JOURNAL OF PROTEOMICS 93 (2013) 234–244

Available online at www.sciencedirect.com

ScienceDirect

www.elsevier.com/locate/jprot

Review

Biofuels as a sustainable energy source: An update of the applications of proteomics in bioenergy crops and algae☆

Bongani Kaiser Ndimba[a,b], *Roya Janeen Ndimba*[c], *T. Sudhakar Johnson*[d], *Rungaroon Waditee-Sirisattha*[e], *Masato Baba*[f,g], *Sophon Sirisattha*[h], *Yoshihiro Shiraiwa*[f,g], *Ganesh Kumar Agrawal*[i,j], *Randeep Rakwal*[i,j,k,l,m,*]

[a]*Proteomics Research and Services Unit, Biotechnology Platform, Agricultural Research Council, Infruitec-Nietvoorbij Campus, Stellenbosch, South Africa*
[b]*Proteomics Research Group, Department of Biotechnology, University of the Western Cape, Bellville 7535, South Africa*
[c]*Materials Research Department, iThemba LABS, National Research Foundation, Old Faure Road, Somerset West, South Africa*
[d]*Natural Remedies Pvt. Ltd, 5B, Veersandra Industrial Area, Electronic City, Bangalore 560100, India*
[e]*Department of Microbiology, Faculty of Science, Chulalongkorn University, Bangkok 10330, Thailand*
[f]*Laboratory of Plant Physiology and Metabolism, Faculty of Life and Environmental Sciences, University of Tsukuba, Tsukuba 305-8572, Japan*
[g]*CREST, JST, Japan*
[h]*Thailand Institute of Scientific and Technological Research (TISTR), Changwat Pathum Thani 12120, Thailand*
[i]*Research Laboratory for Biotechnology and Biochemistry (RLABB), GPO Box 13265, Kathmandu, Nepal*
[j]*GRADE Academy Pvt. Ltd., Adarsh Nagar, Ward 13, Birgunj, Nepal*
[k]*Faculty of Life and Environmental Sciences, University of Tsukuba, Tsukuba 305-8572, Japan*
[l]*Organization for Educational Initiatives, University of Tsukuba, Tsukuba 305-8577, Japan*
[m]*Department of Anatomy I, Showa University School of Medicine, Tokyo 142-8555, Japan*

ARTICLE INFO

Available online 20 June 2013

Keywords:
Sustainable energy
Biofuel crops
Proteomics technology
Biomarkers
Translational proteomics

ABSTRACT

Sustainable energy is the need of the 21st century, not because of the numerous environmental and political reasons but because it is necessary to human civilization's energy future. Sustainable energy is loosely grouped into renewable energy, energy conservation, and sustainable transport disciplines. In this review, we deal with the renewable energy aspect focusing on the biomass from bioenergy crops to microalgae to produce biofuels to the utilization of high-throughput omics technologies, in particular proteomics in advancing our understanding and increasing biofuel production. We look at biofuel production by plant- and algal-based sources, and the role proteomics has played therein.
This article is part of a Special Issue entitled: Translational Plant Proteomics.

☆ This article is part of a Special Issue entitled: Translational Plant Proteomics.

* *Corresponding author at:* Organization for Educational Initiatives, 1-1-1 Tennodai, University of Tsukuba, Tsukuba 305-8577, Japan. Tel.: +81 90 1853 7875; fax: +81 29 853 6614.
E-mail address: plantproteomics@gmail.com (R. Rakwal).

1874-3919/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.jprot.2013.05.041

Contents

1. Introduction

The demand for sustainable energy ranks as one of the most pressing concerns of the 21st century. With dwindling fossil fuel reserves, rising crude oil prices and heightening fears over the effects of climate change, there is an urgent need to promote the use of renewable/alternative energy sources for a sustainable future [1–5]. One alternative to fossil fuels is nuclear powered energy, however, recent catastrophic events as seen in Chernobyl [2] and Fukushima [3], have cast serious doubt over the safety of nuclear energy. Before we move on to the focus of this review — bioenergy — it is important that we know the definition of sustainable energy. Sustainable energy is the sustainable provision of energy that meets the needs of the present without compromising the ability of future generations to meet their needs [6]. Such an approach is avidly being pursued at the level of national and regional policies aimed towards the reduction of fossil fuel emissions, promotion of sovereign energy, security stimulation of job creations and to drive overall economic developments [7]. Towards this end, significant investments are being made in the renewable energy sector, resulting in many promising advancements being made in the fields of solar, wind, geothermal, and biofuel technologies. In the scope of this review the role of biofuels, as a sustainable energy alternative, will be discussed, with particular focus on the role that proteomics and other emerging 'omics' technologies can play in its further development. Here it will be shown, with some examples that proteomics and other related post-genomics techniques namely transcriptomics are beginning to make a vital contribution to the development of our current knowledge and understanding several key bioenergy feed stock plants and algae.

2. Bioenergy — a sustainable energy source

Bioenergy refers to energy produced from biological materials, specifically photosynthetic organisms such as green plants, grasses, and algae. Bioenergy is currently the only alternative energy source able to supply liquid transportation fuels. This can be achieved by i) using fermentation of sugars to produce bio-ethanol (feedstock: sweet *Sorghum*, sugar cane, sweet potato, sugar beet etc.), ii) lignocellulosic biofuels that use all the plant material (feedstock: straw, corn stover, switch grass, poplar, etc.), iii) lipids derived from algae and other oil crops (feedstock: *Jatropha*, *Pongamia*, castor, sunflower, oil palm, etc.) after trans-esterification process, and iv) through the use of syngas obtained from gasification of biomass [8–14]. To date, liquid biofuels have been produced mainly in the USA, Brazil and several European nations. Further, there is a regional preference for biofuel types, with bio-ethanol produced in American and Asian countries, while biodiesel is preferentially produced in European nations and parts of Africa and Asia.

In the past decade, considerable research has been carried out to understand molecular mechanisms of biofuel plants. Rapidly developing post-genomics, systems biology approaches such as transcriptomics [15], proteomics [16], and metabolomics have become essential for understanding how plants respond and adapt to changes in their environment and yield improvement. The utilization of such high-throughput approaches will lead to the production better and high-yielding biomass feedstock which will eventually facilitate the acceleration of production and commercialization of biofuels.

2.1. Plant based: Sorghum and others

Cultivated *Sorghum* (*Sorghum bicolor* L. Moench), the fifth most important grain crop in the world, is a highly versatile cereal that has been selectively bred into four main varieties: grain, sweet stems, high-energy fiber, and for multi-purpose [17]. While the grain sorghums are grown mainly for household food security, the other *Sorghum* types are sought after for their commercial value. *Sorghum* fiber for example can be utilized in the manufacture of various paper and cardboard products [17]. On the other hand, the sweet-stemmed varieties are highly valued for their sucrose content, which is used to produce syrup and bio-ethanol [18]. The multi-purpose

sorghums or sweet *Sorghum* hybrids seem to combine the best of characteristics of both grain and non-grain varieties These varieties are hailed as 'ideal smart crops' because they have the potential to produce food, animal feed, fuel and fiber altogether in one crop [19]. For these reasons, *Sorghum* is the best energy crop for the developing world, particularly for the African continent. In addition to *Sorghum*, sugarcane (*Sacchrum* spp.), maize (*Zea mays*), and sugar beet (*Beta vulgaris*) are alternative sources of sugar-based energy crops.

The multipurpose plant, *Jatropha curcas* (L.) of the Euphorbiaceae family, is perennial in nature and is native to arid and semi-arid tropical regions worldwide. Among its many uses such as for animal (fish and livestock) feeding, *Jatropha* is considered to have great potential for renewable energy, particularly in terms of biodiesel production. Although the *J. curcas* is the most widely studied, other species include *Jatropha gossypifolia*, *Jatropha glandulifera*, *Jatropha multifida*, and *Jatropha podagrica*. *Jatropha* family members also have medicinal benefits. For example, *J. curcas* produces numerous secondary metabolites of medicinal importance; the leaf, fruits, latex and bark contain glycosides, tannins, phytosterols, flavonoids, and steroidal sapogenins exhibiting wide ranging medicinal properties, including anti-bacterial and anti-fungal activities [for review see, 20–23]. Concerning the cultivation and use of *Jatropha* for biodiesel production, Gmunder et al. have comprehensively discussed the cultivation of *J. curcas*, a biodiesel feedstock, which has been identified as suitable for achieving the Indian target of 20% biofuel blending by 2017 in the Indian context of rural development and climate change [22]. It was further shown that the use of *J. curcas* biodiesel generally reduces global warming potential and nonrenewable energy demand as compared to fossil diesel but does not decrease the environmental impacts on acidification, ecotoxicity, eutrophication, and water depletion [22].

2.2. *Algae/cyanobacteria based*

Biofuel production from plant-derived lignocelluloses is a potential way to exploit vast quantities of agricultural and forest residues, the most abundant biological cellulosic biomass on earth — the so called second-generation biofuels (reviewed by [24]). However, this approach has several major drawbacks because the technology to convert lignocelluloses to liquid biofuels efficiently is still lacking and therefore not yet economically viable [10]. During the last decade, scientists have looked for alternative sources and it has been shown that photosynthetic microorganisms such as microalgae and cyanobacteria would serve as an attractive feedstock because of several advantages. For instance, these specific microorganisms have the capacity to directly convert solar energy into biofuel [13]; they have higher growth rates requiring much less land area compared to plants [12]; they have simple nutrient requirements [25]; they are cultivable in aquaculture [26], and they can thrive in areas that cannot usually support mainstream agriculture. Moreover, several strains of cyanobacteria can be genetically engineered relatively easily [25–27].

How do scientists make biofuel from microalgae? The answer lies in the capability of microalgae to synthesize and accumulate high lipids and triglycerides (TAGs) mostly in their cells. Thus, these lipids are further used as feedstocks for direct conversion to biodiesel. Examples of natural lipid accumulator microalgae include *Botryococcus braunii* [28,29], *Chlorella vulgaris* [30], *Nanochloropsis oculata* [31], *Neochloris oleoabundans* [32], *Chlorella zofingiensis*, *N. oleoabundans*, and *Scenedesmus obliquus* [33]. These mostly contain their lipid bodies intracellularly, however, one of them, the green microalga *B. braunii* secretes its oils into the extracellular matrix [28,29]. Thus, this alga has gained a great interest and attention from scientists and the commercial world.

A cyanobacterium is also considered as an advantageous microorganism for biofuel production. Several strains such as *Synechocystis* sp. PCC 6803 and *Synechococcus elongatus* sp. PCC 7942 are naturally transformable; thus, they have potential for metabolic engineering. In recent years, biofuels synthesized from engineered cyanobacteria have been reported. For example, isobutanol, a higher alcohol (>C6), was produced at a high yield from *S. elongatus* sp. PCC 7942 by metabolic engineering of 2-ketoacid-based pathway [34]. Additionally, Lan and Liao reported high production of 1-butanol also in *S. elongatus* sp. PCC 7942 by modifying the Co-A dependent pathway [35]. Given the examples discussed above, the utilization of microalgae and cyanobacteria for production of biofuels provides a promising and sustainable energy source.

3. Proteomics-based discoveries, potential biomarkers, and translational proteomics

Proteomics is a systems biology based approach investigating the whole expressed proteins at a given time point and under certain conditions [36]. The method of choice for proteome analysis is the combination of high resolution protein separation like 2-dimensional gel electrophoresis (2-DGE) with tandem mass spectrometric (MS/MS) identification of proteins (for comprehensive reviews see, [37–41]). Agrawal et al. have recently reviewed the translational proteomics — "an emerging sub-discipline of the proteomics field in the biological sciences" [42]. We quote from the article — "Translational plant proteomics can thus be defined as "applying the outcome of any discovery or technological development in plant proteomics to solve issues related but not limited to the recreational and economic value of plants, food security and safety, energy sustainability, and human health"" [42]. Proteomics identification of target molecules will help increase our understanding of the organism in question (see plant and algae sections below) which may directly lead to their improvement with respect to production of biofuels, the target of this review.

3.1. *Proteomics of biofuel feedstocks*

3.1.1. *Sorghum*

The combination of *S. bicolor*'s natural stress tolerance traits and its recent genome sequencing completion, makes it one of the most logical model plant species for both proteomics and genomics research in cereals [43]. For example, *Sorghum* generally exhibits higher tolerance to environmental stresses, in comparison to maize [44], the world's most cultivated crop. As mentioned elsewhere, *Sorghum* therefore, stands as a fail–safe crop in the hot, dry and relatively saline regions of the

world, where it provides food, feed and fuel supplies for millions of people.

Despite the economic potential of this crop and the promising technique of proteomics approaches in understanding plant biological systems, to our knowledge, *Sorghum* proteomics is still very limited. The first attempt aimed towards *Sorghum* proteome analysis was carried out at the University of the Western Cape (UWC) in Cape Town, South Africa [45]. In that published research work, Ndimba's laboratory established a *Sorghum* cell suspension culture system for subsequent use in the proteomics analysis of both cellular and secreted proteins. The use of cell suspension cultures in *Sorghum* proteomics was largely motivated by the wide application of plant cell cultures in proteomics; the large supply of homogenous plant material provided for by these cultures; as well as the ease with which these cultures may be manipulated under a range of experimental conditions. That study profiled the 2-DGE protein patterns of the total soluble proteins (TSP) and secreted culture filtrate (CF) protein. That attempt culminated in a comprehensive mapping and characterization of the *Sorghum* cell suspension culture secretome [46]. In 2011, Swami and colleagues reported a proteomics analysis of 21 salt stress responsive proteins in a *Sorghum* stress experiment using 2-DGE and MS [47]. A year later, Ngara and colleagues in a similar experimental setup, identified 55 2-DGE separated protein spots [48]. That study used proteomics and bioinformatics tools to classify *Sorghum* leaf proteins into six broad functional categories: carbohydrate metabolism, proton transport, protein synthesis, hydrolytic enzymes, nucleotide metabolism, and detoxifying enzymes. The functional categories and proportional representation of these proteins are graphical representation illustrated in Fig. 1. As expected, carbohydrate metabolism related proteins were the most represented proteins. Proteomics of *Sorghum* (and sugarcane) is reviewed in detail in a book chapter written by Ndimba and Ngara [48,49].

3.1.2. *Sugarcane*

Sugarcane not only produces sugar but is a raw material in high demand for ethanol production [50,51]. Proteomics in sugarcane is at a preliminary stage, and the first proteomics analysis of the sugarcane stalk was performed in 2010 by a group of researchers from the Sugarcane Breeding Institute in Tamil Nadu (India), resulting in the generation of a 2-D gel proteome map [52]. That study identified numerous proteins, but the proteins involved in sugar metabolism (glyceraldehyde-3-phosphate dehydrogenase (GAPDH), glyceraldehyde 3-phosphate, putative UDP-glucose dehydrogenase, GAPDH, UDP-glucose 6-dehydrogenase, and triose-phosphate isomerases) were the most abundant, and these may be targets for further manipulation for increased or modified sugar content [53], with potential benefit in bioethanol production. However, the study is just one small step forward in understanding the total proteome of the sugarcane stalk. Nevertheless, parallel genetic approaches targeting enzymes such as the incorporation of cellulolytic enzymes that hydrolyze lignocellulosic substrates to fermentable sugars with potential application in production of lignocellulosic ethanol [54], and suppression of caffeic acid O-methyltransferase for modifying lignin biosynthesis for reducing the recalcitrance of ligocellulosic biomass to saccharification [55] are also been undertaken. Both genomics and proteomics approaches will therefore be necessary to achieve an understanding of sugar metabolism and other cellular processes involved in the utilization of fermentable sugars to bioethanol.

3.1.3. *Maize*

The utilization of tropical maize as an alternative energy crop is being considered as a feedstock for bioethanol production in the North Central and Midwest United States [56]. Compared to grain corn, there is good reason to use the tropical maize stalks as they contain a large amount of soluble sugars and have greater biomass. To the best of our knowledge there is no report on the proteomics of maize stalks, which remains an open topic for targeted proteomics study. However, a recent article has reviewed the proteomics of maize with respect to its growth and development and stress responses [57], which may serve as base for using the tools and techniques for stalk proteome analysis. As for sugarcane,

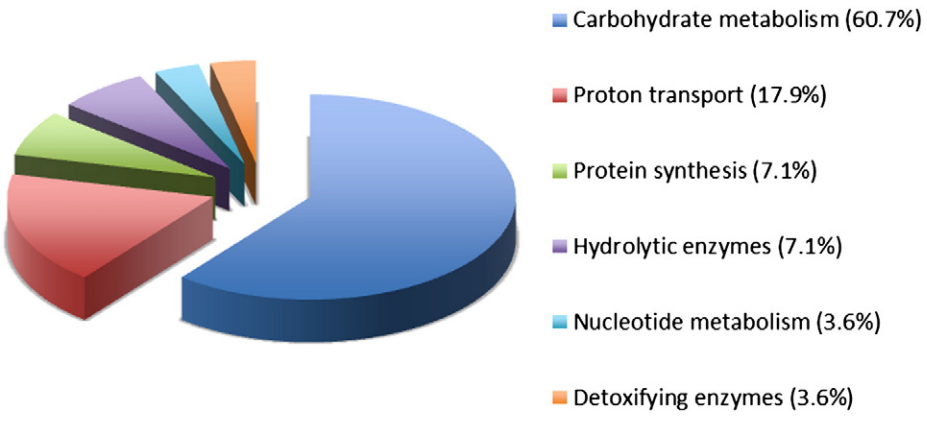

Fig. 1 – Functional distribution of *Sorghum* leaf protein extracts. These proteins were extracted from *Sorghum* seedlings, separated via 2-dimensional gel electrophoresis, visualized with Coomassie Brilliant Blue and identified with MALDI-TOF and MALDI-TOF-TOF MS. Numbers indicated in brackets represent the proportion of proteins within each functional category expressed as a percentage of positively identified protein spots.

genetic engineering approaches are also being used in maize for improving bioethanol production therein. For example, two recent studies used in planta expression of cell wall degrading enzymes (e.g., xylanase) for developing optimized biomass feedstocks that might enable low-cost cellulosic biofuels production [58,59]. The aim of these studies was to reduce the amount of enzymes required for feedstock pretreatment and hydrolysis during bioprocessing to release soluble sugars. Similar to sugarcane, the identification of the total proteome of the maize stalk or seed may help in identifying novel proteins/enzyme (e.g., those involved in starch production, cell wall composition, and biomass conversion enzymes — glucanase and expansin) that could be utilized for producing an appropriate biomass stage for bioethanol production [for review see, 60].

3.1.4. *Sugar beet*

Proteomics studies of sugar beet are also on the increase. Among these studies, one of the key papers is that of Julie Catusse and colleagues from Dominique Job's laboratory. Catusse et al. of France reported a comprehensive proteome-wide characterization of sugar beet seed and seedlings (root, stem, cotyledons, and perisperm) using a combination of 2-DGE and LC MS–MS approaches [61]. In summary, that study identified and quantified 759 sugar beet proteins in the context of their various tissue specific expression profiles. To our knowledge, the most recent sugar beet proteomics publication is from the Institute of Plant Nutrition at Justus Liebig University in Germany. There they reported work that identified nine proteins whose expressions were significantly and reproducibly altered under salt stress conditions [62]. Their claim for this very low number of identified proteins is due to the acquired adaptation of sugar beet to salinity stress. This explanation is however questionable as there is insufficient data to support this claim. The most possible reason for the visualization and identification of only nine statistically significant protein candidates is probably due to experimental design and/or the sensitivity of the chosen proteomics technique.

3.1.5. *Jatropha*

Proteomics analysis in *J. curcas* is mainly confined to characterization of oil bodies and understanding oil biogenesis. Later studies were extended to understanding fatty acid biosynthesis and stress responsive proteins. *Jatropha* meal, which is a rich source of protein, contains toxic phorbol esters and antinutritional factors. Extensive studies to detoxify toxic constituents to make it fit for animal diet have also been conducted [63]. Fig. 2 illustrates the protein types identified in *Jatropha*.

Yang et al. studied oil mobilization during seed germination and post-germination development through proteomics analysis of endosperm in germinating seeds [64]. Results showed that initiation of oil mobilization occurs during germination and subsequently the oil gets consumed during early seedling development. Several pathways such as beta-oxidation, glyoxylate cycle, glycolysis, tricholoroacetic acid cycle (TCA) cycle, gluconeogenesis, and pentose phosphate pathways were found to be involved in oil mobilization. In a different study, proteomics analysis of the soluble proteins derived from embryo and endosperm of mature seeds of *J. curcas* was compared [65]. The results indicated that both tissues include proteins related to stress and signal transduction. The proteins in the endosperm were predominantly catabolism-related enzymes and reserves that provided nutrition for the growing embryo while the embryo-specific proteins were related to anabolism and utilized the nutrition from the endosperm for further growth. In a similar study focused on the proteomics analysis of dry mature seeds of *J. curcas* [66] the 2-DGE profiles of the endosperm and embryo were found to be similar to each other. There are 66 differential proteins between the two seed tissues, in which 28 proteins distributed in nine distinct functional classes have been identified successfully in endosperm or embryo. The major groups of differential proteins are associated with metabolism (25%) and disease/defense (18%). The results demonstrated that in the dry mature *J. curcas* seeds, the proteins involved in oil mobilization, signal transduction, transcription, protein synthesis, and cell cycle which are essential for the seed germination have occurred in endosperm and embryo, reflecting the fact that proteins required for germination are already present in the dry mature seed.

Popluechai et al. studied the proteomics composition of the oil bodies of *J. curcas* and related *Jatropha* species [67]. The oil bodies revealed oleosins as the major components and three oleosins (JcOle1, JcOle2, JcOle3) were isolated and characterized at the gene, transcript, and protein level. The transcript level of JcOle3 was about five-fold higher as compared to the other two oleosins. Interestingly, this oleosin (JcOle3) showed allelic variation and single nucleotide polymorphism in its intron region, which could serve as marker in phylogenetic and molecular breeding studies. Makkar et al. [68] recovered protein concentrate in *Jatropha* seed cake. The presence of phorbol esters and antinutrient factors such as trypsin inhibitor, lectin, and phytate prevents its use in animal diets [68]. Using principles of isoelectric precipitation, above proteins were recovered.

Eswaran and colleagues conducted an in-depth study for understanding the stress responsive proteins [15]. During that study metallothioneins were found to be major class of proteins that are abundantly represented during the stress response. Metallothioneins are low-molecular weight, cys-rich proteins that have a role in metal detoxification. Further, proteins such as aquaporins, plant annexins, and thioredoxins were expressed indicating their role in abiotic stress. Their analysis also identified different classes of transcription factors (TFs) that have been implicated in different regulatory cascades. The TFs identified were AP2/EREBP regulating ethylene, abscisic acid (ABA) and auxin, zinc finger proteins, YABBY, bHLH, bZIP, and WRKY [15]. Overall, the above mentioned studies identified possible factors to understand abiotic stress tolerance in *J. curcas* that can probably be extended to other related energy crops.

Of the most extensive studies, identification of enzymes and their respective genes involved in fatty acid metabolism are noteworthy. The acyl-acyl carrier protein (ACP) thioesterase from *J. curcas* was cloned and expressed in *Arabidopsis*, which resulted in increased levels of saturated fatty acid and reduced levels of unsaturated fatty acids [69]. Further, the heteromeric subunits of *JcACCase* (acetyl CoA carboxylase) were cloned and studied by Gu and colleagues [70]. ACCase is a key enzyme involved in fatty acid synthesis. Other enzymes involved in fatty acid biosynthesis such as KASIII (beta-ketoacyl acyl-carrier

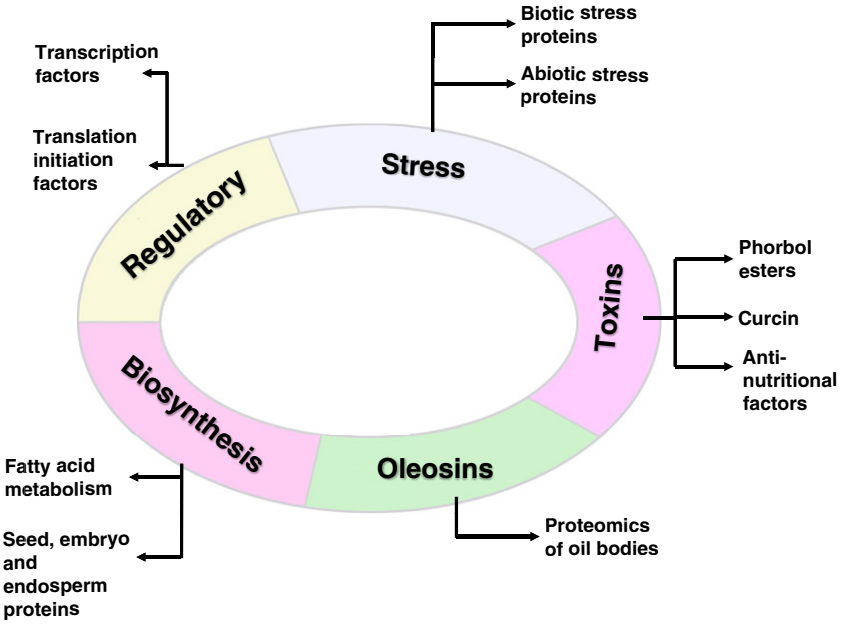

Fig. 2 – Broad description of *Jatropha* proteomics studies. Details are in the text.

protein synthase III) and steroyl-acyl carrier protein desaturase were also studied extensively. Those studies have helped to understand the mechanism of regulation of fatty acid synthesis in *J. curcas*.

Phorbol esters are fatty acid esters of the diterpenoids. Several studies revealed key genes/enzymes involved in diterpenoid biosynthetic pathway. An isoprenoid biosynthesis gene, 3-hydroxy-3-methyl glutaryl coenzyme-A reductase (HMGR) and its protein was characterized by Lin and colleagues [71]. HMGR catalyzes first committed step in mevalonic acid synthesis which leads to production of phorbol esters. De-activation or reduction of toxicity levels to acceptable limits in *Jatropha* seed cake has been target of several studies [63], which can ultimately make it fit for animal consumption, and even to human consumption.

3.2. *Proteomics of biofuel algae/cyanobacteria*

The development of algal proteomics has followed the trends set by progress made in animal and higher plant proteomics. Although there are many studies involving algae for biofuel production, we discuss here only a selected few. Supplementary Table 1 lists the algal proteomics studies.

In microalgal model organisms (e.g., *Synechocystis* sp. PCC 6803 (for review see [72]) and *Chlamydomonas reinhardtii* (for review see [73])), a proteomics approach has been adopted mainly for analyzing the proteins of the subcellular compartments or under stress responses in combination with genome or expressed sequence tag (EST) databases (namely sub-proteomics analysis). On the other hand, proteomics data on non-model organisms is quite sparse and provides only minimal and relatively basic information. Non-genome-sequenced algae can be used for proteomics (namely, using EST databases or by de novo sequencing) but both quality and quantity of data are strongly limited.

Sample preparation is very important for algal samples since polysaccharide-rich specimens hinder separation by SDS-PAGE. In macroalgae, successful analyses were achieved by extracting the protein with organic solvent and purifying the extract using the ethanol/phenol method [74,75], phenol/chloroform method [76], or phenol extraction and desalting steps [77,78]. For useful technical information see review by Loretto Contreras-Porcia and Camilo López-Cristoffanini [79].

Research on biofuels is attracting attention in new areas. Proteomics analysis for hexane resistance of *Synechocystis* sp. PCC 6803 is particularly noteworthy [80]. Alkane biosynthesis by microorganisms reported in older studies was recently revisited in cyanobacteria [81]. Accordingly, a proteomics analysis of the responses of *Synechocystis* cells to hexane, a representative of alkane, has been carried out to develop it as an improved host cell factory. Those authors adopted "isobaric tag for relative and absolute quantification (iTRAQ)" system to analyze the global metabolic response by 0.8% hexane treatment. In this case, cell growth was inhibited approximately by 50%, comparing with no hexane (control), at both 24 h (the middle-exponential phase) and 48 h (the exponential–stationary transition phase). It was noted that intracellular production of hexane may cause much stronger toxicity even at lower concentration. This suggests that hexane resistant ability is important for future algal biofuel production within the cells. By using a cut off of 1.5-fold change (p-value < 0.05), a total of 164 up-regulated and 77 down-regulated proteins were determined. From functional annotation of those proteins and the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analyses, those authors observed that a large number of transporters and membrane-bound proteins, proteins related to sulfur relay system, oxidative stress and photosynthesis were induced and those proteins might be associated with protection mechanisms against hexane.

C. reinhardtii is known to produce hydrogen (H_2) gas under anaerobic conditions, which is remarkable in the context of renewable energy. An elegant comparative proteomics study was carried out to identify the proteins involved during this anaerobic acclimation and to localize proteins and pathways involved in the chloroplasts and mitochondria of *C. reinhardtii* [82]. In that study, stable isotopic labeling of amino acids was used to analyze protein expression quantitatively. Further, localization of major H_2 fermentative components was confirmed at the protein level. The quantitative data not only confirmed previously characterized proteins that were induced at the transcript level [83] but also identified several new proteins of unknown function induced under anaerobic conditions [84]. These unknown proteins may be the most interesting as their functional analysis could provide new direction for the engineering of hydrogen-producing alga strains [82].

Oil is usually stored in an organelle named as lipid droplet (LD, synonym of oleosome, lipid granule, lipid body, and so on). Moellering & Benning first identified a structural protein which is specifically localized in purified LD of *C. reinhardtii*, designated as MLDP, by proteomics [85]. Now specific proteins associated with LD structure and their functions have been identified in algae in addition to animals, yeasts, and higher plants although those proteins are quite distinct among species [86–90]. MLDP is specific to the green algal lineage of photosynthetic organisms. The authors also demonstrated that the knock-down of MLDP gene expression by RNAi resulted in increasing LD size but did not cause any change in triacylglycerol synthesis and metabolism. Recent studies on plant LD revealed new roles for LD, i.e. not only in lipid storage but also in lipid metabolism [91]. The study of LD in microalgae is therefore important for enhancing our present understanding of the overall processes associated with lipid metabolism.

The rate of photosynthetic carbon fixation is surely the most important limiting factor for hydrocarbon productivity, material production, and cell growth rate. The aquatic environment where microalgae live is generally CO_2-limiting (for review see [92]). Therefore, most of microalgae possess the CO_2-concentrating mechanism (CCM) to facilitate the utilization of ambient CO_2 as substrate for photosynthesis. CCM is a low-CO_2-inducible mechanism and therefore is diminished under high-CO_2 environment. Exogenous CO_2 supplementation prevents the CO_2-limitation and accelerates photosynthesis. However, the maximal photosynthetic carbon fixation rate and high-CO_2 tolerance against excess CO_2 supply are dependent on the original character of each microalgal strain. Production of extracellular proteins was shown to be very sensitive to changes in the extracellular CO_2 concentration in *C. reinhardtii*. By proteomics analysis, 22 of the high-CO_2-inducible extracellular proteins including gametogenesis-related proteins and hydroxyproline-rich glycoproteins were identified, although we do not know their physiological roles [93]. Mechanisms regulating the response to environmental CO_2 conditions will be a very important future topic aimed at answering how to increase the total carbon input for biofuel production.

Proteomics-focused studies of non-genome-sequenced algae are still a major challenge. However, there is a recent report focused on the proteomics of an unsequenced microalga, a strain of *C. vulgaris*, by using high-throughput de novo transcriptome data as a guide [94]. This new strategy facilitated the proteomics analysis of a non-model organism of which the complete genome is not available so far, and then the mechanism for accumulation of triacylglycerol biosynthesis components under nitrogen-depleting conditions was reported.

Euglenophyte *Euglena gracilis* is known to produce a unique wax ester under anaerobic conditions [95]. By considering recent progress in understanding of wax ester synthesis, previous proteomics results on metabolic regulation under anaerobic conditions will be revisited [96].

The combination of omics studies (e.g., genomics, proteomics, and lipidomics) is generally considered as one of the strongest tools to study algal metabolism towards the global understanding of metabolic process under various environmental conditions. Model algae are quite useful for use in both metabolomics and proteomics experimental systems towards the understanding of key metabolic networks and its fluxes [97,98]. This data should also be useful to elucidate metabolic mechanism of non-model algae. Establishment of genetic engineering in algae is significant for fundamental and applied science (use), and therefore should be studied together with lipid metabolism [99,100]. Detailed proteomics and lipidomics analyses will help unravel the role of these molecules in production of biofuel, and thus play an important role in promoting algal energy production in the coming years.

4. Concluding remarks

Research initiatives in proteomics for biofuel production are steadily growing. The majority of the research is at the stage of identifying protein components and creating database that can be exploited for plant- and algal-based biofuel production via translational proteomics in combination with other molecular and breeding/plant genetic engineering approaches. Inventory of protein components is growing but still there is a long way to go for identifying and listing most if not all of them associated with biofuel production. For this purpose, complementary proteomics approaches and in-depth proteome analysis will be required with special attention to identity extreme protein components such as highly acidic and basic proteins and low-abundance proteins.

As discussed in this review, proteomics is one way to understand the biology behind biofuel production and also design new approaches and/or modify plant and algal sources for increased biofuel production. Integration of proteomics findings with findings obtained from other approaches such as genomics, transcriptomics and metabolomics can further pave the way for development of more productive energy crops. The draft genome sequences of *Brachypodium*, *Populus*, *Sorghum*, *Jatropha*, *Ricinus*, and maize have been published [43,101–105]. Likewise, the chloroplast genome of date palm and *J. curcas* has been sequenced [106,107]. These resources will be instrumental in developing the tools for functional genomics and proteomics assays and will allow comparative genomics approaches between model species and biofuel crops to become a reality. For example, approaches for gene expression profiling via transcriptomics experiments have been applied to identify enzymes involved in fatty acid metabolism [108], stress tolerance [15,109], and transcription factor proteins for

improvement of *J. curcas* [110–112] and other related energy crops as a source of biodiesel. This review indicates that progress has been made in the direction of biofuel production, but yet there is a long way to have a holistic view at systems biology level.

This proteomics review is a direct evidence for growing interest in biofuel research, and also as per the indication of a recent patent trend [14]. However, a particular standing point is the question of balance between food production and biofuel production [113]. Looking at the plant-based biofuels, it must be remembered that most of the first-generation biofuels have been or are prepared from agricultural commodities [114]. Let us look at one aspect of food production, namely the use of fertilizers. As recently reviewed by Hein and Leemans, all first-generation biofuel production systems require phosphorus (an essential plant nutrient of finite amount) fertilization [114]. Those authors argue that committing scarce P to biofuel production involves a trade-off between climate change mitigation and future food production. This suggestion was based on the finding that around 2% of the global inorganic P fertilizer stock is used up for biofuel production the contribution to P depletion exceeds the contribution to mitigating climate change [114]. Taken together, the study concluded that, with the current production systems first-generation biofuel compromises future food production. However, when we consider the second-generation biofuels, from the microalgae, nutrient recycling and nondestructive extraction of product ("fuel") from cells might constitute an important solution to the avoid the above mentioned nutrient drawback using plant-based sources.

So, how does the use of algae stand out as an alternative feedstock for biofuel production? One reason for the growing attention worldwide to these algal sources lies in their fast growth rate and ability to accumulate high quantity of lipid and carbohydrate inside their cells for biodiesel and bioethanol production, respectively [115]. Lam and Lee argued that algal feedstock offers several environmental benefits, such as effective land utilization, CO_2 sequestration, self-purification if coupled with wastewater treatment and does not trigger food versus fuel feud [115]. However, those authors also caution that "despite having all these 'theoretical' advantages, review on problems and issues related to energy balance in microalgae biofuel are not clearly addressed until now". Thus, we are still far away from the true potential of algal-based commercial biofuel production and its contribution towards energy security.

Taken together, more fundamental studies on both plant and algal sources will be required to gain a better understanding of the biology behind oil production. The practical aspects of biofuel production also deserve more consideration and action to make any significant contribution to the alternative energy sources. Finally, and most importantly, the choice of plant or algae to be utilized for biofuel production has to be carefully considered.

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.jprot.2013.05.041.

Acknowledgments

RR acknowledges the great support of Professor Koji Nomura (Organization for Educational Initiatives, University of Tsukuba) and Professor Seiji Shioda and Dr. Tetsuo Ogawa (Department of Anatomy I, Showa University School of Medicine) in promoting interdisciplinary research and unselfish encouragement. Authors acknowledge the INPPO (www.inppo.com) platform for this initiative in bringing together scientists of different disciplines in constructing this review. Finally, given the vast amount of research in these disciplines and space limitations, many works could not be cited and discussed in this review.

REFERENCES

[1] Hepbasli A. A key review on exergetic analysis and assessment of renewable energy resources for a sustainable future. Renew Sust Energy Rev 2008;12:593–661.
[2] Stone R. Nuclear radiation. Return to the inferno: Chernobyl after 20 years. Science 2006;312:180–2.
[3] Stone R. Devastation in Japan. Fukushima cleanup will be drawn out and costly. Science 2011;331:1507.
[4] Central Intelligence Agency World Factbook. www.cia.gov.
[5] Weart SR. The discovery of global warming: revised and expanded edition (new histories of science, technology, and medicine). Harvard University Press; 2008 .
[6] http://en.wikipedia.org/wiki/Sustainable_energy; 2013 . [accessed January 7th, 2013].
[7] Tester JW, Drake EM, Driscoll MJ, Golay MW. Sustainable energy: choosing among options. Cambridge, MA, USA: The MIT Press; 2005 .
[8] Schmer MR, Vogel KP, Mitchell RB, Perrin RK. Net energy of cellulosic ethanol from switch grass. Proc Natl Acad Sci U S A 2008;105:464–9.
[9] Divakara BN, Upadhyaya HD, Wani SP, Laxmipathi Gowda CL. Biology and genetic improvement of *Jatropha curcas* L.: a review. Appl Energy 2010;87:732–42.
[10] Blanch HW, Simmons BA, Klein-Marcuschamer D. Biomass deconstruction to sugars. Biotechnol J 2011;6:1086–102.
[11] Johnson TS, Eswaran N, Sujatha M. Molecular approaches to improvement of *Jatropha curcas* Linn. as a sustainable energy crop. Plant Cell Rep 2011;30:1573–91.
[12] Malcata FX. Microalgae and biofuels: a promising partnership? Trends Biotechnol 2011;29:542–9.
[13] Parmar A, Singh NK, Pandey A, Gnansounou E, Madamwar D. Cyanobacteria and microalgae: a positive prospect for biofuels. Bioresour Technol 2011;102:10163–72.
[14] Johnson TS, Badri J, Sastry RK, Srivastava A, KaviKishor PV, Sujatha M. Genetic improvement of biofuel plants: recent progress and patents. Recent Pat DNA Gene Seq 2013;7:2–12.
[15] Eswaran N, Sriram P, Balaji S, Raja KK, Bhagyam A, Johnson TS. Generation of expressed sequence tag (EST) library from salt stressed roots of *Jatropha curcas* for the identification of abiotic stress responsive genes. Plant Biol 2012;14:428–37.
[16] Maghuly F, Kogler S, Marzban G, Nöbauer K, Razzazi E, Laimer M. Proteomics, a systems biology based approach to investigations of *Jatropha curcas* seeds. BMC Proc 2011;5(Suppl. 7):P162.
[17] Woods J. The potential for energy production using sweet sorghum in southern Africa. Energy Sustain Dev 2001;5:31–8.
[18] Prasad S, Singh A, Jain N, Joshi HC. Ethanol production from sweet sorghum syrup for utilisation as automotive fuel in India. Energy Fuel 2007;21:2415–20.
[19] Reddy BVS, Ashok Kumar A, Sanjana Reddy P. Recent advances in sorghum improvement research at ICRISAT. Kasetsart J (Nat Sci) 2010;44:499–506.
[20] Abdulla R, Chan ES, Ravindra P. Biodiesel production from *Jatropha curcas*: a critical review. Crit Rev Biotechnol 2011;31:53–64.

[21] Debnath M, Bisen PS. *Jatropha curcas* L., a multipurpose stress resistant plant with a potential for ethnomedicine and renewable energy. Curr Pharm Biotechnol 2008;9:288–306.
[22] Gmunder S, Singh R, Pfister S, Adheloya A, Zah R. Environmental impacts of *Jatropha curcas* biodiesel in India. J Biomed Biotechnol 2012;2012:623070.
[23] Paulillo LC, Mo CL, Isaacson J, Lessa L, Romero-Suarez EL, Brotto L, et al. *Jatropha curcas*: from biodiesel generation to medicinal applications. Recent Pat Biotechnol 2012;6:192–9.
[24] Timilsina G, Shrestha A. How much hope should we have for biofuels? Energy 2011;36:2055–69.
[25] Ruffing AM. Engineered cyanobacteria: teaching an old bug new tricks. Bioeng Bugs 2011;2:136–49.
[26] Golden SS, Brusslan J, Haselkorn R. Genetic engineering of the cyanobacterial chromosome. Methods Enzymol 1987;153: 215–31.
[27] Waditee R, Bhuiyan MN, Rai V, Aoki K, Tanaka Y, Hibino T, et al. Genes for direct methylation of glycine provide high levels of glycinebetaine and abiotic-stress tolerance in *Synechococcus* and *Arabidopsis*. Proc Natl Acad Sci U S A 2005;102:1318–23.
[28] Sakamoto K, Baba M, Suzuki I, Watanabe MM, Shiraiwa Y. Optimization of light for growth, photosynthesis, and hydrocarbon production by the colonial microalga *Botryococcus braunii* BOT-22. Bioresour Technol 2012;110:474–9.
[29] Niitsu R, Kanazashi M, Matsuwaki I, Ikegami Y, Tanoi T, Kawachi M, et al. Changes in the hydrocarbon-synthesizing activity during growth of *Botryococcus braunii* B70. Bioresour Technol 2012;109:297–9.
[30] Thangalazhy-Gopakumar S, Adhikari S, Chattanathan SA, Gupta RB. Catalytic pyrolysis of green algae for hydrocarbon production using H + ZSM-5 catalyst. Bioresour Technol 2012;118:150–7.
[31] Taylor RL, Rand JD, Caldwell GS. Treatment with algae extracts promotes flocculation, and enhances growth and neutral lipid content in *Nannochloropsis oculata* — a candidate for biofuel production. Mar Biotechnol (N Y) 2012;14:774–81.
[32] Rismani-Yazdi H, Haznedaroglu BZ, Hsin C, Peccia J. Transcriptomic analysis of the oleaginous microalga *Neochloris oleoabundans* reveals metabolic insights into triacylglyceride accumulation. Biotechnol Biofuels 2012;5:74.
[33] Breuer G, Lamers PP, Martens DE, Draaisma RB, Wijffels RH. The impact of nitrogen starvation on the dynamics of triacylglycerol accumulation in nine microalgae strains. Bioresour Technol 2012;124:217–26.
[34] Atsumi S, Higashide W, Liao JC. Direct photosynthetic recycling of carbon dioxide to isobutyraldehyde. Nat Biotechnol 2009;27:1177–80.
[35] Lan EI, Liao JC. Metabolic engineering of cyanobacteria for 1-butanol production from carbon dioxide. Metab Eng 2011;3:353–63.
[36] Wilkins MR, Sanchez JC, Gooley AA, Appel RD, Humphery-Smith I, Hochstrasser DF, et al. Progress with proteome projects: why all proteins expressed by a genome should be identified and how to do it. Biotechnol Genet Eng Rev 1995;13:S19–50.
[37] Finnie C. Plant proteomics. In: Finnie C, editor. Hoboken, NJ, USA: John Wiley & Sons, Inc.; 2006.
[38] Thiellement H, Zivy M, Damerval C, Mechin V. In: Thiellement H, editor. Plant proteomics: methods and protocols, vol. 355. Humana Press; 2007.
[39] Agrawal GK, Rakwal R. Plant proteomics: technologies, strategies, and applications. In: Agrawal GK, Rakwal R, editors. Hoboken, NJ, USA: John Wiley & Sons, Inc.; 2008.
[40] Righetti PG, Antonioli P, Simo' C, Citterio A. Gel-based proteomics. In: Agrawal GK, Rakwal R, editors. Plant proteomics: technologies, strategies, and applications. Hoboken, NJ: John Wiley & Sons, Inc.; 2008. p. 11–30.
[41] Jorrín-Novo JV, Maldonado AM, Echevarría-Zomeño S, Valledor L, Castillejo MA, Curto M, et al. Plant proteomics update (2007–2008): second-generation proteomic techniques, an appropriate experimental design, and data analysis to fulfill MIAPE standards, increase plant proteome coverage and expand biological knowledge. J Proteomics 2009;72:285–314.
[42] Agrawal GK, Pedreschi R, Barkla BJ, Bindschedler LV, Cramer R, Sarkar A, et al. Translational plant proteomics: a perspective. J Proteomics 2012;75:4588–601.
[43] Paterson AH, Bowers JE, Bruggmann R, Dubchak I, Grimwood J, Gundlach H, et al. The *Sorghum bicolor* genome and the diversification of grasses. Nature 2009;457:551–6.
[44] Krishnamurthy L, Serraj R, Tom Hash C, Dakheel AJ, Reddy BVS. Screening sorghum genotypes for salinity tolerant biomass production. Euphytica 2007;156:15–24.
[45] Ngara R, Rees J, Ndimba BK. Establishment of sorghum cell suspension culture system for proteomics studies. Afr J Biotechnol 2008;7:744–9.
[46] Ngara R, Ndimba BK. Mapping and characterisation of the sorghum cell suspension culture secretome. Afr J Biotechnol 2011;10:253–66.
[47] Kumar Swami A, Imteyaz Alam S, Sengupta N, Sarin R. Differential proteomic analysis of salt stress response in *Sorghum bicolor* leaves. Environ Exp Bot 2011;71:321–8.
[48] Ngara R, Ndimba R, Borch-Jensen J, Nørregaard Jensen O, Ndimba BK. Identification and profiling of salinity stress-responsive proteins in *Sorghum bicolor* seedlings. J Proteomics 2012;75:4139–50.
[49] Ndimba BK, Ngara R. Sorghum and sugarcane proteomics. In: Paterson AH, editor. Genomics of the saccharinae, plant genetics and genomics: crops and models, 11. New York: Springer Science + Business Media; 2013. p. 141–68 [Chapter 7].
[50] Canilha L, Kumar Chandel A, dos Santos Milessi TS, Fernandes Antunes FA, da Costa Freitas WL, das Graças Almeida Felipe M, et al. Bioconversion of sugarcane biomass into ethanol: an overview about composition, pretreatment methods, detoxification of hydrolysates, enzymatic saccharification, and ethanol fermentation. J Biomed Biotechnol 2012:989572.
[51] Arruda P. Genetically modified sugarcane for bioenergy generation. Curr Opin Biotechnol 2012;23:315–22.
[52] Amalraj RS, Selvaraj N, Veluswamy GK, Ramanujan RP, Muthurajan R, Palaniyandi M, et al. Sugarcane proteomics: establishment of a protein extraction method for 2-DE in stalk tissues and initiation of sugarcane proteome reference map. Electrophoresis 2010;31:1959–74.
[53] Wu L, Birch RG. Doubled sugar content in sugarcane plants modified to produce a sucrose isomer. Plant Biotechnol J 2007;5:109–17.
[54] Harrison MD, Geijskes J, Coleman HD, Shand K, Kinkema M, Palupe A, et al. Accumulation of recombinant cellobiohydrolase and endoglucanase in the leaves of mature transgenic sugar cane. Plant Biotechnol J 2011;9:884–96.
[55] Jung JH, Vermerris W, Gallo M, Fedenko JR, Erickson JE, Altpeter F. RNA interference suppression of lignin biosynthesis increases fermentable sugar yields for biofuel production from field-grown sugarcane. Plant Biotechnol J 2013, http://dx.doi.org/10.1111/pbi.12061 [in press].
[56] Chen MH, Kaur P, Dien B, Below F, Vincent ML, Singh V. Use of tropical maize for bioethanol production. World J Microbiol Biotechnol 2013 [in press, PMID: 23508398].
[57] Pechanova O, Takáč T, Samaj J, Pechan T. Maize proteomics: an insight into the biology of an important cereal crop. Proteomics 2013;13:637–62.
[58] Gray BN, Bougri O, Carlson AR, Meissner J, Pan S, Parker MH, et al. Global and grain-specific accumulation of glycoside hydrolase family 10 xylanases in transgenic maize (*Zea mays*). Plant Biotechnol J 2011;9:1100–8.

[59] Shen B, Sun X, Zuo X, Shilling T, Apgar J, Ross M, et al. Engineering a thermoregulated intein-modified xylanase into maize for consolidated lignocellulosic biomass processing. Nat Biotechnol 2012;30:1131–6.
[60] Torney F, Moeller L, Scarpa A, Wang K. Genetic engineering approaches to improve bioethanol production from maize. Curr Opin Biotechnol 2007;18:193–9.
[61] Catusse J, Strub JM, Job C, Van Dorsselaer A, Job D. Proteome-wide characterisation of sugarbeet seed vigor and its tissue specific expression. Proc Natl Acad Sci U S A 2008;105:10262–7.
[62] Wakeel A, Asif AR, Pitann B, Schubert S. Proteome analysis of sugar beet (*Beta vulgaris* L.) elucidates constitutive adaptation during the first phase of salt stress. J Plant Physiol 2011;168:519–26.
[63] Kumar GRK, Bapat VA, Johnson TS. Phorbol esters and other toxic constituents of *Jatropha curcas* L. In: Carels N, editor. Jatropha, challenges for a new energy crop, vol. 1. NY: Springer Science; 2013. p. 441–60.
[64] Yang MF, Liu YJ, Liu Y, Chen H, Chen F, Shen SH. Proteomic analysis of oil mobilization in seed germination and postgermination development of *Jatropha curcas*. J Proteome Res 2009;8:1441–51.
[65] Liu H, Liu YJ, Yang MF, Shen SH. A comparative analysis of embryo and endosperm proteome from seeds of *Jatropha curcas*. J Integr Plant Biol 2009;51:850–7.
[66] Liu H, Yang Z, Yang MF, Shen S. The differential proteome of endosperm and embryo from mature seed of *Jatropha curcas*. Plant Sci 2011;181:660–6.
[67] Popluechai S, Froissard M, Jolivet P, Breviario D, Gatehouse AMR, Donnell AGO, et al. *Jatropha curcas* oil body proteome and oleosins: L-form JcOle3 as a potential phylogenetic marker. Plant Physiol Biochem 2011;49:352–6.
[68] Makkar HPS, Francis G, Becker K. Protein concentrate from *Jatropha curcas* screw-pressed seed cake and toxic and antinutritional factors in protein concentrate. J Sci Food Agric 2008;88:1542–8.
[69] Wu PZ, Li J, Wei Q, Zheng L, Chen YP, Li MR, et al. Cloning and functional characterization of an acyl-acyl carrier protein thioesterase (JcFATB1) from *Jatropha curcas*. Tree Physiol 2009;29:1299–305.
[70] Gu K, Chiam H, Tian D, Yin Z. Molecular cloning and expression of heteromeric ACCase subunit genes from *Jatropha curcas*. Plant Sci 2011;180:642–9.
[71] Lin J, Jin Y, Zhou M, Zhou X, Wang J. Molecular cloning, characterization and functional analysis of a 3-hydroxy-3-methylglutaryl coenzyme A reductase gene from *Jatropha curcas*. Afr J Biotechnol 2009;8:3455–62.
[72] Pandhal J, Wright PC, Biggs CA. Proteomics with a pinch of salt: a cyanobacterial perspective. Saline Syst 2008;4:1.
[73] Stauber EJ, Hippler M. *Chlamydomonas reinhardtii* proteomics. Plant Physiol Biochem 2004;42:989–1001.
[74] Nagai K, Yotsukura N, Ikegami H, Kimura H, Morimoto K. Protein extraction for 2-DE from the lamina of *Ecklonia kurome* (laminariales): recalcitrant tissue containing high levels of viscous polysaccharides. Electrophoresis 2008;29:672–81.
[75] Yotsukura N, Nagai K, Kimura H, Morimoto K. Seasonal changes in proteomic profiles of Japanese kelp: *Saccharina japonica* (Laminariales, Phaeophyceae). J Appl Phycol 2010;22:443–51.
[76] Wong PF, Tan LJ, Nawi H, Abu Bakar S. Proteomics of the red alga, *Gracilaria changii* (Gracilariales, Rhodophyta). J Phycol 2006;42:113–20.
[77] Ritter A, Ubertini M, Romac S, Gaillard F, Delage L, Mann A, et al. Copper stress proteomics highlights local adaptation of two strains of the model brown alga *Ectocarpus siliculosus*. Proteomics 2010;10:2074–88.
[78] Contreras L, Moenne A, Gaillard F, Potin P, Correa JA. Proteomic analysis and identification of copper stress-regulated proteins in the marine alga *Scytosiphon gracilis* (Phaeophyceae). Aquat Toxicol 2010;96:85–9.
[79] Contreras-Porcia L, López-Cristoffanini C. Proteomics in seaweeds: ecological interpretations, gel electrophoresis — advanced techniques. In: Magdeldin Sameh, editor. InTech. ISBN 978-953-51-0457-5; 2012 [Available from: http://www.intechopen.com/books/gel-electrophoresis-advancedtechniques/proteomics-in-seaweeds-ecological-interpretations].
[80] Liu J, Chen L, Wang J, Qiao J, Zhang W. Proteomic analysis reveals resistance mechanism against biofuel hexane in *Synechocystis* sp. PCC 6803. Biotechnol Biofuels 2012;5:68.
[81] Schirmer A, Rude MA, Li X, Popova E, del Cardayre SB. Microbial biosynthesis of alkanes. Science 2010;329:559–62.
[82] Terashima M, Specht M, Naumann B, Hippler M. Characterizing the anaerobic response of *Chlamydomonas reinhardtii* by quantitative proteomics. Mol Cell Proteomics 2010;9:1514–32.
[83] Mus F, Dubini A, Seibert M, Posewitz MC, Grossman AR. Anaerobic acclimation in *Chlamydomonas reinhardtii*: anoxic gene expression, hydrogenase induction, and metabolic pathways. J Biol Chem 2007;282:25475–86.
[84] Atteia A, van Lis R, Gelius-Dietrich G, Adrait A, Garin J, Joyard J, et al. Pyruvate formate-lyase and a novel route of eukaryotic ATP synthesis in *Chlamydomonas* mitochondria. J Biol Chem 2006;281:9909–18.
[85] Moellering ER, Benning C. RNA interference silencing of a major lipid droplet protein affects lipid droplet size in *Chlamydomonas reinhardtii*. Eukaryot Cell 2010;9:97–106.
[86] Cermelli S, Guo Y, Gross SP, Welte MA. The lipid-droplet proteome reveals that droplets are a protein-storage depot. Curr Biol 2006;16:1783–95.
[87] Walther TC, Farese Jr RV. The life of lipid droplets. Biochim Biophys Acta 2009;1791:459–66.
[88] Athenstaedt K, Zweytick D, Jandrositz A, Kohlwein SD, Daum G. Identification and characterization of major lipid particle proteins of the yeast *Saccharomyces cerevisiae*. J Bacteriol 1999;181:6441–8.
[89] Jolivet P, Roux E, d'Andrea S, Davanture M, Negroni L, Zivy M, et al. Protein composition of oil bodies in *Arabidopsis thaliana* ecotype WS. Plant Physiol Biochem 2004;42:501–9.
[90] Katavic V, Agrawal GK, Hajduch M, Harris SL, Thelen JJ. Protein and lipid composition analysis of oil bodies from two *Brassica napus* cultivars. Proteomics 2006;6:4586–98.
[91] Parthibane V, Rajakumari S, Venkateshwari V, Iyappan R, Rajasekharan R. Oleosin is bifunctional enzyme that has both monoacylglycerol acyltransferase and phospholipase activities. J Biol Chem 2012;287:1946–54.
[92] Baba M, Shiraiwa Y. High-CO_2 response mechanisms in microalgae. In: Najafpour Mohammad, editor. Advances in photosynthesis — fundamental aspects. Rijeka, Croatia: InTech Pub. ISBN 978-953-307-928-8; 2012.
[93] Baba M, Suzuki I, Shiraiwa Y. Proteomic analysis of high-CO_2-inducible extracellular proteins in the unicellular green alga, *Chlamydomonas reinhardtii*. Plant Cell Physiol 2011;52:1302–14.
[94] Guarnieri MT, Nag A, Smolinski SL, Darzins A, Seibert M, Pienkos PT. Examination of triacylglycerol biosynthetic pathways via de novo transcriptomic and proteomic analyses in an unsequenced microalga. PLoS One 2011;6:e25851.
[95] Tucci S, Vacula R, Krajcovic J, Proksch P, Martin W. Variability of wax ester fermentation in natural and bleached *Euglena gracilis* strains in response to oxygen and the elongase inhibitor flufenacet. J Eukaryot Microbiol 2010;57:63–9.
[96] Hoffmeister M, van der Klei A, Rotte C, van Grinsven KW, van Hellemond JJ, Henze K, et al. *Euglena gracilis* rhodoquinone:ubiquinone ratio and mitochondrial

proteome differ under aerobic and anaerobic conditions. J Biol Chem 2004;279:22422–9.
[97] May P, Wienkoop S, Kempa S, Usadel B, Christian N, Rupprecht J, et al. Metabolomics- and proteomics-assisted genome annotation and analysis of the draft metabolic network of *Chlamydomonas reinhardtii*. Genetics 2008;179:157–66.
[98] Wienkoop S, Weiss J, May P, Kempa S, Irgang S, Recuenco-Munoz L, et al. Targeted proteomics for *Chlamydomonas reinhardtii* combined with rapid subcellular protein fractionation, metabolomics and metabolic flux analyses. Mol Biosyst 2010;6:1018–31.
[99] Gong Y, Hu H, Gao Y, Xu X, Gao H. Microalgae as platforms for production of recombinant proteins and valuable compounds: progress and prospects. J Ind Microbiol Biotechnol 2011;38:1879–90.
[100] Radakovits R, Jinkerson RE, Darzins A, Posewitz MC. Genetic engineering of algae for enhanced biofuel production. Eukaryot Cell 2010;9:486–501.
[101] International Brachypodium Initiative. Genome sequencing and analysis of the model grass *Brachypodium distachyon*. Nature 2010;463:763–8.
[102] Tuskan GA, DiFazio S, Jansson S, Bohlmann J, Grigoriev I, Hellsten U, et al. The genome of black cottonwood, *Populus trichocarpa* (Torr.& Gray). Science 2006;313:1596–604.
[103] Sato S, Hirakawa H, Isobe S, Fukai E, Watanabe A, Kato M, et al. Sequence analysis of the genome of an oil-bearing tree, *Jatropha curcas* L. DNA Res 2011;18:65–76.
[104] Chan AP, Crabtree J, Zhao Qi, Lorenzi H, Orvis J, Puiu D, et al. Draft genome sequence of the oilseed species *Ricinus communis*. Nat Biotechnol 2010;28:951–6.
[105] Schnable PS, Ware D, Fulton RS, Stein JC, Wei F, Pasternak S, et al. The B73 maize genome: complexity, diversity and dynamics. Science 2009;326:1112–5.
[106] Yang M, Zhang X, Liu G, Yin Y, Chen K, Yun Q, et al. The complete chloroplast genome sequence of date palm (*Phoenix dactylifera* L.). PLoS One 2010;5:9.
[107] Asif MH, Mantri SS, Sharma A, Sirivastava A, Trivedi I, Gupta P, et al. Complete sequence and organisation of the *Jatropha curcas* (Euphorbiaceae) chloroplast genome. Tree Genet Genomes 2010;6:941–52.
[108] Jiang H, Wu P, Zhang S, Song C, Chen Y, Li M, et al. Global analysis of gene expression profiles in developing physic nut (*Jatropha curcas* L.) seeds. PLoS One 2012;7(5):e36522.
[109] Eswaran N, Sriram P, Balaji S, Bhagyam A, Raja KK, Johnson TS. Yeast functional screen to identify genetic determinants capable of conferring abiotic stress tolerance in *Jatropha curcas*. BMC Biotechnol 2010;10:23.
[110] Chen MS, Wang G-J, Wang R-L, Wang J, Song S-Q, Xu Z-F. Analysis of expressed sequence tags from biodiesel plant *Jatropha curcas* embryos at different developmental stages. Plant Sci 2011;181:696–700.
[111] Costa GGL, Cardoso KC, Del Bem LEV, Lima AC, Cunha MAS, de Campos-Leite L, et al. Transcriptome analysis of the oil-rich seed of the bioenergy crop *Jatropha curcas* L. BMC Genomics 2010;11:462.
[112] Natarajan P, Parani M. De novo assembly and transcriptome analysis of five major tissues of *Jatropha curcas* L. using GS FLX titanium platform of 454 pyrosequencing. BMC Genomics 2011;12:191.
[113] Rodriguez LC, O'Connell D. Biofuels: balance the blend of food and fuel. Nature 2011;476:283.
[114] Hein L, Leemans R. The impact of first-generation biofuels on the depletion of the global phosphorus reserve. Ambio 2012;41:341–9.
[115] Lam MK, Lee KT. Microalgae biofuels: a critical review of issues, problems and the way forward. Biotechnol Adv 2012;30:673–90.