a manner similar to that in wheat, has been shown by Johnson (15) using Agropyron elongatum, and by Riley et al. (41) using Aegilops comosa. Using Hacker's formula (see literature review, p. 11), an attempt has been made in this study to determine the frequency of elimination of the rye chromosome.

Table VII - Distribution of Progenies of selfed (21"W+1'R) plants (extracted from Evans and Jenkins, 1960).

Rye Chromosome Designation	Chromosome Number					Total No. of Plants
	42	43	44	fragments	others	
VI	92	11	1	14	1	119
V	72	10	1	7	0	90
IV	35	8*	1	3	0	47
I	39	12*	2	11	1	65
Total	238	41	5	35	2	321

* One plant 42W+1R+1 telo.

The distribution of progeny from selfed monosomic addition lines (21"W+1'R) involving each of the four rye chromosomes was obtained from the data of Evans and Jenkins (7) and is presented in Table VII. A chi-square test for independence showed that the distribution pattern of all four rye univalents was similar and the results were, therefore, combined. For the purpose of combined data, fragments were regarded as full rye chromosomes, plants with 42W+1 telocentric chromosome were considered as monosomic additions, while those with 42W+1R+1 telocentric were scored as disomic additions. The final combined results used in Hacker's formula were as follows:

Chromosome Number

Class	42	43	44	Total
Freq.	238	74	7	319

The proportion of 42 chromosomes offspring (N) and of 44 chromosomes offspring (U) in the above distribution were determined to be:

$$N = .750789$$

$$U = .022082$$

Substituting the values of N and U in Hecker's formula:

$$2E_{21} = 1.728707 \pm \sqrt{2.988428 - 3.003156}$$

(E_{21} - Proportion of eggs with 21 chromosomes)

Approximating the square root value to zero:

$$E_{21} = .864354, \text{ and}$$

$$E_{22} = .135646$$

On the basis of these calculations, the rye chromosome was absent in 86.4 per cent of the gametes. In comparison, wheat monosomes are lost from 75 per cent of the gametes (48). Using each of these two values for rye and wheat respectively, expected proportions of different gametic products in plants that were double monosomic for a wheat and a rye chromosome were calculated. These results are presented in Table VIII and were used to obtain theoretical values of female and male transmissions, as well as to obtain the theoretical distributions in the progeny of selfed F_1 plants.

(a) Expected transmission of female gametes:

As there can be no competition of gametes on the female side and assuming no gametic or zygotic lethality, the expected transmission of the four types of gametes would be the same as the actual frequency of gametes derived in Table VIII.

Table VIII — Expected frequencies of gametes due to different behaviour of wheat and rye univalents in (20"W+1'W+1'R) plants (only univalent chromosomes shown)

Wheat / Rye	OW .75	1W .25
OR	OWOR	1WOR
.864354	.648265	.216089
1R	OW1R	1W1R
.135646	.101735	.033911

This proportion of different gametes would then be as follows:

Gametes	Per Cent	Ratio
OWOR	64.8	19
OW1R	10.2	3
1WOR	21.6	6
1W1R	3.4	1

(b) **Expected transmission of male gametes:**

Depending upon the functional relationship between the rye chromosome and the corresponding wheat chromosome, one of the two conditions could exist:

(1) <u>Compensating effect</u>, in which the rye chromosome compensates for the missing wheat chromosome. Assuming that nullisomic gametes (OWOR) do not function, the transmission of the remaining three types would be as follows:

Gametes	Per Cent	Ratio
OW1R	28.9	12
1WOR	61.4	25
1W1R	9.6	4

(2) <u>Non-compensating effect</u>, in which the rye chromosome does not functionally compensate for the missing wheat chromosome. Assuming that nullisomic (OWOR) and substitution gametes (OW1R) do not function, the remaining two types of gametes will be transmitted as follows:

Gametes	Per Cent	Ratio
1WOR	86.4	25
1W1R	13.6	4

(c) <u>Expected distribution of the progeny of a selfed F_1</u>:-

Expected distribution in the progeny of a selfed F_1 could be calculated from the theoretical values of male and female transmission. Depending upon whether a rye chromosome does or does not compensate for its wheat counterpart, the frequency distribution in the progeny of a selfed F_1 could vary.

Table IX - Expected frequencies in the progenies of selfed (20"W+1'W+1'R) plants, where rye chromosome compensates for the missing wheat chromosome (only univalent chromosomes shown).

Egg \ Pollen	OW1R .289237	1WOR .614352	1W1R .096411
OWOR .648265	OW1R .187502	1WOR .398263	1W1R .062501
OW1R .101735	OW2R .029426	1W1R .062501	1W2R .009808
1WOR .216089	1W1R .062501	2WOR .132755	2W1R .020833
1W1R .033911	1W2R .009808	2W1R .020833	2W2R .003269

Assuming compensation, the distribution of the progeny of selfed F_1 is shown in Table IX. The eight different types of progeny should be obtained in the following proportions:

OW1R	.187502
1WOR	.398263
1W1R	.187503
OW2R	.029426
2WOR	.132755
1W2R	.019616
2W1R	.041666
2W2R	.003269

Table X - Expected frequencies in the progenies of selfed (20"W+1'W+1'R) plants, where rye chromosome does not compensate for the missing wheat chromosome (only univalent chromosomes shown).

Egg \ Pollen	1WOR .864354	1W1R .135646
OWOR .648265	1WOR .560330	1W1R .087935
OW1R .101735	1W1R .087935	1W2R .013800
1WOR .216089	2WOR .186777	2W1R .029311
1W1R .033911	2W1R .029311	2W2R .004600

If the rye chromosome does not compensate for a missing wheat chromosome, the distribution in progeny of selfed F_1 would be as shown in Table X.

The six types of progeny would be expected in the following proportions:

1W0R	.560330
1W1R	.175870
2W0R	.186777
2W1R	.058622
1W2R	.013800
2W2R	.004600

It is recognized that the above theory is proposed on the basis of certain assumptions, although essentially accurate, may not take into account possible deviations from the expected. For example, a small proportion of nullisomic male gametes may function even under competition. Similarly, a condition of partial compensation may exist, which will be somewhat intermediate to the compensating and non-compensating effect described above.

(d) Origin of telocentrics and Isochromosomes:-

Sears (48) analyzed 335 seedlings from eight monosomic families and found only 10 of them (3 per cent) with a telocentric chromosome. Evans and Jenkins (7) similarly analyzed 470 plants from seven different monosomic rye addition lines and found 86 plants (18.4 per cent) with rye telocentrics. This is an indication that rye univalents may misdivide more often than wheat univalents.

Isochromosomes could not be identified at mitosis except in cases where it was either an isochromosome for a satellited arm, for an arm with a secondary constriction (Fig. 15), or for an unusually long arm. In the present study, none of the wheat chromosomes belonging to the D genome had any of these attributes and therefore an isochromosome for a wheat chromosome could escape detection. Rye isochromosomes could be identified, however, except the possible exception of one involving rye chromosome I.
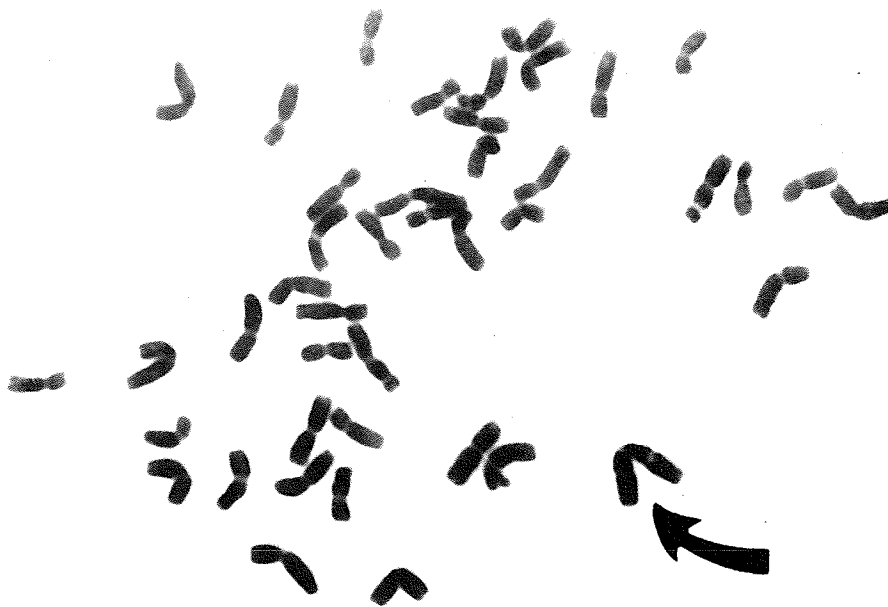
15

Figure 15. Origin of isochromosomes during transmission studies.

Metaphase showing 42W+1 isochromosome for long arm of
rye chromosome V. (Arrow indicates the isochromosome).
(X1500)

B. Practical results:-

In order to apply the theory presented above to the results obtained, the following simplifications were made:

(1) The occasional 40W plant was grouped with 41W plants. Similarly, the few plants with 43W were grouped with 42W plants.

(2) In the analysis of male transmission frequencies, it was assumed that nullisomic gametes do not function, so that the occasional 41W plant was grouped with those having 42W chromosomes. Similarly, in absence of evidence of compensation, occasional 41W+1R plants were grouped with 42W+1R plants.

(3) All telocentrics were regarded as full chromosomes. Telocentric chromosomes which were not identified as belonging to the wheat complement, were considered to be rye telocentrics for following reasons:

(a) During mitosis most of the telocentric chromosomes could be identified as belonging to the rye complement.

(b) There is evidence that rye telocentrics arise much more frequently than those involving wheat chromosomes (see theory, section d).

Results presented in the following section are based on the above simplifications and will be presented and discussed separately for each of the four rye chromosomes studied in this investigation.

Chromosome I:-

Since the chromosome constitution of the line 7DI used was doubtful, the data from only six of the possible seven lines pertaining to this chromosome will be presented.

(a) Female transmission:-

Results of the cross 20"W+1'W+1'R($\female$) x 21"W($\male$) are presented in Table XI. Chi-square test for independence was non-significant (P=.30-.20),

showing that transmission frequencies did not differ from one wheat
chromosome to the other. The data were therefore summed before the
chi-square test for goodness of fit was applied. The summed data gave
a good fit with the theoretical ratio of 19:3:6:1 (P=.10-.50), there-
fore it was concluded that female transmission of rye chromosome I was
as expected on the basis of our knowledge of the behaviour of wheat
and rye univalents.

Table XI - Distribution of Progenies from (20"W+1'W+1'R)♀ x (21"W)♂,
where rye chromosome I is involved.

Wheat Chromosome Monosomic	Chromosome Number				Total
	41W	41W+R	42W	42W+1R	
1D	40	7	8	2	57
2D	25	9	6	4	44
3D	28	5	7	-	40
4D	22	2	6	2	32
5D	25	4	3	-	32
6D	19	1	2	-	22
Total	159	28	32	8	227

(b) Male transmission:-

Results of the cross 21"W (♀) x 20"W+1'W+1'R (♂) are presented in
Table XII. The chi-square value for independence was statistically sig-
nificant (P=< .001), therefore, the results for the six homoeologous
groups were tested individually for goodness of fit to the ratios 12:25:4
and 25:4. Data from homoeologous groups 1 and 3 gave a good fit with the
ratio 12:25:4, while the data from the remaining groups satisfied the
ratio 25:4 (Table XII). It was therefore concluded that rye chromosome I

is able to functionally compensate for 1D as well as for 3D in the male gametophyte.

Table XII   -   Distribution of Progenies from $(21''W)$♀ x $(20''W+1'W+1'R)$♂, where rye chromosome I is involved.

| Wheat Chromosome | Chromosome Number | | | | Ratio(P) | Ratio(P) |
Monosomic	41W+1R	42W	42W+1R	Total	25:4	12:25:4
1D	9	28	4	41	-	.90 - .50
2D	-	49	4	53	.20 - .10	-
3D	13	15	3	31	-	.50 - .20
4D	1	49	10	60	.50 - .20	-
5D	-	51	6	57	.50 - .20	-
6D	-	27	6	33	.50 - .20	-
Total	23	219	33	275		

(c) Progeny of selfed F_1:-

The results from the analysis of the progeny from selfed F_1 plants of the constitution 20''W+1'W+1'R are presented in Table XIII. Since the populations were small for the eight expected classes, analyses for goodness of fit to expected ratios were not conducted. Nevertheless, the presence of disomic substitution for 1D and 40W+1R plants for 1D and 3D wheat chromosomes indicated that some genetic relationship does exist between rye chromosome I and wheat chromosomes 1D and 3D. The relative absence of plants with 40W+1R chromosomes in these two homo-eologous groups is hard to explain, because theoretically in the selfed progeny nearly 18.7 per cent of the progeny is expected to be of this constitution (see Table IX).

Table XIII — Distribution of F$_2$ Progenies from F$_1$ (20"W+1'W+1'R) plants, where rye chromosome I is involved.

Wheat Chromosome Monosomic	Chromosome Number							
	41W	40W +1R	40W +2R	41W +1R	42W +1R	42W	42W +2R	Total
1D	21	1	1	6	3	7	-	39
2D	23	-	-	2	2	5	1	33
3D	21	1	-	5	3	8	-	38
4D	24	-	-	4	10	11	1	50
5D	9	-	-	5	2	3	-	19
6D	8	-	-	4	2	6	-	20
Total	106	2	1	26	22	40	2	199

Riley and Kimber (42) pointed out that the presence of reciprocal translocations in the chromosome arrangement of Secale cereale would enable one rye chromosome to show genetic relationship for more than one homoeologous group of wheat. Such a situation is likely present in the rye chromosome I in the present investigation. Secale montanum which is believed to be ancestral to S. cereale (17) may have chromosomes in the primitive form, so that if S. montanum was used in similar studies, a more precise genetic relationship could be obtained.

Chromosome IV:-

(a) Female transmission:-

The results of the cross 20"W+1'W+1'W (♀) x 21"W (♂) are presented in Table XIV. Test of heterogeneity (P=.10-.05) showed that the results were homogeneous and could be combined. The summed results fit the theoretical ratio 19:3:6:1 (P=.70-.50) and it was therefore concluded that the transmission of different gametes was as expected.

Table XIV - Distribution of Progenies from (20"W+1'W+1'R)♀ x (21"W)♂, where rye chromosome IV is involved.

Wheat Chromosome Monosomic	Chromosome Number				
	41W	41W+1R	42W	42W+1R	Total
1D	25	4	5	4	38
2D	35	-	2	-	37
3D	33	2	9	-	34
4D	19	7	10	2	38
5D	21	4	6	-	31
6D	26	4	6	3	39
7D	16	2	7	1	26
Total	175	23	45	10	253

However, unexpectedly no rye chromosome was transmitted in line 2D, an event that cannot be readily explained. There was no evidence to show that either competition or preferential segregation was the causal factor. It appeared safe to assume, moreover, that the behaviour of two univalents did not differ in the presence of different wheat chromosomes.

(b) Male transmission:-

The results of the cross 21"W (♀) x 20"W+1'W+1'R (♂) are presented in Table XV. Substitution gametes did not function except in line 6D. Chi-square test for independence showed a high degree of heterogeneity, therefore tests for goodness of fit were conducted separately for each of the seven homoeologous groups. The data for homoeologous group 6 satisfied the ratio 12:25:4, while those for all the remaining groups fit the ratio 25:4. It was, therefore, concluded that rye chromosome

IV compensated for the missing wheat chromosome 6D in the male gametophyte, and that there is some genetic relationship between the two.

Table XV - Distribution of Progenies from $(21''W)♀$ x $(20''W+1'W+1'R)♂$, where rye chromosome IV is involved.

Wheat Chromosome Monosomic	Chromosome Number			Total	Ratio(P) 25:4	Ratio(P) 12:25:4
	41W+1R	42W	42W+1R			
1D	-	20	3	23	.95 - .50	-
2D	-	22	2	24	.50 - .20	-
3D	1	48	10	59	.50 - .20	-
4D	-	40	9	49	.50 - .20	-
5D	-	20	4	24	.50 - .20	-
6D	11	36	6	53	-	.50 - .20
7D	1	21	7	29	.10 - .05	-
Total	13	207	41	261		

(c) Progeny of selfed F_1:-

The results of sample analysis of the progeny of selfed 20''W+1'W+1'R plants are presented in Table XVI. Unfortunately, limited size did not permit analysis of goodness of fit. The presence of plants with 40W+1R, which presumably were the result of fertilization between nullisomic female gamete and substitution male gametes, was considered as further confirmation of the compensation effect.

Riley (37) showed that there is homoeologous relationship between chromosome II in rye var. King II and three chromosomes of the homoeologous group 6 in wheat. If such is the situation, then chromosome IV in rye var. Dakold may correspond to chromosome II in rye var. King II. Chromosome II in rye var. King II, however, is the nucleolar organizing chromosome, while in rye var. Dakold, this function is thought to be

governed by chromosome VII (7). To reconcile these apparent differences in regard to the identification of the nucleolar organizing chromosome in rye var. Dakold, further investigation will be required.

Table XVI - Distribution of F_2 Progenies from F_1 (20"W+1'W+1'R) plants, where rye chromosome IV is involved.

Wheat Chromosome Monosomic	Chromosome Number					
	41W	40W +1R	41W +1R	42W	42W +1R	Total
1D	16	-	3	1	-	20
2D	28	-	5	3	2	38
3D	26	-	9	3	1	39
4D	13	-	1	4	1	19
5D	24	-	8+1**	5	1+1*	40
6D	12	2	5	-	-	19
7D	25	-	6	2	1+1*	35
Total	144	2	38	18	8	210

* 42+2R
** 41+2R

If chromosome IV in rye var. Dakold is the nucleolar organizing chromosome and corresponds to chromosome II in the rye var. King II, Dakold IV would then be designated as 6R. That chromosome IV in Dakold belongs to homoeologous group 6 will have to be further confirmed by comparing the corresponding disomic substitution lines with normal Kharkov plants. Meiotic stability and fertility of these disomic substitution plants will have to be compared with those of normal wheat plants. Such a study would give additional information regarding the compensating effect of rye chromosome IV.

Chromosome V:-

(a) <u>Female transmission</u>:-

The results of the cross 20"W+1'W+1'R(♀) x 21"W(o) are presented in Table XVII. Nullisomic gametes, as usual were most frequent. Chi-square tests for heterogeneity gave a probability value of .05 - .02, value that may not be completely free of bias due to the small population size in line 7D. The data were combined, and grouped data satisfied the theoretical ratio 19:3:6:1 (P=.50-.20). It was concluded that the two univalents were distributed as expected on the basis of theorized results.

Table XVII - Distribution of Progenies from (20"W+1'W+1'R)♀ x (21"W)♂, where rye chromosome V is involved.

Wheat Chromosome	Chromosome Number				
Monosomic	41W	41W1R	42W	42W1R	Total
1D	19	3	5	-	27
2D	16	1	3	-	20
3D	14	-	2	2	18
4D	14	6	1	1	22
5D	16	1	6	1	24
6D	38	4	5	1	48
7D	4	4	4	-	12
Total	121	19	26	5	171

(b) <u>Male transmission</u>:-

The results of male transmission from the cross 21"W(♀) x 20"W+1'W+1'R(♂) are presented in Table XVIII. Two plants with 41W+1R did occur, one in each of two homoeologous lines 4D and 6D. These plants

were considered to be the result of occasional functioning substitution gametes and were grouped with those having 42W+1R. Chi-square test for independence (P=.50-.20) showed that the results were homogenous and were therefore combined and found to satisfy the theoretical ratio of 25:4 (P=.20-.10). It was therefore concluded that transmission did not vary from one wheat chromosome to the other, and that rye chromosome V failed to compensate for any of the wheat chromosomes of the D genome.

Table XVIII — Distribution of Progenies from (21"W)♀ x (20'W+1'W=1'R)♂, where rye chromosome V is involved.

Wheat Chromosome	Chromosome Number		
Monosomic	42W	42W1R	Total
1D	28	3	31
2D	29	1	30
3D	24	5	29
4D	36	7	43
5D	37	6	43
6D	47	4	51
7D	29	2	31
Total	230	28	258

(c) Progeny of selfed F_1:-

The results of the analysis of the progeny of selfed 20"W+1'W+1'R are presented in Table XIX. These results are in accordance with what we would expect on the basis of male and female transmission studies.

The behaviour of rye chromosome V showed, therefore, that it had no relationship with any of the seven homoeologous groups of wheat. This is one of the possibilities referred to by Riley and Kimber (42).

Originally this chromosome must have represented one of the seven chromosomes of the diploid prototype, which gave rise to not only the three diploid progenitors of wheat, but also to other related species. It is believed that in the course of time this chromosome was altered due to mutations or complex translocations, so that it no longer exhibits relationship with any of the seven homoeologous groups in wheat.

Table XIX - Distribution of F_2 Progenies from F_1 (20"W+1'W+1'R) plants, where rye chromosome V is involved.

Wheat Chromosome Monosomic	Chromosome Number					
	41W	40W +1R	41W +1R	42W	42W +1R	Total
1D	29	1	8	4	1	43
2D	30	1	7	4	1	43
3D	23	2	8	9	2	44
4D	17	1	5	10	4	37
5D	22	-	5	13	3	43
6D	18	-	13	7	2	40
7D	19	-	8	7	6	40
Total	158	5	54	54	19	290

Chromosome VI

(a) Female transmission

The results of the cross 20"W+1'W+1'R(♀) x 21"W(♂) are presented in Table XX. Chi-square test for independence showed that the results were homogeneous (P=.99-.95), therefore the results were combined. Since the combined results fit the theoretical ratio 19:3:6:1 (P=.50-.20), it was concluded that the transmission of rye chromosome VI did not differ from one line to the other and that the results are in accord with our knowledge of univalent behaviour.

Table **XX** - Distribution of Progenies from (20"W+1'W+1'R)♀ x (21"W)♂, where rye chromosome VI is involved.

Wheat Chromosome Monosomic	Chromosome Number				Total
	41W	41W+1R	42W	42W+1R	
1D	21	5	10	3	39
2D	28	5	8	-	41
3D	38	11	12	2	63
4D	26	4	9	1	40
5D	30	7	9	1	47
6D	35	6	8	1	50
7D	28	4	11	2	45
Total	206	42	67	10	325

(b) Male transmission:-

The results of the cross 21"W(♀) x 20"W+1'W+1'R(♂) are presented in Table **XXI**. The results were almost identical to those obtained in a similar cross involving rye chromosome V, in that apparently male transmission of rye substitution gametes did not occur. Four plants of chromosome formula 41W+1R were observed, two each in line 3D and 5D and were grouped with the class 42W+1R (see practical results p. 38). Chi-square test for independence showed that results could be combined (P=.50-.20) and the summed results gave a good fit with the theoretical ratio 25:4 (P= .99-.95). Obviously rye chromosome VI did not compensate for any of the seven wheat chromosomes of the D genome.

Table XXI - Distribution of Progenies from $(21''W)\female$ x $(20''W+1'W+1'R)\male$, where rye chromosome VI is involved.

Wheat chromosome Monosomic	Chromosome Number		Total
	42W	42W+1R	
1D	81	14	95
2D	57	6	63
3D	44	10	54
4D	53	13	66
5D	53	3	56
6D	28	3	31
7D	64	12	65
Total	380	61	441

(c) Progeny of selfed F_1

Results of the analysis of F_2 progeny from F_1 $(20''W+1'W+1'R)$ plants are presented in Table XXII. Chi-square tests for independence or goodness of fit were not conducted as no ratios were derived for F_2 progenies; however, the frequency of classes appearing in each of the seven lines were very similar. Moreover, the summed results represented all the six possible classes. The frequency of monosomics was very close to the expected 56 per cent, also the proportion of F_1 types (41W+1R) and of disomic normals (42W) was close to the expected 18 per cent (approximately) for each. It was concluded, therefore, that the actual results were in close agreement with those derived on a theoretical basis (see theory p.36).

However, we know much more about rye chromosome VI (designated as chromosome I by O'Mara and Riley) than about any other rye chromosome studied so far. The following evidence from different studies support

the contention that this chromosome belongs to homoeologous group 5
of wheat.

(1) The spontaneous substitution of rye chromosome I for chromosome
5A(IX) of wheat var. Kwan-Do, as recognized by o"Mara (29).

(2) The transmission of alien substitution gametes in a cross
21"W(♀) x 20"W+1'W(5A)+1'R(I)(♂) was reported to be 26 per cent (31).

(3) The compensation by rye chromosome I for wheat chromosome 5A(IX)
as observed by Smith (52).

(4) The high rate of transmission of rye chromosome VI (Secale cereale
var. Dakold) when substituted for wheat chromosome 5D as studied by Ray (34).

Table XXII - Distribution of F_2 Progenies from F_1 (20"W+1'W+1'R) plants,
where rye chromosome VI is involved.

Wheat Chromosome Monosomic	Chromosome Number						Total
	41W	41W +1R	42W	42W +1R	41W +2R	42W +2R	
1D	21	6	7	5	-	-	39
2D	17	4	6	2	-	-	29
3D	23	6	4	3	1	-	37
4D	15	3	8	6	1	1	34
5D	35	5	8	2	-	1	51
6D	14	8	9	3	1	1	36
7D	20	5	7	2	-	-	34
Total	145	37	49	23	3	3	260

In the light of strong evidence available on the degree of com-
pensation which chromosome VI exhibits for the chromosomes of homoeologous
group 5 of wheat, the apparent differences in the results obtained in the
present study are difficult to reconcile. The designation 5R has already
been used for this chromosome (8). The most plausible explanation

possible at this time is that of mistaken identification of wheat mono-
somic 5D. It is possible that univalent shift occurred in this monosomic
line prior to the initiation of this study and unfortunately was not de-
tected. Monosomics used in the present investigation are now being tested
with the use of ditelosomics for their correct identification.

4. <u>Transmission of nullisomic male gametes from F_1 (20"W+1'W+1'R)</u>

In the earlier discussion of section 3, it was assumed that male
nullisomic gametes did not function and therefore the occasional nulli-
somic gamete (0WOR) was included with normal gametes (1WOR). The results
suggested that although nullisomic gametes normally were at a consider=
able disadvantage, they could function to a limited extent. The distri-
bution of nullisomic male gametes from crosses 21"W(♀) x 20"W+1'W+1'R(♂)
is shown in Table XXIII and suggest a uniform distribution. Of the total
of 1235 gametes sampled, 54 were nullisomic gametes, or a 4.4 per cent
transmission rate, which corresponds to 6 per cent transmission frequency
cited by O'Mara (31) from a cross involving chromosome I (corresponds to
rye chromosome VI in the present study) and wheat chromosome 5A(IX). It
was therefore concluded that transmission of nullisomic gametes from the
male side was higher in this material (20"W+1'W+1'R) than in the simple
wheat monosomics which exhibit about 3 per cent transmission through the
male.

5. Transmission of non-compensating rye substitution male gametes from
   F_1 (20"W+1'W+1'R).

Like nullisomic male gametes, alien substitution male gametes in
which the rye chromosome fails to compensate for the missing wheat
chromosome were also transmitted at a very low frequency. In a total
population of 1235 male gametes sampled from 27 different lines involving

four rye chromosomes and seven wheat chromosomes, only 9 non-compensating alien substitution gametes were observed. This represents .74 per cent transmission rate. It was therefore concluded that the presence of a rye chromosome with a non-compensating effect decreased the competitive ability of a male gamete.

Table XXIII - Distribution of Nullisomics in the Progenies of $(21"W)♀$ x $(20"W+1'W+1'R)♂$, involving rye chromosomes I, IV, V and VI.

Wheat Chromosome Monosomic	Rye Chromosome I		IV		V		VI		Total	
	Tot.	Nulli.	Tot.	Nulli.	Tot.	Nulli.	Tot.	Nulli.	Tot.	Nulli.
1D	41	2	23	-	31	3	95	-	190	5
2D	53	-	24	1	30	6	63	2	170	9
3D	31	-	59	3	29	-	54	4	173	7
4D	60	4	49	3	43	5	66	3	218	15
5D	57	-	24	4	43	-	56	6	180	10
6D	33	1	53	1	51	2	31	3	168	6
7D			29	-	31	1	76	1	136	2
Total	275	6	261	12	258	17	441	19	1235	54

Conclusions

Because of its direct bearing on the understanding of the evolutionary relationships in the sub-tribe _Triticinae_, the cytogenetic study of alien chromosome substitution (in wheat) has received considerable attention. This sub-tribe contains five genera, namely: _Triticum_, _Agropyron_, _Secale_, _Aegilops_ and _Haynaldia_. Alien substitutions from _Agropyron_, _Secale_ and _Aegilops_ to wheat are now known. No substitutions from _Haynaldia_ have as yet been made, however, because of low crossability between species of this genus and _T. aestivum_.

Chromosome substitutions from _Agropyron_ for 3D of wheat were identified by Bakshi and Schlehuber (1). Two similar substitutions from _Agropyron_ were recognized by Knott (18, 19), one for an _Agropyron_ chromosome carrying factors governing blue endosperm and the other for an _Agropyron_ chromosome carrying rust resistance. The substitution line having rust resistance was test-crossed and found to have replaced 6A of wheat. Johnson (15) systematically substituted the _Agropyron_ chromosome carrying rust resistance for wheat chromosome 6A and 6D, and believed that this chromosome belonged to homoeologous group 6. These substituting _Agropyron_ chromosomes could not be designated in accordance with the new designations used for wheat chromosomes because the _Agropyron_ genomes from which these chromosomes were derived are not known. Weinhues (57) also produced alien _Agropyron_ substitutions without any special reference to homoeologous relationships.

Secale cereale substitutions were obtained, similarly by o"Mara (29) for 5A of wheat, by Riley for 6A, 6B and 6D and by Driscoll (6) for

telocentric of 2A. If the rye chromosome, to which the substituting
telocentric in Cornell selection 82a1-2-4-7 (6) belongs, is tentatively
designated as 2R, there are three rye chromosomes whose homoeologous
relationship is known, viz: 2R, 5R and 6R. Jenkins (14) reported the
substitution of four rye chromosomes for different wheat chromosomes
representing all seven homoeologous group of wheat. However, he did not
suggest any homoeologous relationship between the substituting rye chromo-
some and the replaced wheat chromosome. Riley et al. (41) reported a
successful substitution from Aegilops for 2A, 2B and 2D of wheat and
designated this alien chromosome as 2M. Although no substitutions have
been made from the genus Haynaldia, recent reports by Halloran (11, 12)
gave evidence of crossability between Triticum aestivum and H. villosa.
He also showed that pairing between chromosomes of these two species
occurs in the wheat - Haynaldia hybrids. It can be concluded, therefore,
that a homoeologous relationship between wheat and Haynaldia chromosomes
also exists. Ultimately, therefore, we should expect to extend our
knowledge of the intraspecific homoeology of wheat chromosomes to inter-
specific homoeology between chromosomes of wheat and other related
species.

Smith (52) using methods similar to those used in the present
investigation, showed that alien substitution gametes (20W+1R) involving
rye chromosome I and wheat chromosome 5A, were not at a competitive dis-
advantage with euploid wheat gametes. This finding indicated that rye
chromosome I (5R) would functionally compensate for missing chromosome 5A.
His explanation that the alien substitution gametes functioned because
they had a normal chromosome number of 21 (20W+1R) may not be correct.
The results from the present study also showed that these techniques

can be successfully used to demonstrate the genetic relationship between alien chromosomes and the chromosomes of wheat. Moreover, it is also clear that the seven chromosomes of S. cereale may not be assigned to seven homoeologous groups due to chromosome interchanges which are present in its complement. Chromosome I in the present investigation showed compensation for 1D and 3D and is therefore believed to be a chromosome which is involved in an interchange. Similarly, chromosome V showed no compensation for any wheat chromosome. If 6R, 5R and 2R as shown previously are other than either chromosome I or chromosome V, it brings the total number of S. cereale chromosomes thus studied to five. Since there are two interchanges in S. cereale complement (17), and if chromosome V is not the one which is involved in these two interchanges, each of the remaining two chromosomes should show compensation for chromosomes of more than one homoeologous group. Riley (35) believed that there is possibly a third interchange present in S. cereale. If it is so, chromosome V may also be involved in such an interchange. Because of these complications, it is realized that if the ancestral species S. montanum was used instead of S. cereale, more precise homoeologous relationship could have possibly been obtained.

A homoeologous relationship between chromosomes of rye and wheat suggests that chromosome pairing between chromosomes of wheat and those of rye under some specific conditions should be possible. Such a pairing even in the absence of the 5B diploidising system was not observed (42). Chromosome pairing between A. squarrosa (D genome) and S. cereale chromosomes was observed by Melnyk and Unrau (23), but chromosomes of D genome when present in a wheat genotype do not show any pairing with rye chromosomes. In the present study, the F_1 material used was ideal for observing

meiotic pairing, there being present one wheat and one rye univalent, but no synapsis between chromosomes of wheat and rye was observed.

In the present investigation, it was difficult to correlate the findings with similar work elsewhere due to lack of information regarding inter-varietal karyotypic or genetic differences in S. cereale. Except the recently designated 6R, which is nucleolar organizing chromosome and 5R which carries the hairy neck marker, very little is known about the remaining five chromosomes to enable a worker to relate his work with that from other laboratories. Therefore, it is deemed necessary that S. cereale material used at Cambridge, Columbia and Winnipeg be studied collectively and the similarities and differences in the chromosomes of these three varieties be recorded. An attempt to relate material from Cambridge and Columbia is in progress at Cambridge (Riley - Oral Communication). The variety used in the present study, viz. Dakold, should be studied in a similar way. As all seven disomic addition lines are available in each of the three varieties, they can be intercrossed and the pairing behaviour of hybrids studied. Similar information can also be derived from extensive trisomic work in S. cereale similar to that in other diploid species, viz. barley, maize, etc.

The availability of a monotelosomic series in wheat var. Kharkov may further help in conducting aneuploid work without the necessity of checking the material for accuracy of its identification. At Columbia, Missouri, the substitution lines are being extracted by using monotelosomic series (6). In the present study the monosomics used could not be checked with ditelosomics. Although identification seems to be essentially correct, there is a definite doubt about 5D monosomic used with rye chromosome VI. In fact a test giving complete certainty cannot be made, because univalent

shift may take place at any time during the study. However, sample
plants from seven monosomics used in the present study are being grown
for tests with ditelosomics.

Although the possibility of making the alien substitutions and
their utility in plant breeding work is now confirmed, there is difference
of opinions regarding the substituting ability of an alien chromosome.
Knott (19), Riley (37), Johnson (15) and Riley et al. (41) developed the
concept of the dependence of substitution on homoeology between alien
substituting chromosome and replaced wheat chromosome. Weinhues (57)
and Jenkins (14) do not believe in such a dependence, and believe that
substitutions can be obtained regardless of the homoeologous relation-
ship between the substituting and the substituted chromosome. As reported
by Riley et al. (41) an alien chromosome would have a _specific substituting_
ability if it substitutes only for homoeologous chromosomes. Alien chromo-
somes with _general substituting ability_ will therefore substitute for
wheat chromosomes irrespective of any homoeologous relationship.

If a successful substitution implies a compensating function on
the part of the alien chromosome the present results indicate that the
occurrence of _general substituting ability_ is doubtful. None of the
four rye chromosomes studied showed compensation for each of the seven
wheat chromosomes used, while chromosome V showed a complete absence of
any compensating effect. However, if non-compensating substitution re-
flects the success of substitution, there is evidence that _general sub-_
stituting ability occurs, and that non-compensating disomic alien sub-
stitution lines can be obtained. These non-compensating substitution
lines would be comparable to the 61 non-compensating nullisomic - tetra-
somics in wheat obtained by Sears and in a similar way would lend further

support to the concept of homoeology between specific chromosomes of

wheat and an alien species. During the present study, it has been ob-

served that non-compensating alien substitution male gametes function

occasionally (.74 per cent) similar to nullisomic male gametes

(4.4 per cent). The male transmission of these alien substitution

gametes can be increased by eliminating competition. Such is the case

in 20"W+1"R+1'W plants in which the formation of zygotes having the

desired non-compensating substitutions cannot be ruled out.

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