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Using Metabolomic Approaches to Explore Chemical Diversity in Rice

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ABSTRACT

Rice (*Oryza sativa*) is an excellent resource; it comprises 25% of the total caloric intake of the world's population, and rice plants yield many types of bioactive compounds. To determine the number of metabolites in rice and their chemical diversity, the metabolite composition of cultivated rice has been investigated with analytical techniques such as mass spectrometry (MS) and/or nuclear magnetic resonance spectroscopy and rice metabolite databases have been constructed. This review summarizes current knowledge on metabolites in rice including sugars, amino and organic acids, aromatic compounds, and phytohormones detected by gas chromatography–MS, liquid chromatography–MS, and capillary electrophoresis–MS. The biological properties and the activities of polar and nonpolar metabolites produced by rice plants are also presented. Challenges in the estimation of the structure(s) of unknown metabolites by metabolomic approaches are introduced and discussed. Lastly, examples are presented of the successful application of metabolite profiling of rice to characterize the gene(s) that are potentially critical for improving its quality by combining metabolite quantitative trait loci analysis and to identify potential metabolite biomarkers that play a critical role when rice is grown under abiotic stress conditions.

Key words: metabolomics, *Oryza sativa*, chemical diversity, mass spectrometry, gene discovery, QTL analysis

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INTRODUCTION

Rice, a member of the Poaceae (Gramineae) family, has a long breeding history. It is one of the major crop grasses particularly in Asian countries because it is a good caloric resource for humans. More than 400 000 rice germplasm accessions are stored in gene banks around the world (Toriyama et al., 2005), suggesting that the chemical diversity across rice varieties is large. Natural products chemistry combined with separation science contributes to the investigation of the diversity of chemicals/compounds produced by rice (Figures 1 and 2). Advances in chromatographic techniques, chemometrics, and structural estimation methods make it possible to use metabolomics to explore its chemical diversity.

Metabolomics is one of the major research fields in “omics” science. The analytical targets of metabolomics are biologically synthesized or degraded compounds in organisms. The ultimate goal of plant metabolomics is quantification of the metabolome

in plants. It has been estimated that the plant kingdom contains more than 200 000 metabolites (Dixon and Strack, 2003). Plants produce essential building blocks such as sugars, amino acids, and phenolics for proteins, starches, and cell wall components that are directly related to agricultural outputs. Various types of bioactive compounds and volatile compounds as secondary metabolites are synthesized to protect plants against biotic and abiotic stress. Their complex metabolism is thought to compensate for their lack of motility. Phytohormones are secondary metabolites that can coordinate cellular activities for germination, growth, protection against stress, and other physiological activities in the life of plants. As crude extracts tend to be used for metabolite profiling in a nontargeted manner, chromatographic separation is usually required (Fukushima and Kusano, 2014), and fractionated compounds

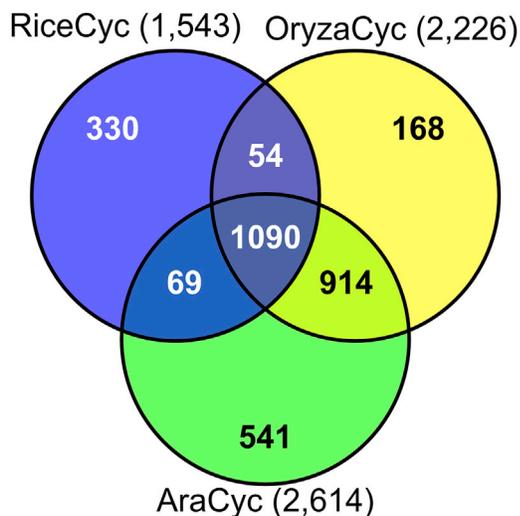


Figure 1. Metabolites Found in RiceCyc and OryzaCyc, but Not in AraCyc.

Based on metabolite IDs (UNIQUE-ID) in RiceCyc (version 3.3, 1543 entries), OryzaCyc (version 1.0, 2226 entries), and AraCyc (version 11.5, 2614 entries), we estimated the proportion of metabolites in rice that was commonly present in the two rice databases. The names of the 54 metabolites in RiceCyc and OryzaCyc are listed in Supplemental Table 1.

are then analyzed by mass spectrometry (MS) and/or nuclear magnetic resonance (NMR) spectroscopy. These analytical methods are most commonly used in not only metabolomics (Kim et al., 2011; Kusano et al., 2011a; Okazaki and Saito, 2012) but also natural products chemistry. MS (Kind et al., 2009; Wishart et al., 2009; Strehmel et al., 2014) and tandem mass spectrometry (MS/MS) spectral libraries (Horai et al., 2010; Sawada et al., 2012; Tautenhahn et al., 2012; Kind et al., 2013) and NMR chemical shift libraries (Steinbeck and Kuhn, 2004; Cui et al., 2008) are available for the provisional identification of known and unknown peaks. The estimation of the chemical structure(s) of unknown metabolites in metabolite profile data continues to present challenges (Nakabayashi and Saito, 2013). Even in the metabolite profiles obtained by gas chromatography (GC) plus MS, approximately half of the peaks remain unidentified because of a lack of reference chemicals. It is impossible or very difficult to estimate the chemical structure(s) of secondary metabolites with only MS-based techniques, in particular for unidentified metabolite peaks. However, ultrahigh-resolution MS (e.g., liquid chromatography [LC]) combined with Fourier transform ion cyclotron resonance-MS increased the possibility of precisely estimating the molecular formula of the peaks of secondary metabolites in the model plant *Arabidopsis thaliana* (Glaser et al., 2014; Morreel et al., 2014) and in onions (Nakabayashi et al., 2013).

In this review, we estimated the number of metabolites in rice by comparing the metabolite names found in publicly available databases (Figure 1). We present the metabolite composition in the kernels and aerial parts of the cultivated rice. Analysis was performed with GC-MS, LC-MS, and capillary electrophoresis (CE)-MS. We also discuss current knowledge on the secondary metabolites produced by rice and their biological activities (Figure 2) as well as the strategies and challenges for using

chemometric approaches to select unidentified peaks as candidates for the further estimation of their chemical structure(s) and to estimate the structure(s) of secondary metabolites by combining high-resolution LC-MS data and algorithms. Lastly, we provide examples of the application of metabolomic approaches to detect metabolites that may be critical for improving the quality of rice by metabolite quantitative trait loci (QTL) analysis and by reverse genetic approaches for the potential enhancement of phosphorus depletion resistance in rice cultivars.

PRIMARY AND SECONDARY METABOLITES IN THE RICE METABOLITE PROFILES

Plants, including the model plant *Arabidopsis* and rice, can produce primary metabolites via similar metabolic pathways. In the production of secondary metabolites, the existence of specific pathways in individual plant species must be considered. To estimate the number of metabolites that are thought to exist as specific metabolites in rice plants, we used three plant metabolic databases. According to Seaver et al. (2012), a comparison of individual metabolic databases revealed clear differences in the stored compounds (Seaver et al., 2012). When we compared metabolite IDs used in the RiceCyc (Jaiswal et al., 2006), OryzaCyc, and AraCyc in Plant Metabolic Network (http://www.plantcyc.org/tools/tools_overview.faces on www.plantcyc.org) (Figure 1), we found more than 1000 metabolites (e.g., primary metabolites) in these three databases. On the other hand, there were 54 metabolites in the two metabolic databases of rice. Of these, oryzalexins (Akatsuka et al., 1985; Kato et al., 1993; Kato et al., 1994) and momilactones (Kato-Noguchi and Peters, 2013) are known to be specific metabolites produced in rice (Supplemental Table 1).

As the huge diversity of rice cultivars renders the investigation of the chemical diversity of all rice cultivars unrealistic, genetic core collections have been established to obtain manageable but representative selections of rice cultivars. The size of the genetic core collection deposited in several gene banks is relatively small. Examples are the Rice Germplasm Core Set from the International Rice Research Institute (623 accessions; <http://iris.irri.org/germplasm/>), the GCore collection (18 collections each of which consists of approximately 130 accessions) (Li et al., 2004), the EMBRAPA Rice Core Collection (242 accessions) (Borba et al., 2009), and the rice diversity research set (67 accessions) (Kojima et al., 2005). Comprehensive metabolite profiling of kernels in the rice diversity research set, two control cultivars, and the Pokkari cultivar used a combination of one- and two-dimensional GC-time-of-flight (TOF)-MS (Kusano et al., 2007) or four different separation techniques applied in TOF-MS, i.e., GC-TOF-MS, CE-TOF-MS, LC coupled to quadruple orthogonal TOF-MS, and LC coupled to ion trap-TOF-MS (Redestig et al., 2011b). Multi-MS-based metabolite profiling covered more than 87% of the chemical diversity of the metabolites when compared to the metabolites listed in RiceCyc (Figure 2). In 70 rice cultivars, 1652 peaks consisting of 156 distinct metabolites and 1496 unknown analytes comprised the metabolite composition of the rice kernels. Among the 156 distinct metabolites, we provisionally identified

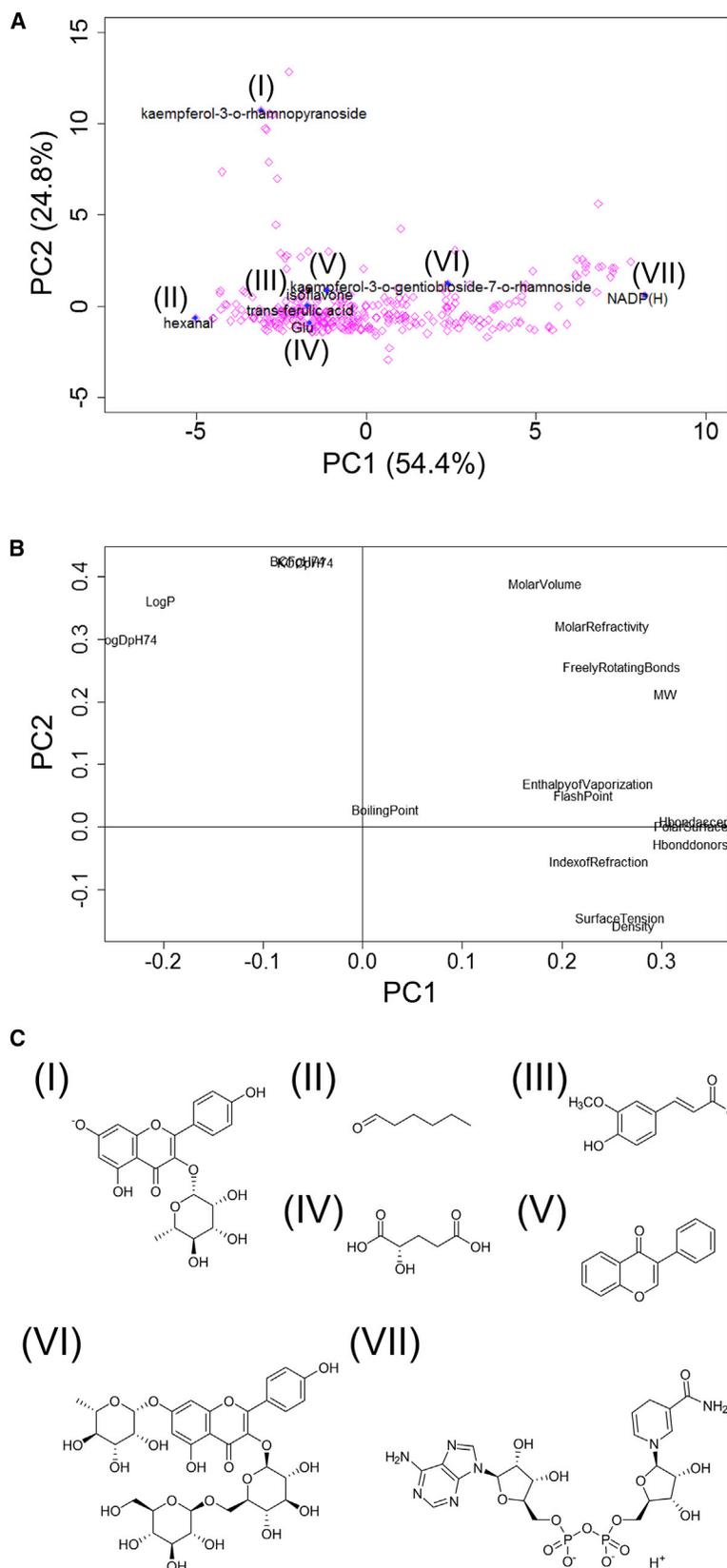


Figure 2. Visualization of the Predicted Chemical Diversity of Metabolites in Rice.

The score scatter (**A**) and loading plots (**B**) of principal component analysis (PCA) to estimate the chemical diversity of the 342 metabolites in the RiceCyc database. We used the 17 predicted physicochemical properties of the 342 metabolites in RiceCyc version 3.3 (<http://pathway.gamene.org/gamene/ricecyc.shtml>) to predict the chemical diversity of rice metabolites by PCA. Of the 1520 compounds in the database, we obtained 342 ChemSpider IDs that could be linked to physicochemical properties, structure information, and chemical identifiers in ChemSpider (<http://www.chemspider.com/>). The loading plots show that principal component 1 (PC1) tends to be dominated by size- and polarity-related properties. We used the 17 predicted physicochemical properties, i.e., the molecular weight (MW), octanol:water partitioning coefficient (LogP), octanol:water solubility distribution coefficient at pH 7.4 (LogDpH74), biological concentration factor at pH 7.4 (KOCpH74), adsorption coefficient at pH 7.4 (KOCpH74), number of H-bond acceptors (Hbondacceptors), number of H-bond donors (Hbonddonors), freely rotating bonds (FreelyRotatingBonds), polar surface area (PolarSurfaceArea), index of refraction (IndexofRefraction), molecular refractivity (MolarRefractivity), molecular volume (MolarVolume), surface tension (SurfaceTension), flash point (FlashPoint), boiling point (BoilingPoint), enthalpy of vaporization (EnthalpyofVaporization), and density (Density). Method details were described by Kusano et al. (2011b). In (**C**), we list the representative metabolites in the RiceCyc database.

I, kaempferol-3-O-rhamnopyranoside; II, hexanal; III, *trans*-ferulic acid; IV, glutamate; V, isoflavone; VI, kaempferol-3-O-gentiobioside-7-O-rhamnoside; and VII, NADP(H).

levels of indole-3-acetic acid and *trans*-zeatin, we could detect these phytohormones in the metabolite profile data. Rice kernels contain not only primary metabolites such as sugars, amino and organic acids, fatty acids, and nucleobases but also various types of secondary metabolites. Classic natural products chemistry provides a solid basis for the identification of secondary metabolites. Trait-metabolite association analysis in rice seeds of the original core collection (51 *Japonica* and 49 *Indica* accessions) was based on the 121 metabolites detected by LC-tandem MS (MS/MS) and GC-MS (Hu et al., 2014). It captured differences in the metabolite abundance and correlations between *Japonica* and *Indica* rice, suggesting that these differences may reflect the extent of metabolic adaptation and the specific phenotypes in *Japonica* and *Indica*.

To quantify the metabolite levels in rice leaves, their metabolite composition was investigated with CE-MS and a CE-diode array detector (Sato et al., 2004). To visualize the metabolic impact of overexpression of the *Arabidopsis* NAD kinase gene in rice plants, Takahara et al. (2010) used CE-MS and Albinsky et al. (2010) applied GC-MS to characterize the transcription factor gene involved in nitrogen metabolism. By

peaks and identified metabolites by comparing their mass spectra and retention times (or indices) with those of the authentic standards. As rice kernels contain relatively higher

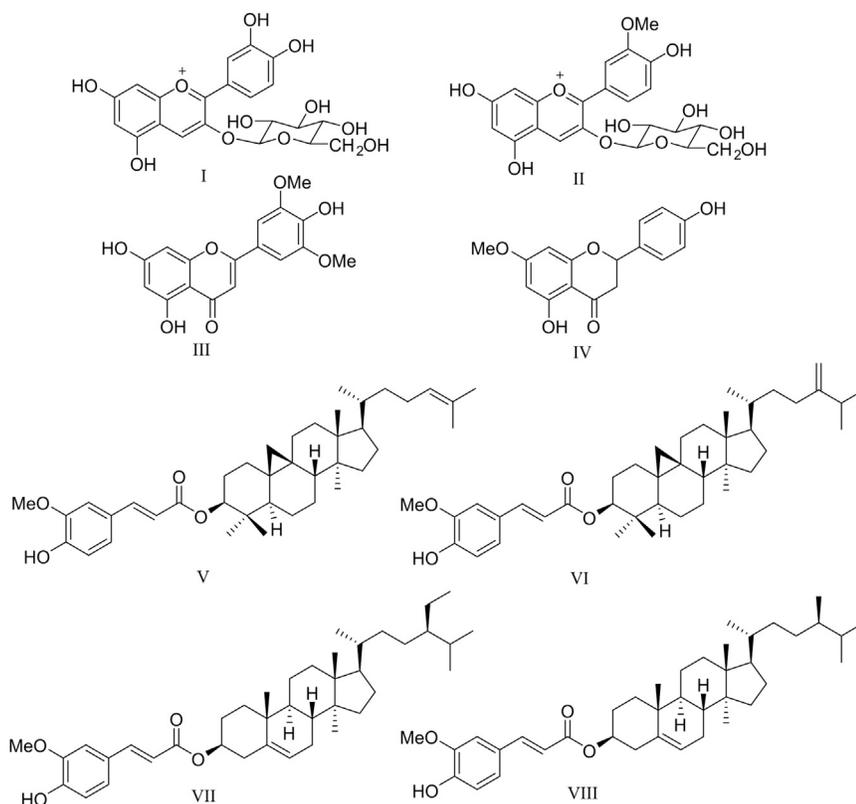


Figure 3. Chemical Structure of Representative Bioactive Metabolites in Rice.

I, cyanidin 3-O-glucoside; II, peonidin 3-O-glucoside; III, tricetin; IV, sakuranetin; V, cycloartenol *trans*-ferulate; VI, 24-methylenecycloartanol *trans*-ferulate; VII, sitosterol *trans*-ferulate; and VIII, campesterol *trans*-ferulate.

These metabolites are introduced in this review. For more details on the bioactive metabolites found in rice, see [Supplemental Tables 2 and 3](#).

combining GC-MS and CE-MS techniques, more than 100 metabolites in central metabolism were detected ([Redestig et al., 2011b](#)). Classic quantification methods or multiple MS-based platforms were applied to explore metabolite changes in the level of primary metabolites and phytohormones in response to abiotic stresses such as drought ([Reguera et al., 2013](#)) and cold or dehydration ([Maruyama et al., 2014](#)).

IDENTIFIED VOLATILES (AROMA) AND PHYTOHORMONES IN RICE

Volatiles and phytohormones are classified as secondary metabolites. More than 300 aroma components have been identified in rice by comparing their mass spectra and those of the authentic standard and/or the mass spectra found in reference libraries ([Maga, 1984](#); [Widjaja et al., 1996](#); [Jezussek et al., 2002](#)). Of these, 2-acetyl-1-pyrroline is a major characteristic aroma predominantly detected in scented rice ([Buttery et al., 1982](#); [Laguerre et al., 2007](#); [Fitzgerald et al., 2009](#)). The concentration of 2-acetyl-1-pyrroline exhibited a positive correlation with the sensory intensity of aromatic and nonaromatic brown rice cultivars ([Itani et al., 2004](#)). Recent developments in headspace collection methods such as solid microphase extraction revealed other flavor components in rice. [Zeng et al. \(2012\)](#) reported a headspace collection of rice bran using solid microphase extraction; the volatiles collected were thermally desorbed by GC-MS ([Zeng et al., 2012](#)). They provisionally identified 43 of 76 compounds based on their mass spectra and temperature-programmed retention indices and attempted to quantify the volatile levels with an overall volume integration technique. Such trials can increase our knowledge of the compo-

sition of aromatic compounds in rice grains. Rice can emit volatile phytohormones such as salicylate, jasmonate ([Iwai et al., 2006](#)), and green leaf volatiles (e.g., (*Z*)-3-hexenal) to resist biotic stress (e.g., pathogen infection) ([Tong et al., 2012](#)). Ethylene, another volatile phytohormone, is released inside the leaf sheath for a rapid internode elongation response when floating rice is submerged in floods or deep water ([Hattori et al., 2009](#)).

For the quantification of gibberellins ([Green et al., 1997](#)) and brassinosteroids ([Noguchi et al., 1999](#); [Yamamoto et al., 2000](#)), GC-MS is used after several fractionations and derivatization. Improvements in the sensitivity of LC-MS have made possible the absolute quantification of 43 molecular species in the four classes of phytohormones: cytokinins, auxins, gibberellins, and abscisic acids ([Kojima et al., 2009](#)). [Ding et al. \(2013\)](#) reported a pretreatment method to quantify brassinosteroids without derivatization using LC-MS ([Ding et al., 2013](#)). Strigolactones, a novel class of phytohormones, can be detected by LC-MS ([Umehara et al., 2008](#)). More than 100 strigolactone derivatives are thought to exist in the plant kingdom ([Akiyama and Hayashi, 2006](#)). Of these, a major form of strigolactones in rice is 2'-epi-5-deoxystrigol ([Xie et al., 2013](#); [Cardoso et al., 2014](#)); it can be released as a major strigolactone from rice roots, especially when the plants are grown under phosphorus-deficient conditions ([Umehara et al., 2010](#)).

BIOACTIVE SECONDARY METABOLITES PRODUCED BY RICE

Rice produces various types of secondary metabolites ([Figure 3](#)). For example, bran, the residue after polishing rice, is brown-black, red, or purple. The pigments are secondary metabolites. Anthocyanins, a subclass of water-soluble flavonoids, are reddish to purple in color and are thought to be major bioactive constituents (e.g., dietary antioxidants) in such colored rice. The primary form of the anthocyanins produced by pigmented rice is cyanidin 3-O-glucoside (I); the secondary form is peonidin 3-O-glucoside (II) ([Figure 3](#)). The total anthocyanin content in black rice cultivars is much higher than that in red rice cultivars ([Terahara et al., 1994](#); [Abdel-Aal et al., 2006](#); [Yawadio et al., 2007](#); [Hiemori et al., 2009](#); [Yao et al., 2009](#); [Kim et al., 2010](#); [Sompong et al., 2011](#)). Other types of anthocyanins were found in colored rice cultivars ([Supplemental Table 2](#)) ([Terahara et al.,](#)

1994; Abdel-Aal et al., 2006; Yao et al., 2009), and cyanidin and malvidin were isolated from dark purple rice (Hyun and Chung, 2004). Pigmented rice cultivars contain a higher amount of phenolics, hydroxycinnamate sucrose esters (Tian et al., 2004), and flavonols such as quercetin, kaempferol, and apigenin (Kim et al., 2010; Sriseadka et al., 2012) than nonpigmented rice. Tricin (III in Figure 3) is one of the flavones produced in the leaves and stems of Gramineae and other plants (Zhou and Ibrahim, 2010). Tricin shows antioxidant activity because of its flavone moiety. Quinolone alkaloids were reported in the aleurone layer of anthocyanin-pigmented rice (Chung and Woo, 2001; Chung and Shin, 2007).

Rice bran oil is a uniquely rich source of commercial and nutritional phytochemicals, such as carotenoids and γ -oryzanol. Carotenoids are yellow-orange pigments mainly found in fruits and vegetables; β -carotene (provitamin A), lutein, and zeaxanthin have been reported in rice (Parkhi et al., 2005; Lamberts and Delcour, 2008; Kim et al., 2010). γ -Oryzanol is a specific component in rice bran; it is a mixture of ferulic acid esters with sterols and triterpene alcohols. The major compounds of γ -oryzanol are cycloartenol *trans*-ferulate (V), 24-methylenecycloartenol *trans*-ferulate (VI), sitosterol *trans*-ferulate (VII), and campesterol *trans*-ferulate (VIII) (Figure 3) (Xu and Godber, 1999; Akihisa et al., 2000; Xu et al., 2001; Fang et al., 2003; Yu et al., 2007).

Rice plants accumulate secondary metabolites to protect against biotic and abiotic stresses (Kodama et al., 1992; Lin and Kao, 1999; Zuther et al., 2007; Kusano et al., 2011c; Maruyama et al., 2014). For example, copper treatment of rice leaves produced significant changes in the level of polyamine, which are thought to play important roles in many physiological processes (Lin and Kao, 1999; Gill and Tuteja, 2010). Sakuranetin (IV in Figure 3), one of the phytoalexins, is synthesized biochemically from naringenin in rice, cherry bark, and other plant species. The level of sakuranetin, barely detectable in healthy rice leaves, is rapidly increased under biotic and abiotic stress stimuli including UV treatment and pathogen attack (Shimizu et al., 2012).

CURRENT STRATEGIES AND CHALLENGES FOR USING CHEMOMETRIC APPROACHES TO ESTIMATE THE ABUNDANCE AND STRUCTURE OF UNKNOWN METABOLITE PEAKS

Rice produces commercially important aromas and bioactive compounds (e.g., antioxidants). The identification of unknown compounds and their biological activities is the first step in the detection of a treasure trove of attractive metabolites in the rice metabolome. Spectroscopic techniques including UV-visible spectroscopy, Fourier transform-infrared spectroscopy, MS, and NMR are used to determine the structure of secondary metabolites. Advances in methods to separate compounds (i.e., chromatography) and molecular ions based on their mass-to-charge ratio (i.e., MS) yield high-quality digital data sets. Chemometrics and bioinformatics are applied to estimate the abundance

(Redestig et al., 2011a) and structure (Wolf et al., 2010; Tohge et al., 2011; Nakabayashi et al., 2013; Yang et al., 2013; Morreel et al., 2014; Zhou et al., 2014) of unknown metabolite peaks in plants. Chemometrics using an artificial biological gradient between two specimens is a powerful approach for the generation of semiquantitative calibration curves for all compounds detected including unknown peaks (Redestig et al., 2011a). This method does not require authentic standards and makes it possible to determine whether the calibration curves fit “ideally” as do clear monotonous and asymptotic curves.

There are three strategies to identify/estimate the chemical structure of unknown peaks. They are the application of (i) chromatographic and spectroscopic techniques for the isolation and the elucidation of their structure (Yang et al., 2014), (ii) chemical isotope labeling methods (Nakabayashi et al., 2013; Glaser et al., 2014; Zhou et al., 2014), and (iii) *in silico* analysis of MS and MS/MS data by computational MS (Wolf et al., 2010; Morreel et al., 2014). The latter is particularly attractive because the separation of all standard compounds, even without labeling, for the identification of unknown metabolites is not possible with strategies (i) and (ii). MetFrag (Wolf et al., 2010) provides a candidate list based on exact mass information stored in the three databases, i.e., KEGG Compound (<http://www.genome.jp/kegg/compound/>), PubChem (<https://pubchem.ncbi.nlm.nih.gov/>), and ChemSpider (<http://www.chemspider.com/>) (with approximately 16 021, 37⁶, and 23⁶ entries, respectively). The required information is the exact mass, which can be obtained by using high-resolution MS or MS/MS; the MetFrag algorithm can generate possible fragmentation patterns of the candidate compounds using the bond dissociation approach. Users can compare the fragmentation patterns generated with their own MS/MS spectra.

Recently, Morreel et al. (2014) developed the candidate substrate-product pair network approach, a novel algorithm that called for the high-throughput structural estimation of 145 secondary metabolites such as glucosinolates, flavonoids, benzenoids, lignans, indoles, and apocarotenoids in the metabolite profiles of *Arabidopsis* obtained by LC-MS. Based on correlations between the peak abundance of a “candidate substrate” and a “candidate product” as well as the difference in the mass-to-charge ratio between them in the profiles, the candidate substrate-product pair algorithm can select possible candidate pairs. This method can be used to elucidate the structure of unknown peaks produced by rice and other organisms by incorporating LC-MS-based profile data into information obtained by MS/MS fragmentation and the retention time.

APPLICATIONS OF RICE METABOLOMICS: ANALYSIS OF QTL-CONTROLLING METABOLIC DIVERSITY AND DISCOVERY OF POTENTIAL METABOLITE BIOMARKERS ASSOCIATED WITH MITIGATION OF ENVIRONMENTAL STRESSES

Various types of secondary metabolites produced by rice have been identified using spectroscopic techniques. Metabolomic

approaches boost metabolite identification, and some of them can be used to improve the quality and yield of rice. As the cultivation and breeding of rice have a long history in Asia, a diverse collection of rice cultivars with different traits including yield, height, and metabolite abundance is available. The development of a strategy for QTL analysis facilitates the identification of useful QTLs that control metabolite accumulation and helps to elucidate systems for the regulation of metabolic networks in various plant species (Fernie and Schauer, 2009; Riedelsheimer et al., 2012; Wen et al., 2014). For gene characterization in primary metabolism, metabolite QTLs of *Arabidopsis* (Keurentjes et al., 2008; Liseč et al., 2008; Rowe et al., 2008), tomato (Schauer et al., 2006; Schauer et al., 2008), and potato (Carreno-Quintero et al., 2012) have been investigated. However, few published mQTL analyses have focused on primary metabolites in rice (Ying et al., 2012). Rather, investigators performed trait-metabolite association analysis (Kusano et al., 2012; Degenkolbe et al., 2013; Zhao et al., 2013; Hu et al., 2014). The effective use of metabolomics and QTL analysis using mapping populations to delineate the genetic control of metabolism in rice has been reported. mQTL analysis of the offspring of particular pairs of rice cultivars helped to discover the genetic regions that determine differences in the level of some metabolites between two particular parental lines. For example, with respect to the characterization of genes in secondary metabolism, we reported metabolite QTL analysis of backcrossed inbred lines produced from Japonica and Indica cultivars and identified a key gene regulating specialized metabolism in the flavonoid biosynthetic pathway that evolved in the Indica-type parental line (Matsuda et al., 2012). Also, mQTL analysis of the 210 recombinant inbred lines generated by crossing two elite rice cultivars using 900 metabolite profile data (Gong et al., 2013) yielded the functional characterization of several genes including two flavone malonyltransferases and unknown genes that are thought to be involved in the flavonoid biosynthetic pathway.

Chen et al. (2014) adapted QTL analysis based on genome-wide association study (GWAS) to link single nucleotide polymorphisms and metabolite levels in rice (Chen et al., 2014). They performed metabolite GWAS using the large matrix consisting of 840 metabolite peaks and 524 accessions and extracted 36 genes as potential candidates involved in metabolite changes of physiological and nutritional importance. They successfully characterized or annotated five genes encoding a methyltransferase, a putrescine/agmatine hydroxycinnamoyl acyltransferase, a putative flavone 5-O-glucosyltransferase, an agmatine hydroxycinnamoyl acyltransferase, and a putative transferase for the production of identified and unknown metabolites. Matsuda et al. (2014) carried out GWAS using the LC-MS-based profile data of 175 Japanese rice accessions leading to identification of 323 associations among 143 single nucleotide polymorphisms and 89 metabolites (Matsuda et al., 2014). Metabolite identification was based on standard compounds, which were isolated and characterized by solid spectroscopic analyses (Yang et al., 2014). Due to the rapid accumulation of whole-genome sequence information on rice (Li et al., 2014), additional trials to associate metabolic diversity with natural variations in various traits in rice populations are under way (Calingacion et al., 2012; Verhoeven et al., 2012; Hu et al., 2014). The findings will help to improve crop designs that

address regional preferences in traits including taste and flavor (Fitzgerald et al., 2009; Calingacion et al., 2014).

When a metabolite or a set of metabolites is differentially accumulated in two subspecies of rice, this may be used as a biomarker. Metabolomics has contributed to the identification of metabolite biomarkers that are associated with tolerance of rice to stress including abiotic stress (Degenkolbe et al., 2013; Maruyama et al., 2014) and nutrition starvation (Masumoto et al., 2010; Okazaki et al., 2013), suggesting metabolomic versatility in rice metabolic studies. According to Vance et al. (2003), crop yields on 30–40% of the world's arable land are reduced because phosphorus is unavailable (Vance et al., 2003). To cope with phosphorus starvation, plants developed various adaptations; lipid remodeling is one of the most intensely studied mechanisms (Härtel et al., 2000; Benning and Ohta, 2005). A typical metabolite change associated with lipid remodeling during phosphorus deprivation is the replacement of membrane phospholipids with nonphosphorus glycerolipids such as sulfoquinovosyl diacylglycerol and digalactosyl diacylglycerol, which promote the remobilization of phosphorus. Our lipidomic profiling of rice grown under phosphorus-controlled conditions showed that a new type of plant lipid, i.e., glucuronosyl diacylglycerol, was inducibly accumulated under phosphorus-limited conditions in rice and *Arabidopsis*, suggesting a shared physiological significance of this novel lipid under conditions of phosphorus depletion in plants (Okazaki et al., 2013).

CONCLUSIONS AND FUTURE PERSPECTIVES

We discussed various types of metabolites in rice, including primary metabolites, volatiles, phytohormones, lipids, and other secondary metabolites such as phenolics, flavonols, carotenoids, and alkaloids. Estimation of the chemical diversity in rice by metabolomic approaches is important for the development of new algorithms for the further elucidation of the chemical structure of unknown peaks. Although phytohormones are classified as secondary metabolites, their production is highly complex and strictly regulated at the transcriptional, translational, and metabolic levels. Such sophisticated regulations triggered by a metabolite were uncovered in *Arabidopsis*, i.e., root elongation via glucose-target-of-rapamycin signaling (Xiong et al., 2013) and trehalose-6-phosphate signaling for flowering (Wahl et al., 2013). Investigation of such mechanisms using “omics” approaches may give rise to the generation of new hypotheses.

Gain-of-function genetic mutations can add new and useful characteristics to improve the quality of rice. For example, “golden rice,” generated with a genetic engineering approach, can accumulate β -carotene in the endosperm (Ye et al., 2000; Paine et al., 2005). The consumption of golden rice by Asian, African, and Latin American children may help to address their vitamin A deficiency. However, field experiments are needed to determine the effects of genetically modified crops grown under various environmental conditions on metabolite composition (e.g., Kusano et al., 2014).

Many investigations have focused on the metabolites of pigmented rice, rice bran, and bran oil because they

have health-promoting properties such as antioxidant, anti-inflammatory, and antihypercholesterolemic activities. The medicinal activities of unique diterpenoid phytoalexins and alkaloids and oryzamutic acids in rice must be investigated for their health-promoting functions. Estimation of the structural diversity of secondary metabolites in rice is important for phytochemical genomics because genetic and metabolomic approaches have already helped to improve the nutritional quality of other crops (Fernie and Schauer, 2009).

SUPPLEMENTAL INFORMATION

Supplemental Information is available at *Molecular Plant Online*.

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