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F. Taguchi-Shiobara · T. Komatsuda · S. Oka

Comparison of two indices for evaluating regeneration ability in rice (*Oryza sativa* L.) through a diallel analysis

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Abstract A full diallel analysis was performed among seven rice cultivars, all of which showed different abilities of regeneration from seed-derived calli. Number of regenerated shoots and regeneration frequency were used as indices of regeneration ability. In both cases, additive effects were significant at the 0.1% level, and dominant genes had a positive effect, that is, they increased regeneration ability. Non-allelic interaction (epistasis) and maternal effects were not detected. Dominance effects were significant at the 1% and the 0.1% level when the number of regenerated shoots and regeneration frequency were used as indices, respectively. Average degree of dominance was 0.531 for the shoot regeneration index and 0.990 for the regeneration frequency index. Since broad-sense heritability was 0.919 for number of regenerated shoots and 0.736 for regeneration frequency, the former was considered to be a better index of regeneration ability than the latter.

Key words Diallel analysis · Regeneration ability · Rice · *Oryza sativa* L. · Heritability

Introduction

Genetic factors are considered to be involved in tissue culture ability because of the different culture responses observed between cultivars or lines. Recently, several genes contributing to regenerable callus formation have been mapped as quantitative trait loci (QTL) in corn, tomato, and barley using many DNA markers (Armstrong et al. 1992; Cowen et al. 1992; Wan et al. 1992; Koornneef et al. 1993; Komatsuda et al. 1993). A higher regeneration ability is expected when these genes are transferred to cultivars of low regeneration ability.

In rice, separate diallel analyses were performed to shed light on the mode of plant regeneration from cultured cells derived from immature embryos (Peng and Hodges 1989), anther (Quimio and Zapata 1990), and mature seed (Abe and Futsuhara 1991; Tsukahara et al. 1995). Additive and dominance effects were significant in all four investigations. However, the direction of the dominance gene effect, the presence or absence of non-allelic interactions (epistasis), and maternal effects were not consistent – discrepancies which can be attributed to cultivar differences and culture conditions used in these experiments. In addition, the measurement of regeneration ability affected some of the results. Peng and Hodges (1989) used two indices to evaluate regeneration ability, the number of regenerated plants per callus and the frequency of plant regeneration. The importance of additive effects and dominance effects was not the same for these two indices.

We performed a genetic analysis of regeneration ability from rice seed calli using a seven-parent full diallel set of crosses. Seed calli were used because it is the most widely used source of material for producing transgenic plants. Seven cultivars of the *japonica*, *indica* and *javanica* ecogeographic races were chosen because of their different phenotypes in regeneration from callus and also their normal F_1 fertility in inter-subspecific crosses (Taguchi-Shiobara et al. in preparation). Our objectives were (1) to search for better indices for evaluating regeneration ability; (2) to clarify the mode of inheritance of regeneration ability from cultured cells of mature seeds.

Materials and methods

A 7×7 diallel set of crosses (including parents and reciprocals) was performed between cultivars originating from Japan, India,

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F. Taguchi-Shiobara (✉) · T. Komatsuda · S. Oka
National Institute of Agrobiological Resources, 2-1-2 Kannondai, Tsukuba, Ibaraki Prefecture 305, Japan

Malaysia, and the Philippines (Table 1). Between 10 and 30 seeds per cultivar were dehusked and sterilized in a 1% sodium hypochlorite solution for 30 min, followed by mild shaking in sterile distilled water for 30 min; this sterilization-washing procedure was repeated one more time. Ten of the sterilized seeds were plated on one 9-cm dish containing 50 ml of the callus-inducing medium. The medium contained salts and vitamins of N6 medium (Chu et al. 1975) and was supplemented with 30 g/l sucrose, 0.3 g/l casein acid hydrolysate, 1.15 g/l proline, 1 mg/l 2,4-dichlorophenoxyacetic acid (2,4-D) (Ozawa and Komamine 1989) solidified with 2.5 g/l gellun gum (pH 5.8). The seeds were incubated in the dark at 28°C for 4 weeks. Calli induced from 1 seed were crushed and transferred to one 100-ml flask containing 20 ml of the subculture medium, the contents of which was the same as that of the callus-inducing medium but without gellun gum. The flasks were kept on a rotary shaker at 133 rpm with a 70 mm stroke, and cultures were incubated in the dark at 30°C for 1 week.

Excess medium on calli was absorbed with layers of filter paper (Tsukahara and Hirosawa 1992). Ten calli 1 mm in diameter originating from 1 seed were plated on a 9-cm dish containing 25 ml of the shoot-inducing medium: salts and vitamins of the N6 medium supplemented with 30 g/l sucrose, 1 mg/l NAA, 5 mg/l kinetin, and 2.5 g/l gellun gum (Wako Pure Chemical Industries Ltd, Osaka, Japan) (pH 5.8) after minor modifications of Ozawa and Komamine (1989). After incubation under light at 28°C for 4 weeks, the average number of regenerated shoots per callus and the regeneration frequency (frequency of regenerated calli against total number of calli) were calculated for each dish, and the mean of both indices for five dishes were used to represent each of the 49 genotypes. These procedures from induction of calli to regeneration of shoots were replicated twice for all 49 genotypes.

Data for the two indices with two replications were analyzed according to the method of Hayman (1954a, b) using a computer program "DIALL" developed by Ukai (1989). Prior to analysis, regeneration frequency (P) data were transformed using arc-sine transformation ($x = P\hat{}0.5$). Broad- and narrow-sense heritabilities were calculated after Mather and Jinks (1971).

Results

The number of regenerated shoots per callus for each of the seven parent cultivars ranged from 0.18 to 6.44, while the regeneration frequency ranged from 0.14 to 0.99 (Table 1). The lowest regeneration ability was observed for 'Koshihikari' and the highest for 'Kasalath', for both indices. Among 42 F_1 combinations, that between 'Koshihikari' and 'Yamahoushi' showed the lowest regeneration ability, while the highest was shown by the F_1 between 'Aus373' and 'Kasalath'.

Analysis of variance of a diallel table for regeneration ability showed that additive and dominance effects were significant (Table 2). Additive effects were significant at the 0.1% level for both indices. The significance level for dominant effects was 1% for number of regenerated shoots per callus, and 0.1% for regeneration frequency. No significant reciprocal differences were observed, suggesting that there were no maternal effects.

Figure 1 shows array variance (Vr) and array parent-offspring covariance (Wr) for regeneration ability based on the full diallel (Table 1) with replications.

Table 1 Diallel table for regeneration ability (average of two replications)

Number of regenerated shoots per callus

Female (Origin)	Male						
	Ecogeographic race						
	japonica			*indica*			*javanica*
	1	2	3	4	5	6	7
1 Nipponbare (Japan)	0.50	0.90	1.12	5.15	2.12	2.21	1.36
2 Koshihikari (Japan)	1.39	0.18	0.65	5.49	3.01	2.30	0.74
3 Yamahoushi (Japan)	0.94	0.72	0.84	3.41	3.52	2.35	1.05
4 Kasalath (India)	2.38	4.72	4.57	6.44	6.89	5.31	5.25
5 Aus373 (India)	3.22	2.93	3.83	7.65	2.92	4.57	1.84
6 CP-SLO (Malaysia)	2.41	2.69	3.18	5.63	6.74	4.23	1.67
7 Calotoc (Philippine)	1.30	1.80	1.78	4.00	2.88	3.30	1.75

Regeneration frequency

Female	Male						
	1	2	3	4	5	6	7
1 Nipponbare	0.34	0.58	0.76	0.92	0.68	0.63	0.53
2 Koshihikari	0.74	0.14	0.45	1.00	0.84	0.88	0.49
3 Yamahoushi	0.53	0.39	0.38	0.77	0.84	0.85	0.61
4 Kasalath	0.87	0.92	0.86	0.99	0.94	0.82	0.81
5 Aus373	0.87	0.84	0.79	0.92	0.58	0.87	0.43
6 CP-SLO	0.87	0.87	0.95	0.84	0.97	0.84	0.59
7 Calotoc	0.58	0.68	0.77	0.68	0.63	0.76	0.70

Table 2 Analysis of variance of diallel tables for regeneration ability after Hayman (1954a)

Source[a]	*df*	Number of regenerated shoots			Regeneration frequency		
		SS	MS	*F*	SS	MS	*F*
a	6	143.76	23.96	75.07***	0.827	0.138	13.62***
b	21	16.04	0.76	2.39**	0.803	0.038	3.78***
b_1	1	2.64	2.64	8.26**	0.208	0.208	20.56***
b_2	6	5.09	0.85	2.66*	0.274	0.046	4.52***
b_3	14	8.32	0.59	1.86	0.321	0.023	2.27*
c	6	2.33	0.39	1.22	0.073	0.012	1.20
d	15	9.89	0.66	2.07*	0.111	0.007	0.73
Error	(48)	0.32			0.010		

*, **, *** Significant at the 0.05, 0.01, and 0.001 probability levels, respectively

[a] a, Additive effect; b, dominance effect; b_1, mean dominance deviation; b_2, dominance deviation due to each parent; b_3, dominance deviation due to each crossing combination; c, maternal effect; d, reciprocal differences not ascribable to "c"

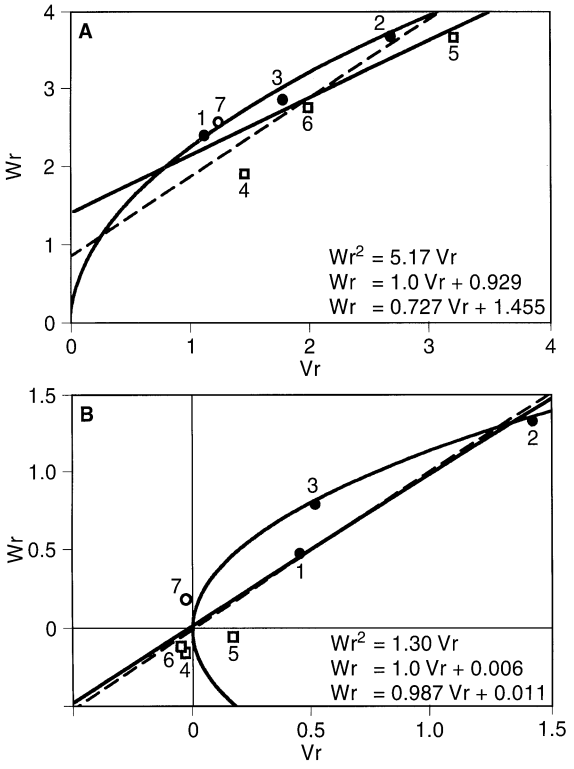

Fig. 1A, B (Vr, Wr) graphs for regeneration ability, from 7×7 diallel table with two replications. *1*, 'Nipponbare', *2* 'Koshihikari', *3* 'Yamahoushi', *4* 'Kasalath', *5* 'Aus373', *6* 'CP-SLO', *7* 'Calotoc'. **A** Number of regenerated shoots per callus, **B** Regeneration frequency. Arc-sine-transformed frequencies were used to obtain values of Vr and Wr. ● *japonica*, □ *indica*, ○ *javanica*

Non-allelic interactions were not recognized because there are no significant differences in the slope of the regression of Wr on Vr from unity and the homogeneity of Wr-Vr. Cultivars plotting close to the upper end of the regression line possess more recessive alleles, while those at the lower end possess more dominant alleles. For number of regenerated shoots per callus, 'Koshihikari' and 'Aus373' possessed more recessive alleles, whereas 'Nipponbare', 'Kasalath', and 'Calotoc' possessed more dominant alleles. 'Yamahoushi' and 'CP-SLO' were intermediate between these cultivars. For regeneration frequency, *indica* and *javanica* cultivars possessed most dominant alleles, and one *japonica* cultivar, 'Koshihikari', had the most recessive alleles. The other two *japonica* cultivars were intermediate. The intercept of the regression line for number of regenerated shoots was above the origin and that for regeneration frequency was at the origin, suggesting partial dominance and compelte dominance, respectively.

Table 3 Genetic parameters for regeneration ability

Parameter	Regeneration ability	
	Number of regenerated shoots	Regeneration frequency
D	5.173	1.302
F	−1.088	1.168
H_1	1.458	1.277
H_2	0.966	0.907
h^2	1.258	1.821
E	0.319	0.171
SQRT(H_1/D)	0.531	0.990
h	1.14	1.41
uv	0.166	0.178
h^2(bs)	0.919	0.736
h^2(ns)	0.858	0.387
r(Pr, Wr + Vr)[a]	−0.264	−0.933

[a] Correlation between Pr and Wr + Vr

Genetic parameters for regeneration ability obtained in this study are shown in Table 3. Additive genetic variance (D) was threefold, larger than the dominance genetic variances (H_1 and H_2) for number of regenerated shoots. For regeneration frequency, the additive and dominance variance were almost the same value.

The degree of dominance ($(H_1/D)\hat{}1/2$) indicated partial dominance for number of regenerated shoots per callus and complete dominance for regeneration frequency. Broad- and narrow-sense heritabilities were 0.919 and 0.858 for number of regenerated shoots, and 0.736 and 0.387 for regeneration frequency, respectively.

The correlation between (Wr + Vr) and the parental values were very close for regeneration frequency (-0.933, $P < 0.01$) but insignificant for the other index (Table 3). The negative correlation suggested a trend that dominant genes increase regeneration frequency. It may be possible to estimate the selection limit for high regeneration frequency from the value of Vr + Wr corresponding to a possible complete dominant genotype in the (Vr, Wr) graph. For the number of regenerated shoots, some dominant genes act positively, while others act negatively.

Discussion

When regeneration ability is evaluated, regeneration frequency is often adopted as an index because it is simple to calculate. In this experiment, two indices, number of regenerated shoots per callus and regeneration frequency, were compared for the first time for their accuracy in evaluating regeneration ability. Number of regenerated shoots per callus gave a higher broad-sense heritability (Table 3) than regeneration frequency and, therefore, we consider it to be a better index for QTL analysis.

Table 4 shows broad- and narrow sense-heritabilities and average degree of dominance calculated from data obtained from previous reports. Number of regenerated plants (=shoots) also gave a higher broad-sense heritability in the report of Peng and Hodges (1989), although the values were much lower than those we report here. Among all of the heritabilities, regeneration frequency of anther calli gave the highest broad-sense heritability, 0.952. It was much higher than other values of heritability for regeneration frequency, i.e. 0.675 (immature embryo calli), 0.81 and 0.736 (seed calli). The mechanism for the regeneration of anther calli might be different from that of calli derived from either the mature or immature embryo.

Broad- and narrow-sense heritabilities for the number of regenerated shoots in this report was the highest among three reports in which calli from mature or immature embryo were used (Table 4). According to a simulation study on diallel analysis by Ukai (1991), the reliability of the results of diallel analysis is low unless the broad-sense heritability is 0.8 or higher. The reliability of our diallel analysis is high since heritability values reached 0.919. The highest narrow-sense heritability for the number of regenerated shoots, 0.858, and absense of epistasis allow us to obtain cultivars or lines of high regeneration ability by gene accumulation. High-regeneration-ability lines would make the production of transgenic plants more efficient and contribute to the use of rice as an experimental plant.

Average degree of dominance for number of regenerated shoots was 0.531, while for regeneration frequency it was 0.990 (Table 3). Peng and Hodges (1989) also reported a difference between these two indices; additive effects and dominance effects were of nearly equal importance for the number of regenerated plants per callus, while for plant regeneration frequency, additive effects were about twofold higher than dominant effects in their analysis of variance. These results suggest that the set of genes controlling number of regenerated shoots is not the same as that controlling regeneration frequency.

Table 4 Heritabilities and average degree of dominance for regeneration ability calculated by "DIALL" using data from five reports

Reporter (Year)	Peng and Hodges (1989)	Quimio and Zapata (1990)	Abe and Futsuhara (1991)	Tsukahara et al. (1995)	Taguchi-Shiobara et al. (this report)
Tissue from which calli derived	Immature embryo	Anther	Seed (mature embryo)	Seed (mature embryo)	Seed (mature embryo)
Number of parents	4	4	5[a]	10[a]	7
Number of replications	1	1	1	3	2
Regeneration frequency					
h^2(bs)	0.675	0.952	0.81	–	0.736
h^2(ns)	0.382	0.863	0.58	–	0.387
SQR(H_1/D)	1.030	0.420	1.06	–	0.990
Number of regenerated shoots/ plants					
h^2(bs)	0.769	–	–	(0.939)[b]	0.919
h^2(ns)	0.290	–	–	(0.645)[b]	0.858
SQR(H_1/D)	1.286	–	–	(1.103)[b]	0.531

[a] Number of parents in subdiallel table from which genetic parameters were calculated

[b] Calculated values based on the data with three replications that the authors kindly provided. They are shown only as references in parentheses because of the significant maternal effect

Additive effects and dominant effects were significant, thereby confirming the results of earlier reports. Both effects were observed consistently despite differences in cultivars, culture conditions, and tissues from which cultured cells were derived.

Maternal effects were not detected in this report, which is inconsistent with the results of Peng and Hodges (1989) and Tsukahara et al. (1995). This may be due to innate differences in the cultivars used.

Direction of the effect of dominant genes was not consistent with the results of other reports. Peng and Hodges (1989) and Abe and Futsuhara (1991) reported positive effects, while Quimio and Zapata (1990) and Tsukahara et al. (1995) reported negative effects. The direction of the dominant genes was not dependent on the tissue from which cultured cells were derived, nor on the ecogeographic race of cultivars. In this report, dominant genes had a positive effect, though the effect was more obvious for regeneration frequency than for number of regenerated shoots per callus. The average degree of dominance depended upon the indices of regeneration ability, which also confirms the hypothesis that different sets of genes affect each index. Further QTL analyses are necessary in order to prove this hypothesis.

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