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Combining Ability and Heritability of Callus Induction and Green-Plant Regeneration in Rice Anther Culture

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Abstract: This study was conducted to determine the inheritance of callus induction and plant regeneration in rice anther culture. Low efficiencies of callus induction and green-plant regeneration have limited the application of anther culture in plant breeding programs. Four parents along with six F_1 genotypes derived from a four-parent incomplete diallel mating set of two commercial lines (Amol2 and Amol3) and two local cultivars (Ghasroddashti and Rashti) were evaluated for their callus induction and plant regeneration abilities. The results showed that the callus induction, green-plant regeneration and the effects of genotype and genotype×medium interaction were significant ($p<0.01$). The local cultivars generally had a higher percent callus induction, plant regeneration and number of calli producing green plants than the commercial lines. The parents showed significant difference in anther callus formation, from 4.01% (Amol2) to 22.26% (Rashti). Combining ability analysis demonstrated the predominance of additive gene effects in the control of both characters with the local cultivars having higher combining ability for green-plant regeneration. Also gene action to be partially dominant for both characters. Combining ability analysis revealed that both additive and dominant gene effects are important in controlling callus induction and green-plant regeneration in rice.

Key words: Callus induction and plant regeneration, diallel analysis, rice

INTRODUCTION

Rice anther culture offers a rapid method of creating homozygous breeding lines from heterozygous breeding lines and allows for an increase in selection efficiency due to better discrimination between genotypes within any generation and efficient retention of desirable alleles in later generations (Croughan, 1995). The theoretical basis for the utilization of androgenic doubled haploids for rice breeding has already been established (Croughan, 1995; He *et al*., 1998).

Anther culture in rice is accomplished and green plants are obtained in two steps; The first step involves the induction of embryogenic calli from microspores and the next step deals with the regeneration of green plants from the calli (Bishnoi *et al*., 2000). Many authors have reported that considerable variation among genotypes has been identified (Omar Faruque *et al*., 1998; Mahmuda Khatun *et al*., 2003). Moreover previous studies also showed that anther culture capacity in rice was highly heritable and was controlled by nuclear genes (Yamagishi *et al*., 1998; Kwon *et al*., 2002). These results suggested that it might be possible to overcome the limitation through genetic recombination and transferring genes controlling anther culturability from high response into poorly responsive lines. Many researchers revealed that callus induction and green-plant regeneration are inherited independently and the two traits are not correlative (Zhu, 1992; He *et al*., 1998).

This technique can be of practical use in plant breeding programs only if it can create sufficient numbers of green plants. Green-plant regeneration is currently recognized as the most limiting step in the process. Consequently, efforts are being made to overcome this limitation through genetic recombination and through manipulations of culture media and cultural conditions (He *et al*., 2006).

For this purpose, a genetic analysis of callus formation ability in rice anther culture using the diallel technique was done earlier by Quimio and Zapata (1990). Four genotypes were evaluated using a single callus induction medium.

In the present study the genetic control of callus induction and green-plant regeneration was investigated using a four-parent incomplete diallel cross. Two callus induction media were used to evaluate the importance of the genotype × medium interaction. In addition, this study was extended to examine the genetic control of green-plant regeneration.

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MATERIALS AND METHODS

Culture conditions

Callus induction: Two commercial lines, Amol2 and Amol3 (indica type) and two Iranian local cultivars, Ghasroddashti and Rashti and six F_1 genotypes derived from a four-parent incomplete diallel mating were used in this study. Ghasroddashti and Rashti are high callus producers (Bagheri *et al*., 2004) while Amol2 and Amol3 are low callus producers (Bagheri *et al*., 2003). At least 12 panicles from each of the F_1s and selfed parents with anthers at the uninucleate microspore stage were used.

Anthers were cultured per the procedure developed by Zapata (1985). Panicles were kept in an incubator at 4 to 8°C during the first 8 day after collection. A total of 1920 anthers was plated per genotype in 8 cm Petri dishes at a density of 30 anthers per 6 mL of medium. These were plated on N_6 medium (Chu, 1978) supplemented with 0.5 mg L^{-1} Kin + 2.0 mg L^{-1} 2,4-D + 0.5 mg L^{-1} NAA + 60.0 g L^{-1} sucrose and on G_1 medium (Gamborg *et al*., 1968) supplemented with 0.75 mg L^{-1} Kin + 2.0 mg L^{-1} 2,4-D + 2.5 mg L^{-1} NAA + 40.0 g L^{-1} sucrose. Each medium was modified to be semisolid (0.8% agar). Two plates were inoculated per medium treatment, one being considered a replicate of the other. Cultures were incubated in the dark at 26±1°C until calli were produced. Percentage of callus induction ability was calculated on the basis of the number of anthers producing callus to total cultured anthers.

Plant Regeneration: Seven-to-15-day-old calli were transferred to two semisolid MS (Murashige and Skoog, 1962) modified media, M_5 medium supplemented with 1.0 mg L^{-1} NAA + 4.0 mg L^{-1} Kin + 40.0 g L^{-1} sucrose and SK_{11} medium supplemented with 1.0 mg L^{-1} NAA + 1.0 mg L^{-1} Kin + 1.0 mg L^{-1} BAP + 40.0 g L^{-1} sucrose. The cultures were incubated at 24±1°C under 10 h light (1.6 W m^{-2}) and 14 h dark conditions for 4 week for plant regeneration. Percentage of plant regeneration was calculated on the basis of the number of calli producing plants to total transferred calli.

Statistical analysis: In each medium culture, means of percentage of callus induction and plant regeneration derived from four replications (Table 1, 2). Also, two media for callus induction (N_6 and G_1) and two media for plant regeneration (SK_{11} and M_5) were used as replications in diallel analysis (Quimio and Zapata, 1990). Callus induction and plant regeneration data were subjected to arcsine transformation prior to statistical analysis. Data presented in the tables are untransformed means unless stated otherwise.

The Hayman (1954) diallel analysis was used to compute the variance (Vr), covariance (Wr) and to construct the Wr, Vr graph. The analysis of variance for general and specific combining abilities was carried out according to Griffing (1956) using Method II (parents and F_1s), Model 1 (fixed effects for genotypes).

RESULTS

Callus induction: Genotypic effects were significant ($p<0.01$) and contributed to the total variation obtained in the analysis of variance in the experiment. Differences in the medium and genotype × medium effects were found to be significant ($p<0.01$), demonstrating that genotypes and media perform differently.

The local cultivars and the crosses involving them as parents were more responsive than the commercial lines (Table 1). The Amol2 had the lowest callus induction, but crosses between this cultivar and Ghasroddashti or Rashti considerably increased callus induction. Less responsive cultivars can thus be improved by crossing with highly responsive ones. Rashti and crosses involving it had higher efficiency of callus induction in N_6 and G_1 media. Also cultivars and cross combinations involving their performed better in N_6 medium.

Plant regeneration: The effect of genotype on green-plant regeneration was found to be significant ($p<0.01$), contributing most to the overall variation in the experiment. Genotypic effects on albino-plant regeneration were significant ($p<0.01$), indicating that this character was affected by genotype.

Percentage of green plant regeneration of Ghasroddashti was highest and lowest in Amol3 (Table 2). Also percentage of albino-plant regeneration was highest in Rashti and lowest in Amol2 (Table 2). Ghasroddashti and Rashti generally had high regeneration rates while Amol2 and Amol3 had very low percent regeneration. This supports previous reports that the local cultivars are more responsive to culture than the commercial lines that are indica types (Bagheri *et al*., 2003, 2004). Crosses with Amol2 as female and Ghasroddashti or Rashti as male greatly increased green-plant regeneration relative to Amol2. Such results are encouraging since Amol2 already has a desirable plant type but does not regenerate many green plants from anther culture. Similar to that in total plant producing, Ghasroddashti gave the highest percentage of calli producing green plants followed by crosses with Rashti. Green plant regeneration in these local cultivars was considerably reduced in crosses involving Amol3.

Table 1: Percentage of callus induction in two media

Female parent	Male parent: Amol2	Ghasroddashti	Rashti	Amol3
			%	
N_6 medium				
Amol2	5.150^{fg}	19.775^{c}	15.450^{e}	9.700^{f}
Ghasroddashti		19.525^{c}	21.275^{b}	14.350^{e}
Rashti			23.375^{a}	16.675^{d}
Amol3				6.500^{g}
G_1 medium				
Amol2	2.875^{g}	16.292^{c}	11.750^{d}	6.775^{f}
Ghasroddashti		17.325^{c}	18.450^{b}	8.325^{e}
Rashti			21.150^{a}	16.425^{c}
Amol3				3.575^{g}

†: Each entry as mean of four replications. For each medium, any two means having a common letter are not significantly different at p = 0.05 based on Duncan's Multiple Range Test

Table 2: Plant regeneration in 10 genotypes derived from a incomplete diallel cross involving four parents

Genotype (female/male)	Calli plated (No.)	Plant regeneration†: Total‡	Green	Albino
		%		
Amol2	120	5.83±0.38^{g}	3.41±0.13^{f}	2.42±0.34^{g}
Amol2/Ghasroddashti	120	9.87±0.25^{e}	6.68±0.24^{c}	3.18±0.18^{f}
Amol2/Rashti	120	9.43±0.78^{e}	4.95±0.22^{d}	4.48±0.68^{d}
Amol2/Amol3	120	7.10±0.39^{f}	1.98±0.17^{h}	5.11±0.28^{c}
Ghasroddashti	120	18.26±0.27^{c}	14.17±0.36^{b}	4.08±0.35^{de}
Ghasroddashti/Rashti	120	20.66±0.45^{b}	17.18±0.31^{a}	3.47±0.25^{ef}
Ghasroddashti/Amol3	120	11.27±0.62^{d}	7.35±0.37^{c}	3.92±0.3^{de}
Rashti	120	24.42±3.54^{a}	4.38±0.32^{e}	20.03±3.77^{a}
Rashti/Amol3	120	9.66±0.40^{e}	2.68±0.40^{g}	6.97±0.25^{b}
Amol3	120	6.47±1.87^{g}	2.10±0.53^{h}	4.37±1.35^{ef}

†: In each column, any two means having a common letter are not significantly different at p = 0.05 based on Duncan's Multiple Range Test, ‡: $\frac{\text{Calli producing green and albino plants}}{\text{Total calli plated}} \times 100$

Combining ability analysis: Analysis of variance for callus induction and green-plant regeneration further partitioned genotypic effects into General Combining Ability (GCA) and Specific Combining Ability (SCA). Both GCA and SCA were significant ($p<0.01$) for the two characters indicating both additive and dominance effects, with additive genotypic variance a primary contributor to the observed responses (Table 3). Furthermore, significant ($p<0.05$) GCA/SCA indicated square ratios of 13.62 for callus induction and 7.081 for green-plant regeneration mean a predominance of additive gene action in the genetic control of these traits. Predominance of additive gene effects confer increased narrow sense heritability of these characters.

The local cultivars had higher GCA values for both traits than the commercial lines, which demonstrated that the former have a good performance in hybrid combination (Table 4). For green-plant regeneration, the GCA value of Ghasroddashti was much higher than that of Rashti, implying that Ghasroddashti performed much better as a parent for green-plant regeneration. The cultivars Amol2 and Amol3 generally gave low performance when in hybrid combinations.

Table 3: Analysis of variance for combining ability effects

Source of variation	df	Mean squares: Callus induction†	Green-plant regeneration
Genotype	9	81.072**	54.342**
GCA	(3)	106.045**	63.524**
SCA	(6)	7.782**	8.970**
Replication	1	41.565**	3.321
Error	9	1.061	0.744

**: significant at p = 0.01 level based on an F-test. †: Data based on the number of anthers producing callus over the total number of anthers plated

Table 4: Combining ability effects for anther culturability

	SCA: Ghasroddashti	Rashti	Amol3	GCA
		%		
Callus induction				
Amol2	4.861**	-0.982	1.712**	-3.464**
Ghasroddashti		-1.084	-1.553*	2.901**
Rashti			2.251**	4.310**
Amol3				-3.747**
Green-plant regeneration				
Amol2	-2.322**	0.270	0.203	-2.002**
Ghasroddashti		5.987**	-0.955*	4.519**
Rashti			-1.288*	0.190
Amol3				-2.706**

*, **: Combining ability estimate significantly different from zero at p = 0.05 and 0.01, respectively, based on Duncan's Multiple Range Test. CV (callus induction) = 7.501%, CV (Green-plant regeneration) = 13.29%

Table 5: Array variances (Vr) and covariances (Wr) for anther culturability

Array	Vr	Wr	Wr + Vr	Wr - Vr	Parent values (Yr)
Callus induction					
Amol2	37.562	48.990	86.552	11.428	4.0125
Ghasroddashti	14.442	23.282	37.725	8.840	18.425
Rashti	14.360	33.610	47.971	19.250	22.262
Amol3	24.028	41.312	65.341	17.283	5.037
Green-plant regeneration					
Amol2	4.083	9.929	14.013	5.845	3.412
Ghasroddashti	26.598	13.781	40.380	-12.816	14.175
Rashti	44.347	36.531	80.879	-7.815	4.387
Amol3	6.575	14.051	20.627	7.476	2.100

Considering of specific effects for callus induction, favorable combinations between Amol2 and Ghasroddashti or Amol3 were observed. The cultivar Amol2 performed poorer when in combination with Rashti than the cross between Amol2 and Ghasroddashti. This differential response in important in choosing cultivars to be crossed with Amol2 for further improvement of its callus induction ability.

Similarly, green-plant regeneration of Amol2 was enhanced when in cross combinations with Rashti and Amol3, but was reduced in cross combination with Ghasroddashti. From the values obtained, Rashti seemed to be a better parental cultivar for crosses with Amol2.

Variance and covariance analysis: The variance (Vr) and covariance (Wr) values on green-plant regeneration (Table 5, Fig. 2), were related by a straight regression line with slope b. Since b = 0.56 and was not significantly

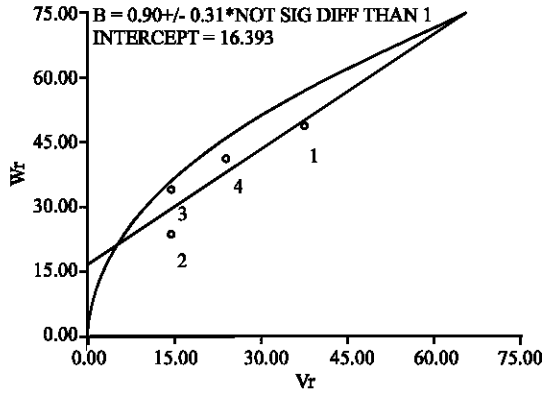

Fig. 1: Variance (Vr), Covariance (Wr) graph on callus induction in a four-parent incomplete diallel cross [1- Amol2, 2- Ghasroddashti, 3- Rashti, 4- Amol3]

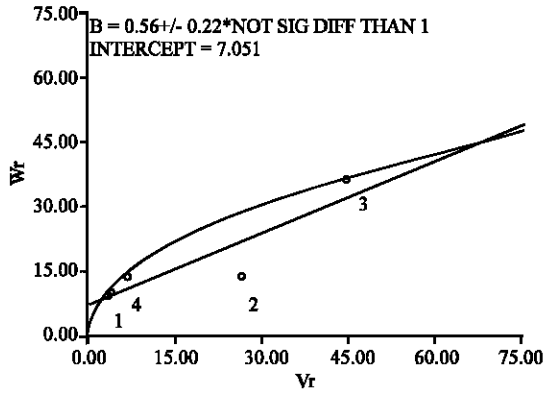

Fig. 2: Variance (Vr), Covariance (Wr) graph on green-plant regeneration in a four-parent incomplete diallel cross [1- Amol2, 2- Ghasroddashti, 3- Rashti, 4- Amol3]

($p>0.05$) different from b = 1.0, the absence of nonallelic interaction and independent distribution of genes were indicated (Fig. 1).

Likewise, the intercept is positive and the actual regression line was slightly above the line of unit slope through the origin, indicating partial dominance in the control of the trait.

The position of points on the regression line depicts the dominance order of the parents. Parents with most dominant genes have their points nearest the origin while the patents with most recessive genes fall farthest from origin. This graph together with Wr + Vr values (Table 5) indicated increased frequency of recessive alleles. The order of dominance was thus Amol2 > Amol3 > Ghasroddashti > Rashti. Rashti, which gave the high percentage of green-plant regeneration, had the most recessive genes while Amol2, which gave the least, had the most dominant genes. The correlation coefficient between parental values (Yr) and parental order of dominance (Wr + Vr) was positive (r = 0.16), indicating that the parents containing most positive genes for the trait had the highest values of Wr and Vr. Therefore, this confirmed the result that the recessive genes must be mostly positive, i.e., they are the beneficial genes contributing to green-plant regeneration.

Values of Wr + Vr for callus induction indicated that increased frequency of dominant alleles. The order of dominance was thus Ghasroddashti > Rashti > Amol3 > Amol2 (Table 5, Fig. 2). Rashti, which gave the highest percentage of callus induction, had the most dominant genes while Amol2, which gave the least, had the most recessive genes. The correlation coefficient between parental values (Yr) and parental order of dominance (Wr + Vr) was negative (r = -0.86) and significant ($p<0.05$), indicating that the parents containing most negative genes for the trait had the highest values of Wr and Vr. Since the value was nearly one, it confirmed the result that the dominant genes must be mostly negative, i.e., they are the beneficial genes contributing to callus induction.

Results of this study imply that for callus induction and green-plant regeneration traits gene action were additive, also parental order of dominance and the combining ability effects were almost identical for both the traits. Therefore, this results can be help in choosing suitable breeding method.

DISCUSSION

This study demonstrated that genetic factors were involved at both steps of the anther culture process, callus induction and green-plant regeneration were traits inherited by quantitative factors. Present results also indicated that additive gene effects controlled the formation of calli. This was similar to previous reports (Quimio and Zapata, 1990; Yan *et al*., 1996; He *et al*., 2006). In case of green-plant regeneration, different results were reported by different authors. Some previous reports revealed the predominance of non-additive gene effects in the control of the character (Taguchi-Shiobara *et al*., 1997; He *et al*., 2006), while Yan *et al*. (1996) concluded it was mainly determined by maternal effects with less influence of additive effects. Present results differed from them. On the contrary, the results presented here confirmed that additive gene effects was more important that non-additive gene effects. The different results could be attributed to different materials used. Parents or one of the parents used in previous studies were japonica genotypes. The parental lines chosen for crosses in this study were from two indica type lines and two Iranian local cultivars.

Under the experimental conditions described, callus induction and plant regeneration traits appeared to be

Table 6: Estimation of different parameters for callus induction and green-plant regeneration traits

Parameter trait	MS(GCA) / MS(SCA)	Gene action	Additive variance	Dominance variance	h_N^2	The best general combiner	The best hybrid combination
Callus induction	13.62**	Additive	32.75	7.25	0.79	Rashti	Amol2/Ghasroddashti
Green-plant regeneration	7.08**	Additive	18.20	8.6	0.75	Ghasroddashti	Ghasroddashti/Rashti

under strong genetic control. More than 85% of the variation was due to genetic variation and just less than 15% was the result of environmental influences (with refer to CV or environmental coefficient of variation for callus induction and green-plant regeneration). These results suggested it could be possibility that transfer of callus induction ability and green-plant regeneration ability from superior responding lines to inferior lines by conventional sexual hybridization. Estimation of narrow sense heritability (h_N^2) may then be used to predict the likely response to selection before embarking on a selection program (He *et al*., 2006). In this study, callus induction and green-plant regeneration parameters showed high heritability (Table 6), implying that these traits can be improved by making selections among the recombinants. Thus, genetic gain can be achieved if breeding strategies are designed for exploiting the anther culture response in rice cultivars. Similar results were reported by Chaudhary *et al*. (2003) and He *et al*. (2006).

Quantitative inheritance of anther culture response could complicate the selection process, analysis of combining abilities help to select suitable parents and the best hybrid combinations. Evaluating a good combination depends on both the value of GCA and SCA. A hybrid combination which has SCA value and good parental GCA values, may be considered as a suitable hybrid combination. For instance, hybrid combination of Ghasroddashti × Rashti for green-plant regeneration (Table 6), had the positive value of SCA and at least one of the parents had the highest GCA value. Therefore, GCA and SCA effects should be taken into account when developing the strategy of the selection of genotypes for obtaining hybrid combination with high callus induction and high green-plant regeneration ability.

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