

Analysis of Developmental Genetics for Phenylalanine Content in *Indica-Japonica* Hybrid Rice (*Oryza sativa* L.) Across Environments

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Summary

Developmental genetics of phenylalanine content in *indica-japonica* hybrid rice (*Oryza sativa* L.) have been studied through 7 *indica* rice crossed with 5 *japonica* rice by using the developmental genetic models and corresponding statistical approaches for quantitative traits of triploid seeds in cereal crops. The unconditional genetic model was used to analyze the cumulative genetic effects (from flowering to a specific time) along the developmental stages, and the conditional genetic model was used to analyze the genetic effects in one specially developmental stage from one filling time to another time ($t-1 \rightarrow t$). Results showed that phenylalanine content of *indica-japonica* hybrid rice was simultaneously controlled by the triploid endosperm effects, cytoplasm effects, diploid maternal effects and their genotype $\times$ environment interaction effects. Endosperm dominance effect, maternal additive effect and cytoplasmic effect were more important at the initial stage of rice development than endosperm additive effect and maternal dominance effect. With regard to the components of heritability, maternal and cytoplasm general heritability and their interaction heritability were higher for phenylalanine content at all 5 developmental stages. Respectively, it occupied 68.3, 92.4, 100.0, 100.0 and 78.9% among all the component of heritability. It was suggested that improving the phenylalanine content of *indica-japonica* hybrid rice would be more efficient when selection was based on maternal plants at early generations in rice breeding program.

Key words: *indica-japonica* hybrid rice; developmental genetics; quality; phenylalanine content; genetic variance; conditional genetic variance; heritability

Introduction

Advantages of *indica* rice and of *japonica* rice could be integrated by planting their F_1 hybrids, especially for their strong tillering ability and larger panicle although some disadvantages existed in F_1 hybrids. Recently, great progress has been made for improving agronomic traits in the utilization of some *indica-japonica* hybrid breeding projects. Emphasis of rice breeding programs in major rice producing countries has shifted to produce a better quality of rice because the yield potential of current rice varieties has reached a rather high plateau (Bolker and Raff, 1996; Chauhan *et al.* 1992). Consumers favor certain varieties and value specific appearances and tastes of milled rice for home cooking. Varieties have different grain configurations and grains differ in chemical composition, influencing milling quality. It is possible to improve rice quality by using different breeding approaches and selecting a good quality of rice. Data are tabulated on varietal impacts on quality characteristics, such as amylose content and composition, grain properties, protein digestibility and nutritive value (Cooper *et al.* 1999).

The expression of genes for phenylalanine content at different filling stages in rice might depend on genotype $\times$ environment interaction effects as well as genetic main effects during developmental stages of grains. So, understanding the dynamics of gene sequential expression across environments would be helpful for improving rice quality. The objective of the present study was to clarify the developmental behavior of gene expression for phenylalanine content of *indica-japonica* hybrid rice under different environments. The developmental dynamic

expression mechanism from different genetic systems was investigated by evaluating the conditional and unconditional genetic effects, and by estimating the heritability components at different filling stages across environments.

In most of the reports, phenotypic value of rice at maturity was used for analyzing quality traits of rice (Kannan *et al.* 2001; Khush and Virk 2000; Kunze 1985; Miller 1974; Peng *et al.* 1994; Pomp *et al.* 1998). However, the developmental processes of these traits could not be revealed. Although the analyzing software for the quantitative traits of seed generated recently makes developmental genetic analysis possible (Ravichandran and Parthiban 1998), but only a few papers on developmental behavior for rice quality has been reported in *indica* rice (Sabelli 19966; Shi *et al.* 1999; Shi *et al.* 2002a) and in *indica-japonica* hybrid rice for rice nutrient qualities (Shi *et al.* 2002b, Zhang, *et al.* 2004). One of the reasons for this is that it is difficult to gain a great number of F₁ seeds for analyzing because they are normally incompatible when *indica* rice is crossed with *japonica* rice.

We created 5 *japonica* wide compatible restores that have higher seed sets when crossed with *indica* sterile lines in a *indica-japonica* hybrid program. It makes this research possible because to make much pure F₁ *indica-japonica* hybrids become so easy by using restore lines and sterile lines.

Materials and methods

Plant materials: 7 *indica* cytoplasmic male sterile lines (A) (Zhe38, Xieqingzao, K17, Zhenshan 97, Zhenong 8010, Jin23 and II-32) and their maintainers (B), and 5 *japonica* wide compatible restorers (R) (T748, T42, R1252, Linhui 422 and Zhong 9308) were used in this experiment.

Field experiments: The mating design used in this experiment was a factorial design. All the F₁ seeds were crossed female parents to male parents (A × R) and were gained at Hainan province (latitude: N18°22'; longitude: E109°45') in the spring season of 2001. Seeds of the parents (A, B and R) and F₁ crosses (A × R) were sown on May 25 in 2001 and 25-day old seedlings were individually transplanted at a spacing of 20 × 26 cm within rows and 36 plants in each plot with two replications on the experimental farm at Zhejiang Academy of Agricultural Sciences (latitude: N30°16'; longitude: 120°12'), China. The second environmental experiment was performed as the above and sown on December 12, 2001 and transplanted on January 10, 2002 at Hainan province, China. According to the developmental stages of rice, the whole filling period was divided into an initial stage (1-7days after flowering), early stage (8-14days), middle stage (15-21days), late stage (22-28 days) and a ripe stage (29-35days). Each panicle from central 16 plants of each plot was labeled and only those flowering grains on that day were grown in the panicle. Seed samples from 12 parents and 35 F₂s in F₁ plants were collected at 7-, 14-, 21-, 28- and 35-day after flowering. F₁ samples analyzed were crossed females (A) to males (R) during the same growing season and harvested in the same way.

Phenylalanine content analysis: All seed hulls including F₁s, F₂s and their parents were taken off by the machine of Yanmar ST50 (Yanmar Co. Ltd, Chayamachi, Kita-ku Osaka, Japan) and milled by a sample miller (model JB-20, Zhejiang province, China). They were further ground to 100 mesh with model 3010-019 cyclone grinder (Fort Collins, Colorado, USA). Phenylalanine content, including free phenylalanine and bound phenylalanine in rice powder, was analyzed by amino acid autoanalyzer (Shi *et al.* 2002b) and duplicated determinations were made for each sample.

Statistical methods: Genotypic differences of phenylalanine content (%) in dry weight at 5 different filling stages from 12 parents and their F_1 and F_2 generations were analyzed by SAS version 9.0 (Proc ANOVA).

Two developmental genetic models, unconditional and conditional models were used in this study.

The unconditional genetic main effects and genotype $\times$ environment interaction effects at different filling stages (t) were analyzed using an extension of the triploid endosperm model for endosperm quantitative traits of cereal crop (Zhu and Weir, 1994). MINQUE (0/1) method (Zhu and Weir, 1994) as used to estimate variances and covariances at different developmental stages (t). For the unconditional analysis, genetic effects were defined as accumulated effects of genes expressed from flowering (0) to a particular time (t). The variance components can be measured into endosperm additive (V_A) and dominance variance (V_D), cytoplasmic variance (V_C), maternal additive (V_{Am}) and dominance variance (V_{Dm}), and their endosperm additive (V_{AE}) and dominance interaction variance (V_{DE}), cytoplasm interaction variance (V_{CE}), maternal additive (V_{AmE}) and dominance interaction variance (V_{DmE}) and residual variance (V_e). Since partial endosperm genes were derived from maternal plants, there might be covariance between endosperm effects and maternal effects including additive ($C_{A.Am}$) and dominance covariance ($C_{D.Dm}$), additive interaction ($C_{AE.AmE}$) and dominance interaction covariance ($C_{DE.DmE}$). The partitioning for the phenotypic variance ($V_{p(t)}$) is:

$$V_{P(t)} = V_{A(t)} + V_{D(t)} + V_{C(t)} + V_{Am(t)} + V_{Dm(t)} + V_{AE(t)} + V_{DE(t)} + V_{CE(t)} + V_{AmE(t)} + V_{DmE(t)} + 2(C_{A.Am(t)} + C_{D.Dm(t)} + 2(C_{AE.AmE(t)} + C_{DE.DmE(t)} + V_{e(t)})$$

For the conditional analysis, the developmental genetic models and statistical methods could be used to estimate conditional variances during the designated filling periods ($t-1 \rightarrow t$). In the experiment, 7d|0d indicated the cumulative effects of gene expression from the initial time to 7 days after flowering, whereas 14d|7d represented the measures at 14 days given the phenotype values measured at 7 days for conditional analysis, and so on. These conditional variance components were $V_{A(t|t-1)}$ (conditional endosperm additive variance), $V_{D(t|t-1)}$ (conditional endosperm dominance variance), $V_{C(t|t-1)}$ (conditional cytoplasmic variance), $V_{Am(t|t-1)}$ (conditional maternal additive variance), $V_{Dm(t|t-1)}$ (conditional maternal dominance variance), $V_{AE(t|t-1)}$ (conditional endosperm additive interaction variance), $V_{DE(t|t-1)}$ (conditional endosperm dominance interaction variance), $V_{CE(t|t-1)}$ (conditional cytoplasm interaction variance), $V_{AmE(t|t-1)}$ (conditional maternal additive interaction variance), $V_{DmE(t|t-1)}$ (conditional maternal dominance interaction variance), $C_{A.Am(t|t-1)}$ (conditional additive covariance), $C_{D.Dm(t|t-1)}$ (conditional dominance covariance), $C_{AE.AmE(t|t-1)}$ (conditional additive interaction covariance), $C_{DE.DmE(t|t-1)}$ (conditional dominance interaction covariance) and $V_{e(t|t-1)}$ (conditional residual variance). The partitioning for the conditional phenotypic variance $V_{p(t|t-1)}$ is:

$$V_{P(t|t-1)} = V_{A(t|t-1)} + V_{D(t|t-1)} + V_{C(t|t-1)} + V_{Am(t|t-1)} + V_{Dm(t|t-1)} + V_{AE(t|t-1)} + V_{DE(t|t-1)} + V_{CE(t|t-1)} + V_{AmE(t|t-1)} + V_{DmE(t|t-1)} + 2(C_{A.Am(t|t-1)} + C_{D.Dm(t|t-1)}) + 2(C_{AE.AmE(t|t-1)} + C_{DE.DmE(t|t-1)}) + V_{e(t|t-1)}$$

Net genetic effects, defined as the effects of genes expressed from flowering to time t , were detected by using unconditional analysis. The estimated total narrow-sense heritability (h^2) at different filling stages were differentiated into general heritability (h_G^2) and interaction heritability (h_{GE}^2). The h_G^2 could be divided into components including endosperm general

heritability ($h_{Go}^2 = (V_A + C_{A \cdot Am})/V_P$), cytoplasm heritability ($h_{Gc}^2 = V_C/V_P$), and maternal general heritability ($h_{Gm}^2 = (V_{Am} + C_{A \cdot Am})/V_P$). The h_{GE}^2 also could be divided into components including endosperm interaction heritability ($h_{GoE}^2 = (V_{AE} + C_{AE \cdot AmE})/V_P$), cytoplasmic interaction heritability ($h_{GcE}^2 = V_{CE}/V_P$) and maternal interaction heritability ($h_{GmE}^2 = (V_{AmE} + C_{AE \cdot AmE})/V_P$). The partitioning for the total narrow-sense heritability was $h^2 = h_G^2 + h_{GE}^2 = (h_{Go}^2 + h_{Gc}^2 + h_{Gm}^2) + (h_{GoE}^2 + h_{GcE}^2 + h_{GmE}^2)$. The Jackknife re-sampling method was employed by sampling generation means of entries for estimating standard errors of estimated components of variances, covariance and heritability (Shi *et al.* 1996; Singer *et al.* 1999). All data were analyzed by programs on a PC computer.

Results

Unconditional analysis for phenylalanine content at different filling stages

Table 1 showed that phenylalanine content was controlled by genetic main effects and by genotype $\times$ environment interaction effects. Among different genetic systems, as compared with the genetic main effects (V_G), phenylalanine content was mainly controlled by the genotype $\times$ environment interaction effects (V_{GE}) at first three developmental stages (7th, 14th and 21st day after flowering) and V_{GE} were accounted for 72.88, 89.63 and 85.68% of total genetic variance. The last two developmental stages were mainly controlled by the genetic main effect and V_G were accounted for 67.97 and 57.74% of total genetic variance.

Table 1 Estimates of unconditional variance components at different developmental stages for phenylalanine content in *indica-japonica* rice

Parameter	Developmental time of rice				
	7d	14d	21d	28d	35d
V_A	0.000	0.000	0.000	0.000	4.496**
V_D	0.595**	0.518**	0.538**	0.000	0.468**
V_C	0.491**	0.000	0.762**	1.983**	1.215**
V_{Am}	2.290**	0.000	0.000	3.467**	5.199**
V_{Dm}	0.000	0.981**	0.000	0.578**	0.000
V_{AE}	3.033**	3.042**	0.000	0.000	0.000
V_{DE}	0.728**	0.754**	0.539**	0.634**	0.945**
V_{CE}	0.964**	2.426**	2.626**	1.458**	2.317**
V_{AmE}	3.238**	5.480**	3.629**	0.000	3.772**
V_{DmE}	1.110**	1.259**	0.985**	0.748**	1.293**
$C_{A \cdot Am}$	0.000	0.000	0.000	-1.290	-1.583
$C_{D \cdot Dm}$	0.000	-0.168	0.000	0.000	0.000
$C_{AE \cdot AmE}$	0.395	-3.013	0.000	0.000	0.000
$C_{DE \cdot DmE}$	-0.160	0.028	0.017	-0.065	-0.032
V_e	0.107**	0.165**	0.160**	0.124**	0.078**

** : significant at 0.01 probability level.

For genetic main effects, it was found that there were endosperm dominance, maternal additive and cytoplasmic effects at the initial stage; endosperm dominance and maternal dominance effects at the early stage; endosperm dominance and cytoplasmic effects at the

middle stage; maternal additive, maternal dominance and cytoplasmic effects at the late stage; and all the genetic main effects except for maternal dominance effect at the ripe stage. For the interaction effects, the genotype $\times$ environment interaction effects were all found at different filling stages except for endosperm additive interaction effect at middle- to ripe-stage and maternal additive interaction effect at the late stage.

According to above results, phenylalanine content was simultaneously controlled by both genetic main effects and their genotype $\times$ environment interaction effects, but mainly controlled by their interaction effects at first and then changed into genetic main effects. In total, the phenylalanine content was mainly controlled by additive effects at the initial stage, then controlled by dominance effects until late stage, at last controlled by additive effect again. Since most of the genetic main covariance ($C_{A \times Am}$ or $C_{D \times Dm}$) or genotype $\times$ environment interaction covariance ($C_{AE \times AmE}$ or $C_{DE \times DmE}$) between the endosperm and maternal effects were all not significant for phenylalanine content, there was no relationship detected between the expression of endosperm and maternal genes at all different filling stage.

Conditional analysis for phenylalanine content at different filling stages

The results in Table 2 showed there was no detection of new genes expression for (1) conditional endosperm additive main effects from initial- to middle-stage, (2) conditional maternal additive main effects at the early stage and at the middle stage, (3) conditional endosperm dominance main effects at the late stage, (4) conditional maternal dominance main effects at the initial stage and at the late stage and (5) conditional cytoplasmic main effects at the early stage and at the ripe stage, since the conditional genetic main variances at these filling stages were not found in this experiment.

Table 2 Estimates of conditional variance components for phenylalanine content at different developmental stages in *indica-japonica* rice

Parameter	Developmental stage of grain				
	7d 0d	14d 7d	21d 14d	28d 21d	35d 28d
$V_A(t t-I)$	0.000	0.000	0.000	1.711**	3.787**
$V_D(t t-I)$	0.595**	0.810**	0.669**	0.000	0.379**
$V_C(t t-I)$	0.491**	0.000	1.137**	0.620**	0.000
$V_{Am}(t t-I)$	2.290**	0.000	0.000	1.833**	3.070**
$V_{Dm}(t t-I)$	0.000	0.769**	0.486**	0.000	0.112**
$V_{AE}(t t-I)$	3.033**	16.016**	0.000	0.000	0.000
$V_{DE}(t t-I)$	0.728**	1.341**	0.000	0.680**	0.710**
$V_{CE}(t t-I)$	0.964**	0.000	0.000	0.000	0.000
$V_{AmE}(t t-I)$	3.238**	10.805**	8.787**	0.000	2.950**
$V_{DmE}(t t-I)$	1.110**	1.347**	0.535**	0.528**	1.139**
$C_{A \times Am}(t t-I)$	0.000	0.000	0.000	0.000	-1.062
$C_{D \times Dm}(t t-I)$	0.000	-0.039	-0.021	0.000	2.242**
$C_{AE \times AmE}(t t-I)$	0.395	-5.299	-5.855	0.000	0.000
$C_{DE \times DmE}(t t-I)$	-0.160	0.526 ⁺	0.000	-0.120	-0.115
$V_e(t t-I)$	0.107**	0.171**	0.167**	0.110**	0.072**

⁺ and **: significant at 0.10 and 0.01 probability level, respectively.

Compared to conditional results in Table 2, it can be assumed that the unconditional cytoplasmic main effect at the ripe stage in Table 1 might be due to the cytoplasmic effect

expressed at the former stage was closed at the late stage, and then continual expression from activated genes at this stage. On the contrary, conditional additive main effect at the late stage was found by conditional analysis, but not found by unconditional analysis, whereas it was found at the ripe stage in Table 1. It probably means that the method of conditional analysis sometimes can understand the expression of gene a little earlier than by unconditional analysis. The results in Table 2 also revealed a phenomenon of gene expression being interrupted during different filling stages and the activation of quantitative genes was gradually carried through the continuous filling period(s). These results were difficult to be detected by the unconditional genetic variance analysis. For the conditional interaction analyzing, it showed similar results with conditional main effect analysis.

There was no significant relationship between the expression of endosperm and maternal genes at different filling stages except for that between conditional endosperm dominance and maternal dominance effects at the ripe stage ($C_{D,Dm}=2.242^{**}$). This result implied that the positive relationship existed for the new expression from endosperm dominance effect and maternal dominance effect at the ripe stage.

Estimation of heritability at different filling stages

The results in Table 3 showed that the general and interaction heritability components from endosperm were all not significant at various filling stages. Significant maternal and cytoplasmic general heritability were found at the initial, middle and late stages except for maternal general heritability at the middle filling stage of rice. Maternal and cytoplasm interaction heritabilities were not found until the early stage. With regard to the components of heritability, maternal and cytoplasm general heritability and their interaction heritability were more important for phenylalanine content at all the developmental stages. It was suggested that the improving for phenylalanine content would be more efficient when selection is based on the performance of maternal plants in *indica-japonica* hybrid rice breeding program.

Table 3 Estimates of heritability components (%) for phenylalanine content at different developmental stages in *indica-japonica* rice

Parameter	Developmental stage of grain				
	7d	14d	21d	28d	35d
h_{Go}^2	0.000	0.000	0.000	0.000	0.176
h_{Gc}^2	0.038**	0.000	0.082**	0.248**	0.073
h_{Gm}^2	0.176**	0.000	0.000	0.272**	0.218
h_{GoE}^2	0.263	0.049	0.000	0.000	0.000
h_{GcE}^2	0.074	0.323**	0.283**	0.182**	0.140**
h_{GmE}^2	0.279	0.275**	0.391**	0.000	0.228**

** : significant at 0.01 probability level

Discussion

This paper revealed that phenylalanine content was mainly controlled by triploid endosperm effect, cytoplasm effect, diploid maternal effect and their genotype $\times$ environment interaction effect both by variance analysis and by heritability analysis. The results of the

conditional genetic variances for phenylalanine content of rice further indicated that there was a phenomenon of interval expression of the endosperm, cytoplasm and maternal genetic factors among rice filling stages. These results might be useful for quantitative trait loci (QTLs) analysis or marker assisted selection (MAS) for improving phenylalanine content, because of the difference on the expression of the genes at different developmental stages.

The genetic analysis further showed that phenylalanine content was simultaneously controlled by the components of different genetic effect from triploid endosperm, cytoplasm, and diploid maternal genetic systems. There were more endosperm additive effects, maternal additive effects and cytoplasmic effects at the initial, late and mature stages by unconditional analysis. Therefore, improvement could be expected by selection mature grains for high phenylalanine content in rice breeding.

This experiment also showed that genotype-environment interaction effects were important for phenylalanine content at most filling stages, which indicated that the sequential expression of genes from endosperm, cytoplasm, and maternal genetic systems was influenced by the environmental conditions. This kind of interaction was the main cause for the genetic differences across environments at various filling stages. These differences could cause the difference of genes expression for phenylalanine content at different environments. Rice breeders could improve the quality trait of rice with small genotype $\times$ environment interaction effects more effectively, which were suitable to different environments in pure-line breeding at different places. It is necessary to consider the variation of rice quantitative traits in different environments because of the varied climatic conditions, planting manner, soil fertilities, and so on.

The genetic basis of the endosperm quantitative traits of rice is more complex than the common plant agronomic traits (Chauhan *et al.* 1992). Rice seeds are small and difficult to be produced by artificial emasculation and pollination in field experiments. It is more difficult to study the developmental genetic variances at the special filling period for the endosperm traits of rice. The genetic models and statistical analysis methods based on the mixed linear model approaches (Zhu and Weir 1994; Zhu and Weir 1996) to analyze the genetic effects only need the means of three generations, such as parents, F_1 and F_2 , among various filling stages of rice at different environments. According to the magnitude of endosperm, cytoplasmic and maternal genetic effects and genotype $\times$ environment interaction effects, it was able to illustrate the genetic mechanism of endosperm quality traits, which are of importance for improving rice quality through breeding.

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