CHROMOSOME ENGINEERING AND ACCELERATION OF GENERATION ADVANCEMENT IN BREEDING RUST RESISTANT WHEAT

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Introduction

Breeding leaf rust resistant wheat cultivars by interspecific and intergeneric crosses is an important objective of our laboratory. A breeding program using alien genera which showed resistance to major races of leaf rust in Japan was started in 1962.

In this case, however, it was impossible to transfer rust resistance of alien genera to wheat by simple breeding methods, because of the lack of chromosomal homology between the parental genomes.

As a possible means of overcoming the difficulty, the breeding methods based on chromosome engineering were employed, as a primary step, for establishing the addition line to wheat of an alien chromosome bearing the gene for leaf rust resistance. As a secondary step, attempts have been made to translocate a small segment of the alien chromosome to a wheat chromosome.

On the other hand, in the field of wheat breeding by interspecific and intergeneric crosses, a number of problems have remained unsolved. One of them is that it takes a long time to breed resistant cultivars which are practically useful. The other problem is the fact that the bred cultivar changes its resistance to susceptibility as a result of physiologic specialization of rust fungi.

One of the means of solving these problems is to accumulate various resistant major genes into a strain in as short a period as possible. To accomplish this, experiments were carried out to shorten the generation time of wheat.

The outline of these studies will be described under the following two headings, namely, the transfer of leaf rust resistance from alien genera to wheat (Mukade et al. 1970, 1975a) and new procedures for accelerating generation advancement in wheat breeding (Mukade et al 1973, 1975b).

The transfer of leaf rust resistance from alien genera to wheat

Because alien genera of wheat such as *Secale* (rye), *Agropyron* and *Aegilops*, have many characters of agronomic interest, including resistance to disease, there has been a great deal of effort directed toward transferring genes from alien genera to wheat.

In our laboratory, with an aim to breed a leaf rust resistant wheat strain, attempts have been made to transfer leaf rust resistance from rye to wheat by intergeneric addition and translocation.

1. Production of the wheat-rye chromosome addition line. The method used to obtain the addition line was basically similar to that proposed by O'Mara (1940). Norin 40 was crossed to 8*X Triticale* (Norin 40 x Petkus) in 1962. Seedlings in the subsequent backcross generation were inoculated with race 21B of leaf rust (*Puccinia recondita* Rob. ex. Desm.) which is the virulent race in Japan. From them, the wheat-rye monosomic addition line bearing leaf rust resistance was selected in 1964. Subsequently, disomic and ditelosomic addition lines were also obtained.

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In the progeny of such disomic addition plant which formed 22 bivalents in PMCs, the different types of aneuploids from the parent occurred in a frequency of over 10 per cent. The instability of the addition lines has previously been reported by many workers. Especially Riley and Kimber (1966) have mentioned that this behavior, which might be termed "addition decay", is probably the most important factor acting against the commercial exploitation of useful alien variation in the form of chromosome additions.

The seedling reactions of the addition lines to four races of leaf rust are shown in Table 1 in comparison with their parents. The inoculation with races 5B, 6A and 21B revealed MR reaction (moderate resistance showing 0;-1) in the monosomic addition, but a highly resistant reaction, showing only flecks, in the disomic addition. With race 37B, however, the reaction types of "X" in the monosomic and of "MR" in the disomic addition line were observed respectively.

Table 1. Seedling reactions of the addition lines to four races of leaf rust

Controls and addition lines	Chromosome configurations	Leaf rust races			
		5B	6A	37B	21B
Triticale	28″	R	R	R	R
Wheat cultivar Norin 40	21″	S	S	S	S
Monosomic addition	21″ + 1′	MR	MR	X	MR
Disomic addition	21″ + 1″	R	R	MR	R

The present results indicate that the added Petkus rye chromosome had a dosage effect for the leaf rust resistance. At least in the case of the naturally-occurring races under field conditions, a high resistance has always been observed on adult plants of the monosomic as well as the disomic addition.

Consequently, this leaf rust resistance is referred to as an adult-plant type of resistance especially in the case of monosomic addition. The leaf rust resistance derived from Petkus rye is potentially of direct use in this breeding program.

2. Transfer by X-ray induced translocation To transfer the small chromosome segment, which carries the resistance, from rye to a wheat chromosome, a portable X-ray apparatus (Hida Elec. Co., Yamato SS-54, 75 KVp, 2.5mA, 249 R/min.) was used for irradiation. X-rays were applied to pollen grains of the disomic addition line (600-900R) and young plants of the monosomic addition line at the VII-VIII stages of spike differentiation (1000R) and then the pollen grains obtained therefrom were used to pollinate the untreated wheat cultivar.

In this experiment Aobakomugi, a leading cultivar in northern Japan, having a resistance to race 37B was used as a parent in the cross to improve the lower level of resistance of the addition line to that race.

The F_1 progenies referred to as X_2 were grown and the seedlings were inoculated with race 6A of leaf rust. Only a total of 42 plants which proved to be resistant were examined cytologically in meiosis for the presence of translocations. As shown in Table 2, seven plants had such chromosome configurations as 21 bivalents (21″), one tetravalent plus 19 bivalents (1″ ″ + 19″), one tetravalent and one trivalent plus 18 bivalents (1″ ″ + 1″ ′ + 18″), one trivalent plus 20 bivalents (1″ ′ + 20″).

As these plants showed no univalent of the rye chromosome, translocation must have occurred between the chromosome of rye and wheat. Out of these seven plants selected cytologically, however, two plants were discarded because of their being inferior in growth. The remaining five were designated as RT 1-5, respectively.

Table 2. Leaf-rust test and cytological selection in X_2 generation

2*n*	Chromosome configurations	No. of plants	
		Resistant	Susceptible
41	20'' + 1', 19'' + 3'	1	1
41	2'' '' + 13'' + 7'		1
41 + 1f	3'' + 12'' + 8' + f'	1	
41 + 1t + 1f	1'' '' + 18'' + 1' + t' + f'	1	
41 + liso	20'' + 1' + i'	3	
41 + liso	1''' + 19'' + i'	1	
42	1^{V} + 1'' '' + 2'' ' + 11'' + 5'	1	
42	1'' '' + 18'' + 2 '		1
42	1'' '' + 19''	(1)	9
42	1'' ' + 18'' + 3'	1	
42	21'', 1'' '' + 19''	(2)	
42	21'', 20'' + 2'	(1)	2
42	21''		42
42 + 1f	19'' + 4' + f'	1	
42 + 1f	1'' '' + 18'' + 2' + f'		2
42 + 1t	1'' '' + 19'' + t'	3	
42 + 1t	21'' + t'	6	1
42 + liso	1'' '' + 19'' + i'	1	
42 + liso	21'' + i'		1
43	1'' '' + 19'' + 1'	6	
43	1'' '' + 1'' ' + 18''	(1)	
43	1'' ' + 20''	(2)	
43	21'' + 1'	9	
	Total	42	60

The RT-1 (1'' '' + 1'' ' + 18'') was obtained from pollen irradiation of the disomic additions and the remaining four RT's were secured from young plant irradiation of the monosomic additions.

The X_3 lines derived from these five plants selected in the X_2 generation were tested with leaf rust race 21B. The data obtained concerning resistance are given in Table 3. Moreover, all 93 plants chosen at random from the resistant plants in each X_3 line were examined cytologically (Table 4). In the X_3 lines which were derived from RT-1 and RT-3, no plant with 21 bivalents was obtained. From the other lines, however, 18 resistant plants with 42 chromosomes (21'') involving the translocation were obtained. From these progenies, at present (X_{10}), eight strains are now being bred and are being subjected to characteristic and yield tests. Some of these strains are superior to the parent cultivar, Aobakomugi, in the yield performance test.

As a special instance for X-ray irradiation, mature pollen grains collected from F_1 plants (8*X Triticale* x wheat cultivar, Shimofusakomugi having resistance to race 37B) were irradiated with 600R and were then used to pollinate Shimofusakomugi. Out of five B_1F_1 plants obtained, a resistant plant with 1'' '' + 19'' was selected. By cytological selection and rust inoculation tests of B_1F_1 to B_3F_7 generations, two homozygous resistant strains (21'') which in other

Table 3. Seedling reactions of X_3 lines to leaf rust race 21B

Chromosome configurations of X_2 plants selected	X_3 Line no.	MR	X	S	No. of plants tested
1″ ″ + 1″ ′ + 18″	1	91		43	134
1″ ′ + 20″	2	82	6	50	138
1″ ′ + 20″	3	45		20	65
21″ , 20″ + 2′	4	53	9	53	115
1″ + 19″	5	94		30	124

Table 4. Cytological selection of X_3 lines

Chromosome configurations	Generation X_3 Line no. 1	2	3	4	5	No. of plants tested
1″ ″ + 1″ ′ + 18″	8					8
1″ ′ + 20″	1	29	18			48
1″ ′ + 20″ , 21″ + 1′				2	1	3
1″ ″ + 19″					2	2
21″ (1″ ″, 1″ ′)					4	4
21″		1		14	3	(18)
21″ + 1″ (heteromorphic)	1	1		1		3
Others ($2n$ = 44 - 41)	2	2	1	1	1	7
Total	12	33	19	18	11	93

characteristics are comparable to wheat cultivars were obtained. These strains were named "Sabikei 31" and "Sabikei 32".

Since the original method was proposed by Sears (1956) using X-ray-irradiation prior to anthesis of monosomic additions of the *Aegilops* chromosome carrying the leaf rust resistance, many other irradiation experiments have been made to compare the effects of different radiation sources such as gamma rays and thermal neutrons, to find out the breeding procedures for detecting desirable translocation. In some cases seeds rather than plants were irradiated (Driscoll and Jensen 1963, Knott 1961, 1971, Sharma and Knott 1966, Sears 1972, Wienhues 1963).

In the present study, pollen grains of the disomic addition line and of F_1 plants (8*X* *Triticale* x Wheat cultivar), and young plants at the VII-VIII stages of spike differentiation of the monosomic addition line were irradiated by using portable X-ray apparatus. It should be noticed here that the scale of this experiment was much smaller as stated above. Under these circumstances, several translocations involving the rye chromosome carrying the gene for leaf rust resistance could be obtained by breeding techniques of chromosome engineering.

In addition, at present the genetic nature of the translocation chromosomes has attracted special interest. In 1968, Knott, in studies using irradiation to translocate a gene for rust resistance from an *Agropyron* to a wheat chromosome, suggested that the translocations were not randomly distributed among the wheat chromosomes. Also in some cases it is assumed that the translocations probably occur between an alien chromosome and a wheat chromosome with the homoeologous relationships.

It will be interesting to identify the wheat chromosome involved in the translocation of "Sabikei 31", "Sabikei 32" and the other translocation strains. This problem remains to be solved.

New procedures for accelerating generation advancement in breeding rust resistant wheat

Most breeders are searching methods to shorten the generation time required to develop a new cultivar. In the present study new procedures for accelerating generations were tested from the standpoint of breeding rust resistant wheat. These involved acceleration of seed maturation, germination of immature seeds, green and seed-green vernalization and other techniques which allowed us to grow successfully four to six consecutive generations within one year. The actual number of generations depends upon the winter growth habit. These procedures can be carried out all the year round by using both the low temperature room and the green-house.

1. Germination technique for immature seeds Emphasis was placed on the development of a rather concrete labor-saving technique for germinating immature seeds. Wheat normally requires about 40 days from the date of anthesis to the full ripening stage. However, it was known that the ripening of wheat kernels is accelerated under high temperature. Thus a part of the green-house was maintained at a high temperature (25–30°C), where plants in the ripening period were placed. With this procedure the seed harvest was efficiently speeded up. Then repeated tests were conducted on the method for drying immature seeds, the concentration of hydrogen peroxide solution, the temperature and the duration of treatment.

In the end, as shown in Table 5, a practical germination technique was established to sow seeds with a germination rate higher than 90% on the 3rd day after the beginning of germination treatment, using seeds harvested 15 to 20 days after anthesis or pollination. The seeds were treated with 1% hydrogen peroxide solution at 25°C for 16–17 hours, then at 11°C for 30 hours.

Table 5. Germination technique applied to immature wheat seeds

1st day	(Morning)	Harvest	: 15–20 days under 25–30°C after anthesis
	(Afternoon)	Drying	: 45°C, two hours
		Threshing	
	(4–5 p.m.)	H_2O_2 treatment	: 1% H_2O_2, 25°C, 16–17 hours
2nd day	(9 a.m.)	H_2O_2 treatment	: 1% H_2O_2, 11°C, 30 hours on germination bed
3rd day	(3 p.m.)	Germination	: Germination bed Tap water 11°C (winter wheat) 25°C (spring wheat)
4th day	(Morning)	Seeding	: Seeds of winter wheat are just sprouting and the spring wheat, rooting.

2. Technique for seed-green vernalization In addition to the so-called seed vernalization using a refrigerator (0°C–2°C), the effect of green vernalization has been reported by several workers (Gott 1957, Chujo 1966). From the standpoint of practical breeding, a low temperature room equipped with special fluorescent lamps (Vitalux-A, 40W, NEC) was constructed for green vernalization, and the effects of temperature, the duration of treatment, the intensity of light, the age of the seedlings, and the difference of cultivars and their cultivation methods, were studied in order to develop the vernalization technique most suited for accelerating generation advancement.

First, green vernalization was carried out at 8°C using first-leaf stage seedlings of two cultivars, Aobakomugi and Miyaginokomugi (also class IV of winter habit), in order to compare the vernalization effect of the Vitalux-A lamp with that of the white fluorescent lamp. The Vitalux-A lamp enhanced flag-leaf emergence by about five to ten days, as compared to the ordinary white fluorescent lamp.

Table 6. Influence of seedling age and culture conditions on the effect of seed-green vernalization (20 plants of Aobakomugi used for each treatment; vernalization carried out for four weeks)

Treat-ment no.	Vernalization treatment		No. of days from seeding to flag leaf emergence	Final leaf No.	Seeds set per head
	Seedling age and seeding conditions when treated	Culture conditions			
1	1st-leaf stage soil-covered	Soil	57.6 ± 0.97	9.0	9.4
2	Sprouted seed soil-covered	Soil	54.2 ± 0.58	8.6	10.4
3	Sprouted seed not soil-covered	Soil	46.8 ± 1.72	6.9	4.5
4	Just sprouted seed not soil-covered	Vermiculite-urethane mat	40.6 ± 1.69	5.2	3.4

In the next experiment, the effect of vernalization on sprouted seeds not covered with soil was examined. Two cultivars, Aobakomugi (class IV) and Nambukomugi (class V) were used. Vernalization was carried out for four and five weeks, respectively. As shown in Table 6, the days required for flag-leaf emergence were 58 days in the case of treatment-1 at the first-leaf stage of Aobakomugi, while in the case of the sprouted seed, 47 days (Treatment-3). This shows a remarkable effect of shortening the emergence of flag-leaf by about ten days. The number of leaves was 9 in the case of the former, while 6.9 in the latter. The same results were obtained with Nambukomugi, in which a sufficient number of seeds per head were obtained.

Since the number of days to flag-leaf emergence close to the number in Treatment-1 was obtained by vernalization which was started after soil-covering of the sprouted seeds (Treatment-2), it may be assumed that the marked vernalization effect was induced by direct action on the apical growing point of sprouting seeds placed at 8°C under continuous illumination with a Vitalux-A lamp (Treatment-3). Therefore, this type of vernalization will be called "Seed-green vernalization".

On the other hand, since the effect of low-nutrient sand culture as a procedure for acceleration of generation advancement had already been known, a further attempt was made to combine it with the seed-green vernalization and to explore the possibility of reducing the generation time.

Aobakomugi was grown on vermiculite instead of on sand, and liquid fertilizer was applied on the 10th day after the end of vernalization. As shown in Table 6 (Treatment-4), the number of days required for flag-leaf emergence was 41 days, which was an additional shortening of about six days, with 3.4 seeds set per head. This was regarded as the maximum enhancement of growth for winter wheat of class IV.

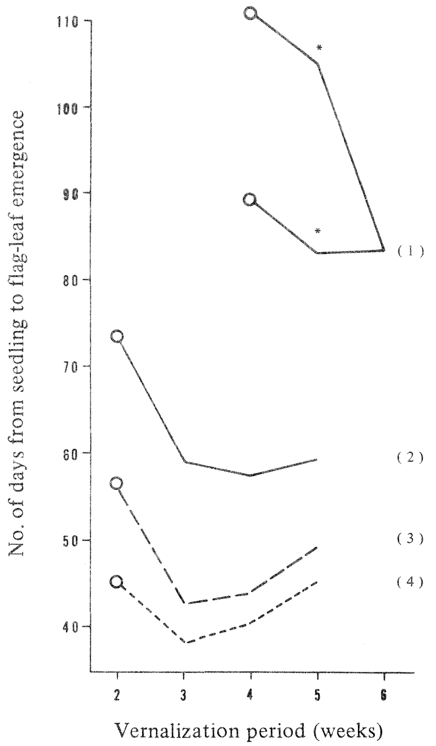

Fig. 1. **Comparison of seed, green and seed-green vernalization** (10 plants of Aobakomugi used for each treatment)
(1) Seed vernalization: at 0–2°C in dark with sprouting seeds, soil culture
(2) Green vernalization: at 8°C under continuous illumination (Vitalux-A) with 1st-leaf stage, soil culture
(3) Seed-green vernalization: at 8°C under continuous illumination (Vitalux-A) with sprouting seeds, not soil covered, soil culture
(4) Seed-green vernalization: at 8°C under continuous illumination (Vitalux-A) with sprouting seeds, not soil covered, vermiculite culture
* : Two types of plants were segregated.

Figure 1 shows the result of another series of tests with Aobakomugi. Seed-green vernalization reduced the days required for flag-leaf emergence by one-half as compared with that of seed vernalization.

Effects of green and seed-green vernalization were compared using 21 cultivars with different degrees of winter habit, including seven standard cultivars used in testing the growth habit. Consequently, it was confirmed that the seed-green vernalization was more effective than green vernalization in enhancing flag-leaf emergence in the cultivars of classes III-VII. However, the effect of seed-green vernalization could not be detected with spring cultivars of classes II-I.

3. Other techniques The materials employed as standard in practical breeding were 10 plants grown in a seedling case (size: 5.5 x 15 x 10 cm) which were successively backcrossed, and 150 plants (seeding rate: 3 x 3 cm) grown in the plant box (size: 34 x 43 x 10 cm) as the bulk-population.

The seedling inoculation test for screening rust resistant individuals was performed efficiently by storing urediospores in liquid nitrogen (Loegering et al. 1966) and by spraying seedlings with a spore suspension in Mobilsol 100. As the inoculation test should be conducted throughout the year for the acceleration of generation advancement, these techniques could be efficiently applied to the present study.

4 Wheat breeding with the acceleration of generation advancement Through the development of these new techniques, as shown in Table 7, breeding materials with the spring habit of class II and those with the winter habit of class IV completed a generation within 60 days (or 6 generations/year) and 80 days (or 4.5 generations/year), respectively.

Table 7. Outline of scheme for acceleration of generation advancement in wheat breeding

Spring wheat (Winter habit : class II)	Days	Winter wheat (Winter habit : class IV)
Harvesting and forcing of sprouting	1st	Harvesting and forcing of sprouting
Seeding	4th	Seeding and starting seed-green vernalization treatment (sprouting stage) in a low temperature room (8°C) with Vitalux-A
Starting green vernalization treatment (1st-leaf stage seedling) in a low temperature room (8°C) with Vitalux-A	7th	
Starting long-day treatment in a greenhouse (20–25°C)	12th	
	32th	Starting long-day treatment in a greenhouse (20–25°C)
(Average date of anthesis) Room temperature (25–30°C)	44th	
Harvesting	60th	(Average date of anthesis) Room temperature (25–30°C)
	80th	Harvesting

With this procedure, both the backcross method and bulk-population method could be efficiently applied. After such progress, many leaf rust resistant strains are now being bred. Furthermore, we are making efforts to breed a highly leaf rust resistant wheat by combining and accumulating various resistant major genes of alien species and genera using the techniques of chromosome engineering as well as adopting actively the acceleration of generation advancement.

Summary

From a stand point of breeding leaf rust resistant cultivars of wheat, especially of combining and accumulating various major genes of alien species and genera in as short a period as possible, the following experiments were carried out.

1) Breeding methods based on chromosome engineering were employed to transfer the leaf rust resistance from rye to wheat. Then several homozygous resistant strains derived from intergeneric translocations induced by X-ray irradiation were obtained.

2) A rather concrete procedure for accelerating generation advancement could be

developed by using the new techniques for germination of immature seeds and for vernalization treatments. With this procedure, a spring wheat of class II and a winter wheat of class IV completed a generation within 60 days (permitting 6 generations/year) and 80 days (4.5 generations/year), respectively. Both the backcross method and the bulk-population method could be efficiently applied.

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Discussion

Y. Watanabe, Japan: I was deeply impressed with your successful development of Sabikei based on the technique of so-called chromosome engineering.

1. When you use these Sabikei strains as a secondary source of resistance is it necessary to make further cytological selection?

2. You quoted the paper of Knott in 1968 on translocations between Agropyron and wheat chromosomes. How is it more convenient to proceed in the breeding work in the two following cases?

a. When translocations occur mainly between homologue chromosomes

b. When they randomly occur between non homologue chromosomes

Answer:

1. All rust resistant lines which are given an accession number in our laboratory are cytologically stable, forming 21 bivalents in meiosis. Furthermore F1 hybrids between these lines and ordinary cultivars also form 21 bivalents. Therefore, I think that there is no need for cytological check in the course of the transfer of their resistant genes to other cultivars.

2. I think that the translocations between the homologue chromosomes which can complement each other are the most favorable for selection for breeding purpose. In fact, more than 30 European wheat cultivars to which the yellow rust resistant gene of rye was transferred carry the translocation between 1B chromosome of wheat and IR chromosome, i.e, its homologue in rye

G. S. Khush, The Philippines: How large are the segments of IR chromosome of rye which are transferred to IB or ID chromosomes of wheat by translocation in the rust resistant lines

Answer: We have not yet studied the size of a translocated segment of the rye chromosome in our materials. However, some researchers in Europe have reported that the short arm of chromosome 1B of wheat was replaced by a segment of 1R chromosome of rye (Zeller, 1973) in the European wheat cultivars having the rust resistant gene of rye.