

RESEARCH ARTICLE

Detecting relationships between amylose content and amino acid contents of *indica* rice with conditional approach

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Abstract

The relationship between the genetic effects of endosperm, cytoplasm and maternal plant on amylose content (AC) and amino acid contents of *indica* rice was studied using unconditional and conditional analysis methods. The results indicated that the protein content (PC) and brown rice weight (WBR) could significantly affect the relationships between AC and amino acid contents of rice. The phenotypic and genotypic covariances between AC and amino acid contents were most significantly negative under the interference of PC or WBR, but most of the relationships for the paired traits were not significant after excluding the influence of PC or WBR on AC. For the conditional genetic relationship analysis of different genetic systems including endosperm, cytoplasm and maternal plant, visible changes were found in many genetic correlation components between AC and amino acid content after eliminating the influences of PC, especially, for the endosperm or maternal additive effects, endosperm additive or dominance interaction effects and maternal additive interaction effects. The relationships of the paired traits conditioned on WBR were mainly controlled by the endosperm dominance or additive interaction effects.

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Introduction

The amylose content (AC) in endosperm is closely correlated with cooking and eating quality of rice and is mainly governed by genetic effects and environmental conditions. Genetic analysis of AC on rice and the relationship between AC and nutrition quality traits have been well documented by traditional statistical analysis. Reddy and Sarala (1979) found that there was no significant correlation between AC and protein content (PC); but Wang *et al.* (2005) found that there was significant negative correlation for this pair of traits in rice. AC was significantly correlated to the alkali digestion value (ADV) or gel consistency (GC) (Reddy and Sarala 1979). Sood and Siddiq (1986) considered that AC had significant positive correlation to the ADV in aromatic rice.

The relationships among the quality traits of rice are complex, since most of them are quantitative traits (Pooni *et al.* 1993; Tang and Khush 1993; Shi *et al.* 1996b, 1997;

Wu *et al.* 2004). Endosperm is the major storage organ of the nutritive materials and the genetic effects of the triploid endosperm nuclear genes could affect the rice quality traits. Since the rice grain is of a new generation that differs from its maternal plant, which provides the nutrient materials for rice grain growth, the genetic effects from diploid maternal plant nuclear genes and cytoplasmic genes are also important for rice quality. Pooni *et al.* (1992) suggested that AC of rice might be related to the genetic effects of the maternal plant or cytoplasmic genes. Significant genetic effects of endosperm and maternal plants were detectable for rice quality traits (Shi and Zhu 1994; Shi *et al.* 1996a,b). Since the variation of gene expression could be observed in different environments, the performance and the correlations of the quantitative traits were also affected by genotype × environment (GE) interaction effects besides the genetic main effects (Paterson *et al.* 1991; Zhuang *et al.* 1997; Shi *et al.* 1997, 1999a,b; Bao and Xia 1999).

The increase in weight of brown rice (WBR) could affect AC of rice (Zhong and Li 1994) and so does PC (Wang

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et al. 2005). Because PC is determined by the content of amino acids in the rice grain, high correlations were found for some paired traits (Souza et al. 1993; Shi et al. 1996a). Hence, the correlations between AC and rice nutrition quality traits might be influenced by WBR and PC. Till date, only few information were found about the correlations between AC and amino acid contents of rice, and the influence of WBR or PC on the relationship between AC and the different amino acid contents was still not detected. The objectives of the present study were to evaluate the genetic relationships between AC and amino acid contents of rice with the unconditional and conditional statistical methods. Comparisons between the unconditional and conditional results could be helpful in providing a clear picture of the genetic relationship between AC and amino acid contents of rice, and provide useful information for carrying out rice breeding with respect to increasing the yield and improving the cooking and nutrition quality of rice.

Materials and methods

Field experiment

The mating design used in the experiment was a factorial design with 12 parents. Seven *indica*-type cytoplasm male sterile lines (CMS or A) including Zhexie 2, Xieqingzao, Zhenan 3, Zhenshan 97, Gangchao 1, V 20 and Zuo 5 were used as females. They were crossed with five restorer lines (R) including T 49, Cezao 2-2, 26715, 102 and 1391 to produce F₁ seeds (A×R) in 1997. The derived F₁ hybrids, together with their parents, were grown in randomized complete plots with two replications in 1998 and 1999. The experiment was conducted at the experimental farm of Zhejiang University, Hangzhou, PR China. The seeds of parents and their F₁ were sown in nursery seedling boxes on 30 March after germination in both years. Thirty-one day old seedlings were transplanted to the same piece of rice paddy field on a silty-loamy soil with a single plant per hill spaced at 20 cm within a row. Each plot consisted of six rows with 36 plants. All the management practices on the rice field were similar to conventional practice of local normal cultivated methods of rice during the course of the experiments. Seeds of parents and F₂ from F₁ plants were sampled for a batch of 16 plants in the middle part of each plot at maturity. The F₁ seeds harvested were obtained by crossing CMS lines to restoring lines during the same growing season. All brown rice were milled by a sample miller (model JB-20, Huangyan, China) and further ground into 100-mesh powder with a model 3010-019 cyclone grinder (UDY Corporation, Fort Collins, USA). AC, PC and 16 different amino acid contents of rice including aspartic acid (Asp), threonine (Thr), serine (Ser), glutamic acid (Glu), glycine (Gly), alanine (Ala), cysteine (Cys), valine (Val), isoleucine (Ile), leucine (Leu), tyrosine (Tyr), phenylalanine (Phe), lysine (Lys), histidine (His), arginine (Arg) and proline (Pro) were measured using the method of near-

infrared reflectance spectroscopy with a NIR systems model 5000 monochromator (NIR Systems, Silver Spring, USA) (Wu et al. 2002). Each sample (3 g) was scanned in a small ring cup of 3.6 cm inner diameter. All samples from each parent, F₁ and F₂ were measured with two replications.

Statistical analysis

The MINQUE (0/1) method (Zhu and Weir 1994) was used to estimate variances and covariances. The unconditional phenotypic covariances (C_P) between AC and amino acid contents were obtained by the mixed model approaches for the unconditional analysis of quantitative traits (Zhu and Weir 1994). Since the performance of the quality traits of rice was simultaneously controlled by genetic main effects and GE interaction effects from triploid endosperm nuclear genes, cytoplasmic genes and diploid maternal plant nuclear genes, C_P can be partitioned as,

$$\begin{aligned} C_P &= C_G + C_{GE} + C_e \\ &= C_{Ge} + C_C + C_{Gm} + 2C_{Ge-Gm} + C_{GeE} + C_{CE} \\ &\quad + C_{GmE} + C_{GeE-GmE} + C_e \\ &= C_{Ae} + C_{De} + C_C + C_{Am} + C_{Dm} + 2(C_{Ae-Am} + C_{De-Dm}) \\ &\quad + C_{AeE} + C_{DeE} + C_{CE} + C_{AmE} + C_{DmE} \\ &\quad + 2(C_{AeE-AmE} + C_{DeE-DmE}) + C_e, \end{aligned}$$

where C_P , phenotypic covariance; C_G , genotypic covariance; C_{GE} , GE interaction covariance; C_e , residual covariance; C_{Ge} , endosperm genetic main covariance; C_C , cytoplasm genetic main covariance; C_{Gm} , maternal genetic main covariance, C_{Ge-Gm} , covariance between endosperm and maternal genetic main effects; C_{Ae} , endosperm additive main covariance, C_{De} , endosperm dominance main covariance, C_{Am} , maternal additive covariance, C_{Dm} , maternal dominance covariance, C_{Ae-Am} , covariance between endosperm and maternal additive effects, C_{De-Dm} , covariance between endosperm and maternal dominance effects, and C_{GeE} , C_{CE} , C_{GmE} , C_{AeE} , C_{DeE} , C_{AmE} , C_{DmE} , $C_{GeE-GmE}$, $C_{AeE-AmE}$, $C_{DeE-DmE}$ means their corresponding GE interaction covariances, respectively.

Conditional phenotypic covariance ($C_{P(T_1|T_2)}$) between AC|PC or AC|WBR and amino acid contents for phenotypic value ($y_{(T_1|T_2)}$) were obtained by the mixed model approaches of conditional genetic analysis for quantitative traits (Zhu 1995). For the quantitative traits of rice quality which were simultaneously affected by endosperm, cytoplasmic and maternal plant, $C_{P(T_1|T_2)}$ can be further partitioned as,

$$\begin{aligned} C_{P(T_1|T_2)} &= C_{G(T_1|T_2)} + C_{GE(T_1|T_2)} + C_{e(T_1|T_2)} \\ &= C_{Ge(T_1|T_2)} + C_{C(T_1|T_2)} + C_{Gm(T_1|T_2)} \\ &\quad + 2C_{Ge-Gm(T_1|T_2)} + C_{GeE(T_1|T_2)} + C_{CE(T_1|T_2)} \\ &\quad + C_{GmE(T_1|T_2)} + 2C_{GeE-GmE(T_1|T_2)} + C_{e(T_1|T_2)} \\ &= C_{Ae(T_1|T_2)} + C_{De(T_1|T_2)} + C_{C(T_1|T_2)} + C_{Am(T_1|T_2)} \\ &\quad + C_{Dm(T_1|T_2)} + 2(C_{Ae-Am(T_1|T_2)}) + C_{De-Dm(T_1|T_2)} \end{aligned}$$

$$\begin{aligned}
 &+ C_{AeE(T1|T2)} + C_{DeE(T1|T2)} + C_{CE(T1|T2)} \\
 &+ C_{AmE(T1|T2)} + C_{DmE(T1|T2)} + 2(C_{AeE-AmE(T1|T2)} \\
 &+ C_{DeE-DmE(T1|T2)}) + C_{e(T1|T2)},
 \end{aligned}$$

where T1|T2 denotes trait 1 conditioned on trait 2, it means the conditional phenotypic value when excluding the impacts of trait 2 on trait 1(AC|PC, amylose content conditioned on protein content, AC|WBR, amylose content conditioned on weight of brown rice). $C_{P(T1|T2)}$, conditional phenotypic covariance of trait 1 conditioned on trait 2; $C_{G(T1|T2)}$, conditional genotypic main covariance of trait 1 conditioned on trait 2; $C_{GE(T1|T2)}$, conditional GE interaction covariance of trait 1 conditioned on trait 2; $C_{e(T1|T2)}$, conditional residual covariance of trait 1 conditioned on trait 2; $C_{Ge(T1|T2)}$, conditional endosperm main covariance of trait 1 conditioned on trait 2; $C_{C(T1|T2)}$, conditional cytoplasm main covariance of trait 1 conditioned on trait 2; $C_{Gm(T1|T2)}$, conditional maternal main covariance of trait 1 conditioned on trait 2; $C_{Ge-Gm(T1|T2)}$, conditional covariance between endosperm and maternal main effects of trait 1 conditioned on trait 2; $C_{Ae(T1|T2)}$, conditional endosperm additive main covariance of trait 1 conditioned on trait 2; $C_{De(T1|T2)}$, conditional endosperm dominance main covariance of trait 1 conditioned on trait 2; $C_{Am(T1|T2)}$, conditional maternal additive main covariance of trait 1 conditioned on trait 2; $C_{Dm(T1|T2)}$, conditional maternal dominance main covariance of trait 1 conditioned on trait 2; $C_{Ae-Am(T1|T2)}$, conditional covariance between endosperm and maternal additive main effects of trait 1 conditioned on trait 2; $C_{De-Dm(T1|T2)}$, conditional covariance between endosperm and maternal dominance main effects of trait 1 conditioned on trait 2; and $C_{GeE(T1|T2)}$, $C_{GcE(T1|T2)}$, $C_{GmE(T1|T2)}$, $C_{AeE(T1|T2)}$, $C_{DeE(T1|T2)}$, $C_{CE(T1|T2)}$, $C_{AmE(T1|T2)}$, $C_{DmE(T1|T2)}$, $C_{AeE-AmE(T1|T2)}$, $C_{DeE-DmE(T1|T2)}$ means their cor-

responding conditional GE interaction effect covariances, respectively.

The Jackknife re-sampling method was used with sampling generation means of entries to derive the standard errors of estimated components of unconditional or conditional covariances (Miller 1974; Zhu and Weir 1996).

Results

The covariance analysis between AC and PC or AC and WBR of rice

Phenotypic (C_P) and genotypic (C_G) covariances between AC and PC or AC and WBR indicated that the relationship for AC and PC ($C_P = -1.62^{**}$, $C_G = -1.63^{**}$) or AC and WBR ($C_P = 2.90^{**}$, $C_G = 2.99^{**}$) was significant. The genetic covariances for both paired traits were similar to or larger than the phenotypic covariances, which implied the existence of true genetic associations between AC and PC or AC and WBR. Since the estimated residual covariances (C_e) were all not significant, the influence from sampling errors was not vital for the relationship between AC and PC ($C_e = 0.01$) or AC and WBR ($C_e = -0.09$) in the present experiment. Therefore, PC or WBR was closely related to AC of *indica* rice. The conditional analysis of the relationship between AC and amino acid contents of rice conditioned on PC or WBR could be carried out after excluding the effects of PC or WBR on the relationship between AC and the amino acid contents.

Unconditional and conditional analysis for the phenotypic and genotypic covariances between AC and amino acid contents

Components of unconditional and conditional phenotypic covariance, genotypic covariance and residual covariance between AC and amino acid contents are summarized in table 1.

Table 1. The estimates of unconditional and conditional phenotypic and genotypic covariance components between amylose content (AC) and the amino acid contents of rice.

| Trait | C_P | $C_{P(AC PC)}$ | $C_{P(AC WBR)}$ | C_G | $C_{G(AC PC)}$ | $C_{G(AC WBR)}$ | C_e | $C_{e(AC PC)}$ | $C_{e(AC WBR)}$ |
|-------|----------|----------------|-----------------|----------|----------------|-----------------|---------|----------------|-----------------|
| Asp | -11.90* | 3.06 | -3.35 | -11.87* | 3.24 | -3.31 | -0.03 | -0.18 | -0.04 |
| Thr | -6.05** | 0.68 | -2.32 | -6.05** | 0.74 | -2.31 | 0.01 | -0.06 | 0.01 |
| Ser | -9.25** | 1.24 | -3.51 | -9.23** | 1.36 | -3.49 | -0.02 | -0.12 | -0.02 |
| Glu | -29.00** | 1.75 | -10.40 | -28.56** | 2.47 | -9.94 | -0.44** | -0.72 | -0.46** |
| Gly | -6.69 | 4.25 | -1.21 | -6.80* | 4.25 | -1.31 | 0.11 | 0.00 | 0.10 |
| Ala | -17.19** | -0.89 | -8.78 | -17.00** | -0.53 | -8.58 | -0.19 | -0.36 | -0.20 |
| Cys | -1.59** | -0.16 | -0.58 | -1.60** | -0.15 | -0.57 | 0.01 | -0.01 | 0.01 |
| Val | -10.85* | 1.71 | -4.77 | -10.41* | 2.31 | -4.33 | -0.44 | -0.60 | -0.44 |
| Ile | -7.88** | 0.11 | -2.56 | -7.74** | 0.32 | -2.41 | -0.14** | -0.21 | -0.15** |
| Leu | -14.92** | 0.44 | -5.18 | -14.68** | 0.82 | -4.93 | -0.24** | -0.38 | -0.25** |
| Tyr | -7.94** | -0.62 | -2.32 | -7.78** | -0.38 | -2.16 | -0.16* | -0.24 | -0.16* |
| Phe | -8.03** | 0.14 | -3.15 | -7.98** | 0.27 | -3.09 | -0.05 | -0.13 | -0.06 |
| Lys | 0.39 | 3.96* | 1.62 | 0.13 | 3.74* | 1.37 | 0.26 | 0.22 | 0.25 |
| His | -3.23** | -0.09 | -0.83 | -3.13** | 0.03 | -0.73 | -0.10** | -0.12 | -0.10** |
| Arg | -10.94** | 1.44 | -3.62 | -10.83** | 1.67 | -3.50 | -0.11** | -0.23 | -0.12** |
| Pro | -9.05** | 0.59 | -3.39 | -9.03** | 0.69 | -3.37 | -0.01 | -0.10 | -0.02 |

* And ** significant at 0.05 and 0.01 probability levels, respectively.

Unconditional analysis: The C_P were significantly negative between AC and other amino acid contents of rice, except for the covariance between AC and Gly or Lys contents. These results revealed that decreasing the amylose content could notably increase most of the amino acid contents of *indica* rice. For the (C_G), these paired traits were significantly negative, except for C_G between AC and Lys content, which means that the genetic relationship can also be found between AC and Gly content in spite of the non-significant C_P . The significant residual covariances (C_e) between AC and Glu, Ile, Leu, Tyr, His or Arg indicated that the relationships for these paired traits were also influenced by sampling errors, but because of the small value of estimated C_e it is concluded that the relationships between AC and nutrition quality traits of rice were mainly controlled by genetic effects.

Conditional analysis: Although the genotypic covariances estimated by unconditional analysis revealed the relationships of the paired traits under the interference of other quality traits such as PC or WBR, the results could not clarify the actual relationships between AC and nutrition quality traits of rice because of the influences from PC or WBR of rice. To explain the correlations of the paired traits more effectively, conditional analysis approaches, which could exclude the interference of other quality traits, are required.

When AC was conditioned on PC (AC|PC), most of $C_{P(AC|PC)}$ and $C_{G(AC|PC)}$ between AC|PC and amino acid contents were non-significantly positive, except for the negative covariances between AC|PC and Ala, Cys, Tyr or His. After conditioning on PC, the $C_{P(AC|PC)}$ and $C_{G(AC|PC)}$ between AC|PC and Lys became significantly positive as compared to C_P and C_G analysed by using the unconditional approach. When AC was conditioned on WBR (AC|WBR), $C_{P(AC|WBR)}$ and $C_{G(AC|WBR)}$ between AC|WBR and amino acid contents both became insignificantly negative, except for the covariance between AC and the Lys content, which was positive but not significant. Hence, the performance of PC or WBR could significantly affect the correlations between AC and the amino acid contents of *indica* rice. Since the AC and PC traits of rice are negatively correlated, PC could negatively affect the covariation between AC and amino acid contents. But WBR being positively correlated with AC could positively affect the covariation between AC and amino acid contents (table 1).

Unconditional and conditional analysis for the covariance components from different genetic systems between AC and the amino acid contents of rice

Unconditional analysis: The covariance components of triploid endosperm effects, cytoplasmic effects and diploid maternal effects between AC and the amino acid contents analysed by unconditional method are given in table 2. Unconditional covariance analysis revealed that the relationships between AC and the amino acid contents were mainly from the endosperm and the maternal effects; especially, for

the endosperm additive covariance (C_{Ae}), endosperm dominance covariance (C_{De}) and maternal additive covariance (C_{Am}). Most of C_{Ae} were significantly negative, but C_{De} and C_{Am} were in most cases significantly positive. The relationships of cytoplasmic covariance (C_C) for the pair of AC and Thr and maternal dominance covariance (C_{Dm}) for the pair of AC and Lys were notable in this experiment.

Although most of GE interaction covariances (C_{GE}) were not significant between AC and the amino acid contents, endosperm additive \times environment interaction covariances (C_{AeE}) between AC and Gly, Cys or His, and the maternal dominance \times environment interaction covariances (C_{DmE}) between AC and Cys were significantly negative in present experiment. This result indicated that the environment factors had small effects on the correlations between AC and the amino acid contents using the unconditional analysis method.

No significant relationships were found for the covariances between endosperm effects and maternal effects or between endosperm interaction effects and maternal interaction effects (C_{Ae-Am} , C_{De-Dm} , $C_{AeE-AmE}$ and $C_{DeE-DmE}$), since the significant covariances were not found for the paired traits of rice, except in C_{Ae-Am} between AC and Cys.

Conditional analysis: Because, the relationships from different genetic systems between AC and the amino acid contents could be affected by PC or WBR; it is necessary to reveal the correlations of the paired traits when the influence of PC or WBR was excluded. For the relationships between AC and the amino acid contents when AC was conditioned on PC, $C_{Ae(AC|PC)}$ between AC|PC and Gly or Val was not visible (table 3), while C_{Ae} were significantly negative by unconditional analysis method (table 2). Except the covariances between AC|PC and Cys or Lys, most of $C_{De(AC|PC)}$ changed to insignificantly positive. $C_{C(AC|PC)}$ between AC|PC and Ala, Cys or Val became significant, while $C_{C(AC|PC)}$ between AC|PC and Thr was not remarkable in the conditional analysis. The entire $C_{Am(AC|PC)}$ for the paired traits of rice changed to significantly negative, while C_{Am} were most significantly positive by unconditional analysis method in table 2. Hence, protein content in rice could notably affect the relationships of the paired traits from the maternal additive effects. When AC was conditioned on PC, the components of $C_{GE(AC|PC)}$ were very important for the relationships between AC and the amino acid contents of rice. The conditional GE interaction covariances became larger and more significant than those from unconditional analysis method; especially, for $C_{AeE(AC|PC)}$ and $C_{AmE(AC|PC)}$, which were all significantly positive, but they were mostly insignificantly negative in table 2. These results showed that the protein content of rice could visibly affect the correlations between AC and the amino acid contents by the endosperm additive interaction effects, cytoplasmic environment interaction effects and maternal additive interaction effects. Since all of $C_{Ae-Am(AC|PC)}$ changed

Table 2. The estimates of unconditional covariance components from different genetic systems between amylose content (AC) and the amino acid contents of rice.

| Trait | C_G | | | | | | | C_{GE} | | | | | | |
|-------|----------|----------|--------|----------|----------|-------------|-------------|-----------|-----------|----------|-----------|-----------|---------------|---------------|
| | C_{Ae} | C_{De} | C_C | C_{Am} | C_{Dm} | C_{Ae-Am} | C_{De-Dm} | C_{AeE} | C_{DeE} | C_{CE} | C_{AmE} | C_{DmE} | $C_{AeE-AmE}$ | $C_{DeE-DmE}$ |
| Asp | -16.32** | 2.58** | -3.87 | 28.97* | -0.59 | -10.63 | -0.12 | -18.72 | 1.28 | 2.44 | -19.89 | -3.23 | 18.29 | 0.20 |
| Thr | -8.00** | 0.74** | -1.14* | 11.41 | -0.54 | -3.85 | 0.06 | -7.55 | 0.73 | -0.05 | -8.70 | -1.09 | 7.89 | -0.02 |
| Ser | -13.32** | 1.21** | -1.03 | 16.74* | -0.97 | -5.29 | 0.16 | -11.39 | 1.14 | -0.43 | -13.30 | -1.50 | 12.09 | -0.16 |
| Glu | -40.03** | 4.05** | -6.01 | 47.93* | -2.00 | -14.65 | 0.08 | -31.98 | 1.84 | 2.46 | -34.75 | -5.39 | 32.12 | 0.11 |
| Gly | -9.21** | 1.87** | -4.54 | 22.17* | 0.05 | -7.83 | -0.35 | -13.74* | 0.85 | 2.58 | -14.22 | -2.77 | 12.90 | 0.36 |
| Ala | -16.26** | 0.84 | -9.39 | 23.78** | 0.20 | -7.41 | -0.40 | -14.37 | -0.05 | 3.89 | -13.73 | -3.33 | 12.98 | 0.55 |
| Cys | -0.48** | 0.12** | -0.64 | 2.69* | 0.10 | -1.58* | -0.07 | -1.62* | 0.04 | 0.20 | -1.51 | -0.49* | 1.54 | 0.10 |
| Val | -10.65** | 0.82 | -4.37 | 23.07* | -0.48 | -8.90 | 0.03 | -13.03 | -0.09 | 2.05 | -14.80 | -2.37 | 13.23 | 0.37 |
| Ile | -10.39** | 1.18** | -2.40 | 12.56* | -0.26 | -3.84 | -0.11 | -8.05 | 0.10 | 1.64 | -8.11 | -1.80 | 7.61 | 0.23 |
| Leu | -20.33** | 2.11** | -3.59 | 24.00* | -0.86 | -7.21 | -0.05 | -15.54 | 0.54 | 1.96 | -16.56 | -3.03 | 15.34 | 0.24 |
| Tyr | -7.51** | 1.09** | -4.76 | 13.20* | 0.23 | -4.77 | -0.38 | -6.70 | -0.40 | 3.15 | -6.19 | -2.12 | 5.62 | 0.64 |
| Phe | -9.57** | 1.21** | -2.40 | 15.18* | -0.42 | -5.66 | -0.02 | -9.73 | 0.43 | 1.25 | -10.39 | -1.79 | 9.67 | 0.13 |
| Lys | -1.52 | 0.91* | -1.98 | 8.02* | 0.55* | -2.39 | -0.27 | -3.27 | 0.28 | 1.16 | -3.77 | -1.25 | 2.93 | 0.24 |
| His | -4.16* | 0.58* | -0.69 | 4.91* | 0.05 | -1.75 | -0.10 | -4.14** | -0.13 | 1.00 | -3.38 | -1.02 | 3.61 | 0.17 |
| Arg | -15.43** | 1.67** | -2.40 | 19.90* | -0.80 | -6.16 | -0.01 | -12.44 | 0.83 | 0.78 | -14.05 | -2.10 | 12.68 | 0.09 |
| Pro | -11.85** | 1.05** | -2.18 | 15.62* | -0.71 | -5.05 | 0.07 | -10.31 | 0.82 | 0.64 | -11.39 | -1.62 | 10.41 | 0.01 |

* And ** significant at 0.05 and 0.01 probability levels, respectively.

to significantly positive and $C_{AeE-AmE(AC|PC)}$ became significantly negative by using the conditional analysis method, the relationships could also be significantly affected by the associated additive or additive interaction effects from endosperm and maternal plant for all paired traits of rice. $C_{De-Dm(AC|PC)}$ and $C_{DeE-DmE(AC|PC)}$ were not visible except for the significant $C_{DeE-DmE(AC|PC)}$ between AC and Thr or Ser.

The conditional covariance components between AC|WBR and the amino acid contents from different genetic systems including triploid endosperm, cytoplasm and diploid maternal plant are shown in table 4. For the relationships of the paired traits when conditioned on WBR, some significant covariance components were found between AC|WBR and the amino acid contents. Almost all of the endosperm additive covariances ($C_{Ae(AC|WBR)}$) become insignificantly positive; whereas C_{Ae} were all significantly negative when analysed by unconditional method in table 2. This means the brown rice weight could significantly affect the relationships of the paired traits from the endosperm additive effects. The same phenomena could be observed in $C_{De(AC|WBR)}$, $C_{C(AC|WBR)}$, $C_{Am(AC|WBR)}$ and $C_{Dm(AC|WBR)}$. For C_{GE} of the paired traits, the influence of WBR on the relationships between AC and other amino acid contents was smaller than that from C_G . Among the paired traits, only several C_{GE} components including $C_{AeE(AC|WBR)}$ between AC|WBR and Asp, Gly, Cys, Lys or His, $C_{DeE(AC|WBR)}$ between AC|WBR and Lys, $C_{DmE(AC|WBR)}$ between AC|WBR and Cys, Tyr or His were significant when AC conditioned on WBR of rice. Hence, the WBR might have less effect on the relationships between AC and the amino acid contents of rice.

Discussion

Statistical analysis method plays a key role in classical quantitative genetics. With the rapid development in quantitative genetics, the statistical analysis methodology becomes more important for revealing the genetic mechanisms of quantitative traits. Stimulated by the development of biology, a number of statistical approaches with varied degrees of theoretical sophistication and practical utility have been developed for studying the correlations between the complex quantitative traits of crops (Griffing 1956; Zhu 1993; Zhu and Weir 1994). Some experiments have been done for investigating the relationships among the quality traits of seeds (Shi and Zhu 1995; Shi *et al.* 1997). But these genetic models and statistical analysis methods can only identify the correlations of complex quantitative traits based on the composite effects from the different components and can not exclude the influence of the other related trait(s) to the relationships between two quantitative traits.

Since rice is one of the staple food in the world and the cooking or nutrition quality is very important for customers, studying the genetic mechanism and the correlations among the quality traits will be helpful in rice quality breeding. Although the complex relationships of rice-quality traits have been found from the conventional statistical genetic analysis, the results only revealed the correlations of the paired traits under the interference of other traits such as PC or WBR. Therefore, the results of genotypic covariances estimated by unconditional analysis methods could not clarify the actual relationships among the quality traits of rice. The conditional analysis approach proposed by Zhu (1995) can be used for excluding the causal influences from the extra trait which affected the genetic relationships between the target trait and

Table 3. The estimates of unconditional covariance components from different genetic systems between amylose content (AC) and the amino acid contents of rice.

| Trait | Genetic main covariance | | | | | | | | | | GE interaction covariance | | | | | | | | | |
|-------|-------------------------|-----------------|----------------|-----------------|-----------------|--------------------|-----------------|--------------------|-----------------|-----------------|---------------------------|------------------|------------------|----------------------|----------------------|--|--|--|--|--|
| | $C_{Ac}(AC PC)$ | $C_{Dc}(AC PC)$ | $C_{C}(AC PC)$ | $C_{Am}(AC PC)$ | $C_{Dm}(AC PC)$ | $C_{Ae-Am}(AC PC)$ | $C_{Ae}(AC PC)$ | $C_{Dc-Dm}(AC PC)$ | $C_{Ae}(AC PC)$ | $C_{Dc}(AC PC)$ | $C_{CE}(AC PC)$ | $C_{AmE}(AC PC)$ | $C_{DmE}(AC PC)$ | $C_{AeE-AmE}(AC PC)$ | $C_{DcE-DmE}(AC PC)$ | | | | | |
| Asp | -30.00* | 1.62 | -10.56 | -71.32** | -0.69 | 57.25** | 0.07 | 126.82** | -5.95** | 21.74 | 165.46** | 1.87 | -154.11** | -1.07 | | | | | | |
| Thr | -13.56* | 0.64 | -5.12 | -28.32** | -0.75 | 23.45** | 0.21 | 51.85** | -2.58** | 9.83* | 66.59** | 1.25 | -62.60** | -0.61* | | | | | | |
| Ser | -20.19* | 1.04 | -7.37 | -41.40** | -1.21 | 34.39** | 0.40 | 76.48** | -3.90** | 14.67* | 97.10** | 2.00 | -91.65** | -1.07* | | | | | | |
| Glu | -65.23* | 2.58 | -20.68 | -133.20** | -2.51 | 109.78** | 0.76 | 235.79** | -11.67** | 41.62* | 304.06** | 4.41 | -284.30** | -2.56 | | | | | | |
| Gly | -16.56 | 1.26 | -9.02 | -52.24** | -0.39 | 40.78* | -0.25 | 97.12** | -5.02** | 17.58 | 125.69** | 1.68 | -117.94** | -0.51 | | | | | | |
| Ala | -31.01* | 0.49 | -18.61* | -70.07** | -1.76 | 58.02** | 0.40 | 124.79** | -7.03** | 25.17* | 161.55** | 4.61 | -151.52** | -1.22 | | | | | | |
| Cys | -3.58** | 0.20** | -1.46* | -7.68** | 0.01 | 6.18** | -0.05 | 12.19** | -0.74** | 2.69* | 16.90** | 0.00 | -15.43** | -0.03 | | | | | | |
| Val | -23.32 | 0.63 | -12.95* | -57.01** | -2.03 | 46.53** | 0.58 | 108.15* | -6.39** | 21.60* | 137.15** | 4.38 | -129.86** | -1.20 | | | | | | |
| Ile | -18.15* | 0.60 | -5.24 | -38.09** | -0.33 | 30.99** | 0.05 | 65.46** | -3.25** | 10.88 | 85.93** | 0.61 | -79.66** | -0.43 | | | | | | |
| Leu | -33.34* | 1.23 | -10.22 | -69.16** | -1.07 | 56.60** | 0.29 | 121.56** | -6.11** | 20.87* | 157.98** | 1.72 | -147.15** | -1.07 | | | | | | |
| Tyr | -17.99** | 0.53 | -6.00 | -42.18** | 0.00 | 33.39** | -0.29 | 68.52** | -3.50** | 11.33 | 94.94** | 0.32 | -86.34** | 0.05 | | | | | | |
| Phe | -18.35** | 0.72 | -6.34 | -38.50** | -0.58 | 31.60** | 0.19 | 67.90** | -3.20** | 11.49 | 88.57** | 1.04 | -82.38** | -0.65 | | | | | | |
| Lys | -0.77 | 0.99* | -4.23 | -17.43* | 0.05 | 12.38 | -0.29 | 31.59** | -2.59* | 8.31 | 43.66** | 0.79 | -40.41** | 0.00 | | | | | | |
| His | -9.77* | 0.16 | -1.35 | -18.12** | 0.24 | 14.98** | -0.05 | 30.42** | -1.27** | 3.84* | 38.50** | -0.45 | -35.95** | -0.08 | | | | | | |
| Arg | -23.94* | 1.30 | -8.80 | -52.77** | -1.11 | 42.91** | 0.23 | 94.76** | -5.05** | 17.91* | 123.12** | 2.08 | -115.08** | -0.96 | | | | | | |
| Pro | -20.05* | 0.67 | -6.88 | -41.41** | -1.00 | 34.17** | 0.28 | 73.34** | -3.52** | 13.31* | 95.40** | 1.64 | -89.05** | -0.81 | | | | | | |

* And ** significant at 0.05 and 0.01 probability levels, respectively.

another quality trait of rice, and the actual genetic correlation between the paired traits can be effectively detected. By using this analysis approach, the conditional correlations between quality traits of *indica* rice have been effectively analysed (Ge *et al.* 2007a,b, 2008). In an indirect selective process of breeding, these kinds of net genetic correlations detected from conditional analysis might be more important and could improve the selective efficiency for the quality traits of rice. Moreover, the conditional analysis method can also distinguish the genetic main effects and GE interaction effects from the different genetic systems including triploid endosperm nuclear genes, cytoplasmic genes and diploid maternal plant nuclear genes.

The results in the present experiment showed that the correlations between AC and the amino acid contents were complex and simultaneously affected by different genetic systems. When analysed by unconditional approaches, the genetic main effects and the GE interaction effects of endosperm, cytoplasm and maternal plant were all important for the relationships between AC and the amino acid contents of rice. The phenotypic and genotypic covariances of the paired traits were most significantly negative, but the results from conditional analysis method indicated that most of the relationships for the paired traits were not significant after excluding the influence of PC or WBR to AC. Hence, WBR and PC of rice could visibly affect the relationship between AC and the nutrient quality traits. The correlations among the quality traits of rice could be analysed after excluding the causal influences from WBR and PC which affected the genetic relationships between AC and the nutrient quality of rice. The better indirect selective effects for simultaneously improving the AC and different amino acids might be expected in rice quality breeding, since the conditional analysis detected that the genetic relationships for most paired traits in the present experiment were turned to not significant by excluding the influence of PC or WBR on AC. Among the different genetic systems, the relationships excluded the influence of PC were mainly negatively affected by the endosperm and maternal additive effects and dominance interaction effects, and positively affected by endosperm additive and maternal additive interaction effects, while the relationships excluded the influence of WBR were positively influenced by the endosperm dominance effects and negatively influenced by the endosperm additive interaction effects between AC and the amino acid contents of rice. Therefore, the conditional analysis method used in the experiment can provide a more useful tool and a new strategy for understanding the actual genetic relationships among the complex quantitative traits for the cereal crops such as rice, wheat, corn, barley and sorghum.

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Table 4. The estimates of conditional covariance components from different genetic systems between AC|WBR (amylose content conditioned on weight of brown rice) and the amino acid contents of rice.

| Trait | Genetic main covariance | | | | | | |
|-------|-------------------------|------------------|-----------------|------------------|------------------|---------------------|---------------------|
| | $C_{Ae(AC WBR)}$ | $C_{De(AC WBR)}$ | $C_{C(AC WBR)}$ | $C_{Am(AC WBR)}$ | $C_{Dm(AC WBR)}$ | $C_{Ae-Am(AC WBR)}$ | $C_{De-Dm(AC WBR)}$ |
| Asp | 10.43* | 1.82 | -6.59 | 8.87 | 0.93 | -8.01 | -0.40 |
| Thr | 3.45 | 0.40 | -2.37 | 3.90 | 0.03 | -3.07 | -0.03 |
| Ser | 5.98 | 0.60 | -3.19 | 6.17 | -0.10 | -5.21 | 0.03 |
| Glu | 19.84 | 2.32 | -11.04 | 17.24 | 0.41 | -15.79 | -0.23 |
| Gly | 5.45 | 1.67 | -6.48 | 3.69 | 1.23 | -2.06 | -0.56 |
| Ala | 10.35 | 0.74 | -9.46 | 5.29 | 1.09 | -5.17 | -0.50 |
| Cys | -0.14 | 0.20* | -0.83 | 0.23 | 0.20* | -0.01 | -0.10 |
| Val | 5.46 | 0.77 | -6.40 | 4.12 | 0.55 | -2.53 | -0.16 |
| Ile | 5.15 | 0.80* | -3.48 | 4.04 | 0.35 | -3.71 | -0.16 |
| Leu | 9.43 | 1.33 | -6.09 | 8.19 | 0.33 | -7.22 | -0.18 |
| Tyr | 2.94 | 1.04* | -5.11 | 2.79 | 0.78 | -1.63 | -0.43 |
| Phe | 5.72* | 0.79* | -3.45 | 5.35 | 0.30 | -5.05 | -0.14 |
| Lys | 1.30 | 0.97 | -3.24 | -0.72 | 1.04** | 1.61 | -0.38 |
| His | 2.53 | 0.50* | -1.26 | 1.32 | 0.28 | -1.57 | -0.11 |
| Arg | 6.65 | 1.06 | -4.87 | 6.40 | 0.23 | -5.12 | -0.14 |
| Pro | 5.69 | 0.52 | -3.45 | 5.64 | 0.06 | -4.85 | -0.04 |

| Trait | GE interaction covariance | | | | | | |
|-------|---------------------------|-------------------|------------------|-------------------|-------------------|-----------------------|-----------------------|
| | $C_{AeE(AC WBR)}$ | $C_{DeE(AC WBR)}$ | $C_{CE(AC WBR)}$ | $C_{AmE(AC WBR)}$ | $C_{DmE(AC WBR)}$ | $C_{AeE-AmE(AC WBR)}$ | $C_{DeE-DmE(AC WBR)}$ |
| Asp | -24.43* | 0.56 | 1.77 | 0.60 | -4.79 | 11.58 | 0.60 |
| Thr | -10.42 | 0.43 | 0.08 | -0.49 | -1.76 | 5.16 | 0.16 |
| Ser | -15.89 | 0.71 | -0.36 | -0.50 | -2.58 | 7.88 | 0.13 |
| Glu | -44.38 | 0.62 | 1.47 | 1.19 | -8.25 | 20.46 | 0.88 |
| Gly | 17.60* | 0.23 | 1.71 | 2.12 | -3.95 | 7.29 | 0.64 |
| Ala | -19.56 | -0.91 | -1.64 | 4.82 | -4.21 | 7.28 | 0.85 |
| Cys | -1.66* | -0.04 | 0.51 | -0.26 | -0.55** | 0.89 | 0.12 |
| Val | -18.74 | -0.71 | -0.56 | 2.69 | -3.46 | 8.01 | 0.64 |
| Ile | -10.98 | -0.22 | 1.32 | 0.86 | -2.48 | 4.59 | 0.40 |
| Leu | -21.46 | -0.09 | 1.57 | 1.09 | -4.42 | 9.40 | 0.60 |
| Tyr | -9.39 | -0.67 | 3.66 | 0.71 | -2.62* | 3.20 | 0.72 |
| Phe | -12.58 | 0.04 | 0.56 | 0.32 | -2.54 | 6.05 | 0.34 |
| Lys | -5.78* | 0.14* | 1.37 | 2.37 | -1.70 | 1.25 | 0.33 |
| His | -3.99* | -0.37 | 0.24 | 0.49 | -1.24* | 1.82 | 0.24 |
| Arg | -17.91 | 0.37 | 1.21 | 0.29 | -3.31 | 8.07 | 0.39 |
| Pro | -14.55 | 0.45 | 0.46 | -0.30 | -2.51 | 6.96 | 0.24 |

* And ** significant at 0.05 and 0.01 probability levels, respectively.

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