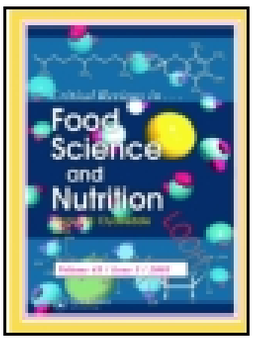

Taylor & Francis
Taylor & Francis Group

Critical Reviews in Food Science and Nutrition

ISSN: 1040-8398 (Print) 1549-7852 (Online) Journal homepage: http://www.tandfonline.com/loi/bfsn20

Metabolomics assisted biotechnological interventions for developing plant-based functional foods and nutraceuticals

Arun Kumar, Kareem A. Mosa, Liyao Ji, Udaykumar Kage, Dhananjay Dhokane, Shailesh Karre, Deepa Madalageri & Neemisha Pathania

To cite this article: Arun Kumar, Kareem A. Mosa, Liyao Ji, Udaykumar Kage, Dhananjay Dhokane, Shailesh Karre, Deepa Madalageri & Neemisha Pathania (2017): Metabolomics assisted biotechnological interventions for developing plant-based functional foods and nutraceuticals, Critical Reviews in Food Science and Nutrition, DOI: 10.1080/10408398.2017.1285752

To link to this article: http://dx.doi.org/10.1080/10408398.2017.1285752

Metabolomics assisted biotechnological interventions for developing plant-based functional foods and nutraceuticals

Arun Kumar[1]*, Kareem A. Mosa[2,3], Liyao Ji[4], Udaykumar Kage[4], Dhananjay Dhokane[4], Shailesh Karre[4], Deepa Madalageri[5], Neemisha Pathania[6]

[1]Department of Horticulture, University of Wisconsin-Madison, 1575 Linden Drive, Madison, Wisconsin 53706 USA

[2]Department of Applied Biology, College of Sciences, University of Sharjah, P.O. Box 27272, Sharjah, United Arab Emirates

[3]Department of Biotechnology, Faculty of Agriculture, Al-Azhar University, Cairo, Egypt

[4]Plant Science Department, McGill University, Ste.-Anne-de-Bellevue, Quebec, H9×3V9, Canada

[5]Department of Food Science and Nutrition, College of Home Science, University of Agricultural Science, Dharwad, India 580005

[6]Department of Soil Sciences, Punjab Agricultural University, Ludhiana

[*]**Author to whom correspondence should be addressed: Dr. Arun Kumar** Department of Horticulture, University of Wisconsin-Madison, USA. **Email**: arunihbt@gmail.com, akumar68@wisc.edu

Abstract

Today, the dramatic changes in types of food consumed have led to an increased burden of chronic diseases. Therefore, the emphasis of food research is not only to ensure quality food that can supply adequate nutrients to prevent nutrition related diseases, but also to ensure overall physical and mental-health. This has led to the concept of functional foods and nutraceuticals

(FFNs), which can be ideally produced and delivered through plants. Metabolomics can help in getting the most relevant functional information, and thus has been considered the greatest -- OMICS technology to date. However, metabolomics has not been exploited to the best potential in plant sciences. The technology can be leveraged to identify the health promoting compounds and metabolites that can be used for the development of FFNs. This article reviews (i) plant-based FFNs-related metabolites and their health benefits; (ii) use of different analytic platforms for targeted and non-targeted metabolite profiling along with experimental considerations; (iii) exploitation of metabolomics to develop FFNs in plants using various biotechnological tools; and (iv) potential use of metabolomics in plant breeding. We have also provided some insights into integration of metabolomics with latest genome editing tools for metabolic pathway regulation in plants.

Keywords

Functional foods and nutraceuticals, metabolomics, metabolic pathway regulation, metabolomics assisted breeding, metabolic engineering, secondary metabolites

INTRODUCTION

Nowadays, a large population around the world is more conscious about health and food, more than ever before. The proverb "Prevention is better than cure" is highly commendable and ardently followed. Changes in lifestyle, from increased pressures in the workplace to changes in eating habits from busy schedules, can lead to malnutrition and disease. Functional foods and nutraceuticals (FFNs) are receiving more attention from people around the world due to their numerous health benefits. Functional foods are defined as: "products those have a relevant effect on well-being and health or result in a reducing the risk of diseases" Roberfroid (1999). Health Canada defines functional food as is similar in appearance to, or may be, a conventional food that is consumed as part of a usual diet, and is demonstrated to have physiological benefits and/or reduce the risk of chronic diseases beyond basic nutritional functions, i.e. they contain bioactive compounds" (HealthCanada, 1998a). Meanwhile, nutraceuticals are "any non-toxic food extract supplement that has scientifically proven health benefits for both disease treatment and prevention" (DeFelice, 1995). Health Canada's definition for nutraceutical is "a product isolated or purified from foods that is generally sold in medicinal forms (pills, capsules or liquids) not usually associated with foods" (HealthCanada, 1998a, 1998b). The bioactive agents that possess demonstrated physiological benefits need to be identified for their further use as functional food ingredients and/or production of nutraceuticals.

FFNs are receiving increasing attention and have been accepted by governing bodies as a possible therapy in mainstream medical education and health. There is also a shift of population from traditional medical treatment to the therapy of FFNs against many types of cancers and human disorders (Dillard and German, 2000). FFNs are bioactive food components that provide

medical or health benefits (Harborne, 1999). In addition to essential nutrients such as carbohydrates, proteins, fatty acids, minerals, and vitamins, there are various non-essential bioactive food components capable of modulating cellular processes. Food we eat contain several health benefiting FFNs, for example: phenolics, flavonoids, folates, polyamines, anthocyanins, and carotenoids. These FFNs are of enormous significance because of their human health beneficial effects, including protection against diseases or disorders such as cancer, cardiovascular disease (CVD), obesity, and type II diabetes, inflammation, etc. (Salunkhe et al., 1983). For example, epidemiological studies have reported that phenolics, which are found largely in fruits, vegetables, cereals and beverages, reduce the risks of CVDs (Hertog et al., 1997) and several kinds of cancers (Hertog et al., 1994; Hertog et al., 1995). Flavonoids are a subclass of plant phenols and have been shown to have many health benefits, such as, antioxidant, anticancer, anti-allergic, anti-inflammatory (Ren et al., 2003). Folate has been shown to play a role in colorectal cancer prevention (Hubner and Houlston, 2008) and cardiovascular diseases (Bailey et al., 2003). Polyamines influence cellular proliferation, apoptosis, gut maturation (Larqué et al., 2007) and the immune system. Carotenoids have been proposed for playing a role in human health including reducing the risks of lung cancer (Ziegler et al., 1996), breast cancer (Zhang et al., 1997), prostate cancer (Mills et al., 1989) and protecting from eye diseases (Yeum et al., 1995). Capsaicin, which is a bioactive compound in red pepper and ginger exhibited anticarcinogenic and antimutagenic effects (Prakash and Sharma, 2014). Plenty of studies showed a positive relation between high amount consumption of isoflavonoids and the lower incidences of various types of cancers such as breast, prostate and colon cancer (Prakash and Sharma, 2014). For example, the compound genistein, one of the major

isoflavonoids in soybean, has exhibited antiproliferative effects for human breast cancer cells in culture (Prakash et al., 2007). Isoflavonoids or soy products also play a role in reducing the risks of cardiovascular diseases by lowering total and low-density lipoprotein (LDL) cholesterols and raising (high-density lipoprotein) HDL cholesterol (Prakash and Sharma, 2014).

Understanding the relevance of the scientific principles in determining the safety and effectiveness of FFNs is essential (Bagchi et al., 2015) and requires technological resources to facilitate it. Although various --OMICS tools like genomics (nutrigenomics and nutrigenetics), and proteomics offer opportunities to unravel complex interactions among genes, gene products, genetic polymorphisms, and functional food components; metabolomics has emerged as one of the latest technologies with large potential to provide insight into biochemical changes after dietary interventions (Bagchi et al., 2015; Lau et al., 2010). Genes and proteins are subject to post- transcriptional/translational modifications and therefore, it is very difficult to relate them to any phenotypic change. On the other hand, metabolites are the end products of biochemical reactions, and serve as functional readout of cellular biochemistry. Therefore, it is easier to correlate metabolites with the phenotype (Patti et al., 2012). Metabolomics is the non-biased identification and quantification of all metabolites in a biological system at a given time and under a specified condition (Ellis et al., 2007). Metabolomics has been demonstrated to be a powerful tool for the analysis and comparison of FFNs for the improvement of food crops, which has a very wide application in food and nutrition science. Metabolomics has been widely exploited as a tool for the identification of genes and explanation of gene functions, discover biomarkers associated with disease phenotypes, safety assessment of genetically modified crops, QTL analysis, stress resistance (abiotic and biotic) and trait improvement in plants (Kumar, et

al., 2016; Kumaraswamy et al., 2011; Okazaki and Saito, 2012; Pushpa et al., 2014; Schauer and Fernie, 2006; Yogendra et al., 2015). Metabolomics assisted breeding, in conjunction with genomics and proteomics, is offering many opportunities for nutraceutical breeding (Saxena and Cramer, 2013).

Different metabolomics platforms such as liquid chromatography-mass spectrometry (LC-MS), gas chromatography-mass spectrometry (GC-MS), nuclear magnetic resonance (NMR) coupled to various bioinformatics tools have greatly facilitated metabolomics approach to nutrition research (Hall, 2006; Trujillo et al., 2006). High-performance liquid chromatography (HPLC) was used to quantify soybean lutein (a lipophilic antioxidant vitamin that protects against cataracts, macular degeneration) content, which showed high variation among 20 different genotypes (Seguin et al., 2011). In raspberry, 300 individual plants were analyzed based on LC-MS approach to identify significant environmental and genetic effects on metabolite concentrations (Stewart et al., 2007). A metabolic analysis using a GC-MS approach on potato was done to detect the variation between cultivars and landraces for breeding purposes (Dobson et al., 2008). The metabolic profiles of two genetically modified tomato lines were compared with the metabolic profiles of commercial tomato cultivars using GC-MS, LC-MS and CE-MS to identify the significant differences between them (Kusano et al., 2011). Keeping in view the potential applications of metabolomics, this review article focuses on the plant-based FFNs and their advantages, metabolite analysis, biosynthesis pathways, as well as biotechnological perspective for their development.

1. FUNCTIONAL FOODS AND NUTRACEUTICALS RELATED METABOLITES AND THEIR HEALTH BENEFITS

Worldwide natural products have gained popularity for promoting wellbeing and healthcare, as well as disease prevention. The plant FFNs related metabolites are classified into several major classes such as terpenoids, phenolic metabolites, alkaloids, flavonoids and fatty acids, and their health benefits are discussed in detail below and also presented in Table 1.

1.1 Terpenoids

The terpenoids are a large and diverse class of naturally occurring organic compounds derived from five-carbon C5 precursor isopentenyl diphosphate (IPP) and its allylic isomer dimethylallyl diphosphate (DMAPP), assembled and modified in thousands of ways (Jia and Chen, 2016). Terpenoids are the largest class of phytonutrients in green foods, soy plants and grains. They are known for their antioxidant, antibacterial, antifungal, anti-inflammatory, antileishmanial, cytotoxic and antitumor activities (Singh et al., 1999). The compounds tocotrienols and tocopherols are effective apoptotic inducers for human cancer cells (Yu et al., 1999) and they play a role in suppressing the growth of tumor cell lines (Mo and Elson, 1999). Another extensively studied terpene group is carotenoids, they protect humans against many types of cancers including uterine, prostate, breast, colorectal, lung and digestive tract cancer (Franceschi et al., 1994). Carotenoids have been proposed for playing a role in human health. β-Carotene has shown the capacity to reduce the risks of lung cancer (Ziegler et al., 1996). Lycopene has been reported to be able to reduce the risks of breast cancer (Zhang et al., 1997) and prostate cancer (Giovannucci et al., 1995). Lutein and zeaxanthin have been proposed to have the capacity to protect eye diseases (Yeum et al., 1995). Limonoids are a group of metabolites mainly found in citrus fruit. They were reported to play a role in inhibiting pancreatic carcinogenesis and provide protection to lung tissues (Nakaizumi et al., 1997).

1.2 Polyphenols

Polyphenols are secondary metabolites naturally occurring in fruits, vegetables, cereals and beverages (Pandey and Rizvi, 2009). Polyphenols are generally involved in plant defense against ultra violet radiation and pathogen attack (Beckman, 2000). In food, polyphenols influence the sensory attributes such as taste, color, odor, flavor and oxidative stability (Pandey and Rizvi, 2009). In the last decade, dietary polyphenols have received tremendous attention among nutritionists, food scientists, food manufacturers and consumers due to their major roles in human health and wellbeing. The health benefits of polyphenols dependent on the total amount of polyphenols consumed and their bioavailability (Manach et al., 2004). The extensive work on polyphenols have proven its role in maintenance of health and lowering the risk of degenerative diseases, particularly cancers, cardiovascular diseases, atherosclerotic, and neurodegenerative diseases (Middleton et al., 2000; Puupponen-Pimiä et al., 2001; Scalbert et al., 2005) by several mechanisms like free-radical neutralization; protection and regeneration of other dietary antioxidants (i.e. vitamin E); and the chelating of pro-oxidant metal ions (Watson and Preedy, 2010). Hence, the antioxidant properties of polyphenols help to scavenge free radicals, quench lipid peroxidation, prevent DNA oxidative damage, change gene expression, influence the immune system, and prevent inhibition of cell communication (Cao and Cao, 1999; Sigler and Ruch, 1993). However, there is no precise information available on dietary requirements and recommendations of polyphenols for normal and therapeutic conditions. In some of the studies, no acute or lethal toxicity was observed when 1g/day of total polyphenols consumed orally (Scalbert and Williamson, 2000). Phenolic compounds constitute one of the most extensive groups of chemicals and ubiquitous secondary metabolites in the plant kingdom. Plant phenolic

compounds occur primarily in conjugated forms with sugar residues linked to hydroxyl groups, or directly linked to aromatic carbon. Phenolics can also associate with other compounds like carboxylic and organic acids, amines, lipids and linkage with other phenol (Kondratyuk and Pezzuto, 2004). Whereas, polyphenols may be classified into different groups as a function of the number of phenol rings that they contain and on the basis of structural elements that bind these rings to one another. Polyphenols are divided into following groups; phenolic acids, flavonoids lignans, stilbenes, and tannins.

1.2.1 Phenolic acids

Phenolic acids are classified into two classes: benzoic acid derivative and cinnamic acid derivatives. The hydroxybenzoic acids are rich in certain red fruits, black radish, and onions (Shahidi and Naczk, 1995). Tea is a good source of gallic acid (Shahidi and Naczk, 1995). Hydroxycinnamic acids are more common than are hydroxybenzoic acids and mainly consists of caffeic acid, coumaric acid, ferulic acid and sinapic acid (Pandey and Rizvi, 2009). Normally, hydroxycinnamic acids are found in bound form as glycosylated derivatives, or esters, of quinic acid, tartaric acid, and shikimic acid, but are in free forms especially in the processed foods (Manach, et al., 2004). Caffeic acid together with quinic acid forms chlorogenic acid, which is found in coffee and many fruits. Fruits such as blueberries, kiwis, plums, cherries and apples contain high concentrations of hydroxycinnamic acids (Macheix et al., 1990). Ferulic acid is most abundant hydroxycinnamic acid found in cereals, it account up to 90% of total polyphenols (Lempereur et al., 1997). Whereas, caffeic acid is abundant phenolic acid in fruits and represents 75 -- 100% of total fruit hydroxycinnamic acid (Manach, et al., 2004). Coumaric acid is commonly found in various edible plants, such as carrots, tomatoes and cereals (Boz, 2015).

Phenolic acids have broader role in human health benefits. For instance, ferulic acid has wide range of health benefits including anti-inflammatory, therapeutic usage, anti-diabetic, anti-cancer, anti-apoptotic, anti-ageing, hepatoprotective, neuroprotective, radioprotective, pulmonary protective, hypotensive effect and anti-atherogenic (Srinivasan et al., 2007). Caffeic acid and ellagic acids had anti-inflammatory and anti-glycative effect in kidney of diabetic mice (Chao et al., 2010). Highly active antiretroviral therapy (HAART) was designed to rapidly control HIV replication. Caffeic acid and its derivatives act as antiviral compounds; such compounds can be used as potent nutritional therapeutic supplementation source for HIV or other viral diseases (Bailly and Cotelle, 2005). Cinnamic acid and its derivatives, in addition to having antioxidant and antibacterial property, inhibit the activity of oncogenic protein kinase, involved in causing cancer (Sova, 2012; Mielecki and Lesyng, 2016). Coumaric acid has anti-inflammatory, anticancer, antimicrobial and antioxidant effects (Boz, 2015). By decreasing low-density lipoprotein (LDL) peroxidation, coumaric acid plays a protective role against heart diseases (Garrait et al., 2006).

1.2.2 Flavonoids

Flavonoids occur ubiquitously in foods of plant origin and are most abundant in our diets. Fruits, vegetables, green tea, red wine, soy beans, and legumes are the major food sources of flavonoids (Hollman and Katan, 1999; Scalbert et al., 2005). Over 5,000 flavonoid metabolites have been identified so far (Yao et al., 2004). Once thought to be vitamins, because they have vitamin-like properties, flavonoids were given such names as vitamin P and vitamin C2. Some plant secondary metabolites such as catechin, epicatechin, erodictyol, kaempeferol and naringenin belong to this chemical group (Brown, 2005). Flavonoids were classified into six types of

scaffolds namely, flavone, flavonol, flavanone, flavanol, isoflavone, and anthocyanidin (Kinoshita et al., 2006). The antioxidant properties of flavonoids can protect the tissues against damage by oxygen free radicals by preventing lipid peroxidation (Kandaswami and Middleton Jr, 1994). Free radical mediated lipid peroxidation is a major cause of degenerative diseases such as atherosclerosis, cancer, cardiovascular diseases and chronic inflammation (Ren et al., 2003). Flavonoids can also help reduce the risk of coronary artery disease (Naderi et al., 2003) and prevent oxidation of low density lipoproteins (LDL) (Duthie et al., 2000). For instance, the metabolite kaempferol showed anti-inflammatory and antibacterial effects (Harborne and Baxter, 1993). The compounds of catechins, mainly found in grapes and green tea, can protect against free radicals (Pace-Asciak et al., 1995). Quercetin, a potent antioxidant, played a role in protecting against cardiovascular disease (Finotti and Di Majo, 2003), diabetes, and human immunodeficiency virus (HIV) (Li et al., 2000). Baicalin possessed the anti-inflammatory and anti-HIV-1 capacity (Li et al., 2000). On the other hand, anthocyanins are a group of water soluble flavonoids found in the pigments of red fruits (Frankel et al., 1995), which showed antioxidant effects *in vitro* (Pool-Zobel et al., 1999).

1.2.3 Lignans

Lignans are non-nutrient, bioactive, non-caloric phenolic compounds (Peterson et al., 2010). They are diphenolic in nature, derived from hydroxycinnamic acids (p-coumaric, ferulic, and sinapic acids), are either dimerized to lignans (Peterson et al., 2010). Lignans are usually found in free form (lariciresinol, matairesinol, pinoresinol, and secoisolariciresinol) or bound to sugars (diglucosides of pinoresinol, secoisolariciresinol, syringaresinol, sesaminoltriglucoside and sesaminoldiglucoside) (Katsuzaki et al., 1994; Heinonen et al., 2001; Smeds et al., 2007).

Lignan content of food and consumption of lignans are very low (Peterson et al., 2010). However, several plant sources have considerable amount of lignans. Secoisolariciresinol, matairesinol and lariciresinol are found in coffee, red wine, tea, wheat, buckwheat, oat, rye, rice, barley, strawberry, cranberry, pineapple, kiwi, flax seed, sesame seed, peas, chickpea, asparagus, and soybean. Here coffee, red wine and tea are exceptions for lariciresinol. Pinoresinol, medioresinol, syringaresinol are abundantly found in wheat, buckwheat, oat, rye, barley, grapes, pineapple, lemon, oranges, flax seed, sesame seed, asparagus, eggplant, radish and tomato (Peterson et al., 2010). Generally, flax seed (335 mg/100g) and sesame seeds (373 mg/100g) are considered as richest dietary sources of lignans (Mannach et al., 2004; Pandey and Rizvi, 2009; Peterson et al., 2010). Lignans were believed to have risk-reducing properties of breast cancer, and prostate cancer (Cotterchio et al., 2006; Hooper and Cassidy, 2006). Secoisolariciresinol diglucoside from flax seed and sesamin from sesame seed can reduce risk of cardiovascular diseases (Pan et al., 2009). Secoisolariciresinol glucoside has reduced diastolic blood pressure in middle-aged hypertensive Canadian men but had no association with systolic blood pressure (Conrnish et al., 2009).

1.2.4 Stilbenes

Stilbenes are 1,2-diarylethenes which contain 2 rings; ring A usually carries two hydroxyl groups in the m-position, while ring B is substituted by hydroxy and methoxy groups in the o-,m- and/or p-position. Stilbenes are made up of two phenyl moieties linked by a two-carbon methylene bridge (Pandey and Rizvi, 2009). The subclass of stilbenes distributed in plants are in the form of monomeric and dimeric, trimeric and polymeric and they so-called as viniferins. Stilbenes in human diet are quite low as they are widely found in liverworts and higher plants (Gorham,

1989) and one of the major active compounds, and most studied plant based monomeric stilbenes is resveratrol (3,4′,5-trihydroxystilbene), found in grapes (Manach et al., 2004). However, stilbenes are commonly found in plants that are not habitually consumed or in the non-edible tissue but according to the available literature chief dietary sources of stilbenes are grapes, grape juices and wine, and peanuts and peanut butter. Red wines are the higher (8 mg/L) in total resveratrol content than other wines, such as rose wines (2.93 mg/L), white wine (0.1 mg/L), or sparkling wine (1.2 mg/L) (Romero-Perez et al., 1996a and Romero-Perez et al., 1996b) and grape juices like red juices (0.69 -14.47 mg/L mean 4.73 mg/L) and in white juice (1.44 mg/L) depending up on the variety of grapes used (Lamuela-Raventos et al.,1995 and Romero-Perez et al., 1999). Resveratrol has anti-ageing, anti-cancer, anti-diabetic, cardioprotective and neuroprotective effects (Pandey and Rizvi, 2009). Resveratrol has many neurological benefits: it protects embryonic mesencephalic cells (Karlsson et al., 2000) as well as spinal cord from ischemia-reperfusion injury (Kiziltepe et al., 2004), decreases carrageenan induced hyperalgesia (Gentilli et al., 2001), reduces infarct size and focal ischemia damage (Huang et al., 2001 and Sinha et al., 2002).

1.2.5 Tannins

Tannins are water soluble polyphenols which are widely distributed in leguminous forages and seeds. There are two different groups of tannins: hydrolyzable tannins and condensed tannins. Hydrolysable tannins are compounds that are made of a central core of glucose or another polyol, and esterified with gallic acid (gallotannins), or with hexahydroxydiphenic acid (ellagitannins) (Dai and Mumper, 2010). Important examples of gallotannins are Chinese tannin (tannic acid, extracted from nutgall), Turkish tannin (extracted from Aleppo oak), tara tannin (extracted from

tara, is a small leguminous tree or thorny shrub native to Peru), acer tannin (Korean Maple), and hamamelis tannin (hazel) (Chung et al., 1998). Ellagitannins are Corilagin (from *Caesalpinia coriaria*, *Terminalia chebula*, *Schinopsis* species, and *Eucalyptus sieberiana*), Brevilagins are other tannins obtained from the extracts of *Casesalpinia brevifolia* and dehydridigallic acid extracted from Sweet chestnut. Hydrolyzable tannins found in seed pods, wood and bark, leaves, and fruits or galls of plants belonging to the family Leguminosae, Combretaceae, Anacardiaceae, and Fabaceae (Chung et al., 1998). Condensed tannins, also referred as proanthocyanidins, are oligomers or polymers of flavan-3-ols linked through an interflavan carbon bond (Dai and Mumper, 2010). Condensed tannins are widely distributed in fruits, vegetables, plants, forage, red wine, cocoa, and certain food grains, such as finger millets, sorghum, and legume (Chung et al., 1998). Condensed tannins were shown to have anticarcinogenic, cardiovascular, cholesterol-lowing properties (Dykes and Rooney, 2006; Gondim Junior et al., 2005). Gallic acid, ellagic acid, tannic acid and quercitin, epigallocatechin gallate (EGCG), and hydrolyzable tannins, including agrimoniin, oenothein B, and coriariin A, have anticarcinogenic effects in humans (Chung et al., 1998). Polyphenols like epigallocatechin, EGCG, epicatechin gallate, gallic acid, tannic acid, epicatechin gallate, geraniin quercetin and rutin are found to be antimutagenic in nature. Tannins also have antimicrobial activity against bacteria, fungi and viruses and other biological activities, such as accelerating blood clotting and reducing blood pressure (ellagic acid) (Ratnoff et al., 1964; Bhargava et al., 1969). Tannic acid is found to be a hepatotoxin; it produces hepatic necrosis in humans and grazing animals and also neutralizes snake venom (Kuppusamy et al., 1993; Wells et al., 1942).

1.3 Alkaloids

Alkaloids are present primarily as a class of nitrogen-containing organic compounds in many organisms including plants, fungi, bacteria, and animals. Some of the important and naturally-derived alkaloids having health benefits are berberine, matrine, piperine, fritillarine, and rhynchophylline (Shi et al., 2014). The majority of alkaloids are present in higher plants, especially in dicots, and only some exist in the lower plants (Dang et al., 2012). Glusosinolates which are mainly found in cruciferous vegetables can protect against carcinogenesis, mutagenesis and other forms of toxicity (Fahey et al., 1997). Indole-3-carbinol, a plant secondary metabolite of glucosinolate, can provide inhibition to organ-site carcinogenesis in rodent models (Telang et al., 1997). Pepperin, an alkaloid present in black pepper, showed antihyperglycemic activity in blood glucose studies (Atal et al., 2012). Studies have shown its ability in preventing human mammary carcinogenesis through regulating cell cycle progression, increasing the formation of antiproliferative estradiol metabolite and inducing cellular apoptosis (Cartea and Velasco, 2008; Traka and Mithen, 2009). Natural alkaloids were proposed to be excellent promise drugs for the therapy of diseases such as cancer, fighting, neurological dysfunction, viral hepatitis, -inflammatory, bacterial and viral infections and Hypoglycaemia (Shi et al., 2014).

1.4 Fatty acids

A fatty acid is a carboxylic acid with a long aliphatic tail (chain), which is either saturated or unsaturated. Omega-3 and omega-6 fatty acids are two main essential fatty acids and they belong to polyunsaturated fatty acids (PUFAs). The three types of omega-3 fatty acids (FA) involved in human physiology are α-linolenic acid (ALA; 18:3 omega-3), eicosapentaenoic acid (EPA; 20:5 omega-3), and docosahexaenoic acid (DHA; 22:6 omega-3). ALA is a major omega-3 FA in plants. Flaxseed (linseed) is the richest source of omega-3 FA (contains around 55% of ALA),

followed by perilla, walnut, canola, soy etc. (Asif, 2011). EPA and DHA are mainly found in fish oil. DHA shows anti-inflammatory and anti-arrhythmic effect (Serhan et al., 2002) and contributes to brain formation and development, therefore it is essential for women during the pregnancy and lactation (Brenna et al., 2007). Linoleic acid (LA) is omega-6 FA which plays a role in mammary and prostate cancer protection (Belury, 2002b), and shows antidiabetogenic, anticarcinogenic activities (Belury, 2002a). Both LA and ALA can reduce the cardiovascular risk by decreasing the LDL-cholesterol level (Bloedon et al., 2008). γ-Linolenic acid shows anti-inflammation, anti-diabetic activities and helps reduce cardiovascular disorders and reproductive disorders (Borgeat et al., 1976).

2. METABOLOMICS FOR NUTRITION RESEARCH

2.1 Analytic platforms for targeted and non-targeted metabolite profiling

As the end product of genome, the plant metabolome is highly complex constituting amalgamation of primary and secondary metabolites. The detection and quantification of all the metabolites, constituting a metabolome of an individual, is very important to understand complex biochemical networks, and the underlying biosynthetic genes. However, no single analytical platform to date, can detect all the metabolites in an individual due to the broad chemical assortment of plant primary and secondary metabolites. The total number of primary and secondary metabolites in plants is estimated between 100,000 to 200,000. However, technological advancements, rapid development of metabolite databases, and novel software innovations with pattern recognition techniques have boosted the unbiased identification and absolute quantification of several metabolites.

Metabolomics has been demonstrated to be a powerful tool for the analysis and comparison of metabolites for the improvement of food crops. Major approaches used in plant metabolomics studies include metabolite profiling, targeted metabolite analysis, and metabolic fingerprinting (Fiehn, 2001, 2002; Nielsen and Oliver, 2005). So depending on the objectives of the study, one has to use the specific approach or combination of approaches. The aim of metabolite profiling is to detect the relative abundances of as many metabolites as possible within a structurally related predefined group, but not necessarily to detect the exact concentrations. On other hand, targeted metabolite analysis aims to detect the absolute concentrations of the metabolites involved in a particular biosynthetic pathway. It is a highly quantitative method that is very effective for the detection of known metabolites. Targeted metabolite analysis employs optimized measurements of preselected metabolites characterized by mass spectrum and retention time (Halket et al., 2005; Shulaev, 2006). In contrast, metabolic fingerprinting is employed to identify metabolic signatures or patterns associated under specific conditions without identification or precise quantification of all the metabolites. Various analytical platforms are used to study different approaches in plant metabolomics.

To date, several analytical platforms like liquid chromatography - mass spectrometry (LC-MS), gas chromatography - mass spectrometry (GC-MS), capillary electrophoresis - mass spectrometry (CE-MS), Fourier transform - ion cyclotron resonance - mass spectrometry (FT-ICR-MS), nuclear magnetic resonance (NMR) are deployed for metabolome profiling. Each platform has associated advantages and limitations (Table 2). Therefore, a combination of different analytical techniques must be used to gain comprehensive view of a metabolome. Among various techniques used for metabolome analysis, MS and NMR are considered the most

universal approaches. MS provides mass to charge ratio information that helps in determining the structure, and owing its sensitivity, selectivity and wide dynamic range makes MS the technique of choice. MS is often connected to chromatographic separation techniques like LC, GC, or CE to increase the number of compounds detected by reducing the complexity of mass spectra and the matrix effect. LC-MS is used to analyze a broader range of metabolites, including thermally labile, non-volatile, high molecular weight compounds (Lu et al., 2008). LC-MS is used to profile secondary metabolites like phenylpropanoids, flavonoids, saponins, and lipids. More advanced approaches, such as combining LC with ultra-high-resolution mass spectrometry (LC-HRMS) and tools like LC-NMR-MS (Breitling et al., 2006; Peterman et al., 2006) will benefit the process of identifying diverse plant metabolites. Additionally, improved separation technologies such as ultra-performance LC (UPLC) coupled to MS (Laaksonen et al., 2006; Nordström et al., 2006) can show significantly better precision. The use of LC-HRMS, such as Orbitrap, which has the ability to enhance the separation of unknown compounds, has the potential to detect thousands of metabolites in plants (Pushpa et al., 2014; Yogendra et al., 2014). Gas chromatography-mass spectrometry (GC-MS) is used to profile low molecular weight metabolites, which are volatile or derivatized (Pasikanti et al., 2008), especially primary metabolites such as fatty acids, sterols, sugars, organic acids, etc. (De Vos et al., 2007; Kopka et al., 2004). GC-MS has been regarded as the "gold standard" in metabolomics because of its separation ability, quantification methods and metabolites identification (Harrigan, 2002). GC-MS has been applied to the metabolite profiling and target analysis and established protocols are available for machine setup, data mining, and interpretation. Besides, it has the lowest expenses compared with the other platforms (Kanani et al., 2008). NMR is one of the best techniques used

as it is non-destructive, non-biased, highly quantitative, provides chemical and structural information and doesn't require prior separation and derivatization. NMR spectroscopy is generally used to profile low molecular weight metabolites. However, due to its low sensitivity, NMR can be appropriate for detecting metabolites from large sample volumes (Biais et al., 2010). Fourier transform-ion cyclotron resonance mass spectrometry (FT-ICR-MS) is a new platform and has very high sensitivity and resolution compared to other techniques (Okazaki and Saito, 2012).

2.2 Metabolite identification and databases

Metabolite identification/annotation is one of the major technical challenges encountered in metabolomics (Matsuda et al., 2009). Metabolites are identified based on following basic criteria: i) Accurate mass match with metabolites in databases such as PlantCyc, METLIN, KEGG, KNApSAcK, MetaCyc, NIST and LIPID MAPS, with accurate mass error of, AME < 5ppm (Tohge and Fernie, 2010); ii) Fragmentation pattern match with available databases, literature and in-house fragmentation of a few pure compounds (Matsuda et al., 2009); iii) *In-silico* confirmation of fragmentation based on Masspec scissor in Chemsketch (ACD labs, Toronto) (Matsuda et al., 2009); iv) Number of predicted carbons in the molecule based on ratio of isotopic ion intensity (Bollina et al., 2010) using the formula: n = (^{13}C isotopic ion intensity / ^{12}C isotopic ion intensity) * (0.9893 / 0.0107), where n is the number of carbon in the molecular formula; v) Metabolites also can be identified based on NMR spectra (Okazaki and Saito, 2012).

Some of the important metabolite databases available are: i) **PlantCyc** is a source for compounds from 350 plant species (http://pmn.plantcyc.org/cpd-search.shtml); ii) **METLIN** contains data on known endogenous plant metabolites and drug metabolites (Smith et al., 2005); iii) **KNApSAcK**

contains plant metabolites (Afendi et al., 2012); iv) **KEGG** is large collection of chemicals from plants and animals (Kanehisa et al., 2002); v) **MetaCyc** is the largest curated non redundant reference database of small-molecules which are experimentally validated and reported in the scientific literature (Caspi et al., 2010); vi) **LIPID MAPS** is a rich collection of information on different categories of lipid molecules such as fatty acyls, glycerolipids, glycerophospholipids, sphingolipids, sterol lipids, and prenol lipids (http://www.lipidmaps.org/). vii) **NIST** (National institute of standards and technology) **reference database** is fully evaluated in house MS and MS/MS spectra library of authentic compounds. In addition, some of these databases also contain metabolic pathways and link to biosynthetic enzymes and genes. A list of various resources useful for non-target metabolomics of FFNs is given in Supplementary Table S1.

2.3 Experimental considerations and design

Plants can survive in various environmental conditions by adjusting their metabolite constitution to achieve metabolite homeostasis and steady state. Researchers usually study the specific stress perturbation to understand the cause and effect relationships; such experiments are commonly conducted under deliberate and controlled environmental conditions to study systems response in systematic way. Therefore, it is important to have a good experimental design to study the plant response to the stress perturbation without confounding the results (Gibon and Rolin, 2012). All possible care should be taken to reduce the errors arising out of biological and technical variations. Metabolite constitution is very sensitive and variable in response to perturbations, and it highly depends on experimental considerations, metabolite extraction and analytical methods. Therefore, before embarking on main experiment, a pilot study should be conducted to optimize the experimental conditions, adequate biological or technical replications, sample size and time

of sample collection required for our piece of research. Furthermore, the metabolomics research community has framed some important minimal standards for metabolomics experiments to aid in supporting analysis and comparison of metabolite datasets (Fiehn et al., 2008). Factorial or randomized experimental designs can be used to ensure minimal confounding experimental effects (Rai et al., 2013).

2.4 Post analytical considerations

High throughput metabolomics experiments generate tremendously large amount of data. Handling and working with such complex data is very challenging and has great impact on resulting biological interpretations. Extracting relevant information from such huge data is key intellectual for knowledge discovery in this field (Boccard et al., 2010). Systematic errors in high throughput metabolomics are very high during a whole process of research, from starting till data analysis. Hence, data quality check is a very critical step for checking experimental errors; and necessary in capable data analysis. Before data analysis, data should be processed and normalized to minimize the noise. Required robust statistical analysis should be carried out to know the experimental variations (Eliasson et al., 2011; Hendriks et al., 2011). Several software packages for metabolite analysis and statistical techniques are available for to process the high throughput metabolite datasets. The next step in exploiting the metabolomics information for the manipulation of metabolites in plants is the identification of biochemical pathways and the genetic mechanism. It is one of the prerequisites for developing biotechnological strategies to enhance the quality and quantity of those metabolites and has been discussed in detail in the section below with details on metabolic pathway regulation, biotechnological interventions, integration of metabolomics with other -omics approaches for breeding and genome editing etc.

3. UNDERSTANDING FUNCTIONAL FOODS RELATED METABOLITIC PATHWAY REGULATION FOR RAISING NEXT GENERATION CROPS

3.1 Pathways and regulation of functional foods metabolite biosynthesis in plants

Considerable efforts have been made to illustrate the metabolic pathways with its catalytic enzymes and the genes involved in the synthesis of nutraceuticals and functional foods compounds (Kumar et al., 2014). Plant-health promoting metabolites belong to different important biosynthesis pathways, including phenylproponoid biosynthesis pathway, flavonoid pathway, terpenoid biosynthesis pathway, and others. Figure 1 illustrates the major general steps involved in FFNs metabolites production. However, more details about specific metabolites of interest and its pathways can be extracted from the plant-specific metabolic pathway database (PlantCyc): http://pmn.plantcyc.org/PLANT/organism-summary?object = PLANT. Different transcription factors such as MYB, WRKY, and basic helix--loop--helix (bHLH) are known to regulate the biosynthesis of FFNs metabolites in plants. Some of these are illustrated in the examples provided below.

Phenylpropanoid biosynthesis pathway is considered as the starting point of the production of many important FFNs metabolites. The pathway begins with phenylalanine, which is then converted into cinnamic acid by phenylalanine ammonia lyase (PAL) enzyme (Yogendra et al., 2014). Cinnamic acid is then converted into p-coumaric acid and p-coumaroyl-CoA by Cinnamate 4-hydroxylase (C4L) and 4-coumarate-CoA ligase (4CL). Caffeic acid is then produced, among other important synthesized metabolites are chlorogenic acid, coumarin, rosmarinic acid, and 6-gingerol. Phenylpropanoid biosynthesis pathway has been shown to be regulated by different transcription factors and regulatory genes. For example, MYB

transcription factors are key regulators of many phenylpropanoid compounds biosynthesis. Phenylpropanoid pathway repression and reduced production of phenylpropanoid metabolites is demonstrated by members of MYB subgroup 4 (Zhang et al., 2013a). Another important gene in the phenylpropanoid pathway is the *Reduced Epidermal Fluorescence 4* (*REF4*) gene. It has been reported that decreased accumulation of phenylpropanoids can be caused by the *REF4* gene. In contrast, increased accumulation of many phenylpropanoids downstream products and enhanced expression of many biosynthetic phenylpropanoids genes is achieved by the disruption of *REF4* (Bonawitz *et al.*, 2012).

Flavonoid biosynthesis pathway is linked to phenylpropanoid pathway (Figure 1) by phenylalanine as the starting compound (Ferreyra et al., 2012). The first gene involved in the flavonoid biosynthesis pathway is *CHS*, which encodes the chalcone synthase enzyme, leading to the production of all flavonoids. Different enzymes are involved in the biosynthesis of the flavonoids subclasses such as isomerases, reductases, and hydroxylases (Martens et al., 2010). Flavonoid biosynthetic pathway is regulated by MYB, bHLH, and WD40-type transcription factors (Hichri et al., 2011). For example, expression of Arabidopsis *MYB12* in tomato resulted in the activation and regulation of caffeoylquinic acid and flavonol biosynthetic pathway, and the tomato fruits showed very high levels of different polyphenolic antioxidants (Luo et al., 2008). One of the most important flavonoids health-promoting pigments are anthocyanins, which have been shown to be regulated by different members of MYB transcription factors (Jaakola, 2013). *MYBA1* and *MYBA2* regulate the biosynthesis of anthocyanins in grapevine berries (Azuma et al., 2008). A recent study revealed that anthocyanin accumulation in red-skinned peach is regulated by *MYB10.1* transcription factor (Tuan et al., 2015). Furthermore, *MYB10* has been

reported to promote anthocyanins synthesis and enhanced anthocyanin accumulation in crabapple (Tian et al., 2015).

Mevalonic acid (MVA) pathway is the source of the terpenoid biosynthesis pathway, mainly triterpenoids and sesquiterpenoids are biosynthesized through the MVA pathway. Beta-amyrin, thalianol, and sterols are synthesized by cyclization of 2,3-oxidosqualene. Different important functional foods metabolites are then synthesized (Sawai and Saito, 2011). Many terpenoid indole alkaloids (TIAs) with anticancer properties have been reported in *Catharanthus roseus* plants. *Tryptophan decarboxylase* (*TDC*) gene, a key gene in the terpenoid pathway, was up-regulated by the overexpression of *WRKY1* transcription factor in *C. roseus* hairy root. *C. roseus* hairy roots overexpressing *WRKY1* accumulated high levels of tryptamine and serpentine compounds, as compared with control roots (Suttipanta et al., 2011). A recent study suggested that triterpene saponin biosynthesis in *Medicago truncatula* is regulated by the *basic helix-loop-helix* (*bHLH*) transcription factors of Triterpene Saponin biosynthesis Activating Regulator (TSAR). Hairy roots of *M. truncatula* overexpressing TSAR exhibited increased transcript levels of triterpene saponin biosynthetic genes and accumulated high levels of triterpene saponins (Goossens et al., 2015).

3.2 Biotechnological interventions for developing FFNs

Enriched staple crops with health-promoting nutrients are better than nutritional supplements due to sustainability reasons. Developing staple crops enriched with health- promoting compounds has become the focus of several scientists. The recent interest in study of phytochemicals has driven a surge in industrial and academic research in plants. Plant metabolites are characterized by an enormous chemical diversity; every plant has its own complex set of metabolites.

Spectacular advances in plant metabolomics offer new possibilities, together with the aid of systems biology, to explore the extraordinary complexity of the plant biochemical capacity (Ji et al., 2016). State-of-the art genomics tools can be combined with metabolic profiling to identify key genes that could be engineered to produce improved crop plants (Oksman-Caldentey and Saito, 2005). Application of biotechnology, particularly via metabolic engineering strategies in plants by the expression/ overexpression/ co-expression of key genes and transcription factors in the target pathway, may lead to incresed levels of desired FFNs. Examples cited below further represent some of these biotechnological approaches used to enhance the content of specific FFNs.

Chlorogenic acid (CA), belonging to the phenylpropanoid biosynthetic pathway, is a well-known compound for its wide biological effects, including antitumor, antioxidant, anti-inflammatory, antibacterial, and antiviral properties (Liu and Qiu, 2003). Overexpression of *hydroxycinnamoyl transferase* (*HQT*) in tomato showed higher levels of CA accumulation (Niggeweg et al., 2004). Another approach for the production of three important metabolites caffeic, chlorogenic and rosmarinic acids, was reported by using suspension culture of the medicinal plant *Glechoma hederacea* (Döring and Petersen, 2014).

In one of the attempts to engineer flavonoid biosynthetic pathway, the *leaf colour* (*Lc*) regulatory gene from maize was overexpressed in apple (*Malus domestica Borkh.*). The apple transgenic lines exhibited 12-fold, 14-fold, 41-fold, and 7 to 134-fold increase in the anthocyanidn, flavan 3-ol epicatechin, isomeric catechin, and dimeric proanthocyanidins, respectively (Li et al., 2007). The antioxidant activity of *Codonopsis lanceolata* medicinal plant was improved by overexpressing the γ-tocopherolmethyltransferase (γ-tmt) gene. Leaf and root extracts of the

Codonopsis lanceolate transgenic plants showed significant increase in 12 major phenolic acids and flavonoids (Ghimire et al., 2011). Similarly, enhanced antioxidant activity of *Perilla frutescens* medicinal plant was reported by the overexpression of γ-tmt gene (Ghimire et al., 2015).

Overexpression of R2R3-MYB transcription factor *CsMYBF1* in tomato led to an up-regulation of genes involved in primary metabolism and the phenylpropanoid pathway, and led to high accumulation of hydroxycinnamic acid compounds (Liu et al., 2016). Transgenic tomato expressing two transcription factors; *Delila* (*Del*) and *Rosea1* (*Ros1*) from snapdragon exhibited high level of anthocyanin and antioxidant capacity. Interestingly, cancer-susceptible mice fed with these transgenic tomatoes demonstrated higher life expectancy (Butelli et al., 2008). In another study, transgenic rice seeds accumulating high levels of different health promoting flavonoids; naringenin, kaempferol, genistein, and apigenin, were successfully produced by expressing their biosynthetic genes under the control of seed-specific promoters (Ogo et al., 2013).

A successful approach to increase carotenoid and provitamin A levels in wheat grains, which naturally contains very low levels of both compounds, has been described (Wang et al., 2014). Co-expression of bacterial *phytoene synthase gene* (*CrtB*) and *carotene desaturase* gene (*CrtI*) in wheat cultivar Bobwhite showed 8-fold, 65-fold, and 76-fold increase in carotenoid, β-carotene, and provitamin A content, respectively (Wang et al., 2014). Artemisinin is an important metabolite in the terpenoid biosynthetic pathway due to its potential anti-malarial effect. Transgenic *Artemisia annua* plants with high artemisinin content and high drought tolerance were developed by overexpressing the *A. annua* ABA receptor orthologue *AaPYL9* (Zhang et al.,

2013). Overexpression of *WRKY1* transcription factor in *A. annua* activated the transcription of the *CYP71AV1* gene, and significantly increased artemisinin levels (Han et al., 2014).

Recently, plant hairy root cultures are widely used as an efficient strategy for the production of different health promoting metabolites. For example, high levels of different flavones (i.e., baicalin, baicalein, and wogonin) were produced in transgenic hairy root lines of the medicinal plant *Scutellaria baicalensis* by the overexpression of *phenylalanine ammonia-lyase* (*PAL*) (Park et al., 2012). Transgenic hairy root lines of *Salvia miltiorrhiza* medicinal plant were developed to accumulate high level of tanshinone, a cardio-cerebrovascular disease treatment, by overexpressing two key enzyme genes involved in the tanshinone biosynthetic pathway; *SmHMGR* (*3-hydroxy-3-methylglutaryl CoA reductase*) and *SmDXR* (*1-deoxy-d-xylulose 5-phosphate reductoisomerase*) (Shi et al., 2014). Recently, the accumulation of bioactive compound ginsenoside Rg1 was improved in transgenic hairy root culture of *Panax ginseng* herbal plant by overexpressing the *α-l-rhamnosidase* gene from *Bifidobacterium breve* (*BbRha*). The transgenic lines showed 2.2-fold ginsenoside Rg1 increase in comparison to the control (Zhang et al., 2015). In rice, studies have focused on metabolic engineering of β-carotene and tryptophan since high content of both can serve as a functional food in rice. *Anthranilate synthase* (*AS*) is an important enzyme in feedback inhibition by tryptophan and hence transgenic rice were produced by expressing a feedback-insensitive *OASA1* gene (*OASA1D*) in which aspartate-323 is replaced by asparagine (Tozawa et al., 2001). *OASA1D* transgenic rice revealed increased level of free tryptophan in the calli, seedlings, and seeds up to 35- to 300-folds (Tozawa et al., 2001; Wakasa et al., 2006). Since transgenic plants may have some other altered traits, metabolomic approaches, along with other -omics approaches, can be employed to study

these changes. Microarray and targeted metabolite analyses of *OASA1D* rice seedlings showed limited effect of the transgene, which was thought to be due to the low activity of the tryptophan-utilizing pathways in rice seedlings (Dubouzet et al., 2007). Profiling analyses of UV-active metabolites in the rice calli and seeds expressing the *OASA1D* transgene also demonstrated little effects on the profiles of other UV-active metabolites, except for the accumulation of a few minor indole alkaloids in these tissues (Wakasa et al., 2006).

Although, transgenic approaches appear viable solution for food and nutritional security, the environmental and food safety, and public concerns and international trade issues, can still be stumbling blocks for approval to field releases and commercialization of transgenic crops. To ensure public acceptance of transgenic crops, the regulatory agencies have to be careful until there are enough studies on the safety issues on consumption of transgenic crops. Molecular breeding approaches can be very handy when dealing with the said issues and has been discussed in detail in the section below.

3.3 Integration of metabolomics with other -omics approaches to uncover important traits and exploit them in breeding programs

Use of genomics assisted-breeding, also known as molecular breeding, is rapidly increasing to enhance crop yields and qualities. However, since costs incurred in phenotyping are very high, breeding coupled to genomics and metabolomics is recently becoming very handy and successful in many cases. Biosynthesis of secondary metabolites is a quantitative trait, governed by quantitative trait loci (QTLs), and is influenced by epistatic interactions resulting in phenotypic variations (Fernie and Schauer, 2009). Metabolite profiling coupled with introgression breeding from an interspecific cross between cultivated *Solanum lycopersicum* and its wild relative *S.*

pennellii led to identification of 322 putative QTLs affecting metabolite accumulation in tomato fruit (Schauer et al., 2006). They also studied the mode of inheritance of metabolic QTL and the consequences of mode of inheritance with respect to the breeding of specific traits. It was found that putative wild species QTL showed an increasing effect on metabolite content relative to the reference cultivated *S. lycopersicum* line and were inherited in a dominant or additive manner (Schauer et al., 2006). Studies on metabolites heritability of 25-35% are also facilitating the future breeding efforts (Eckardt, 2008; Fernie and Schauer, 2009). A multi-platform metabolomic analysis (using NMR, mass spectrometry and HPLC), of introgression lines of *Solanum pennellii* with a domesticated line led to identification of QTL for health-related antioxidant carotenoids and tocopherols, as well as molecular signatures for some 2000 compounds (Perez-Fons et al., 2014). To identify components of fruit metabolic composition, tomato introgression lines containing chromosome segments of a wild species in the genetic background of a cultivated variety were phenotyped, leading to identification of 889 quantitative fruit metabolic loci and 326 loci that modify yield-associated traits. The mapping analysis also indicated that at least 50% of the metabolic loci were associated with quantitative trait loci (QTLs) that modify whole-plant yield-associated traits. The results demonstrated the power of genome-wide metabolic profiling and detailed morphological analysis for uncovering traits with potential for crop breeding (Schauer and Fernie, 2006).

Screening of a tomato introgression line population, harboring introgression of the wild species *Solanum pennellii*, led to the identification of multiple QTL for total soluble solid content. One of these introgression lines (Brix9-2-5), was delimited to a single base-pair change in *LIN5*, an apoplastic invertase coding sequence and the line containing the allele from the wild species,

which had a greater ability to bind sucrose and, hence, an increased sugar yield (Fridman et al., 2004). The tomato hybrid AB2 harbours a QTL from *S. pennellii* and is currently a leading processing variety.

Studies in maize were conducted to study the oil and vitamin A, which didn't heavily rely on metabolomics. One of these used a combination of QTL map-based cloning, transgenics and association mapping, to dissect the molecular basis of oil QTLs. A high-oil QTL (qHO6) affecting maize seed oil and oleic-acid contents was dissected in this study. This QTL was found to encode acyl-CoA:diacylglycerol acyltransferase (DGAT1-2) enzyme, which catalyzes the final step of oil synthesis. Furthermore, it shown that a phenylalanine insertion in DGAT1-2 allele at position 469 (F469) was responsible for the increased oil and oleic-acid contents. Ectopic expression of the high-oil DGAT1-2 allele increased oil and oleic-acid contents by up to 41% and 107%, respectively. This study provided insights into the molecular basis of natural variation of oil and oleic-acid contents in plants and highlighted DGAT as a promising target for increasing oil and oleic-acid contents in other crops (Zheng et al., 2008). Another study in maize, using association analysis, linkage mapping, expression analysis, and mutagenesis, showed that variation at the *lycopene epsilon cyclase* (*lcyE*) locus alters flux down alpha-carotene versus beta-carotene branches of the carotenoid pathway. Selection of favorable lcyE alleles using molecular markers can enable breeders to more effectively produce maize grain with higher provitamin A levels (Harjes et al., 2008).

3.4 Engineering secondary metabolic pathways using genome editing tools

Engineering cellular metabolism for improved production of valuable chemicals requires extensive modulation of genome to explore complex genetic spaces (Li et al., 2015). Various

types of genetic modifications in genome engineering include gene deletion, overexpression, and precise regulation, which are vital in improving pathway efficiency and product yield (Esvelt and Wang, 2013; Wang et al., 2009; Woodruff and Gill, 2011). Genome editing is the precise editing of genetic information at defined genomic locations that enable the rational engineering and perturbation of biological systems (Esvelt and Wang, 2013). There are several genome-editing technologies available like: zinc finger nucleases (ZFNs), transcription activator-like effectors (TALEs) and Clusterly regularly interspaced short palindromic repeats (CRISPR)-Cas9 (CRISPR-Cas9). Latter is one of the most emerging techniques that have potential to precisely edit the genomes with ease. Detailed discussion about these techniques is beyond the scope of this review; the intent is to discuss the importance and impact of these fast-emerging technologies on the metabolic pathway engineering. CRISPR-Cas9 technology has been successfully applied to engineer metabolism in yeast as well as *E. coli*. CRISPR-Cas9 based technique was successfully used for iterative genome editing and metabolic engineering β-carotene synthetic pathway in *E. coli* (Li et al., 2015). Transcriptional regulation of metabolic pathways is highly complex and any perturbation can lead to activation of a new set of enzymes while repressing previously expressed enzymes. Several transcriptional activators, repressors, or other regulators that assemble at specific sites in the genome are involved in metabolic regulation (Zalatan et al., 2015). Modified CRISPR-Ca9 system that utilizes a modular RNA-based system for locus-specific transcriptional programming was applied to flexibly redirect flux through a complex branched metabolic pathway in yeast (Zalatan et al., 2015). In one of the studies, an integrated approach of metabolomics and CRISPR-Cas9 based genomic editing was used to decipher the mechanism for the improved xylose metabolism by the *pho13Δ* mutant of

engineered *S. cerevisiae* (Xu et al., 2016). Similar kind of approaches can be extended to engineer metabolic pathways in plants for production of FFNs. These engineered crops are expected to have better public acceptance compared to transgenic crops. However, performance of the engineered crops, public perception, and the regulatory frameworks will determine the extent to which these authoritative technologies can be exploited to meet world food security (Voytas and Gao, 2014).

4. SUMMARY AND FUTURE PERSPECTIVES

The increased attention to FFNs from the scientific community parallels the increased consumers public health awarness in the recent years. It is better for consumers to rely on foods rather than regular medicine for preventing diseases as well as for enhancing the overall physical fitness. Plants are perfect source to deliver FFNs, as these contain thousands of naturally beneficial phytochemical compounds and metabolites, which can be increased through genetic improvement. With its added advantage as the final link in the biological phenotypes, metabolomics has recently been applied to different biological fields aiming for high throughput analysis of diverse biochemical metabolites and compounds. Significant developments in plant metabolomics techniques have accelerated the measurement and identification of hundreds of non-targeted metabolites (Allwood and Goodacre 2010; Kumar et al., 2014). The most commonly utilized tools that can be used for FFNs identification relies heavily on LC-MS, GC-MS, and NMR and its advanced technologies. Promising approaches for developing new plant varieties as functional foods to ensure food and nutrition security includes biotechnologies and conventional plant breeding. Generally, plants accumulate very low content of the metabolites. Thus, plant metabolic engineering and molecular breeding may offer prospects for enhancing the

production of the desired metabolites. Identifying the genetic controls for the biosynthesis of plant functional foods metabolites and the use of different biotechnological strategies represent a viable alternative to meet the production limitations of these compounds. Recent advances in plant biotechnology fields such as cisgenics, and genome editing using TALENs technology, or the CRISPR-Cas9 system can be used to design and produce the desired plant with desired health promoting compounds and metabolites. In conclusion, we have shown a workflow for the use of metabolomics for developing plant-based functional foods and nutraceuticals (Figure 2) and how metabolomics combined with other omics technologies can play a central role in biotechnological quests for developing plant-based FFNs.

REFERENCES

Afendi, F. M., Okada, T., Yamazaki, M., Hirai-Morita, A., Nakamura, Y., Nakamura, K., Ikeda, S., Takahashi, H., Altaf-Ul-Amin, M., and Darusman, L. K. (2012). KNApSAcK family databases: integrated metabolite–plant species databases for multifaceted plant research. *Plant Cell Physiol.*, **53**(2), e1-e1.

Ahn, J. H., Kim, E. S., Lee, C., Kim, S., Cho, S. H., Hwang, B. Y., and Lee, M. K. (2013). Chemical constituents from *Nelumbo nucifera* leaves and their anti-obesity effects. *Bioorg. Med. Chem. Lett.*, **23**(12), 3604-3608.

Allwood, J. W., and Goodacre, R. (2010). An introduction to liquid chromatography–mass spectrometry instrumentation applied in plant metabolomic analyses. *Phytochem. Anal.*, **21**(1), 33-47.

Al-Taweel, A. M., Perveen, S., El-Shafae, A. M., Fawzy, G. A., Malik, A., Afza, N., Iqbal, L., and Latif, M. (2012). Bioactive phenolic amides from Celtis africana. *Molecules*, **17**(3), 2675-2682.

Asif, M., 2011. Health effects of omega-3, 6, 9 fatty acids: *Perilla frutescens* is a good example of plant oils. *Orient. Pharm. Exp. Med.*, 11(1), 51-59.

Atal, S., Agrawal, R. P., Vyas, S., Phadnis, P., and Rai, N. (2012). Evaluation of the effect of piperine per se on blood glucose level in alloxan-induced diabetic mice. *Acta Pol. Pharm.*, **69**(5), 965-969.

Azuma, A., Kobayashi, S., Mitani, N., Shiraishi, M., Yamada, M., Ueno, T., Kono, A., Yakushiji, H. and Koshita, Y. (2008). Genomic and genetic analysis of Myb-related

genes that regulate anthocyanin biosynthesis in grape berry skin. *Theor. Appl. Genet.*, **117**(6), 1009-1019.

Bagchi, D., Swaroop, A., and Bagchi, M. (2015). Genomics, proteomics and metabolomics in nutraceuticals and functional foods. *In: Bagchi D, Lau FC, Bagchi M, editors. Ames: Wiley-Blackwell. pp 3–338. John Wiley & Sons.*

Bailey, L. B., Rampersaud, G. C., and Kauwell, G. P. (2003). Folic acid supplements and fortification affect the risk for neural tube defects, vascular disease and cancer: evolving science. *J. Nutr.*, **133**(6), 1961S-1968S.

Bailly, F., and Cotelle, P. (2005). Anti-HIV activities of natural antioxidant caffeic acid derivatives: toward an antiviral supplementation diet. *Curr. Med. Chem.*, **12**(15), 1811-1818.

Beckman, C. H. (2000). Phenolic-storing cells: keys to programmed cell death and periderm formation in wilt disease resistance and in general defence responses in plants? *Physiol. Mol. Plant P.*, **57**(3), 101-110.

Belury, M. A. (2002a). Dietary conjugated linoleic acid in health: Physiological effects and mechanisms of action. *Annu. Rev. Nutr.*, **22**(1), 505-531.

Belury, M. A. (2002b). Inhibition of carcinogenesis by conjugated linoleic acid: potential mechanisms of action. *J. Nutr.*, **132**(10), 2995-2998.

Bhargava, U. C. and Westfall, B. A. (1969). The mechanism of blood pressure depression by ellagic acid. *Exp. Biol. Med.*, **132**(2), 754-756.

Biais, B., Beauvoit, B., Allwood, J. W., Deborde, C., Maucourt, M., Goodacre, R., Rolin, D., and Moing, A. (2010). Metabolic acclimation to hypoxia revealed by metabolite gradients in melon fruit. *J. Plant Physiol.*, **167**(3), 242-245.

Bloedon, L. T., Balikai, S., Chittams, J., Cunnane, S. C., Berlin, J. A., Rader, D. J., and Szapary, P. O. (2008). Flaxseed and cardiovascular risk factors: results from a double blind, randomized, controlled clinical trial. *J. Am. Coll. Nutr.*, **27**(1), 65-74.

Boccard, J., Veuthey, J. L., and Rudaz, S. (2010). Knowledge discovery in metabolomics: an overview of MS data handling. *J. Sep. Sci.*, **33**(3), 290-304.

Bollina, V., Kumaraswamy, G. K., Kushalappa, A. C., Choo, T. M., Dion, Y., Rioux, S., Faubert, D., and Hamzehzarghani, H. (2010). Mass spectrometry-based metabolomics application to identify quantitative resistance-related metabolites in barley against Fusarium head blight. *Mol. Plant Pathol.*, **11**(6), 769-782.

Bonawitz, N. D., Soltau, W. L., Blatchley, M. R., Powers, B. L., Hurlock, A. K., Seals, L. A., Weng, J.-K., Stout, J., and Chapple, C. (2012). REF4 and RFR1, subunits of the transcriptional coregulatory complex mediator, are required for phenylpropanoid homeostasis in Arabidopsis. *J. Biol. Chem.*, **287**(8), 5434-5445.

Borgeat, P., Hamberg, M., and Samuelsson, B. (1976). Transformation of arachidonic acid and homo-gamma-linolenic acid by rabbit polymorphonuclear leukocytes. Monohydroxy acids from novel lipoxygenases. *J. Biol. Chem.*, **251**(24), 7816-7820.

Boz, H. (2015). p-Coumaric acid in cereals: presence, antioxidant and antimicrobial effects. *Int. J. Food Sci. Tech.*, **50**(11), 2323-2328.

Bratt, K., Sunnerheim, K., Bryngelsson, S., Fagerlund, A., Engman, L., Andersson, R. E., and Dimberg, L. H. (2003). Avenanthramides in oats (*Avena sativa* L.) and structure-antioxidant activity relationships. *J. Agric. Food Chem.,* **51**(3), 594-600.

Breitling, R., Pitt, A. R., and Barrett, M. P. (2006). Precision mapping of the metabolome. *Trends Biotechnol.,* **24**(12), 543-548.

Brenna, J. T., Varamini, B., Jensen, R. G., Diersen-Schade, D. A., Boettcher, J. A., and Arterburn, L. M. (2007). Docosahexaenoic and arachidonic acid concentrations in human breast milk worldwide. *Am. J. Clin. Nutr.,* **85**(6), 1457-1464.

Brown, C. (2005). Antioxidants in potato. *Am. J. Potato Res.,* **82**(2), 163-172.

Butelli, E., Titta, L., Giorgio, M., Mock, H.-P., Matros, A., Peterek, S., Schijlen, E. G., Hall, R. D., Bovy, A. G., and Luo, J. (2008). Enrichment of tomato fruit with health-promoting anthocyanins by expression of select transcription factors. *Nat. Biotechnol.,* **26**(11), 1301-1308.

Cao, Y., and Cao, R. (1999). Angiogenesis inhibited by drinking tea. *Nature*, **398**(6726), 381-381.

Cartea, M. E., and Velasco, P. (2008). Glucosinolates in Brassica foods: bioavailability in food and significance for human health. *Phytochem. Rev.,* **7**(2), 213-229.

Caspi, R., Altman, T., Dale, J. M., Dreher, K., Fulcher, C. A., Gilham, F., Kaipa, P., Karthikeyan, A. S., Kothari, A., and Krummenacker, M. (2010). The MetaCyc database of metabolic pathways and enzymes and the BioCyc collection of pathway/genome databases. *Nucleic Acids Res.,* **38**(suppl 1), D473-D479.

Chao, C. Y., Mong, M. C., Chan, K. C. and Yin, M. C (2010). Anti-glycative and anti-inflammatory effects of caffeic acid and ellagic acid in kidney of diabetic mice. *Mol. Nutr. Food Res.,* **54**(3), 388-395.

Chung, K.-T., Wei, C.-I., and Johnson, M. G. (1998). Are tannins a double-edged sword in biology and health? *Trends Food Sci. Technol.,* **9**(4), 168-175.

Cornish, S. M., Chilibeck, P. D., Paus-Jennsen, L., Biem, H. J., Khozani, T., Senanayake, V., Vatanparast, H., Little, J. P., Whiting, S. J., Pahwa, P. (2009). A randomized controlled trial of the effects of flaxseed lignan complex on metabolic syndrome composite score and bone mineral in older adults. *Appl. Physiol. Nutr. Metab.*, **34**(2), 89–98.

Cotterchio, M., Boucher, B. A., Manno, M., Gallinger, S., Okey, A., and Harper, P. (2006). Dietary phytoestrogen intake is associated with reduced colorectal cancer risk. *J. Nutr.,* **136**(12), 3046-3053.

Dai, J., and Mumper, R. J. (2010). Plant phenolics: extraction, analysis and their antioxidant and anticancer properties. *Molecules,* **15**(10), 7313-7352.

Dang, T. T. T., Onoyovwi, A., Farrow, S. C., and Facchini, P. J. (2012). 11 Biochemical Genomics for Gene Discovery in Benzylisoquinoline Alkaloid Biosynthesis in Opium Poppy and Related Species. *Methods Enzymol.,* **515**, 231.

De Vos, R. C., Moco, S., Lommen, A., Keurentjes, J. J., Bino, R. J., and Hall, R. D. (2007). Untargeted large-scale plant metabolomics using liquid chromatography coupled to mass spectrometry. *Nat. Protoc.,* **2**(4), 778-791.

DeFelice, S. L. (1995). The nutraceutical revolution: its impact on food industry R&D. *Trends Food Sci. Technol.*, **6**(2), 59-61.

Dillard, C. J., and German, J. B. (2000). Phytochemicals: nutraceuticals and human health. *J. Sci. Food Agric.*, **80**(12), 1744-1756.

Dobson, G., Shepherd, T., Verrall, S. R., Conner, S., McNicol, J. W., Ramsay, G., Shepherd, L. V. T., Davies, H. V., and Stewart, D. (2008). Phytochemical Diversity in Tubers of Potato Cultivars and Landraces Using a GC-MS Metabolomics Approach. *J. Agric. Food Chem.*, **56**(21), 10280-10291. doi:10.1021/jf801370b.

Döring, A. S., and Petersen, M. (2014). Production of caffeic, chlorogenic and rosmarinic acids in plants and suspension cultures of Glechoma hederacea. *Phytochem. Lett.*, **10**, cxi-cxvii.

Dubouzet, J. G., Ishihara, A., Matsuda, F., Miyagawa, H., Iwata, H., and Wakasa, K. (2007). Integrated metabolomic and transcriptomic analyses of high-tryptophan rice expressing a mutant anthranilate synthase alpha subunit. *J. Exp. Bot.*, **58**(12), 3309-3321.

Duthie, G. G., Duthie, S. J., and Kyle, J. A. (2000). Plant polyphenols in cancer and heart disease: implications as nutritional antioxidants. *Nutr. Res. Rev.*, **13**(01), 79-106.

Dykes, L., and Rooney, L. W. (2006). Sorghum and millet phenols and antioxidants. *J. Cereal Sci.*, **44**(3), 236-251.

Eckardt, N. A. (2008). Heritability of the tomato fruit metabolome. *The Plant Cell*, **20**(3), 501-501.

Eliasson, M., Rannar, S., and Trygg, J. (2011). From data processing to multivariate validation-essential steps in extracting interpretable information from metabolomics data. *Curr. Pharm. Biotechnol.*, **12**(7), 996-1004.

Ellis, D. I., Dunn, W. B., Griffin, J. L., Allwood, J. W., and Goodacre, R. (2007). Metabolic fingerprinting as a diagnostic tool. *Pharmacogenomics*, 8(9):1243-1266.

Esvelt, K. M., and Wang, H. H. (2013). Genome-scale engineering for systems and synthetic biology. *Mol. Syst. Biol.*, **9**(1), 641.

Fahey, J. W., Zhang, Y., and Talalay, P. (1997). Broccoli sprouts: an exceptionally rich source of inducers of enzymes that protect against chemical carcinogens. *Proc. Natl. Acad. Sci. U. S. A.*,**94**(19), 10367-10372.

Fernie, A. R., and Schauer, N. (2009). Metabolomics-assisted breeding: a viable option for crop improvement? *Trends Genet.*, **25**(1), 39-48.

Ferreyra, M. F., Rius, S. P., and Casati, P. (2012). Flavonoids: biosynthesis, biological functions, and biotechnological applications. *Front. Plant Sci.*, **3**(222), 1-15.

Fiehn, O. (2001). Combining genomics, metabolome analysis, and biochemical modelling to understand metabolic networks. *Comp. Funct. Genomics*, **2**(3), 155-168.

Fiehn, O. (2002). Metabolomics–the link between genotypes and phenotypes. *Plant Mol. Biol.*, **48**(1-2), 155-171.

Fiehn, O., Wohlgemuth, G., Scholz, M., Kind, T., Lee, D. Y., Lu, Y., Moon, S., and Nikolau, B. (2008). Quality control for plant metabolomics: reporting MSI-compliant studies. *The Plant J.*, **53**(4), 691-704.

Finotti, E., and Di Majo, D. (2003). Influence of solvents on the antioxidant property of flavonoids. *Food/Nahrung*, **47**(3), 186-187.

Franceschi, S., Bidoli, E., Vecchia, C. L., Talamini, R., D'Avanzo, B., and Negri, E. (1994). Tomatoes and risk of digestive-tract cancers. *Int. J. Cancer*, **59**(2), 181-184.

Frankel, E. N., Waterhouse, A. L., and Teissedre, P. L. (1995). Principal phenolic phytochemicals in selected California wines and their antioxidant activity in inhibiting oxidation of human low-density lipoproteins. *J. Agric. Food Chem.,* **43**(4), 890-894.

Fridman, E., Carrari, F., Liu, Y.-S., Fernie, A. R., and Zamir, D. (2004). Zooming in on a quantitative trait for tomato yield using interspecific introgressions. *Science,* **305**(5691), 1786-1789.

Gentilli, M., Mazoit, J. X., Bouaziz, H., Fletcher, D., Casper, R. F., Benhamou, D., Savouret, J. F. (2001). Resveratrol decreases hyperalgesia induced by carrageenan in the rat hind paw. *Life Sci.* **68** (11), 1317–1321.

Ghimire, B. K., Seong, E. S., Goh, E. J., Kang, E. Y., Ahn, J. K., Yu, C. Y., and Chung, I. M. (2011). Improving antioxidant activity in transgenic *Codonopsis lanceolata* plants via overexpression of the γ-tocopherol methyltransferase (γ-tmt) gene. *Plant Growth Regul.,* **63**(1), 1-6.

Ghimire, B. K., Seong, E. S., Lee, C. O., Lee, J. G., Yu, C. Y., Kim, S. H., and Chung, I. M. (2015). Improved antioxidant activity in transgenic *Perilla frutescens* plants via overexpression of the γ-tocopherol methyltransferase (γ-tmt) gene. *Protoplasma,* **252**(5), 1285-1290.

Gibon, Y., and Rolin, D. (2012). Aspects of experimental design for plant metabolomics experiments and guidelines for growth of plant material. *Methods Mol. Biol*., 13-30.

Giovannucci, E., Ascherio, A., Rimm, E. B., Stampfer, M. J., Colditz, G. A., and Willett, W. C. (1995). Intake of carotenoids and retino in relation to risk of prostate cancer. *J. Natl. Cancer Inst.*, **87**(23), 1767-1776.

Gondim Junior, M. G. C., Barros, R., Silva, F. R. d., and Vasconcelos, G. J. N. d. (2005). Occurence and biological aspects of the clitoria tree psyllid in Brazil. *Sci. Agric.*, **62**(3), 281-285.

Goossens, A., Mertens, J., Pollier, J., Bossche, R. V., López-Vidriero, I., and Franco-Zorrilla, J. M. (2015). The bHLH Transcription Factors TSAR1 and TSAR2 Regulate Triterpene Saponin Biosynthesis in Medicago truncatula. *Plant Physiol.*, pp. 01645.02015.

Gorham, J. (1989). Stilbenes and phenanthrenes. *In*: *Harborne JB* (ed). *Methods in Plant Biochemistry*, vol 1, plant phenolics. Academic Press, London, pp 159–196.

Halket, J. M., Waterman, D., Przyborowska, A. M., Patel, R. K. P., Fraser, P. D., and Bramley, P. M. (2005). Chemical derivatization and mass spectral libraries in metabolic profiling by GC/MS and LC/MS/MS. *J. Exp. Bot.*, **56**(410), 219-243.

Hall, R. D. (2006). Plant metabolomics: from holistic hope, to hype, to hot topic. *New Phytol.*, **169**(3), 453-468. doi:10.1111/j.1469-8137.2005.01632.x

Han, J., Wang, H., Lundgren, A., and Brodelius, P. E. (2014). Effects of overexpression of *AaWRKY*1 on artemisinin biosynthesis in transgenic *Artemisia annua* plants. *Phytochemistry*, **102**, 89-96.

Harborne, J. B. (1999). Classes and functions of secondary products from plants. **In:** *Chemicals from Plants—Perspectives on Plant Secondary Products.* Walton, J.N. and Brown, D.E., Eds., Imperial College Press, London, UK, pp. 1–25.

Harborne, J. B., and Baxter, H. (1993). *Phytochemical dictionary. A handbook of bioactive compounds from plants*: Taylor & Francis Limited.

Harjes, C. E., Rocheford, T. R., Bai, L., Brutnell, T. P., Kandianis, C. B., Sowinski, S. G., Stapleton, A. E., Vallabhaneni, R., Williams, M., and Wurtzel, E. T. (2008). Natural genetic variation in lycopene epsilon cyclase tapped for maize biofortification. *Science*, **319**(5861), 330-333.

Harrigan, G. (2002). Metabolic profiling: pathways in drug discovery. *Drug Discov. Today*, **7**(6), 351-352.

HealthCanada. (1998a). Nutraceuticals/functional foods and health claims on foods. *HealthCanada, Policy paper*, **Section 2.2**(ed, Ottawa).

HealthCanada. (1998b). Nutraceuticals/functional foods and health claims on foods. *HealthCanada*, *Policy paper*, **Section 2.2** (ed, Ottawa).

Heinonen, S., Nurmi, T., Liukkonen, K., Poutanen, K., Wahala, K., Deyama, T., Nishibe, S., Adlercreutz, H. (2001). *In vitro* metabolism of plant lignans: new precursors of mammalian lignans enterolactone and enterodiol. *J. Agric. Food Chem.*, **49**(7):3178–3186.

Hendriks, M. M., van Eeuwijk, F. A., Jellema, R. H., Westerhuis, J. A., Reijmers, T. H., Hoefsloot, H. C., and Smilde, A. K. (2011). Data-processing strategies for metabolomics studies. *TrAC, Trends Anal. Chem.*, **30**(10), 1685-1698.

Hertog, M. G., Feskens, E. J., Hollman, P. C., Katan, M. B., and Kromhout, D. (1994). Dietary flavonoids and cancer risk in the Zutphen Elderly Study. *Nutr. Cancer*, **22**:175–184.

Hertog, M. G., Kromhout, D., Aravanis, C., Blackburn, H., Buzina, R., Fidanza, F., Giampaoli, S., Jansen, A., Menotti, A., and Nedeljkovic, S. (1995). Flavonoid intake and long-term

risk of coronary heart disease and cancer in the seven countries study. *Arch. Intern. Med.*, **155**(4), 381-386.

Hertog, M., Sweetnam, P. M., Fehily, A. M., Elwood, P. C., and Kromhout, D. (1997). Antioxidant flavonols and ischemic heart disease in a Welsh population of men: the Caerphilly Study. *Am. J. Clin. Nutr.*, **65**(5), 1489-1494.

Hichri, I., Barrieu, F., Bogs, J., Kappel, C., Delrot, S., and Lauvergeat, V. (2011). Recent advances in the transcriptional regulation of the flavonoid biosynthetic pathway. *J. Exp. Bot.*, erq442.

Hollman, P. C. H., and Katan, M. (1999). Dietary flavonoids: intake, health effects and bioavailability. *Food Chem. Toxicol.*, **37**(9), 937-942.

Hooper, L., and Cassidy, A. (2006). A review of the health care potential of bioactive compounds. *J. Sci. Food Agric.*, **86**(12), 1805-1813.

Huang, S. S., Tsai, M. C., Chih, C. L., Hung, L. M., Tsai, S. K. (2001). Resveratrol reduction of infarct size in Long-Evans rats subjected to focal cerebral ischemia. *Life Sci.*, **69**(9), 1057–1065.

Hubner, R., and Houlston, R. (2008). Folate and colorectal cancer prevention. *Br. J. Cancer,* **100**(2), 233-239.

Jaakola, L. (2013). New insights into the regulation of anthocyanin biosynthesis in fruits. *Trends Plant Sci.*, **18**(9), 477-483.

Ji, L., Yogendra, K. N., Mosa, K. A., Kushalappa, A. C., Piñeros-Niño, C., Mosquera, T., and Narváez-Cuenca, C. E. (2016). Hydroxycinnamic acid functional ingredients and their

biosynthetic genes in tubers of *Solanum tuberosum* Group Phureja. *Cogent Food & Agriculture*, **2**(1), 1138595.

Jia, Q., and Chen, F. (2016). Catalytic Functions of the Isoprenyl Diphosphate Synthase Superfamily in Plants: A Growing Repertoire. *Mol. Plant.*, **9(**2), 189-191.

Kadan, S., Saad, B., Sasson, Y., and Zaid, H. (2016). In vitro evaluation of anti-diabetic activity and cytotoxicity of chemically analysed Ocimum basilicum extracts. *Food Chem.*, **196**, 1066-1074.

Kanani, H., Chrysanthopoulos, P. K., and Klapa, M. I. (2008). Standardizing GC–MS metabolomics. *J. Chromatogr. B,* **871**(2), 191-201.

Kandaswami, C., and Middleton Jr, E. (1994). Free radical scavenging and antioxidant activity of plant flavonoids. *Free Radicals in Diagnostic Medicine* (pp. 351-376): Springer.

Kanehisa, M., Goto, S., Kawashima, S., and Nakaya, A. (2002). The KEGG databases at GenomeNet. *Nucleic Acids Res.*, **30**(1), 42-46.

Karlsson, J., Emgard, M., Brundin, P., and Burkitt, M. J. (2000). Trans-resveratrol protects embryonic mesencephalic cells from tert-butyl hydroperoxide: Electron paramagnetic resonance spin trapping evidence for a radical scavenging mechanism. *J. Neurochem.*, **75**(1), 141–150.

Katsuzaki, H., Kawakishi, S., Osawa, T. (1994). Sesaminol glucosides in sesame seeds. *Phytochemistry*, **35**(3):773–776.

Kinoshita, T., Lepp, Z., Kawai, Y., Terao, J., and Chuman, H. (2006). An integrated database of flavonoids. *BioFactors*, **26**(3), 179-188.

Kiziltepe, U., Turan, N. N., Han, U., Ulus, A. T., Akar, F. (2004). Resveratrol, a red wine polyphenol, protects spinal cord from ischemia-reperfusion injury. *J. Vasc. Surg.*, **40**(1), 138–145.

Kondratyuk, T. P., and Pezzuto, J. M. (2004). Natural product polyphenols of relevance to human health. *Pharm. Biol.*, **42**(sup1), 46-63.

Kopka, J., Fernie, A., Weckwerth, W., Gibon, Y., and Stitt, M. (2004). Metabolite profiling in plant biology: platforms and destinations. *Genome Biol.*, **5**(6), 109.

Kumar, A., Kage, U., Mosa, K., and Dhokane, D. (2014). Metabolomics: A Novel Tool to Bridge Phenome to Genome under Changing Climate to Ensure Food Security. *Med. Aromat. Plants*, **3**, e154.

Kumar, A., Yogendra, K. N., Karre, S., Kushalappa, A. C., Dion, Y., and Choo, T. M. (2016). WAX INDUCER1 (HvWIN1) transcription factor regulates free fatty acid biosynthetic genes to enforce cuticle to resist fusarium head blight in barley spikelets. *J. Exp. Bot.*, erw187.

Kumaraswamy, G. K., Bollina, V., Kushalappa, A. C., Choo, T. M., Dion, Y., Rioux, S., Mamer, O., and Faubert, D. (2011). Metabolomics technology to phenotype resistance in barley against Gibberella zeae. *Eur. J. Plant Pathol.*, **130**(1), 29-43.

Kuppusamy, U. R. and Das, N. P. (1993). Protective effects of tannic acid and related natural compounds on *Crotalus adamenteus* subcutaneous poisoning in mice. *Pharmacol. Toxicol.*, **72**(4-5), 290-295.

Kusano, M., Redestig, H., Hirai, T., Oikawa, A., Matsuda, F., Fukushima, A., Arita, M., Watanabe, S., Yano, M., and Hiwasa-Tanase, K. (2011). Covering chemical diversity of

genetically-modified tomatoes using metabolomics for objective substantial equivalence assessment. *PLoS One,* **6**(2), e16989.

Laaksonen, R., Katajamaa, M., Päivä, H., Sysi-Aho, M., Saarinen, L., Junni, P., Lütjohann, D., Smet, J., Van Coster, R., and Seppänen-Laakso, T. (2006). A systems biology strategy reveals biological pathways and plasma biomarker candidates for potentially toxic statin-induced changes in muscle. *PLoS One,* **1**(1), e97.

Lamuela-Raventos, R. M., Romero-Perez, A. I., Waterhouse, A. L., and de la Torre-Boronat, M. C. (1995). Direct HPLC analysis of cis and trans-resveratrol and piceid isomers in Spanish red *Vitis vinifera* wines. *J. Agric. Food Chem.,* **43**(2), 281–283.

Larqué, E., Sabater-Molina, M., and Zamora, S. (2007). Biological significance of dietary polyamines. *Nutrition,* **23**(1), 87-95.

Lau, F. C., Bagchi, D., Zafra-Stone, S., and Bagchi, M. (2010). Recent advances in nutraceuticals and functional foods. *Genomics, Proteomics and Metabolomics in Nutraceuticals and Functional Foods*, 1. Bagchi, D., Lau, F.C., and Bagchi, M.., Eds.,Wiley–Blackwell, Oxford, UK.

Lempereur, I., Rouau, X. and Abecassis, J. (1997). Genetic and agronomic variation in arabinoxylan and ferulic acid contents of durum wheat (*Triticum durum* L.) grain and its milling fractions. *J. Cereal Sci.*, **25**(2), 103–110.

Li, B. Q., Fu, T., Dongyan, Y., Mikovits, J. A., Ruscetti, F. W., and Wang, J. M. (2000). Flavonoid baicalin inhibits HIV-1 infection at the level of viral entry. *Biochem. Biophys. Res. Commun.,* **276**(2), 534-538.

Li, H., Flachowsky, H., Fischer, T. C., Hanke, M.-V., Forkmann, G., Treutter, D., Schwab, W., Hoffmann, T., and Szankowski, I. (2007). Maize Lc transcription factor enhances biosynthesis of anthocyanins, distinct proanthocyanidins and phenylpropanoids in apple (*Malus domestica* Borkh.). *Planta*, **226**(5), 1243-1254.

Li, Y., Lin, Z., Huang, C., Zhang, Y., Wang, Z., Tang, Y.-j., Chen, T., and Zhao, X. (2015). Metabolic engineering of *Escherichia coli* using CRISPR–Cas9 meditated genome editing. *Metab. Eng.*, **31**, 13-21.

Liu, C., Long, J., Zhu, K., Liu, L., Yang, W., Zhang, H., Li, L., Xu, Q., and Deng, X. (2016). Characterization of a Citrus R2R3-MYB Transcription Factor that Regulates the Flavonol and Hydroxycinnamic Acid Biosynthesis. *Sci. Rep.* doi:doi:10.1038/srep25352

Liu, J. H., and Qiu, A. Y. (2003). Application and extraction, purifying technology of chlorogenic acid [J]. *Journal of Cereals & Oils*, **9**, 017.

Luo, J., Butelli, E., Hill, L., Parr, A., Niggeweg, R., Bailey, P., Weisshaar, B., and Martin, C. (2008). AtMYB12 regulates caffeoyl quinic acid and flavonol synthesis in tomato: expression in fruit results in very high levels of both types of polyphenol. *The Plant J.*, **56**(2), 316-326.

Macheix, J.-J., Fleuriet, A., and Billot, J. (1990). *Fruit Phenolics*. Boca Raton, FL: CRC Press.

Manach, C., Scalbert, A., Morand, C., Rémésy, C., and Jiménez, L. (2004). Polyphenols: food sources and bioavailability. *Am. J. Clin. Nutri.*, **79**(5), 727-747.

Manach, C., Scalbert, A., Morand, C., Rémésy, C., and Jiménez, L. (2004). Polyphenols: food sources and bioavailability. *Am. J. Clin. Nutr.*, **79**(5), 727-747.

Martens, S., Preuß, A., and Matern, U. (2010). Multifunctional flavonoid dioxygenases: Flavonol and anthocyanin biosynthesis in *Arabidopsis thaliana* L. *Phytochemistry,* **71**(10), 1040-1049.

Matsuda, F., Yonekura-Sakakibara, K., Niida, R., Kuromori, T., Shinozaki, K., and Saito, K. (2009). MS/MS spectral tag-based annotation of non-targeted profile of plant secondary metabolites. *The Plant J.,* **57**(3), 555-577.

Middleton, E., Kandaswami, C., and Theoharides, T. C. (2000). The effects of plant flavonoids on mammalian cells: implications for inflammation, heart disease, and cancer. *Pharmacol. Rev.,* **52**(4), 673-751.

Mielecki, M., and Lesyng, B. (2016). Cinnamic acid derivatives as inhibitors of oncogenic protein kinases–structure, mechanisms and biomedical effects. *Curr. Med. Chem.,* **23**(10), 954-982.

Mills, P. K., Beeson, W. L., Phillips, R. L., and Fraser, G. E. (1989). Cohort study of diet, lifestyle, and prostate cancer in Adventist men. *Cancer,* **64**(3), 598-604.

Mo, H., and Elson, C. E. (1999). Apoptosis and cell-cycle arrest in human and murine tumor cells are initiated by isoprenoids. *J.Nutri.,* **129**(4), 804-813.

Naderi, G. A., Asgary, S., Sarraf-Zadegan, N., and Shirvany, H. (2003). Anti-oxidant effect of flavonoids on the susceptibility of LDL oxidation. *Mol. Cell. Biochem.,* **246**(1-2), 193-196.

Nakaizumi, A., Baba, M., Uehara, H., Iishi, H., and Tatsuta, M. (1997). d-Limonene inhibits N-nitrosobis (2-oxopropyl) amine induced hamster pancreatic carcinogenesis. *Cancer Lett.,* **117**(1), 99-103.

Nielsen, J., and Oliver, S. (2005). The next wave in metabolome analysis. *Trends Biotechnol.*, **23**(11), 544-546.

Niggeweg, R., Michael, A. J., and Martin, C. (2004). Engineering plants with increased levels of the antioxidant chlorogenic acid. *Nat. Biotechnol.*, **22**(6), 746-754.

Nordström, A., O'Maille, G., Qin, C., and Siuzdak, G. (2006). Nonlinear data alignment for UPLC-MS and HPLC-MS based metabolomics: quantitative analysis of endogenous and exogenous metabolites in human serum. *Anal. Chem.*, **78**(10), 3289-3295.

Ogo, Y., Ozawa, K., Ishimaru, T., Murayama, T., and Takaiwa, F. (2013). Transgenic rice seed synthesizing diverse flavonoids at high levels: a new platform for flavonoid production with associated health benefits. *Plant Biotechnol. J.*, **11**(6), 734-746.

Okazaki, Y., and Saito, K. (2012). Recent advances of metabolomics in plant biotechnology. *Plant Biotechnol.Rep.*, **6**(1), 1-15.

Oksman-Caldentey, K.-M., and Saito, K. (2005). Integrating genomics and metabolomics for engineering plant metabolic pathways. *Curr. Opin. Biotechnol.*, **16**(2), 174-179.

Pace-Asciak, C. R., Hahn, S., Diamandis, E. P., Soleas, G., and Goldberg, D. M. (1995). The red wine phenolics trans-resveratrol and quercetin block human platelet aggregation and eicosanoid synthesis: implications for protection against coronary heart disease. *Clin. Chim. Acta*, **235**(2), 207-219.

Pan, A., Yu, D., Demark-Wahnefried, W., Franco, O. H., Lin, X. (2009). Meta-analysis of the effects of flaxseed interventions on blood lipids. *Am. J. Clin. Nutr.*, **90**(2), 288–97.

Pandey, K. B., and Rizvi, S. I. (2009). Plant polyphenols as dietary antioxidants in human health and disease. *Oxid. Med. Cell Longev.*, ***2***(5), 270-278.

Park, N. I., Xu, H., Li, X., Kim, Y. S., Lee, M. Y., and Park, S. U. (2012). Overexpression of phenylalanine ammonia-lyase improves flavones production in transgenic hairy root cultures of *Scutellaria baicalensis*. *Process Biochem.,* **47**(12), 2575-2580.

Pasikanti, K. K., Ho, P., and Chan, E. (2008). Gas chromatography/mass spectrometry in metabolic profiling of biological fluids. *J. Chromatogr. B,* **871**(2), 202-211.

Patti, G. J., Yanes, O., and Siuzdak, G. (2012). Innovation: Metabolomics: the apogee of the omics trilogy. *Nat. Rev. Mol. Cell Biol.,* **13**(4), 263-269.

Perez-Fons, L., Wells, T., Corol, D. I., Ward, J. L., Gerrish, C., Beale, M. H., Seymour, G. B., Bramley, P. M., and Fraser, P. D. (2014). A genome-wide metabolomic resource for tomato fruit from *Solanum pennellii*. *Sci. Rep.,* **4**.

Peterman, S. M., Duczak Jr, N., Kalgutkar, A. S., Lame, M. E., and Soglia, J. R. (2006). Application of a linear ion trap/orbitrap mass spectrometer in metabolite characterization studies: examination of the human liver microsomal metabolism of the non-tricyclic anti-depressant nefazodone using data-dependent accurate mass measurements. *J. Am. Soc. Mass Spectrom.,* **17**(3), 363-375.

Peterson, J., Dwyer, J., Adlercreutz, H., Scalbert, A., Jacques, P., and McCullough, M. L. (2010). Dietary lignans: physiology and potential for cardiovascular disease risk reduction. *Nutrition Rev.,* **68**(10), 571-603.

Pool-Zobel, B., Bub, A., Schröder, N., and Rechkemmer, G. (1999). Anthocyanins are potent antioxidants in model systems but do not reduce endogenous oxidative DNA damage in human colon cells. *Eur. J. Nutr.,* **38**(5), 227-234.

Prakash, D., and Sharma, G. (2014). *Phytochemicals of nutraceutical importance*: CABI, http://www.cabi.org/bookshop/book/9781780643632.

Prakash, D., Upadhyay, G., Singh, B. N., and Singh, H. (2007). Antioxidant and free radical-scavenging activities of seeds and agri-wastes of some varieties of soybean (Glycine max). *Food Chem.*, **104**(2), 783-790.

Pushpa, D., Yogendra, K. N., Gunnaiah, R., Kushalappa, A. C., and Murphy, A. (2014). Identification of Late Blight Resistance-Related Metabolites and Genes in Potato through Nontargeted Metabolomics. *Plant Mol. Biol. Rep.*, **32**(2), 584-595.

Puupponen-Pimiä, R., Nohynek, L., Meier, C., Kähkönen, M., Heinonen, M., Hopia, A., and Oksman-Caldentey, K. M. (2001). Antimicrobial properties of phenolic compounds from berries. *J. Appl. Microbiol.*, **90**(4), 494-507.

Rai, R. K., Azim, A., Sinha, N., Sahoo, J. N., Singh, C., Ahmed, A., Saigal, S., Baronia, A. K., Gupta, D., and Gurjar, M. (2013). Metabolic profiling in human lung injuries by high-resolution nuclear magnetic resonance spectroscopy of bronchoalveolar lavage fluid (BALF). *Metabolomics*, **9**(3), 667-676.

Rakshit, S., Mandal, L., Pal, B. C., Bagchi, J., Biswas, N., Chaudhuri, J., Chowdhury, A. A., Manna, A., Chaudhuri, U., and Konar, A. (2010). Involvement of ROS in chlorogenic acid-induced apoptosis of Bcr-Abl+ CML cells. *Biochem. Pharmacol.*, **80**(11), 1662-1675.

Ratnoff, O. D. and Crum, J. D. (1964). Activation of Hageman factor by solutions of ellagic acid. *J. Lab. Clin. Med.*, **63**(3), 359-377.

Ren, W., Qiao, Z., Wang, H., Zhu, L., and Zhang, L. (2003). Flavonoids: promising anticancer agents. *Med. Res. Rev.,* **23**(4), 519-534.

Roberfroid, M. B. (1999). Concepts in functional foods: the case of inulin and oligofructose. *J.Nutri.,* **129**(7 Suppl), 1398S-1401S.

Romero-Perez, A. I., Ibern-Gomez, M., Lamuela-Raventos, R. M., and de la Torre-Boronat, M. C. (1999). Piceid, the major resveratrol derivative in grape juices. *J. Agric. Food Chem*. **47**(4), 1533–1536.

Romero-Perez, A. I., Lamuela-Raventos, R. M., Buxaderas, S., and de la Torre-Boronat, M. C. (1996b). Resveratrol and piceid as varietal markers of white wines. *J. Agric. Food Chem.* **44**, 1975–1978.

Romero-Perez, A. I., Lamuela-Raventos, R. M., Waterhouse, A. L., and de la Torre-Boronat, M. C. (1996a). Levels of cis- and trans resveratrol and piceid isomers in rose and white *Vitis vinifera* wines. *J. Agric. Food Chem.,* 44, 2124–2128.

Ross, A. B., Kamal-Eldin, A., and Åman, P. (2004). Dietary Alkylresorcinols: Absorption, Bioactivities, and Possible Use as Biomarkers of Whole-grain Wheat–and Rye–rich Foods. *Nutr. Rev.,* **62**(3), 81-95.

Salunkhe, D., Jadhav, S., Kadam, S., Chavan, J., and Luh, B. (1983). Chemical, biochemical, and biological significance of polyphenols in cereals and legumes. *Crit. Rev. Food Sci. Nutr.,* **17**(3), 277-305.

Sawai, S., and Saito, K. (2011). Triterpenoid biosynthesis and engineering in plants. *Front. Plant. Sci.,* **2**(25), 1-8.

Saxena, A., and Cramer, C. S. (2013). Metabolomics: a potential tool for breeding nutraceutical vegetables. *Adv. Crop Sci. Tech.,* **1**(106), 2.

Scalbert, A., and Williamson, G. (2000). Dietary intake and bioavailability of polyphenols. *J Nutri.,* **130**(8), 2073S-2085S.

Scalbert, A., Manach, C., Morand, C., Rémésy, C., and Jiménez, L. (2005). Dietary polyphenols and the prevention of diseases. *Crit. Rev. Food Sci. Nutr.*, **45**(4), 287-306.

Schauer, N., and Fernie, A. R. (2006). Plant metabolomics: towards biological function and mechanism. *Trends Plant Sci.,* **11**(10), 508-516.

Schauer, N., Semel, Y., Roessner, U., Gur, A., Balbo, I., Carrari, F., Pleban, T., Perez-Melis, A., Bruedigam, C., Kopka, J. and Willmitzer, L. (2006). Comprehensive metabolic profiling and phenotyping of interspecific introgression lines for tomato improvement. *Nature Biotechnol.,* **24**(4), 447-454.

Seguin, P., Tremblay, G., Pageau, D., Liu, W., and Turcotte, P. (2011). Soybean lutein concentration: impact of crop management and genotypes. *Crop Sci.*, **51**(3), 1151-1160.

Serhan, C. N., Chiang, N., and Van Dyke, T. E. (2008). Resolving inflammation: dual anti-inflammatory and pro-resolution lipid mediators. *Nat. Rev. Immunol.,* **8**(5), 349-361.

Serhan, C. N., Hong, S., Gronert, K., Colgan, S. P., Devchand, P. R., Mirick, G., and Moussignac, R.-L. (2002). Resolvins a family of bioactive products of omega-3 fatty acid transformation circuits initiated by aspirin treatment that counter proinflammation signals. *J Exp.Med.,* **196**(8), 1025-1037.

Shahidi, F., and Naczk, M. (1995). Food phenolics, sources, chemistry, effects, applications. *Lancaster, PA: Technomic Publishing Co Inc*.

Shi, M., Luo, X., Ju, G., Yu, X., Hao, X., Huang, Q., Xiao, J., Cui, L., and Kai, G. (2014). Increased accumulation of the cardio-cerebrovascular disease treatment drug tanshinone in Salvia miltiorrhiza hairy roots by the enzymes 3-hydroxy-3-methylglutaryl CoA reductase and 1-deoxy-D-xylulose 5-phosphate reductoisomerase. *Funct. Integr. Genomics,* **14**(3), 603-615.

Shi, Q., Hui, S., ZHANG, A.-H., Hong-Ying, X., Guang-Li, Y., Ying, H., and Xi-Jun, W. (2014). Natural alkaloids: basic aspects, biological roles, and future perspectives. *Chin. J. Nat. Med.,* **12**(6), 401-406.

Shulaev, V. (2006). Metabolomics technology and bioinformatics. *Brief. Bioinform.,* **7**(2), 128-139.

Sigler, K., and Ruch, R. J. (1993). Enhancement of gap junctional intercellular communication in tumor promoter-treated cells by components of green tea. *Cancer Lett.,* **69**(1), 15-19.

Singh, M., Pal, M., and Sharma, R. (1999). Biological activity of the labdane diterpenes. *Planta Med.* **65**(01), 002-008.

Sinha, K., Chaudhary, G., Gupta, Y. K. (2002). Protective effect of resveratrol against oxidative stress in middle cerebral artery occlusion model of stroke in rats. *Life Sci*., **71**(6): 655–665.

Smeds, A. I., Willfor, S. M., Pietarinen, S. P., Peltonen-Sainio, P., Reunanen, M. H. (2007). Occurrence of "mammalian" lignans in plant and water sources. *Planta*, **226**(3):639–46.

Sova, M. (2012). Antioxidant and antimicrobial activities of cinnamic acid derivatives. *Mini Rev. Med.Chem.,* **12**(8), 749-767.

Srinivasan, M., Sudheer, A. R. and Menon, V.P. (2007). Ferulic acid: therapeutic potential through its antioxidant property. *J. Clin. Biochem. Nutr.*, **40**(2), 92-100.

Stewart, D., McDougall, G. J., Sungurtas, J., Verrall, S., Graham, J., and Martinussen, I. (2007). Metabolomic approach to identifying bioactive compounds in berries: advances toward fruit nutritional enhancement. *Mol. Nutr. Food Res.*, **51**(6), 645-651.

Suttipanta, N., Pattanaik, S., Kulshrestha, M., Patra, B., Singh, S. K., and Yuan, L. (2011). The transcription factor CrWRKY1 positively regulates the terpenoid indole alkaloid biosynthesis in Catharanthus roseus. *Plant Physiol.,* **157**(4), 2081-2093.

Telang, N. T., Katdare, M., Bradlow, H. L., Osborne, M. P., and Fishman, J. (1997). Inhibition of proliferation and modulation of estradiol metabolism: novel mechanisms for breast cancer prevention by the phytochemical indole-3-carbinol. *Proc. Soc. Exp. Biol. Med.*, **216**(2), 246-252.

Tian, J., Peng, Z., Zhang, J., Song, T., Wan, H., Zhang, M., and Yao, Y. (2015). McMYB10 regulates coloration via activating McF3′ H and later structural genes in ever-red leaf crabapple. *Plant Biotechnol. J.*, **13**(7), 948-961.

Tohge, T., and Fernie, A. R. (2010). Combining genetic diversity, informatics and metabolomics to facilitate annotation of plant gene function. *Nat. Protoc.*, **5**(6), 1210-1227.

Tozawa, Y., Hasegawa, H., Terakawa, T., and Wakasa, K. (2001). Characterization of Rice Anthranilate Synthase α-Subunit Genes *OASA1* and *OASA2*. Tryptophan Accumulation in Transgenic Rice Expressing a Feedback-Insensitive Mutant of *OASA1*. *Plant Physiol.*, **126**(4), 1493-1506.

Traka, M., and Mithen, R. (2009). Glucosinolates, isothiocyanates and human health. *Phytochem. Rev.*, **8**(1), 269-282.

Trujillo, E., Davis, C., and Milner, J. (2006). Nutrigenomics, proteomics, metabolomics, and the practice of dietetics. *J. Am. Diet. Assoc.,* **106**(3), 403-413.

Tuan, P. A., Bai, S., Yaegaki, H., Tamura, T., Hihara, S., Moriguchi, T., and Oda, K. (2015). The crucial role of PpMYB10. 1 in anthocyanin accumulation in peach and relationships between its allelic type and skin color phenotype. *BMC Plant Biol.,* **15**(1), 1.

Voytas, D. F., and Gao, C. (2014). Precision genome engineering and agriculture: opportunities and regulatory challenges. *PLoS Biol.,* **12**(6), e1001877.

Wakasa, K., Hasegawa, H., Nemoto, H., Matsuda, F., Miyazawa, H., Tozawa, Y., Morino, K., Komatsu, A., Yamada, T., and Terakawa, T. (2006). High-level tryptophan accumulation in seeds of transgenic rice and its limited effects on agronomic traits and seed metabolite profile. *J. Exp. Bot.,* **57**(12), 3069-3078.

Wang, C., Zeng, J., Li, Y., Hu, W., Chen, L., Miao, Y., Deng, P., Yuan, C., Ma, C., and Chen, X. (2014). Enrichment of provitamin A content in wheat (*Triticum aestivum* L.) by introduction of the bacterial carotenoid biosynthetic genes *CrtB* and *CrtI*. *J. Exp. Bot.,* **65**(9), 2545-2556.

Wang, H. H., Isaacs, F. J., Carr, P. A., Sun, Z. Z., Xu, G., Forest, C. R., and Church, G. M. (2009). Programming cells by multiplex genome engineering and accelerated evolution. *Nature,* **460**(7257), 894-898.

Watson, R. R., and Preedy, V. R. (2010). *Bioactive foods in promoting health: probiotics and prebiotics*: Academic Press.

Wells, D. B., Humphrey, H. D. and Coll, J. J. (1942). The relation of tannic acid to the liver necrosis occurring in burns. *N. Engl. J. Med*., **226**(16), 629-636.

Woodruff, L. B., and Gill, R. T. (2011). Engineering genomes in multiplex. *Curr. Opin. Biotechnol.,* **22**(4), 576-583.

Xu, H., Kim, S., Sorek, H., Lee, Y., Jeong, D., Kim, J., Oh, E. J., Yun, E. J., Wemmer, D. E., and Kim, K. H. (2016). PHO13 deletion-induced transcriptional activation prevents sedoheptulose accumulation during xylose metabolism in engineered Saccharomyces cerevisiae. *Metab. Eng.,* **34**, 88-96.

Yao, L. H., Jiang, Y., Shi, J., Tomas-Barberan, F., Datta, N., Singanusong, R., and Chen, S. (2004). Flavonoids in food and their health benefits. *Plant Foods Hum. Nutr.,* **59**(3), 113-122.

Yeum, K.-J., Taylor, A., Tang, G., and Russell, R. M. (1995). Measurement of carotenoids, retinoids, and tocopherols in human lenses. *Invest. Ophthalmol. Vis. Sci.,* **36**(13), 2756-2761.

Yogendra, K. N., Kumar, A., Sarkar, K., Li, Y., Pushpa, D., Mosa, K. A., Duggavathi, R., and Kushalappa, A. C. (2015). Transcription factor StWRKY1 regulates phenylpropanoid metabolites conferring late blight resistance in potato. *J. Exp. Bot.,* **66**(22), 7377-7389.

Yogendra, K., Pushpa, D., Mosa, K., Kushalappa, A., Murphy, A., and Mosquera, T. (2014). Quantitative resistance in potato leaves to late blight associated with induced hydroxycinnamic acid amides. *Functional & Integrative Genomics,* **14**(2), 285-298. doi:10.1007/s10142-013-0358-8.

Yu, W., Simmons-Menchaca, M., Gapor, A., Sanders, B. G., and Kline, K. (1999). Induction of apoptosis in human breast cancer cells by tocopherols and tocotrienols. *Nutr.*Cancer, **33**(1),26–32.

Zalatan, J. G., Lee, M. E., Almeida, R., Gilbert, L. A., Whitehead, E. H., La Russa, M., Tsai, J. C., Weissman, J. S., Dueber, J. E., and Qi, L. S. (2015). Engineering complex synthetic transcriptional programs with CRISPR RNA scaffolds. *Cell,* **160**(1), 339-350.

Zhang, F., Lu, X., Lv, Z., Zhang, L., Zhu, M., Jiang, W., Wang, G., Sun, X., and Tang, K. (2013b). Overexpression of the Artemisia orthologue of ABA receptor, *AaPYL9*, enhances ABA sensitivity and improves artemisinin content in *Artemisia annua* L. *PLoS One*, **8**(2), e56697.

Zhang, R., Zhang, B. L., Li, G. C., Xie, T., Hu, T., and Luo, Z. Y. (2015). Enhancement of ginsenoside Rg1 in *Panax ginseng* hairy root by overexpressing the α-l-rhamnosidase gene from *Bifidobacterium breve*. *Biotechnol. Lett.*, **37**(10), 2091-2096.

Zhang, S., Ma, P., Yang, D., Li, W., Liang, Z., Liu, Y., and Liu, F. (2013a). Cloning and characterization of a putative R2R3 MYB transcriptional repressor of the rosmarinic acid biosynthetic pathway from *Salvia miltiorrhiza*. *PLoS One,* **8**(9), e73259.

Zhang, S., Tang, G., Russell, R. M., Mayzel, K. A., Stampfer, M. J., Willett, W. C., and Hunter, D. J. (1997). Measurement of retinoids and carotenoids in breast adipose tissue and a comparison of concentrations in breast cancer cases and control subjects. *Am. J. Clin. Nutr*., **66**(3), 626-632.

Zheng, P., Allen, W. B., Roesler, K., Williams, M. E., Zhang, S., Li, J., Glassman, K., Ranch, J., Nubel, D., and Solawetz, W. (2008). A phenylalanine in DGAT is a key determinant of oil content and composition in maize. *Nat. Genet.*, **40**(3), 367-372.

Ziegler, R. G., Mayne, S. T., and Swanson, C. A. (1996). Nutrition and lung cancer. *Cancer Causes Control*, **7**(1), 157-177.

Table 1. Plant functional foods related metabolites, their sources and health benefits.

Plant metabolites	Plant source	Health benefits	References
Alkylresorcinols	wheat, rye, barley	antibacterial and antifungal properties	(Ross et al., 2004)
Anthocyanidins	blackberries, grapes etc.	antioxidant effects	(Pool-Zobel et al., 1999)
Avenanthramides	oats	anti-inflammatory and antioxidant	(Bratt et al., 2003)
Baicalin	Scutellaria, including *Scutellariabaicalensis* and *Scutellarialateriflora*	anti-inflammatory, anti-HIV-1	(Li et al., 2000)
Capsaicin	red pepper and ginger	anticarcinogenic and antimutagenic	(Prakash and Sharma, 2014)
Catechins	grapes and green tea	protect against free radicals	(Pace-Asciak et al., 1995)
Chlorogenate	bamboo, peach, prunes, green coffee bean extract	antioxidant, anti-lipidemic anti-carcinogenic effects, anti-diabetic	(Rakshit et al., 2010)
Docosahexaenoic acid (DHA)	algae	anti-inflammatory and anti-arrhythmic effect, contributes to brain formation and development	(Serhan et al., 2008)
Genistein	soy	antiproliferative effects for human breast cancer cells	(Prakash et al., 2007)

Hexadecanoic acid	palm oil	inhibition of HIV-1 infection, type 2 antidiabetic effects	(Kadan et al., 2016)
Indole-3-carbinol	cruciferous vegetables such as broccoli, cabbage, cauliflower, brussels sprouts, collard greens and kale	inhibition to organ-site carcinogenesis	(Cartea and Velasco, 2008; Traka and Mithen, 2009)
Kaempferol	Pteridophyta, Pinophyta and Angiospermae	anti-inflammatory and antibacterial effects	(Harborne and Baxter, 1993)
Lignans	flax seed and sesame seed	risk-reducing properties of breast cancer, and prostate cancer	(Hooper and Cassidy, 2006)
Limonoids	citrus fruit	inhibiting pancreatic carcinogenesis, providing protection to lung tissues.	(Nakaizumi et al., 1997)
Linoleic acid (LA)	salocorn oil, safflower oil, evening primrose oil, poppyseed oil, grape seed oil and sunflower oil	mammary and prostate cancer protection, antidiabetogenic, anticarcinogenic activities	(Belury, 2002a)
Lutein	kiwi fruit, grapes, spinach, orange juice, zucchini	protect eye diseases	(Yeum et al., 1995)
Lycopene	tomatoes, red carrots, watermelons, gac, and papayas,	reduce the risks of breast cancer, prostate cancer	(Giovannucci et al., 1995)

N-Feruloyltyramine	Garlic	antioxidant and radical scavenging properties,, anti-inflammatory effects and anti-obesity properties	(Ahn et al., 2013; Al-Taweel et al., 2012)
Quercetin	many fruits, vegetables, leaves and grains	Antioxidant, protecting against cardiovascular disease, diabetes, and human immunodeficiency virus (HIV)	(Finotti and Di Majo, 2003)
Condensed tannins	virtually all families of plants	anticarcinogenic, cardiovascular, cholesterol-lowing properties	(Dykes and Rooney, 2006; Gondim Junior et al., 2005)
Tocotrienols and tocopherols	vegetable oils, including rice bran oil and palm oil	apoptotic inducers for human cancer cells	(Yu et al., 1999)
α-Linolenic acid (ALA)	Flaxseed	reduce the cardiovascular risk	(Bloedon et al., 2008)
β-Carotene	carrots, pumpkins and sweet potatoes	reduce the risks of lung cancer	(Ziegler et al., 1996)
γ-Linolenic acid	vegetable oils such as evening primrose oil blackcurrant seed oil, borage seed oil, and hemp seed oil.	anti-inflammation, anti-diebetic activities and helps reduce cardiovascular disorders and reproductive disorders	(Borgeat et al., 1976)

Table 2. State of the art and envisioned technologies for metabolic profiling! (Reprinted from Trends in Genetics, 25/1, Alisdair R. Fernie and Nicolas Schauer, Metabolomics-assisted breeding: a viable option for crop improvement?, 39-48, Copyright (2015), with permission from Elsevier.

Technology	Application	Properties
GC-MS	Analyses of polar or lipophilic compounds (e.g. sugars, organic acids, tocopherols, vitamins).	Accuracy: <50 ppm Mass range: <350 Da
GC × GC-MS	Similar to GC-MS, but with better separation of co-eluting compounds and increased sensitivity owing to GC × GC.	Accuracy: <50 ppm Mass range: <350 Da
SPME GC-MS	Analyses of volatile compounds (e.g. aroma components, repellents).	Accuracy: <50 ppm Mass range: <350 Da
CE-MS	Analyses of polar compounds (e.g. amino acids, CoA-derivates, sugars, organic acids, tocopherols, vitamins).	Accuracy: <50 ppm Mass range: <1000 Da
LC-MS	Analyses of mainly secondary metabolites (e.g. carotenoids, flavonoids, glucosinolates, vitamins).	Accuracy: 50--100 ppm Mass range: <1500 Da
FT-ICR-MS	High-resolution MS in combination with LC is highly powerful. Enables the identification of unknown metabolites by m/z mass to charge ratio.	Accuracy: <1 ppm Mass range: <1500 Da
NMR	Non-destructive analyses of abundant metabolites in a sample.	Mass range: <∼50 kDa
Direct-injection-MS	Non separative technique giving a fingerprint of the metabolic content in a biological sample.	Accuracy: 50--100 ppm Mass range: <1500 Da
FAIMS-MS	Next generation hyphenation technology to	Accuracy: 50--100 ppm

	MS. Enables selection of specific ions, reducing ion suppression and matrix effects. FAIMS enables the separation of isobaric compounds in combination with selective MS.	Mass range: <1500 Da

[1]Abbreviations: Da, Dalton; FT-ICR, fourier transform ion-cyclotron resonance; FAIMS, field asymmetric waveform ion mobility spectrometry; ppm, parts per million; SPME, solid phase micro extraction.

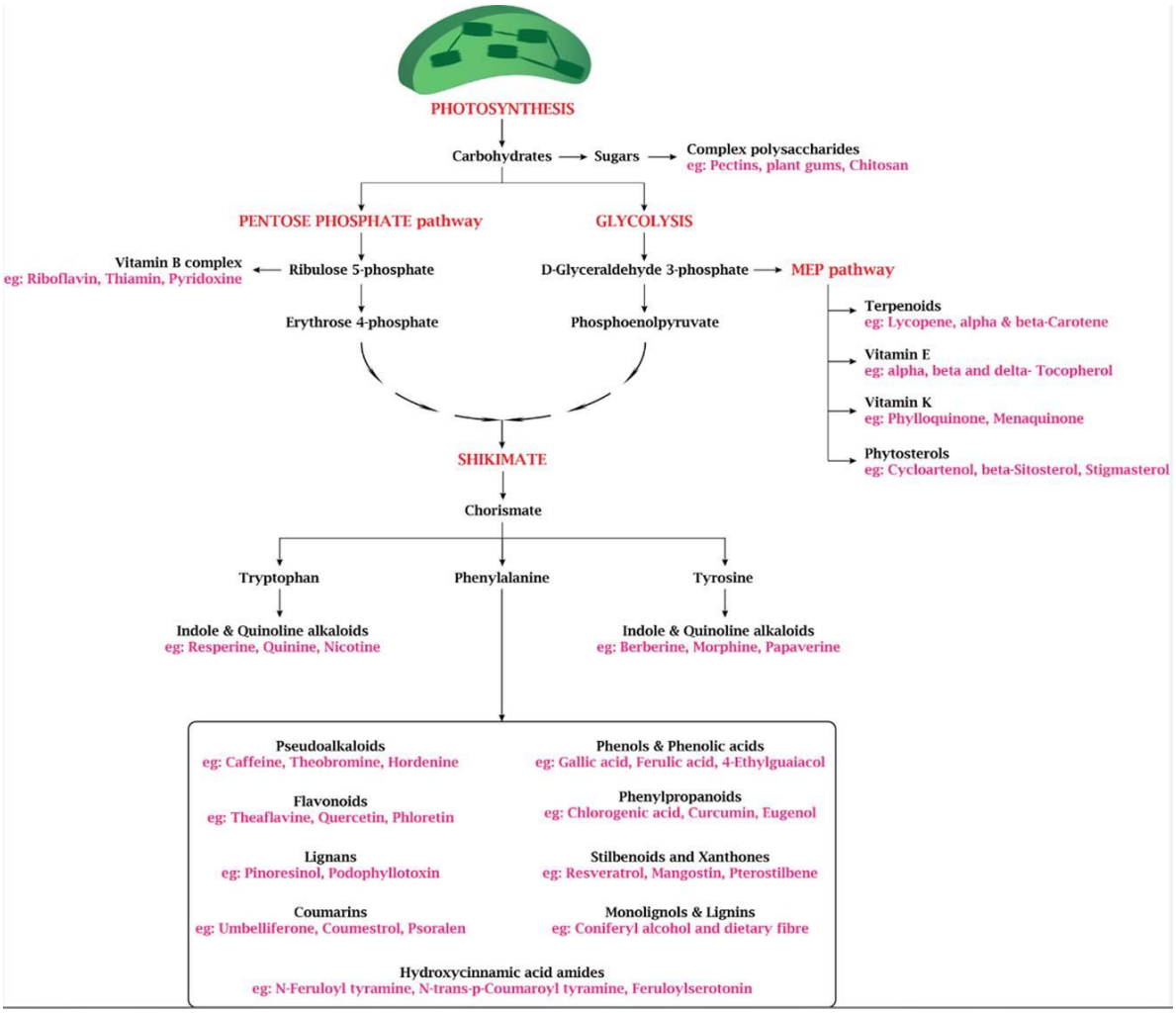

Figure 1. Simplified schematic representation of the important metabolic pathways involved in production of functional foods and nutraceuticals metabolites. Figure represents the metabolic pathways for biosynthesis of various functional foods related metabolites (shown in pink color). More details about plant metabolites and its pathways can be extracted from the plant-specific metabolic pathway database (PlantCyc): http://pmn.plantcyc.org/PLANT/organism-summary?object = PLANT.

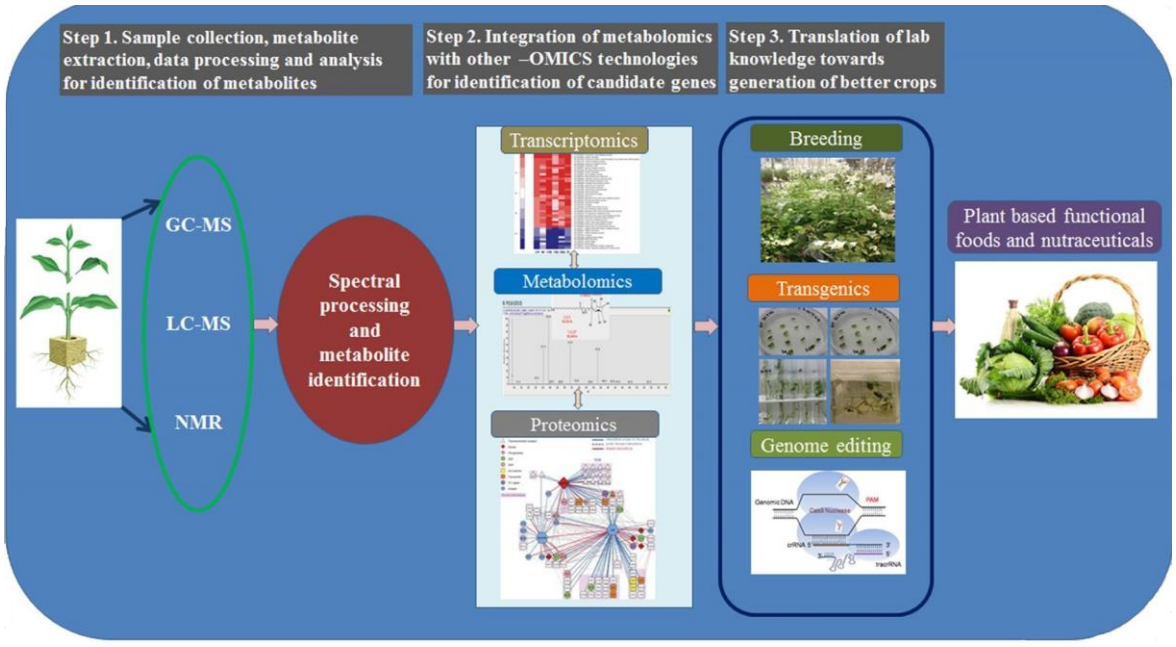

Figure 2. Workflow and schematic representation of application of metabolomics towards development of plant based functional foods and nutraceuticals.