The utility of metabolomics as a tool to inform maize biology

David B. Medeiros1, Yariv Brotman2,.* and Alisdair R. Fernie1,.*
1Max Planck Institute of Molecular Plant Physiology, Potsdam, Germany
2Department of Life Sciences, Ben-Gurion University of the Negev, Beersheva, Israel
*Correspondence: Yariv Brotman (brotmany@post.bgu.ac.il), Alisdair R. Fernie (fernie@mpimp-golm.mpg.de)
https://doi.org/10.1016/j.xplc.2021.100187

ABSTRACT

With the rise of high-throughput omics tools and the importance of maize and its products as food and bioethanol, maize metabolism has been extensively explored. Modern maize is still rich in genetic and phenotypic variation, yielding a wide range of structurally and functionally diverse metabolites. The maize metabolome is also incredibly dynamic in terms of topology and subcellular compartmentalization. In this review, we examine a broad range of studies that cover recent developments in maize metabolism. Particular attention is given to current methodologies and to the use of metabolomics as a tool to define biosynthetic pathways and address biological questions. We also touch upon the use of metabolomics to understand maize natural variation and evolution, with a special focus on research that has used metabolite-based genome-wide association studies (mGWASs).

Key words: GWAS, metabolism, metabolite profiling, primary metabolites, secondary metabolites, Zea mays

Medeiros D.B., Brotman Y., and Fernie A.R. (2021). The utility of metabolomics as a tool to inform maize biology. Plant Comm. 2, 100187.

INTRODUCTION

Maize (Zea mays L.), was first domesticated about 9,000 years ago from its wild relative, the lowland grass teosinte, in southwestern Mexico (Matsuoka et al., 2002; Piperno et al., 2009). In recent decades, it has become the most widely cultivated grain in the world, with a global production of about one billion metric tons in 2018 (Food and Agriculture Organization Corporate Statistical Database, http://www.fao.org/faostat/en/). Despite a loss of genetic diversity upon domestication, modern maize remains relatively rich in genetic variation, facilitating its cultivation in diverse environmental conditions (Vigouroux et al., 2002). The popularity of maize results not only from an increase in direct human consumption as food but also from the ever-increasing production of corn bioethanol, farm animal feed, and additional products such as syrup, oil, and cornmeal. Maize research, driven to a great extent by the crop’s growing economic importance, has also risen dramatically. Several aspects of maize biochemistry, genetics, physiology, and ecology have been thoroughly explored in recent years, but needless to say, many knowledge gaps remain.

The rise of high-throughput genomic, transcriptomic, proteomic, and metabolomic tools constitutes one of the hallmarks of modern biological research. Maize has profited from the emergence of omics tools, with numerous recent studies delving into its systems biology. Given that maize and its products are used as both food and bioethanol, its metabolism has been given special attention. Several aspects of maize metabolism have received considerable attention, including (1) the role of the metabolome in the context of its participation in basic molecular processes and in responses to biotic and abiotic stresses and beneficial biotic interactions; (2) the nutritional composition of maize kernels and the molecular mechanisms that underlie the production of specific metabolites; (3) the means by which the metabolome and metabolic models link to leaf physiology and crop yield; (4) the metabolic alterations brought about by genetic modifications; and (5) the extent of natural variation in metabolism and its potential utility in breeding efforts. In addition, several further questions that cannot be strictly categorized have been addressed by maize metabolomics in recent studies on how pesticides influence the maize metabolome (Blondel et al., 2016); how phloem sap metabolites correlate with kernel yield (Yesbergenova-Cuny et al., 2016); how exposure of maize to polycyclic aromatic hydrocarbons (toxic organic pollutants) affects the metabolome (Sivaram et al., 2019); and the metabolic mechanisms that underlie plant root growth stimulation by smoke (Çatav et al., 2018).

Taking this broad basis into account, in this review we discuss both recent advances and trends in maize metabolomics,
Plant Communications

focusing on methodologies and on the contribution of metabolomics to defining metabolic pathways and addressing relevant biological questions. Finally, we discuss the identification of key genes that control maize metabolism.

METHODOLOGIES

The maize metabolome is analyzed using essentially the same variety of methods used in other plant metabolomics studies, namely hyphenated mass spectrometry (MS) and nuclear magnetic resonance (NMR) (Obata and Fernie, 2012). Aside from several techniques rarely used in maize, such as infrared spectroscopy (Pavlik et al., 2010), two major approaches dominate: MS (e.g., Václavík et al., 2013) and NMR (e.g., Çatav et al., 2018). The former is more sensitive, whereas the latter can better quantify metabolites and detect conformational isomers (Obata and Fernie, 2012; Alseekh and Fernie, 2018). Although only a few research groups (Barros et al., 2010; Walker et al., 2011; Marti et al., 2013; Vinci et al., 2018) are able to use both approaches in parallel, it is generally agreed that they complement and enhance one another, with several studies dedicated to demonstrating just that (Venkatesh et al., 2016). MS tends to be the method of choice in most maize studies. MS/MS (tandem MS), an extended form of MS that uses ion fragmentation to enable superior identification, is increasingly used in maize metabolomics (Mesnage et al., 2016; Cocuron et al., 2019). Blondel et al. (2016) represents an example of the application of a little-used NMR variation, high-resolution magic-angle spin (1H-HRMAS), that enables metabolite detection in heterogeneous tissues or solutions without any extraction. In this case, the toxic effects of organochlorine pesticides in maize root tips were assessed by 1H-HRMAS, revealing profound alterations in the glycolysis/gluconeogenesis balance, inactivation of the tricarboxylic acid (TCA) cycle, and changes in internal nitrogen distribution, indicating that 1H-HRMAS NMR metabolomics can be a sensitive tool for understanding molecular disturbances.

In the most common metabolomics approaches used in maize, namely hyphenated MS methods, samples are separated into their components by gas chromatography (GC), liquid chromatography (LC), and frequently both, either in the same study, e.g., Tang et al. (2017), or in consecutive studies, e.g., Asiago et al. (2012) and its follow-up (Baniadisi et al., 2014); by capillary electrophoresis (CE) (Levandi et al., 2008; Leon et al., 2009); or, less ideally, by direct injection without prior separation (Garcia-Flores et al., 2012). Although chromatography requires relatively lengthy extraction (and, in the case of GC, derivatization to render the metabolites volatile) in return for highly detailed results, direct injection provides rough profiles that are mainly useful for comparative purposes; however, the approach does have the advantage of requiring minimal preparation.

GC-MS is widely used for plant metabolomics and facilitates the identification and robust quantification of a few hundred metabolites in plant samples. Among these, sugars, sugar alcohols, amino acids, organic acids, and polyamines are often annotated, resulting in relatively comprehensive coverage of central primary metabolism. The great advantage of this approach is that highly stable protocols have been established for the setup and maintenance of machines and the evaluation and interpretation of chromatograms, meaning that libraries of retention times and mass spectral data for standard compounds can be shared among laboratories (Schauer et al., 2005). However, the use of GC-MS is limited to thermally stable volatile, or at least volatileizable, compounds. By contrast, LC does not require prior sample treatment and separates the components in a liquid phase. LC can analyze a variety of metabolites based on their chemical properties and the choice of columns, which include reversed phase, ion exchange, and hydrophobic interaction columns (Obata and Fernie, 2012). A crucial advantage of LC-MS is that, taking advantage of a variety of methods, we can analyze a wide array of metabolites, including those with high molecular mass and low thermostability. On the other hand, this flexibility presents difficulties in establishing mass spectral libraries for peak identification because mass spectra and retention times are dependent on instrument type (Moco et al., 2006). Finally, CE can separate a diverse range of chemical compounds and is more powerful than LC with respect to separation efficiency. One of the unique properties of CE-MS is the small amount of sample required for analysis; only nanoliters of sample are introduced into the capillary. A downside of CE is the poor migration time reproducibility and the absence of reference libraries, which can be only partially overcome by the prediction of migration time (Zhang and Ramautar, 2020).

Aside from these canonical methods generally used for non-targeted profiling, specific classes of metabolites lend themselves to analysis by other methods. For instance, carotenoids have been analyzed with a high-performance liquid chromatography (HPLC) photodiode array detector (Owens et al., 2014), used in this case for an association mapping study that correlated carotenoid levels with kernel color. In Decourcelle et al. (2015), carotenoids were profiled using HPLC, while at the same time the general metabolome was profiled using GC-MS. Similarly, targeted provitamin A and tocopherol determination protocols based on ultraperformance liquid chromatography (UPLC) have been used to screen maize germplasm (Wang et al., 2018a; Zhan et al., 2019).

The spatial distribution of metabolites within organisms has been an intriguing topic for decades, with implications related to biochemistry, kinetics, flux analysis, and physiology in general. In the past decade, however, matrix-assisted laser desorption-ionization MS imaging (MALDI-MSI) has been used as an analytical tool to visualize metabolites directly on plant tissues, and it has undergone important technical improvements in resolution, sensitivity, and chemical versatility (Sturtevant et al., 2016), and it has been used to image maize metabolites at the cellular and subcellular levels in leaves (Korte et al., 2015; Dueñas et al., 2016, 2017), seeds (Feenstra et al., 2017a), and roots (Feenstra et al., 2017b; O’Neill and Lee, 2020). The same group also reported that they were able to achieve non-targeted profiling using MALDI-MSI, overcoming one of the main limitations of the method (Feenstra et al., 2015).

DEFINING BIOCHEMICAL PATHWAYS

Primary metabolism

Metabolites are traditionally divided into primary metabolites, which promote cell viability, and secondary/specialized...
Metabolomics approaches have also been used as the main tool for deciphering the importance of the main pathways of primary metabolism. For instance, the responses of the leaf metabolome in different growth zones of maize leaves (cell division, elongation, and mature) and under carbon depletion were also investigated and linked to the rate of leaf elongation and protein synthesis. Central metabolism was shown to differ markedly between the growth and mature zones, and metabolic response during carbon depletion was less pronounced and was delayed in the growth zones compared with mature tissue. Interestingly, leaf growth largely followed sucrose content in the growth zones (Czedik-Eysenberg et al., 2016).

GC-MS-based metabolite profiling has been used to uncover the genetic basis for differences in primary metabolism and its relationship to plant performance in maize inbred populations (Wen et al., 2015, 2018; Cañas et al., 2017). In addition, a time-series metabolome analysis integrated with proteome data from maize hybrids and their inbred parents revealed that hybrids can better tolerate photoinhibition stress, maintaining higher photosynthesis without excessive elevation of photorespiration compared with the inbred lines. This highlights the roles of photosynthetic and photorespiratory pathways in maize seedling heterosis and provides advances for the biotechnological improvement of hybrid crops (Li et al., 2020b).

Maize uses a specialized photosynthetic pathway, C₄ metabolism (Hatch, 1987), as opposed to most other plants, including grasses such as wheat and rice, which use C₃ metabolism. C₄ plants spatially divide their photosynthetic process into two cell types, the mesophyll and bundle sheath cells, thereby fixing CO₂ and using water more efficiently (Schlüter and Weber, 2020). This allows higher yields in warmer climates; therefore, the integration of C₄ traits into C₃ plants to increase yield and environmental tolerance has been the new challenge. In fact, the installation of a C₄ photosynthetic pathway in rice has been predicted to increase rice yields by up to 50% (Hibberd et al., 2008), and attempts to install a partial C₄ pathway into rice using maize enzymes have been successfully metabolically confirmed using LC-MS methods (Emakova et al., 2020; Lin et al., 2020). Comparative metabolic analyses combined with transcriptomic data from C₃ (rice) and C₄ (maize) plants were used either to identify differences between the two photosynthetic mechanisms (Wang et al., 2014a) or to draw the evolutionary histories of both groups (Deng et al., 2020). Arrivalet al. (2019) profiled the abundance of Calvin-Benson cycle (CBC) metabolites from five C₃ plants (including rice) and four C₄ plants (including maize). They discovered substantial differences not only between C₃ and C₄ groups but also within each group, especially among the five C₃ species, suggesting independent evolution of CBC regulation in different plant lineages.

C₄ plants are traditionally classified into three distinct subtypes based on the enzyme that performs the primary decarboxylation reaction in the bundle sheath cells: plastidial nicotinamide adenine dinucleotide phosphate (NADP)-dependent malic enzyme (NADP-ME), mitochondrial nicotinamide adenine dinucleotide (NAD)-dependent malic enzyme (NAD-ME), and cytosolic phosphoenolpyruvate carboxykinase (PEPCK). Maize is predominantly categorized as the NADP-ME subtype (Figure 1A), but recent evidence indicates that the C₄ cycle functions as a branched rather than a linear pathway (Figure 1B), providing flexibility between the different decarboxylation pathways that may be controlled by developmental and environmental cues (Pick et al., 2011; Wang et al., 2014b). Pick et al. (2011) showed that there is no evidence to suggest switches between the decarboxylation pathways within the age gradient of a single leaf, but a previous study (Wingler et al., 1999) reported higher activity of PEPCK in older leaves, pointing to the developmental regulation of decarboxylation pathways. This idea is supported by the metabolic characterization of maize DCT2 mutants, which show impaired malate transport into bundle sheath cells (Weissmann et al., 2016), and by following the carbon flux through C₄ photosynthesis of maize (Arrivalet al., 2016). Both studies used isotopic labeling experiments combined with metabolomics approaches and concluded that the maintenance of different C₄ decarboxylation pathways may robustly afford high photosynthetic efficiency under a broad range of environmental conditions.

Secondary metabolism

Secondary metabolites are fascinating because of their chemical and functional diversity. For each way that the plant interacts with its environment, there are thousands of metabolites that serve as mediators. Thanks to clear chemical classification, many studies focus on a single class of metabolites, with specific goals. For a comprehensive review of carotenoids and anthocyanins in maize kernels, see Ranilla (2020). For instance, studies have focused on carotenoids, with the aim of increasing their abundance to help prevent dietary vitamin A deficiency (Owens et al., 2014).
Figure 1. C₄ metabolism model in maize

(A) NADP-ME decarboxylation pathway. The first step in the C₄ cycle is the assimilation of CO₂ into oxaloacetate (OAA) by phosphoenolpyruvate carboxylase (PEPC). In the NADP-ME cycle, OAA is imported into the chloroplasts of mesophyll cells (MCs) and reduced to malate. Malate diffuses along its concentration gradient into the bundle sheath cells (BSCs), where it is imported into the chloroplasts and decarboxylated by NADP-ME. This reaction yields one molecule each of CO₂, reduced NADP (NADPH), and pyruvate. CO₂ is assimilated by ribulose-1,5-bisphosphate carboxylase-oxygenase (Rubisco), yielding two molecules of 3-phosphoglyceric acid (3PGA) that can enter the CBC in either the BSCs or the MCs. The latter requires the shuttling of 3PGA and triose phosphate (TrioseP) between the BSCs and MCs. Pyruvate is exported from the BSCs to MCs and taken up into the chloroplasts, where it is converted to phosphoenolpyruvate (PEP) by PPDK. This reaction consumes adenosine triphosphate (ATP) and phosphate (Pi).

(legend continued on next page)
Metabolomics in maize biology

tocochromanols, with an identical aim concerning vitamin E (Lipka et al., 2013) and flavonoid derivatives, for both their antioxidant power (Lago et al., 2014; Li et al., 2021) and their contribution to lignin deposition to improve biomass processing (Eloy et al., 2017).

Questions that accompany these main topics have also been studied. For instance, although maize that produces enhanced amounts of carotenoids was generated some time ago, the global effects of this alteration were only explored years later in a representative example of a triple-omics approach (Decourcelle et al., 2015). In the same vein, maize engineered for the production of astaxanthin, a rare and exceptionally desired carotenoid that does not occur naturally in maize, was metabolically profiled (Farré et al., 2016); certain perturbations in central metabolism were observed, and these were within the natural variation of the parental plants.

Biotic stress is a major constraint to productivity in modern maize varieties (also see section, “metabolomics of stress response”). In recent years, several studies have used quantitative genetics and metabolomics approaches to help identify several genes and secondary metabolic pathways involved in maize native resistance mechanisms to biotic threats (Table 1). For instance, maysin, the major C-glycosyl flavone in silks of most maize varieties, is synthesized in a branch of the flavonoid pathway and confers maize resistance to the corn earworm, an important insect pest in maize and other crops (Waiss et al., 1979; Elliger et al., 1980). Since its discovery, the genetic background for maysin biosynthesis has been extensively studied and was recently completely described (Casas et al., 2016). Interestingly, maize varieties adapted to high altitudes exhibited higher accumulation of maysin after UV-B exposure not only in silks but also in leaves (Casati and Walbot, 2005), suggesting that this specific environmental condition may trigger specific plant metabolic responses to biotic attacks (see section, “how combined stresses affect the maize metabolome”).

Maize also produces a range of herbivore-induced terpene volatiles and pathogen-induced non-volatile terpenoids that play significant defensive and developmental roles. Terpenoids, also known as isoprenoids, originate from the conjugation of dimethylallyl diphosphate and its isomer isopentenyl diphosphate. Subsequent reactions catalyzed by prenyl transferases and prenyl diphosphatases yield the different classes of terpenes.

Rearrangements of terpene molecules by terpene synthases (TPSs) and cytochrome P450 enzymes result in a myriad of terpenoids (Tholl, 2015). Recent studies have characterized about half of the 30 TPS genes present in the maize genome (reviewed by Block et al., 2019). The volatile products of TPSs have been detected in different maize tissues, but their composition and content depend strongly on the genetic background (Degen et al., 2004), developmental stage, and organ (Köllner et al., 2004), as well as on abiotic and biotic stress (Gouguenue and Turlings, 2002; Becker et al., 2014; Block et al., 2017; Chiriboga et al., 2018). Upon biotic attack, maize plants can also produce different classes of non-volatile terpenoid phytoalexins. For instance, kauralexins and dolabralexins are two major diterpenoid groups that have important roles in responses to biotic stress. Kauralexins mediate defense responses against fungal pathogens (Christie et al., 2017; Meyer et al., 2017; Christensen et al., 2018b) and insect herbivory (Dafoe et al., 2011), whereas dolabralexins not only inhibit pathogenic fungi (Mafu et al., 2018) but also affect the rhizosphere microbial community (Murphy et al., 2021). Zealexins, non-volatile sesquiterpenoid phytoalexins, are also elicited in response to herbivory (Christensen et al., 2018a) and infection with diverse fungal pathogens (Basse, 2005; Köllner et al., 2008; Huffaker et al., 2011; Christensen et al., 2018a). Interestingly, the accumulation of zealexin A4 seems to be attenuated by high CO₂ levels, supporting the idea that elevated CO₂ has a negative impact on maize chemical defense against biotic stress (Vaughan et al., 2014, 2016).

Grasses synthesize a unique class of secondary metabolites known as benzoxazinoids. They are among the most agriculturally relevant groups of plant specialized metabolites because, since their identification in the 1950s, benzoxazinoids have been associated with defense against insect herbivores, microbial pathogens, and competing plant species. Recently, they have also been associated with signaling events (Zhou et al., 2018). These ~20 natural defense chemicals share the 2-hydroxy-2H-1,4-benzoxazin-3(4H)-one skeleton (HBOA) and are found mainly as inactive glucoside-bound precursors stored in the vacuole (Frey et al., 2009). The core biosynthetic pathway of the major maize benzoxazinoid (Figure 2), 2-(2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one)-beta-d-glucopyranose (DIMBOA-Glc), has been characterized (reviewed by Zhou et al., 2018). However, only recently have later biosynthetic steps been characterized in maize (Handrick et al., 2016), and the transcriptional regulation of the biosynthetic pathway has only begun to be revealed (Zhang

Plant Communications 2, 100187, July 12 2021 © 2021 The Authors. 5
| Trait                                      | Measurement | Candidate gene, locus, or encoding enzyme | Analysis                                   | Reference               |
|--------------------------------------------|-------------|-------------------------------------------|--------------------------------------------|-------------------------|
| Carotenoids in kernels                     | LC          | $y_1$, vp5, and QTL                       | linkage mapping                            | Wong et al. (2004)      |
| Maysin and chlorogenic acid in silks       | LC          | $p$, a1, c2, and whp1                     | linkage mapping                            | Szalma et al. (2005)    |
| Oleic acid in kernels                      | GC          | fad2                                      | linkage mapping                            | Beloé et al. (2008)     |
| Carotenoid composition and content in kernels | LC          | lcyE                                      | association and linkage mapping            | Harjes et al. (2008)    |
| Oil content and fatty acid composition in seeds and embryos | NMR and GC | dgat1-2                                   | QTL mapping                               | Zheng et al. (2008)     |
| β-carotene in grains                       | LC          | lcyE and crtRB1                           | QTL and linkage mapping                    | Yan et al. (2010)       |
| Oil content and fatty acid composition in kernels | GC          | multiple candidate genes                  | QTL and linkage mapping                    | Yang et al. (2010)      |
| Palmitic acid content in kernels           | GC          | fatb                                      | QTL, association, and linkage mapping      | Li et al. (2011)        |
| Carbohydrates and ABA metabolites during stress in ears, silks, and leaves | ELISA and spectrophotometry | multiple candidate genes                  | association mapping          | Setter et al. (2011)    |
| Anthocyanin in kernels                     | LC          | f3'h1                                     | linkage mapping                            | Sharma et al. (2011)    |
| Oil content and fatty acid composition in kernels | NMR and GC | dgat1-2                                   | linkage mapping                            | Chai et al. (2012)      |
| Starch, protein, and oil content in kernels | NIRS        | multiple candidate genes                  | linkage mapping and GWAS                  | Cook et al. (2012)      |
| α-tocopherol content in kernels            | LC          | vte4                                      | linkage mapping and GWAS                  | Li et al. (2012)        |
| Leaf metabolome                            | GC-MS       | multiple candidate genes                  | GWAS                                      | Riedelsheimer et al. (2012) |
| α-carotene in kernels                      | LC          | crrRB3                                    | QTL and linkage mapping                    | Zhou et al. (2012)      |
| Carotenoid content in grains               | LC          | psy1                                      | QTL and linkage mapping                    | Fu et al. (2013)        |
| Carotenoid composition and concentration in grains | LC          | multiple candidate genes                  | QTL and linkage mapping                    | Kandianis et al. (2013) |
| Oil biosynthesis in kernels                | GC          | multiple candidate genes                  | linkage mapping and GWAS                  | Li et al. (2013)        |
| Tocochromanols in grains                   | LC          | hgt1 and GRMZM2G437912                    | GWAS                                      | Lipka et al. (2013)     |
| Aphid resistance/ benzoaxacinoid content in leaves | LC-MS      | bx10a, bx10b, and bx10c                   | QTL and association mapping               | Meihls et al. (2013)    |
| Leaf lipidome                              | LC-MS       | multiple candidate genes                  | GWAS                                      | Riedelsheimer et al. (2013) |
| Carotenoids in kernels                     | LC          | multiple candidate genes                  | GWAS                                      | Owens et al. (2014)     |
| Metabolic diversity of kernels             | LC-MS       | multiple candidate genes                  | linkage mapping and GWAS                  | Wen et al. (2014)       |
| Carotenoids in kernels                     | LC          | multiple candidate genes                  | GWAS                                      | Suwarno et al. (2015)   |
| Primary metabolism in leaves and kernels   | GC-MS       | multiple candidate genes                  | QTL and linkage mapping                    | Wen et al. (2015)       |
| Carbon and nitrogen metabolism in leaves   | spectrophotometry | multiple candidate genes                  | linkage mapping and GWAS                  | Zhang et al. (2015)     |
| Ratio of tocotrienols*                     | LC          | vte1                                      | GWAS                                      | Chen and Lipka, (2016)  |

Table 1. Genetic mapping studies on metabolic traits in maize.

(Continued on next page)
| Trait                                             | Measurement | Candidate gene, locus, or encoding enzyme | Analysis                        | Reference               |
|--------------------------------------------------|-------------|-------------------------------------------|---------------------------------|-------------------------|
| Starch content in kernels                        | NIRS        | multiple candidate genes                  | GWAS                            | Liu et al. (2016b)      |
| Metabolic diversity in mature kernels            | LC-MS       | multiple candidate genes                  | QTL, linkage mapping, and GWAS  | Wen et al. (2016b)      |
| Carbohydrates and ABA metabolites during stress in ears, silks, and leaves | ELISA and LS | multiple candidate genes                  | GWAS                            | Zhang et al. (2016)     |
| Amino acids in kernels                           | CEC and spectrophotometry | multiple candidate genes                  | QTL, linkage mapping, and GWAS  | Deng et al. (2017)      |
| Root volatiles                                   | GC-MS and GC-FID | tps21                                     | QTL, linkage mapping, GWAS      | Ding et al. (2017)      |
| Flavonoid biosynthesis in kernels                | LC-MS       | multiple candidate genes                  | linkage mapping and GWAS        | Jin et al. (2017)       |
| Nitrogen metabolism in leaves                    | spectrophotometry | multiple candidate genes                  | QTL mapping                      | Trucillo Silva et al. (2017) |
| Carotenoid content in kernels                    | LC          | multiple candidate genes                  | GWAS                            | Azmach et al. (2016)    |
| Tocochromanol content in kernels                 | LC          | multiple candidate genes                  | QTL and linkage mapping         | Fenton et al. (2018)    |
| Nitrogen metabolism in roots                     | spectrophotometry | multiple candidate genes                  | QTL mapping                      | Trucillo Silva et al. (2018) |
| Tocopherol content in kernels                    | LC          | multiple candidate genes                  | linkage mapping and GWAS        | Wang et al. (2018a)     |
| Primary metabolism in leaves and kernels         | GC-MS       | multiple candidate genes                  | GWAS                            | Wen et al. (2018)       |
| Kernel composition and flour pasting behavior    | NIRS        | multiple candidate genes                  | GWAS                            | Alves et al. (2019)     |
| Tocochromanols in kernels                        | LC          | vte1, vte4, hggt1, sh2, su1              | GWAS                            | Baseggio et al. (2019)  |
| Diterpenoid defenses                             | GC-MS and LC-MS | multiple candidate genes                  | GWAS                            | Ding et al. (2019)      |
| Oil and fatty acid composition in kernels[^3]    | GC          | multiple candidate genes                  | GWAS and pathway analysis       | Li et al. (2019a)       |
| Primary metabolites in leaves and kernels        | GC-MS and spectrophotometry | QTL                                     | QTL and linkage mapping         | Li et al. (2019b)       |
| Starch content in kernels                        | NIRS        | GRMZM2G110929, GRMZM5G852704              | linkage mapping and GWAS        | Lin et al. (2019)       |
| Cell wall-bound hydroxycinnamates in stems       | LC and spectrophotometry | multiple candidate genes                  | GWAS                            | López-Malvar et al. (2019) |
| Mechanisms of phosphorus deficiency in leaves and roots | LC-MS and GC-MS | GRMZM2G051806, GRMZM2G025854, GRMZM2G039588, GRMZM2G050570, GRMZM5G841893 | GWAS                            | Luo et al. (2019)       |
| Tocopherol content in leaves and kernels         | ELISA and LC | porb2                                     | QTL and association mapping     | Zhan et al. (2019)      |
| Antibiotic biosynthesis (zealexin)               | GC-MS and LC-MS | multiple candidate genes                  | GWAS                            | Ding et al. (2020)      |
| Secondary metabolites in leaves                  | LC-MS       | multiple candidate genes                  | GWAS                            | Zhou et al. (2019)      |

Table 1. Continued (Continued on next page)
Plant Communications

Metabolomics in maize biology

Table 1. Continued

| Trait | Measurement | Candidate gene, locus, or encoding enzyme | Analysis | Reference |
|-------|-------------|------------------------------------------|----------|-----------|
| Volatile composition in wholemeal flour | GC-MS | multiple candidate genes | GWAS | Alves et al. (2020a) |
| Antioxidant content in kernels | LC and spectrophotometry | multiple candidate genes | GWAS | Alves et al. (2020a) |
| Carotenoids in kernels | LC | multiple candidate genes | GWAS | Basegge & et al. (2020) |
| Carotenoids in kernels | HPLC | multiple candidate genes | linkage mapping and GWAS | Diepenbrock et al. (2020) |
| Anthocyanin in kernels | HPLC and spectrophotometry | multiple candidate genes | GWAS | Chatham and Juvik, (2021) |
| Metabolite biomarkers for salt tolerance | LC-MS | cts3, cyp709b2, ugt, and multiple candidate genes | GWAS | Liang et al. (2021) |

et al., (2021). Upon herbivore-mediated tissue damage, glucosidases cleave the glucoside moiety of the benzoxazinoid glucosides, producing biocidal aglucone benzoxazinoids (Morant et al., 2008). It has also been proposed that benzoxazinoids such as 6-methoxy-benzoxazolin-2-one (MBOA) and 2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one (DIMBOA) may regulate belowground and aboveground biotic interactions (Mehlis et al., 2013; Hu et al., 2018). In fact, benzoxazinoids were shown to affect microbial communities in shoots and roots, as well as the rhizosphere (Kudjordje et al., 2019). The negative correlation between benzoxazinoids and fungal pathogen genera in shoots could indicate a potential for these compounds to serve as a control for pathogenic fungal infections. Further evaluation of the regulatory activity of benzoxazinoids on the maize metabolome and associated microbial communities revealed that benzoxazinoids influence the rhizobium through the endogenous regulatory activity of plant-derived rhizosphere signals such as flavonoids (Cotton et al., 2019). Benzoxazinoids are often identified by targeted HPLC profiling, but in-depth studies that attempted to gain comprehensive insights into metabolic alterations upon herbivore attack have used non-targeted approaches (Glauser et al., 2011). In this respect, these studies and those that define the pathways of flavonoid biosynthesis (Wen et al., 2020) are distinct from studies of primary metabolism, as they define the pathway structure—and in some cases the function—of the metabolite rather than the state of metabolic regulation, and such information is still lacking for many secondary metabolites.

Lipids and oils

Although starch is the most commercially and nutritionally interesting component of maize, its oil is gaining importance for cooking, as a component of foods such as margarine, and for products such as soap, ink, and paint. The oil content of maize is low, about 3%, but it can reach up to 7% in high-oil corn genotypes (Singh et al., 2014). Maize seeds possess high oleic acid contents and, although it is beneficial to human health, oleic acid is sensitive to oxidation and unstable at high temperatures (Du et al., 2016). Attempts have been made to increase oil content, with metabolomics playing an important confirmatory role (Pouvreau et al., 2011). Increased oil production in maize is also desirable for reasons other than direct use of the oil. Indeed, the astaxanthin-producing maize line was crossed with a high-oil producing line to facilitate the storage and easy extraction of the lipophilic carotenoid (Farré et al., 2016). Moreover, the fatty acid composition of maize oil has also been a target for manipulation for a variety of nutritional and commercial purposes (White et al., 2007; Du et al., 2016).
growth and impair productivity. In these unfriendly growth environments, plants need to adapt to survive. In such scenarios, plant metabolism is perturbed, and the metabolic network must be reprogrammed. Metabolomics is a powerful tool for gaining a comprehensive perspective on how metabolic networks are regulated, and it has been used extensively in maize stress response research. It is established that plants adjust their metabolite production in response to stress, but the reasons, mechanisms, and regulation are only partially known. Common stresses that plants experience in nature include herbivory and pathogen infection, as well as water-deficit stress, toxicity (including salinity), nutrient deficiency, radiation, low or high temperatures, and excess or deficient light. All these stresses have been studied under both field and controlled conditions, frequently showing that the responses in controlled growth conditions are not always faithful replicates of those in the field (Casati et al., 2011; Witt et al., 2012). Below we summarize the most recent findings on stress metabolomics studies that directly address the following questions.

**Which organs are most affected?**

Leaf blades were demonstrated to be the sites of greatest metabolic change following drought (Witt et al., 2012; Obata et al.,...
When leaves are infested by the African cotton leafworm moth, many metabolite changes are observed at the site of infection; defense-related metabolites increase in the vascular sap and root exudates, but only a few metabolites are changed in the roots (Martí et al., 2013). The effect of the smut caused by the fungus *Ustilago maydis* on maize root metabolism has been characterized in detail (Djamei et al., 2011). Among the 150 proteins with known functions in the *U. maydis* secretome, chorismate mutase seems to be a virulence factor. It has been suggested that chorismate mutase enters the plant cell and channels chorismate into the phenylpropanoid pathway, preventing its flow toward salicylic acid biosynthesis, probably as a mechanism to reduce maize resistance to *U. maydis*. This illustrates a reprogramming of maize root metabolism by a fungal effector to favor the fungus’s requirements. The suppression of salicylic acid by *U. maydis* was further demonstrated by the identification of a cytoplasmic *U. maydis* salicylate hydroxylase that is induced during plant colonization (Rabe et al., 2013). Another *U. maydis* secreted effector, Tin2, was shown to induce changes in anthocyanin biosynthesis (Tanaka et al., 2014). Tin2 translocates into plant cells and targets the cytoplasmic protein kinase ZmTTK1, which is stabilized, leading to anthocyanin formation as a strategy to compete with lignification of the colonized tissue. This was later characterized as a neofunctionalization of Tin2, probably related to the rare ability of *U. maydis* to induce leaf tumors by lowering lignification that might otherwise restrict fungal proliferation (Tanaka et al., 2019).

**Which pathways are mainly affected?**

Alvarez et al. (2008) were among the pioneers in studying the maize stress response using metabolomics, particularly HPLC-MS/MS fragmentation. Because no libraries existed at the time, the authors screened their results against 64 standards of compounds thought to exist in the xylem sap. Changes were found in hormones associated with stomatal movements, such as abscisic acid (ABA) and cytokinins, highlighting the functioning of root-to-shoot signals. In addition, increases in the content of phenylpropanoid pathway intermediates were also observed. Although this is probably related to impacts on lignin and anthocyanin biosynthesis under water stress, it may also affect flavonol levels with effects on stomatal aperture, as suggested for other species (reviewed by Medeiros et al., 2020). In fact, just recently, Li et al. (2021) showed that increased flavonol content in guard cells improves the water use efficiency of a drought tolerant maize genotype (*drought overly insensitivity 57*) by both increasing antioxidant capacity and downregulating stomatal closure in the mutant plants under drought stress.

Schlüter et al. (2013) found similarities in the regulation of carbon metabolism in source leaves under low temperature and low nitrogen stress and corresponding impacts on plant growth. Accumulation of carbohydrates under these two conditions indicated that growth was limited by a feedback downregulation of photosynthesis. Moreover, phosphorus deficiency directly influenced carbon and energy metabolism: photosynthesis dropped dramatically, and a decrease in carbohydrate levels was observed. However, nitrate assimilation was the only primary pathway downregulated under all three conditions (low temperature, low nitrogen, and low phosphorus stress). The coordination of carbon and nitrogen metabolism is known to affect plant growth, and a mixed supply of nitrate (NO$_3^-$) and ammonium (NH$_4^+$) can maximize plant growth compared with a sole NO$_3^-$ or NH$_4^+$ supply. However, only recently, Wang et al. (2019b) shed light on this observation by showing that a mixed nitrogen source enhances auxin synthesis by the shikimic acid pathway, increasing carbon and nitrogen utilization. Commonality in metabolic responses to stress is to be expected, and comparative analysis of different stresses has revealed considerable overlap (Obata et al., 2015).

Autophagy, a constitutive cellular process of homeostatic recycling, is exacerbated under nutrient deficiency. Although it is one of the most studied cell biology topics in recent times, research on autophagy in maize using metabolomics is limited. In a pioneer study that combined both, McLoughlin et al. (2018) applied a multi-omics approach to nitrogen-starved maize and identified numerous metabolic alterations, mainly in lipid and secondary metabolism.

Organochlorine pesticides were shown to alter the glycolysis/gluconeogenesis balance, inactivate the TCA cycle, redistribute nitrogen compounds, and increase fatty acid production and oxidation in maize roots (Blondel et al., 2016). Smoke, not a stressor per se but an inducer of plant persistence and recolonization after wildfires, was shown to affect carbohydrate and energy pathways in young maize roots (Çatav et al., 2018).

**How do combined stresses affect the maize metabolome?**

In the last decade, maize metabolic responses to single abiotic stresses have been relatively well documented, with studies covering salinity (Gavaghan et al., 2011; Henry et al., 2015; Richter et al., 2015; Forieri et al., 2016; Li et al., 2018), water deficit (Alvarez et al., 2008; Virlouvet et al., 2011; Witt et al., 2012; Barnaby et al., 2013; Benevenuto et al., 2017; Yang et al., 2018b), cold (Noblet et al., 2017; Li et al., 2019c; Urrutia et al., 2021), and high temperature (Suwa et al., 2010; Sun et al., 2016a). However, in natural habitats, plants often experience a combination of stress conditions, and the effects of these interactions on the maize metabolome is less well characterized. The plasticity of maize molecular responses to combinations of different abiotic stresses has received some attention due to their potentially highly damaging effect compared with isolated stress conditions. For instance, maize plants subjected to a combination of water deficiency with salt or heat stress presented metabolic responses distinct from those of plants subjected to one stress alone, suggesting that...
maize exhibits metabolic plasticity in response to different stress conditions (Sun et al., 2015, 2016b). The metabolome analysis of plants grown under elevated CO2 and subjected to sudden heat shock stress identified malate metabolism as a key player in the recovery of photosynthetic activity after a short-term heat wave (Qu et al., 2018). In addition, elevated CO2 was shown to eliminate early responses of maize leaf metabolites under water deficit (Sicher and Barnaby, 2012). Moreover, GC-MS-based metabolite profiles of leaves from 10 tropical maize hybrids with diverse abiotic stress tolerances were analyzed after exposure to drought, heat, and both stresses simultaneously in field trials (Obata et al., 2015). Interestingly, most metabolic changes in the combined treatment (drought and heat) could be predicted from the sum of responses to the individual stresses. This study also identified metabolite signatures closely related to grain yield under abiotic stress conditions, specifically highlighting myo-inositol and raffinose as promising metabolic markers for breeding purposes.

The metabolic changes in maize plants caused by combined abiotic and biotic stress treatments remain largely unknown. A few studies have recently touched upon this interesting topic, revealing that different abiotic stresses can have distinct effects on biotic threats. For instance, elevated CO2 was observed to reduce phytoalexin accumulation, enhancing the mycotoxicogenic effects of Fusarium verticillioides in maize (Vaughan et al., 2014). However, when combined with drought stress, high CO2 levels increased phytoalexin content, thereby enhancing maize phytochemical defenses against F. verticillioides (Vaughan et al., 2016). Elevated CO2 alone was also shown to compromise maize metabolic defenses against Aspergillus flavus by reducing the levels of the keto-acidic sesquiterpenoid zealexin A4 (Christensen et al., 2018a). Additive and synergistic effects of flooding and anti-insect defense responses were also observed against Spodoptera frugiperda (fall armyworm) infestation in maize. In this case, the combined stress led to elevated production of salicylic acid, which did not occur in the individual stresses, resulting in extra salicylic acid-dependent protection against S. frugiperda (Block et al., 2020). Moreover, the combination of flooding and herbivory led to a remodeling of the phenylpropanoid pathway, which in turn increased maesin accumulation by 2-fold compared with the control non-infested plants (Block et al., 2020). Interestingly, heat stress applied prior to fungal inoculation had a negative effect on maize resistance to Cochliobolus heterostrophus, and targeted metabolome analysis revealed that deficiency in the hydroxycinnamic acid p-coumaric acid may have contributed to the observed heat-induced susceptibility to the fungus (Christensen et al., 2021). These findings highlight the phenotypic variation observed in maize plants under different stress combinations and demonstrate the complexity of plant–environment relationships. More intriguingly, they point to the fact that abiotic stresses can also predispose crops to more severe biotic threats.

Changes in the maize metabolome under beneficial biotic interactions

Plant–microbe interactions are ubiquitous and are important for the health of plants and soil. Many of these interactions occur in the rhizosphere and often result in positive effects when plants associate with beneficial microorganisms such as plant-growth-promoting bacteria, mycorrhizal fungi, rhizobia, and endophytes (Kaur and Suseela, 2020).

Plant Communications
the priming effect observed in maize leaves is a mycorrhiza-specific response (Gerlach et al., 2015).

GENETICALLY MODIFIED MAIZE

Approximately a third of the worldwide maize crop area is dedicated to genetically modified (GM) maize (OECD, 2018). As part of the safety assessment that GM crops undergo before introduction to the market, so-called substantial equivalence to wild-type counterparts must be shown. For decades, targeted analysis of several key metabolites has been the standard. The bias and limitations of this approach are clear, and it is gradually being replaced with non-targeted analyses. One of the first such studies in maize used the insect-resistance gene Cry1Ab as a model and employed for the first time the partial least square discriminant analysis (PLS-DA) statistical method (Manetti et al., 2006), which has since appeared in countless metabolomics publications. Another early example indicated that, for the transgenic events studied, (1) metabolites not included on the list of key metabolites to be tested showed much higher variability than those recommended by the OECD (Harrigan et al., 2007); and (2) the impact of GM trait insertion on the grain metabolome variation in hybrids derived from the glyphosate-tolerant maize strain NK603 was negligible compared with corresponding GM trait-negative hybrids, supporting the hypothesis that residual genetic variation due to the conventional breeding process accounted for the observed differences between GM and non-GM segregants (Harrigan et al., 2016). However, comprehensive non-targeted metabolomics revealed that the NK603 strain was not substantially equivalent to its nearest isogenic non-GM strain, DK2675 (Mesnage et al., 2016). An earlier study assessed the contributions of two genetic modifications (glyphosate tolerance and Bacillus thuringiensis insect resistance) relative to those of environmental factors such as growth conditions and location (Frank et al., 2012). The authors concluded that most differences were related to natural variability rather than to genetic modification, with a substantial contribution from environmental factors. A similar conclusion was reached in a study of stacked GM maize that combined insect resistance (Cry) with glyphosate tolerance (Epsps) genes in a single strain (Wang et al., 2018b). Although these studies provided important information that could inform regulatory frameworks established to determine whether transgenics could generally be regarded as safe, it must be borne in mind that such studies must be performed empirically for each specific transgenic event, and one cannot merely generalize from them.

GM is mediated, among other things, through transformation into embryonic callus, a tissue that is ideal for this purpose thanks to its inducible totipotency. However, the relatively low rate of embryonic callus induction and regeneration in maize has hampered genetic engineering in this species. A recent study offers one of the most comprehensive investigations to date of the factors that control this process in maize, combining proteomics and metabolomics of different lines and induction stages (Ge et al., 2017). These analyses revealed that differences in the capacity to produce embryonic callus involve various metabolic pathways. The induction of amino acids, lipids, and sugar metabolism, as well as the regulation of hormone synthesis, including that of auxin, cytokinin, jasmonic acid, and brassinosteroids, seems to be associated with a higher rate of embryonic callus induction.

In the last decade, the RNA-guided CRISPR/Cas9 (clustered regularly interspersed short palindromic repeats/CRISPR-associated protein 9) system has been applied to plant genome editing and represents a massive breakthrough. The CRISPR/Cas9 system introduces stable mutations at specific sites dictated by a single guide RNA, with a much cleaner genetic background. It has been increasingly used in maize studies and is a great advance for both functional research and breeding of maize (Liu et al., 2020a). Despite the great advances in gene discovery and trait development in crops brought about by CRISPR/Cas9, this technology shares major challenges with classic genetic modification methods, e.g., polyploidy, transformation efficiency, and tissue regeneration, which remain concerns in the development and application of crop genome editing (Mao et al., 2019). Due to its ability to determine global metabolic changes, metabolomics has been proposed as a route for the identification of gene-edited individuals (Fraser et al., 2020). Although this is clearly dependent on the gene-editing event having metabolic consequences, there is no doubt that metabolomics can help to discriminate gene-edited from non-edited plants during regulatory assessment to identify both intended and unanticipated detectable metabolic outcomes.

INTEGRATION OF OMICS DATA

Rapid advances in omics technology, together with falling costs, especially of sequencing, have led to the parallel application of several omics techniques in many studies. In this regard, parallel application is distinguished from bona fide data integration. In the former, metabolomics is accompanied by either proteomics (Ge et al., 2017), transcriptomics (Xu et al., 2019), or both (Barros et al., 2010; Casati et al., 2011; Amiour et al., 2014; McLoughlin et al., 2018). An important example is the proteome and metabolome profiles of glyphosate-tolerant GM maize, which show that despite previous claims, it is not in fact equivalent to its isogenic counterpart in protein and metabolite content (Mesnage et al., 2016). In studies that implement bona fide integration, data from the different sources are mathematically combined, often in the form of network analysis, to unravel correlations that are not otherwise immediately apparent (Xu et al., 2019). The use of metabolomics alongside more exotic omics datasets has also gained attention; these include fluxomics, which determines the rates of metabolic reactions (Cocuron et al., 2019); exometabolomics, also known as metabolic footprinting, which studies changes in extracellular metabolites (Zha et al., 2014); and ionomics, the analysis of mineral nutrient and trace element composition (Guo et al., 2017).

The integration of phenotypic data, mostly relevant agronomic traits, with the metabolome is present in several studies and goes hand in hand with the aim of yield improvement. For instance, in a seminal study, GC-MS-based metabolite profiling was integrated with enzymatic activity profiles of 29 key central-metabolism enzymes, as well as agronomic traits (Cañas et al., 2017). This study, conducted on a panel of 19 genetically and geographically distant maize lines, yielded a “maize ideotype,” a hypothetical strain optimized for high yield, and several metabolites were identified as excellent predictors of kernel size, e.g., chlorogenates.

The optimal methodology for integrating omics data for hybrid selection and prediction has been thoroughly tested and discussed.
Metabolomics in maize biology

It has been shown that genomic, and more so transcriptomic, data alone are superior to metabolomic data (proteomics was not included) with regard to predictive power for complex and highly heterotic traits (Westhues et al., 2017). Despite this fact, Westhues et al. (2017) still acknowledge the potential (and practical) contribution of metabolites, by force of their “physiological proximity to the phenotype, which provides information that is impossible to infer from DNA or proteins.” In this context, it is important to mention a study that assayed the robustness of metabolite levels in inbred lines versus hybrids using metabolomics alone. The hybrids displayed greater robustness, underscoring the much-studied phenomenon of heterosis and providing a predictive model for the performance of new hybrids (de Abreu E Lima et al., 2017). In their study, Amiour et al. (2012) stated: “It was also found that the integration of the three ‘omics’ studies is not straightforward, since different levels of regulation seem to occur in a stepwise manner from gene expression to metabolite accumulation.” With this in mind, considerable achievements have been made using omics integration. A notable example is the study by Rao et al. (2014), which provided the research community with a comprehensive metabolic map of maize kernels. Non-targeted profiling conducted on 14 representative maize lines showed that several metabolites could be used to distinguish between the lines.

Some initiatives, namely OPTIMAS-DW (http://www.optimas-bioenergy.org/optimas_dw; Colmsee et al., 2012), MaizeGDB (https://maizegdb.org/; Andorf et al., 2015), MODEM (http://modem.hzau.edu.cn/; Liu et al., 2016a), and most recently ZEAMAP (http://www.zeamap.com/; Gui et al., 2020), have aimed to integrate maize omics results, including metabolomics, in more comprehensive databases, providing useful tools for the search, analysis, and visualization of these rich datasets. In addition, since the development of the C4GEM, an early attempt to build a genome-scale metabolic model to study C4 metabolism (Dal'Molin et al., 2010), some in silico reconstructions of maize metabolism have been presented as ideal tools for the integration of different omics approaches. For instance, the Zea mays iRB1563 model comprises 1,563 genes and 1,825 metabolites that participate in 1,985 reactions from both primary and secondary metabolism. It revealed unique reactions and metabolites compared with the AraGEM model for Arabidopsis and the C4GEM (Saha et al., 2011). A second-generation genome-scale metabolic model for the maize leaf, approximately four times broader than the earlier iRS1563, was generated to capture C4 carbon fixation and investigate nitrogen assimilation by modeling the interactions between the bundle sheath and mesophyll cells (Simons et al., 2014). Moreover, a tissue-specific metabolic model (Seaver et al., 2015) and one that describes mesophyll and bundle sheath cells in different segments of the developing maize leaf (Bogart and Myers, 2016) have substantially increased the accuracy of predictions of the spatial variation in metabolic state and metabolic fluxes from expression data.

THE ORIGIN, EVOLUTION, AND NATURAL VARIATION OF MAIZE THROUGH A METABOLOMIC LENS

Similar to other crops with a long history of human cultivation, many favorable genes that were lost during the domestication of maize, including those related to nutritional value and stress tolerance, remain hidden in wild ancestors. Therefore, much research has been dedicated to understanding the origin, evolution, and natural variation of maize. Pioneering studies have reported the metabolite profiling of various maize strains, lines, and crosses derived from or grown in different locations, demonstrating the considerable impact of origin on metabolic composition (Rohlig et al., 2009; Skogerson et al., 2010).

The influence of teosinte on the genetics, ecology, and composition of domesticated maize has also attracted considerable attention. For instance, a large-scale metabolite-based quantitative trait loci (mQTL) analysis in a population generated from crossing teosinte with the maize inbred line Mo17 demonstrated massive metabolic variations (Li et al., 2019b). Most of the metabolites analyzed displayed an additive effect in the presence of alleles from the teosinte genome, whereas the opposite pattern was observed for grain yield and shape trait quantitative trait loci (QTL). Another comprehensive metabolic analysis was conducted on teosinte and tropical and temperate maize, as well as on a teosinte maize cross. Lipids, alkaloids, and terpenoids mostly differed between teosinte and tropical maize, whereas benzoxazinoids differed between tropical and temperate maize. Further integration with transcriptomics led to the identification of several genes responsible for the metabolic divergence (Xu et al., 2019). The genetic architecture of oil and carotenoid traits in a teosinte-maize population has also been targeted. A trait-QTL network was constructed to assess the genetic relationships among 33 oil- and carotenoid-related traits. The evolutionary trajectories of the genes or QTLs responsible for variations in oil and carotenoid traits revealed that these traits caused diverse selection events during maize domestication. This indicates the complex selection patterns of the genes that underlie maize kernel nutritional traits and shows that teosinte alleles can also be valuable for improving those traits (Fang et al., 2020).

In fact, most agriculturally and economically important traits have a complex genetic basis (i.e., they are determined by multiple QTLs); therefore, precisely locating and characterizing the functional loci are extremely important for crop improvement (Wen et al., 2016a; Liu et al., 2020b). Linkage mapping based on an F2 or recombinant inbred line (RIL) from crosses between two or more parental accessions is a well-known approach for locating QTLs. However, only a few QTLs are usually detected by linkage mapping in each experiment; further fine mapping to obtain a more precise genetic position is needed, and larger secondary populations are required to achieve sufficient map resolution (Xiao et al., 2017). Even with the introduction of high-density maps generated by next-generation sequencing, which increase the mapping resolution of mQTLs, this approach is not scalable for exploring variation in abundant diverse germplasm (Luo, 2015).

In this context, genome-wide association studies (GWASs) have been used in diverse populations as a strategy for fine mapping QTLs. Although the first association mapping study in maize was performed about 20 years ago (Thornsberry et al., 2001), only considerably later was this approach used to study maize metabolism, revealing that allelic variance in FAD2, which encodes a fatty acid desaturase, is responsible for differences
in the oleic acid content of kernels (Belé et al., 2008). Since then, GWAS in plants has undoubtedly gained popularity, and maize is no exception (Xiao et al., 2017). The diploid genome and the cultivation history of maize account for the impressive phenotypic diversity that lends itself to association studies.

In recent years, GWAS and linkage analysis have been successfully conducted to dissect the diversity of maize metabolic traits in populations of either natural variation (accessions) or generated variation (introgression lines, RILs, and backcross inbred lines) (Table 1). Metabolite-based GWASs (mGWASs) are classic applications of omics integration in which metabolite levels, serving as quantitative traits, are correlated with genomic marker data (single nucleotide polymorphisms [SNPs]) from a population. One of the major advantages of mGWAS is its ability to help identify novel genes in metabolic pathways. Due to the large diversity across experimental populations and precise evaluation of metabolite levels, it is much easier to identify the genetic variants that control the accumulation of metabolites rather than QTLs related to agricultural performance, which usually have a moderate or low effect (Fang and Luo, 2019). However, factors such as the degree of diversity in the population, the density and quality of the SNP data, and the tissue selected for metabolite profiling may greatly influence the results.

Natural variation in the maize metabolome has been widely explored by mQTL research (Liu et al., 2020b). For instance, the Goodman Diversity Panel (Flint-Garcia et al., 2005) has been used numerous times in various studies. The panel consists of 302 accessions and captures a large proportion of the alleles in cultivated maize. GWAS analyses focusing on vitamin E in maize kernels demonstrate how consecutive studies build upon each other with incremental but significant improvements, with, in this case, the expansion of the profiled metabolites (from only several tocopherols to all tocochromanolos), the use of better populations, and, in the final study, the comparison of two different populations created specifically to reflect two each other with incremental but significant improvements, with, in this case, the expansion of the profiled metabolites (from only several tocopherols to all tocochromanolos), the use of better populations, and, in the final study, the comparison of two different populations created specifically to reflect two different vitamin E traits (Li et al., 2012; Lipka et al., 2013; Diepenbrock et al., 2017; Fenton et al., 2018). In addition, non-targeted metabolite profiling identified almost 4,000 metabolic features in leaf bases and tips (Zhou et al., 2019) and found an interesting bimodal metabolite distribution. The vast majority of metabolites were present in less than half the lines, bases, and tips differed in flavonoid content, and different maize varieties differed in benzoazinoid content. The analysis of root volatiles and the application of mGWAS enabled the identification of the terpene synthase21 (tps21) gene as an important player in a previously unrecognized β-cassic acid pathway in maize that contributes to fungal pathogen resistance (Ding et al., 2017). Later, an integrative study used association mapping, pan-genome multi-omic correlations, enzyme structure-function, and targeted CRISPR-Cas9 mutations to identify genes involved in hormone pathways that partition diterpenoid defenses (Ding et al., 2019). Kauralexin biosynthesis was shown to use ent-kaurene formed by diterpene synthases recruited from gibberellin metabolism. This mechanism minimizes the unregulated accumulation of gibberellin precursors, which could affect hormone signaling during the defense response to biotic stress (Ding et al., 2019). Using the same approaches, Ding et al. (2020) also identified 10 genes in three zealexin gene clusters that encode four sesquiterpene synthases and six cytochrome P450 proteins. The findings from this elegant work suggest a so-called hourglass-shaped biosynthetic network in maize defensive terpenoid metabolism in which terpene synthase-derived metabolites meet at a single cytochrome P450 monoxygenase enzyme node, with subsequent diversification via pathway-specific enzymes (Ding et al., 2020).

Another important population used for the genetic mapping of metabolic traits in maize is the MaizeGo panel (http://www.maizego.org/Resources.html), consisting of 540 lines (Yang et al., 2011). mGWAS has been applied in studies using this population or a subset of this population, and several QTLs and, subsequently, genes have been identified. For instance, the identification of two insertion/deletions within a gene encoding γ-tocopherol methyltransferase (ZmVTE4) and an SNP located ~85 kb upstream of this gene revealed that ZmVTE4 is a major gene involved in natural phenotypic variation in α-tocopherol of maize kernels (Li et al., 2012). Later, a combination of linkage and association analyses suggested a role for non-tocopherol pathway genes in the modulation of natural tocopherol variation, including genes involved in fatty acid metabolism, chlorophyll metabolism, and chloroplast function (Wang et al., 2018a). The genetic basis for natural variation in oil biosynthesis, fatty acid composition (Li et al., 2013), and amino acid metabolism in kernels has also been examined in this population (Deng et al., 2017). Broader analyses of the maize metabolome in different tissues have identified novel genes involved in key processes in the formation of phenolamides and flavonoids (Wen et al., 2014), as well as variations underlying the trehalose, aspartate, and aromatic amino acid pathways (Wen et al., 2018).

Recently, some maize studies have used a new approach that combines metabolic pathway analysis with GWAS to determine the cumulative effects of several genes clustered according to their shared biological function. This approach can potentially find new clues to the genetic basis of a trait by revealing biological insights that may not appear when focusing on only one or a few genes that are most significantly associated with a certain trait (Li et al., 2019a). The pathway-based approach, first developed to study human disease, has just begun to be applied to plants and maize. It has been used specifically to study corn earworm resistance (Warburton et al., 2018) and lipid biosynthesis (Li et al., 2019a). It seems likely, based on the success of these studies, that this approach will gain further utility in maize and indeed in other species.

**CONCLUDING REMARKS**

Recent years have been characterized by impressive advances in the identification of maize metabolites. Despite this progress, as in general for the plant metabolome, it is clear that the majority of maize metabolites cannot be accessed by current profiling methods. Therefore, metabolite identification remains one of the main challenges in metabolomics, regardless of the organism analyzed. As described in this review, several metabolomics studies have performed targeted analysis of specific metabolites or classes, in which case standards can often be used to confirm metabolite identities. Other cases of a more comparative nature focus on features or peaks (their existence, absence, or patterns of abundance across different plant lines), obviating the need for
metabolite identification (Baniasadi et al., 2014). Moreover, the integration of publicly available metabolomics data (see section “integration of omics data”) is currently proving highly informative, with network analysis such as that carried out using Global Natural Product Social Molecular Networking (GNPNS) being applied to LC-MS (Wang et al., 2016) and more recently GC-MS (Aksenov et al., 2020) datasets.

In addition to the identification of new metabolites, the spatial distribution of already known metabolites within organisms has also been of special interest. For instance, as a C4 plant, maize spatially separates its photosynthesis in a well-characterized mechanism for concentrating CO2 in the bundle sheath cells (Figure 1). The operation of such a two-cell pathway and the dynamic subcellular compartmentalization of the maize metabolome underscore the usefulness and need for the development of imaging methods such as MALDI-MSI (see section “methodologies”) that will help us better understand metabolic compartmentalization in this species. The further development of single-cell metabolomics is likely to advance our understanding of how cells work in concert to achieve organismal function.

Metabolomics approaches have been used as an important tool for the identification and functional characterization of metabolism-associated genes in maize (Table 1). The combination of metabolomics and quantitative genetics approaches represents a highly powerful instrument for characterizing the genetic architecture associated with the accumulation of metabolites that are important for plant performance or for the biofortification of maize for human and animal use. The analysis of mutants for candidate genes, linkage mapping, and mGWAS have been used extensively for this purpose. Of particular note in this light is that improving the nutritional composition of what is now the major crop worldwide will be greatly facilitated by metabolomics studies of maize kernels from broad populations and biofluid metabolomics of human cohorts. It will ultimately be possible to better distinguish nutritional benefits by comparing individuals who eat maize of a particular type with those who do not and determining whether this corresponds to their relative incidence of chronic disease.

Continued advances in functional genomics and genetics, the design of new, highly genetically diverse maize populations, and the characterization of the pan-panicoid metabolome should be targets in the near future. This will certainly deepen our understanding of maize metabolism and evolution, subsequently contributing to the improvement of maize toward a more ideal crop by either the de novo domestication of wild relatives, as recently proposed by Fernie and Yan (2019), or by the manipulation of only a specific set of genes.

**FUNDING**

This work was supported by the Bundesministeriums für Bildung und Forschung (BMBF, German Federal Ministry of Education and Research) under the FullThrottle (031B0205B) and Reconstruct (031B0200E) projects.

**ACKNOWLEDGMENTS**

We apologize to those authors whose work could not be discussed due to space limitations. The authors declare no competing interests.

**REFERENCES**

Aksenov, A.A., Laponogov, I., Zhang, Z., Doran, S.L.F., Belluomno, I., Veselkov, D., Bittremieux, W., Nothias, L.F., Nothias-Esposito, M., Maloney, K.N., et al. (2020). Auto-deconvolution and molecular networking of gas chromatography–mass spectrometry data. Nat. Biotechnol. https://doi.org/10.1038/s41587-41020-40700-41583.

Alseekh, S., and Fernie, A.R. (2018). Metabolomics 20 years on: what have we learned and what hurdles remain? Plant J. 94:933–942.

Alvarez, S., Marsh, E.L., Schroeder, S.G., and Schachtman, D.P. (2008). Metabolomic and proteomic changes in the xylem sap of maize under drought. Plant Cell Environ. 31:325–340.

Alves, M.L., Bento-Silva, A., Carbas, B., Gaspar, D., Paulo, M., Brites, C., Mendes-Moreira, P., Brites, C.M., Bronze, M.D.R., Malosetti, M., et al. (2020a). Alleles to enhance antioxidant content in maize - a genome-wide association approach. J. Agric. Food Chem. 68:4051–4061.

Alves, M.L., Bento-Silva, A., Gaspar, D., Paulo, M., Brites, C., Mendes-Moreira, P., Bronze, M.D.R., Malosetti, M., van Eeuwijk, F., and Vaz Patto, M.C. (2020b). Volatiome - genome-wide association study on wholemeal maize flour. J. Agric. Food Chem. 68:7809–7818.

Alves, M.L., Carbas, B., Gaspar, D., Paulo, M., Brites, C., Mendes-Moreira, P., Brites, C.M., Malosetti, M., van Eeuwijk, F., and Vaz Patto, M.C. (2019). Genome-wide association study for kernel composition and flour pasting behavior in wholemeal maize flour. BMC Plant Biol. 19:123.

Amiour, N., Imbaud, S., Clément, G., Agier, N., Zivy, M., Valot, B., Balliau, T., Armengaud, P., Quilleré, I., Cañas, R., et al. (2012). The use of metabolomics integrated with transcriptomic and proteomic studies for identifying key steps involved in the control of nitrogen metabolism in crops such as maize. J. Exp. Bot. 63:5017–5033.

Amiour, N., Imbaud, S., Clément, G., Agier, N., Zivy, M., Valot, B., Balliau, T., Quilleré, I., Tercé-Laforgue, T., Dargel-Graffin, C., et al. (2014). An integrated “omics” approach to the characterization of maize (Zea mays L.) mutants deficient in the expression of two genes encoding cytosolic glutamine synthetase. BMC Genomics 15:1005.

Andorf, C.M., Cannon, E.K., Portwood, J.L., II, Gardiner, J.M., Harper, L.C., Schaeffer, M.L., Braun, B.L., Campbell, D.A., Vinnakota, A.G., Sribalusu, V.V., et al. (2015). MaizeGDB update: new tools, data and interface for the maize model organism database. Nucleic Acids Res. 44:D1195–D1201.

Arrivault, S., Alexandre Moraes, T., Obata, T., Medeiros, D.B., Fernie, A.R., Boulouis, A., Ludwig, M., Lunn, J.E., Borghi, G.L., Schlereth, A., et al. (2019). Metabolite profiles reveal interspecific variation in operation of the Calvin-Benson cycle in both C4 and C3 plants. J. Exp. Bot. 70:1843–1858.

Arrivault, S., Obata, T., Szewczówka, M., Mengin, V., Guenther, M., Hoehne, M., Fernie, A.R., and Stitt, M. (2016). Metabolite pools and carbon flow during C4 photosynthesis in maize: 13CO2 labeling kinetics and cell type fractionation. J. Exp. Bot. 68:283–298.

Asiago, V.M., Hazebroek, J., Harp, T., and Zhong, C. (2012). Effects of genetics and environment on the metabolome of commercial maize hybrids: a multisite study. J. Agric. Food Chem. 60:11498–11508.

Azmach, G., Menkir, A., Spillane, C., and Gedil, M. (2018). Genetic loci controlling carotenoid biosynthesis in diverse tropical maize lines. G3 (Bethesda) 8:1049–1065.

Baniasadi, H., Vlahakis, C., Hazebroek, J., Zhong, C., and Asiago, V. (2014). Effect of environment and genotype on commercial maize,
Plant Communications

hybrids using LC/MS-based metabolomics. J. Agric. Food Chem. 62:1412–1422.

Barnaby, J.Y., Kim, M., Bauchan, G., Bunce, J., Reddy, V., and Sicher, R.C. (2013). Drought responses of foliar metabolites in three maize hybrids differing in water stress tolerance. PLoS One 8:e77145.

Barros, E., Lezar, S., Anttonen, M.J., Van Dijk, J.P., Rohlig, R.M., Kok, E.J., and Engel, K.-H. (2010). Comparison of two GM maize varieties with a near-isogenic non-GM variety using transcriptomics, proteomics and metabolomics. Plant Biotechnol. J. 8:436–451.

Baseggio, M., Murray, M., Magallanes-Lundback, M., Kaczmar, N., Chamness, J., Buckler, E.S., Smith, M.E., DellaPenna, D., Tracy, W.F., and Gore, M.A. (2019). Genome-wide association and genomic prediction models of tocotrienols in fresh sweet corn kernels. Plant Genome 12. https://doi.org/10.3835/plantgenome2018.3806.0038.

Baseggio, M., Murray, M., Magallanes-Lundback, M., Kaczmar, N., Chamness, J., Buckler, E.S., Smith, M.E., DellaPenna, D., Tracy, W.F., and Gore, M.A. (2020). Natural variation for carotenoids in fresh kernels is controlled by uncommon variants in sweet corn. Plant Genome 13:e20008.

Basse, C.W. (2005). Dissecting defense-related and developmental transcriptional responses of maize during Ustilago maydis infection and subsequent tumor formation. Plant Physiol. 138:1774–1784.

Becker, E.-M., Herrfurth, C., Irimisch, S., Kollner, T.G., Feussner, I., Karlovsky, P., and Splivallo, R. (2014). Infection of corn ears by Fusarium spp. induces the emission of volatile sesquiterpenes. J. Agric. Food Chem. 62:5226–5236.

Belò, A., Zheng, P., Luck, S., Shen, B., Meyer, D.J., Li, B., Tingley, S., and Rafalski, A. (2008). Whole genome scan detects an allelic variant of fad2 associated with increased oleic acid levels in maize. Mol. Genet. Genomics 279:1–10.

Benevenuto, R.F., Agapito-Tenfen, S.Z., Vilperte, V., Wikmark, O.G., Block, A.K., Hunter, C.T., Sattler, S.E., Rering, C., McDonald, S., Basset, G.J., and Christensen, S.A. (2017). Molecular responses of genetically modified maize to abiotic stresses as determined through proteomic and metabolomic analyses. PLoS One 12:e0173069.

Block, A., Vaughan, M.M., Christensen, S.A., Alborn, H.T., and Tumlinson, J.H. (2017). Elevated carbon dioxide reduces emission of herbivore-induced volatiles in Zea mays. Plant Cell Environ. 40:1725–1734.

Block, A.K., Hunter, C.T., Sattler, S.E., Rering, C., McDonald, S., Basset, G.J., and Christensen, S.A. (2020). Fighting on two fronts: elevated insect resistance in flooded maize. Plant Cell Environ. 43:223–234.

Block, A.K., Vaughan, M.M., Schmelz, E.A., and Christensen, S.A. (2019). Biosynthesis and function of terpenoid defense compounds in maize (Zea mays). Planta 249:21–30.

Blondel, C., Khelalfa, F., Reynaud, S., Fauvette, F., and Raveton, M. (2016). Effect of organochlorine pesticides exposure on the maize root metabolome assessed using high-resolution magic-angle spinning 1H NMR spectroscopy. Environ. Pollut. 214:539–548.

Bogart, E., and Myers, C.R. (2016). Multiscale metabolic modeling of C4 plants: connecting nonlinear genome-scale models to leaf-scale metabolism in developing maize leaves. PLoS One 11:e0151722.

Broyart, C., Fontaine, J.X., Moliné, R., Caillieu, D., Tercé-Laforgue, T., Dubois, F., Hirel, B., and Mesnard, F. (2010). Metabolic profiling of maize mutants deficient for two glutamine synthetase isoenzymes using 1H-NMR-based metabolomics. Phytochem. Anal. 21:102–109.

Brusamarello-Santos, L.C., Gilard, F., Brulé, L., Quilleré, I., Gourion, B., Ratet, P., Maltemps de Souza, E., Lea, P.J., and Hirel, B. (2017). Metabolic profiling of two maize (Zea mays L.) inbred lines inoculated with the nitrogen fixing plant-interacting bacteria Herbaspirillum seropedicae and Azospirillum brasilense. PLoS One 12:e0174576.

Cañas, R.A., Yesbergeno-Can, S., Simons, M., Chardon, F., Armengaud, P., Quilleré, I., Cukier, C., Gibon, Y., Limami, A.M., Nicolas, S., et al. (2017). Exploiting the genetic diversity of maize using a combined metabolic, enzyme activity profiling, and metabolic modeling approach to link leaf physiology to kernel yield. Plant Cell 29:919–943.

Casas, M.I., Falcone-Ferreya, M.L., Jiang, N., Mejía-Guerra, M.K., Rodriguez, E., Wilson, T., Engelmeier, J., Casati, P., and Grotswold, E. (2016). Identification and characterization of maize salmon silks silks genes involved in insecticidal maysin biosynthesis. Plant Cell 28:1297–1309.

Casati, P., Campi, M., Morrow, D.J., Fernandes, J.F., and Walbot, V. (2011). Transcriptomic, proteomic and metabolomic analysis of UV-B signaling in maize. BMC Genomics 12:321.

Casati, P., and Walbot, V. (2005). Differential accumulation of maysin and rhamnosylisorientin in leaves of high-altitude landraces of maize after UV-B exposure. Plant Cell Environ. 28:788–799.

Çatav, S.S., Elgin, E.S., Daş, Ç., Stark, J.L., and Kuçukakaykuz, K. (2018). NMR-based metabolomics reveals that plant-derived smoke stimulates root growth via affecting carbohydrate and energy metabolism in maize. Metabolomics 14:143.

Chai, Y., Hao, X., Yang, X., Allen, W.B., Li, J., Yan, J., Shen, B., and Li, J. (2012). Validation of DQAT1-2 polymorphisms associated with oil content and development of functional markers for molecular breeding of high-oil maize. Mol. Breed. 29:939–949.

Chatham, L.A., and Juvik, J.A. (2021). Linking anthocyanin diversity, hue, and genetics in purple corn. G3 (Bethesda) 11:jkaa062.

Chen, A.H., and Lipka, A.E. (2016). The use of targeted marker subsets to account for population structure and relatedness in genome-wide association studies of maize (Zea mays L.). G3 (Bethesda) 6:2365–2374.

Chiriñoga, M., Guo, X.H., Campos-Herrera, R., Röder, G., Imperiali, N., Keel, C., Maurhofer, M., and Turlings, T.C.J. (2018). Root-colonizing bacteria enhance the levels of (E)-l-caryophyllene produced by maize roots in response to rootworm feeding. Oecologia 187:459–468.

Christensen, S.A., Huffaker, A., Sims, J., Hunter, C.T., Block, A., Vaughan, M.M., Willett, D., Romero, M., Mylroie, J.E., Williams, W.P., et al. (2018a). Fungal and herbivore elicitation of the novel maize sesquiterpenoid, zealexin A4, is attenuated by elevated CO2. Planta 247:863–873.

Christensen, S.A., Sims, J., Vaughan, M.M., Hunter, C., Block, A., Willett, D., Alborn, H.T., Huffaker, A., and Schmelz, E.A. (2018b). Commercial hybrids and mutant genotypes reveal complex protective roles for inducible terpenoid defenses in maize. J. Exp. Bot. 69:1693–1705.

Christensen, S.A., Santana, E.I.A., Alborn, H.T., Block, A.K., and Chamberlain, C.A. (2021). Metabolomics by UHPLC-HRMS reveals the impact of heat stress on pathogen-elicted immunity in maize. Metabolomics 17:6.

Christie, N., Myburg, A.A., Joubert, F., Murray, S.L., Carstens, M., Lin, Y.-C., Meyer, J., Crampton, B.G., Christensen, S.A., Ntuli, J.F., et al. (2017). Systems genetics reveals a transcriptional network associated with susceptibility in the maize–grey leaf spot pathosystem. Plant J. 89:746–763.

Cocuron, J.-C., Koubaa, M., Kimmelfield, R., Ross, Z., and Alonso, A.P. (2019). A combined metabolomics and fluxomics analysis identifies steps limiting oil synthesis in maize embryos. Plant Physiol. 181:961–975.
Metabolomics in maize biology

Colmsee, C., Mascher, M., Czauderna, T., Hartmann, A., Schlüter, U., Zellerhoff, N., Knuth, A., Brüggemann, A., Pick, T.R., Alter, P., et al. (2012). OPTIMAS-DW: a comprehensive transcriptomics, metabolomics, ionomics, proteomics and phenomics data resource for maize. BMC Plant Biol. 12:245.

Cook, J.P., McMullen, M.D., Holland, J.B., Tian, F., Bradbury, P., Ross-Ibarra, J., Buckler, E.S., and Flint-Garcia, S.A. (2012). Genetic architecture of maize kernel composition in the nested association mapping and inbred association panels. Plant Physiol. 158:824–834.

Cotton, T.E.A., Périat, R., Cameron, D.D., Meselmi, M.A., Schwarzenbacher, R., Rolfe, S.A., and Ton, J. (2019). Metabolic regulation of the maize rhizobiome by benzoxazinoids. ISME J. 13:1647–1658.

Czedik-Eysenberg, A., Arrivault, S., Lohse, M.A., Feil, R., Krohn, N., Encke, B., Nunes-Nesi, A., Fernie, A.R., Lunn, J.E., Sulprise, R., et al. (2016). The interplay between carbon availability and growth in different zones of the growing maize leaf. Plant Physiol. 172:943–967.

Dafoe, N.J., Huffaker, A., Vaughan, M.M., Duehl, A.J., Teal, P.E., and Schmelz, E.A. (2011). Rapidly induced chemical defenses in maize stems and their effects on short-term growth of Ostrinia nubilalis. J. Chem. Ecol. 37:984–991.

Dal’Molin, C.G.d.O., Quek, L.-E., Paflreyman, R.W., Brumbley, S.M., and Nielsen, L.K. (2010). C4GEM, a genome-scale metabolic model to study C4 plant metabolism. Plant Physiol. 154:1871–1885.

de Abreu E Lima, F., Westhues, M., Cuadros-Inostroza, Á., Willmitzer, L., Melchinger, A.E., and Nikoloski, Z. (2017). Metabolic robustness in young roots underpins a predictive model of maize hybrid performance in the field. Plant J. 90:319–329.

Decourcelle, M., Perez-Fons, L., Baulande, S., Steiger, S., Couvelard, L., Hem, S., Zhu, C., Capell, T., Christou, P., Fraser, P., et al. (2015). Combined transcript, proteome, and metabolite analysis of transgenic maize seeds engineered for enhanced carotenoid synthesis reveals pleiotropic effects in core metabolism. J. Exp. Bot. 66:3141–3150.

Degen, T., Dillmann, C., Marion-Poll, F., and Turlings, T.C.J. (2004). High genetic variability of herbivore-induced volatile emission within a broad range of maize inbred lines. Plant Physiol. 135:1928–1938.

Deng, M., Li, D., Luo, J., Xiao, Y., Liu, H., Pan, Q., Zhang, X., Jin, M., Zhao, M., and Yan, J. (2017). The genetic architecture of amino acids dissection by association and linkage analysis in maize. Plant Biotechnol. J. 15:1250–1263.

Deng, M., Zhang, X., Luo, J., Liu, H., Wen, W., Luo, H., Yan, J., and Xiao, Y. (2020). Metabolomics analysis reveals differences in evolution between maize and rice. Plant J. 103:1710–1722.

Dhawi, F., Datta, R., and Ramakrishna, W. (2015). Mycorrhiza and PGPB modulate maize biomass, nutrient uptake and metabolic pathways in maize grown in mining-impacted soil. Plant Physiol. Biochem. 97:390–399.

Diepenbrock, C.H., Illut, D.C., Magallanes-Lundback, M., Kandianis, C.B., Lipka, A.E., Bradbury, P.J., Holland, J.B., Hamilton, J.P., Wooldridge, E., Vaillancourt, B., et al. (2020). Eleven biosynthetic genes explain the majority of natural variation in carotenoid levels in maize grain. Plant Cell, koab032. https://doi.org/10.1093/plcell/koab032.

Diepenbrock, C.H., Kandianis, C.B., Lipka, A.E., Magallanes-Lundback, M., Vaillancourt, B., Góngora-Castillo, E., Wallace, J.G., Cepela, J., Mesberg, A., Bradbury, P.J., et al. (2017). Novel loci underlie natural variation in vitamin E levels in maize grain. Plant Cell 29:2374–2392.

Ding, Y., Huffaker, A., Kollner, T.G., Weckwerth, P., Robert, C.A.M., Spencer, J.L., Lipka, A.E., and Schmelz, E.A. (2017). Selinane volatiles are essential precursors for maize defense promoting fungal pathogen resistance. Plant Physiol. 175:1455–1468.

Ding, Y., Murphy, K.M., Poretsky, E., Mafu, S., Yang, B., Char, S.N., Christensen, S.A., Saldivar, E., Wu, M., Wang, Q., et al. (2019). Multiple genes recruited from hormone pathways partition maize diterpenoid defenses. Nat. Plants 5:1043–1056.

Ding, Y., Weckwerth, P.R., Poretsky, E., Murphy, K.M., Sims, J., Saldivar, E., Christensen, S.A., Char, S.N., Yang, B., Tong, A.-d., et al. (2020). Genetic elucidation of interconnected antibiotic pathways mediating maize innate immunity. Nat. Plants 6:1373–1388.

Djamei, A., Schipper, K., Rabe, F., Ghosh, A., Vincon, V., Kaht, J., Osorio, S., Tohge, T., Fernie, A.R., Feussner, I., et al. (2011). Metabolic priming by a secreted fungal effector. Nature 478:395–398.

Duan, H., Huang, M., Hu, J., and Li, J. (2016). Modification of the fatty acid composition in Arabidopsis and maize seeds using a stearyl-acyl carrier protein desaturase-1 (ZmSAD1) gene. BMC Plant Biol. 16:137.

Dueñas, M.E., Carlucci, L., and Lee, Y.J. (2016). Matrix recrystallization for MALDI-MS imaging of maize lipids at high-spatial resolution. J. Am. Soc. Mass Spectrom. 27:1575–1578.

Dueñas, M.E., Klein, A.T., Alexander, L.E., Yandeval-Nelson, M.D., Nikolau, B.J., and Lee, Y.J. (2017). High spatial resolution mass spectrometry imaging reveals the genetically programmed, developmental modification of the distribution of thylakoid membrane lipids among individual cells of maize leaf. Plant J. 89:825–838.

Elliger, C.A., Chan, B.C., and Waiss, A.C. (1980). Flavonoids as larval growth inhibitors. Naturwissenschaften 67:358–360.

Eloy, N.B., Voorend, W., Lan, W., Saleme, M.D.S., Cesario, I., Vanholme, R., Smith, R.A., Goeminne, G., Pallidis, A., Morreel, K., et al. (2017). Silencing CHALCONE SYNTHASE in maize impedes the incorporation of tricin into lignin and increases lignin content. Plant Physiol. 173:998–1016.

Ermakova, M., Arrivault, S., Giuliani, R., Danila, F., Alonso-Cantabrana, H., Vlad, D., Ishihara, H., Feil, R., Guenther, M., Borghi, G.L., et al. (2020). Installation of C4 photosynthetic pathway enzymes in rice using a single construct. Plant Biotechnol. J. https://doi.org/10.1111/pbi.13487.

Fang, C., and Luo, J. (2019). Metabolic GWAS-based dissection of genetic bases underlying the diversity of plant metabolism. Plant J. 97:91–100.

Fang, H., Fu, X., Wang, Y., Xu, J., Feng, H., Li, W., Xu, J., Jitham, O., Zhang, X., Zhang, L., et al. (2020). Genetic basis of kernel nutritional traits during maize domestication and improvement. Plant J. 101:278–292.

Farré, G., Perez-Fons, L., Decourcelle, M., Breitenbach, J., Hem, S., Zhu, C., Capell, T., Christou, P., Fraser, P.D., and Sandmann, G. (2016). Metabolic engineering of astaxanthin biosynthesis in maize endosperm and characterization of a prototype high oil hybrid. Transgenic Res. 25:477–489.

Feenstra, A.D., Alexander, L.E., Song, Z., Korte, A.R., Yandeau-Nelson, M.D., Nikolau, B.J., and Lee, Y.J. (2017a). Spatial mapping and profiling of metabolite distributions during germination. Plant Physiol. 174:2532–2548.

Feenstra, A.D., Dueñas, M.E., and Lee, Y.J. (2017b). Five micron high resolution MALDI mass spectrometry imaging with simple, interchangeable, multi-resolution optical system. J. Am. Soc. Mass Spectrom. 28:434–442.

Feenstra, A.D., Hansen, R.L., and Lee, Y.J. (2015). Multi-matrix, dual polarity, tandem mass spectrometry imaging strategy applied to a germinated maize seed: toward mass spectrometry imaging of an untargeted metabolome. Analyst 140:7293–7304.
Fenton, M.E., Owens, B.F., Lipka, A.E., Ortiz, D., Tiede, T., Mateos-Hernandez, M., Ferruzzi, M.G., and Rocheford, T. (2018). High-density linkage mapping of vitamin E content in maize grain. Mol. Breed. 38:31.

Fernie, A.R., and Yan, J. (2019). De novo domestication: an alternative route toward new crops for the future. Mol. Plant 12:615–631.

Flint-Garcia, S.A., Thuillet, A.C., Yu, J., Pressoir, G., Romero, S.M., Mitchell, S.E., Doebely, J., Kresovich, S., Goodman, M.M., and Buckler, E.S. (2005). Maize association population: a high-resolution platform for quantitative trait locus dissection. Plant J. 44:1054–1064.

Forieri, I., Hildebrandt, U., and Rostás, M. (2016). Salinity stress effects on direct and indirect defence metabolites in maize. Environ. Exp. Bot. 122:68–77.

Frank, T., Rohlig, R.M., Davies, H.V., Barros, E., and Engel, K.H. (2012). Metabolite profiling of maize kernels–genetic modification versus environmental influence. J. Agric. Food Chem. 60:3005–3012.

Fraser, P.D., Aharoni, A., Hall, R.D., Huang, S., Giovannoni, J.J., Frey, M., Schullehner, K., Dick, R., Fiesselmann, A., and Gierl, A. (2020). Metabolomics should be deployed in the identification and characterization of gene-edited crops. Plant J. 102:897–902.

Frey, M., Schullehner, K., Dick, R., Fiesselmann, A., and Gierl, A. (2009). Benzoxazinoid biosynthesis, a model for evolution of secondary metabolic pathways in plants. Phytochemistry 70:1645–1651.

Fu, Z., Chai, Y., Zhou, Y., Yang, X., Warburton, M.L., Xu, S., Cai, Y., Zhang, D., Li, J., and Yan, J. (2013). Natural variation in the sequence of PSY1 and frequency of favorable polymorphisms among tropical and temperate maize germplasm. TAG. Theoretical and applied genetics. Theoretische Angew. Genet. 126:923–935.

Ganie, A.H., Ahmad, A., Pandey, R., Aref, I.M., Yousuf, P.Y., Ahmad, S., and Iqbal, M. (2015). Metabolite profiling of low-P tolerant and low-P sensitive maize genotypes under phosphorus starvation and restoration conditions. PLoS One 10:e0129520.

García-Flores, M., Juárez-Colunga, S., Montero-Vargas, J.M., López-Arciniega, J.A., Chagolla, A., Tiessen, A., and Winkler, R. (2012). Evaluating the physiological state of maize (Zea mays L.) plants by direct-injection electrospray mass spectrometry (DIESI-MS). Mol. Biosyst. 8:1688–1690.

Gavaghan, C.L., Li, J.V., Hadfield, S.T., Hole, S., Nicholson, J.K., Wilson, I.D., Howe, P.W.A., Stanley, P.D., and Holmes, E. (2011). Application of NMR-based metabolomics to the investigation of salt stress in maize (Zea mays). Phytochem. Anal. 22:214–224.

Ge, F., Hu, H., Huang, X., Zhang, Y., Wang, Y., Li, Z., Zou, C., Peng, H., Li, L., Gao, S., et al. (2017). Metabolicomic and proteomic analysis of maize embryonic callus induced from immature embryo. Sci. Rep. 7:1004.

Gerach, N., Schmitz, J., Polatajko, A., Schluter, U., Fahnenstich, H., Witt, S., Fernie, A.R., Uroic, K., Scholz, U.W.E., Sonnewald, U.W.E., et al. (2015). An integrated functional approach to dissect systemic responses in maize to arbuscular mycorrhizal symbiosis. Plant Cell Environ. 38:1591–1612.

Glauser, G., Marti, G., Villard, N., Doyen, G.A., Wolfender, J.L., Turlings, T.C., and Erb, M. (2011). Induction and detoxification of maize 1,4-benzoxazin-3-ones by insect herbivores. Plant J. 68:901–911.

Gouinguene, S.P., and Turlings, T.C.J. (2002). The effects of abiotic factors on induced volatile emissions in corn plants. Plant Physiol. 129:1296–1307.

Gui, S., Yang, L., Li, J., Luo, J., Xu, X., Yuan, J., Chen, L., Li, W., Yang, X., Wu, S., et al. (2020). ZEAMAP, a comprehensive database adapted to the maize multi-omics era. iScience 23:101241.

Guo, R., Shi, L., Yan, C., Zhong, X., Gu, F., Liu, Q., Xia, X., and Li, H. (2017). Ionomic and metabolic responses to neutral salt or alkaline salt stresses in maize (Zea mays L.) seedlings. BMC Plant Biol. 17:41.

Handrick, V., Robert, C.A., Ahern, K.R., Zhou, S., Machado, R.A., Maag, D., Glauser, G., Fernandez-Penny, F.E., Chandran, J.N., Rodgers-Melnik, E., et al. (2016). Biosynthesis of 8-O-methylated benzoxazinoid defense compounds in maize. Plant Cell 28:1682–1700.

Harjes, C.E., Rocheford, T.R., Bai, L., Brutnell, T.P., Kandianis, C.B., Sowiński, S.G., Stapleton, A.E., Vallabhāneni, R., Williams, M., Wurtzel, E.T., et al. (2008). Natural genetic variation in lycopene epsilon cyclase tapped for maize biofortification. Science 319:330–333.

Harrigan, G.G., Stork, L.G., Riordan, S.G., Reynolds, T.L., Ridley, W.P., Masucci, J.D., Maclusa, S., Halls, S.C., Orth, R., Smith, R.G., et al. (2007). Impact of genetics and environment on nutritional and metabolite components of maize grain. J. Agric. Food Chem. 55:6177–6185.

Harrigan, G.G., Venkatesh, T.V., Leibman, M., Blankenship, J., Perez, T., Halls, S., Chassy, A.W., Fiehn, O., Xu, Y., and Goodacre, R. (2016). Evaluation of metabolomics profiles of grain from maize hybrids derived from near-isogenic GM positive and negative segregant inbreds demonstrates that observed differences cannot be attributed unequivocally to the GM trait. Metabolomics 12:82.

Hatch, M.D. (1987). C4 photosynthesis: a unique eld of modified biochemistry, anatomy and ultrastructure. Biochim. Biophys. Acta 895:81–106.

Henry, C., Bledsoe, S.W., Griffiths, C.A., Kollman, A., Paul, M.J., Sakr, S., and Lagrimini, L.M. (2015). Differential role for trehalose metabolism in salt-stressed maize. Plant Physiol. 169:1072–1089.

Hibberd, J.M., Sheehy, J.E., and Langdale, J.A. (2008). Using C4 photosynthesis to increase the yield of rice-rationale and feasibility. Curr. Opin. Plant Biol. 11:228–231.

Hu, L., Robert, C.A.M., Cadot, S., Zhang, X., Ye, M., Li, B., Manzo, D., Chervet, N., Steinger, T., van der Heijden, M.G.A., et al. (2018). Root exudate metabolites drive plant-soil feedbacks on growth and defense by shaping the rhizosphere microbiota. Nat. Commun. 9:2738.

Hu, Y., Xie, W., and Chen, B. (2020). Arbuscular mycorrhiza improved drought tolerance of maize seedlings by altering photosystem II efficiency and the levels of key metabolites. Chem. Biol. Technol. Agric. 7:20.

Huffaker, A., Kaplan, F., Vaughan, M.M., Dafoe, N.J., Ni, X., Rocca, J.R., Alborn, H.T., Pea, P.E., and Schmelz, E.A. (2011). Novel acidic sesquiterpenoids constitute a dominant class of pathogen-induced phytoalexins in maize. Plant Physiol. 156:2082–2097.

Jin, M., Zhang, X., Zhao, M., Deng, M., Du, Y., Zhou, Y., Wang, S., Tohge, T., Fernie, A.R., Willmitzer, L., et al. (2017). Integrated genomics-based mapping reveals the genetics underlying maize flavonoid biosynthesis. BMC Plant Biol. 17:17.

Kandianis, C.B., Stevens, R., Liu, W., Palacios, N., Montgomery, K., Pixley, K., White, W.S., and Rocheford, T. (2013). Genetic architecture controlling variation in grain carotenoid composition and concentrations in two maize populations. TAG. Theoretical and applied genetics. Theoretische Angew. Genet. 126:2879–2895.

Kaur, S., and Suseela, V. (2020). Unraveling arbuscular mycorrhiza-induced changes in plant primary and secondary metabolome. Metabolites 10:335.

Kollner, T.G., Schnee, C., Gershenzon, J., and Degenhardt, J. (2004). The sesquiterpene hydrocarbons of maize (Zea mays) form five groups with distinct developmental and organ-specific distributions. Phytochemistry 65:1895–1902.

Kollner, T.G., Schnee, C., Li, S., Svatoš, A., Schneider, B., Gershenzon, J., and Degenhardt, J. (2008). Protonation of a neutral
Metabolomics in maize biology

(S)-β-bisabolene intermediate is involved in (S)-β-macrocarpene formation by the maize sesquiterpene synthases TPS6 and TPS11*. J. Biol. Chem. 283:20779–20788.

Korte, A.R., Yandeau-Nelson, M.D., Nikolau, B.J., and Lee, Y.J. (2015). Subcellular-level resolution MALDI-MS imaging of maize leaf metabolites by MALDI-linear ion trap-Orbitrap mass spectrometer. Anal Bioanal. Chem. 407:2301–2309.

Kudjordjie, E.N., Sapkota, R., Steffensen, S.K., Fomsgaard, I.S., and Nicolaelsen, M. (2019). Maize synthesized benzoazinoids affect the host associated microbiome. Microbiome 7:59.

Lago, C., Cassani, E., Zanzi, C., Landoni, M., Trovato, R., and Piliu, R. (2014). Development and study of a maize cultivar rich in anthocyanins: coloured polenta, a new functional food. Plant Breed 133:210–217.

Leon, C., Rodriguez-Meizoso, I., Lucio, M., Garcia-Cañas, V., Ibáñez, E., Schmitt-Kopplin, P., and Cifuentes, A. (2009). Metabolomics of transgenic maize combining Fourier transform-ion cyclotron resonance-mass spectrometry, capillary electrophoresis-mass spectrometry and pressurized liquid extraction. J. Chromatogr. A 1218:7314–7323.

Levandi, T., Leon, C., Kaljurand, M., García-Canas, V., and Cifuentes, A. (2008). Capillary electrophoresis time-of-flight mass spectrometry for comparative metabolomics of transgenic versus conventional maize. Anal Chem. 80:6329–6335.

Li, B., Fan, R., Sun, G., Sun, T., Fan, Y., Bai, S., Guo, S., Huang, S., Liu, J., Zhang, H., et al. (2021). Flavonoids improve drought tolerance of maize seedlings by regulating the homeostasis of reactive oxygen species. Plant Soil https://doi.org/10.1007/s11104-020-04814-11108.

Li, H., Peng, Z., Yang, X., Wang, W., Fu, J., Wang, J., Han, Y., Chai, Y., Guo, T., Yang, N., et al. (2013). Genome-wide association study dissects the genetic architecture of oil biosynthesis in maize kernels. Nat. Genet. 45:43–50.

Li, K., Pipatala, V.R., Shaik, R., Datta, R., and Ramakrishna, W. (2014). Integrated metabolomic and proteomic approaches dissect the effect of metal-resistant bacteria on maize biomass and copper uptake. Environ. Sci. Technol. 48:1184–1193.

Li, H., Thrash, A., Tang, J.D., He, L., Yan, J., and Warburton, M.L. (2019a). Leveraging GWAS data to identify metabolic pathways and networks involved in maize lipid biosynthesis. Plant J. 98:853–863.

Li, K., Wen, W., Alseekh, S., Yang, X., Guo, H., Li, W., Wang, L., Pan, Q., Zhan, W., Liu, J., et al. (2019b). Large-scale metabolite quantitative trait locus analysis provides new insights for high-quality maize improvement. Plant J. 99:216–230.

Li, M., Sui, N., Lin, L., Yang, Z., and Zhang, Y. (2019c). Transcriptomic profiling revealed genes involved in response to cold-maize. Funct. Plant Biol. 46:830–844.

Li, L., Li, H., Li, Q., Yang, X., Zheng, D., Warburton, M., Chai, Y., Zhang, P., Guo, Y., Yan, J., et al. (2011). An 11-bp insertion in Zea mays fatb reduces the palmitic acid content of fatty acids in maize grain. PLoS One 6:e24699.

Li, P., Wang, A., Du, W., Mao, L., Wei, Z., Wang, S., Yuan, H., Ji, R., and Zhao, L. (2020a). Insight into the interaction between Fe-based nanomaterials and maize (Zea mays) plants at metabolic level. Sci. Total Environ. 738:139795.

Li, Z., Zhu, H., Song, Q., Chen, H.Y., Harmon, F.G., and Chen, Z.J. (2020b). Temporal regulation of the metabolome and proteome in photosynthetic and photosynthetic respiratory pathways contributes to maize heterosis. Plant Cell 32:3706–3722.

Li, Q., Wang, Z., Li, S., Qiu, Y., Yang, X., Xu, J., Zhang, D., Han, Y., Li, L., Zhang, Z., Gao, S., Li, J., et al. (2012). Genome-wide association studies identified three independent polymorphisms associated with tocopherol content in maize kernels. PLoS One 7:e36807.

Li, Y., Li, P., Wang, T., Zhang, F., Huang, X.X., and Hou, B.K. (2018). The maize secondary metabolism glycosyltransferase UFGT2 modifies flavonols and contributes to plant acclimation to abiotic stresses. Ann. Bot-london 122:1203–1217.

Liang, L., Liu, S., Wang, T., Li, F., Cheng, J., Lai, J., Qin, F., Li, Z., Wang, X., and Jiang, C. (2021). Metabolomics-driven gene mining and genetic improvement of tolerance to salt-induced osmotic stress in maize. New Phytol. https://doi.org/10.1111/nph.17323.

Lin, F., Zhou, L., He, B., Zhang, X., Dai, H., Qian, Y., Ruan, L., and Zhao, H. (2019). QTL mapping for maize starch content and candidate gene prediction combined with co-expression network analysis. TAG. Theor. Appl. Genet. Theoretische Angew. Genetik 132:1931–1941.

Lin, H., Arrivault, S., Coe, R.A., Karki, S., Covshoff, S., Bagunu, E., Lunn, J.E., Stitt, M., Furbank, R.T., Hibberd, J.M., et al. (2020). A partial C4 photosynthetic biochemical pathway in rice. Front Plant Sci. 11:564463.

Lipka, A.E., Gore, M.A., Magallanes-Lundback, M., Mesberg, A., Lin, H., Tiede, T., Chen, C., Buell, C.R., Buckler, E.S., Rocheford, T., et al. (2013). Genome-wide association study and pathway-level analysis of tocochromanol levels in maize grain. G3 (Bethesda, Md. 3:1287–1299.

Liu, H.-J., Liu, X., Xu, J., Zhang, Q., Zhang, M., Jin, M., Peng, Y., Yan, J., Han, B., Liu, J., et al. (2020a). High-throughput CRISPR/Cas9 mutagenesis streamlines trait gene identification in maize. Plant Cell 32:1397–1413.

Liu, J., Fernie, A.R., and Yan, J. (2020b). The past, present, and future of maize Improvement: domestication, genomics, and functional genomic routes toward crop enhancement. Plant Commun. 1:100010.

Liu, H., Wang, F., Xiao, Y., Tian, Z., Wen, W., Zhang, X., Chen, X., Liu, N., Li, W., Liu, L., et al. (2016a). MODEM: multi-omics data envelopment and mining in maize. Database https://doi.org/10.1093/database/baw1117.

Liu, N., Xue, Y., Guo, Z., Li, W., and Tang, J. (2016b). Genome-wide association study identifies candidate genes for starch content regulation in maize kernels. Front. Plant Sci. 7:1046.

López-Malvar, A., Butrón, A., Samayoa, L.F., Figueroa-Garrido, D.J., Malvar, R.A., and Santiago, R. (2019). Genome-wide association analysis for maize stem cell wall-boundhydroxycinnamates. BMC Plant Biol. 19:519.

Luo, B., Ma, P., Nie, Z., Zhang, X., He, X., Ding, X., Feng, X., Lu, Q., Ren, Z., Lin, H., et al. (2019). Metabolite profiling and genome-wide association studies reveal response mechanisms of phosphorus deficiency in maize seedling. Plant J. 97:947–969.

Luo, J. (2015). Metabolite-based genome-wide association studies in plants. Curr. Opin. Plant Biol. 24:31–38.

Mafu, S., Ding, Y., Murphy, K.M., Yaacoobi, O., Addison, J.B., Wang, Q., Shen, Z., Briggs, S.P., Bohlmann, J., Castro-Falcon, G., et al. (2018). Discovery, biosynthesis and stress-related accumulation of dolabradiene-derived defenses in maize. Plant Physiol. 176:2677–2690.

Manetti, C., Bianchetti, C., Casciani, L., Castro, C., Di Cocco, M.E., Michelli, A., Motto, M., and Conti, F. (2006). A metabonomic study of transgenic maize (Zea mays) seeds revealed variations in osmylotes and branched amino acids. J. Exp. Bot. 57:2613–2625.

Mao, Y., Botella, J.R., Liu, Y., and Zhu, J.-K. (2019). Gene editing in plants: progress and challenges. Natl. Sci. Rev. 6:421–437.

Marti, G., Erb, M., Boccard, J., Glauser, G., Doyen, I., Villard, N., Robert, C.A.M., Turlings, T.C.J., Rudaz, S., and Wollender, J.-L. (2013). Metabolomics reveals herbivore-induced metabolites of resistance and susceptibility in maize leaves and roots. Plant Cell Environ. 36:621–639.
Plant Communications

Matsuoka, Y., Vigouroux, Y., Goodman, M.M., Sanchez, G.J., Buckler, E., and Doebley, J. (2002). A single domestication for maize shown by multilocus microsatellite genotyping. Proc. Natl. Acad. Sci. U S A 99:6080–6084.

McLoughlin, F., Augustine, R.C., Marshall, R.S., Li, F., Kirkpatrick, L.D., Otegui, M.S., and Vierstra, R.D. (2018). Maize multi-omics reveal roles for autophagic recycling in proteome remodelling and lipid turnover. Nat. Plants 4:1056–1070.

Medeiros, D.B., Barros, J.A.S., Fernie, A.R., and Araujo, W.L. (2017). Metabolomics in maize biology. Plant Communications 2, 100187, July 12 2021.

Meihls, L.N., Handrick, V., Glauser, G., Barbier, H., Kaur, H., Haribal, M.M., Lipka, A.E., Gershenzon, J., Buckler, E.S., Erb, M., et al. (2013). Natural variation in maize aphid resistance is associated with 2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one glucoside methyltransferase activity. Plant Cell 25:2341–2355.

Mesnage, R., Agapito-Tenfen, S.Z., Vilperte, V., Renney, G., Ward, M., Seralini, G.-E., Rodari, R.O., and Antoniou, M.N. (2016). An integrated multi-omics analysis of the NK603 Roundup-tolerant GM maize reveals metabolism disturbances caused by the transformation process. Sci. Rep. 6:37855.

Meyer, J., Berger, D.K., Christensen, S.A., and Murray, S.L. (2017). RNA-seq analysis of resistant and susceptible sub-tropical maize lines reveals a role for kauralexins in resistance to grey leaf spot disease, caused by Ceratocystis zeina. BMC Plant Biol. 17:197.

Moco, S., Bino, R.J., Vorst, O., Verhoeven, H.A., de Groot, J., van Beek, T.A., Vervoort, J., and de Vos, C.H. (2006). A liquid chromatography-mass spectrometry-based metabolome database for tomato. Plant Physiol. 141:1205–1218.

Morant, A.V., Jørgensen, K., Jørgensen, C., Paquette, S.M., Sanchez-Perez, R., Moller, B.L., and Bak, S. (2008). beta-Glucosidasases as detonators of plant chemical defense. Phytochemistry 69:1785–1813.

Murphy, K.M., Edwards, J., Louie, K.B., Bowen, B.P., Sundaresan, V., Northen, T.R., and Zerbe, P. (2021). Bioactive diterpenoids impact the composition of the root-associated microbiome in maize (Zea mays). Sci. Rep. 11:333.

Noblet, A., Leymarie, J., and Bailly, C. (2017). Chilling temperature models remodel phospholipidome of Zea mays seeds during imbibition. Sci. Rep. 7:8886.

O’Neill, K.C., and Lee, Y.J. (2020). Visualizing genotypic and developmental differences of free amino acids in maize roots with mass spectrometry imaging. Front. Plant Sci. 11:639.

Obata, T., and Fernie, A.R. (2012). The use of metabolomics to dissect plant responses to abiotic stresses. Cell Mol. Life Sci. 69:3225–3243.

Obata, T., Witt, S., Lisej, J., Palacios-Rojas, N., Florez-Sarasa, I., Yousfi, S., Araus, J.L., Cairns, J.E., and Fernie, A.R. (2015). Metabolite profiles of maize leaves in drought, heat, and combined stress field trials reveal the relationship between metabolism and grain yield. Plant Physiol. 169:2665–2683.

OECD. (2018). Concentration in Seed Markets: Potential Effects and Policy Responses (Paris: OECD Publishing).

Owens, B.F., Lipka, A.E., Magallanes-Lundback, M., Tiede, T., Diepenbrock, C.H., Kandianis, C.B., Kim, E., Cepela, J., Mateos-Hernandez, M., Buell, C.R., et al. (2014). A foundation for provitamin A biofortification of maize: genome-wide association and genomic prediction models of carotenoid levels. Genetics 198:1699–1716.

Pavlík, M., Pavlíková, D., and Pavlíková, S. (2010). Infrared spectroscopy-based metabolic analysis of maize growing under different nitrogen nutrition. Plant Soil Environ. 56:533–540.

Pick, T.R., Brautigam, A., Schluter, U., Denton, A.K., Colmsee, C., Scholz, U., Fahnentich, H., Pieruschka, R., Rascher, U., Sonnewald, U., et al. (2011). Systems analysis of a maize leaf developmental gradient redefines the current C4 model and provides candidates for regulation. Plant Cell 23:4208–4220.

Piperno, D.R., Ranere, A.J., Holst, I., Iriarte, J., and Dickau, R. (2009). Starch grain and phytolith evidence for early ninth millennium B.P. maize from the Central Balsas River Valley, Mexico. Proc. Natl. Acad. Sci. U S A 106:5019–5024.

Pouvreau, B., Baud, S., Vernoud, V., Morin, V., Py, C., Hendrot, G., Pichon, J.-P., Rouster, J., Paul, W., and Rogowsky, P.M. (2011). Duplicate maize Wrinkled1 transcription factors activate target genes involved in seed oil biosynthesis. Plant Physiol. 156:674–686.

Qu, M., Chen, G., Bunce, J.A., Zhu, X., and Sicher, R.C. (2018). Systematic biology analysis on photosynthetic carbon metabolism of maize leaf following sudden heat shock under elevated CO2. Sci. Rep. 8:7849.

Rabe, F., Ajami-Rashidi, Z., Doeblemann, G., Kakhm, R., and Djamei, A. (2013). Degradation of the plant defence hormone salicylic acid by the biotrophic fungus Ustilago maydis. Mol. Microbiol. 89:179–188.

Randilla, L.G. (2020). The application of metabolomics for the study of cereal corn (Zea mays L.). Metabolites 10:300.

Rao, J., Cheng, F., Hu, C., Quan, S., Lin, H., Wang, J., Chen, G., Zhao, X., Alexander, D., Guo, L., et al. (2014). Metabolic map of mature maize kernels. Metabolomics 10:775–787.

Richter, J.A., Erban, A., Kopka, J., and Zörb, C. (2015). Metabolic contribution to salt stress in two maize hybrids with contrasting resistance. Plant Sci. 233:107–115.

Riedelsheimer, C., Brotman, Y., Méret, M., Melchinger, A.E., and Willmitzer, L. (2013). The maize leaf lipidome shows multilevel genetic control and high predictive value for agronomic traits. Sci. Rep. 3:2479.

Riedelsheimer, C., Lisec, J., Czedik-Eysenberg, A., Altmann, T., Stitt, M., Willmitzer, L., and Melchinger, A.E. (2012). Genome-wide association mapping of leaf metabolic profiles for dissecting complex traits in maize. Proc. Natl. Acad. Sci. U S A 109:8872–8877.

Rohlig, R.M., Eder, J., and Engel, K.H. (2009). Metabolite profiling of maize grain: differentiation due to genetics and environment. Metabolomics 5:459.

Rozier, C., Erban, A., Hamzaoui, J., Prigent-Combaret, C., Comte, G., Kopka, J., Czernas, S., and Legendre, L. (2016). Xylem sap metabolite profile changes during phytostimulation of maize by the plant growth-promoting rhizobacterium, Azospirillum lipoferus CRT1. Metabolomics 6:182.

Rozier, C., Hamzaoui, J., Lemoine, D., Czernas, S., and Legendre, L. (2017). Field-based assessment of the mechanism of maize yield enhancement by Azospirillum lipoferus CRT1. Sci. Rep. 7:7416.

Saha, R., Suthers, P.F., and Maranas, C.D. (2011). Zea mays iRS1563: a comprehensive genome-scale metabolic reconstruction of maize metabolism. PLoS One 6:e21784.

Schafer, N., Steinhauser, D., Strelkov, S., Schomburg, D., Allison, G., Moritz, T., Lundgren, K., Roessner-Tunali, U., Forbes, M.G., Willmitzer, L., et al. (2006). GC-MS libraries for the rapid identification of metabolites in complex biological samples. FEBS Lett. 579:1332–1337.

Schluter, U., Colmsee, C., Scholz, U., Brautigam, A., Weber, A.P.M., Zellerhoff, N., Bucher, M., Fahnentich, H., and Sonnewald, U. (2013). Adaptation of maize source leaf metabolism to stress related disturbances in carbon, nitrogen and phosphorus balance. BMC Genomics 14:442.

Schluter, U., and Weber, A.P.M. (2020). Regulation and evolution of C4 photosynthesis. Annu. Rev. Plant Biol. 71:183–215.
Metabolomics in maize biology

Seaver, S.M.D., Bradbury, L.M.T., Frelin, O., Zarecki, R., Ruppin, E., Hanson, A.D., and Henry, C.S. (2015). Improved evidence-based genome-scale metabolic models for maize leaf, embryo, and endosperm. Front. Plant Sci. 6:142.

Setter, T.L., Yan, J., Warburton, M., Ribaut, J.M., Xu, Y., Sawkins, M., Buckler, E.S., Zhang, Z., and Gore, M.A. (2011). Genetic association mapping identifies single nucleotide polymorphisms in genes that affect asbiscis acid levels in maize floral tissues during drought. J. Exp. Bot. 62:701–716.

Sharma, M., Cortes-Cruz, M., Ahern, K.R., McMullen, M., Brutnell, T.P., and Chopra, S. (2011). Identification of the pr1 gene product completes the anthocyanin biosynthesis pathway of maize. Genetics 188:69–79.

Sheng, M., Tang, M., Zhang, F., and Huang, Y. (2011). Influence of arbuscular mycorrhiza on organic solutes in maize leaves under salt stress. Mycorrhiza 21:423–430.

Sicher, R.C., and Barnaby, J.Y. (2012). Impact of carbon dioxide enrichment on the responses of maize leaf transcripts and metabolites to water stress. Physiol. Plant 144:238–253.

Simons, M., Saha, R., Amiour, N., Kumar, A., Guillard, L., Clément, G., Miquel, M., Li, Z., Mouille, G., Lea, P.J., et al. (2014). Assessing the metabolic impact of nitrogen availability using a compartmentalized maize leaf genome-scale model. Plant Physiol. 166:1659–1674.

Singh, N., Vasudev, S., Yadava, D.K., Chaudhary, D.P., and Prabhu, K.V. (2014). Oil improvement in maize: potential and prospects. In: Biotechnology of Isoprenoids, J. Schrader and J. Nielsen, eds. (New Delhi: Springer), pp. 77–82.

Suwarno, W.B., Pixley, K.V., Palacios-Rojas, N., Kaeppler, S.M., and Babu, R. (2015). Genome-wide association analysis reveals new targets for carotenoid biofortification in maize. TAG. Theoretical and applied genetics. Theoretische Angew. Genetik 128:851–864.

Suzuki, M., Wu, S., Mimura, M., Alseekh, S., Fernie, A.R., Hanson, A.D., and McCarty, D.R. (2020). Construction and applications of a B vitamin genetic resource for investigation of vitamin-dependent metabolism in maize. Plant J. 101:442–454.

Szalma, S.J., Buckler, E.S., Snook, M.E., and McMullen, M.D. (2005). Association analysis of candidate genes for masin and chlorogenic acid accumulation in maize silks. TAG. Theoretical and applied genetics. Theoretische Angew. Genetik 110:1324–1333.

Tanaka, S., Brefort, T., Neidig, N., Djamei, A., Kahnt, J., Vermorris, W., Koenig, S., Feussner, K., Feussner, I., and Kahmann, R. (2014). A secreted Ustilago maydis effector promotes virulence by targeting anthocyanin biosynthesis in maize. eLife 3:e01355.

Tanaka, S., Schweiger, G., Rossel, N., Fukada, F., Thines, M., and Kahmann, R. (2019). Neofunctionalization of the secreted Tin2 effector in the fungal pathogen Ustilago maydis. Nat. Microbiol. 4:251–257.

Tang, W., Hazebroek, J., Zhong, C., Harp, T., Vlahakis, C., Baumhover, B., and Asiago, V. (2017). Effect of genetics, environment, and phenotype on the metabolome of maize hybrids using GC/MS and LC/MS. J. Agric. Food Chem. 65:5215–5225.

Tholl, D. (2015). Biosynthesis and biological functions of terpenoids in plants. In: Biotechnology of Isoprenoids, J. Schrader and J. Bohmann, eds. (Cham: Springer International Publishing), pp. 63–106.

Thornsberry, J.M., Goodman, M.M., Doebely, J., Kresovich, S., Nielsen, D., and Buckler, E.S. (2001). Dwarf8 polymorphisms associate with variation in flowering time. Nat. Genet. 28:286–289.

Trucillo Silva, I., Abbaraju, H.K.R., Fallis, L.P., Liu, H., Lee, M., and Dhugga, K.S. (2017). Biochemical and genetic analyses of N metabolism in maize testcross seedlings: 1. Leaves. TAG. Theoretical and applied genetics. Theoretische Angew. Genetik 130:1453–1466.

Trucillo Silva, I., Abbaraju, H.K.R., Fallis, L.P., Liu, H., Lee, M., and Dhugga, K.S. (2018). Biochemical and genetic analyses of N metabolism in maize testcross seedlings: 2. Roots. TAG. Theoretical and applied genetics. Theoretische Angew. Genetik 131:1191–1205.

Urrutia, M., Blein-Nicolas, M., Prigent, S., Bernillon, S., Deborde, C., Balliau, T., Maucourt, M., Jacob, D., Ballias, P., Bénard, C., et al. (2021). Maize metabolome and proteome responses to controlled cold stress partly mimic early-sowing effects in the field and differ from those of Arabidopsis. Plant Cell Environ., 1–18.

Václavík, L., Ovsená, J., Kucera, L., Hodek, J., Demnerová, K., and Hajšlová, J. (2013). Application of ultra-high performance liquid chromatography-mass spectrometry (UHPLC-MS) metabolic fingerprinting to characterise GM and conventional maize varieties. Czech J. Food Sci. 31:368–375.

Vaughan, M.M., Huffaker, A., Schmelz, E.A., Dafoe, N.J., Christensen, S., Sims, J., Martins, V.F., Sweribilow, J.A.Y., Romero, M., Albom, H.T., et al. (2014). Effects of elevated [CO2] on maize defence against mycotoxicogenic Fusarium verticilloides. Plant Cell Environ. 37:2691–2706.

Vaughan, M.M., Huffaker, A., Schmelz, E.A., Dafoe, N.J., Christensen, S.A., McAuslane, H.J., Albom, H.T., Allen, L.H., and Teal, P.E. (2016). Interactive effects of elevated [CO2] and drought on the maize phytochemical defence response against mycotoxicogenic Fusarium verticilloides. PLoS One 11:e0159270.

Venkatesh, T.V., Chassy, A.W., Fiehn, O., Flint-Garcia, S., Zeng, Q., Skogerson, K., and Harrigan, G.G. (2016). Metabolomic assessment of key maize resources: GC-MS and NMR profiling of grain from B73 hybrids of the nested association mapping (NAM) founders and of geographically diverse landraces. J. Agric. Food Chem. 64:2162–2172.
Plant Communications

Vigouroux, Y., McMullen, M., Hittinger, C.T., Houchins, K., Schulz, L., Kresovich, S., Matsuoka, Y., and Doebly, J. (2002). Identifying genes of agronomic importance in maize by screening microsatellites for evidence of selection during domestication. Proc. Natl. Acad. Sci. U S A 99:9650–9655.

Vinci, G., Cozzolino, V., Mazzei, P., Monda, H., Savvy, D., Drosos, M., and Piccolo, A. (2018). Effects of Bacillus amylolyticus and different phosphorus sources on maize plants as revealed by NMR and GC-MS based metabolomics. Plant Soil 429:437–457.

Virlouvet, L., Jacquetmot, M.P., Gerentes, D., Corti, H., Bouton, S., Gilard, F., Valot, B., Trouverie, J., Tcherkez, G., Falque, M., et al. (2011). The ZmASR1 protein influences branched-chain amino acid biosynthesis and maintains kernel yield in maize under water-limited conditions. Plant Physiol. 157:917–936.

Waiss, A.C., Jr., Chan, B.G., Elliger, C.A., McMullan, B.R., McMillian, W.W., Widstrom, N.W., Zuber, M.S., and Keaster, A.J. (1979). Maysin, a flavone glycoside from corn silks with antibiotic activity toward corn earworm. J. Econ. Entomol. 72:256–258.

Walker, V., Bertrand, C., Bellvert, F., Moënne-Loccoz, Y., Bally, R., Vinci, G., Cozzolino, V., Mazzei, P., Monda, H., Savy, D., Drosos, M., Wang, Y., Br, Wang, M., Carver, J.J., Phelan, V.V., Sanchez, L.M., Garg, N., Peng, Y., Xu, S., Fan, Y., Liu, N., Zhan, W., Liu, H., Xiao, Y., Li, K., Pan, W., Shi, J., Xie, Q., Jiang, Y., Yu, N., and Wang, E. (2017). Nutrient exchange and regulation in arbuscular mycorrhizal symbiosis. Mol. Plant 10:1147–1158.

Wang, H., Xu, S., Fan, Y., Liu, N., Zhan, W., Liu, H., Xiao, Y., Li, K., Pan, Q., Li, W., et al. (2018a). Beyond pathways: genetic dissection of tocopherol content in maize kernels by combining linkage and association analyses. Plant Biotechnol. J. 16:1464–1475.

Wang, X.-J., Zhang, X., Yang, J.-T., and Wang, Z.X. (2018b). Effect on transcriptome and metabolome of stacked transgenic maize containing insecticidal cry and glyphosate tolerance epsps genes. Plant J. 93:1007–1016.

Warburton, M.L., Womack, E.D., Tang, J.D., Thrash, A., Smith, J.S., Xu, W., Murray, S.C., and Williams, W.P. (2018). Genome-wide association and metabolic pathway analysis of corn earworm resistance in maize. Plant Genome 11:170069.

Weissmann, S., Ma, F., Furuyama, K., Gierse, J., Berg, H., Shao, Y., Taniguchi, M., Allen, D.K., and Brutnell, T.P. (2016). Interactions of 



Metabolomics in maize biology

Wen, W., Alseekh, S., and Fernie, A.R. (2020). Conservation and diversification of flavonoid metabolism in the plant kingdom. Curr. Opin. Plant Biol. 55:100–108.

Wen, W., Brotman, Y., Willmitzer, L., Yan, J., and Fernie, A.R. (2016a). Broadening our portfolio in the genetic improvement of maize chemical composition. Trends Genet. 32:459–469.

Wen, W., Liu, H., Zhou, Y., Jin, M., Yang, N., Li, D., Luo, J., Xiao, Y., Pan, Q., Tohge, T., et al. (2016b). Combining quantitative genetics approaches with regulatory network analysis to dissect the complex metabolism of the maize kernel. Plant Physiol. 170:136–146.

Wen, W., Jin, M., Li, K., Liu, H., Xiao, Y., Zhao, M., Alseekh, S., Li, W., de Abreu e Lima, F., Brotman, Y., et al. (2019). An integrated multi-layered analysis of the metabolic networks of different tissues uncovers key genetic components of primary metabolism in maize. Plant J. 93:1116–1128.

Wen, W., Li, D., Li, X., Gao, Y., Li, W., Li, H., Liu, J., Liu, H., Chen, W., Luo, J., et al. (2014). Metabolome-based genome-wide association study of maize kernel lead to novel biochemical insights. Nat. Commun. 5:3438.

White, P.J., Pollak, L.M., and Duvick, S. (2007). Improving the fatty acid composition of corn oil by using germplasm introgression. Lipid Technol. 19:35–38.

Wingler, A., Walker, R.P., Chen, Z.-H., and Leegeood, R.C. (1999). Phosphoenolpyruvate carboxylase is involved in the decarboxylation of aspartate in the bundle sheath of maize. Plant Physiol. 120:539–545.

Witt, S., Galicia, L., Lisej, J., Cairns, J., Tiessen, A., Araus, J.L., Palacios-Rojas, N., and Fernie, A.R. (2012). Metabolic and phenotypic responses of greenhouse-grown maize hybrids to experimentally controlled drought stress. Mol. Plant 5:401–417.

Wong, J.C., Lambert, R.J., Wurtzel, E.T., and Rocheford, T.R. (2004). QTL and candidate genes phytorepine synthase and ζ-carotene desaturase associated with the accumulation of carotenoids in maize. TAG. Theor. Appl. Genet. Theoretische Angew. Genetik 108:349–359.

Xiao, Y., Liu, H., Wu, L., Warburton, M., and Yan, J. (2017). Genome-wide association studies in maize: praise and stargaze. Mol. Plant 10:359–374.

Xu, G., Cao, J., Wang, X., Chen, Q., Jin, W., Li, Z., and Tian, F. (2019). Evolutionary metabolomics identifies substantial metabolic divergence between maize and its wild ancestor, Teosinte. Plant Cell 31:1990–2009.

Yan, J., Kandasivis, C.B., Harjes, C.E., Bai, L., Kim, E.-H., Yang, X., Skinner, D.J., Fu, Z., Mitchell, S., Li, Q., et al. (2010). Rare genetic variation at Zea mays crg81R1 increases ζ-carotene in maize grain. Nat. Genet. 42:322–327.

Yan, L., Li, P., Zhao, X., Ji, R., and Zhao, L. (2020). Physiological and metabolic responses of maize (Zea mays) plants to Fe(3O)4 nanoparticles. Sci. Total Environ. 718:137400.

Yang, J., Fu, M., Ji, C., Huang, Y., and Wu, Y. (2018a). Maize oxalyl-CoA decarboxylase1 degrades oxalate and affects the seed metabolism and nutritional quality. Plant Cell 30:2447–2462.
Yang, L., Fountain, J.C., Ji, P., Ni, X., Chen, S., Lee, R.D., Kemerait, R.C., and Guo, B. (2018b). Deciphering drought-induced metabolic responses and regulation in developing maize kernels. Plant Biotechnol. J. 16:1616–1628.

Yang, X., Gao, S., Xu, S., Zhang, Z., Prasanna, B.M., Li, L., Li, J., and Yan, J. (2011). Characterization of a global germplasm collection and its potential utilization for analysis of complex quantitative traits in maize. Mol. Breed 28:511–526.

Yang, X., Guo, Y., Yan, J., Zhang, J., Song, T., Rocheford, T., and Li, J.-S. (2010). Major and minor QTL and epistasis contribute to fatty acid compositions and oil concentration in high-oil maize. TAG. Theoretical and applied genetics. Theoretische Angew. Genetik 120:665–678.

Yesbergenova-Cuny, Z., Dinant, S., Martin-Magniette, M.L., Quillere, I., Armengaud, P., Monfalet, P., Lea, P.J., and Hirel, B. (2016). Genetic variability of the phloem sap metabolite content of maize (Zea mays L.) during the kernel-filling period. Plant Sci. 252:347–357.

Zhao, L., Zhang, H., White, J.C., Chen, X., Li, H., Qu, X., and Ji, R. (2019). Metabolomics reveals that engineered nanomaterial exposure in soil alters both soil rhizosphere metabolite profiles and maize metabolic pathways. Environ. Sci. Nano 6:1716–1727.

Zheng, P., Allen, W.B., Roesler, K., Williams, M.E., Zhang, S., Li, J., Glassman, K., Ranch, J., Nubel, D., Solawetz, W., et al. (2008). A phenylalanine in DGAT is a key determinant of oil content and composition in maize. Nat. Genet. 40:367–372.

Zhou, S., Richter, A., and Jander, G. (2018). Beyond defense: multiple functions of benzoxazinoids in maize metabolism. Plant Cell Physiol 59:1528–1537.

Zhou, Y., Han, Y., Li, Z., Fu, Y., Fu, Z., Xu, S., Li, J., Yan, J., and Yang, X. (2012). ZmcrtRB3 encodes a carotenoid hydroxylase that affects the accumulation of α-carotene in maize kernel. J. Integr. Plant Biol. 54:260–269.