CYTOGENETIC EVALUATION OF A WHEAT-RYE DERIVATIVE FOR RESISTANCE TO LEAF RUST

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By

Rafael Antonio Bravo

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By Rafael Antonio Bravo

The cytology of selection 82 a 1-2-6-2, a wheat-rye derivative which has excellent mature plant resistance to leaf rust at East Lansing, Michigan, was investigated. This wheat-like selection, as shown by Sebesta (1958), has 44 chromosomes with the two extra chromosomes being telocentric and carrying a factor(s) for leaf rust resistance.

To obtain some information on the homology of the extra chromosomes, the wheat and the rye complements, crosses were made between 82 a 1-2-6-2 and two of its ancestors, Rosen rye and Cornell 595 wheat. The formation of heteromorphic bivalents in the first type of cross may be taken as evidence for a degree of homology between the extra telocentric and the rye chromosomes. Tetravalent formation was observed in 13 % of the pollen mother-cells of the F_1 between 82 a 1-2-6-2 x Cornell 595. This was attributed to possible rearrangements within the wheat genome, as three different varieties (Honor, Yorkwin, and Cornell 595) were used in the evolutionary process of this selection.

Rafael Antonio Bravo

Selection 82 a 1-2-6-2 has shown some variation in leaf rust resistance at East Lansing due to the instability of the two telocentric chromosomes. Methods of transferring a small segment of the telocentric chromosome containing the leaf rust resistance to a wheat chromosome were studied. Dry seed of several plant selections of 82 a 1-2-6-2 were irradiated with the source of fast neutrons at Oak Ridge National Laboratory. The dosage delivered was 72 minutes at a total flux of 7.7×10^8 N/ cm.^{2}/ sec. This treatment proved very effective in inducing translocations, as judged from the number of multivalent associations found in both N_1 and N_2. In comparison, no multivalent configurations were found in control (non-irradiated) plants. The N_2 was inoculated with an oil suspension of leaf rust spores. One resistant plant (4-35) with 42 chromosomes was found in this second generation. Its resistance was attributed to a possible transfer of the factor(s) for leaf rust resistance from the telocentric "rye" chromosomes to the wheat complement. The possibility of the telocentric chromosomes being different in some cases is suggested. Leaf rust would depend not only on the presence of one or two extra telocentric chromosomes, but also on the kind present.

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INTRODUCTION

The transfer of desirable characteristics such as disease resistance to wheat through intergeneric and interspecific crosses, has been of particular interest to cytogeneticists, especially since genome homologies were demonstrated.

In 1930 McFadden suggested the possibility of transferring desirable characters from emmer, durum, einkorn, rye, Aegilops and other related grasses to common wheat. Such transfers are very difficult to execute by conventional hybridization methods, as emphasized by Riley (1960) and Unrau (1960) and different approaches are required.

New possibilities in the intergeneric and interspecific transfer of characters to wheat species have become available through the use of alien addition and substitution races. The process, by which a nonhomologous chromosome is substituted into the genome of another species is known as alien-chromosome substitution, whereas the addition of an alien chromosome to the normal complement of the host species is the alternative. Several investigators including Florell (1931), O'Mara (1940, 1947, 1951, 1953), Sears (1953), Hyde (1953) Chapman and Riley (1955) and Jenkins (1958) have contributed to the development of this approach.

Whole chromosome substitutions or additions have usually been found to be unsatisfactory from a practical point of view, apparently because so much genetic material is introduced from the donor species that undesirable characters are included with desirable factors. Genetic recombination of this desired character with the desirable genes cannot be effected due to the lack of chromosome pairing. In the transfer of leaf rust resistance from *Aegilops umbellulata* to common wheat and stem rust resistance from *Agropyron elongatum* to common wheat, Sears (1956) and Elliott (1957) have demonstrated a method of introducing small segments of chromosomes from alien species, by irradiation techniques.

For several years a wheat-rye selection 82 a 1-2-6-2, originating at Cornell University, has demonstrated a high degree of resistance to leaf rust, *Puccinia rubigo-vera tritici* (Eriks) Carl.,at East Lansing, Michigan. Sebesta (1958) found that this selection, a wheat-rye derivative, has two extra telocentric chromosomes, presumably derived from the rye parent. He further demonstrated that the telocentric chromosomes carry a factor(s) for leaf rust resistance. Research at East Lansing has demonstrated that this selection loses its leaf rust resistance due to the instability

of these two extra telocentric chromosomes. It would be highly desirable to transfer the segment(s) containing the factor(s) for leaf rust resistance to the wheat complement to stabilize the character. Furthermore the quality of this selection may be enhanced by the loss of the extra rye chromatin linked with the resistance factor(s).

One of the objectives in the present work was to study the relationship between the extra chromosomes and the wheat complement, through the analysis of the F_1 of the cross between the 44-chromosome material and Cornell 595, a wheat variety with 42 chromosomes. Similarly the study of the homology between the extra telocentric and the rye complement was an objective of this study, by an analysis of the F_1 of the cross between selection 82 a 1-2-6-2 and Rosen rye, one of the original parents of this selection.

Another objective was to study methods of transferring the factor(s) for leaf rust resistance from the extra telocentric chromosomes in selection 82 a 1-2-6-2 to the wheat complement, through possible induction of translocations by means of neutron irradiation.

REVIEW OF LITERATURE

The cytology of wheat and related species included in the Sub-Tribe Triticinae was reviewed by Sears (1948). Genome homologies are given for the genera Triticum, Aegilops, Agropyron, Secale and Haynaldia.

Sebesta (1958) reviewed the cytology of wheat, rye, and the F_1 between them, as well as the constant intermediate hybrids, and hybrids of later generations. Certain aspects of wheat and rye cytology pertinent to this thesis, including meiotic behavior in the F_1 hybrids and haploids in both wheat and rye will be reviewed.

Haploids in wheat and rye.

Since Gaines and Aase (1926) described a haploid plant in Triticum compactum, a number of such plants have been found, not only in Triticum compactum but also in T. monococcum and T. vulgare. Sebesta (1958) reviewed the early work with haploid wheat plants. McGinnis and Unrau (1960) found that in a haploid plant of Triticum vulgare, 57.6 % of the metaphase cells showed one to three bivalents. Homologous pairing was assumed if a chiasma was actually observed or if the appearance of a disjoining pair indicated the previous formation of a chiasma. Person (1955)

made an analytical study of chromosome behavior in a haploid wheat plant. He describes two types of chromosome associations: 1. Side-by-side (s-s) in twos ("secondary association," "secondary pairing"); 2. End-to-end association (e-e) in groups of two or more. He showed that the proportion of total univalents forming e-e associations was relatively unaffected by changes in the number of bivalents and concluded that homology was not a factor in the formation of e-e- associations. Instead, homology was considered a determining factor in s-s associations, which divide at the metaphase plate.

Riley and Chapman (1957) also dealt with the problem of homologies in pairing in haploids and polyhaploids of Aegilops and Triticum. They concluded that the presence of chiasmata associations in this type of material may be accepted as evidence of some degree of segmental correspondence, and accept Person's view that the end-to-end associations do not depend upon homology. That this type of association may be due to the fusion of heterochromatin, was suggested by Kostoff (1938). Four kinds of haploids are mentioned by Riley (1958) in Triticum vulgare var.

Holdfast, as derived from lines used in obtaining additions and substitutions of single pairs of rye chromosomes.

Haploid plants have also been found in rye. Muntzing (1937), in temperature induced doubling studies, found a haploid rye plant among the progeny from five spikes exposed to -3°C for 30 minutes after cross pollination. Nordenskiold (1939) also found and described the cytology of a haploid rye plant. Generally no associations were found between the seven univalents, but in some cases, there was some bivalent formation. The bivalents were rod-shaped and the two chromosomes were joined by a chiasma. Levan (1942) described the meiotic process in a haploid rye plant, finding mostly bivalents and trivalents. Quadrivalents were found in 4 out of 2628 cells. The results were interpreted as a consequence of non-homologous pairing.

Wheat x rye hybrids.

Since Wilson (1876) described the first wheat x rye hybrids, a number of workers have been interested in this type of cross. A review of the early work is given by Vakar and Krot (1934), Aase (1935 and 1946) and Sebesta (1958).

Thompson (1927), studying the cytology of hybrids between wheat and rye, found that although occasionally 1 or 2, and more rarely 3, bivalents were formed, the usual feature was the complete absence of chromosome pairing.

Longley and Sando (1930) found no bivalent formation in a hybrid between Triticum vulgare and Secale cereale. Kagawa and Chizaki (1934) made a cytological analysis of hybrids between Triticum compactum and T. spelta in crosses with Secale cereale. In most cases they observed 28 univalent chromosomes at metaphase, but often found from 1-3 bivalents, formed by two chromosomes of similar size, paired end to end.

Ledingham and Thompson (1938) studied the cytology of non-amphidiploid derivatives of wheat-rye hybrids. In the F_1 they found occasional bivalents, of the "loose" type, which separated very slowly. In the F_2 they found 3 groups of material, with 28, 42 and 49 chromosomes respectively. In the 28-chromosome group a few bivalents of the end-to-end type were formed. Secondary pairing of bivalents was also observed, and it was explained as due in some cases to duplication of wheat chromosomes. They found neither evidence that the individual rye chromosomes could be "added

permanently to the wheat complement, or substituted for wheat chromosomes, nor that by crossing over or translocation composite wheat-rye chromosomes can be produced or at least cause a visible effect."

Chin (1946) crossed a variety of Chinese wheat and rye, finding at meiosis from 0-3 bivalents, although most of the 28 univalents remained unpaired. He suggests that the bivalent formation may be due to pairing of intrageneric genomes. Nakajima (1952) makes an analysis of intergeneric F_1 hybrids between _Triticum_ and _Secale_. He used _Triticum durum_, _T. spelta_ and _T. vulgare_, in crosses with _Secale cereale_. One plant of the cross _Triticum spelta_ x _Secale cereale_ was studied and he found at first metaphase from 0-4 bivalents and 20-28 univalents. Some trivalents but no tetravalents were found. In the cross _Triticum vulgare_ x _Secale cereale_ bivalents were observed at M I, in a range of 0-4. In rare occasions ring bivalents were observed, and formation of interstitial chiasma was infrequent. He thought that the bivalents were due to the autosyndesis between the genomes of _Triticum_. Of the 11 bivalents found in this last hybrid, 7 are explained as the result of autosyndesis between A and B genomes of _Triticum_, 3 are formed from the conjugation of

R genome of *Secale* and 1 bivalent by the conjugation of chromosomes in the D genome of *Triticum*. This means that there is no syndesis between genomes of *Triticum* and *Secale*. Nakajima (1953) in crosses between *Triticum pyramidale* (28 chromosomes) and *Secale cereale* found from 0-4 bivalents at M I., and from 21-13 univalents. The occurrence of bivalents is explained as the product of autosyndesis between the AB genomes of *Triticum pyramidale*.

Behavior of telocentrics and iso-chromosomes.

The origin and behavior of telocentrics and iso-chromosomes has been reviewed by Darlington (1939, 1940) and by Sears (1944, 1952a, b). Only a few pertinent citations will be made here.

According to Darlington (1939) telocentrics originate by misdivision of the centromere in univalent chromosomes. He points out several types of misdivision leading to the formation of telocentrics, that is, of chromosomes with a terminal centromere. These telocentrics are functional, as they can divide normally at both first and second anaphase, but they are unstable. The telocentric may give origin to iso-chromosomes with identical arms, by a process described by Darlington (1940).

Sears (1944) found both telocentric and iso-chromosomes in the offspring of trisomic and monosomic plants of Triticum vulgare. He points out that the frequency of telocentrics and iso-chromosomes among the offspring of certain monosomics is rather high, and might result from pollen competition rather than from a high frequency of misdivision. In another paper (1952a) he describes the manner of origin of telocentrics and iso-chromosomes in wheat, and states that they are due to a number of types of misdivision of univalent chromosomes at the first division of meiosis. At the second division some iso-chromosomes might give rise to telocentrics by a new misdivision, and new telocentrics may be found by misdivision of chromosomes that divided normally during the first division. The behavior of these chromosomes was also analyzed by Sears (1952b) using iso-chromosomes and telocentrics for the long arm of chromosome IX of Chinese Spring wheat. He found that telocentric chromosomes behave normally when they are present as a pair, or when a normal homologue is present. There might be post-meiotic conversion of a telocentric into an iso-chromosome and also somatic loss of both telocentrics and iso-chromosomes.

Transfer of characters from related species and genera to hexaploid wheat.

As the chromosome homologies between different species of Triticum and related species were studied and clarified, the possibility of the transfer of desirable characteristics from those species to hexaploid wheats has been of special interest in wheat breeding.

Hayes (1920) found that by growing large populations it was possible to obtain stem rust resistance in crosses between *Triticum vulgare* and both *Triticum durum* and *Triticum dicoccum*. Harrington and Smith (1929) also emphasized the need for large segregating populations in order to obtain 42-chromosome hybrids resistant to stem rust, in the early segregating generations of a *T. dicoccum* x *T. vulgare* cross. McFadden (1930) using hybridization was successful in transferring desirable characteristics from Yaroslav emmer to common wheat.

In the search for new sources of desirable characteristics, crosses between species of *Triticum* and related genera have been made. As the common methods of hybridization are effective only when there is pairing of the parental chromosomes, the synthesis of alien addition races has been

suggested as a method of securing the addition of foreign germplasm to the wheat genome in a stable manner. Florell (1931a, b) found cytological evidence for the possibility of adding a pair of rye chromosomes to the wheat complement in a constant combination, through hybridization between wheat and rye and backcrossing to the wheat parent. O'Mara (1940) discusses the possibility of combining characters of two species or genera by the addition of one or more chromosomes from one species to the diploid complement of the other. The proposed method involves the production of an amphidiploid between two species or genera, A and B, and subsequent backcrossing to one of the parents. By this method he secured plants which had the normal wheat complement plus a disomic rye chromosome.

As Haynaldia shows a marked resistance to stem and leaf rust of wheat, Hyde (1953) was interested in obtaining the addition of Haynaldia chromosomes to common wheat. He accomplished this by the use of an amphidiploid (AABBVV) between Triticum dicoccoides and Haynaldia villosa as a bridge for the cross with Triticum aestivum (= Triticum vulgare). A series of backcrosses to Triticum aestivum and selfing of different types of progeny, gave origin to

"addition monosomics" and "alien addition races." Sears (1953) used the same bridge technique for adding the chromosomes of Haynaldia villosa (L) Schur. to common wheat, a technique that he also used to add individual chromosomes to wheat from Aegilops umbellulata.

Chapman and Riley (1955) found in the progeny of an amphidiploid between Triticum vulgare var. Holdfast and Secale cereale var. King II, a 28-chromosome plant that arose parthenogenetically; this plant was crossed with T. vulgare and a single F_1 plant was obtained, with 49 chromosomes (21 II from wheat and 7 I from rye). Selfing of this gave origin to a 44-chromosome plant, with two identical rye chromosomes. Evans and Jenkins (1960) described the addition of individual "Dakold" fall rye chromosomes to "Kharkov" winter wheat and their subsequent identification. Bhattacharyya et al. (1961) made a detailed karyotypic analysis of the Dakold rye chromosome additions to Kharkov winter wheat and identified these addition lines in relation to the wheat karyotype.

As obtaining addition and substitution lines does not assure that genetic recombination will occur between the alien material and the wheat chromosomes, new methods have

been used to secure the transfer of desirable characteristics, mainly disease resistance, from the foreign material to the wheat complement. Sears (1956), after using the amphiploid of *Triticum dicoccoides* x *Aegilops umbellulata* as a bridge for combining the genomes of this last species and *Triticum aestivum*, obtained plants with the whole wheat complement and one *Aegilops* iso-chromosome. X-rays were used to irradiate these plants and both reciprocal and intercalary translocations were obtained. Plants homozygous for the small intercalary translocation were secured and they carried the factor for leaf rust resistance. Elliott (1957) reported the transfer of resistance to stem rust from *Agropyron elongatum* to hexaploid wheat, after the irradiation of a 49-chromosome plant obtained by crossing a resistant octoploid plant (derived from the original cross between *Agropyron elongatum* and common wheat) and the susceptible variety Idaed.

Another method for obtaining genetic recombination is proposed by Riley and Chapman (1958) who suggest that "intergeneric pairing may well take place in the absence of the HH chromosome and alien genes could then be introduced into the wheat chromosomes by normal recombination." The H

chromosome (in the variety Holdfast) is supposed to control the normal diploid behavior in hexaploid wheats.

Previous work with selection 82 a 1-2-6-2 and related lines.

Since the original cross was made at Cornell University, different lines of wheat-rye hybrids have been obtained possessing a high degree of resistance to leaf rust.

Originally the wheat variety Honor, susceptible to leaf rust, was crossed with the resistant rye variety Rosen, as seen in the pedigree in Fig. 1. The F_1 was backcrossed to Honor and selection 369 a 2-2-44 was obtained in the F_4 generation. This selection was crossed with Yorkwin in 1935 and a single disease resistant plant, selection 779 b 10-8-6 was obtained. The progeny of a cross made in 1942 between Selection 779 b 10-8-6 and Cornell 595, gave origin to selections 82 a 1-2-4-7 and 82 a 1-2-6-2 (Sebesta, 1958). Both of these lines have been selfed more than 15 generations.

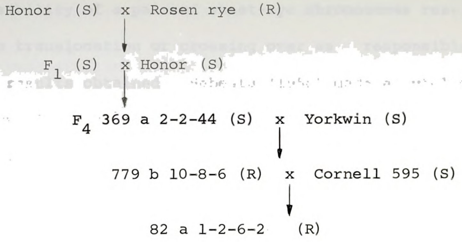

Figure 1. Pedigree of leaf rust resistant selection 82 a 1-2-6-2 used in this study. (R) = resistant and (S) = susceptible leaf rust reactions.

Jensen and Kent (1952), trying to explain the disease resistance (leaf rust and mildew) of selection 82 a 1-2-4-7 proposed "the transfer, in the original hybrid, of one or more segments of rye chromatin by crossing over or reciprocal translocation, followed by a novel recombination of genes in the final hybrid." Jones and Jensen (1954) made crosses between Alaskan and each one of three wheat-rye selections derived from the head selections of 779 b 10-8-6. They concluded that the hairy neck character was inherited as a simple Mendelian character, and suggested the

possibility of a pair of wheat-rye chromosomes resulting from translocation or crossing over as responsible for the results obtained. Sebesta (1958) made a cytological study of the selections 82 a 1-2-4-7 and 82 a 1-2-6-2, discovering that completely leaf rust resistant, moderately resistant and completely susceptible plants of these selections had 44, 43 and 42 chromosomes, respectively. In the case of the 44 and 43-chromosome plants, the extra chromosomes were telocentric, with the exception of their being iso-chromosomes in the case of some completely resistant plants with 43 chromosomes. In the progeny from moderately resistant plants with 43 chromosomes, he found resistant, moderately resistant and susceptible plants. The latter ones had lost the extra telocentric chromosomes. Sebesta concluded that the extra chromosomes were apparently the same in both selections 82 a 1-2-6-2 and 82 a 1-2-4-7, and that they were the carriers for the factor or factors, for leaf rust resistance.

MATERIALS AND METHODS

Several plant selections of 82 a 1-2-6-2 were used in the course of this work. Approximately 200 seeds from each one of them were irradiated with fast neutrons at the Oak Ridge, Tennessee, Laboratory of the Atomic Energy Commission, through the courtesy of Dr. T. S. Osborne. The dosage delivered was 72 minutes of fast neutrons at a total flux of 7.7 $x\ 10^{8}$ $N/cm.^{2}/sec.$ A tabulation of technical details concerning the source of irradiation is given by Heinz (1961). Seed of the irradiated material and the non-irradiated (control) was space planted in the field in September, 1960, at a distance of one foot between rows and plants. Samples for meiotic studies were taken in the summer of 1961 and fixed in Newcomer's solution.

Whole spikes were collected at the early boot stage, three to four hours after sunrise and stored in a refrigerator until further use.

Spikes of the N_1 and control populations were harvested in late summer of 1961. Four or five seeds of each spike were planted in small peat pots in early September of 1961 and vernalized for 7-8 weeks in a cold room at 38°F., and

transplanted to 4-inch pots in the greenhouse. Meiotic samples were taken in December 1961, and fixed as whole spikes in Newcomer's solution. Samples were taken after three to four hours of illumination each day, and stored in the refrigerator until needed. After heading, the N_2 population was inoculated with leaf rust spores from a field collection that had been increased on the susceptible wheat variety Little Club. The method of inoculation was described by Rowell and Olien (1957).

Plants of susceptible varieties (Little Club, Genesee) were included among N_2 plants to serve as a control for the inoculation procedure.

In the summer of 1961 flowers of non-irradiated material of selection 82 a 1-2-6-2 were emasculated and pollinated with pollen from Rosen rye. Out of 693 flowers pollinated, 15 hybrid seeds were obtained. These seeds were planted in small peat pots, vernalized for two months in a cold room at 38° F and then transplanted to 4 inch pots and grown under greenhouse or growth chamber conditions. Meiotic samples were taken after three to four hours of illumination each day, in the growth chamber, and fixed in Newcomer's until examined.

Crosses between control plants with 44 chromosomes and Cornell 595, a wheat variety with 42 chromosomes were also made and handled in the same way as the wheat-rye hybrids.

From anthers of all the material fixed in Newcomer's (control population, N_1, N_2, 82 a 1-2-6-2 x rye F_1 and 82 a 1-2-6-2 x Cornell 595 F_1), temporary slides were prepared with propionic carmine and meiotic associations analyzed. Photographs were taken of suitable material. Root tips were secured from some of the hybrid 82 a 1-2-6-2 x rye plants and pretreated with hydroxyquinoline for 24 hours (Markarian, 1957). They were then fixed in 3:1 (absolute alcohol: glacial acetic acid) and stained in Feulgen. The stain was prepared following the modification suggested by Wilson (personal communication):

1 gram of basic fuchsin was dissolved by pouring over it 200 c.c. of boiling distilled water; this was shaken and cooled to 50° C. and filtered; to the filtrate 30 c.c. of 1/N HCl were added and then 3 grams of potassium metabisulphite. The solution was allowed to bleach for 24 hours in the dark, in a stoppered bottle, and then 0.5 grs. of Norit-A were added and after some time it was filtered again.

Seed from both resistant and susceptible N_2 plants were germinated in moist blotting paper in Petri dishes in a germinator under controlled light and temperature conditions (8 hours light, 16 darkness at 30 and 20° C of temperature respectively). When primary root tips were about 4-6 mm. long, they were excised and pretreated with actidione for 12 hours (Wilson, 1950; Bowen and Wilson, 1954) or with hydroxyquinoline for 24 hours. Both pretreatments gave satisfactory results. The root tips were then fixed in 3:1 absolute alcohol: glacial acetic acid for a minimum of 12 hours and were stained in Feulgen, after hydrolysis for 10-12 minutes in 1/N HCl at 60° C.

Figure 2. Spikes from selection 82 a 1-2-6-2 on the left and from a 82 a 1-2-6-2 x rye hybrid plant on the right.

RESULTS

Observations on Rosen rye and Cornell 595.

No detailed analyses were made for meiotic associations on Rosen rye or Cornell 595, but meiotic behavior was observed in some plants of both species. Rosen rye has 14 somatic chromosomes. Meiosis is normal, seven bivalents always being found at Metaphase I, and the chromosome separation at Anaphase I being regular. The 42 chromosomes of Cornell 595 form 21 bivalents at Metaphase I, the chromosome distribution at Anaphase I also being normal.

Observations in plants of selection 82 a 1-2-6-2.

Plants of the control population of selection 82 a 1-2-6-2 were found to have 43 or 44 chromosomes, the extra chromosomes being telocentric. Of 20 plants that were examined, 6 had 43 and 14 had 44 chromosomes. In 238 cells analyzed to determine meiotic associations, only univalents and bivalents were found. No multivalent configurations were present in the cells studied. The wheat chromosomes almost always formed 21 pairs at Metaphase I, with very few exceptions where more than two univalents were found. The telocentric chromosomes (1 or 2) were usually found off the

metaphase plate, unpaired in the case of two extra telocentric being present, but a few plants showed a tendency for them to form pairs, in which case 22 pairs were found at M I. Sometimes one pair of telocentrics showing an end-to-end association was found slightly off the equatorial plate. At early anaphase I the distribution of chromosomes was normal, almost always being 22-21 or 22-22, in 43 or 44-chromosomes plants, respectively.

At advanced anaphase or telophase I the univalents, already divided or dividing, showed a tendency to lag. The second division was not observed in detail, but the presence of micronuclei at the tetrad stage indicated that the lagging chromosomes sometimes were not included in the daughter nuclei.

82 a 1-2-6-2 x rye hybrids.

Of 693 flowers of control plants of 82 a 1-2-6-2 pollinated with Rosen rye (2n = 14), a total of 15 hybrid seeds were obtained, all with characteristic shrunken endosperms. Ten of them germinated, were wheat-like in appearance and grew normally, showing the hairy neck characteristic and being completely sterile. The spikes of these

hybrids were intermediate in length between wheat and rye, with awns of intermediate length, in contrast with the spikes of plants of selection 82 a 1-2-6-2, which were awnless. Figure 2 shows the differences in spike length, presence of awns and general morphology of spikes of 82 a 1-2-6-2 and of one of the hybrids. Some of these hybrids had 28 and some 29 chromosomes. Figure 3 shows the 29 somatic chromosomes from plant No. 9. Meiotic associations were analyzed for several of the hybrid plants. In most of the cells the univalent chromosomes did not pair at prophase and were scattered throughout the cell. No regular metaphase plates were observed, except in one case where the chromosomes were well aligned at the equatorial plate, as seen in Figure 7.

At "anaphase" of the first division the univalents went to either pole, frequently in about the same numbers. When associations of two chromosomes forming true chiasmata were observed, they frequently remained at the equatorial region after the rest of the univalents had moved to the poles. When the members of these associations began separation, they were considerably stretched, forming a long

chromatin thread until separated. Usually there was no division of the univalents during the first division of meiosis.

In rare cases chromosome bridges were observed at telophase I. Chromosome behavior at the second division was not observed in detail, but some irregularities were found. Formation of micronuclei was particularly notable, their number per tetrad ranging from 0-9. Although most tetrads were normal in appearance "tetrads" with more than four cells were commonly observed.

Out of 941 cells, from 4 different plants observed, 210 (22.32 %) had bivalent or multivalent associations. The total number of associations recorded was 286, distributed as shown in Table I.

Table I. Distribution of bivalent and multivalent associations in 82 a 1-2-6-2 x Rosen rye.

Type of association	Total number	%	Mean No. per cell
II	235	82.17	.48
III	48	16.78	.098
IV	2	.70	.004
V	1	.35	.002

Of the 235 bivalents found, 54 (22.98 %) had chiasmata formation and 181 (77.02 %) were of the end-to-end type. Of the 54 "true" bivalents formed (with chiasmata) 25 were heteromorphic, having one arm shorter than the other.

Observations in 82 a 1-2-6-2 x Cornell 595.

Ten plants from this type of cross were studied cytologically and meiotic associations determined. All of them had 43 chromosomes, one univalent always being found at M I. Anaphase distribution was normal and the telocentric chromosome was found lagging at advanced anaphase and in telophase I. Of 138 cells examined for meiotic associations at diakinesis or M I, 18 multivalent configurations (13 %) were found, 16 being open tetravalents, one a ring of four chromosomes and one a trivalent. The mean number of tetravalent associations per cell was 0.1232, the mean frequency for the trivalent being 0.007 per cell.

Observations in N_1 plants.

Out of 493 cells observed in 30 plants of the N_1, it was found that 277 (56.19 %) had trivalents or associations of higher order. The total number of multivalent associations was 411, distributed as shown in Table II.

Table II. Distribution of multivalent associations in N_1 plants.

Type of association	Total number	%	Mean No. per cell
III	14	3.41	.028
IV	350	85.16	.71
VI	23	5.60	.047
VIII	14	3.41	.028
X	6	1.46	.012
XII	4	.97	.008

Presence of fragments was observed in 23 cells from 11 plants. A total of 28 fragments was observed, the average number of fragments per cell being .057.

The anaphase I was rather regular and normal, the chromosomes being distributed in about the same numbers, but in some cases there was some chromosome stickiness. This was probably responsible for the tendency of some associations to move to a given pole as a group.

Observations in the second generation (N_2).

Of 941 cells from 70 plants of the second generation which were analyzed for meiotic associations, 396 (42.08 %)

had associations of three or more chromosomes. The total number of multivalents scored was 480 and their distribution is given in Table III.

Table III. Distribution of multivalent associations in N_2 plants.

Type of association	Total number	%	Mean No. per cell
III	27	5.63	.029
IV	407	84.78	.43
VI	14	2.92	.015
VIII	23	4.79	.024
X	6	1.25	.006
XII	2	0.42	.002
XVI	1	0.21	.001

Meiosis in the N_2 was normal and the chromosome distribution at A I was regular, although univalent chromosomes and fragments were found lagging at late anaphase and telophase I. Fragments were found in 53 cells from 18 plants. Up to 4 fragments per cell were found in one single cell. A total of 67 fragments were found in the 941 cells examined, this giving an average number of .07 fragments per

cell. About 10 % sterility was found in plants of this second generation at harvesttime.

Results of the inoculation of N_2 plants with leaf rust.

Whereas all of the plants of susceptible varieties (Little Club, Genesee) used as controls for the inoculation procedure developed leaf rust, only 39 plants, of a population of approximately 2,200 plants of the second generation developed leaf rust pustules. Some plants were only slightly affected, but several presented a heavy infection. All of the plants with pustules on the leaves were classified as susceptible, some of them being classified as "very susceptible."

Due to sterility of some plants and to environmental conditions (mostly cloudy days) when meiotic samples were taken, only a few of the susceptible plants were useful in the determination of chromosome number and meiotic behavior. Germinating seeds from some were used to ascertain chromosome numbers in the N_3. Meiosis was observed in pollen mother-cells from plant 5-121, classified as very susceptible. This plant was found to possess 43 chromosomes, one being a univalent. Two multivalent associations (one chain

of 4 and one chain of 10) were observed among others. Plants 2-391, 4-29, 4-40 and 5-177, the first classified as susceptible and the last three as slightly susceptible, also showed 43 chromosomes.

Of 69 resistant plants analyzed for meiotic behavior in N_2, one was found to have 42 chromosomes (plant 4-35), 22 had 43 chromosomes and 46 had 44 chromosomes. Meiotic behavior in these plants was summarized before, as they were the basis for the analysis of the multivalent associations in the second generation. Chromosome number was determined at early M I or at A I.

As stated before, a 42-chromosome resistant plant was found in this N_2 population. Chromosome number for this plant was first determined from N_3 germinating seeds, and this number was confirmed upon cytological examination of pollen mother-cells from one spike of the second generation. In most cells two univalents were found, but up to seven were observed in one single cell, which indicates a rather high degree of asynapsis between some chromosomes. As usual, from one to three open bivalents were observed, but in one cell five were found. Chains and rings of four chromosomes were observed, this being an indication of

segmental interchanges in this plant. Figures 21 and 22 show some aspects of the meiotic process in this leaf rust resistant, 42-chromosome plant, in the N_2.

Observations in mitosis in N_3 seeds.

Root tips of N_3 germinating seeds were examined for chromosome number in both resistant and susceptible plants. In seeds from 15 susceptible plants 3 had 42, 7 had 43 and 5 had 44 chromosomes. Mitotic chromosomes of some of these plants are shown in Figure 24 through Figure 26.

In seeds from 92 resistant plants, 2 had 42, 18 had 43 and 72 had 44 chromosomes. Mitotic chromosomes of one of these resistant plants are shown in Figure 27. Of the 42-chromosome plants found, this chromosome number could be confirmed in the N_2 meiotic sample of one plant (4-35).

Root tip cells from germinating seed of some of the control plants, 82 a 1-2-6-2,were also examined, which were inoculated at the same time as N_2 plants. One susceptible plant was found in this group and the chromosome number was 44. Chromosome number from eleven of these control resistant plants was also 44.

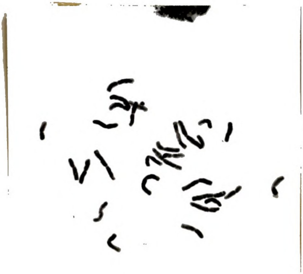

Fig. 3. Somatic chromosomes in a root tip cell from the 82 a-1-2-6-2 x rye plant No. 9. 750X

Fig. 4. Prophase I showing 21 I, 2 II and 1 III in a microsporocyte from hybrid plant No. 3. 970X

Fig. 5. Prophase I showing 24 I, and 2 II in a microsporocyte from hybrid plant No. 3. 970X

Fig. 6. Prophase I showing 23 I, 1 II and 1 III in a microsporocyte from hybrid plant No. 3. 970X

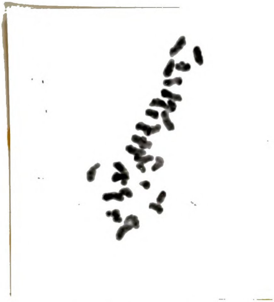

Fig. 7. Organized metaphase plate showing 28 chromosomes in hybrid plant No. 10. 970X

Fig. 8. Prophase I showing 27 I and 1 II in a microsporocyte from hybrid plant No. 2. 970X

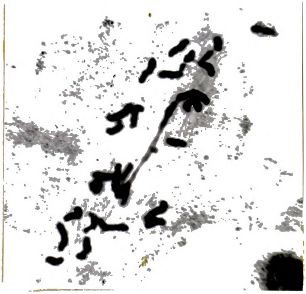

Fig. 9. Prophase I showing 25 I and 1 chain of 4 chromosomes in a microsporocyte from hybrid plant No. 2. 970X

Fig. 10. Prophase I showing 20 I, 2 II and 1 association of 5 chromosomes in microsporocyte from hybrid plant No. 5. 970X

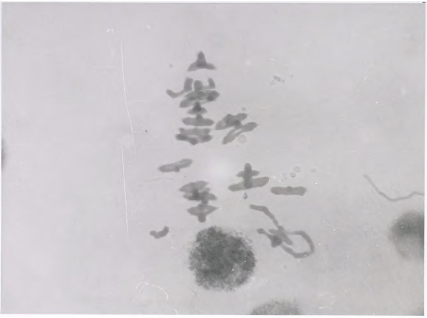

Fig. 11. Metaphase I showing 1 I, 19 II and 1 IV in a microsporocyte from 82 a 1-2-6-2 x Cornell 595. 1455X

Fig. 12. Metaphase I showing 20 II and 1 IV in a microsporocyte from N_1 plant 2-15-1. 970X

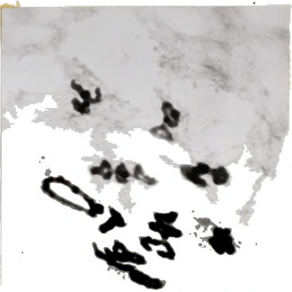

Fig. 13. Metaphase I showing 20 II and 1 ring of 4 in N_1 plant 1-6-3. 970X

Fig. 14. Metaphase I showing 1 fragment, 1 I, 18 II, 1 chain of 6. N_1 plant 2-15-1. 970X

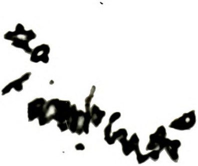

Fig. 15. Metaphase I showing 1 fragment, 14 II, 1 III and 1 chain of 12. N_1 plant 1-17-3. 970X

Fig. 16. Metaphase I showing 1 I, 10 II, 1 III and 2 chains of 10 chromosomes in a microsporocyte from N_1 plant 2-24-7. 1455X

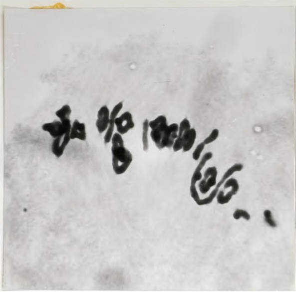

Fig. 17. Metaphase I showing 2 I, 17 II, 1 ring of 4 and 1 chain of 4 in a microsporocyte from N_2 plant 3-123. 970X

Fig. 18. Metaphase I showing 2 I, 18 II and 1 chain of 6. N_2 plant 5-114. 970X

Fig. 19. Metaphase I showing 18 II, and 1 chain of 8 chromosomes. N_2 plant 3-101. 970X

Fig. 20. Metaphase I showing 14 II and 1 chain of 16 chromosomes. N_2 plant 3-205. 970X

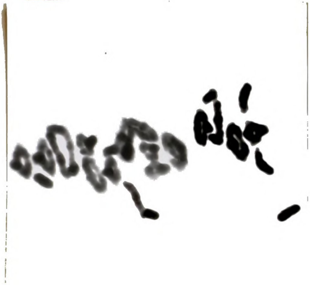

Fig. 21. Metaphase I showing 2 I, 15 II, 1 IV and 1 chain of 6 in N_2 resistant plant 4-35. 970X

Fig. 22. 42 chromosomes (1 divided) at Anaphase I in N_2 resistant plant 4-35. 750X

Fig. 23. 42 somatic chromosomes in a root tip cell from a germinating seed from plant 4-35. 750X

Fig. 24. 42 chromosomes in a root tip cell from a germinating seed from N_2 susceptible plant 4-70. 750X

Fig. 25. 43 chromosomes in a root tip cell from a germinating seed from N_2 susceptible plant 5-125. 750X

Fig. 26. 44 chromosomes in a root tip cell from a germinating seed from N_2 susceptible plant 5-126. 750X

Fig. 27. 44 chromosomes in a root tip cell from a germinating seed from N_2 resistant plant 2-259. 1455X

Fig. 28. Double "satellited" iso-chromosome in a root tip cell from a germinating seed from N_2 resistant plant 5-180. 1455X

DISCUSSION

Cytological observations in Rosen rye and Cornell 595 wheat indicated that structural homozygosity is a common feature in these two varieties. Only bivalents are found at Metaphase I and the meiotic process is completely normal. No multivalent associations, indicating any degree of structural heterozygosity were found in this material.

Observations in control plants of selection 82 a 1-2-6-2 indicate that through the process of crossing, backcrossing, selfing and selection, after the original cross Honor wheat x Rosen rye was made, it has reached a state of structural homozygosity. Only univalents, usually 1 or 2, and 21 bivalents were found at meiosis. These results agreed with those found by Sebesta (1958) in several wheat-rye selections derived from the same original cross. The extra univalents are usually telocentric and presumably they are derived from the original rye parent. In the course of the evolution of this wheat-rye derivative some interchange between the rye and the wheat chromatin may have taken place. This was suggested by Jensen and Kent (1952) and is evidenced by the presence of the hairy neck character

in plants with 42 chromosomes. Therefore the telocentric chromosomes might have some structural heterozygosity and will be referred to as "rye" chromosomes. Even if some chromosome rearrangements have taken place in this material since the cross was made, they now seem to be in a state of structural homozygosity. No special configurations indicating some degree of structural heterozygosity are seen at meiosis in plants of the non-irradiated (control) population of this leaf-rust resistant material. The wheat chromosomes always form 21 pairs at Metaphase I with very rare instances when some asypnasis occurs and several univalents (besides the extra telocentric "rye" chromosomes) are present. Even in the case of the extra chromosomes, their lack of pairing is not unexpected as they are operating in a foreign genome and under this condition may lack their characteristic pairing ability (O'Mara, 1940).

Although the loss of these telocentrics should occur frequently (Darlington, 1940; Sears, 1952a) they have shown some stability in the selection 82 a 1-2-6-2 and they are usually transmitted to the progeny of plants possessing them. This persistence might be due to some

selective advantage, other than leaf rust resistance, which is not critical for wheat survival. The extra telocentric "rye" chromosome may have received a small wheat segment carrying some factor necessary to the normal functioning of the wheat complement.

In an attempt to establish any homology between the telocentric chromosomes and both the wheat and the rye complements, crosses between 82 a 1-2-6-2 control plants and Rosen rye, and also Cornell 595 were made. The results of the cross to Rosen rye established a relatively high number of cells (22.32 %) with bivalent and multivalent associations. Bivalent formation was especially notable, 82.17 % of the total number of associations found. This could be taken as an indication of a degree of homology between the extra "rye" chromosome (in 29 chromosome hybrids) and one of the 7 normal rye chromosomes, if always 27 I and one heteromorphic bivalent were formed. However this was not the case, as not only bivalents but other types of associations were observed (see Table I). If the extra "rye" chromosome was involved in bivalent association, some autosyndetic pairing between the wheat or the rye chromosomes or some pairing between normal wheat

and rye chromosomes occurred. Allosyndetic pairing is not likely in wheat-rye hybrids, as established by many investigators. Although hybrids between wheat and rye are made with relative ease, no homology between wheat and rye chromosomes have been demonstrated, and it seems that normal pairing and recombination between them is almost impossible (Unrau, 1960) and all attempts to transfer desirable characteristics from Secale to Triticum by conventional methods have failed.

Some of the pairing found in 82 a 1-2-6-2 x Rosen rye may be attributed to autosyndesis. As only one bivalent per cell would be expected to form between the telocentric and one normal rye chromosome, and as many as three bivalents in the same cell were found, the two other associations have to be explained on the basis of autosyndetic pairing. Bivalent and multivalent associations have often been reported in haploid wheat and rye plants, as well as in 28 chromosome wheat-rye hybrids. As pointed out by Darlington (1937) in "haploid," "triploid" and hybrid derivatives from allopolyploid species, "chromosomes derived from the same parental gamete pair with one another," even if this pairing is not frequent in the polyploid parent.

So it is clear that autosyndesis may occur between homoeologous chromosomes that normally do not pair with each other in the normal polyploid. The absence of homologous chromosomes in the case of the haploids and in interspecific hybrids may promote "abnormal" pairing of homoeologous chromosomes. This autosyndetic type of pairing has been given as an explanation for the formation of bivalent and multivalent associations in wheat and in rye haploids and in wheat-rye hybrids by many investigators, including Chin (1946), Kihara and Nishiyama (1928, 1937), Levan (1942), Nakajima (1952, 1953), O'Mara (1953) and Thompson (1931). However, Sears and Okamoto (1958) point out that homoeologous chromosomes show little tendency to pair, as the bivalent frequency in haploids normally averages about one. The presence of heteromorphic bivalents would support the assumption of pairing between a telocentric chromosome and a normal rye chromosome. Out of 54 "true" bivalents (with chiasma formation) found, 25 were heteromorphic, with one arm shorter than the other. This may be taken as evidence for the homology between the telocentric and one normal rye chromosome. However, the possibility of a heteromorphic wheat bivalent, formed by intragenomic

pairing cannot be discarded as Love (1939) found a number of chromosome rearrangements, including the formation of heteromorphic bivalents, in advanced generations from vulgare-like derivatives of pentaploid wheat crosses.

The results obtained in the cross 82 a 1-2-6-2 x Cornell 595, where tetravalent associations, almost always of the "chain" type were found in 13 % of the cells studied, lead to the conclusion that some structural rearrangement has taken place in the wheat complement, at least in relation to Cornell 595, the last of the wheat ancestors in the pedigree of selection 82 a 1-2-6-2. Even in the case that no transfer of rye to wheat chromatin has become involved in the evolution of this selection, chromosome interchanges between Honor, Yorkwin, and Cornell 595, successive wheat parents of this wheat-rye derivative, might lead to multivalent configurations, as pointed out by Elliott (1958) and demonstrated by Love (1951), who found some multiple associations in plants of intervarietal wheat crosses. The three possible crosses among the varieties Honor, Yorkwin and Cornell 595 could be made and the respective F_1s studied for evidence of structural differences. These would be reflected in the formation of

multivalent associations at Metaphase I. But even in the case of structural homology between these varieties, as they exist today, chromosome rearrangements between them might have taken place, in the evolutionary process of 82 a 1-2-6-2 and this arrangement would be reflected, again, in multivalent configurations in the cross 82 a 1-2-6-2 x Cornell 595.

That some transfer of rye chromatin to the wheat has occurred, was first suggested by Jensen and Kent (1952), who proposed that leaf rust as well as mildew resistance of selection 779 b 10-8-6 (parent of 82 a 1-2-6-2) could have developed through the transfer of "one or more segments of rye chromatin by crossing over or reciprocal translocation, followed by a novel recombination of genes in the final hybrid." The fact that 42-chromosome derivatives of these wheat-rye selections show the hairy neck characteristic, normally carried in one rye chromosome (O'Mara, 1951; Riley and Chapman, 1958) supports this hypothesis that a segment of rye chromatin has been transferred to the wheat chromatin. However Sebesta (1958) demonstrated that leaf rust resistance was carried in the extra telocentric chromosomes and that there was no

association between leaf rust and the hairy neck characteristic. Although the possibility of the transfer of a small segment of wheat chromatin to the telocentric rye chromosome cannot be ruled out, the results obtained in the present work do not give clear evidence for this hypothesis. As pointed out before, the tetravalent associations found can be due to structural rearrangements within the wheat genome, modified by possible chromosome rearrangements between the three wheat parents of 82 a 1-2-6-2. Assuming that a small segment was translocated from the wheat chromatin to the "rye" telocentric, it would have been so small as to not have a competitive ability for pairing with the normal wheat chromosomes. No pairing between the extra univalent and the wheat chromosomes was observed in the hybrid plants 82 a 1-2-6-2 x Cornell 595. If a definite homology existed between the extra chromosome and those from wheat, trivalent formation would be expected, which occurred in only one case.

The results obtained from the cytological analysis of microsporocytes of the N_1 and N_2 generations, after neutron irradiation of 82 a 1-2-6-2, proves the effectiveness of this type of radiation to induce chromosome breaks and

rearrangements. The dosage delivered to dry seeds was effective in inducing translocations as judged by the number of multivalent associations found in the N_1 where, out of 493 cells studied, 56.19 % had associations of more than three chromosomes. Especially notable was the number of tetravalents formed, as 85.16 % of the multivalent associations were of this type, indicating that one interchange had taken place in most of the cases. In comparison, no multivalent configurations were found in 238 cells of control plants examined during meiosis. The presence of associations of 6 and 8 chromosomes in chains or rings, demonstrated that two or three interchanges had taken place, as indicated by Osborne and Elliott (1955). Associations of 10 and 12 chromosomes were also found although their formation was not too frequent (1.46 and 0.97 %). This high number of interchanges between chromosome segments in the irradiated population of 82 a 1-2-6-2 increased the probability of transferring the desirable portion of the telocentric "rye" chromosome carrying the gene(s) for leaf rust resistance to any of the wheat chromosomes.

It was observed that the N_1 had a decreased vigor in relation to the control population. This was perhaps due

to an overall effect of the reshuffling of chromosome segments, when some favorable linkage groups were broken. Some sterility was noted but the amount not calculated.

In the second generation 42.08 % of the cells analyzed presented trivalents or associations of higher order. This high a percentage of residual heterozygosity in the N_2 reflects the fact that many different translocations were induced by the neutron radiation. The percentage of tetravalents was highest, being 84.78 % of all associations found.

The results of inoculating the N_2 population with an oil suspension of leaf rust spores confirmed the high degree of resistance possessed by the wheat-rye selection 82 a 1-2-6-2. The resistance to leaf rust in this type of material was shown to be due to the presence of extra telocentric "rye" chromosomes by Sebesta (1958). Only five meiotic samples from susceptible N_2 plants were suitable for study. All of these plants had 43 chromosomes; one was classified as very susceptible, one as susceptible, and the other three as slightly susceptible (or moderately resistant). According to Sebesta the first two plants should have been moderately resistant. In this study some

43-chromosomes N_2 plants were found to be highly resistant to leaf rust. This observation does not agree with Sebesta's results for he found these plants were only moderately resistant. These 43-chromosome plants occurred in the N_2 population which had a high number of interchanges as seen above. Sebesta worked with non-irradiated material such as the control in this experiment. It appears that these plants are a product of chromosomal rearrangements. The results also indicate that the degree of expression of leaf rust in 43-chromosome plants might be affected by the environment. Although the environmental conditions under which the inoculation was done were kept as constant as possible, the possibility of slight changes in humidity, light, temperature, etc., cannot be ruled out. This might have influenced the degree of expression of leaf rust.

The possibility also exists that the telocentric chromosomes of plants with 43 and 44 chromosomes are not always the same. Not only the number of chromosomes but the kind of extra telocentric(s) carried by a plant should determine its degree of resistance to leaf rust. The factor(s) for resistance, originally carried in one arm of a rye chromosome, may be present or absent in a

telocentric chromosome. Only when this factor is present will the plant be resistant. This also would explain the finding of several 43-chromosome plants among the N_2 resistant material. Although the resistance of 43-chromosome plants might be due to the presence of one iso-chromosome (Sebesta, 1958), the frequency of this type of chromosome was not common. Resistance of 43-chromosome plants cannot be always attributed to the presence of iso-chromosomes. More likely the telocentric chromosomes might be different and the factor(s) for leaf rust resistance may be present or absent in a given telocentric, as explained before.

In root tip cells from germinating seeds of the N_3 generation, a variable number of chromosomes was found, this being an indication of the lack of complete stability of the 44-chromosome number in this type of material. When plants with 43 or 44 chromosomes are selfed, they may give origin to 42, 43 or 44-chromosome plants. It must be recalled that leaf rust readings were made only in the N_2 generation. Chromosome numbers in germinating seeds of the N_3 generation were determined, but no leaf rust readings were made in this third generation. In

seeds from 15 N_2 susceptible plants, it was found that 3 had 42, 7 had 43 and 5 had 44 chromosomes. The first three very likely came from 42-chromosome susceptible plants and the last ones from 43-chromosome plants (slightly susceptible).

Progeny from resistant plants also showed a variable chromosome number. Out of 92 examined, only two showed 42 chromosomes, all the others having 43 or 44. This indicates that most of these plants came from N_2 plants having at least 43 but more likely 44 chromosomes, unless they came from 42-chromosome plants fertilized by pollen carrying 22 chromosomes which is unlikely as outcrossing in wheat is very infrequent under greenhouse conditions.

Of special importance to this study was the finding of a leaf rust resistant plant having 42 chromosomes. This number was found in cells from root tips of the N_3 generation and also in microsporocytes of the N_2. Since all 42 chromosome plants not carrying the extra chromosomes which confer the leaf rust resistance of 82 a 1-2-6-2 are susceptible, it can be concluded that at least one interchange took place between the telocentric "rye" chromosome and one of the wheat chromosomes and that it is

possible that the gene(s) for such resistance were incorporated in the wheat complement. Although the results must be interpreted with caution, when consideration is given to the high number of translocations observed in both N_1 and N_2 populations, and the meiotic irregularity of this plant (4-35) in the N_2, it is possible that the desired type of interchange took place. An N_3 population with seed from this N_2 resistant plant should be grown and inoculated with leaf rust to see if both its disease resistance and chromosome number remain constant. After a few generations the desired translocation should become homozygous and a stable, 42-chromosome, leaf-rust resistant line should be available. It could be crossed with susceptible, 42-chromosome wheat varieties to study leaf rust reaction in F_1 and subsequent generations.

The occurrence of a point mutation, resulting in the resistance to leaf rust of plant 4-35 cannot be ruled out. Although a point mutation could not be distinguished cytologically from a very small translocation, the two could be identified by the coverage of resistance to different physiologic races of the pathogen. As pointed out by Jensen and Kent (1952), selection 82 a 1-2-6-2 is highly

resistant (in the seedling stage) to races 9 and 19, and susceptible to races 5, 11, 15, 58, 93, 105 and 126, and segregating for susceptibility to races 21 and 45. If resistance of plant 4-35 is due to a translocation, its segregates should show the same pattern of resistance to the mentioned races; if due to a point mutation, the reaction to physiologic races of leaf rust should be different.

As stated before, the possibility exists that the telocentric chromosomes found in selection 82 a 1-2-6-2 are not always the same. There might be at least two types, corresponding to the original arms of the rye chromosome responsible for leaf rust resistance. This may be supported by the finding of a pair of double satellited isochromosomes (satellites at both ends, see Figure 28) in cells from root tips of germinating seed from N_2 plant 5-180. Unfortunately, the whole complement could not be studied in a single field. A very careful study of the karyotype of both 82 a 1-2-6-2 and of Rosen rye would be necessary to determine the possible origin of the telocentric involved in leaf rust resistance in this selection.

As suggested before, the problem could be clarified in two ways:

1. By making crosses between 82 a 1-2-6-2 and susceptible wheat varieties, inoculating the F_2 or backcrosses with leaf rust to study segregation and by cytological study of the segregates carrying the extra telocentric "rye" chromosome. All 42-chromosome plants in this segregate should be susceptible.

2. By obtaining a series of monosomic addition lines from Rosen rye to a susceptible hexaploid wheat variety. This would help in identifying the rye chromosome responsible for leaf resistance (if this variety is still resistant as it was when chosen as parent in the original wheat-rye cross), and would provide new germplasm for further work in the problem of transfer of disease resistance, especially by the radiation-induced translocation technique demonstrated by Sears (1956) and Elliott (1957).

SUMMARY AND CONCLUSIONS

The cytology of selection 82 a 1-2-6-2, a wheat-rye derivative which has excellent mature plant resistance to leaf rust at East Lansing, Michigan, was investigated. This wheat-like selection, as shown by Sebesta (1958), has 44 chromosomes, with the two extra chromosomes being telocentric and carrying a factor(s) for leaf rust resistance.

Crosses between plants of this selection and both Rosen rye and Cornell 595 wheat were made, in an attempt to determine the homologies between the extra chromosomes and the wheat and rye complements. Associations of two, three, four, and five chromosomes were observed in the cross 82 a 1-2-6-2 x Rosen rye. The formation of bivalents was predominant (82.17 %). Chiasmata were observed in 54 of the bivalents and 25 of them were heteromorphic. The presence of this type of bivalent may be taken as evidence for a degree of homology between the extra telocentric chromosome and one of the rye chromosomes. However the possibility of heteromorphic wheat-rye bivalents cannot be ruled out, and it was very difficult to make a distinction between rye and wheat chromosomes at meiosis of the hybrid plants.

Tetravalent formation was observed in 13 % of cells analyzed from the cross 82 a 1-2-6-2 x Cornell 595. It was concluded that homologies between the extra telocentric and the wheat genome were not apparent, since the extra univalent was unpaired in each cell examined, except in one with a trivalent. The tetravalent formation was probably due to structural heterozygosity in the wheat complement of 82 a 1-2-6-2, in relation to Cornell 595 chromosomes.

Selection 82 a 1-2-6-2 has shown some variation in leaf rust resistance at East Lansing due to the instability of the two telocentric chromosomes. Methods of transferring a small segment of the telocentric chromosomes containing the leaf rust resistance to a wheat chromosome and eliminating the remainder of the telocentric chromosomes were studied. Dry seed from several plant selections of 82 a 1-2-6-2 were irradiated in an attempt to transfer leaf rust resistance to the wheat complement. As determined from the analysis of multivalent associations in pollen mother-cells from the N_1, 56.19 % of them had trivalents or associations of high order, with tetravalents being predominant (58.16 %). In the N_2 the situation was

similar, with 42.08 % of the cells studied showing multivalent associations and again tetravalents being predominant (84.78 %). This proved that at least one interchange was obtained in a high percentage of the cells analyzed.

The results of the inoculation of N_2 plants with an oil suspension of leaf rust spores demonstrated the high degree of resistance of this type of material, as about 98 % of the population was resistant. When chromosome number in root tip cells of germinating seeds from N_2 resistant plants was examined, seeds from plant 4-35 proved to have 42 somatic chromosomes. This chromosome number was confirmed in the meiotic sample from the N_2 of this plant. A number of irregularities, as multivalent associations, unpaired univalents at M I and lagging chromosomes at A I and T I were found. This suggests the possibility that some chromosomal rearrangements have taken place as a result of the neutron irradiation and that the desired transfer of the leaf rust resistance factor(s) from the "rye" telocentrics to the wheat complement may have taken place. N_3 and further generations of this plant should be grown and inoculated with leaf rust to determine if it keeps its resistance and if the 42 chromosome number is

stable. If this is the case, it would be a useful source of germplasm for further work.

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