JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Article

Enhancing Contents of Gamma-Aminobutyric Acid (GABA) and Other Micronutrients in Dehulled Rice during Germination under Normoxic and Hypoxic Conditions

Junzhou Ding, Tewu Yang, Hao Feng, Mengyi Dong, Margaret Slavin, Shan-bai Xiong, and Si-ming Zhao

J. Agric. Food Chem., **Just Accepted Manuscript** • DOI: 10.1021/acs.jafc.5b04859 • Publication Date (Web): 14 Jan 2016

Just Accepted

ACS Publications

Journal of Agricultural and Food Chemistry is published by the American Chemical Society. 1155 Sixteenth Street N.W., Washington, DC 20036

Enhancing Contents of Gamma-Aminobutyric Acid (GABA) and Other Micronutrients in Dehulled Rice during Germination under Normoxic and Hypoxic Conditions

Junzhou Ding[1,2], Tewu Yang[3*], Hao Feng[2], Mengyi Dong[4], Margaret Slavin[4], Shanbai Xiong[1*], Siming Zhao[1]

[1]*College of Food Sciences and Technology, Huazhong Agricultural University, Wuhan 430070, China*

[2]*Department of Food Science and Human Nutrition, University of Illinois at Urbana-Champaign, Urbana, IL 61801, USA*

[3]*MOA Key Laboratory of Crop Ecophysiology and Farming System in the Middle Reaches of the Yangtze River, College of Plant Sciences and Technology, Huazhong Agricultural University, Wuhan 430070, China*

[4] *Department of Nutrition and Food Studies, George Mason University, Fairfax, VA, 22030, USA*

*Corresponding author:

E-mail: Shanbai Xiong, xiongsb@mail.hzau.edu.cn; Tewu Yang, yangtewu@mail.hzau.edu.cn;

Abstract: Biofortification of staple grains with high contents of essential micronutrients is an important strategy to overcome micronutrient malnutrition. However, few attempts have targeted at γ-aminobutyric acid (GABA), a functional nutrient for aging populations. In this study, two rice cultivars, Heinuo and Xianhui 207, were used to investigate changes in GABA and other nutritional compounds of dehulled rice after germination under normoxic and hypoxic conditions. Forty-one metabolites were identified in both cultivars treated by normoxic germination, whereas the germinated dehulled rice of Heinuo and Xianhui 207 under hypoxic treatment had 43 and 41 metabolites identified, respectively. GABA increased in dehulled rice after germination, especially under hypoxia. Meanwhile, a number of other health-beneficial and/or flavor-related compounds such as lysine and D-mannose increased after the hypoxic treatment. The accumulation of GABA exhibited genotype-specific modes in both normoxic and hypoxic treatments. With regard to GABA production, Xianhui 207 was more responsive to germination process than the Heinuo, while Heinuo was more responsive to hypoxia than Xianhui 207. This study provides a promisingly approach to biofortify dehulled rice with increased GABA and other nutrients through metabolomic-based regulation.

Keywords Dehulled rice, γ-aminobutyric acid, metabolites, germination, hypoxic treatment, GC/MS

Introduction

Biofortification is the endogenous fortification of nutrients in foods by biological manipulation of the crop. Major strategies used in biofortification include agronomic interventions, conventional breeding, and genetic modification in crops. Plants often show genetic variation in nutrient contents, and conventional cross-breeding can be employed to select cultivars with enhanced nutrient content.[1] Because this approach uses intrinsic properties of a crop, it receives widespread acceptance and few regulatory constraints. However, the success of this method relies on the limited natural genetic variation or special germplasm.[2] Meanwhile, the process of introducing a new trait into commercial cultivars is usually costly and time-consuming. Alternatively, another strategy for biofortification utilizes transgenic techniques to engineer plant metabolism. This genetic engineering has shown an appealing potential to speed up the process in biofortification efforts, based on increased understanding of metabolic pathways and gene expression patterns.[2] The transgenic approach may also allow introduction of metabolic pathways from bacteria and other organisms into crops. However, these metabolic alterations may affect the agronomic performances or stress tolerance of crops. Moreover, the public concerns on the safety of genetically modified (GMO) foods have hampered some commercialization attempts using this technology.[2] In this context, a multi-tier coordinated strategy, including development of new strategies, is required to achieve the desired results for the biofortification of various nutrients.

One compound that contributes to enhance the nutritional value of foods is γ-aminobutyric acid (GABA), which is naturally present in rice. GABA is widely recognized as one of the major neurotransmitters in the brain, and ingestion of GABA has been recognized to influence several important physiological functions in higher animals, including improvement of brain function

and postponement of intelligence degradation,[3] relief of nervous tension,[4] mitigation of hypertension,[5] and regulation of hepatic cholesterol metabolism.[6] Thus, GABA is considered to be a functional nutrient. Though it is not physiologically necessary to consume GABA in the diet due to endogenous production from L-glutamate, it is important to consider that the synthetic capacity of GABA *in vivo* declines gradually with aging.[7] Therefore, the dietary supplementation with GABA may be considered in aging populations. In higher plants, including rice, GABA is biosynthesized via a pathway known as the GABA shunt (Fig. 1). It is produced directly from glutamate catalyzed by glutamate decarboxylase (GAD; EC 4.1.1.15). Simultaneously, GABA can be converted reversibly to succinic semialdehyde (SSA) by the action of GABA transaminase (GABA-T; EC 2.6.1.19) using either pyruvate, and SSA irreversibly oxidized to succinate by succinate semialdehyde dehydrogenase (SSADH; EC 1.2.1.16). Succinate then enters into Krebs cycle for catabolism.[8] Since the decreases in respiration, the NAD:NADH ratio, the SSADH reaction, and the entry of carbon into the Krebs cycle are limited during hypoxia, which may cause GABA accumulation in plant.[9] GABA accumulation has been shown to relate closely to the expression of genes *GADs*, *GABA-Ts* and *SSADHs* in vegetable soybean,[10] and the genes for *GAD*, *GABA-T*, *GLYR1-2* (*glyoxylate reductase/succinic semialdehyde reductase 1&2*) and *GABP (GABA permease*) in *Arabidopsis* seeds.[11] Thus, identification of the genes encoding essential enzymes involved in the GABA shunt will make it possible to engineer the metabolic flux to increase GABA through genetic manipulation in plants.

It has been shown that GABA increases in plants under various environmental stress such as hypoxia,[11] e.g., in the roots of rice[12] and *Arabidopsis*[13] and stem of ginseng (*Panax ginseng* C.A. Meyer) [14], and functions as a signal to induce higher stress resistance of plants.[15] Accumulation of GABA is attributed to the expression of genes associated with the anabolic pathway, which is

more responsive to stresses than those genes associated with the catabolic phase of GABA-shunt.[11] Moreover, GABA has also been found to increase in brown rice after germination.[16,17] In a preliminary study, we found that GABA accumulation was inhibited initially but was ultimately promoted after 60 h germination with the highest content being detected at 72 h in germinating brown rice under hypoxic conditions. Given this, the combination of germination process with hypoxic treatment may provide a promising strategy for cost-effective and rapid biofortification of dehulled rice to contain higher GABA. However, there is neither metabolomic evidence concerning the GABA changes, nor any understanding of the responses of GABA in different rice genotypes during germination.

This study was conducted to understand the regulatory mechanism of metabolism under different oxygen conditions, and to assist the biofortification attempts to enhance the metabolites of interest, GABA in particular. Gas chromatography/mass spectrometry (GC/MS) was used to achieve such purpose, with the help of multivariate statistical analysis to analyze the high-throughput data.[18,19] We determined the changes in free amino acids, small molecule sugars, organic acids, and other related metabolites in dehulled rice after normoxic germination and in germinated dehulled rice after hypoxic treatment, using two rice cultivars and GC/MS as the primary analytical technique. The aim of this study was to understand the metabolomic alternations associated with the GABA shunt in dehulled rice after germination under normoxic and hypoxic conditions, to provide insight into the development of a new GABA biofortification strategy in grains based on regulation of plant metabolism.

Materials and Methods

Materials and chemicals

Two *Oryza sativa* L. subsp. *indic*a rice cultivars, Heinuo, a black glutinous landrace that is grown locally and regarded traditionally as a nutritious food, and Xianhui 207, a white rice that is widely used as a restorer line in hybridization breeding program in southern China, were used in the treatments. The two rice genotypes were grown and harvested under the same management and conditions on the experimental farm of Huanggang Academy of Agricultural Sciences (114º53' E, 30º26' N), Hubei Province of China, in 2013. GABA, bis methylsilane trifluoroacetamide (BSTFA derivatization reagent), methoxy pyridine hydrochloride were purchased from Sigma Aldrich (St. Louis, MO).

Material treatment and sample preparation

Dehulled rice was prepared and the sample prior to germination was marked with A_0 (black/purple rice) for Heinuo and B_0 (brown rice) for Xianhui 207, respectively. Ungerminated dehulled rice (100 g) of each cultivar was sampled and soaked in water at 30 ºC for 12 h, and then placed in a germination tray and allowed to germinate in a plant growth chamber (HP 250G, Wuhan Ruihua Instrument Equipment Co., LTD) under the condition of normoxia and moisture supplied with an ultrasonic humidifier at 28 ºC for 72 h. For the normoxic treatment, the dehulled rice was kept in the growth chamber to germinate for 72 h. This sample was termed as marked with A_1 for Heinuo and B_1 for Xianhui 207, respectively. For the hypoxic treatment, following germination for 66 h under the same normoxia- germinated conditions, the germinated rice together with the germination tray was transferred into a sealed plastic bag filled with high purity CO_2 (2.0-2.5 kPa) to be treated for 6 h at 28 ºC using an automatic tray sealer with vacuum and gas flushing system. This hypoxia- germinated rice was marked with A_2 for Heinuo and B_2 for Xianhui 207, respectively. After treatments, all samples were rinsed with deionized

water for 3 seconds, then ground to powder in liquid nitrogen immediately and freeze-dried under vacuum prior to testing. The experiment was replicated three times. Data are presented as means ± standard deviation *(SD)*.

Metabolites determination using GC/ MS

The metabolites in samples were extracted, derivatized, and identified and quantified using the method of Roessner *et al.*[20] with slight modification. Fifty mg of dried rice powder was weighed into a 1.5 mL microcentrifuge tube and 1 mL of pre-cooled 100% methanol added. Following vortexing and homogenization, the mixture was ultrasonically extracted in an ultrasonic cleaner at 60 ºC for 15 min and then centrifuged for 10 min at 6000 g. An aliquot of 0.4 mL supernatant was pipetted to another microcentrifuge tube and 0.2 mL of cold acetonitrile and 0.4 mL of ultrapure water added. The mixture was centrifuged for 15 min at 10000 g, then 200 µL of the supernatant was drawn and dried in a glass bottle with nitrogen gas. After 30 µL of 20 mg/mL methoxy pyridine hydrochloride solution was added, the bottle was strongly shaken for 30 s, then kept at 37 ºC for oximation for 90 min. Afterwards, 30 µL of BSTFA derivatization reagent containing 1 % tri methyl chloro silane was added and allowed to react at 70 ºC for 60 min. Following that, the mixture was cooled at room temperature for 30 min prior to GC/MS analysis.

The GC/MS was performed using an Agilent 7890A /5975C GC/MS system with a HP-5ms capillary column (30 m×0.25 mm with a 0.25 µm film thickness) (Agilent J & W Scientific Inc.). The temperature of inlet, EI ion source and quadrupole was set to 280, 230 and 150ºC, respectively. Helium (> 99.99%) was used as carrier gas without split stream. Injection volume was 1.0 µL. Oven temperature started at 80 ºC and held for 2 min, and then increased to 320 ºC

by 10 ºC/min and held for 6 min. Full scan mode was used for mass spectrometry analysis with a detection range of 50-550 m/z. A randomized sequence of consecutive samples was used to minimize the influence of instrumental signal fluctuations. The chromatograms were then subjected to noise reduction and the relative intensity of each peak was calculated before statistical analysis. GC/MS mass spectra were identified with NIST. Evaluation and qualification of metabolites were carried out using the workstation in Shanghai Sensichip Infotech Co., Ltd, (Shanghai, China). The precise quantification of GABA in samples was determined in a linear range of 6.73-673.08 mg /100g using GABA as standard and the above GC/MS method.

Statistical analysis

The statistical analysis methods of Meng *et al.*[19] and Zhang *et al.*[18] were applied to discriminate and identify the differential metabolites in various samples detected by GC/MS. A data matrix was obtained for characterizing the biochemical pattern of each sample. All data was Unit Variance scaled and mean-centered in SIMCA-P 11.0, and data analysis was performed using XCMS files under R software as an add-in for Excel 2007. Principal Component Analysis (PCA) was performed to visualize the trends of samples and Partial Least Squares Discriminant Analysis (PLS-DA) was used to discriminate the differences in detected metabolites among dehulled rice, normoxia- and hypoxia- germinated rice, based on the Variable Importance in the Projection (VIP) of the first principal component in the PLS-DA model > 1 and *t*-test at $P = 0.05$.

The relative value of differential metabolites was calculated to show the change in dehulled rice after germination under normoxic conditions of the same cultivar (A_1/A_0 and B_1/B_0 for Heinuo and Xianhui 207, respectively), after germination with hypoxia-treatment of the same

cultivar (A_2/A_1 and B_2/B_1 for Heinuo and Xianhui 207, respectively), and in normoxia-germinated rice (B_1/A_1) and hypoxia- germinated rice (B_2/A_2) between cultivars. The specific values of metabolites between different samples were calculated by the ratio of peak area. Data on metabolite levels of all treatments were analyzed by one-way ANOVA at $P = 0.05$ using IBM SPSS Statistics 2.0 (SPSS, Inc., Chicago, IL, USA), and t-Test was used to separate the differences of metabolite levels between dehulled rice and normoxia- germinated rice (A_0 vs. A_1 for Heinuo, B_0 vs. B_1 for Xianhui 207), dehulled rice and hypoxia- germinated rice (A_0 vs. A_2 for Heinuo, B_0 vs. B_2 for Xianhui 207) at $P = 0.05$.

Results

Quantity of differential metabolites in dehulled rice after germination under different conditions

Fig. 2 shows the Total Ion Chromatograms (TIC) of dehulled rice before germination and germinated dehulled rice under normoxic and hypoxic conditions of two cultivars. There were 1059 (A_0) and 1027 (B_0) peaks detected in ungerminated dehulled rice, and 1144 (A_1) and 1143 (B_1) peaks in normoxia- germinated rice for Heinuo and Xianhui 207, respectively. The hypoxia-germinated rice had 1145 peaks detected for both cultivars. Thus, the Heinuo cultivar had 32 more detectable metabolites than Xianhui 207 in the ungerminated dehulled rice. This difference in total number of metabolites between cultivars diminished after normoxic germination and after hypoxic treatment.

Distinct metabolic patterns were revealed in the samples between ungerminated dehulled rice and normoxia- germinated rice (A_0 vs. A_1 for Heinuo, B_0 vs. B_1 for Xianhui 207), ungerminated dehulled rice and hypoxia- germinated rice (A_0 vs. A_2 for Heinuo, B_0 vs. B_2 for Xianhui 207).

The parameter of PCA (R^2X) was 0.888 for A_0 vs. A_1, 0.886 for B_0 vs. B_1, 0.893 for A_0 vs. A_2 and 0.879 for B_0 vs. B_2. All parameters were larger than 0.85, suggesting significant impacts of germination and hypoxic treatment on metabolic products. However, no significant difference in metabolic patterns was detected between normoxia- and hypoxia-germinated rice for both cultivars.

Qualitative identification of differential metabolites in dehulled rice under different germination conditions

Table 1 shows the name and peak area of differential metabolites as determined by multivariate statistical analysis and identified by NIST mass spectra library. There were 45 differential metabolites expressed in normoxia-germinated rice (A_1 & B_1) compared to ungerminated dehulled rice (A_0 & B_0) for both cultivars. These included 15 amino acids, 11 carbohydrates, 7 organic acids, 2 fatty acids, 2 alcohols, 3 amines and 5 other compounds. Compared to ungerminated dehulled rice (A_0 & B_0), there were 47 differential metabolites detected in the hypoxia- germinated rice (A_2 & B_2) for both cultivars, which included 16 amino acids, 9 carbohydrates, 7 organic acids, 3 fatty acids, 2 alcohols, 3 amines and 5 other compounds (Table 1). These suggested that the metabolic alternations were mainly related to amino acid, carbohydrate and lipid pathways in dehulled rice after normoxic germination and under hypoxic treatment during germination. Nevertheless, the variation in metabolites is relatively small between normoxic and hypoxic conditions.

Among the detected differential metabolites, most compounds increased upon germination, and some increased by orders of magnitude in normoxia-germinated rice compared to ungerminated dehulled rice, for both cultivars. Meanwhile, there were significant decreases from

ungerminated dehulled rice to normoxia-germinated rice for both cultivars in sucrose (M41), glycine (M5), and several organic acids or fatty acids including oxalic acid (M3), D-glucuronic acid (M38), protocatechuic acid (M25), palmitic acid (M32), linoleic acid (M34) and oleic acid (M35), lipids including phosphate acid propyl ester (M24), myo-inositol phosphate (M39) and 1-monopalmitin ether (M40), polyamines including cadaverine (M14) and putrescine (M15) (Table 1). The changes in differential metabolites of the HGDR showed almost similar trends to that of the normoxia- germinated rice. However, after hypoxic treatment for both cultivars there were higher amounts of the following metabolites in germinated rice compared to normoxia-germinated rice: 6 essential amino acids including lysine (M26), L-phenylalanine (M23), threonine (M13), isoleucine (M9), methionine (M18) and valine (M4), and other health-benefiting amino acids including GABA (M20), proline (M10), serine (M12), L-alanine (M2), and small molecule carbohydrates including D-glucose (M30), D-mannose (M37), and melibiose (M44), as well as glycerol (M7), phosphoric acid (M8) and succinic acid (M11). Still, hypoxia-germinated rice contained lower amounts than normoxia- germinated rice of a few compounds: D-fructose (M29), maltose (M42), D-turanose (M43), tyrosine (M28) and 1-monopalmitin ether (M40) (Table 1).

The two cultivars responded differently after normoxic germination and/or in hypoxic germination, as indicated in Table 1. In the normoxia-germinated rice, lactic acid (M1), phosphoric acid (M8) and L-glutamate (M22) increased in Heinuo only; ethanolamine (M6), lactose (M46) and D-glucose-L-mannopyranosyl (M47) increased in Xianhui 207 but decreased in Heinuo, compared to the ungermianted dehulled rice (Table 1). Serine (M12), L-asparagine (M21), lysine (M26), malic acid (M16), pyroglutamic acid (M19), inositol (M33), furanone (M17) and D-mannose (M37) increased more in the normoxia-germinated rice of Heinuo than in

that of Xanhui 207; GABA (M20), L-phenylalanine (M23), L-tryptophan (M36), D-galactose (M31), maltose (M42), D-turanose (M43) and melibiose (M44) increased more in the normoxia-germinated rice of Xianhui 207 than in that of Heinuo, in comparison with the dehulled rice (Table 1). In the normoxia- germinated rice, palmitic acid (M32) and myo-inositol phosphate (M39) decreased more in Heinuo, whereas linoleic acid (M34) and 1-monopalmitin ether (M40) decreased more in Xianhui 207, compared to the ungermianted dehulled rice (Table 1),

After hypoxic treatment, the content of lactic acid (M1), lactose (M46) and D-glucose-L-mannopyranosyl (M47) increased, but malic acid (M16), pyroglutamic acid (M19) and talose (M45) decreased in the germinated rice of Heinuo only. Phosphoric acid (M8), protocatechuic acid (M25), L-asparagine (M21), L-glutamate (M22), furanone (M17) and inositol (M33) increased in hypoxia- germinated rice of Xianhui 207 only, compared to the ungermianted dehulled rice (Table 1). While, L-methionine (M18) and melibiose (M44) increased more in the hypoxia- germinated rice of Xianhui 207 than in that of Heinuo, but D-fructose (M29) and maltose (M42) decreased more. L-tryptophan (M36) increased in the hypoxia- germinated rice of Heinuo but decreased in Xianhui 207, in comparison with the ungerminated dehulled rice (Table 1).

Changes in contents of key metabolites associated with GABA shunt in dehulled rice under different germination conditions

There were considerable changes in GABA and other metabolites associated with the GABA shunt in the dehulled rice before and after germination in normoxic and hypoxic conditions (Table 1 & 2). The precise quantification revealed that GABA content was 38.5 and 37.6 times high in the hypoxia- germinated rice, and 2.8 and 6.4 times high in the normoxia, as that in the

ungerminated dehulled rice, for Heinuo and Xianhui 207, respectively (Table 2). Heinuo had a slightly higher GABA level in the ungerminated dehulled rice, but the GABA was higher in the normoxia- germinated rice of Xianhui 207 than in that of Heinuo (Table 2). Nevertheless, no significant difference in GABA content was detected in the hypoxia- germinated rice between cultivars (Table 2). Succinic acid (M11) content increased in the hypoxia- germinated rice compared to that in the ungerminated dehulled rice and normoxia-germinated rice for both cultivars (Table 1). A similar change was also found in L-glutamate (M22) for Xianhui 207: its content increased in the hypoxia- germinated rice but did not in the normoxia-germinated rice, as compared to the ungerminated dehulled rice (Table 1). In Heinuo, L-glutamate content increased in both of normoxia- and hypoxia-germinated rice compared to that in the ungerminated dehulled rice (Table 1), but was similarly elevated in both germination conditions.

Discussion

Rice is a staple food for almost half of the population across the world. White rice, from which the testa and bran have also been removed and therefore consists primarily of starchy endosperm, is the most commonly consumed type in the world.[21] The testa layer of the rice grain exists in various colors, such as black, purple, red, and brown, evidenced by the colors visible after the de-hulling, or removal of the outer husk. The colorful dehulled rice types contain anthocyanin pigments which endow them with antioxidant and anti-inflammatory properties.[22] Germinated dehulled rice has the potential to be a GABA-enriched functional grain food,[17] which may be instrumental to the glycemic control for type 2 diabetes[23] and reducing cardiovascular disease risk.[6] In addition, germinated dehulled rice products contain abundant fibers, minerals, vitamins.[24] Therefore, biofortification of germinated dehulled rice with vital

micronutrients is important for the population that depends on rice as daily diet to overcome micronutrient malnutrition.

In this study, we found that GABA, as well as several essential amino acids and other health-favorable nutrients increased in dehulled rice after germination and to greater extents when germination was conducted under hypoxic conditions, thereby providing a promising strategy for micronutrient biofortification of rice. This approach is cost- and time-effective compared to conventional crop breeding programs to promote the content of micronutrients, and avoids the problem of low public acceptance of genetically modified foods and of food additives.

However, GABA accumulation showed a genotype-specific effect on germination of dehulled rice in response to normoxic germination and hypoxic germination. Xianhui 207 was more responsive to the germination process, whereas Heinuo was more responsive to hypoxic treatment. This genotype-specific effect was also found in the accumulation of some essential amino acids and health-favorable nutrients. For instance, the accumulation of mannose and lysine was more responsive to the germination process in dehulled rice of Heinuo, whereas, Xianhui 207 was more responsive to hypoxic treatment. An earlier studyfound that GABA accumulation in the germ of different rice cultivars varied considerably during imbibition.[25] Therefore, in continued attempts to biofortify GABA in rice, both germination conditions and genotype should be considered.

The significant increases of small molecule carbohydrates and amino acids (with the exception of sucrose and glycine which decreased in both cultivars) in normoxia-germinated rice indicated active catabolism of starch/sucrose and proteins in rice grains during normoxic germination in this study. Decreases of fatty acids in normoxia-germinated rice may imply their consumption involved in the synthesis of membrane lipids for sprouts' growth during normoxic

316 germination. The addition of hypoxic treatment during the germination process promoted most
317 amino acids and small molecule carbohydrates to increase, which may have resulted from an
318 inability to synthesize protein and conduct low energy metabolism under anaerobic conditions.
319 Generally, anoxia-tolerant plant species including rice strengthen fluxes through glycolytic and
320 fermentative pathways to compensate for the loss in aerobic ATP.[26] Transcriptomic analysis by
321 Narsai et al.[27] revealed that carbohydrate and energy metabolism are underrepresented in
322 anaerobic germinated rice. A number of metabolite responses, including increases in the amino
323 acids alanine, valine, glycine, leucine, arginine, tyrosine, phenylalanine, proline and GABA, the
324 organic acids succinate and lactate, and polyamine putrescine, and decreases in glutamate,
325 asparagine and 2-oxoglutarate, have been reported in oxygen-deprived rice seedlings in previous
326 studies.[28] Similar changes in GABA, alanine, valine, phenylalanine and succinate were also
327 found in hypoxia- germinated rice. However, glutamate, asparagine, and inositol results diverged
328 from the decreases observed in the previous work, [28] and were observed to increase in hypoxia-
329 germinated rice of Xianhui 207 in this study. This may be due to the differences in rice genotypes
330 and germination procedure in our study.

331 Both gene-dependent and -independent processes may be involved in the response of the
332 GABA shunt to abiotic stresses.[11] Glutamate, the direct precursor of GABA biosynthesis, can
333 enhance the activity of GAD, the key enzyme to catalyze glutamate decarboxylation, which
334 causes GABA accumulation in plants.[29] In this study, glutamate increased in hypoxia-
335 germinated rice compared to ungerminated dehulled rice for both cultivars, which suggest that
336 the synthetic pathway of the GABA shunt is dominant in germinating dehulled rice under
337 hypoxic conditions, the significant increase may contribute to the remarkable increase of GABA[9].
338 However, glutamate accumulation occurred differently in the two cultivars during normoxic

conditions (Table 1). In Heinuo, glutamate levels were similar between normoxia- and hypoxia-germinated rice, but GABA still increased significantly in hypoxia- germinated rice compared to normoxia-germinated rice. In Xianhui 207, both glutamate levels and GABA levels increased in hypoxia- germinated rice as compared to normoxia-germinated rice. These imply that the substrate increase may not totally explain the variations of GABA accumulation in dehulled rice after germination in normoxic and hypoxic treatment conditions among different cultivars. Meanwhile, succinic acid increased in hypoxia- germinated rice compared with normoxia-germinated rice and ungerminated dehulled rice for both cultivars. This may result from the inhibition of its catabolism due to oxygen deprivation or enhanced catabolism of GABA. [11]

The concentrations of several organic acids and phosphoric acid increased, which may cause cytosolic acidification in normoxia-germinated rice and hypoxia- germinated rice in this study. Cellular acidification has been shown to stimulate GAD activity resulting in a transient increase of GABA in plants, because the enzyme exhibits a sharp acidic pH optimum of about 5.8.[9] In this regard, the increases of organic acids and phosphoric acid, together with amino acids may contribute to GABA accumulation in normoxia- and hypoxia-germinated rice. However, organic acids and phosphoric acid showed different responses to the germination process and hypoxic treatment in the two cultivars. Phosphoric acid increased in both normoxia- and hypoxia-germinated rice compared to ungerminated dehulled rice, as well as in hypoxia compared to normoxia for both cultivars. Lactic acid did so for Heinuo only. Succinic acid increased in germinated rice only in response to hypoxic treatment, while, malic acid and octanedioic acid increased in germinated rice only under normoxic condition in this study. Whether these changes are related to GABA accumulation in dehulled rice in response to germination conditions cannot be deduced from the present data. Further investigation is required. Meanwhile, GABA

accumulation over a long term is typically associated with elevated levels of *GADs* expression.[11] Further studies on the enzymatic activity and gene expression of the GABA pathway may bring a deeper insight into the biochemical mechanisms of GABA accumulation in dehulled rice after germination in normoxic and hypoxic conditions, and among different cultivars.

Free amino acids were reported to function as an osmotic regulator in cells for adapting to stress, and to meet the energy and nutritional needs in plants after the plants relieving from abiotic stresses.[30] Reggiani et al. (1988) found that the interconversion between free amino acids and glutamate synthesis contributed ,to the increase in GABA and alanine contents in hypoxia-treated rice roots. [12] The significant increases of several free amino acids in the hypoxia-germinated rice may be also attributed to the adaptation of germinated rice to hypoxic stress in this study. Mannose was also shown to be involved in stress adaptation by protecting cells from the damage by reactive oxygen species (ROS) in plants.[30] In this study, D-mannose increased sharply in the normoxia- and hypoxia- germinated rice of Heinuo, but did not do so with Xianhui 207, suggesting different adaptive mechanisms to stresses among rice genotypes. Further investigations on genotype-specific accumulative modes of various functional nutrients in response to germination and hypoxic treatment will facilitate the attempts using this biofortification strategy.

Acknowledgements

We thank Prof. Qiang Zhou at the Huanggang Academy of Agricultural Sciences in Hubei Province for growing the rice materials for this study, and thank the technical staff at Shanghai Sensichip Infotech Co., Ltd. for their assistance in data analyses. Our thanks are also given to Dr. Kang-Mo Ku in the Division of Plant & Soil Sciences at West Virginia University for his assistance on providing metabolites information.

Supporting Information Available

The PCA Scores Plot of normoxia- and hypoxia-germinated rice compared to the ungerminated dehulled rice of different cultivars. This material is available free of charge via the Internet at http://pubs.acs.org.

Funding Sources

This research was partly supported by research grants from the Key Sciences & Technology Research Innovative Programs (Industrialization) of Hubei Province (2014ABC009), China.

References

(1) Messias, R. da S.; Galli, V.; Silva, S. D. D. A. E.; Schirmer, M. A.; Rombaldi, C. V. Micronutrient and functional compounds biofortification of maize grains. *Crit. Rev. Food Sci. Nutr.* **2015**, *55* (1), 123–139.

(2) Hirschi, K. D. Nutrient biofortification of food crops. *Annu. Rev. Nutr.* **2009**, *29* (1), 401–421.

(3) Palmer, L. M.; Schulz, J. M.; Murphy, S. C.; Ledergerber, D.; Murayama, M.; Larkum, M. E. The cellular basis of $GABA_B$-mediated interhemispheric inhibition. *Science* **2012**, *335* (6071), 989–993.

(4) Abdou, A. M.; Higashiguchi, S.; Horie, K.; Kim, M.; Hatta, H.; Yokogoshi, H. Relaxation and immunity enhancement effects of gamma-aminobutyric acid (GABA) administration in humans. *BioFactors Oxf. Engl.* **2006**, *26* (3), 201–208.

(5) Yoshimura, M.; Toyoshi, T.; Sano, A.; Izumi, T.; Fujii, T.; Konishi, C.; Inai, S.; Matsukura, C.; Fukuda, N.; Ezura, H.; et al. Antihypertensive effect of a gamma-aminobutyric acid rich tomato cultivar "DG03-9" in spontaneously hypertensive rats. *J. Agric. Food Chem.* **2010**, *58* (1), 615–619.

(6) Imam, M. U.; Ishaka, A.; Ooi, D.-J.; Zamri, N. D. M.; Sarega, N.; Ismail, M.; Esa, N. M. Germinated brown rice regulates hepatic cholesterol metabolism and cardiovascular disease risk in hypercholesterolaemic rats. *J. Funct. Foods* **2014**, *8*, 193–203.

(7) Leventhal, A. G.; Wang, Y.; Pu, M.; Zhou, Y.; Ma, Y. GABA and its agonists improved visual cortical function in senescent monkeys. *Science* **2003**, *300* (5620), 812–815.

(8) Shelp, B. J.; Bozzo, G. G.; Trobacher, C. P.; Chiu, G.; Bajwa, V. S. Strategies and tools for studying the metabolism and function of gamma-aminobutyrate in plants. I. Pathway

structure. *Botany* **2012**, *90* (8), 651–668.

(9) Shelp, B. J.; Bown, A. W.; McLean, M. D. Metabolism and functions of gamma-aminobutyric acid. *Trends Plant Sci.* **1999**, *4* (11), 446–452.

(10) Takahashi, Y.; Sasanuma, T.; Abe, T. Accumulation of gamma-aminobutyrate (GABA) caused by heat-drying and expression of related genes in immature vegetable soybean (edamame). *Breed. Sci.* **2013**, *63* (2), 205–210.

(11) Shelp, B. J.; Bozzo, G. G.; Zarei, A.; Simpson, J. P.; Trobacher, C. P.; Allan, W. L. . Strategies and tools for studying the metabolism and function of gamma-aminobutyrate in plants. II. Integrated analysis. *Botany* **2012**, *90* (9), 781–793.

(12) Reggiani, R.; Cantu, C. A.; Brambilla, I.; Bertani, A. Accumulation and interconversion of amino acids in rice roots under anoxia. *Plant Cell Physiol*. **1988**, 29(6), 981–987.

(13) Klok, E. J.; Wilson, I. W.; Wilson, D.; Chapman, S. C.; Ewing, R. M.; Somerville, S. C.; Peacock, W. J.; Dolferus, R.; Dennis, E. S. Expression profile analysis of the low-oxygen response in *Arabidopsis* root cultures. *Plant Cell* **2002**, *14* (10), 2481–2494.

(14) Lee, J. H.; Kim, Y. J.; Jeong, D. Y.; Sathiyaraj, G.; Pulla, R. K.; Shim, J. S.; In, J. G.; Yang, D. C. Isolation and characterization of a Glutamate decarboxylase (GAD) gene and their differential expression in response to abiotic stresses from *Panax ginseng* C. A. Meyer. *Mol. Biol. Rep.* **2010**, *37* (7), 3455–3463.

(15) Ramesh, S. A.; Tyerman, S. D.; Xu, B.; Bose, J.; Kaur, S.; Conn, V.; & Gillham, M. GABA signalling modulates plant growth by directly regulating the activity of plant-specific anion transporters. *Nat. Commun.***2015**, *6*, 7879

(16) Moongngarm, A.; Saetung, N. Comparison of chemical compositions and bioactive compounds of germinated rough rice and brown rice. *Food Chem.* **2010**, *122* (3), 782–788.

(17) Zhang, Q.; Xiang, J.; Zhang, L.; Zhu, X.; Evers, J.; van der Werf, W.; Duan, L. Optimizing soaking and germination conditions to improve gamma-aminobutyric acid content in japonica and indica germinated brown rice. *J. Funct. Foods* **2014**, *10*, 283–291.

(18) Zhang, H.; Wu, L.; Xu, C.; Xia, C.; Sun, L.; Shu, S. Plasma metabolomic profiling of dairy cows affected with ketosis using gas chromatography/mass spectrometry. *BMC Vet. Res.* **2013**, *9* (1), 186.

(19) Meng, J.; Zhang, X.; Wu, H.; Bu, J.; Shi, C.; Deng, C.; Mao, Y. Morphine-induced conditioned place preference in mice: Metabolomic profiling of brain tissue to find "molecular switch" of drug abuse by gas chromatography/mass spectrometry. *Anal. Chim. Acta* **2012**, *710*, 125–130.

(20) Roessner, U.; Wagner, C.; Kopka, J.; Trethewey, R. N.; Willmitzer, L. Technical advance: simultaneous analysis of metabolites in potato tuber by gas chromatography-mass spectrometry. *Plant J. Cell Mol. Biol.* **2000**, *23* (1), 131–142.

(21) Babu, V. Ravindra. Importance and advantages of rice biofortification with iron and zinc. *J. SAT Agric. Res.* **2013**, *11*, 1.

(22) Finocchiaro, F.; Ferrari, B.; Gianinetti, A.; Dall'asta, C.; Galaverna, G.; Scazzina, F.; Pellegrini, N. Characterization of antioxidant compounds of red and white rice and changes in total antioxidant capacity during processing. *Mol. Nutr. Food Res.* **2007**, *51* (8), 1006–1019.

(23) Imam, M. U.; Ismail, M. Nutrigenomic effects of germinated brown rice and its bioactives on hepatic gluconeogenic genes in type 2 diabetic rats and HEPG2 cells. *Mol. Nutr. Food Res.* **2013**, *57* (3), 401–411.

(24) Adhikari, K. B.; Tanwir, F.; Gregersen, P. L.; Steffensen, S. K.; Jensen, B. M.; Poulsen, L.

K.; Nielsen, C. H.; Høyer, S.; Borre, M.; Fomsgaard, I. S. Benzoxazinoids: Cereal phytochemicals with putative therapeutic and health-protecting properties. *Mol. Nutr. Food Res.* **2015**, *59* (7), 1324–1338.

(25) Saikusa, T.; Horino, T.; Mori, Y. Accumulation of γ-aminobutyric acid (GABA) in the rice germ during water soaking. *Biosci. Biotech. Bioch.* **1994**, 58(12), 2291–2292.

(26) Bailey-Serres, J.; Voesenek, L. a. C. J. Flooding stress: acclimations and genetic diversity. *Annu. Rev. Plant Biol.* **2008**, *59*, 313–339.

(27) Narsai, R.; Howell, K. A.; Carroll, A.; Ivanova, A.; Millar, A. H.; Whelan, J. Defining core metabolic and transcriptomic responses to oxygen availability in rice embryos and young seedlings. *Plant Physiol.* **2009**, *151* (1), 306–322.

(28) Magneschi, L.; Perata, P. Rice germination and seedling growth in the absence of oxygen. *Ann. Bot.* **2009**, *103* (2), 181–196.

(29) Scott-Taggart, C. P.; Van Cauwenberghe, O. R.; McLean, M. D.; Shelp, B. J. Regulation of-aminobutyric acid synthesis in situ by glutamate. *Physiol. Plantarum*. **1999**, *106*, 363–369

(30) Wu, D.; Cai, S.; Chen, M.; Ye, L.; Chen, Z.; Zhang, H.; Dai, F.; Wu, F.; Zhang, G. Tissue metabolic responses to salt stress in wild and cultivated barley. *PLoS ONE* **2013**, *8* (1), e55431.

List of figures

Fig. 1 Schematic depiction of the GABA shunt in plants (adapted from Shelp et al. 2012). [8,11]

Fig. 2 Total Ion Chromatograms (TIC) detected by GC/MS in ungerminated dehulled rice and germinated dehulled rice under normoxic condition (NGDR) and after hypoxic treatment (HGDR) of different cultivars. A_0: ungerminated dehulled rice for Heinuo; B_0: ungerminated dehulled rice for Xianhui 207; A_1: NGDR for Heinuo; B_1: NGDR for Xianhui 207; A_2: HGDR for Heinuo; B_2: HGDR for Xianhui 207. Fig.2 a, b, c, d, e, f is TIC for A_0, B_0, A_1, B_1, A_2 and B_2 respectively.

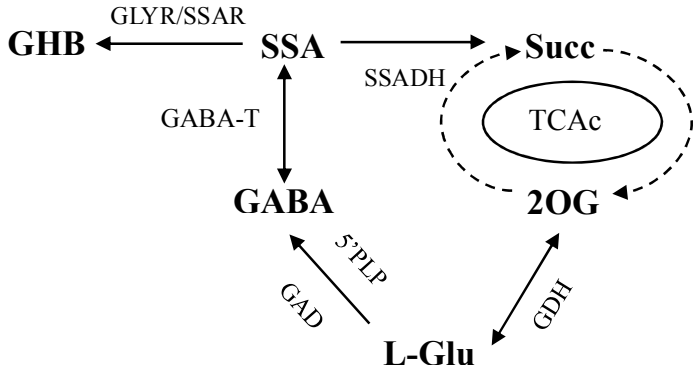


Fig. 1

*Abbreviations: GABA, γ-aminobutyrate; L-Glu, L-glutamate; SSA, succinic semialdehyde; Succ, succinate; 2OG, 2-oxoglutarate; TCAc, tricarboxylic acid cycle; GAD, glutamate decarboxylase; GABA-T, GABA transaminase; SSADH, succinic semialdehyde dehydrogenase; GDH, glutamate dehydrogenase; 5'PLP, pyridoxal-5'-phosphate; GLYR, glyoxylate reductase; SSAR, succinic semialdehyde reductase; GHB, 4-hydroxybutyrate

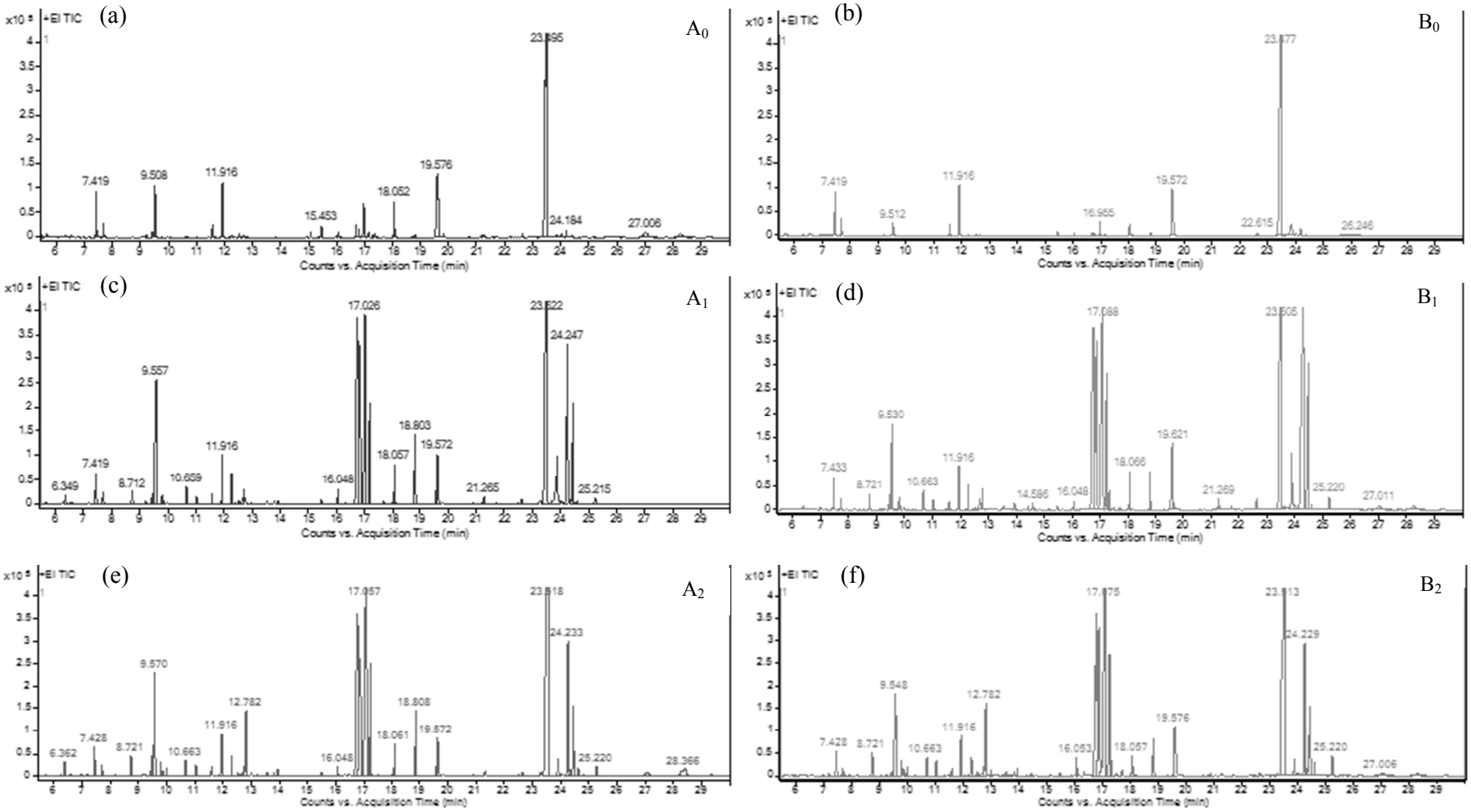


Fig. 2

Table 1 Differential metabolites in ungerminated dehulled rice and germinated dehulled rice under the normoxic condition (NGDR) and after hypoxic treatment (HGDR) of different cultivars. A_0: ungerminated dehulled rice for Heinuo; B_0: ungerminated dehulled rice for Xianhui 207; A_1: NGDR for Heinuo; B_1: NGDR for Xianhui 207; A_2: HGDR for Heinuo; B_2: HGDR for Xianhui 207; VIP: Variable importance in the projection; ND: Not detectable.

M#	VIP	Differential Metabolites	A_0	A_1	A_2
			B_0	B_1	B_2
1	1.013	Lactic acid	11.6±0.8 $^{c(y)}$	52.3±11.6 $^{b(x)}$	96.7±11.2 $^{a(x)}$
			15.7±2.4 $^{b(x)}$	17.0±1.1 $^{ab(y)}$	18.2±1.9 $^{a(y)}$
2	1.013	L-Alanine	ND	ND	630.5±93.1 $^{(x)}$
			ND	ND	450.2±88.4 $^{(x)}$
3	1.016	Oxalic acid	244.2±33.28 $^{a(y)}$	89.6±5.9 $^{b(x)}$	86.6±10.9 $^{b(x)}$
			346.0±35.7 $^{a(x)}$	86.7±13.3 $^{b(x)}$	56.7±7.5 $^{b(y)}$
4	1.06	Valine	141.4±12.8 $^{c(x)}$	1490.6±107.8 $^{b(x)}$	2792.8±146.3 $^{a(x)}$
			152.2±23.3 $^{c(x)}$	1425.7±120.1 $^{b(x)}$	2795.2±71.1 $^{a(x)}$
5	1.065	Glycine	450.3±25.4 $^{a(x)}$	117.0±11.9 $^{b(x)}$	88.3±9.2 $^{b(x)}$
			524.2±44.9 $^{a(x)}$	99.3±10.8 $^{b(x)}$	71.4±10.5 $^{b(x)}$
6	1.012	Ethanolamine	1750.1±44.7 $^{a(x)}$	759.2±48.0 $^{b(x)}$	862.8±60.2 $^{b(x)}$
			382.4±22.7 $^{b(y)}$	791.1±20.6 $^{a(x)}$	829.2±36.9 $^{a(x)}$
7	1.063	Glycerol	123.8±10.0 $^{c(x)}$	2066.0±278.0 $^{b(x)}$	4092.2±454.2 $^{a(x)}$
			107.6±2.6 $^{c(x)}$	2274.3±155.5 $^{b(x)}$	4348.6±744.5 $^{a(x)}$
8	1.012	Phosphoric acid	522.7±73.5 $^{b(x)}$	1427.9±108.6 $^{a(x)}$	1624.0±157.5 $^{a(x)}$
			213.1±58.4 $^{c(y)}$	582.7±12.4 $^{b(y)}$	861.7±131.7 $^{a(y)}$
9	1.058	Isoleucine	99.3±14.2 $^{c(x)}$	1173.8±160.2 $^{b(x)}$	1752.3±278.2 $^{a(x)}$
			100.2±7.4 $^{c(x)}$	1202.4±133.2$^{b(x)}$	1974.8±458.0 $^{a(x)}$
10	1.067	Proline	75.6±9.0 $^{c(x)}$	697.8±77.4 $^{b(x)}$	1113.7±76.6 $^{a(x)}$
			92.4±7.3 $^{c(x)}$	708.6±25.6 $^{b(x)}$	1246.8±265.2 $^{a(x)}$

11	1.058	Succinic acid	ND	ND	188.8±13.6 $^{(x)}$
			ND	ND	207.5±39.8 $^{(x)}$
12	1.088	Serine	58.4±9.3 $^{b(y)}$	1283.7±88.5 $^{a(x)}$	1469.2±101.0 $^{a(x)}$
			191.3±8.2 $^{c(x)}$	1018.0±10.6 $^{b(y)}$	1372.8±190.5 $^{a(x)}$
13	1.063	L-Threonine	12.4±5.0 $^{b(x)}$	129.6±13.5 $^{a(x)}$	194.2±22.7 $^{a(x)}$
			16.2±5.1 $^{c(x)}$	134.3±6.5 $^{b(x)}$	238.3±53.5 $^{a(x)}$
14	1.044	Cadaverine	4622.3±390.3$^{a(x)}$	1158.9±71.0 $^{b(x)}$	1504.1±215.2 $^{b(x)}$
			5112.1±320.2 $^{a(x)}$	1137.5±49.9 $^{b(x)}$	1130.0±152.6 $^{b(x)}$
15	1.041	Putrescine	1968.5±137.6 $^{a(x)}$	471.6±80.1 $^{b(x)}$	665.6±108.2 $^{b(x)}$
			2328.1±227.8 $^{a(x)}$	631.7±68.2 $^{b(x)}$	520.9±109.2 $^{b(x)}$
16	1.09	Malic acid	42.2±4.8 $^{c(x)}$	308.1±10.2 $^{a(x)}$	240.0±21.0 $^{b(x)}$
			47.0±8.6 $^{b(x)}$	231.2±14.0 $^{a(y)}$	255.7±3.6 $^{a(x)}$
17	1.039	Furanone	24.2±0.8 $^{b(x)}$	107.9±11.1$^{a(x)}$	101.5±9.9 $^{a(y)}$
			32.8±7.0 $^{c(x)}$	74.5±1.8 $^{b(y)}$	129.3±12.7 $^{a(x)}$
18	1.064	L-Methionine	17.3±11.7 $^{c(y)}$	77.7±8.8 $^{b(x)}$	108.3±1.4 $^{a(y)}$
			43.6±9.8 $^{c(x)}$	89.1±3.6 $^{b(x)}$	153.2±29.8 $^{a(x)}$
19	1.044	Pyroglutamic acid	340.3±27.4 $^{c(x)}$	2234.0±320.9 $^{a(x)}$	1773.0±155.2 $^{b(x)}$
			269.7±15.6 $^{b(y)}$	1432.7±114.3 $^{a(y)}$	1576.7±237.5 $^{a(x)}$
20	1.004	GABA	257.5±59.6 $^{c(x)}$	884.0±46.0 $^{b(y)}$	8840.7±49.6 $^{a(x)}$
			248.7±15.2 $^{c(x)}$	1609.1±31.5 $^{b(x)}$	8183.2±1002.1 $^{a(x)}$
21	1.078	L-Asparagine	21.7±1.4 $^{b(x)}$	175.5±8.1 $^{a(x)}$	197.4±10.9 $^{a(x)}$
			20.7±3.6 $^{c(x)}$	88.1±7.3 $^{b(y)}$	179.9±16.8 $^{a(x)}$
22	1.018	L-Glutamate	65.5±10.3 $^{b(y)}$	259.1±12.7 $^{a(x)}$	256.7±35.8 $^{a(y)}$
			143.3±15.6 $^{b(x)}$	107.1±13.0 $^{b(y)}$	356.9±46.1 $^{a(x)}$
23	1.066	L-Phenylalanine	3.6±2.0 $^{c(x)}$	185.4±6.6 $^{b(y)}$	448.1±37.2 $^{a(x)}$
			3.5±2.4 $^{c(x)}$	242.6±31.5 $^{b(x)}$	431.6±59.6 $^{a(x)}$
24	1.048	Phosphoric acid propyl ester	222.1±5.9 $^{a(x)}$	32.2±7.0 $^{b(x)}$	35.4±5.1 $^{b(x)}$

			110.6±27.4 $^{a(y)}$	22.4±4.8 $^{b(x)}$	31.6±4.3 $^{b(x)}$
25	1.02	Protocatechuic acid	209.4±15.3 $^{a(x)}$ 2.1±0.14 $^{b(y)}$	14.3±2.4 ^{b} ND	12.6±1.7$^{b(y)}$ 17.6±1.5 $^{a(x)}$
26	1.054	Lysine	3.4±1.0 ^{c} ND	28.1±3.1 $^{b(x)}$ 19.1±1.2 $^{b(y)}$	62.2±8.2 $^{a(x)}$ 77.6±7.6$^{a(x)}$
27	1.059	Octanedioic acid	0.6±0.1 $^{b(y)}$ 2.0±0.2 $^{b(x)}$	145.9±18.6 $^{a(x)}$ 124.9±15.3 $^{a(x)}$	112.4±8.5 $^{a(x)}$ 124.8±22.7 $^{a(x)}$
28	1.061	Tyrosine	61.2±8.8 $^{c(x)}$ 35.3±8.3 $^{c(y)}$	1474.6±222.8 $^{a(x)}$ 1705.0±93.8 $^{a(x)}$	275.1±30.1 $^{b(x)}$ 270.5±21.0 $^{b(x)}$
29	1.106	D-Fructose	1629.0±128.1 $^{c(x)}$ 631.9±125.2 $^{c(y)}$	26436.5±232.2 $^{a(y)}$ 45436.6±163.5 $^{a(x)}$	24693.9±584.9 $^{b(y)}$ 38214.4±959.8 $^{b(x)}$
30	1.105	D-Glucose	1856.0±121.4 $^{c(x)}$ 937.7±66.6 $^{c(y)}$	8557.2±162.9 $^{b(y)}$ 11088.2±221.4 $^{b(x)}$	12207.2±244.8 $^{a(x)}$ 11717.4±84.0 $^{a(y)}$
31	1.102	D-Galactose	81.5±6.4 $^{c(x)}$ 68.0±10.0 $^{b(x)}$	2025.8±55.2 $^{b(y)}$ 2620.8±68.9 $^{a(x)}$	2738.0±77.4 $^{a(x)}$ 2809.0±17.4 $^{a(x)}$
32	1.064	Palmitic acid	761.7±50.7 $^{a(x)}$ 435.7±41.0 $^{a(y)}$	316.6±17.3 $^{b(x)}$ 255.1±10.3 $^{b(y)}$	292.9±10.2 $^{b(x)}$ 205.5±9.8 $^{b(y)}$
33	1.095	Inositol	316.3±45.3 $^{b(y)}$ 663.5±300.8 $^{c(x)}$	3298.0±203.9 $^{a(x)}$ 1472.0±46.5 $^{b(y)}$	3396.0±212.0 $^{a(x)}$ 2055.6±320.8 $^{a(y)}$
34	1.064	Linoleic acid	1873.5±44.8 $^{a(y)}$ 2219.8±115.2 $^{a(x)}$	640.6±50.9 $^{b(x)}$ 535.3±46.1 $^{b(y)}$	553.1±27.7 $^{b(x)}$ 532.8±25.7 $^{b(x)}$
35	1.067	Oleic acid	380.9±25.3 $^{a(x)}$ 340.9±36.7 $^{a(x)}$	165.3±12.8 $^{b(x)}$ 159.7±11.4 $^{b(x)}$	129.3±22.1 $^{b(x)}$ 116.1±19.6 $^{b(x)}$
36	1.119	L-Tryptophan	26.8±1.3 $^{c(x)}$ 2.3±0.2 $^{c(y)}$	93.4±13.4 $^{b(y)}$ 233.6±1.1 $^{a(x)}$	157.1±2.5 $^{a(x)}$ 144.1±6.2 $^{b(y)}$
37	1.03	D-Mannose	ND 9.3±2.8 ^{b}	122.0±11.6 $^{b(x)}$ 35.9±3.2 $^{b(y)}$	160.7±23.7 $^{a(x)}$ 156.4±42.4 $^{a(x)}$

38	1.031	D-Glucuronic acid	58.8±8.1 $^{a(x)}$	17.7±3.6 $^{b(x)}$	18.3±3.2 $^{b(x)}$
			61.1±6.3 $^{a(x)}$	13.0±1.3 $^{b(x)}$	13.4±1.7 $^{b(x)}$
39	1.015	Myo-Inositol Phosphate	97.2±5.4 $^{a(x)}$	24.8±3.1 $^{b(y)}$	25.6±4.2 $^{b(x)}$
			68.2±6.7 $^{a(y)}$	21.9±1.8 $^{b(x)}$	17.0±1.5 $^{b(y)}$
40	1.061	1-Monopalmitin ether	245.9±4.0 $^{a(y)}$	126.6±4.6 $^{b(y)}$	114.2±4.7 $^{b(x)}$
			366.8±28.0 $^{a(x)}$	178.7±13.5 $^{b(x)}$	105.9±14.8 $^{c(x)}$
41	1.083	Sucrose	4272.5±251.0$^{a(x)}$	1206.4±117.5 $^{b(x)}$	1233.9±95.5 $^{b(x)}$
			4173.9±176.7 $^{a(x)}$	1219.0±36.9 $^{b(x)}$	1297.2±177.4$^{b(x)}$
42	1.099	Maltose	2340.2±116.4 $^{c(y)}$	12292.1±170.8 $^{a(y)}$	11555.0±282.4 $^{b(x)}$
			3001.6±157.3 $^{c(x)}$	14576.2±448.7 $^{a(x)}$	10891.6±233.2 $^{b(y)}$
43	1.102	D-Turanose	28.5±11.1$^{c(y)}$	732.6±10.3 $^{a(y)}$	587.0±10.4 $^{b(x)}$
			54.1±3.0 $^{c(x)}$	1297.9±38.7 $^{a(x)}$	623.8±54.5 $^{b(x)}$
44	1.015	Melibiose	72.9±7.2 $^{c(x)}$	362.9±45.8 $^{b(y)}$	597.5±67.0 $^{a(y)}$
			76.8±3.4 $^{c(x)}$	618.9±50.6 $^{b(x)}$	1513.1±304.2$^{a(x)}$
45	1.075	Talose	ND	12.3±2.7 $^{a(x)}$	4.7±0.6 $^{b(x)}$
			ND	9.7±0.6 $^{a(x)}$	9.0±3.0 $^{a(x)}$
46	1.001	Lactose	456.1±31.9 $^{a(x)}$	0.4±0.2 $^{c(y)}$	229.1±23.4 $^{b(x)}$
			2.6±0.5 $^{b(y)}$	141.1±14.8 $^{a(x)}$	119.0±16.2 $^{a(y)}$
47	1.056	D-Glucose-L-mannopyranosyl	443.1±59.3 $^{a(x)}$	0.8±0.1 $^{b(y)}$	599.4±48.5 $^{a(x)}$
			0.9±0.1 $^{b(y)}$	175.5 ±8.2 $^{a(x)}$	128.8 ±7.2 $^{b(y)}$

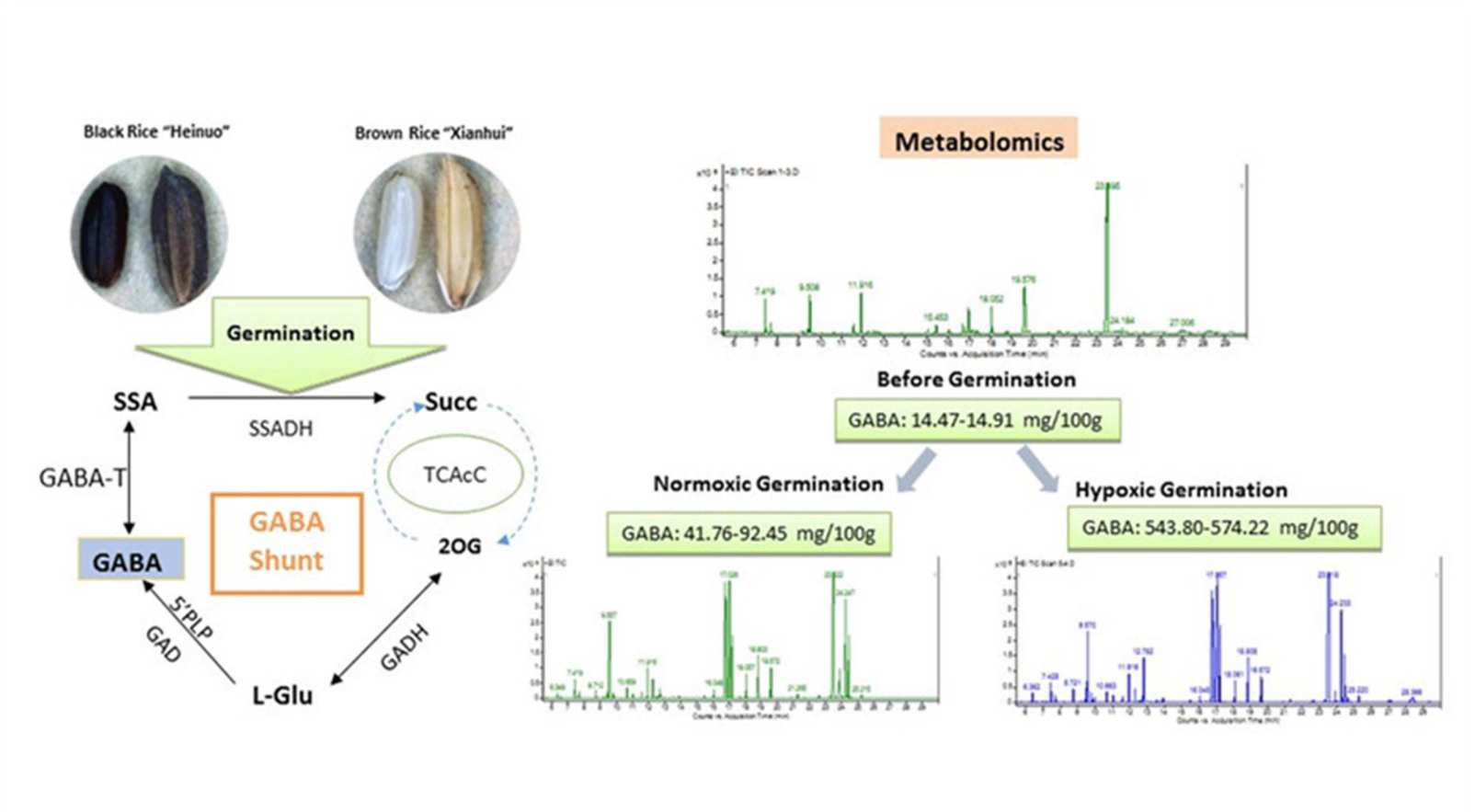
a~c: Different letters indicate significant differences among samples at $P < 0.05$.
x~y: Different letters indicate significant differences between cultivars at $P < 0.05$.

Table 2 GABA content (mg/100 g DW) in ungerminated dehulled rice and germinated dehulled rice under normoxic condition (NGDR) and after hypoxic treatment (HGDR) of different cultivars

Cultivar	Dehulled rice	NGDR	HGDR
"Heinuo"	$14.91\pm0.12^{b(x)}$	$41.76\pm2.49^{b(y)}$	$574.22\pm32.10^{a(x)}$
"Xianhui 207"	$14.47\pm0.15^{c(y)}$	$92.45\pm4.56^{b(x)}$	$543.80\pm28.19^{a(x)}$

a~c: Different letters indicate significant differences among different samples at $P < 0.05$.
x~y: Different letters indicate significant differences between cultivars at $P < 0.05$.

TOC Graphic


Black Rice "Heinuo"
Brown Rice "Xianhui"
Germination
SSA
SSADH
Succ
GABA-T
TCAcC
GABA
GABA Shunt
2OG
5'PLP
GAD
GADH
L-Glu
Metabolomics
Before Germination
GABA: 14.47-14.91 mg/100g
Normoxic Germination
GABA: 41.76-92.45 mg/100g
Hypoxic Germination
GABA: 543.80-574.22 mg/100g