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Metabolic variation between *japonica* and *indica* rice cultivars as revealed by non-targeted metabolomics

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121

R (*Oryza sativa* .) 50% (*Oryza rufipogon*) *japonica* *indica* *japonica* *indica* *japonica* *indica* *et al* (2007) 10 62 (2010) 3,0 7 10 (2011) 41 48 (2012) 52 (2012) 100 *japonica* *indica* *japonica* *indica*

[8,14] - - (. 1 1)

-

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Results

Metabolic profiles of rice seeds.

japonica *indica* .

121 ,

, , ,

(,) .

et al. (2011) *et al.* (2012),

x.

[11,13] .

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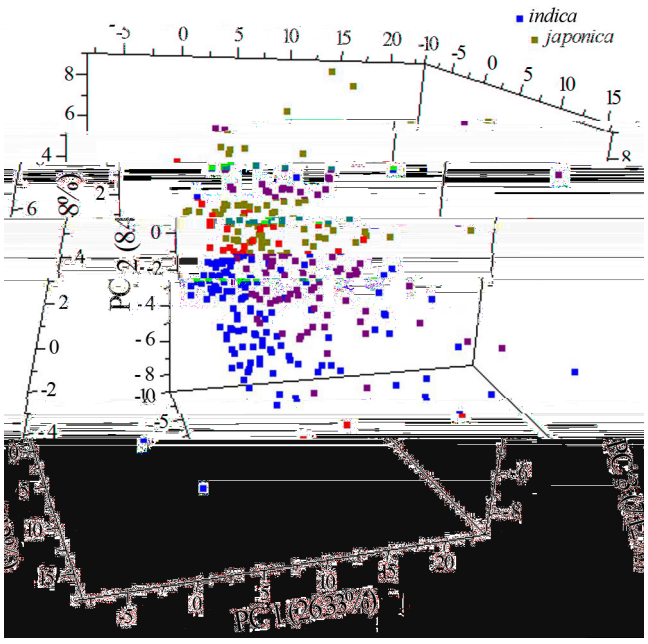


F 2 P (PC)
. The first two PCs explain 34.81% of variance separating *japonica* from *indica* cultivars.

16

japonica *indica* - ,

(A)

japonica *indica*

(. 2),

(. 1).

japonica *indica*

japonica *indica* -

, A A

2

28 , 23 , 22 ,

12 , x -

japonica *indica*

66 *japonica* 26

indica (. 1 1).

japonica *indica*, 4%

14

japonica *indica* ,

(1). 30

,

A A,

japonica *indica*

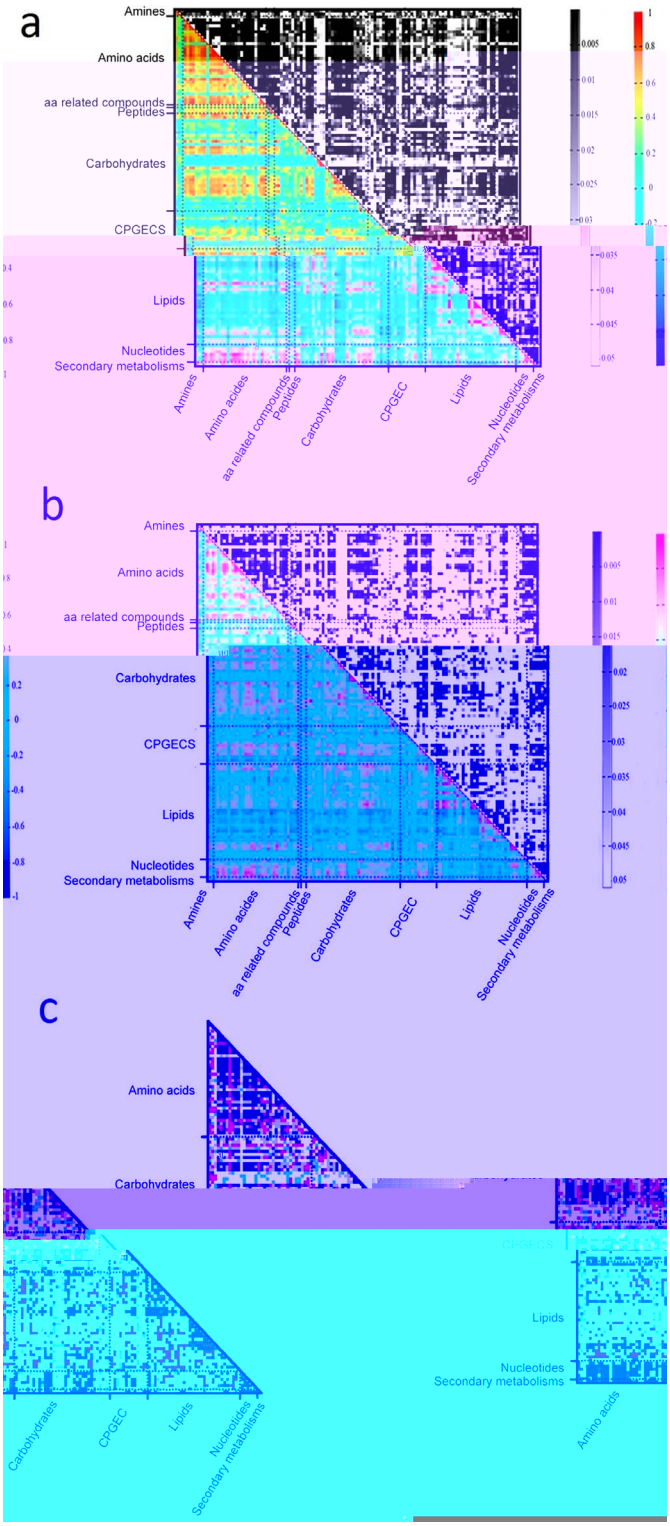
japonica *indica*

Table 1 | The top 30 metabolites contributing significantly to the correct classification of *japonica* and *indica* samples generated by Random Forest ranking

Biochemical Name	Mean Decrease Accuracy	*Japonica/indica*	P-value	FDR-value	Super Pathway
asparagine	9.4445	**0.68**	3.62E-09	6.71E-09	Amino acid
4-guanidinobutanoate	9.1961	**0.68**	1.00E-14	8.50E-14	Amino acid
alanine	9.0173	**1.73**	1.00E-15	1.50E-14	Amino acid
trigonelline	8.435	**2.63**	1.00E-15	1.90E-14	CPGEC
gamma-tocopherol	8.2808	**0.50**	8.90E-14	4.93E-13	CPGEC
glutamate	8.2736	**1.38**	1.20E-14	8.50E-14	Amino acid
phytate	7.7472	**1.54**	1.26E-06	1.52E-06	Carbohydrate
13-HODE-9-HODE	6.263	**1.88**	4.36E-11	1.16E-10	Lipids
agmatine	6.2329	**2.10**	8.06E-13	3.20E-12	Amino acid related compound
putrescine	6.1999	**2.07**	1.16E-11	3.58E-11	Amine
gamma-aminobutyrate	6.1925	**2.29**	1.04E-10	2.40E-10	Amino acid
glycine	6.0299	**1.35**	4.95E-06	4.92E-06	Amino acid
adenine	5.8866	**1.75**	9.07E-12	3.15E-11	Nucleotide
trans-4-hydroxyproline	5.5906	**0.60**	0.0002	0.0001	Amino acid
serine	5.4119	**1.43**	2.17E-08	3.41E-08	Amino acid
gluconate	4.9611	**2.89**	5.96E-13	2.76E-12	Carbohydrate
carnitine	4.8728	**0.69**	3.91E-07	5.44E-07	CPGEC
sucrose	4.826	**1.19**	1.07E-09	2.28E-09	Carbohydrate
inositol-1-phosphate	4.8148	**0.83**	0.0006	0.0004	Carbohydrate
nicotianamine	4.5049	**1.22**	7.41E-07	9.37E-07	CPGEC
citrate	4.4552	**0.80**	0.0002	0.0001	Carbohydrate
1,3-dihydroxyacetone	4.114	**1.43**	2.21E-08	3.41E-08	Carbohydrate
tyrosine	4.0544	**1.29**	6.31E-06	6.02E-06	Amino acid
pipecolate	4.054	**0.78**	9.28E-05	6.98E-05	Amino acid
arginine	3.921	**1.15**	0.0097	0.0042	Amino acid
pyridoxate	3.9206	**0.84**	0.0004	0.0002	CPGEC
guanosine	3.9175	**1.10**	0.0163	0.0067	Nucleotide
stigmasterol	3.8728	**1.18**	2.31E-09	4.59E-09	Lipids
mannitol	3.8254	**0.70**	0.0064	0.003	Carbohydrate
spermidine	3.8041	**1.47**	1.94E-06	2.16E-06	Amine

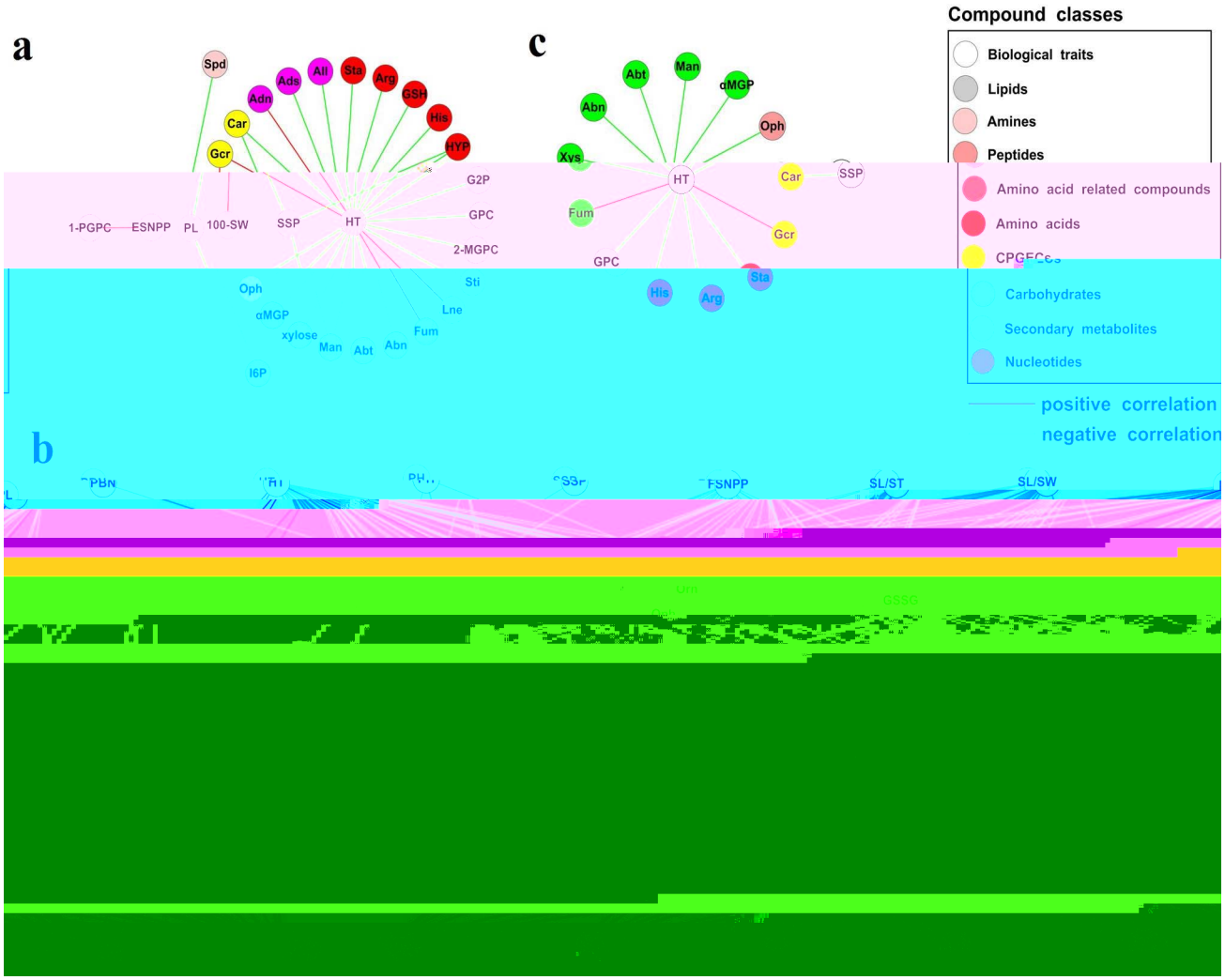
Higher values of mean decrease accuracy correspond to a larger importance of the metabolite in classifying *japonica* from *indica* cultivars. The column of *japonica/indica* shows the ratios of relative metabolite levels between *japonica* and *indica*. Red and green shaded cells indicate that the mean values are significantly higher in *japonica* and *indica*, respectively. P-value and FDR-value indicate the significance and false discovery rate of difference of the relative metabolite levels between *japonica* and *indica*, respectively. CPGEC: Cofactors, Prosthetic Groups, and Electron Carriers.

japonica *indica* (1). *japonica* *trans*-4- x 4- *indica* (1).



F 3 C
. (a) Heatmap of metabolite-metabolite correlation and significance in *inidca*. In the colored area, rectangles represent Pearson correlation coefficient (r) values of metabolite pairs (see correlation color key). In the black and white area, rectangles represent the respective p-values (see significance color key). (b) Heatmap of metabolite-metabolite correlation and significance in *japonica*. (c) Fisher's z-transformation analysis of differential metabolite-metabolite correlations between *japonica* and *indica* subspecies. Red rectangles indicate r-values of *indica* that are significantly bigger than those of *japonica*. Green rectangles indicate r-values of *indica* that are significantly smaller than those of *japonica*. Blue rectangles indicate r-values that are significant in both *indica* and *japonica*, but not significantly different between *indica* and *japonica*. Grey rectangles indicate r-values that are at least significant in one subspecies, but not significant between *indica* and *japonica*. White rectangles indicate r-values that are significant neither in indica nor in *japonica*, and not significantly different between *indica* and *japonica*.

117 (*i.e.*), (*i.e.*, , , - , -). *Japonica* x , 11 , (*i.e.*, , -) (. 5). , - . *indica* *japonica* - 121 , 3 34 , 572 - , - 11 , 21 , 13 - . - *japonica* *indica* . 13 (. 3 2). x , 1 (-), - (. 3 1). A , 100 ( ), 21 *japonica* *indica* . , 17 21 *indica* , , , , , , *N*- , , *N*- , 1,3- x - , , ( ), , (*N*-), , 13- - , - , . *japonica* , . ., - , . - , , *indica* , 13 , *japonica* , 12 10 (. 4). , , - , , , x / - . 12 (*i.e.*, x /) - , , - , , - (3). x - . A , , - .



F 4 C w . Full names of the abbreviation of metabolites and morphological traits refer to Supplementary Table S1 and Methods, respectively. Details about the associations are listed in Supplementary Data 1. Positive and negative correlations are represented by red and green edges, respectively. Each color denotes a compound class as shown in the top right legend. (a) Six positive and 23 negative correlations observed in *japonica* cultivars. (b) 49 positive and 142 negative correlations determined in *indica* cultivars. (c) 13 correlations shared between *japonica* and *indica* cultivars.

(*i.e.*) (2).

2 *indica* *japonica* , x, *indica* , *japonica* .

Metabolomics profiles support isolation-by-distance model.

x 2 ,30

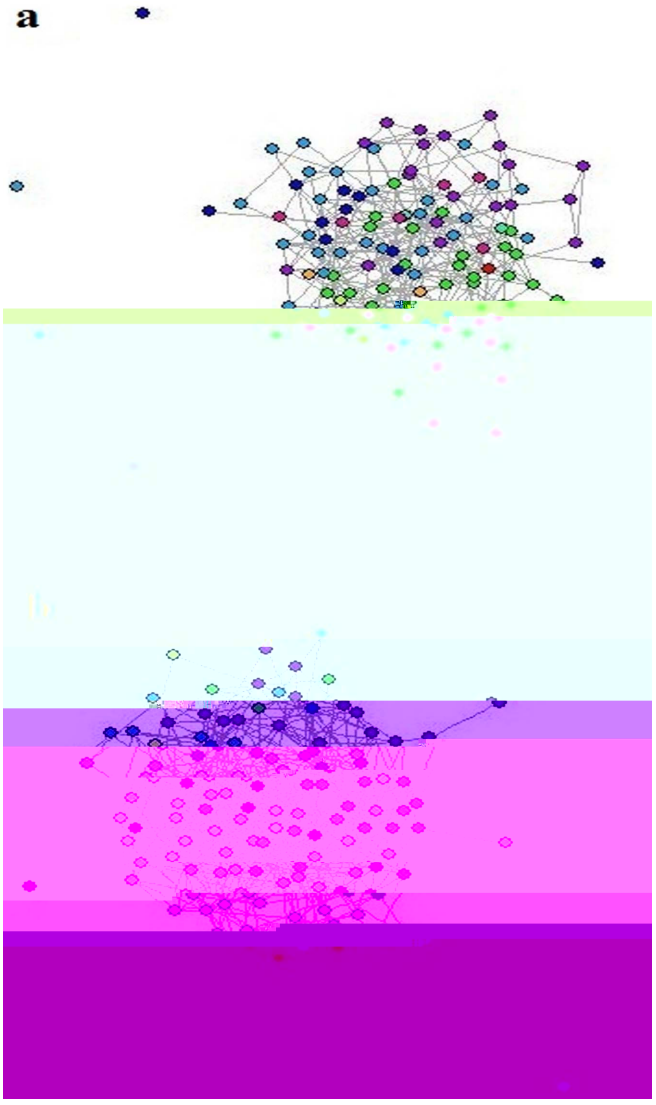
, - . ' *I* (0.57 0.5), ' *C* (0.40 0.42), (0.022) (4).

Discussion

(. .,)[31].

/ /

japonica *indica*



F 5 D **5**

Different colors of nodes represent different types of metabolites: green, amino acid; light green, secondary metabolite; dark pink, nucleotide; orange, amine; cambridge blue, carbohydrate; blue, cofactors and prosthetic groups; red, amino acid related metabolite; purple, lipid; turquoise, peptide. (a) The *japonica* network consists of five connected components, the largest of which includes 117 nodes and 419 edges, and the remaining four are isolated nodes. (b) The *indica* network consists of three connected components, the largest of which includes 119 nodes and 339 edges, and the remaining two are isolated nodes.

japonica *indica* *vs* (18 : 3) (18 : 2)[38]. *indica*, *japonica* *indica* *japonica*[22]. *japonica* *indica*. *japonica* *indica* *japonica* *indica*. *japonica* *indica*, *indica* *japonica*

Methods

Materials.

Metabolite profiling.

Data analysis.

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Acknowledgments

A ., ,

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Author contributions

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Additional information

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C :
H 5 : , .Y. *et al.* *japonica* *indica* . *Sci. Rep.* **4**, 5067 10.1038/ 05067 (2014).