A GENETIC METHOD FOR DETECTING INDUCED INTERGENERIC TRANSLOCATIONS¹

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Received September 4, 1962

MANY characters which would be desirable in cultivated crops are known to exist in related species and genera. The most striking examples of such characters are the resistances to various diseases. Where hybridization can be achieved between the pertinent genera, attempts have been made to transfer genes for these desired characters into the genome of the cultivated species. Partially for this reason *Triticum aestivum* L. has been hybridized with species of the other four genera of the sub-tribe Triticinae—viz., *Aegilops*, *Agropyron*, *Secale* and *Haynaldia*. However, completion of such incorporation usually has not been achieved owing to a lack of synapsis and crossing-over between the introduced chromosomes and the host chromosomes. The end result in a number of these cases has been an addition line in which the added foreign chromosome is present as an intact or telocentric chromosome or as an isochromosome. The added chromosome may be present as a monosome or as an homologous pair. Such addition lines containing foreign chromatin in addition to the normal complement are usually unsuitable because of undesirable genes present on the added chromosome and/or partial desynapsis of the added pair.

Irradiation of addition lines has been employed in order to transfer the desired alien chromatin to a wheat chromosome. SEARS (1956) incorporated into the wheat genome a segment of *Aegilops umbellulata* Zhuk. chromatin, which confers resistance to wheat leaf rust (*Puccinia recondita* Rob. ex Desm.). This was accomplished by irradiating plants which possessed the normal 42 wheat chromosomes plus an added *Aegilops* isochromosome consisting of the reduplicated chromosome arm which carried the gene or genes for wheat leaf-rust resistance. Pollen from X-irradiated plants was used in crosses with the wheat variety Chinese Spring, and resistant F₁ plants were examined cytologically. One line was isolated which possessed an intercalary translocation in which a piece of *Aegilops* chromatin, carrying the factor for resistance, had been inserted into a wheat chromosome. The newly synthesized chromosome exhibited normal male transmission.

¹ Paper No. 423 in the Plant Breeding Series, Cornell University, Ithaca, New York. Adapted from a thesis submitted to Cornell University in partial fulfillment of the requirements for the Ph.D. degree.

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KNOTT (1961) irradiated a 43-chromosome addition line of wheat involving a chromosome of *Agropyron elongatum* (Host) Beauv. that carries a gene (or genes) conferring resistance to wheat stem rust. Following hybridizations with the wheat variety Thatcher, a line was isolated in which the *Agropyron* resistance had been transferred to a wheat chromosome.

The present paper reports on the application of a new method of detecting and isolating induced intergeneric translocations.

MATERIALS AND METHODS

The test material chosen to examine the possibilities of this method was the Cornell Wheat Selection 82a1-2-4-7. This selection is of the following pedigree: (Honor $\times$ Rosen Rye) $\times$ Honor $\rightarrow$ Selection 369a2-2-44; Selection 369a2-2-44 $\times$ Yorkwin $\rightarrow$ Selection 779b10-8-6; Selection 779b10-8-6 $\times$ Cornell 595 $\rightarrow$ 82a1-2-4-7. Honor, Yorkwin and Cornell 595 are varieties of common hexaploid wheat. Selection 82a1-2-4-7 has been resistant in the adult stage to all races of leaf rust at Ithaca, New York, for many years. In describing the production of this selection, JENSEN and KENT (1952) reported on linkage between the gene(s) for leaf-rust resistance and a gene (or genes) for adult plant resistance to powdery mildew of wheat (*Erysiphe graminis tritici* March.).

SEBESTA (1958) found that this line possessed 21 bivalents plus a bivalent consisting of two telocentric chromosomes derived from *Secale cereale* L. These telocentrics carry the gene(s) for resistance to wheat leaf rust. It was found that this line normally bred true in this respect; however, segregation occasionally occurred, originating from infrequent desynapsis and loss of the rye telocentrics. The progenies of 43-chromosome plants were observed to segregate such that susceptible plants constituted 57 percent of the population.

SEARS (private communication) detected that one of the wheat chromosomes of this line was also telocentric. The telocentric involved was the left arm of chromosome 2A (XIII). As the right arm of chromosome 2A is essential for normal vigor, it was deduced that the added rye telocentric was homoeologously compensating for this missing arm.

The method for inducing transfer of the resistance involved the irradiation of the 44-chromosome line. If by such irradiation a translocation should be induced involving a wheat chromosome and one of the rye chromosomes, multivalent formation or incompletely homologous bivalent formation could give rise to gametes that do not carry the added gene(s) for resistance. Following selfing of the R_1 plants (i.e., those which originate from the irradiated grain), fusion of such gametes would give rise to individuals which lack resistance. Thus segregation for resistance-susceptibility would be observed in the R_2 generation. Induced changes other than the translocation postulated above could also lead to segregation in the generation referred to, but it was expected that determination of breeding behavior in later generations would identify these other changes. Progeny testing of resistant plants from segregating lines would be particularly useful in this respect.

Dormant seed of the 44-chromosome line was irradiated at the Brookhaven National Laboratory, New York, in September 1959. Three separate treatments were applied: 1. X rays (15,000r), 2. X rays (20,000r), 3. Thermal neutrons (dose equivalent to four hours at 7.5×10^8 thermal neutrons/cm²/sec). From each treatment approximately 150 R₁ plants were harvested on a single-plant basis along with nonirradiated control plants. All R₁ plants were threshed on a single-tiller basis. The R₂ nursery was sown as space-planted head rows, the rows originating from a common R₁ plant being sown adjacently. Fifty-two randomly placed check rows of a bulk stock of nonirradiated 82a1-2-4-7 were also included in the R₂ nursery.

An artificial leaf-rust epiphytotic was originated by hypodermically inoculating plants in spreader rows with a suspension of urediospores. This inoculum contained races 6, 15, and 58 (C. O. JOHNSTON, private communication). After development of a heavy epiphytotic, R₂ plants were classified for reaction types. Three types were observed on the flag leaves of plants in segregating rows. These were resistant (range "0" to "1"), susceptible ("3" to "4") and an intermediate reaction ("X") in which both resistant and susceptible type pustules existed on the same leaf (Figure 1). Reaction types employed in these investigations conform with those described by MAINS and JACKSON (1926).

Twenty-three R₂ individuals of the "X" reaction type were selected from segregating head rows. The ensuing R₃ and R₄ progenies were examined for segregation of reaction types by inoculating seedlings with a pure culture of race 58 of leaf rust under greenhouse conditions. Selection 82a1-2-4-7 does not possess any resistance to *P. recondita*, race 58, other than that carried by the added rye telocentrics.

Cytological observations of meiosis in pollen mother cells were carried out by the aceto-carmin squash technique, material having been fixed in a solution of six parts 95 percent alcohol: three parts chloroform: one part glacial acetic acid.

RESULTS

With this method no observations are necessary on the R₁ plants. In the R₂ generation, 51 of the 52 control rows consisted entirely of resistant plants. The remaining row contained one susceptible plant. This individual exhibited the other genetic markers of 82a1-2-4-7 and thus was not considered an admixture. Significantly this plant was very stunted, a result which may have been predicted, as the loss of resistance is a reflection of the loss of the rye chromosomes which prevent the expression of the nullisomic condition of the right arm of chromosome 2A.

Of the 150 groups of R₂ head rows originating from the 15,000r treatment, one was eliminated as an original mixture, 114 consisted entirely of resistant plants, four contained homogeneous resistant rows and homogeneous susceptible rows, and 31 exhibited segregation for leaf-rust reaction types in at least one head row. These 31 groups collectively embodied 113 homogeneous resistant rows, one homogeneous susceptible row, and 66 segregating rows.

FIGURE 1.—A representative of each of the three leaf-rust reaction types observed in the R_2 field. Left: the resistant reaction "0" to "1." Center: the mesothetic reaction "X." Right: the susceptible reaction "3" to "4." (ca. 2 $\times$.)

Of the 151 groups of R_2 head rows originating from the 20,000r treatment, 110 consisted entirely of resistant plants, and 41 contained at least one segregating row. These 41 groups collectively contained 159 homogeneous resistant rows, one homogeneous susceptible row, and 90 rows which exhibited segregation.

Of the 123 groups of R_2 head rows originating from the neutron treatment, five were eliminated as being derived from original mixtures, 85 consisted entirely of resistant plants, four contained head rows that were entirely of resistant plants and head rows that were entirely of susceptible plants, and 29 contained at least one segregating row. These 29 groups contained 117 homogeneous resistant rows, nine homogeneous susceptible rows, and 110 segregating rows.

The 266 segregating rows observed in the R_2 generation exhibited a wide range of segregating ratios, showing all gradations from almost all resistant plants to almost all susceptible plants (DRISCOLL 1962). In all three treatments it was very

obvious that the vast majority of susceptible plants were stunted and poorly fertile (Figure 2). From 80 of the 101 R_1 plants which produced at least one segregating row, all the susceptible R_2 plants were stunted and partially sterile. Of the other 21 groups, 19 contained stunted susceptible plants and susceptible plants of normal vigor, and three groups contained only the latter type. It is apparent that the loss of the resistance usually results in the loss of the compensating rye chromatin, and this loss allows the nullisomic condition of the right arm of chromosome 2A to manifest itself. The occurrence of susceptible plants of full vigor is of interest, for this suggests separation of the gene(s) for

FIGURE 2.—The R_2 progeny N-4-8 illustrating the very common occurrence in the R_2 field of stunted susceptible plants (left and center) and vigorous resistant plants (right). (ca. 0.13 $\times$.)

resistance from the compensating rye chromatin, indicating fractionation of the rye telocentric.

From the distribution of plants exhibiting the intermediate leaf-rust reaction type, it was deduced that all "X" type plants in this particular R_2 nursery possessed a single dose of resistance. If plants with two doses of resistance had also exhibited the "X" reaction, this reaction would have been observed in the control rows and the R_2 rows which were not segregating. No "X" types were observed in the 52 control rows, and of the 216 plants of the "X" reaction type all but three were in groups of head rows which included susceptible plants. Each of these three was in a small R_2 population that was derived from an R_1 plant that possessed only one tiller. Thus the "X" reaction type was only observed when segregation occurred, and it was not displayed by plants possessing two doses of resistance. Therefore it was concluded that all plants of the "X" reaction in the R_2 nursery possessed a single dose of resistance.

Results are at hand which demonstrate that the reaction type of this resistance is also dependent on the developmental stage of the plant. These results will be elaborated on in a further publication, and it will suffice to say that independent of dosage an "X" reaction is obtained at the first-leaf stage. As R_3 progenies were tested at various stages of maturity, the results are presented as resistant ("0" to "X") and susceptible ("3" to "4").

The 23 R_2 plants selected for progeny testing were all of the "X" reaction type at the flag-leaf stage, thus each possessed only one dose of resistance. If these plants possess the desired translocation, and if the male transmission rate of the newly formed chromosome is not impaired, their progenies would be expected to segregate in conformance with a ratio of three resistant: one susceptible. If a translocation with a low male transmission rate is involved, a progeny with a proportion of susceptible plants in excess of 25 percent would ensue. If one rye telocentric had been eliminated by the effects of the irradiation, a progeny in which susceptible plants would constitute approximately 57 percent of the population would be expected (see MATERIALS AND METHODS).

In 21 of the 23 R_3 families, the number of susceptible segregants significantly exceeded 25 percent of the population at the .05 level of significance (Table 1). Families with no resistant plants indicate a chimeric condition in which the resistance factor was not present in the germ line of the R_2 plant. As all of the situations leading to an excessive number of susceptible offspring are undesirable, the plants in these 21 families were discarded without further observations being taken. As the other two populations—*viz.*, 15r-137-1-I1 and 20r-148-5-I1—segregated in accordance with a 3:1 ratio, it is possible that they possess a desired translocation.

Further observations have been made on the first of these two populations only. At the heading stage it was obvious that a perfect correlation existed between susceptibility and stunting. Thus, the factor for resistance was still attached to the compensating rye chromatin. Cytological observations on the resistant plants showed that these individuals possessed either 43 or 44 chromosomes. The monosome involved in the 43-chromosome plants was observed to be telocentric (Fig-

TABLE 1

The R_3 segregation ratios obtained in selfed progenies of 23 R_2 plants which were constituents of segregating R_2 progenies and which exhibited the "X" leaf-rust reaction type at the flag-leaf stage

Pedigree	Leaf-rust reaction type		Chi-square (3:1 ratio)	P
	"0" to "X"	"3" to "4"		
15r-7-2-I1	12	21	26.27	<.01
15r-25-3-I1	15	19	17.29	<.01
15r-56-1-I1	5	11	16.33	<.01
15r-79-1-I1	0	67	201.00	<.01
15r-117-5-I1	11	13	10.89	<.01
15r-121-4-I1	0	49	147.00	<.01
15r-137-1-I1	90	30	0.00	1.0
20r-4-4-I1	31	21	6.56	.02-.01
20r-5-2-I1	13	15	12.19	<.01
20r-23-2-I1	1	25	58.24	<.01
20r-27-1-I1	1	5	10.89	<.01
20r-62-1-I1	3	207	606.23	<.01
20r-74-8-I1	13	27	38.53	<.01
20r-116-1-I1	0	57	171.00	<.01
20r-126-5-I1	3	71	198.65	<.01
20r-144-4-I1	39	26	7.80	<.01
20r-148-5-I1	78	21	0.76	.50-.30
N-2-3-I1	18	20	15.47	<.01
N-6-1-I1	29	24	11.63	<.01
N-27-1-I1	0	87	260.08	<.01
N-52-4-I1	0	16	48.00	<.01
N-60-9-I1	2	53	149.39	<.01
N-149-4-I1	19	26	25.79	<.01

ures 3 and 4). The susceptible plants of this population were unsuitable for cytological examination, and only one individual provided observable chromosomes. This individual possessed 43 chromosomes including the telocentric, which is evidence of the removal of the gene(s) for resistance from this chromosome. In some pollen mother cells of this plant the telocentric chromosome was observed to be incorporated in a trivalent with two dibrachial chromosomes. This indicates that the monosome is not simply the rye telocentric from which the gene(s) for resistance has been deleted, but rather that it contains a segment of wheat chromatin.

The progenies of 11 resistant R_3 plants were tested with leaf rust (Table 2). Three of the 11 progenies were homozygous resistant; the other eight families each segregated in conformance with a 3:1 ratio. Excluding the three homozygous resistant lines, the total segregation ratio was 86:31, which is in good agree-

FIGURE 3 (left).—Pollen mother cell at metaphase I of R_3 plant 15r-137-1-I1-R13. One telocentric univalent present. (ca. 1015 $\times$.)

FIGURE 4 (right).—Pollen mother cell at anaphase I of R_3 plant 15r-137-1-I1-R13. The telocentric univalent has divided late, and the daughter half at the top clearly exhibits a terminal spindle attachment. (ca. 910 $\times$.)

TABLE 2

The R_3 segregation ratios obtained in selfed progenies of 11 R_3 plants of the family 15r-137-1-I1

R_4 progeny no.	Chromosome no. of R_3 plant	Resistant "0" to "X"	Susceptible "3" to "4"
3	43	3	1
6	43	3	3
8	43	17	7
13	43	15	4
1	44	19	0
2	44	19	0
4	44	14	6
7	44	14	5
9	44	10	3
14	44	10	2
16	44	20	0

ment with a monofactorial ratio. Also, three homozygous resistant: eight segregating is a satisfactory fit to the 1:2 ratio expected with monofactorial inheritance.

DISCUSSION

The method employed in this study eliminates a large proportion of the irradiated material in the early selfed generations. Only testing for resistance is

required. Lines which conform with the designated restrictive behaviors can then be subjected to further detailed study. Thus this method should be viewed as a technique of detecting the small number of lines that warrant detailed cytological observations.

Two lines have been detected in which monofactorial inheritance is simulated in the R_1 generation. One of these lines has been examined in the R_4 generation, and the inheritance pattern still indicated that a desired translocation may be involved. The necessary steps to be taken are to cross individuals of these lines with the wheat variety Chinese Spring, which represents the commonly acknowledged standard karyotype, and to observe chromosome pairing in the F_1 hybrid.

It appears that this method can be applied to other disomic addition lines, although amendments to the procedure may be desirable. The most efficient method of selecting from the R_1 generation would be to select only one head from each R_1 plant. Although this would necessitate growing more R_1 plants—perhaps 1,000—duplication of identical induced changes would be circumvented. The R_2 head progenies would be tested for segregation of the character involved. Resistant plants of segregating rows, in which the susceptible segregants do not exceed 25 percent, would then be progeny tested for simulated monofactorial inheritance. If the character involved can be recognized in the seedling stage, the R_2 observations would best be carried out under greenhouse conditions. If the character involved shows a dosage effect, the selection of R_2 plants exhibiting the effect of a single dose of the alien gene would be highly desirable.

One favorable feature of this procedure is the fact that cases of inadvertent outcrossing to a 42-chromosome line carrying another type of resistance are self eliminating, as the first guide to a translocation is a susceptible segregant, not a resistant plant.

SUMMARY

A disomic addition line of wheat, involving a pair of rye chromosomes bearing the gene(s) for wheat leaf-rust resistance, was subjected to a method of detecting induced translocations between wheat and rye chromosomes. The main advantage of the method used was that a large proportion of the irradiated material could be excluded as unsuccessful, simply by observing the segregation patterns of leaf-rust reactions in selfed generations. Only the R_2 head progenies that exhibited segregation of resistance-susceptibility were retained, and resistant plants in a number of these populations were progeny tested. Two R_3 lines were observed to segregate in conformance with a three resistant:one susceptible ratio. Observations on one of these lines in the R_4 generation also conformed with that expected with monofactorial inheritance. Thus it is possible that two acceptable translocations to a wheat chromosome, of the gene(s) for resistance, have been detected. However, confirmation of such translocations requires cytological observations on F_1 hybrids following crossing with normal wheat.

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