Metabolic prediction of important agronomic traits in hybrid rice (Oryza sativa L.)

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Hybrid crops have contributed greatly to improvements in global food and fodder production over the past several decades. Nevertheless, the growing population and changing climate have produced food crises and energy shortages. Breeding new elite hybrid varieties is currently an urgent task, but present breeding procedures are time-consuming and labour-intensive. In this study, parental metabolic information was utilized to predict three polygenic traits in hybrid rice. A complete diallel cross population consisting of eighteen rice inbred lines was constructed, and the hybrids’ plant height, heading date and grain yield per plant were predicted using 525 metabolites. Metabolic prediction models were built using the partial least square regression method, with predictive abilities ranging from 0.858 to 0.977 for the hybrid phenotypes, relative heterosis, and specific combining ability. Only slight changes in predictive ability were observed between hybrid populations, and nearly no changes were detected between reciprocal hybrids. The outcomes of prediction of the three highly polygenic traits demonstrated that metabolic prediction was an accurate (high predictive abilities) and efficient (unaffected by population genetic structures) strategy for screening promising superior hybrid rice. Exploitation of this pre-hybridization strategy may contribute to rice production improvement and accelerate breeding programs.

The continuously growing global population and changing climate have pushed crop breeders to find effective breeding strategies for meeting food and energy demands. Although hybrid crops have made tremendous contributions to yield improvements over the past several decades, breeding new elite hybrid combinations is urgently required to guarantee food security. Quantitative hybridization trials and hybrid performance evaluation, which are time-consuming and laborious, form the foundation of present hybrid breeding programs. Breeding procedures are even tougher for self-pollinated plants such as rice and wheat compared with cross-pollinated plants. Prediction of hybrid performance based on parental information appears to be a practicable method to increase breeding efficiency. However, parental phenotypes have exhibited low accuracy for the prediction of hybrid performance\textsuperscript{1,2}. Additionally, isozymes have proven to be unreliable for the prediction of $F_1$ yields and heterosis\textsuperscript{3}. Then, parental genetic distances based on AFLP (Predictive abilities: 0.0–0.97, 0.161–0.699 and 0.06–0.8, respectively)\textsuperscript{4,5}, InDel (0.049–0.21)\textsuperscript{7}, RAPD (0.069–0.785)\textsuperscript{2}, RFLP (–0.028–0.773)\textsuperscript{5}, SNP (0.26–0.56 and 0.3–0.46)\textsuperscript{1,10}, and SSR (0.06–0.82, 0.069–0.785, and –0.028–0.773, respectively)\textsuperscript{6,8} DNA markers have been widely used to predict hybrid phenotypes, heterosis or combing ability in maize (Zea Mays L.)\textsuperscript{5,6,10}, rice (Oryza Sativa L.)\textsuperscript{7–9}, sunflowers (Helianthus annuus L.)\textsuperscript{4}, and Arabidopsis thaliana\textsuperscript{1}. Some prediction methods based on DNA markers produced no significant correlation or low predictive ability between parental genetic distance and hybrid performance\textsuperscript{9,10}. Meanwhile, DNA markers were found to be appropriate for the prediction of specific traits\textsuperscript{6,8} and had high predictive ability for populations consisting of genetically related parental lines\textsuperscript{6,8}. Additionally, parental transcriptome-based predictions of hybrids showed high predictive ability for intra-pool crosses but not inter-pool crosses\textsuperscript{11}. Generally speaking, although various types of biomarkers, populations and mathematical models have been tested in various species, problems of low predictive ability or limited application ranges of the prediction approaches remain. And worldwide, numerous studies of hybridization are still performed every year.

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The metabolome has manifested potential roles in facilitating crop breeding strategies over the past decades. The results from a tomato IL population showed that 73% of the total analysed metabolites were significantly associated with the whole-plant phenotypes. Arabidopsis thaliana biomass could be described as a function of metabolic compositions with high predictive power. Moreover, metabolite profiling of a rice recombinant inbred line population and diverse accessions showed promise in bridging the gap between the genome and phenotype. Importantly, parental metabolites as biomarkers have been tested for the prediction of traits such as yield in maize, biomass and tolerance in Arabidopsis thaliana and postharvest quality traits in potatoes (Solanum tuberosum L.).

In the present study, a complete diallel cross population was built with eighteen rice inbred lines as the parents. Three polygenic traits—yield per plant, heading date and maturation stage plant height—were predicted in the hybrids using 525 metabolites with the partial least square regression method. The predictive abilities ranged from 0.858 to 0.977 for the three traits. The population structure and cytoplasmic effects had slight influences on the predictive ability.

Results
Population structure and hybrid performance. Eighteen rice inbred lines were chosen as parental lines for the complete diallel cross design (see Supplementary Table S1). All the parental lines were divided into equal indica (Fi values from 0.39 to 1) and japonica (Fi values from 0 to 0.33) groups based on the InDel marker estimating method (see Supplementary Table S1). Principal component analysis (PCA) was also applied to the parental phenotypic and metabolic data to discriminate between the parental lines. PCA of the parental phenotypic performance showed that three indica varieties were divided into the japonica group and three japonica varieties were in the indica group (Fig. 1a). At the metabolic level, a total of 525 analytes were detected in all eighteen inbred lines. Unexpectedly, PCA for the parental metabolic parameters produced a similar grouping result as the consequences of the InDel markers and was also highly similar to the dendrogram (Fig. 1b,c).

Three important agronomic traits were measured: yield per plant (YPP), maturation stage plant height (MSPH), and heading date (HD). Prior to the analysis, the hybrids were divided into an indica-indica group (i-group), a japonica-japonica group (j-group) and an indica-japonica group (ij-group) based on the group to which the corresponding parental lines belonged. For the hybrid phenotypes (trait values per se), the highest mean YPP was in the i-group, the lowest was in the j-group, and the ij-group was between the i- and j-group (Fig. 1d). For MSPH and HD, the highest means were observed for the ij-group, which was consistent with the phenomenon that hybridization between inter-subspecific rice has stronger biomass heterosis (increased plant height) and a later heading date. Box plots depicting the relative low-parent heterosis (LPH), mid-parent heterosis (MPH), better-parent heterosis (BPH) and specific combining ability (SCA) for the three traits are shown in Supplementary Fig. S1a,b. For YPP, the hybrid trait values per se had the closest relationship with SCA (see Supplementary Table S2). Trait values of MSPH per se had the closest relationship with BPH, and HD was most closely associated with MPH.

Predictions of hybrid performance based on parental traits and genetic distance. First, the sum trait values of the parents (sum), the differences between the parents (difference) and the ratios of the parents (ratio) were used as predictive variables. The highest correlation coefficient (0.714) was obtained between the HD and the sum values of the parents (see Supplementary Table S3). After performing linear regression analysis of the sum HD values of the parents and the hybrid HD values, the predicted HD values and measured true values displayed dramatic deviances (see Supplementary Fig. S2). Therefore, the parental traits were not appropriate for the prediction of hybrid performance.

Second, to evaluate whether genetic distance based on parental metabolic data was suitable for predicting hybrid performance, squared Euclidean distances based on all 525 analytes were calculated to analyse the correlations (see Supplementary Table S4). The Pearson correlation coefficients between genetic distance and hybrid performance shown in Supplementary Table S5 suggested that, although genetic distance was significantly correlated with some traits (such as YPP and MSPH), the predictive abilities were quite low, and some traits were not closely correlated with genetic distance.

Stepwise linear regression and partial least square (PLS) regression with metabolic data and hybrid performance. Next, the sums, differences and ratios of the parents' relative metabolite levels were calculated, and stepwise linear regression was used to identify the appropriate metabolic predictors. The adjusted R² values in Supplementary Table S6 shows that the highest value was 0.677 for HD, while no metabolite could be used for the regression of traits such as SCA-YPP. Furthermore, the predictive abilities of different traits varied widely, and they were not sufficiently high for most traits.

Then, partial least square regression was used to create a prediction model with metabolic data of the three traits. In Fig. 2a, 17 latent factors were extracted for all the traits using the sums and differences of the parents' relative metabolite levels as variables. Unfortunately, the highest R² value obtained was 0.6, and some hybrid traits could not be predicted using these parental variables.

Surprisingly, however, high R² values were obtained when the ratios of parental metabolic data were used as predictive variables (Fig. 2b). For this analysis, 104 to 107 latent factors were extracted from the metabolic data; most of the traits exhibited the highest R² values at approximately 50 latent factors. Then, the number of latent factors at the top R² value for each trait was fixed to obtain the values of variable importance in the projection (VIP) of each variable. Because Latent Factor 1 explained the largest proportion of the variance, the VIP values of Latent Factor 1 were reordered to reduce the number of variables. After excluding variables whose VIP values were smaller than 1, the remaining variables were used for PLS regression. Finally, although more than 300 variables were excluded from the regression model, and only slight decreases were observed in R² values compared
with the values obtained using all 525 metabolites (Table 1). Among the 15 predicted traits, LPH-YPP used the fewest (149) variables and LPH-HD used the most variables (196).

Considering that 149–196 is still a quite large number of variables and might result in overfitting

The coefficient of each variable in the PLS regression results was used to build the equations (see Supplementary Table S7). Then, a predicted value was assigned for every trait in each hybrid. The relationship between the observed YPP (Fig. 3a), MPH-YPP (Fig. 3b), BPH-YPP (Fig. 3c), and SCA-YPP (Fig. 3d) values and the predicted values demonstrated the high accuracy for this metabolite-based prediction method. The lowest predictive ability was 0.858 with SCA-YPP and the highest was 0.924 with YPP. High predictive abilities were also achieved through the PLS regression approach for the remaining traits (Table 2).

Because the complete diallel cross population consisted of rice inbred lines ranging from typical indica to typical japonica, the predictive abilities might vary with different hybridization groups. Thus, the predictive abilities for the i-group, j-group and ij-group were calculated to validate the stability of the prediction model (Table 2). As shown in Fig. 4a,b, the predictive abilities of YPP in the i-group and j-group were 0.849 and 0.806, respectively. Surprisingly, the predictive ability of the ij-group was as high as 0.948 (Fig. 4c). Moreover, the predictive abilities of relative heterosis and SCA of YPP were all the highest for the ij-group, thereby showing a promising approach for exploiting inter-subspecific heterosis:

**Metabolic prediction of agronomic traits by PLS regression.** The coefficient of each variable in the PLS regression results was used to build the equations (see Supplementary Table S7). Then, a predicted value was assigned for every trait in each hybrid. The relationship between the observed YPP (Fig. 3a), MPH-YPP (Fig. 3b), BPH-YPP (Fig. 3c), and SCA-YPP (Fig. 3d) values and the predicted values demonstrated the high accuracy for this metabolite-based prediction method. The lowest predictive ability was 0.858 with SCA-YPP and the highest was 0.924 with YPP. High predictive abilities were also achieved through the PLS regression approach for the remaining traits (Table 2).

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Finally, the cytoplasm from the reciprocal parental lines might have different influences on hybrid performance; these differences might be obstacles to applying the metabolic prediction strategy in screening for potential sterile and restorer lines. Hence, the whole population was divided into two sets of reciprocal hybrids to test whether cytoplasmic effects affected the predictive ability. The results demonstrated that different cytoplasms had weak influences on the predictive ability (see Fig. 4d,e and Supplementary Table S8).

Discussion

Accuracy and efficiency are two pivotal indicators for the application of a biotechnology-assisted prediction model in breeding. Because predictive ability might be heavily influenced in populations consisting of genetically distant lines\(^1,7\); therefore, assessing whether a prediction model is appropriate is largely determined by its predictive abilities in different genetic structures. To avoid incomplete bias prediction, the predictive model should be built on representative inbred lines. In addition, the richer the information on these inbred lines, the higher the potential accuracy of the model. In this experiment, eighteen rice inbred lines were used to build a prediction model. These lines were collected from a wide range of locations (China, Italy and India) and possessed different degrees of \textit{indica} or \textit{japonica} contents and variable general combining abilities (see Supplementary Table S1).
To some extent, they represented a large range of rice accessions that were suitable for building the prediction model. Therefore, this model may also be applicable to rice inbred lines that were not included in this experiment. 

The relationship between parental metabolite profiling and hybrid performance should be nonlinear and was treated as a megavariate quantitative structure-activity relationship in this study. Because of its powerful multi-dimensional information regression ability, partial least squares projections to latent structures were applied to build the hybrid trait prediction model. Meanwhile, because cross-validation is built into the PLS algorithms, the predictive significance is assessed and predictive abilities can be insured for every trait. In addition, taking advantage of the values of variable importance in the projection, the low contribution variables were excluded and the representative variables were reserved for the equations. The high predictive abilities (0.858–0.977) in Table 2 demonstrate that PLS matched the metabolic prediction model quite well.

Metabolites have close connections with phenotypes. Before determining the exact function of a metabolite, correlation analysis between the relative metabolite levels and phenotypes can be used as a type of rough estimating method. In this study, different groups of metabolites were exploited to predict various traits. Because specific metabolites may have single or multiple functions in different pathways, metabolites such as saccharic acid, p-Cresol, and triacontanoic acid methyl ester were found to be predictive variables for two or more traits (see Supplementary Table S9). Different correlation levels were detected for the metabolites that were predictive variables for all three traits (see Supplementary Table S10). Some of these overlapping metabolites manifested significant positive and negative correlations with different traits, which might be the origin of the balance between component traits or outcomes of feedback regulation of the biological networks involved in complex traits. Meanwhile, only a single value was used to represent reciprocal hybrids when calculating the ratios of relative parental metabolite levels. Ignorance of the relationship between the female parent and male parent might decrease the predictive abilities for the reciprocal hybrids. However, almost no predictive ability change.

**Figure 3.** Observed and predicted values of YPP, MPH-YPP, BPH-YPP, and SCA-YPP. (a) Relationships between observed yield per plant and predicted yield per plant. Predicted values were calculated with the equations based on variable coefficients in the PLS regression results. The horizontal axis represents the observed values, and the vertical axis represents the predicted values. The green solid line represents the total fit line, and the black dotted line is y = x. (b–d) Relationships between the observed relative mid-parent heterosis, better-parent heterosis, specific combining ability of YPP and the corresponding predicted values. YPP = Yield per plant, LPH = Low-parent heterosis, MPH = Mid-parent heterosis, BPH = better-parent heterosis, SCA = Specific combining ability.
was found between the reciprocal hybrids. Therefore, close balances might exist between metabolites and their associations might be controlled by the rules of chemistry26. The influences of the complex associations between metabolites on the hybrid trait prediction model remain unknown.

Nevertheless, predictable metabolites in the prediction model might simply have high predictive abilities for the traits. Therefore, biological functions of the predictive variables in this study were analysed to verify their contributions to the prediction model. Fructose (a predictive variable for yield per plant and maturation stage plant height) contributes strongly to the metabolic efficiency of rosette fresh weight and protein concentration in Arabidopsis plant height) contributes strongly to the metabolic efficiency of rosette fresh weight and protein concentration in Arabidopsis. Spermidine (a predictive variable for heading date and yield per plant) is associated with contributions to the metabolic efficiency of rosette fresh weight and protein concentration in Arabidopsis. Ferulic acid, a phenolamide32, plays an important role in plant development33 and is a predictive variable for plant height. Spermidine (a predictive variable for heading date and yield per plant) is associated with contributions to the metabolic efficiency of rosette fresh weight and protein concentration in Arabidopsis.

In summary, a reliable and efficient metabolic prediction strategy was provided by combining parental metabolite profiling with a PLS regression method for rice hybrids. The high predictive abilities for three agronomic traits were implemented with respect to hybrid phenotypes, relative heterosis and specific combining ability. The predictive abilities for the three traits were only slightly influenced by population structures (genetic relatedness) for hybrid traits. Thus, the metabolite profiling data were only a snapshot of the whole plant growth process, and the performance of a plant in nature is the consequence of the combination of genetic information and environmental influences; thus, the effects of environmental inputs on metabolome-assisted breeding strategies might need to be taken into account26. Metabolite profiling data of naturally growing parental seedlings in a specific field environment should be more accurate for the prediction of hybrid performances in corresponding locations.

In summary, a reliable and efficient metabolic prediction strategy was provided by combining parental metabolite profiling with a PLS regression method for rice hybrids. The high predictive abilities for three agronomic traits were implemented with respect to hybrid phenotypes, relative heterosis and specific combining ability. The predictive abilities for the three traits were only slightly influenced by population structures (genetic relatedness) or cytoplasmic effects. Additionally, metabolite-based prediction might be more suitable for traits such as resistance (susceptibility) or tolerance ability21,22,37 because small molecules function more directly in these defence processes by confronting biotic/abiotic pressures.

**Methods**

**Plant materials.** The eighteen rice inbred lines were selected based on their proportions of indica-japonica content using the InDel marker estimation method24. A complete diallel cross design was used. All the hybrid seeds were produced through manual emasculation at the Hybrid Rice Hainan Experimental Base of Wuhan University in Lingshui (N18° 30′, E110° 2′), Hainan Province, China, in 2012. Seedlings of the eighteen inbred lines and 306 hybrids were planted with a randomized block design in three replicates at the Hybrid Rice Ezhou Experimental Base of Wuhan University in Ezhou (N30° 22′, E114° 44′), Hubei Province, China, in June 2012. Ten plants were planted per row with a spacing of 16.5 × 26.4 cm. Four cytoplasmic male-sterile plants (named YTA) were planted around these experimental plants to decrease marginal effects. The four cytoplasmic male-sterile plants (named YTA) were planted around these experimental plants to decrease marginal effects. The four cytoplasmic male-sterile plants (named YTA) were planted around these experimental plants to decrease marginal effects. The four cytoplasmic male-sterile plants (named YTA) were planted around these experimental plants to decrease marginal effects. The four cytoplasmic male-sterile plants (named YTA) were planted around these experimental plants to decrease marginal effects. The four cytoplasmic male-sterile plants (named YTA) were planted around these experimental plants to decrease marginal effects.

![Table 2. Predictive abilities of the whole population and three subgroups for the three polygenic traits.](image)

| Traits       | Whole population | i-group | j-group | ij-group |
|--------------|------------------|---------|---------|----------|
| YPP          | 0.924            | 0.849   | 0.806   | 0.948    |
| MSPH         | 0.951            | 0.911   | 0.901   | 0.956    |
| HD           | 0.977            | 0.987   | 0.937   | 0.977    |
| LPH-YPP      | 0.931            | 0.927   | 0.906   | 0.940    |
| LPH-MSPH     | 0.954            | 0.931   | 0.897   | 0.965    |
| LPH-HD       | 0.964            | 0.963   | 0.894   | 0.968    |
| MPH-YPP      | 0.921            | 0.883   | 0.911   | 0.940    |
| MPH-MSPH     | 0.938            | 0.933   | 0.856   | 0.948    |
| MPH-HD       | 0.954            | 0.963   | 0.838   | 0.957    |
| BPH-YPP      | 0.916            | 0.848   | 0.926   | 0.935    |
| BPH-MSPH     | 0.949            | 0.933   | 0.904   | 0.949    |
| BPH-HD       | 0.931            | 0.953   | 0.948   | 0.913    |
| SCA-YPP      | 0.858            | 0.783   | 0.873   | 0.898    |
| SCA-MSPH     | 0.885            | 0.940   | 0.736   | 0.868    |
| SCA-HD       | 0.940            | 0.963   | 0.916   | 0.927    |

*YPP = Yield per plant, MSPH = Maturation stage plant height, HD = Heading date, LPH = Relative low-parent heterosis, MPH = Relative mid-parent heterosis, BPH = Relative better-parent heterosis, SCA = Specific combining ability.*
middle five plants of each replicate were chosen for data collection. Three agronomic traits were evaluated: grain yield per plant, heading date and maturation stage plant height. Means among the replications were calculated for each trait and used in the data analysis.

For metabolite profiling analysis, seeds of the eighteen rice inbred lines were first submerged in water for two days at 28 °C. Then, the seeds were placed in an incubator at 28 °C for 24 h to accelerate germination. Next, seedlings at approximately the same stage were transferred to soil containers. Three random replicates were applied for each inbred line; the spacing between seedlings was 2 × 2 cm. Finally, all the seedlings were placed in a phytotron with a temperature of 28 °C, 70% relative humidity and an 8 h light/16 h dark photoperiod. On day 15, 100 mg of the seedlings (root excluded) were collected into 2 ml EP tubes for each replicate and immediately frozen in liquid nitrogen.

**Gas chromatography mass spectrometry–based metabolite profiling.** For each sample, 0.4 ml of methanol-chloroform (\(V_{\text{methanol}} : V_{\text{chloroform}} = 3:1\)) and 20 μl of ribitol (0.2 mg/ml stock in dH\(_2\)O, Sigma-Aldrich Co. LLC., USA) were added as internal standards. After vortex mixing for 10 s, steel balls were placed into EP tubes and the samples were homogenized with a ball mill (JXFSTPRP-24, Shanghai Jingxin Experimental Technology, Shanghai, China) for 5 min at 55 Hz. Then, the samples were centrifuged for 15 min at 12,000 rpm at 4 °C. The supernatant (approximately 0.4 ml) was transferred to a new 2 ml GC/MS glass vial. An equal volume of approximately 13 μl (based on the number of samples) from each sample was transferred into a new 2 ml GC/MS glass vial as a mixed sample for quality control. Then, the extracts were dried in a vacuum concentrator without heating at 30 °C for approximately 1.5 h. Next, 80 μl of methoxymethyl amine salt (dissolved in pyridine, final concentration of 20 mg/ml) was added to the dried extracts and incubated at 37 °C for 2 h in an oven after mixing and sealing. The lids were opened, and 100 μl of BSTFA (containing 1% TCMS, v/v, Regis Technologies, Inc., USA) was added to each sample; then, the samples were resealed and incubated at 70 °C for an hour. When the samples had cooled to room temperature, 10 μl of FAMEs (standard mixture of fatty acid methyl esters, 1 mg/ml C8-C16 and 0.5 mg/ml C18-C30 in chloroform) was added to the mixed sample. Then, the sample was mixed well for GC-MS analysis.

GC/TOF MS analysis was performed using an Agilent 7890 (Agilent Technologies, USA) gas chromatograph system coupled with a Pegasus HT time-of-flight mass spectrometer (LECO Corporation, USA). The system utilized a DB-5 MS capillary column coated with 5% diphenyl cross-linked with 95% dimethyl polysiloxane (30 m × 250 μm inner diameter, 0.25 μm film thickness; J&W Scientific, Folsom, CA, USA). A total of 1 μl of the
sample was injected in the splitless mode with helium as the carrier gas. The front inlet purge flow was 3 ml min⁻¹, and the gas flow rate through the column was 20 ml min⁻¹. The initial temperature was held constant at 50 °C for 1 min, followed by a 10 °C per min ramp up to 330 °C, and then maintained for 5 min at 330 °C. The injection, transfer line, and ion source temperatures were 280 °C, 280 °C, and 220 °C, respectively. The energy was ~70 eV in electron impact mode. Spectra were recorded in full-scan mode with an m/z range of 85–600, at a rate of 20 spectra per second after a solvent delay of 366 s.

The Chroma TOF 4.3X software from LECO Corporation and the LECO-Fiehn Rtx5 database were used for raw peak extraction, data baseline filtering and calibration, peak alignment, deconvolution analysis, peak identification and integration of the peak area. The RI (retention time index) method was used for peak identification; the RI tolerance was 5000.

**Data analysis.** Relative low-parent heterosis (LPH), mid-parent heterosis (MPH), better-parent heterosis (BPH) and specific combining ability (SCA) for each trait were calculated using the following equations:

\[
\text{LPH} = (F_1 - P_{\text{low}})/P_{\text{mean}}, \quad \text{MPH} = (F_1 - P_{\text{mean}})/P_{\text{mean}}, \quad \text{BPH} = (F_1 - P_{\text{high}})/P_{\text{high}} \text{,}
\]

where the suffixes −low, mean and high correspond to the low value, mean value and high value of the two corresponding parents, respectively. Female P represents the mean of the female parent values and male P represents the mean of the male parent values. When parental metabolic data were used as predictive variables, only single sum, difference and ratio values were calculated for the reciprocal hybrids. For example, for reciprocal hybrids YB/Balilla and Balilla/YB, if the relative level of Analyte X is a1 for YB and a2 for Balilla, the sum, difference, and ratio values for Analyte X of these reciprocal hybrids are \((a1 + a2)\), \((a1 - a2)\), and \((a1/a2)\), respectively. The reason for this calculation approach is explained in the Discussion. Correlation analyses and regression analyses were performed with IBM SPSS Statistics 20 (IBM, USA). Average linkage between groups was chosen as the cluster method in the hierarchical cluster analysis. Squared Euclidean distance was calculated as the genetic distance. Principle component analysis was achieved through factor analysis, and eigenvalues greater than 1 were extracted; maximum iterations for convergence was 25; and the non-rotation method was applied for factor analysis. In stepwise linear regression, the probability of F was used as the stepping method criteria. The entry value was 0.05, and the removal value was 0.10. Partial least squares regression was conducted with the PLS extension bundle for SPSS. Main effects were used to specify model effects. Maximum numbers of latent factors were adjusted until no more latent factors could be extracted. Then, in the Proportion of Variance Explained table, the number of latent factor to a trait was determined where the corresponding adjusted R-square value was at the highest. After the number of latent factor was fixed, all the 525 parental metabolic data were applied to PLS again. And variable importance in the projection of Factor 1 was used for reordering. Parameters of independent variables were used to calculate the corresponding values of the dependent variables. Predictive ability was defined based on the Pearson correlation (2-tailed) coefficient of the predicted and observed values.

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et al.: Dan, Z. Oryza Competing financial interests: The authors declare no competing financial interests.

How to cite this article: Dan, Z. et al. Metabolic prediction of important agronomic traits in hybrid rice (Oryza sativa L.). Sci. Rep. 6, 21732; doi: 10.1038/srep21732 (2016).

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