ANALYSIS OF GENETIC AND GENOTYPE × ENVIRONMENT INTERACTION EFFECTS FOR AGRONOMIC TRAITS OF RICE (*ORYZA SATIVA* L.) IN SALT TOLERANCE

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Abstract

A diallel cross experiment of 4 rice (*Oryza sativa* L.) female and 6 male varieties was conducted to study the genetic effects and their interaction with salt-stress condition of 7 agronomic traits in normal and salt-stressed planting conditions. The panicle length (PL), effective number of panicles per plant (ENP), plumped number of grains per panicles (PNG), total number of grains per panicles (TNG), 1000-grain weight (W), seed setting ratio (SSR) and grain weight per plant (PGW), were investigated. A genetic model including additive effect, dominance effect and their interaction effects with environment (*ADE*) was employed for analysis of data. It was observed that significant (p<0.05) additive effects, dominance effects, additive × environment interaction effects and dominance × environment interaction effects exist for most of the agronomic traits of rice. In addition, significant (p<0.05) narrow sense heritabilities of ENP, PNG, TNG, W and PGW were found, indicating that the genetic performance of these traits are greatly affected by salt stress condition. A significant (p<0.05) negative correlations in the additive effects and additive × environment interaction effects detected between ENP and PNG suggesting that selection on increasing of ENP can reduce PNG. In addition, there exist a highly significant (p<0.01) positive dominance correlation among the dominance effects of the ENP, PNG and TNG, which shows that it is possible to breed salt-tolerant rice variety by coordinating large panicle and multi-panicle in utilization of heterosis.

Introduction

Salt injury is the most important issue which can adversely influence the non-biological agricultural production (Rhoades & Loveday, 1990). About 20% cultivated land and nearly half of the fields irrigated are spoiled by the salt stress in the world. With the increasing trend of global warming, the sea-level has been rising increasingly in the past decades. The latest satellite altimetry data on ocean showed that the rising-rate of global average sea-level has reached 3.2 mm/a since 1993 (Wu *et al.*, 2006; Li *et al.*, 2004). The rising of sea-level will lead to sea-water intrusion, soil salinization and consequently, the salinization of cultivated land is aggravated. The salt tolerance is crucial physiological characteristics of crops (Yeo & Flowers, 1986). Currently used measurement indicators of salt tolerance, generally, have low heritability (Akbar *et al.*, 1986; Gregorio & Senadhira, 1993), as a result, there is a lack of ideal salt-tolerant indicators for rice. In breeding practice, the salt-tolerance of rice has to be evaluated indirectly by measuring the response of agronomic characters to the stress of salt. Rice is

a kind of crop with middle salt-sensitiveness. Rice salt–tolerance, as a quantitative trait, is mainly controlled by multiple genes and easily affected by the environmental condition (Yeo *et al.*, 1990). Under salt stress condition, the phenotypes of agronomic traits are affected not only by its salt-tolerance but also by its own genetic mechanism. In addition, improving salt tolerance of crops is necessary for sustainable food production in different saline regions (Pitman & Lauchli, 2002). Therefore, it is necessary to understand the genetic mechanism on the response of different agronomic traits to salt stress, in order to improve the accuracy of genetic evaluation and selection efficiency of salt tolerance (Gu *et al.*, 1999).

The salt tolerance is important for rice scientists (Khatun *et al.*, 1995; Flowers & Yeo, 1995; Sahi *et al.*, 2006; Babu *et al.*, 2007). Rice exhibits different salt tolerance at different growth stages and the researchers are focusing more on seedling stage than on middle or late stage in studying the salt tolerance (Khatun *et al.*, 1995; Gu *et al.*, 1999). Among cereal crops, rice is a major source of food after wheat for more than 2.7 billion people. It is planted on about one-tenth of the earth's arable land and is the single largest source of food energy to half of the humanity (Naheed *et al.*, 2007). So, it is important to conduct genetic analysis to understand the genetic mechanism of salt tolerance of rice, so as to design better method for breeding rice variety of salt tolerance and effectively use salt-alkali land to enlarge the crop area for increasing yield production.

Materials and Methods

Experimental materials and design: A diallel cross was conducted between 4 female parents viz., FO2123 (P_1), RO2218 (P_2), GD1S (P_3), FO2124 (P_4) and 6 male parents RO2190 (P_5), RO2194 (P_6), RO2179 (P_7), RO2184 (P_8), RO2213 (P_9), and RO2210 (P_{10}). The hybrid offsprings (F_1) and all parents were planted in two different environments E1 (normal rice planting condition) and E2 (planting environment irritated by 0.5% salt water). The cross information of parents are presented in Table 1. The seeds of parents and F_1 hybrid were sown in the rice field of Guang-dong Ocean University experimental farm in late of the year 2004. Randomized block design was employed with three replications, taking 8 lines per plot with 10 plants in each line by taking a line distance of 15cm × 23 cm. The field was managed in a conventional procedure and all other agronomic practices were kept consistent. Ten plants were sampled from each plot for investigation of agronomic traits and the average value was used in statistical analysis. The investigated agronomic traits are the PL (panicle length), ENP (effective number of panicles per plant), PNG (plumped number of grains per panicles), TNG (total number of grains per panicles), W (1000-grain weight), SSR (seed setting ratio) and PGW (grain weight per plant).

Table 1. Varieties as parents and their F_1 progenies in diallel cross experiment.

	RO2190 (P₅)	RO2194 (P₆)	RO2179 (P₇)	RO2184 (P₈)	RO2213 (P₉)	RO2110 (P₁₀)
FO2123(P_1)	RO2185		RO2175	RO2180	RO2212	RO2108
RO2218(P_2)	RO2186	RO2192	RO2172	RO2183		
GD1S(P_3)		RO2191	RO2171	RO2182	RO2211	
F02124(P_4).	RO2189	RO2193	RO2176	RO2181		RO2102

P_1, P_2, P_3, and P_4 are female parents in diallel cross, while, P_5, P_6, P_7, P_8, P_9 and P_{10} are male parents

Statistical analysis: A genetic model including genotypic effects and genotype by environment interaction effects (Zhu, 1997) was used for studying the inheritance of aforementioned agronomic traits. Mixed linear model approach was applied for estimation of variance and prediction of random effects of various genetic components in the model. The total phenotypic variance (V_P) was partitioned into variances of general combining ability effect (σ_g^2), specific combining ability effect (σ_s^2), and their interaction effects with environment ($\sigma_{ge}^2, \sigma_{se}^2$). Using the estimated variances, the additive variance was estimated by $\hat{V}_A = 2\hat{\sigma}_g^2$, the dominance variance by $\hat{V}_D = \hat{\sigma}_s^2$, variance of additive by environment interaction effect by $\hat{V}_{AE} = 2\hat{\sigma}_{ge}^2$, variance of dominance × environment interaction effect by $\hat{V}_{DE} = \hat{\sigma}_{se}^2$. After obtaining each genetic variance components, the general narrow sense heritability was estimated by $\hat{h}_A = \hat{V}_A / \hat{V}_P$, the general narrow sense interaction heritability by $\hat{h}_{AE} = \hat{V}_{AE} / \hat{V}_P$ (Zhu, 1997). The same procedure was adopted for estimating genetic variance components and genetic covariance between two different traits (Zhu & Weir, 1994). Correlation coefficients were calculated, respectively, for additive effects, dominance effects, additive by environment interaction effects and dominance by environment interaction effects. Jackknife resampling technique was used to test the significance of genetic parameters in the model. All the analyses were performed by a software package QGAStation (http://ibi.zju.edu.cn/software/ qga/index_c.htm).

Results

Genetic variance components analysis: The estimates of all genetic variance components have been presented in Table 2. It is clear that the additive variance and the dominant variance reach the significance of 5% or 1%, as well as their interaction variances with environment for all the studied traits, indicating the prevalence of genotype by environment interaction. Therefore, the genetic model including both the genotypic effects and genotype × environment interaction effects, not the simple additive-dominance genetic model, is appropriate to analyze these surveyed traits.

It is evident that the variances of additive and dominance genetic effects are apparently larger as compared to their interaction effects with environment for the PNG, TNG and SSR, showing that the additive as well as the dominance effects are major genetic effects. On the other hand, the genetic variation in PL, ENP, W and PGW are mainly controlled by the genotype × environment interaction effects with exception of W which has larger and significant (p<0.01) additive variance (Table 2).

In terms of genetic variance components, the ENP and SSR are mainly controlled by the dominance effects and much stronger heterosis could be observed. In contrast to these two traits, the PL, TNG, W, PGW are mainly controlled by the additive effects, with relatively weaker heterosis, indicating much better effect can be expected in improvement of these traits by selection of advanced hybrid population.

In case of the variance of genotype × environment interaction effects, all dominance × environment interaction variances were significant and much larger as compared to the additive × environment interaction variances for all the studied traits. It shows that the heterosis of rice major agronomic traits are significantly (p<0.05) affected by salt stress and significant differences in heterosis can be found between different intensity of salt stress, especially for the PNG, TNG, SSR and PGW traits. All additive × environment interaction variances reached significant (p<0.05) or highly significant (p<0.01) levels for the aforementioned seven traits. For the ENP and TNG, highly significant (p<0.01) V_{AE} was recorded, showing that there will be more difference in phenotype for same variety or different cross combination of parents under condition of salt stress (es).

Table 2. Estimates of genetic variance components for seven major agronomic characters of rice.

Traits	$\hat{V}_A$	$\hat{V}_D$	$\hat{V}_{AE}$	$\hat{V}_{DE}$	$\hat{V}_\varepsilon$
PL	13.749**	8.274*	10.845*	12.846**	5.083[NS]
ENP	1.356**	2.036*	3.513**	4.136**	1.022*
PNG	89.519**	77.571**	14.532*	34.682**	8.254[NS]
TNG	551.803**	448.371**	103.742**	127.756**	81.265*
W	8.358**	1.246*	1.057*	1.568*	0.057[NS]
SSR	86.228*	169.657**	24.744*	51.890*	20.614*
PGW	19.364**	13.885**	11.064*	48.137**	12.963*

PL=Panicle length, ENP= Effective number of panicles per plant, PNG= Plumped number of grains per panicles, TNG=Total number of grains per panicles, W=1000-grain weight, SSR= Seed setting ratio, PGW= Grain weight per plant; $\hat{V}_A$ = estimator of additive variance, $\hat{V}_D$ = estimator of dominance variance, $\hat{V}_{AE}$ =estimator of additive-environment interaction variance, $\hat{V}_{DE}$ =estimator of dominance-environment variance, $\hat{V}_\varepsilon$ = residual variance; * and ** are significant at 0.05 and 0.01 levels of probability, respectively; NS indicate non significant.

Table 3. Estimates of heritability for major agronomic characters of rice.

Traits	$\hat{h}_G^2$ (%)	$\hat{h}_{GE}^2$ (%)	$\hat{h}_N^2$ (%)
PL	43.28**	20.92**	64.20**
ENP	13.97*	29.54**	43.51**
PNG	33.62**	16.01**	49.63**
TNG	45.28**	8.17	54.45**
W	60.52**	9.27	69.79**
SSR	39.25**	5.81	45.06**
PGW	19.15**	31.84**	50.99**

Traits are same as in Table 2; $\hat{h}_G^2$ = general heritability in narrow sense, $\hat{h}_{GE}^2$ = interaction heritability in narrow sense; $\hat{h}_N^2$ = heritability in narrow sense; * and ** indicate significance at 0.05 and 0.01 levels, respectively

Heritability estimates: The heritability (h_N^2) in narrow sense is generally referred to as the ratio of the additive variance to the phenotypic variance ($h_N^2 = V_A/V_P$). According to the Table 3, the h_N^2 of each of the studied trait reached the significant level of 0.01, which indicates that these traits have significant genetic selection effect and are mainly affected by the genetic additive effect. Except the ENP and the PGW, the heritability of other five agronomic traits is mainly determined by the general heritability in narrow sense in combination with the relatively small interaction narrow sense heritability. However, the interaction heritabilities in narrow sense (h_{GE}^2) of PL, ENP, PNG and PGW were detected significant at 1% level of probability. It indicates that these traits are largely affected by the salt stress environments and the selection response for these traits may be different under different environmental conditions.

Table 4. Estimates of genetic correlations between major agronomic traits of rice.

	Correlation type	PNG	TNG	W	PGW
ENP	r_A	-0.528**	0.525**	-0.285*	0.537**
	r_D	0.495**	0.663**	-0.176	0.654**
	r_{AE}	-0.319*	-0.529**	-0.202	-0.236*
	r_{DE}	0.179	0.594**	0.136	0.315*
PNG	r_A		-0.536**	-0.199	0.347*
	r_D		-0.486**	-0.328*	0.812**
	r_{AE}		0.438**	-0.491**	0.552**
	r_{DE}		-0.328*	0.185	0.168
TNG	r_A			-0.252*	0.327*
	r_D			-0.203	0.487**
	r_{AE}			0.332*	0.257*
	r_{DE}			0.193	0.115
W	r_A				0.531**
	r_D				0.602**
	r_{AE}				0.715**
	r_{DE}				0.157

The traits are same as in above table; r_A, r_D, r_{AE} and r_{DE} are correlation coefficients in the additive effects, the dominance effects, the additive × environment interaction effects, and the dominance × environment interaction effects between traits, respectively; * and ** indicate the correlation are detected at significant level of 0.05 and 0.01, respectively.

Genetic correlation between yield component traits: Genetic correlations among the agronomic/yield component traits are presented in Table 4. It reveals positive genetic correlations in the dominance effects among ENP, PNG, TNG, W and PGW reached the significance level of 0.05 or 0.01, indicating that the yield of single plant can be effectively increased by improving any of the yield component traits in the salt stress environment. However, ENP and PGW showed significantly negative additive × environment interaction correlation, as a result, the genetic correlation between ENP and yield of single plant is weakened. It can also be found there are significant positive correlations (additive × environment interaction) between PNG, TNG, W and PGW. The partition of total genetic correlation into correlation of different effects of genetic components will help us to uncover the essential of genetic correlation between paired traits, and provide theoretical proof for improving the comprehensive traits of rice and creating cultivation measures for high yield under salt stress conditions. Furthermore, it also shows a significant (p<0.05) or highly significant (p<0.01) negative correlation between the additive effects and additive × environment interaction effects of ENP and

PNG, while, the additive effects between ENP and TNG, the dominance effects between ENP and PNG and dominance × environment interaction effects between ENP and TNG exhibited remarkable positive correlation. Therefore, the breakthrough of rice yield can be achieved by utilization of heterosis in large-panicle and multi-panicles. However, due to the fact that there are significant or highly significant positive or negative correlations between yield component traits of rice, it is an important and difficult task to achieve high and stable rice yield by coordinating main yield component traits.

Discussion

The quantitative traits of crops, whose performance in terms of phenotype is the final result of a mixture effects of genes, environments and their complicated interactions. Existence of genotype × environment interaction is very prevalent in phenomena in biological processes. The genetic performance of hybrid in one specific environment is determined by following two genetic component effects, one is the genotypic values, and the other is the genotype × environment interaction effects. Genotypic value (or general heterosis) measures the average performance of a variety in all environments. In contrast, the genotypic × environment interaction effect (or interaction heterosis) measures the deviation in specific environment from the average performance in all environments (Wu & Zhu, 1994). It is necessary to understand the effect of each genetic component and their interaction with environment for obtaining efficiently breeding superior cross combination with high and stable yield. In the present study, we used the genetic model containing additive, dominance effects and their interaction effects with environment (*ADE*) (Zhu, 1997) to analyze the genetic effects of rice agronomic traits under salt stress environment.

The heritability is an important parameter defined by the ratio of genetic variance to phenotypic variance. If the phenotypic variation of trait is mainly composed of genetic variance, expected improvement of trait could be achieved by selection on genetic effects. If the additive variance is the major constitution in total genetic variance, selection on population should be carried out in early generation. Under normal or salt stress environment, significant genetic variances for the agronomic traits of rice can be detected, which shows the existence of wide genetic variation for these characters. They have relatively high and significant heritability in narrow sense, indicating that these traits are mainly governed by the additive effects of genes. Based on the obtained results, therefore, it is suggested to breed salt-tolerant variety with high yield by selection of additive effects.

Both the additive × environment interaction and the dominance × environment interaction were found significant for all the studied agronomic traits, which indicate the prevalence of genotype × environment interaction. The results also clearly showed that the dominance × environment interaction was greater as compared to additive × environment interaction for each of the studied trait. It indicates that there could be more different heterosis between environments, thus, in the procedure of testing and distinguishing the cross combination with strong heterosis, experiment conducted in several years and locations should be paid more attention. The breeding potential of cross combination will be truly evaluated only by integrated analysis on the data from experiments conducted in multiple environments experiment.

In the present study, the interaction heritability in narrow sense is larger than the general heritability in narrow sense for the ENP and the PGW, which indicate that

selection response on these two traits will exhibit greater differences between different salt stress environments, selection under one can not be applied in another salt stress environment. The h^2_{GE} of the PL, ENP, PNG and PGW reached highly significant level, suggesting that the genetic variation of these traits are largely affected by environment, as a result, different selection response might be expected under different environments.

In the present investigation, we partitioned the total genetic correlation into the correlations of the additive effects, dominance effects, additive or dominance × environment interaction effects. This partitioning is necessary to disclose the essential of genetic correlation between agronomic traits under different salt stress conditions. In the practice of high-yield breeding and cultivation for rice, improvement of the main component traits of the rice yield are usually in confliction with each other and difficult to coordinate. By further decomposition of total genetic correlation into different genetic components correlations, it is necessary to clearly understand the relationship between traits, so as to seek appropriate direct or indirect selection strategy to improve them simultaneously. The present findings shows that the additive effects and additive × environment interaction effects of the ENP and PNG are significantly ($p<0.05$) negatively correlated indicating that selection on ENP could reduce the PNG. At the same time, there exist a highly significant positive dominance correlation among the dominance effects of ENP and, PNG, and TNG, indicating that it is possible to breed salt-tolerant rice variety by coordinating large panicle and multi-panicle in utilization of heterosis.

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