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High-throughput cereal metabolomics: Current analytical technologies, challenges and perspectives

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ABSTRACT

Metabolomics attempts to answer questions that lie beyond the powers of genomics, transcriptomics and proteomics to facilitate an understanding and assessment of the phenotype based on the metabolome. Metabolomics can serve as (1) a direct tool to explicit secondary metabolites, (2) as an epigenetic gene amplification on the whole phenome level to define the whole genotype by a metabolome marker pattern and (3) as a marker for optimal adaptation of a specific genotype to the environment. Several biologically important questions such as influence of genetic engineering, breeding, climate change, fertilizers, biotic and abiotic stresses in bioactive components and nutritional properties of crop plants have been addressed by using metabolomic approaches. This article focusses on application of high-throughput metabolomics in cereals. Cereal metabolomics is a newly emerged and rapidly developing 'omics' area that assists in the evaluation of cereals and cereal products and plays a key role in the development and improvement of cereal cultivars, by quantitative (and qualitative) global metabolome analysis of phenotypes. In this review, all steps of the metabolomic workflow, from sample harvesting to data analysis are discussed in detail. Main sources of errors that lead to an increase in non-sample-related variations are addressed and current recommended solutions are highlighted. Analytical platforms are discussed and compared in terms of their sensitivity, resolution and applications. Several raw metabolomic data pre-processing and analyses methods are illustrated with examples and their advantages and limitations are addressed. Finally, selected metabolomic studies applied to main cereals are summarized and discussed with emphasis on analytical technologies and protocols focussing on targeted and untargeted metabolomics.

1. Introduction

Cereals are grown primarily for food and feed. Cereals such as rice, maize, wheat, barley, rye and oat are grown in many different geographical regions of the world. In each region, several different cereal varieties may be grown which have been bred to grow under local climatic conditions and to give high yields. The same variety of a cereal grown in two different regions will have phenotypic differences, and these differences will become even more pronounced if the growing conditions in two regions differ significantly. Unfortunately, the rapidly increasing climate changes and climatic fluctuations may limit growth and diminish nutritional value. The current trends in climate change and increasing fluctuations such as drought, flooding and extreme temperatures challenge cereal production and the value of the important agricultural varieties grown today. Therefore, it is of prime importance to develop new varieties that are resistant and/or adaptable to such changes and maintain their health benefits. Moreover, there is a huge demand for increasing the production of cereal crops and at the same time to reduce the use of fertilizers, pesticides and water.

The major approach to adapt cereal genotypes to changing environments is by classical plant breeding, employing mutation and selection in the field and in the laboratory. In addition, molecular scientists employ state-of-the-art techniques such as genomics, proteomics, transcriptomics and metabolomics. Metabolomics is a recent approach, which has found wide application after the development of high-throughput hyphenated analytical techniques. Metabolomics was established as a powerful screening approach in toxicology (Nicholson et al., 1999), but has now become a key tool to investigate biological questions that are not easily addressed by applying other 'omics' technologies. State-of-the-art metabolomic techniques allow the detection of hundreds of

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different cell metabolites at the given state of the cell. Since metabolites are synthesized and turned over in cells within a very short time, the metabolomic equilibrium of organisms (the metabolome) is constantly changing. For example, the plant leaf metabolome changes according to the season and to the time and temperature of the day. Metabolomic changes become more pronounced when the plant is challenged with biotic or abiotic stresses. Moreover, the metabolome reflects the changes that occur due to breeding and/or genetic engineering. Therefore, metabolites and/or metabolite patterns are effective biomarkers for evaluating the effects of stresses. Several studies have used metabolomics in crop breeding, genetic modification and biomarker discovery, to evaluate intended/unintended changes and to assess the quality of the final products (Fernie and Schauer, 2009; Kusano and Saito, 2012; Larkin and Harrigan, 2007).

Metabolomic analyses of biological systems consist of several steps that are equally important in order to draw meaningful conclusions. Metabolomic analyses follow experimental design, sample preparation, metabolite extraction, data acquisition, data pre-treatment, data analysis and interpretation. In this review, we focus on each step of the metabolomic workflow that is part of most cereal metabolomic studies (Fig. 1).

Chemical composition is one of the most important characteristics that define the value of the product. A major part of the global cereal production is utilized as animal feed where amino acid (protein) composition and metabolisable energy are the most important quality traits. Quantitative and qualitative analyses of beneficial components such as dietary fibre, proteins, vitamins, sterols, polyphenols and other primary and secondary metabolites largely determine the phenotype and thus are fundamental in most cereal science studies focussing on human foods. Most metabolomic studies related to cereals aim to determine the chemical composition of cereals and/or cereal products and to understand the plants' response to internal and/or external factors. This review summarizes current cereal metabolomic studies, differentiates purpose-orientated metabolomics from untargeted approaches and highlights the role of the current analytical platforms including their advantages and limitations. The main steps in the quantitative metabolomics technology workflow are discussed in the order in which they are performed, the minimization of non-sample-related variation is illustrated and main sources of experimental errors are highlighted. A concise tutorial on the preparation of raw metabolomic data for multivariate data analysis is provided. The main purpose of each step of metabolomic data pre-processing is explained with examples and useful tools being described in detail. Advantages and limitations of the state-of-the-art, semi-automated complex chromatographic data processing tools are also described.

Interpretation of metabolomic data and subsequent biological interpretation require an appropriate statistical treatment. Some commonly applied statistical approaches are discussed and useful recommendations are provided. Sources of common errors that lead to misinterpretations are illustrated. The most frequently used classification methods such as PCA, OPLS-DA, PLS-DA, SIMCA and ECVA are demonstrated with examples and their advantages and drawbacks are compared. In addition, the review compiles recent studies conducted on metabolomic analysis of the main cereals, maize, rice, wheat, barley, oat and rye. The current trends in metabolomics are finally set in perspective of future developments of integrated cereal phenomics laboratories.

2. Cereal metabolomics

2.1. Background, definition and motivation

Cereal metabolomics comprise various qualitative and/or quantitative measurements of metabolites from cereal plants such as maize, rice, wheat, barley, rye and oat (Fig. 1). Early chemical analyses of cereals focused on measurement of nitrogen-containing compounds (Teller, 1935), phosphorus-containing compounds (Anderson, 1912; Rooke et al., 1949), dietary fibre (Vandekamer and Vanginkel, 1952), sugars (Clegg, 1955; Ponte et al., 1969) and protein content (Bietz and Kruger, 1988; Wiser and Jones, 1971). From the middle of the 20th century, the analysis of cereal plants was significantly broadened by the development of chromatography, mass spectrometry and various spectroscopic techniques. In the 1950s, substantial amounts of research were performed on wheat plants to understand the regulation of proteins (Bilinski and Mcconnell, 1958b), carbohydrates (McConnell et al., 1958) and energy expenditure systems (Bilinski and Mcconnell, 1958a; McConnell, 1959) by using ^{14}C labelling, while an early phenolic profile screen of various cereal plants was reported in 1962 (Bardinskaya and Shubert, 1962). Until 2000, most cereal metabolomic studies were based on targeted analysis of components such as vitamins (Sampson et al., 1996), sterols (Berry et al., 1968; Kemp and Mercer, 1968), phenolics (Collins et al., 1991; Maier et al., 1995; Sridhar and Ou, 1974), volatile compounds (Withycom et al., 1974) and metabolites that are related to responses to biotic and/or abiotic stresses (Baker and Smith, 1977; Tsai and Tood, 1972).

The first comprehensive metabolomic analyses of cereals were metabolomic fingerprinting of field-grown transgenic wheat samples by using 1D ^{1}H NMR and GC–MS (Baker et al., 2006) and metabolomic profiling of rice plants during plant development by using GC–MS (Tarpley et al., 2005). Continuous development of analytical techniques and advances in the analysis and subsequent interpretation of highly complex metabolomic datasets have significantly broadened the role of metabolomics in cereal science and today allow the elucidation of important biological phenomena that a few years ago could not be effectively studied.

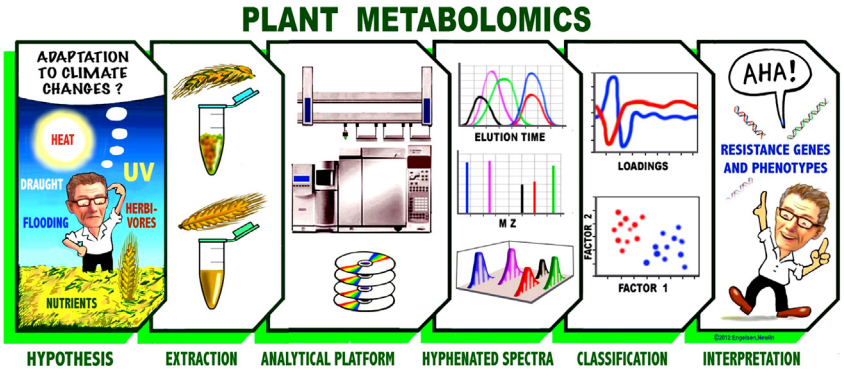

Fig. 1. General overview of plant metabolomics studies.