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Genetic studies of anther culture ability in rice (*Oryza sativa*)

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Abstract

Inheritance of three anther and culture characters, callus induction, green plant regeneration and culture efficiency was studied using incomplete diallel crosses with a gamete model. It was suggested that callus induction was mainly controlled by gametic additive effects and with less effect of the maternal effects. Green plant regeneration was mainly determined by maternal effects with less influence of gametic additive effects. Culture efficiency was controlled by gametic additive, maternal and cytoplasmic effects. Cultivar Lunhui 422 showed positive genetic effects for all three traits and was a very good parent for rice anther culture breeding. Significant positive heterosis was observed for callus induction. Both gametic additive and maternal correlations contributed to the significant genotypic and phenotypic correlations between callus induction and green plant regeneration suggesting these two traits to be linked.

Abbreviations: 2,4-D – 2,4-dichlorphenoxyacetic acid; NAA – α-napthaleneacetic acid

Introduction

Anther culture may reduce the time needed to reach homozygosity by spontaneous or induced doubling of the haploid chromosome number. It allows for an increase in selection efficiency due to better discrimination between genotypes within any generation and efficient retention of desirable genes in later generation (Dunwell, 1986; Li, 1991). The creation of sufficient numbers of green plant is a prerequisite for the practical use of this technique (Zhu et al., 1990; Li, 1991; Cao et al., 1992). Anther culture of rice is influenced by the genotypes of the explant (Niizeki and Oono, 1968; Shen et al., 1982; Li, 1991), the growth condition of the donor plants (Chen, 1988), the developmental stage of the microspores (Chen, 1977; Genovesi and Magill, 1979), pre-treatment (Qu and Chen, 1983), the culture methods (Yang and Zhou, 1979; Chen, 1988), the media (Chen, 1988; Sun et al., 1990) and the culture conditions (Wang et al., 1977; Qu and Chen, 1983). Among these influencing factors, the genotype of the donor plants has been reported to be the most important factor in anther culture (Chen, 1988; Henry et al., 1994). Considerable variation in anther culture among rice genotypes has been identified (Niizeki and Oono, 1968; Mukherjee, 1973; Oono, 1975; Shen et al., 1982; Chen, 1988; Li, 1991) and a general trend has been reported as follows: japonica/waxy > japonica/japonica > japonica > indica/japonica > indica/indica > indica (Shen et al., 1982). Genetic effects on callus induction, green plant regeneration and culture efficiency, which was derived directly from the product of callus induction and green plant regeneration, contribute to the variation observed among rice genotypes with or without cytoplasmic effects (Quimio and Zapata 1990; Zhu et al 1990; Henry et al., 1994).

In this study the genetic control of callus induction, green plant regeneration and culture efficiency of rice (*Oryza sativa* L.) anther culture was investigated using incomplete diallel (3×6) crosses with a gamete model proposed in this paper

Material and methods

Anther culture

Incomplete diallel crosses were made by using three wide-compatible varieties, 02428 (Lu and Pan, 1992), CPSLO17 (Ikehashi, 1991), TG7 (Yan and Xue, 1995) as female parents and three japonica varieties, T1950, Lunhui 422, WL1312 and three indica ones, Minghui 63, Milyang 46, Erjiufeng as male parents. F_1 hybrids and their parents were grown in experimental plots in Zhejiang Agricultural University. Young ears of donor plants with anthers at the uninucleate stage of microspore development were collected and pretreated in the dark for 7–10 days at 80 °C. Spikes were surface sterilized with 10.0% (V/V) calcium-hypochlorite for 17–20 min and 3–4 rinses in sterile distilled water. Anthers of spiklets in the middle spikes were aseptically removed and cultured on 0.7% (W/V) agar solidified N6 medium (Chu, 1978) supplemented with 2.0 mg l^{-1} 2,4-D and 5.0% (W/V) sucrose at pH 5.8. 20–30 test tubes containing 25 ml medium with about 60 anthers per test tube, were cultured per genotype and randomly kept in two groups. All cultures were incubated in a growth chamber at 25±1°C in the dark until calluses were produced. The callus induction percentage was calculated as the number of anthers producing calluses per number of anthers cultured.

One to two-week old calluses were transferred to agar solidified MS medium (Murashige and Skoog, 1962) containing 2.0 mg l^{-1} kinetin, 1.0 mg l^{-1} NAA and 3.0%(W/V) sucrose at pH 5.8. The cultures were incubated under continuous light (1500lx) at 25±1°C until the regenerated plants were 2–3 weeks old. Green plant regeneration was calculated based on the number of calluses producing green plants. The culture efficiency was the number of calluses which differentiated into green plants divided by total number of anthers cultured.

Statistical methods

In the gamete model with the assumption of no epistatic effects and no interaction between genetic and environmental effects, phenotype mean of F_1 ($i \neq j$) or parent ($i = j$) in a diallel experiment from the i-th maternal line and the j-th paternal line in the k-th block can be expressed by a linear model:

$$\begin{aligned} Y_{ijk} &= \mu + G_{ij} + e_{ijk} \\ &= \mu + 0.5A_i + 0.5A_j + M_{ij} + C_i + e_{ijk} \end{aligned}$$

where μ is the population mean, A_i or A_j is the gametic additive effect, M_{ij} is the maternal plant effect of genotype ixj, C_i is the cytoplasmic effect of parent i, e_{ijk} is the residual error. The total genetic effect (G_{ij}) can be expressed by its components as:

$$G_{ij} = 0.5A_i + 0.5A_j + M_{ij} + C_i$$

The MINQUE(1) method (Zhu, 1992; Zhu and Weir, 1996), which is a MINQUE method (Rao, 1971) with all prior values setting 1, was used to estimate variance components for each trait and for covariance components between two traits. Phenotypic and genotypic correlations as well as additive, maternal, cytoplasmic and residual correlations among three traits were then estimated. Random genetic effects were predicted by the Adjusted Unbiased Prediction (AUP) method (Zhu, 1993; Zhu and Weir, 1996). The Jackknife method was applied for obtaining estimates or predictors and their standard errors in a t-test for parameters (Miller, 1974).

There are two types of maternal plants (M_{ii} for parents and M_{ij} for F_1 hybrids) from which gametes are produced. Since $\sum_i M_{ii} + \sum_i \sum_j M_{ij} = 0$ and $-\sum_i M_{ii} = \sum_i \sum_j M_{ij}$, the standard maternal heterosis $\Delta = -\sum_{i=1}^{p} M_{ii}/\sqrt{p\sigma_M^2}$ can be used for convenience in comparison of heterosis for maternal plants (Zhu et al., 1993). If heterozygote maternal effects (M_{ij}) are mostly positive, Δ will be larger than 0 and heterosis is expected with positive direction. Negative heterosis will be predicted by the result of $\Delta < 0$. If there is no maternal variation M_{ii} and M_{ij} as well as Δ will be 0.

Results

Genetic conponents of variation

In Table 1, variance components were listed for callus induction, green plant regeneration and culture efficiency. The variation of callus induction was mainly contributed by gametic additive effects with less importance of maternal effects. Maternal variance was higher than gametic additive variance and no cytoplasmic effect was detectable for green plant regeneration. Gametic additiive, maternal and cytoplasmic effects were all significant for culture efficiency with gametic additive effects being more important than the other two. Although the residual effects were also significant for culture efficiency, the proportional values were very

Table 1. Variance components for anther culture characters.

Parameter	Estimated proportional values ±SE		
	Callus induction	Green plant regeneration	Culture efficiency
V_A/V_P	0.805 ± 0.088**	0.356 ± 0.088**	0.554 ± 0.080**
V_M/V_P	0.188 ± 0.083*	0.627 ± 0.085**	0.217 ± 0.070**
V_C/V_P	0	0	0.2I7 ± 0.067**
Ve/V_P	0.008 ± 0.055	0.017 ± 0.054	0.012 ± 0.002**

*, **: Significant at 5%, 1% level, respectively.
V_A, V_M, V_C, Ve and V_P are gametic additive, maternal, cytoplasmic, residual and phenotypic variances, respectively.

small, suggesting that all three characters were mainly controlled by genetic effects with little influence of the random errors.

Predicated genetic effects of anther culture traits

The predicated genetic effects (Table 2) indicated that Lunhui 422 and Erjiufeng had significant gametic additive effects on increasing or decreasing callus induction, respectively. Additive effects of 02428 could increase green plant regeneration, but that of TG7 and Erjiufeng could decrease it. Lunhui 422 had maternal effects for increasing callus induction, but CPSL017, TG7 and WL1312 had the opposite effects. T1950, Lunhui 422 and TG7 had significant cytoplasmic effects for increasing culture efficiency, but WL1312 and Minghui 63 showed the opposite effects. It was also showed by results that one parent might have the additive and maternal or cytoplasmic effects for a particular trait in the same direction, e.g., Erjiufeng and Lunhui 422 or in the opposite direction, e.g., WL1312 that might cause counteraction of these two kinds of effects. Considering gametic additive, maternal and cytoplasmic effects for three characters, Lunhui 422 had genetic effects all for increasing callus induction, green plant regeneration, and culture efficiency, and was a very good parent in rice anther culture breeding. Erjiufeng showed genetic effects for decreasing the three traits and should not be included in anther culture breeding plans.

The standard maternal heterosis (Δ) for callus induction was predicted to be 1.778, significant at 0.1 levels, indicating that positive heterosis might exist for this trait in the whole. Directions of heterozygote maternal effects were various among different crosses, suggesting both negative and positive heterosis exists in different crosses (Table 2.). Although WL1312 had negative maternal effects itself, it showed a significant positive maternal effects in F_1 hybrids which indicated that WL1312 if used as parent will have higher heterosis for callus induction. The predicted Δ was -0.075 and 0.711 and not significant for green plant regeneration and culture efficiency, respectively, implying that no heterosis existed for these two traits. There were differences among crosses, e.g., CPSLO17/Minghui 63, TG7/WL1312 had a significant positive heterosis for these two traits. 02428/Lunhui 422 showed negative heterosis, although the parents themselves both had positive maternal effects. Therefore, anther culture ability of both parents themselves and their F_1 hybrids should be considered in the choice of parents in rice anther culture breeding.

Genetic correlations between anther culture characters

Phenotypic and genotypic correlations among the three anther culture characters (callus induction, green plant regeneration and culture efficiency) are shown in Table 3. Both genotypic and phenotypic correlations were significant between callus induction and culture efficiency, and were less important between callus induction and green plant regeneration or between green plant regeneration and culture efficiency. The same tendency of genotypic and phenotypic correlations between two of the three characters suggested that it may be possible to obtain better genotypes by selecting phenotypes. Further analysis of genetic correlation showed that gametic additive and maternal correlations were important between callus induction and green plant regeneration. Between callus induction and culture efficiency, gametic additive, maternal and residual correlations were all significant, indicating that correlations of genetic effects and random errors all con-

Table 2. Predicated genetic effects of anther culture characters in rice.

Parameter	Variety(i)								
	i=1	i=2	i=3	i=4	i=5	i=6	i=7	i=8	i=9
Callus induction									
A_i	-1.80	-6.90	-7.32	-7.67	-8.95+	-12.05*	15.83+	-3.93	32.79*
M_i	-5.43	-12.61+	-13.22+	-5.23	-2.98	-3.36	-11.16+	-0.14	15.27
M_{i1}				-4.45	-4.54	-10.12	24.49+	-3.40	5.81
M_{i2}				6.09+	3.90+	-0.64	1.09	-2.37	7.11
M_{i3}				-2.41	-6.63		19.28+		5.66
Green plant regeneration									
A_i	12.24+	-1.44	-5.52	4.57	-0.42	-24.46	0.89	9.05	5.12
M_i	20.98+	-6.23	-11.15+	-17.10+	0.71	-3.27	-1.57	9.77	+10.52+
M_{i1}				-9.74	15.09+	-15.58	6.05	-5.65	-14.20+
M_{i2}				49.74+	-9.11	-9.94	-14.20	1.66	-9.99
M_{i3}				-8.50	-6.86		13.99+		14.77+
Culture efficiency									
A_i	0.34	-1.28	-1.56	-0.74	-2.02+	-2.77*	2.44	-0.41	6.01+
M_i	0.65	-1.82	-2.83	-1.66	-0.69	-0.64	-2.52+	0.15	5.56+
M_{i1}				-1.57	0.28	-1.81	5.27+	-0.83	-2.38+
M_{i2}				5.40+	0.08	0.80	-1.67	-0.13	-1.22
M_{i3}				-1.89	-1.79		4.69+		2.18
C_i	0.13	0.12	1.04+	-1.82+	-0.78	-0.80	-2.39+	0.35**	4.16+

+, *, **: Significant at 10%, 5% and 1% level, respectively.
1=02428; 2=CPSLO17; 3=TG7; 4=Minghui 63; 5=Milyang 46; 6=Erjiufeng 7=WL1312; 8=T1950; 9=Lunhui 422.

Table 3. Genotypic and phenotypic correlations between anther culture characters.

Correlation	CI & GP	CI & CE	GP & CE
Phenotype	0.295+	0.597**	0.221+
Genotype	0.296+	0.598**	0.258+
Additive	0.310**	0.621**	0.136
Maternal	0.255**	0.540**	0.691**
Cytoplasmic	0	0	0
Residual	0.235	0.819**	0.570**

+, *, **: Significant at 10%, 5% and 1% level, respectively.
CI: callus induction. GP: green plant regeneration. CE: culture efficiency.

tributed to this positive correlation. The significance of maternal and residual correlations between green plant regeneration and culture efficiency indicated that this correlation was mainly influenced by F_1 hybrids with residual effects.

Discussion

There have been several previous studies on the genetic control of anther culture in rice (Henry et al., 1994). Callus induction was inherited as a recessive character controlled by single block of genes and additive gene effects were predominant (Miah et al., 1985). The main additive genetic effect was in the control of both callus induction and green plant regeneration with no involvement of cytoplasmic effects (Zhang and Chu, 1985; Quimio and Zapata, 1990), or with cytoplasmic effects (Zhu et al., 1990). Additive genetic variance was higher than non-additive variance for anther response and frequency of callus induction, but lower for callus differentiation and culture efficiency by analysing the data of six generations (Chen and Chen, 1993).

In all these previous reports, it was assumed that rice anther culture characters were sporophyte inheritance without gametophyte effects. Rice anther includes two parts, one is the diploid sporophyte tissue of the anther wall, the other is the haploid gametophyte pollen. The maternal plants provide nutrients for the development of pollen. In rice anther culture, calluses were produced from haploid gametes with nutrients transferring from the anther wall. Therefore, anther culture traits may be controlled by both haploid gamete and diploid maternal tissues. So the assumption of no gametophyte effects may not be satisfied for the genetic study of rice anther culture characters.

In the present study, it was assumed that anther culture traits were controlled by both gametophyte and sporophyte genotypes. The genetic effects were further partitioned into gametic additive, maternal and cytoplasmic effects. Results confirmed that gametic additive and maternal effects were all significant for callus induction, green plant regeneration and culture efficiency, although the importance of these effects had some differences among the three traits. The significant influence of both gametic and maternal effects on rice anther culture traits has not been previously reported. This also gives a very good explanation of previous observations that most pollen plants derived from indica/japonica hybrids by anther culture are japonica type or tended towards the japonica type, and few are indica or intermediate types (Zhang, 1989; Guiderdoni et al., 1989). Japonica varieties had higher gametic additive effects for anther culture traits than indica ones (Table 2). In anther culture of F_1 hybrids between indica and japonica, those pollens with more genetic substances from japonica parent may have higher ability to form callus and differentiate to green plants, resulting in the distorted distribution of indica and japonica type among pollen plants. Results also indicated that gametic selection existed in rice anther culture.

The major limitation of successful incorporation of anther culture technique to commercial rice breeding plans was the difficulty in obtaining doubled haploid populations large enough for all kinds of genetic recombination. Consequently, efforts are being made to overcome this limitation through manipulations of culture media and culture conditions; genetic recombination of higher culture ability and superior agronomic traits (Li, 1991). The higher heritability estimates for callus induction and culture efficiency suggests that relatively rapid genetic gain can be reached by transferring this trait from high culture ability to low culture ability germplasm. The significant gametic additive and maternal correlations that contribute to the positive genetic correlation between callus induction and green plant regeneration suggest that these two traits which together determine overall anther culture efficiency are linked. This differs from some previous reports that callus induction and green plant regeneration are inherited independently (Zhu et al., 1992). Therefore, the selection for higher callus induction should be sufficient to improve anther culture efficiency.

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