Breeding of Candidate Lines for Male Sterility-Maintainer by Anther Culture for Hybrid Wheat Production Using an S^v Type Cytoplasm and a 1BL-1RS Chromosome

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Summary

We previously proposed a new breeding scheme to develop hybrid wheat utilizing male sterility induced by the interaction between the S^v type cytoplasm of *Ae. kotschyi* and the 1BL-1RS chromosome (Toriyama *et al.* 1991, 1993). In our proposed scheme male sterility-maintainer lines can be produced by two ways, i. e. the backcrossing method (Nonaka *et al.* 1993) and anther culture. This article reports the results of anther culture. Donor for the 1BL-1RS chromosome, namely, the strain 911-B-8-10 (abbreviation, st. 911) was crossed to 13 Japanese wheat cultivars. The anther culture was conducted using the F₁ and B₁F₁ plants. Frequency of embryo formation in the F₁s was higher than that in the parental Japanese cultivars. The frequency observed in the B₁F₁s was lower than that in the F₁s. High frequencies of embryo formation in st. 911 and the F₁s were attributable in part to the 1BL-1RS chromosome. Since the leaf rust resistance gene *Lr 26* was located on the 1RS chromosome arm, regenerated haploid seedlings were artificially inoculated by leaf rust (race 21B) to select resistant ones. Doubled haploids with resistance were crossed with (S^v)-Chinese Spring having the 1B chromosome and with (S^v)-Salmon having the 1BL-1RS chromosome. The F₁s of the former cross showed high selfed fertility, while the F₁s of the latter showed complete male sterility. This means that resistant doubled haploids obtained by anther culture can be used as a maintainer for developing male-sterile line.

Key Words : *Aegilops kotschyi* cytoplasm, anther culture, hybrid wheat, 1BL-1RS translocation, cytoplasmic male sterility, fertility restoration.

Introduction

Breeding hybrid wheat has not been successful despite more than 30 years of research efforts, where the G type cytoplasm of *Triticum timopheevi* was used as the source of male sterility. Alternatively, the S^v type cytoplasm of *Aegilops kotschyi* was proposed to be a

promising male sterility source for hybrid wheat breeding by Mukai and Tsunewaki (1979). They found that male sterility was expressed by the S^v type cytoplasm in a 6x derivative of an 8x triticale, named Salmon, that has a 1BL-1RS translocation chromosome instead of the normal 1B wheat chromosome, and that ordinary wheat cultivars had a fertility-restoring gene *Rfv1* on the short arm of the 1B chromosome. The breeding scheme for hybrid wheat utilizing male sterility induced by the S^v type cytoplasm has an extreme advantage that ordinary wheat cultivars having normal 1B chromosome can be used as a restorer, because the degree of freedom in selecting pollen parents for hybrid wheat breeding is quite large. Taking this advantage, we previously proposed a new breeding scheme for hybrid wheat utilizing the interaction between the S^v cytoplasm and the 1BL-1RS chromosome (Toriyama *et al.* 1991, 1993). In this proposal male sterility-maintainer lines are produced by two ways, i. e., the backcross method and anther culture technique. Results obtained by the former method were already reported by Nonaka *et al.* (1993). In this article we report the results obtained by anther culture and discuss some problems related to this technique.

Materials and Methods

As a donor of the 1BL-1RS chromosome to male sterility-maintainer lines, strain 911-B-8-10 (abbreviation, st. 911) was used, that was developed by a Bulgarian wheat breeder, I. Panayotov (1991). The 1BL-1RS chromosome in st. 911 was derived from cv. Skorospelka 35, an offspring of a rye-wheat hybrid. In this chromosome the short arm of the 1B wheat chromosome with the *Rfv1* gene was replaced with the short arm of the 1R rye chromosome by spontaneous translocation. Therefore, (S^v)-911 shows male sterility, and st. 911 is used as the maintainer line of (S^v)-911. They are used in Bulgaria to develop hybrid wheat (Tsujiimoto *et al.* 1985).

St. 911 was crossed to 13 Japanese wheat cultivars belonging to very early to medium (degree of spring habit I ~ IV) maturity classes as recurrent pollen

parents. These are cv. Aobakomugi (IV), Abukumakase (I ~ II), Asakazekomugi (II), Daichinominori (I ~ II), Ebisukomugi (IV), Fukuhokomugi (II), Fukuwasekomugi (II), Nishikazekomugi (I ~ II), Norin 26 (I ~ II), Norin 61 (I ~ II), Shiranekomugi (IV), Shirasagikomugi (II) and Shiroganekomugi (II). They are expected to have the *Rfv1* gene and to express normal fertility in the presence of the S^v cytoplasm. The crosses were made in greenhouse at Ishikawa Agricultural College in 1988, and the F_1 s were backcrossed with the 13 cultivars to produce B_1F_1 seeds in the same year by accelerating generation advancement.

Potato-2 solid medium was used for anther culture to induce haploid plants after Shimada and Otani (1988). Both 2 steps and 1 step culture methods were applied to induce plant regeneration from the anthers of the F_1 plants in 1988, and 1 step culture method to those of the B_1F_1 plants in 1989.

To examine the difference in anther-culture responses between reciprocal crosses, st. 911 was crossed reciprocally with three cultivars, Kitakamikomugi (IV), Nanbukomugi (IV) and Norin 61, in 1990, and the anthers of these reciprocal F_1 s were cultured.

Transmission rate of the 1BL-1RS chromosome to the regenerants from cultured anthers was examined using anthers of the F_1 s between st. 911 and three cultivars, Daichinominori, Nishikazekomugi and Norin 61, in 1991. Culture of the anthers of these F_1 s was conducted with the C_{17} liquid medium in which maltose was added in place of sucrose.

Reaction of young seedlings to leaf rust was determined by artificial inoculation at the 1.5 leaf age with spores of a leaf rust race 21B that was provided by the Tohoku National Agricultural Experiment Station. Doubled haploids were obtained from regenerated green

haploids by 0.01 % colchicine treatment and/or by spontaneous chromosome doubling, and agronomic traits of the doubled haploid strains were evaluated at the Chikugo field of the Kyushu National Agricultural Experiment Station.

The male sterility-inducing effect of the S^v type cytoplasm on the doubled haploid strains was examined by fertility of the F_1 plants between the doubled haploid strains and fertile (S^v)-Chinese Spring and male sterile (S^v)-Salmon as female parent. (S^v)-Chinese Spring was developed by repeated backcrossing *Ae. kotschy* with Chinese Spring normal 1B chromosome and the *Rfv1* gene as recurrent pollen parent. (S^v)-Salmon was developed by backcrossing *Ae. kotschy* with Salmon having the 1BL-1RS chromosome without the *Rfv1* gene as recurrent pollen parent (Tsunewaki 1987).

Results

Breeding of new candidate lines for male sterility-maintainer by anther culture

The results of anther culture applied to the F_1 s between st. 911 and 13 Japanese cultivars were shown in Table 1. Though the number of regenerated green plants per cross differed from 6 to 38 in the 2 steps culture method and 1 to 15 in the 1 step culture method, a total number of regenerated green plants reached 223 (1.6 %) in the former method and 52 (0.5 %) in the latter method. Apparently, the former was more effective than the latter in obtaining green regenerants. During colchicine treatment and/or successive vernalization treatment, about two-thirds of the green regenerants died, and 97 individuals reached maturity. Finally only 11 individuals produced seeds. Offsprings of 9 out of these 11 individuals were resistant to leaf rust, and offsprings of 2 other individuals were

Table 1. Results of anther culture using the F_1 s between 911-B-8-10 and Japanese wheat cultivars

Cultivar crossed to st.911	2 steps culture				1 step culture			
	No. of anthers cultured	No. of embryos formed	No. of green plants	No. of albino plants	No. of anthers cultured	No. of embryos formed	No. of green plants	No. of albino plants
		(%)	(%)	(%)		(%)	(%)	(%)
Aobakomugi	988	124 (12.6)	6 (0.6)	17 (1.7)	—	—	—	—
Abukumawase	1204	121 (6.3)	6 (0.5)	15 (1.2)	1098	28 (2.6)	2 (0.2)	4 (0.4)
Asakazekomugi	1187	98 (8.2)	15 (1.3)	7 (1.4)	439	12 (2.7)	5 (1.1)	2 (0.5)
Daichinominori	1016	243 (23.9)	38 (3.7)	39 (3.8)	—	—	—	—
Ebisukomugi	1233	98 (7.9)	12 (1.0)	11 (0.9)	—	—	—	—
Fukuhokomugi	925	103 (11.1)	7 (0.8)	20 (2.2)	1474	58 (3.9)	6 (0.4)	8 (0.5)
Fukuwasekomugi	1283	237 (18.5)	23 (1.8)	50 (3.9)	765	46 (6.0)	8 (1.0)	7 (0.9)
Nishikazekomugi	1006	150 (14.9)	28 (2.8)	20 (2.0)	2299	120 (5.2)	11 (0.5)	17 (0.7)
Norin 26	619	97 (14.0)	9 (1.3)	17 (2.5)	—	—	—	—
Norin 61	1158	330 (28.5)	28 (3.3)	44 (3.8)	1181	98 (8.3)	15 (1.3)	13 (1.1)
Shiranekomugi	1138	178 (15.6)	27 (2.4)	62 (5.4)	—	—	—	—
Shirasagikomugi	1168	108 (9.2)	6 (0.5)	13 (2.5)	1798	75 (4.2)	4 (0.2)	7 (0.4)
Shiroganekomugi	1093	96 (8.8)	8 (0.7)	17 (1.6)	1683	54 (3.2)	1 (0.1)	12 (0.7)
Total	14090	1983 (14.1)	223 (1.6)	342 (2.4)	10737	491 (4.6)	52 (0.5)	70 (0.7)

susceptible.

Nine doubled haploid strains with leaf rust resistance did not show any segregation in their agronomic traits. Generally speaking, they were short in stature, somewhat late in maturity, resistant to powdery mildew and susceptible to scab. Based on their plant type, culm length, ear type and reaction to scab, 4 strains were selected for further tests. All 4 strains were reconfirmed to be resistant to leaf rust, and were judged to be the 1BL-1RS chromosome carriers. These doubled haploid strains, Therefore, were used as a new maintainer line to develop male-sterile lines by backcrossing them as recurrent parent to (S^v)-Norin 26.

For developing maintainer lines with closer genetic background to Japanese cultivars than single cross derivatives, anther culture was applied to the B₁F₁ plants in 1989 summer. As shown in Table 2, percentages of embryo formation and green plant regeneration in the B₁F₁s decreased considerably, comparing with those in the F₁s (ref. Table 1). For example, percentages of embryo formation and green plant regeneration in the F₁ of 911/Norin 61 were 28.5 % and 3.3 %, but those in the B₁F₁ were 8.3 % and 0.6 %, respectively. The low percentages observed in the

B₁F₁ became close to those of recurrent parent Norin 61.

In total, 27 individuals derived from the B₁F₁s reached maturity, and eight of them produced seeds. Of them, one individual showed segregation in glume color indicating its origin from somatic tissue. The remaining seven were considered to be doubled haploid induced by spontaneous chromosome doubling. Of seven doubled haploid strains, only three were determined to be 1BL-1RS chromosome carriers by their resistant reaction to leaf rust. They were used as a maintainer line to develop new male-sterile lines possessing close genetic background to Japanese cultivars than single cross derivatives.

Male sterility-inducing effect of the S^v type cytoplasm on doubled haploid strains

Of the 11 doubled haploid strains originated from the F₁s in the first experiment, six were crossed as pollen parent with (S^v)-Chinese Spring and (S^v)-Salmon in 1989. Selfed and crossed seed fertilities of the F₁s are shown in Table 3. All four doubled strains with leaf rust resistance produced highly self-fertile F₁s when

Table 2. Results of anther culture using the B₁F₁s between 911-B-8-10 and Japanese wheat cultivars as the backcross parent

Backcross parent	No. of anthers cultured	No. of embryos formed	No. of green plants	No. of albino plants
		(%)	(%)	(%)
Aobakomugi	951	124 (5.7)	4 (0.4)	1 (0.1)
Abukumawase	1005	24 (2.4)	0 (0)	2 (0.2)
Asakazekomugi	1611	39 (2.4)	0 (0)	2 (0.1)
Daichinominori	2884	212 (7.4)	7 (0.2)	30 (1.0)
Ebisukomugi	1271	47 (3.7)	2 (0.2)	0 (0)
Fukuhokomugi	991	53 (4.3)	2 (0.2)	11 (1.1)
Fukuwasekomugi	1351	58 (4.3)	1 (0.1)	8 (0.6)
Nishikazekomugi	813	31 (3.8)	0 (0)	2 (0.2)
Norin 26	798	37 (4.6)	0 (0)	3 (0.4)
Norin 61	1253	104 (8.3)	8 (0.6)	36 (2.9)
Shiranekomugi	1230	65 (5.3)	3 (0.2)	6 (0.5)
Shirasagikomugi	1987	40 (2.6)	0 (0)	2 (0.1)
Shiroganekomugi	1868	49 (2.6)	0 (0)	11 (0.6)
Total	18013	883 (4.90)	27 (0.15)	114 (0.63)

Table 3. Selfed seed fertility and leaf rust reaction of doubled haploid strains produced by anther culture, and selfed seed fertility of the F₁s between them and (S^v)-Chinese Spring or (S^v)-Salmon as female parent

Strain no.	Origin	Doubled haploid Self-fertility		Leaf rust reaction	Self-fertility of F ₁ (1990)	
		1989	1990		(S ^v)-Chinese Spring	(S ^v)-Salmon
		(%)	(%)		(%)	(%)
R2-20	911/Norin 26	59.8	65.3	R	49.3	0.0
R5-1	911/Shirasagikomugi	68.3	62.4	R	47.8	0.0
R6-3	911/Shiroganekomugi	96.2	79.0	R	69.1	0.0
R9-5	911/Fukuwasekomugi	93.4	84.0	R	60.1	0.0
R10-30	911/Nishikazekomugi	83.8	86.4	S	95.0	—
R13-21	911/Daichinominori	82.2	72.9	S	77.3	54.3

crossed with (S^v)-Chinese Spring, whereas completely self-sterile F₁s when crossed with (S^v)-Salmon. This indicates that the resistant doubled haploid strains are the 1BL-1RS chromosome carrier. On the contrary, susceptible doubled haploid strains produced fully self-fertile F₁s crossed to both (S^v)-Chinese Spring and (S^v)-Salmon. This indicates that the susceptible doubled haploid strains have the 1B chromosome with the *Rfv1* gene.

Difference between reciprocal crosses in regeneration frequency in anther culture

Of three Japanese cultivars used in this experiment, Nanbukomugi and Norin 61 are characterized by high ability of embryo formation but low frequency of green plant regeneration in anther culture, and Kitakamikomugi is characterized by low ability of embryo formation. While the common parent, st. 911, has extremely high ability of embryo formation with low frequency of green plant regeneration. No difference between reciprocal crosses was detected as shown in Table 4.

Embryo formation ability of the reciprocal F₁s of st. 911 with Nanbukomugi and Norin 61 was at the nearly same, high level as that in st. 911, and that of the F₁s

with Kitakamikomugi was intermediate between those of the two parents. While, frequency of green plant regeneration increased obviously in the F₁s with Norin 61, but did not differ in the F₁s with Kitakamikomugi and Nanbukomugi comparing to those of their parents. These results indicate that high ability of embryo formation in st. 911 is a dominant character, and that increased green plant regeneration frequency in the F₁s is determined by interaction between genes in both parents, i. e., st. 911 and Japanese cultivars.

Transmission of the 1BL-1RS chromosome to plants regenerated from microspore by anther culture

Transmission rate of the 1BL-1RS chromosome was estimated from the frequency of leaf rust resistant individuals among regenerants from cultured anthers. Since the 1BL-1RS chromosome carriers have the leaf rust resistance gene *Lr 26* on the 1RS chromosome arm, frequency of the leaf rust resistant individuals should give transmission rate of the 1BL-1RS chromosome to plants regenerated from microspore by anther culture.

In this experiment C₁₇ liquid medium containing maltose as a carbohydrate source was used, that improved greatly frequency of green plant regeneration comparing to the former experiments using Potato-2

Table 4. Abilities of embryo formation and green plant regeneration of reciprocal F₁s between st. 911 and Norin 61, Nanbukomugi or Kitakamikomugi

Parent and F ₁	No. of anthers cultured	No. of anthers with embryo	No. of embryos formed	No. of embryos inoculated	No. of plants regenerated	
					green	albino
		(%)	(%)		(%)	(%)
911	593	115 (19.4)	381 (64.2)	415	2 (0.5)	143 (34.5)
Kitakamikomugi	669	5 (0.7)	6 (0.9)	8	0 (0)	2 (25.0)
911/Kitakamikomugi	575	46 (8.0)	79 (13.7)	111	2 (1.8)	24 (21.6)
Kitakamikomugi/911	593	28 (4.7)	149 (8.3)	124	4 (3.2)	29 (23.4)
Nanbukomugi	677	64 (9.5)	152 (22.5)	149	2 (1.3)	13 (8.7)
911/Nanbukomugi	579	94 (16.2)	316 (54.6)	368	2 (0.5)	75 (20.4)
Nanbukomugi/911	581	125 (21.5)	354 (60.9)	372	10 (2.7)	75 (20.2)
Norin 61	580	74 (12.8)	201 (34.0)	103	0 (0)	19 (18.4)
911/Norin 61	582	143 (24.6)	369 (63.4)	415	17 (4.1)	124 (29.9)
Norin 61/911	927	207 (22.3)	675 (72.8)	632	43 (6.8)	203 (32.1)

Table 5. Results of anther culture and leaf rust reaction of the regenerants from cultured anthers of st. 911, three Japanese cultivars and their hybrids

Parent and F ₁	No. of anthers cultured	No. of embryos formed	No. of embryos transferred	No. of green plants	No. of albino plants	No. of plants		
						Tested	Resistant	Susceptible
		(%)		(%)	(%)			
Daichinominori	106	49 (46.2)	33	3 (9.0)	4 (12.1)	3	0	3
Nishikazekomugi	164	22 (13.4)	24	3 (12.5)	7 (29.2)	1	0	1
Norin 61	411	183 (44.5)	138	1 (0.7)	62 (44.9)	—	—	—
911	333	538 (161.6)	220	80 (36.4)	26 (11.8)	23	23	0
Daichinominori/911	321	619 (192.8)	388	141 (36.3)	9 (2.3)	72	31	41
Nishikazekomugi/911	279	124 (44.4)	108	19 (17.6)	11 (10.2)	19	4	15
Norin 61/911	456	1372 (300.9)	546	225 (41.2)	40 (7.3)	152	76	76
Subtotal	1056	2115 (200.3)	1042	385 (36.9)	60 (5.8)	243	111	132

solid medium as shown in Table 5 (ref. Table 1, 2 and 4). Results of testing reactions of young seedlings to leaf rust were also shown in Table 5.

All regenerants from Japanese cultivars were susceptible to leaf rust indicating presence of the 1B chromosome without *Lr 26* gene in them. On the contrary, all regenerants from st. 911 were resistant indicating presence of the 1BL-1RS chromosome with the *Lr 26* gene in this strain. From the leaf rust reaction data on the F_1 s (Table 5), transmission rate of the 1BL-1RS chromosome was estimated to be 45.7%. This frequency suggests that the 1BL-1RS chromosome is transmitted at an equal frequency as the 1B chromosome to plants regenerated from microspores by anther culture.

Discussion

Anther culture technique has great advantage in producing homozygous lines for plant breeding and offers an efficient method of isolating recessive mutants. Among common wheat cultivars, genotypic variation in anther culture response has been noticed and the ability of microspores to produce pollen embryos and that of the embryos to regenerate into green plantlets have been shown to be controlled by nuclear genes (Lazar *et al.* 1984, Deaton *et al.* 1987).

There have been several attempts made to investigate the influence of specific chromosomes and chromosome arms on anther culture response through the use of chromosome substitution and translocation lines (Henry and DeBuyser 1985, Agache *et al.* 1989, Ikeguchi 1989, Muller *et al.* 1989, 1990). Some of them noticed the effect of rye chromosomes incorporated into wheat : lines containing the 1BL-1RS translocated chromosome give a good androgenetic response, and one of the genetic factors involved in anther culture response is suggested to be on the 1RS chromosome arm. On the other hand, Lazar *et al.* (1987) observed that 4R chromosome of rye significantly improved anther culture response, whereas 1R chromosome did not.

We found that percentages of embryo formation and green plant regeneration were extremely high in st. 911 that carries the 1BL-1RS chromosome, and in the F_1 s between st. 911 and Japanese cultivars, but they dropped considerably in the B_1F_1 s to almost the same level as those of Japanese cultivars used as recurrent parent. Extremely high percentages of embryo formation and green plant regeneration in st. 911 and the F_1 s may be determined by the 1BL-1RS chromosome as suggested by Ikeguchi (1989). However, the F_1 s from different cross combinations showed different anther culture response including green plant regeneration ability (Table 4). These facts suggested that the genotypes of both parents are involved in determining the anther culture responses.

Relative transmission rates of the 1BL-1RS chromosome and the normal 1B chromosome were

almost the same (45.7 % vs. 54.3 %) From this we can expect that about half of the plants regenerated from anther culture will be used as male sterility-maintainer.

There are some important genetic problems related to hybrid wheat breeding. One is complete fertility restoration by single dose of the *Rfv1* gene at the presence of the S^v type cytoplasm. Mukai (1983) reported that single dose of the *Rfv1* gene was not sufficient to restore complete male fertility when a 1BS chromosome arm was deficient. We previously reported that fertility restoration attained by single dose of the *Rfv1* gene was nearly complete when an Asian cultivar population was used as basic germplasm for hybrid, while was not sufficient enough when an European cultivar population was used (Nonaka *et al.* 1993). In this work we also observed high fertility of the F_1 s between (S^v)-Chinese Spring and doubled haploid strains with the 1BL-1RS chromosome.

Though production of male-sterile lines by back-crossing was successfully conducted (Nonaka *et al.* 1993), the genotype of these male sterility-maintainers must be nearly same genotype of the recurrent parent except the 1BL-1RS chromosome. However, the male sterility-maintainers produced by anther culture are expected to be new genotype other than existing cultivars, but number of green plants regenerated through anther culture using Potato-2 solid medium was not large enough for practical breeding work. Otani and Shimada (1990) reported that C_{17} liquid medium containing maltose as a carbohydrate source gave better results than Potato-2 solid medium for anther culture of wheat. Our results confirmed their finding. Our results also indicated that high fertility restoration ability of the F_1 s between (S^v)-Chinese Spring and doubled haploids, encouraging use of the latter as the male sterility-maintainer. Consequently, in addition to the improved anther culture technique, other efficient methods to develop doubled haploid including use of wheat $\times$ maize crosses (Suenaga and Nakajima 1989) must be applied for adapting practical breeding by getting a large number of doubled haploids.

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