Efficient Anther Culture of F₁ Plants Derived from Koshihikari and its Related Cultivars in the Japonica Rice

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The efficiency of anther culture of Koshihikari and its related cultivars with high eating quality i.e., Hitomebore, Akitakomachi and Kinuhikari was improved by using DKN medium as basal medium in the three-step anther culture method. The anther culture efficiency of F₁ plants derived from crosses between these cultivars was also improved. In this culture method, a positive correlation was recognized between the anther culture efficiency and the coefficient of parentage of the donor plant to Koshihikari. Therefore, this method was an effective means for the anther culture of materials derived from crossings between cultivars related to Koshihikari. On the other hand, the results implied that F₁ pollen which has a definite genetic background was regenerated preferentially. To examine the segregation of characteristics both in the anther culture-derived population and F₂ population, two populations were produced from the F₁ plant derived from a cross between the long-culmed medium-ripening cultivar Koshihikari and short-culmed early-ripening cultivar Niigatawase. No significant differences were observed in the segregation of heading date or culm length between the two populations. It is concluded that the anther culture method developed in this study is a useful tool for breeding of rice by using Koshihikari and its related cultivars as a crossing parent.

Key Words: *Oryza sativa*, Koshihikari, anther culture, high eating quality, coefficient of parentage, rice breeding.

Introduction

The japonica rice cultivar Koshihikari is highly evaluated for palatability in the market, and is planted widely in western area of Japan. After awarded as the best rice in 1979, the planted acreage of Koshihikari in Niigata Prefecture has kept increasing and the ratio of its planted acreage to the whole cultivation area of all nonglutinous cultivars became 80% or more in 1998. Nowadays, it is impossible to harvest at the most suitable ripening period because the harvest work is concentrated over a certain short period and

there is anticipation that grain quality and eating quality will be deteriorated. It is not a desirable situation for rice cultivation, because the expansion of one cultivar would increase the risk of disease injury, insect injury, and weather disaster. Therefore, development a new early-ripening cultivar which has high eating quality equivalent to Koshihikari is awaited.

In order to shorten the period needed for producing a new rice cultivar, the anther culture method has been used. We have reported an efficient method for anther culture of Koshihikari (Daigen *et al.* 2000). The culture method is characterized by the application of DKN medium developed for Koshihikari callus culture as the basal medium in the three-step anther culture method. In this study, the efficiency of anther culture of Koshihikari, its related cultivars and F₁ plants derived from a cross between these cultivars was investigated. Then, the relationship between anther culture efficiency of donor plant and its coefficient of parentage to Koshihikari were investigated, and whether the anther culture method was able to be used for rice breeding was discussed.

Yamagishi *et al.* (1996) examined whether parental alleles were randomly transmitted from heterozygous donor plants to anther culture-derived plants by RFLP analysis, and indicated that parental alleles were not randomly transmitted from the F₁ plant to the anther culture-derived plants. Because DKN medium was developed for Koshihikari callus culture, we suspected that definite pollen grains in F₁ plant were regenerated preferentially through the anther culture. Thus, we examined the distributions of the culm length and the heading date in the anther culture-derived population and F₂ population.

Materials and Methods

Anther culture efficiency of Koshihikari, its relatives and their F₁ plants

Koshihikari and 4 high-eating quality rice cultivars, i.e., Hitomebore, Akitakomachi, Kinuhikari and Niigatawase, were grown in the field. The anther culture of these cultivars and 6 F₁ plants derived from the crosses between the cultivars i.e., Koshihikari×Hitomebore, Kinuhikari×Hitomebore, Koshihikari×Niigatawase, Kinuhikari×Akitakomachi, Niigatawase×Hitomebore, Niigatawase×Akitakomachi, was carried out by the three-step culture method (Tsugawa 1992) in which DKN medium or 1/2R2 medium were used as basal medium. The details of these media were as reported separately (Daigen *et al.* 2000).

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Comparison of distribution of culm length and heading date in anther culture-derived population and F₂ population

The anthers of F₁ plant derived from a cross between Koshihikari and Niigatawase were cultured by the three-step culture method in which DKN medium was used as basal medium. The green plants (A₁) which regenerated from the anthers were planted in potted soils and grown in a greenhouse, and A₂ seeds were yielded from the fully fertile regenerants (here 'A' represents regenerants. A₂ means the 2nd generation of regenerants). Fourteen seeds on each panicle of the regenerants were raised as a panicle line. One hundred and four lines were grown to maturity in a paddy field and the heading date and the culm length were determined.

F₂ seeds (100 individuals) derived from a cross between Koshihikari and Niigatawase were also grown in the same field, and the heading date and the culm length of each individual were investigated. Fertilizers applied were 0.5 kg/a for N, P₂O₅ and K₂O, respectively, and plant spacing was 15×30 cm. This experiment was carried out in 1996. Seeds were sown on April 22 and seedlings were transplanted on May 22, respectively.

Results and Discussion

Anther culture efficiency of Koshihikari, its related cultivars and their F₁ plants

Table 1 shows the results of anther culture of 5 cultivars and F₁ plants derived from 6 crosses by using the three-step anther culture method in which DKN medium or 1/2R2 medium was used as the basal medium. In the cultivars except Niigatawase, both the callus formation rate and plant regeneration rate observed when DKN medium was used as the basal medium were higher than those observed when 1/2R2 medium was used. The green plant regeneration rate per anther, that is, the index of the work efficiency (G/A ratio), was 2.7 to 35 times higher in cultures using DKN medium than in cultures using 1/2R2 medium. G/A ratios of F₁ plants cultured with DKN medium were higher than or equal to those with 1/2R2 medium in all crosses.

The results also indicated that the efficiency of culture was highly variable with the cultivars used. As DKN medium was developed for anther culture of Koshihikari in which cell culture had been unsuccessful (Daigen *et al.* 2000), it was thought that the efficiency of anther culture depended on the genotype. Then, the relationship between the efficiency and the coefficient of parentage to Koshihikari were tested. The coefficients of parentage to Koshihikari were computed

Table 1. Anther culture efficiency of high eating quality cultivars and F₁ plants in the three-step culture method and comparison of DKN medium and 1/2R2 medium

Materials	Name of cultivar and crossing parent ¹⁾	Coefficient parentage to Koshihikari	Basal medium	No. of anthers inoculated (A)	No. of calli formed (C)	Callus formation rate (%)	No. of calli regenerated			G/C (%)	G/A (%)
							Green plant (G)	Albino plant (al)	Total (G)+(al)		
Cultivar	Koshihikari	1.000	DKN	500	1200	240.0	193	194	387	16.1	38.6
			1/2R2	450	158	35.1	8	16	24	5.1	1.8
	Hitomebore	0.796	DKN	500	770	154.0	185	206	391	24.0	37.0
			1/2R2	500	510	102.0	23	55	78	4.5	4.6
	Akitakomachi	0.617	DKN	500	993	198.6	105	377	482	10.6	21.0
			1/2R2	500	540	108.0	3	10	13	0.6	0.6
	Kinuhikari	0.534	DKN	500	400	80.0	123	60	183	30.8	24.6
			1/2R2	500	164	32.8	46	12	58	28.0	9.2
	Niigatawase	0.238	DKN	500	248	49.6	42	45	87	16.9	8.4
			1/2R2	500	246	49.2	74	21	95	30.1	14.8
F ₁ plant	Ko×Hi	0.898	DKN	500	1425	285.0	178	218	396	12.5	35.6
			1/2R2	500	293	58.6	8	3	11	2.7	1.6
	Ki×Hi	0.665	DKN	450	375	83.3	54	85	139	14.4	12.0
			1/2R2	500	194	38.8	38	21	59	19.6	7.6
	Ko×Ni	0.619	DKN	500	406	81.2	61	167	228	15.0	12.2
			1/2R2	500	278	55.6	31	86	117	11.2	6.2
	Ki×Ak	0.576	DKN	500	575	115.0	105	63	168	18.3	21.0
			1/2R2	500	775	155.0	107	82	189	13.8	21.4
	Ni×Hi	0.517	DKN	500	368	73.6	37	87	124	10.1	7.4
			1/2R2	500	200	40.0	9	40	49	4.5	1.8
	Ni×Ak	0.428	DKN	500	759	151.8	92	224	316	12.1	18.4
			1/2R2	500	535	107.0	22	63	85	4.1	4.4

¹⁾ Ko: Koshihikari, Hi: Hitomebore, Ki: Kinuhikari, Ni: Niigatawase, Ak: Akitakomachi

by Yoshida's method (Oosato and Yoshida, 1996). Fig. 1 shows the relationship between the callus formation rate and the coefficient of parentage to Koshihikari. The correlation coefficient was 0.758 and significant at the 1% level. Fig. 2 shows the relationship between the green plant regeneration rate per anther and the coefficient of parentage to Koshihikari. The correlation coefficient was 0.802 and significant at the 1% level. These results indicate that the efficiency of anther culture by using DKN medium is positively correlated to the coefficient of parentage to Koshihikari.

It is said that the callus induction and the plant regeneration from the callus is difficult in cv. Koshihikari (Iwai *et al.* 1990, Abe 1992). Recently, Yoshida and Oosato (1998)

Fig. 1. Relationship between callus formation rate and coefficient of parentage to Koshihikari.

Fig. 2. Relationship between green plant regeneration rate and coefficient of parentage to Koshihikari.

found a negative correlation in the root regeneration rate from embryo callus on N₆ medium and the coefficient of parentage to Koshihikari. The anther culture method developed by us will make a breakthrough in developing fixed lines after crossing between high eating quality cultivars, i.e., Koshihikari and its related cultivars.

Comparison of distribution of culm length and heading date in anther culture-derived population and F₂ population

The findings that the efficiency of anther culture was positively correlated with the coefficient of parentage to Koshihikari, however, aroused our suspicion that F₁ pollen which had a definite genetic background, that is, the pollen transmitted more chromosomes from one parent "Koshihikari" than from the other parent, regenerated preferentially in the medium used. Thus, we examined the variation of the characteristics (heading date and culm length) of fixed lines in A₂ generation. Fourteen individuals in each panicle of A₁ plants were raised as a panicle line. When all 14 individuals in a line had the same heading date and culm length, the line was defined as a fixed line, that is, the regenerant (A₁ plant) which gave the line was a doubled haploid. Sixty three lines in 104 lines were determined as fixed lines. The mean heading date and the culm length of 14 individuals of 63 fixed lines and heading dates and culm lengths of 100 individuals in F₂ population were plotted in Fig. 3. The distribution of the heading date and the culm length in anther culture-derived population was similar to that in the F₂ population.

Yamagishi *et al.* (1996) found by RFLP analysis that parental alleles were not randomly transmitted from the F₁ plant between distantly related cultivars of Nipponbare (*japonica* type) and Milyang 23 (*indica* type) to the anther culture-derived plants. In the present study in which two *japonica* rice cultivars were used as a crossing parent,

Fig. 3. Distribution of heading date and culm length in anther culture-derived population and F₂ population.

however, the distribution of culm length and heading date in the anther culture-derived population and that in the F_2 population shown in Fig. 3 suggest that there was no segregation distortion of characteristics examined in regenerated plants derived from anther culture of an F_1 hybrid of Koshihikari and Niigatawase. Though the efficiency of anther culture by using DKN medium in the three-step culture method was positively correlated with the coefficient of parentage to Koshihikari, it was clear that the definite pollen in the F_1 plant was not regenerated preferentially through this anther culture method.

The ripening period of Koshihikari is comparatively late and its lodging resistance is very low. Therefore, the development of a new early-ripening cultivar which has eating quality as high as Koshihikari and high lodging resistance is eagerly awaited in Niigata Prefecture. The anther culture method in this study will be a useful tool for breeding of rice by using Koshihikari and its related cultivars as a crossing parent.

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