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

Juqiang Yan, Qingzhong Xue & Jun Zhu

*Department of Agronomy, Zhejiang Agricultural University, Hangzhou, 310029, China*

Received 22 November 1995; accepted in revised form 10 June 1996

**Key words:** anther culture, gamete model, genetic analysis

**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-dichlorophenoxyacetic 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 x 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. FI 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 pre-treated 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 254 ± 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 F1 (i ≠ 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:

\[ Y_{ijk} = \mu + G_{ij} + e_{ijk} \]

where \( \mu \) is the population mean, \( A_i \) or \( A_j \) is the gametic additive effect, \( M_{ij} \) is the maternal plant effect of genotype i\(x\)j, \( C_i \) 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 covariation 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 F1 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 \( \Delta = -\frac{\sum_{i=1}^{p} M_{ii}}{\sqrt{p\sigma^2_M}} \) 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, \( \Delta \) 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 \( \Delta \) will be 0.

Results

Genetic components 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 additive, 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