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Inheritance of callus formation ability in anther cultures of rice, *Oryza sativa* L.

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Summary. Inheritance of ability to form callus in rice anther culture was studied using the diallel technique. Anthers containing uninucleate microspores from two *japonica* cultivars ('Minehikari' and 'Taipei 309'), two *indica* cultivars ('Mingolo' and 'Suweon 290'), and 12 F_1's of the diallel crosses involving these four parents were cultured on Chaleff's R_2 medium and evaluated for callus induction. The parents showed significant differences in anther callus formation, from 41.9% ('Taipei 309') to 0% ('Suweon 290'). Callus induction ability was inherited as a recessive character conditioned by a single block of genes. Additive gene effects were predominant. The *japonica* types seemed to be good combiners for callus induction. The order of dominance among the four parents was 'Suweon 290', 'Mingolo', 'Minehikari' and 'Taipei 309'.

Key words: Microspore – Anther culture – Callus formation – Diallel analysis

Introduction

Crop improvement is based on the manipulation of genetic potential through cross breeding of diverse parents and selection in the segregating populations. Innovative in vitro approaches that could be incorporated into plant breeding programs include anther (microspore) culture, ovary culture, in vitro fertilization, embryo culture, regeneration from cell culture, and protoplast fusion. Anther culture and regeneration of plants from microspores greatly facilitate the subsequent selection of recombinants following a hybridization program. Moreover, anther culture reduces the time needed to reach homozygosity since spontaneous or induced doubling of the haploid chromosome number results in homozygous diploid plants. Screening of haploid cells against salinity, heavy metals, phytotoxins, and other selective agents before plant regeneration is also feasible.

Reports of successful rice anther culture include those of Niizeki and Oono (1968); Guha et al. (1970); Guha-Mukherjee (1973); Chu (1975); Chen and Lin (1976); Song et al. (1978); Chaleff (1979); Rush and Shao (1980); Chaleff and Stolarz (1981); and Schaeffer (1983). Many of these authors have reported varietal differences in the formation of callus from cultured anthers, the first step toward plant regeneration. However, very little information on the inheritance of this trait is available.

The present study investigates the mode of inheritance of anther callus formation ability via a four-parent diallel analysis.

Materials and methods

Two *japonica* rice cultivars, 'Minehikari' and 'Taipei 309', and two *indica* rice cultivars, 'Mingolo' and 'Suweon 290', were used to secure full diallel matings. Five panicles with anthers at the uninucleate stage of microspore development were collected in the morning from each parent and F_1 and cold-treated at 7 °C for 7 days. Spikelets were surface-sterilized in 7% calcium hypochlorite + 0.1% sodium lauryl sulphate and rinsed with distilled water. Individual anthers were dissected out and placed on agar-solidified Chaleff's R_2 medium containing 2 mg/l α-naphthaleneacetic acid and 0.3 mg/l kinetin (Chaleff and Stolarz 1981). At least 900 anthers of each genotype were cultured, 60/plate, at 28 °C in the dark. The percentage of callus formation was calculated on the basis of calli formed per anther plated after four to six weeks of incubation.

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Statistical analysis

Hayman's diallel analysis procedure (1954a) was used to compute variance (Vr) and covariance (Wr) in the analysis of F_1 data, and the appropriate Vr, Wr graph was constructed. The relationship between the order of dominance (Vr+Wr), the mean performance, and callus induction ability was determined by the standardized deviation graph of parental measurements (Yr) and the order of dominance of the parents (Johnson 1963; Johnson and Aksel 1959). Narrow and broad sense heritabilities were estimated using the formula of Mather and Jinks (1971). The analysis of variance for general and specific combining ability was carried out according to Griffing (1956) using method I, model I (fixed effects for genotypes).

Results and discussion

The percentage of callus formation in anther cultures of the four parents and their F_1 crosses and reciprocals are given in Table 1. The *japonica* material was more responsive than the *indica* types, confirming earlier observations (Chaleff 1979). 'Taipei 309' gave best callus formation, followed by 'Minehikari', 'Mingolo' and 'Suweon 290'. F_1's involving these parents had callus formation values between the parental ones except for the F_1 of 'Taipei 309'×'Minehikari' where it exceeded both the parents. Analysis of variance for callus induction in the parents, F_1 hybrids and their reciprocals is presented in Table 2. Differences between means of the genotypes were highly significant so further analyses were done to estimate the effects of variances and covariance associated with genetic effects for callus formation.

Variance and covariance

The variance and covariance (Vr, Wr) graph of the callus formation is shown in Fig. 1. The position of Vr, Wr on the line reveals the relative proportions of dominant and recessive genes in the parents. In the present study parent 4 occupied a position near the point of origin, indicating a predominance of dominant genes. Parent 2, located at a position away from the point of origin, indicated a relative excess of recessive genes. This was also true for the parent 'Minehikari'. The parent 'Mingolo' appears to have a higher proportion of recessive genes than dominant ones. As the regression line passes above the point of origin, it indicates the influence of partial dominance in the inheritance of callus formation. The Wr+Vr graph (Fig. 2) also confirmed the above results as the parents 1 and 2 occupied their positions in the first quadrant

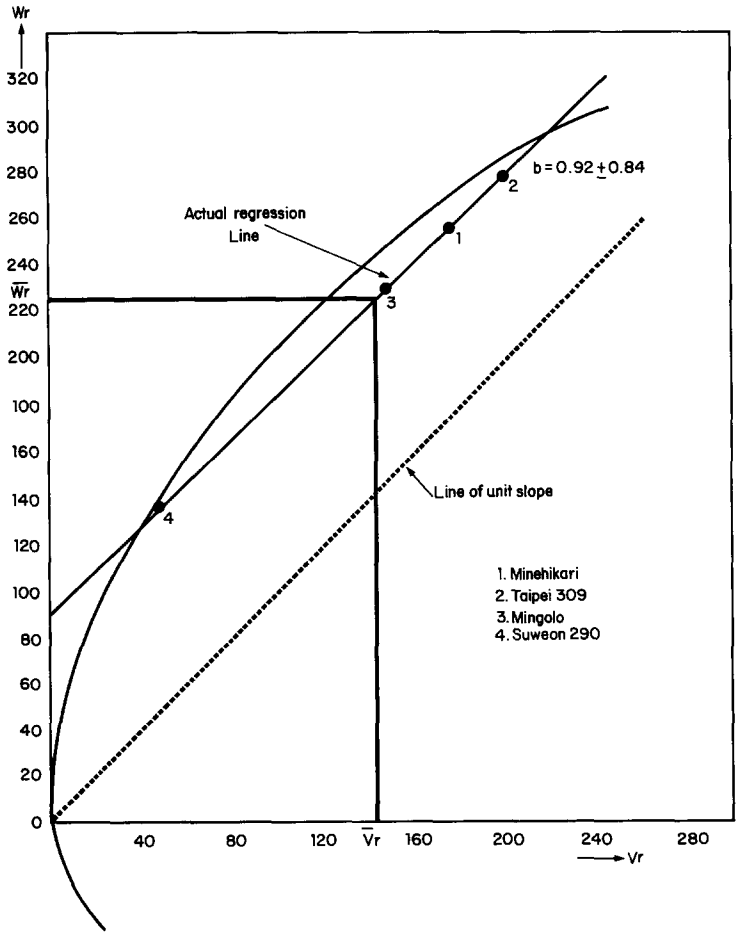

Fig. 1. *Vr, Wr* graph of the four-parent diallel analysis for callus induction

Table 1. Callus formation in anther cultures of a four-parent diallel cross

Cross no.	Parents/cross	Anthers plated (no.)	Callus formation (%)
1	P_1 ('Minehikari')	954	33.4
2	P_2 ('Taipei 309')	1,004	43.7
3	P_3 ('Mingolo')	962	8.9
4	P_4 ('Suweon 290')	900	0.0
5	'Minehikari'/'Taipei 309'	1,020	36.9
6	'Minehikari'/'Mingolo'	1,080	29.1
7	'Menehikari'/'Suweon 290'	1,230	8.2
8	'Taipei 309'/'Mingolo'	1,250	21.1
9	'Taipei 309'/'Suweon 290'	1,080	17.3
10	'Mingolo'/Suweon 290'	1,402	0.4
11	'Taipei 309'/'Minehikari'	1,026	48.3
12	'Mingolo'/'Minehikari'	960	29.2
13	'Suweon 290'/'Minehikari'	1,050	9.2
14	'Mingolo'/'Taipei 309'	1,196	22.1
15	'Suweon 290'/'Taipei 309'	1,134	18.0
16	'Suweon 290'/'Mingolo'	1,476	1.2
		17,524	19.4

Table 2. Analysis of variance for callus formation

Source of variation	DF	SS	Mean squares	F value
Replication	4	124.35	31.08	1.13 ns
Genotype	15	16,302.40	1,086.82	39.65**
Error	60	1,644.46	27.40	
Total	79	18,071.22		

C.V. = 26.5%
** = Significant at 1% level
ns = not significant

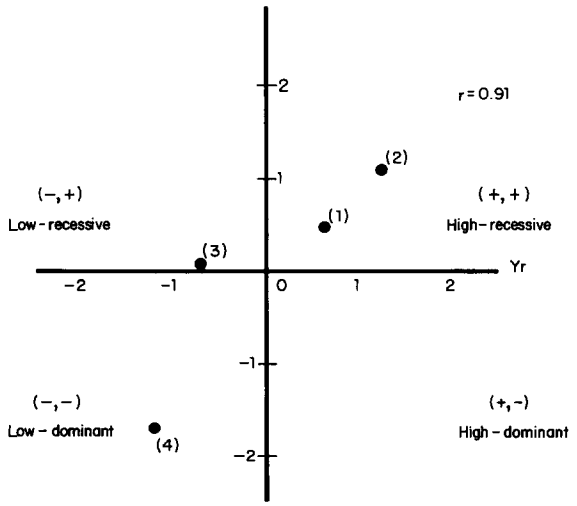

Fig. 2. Standardized deviation graph of the four-parent diallel analysis for callus induction. Array *1* = 'Minehikari'; *2* = 'Taipei 309'; *3* = 'Mingolo'; *4* = 'Suweon 290'

Table 3. Genetic components of variation for callus formation in a four-parent diallel cross

Notation[a]	Estimated value ± SE
D	411.99± 7.37**
F	−69.51±18.95**
H_1	73.51±21.44**
H_2	57.40±19.80**
h^2	8.20±13.43 ns
E	5.52± 3.30 ns
	Proportional values
$(H_1/D)^{1/2}$[b]	0.42
$H_2/4H_1$[c]	0.19
$\frac{(4DH_1)^{1/2}+F}{(4DH_1)^{1/2}-F}$[d]	0.66
h_2/H_2[e]	0.14
h(ns)[f]	0.92
h(bs)[g]	0.97

** = Significant at 1% level
ns = not significant
[a] D = component of variation due to additive gene effects; F = mean of covariance of additive and dominance effects over the arrays; H_1 = component of variation due to dominant gene effects; $H_2 = H_1[1-(u-v)^2]$ where u and v are proportion of positive and negative genes in the parents and where u + v = 1, h^2 = dominance effect (as the algebraic sum over all loci in heterozygous phase in all crosses); E = component of variation due to environmental effects
[b] Mean degree of dominance at each locus
[c] Ratio of genes with positive and negative effects in the parents
[d] Ratio of dominant and recessive genes in the parents
[e] Number of groups of genes which control the trait and exhibit dominance
[f] Heritability, narrow-sense
[g] Heritability, broad sense

indicating predominance of recessive genes which gave relatively high callus formation. Parent 3 occupied its position in the second quadrant, and, although controlled by recessive and dominant genes, had low callus production. Parent 4 occupied its position in the third quadrant, suggesting lower callus formation by dominant genes.

Genetic components of variation

The estimated genetic components of variation for callus formation are presented in Table 3. Ratios computed from the genetic components provide information on the degree, order, and direction of dominance and/or recessiveness in the inheritance of callus formation ability. The low value of $(H_1/D)^{1/2}$ indicated predominance of additive gene effects on callus induc-

Table 4. Analysis of variance for combining ability for callus formation

Source of variation	DF	Variance	F value
GCA	3	1,000.75	182.57**
SCA	6	34.22	6.24**
Reciprocals	6	8.80	1.61 ns
Error	60	5.48	

** = significant at 1% level
ns = not significant

Table 5. Combining ability effects for callus formation

Parents/cross	SCA	GCA[a]
'Minehikari'/'Taipei 309'	2.188	P_1 7.95
'Minehikari'/'Mingolo'	4.921	P_2 10.34
'Minehikari'/'Suweon 290'	−4.847	P_3 −4.82
'Taipei 309'/'Mingolo'	−3.400	P_4 − 13.47
'Taipei 309'/'Suweon 290'	−2.010	
'Mingolo'/'Suweon 290'	−0.366	

[a] P_1 = 'Minehikari'; P_2 = 'Taipei 309'; P_3 = 'Mingolo'; P_4 = 'Suweon 290'

tion. The low value of $H_2/4H_1$ (less than 0.25) suggested unequal mean allelic frequencies at the loci influencing this trait, i.e. positive and negative genes were not present in equal proportions. The negative value of F and the ratio of dominant to recessive alleles $[(4DH_1)^{1/2}+F]/[(4DH_1)^{1/2}-F]$ indicated that the parents used in this study had more recessive alleles than the dominant ones for callus formation. The low h^2/H_2 value suggested that only one block of genes was involved in parental differences for callus formation.

The narrow-sense heritability [h (ns)] was quite high due to the major role of additive gene effects and the low role of non-additive gene effects and environmental effects in the control of callus formation. The estimate of broad-sense heritability [h (bs)] value was only slightly higher than that of h (ns) because of the low importance of dominance effects.

Combining ability

The analysis of variance for combining ability is given in Table 4. Although the variance both for GCA and SCA was significant, yet relatively higher value for GCA variance suggests preponderance of additive gene effects as compared to dominance effects. These results are in conformity with those obtained by Hayman analysis.

The perusal of Table 5 indicates that both the *japonica* parents ('Minehikari' and 'Taipei 309') showed high GCA effects whereas the *indica* parents showed low GCA effects. The cross between *japonica* varieties showed positive SCA effects. All other crosses except 'Minehikari' × 'Mingolo' showed negative SCA effects. Cross 1 × 3 showed a high and positive SCA effect.

These results suggest that in *japonica* × *indica* crosses segregants with higher callus induction ability may be selected in segregating generations even in the *indica* type background.

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