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Research opportunities on the coconut (*Cocos nucifera* L.) using new technologies

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ABSTRACT

A member of the Arecaceae family, coconut *Cocos nucifera* L. is cultivated in tropical regions worldwide. Humans have exploited the different structures of this palm for millennia. Although the trunk and leaves are used, mainly as construction material, by far the most valuable element is the fruit. It is the source of edible components such as coconut water, virgin coconut oil, copra, and coconut milk, as well as natural fiber (husk) and activated charcoal (nutshell). Today, all of them are at high demand in the international markets. Thus represent a commerce valued in ~11.5 billion dollars, and it is expected to reach ~31.1 billion by 2026. The global market of coconut derived products used in food applications must meet an increase stringent food quality and security parameters. Application of new technologies and research strategies such as metabolomics, proteomics, genomics and transcriptomics to coconut fruit is generating exciting data that will help improve management and marketing of this valuable crop. This in turn will lead to progressive genetic improvement of *C. nucifera* while allowing current producers to meet market demands. This review condenses the most outstanding current research on coconut fruit involving these technologies and approaches.

1. Introduction

The coconut palm *Cocos nucifera* L. is a tropical tree belonging to the family Arecaceae with a 2n=32 ploidy. It is thought to have evolved in the region encompassing the Malayan Peninsula and Archipelago, New Guinea and the Bismarck Archipelago, from where it spread throughout the tropics. Today its known distribution includes West and East Africa, Southeast Asia, the Pacific Islands and North, Central and South America (Prades et al., 2016; Xiao et al., 2017). Worldwide coconut cultivation covers 12.3 million hectares, which annually produce 61.4 million metric tons of coconut fruit (Statista, 2020). The species' early and successful spread to and colonization of distant areas is probably due the characteristics of its fruit. In the immature (green) and mature (brown and dry) states the exocarp is impermeable and the mesocarp is dense fiber, making the fruit extremely buoyant. This property allows it to remain viable for long periods as it floats with marine currents until deposited in an environment where it can germinate (Harries, 2012). Immature and mature fruit also contain fresh water in their center, making it an effective way of transporting fresh water. Sailors would have carried them to new lands as part of their voyages (Harries, 2012). Today, coconut is a fundamental commodity in tropical areas worldwide. It is trading and increase value of coconut food-derived products every year, although non-food derivates with or without processing also provides an income (Prades et al., 2016). The rising social demands to the global market to substitute the highly polluting oil-derived products, e.g., plastics and diesel, among others, by eco-friendly alternatives, allow the research-educative institutions from India, New England, Indonesia, Philippines, Thailand and Latin America, establish start-ups, where the new developments or improved strategies to exploit the coconut products not used as foods, are applied (Kek, 2018). Those strategies include the biodiesel production from C8-C12 fatty acids (Rasyid et al., 2018) and the extraction of fibers from husk to process them into raw renewable materials, as the ecocoboard (Ben University of Applied Sciences, 2021), the coco green walls (Universiti Malaysia Sarawak, 2019) and geotextiles (Rao et al., 2020), whose use in agriculture and civil engineering to reinforce soil and the eco-green cities, are increasing (Prades et al., 2016; Rao et al., 2020). Although, those start-ups are a beginning to reach an optimal exploitation of coconut, most of them remain as artisanal and semi-industrial processing businesses (Prades et al., 2016; Kek, 2018; Rao et al., 2020). As coconut derivatives become increasingly global products these smaller processers face challenges such as developing new technologies to speed product extraction, and, in the case of products destined for the food industry, fulfilling international requirements for food innocuity and defining specific components in

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functional foods (Prades et al., 2012; Kawashima et al., 2013; Prades et al., 2016; Kumar et al., 2021).

2. Coconut varieties

The number of *C. nucifera* varieties is not definitively known, but it is generally accepted that all current coconut varieties derive from this one species. Natural and artificial selection have led to creation of three main varietal groups: tall or typical; dwarf or nana; and hybrids (Central Plantation Crops Research Institute, 2014; Ribeiro et al., 2010; Ekanayake et al., 2010). Tall varieties (e.g., Jamaican and East and West coast varieties) can grow to 15 – 24 m in height. At six to ten years post-germination they produce fruit of medium to large size which contain high-quality kernels commonly used in coconut oil production. They can produce nuts for eighty or more years (Ribeiro et al., 2010). Compared to the tall varieties, the dwarf varieties (e.g., Malayan, Fiji, Brazilian green, Macapuno and Cameroon Red, among others) are shorter in height (5 – 18 m), produce smaller fruit and begin fruit production three to five years after planting (Chan and Elevitch, 2006). Fruit from dwarf varieties is largely used as a source of fresh coconut water, although specific varieties (i.e., Makapuno and Lono) are more sought after for the taste of the gelatinous endosperm they produce, which is valued for use in desserts (Angeles et al., 2018). Hybrid varieties (e.g., Maypan) are crosses between the Malayan Yellow Dwarf (MYD) and Panama Tall (PNT) varieties. They generally produce more fruit than the parent varieties, the size of which is intermediate between the progenitors (Baudouin et al., 2007). Hybrids are generally more resistant to pests and diseases, than their parents, but the characteristic are highly dependent on the variety, i.e., tall, dwarf or hybrid, that is used in the crosses as pollen receptor (female) and pollen donator (male) (Baudouin et al., 2009; Niral et al., 2019). Hybrids begin fruit production at three to six years of age and fruit color in any of the varieties is most commonly green or yellow, although it can also be red, orange or mixtures of orange-red or green-yellow.

3. Traditional uses

Described as one of the most cultivated palm trees species worldwide, coconut is often called the “tree of life” or “the tree of a thousand uses” (Angeles et al., 2018). Every part of the palm is useful. Its trunk and leaves are used as building material for houses and fences, and are widely utilized in handcrafts (Prades et al., 2012). The fruit’s fibrous mesocarp, called the husk, is processed into carpets, geotextiles, rope and compressed wood, as well as being used as an inert sterile support medium for growing plants, a nutritional fiber source, and as seat padding in vehicles such as trucks and trains (Rencoret et al., 2013). The extremely hard endocarp, known as the shell, is used to produce activated charcoal. The endosperm consists of liquid and solid portions (Prades et al., 2016). The liquid endosperm is in high demand as a fresh water or sports beverage product attributed isotonic properties due to its mineral, sugar and vitamin contents (Yong et al., 2009; Prades et al., 2016; Hidalgo, 2017). Fresh coconut water is a growing market supplied by a craft industry worth hundreds of thousands of dollars which is expanding in countries like Brazil and the Philippines (Prades et al., 2016; Hidalgo, 2017). The solid endosperm forms a white layer lining in the inside of the shell and when it is extracted from mature coconut and dried, it is known as copra, which commonly is used as source of coconut oil (Patil et al., 2017). When extracted from young fruit the solid endosperm is gelatinous, a consistency which makes it useful in producing desserts or as a food supplement. No matter if it is grown and/or processed on artisanal or industrial scales, coconut fruit is a vital income source for coconut growers in producing countries (Angeles et al., 2018).

4. Research into additional applications

Developing possible uses for coconut fruit beyond the traditional ones has piqued the interest of the scientific community (Angeles et al., 2018; Burns et al., 2020). Studies have been done into how water accumulates in the fruit, and how in early development stages its biochemistry promotes deposition of the carbohydrates and minerals that provide flavor to the water (Burns et al., 2020). Additional research has been done into the regulatory processes that occur when fruits are immature and at intermediate ripeness. These processes coordinate increased fatty acid deposition and decreased sugar production in the solid endosperm (López-Villalobos et al., 2001; Appaiah et al., 2015). The food and chemical industries carry out ongoing research into lauric acid (12:0), a medium-chain saturated fat extracted from the endosperm for use in manufacturing margarine and soap, among other uses (Dayrit, 2014; Ghosh et al., 2014; Suryani et al., 2020). Lauric acid is particularly interesting in food applications since it is quickly incorporated into the body and easily transported to the liver where it is consumed as an energy precursor rather than being stored as fat (Dayrit, 2014). Coconut water composition and its properties have also been studied to quantify sugar and mineral contents, solids composition and water proportion, among other parameters (Costa et al., 2015; Hidalgo, 2017). This attention is timely since the fresh coconut water market has grown by more than 300% in recent years (Prades et al., 2016; Hidalgo, 2017; Burns et al., 2020). However, this growth has been accompanied by new regulations demanding nutritional data for water-derived products, especially those exported to the principal markets in the United States and Europe (Food and Drug Administration, importing food and beverages into the United States, 2018, Statista 2020; Privacy Shield Framework, 2020). This constitutes another reason to better understand the raw coconut material. Today, companies producing coke and caloric juices worldwide, are fighting hard to be not displaced of their beverage global market domination. However, demand for healthy alternatives to those beverages, has positioned the America’s, Europe, South America and Asia-Pacific as the areas where coconut water is gaining acceptation in its consumption (Businesswire, 2020; Fior-Markets, 2021). An unexpected worldwide challenge to the market of coconut water emerged with the COVID-19 pandemic, because disease interrupted the coconut water chained-market. By affecting water production and demand, impacting the progress and economy of producers and firms involved in coconut water processing and more important, negatively impacting the development of strategies to better support and improve the coconut water (More, 2021). We do not know what is going to happen when COVID 19 pandemic ends but it might be expected that after a short time, global market of coconut water recovers its dynamic of offer-demand. Moreover, it would be mandatory that key players in the industry of coconut water, focus on adopting new technology, support product innovations, establish new alliances and partnerships with the goal to improve their market position in the global coconut water commerce.

5. New technologies promise new alternatives for coconut fruit

The booming worldwide market for alternative health foods has enthusiastically incorporated coconut water, virgin coconut oil and coconut sugar. However, consumers in this market demand that natural products be guaranteed as safe (Prades et al., 2016; Hidalgo, 2017). For coconut water various strategies have been used, including microwave heating (Arzeta-Ríos et al., 2020), filtration and refrigeration (Holdsworth, 1997), ultra-high temperature sterilization (Awuah et al., 2007), and low and high temperature treatments in conjunction with added sulphites (Sucupira et al., 2017). However, these techniques do not always meet market demands because they might modify the natural compounds in coconut fresh water

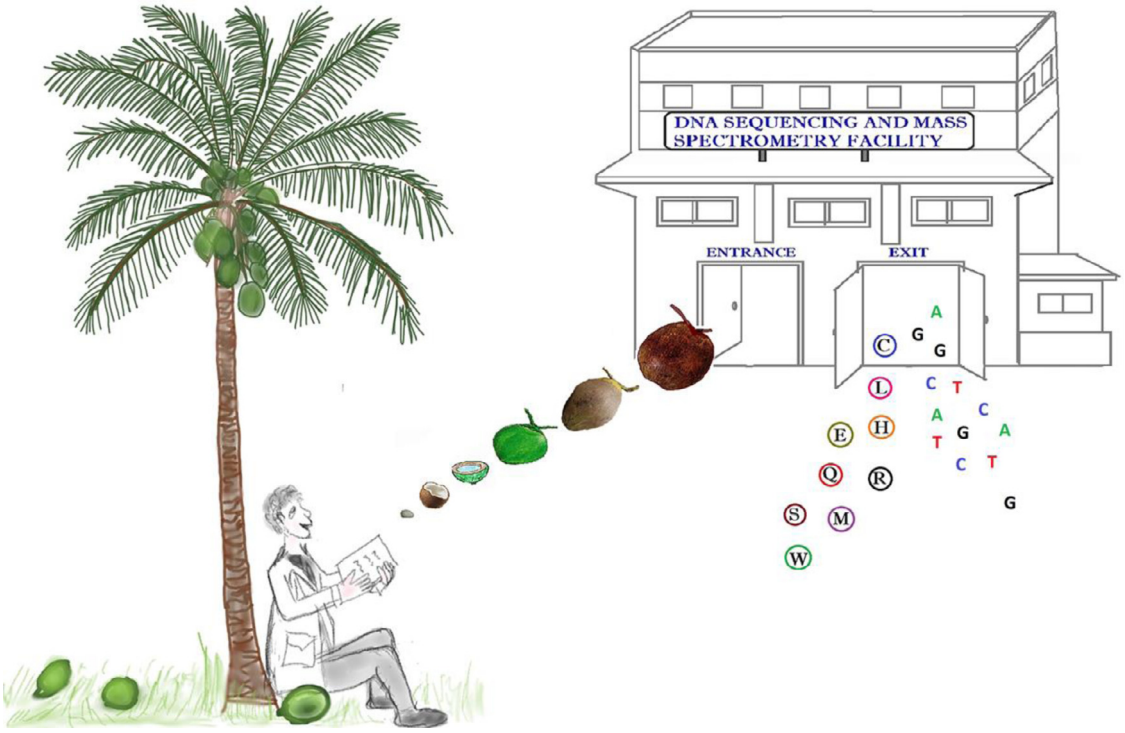

Fig. 1. New technologies are unlocking the secrets of the coconut palm. The combination of genomics, transcriptomics, proteomics and metabolomics with bioinformatics is generating a new understanding of the coconut that will revolutionize its cultivation and use in the future.

(Sucupira et al., 2017). Earlier efforts to demonstrate that physical or chemical treatments do not represent a risk for consumers, faced with the fact that most of the equipment used for water analysis has low resolution to differentiate between the natural and modified compounds (Costa et al., 2015). In response, to the challenge more recent research involves newer, advanced technologies to decipher the secrets of coconut (Fig. 1). In case of coconut water, the study at low scale of their metabolite composition, was replaced by high-throughput metabolomics analyses because consumer demand to know what are they ingesting.

5.1. a) Metabolomics

Metabolomics is the large-scale study of small molecules, commonly known as metabolites, to establish the metabolic network and understand an organism's condition at a certain moment or in a specific environment (Pinu et al., 2019). One of the first metabolomics studies of coconut water quality was done using magnetic resonance imaging (MRI) to verify the sugar regions (δ 5.1-5.5) in water extracted from mature coconuts (Jagannathan et al., 1995). Later, an instrumental metabolomics study of coconut water was published by Sucupira et al. (2017). In it, ^{1}H nuclear magnetic resonance (^{1}H NMR) spectroscopy was used together with chemometrics to analyze chemical variation in coconut water treated with different methods, e.g., with and without sulphyte and thermal treatment, among others. The chemometrics and ^{1}H NMR spectra from non-treated water (control), generally recorded compounds in two different regions, showing that coconut water contains high sugar levels and aliphatic structures. In contrast, findings describing decreasing concentrations and chemical variations in those compounds, in response to treatments, were explained and supported by mathematical description and principal component analysis (PCA) at a 95% confidence level (Sucupira et al., 2017).

In another approach, electrospray ionization combined with Fourier transform ion cyclotron resonance mass spectrometry [ESI-FT-ICR-MS] was used to monitor the physicochemical degradation of coconut water from 0 to 15 days (Costa et al., 2015). After three days, observed changes in the chemical profile included decreases in the mass/charge (m/z) 150-250 and 350-450 regions. Correlated with these decreases were the appearance of new spectra identified as citric (m/z 191), galacturonic (m/z 193), gluconic (m/z 195) and saccharic (m/z 209) acids. This technique is clearly effective for describing physicochemical properties in coconut water (costa et al., 2015).

In another study, an electronic tongue, a device composed of several chemical sensors, was used to monitor changes in fresh coconut milk (Yan et al., 2017). Data analysis using PCA, cluster analysis and similarity analysis showed that in fresh coconut milk the values of quality indices (i.e., acidity, pH and microorganisms) changed significantly two to three hours after extraction; these results were confirmed with chemical and microbiological analyses done in parallel samples (Yan et al., 2017). The electronic tongue was found to be a reliable method for monitoring changes in coconut milk or water quality (Yan et al., 2017).

Ultra-performance liquid chromatography coupled to mass spectroscopy [UPLC-MS] and multivariate statistical analysis have been applied to water from ripe coconuts to document the changes in metabolites that occur during storage (Chen et al., 2018). UPLC-MS data sets were collected from 34 coconut water samples after 0, 1, 2, 3, 4, and 5 months storage, and these data processed with PCA, partial least squares-discriminative analysis (PLS-DA) and orthogonal projections to latent structures discriminant analysis (OPLS-DA). All samples clearly separated into two clusters in the OPLS-DA model, indicating that maximum postharvest storage period of coconut water cannot exceed three months. After this period the TCA cycle, protein hydrolysis and metabolite interconversion accelerate, reducing coconut water quality (Chen et al., 2018). An additional study, using UPLC-MS/MS-based metabolomics in 35 tender coconut water samples stored at 4 C for 0-6 weeks, to analyze the deterioration mechanism and storage time limit of tender coconut water at storage, showed the metabolites that were present in water (Zhang et al., 2020). Moreover, analysis of the obtained dataset with multivariate

statistical analysis, *p*-value and fold change, revealed that 72 metabolites were differentially expressed at all that conditions, while the OPLS-DA score chart showed well grouped samples (Zhang et al., 2020). Search in the Kyoto Encyclopedia of Genes and Genomes (KEGG) database and the MetaboAnalyst identify the metabolic pathway where differential metabolites were involved. This showed thirty-one metabolic pathways enriched in samples from week 0-1, with amino acid metabolism having the biggest changes. Together, these results suggest that alterations in the amino acid metabolic pathway is one of the causes for the coconut water deterioration and that 1 week is the maximum time for storage at 4 C of the water from tender coconut (Zhang et al., 2020). The most recent study in coconut metabolomics used a combination of gas chromatography coupled to mass spectrometry (GC-MS) and UPLC analysis to characterize the major physico-chemical, nutritional, and metabolomics changes occurring in coconut water from two varieties, the "Chowghat Orange Dwarf" (COD) and "Malayan Yellow Dwarf" (MYD) at four different nut developmental stages (Kumar et al., 2021). Remarkable changes in metabolites free amino acids, sugars, and ascorbic acid, among others, were observed in each variety during the process of their nut maturation. After metabolite identification, authors used multivariate PLS-DA to discriminate among their abundance at each nut developmental stage and based on their results, they proposed 8 metabolites, e.g., chlorogenic acid, ferulic acid, lauric acid, succinic acid, shikimic acid, sucrose, among others, that may be used as biomarkers to distinguish between the nut maturity stages (Kumar et al., 2021). Findings are relevant because these biomarkers should help to define the maturity stage of coconut; today a real challenge in biology, but most important for the coconut water market, the coconut industry should select coconut at appropriate maturity stages to offer and customize water in request to specific requirements (Kumar et al., 2021).

5.2. b) Proteomics

As the functional agents created by genomic transcripts and the final products of gene expression, proteins function through an integral and coordinated network to regulate metabolism in cells, tissues and the organism as a whole (Kumar et al., 2017). Various studies have analyzed the individual enzymatic functions of proteins in coconut fruit. They have addressed tyrosine kinase activity during zygotic or somatic embryo development (Islas-Flores et al., 1998; 2000), polyphenol oxidase activity (Tan et al., 2014), and lipase activity (Ejedegba et al., 2007), during coconut fruit development, and the function of protein reserves in the coconut kernel (García et al., 2005). Although informative, these studies are limited because they focus only on specific proteins or small numbers of protein groups.

The broader approach of proteomics is being increasingly used to analyze coconut fruit. This technology allows protein analysis at a superior level of integration due to its large-scale and deep analysis techniques. These can describe how protein composition, structure, function, interaction and modification direct and coordinate cell activities over a given time or under specific environmental cell conditions (Kumar et al., 2017). One study using this approach employed an UltiMate 3000 RSLC nano chromatographic system, coupled to an LTQ-XL mass spectrometer equipped with a nano spray ion source to analyze coconut milk titrated at pH 7.0 and 9.3 or conjugated to combinatorial peptide ligand libraries (CPLL) (D'Amato et al., 2012). Using the Mascot search engine and full scan mass spectra (350 to 1800 Da m/z mass range) to search in the Uniprot-viridiplantae database, these researchers identified a total of 307 unique proteins: 200 captured with CPLL; 137 in the untreated control; and 30 held in common among the treatments. This remains the first extensive proteomic analysis of coconut milk. Prior to it only a dozen proteins were known in coconut milk, all belonging to the most abundant groups, and the total coconut protein database contained only 106 proteins (D'Amato et al., 2012). Two other proteomic profiles of coconut were generated using two-dimensional electrophoresis (2-DE; (Huang et al., 2016) or SDS-PAGE (Lin et al., 2020), and matrix-assisted laser desorption ionization-time of flight/time of flight-mass spectrometry [MALDI-TOF/TOF-MS]. They identified at least 200 protein spots in 2-DE, and the six major protein bands in SDS-PAGE. Further bioinformatics analysis showed that many of the spots and protein bands were associated with 11S and 7S globulin, glutelin and a putative receptor-like protein kinase (Huang et al., 2016; Lin et al., 2020). Although these two studies do not include as many identified proteins as D'Amato et al. (2012), they are nonetheless very informative in that many of the proteins in coconut endosperm were found to be "storage proteins". The "storage proteins" are a group of plant structurally conserved polypeptides, lacking of catalytic activities, but along the seed development, they accumulate in the endosperm, and further, during seed germination, they are catabolized to be used as a nitrogen source to support the growth of the seedling (Balasundaresan et al., 2002). Today, coconut storage proteins are attracting increasing attention due to their nutritional and health benefits (Balasundaresan et al., 2002; Lin et al., 2020). The most recent study in coconut seedling leaf proteomics employed isobaric tags for relative and absolute quantification (iTRAQ) using a nanoHPLC coupled to a ESI-MS/MS to analyze the cold stress response of two seedling coconut varieties, Hainan Tall BenDi (BD) and the aromatic XiangShui (XS) (Yang at al., 2020). After two days at 8 °C, up and down regulation were exhibited in both BD (193 up/134 down) and XS (140 up/155 down). Evaluation at five days post-cold treatment identified a higher abundance of up-regulated proteins in BD than in XS. Gene ontology (GO) bioinformatics analysis showed that, in both varieties, 22 of the differential expressed proteome (DEP) categories were related to biological processes, twelve to the cellular component and fourteen to molecular function. Analysis of the DEP data for metabolic pathways in the Kyoto encyclopedia of genes and genomes (KEGG) database showed that four major categories, including metabolism, stress response, photosynthesis and respiration increased their abundance in both varieties. However, only the stress-responsive proteins were more up-regulated in BD than in XS. This increased abundance of stress-responsive proteins in BD suggests that this variety is tolerant to cold-stress while the XS is susceptible. Together, these results showed that iTRAQ analysis has the resolution to differentiate fine proteome differences in response to cold stress, but can also provide the basis for further study of different kinds of biotic and abiotic stresses. This may help to identify the molecular and biochemical mechanisms that coconut and other tropical plants use to adapt and respond to adverse environment conditions.

5.3. c) Genomics

Many molecular biology techniques are currently available to assess genetic resources; for example, restriction fragment length polymorphisms (RFLP); random amplified polymorphic DNA (RAPD); amplified fragment length polymorphism (AFLP); and sequence-tagged microsatellites (SSRs). In coconut, however, these have only been applied at low to medium scales (Teulat et al., 2000; Sankaran et al., 2012). Until recently, the identified *C. nucifera* molecular marker candidates for improvement of agricultural and quality traits could not be tested or compared, mostly because the genome sequence data was unavailable. The first RNA-seq analysis from mixed *C. nucifera* samples allowed use of the first global transcriptome sequencing and *de novo* assembly for coconut in molecular functional genomics (Fan et al., 2013). The assembled transcriptome contains 57,304 unigenes with an average length of 752 base pairs (bp). In this study, the assembled unigenes containing *C. nucifera* cDNA sequences available from different databases were compared to the sequences of oil palm *Elaeis guineensis* (a coconut relative); the resulting assembled sequences were high quality and almost all were

classified as novel genes. Further analysis showed that 347 of these unigenes are involved in the five steps of fatty acid metabolism, 121 are involved in fatty acid biosynthesis, and 2,404 are involved in carbohydrate metabolism. This technique can clearly describe gene expression in *C. nucifera*.

To date, complete genome sequences have been generated for two coconut varieties: "Hainan Tall" (Xiao et al., 2017), and "Catigan Green Dwarf" (Lantican et al., 2019). In the "Hainan Tall" variety genomic DNA was obtained from healthy young leaflets and sequenced using the Illumina 2000 HiSeq platform. Predicted genome length was 2.42 Gbp with 28,039 protein-coding genes, but total scaffold length turned out to be 2.20 Gbp, representing 90.91% of the genome with an estimated x173.32 read depth. Bioinformatic analysis using BUSCO programs found the coconut annotation to be 74.1% complete. Moreover, 72.75% of the coconut genome was identified as containing transposable elements. Long-terminal repeat retrotransposons elements (LTRs) accounted for the largest proportion, but significant gene expansion was also observed in others, including the antiporter gene family (e.g., Na+/H+ and carnitine/acylcarnitine translocase, among others) and the ion channel families (i.e., potassium channel) (Xiao et al., 2017). Again, using genomic DNA from healthy young leaflets, the "Catigan Green Dwarf" variety was sequenced using the PacBio SMRT sequencing platform and corrected with the Illumina paired-end MiSeq, using reads of the same genome. Predicted genome length was 2.15 Gbp, of which 2.1 Gbp were assembled, representing 97.6% of the estimated genome size. A total of 34,958 gene coding proteins were predicted, with many associated with pest and disease resistance, oil biosynthesis and putative transcription factors. These varieties' genomes were compared by aligning the "Hainan Tall" whole genome sequences with the "Catigan Green Dwarf" non-repetitive regions of the assembled genome. This identified 7,139 unique simple sequence repeats (SSR) markers and 58,503 variants in the "Catigan Green Dwarf" genome which could help to improve coconut yield and quality (Lantican et al., 2019).

5.4. d) Comparative genomics or transcriptomics

Only preliminary transcriptome data is available for *C. nucifera*, although comparative genomics or transcriptomics has been done between the related *E. guineensis* and date palm (*Phoenix dactylifera*) (Bourgis et al., 2011). This is an interesting comparison because in *E. guineensis* fruit metabolism is almost exclusively focused on oil and fatty acids production in almost all fruit development stages (Bourgis et al., 2011), while in *P. dactylifera* fruit metabolism is entirely destined towards sugar production and accumulation in all fruit development stages (Al-shahib and Marshall, 2009). Carried out during mesocarp development in both species, the analysis found higher transcript levels in *E. guineensis* for all fatty acid synthesis, specific plastid transporters, and key enzymes of plastidial carbon metabolism, including phosphofructokinase, pyruvate kinase, and pyruvate dehydrogenase, all associated with high oil content. Unexpectedly, despite a more than 100-fold difference in flux to lipids between the species, most triacylglycerol assembly enzymes were expressed at similar levels in both. Transcript levels for all the cytosolic enzymes of glycolysis were also comparable between them. These data points suggest that control over oil storage in the *E. guineensis* mesocarp occurs through fatty acids synthesis and pyruvate supply in the plastid, rather than assembly of acyl into triacylglycerol. In addition, fatty acids synthesized from acetyl-CoA in the plastid are exported as acyl-CoA esters to the endoplasmic reticulum where they enter glycerolipid metabolism.

An initial comparison of the *C. nucifera* transcriptome to those of *E. guineensis* and *P. dactylifera* suggests that the coconut genome contains fewer protein-coding genes (28039) than either *P. dactylifera* [PDK30: 28889; DPV01: 41660] or *E. guineensis* (EG5: 34802) (Xiao et al., 2017; Angeles et al., 2018). A detailed genomic or transcriptomic comparison between these palm species would be very informative. In contrast to *E. guineensis* and *P. dactylifera*, carbohydrate biosynthesis predominates in young *C. nucifera* fruit but transitions to fatty acid biosynthesis in intermediate and mature stage fruit. An additional advantage is that the transcriptomes for *E. guineensis* and *P. dactylifera* are in the public domain (Dussert et al 2013; Radwan et al., 2015).

The combination of comparative genomics with bioinformatics can be a powerful tool. It can be used to more efficiently describe the genome of as yet unsequenced species by comparing it to closely related species with completely known genomes. One study applied *in silico* comparative genomics by comparing the *E. guineensis* genome as a "reference species" to the *C. nucifera* genome as the "target species" to purify and confidently assemble the coconut transcriptome and proteome (Armero et al., 2017). This translational genomics from oil palm to coconut effectively used the relatively well-known *E. guineensis* genome as a "reference" to purify and assemble *de novo* the little known *C. nucifera* transcriptome and proteome (i.e. the "target"). Using the BRANCH software, bioinformatics showed that this approach generated 29,366 proteins for *C. nucifera*, much more than the 28,039 predicted previously (Xiao et al., 2017). The additional proteins (1,246) were assigned as new contigs. Further analysis found the coconut proteome to have a functional profile like that observed in rice, in that many metabolic pathways were related to secondary metabolism, and the new transcriptome sequences were enriched in functions related to biotic stress.

6. Conclusions and perspectives

For such an important agricultural species *C. nucifera* has received only limited study. This palm species has a years-long developmental period before beginning reproduction, which hinders conventional or assisted breeding programs. Another challenge in improving *C. nucifera* is the lack of research focused on deciphering genomic characteristics and potential gene-marker candidates. Most traditional studies have focused on analyzing morphological and productive characteristics of the tree and fruit. Various studies have been done characterizing the physicochemical composition of coconut fruit water, kernel and derived products (e.g., virgin oil and coconut milk). However, extensive further research is necessary, so any contributions are welcome, be they morphological, biochemical or molecular studies.

The present review mentions the most novel research strategies that have been applied to *C. nucifera*. Approaches such as MRI, ^{1}H NMR spectroscopy together with chemometrics, ESI-FT-ICR-MS, electronic tongue and UPLC-MS MS have been used to study the metabolomic state of coconut water, coconut milk and coconut oil. All revealed specific chemical variations in response to treatments. Compounds that decrease during aging or long-term storage include carbohydrates and aliphatic compounds, although new chemical species also arise such as citric, gluconic, saccharic and galacturonic acids. As a whole these dynamic changes impact the metabolic interrelations in coconut fruit thus affecting their quality in response to storage time or developmental stage. These changes are mediated by enzymatic activities, another key regulator closely linked to metabolism in coconut fruit. Assuming that one of the goals on this area of research is preservation of coconut water quality (nutritional benefits, taste and flavor) for human consumption, future research should then focus on identifying the natural enzyme inhibitors which prevent the deleterious enzyme activities negatively impacting coconut water quality. Enzyme inhibitors must be present in the endosperms (solid and liquid) and the embryo. Though challenging, recent advances in bioinformatics and machine learning will support the search for and identification of enzyme inhibitor candidates in the recently sequenced coconut genome, the reported transcriptome and the initial proteomics findings. Enzyme inhibition or suppression in coconut water could be a promising and dynamic area for research.

Only four reports exist to date using proteomics in coconut. Three involved fruit analyzed with a nanoHPLC coupled to a LTQ-XL or MALDI-TOF-TOF-MS mass spectrometer. The fourth was done with coconut seedling leaves using an iTRAQ nanoHPLC coupled to an ESI-MS/MS. The results from the first three studies are quite informative because they identified glutelin and 7s globulin, both related to storage proteins, and whose accumulation during fruit development is key for embryo growth and survival. Moreover, they doubled the number of known proteins in coconut milk from 106 to at least 307 unique proteins related to coconut milk quality. Although these studies provide limited data, they represent the beginning of proteomic analysis in the search for how proteins participate in coconut fruit metabolism at different developmental stages, or how they participate in synthesis of the flavor or aromatic compounds characteristic of coconut milk or water. The fourth proteomic analysis did not identify many more proteins (~350) than the total found in the first three studies. However, the use of isobaric tags to label proteomes from different varieties clearly provided enough sensitivity to reveal the differences in the proteomic response of these two varieties in coping with cold stress. Future proteomic research using iTRAQ to study the coconut's response to different biotic and abiotic challenges (e.g., nutritional conditions, plant age, water availability and climate change, among others) could contribute to better understanding the molecular mechanisms used by tropical plants in their response and adaption. Proteomics clearly has the potential to revolutionize and potentiate research on how proteins function as the key metabolic pathway regulators and integrators in coconut fruit.

Successful genome sequencing has been done for the "Hainan Tall" and "Catigan Green Dwarf" varieties. Most of the genome was sequenced in both cases, with predicted genes coding for at least 28,000 proteins. The transcriptome of coconut under stress has also been sequenced, documenting the transcripts from expressed genes while adding another powerful, high-resolution strategy to genomic analysis of *C. nucifera*. The increasing availability of coconut genome data provides the opportunity to make elaborate genomic comparisons between coconut varieties and/or with other plant species to identify the biochemical and molecular differences that determine metabolome, proteome and genome functions. The recent *in silico* combination of genomics and transcriptomics with bioinformatics has identified transcript candidates to be key regulators in coconut carbohydrate, lipid, amino acid, nucleotide and energy metabolism. Future research needs to confirm if these or additional candidates are expressing in coconut fruit and how their protein products impact fruit quality and interrelations between metabolic pathways. Another option is to explore the *in vitro* heterologous expression of these transcripts to analyze and characterize their enzyme catalytic properties. Combining metabolomics, proteomics, genomics, transcriptomics and bioinformatic tools will clearly accelerate research on the *C. nucifera* genome. What is revealed will push knowledge on the coconut firmly into the future.

Authors' contributions

I.-F.I., conceived, designed and wrote the review, T.-S.M., wrote and improve the review. Both authors read and approved the final version of the manuscript

Declaration of Competing Interest

All authors declare no competing financial interests.

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