New Procedures for Accelerating Generation Advancement in Wheat Breeding

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Breeding leaf-rust resistant wheat cultivars by interspecific and intergeneric crosses is an important objective of our laboratory. However, there still remains a number of problems. 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 the physiologic specialization of rust fungi.

One of 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 procedures for accelerating generation advancement have been extensively studied up to date. However, a number of problems remained unsolved[6,8,11].

To solve such problems, new procedures for accelerating generations were studied. These involve acceleration of seed maturation, germination of immature seeds, green and seed-green vernalization, and other techniques which allowed us to most favorably grow four to six consecutive generations within one year. The actual number of generations depends upon the winter growth habit, which can be carried out all the year round by using both the low-temperature room and the greenhouse.

Germination technique for immature seeds

Emphasis was placed on the development of a rather concrete labor-saving technique for germinating immature seeds.

It was known that the ripening of wheat kernels is accelerated under high temperature[5]. So part of the greenhouse 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. And the comparative tests were repeatedly conducted on the method for

Table 1. Germination technique applied to immature wheat seeds

1st day (Morning)	Harvest	: 15–20 days under 25–30°C after anthesis:
(Afternoon)	Drying	: 45°C, 2 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

drying immature seeds, the concentration of hydrogen peroxide (H_2O_2) solution, the temperature and the duration for treatment.

Eventually, the practical germination technique was established to sow the seeds with higher germination rate than 90% on the 3rd day from the beginning of germination treatment by using the 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. Details of these procedures are shown in Table 1.

The present method gave a higher germination efficiency than that reported by Robertson and Curtis[12].

Techniques for green and seed-green vernalization

Beside the so-called seed vernalization using a refrigerator (0°–2°C), green vernalization has also been reported[3]. Particularly to be mentioned are the detailed studies of Chujo[1),2)] on the effects of various temperatures, fretlizer and light on vernalization.

By referring to his results, the low temperature room equipped with special fluorescent lamps (Vitalux-A, 40 W, NEC) for green vernalization was constructed, in which the effects of temperature and duration of treatment, the intensity of light, the age of seedling, and the difference in cultivars and their cultivation methods, could be studied in order to develop the vernalization technique most suited for accelerating generation advancement.

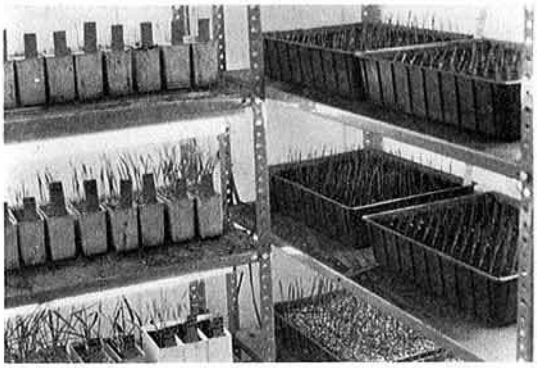

Fig. 1. Green and seed-green vernalization treatment in a low temperature room

1) Effect of the light source on green vernalization

Green vernalization was conducted 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.

As the effect of red light on the vernalization process of winter wheat has been reported by Kruzsilin and Schwedskaya[7], it was assumed that the Vitalux-A lamp with spectral emission of red light elicited the particular effect.

2) Comparison of seed and green vernalization

A test was conducted using seven standard cultivars employed in testing the growth habit (I–VII) to compare green vernalization with seed vernalization during the treatment period of 0–8 weeks with intervals of one week.

Green vernalization was more effective than seed vernalization in enhancing flag-leaf emergence in all the cultivars tested. The optimum treatment periods for green vernalization in each cultivars with different degrees of winter habit are shown in Table 2.

Table 2. Effect of seed, green and seed-green vernalization on the optimum treatment period for vernalization in cultivars with different degrees of winter habit

Vernalization treatment	Degrees of winter habit and optimum treatment period (No. weeks) for vernalization					
	VII–VI	V	IV	III	II	I
(1) Seed V.	7	7	6–5	4	1	0
(2) Green V.	7	5	4	3	1	0
(3) Seed-green V.	5	5–4	3	2	1	0

3) Influence of seedling age and seeding condition on the effect of green vernalization

In the above green vernalization, treatment was started with first-leaf stage seedlings. In this experiment, however, the effect of vernalization on sprouted seeds not covered with soils was examined. Two cultivars, Aobakomugi (class IV) and Nambukomugi (class V) were used. Vernalization was carried out for 4 and 5 weeks, respectively.

Results with Aobakomugi are shown in Table 3. Green vernalization of uncovered, sprouting seeds (Treatment 3) was more effective than that of first-leaf stage seedlings (Treatment 1), shortening by about ten days the date of flag-leaf emergence. The same results were obtained with Nambukomugi.

Since this experiment demonstrated a marked vernalization effect in sprouting seeds placed at 8°C under continuous illumination with a Vitalux-A lamp, this type of vernalization will be called "seed-green vernalization".

On the other hand, since the effect of the low-nutrient sand culture as the procedure for accelerating generation advancement has already been known[4], further attempt was tried 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 (Sumitomo No. 2, 9-5-7, diluted 150 times) was applied on the 10th day after the end of vernalization. As shown in Table 3 (Treatment 4), the number of days required for flag-leaf emergence was 40 days, which was 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.

Figure 2 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.

Table 3. 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 4 weeks)

Treatment No.	Vernalization treatment: Seedling age and seeding condition when treated	Vernalization treatment: Culture condition	No. of days from seeding to flag-leaf emergence	Final leaf number	Seeds set per head
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

4) Effect of temperature on seed-green vernalization

The above mentioned experiments on seed-green vernalization were carried out at a temperature of 8°C. Here, another series of tests was conducted to evaluate the effects of different temperatures on Aobakomugi grown in soils with the standard application of fertilizers.

The number of days to flag-leaf emergence was increased by 2 and 11 days with treatments at 4°C and 12°C, respectively, as compared with the treatment at 8°C. Hence, the temperature of 8°C was regarded as the most suitable for seed-green vernalization.

5) Effect of green and seed-green vernalization on cultivars with different degrees of winter habit

Effects of green and seed-green vernali-

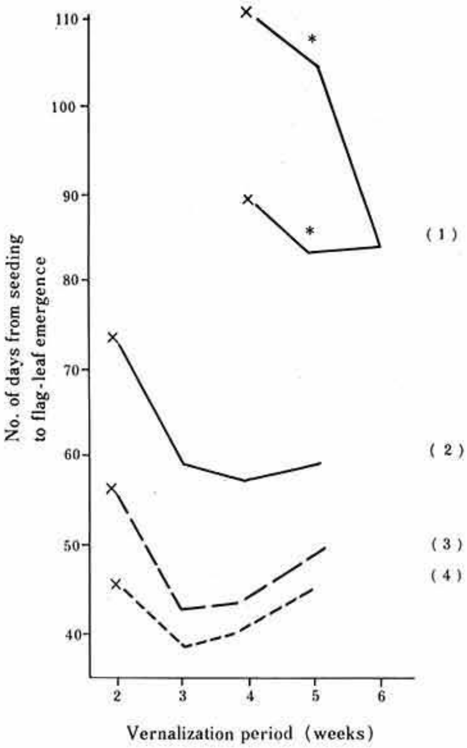

Fig. 2. Comparison of seed, green and seed-green vernalization (10 plants of Aobakomugi used for each treatment)

(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

zation 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. The optimum treatment period for seed-green vernalization was further reduced than that of green vernalization (Table 2).

Other procedures for accelerating generation advancement

The materials employed as standard in the practical breeding were 10 plants grown in a seedling case (size: 5.5 × 15 × 10 cm) which were successively backcrossed, and 150 or 182 plants (seeding rate: 3 × 3 or 2.5 × 3 cm) grown in the plant bed (size: 34 × 43 × 10 cm) as the bulk-population. The standard amount of fertilizer was 50 g of compound fertilizer (8-15-8) per 0.15 m^2 of volcanic ash soil.

The pollen parent for successive backcross is subjected to early seeding on about the 15th day before the harvest of the hybrid seeds to promote tillering and to control the flowering time.

Summarized procedures

The procedure eventually developed consists of the following steps: (1) acceleration of seed ripening by high temperature (25°–30°C); (2) hastening of germination of immature seeds by H_2O_2 treatment (1% at 25°C for 16–17 hours then at 11°C for 30 hours); (3) green (spring wheat) and seed-green vernalization (winter wheat) at 8°C under continuous light from a Vitalux-A lamp; and (4) long day treatment with a metal-halide lamp (400 W/17.5 m^2) in a greenhouse

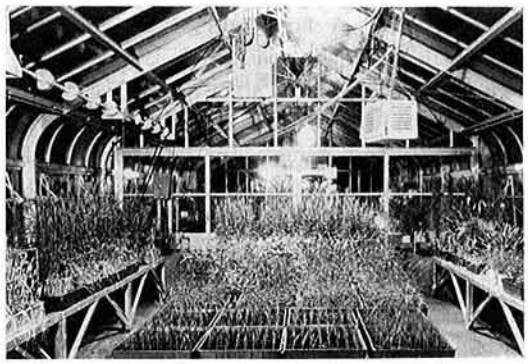

Fig. 3. Long-day treatment in a greenhouse

Table 4. 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	
	32nd	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

(20°–25°C).

Through the development of these new procedures, a spring wheat of class II and a winter wheat of class IV completed a generation within 60 days (namely 6 generations/years) and 80 days (4.5 generations/years), respectively (Table 4). Both the backcross and the bulk-population methods could be efficiently applied.

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