agronomy

MDPI

Hypothesis

Towards the Understanding of Important Coconut Endosperm Phenotypes: Is there an Epigenetic Control?

Jorge Gil C. Angeles [1,*], Jickerson P. Lado [2], Evangeline D. Pascual [2], Cristeta A. Cueto [3], Antonio C. Laurena [1,4] and Rita P. Laude [2]

1 Philippine Genome Center—Program for Agriculture, Livestock, Forestry and Fisheries, Office of the Vice-Chancellor for Research and Extension, University of the Philippines Los Baños, Los Baños, 4031 Laguna, Philippines; aclaurena@yahoo.com (A.C.L.)
2 Genetics and Molecular Biology Division, Institute of Biological Sciences, College of Arts and Sciences, University of the Philippines Los Baños, Los Baños 4031 Laguna, Philippines; jplado@up.edu.ph (J.P.L.); edpascual2@up.edu.ph (E.D.P.); ritalaude50@gmail.com (R.P.L.)
3 Philippine Coconut Authority—Albay Research Center, Guinobatan, 4503 Albay, Philippines; kristicueto@gmail.com
4 Biochemistry Laboratory, Institute of Plant Breeding, College of Agriculture and Food Science, University of the Philippines Los Baños, Los Baños, 4031 Laguna, Philippines
* Correspondence: jgcangeles.pgc.uplb@gmail.com; Tel.: +63-49-536-5326 or +63-49-576-0961

Received: 24 July 2018; Accepted: 28 September 2018; Published: 13 October 2018

Abstract: The coconut is a major crop of many tropical countries, with the endosperm being one of its main products. The coconut soft-endosperm variants, the Makapuno and the Lono, are emerging as economically important. This review describes this crop, its salient endosperm phenotypes and the prevailing hypotheses associated with these. We also collate the literature on the Makapuno and provide a comprehensive review of the scarce information on the Lono. We review the current tenets of plant DNA methylation and provide examples of altered phenotypes associated with such methylation changes. We explore how the changes in the methylome affect endosperm development and the tissue culture process. We further cite the epigenetic basis of an altered endosperm phenotype of a closely related species to the coconut, the oil palm. We discuss how such modifications could affect coconut endosperm development, yielding the Makapuno and Lono phenotypes.

Keywords: coconut; Makapuno; Lono; epigenomics; endosperm development; tissue culture; virgin coconut oil

1. The Coconut Endosperm, Its Development and Fatty Acid Composition

The coconut (*Cocos nucifera* L.) (Palmae: Arecaceae, monocotyledons) is an economically important crop for many tropical and sub-tropical regions [1–5] and can grow in various soil types [1]. The coconut plant is monocieous, with both the male and female flowers growing on the same tree [1,6]. The coconut has a large seed, growing up to 8 kg [7], which is the second largest in the world, with only the coco de mer or the double coconut palm, *Lodoicea maldivica*, endogenous to the Seychelles, having bigger seeds of up to 20 kg [8,9]. The coconut (Arecaceae: Arecoideae) and the coco de mer (Arecaceae: Coryphoideae) are related species that differ starting at the subfamily level. In general, there are two distinct types of coconut worldwide, the tall and the dwarf, that differ with respect to plant height, flower biology and the timing of flowering [3]. In the tall variety, the male and female flowers on the same plant (monoecious) bloom at different times, resulting in cross-pollination. The tall variety produces 12–14 inflorescences per plant a year and is fertile earlier than the dwarf variety. The dwarf

variety is self-pollinating as the male and female flowers flower simultaneously, it produces about 18 inflorescences a year [1,4,6,10].

The coconut is often called the "Tree of Life" since it yields fruit almost every month and its different parts like the copra (dried meat of the coconut seed) and coconut oil from the matured coconut kernels are of economic interest [1,11]. The main product is the endosperm, which includes the liquid coconut endosperm and the solid coconut endosperm or meat and its by-products. The solid endosperm on average contains 46% oil that is primarily composed of lauric acid (C12 fatty acid) and alpha tocopherol [3,12,13]. Coconut oil is used in food products, for cosmetics and toiletries, detergents, surfactants and other industrial uses [1,14–17]. Child (1974) further listed the various properties of coconut oil, such as its melting point (between 20 through 27 C) and an iodine value of 7.7, which is one of the lowest among commercially available vegetable oils [1] and indicates a low concentration of unsaturated fatty acids compared to other plant oils (Table 1).

Table 1. A meta-analysis of the average % composition of different fatty acids in various edible, commercial vegetable seed oils [16,18–20]. Numbers in bold indicate the fatty acid with the highest proportion for each vegetable oil.

	Coconut	Canola	Corn	Cotton	Flaxseed	Olive	Oil Palm	Peanut	Soybean	Sunflower
C6 (caproic)	0.083	0		0	0	0	0	0	0	0.26
C8 (caprylic)	5.19	0	4	0	0	0	3.72	0	0	0.13
C10 (capric)	5.84	0	7	0	0	0	4.12	0	0	0.34
C12 (lauric)	**48.04**	0		0	0	0	**43.57**	0	0	0.07
C14 (myristic)	19.04	0.06	0.6	0.69	0	0.35	16.09	0.03	0.21	5.57
C16 (palmitic)	9.42	6.48	10.94	21.76	5.5	8.34	8.33	8.45	10.05	0.13
C16:1 (palmitoleic)			1.47	1.84	0		0	0	0	0.05
C17 (margaric acid)		3.78	0.08	0.08	0	0.11	0	0.12	0.11	3.714
C18 (stearic)	3.06	1.87	2.42	2.35	3.5	2.83	2.14	3.58	4.04	29.56
C18:1 (oleic)	7.92	**41.35**	29.39	33.69	22.1	**78.4**	22.5	**58.5**	26.63	**59.55**
C18:2 (linoleic)	1.38	17	**48.49**	**46.91**	20.5	7	1.25	20	**51.83**	0.24
C18:3 (linolenic acid)	0.067	27.95	0.76	0.35	**47.5**		0	0	6.58	0.64
C20 (arachidonic)	0.14	0.64	0.50	0.34	0.65	0.29	0.15	2.19	0.38	0.65
C22 (behenic)		0.35	0.49	0.35	0	0.13	0	3.14	0.58	0.6
C22:1 (erucic acd)				0	0		0	0	0	0.21
C24 (lignoceric)	0.03	0.27	0.29	0.22	0	0.03	0.3	1.66	0.23	0

In plants, the double fertilization process yields seeds that contain a diploid embryo (2n) and a triploid endosperm (3n), which serves as a nutrient resource for the germinating embryo in the cereal and coconut monocots and persists in the mature seed [9]. In mature coconut seeds, the endosperm can be 100 times the weight of the embryo [9].

Tammes (1955) and Child (1974) outlined the development of the coconut plant and its endosperm (Figure 1) [1,6]. The coconut plant begins flowering five years after planting and does so at monthly intervals. As the fruit matures, its embryo sac increases in size, while leaving a large vacuole at the center [21]. In a bunch of developing fruits, the least mature ones yield high lauric, oleic and linoleic acids but their levels decrease rapidly as the bunch matures [22]. It takes 12–13 months from fertilization until a mature coconut fruit that consists of 70–75% oil develops [1,23]. During the first six months of fruit development, the fruit increases in volume as the cavity or the embryo sac remains filled with the liquid endosperm, while the solid part begins to form starting from the end opposite of the stalk, then gradually extending into the endosperm's interior to form a layer around it. The solid endosperm layer develops rapidly from the seventh month through the ninth month starting as an initial thin jelly-like layer to form a firm and solid kernel with about 75% dry weight by the ninth month, and then finally forming a hard, white flesh due to the intracellular deposition of oil by 13 months.

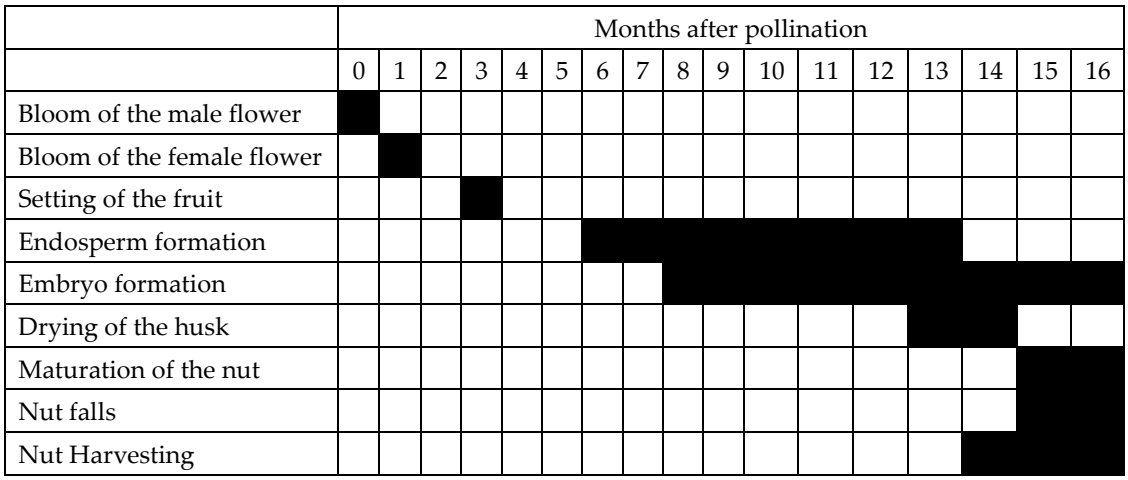

Figure 1. Timeline of the coconut nut development (adapted from Tammes, 1955 [6]). Blocks illustrated in black indicate the age (months after pollination) associated with a particular stage of the coconut nut development.

Virgin coconut oil (VCO) is highly composed of medium chain triglycerides and has recently been popular due to its potential health benefits. VCO has properties that are beneficial for human health, as shown by studies related to chronic inflammation, osteoporosis, blood pressure control, nitric oxide deactivation, sugar level control, wound healing, and studies in rat models testing for analgesic and antipyretic characteristics [1,11,24–27]. VCO is resistant to lipid peroxidation, and increases important antioxidant enzymes (such as superoxide mutase, catalase, glutathione peroxidase and glutathione reductase), hepatic antioxidant enzymes and lipid-controlling enzymes (such as paraoxonase-1) that could protect against coronary heart disease [11,28–31]. A comparison of the fatty acid composition in edible vegetable oils derived from coconut, oil palm, canola, corn, cottonseed, flax or linseed, olive, peanut, soybean and sunflower shows that coconut oil and palm oil are rich sources of lauric acid (C12) (Table 1) and are considered to be of high economic value [3,5,16,18–20]. Lauric acid is a medium-chain triglyceride that is beneficial for human health and nutrition [16]. The FAO predicts that 44% of the calories needed by the postulated nine billion global population in 2020 will come from oil crops; hence the high economic impact of coconut oil [32]. Child (1955) recommends that the oil's yield is a good criterion for enhanced coconut breeding and germplasm maintenance [1].

The coconut fruit is a fibrous drupe and is made up of three main layers: The most external exocarp layer is smooth and varies in color from green to red-brown, the middle mesocarp layer has a white and tough texture in the young coconut and becomes the fibrous husk as the coconut ripens, and the most internal endocarp layer has a hard, strong shell and encloses the kernel or endosperm. In between the endocarp and the endosperm is the testa, a thin, brown seed-coat that is firmly attached to the kernel [1]. There are three main parts of the coconut endosperm: The inner layer that is nearest to the coconut water and is about 16% (% weight) fat, the middle layer has 46% fat while the outer layer nearest to the testa has 62% fat and is predominated by lauric acid (C12) [33].

During the various stages of endosperm development, the type and amount of fatty acid produced varies. Fat synthesis starts by the 4th to 7th month when the solid endosperm begins to form and this rate increases until the 12th month [1,6,22,34–36]. The shorter chain fatty acids (C6, C8 and C10) increase along with the development of the liquid and solid coconut endosperm, while the longer chain fatty acids (C18:3 and C22) are found in all of the stages of the liquid endosperm but not in the kernel [22,33]. At 6–7 months, the endosperm is mainly composed of linoleic acid (C18:1) at 29%, at 11–12th month, but by the 13–14th month shifts to become predominantly lauric acid (C12) at 35% and 32%, respectively [1]. Child (1974) further stated that during the 6–7th month, the endosperm is still synthesizing C10 through C14 fatty acids but these are completely synthesized by the 11–12th month stage, yielding an endosperm that is about 50% C12 (lauric) and C14 (myristic) acids [1]. Caprylic

(C8) and C12 (lauric) acid levels significantly increase starting from 7 to 11 months after fertilization. The longer chain fatty acids C16 and C18:1 significantly decrease from 14% and 23% in six-month old endosperms to 9% and 6%, respectively, in 12-month old endosperms. Palmitoleic (C16:1) and linoleic acids (C18:2) were found in low quantities in six-month old endosperm but further decrease as the nut develops [33].

Plant seeds begin oil biosynthesis in the plastids and continue in the cytoplasm either through the acyl-CoA dependent or the acyl-CoA independent pathway utilizing the phospholipid:diacylglycerol transferase (PDAT). The resulting oil is stored in various tissues such as in the mesocarp, perisperm, endosperm (such as in the coconut) and embryo in discrete cellular bodies called oleosomes. The oil is stably held by the oleosin proteins (also known as caleosins in oil palm and stereolosin) in the oleosomes [37–39].

2. The Makapuno and Lono Endosperm Phenotypes

2.1. Makapuno

The 'Makapuno' is a natural coconut cultivar with an over-proliferating solid endosperm and was initially detected in Laguna, the Philippines and in Java, Indonesia [40]. The Makapuno fruit consists of very thick and fluffy solid endosperm almost filling up the whole cavity and has little or no water in its central space [41]. A viscous, white, translucent jelly nearly fills the remaining core of the fruit. In contrast, the normal coconut has a hard, compact, and crisp solid endosperm with lots of coconut water, or liquid endosperm. With the exception of the endosperm, Makapuno and normal coconut trees are similar in all morphological aspects [40,42]. The naturally thick endosperm of Makapuno is four to five times more expensive than the normal coconut, it is utilized for dessert preparation, delicacies, and has other industrial and food applications; hence it has economic benefit.

Before the 1960s, the Makapuno was propagated by planting the non-Makapuno nuts harvested from Makapuno-bearing trees instead of the Makapuno nuts themselves, as these were non-viable and do not grow into shoots. The Makapuno nut cannot be propagated in seed beds or nurseries, unlike the normal coconut. Today, Makapuno nuts can be harvested from Makapuno-bearing palms which have been produced through the embryo-culture technology that was successfully developed by De Guzman in the 1960s after a decade of experimentation [43,44]. The de Guzman technology was improved at the Philippine Coconut Authority—Albay Research Center [45] and paved the way for the commercial production of embryo culture seedlings in the Philippines [46]. To date, there have been several laboratories engaged in mass production of embryo-cultured Makapuno seedlings including the University of the Philippines Los Banos; the Philippine Coconut Research & Development Foundation (PCRDF); National Coconut Research Centre, Leyte State University, Baybay, Leyte; Lipa Agricultural Experiment Station in Lipa, Batangas, Philippines; and the Philippine Coconut Authority—Albay, Davao and Zamboanga Research Centers.

The initial studies of Torres (1937) and Zuñiga (1953) have provided evidence for the Makapuno endosperm genetics or mode of inheritance. The Makapuno phenomenon is hypothesized to be controlled by a recessive *m* gene and is expressed as a homozygous condition derived either from embryo-rescued Makapuno (*mmm*) endosperms or from existing Makapuno trees either with a heterozygous *MMm* or *Mmm* genotype (Table 2) [42,47–49]. Thus, a pure Makapuno palm does not exist naturally and is often cultivated via tissue culture [43,44]. Normally, there is a 25% probability of producing Makapuno nuts if the nuts are obtained from Makapuno-bearing parent plants [47,50] but this possibility could increase to 85% (ranging between 50 to less than 100%) if these are harvested from embryo-rescued Makapuno palms [43,44]. Copeland (1931) notes that these Makapuno nuts will not germinate but the palms would produce only a proportion of the Makapuno nut [40]. Cedo et al., (1984) noted an all Makapuno nut yield from self-pollination or cross-pollination of embryo-cultured Makapuno with the same type, while cross-pollination of an embryo-cultured Makapuno with a normal coconut produced all normal nuts [50]. Hence, this study inferred a possible homozygosity

(*mm*) of the Makapuno character in the embryo-cultured Makapuno coconut and that the presence of the dominant factor (*M*) restores the normal endosperm type. Results of genetic studies were indeed a concrete basis for postulating that a single recessive gene controls the Makapuno character, and that Makapuno-bearing coconuts express heterozygosity for the endosperm phenotype.

Table 2. Makapuno embryo and endosperm genotypes postulated [48,49].

♀ ♂	**EGG NUCLEUS (n)**		**POLAR NUCLEI (2n)**	
Sperm nucleus (n)	*M*	*m*	*MM*	*mm*
M	*MM* Germinating embryo	*Mm* Germinating embryo	*MMM* Normal endosperm	*Mmm* Normal endosperm
m	*Mm* Germinating embryo	*mm* Non-germinating embryo	*MMm* Normal endosperm	***mmm*** ***Makapuno endosperm***

One school of thought postulates that the xenia effect contributes to the Makapuno endosperm phenotype though no literature confirming this has been published. The xenia effect is the appearance of phenotypic characteristics controlled by a single recessive gene and is affected by the genotype of donor pollen influencing the resulting fruits. The xenia effect has been found in dates, maize and coconuts [51]. The xenia effect affects coconut fruits producing increased nut size, copra weight and hybrid vigor [52].

The Makapuno nut is classified into three types based on the appearance of the solid and liquid endosperms, with type A having the same thickness as the normal and its liquid endosperm being slightly viscous, type B with a thicker solid endosperm having a soft inner layer and a very viscous, translucent liquid endosperm, and type C having the thickest solid endosperm and a soft inner proliferating layer filling up the whole cavity while its liquid endosperm is almost non-existent and is replaced by an oily semi-solid endosperm [53,54]. There is no current explanation on how these intra-variations in the Makapuno endosperm arise.

Depending on the study, proteins, fats, and sugars levels were similar or different between Makapuno and the normal coconut endosperm [55,56]. Ramirez and Mendoza (1998) summarized that the protein and carbohydrate content of Makapuno is higher as compared to the normal coconut but depends on the developmental stage [42]. Recently, higher levels of ketoacyl-acyl carrier protein synthase I (KASI), involved in fatty acid biosynthesis, were shown in Makapuno compared to the normal counterpart [57].

The characteristic viscous and jelly-like Makapuno endosperm is generally attributed to the increased galactomannan content compared to the normal coconut and depends on the developmental stage [58]. These galactomannans are made up of 1:3 galactose-mannose residues [55,59]. Galactomannan degradation is sequentially controlled by three enzymes: alpha-D-galactosidase (AGAL), beta-mannanase, and mannosidase. Various studies found that alpha-D-galactosidase and mannosidase transcript and/or enzyme activity levels are much lower in Makapuno than in normal coconut [60–63] and that there is no significant difference in beta-mannanase activity [62,63]. AGAL activity was hardly detected in almost all stages of development except at the mature stage (11–12 months after pollination) having 8268-fold lower activity than normal. The deficiency in AGAL activity was postulated to cause the aberrant cellular behavior and properties of Makapuno, presumably through a metabolic block in the normal galactomannan degradation in the endosperm. The increasing alpha-D-galactosidase enzyme activity in the normal coconut over the different developmental stages produces more mannans, resulting in the more solid and crisp characteristic endosperm [60,62].

Two forms of AGAL were also observed by Mujer et al., (1984) [60]. Both monomeric isoenzymes A (MW = 23,000 Da) and B (MW 26,600 Da) exhibited optimum activity at pH 7.5. Enzyme kinetics of

normal and Makapuno AGAL from the endosperms revealed no difference between their Km or Vmax values. Such findings led them to postulate that there is no possible structural mutation present in the AGAL gene, but instead, a continuous repression of enzyme synthesis or specific inhibitors maybe at play. AGAL isoform A was shown to be strongly inhibited by D-galactose followed by myo-inositol, glucose-6-phosphate, arabinose, and melibiose [60]. Sulhydryl specific reagents like iodoacetic acid also showed inhibition, suggesting the participation of sulfhydryl group during enzyme catalysis.

Recently, Dela Cruz et al., (2013) reported the cloning of partial cDNAs from normal and Makapuno coconut solid endosperms [57]. Of the 13 cDNAs, they have noted seven genes and isoforms to be involved in carbon metabolism, two in hormone biosynthesis, and four are involved in the regulation of transcription, translation, and cell division. Sequence alignment results revealed identical partial sequences between normal and Makapuno endosperms, including AGAL isoforms. Interestingly, several deletions, insertions, substitutions were detected in the alpha-D-galactosidase gene in Makapuno [64]. De la Cruz and Bugayong (2016) suggested these differences in the genomic sequence as possible major causes of the Makapuno phenotype. The current working hypothesis is that the Makapuno phenotype is due to increased galactomannan content owing to decreased activity of the alpha-D-galactosidase and mannosidase enzymes, which are important for galactomannan degradation [58].

2.2. Lono

The Lono cultivar is a high-value, soft-endosperm coconut [65,66] and is described to initially originate from La Union [67]. The cultivar has high nut yield and qualities such as tender nut water quality, soft endosperm meat and high oil content similar to other cultivars such as the Malayan Green Dwarf variety and the tall varieties, including the East and West African, Seychelles, Philippine Ordinary, Panama, Borneo and Guam [68]. The Lono cultivar is closely related to the Philippine Ordinary and Laguna Tall varieties and is also closely related to the Cochin China Tall, the San Ramon tall and the Borneo and Fiji tall genotypes [69,70]. Currently, Lono is observed among typica cultivars, specifically the Laguna Tall variety [2]. Padolina (1985) reported that these Lono cultivars produce both small and large fruits [67]. The soft endosperm of the Lono can be eaten fresh and like the Makapuno cannot be made into copra.

Lono trees are about 6–6.5 m tall and measures 91 cm in its stem while its trunk's diameter is 1 m from the base of the plant. The leaves are long and slender (125 cm long and 6 cm wide). Inflorescence production starts about 10–11 years after planting. These inflorescences are 109 cm in length and 61 cm wide [7]. The Lono inflorescence has a Type IIA flowering pattern, since this cultivar shows mixed and indirect allogamy wherein the male and female flowers do not overlap in the same inflorescence but these phases could overlap between subsequent florescences [71,72]. Similar to the Tall variety, the mean male phase in Lono is 20.0 days and 5.0 days for the female phase with a gap phase of 3.0 days between the two flower reproductive systems, thus, the male and female flowering periods are non-overlapping [1,6]. These values lie in the upper quartile for the length of these male, female and gap phases considering the 54 coconut cultivars tested [72,73]. Lono nuts are large (about 1.5 kg fresh weight), yellow-green, and are oval in shape with three characteristic ridges that become more prominent along the base. Dehusked nuts have a flattened bottom end with a distinct round projection at its posterior end. The Lono kernel is distinctively thick, though its shell is thin.

Based on observations from the Philippine Coconut Authority—Albay, the possibility of obtaining a Lono nut from a Laguna Tall tree ranges from 0 to 25%. It is more difficult to source Lono nuts than Makapuno coconuts. Similar to the Makapuno, the Lono nut could only be distinguished from other nuts upon splitting of the nut. Because of the soft nature of the endosperm, the embryo does not germinate in situ, hence, it has to be rescued. Embryo rescue of Lono embryos and hand-pollination of embryo-cultured Lono palms are planted at the Philippine Coconut Authority—Albay Research Center for mass production of Lono coconuts [65,74]. Several Lono cultivar plantations are found in the Asian region. Lono cultivars are planted at the International Coconut Gene Bank for South Asia (88 plants)

for conservation of the germplasm [7,75,76], in AIRCP (Palms) Centres [7,77], at the University of the Philippines Los Banos, and at the Philippine Coconut Authority—Albay Research Center (PCA-ARC).

Analysis of the oil content of the Lono nut shows that 64.5% of the solid endosperm is oil. Gas chromatography analysis reveal that this Lono-derived oil has 93.2% saturated and 6.8% unsaturated fatty acids. Of the saturated fatty acids, 60.23% are medium chain fatty acids while the long chain fatty acids account for 32.98%. There are also detected levels of unsaturated fatty acids (Table 3) [3,15,17]. Fatty acids are stored in discrete cellular structures called oleosomes or oil bodies [37] that are large in Lono [78].

Table 3. Average % composition of different fatty acids in normal Laguna Tall and the Makapuno and Lono Laguna Tall phenotypes.

	Laguna Tall [14]	**Makapuno [42,79]**	**Lono [3,15–17]**
C6	0.56	0.61	0.18
C8	7.64	7.14	5.12
C10	6.55	7.34	5.1
C12	49.70	50.06	49.85
C14	18.07	18.36	20.55
C16	8.34	7.34	9.12
C16:1			0.06
C18	6.02	3.06	2.97
C18:1		3.06	5.85
C18:2	3.13		0.89
C18:3			0.07
C20			0.08
C24			0.06

Interestingly, the Lono cultivar is reported to be acted on by the eriophyid mite and root (wilt) disease. The mite causes scarring in the growing nuts especially 2–3 months before maturation stage since the mite inhabits the basal portion of the nut below the perianth. Eriophyid mite infestation is prevalent in India [80]. Various reports have shown that the Lono cultivar ranges from susceptible [81] to moderately resistant [80] against the eriophyid mite (*Aceria guerreronis* Keifer), while the cultivar had been also shown to be 71% resistant, and is the second most resistant variety following the Chowghat Green Dwarf, against the root wilt disease [82].

The occurrence of the Lono endosperm phenotype and its relatively high fatty acid profile, might have occurred by spontaneous mutation [52,83], but there is no molecular basis for such a mutation. However, given the Makapuno and Lono phenotypes are primarily propagated through tissue-culture methods which are known to cause epigenetic changes, another hypothesis postulates that altered epigenetics could be responsible for the Makapuno and Lono phenotypes.

3. Molecular Characterization of the Coconut

Some important coconut genes deposited in the NCBI nucleotide database so far are those for fatty acid biosynthesis and oil storage, carbohydrate metabolism, plant defense, hormone synthesis, and for plant development.

There are various efforts to sequence the coconut genome (Table 4), including those using the Hainan Tall [84] and the Catigan Dwarf [85] varieties. Lantican et al.'s (2018) efforts elucidated that the coconut's genome is 2.1 GB with a scaffold N50 of 570 kb. Lactican's endeavor led in covering 98% of the genome of the coconut. Table 4 also show that the coconut genome (2.1 GB) is slightly bigger than other related palm species, *Elaeis guineensis* (African oil palm) (1.54 GB), *Elaeis oleifera* (American oil palm) (1.4 GB) and *Phoenix dactylifera* (date palm) (0.558 GB).

Table 4. Various efforts to sequence the coconut genome and other closely related palm species.

Organism	Variety	Sequencing Technology	Genome Size (GB)	Genome Coverage (%)	Number of Scaffolds [1]	Number of Gaps	Scaffold N50 [2] (kb)	Reference
Cocos nucifera	Hainan Tall	HiSeq 2000	2.2	90.91			418.07	[84]
	Catigan Dwarf	PacBio SMRT, MiSeq and Dovetail Genomics	2.1	98	7998	12,106	570	[85]
Elaeis guineensis	AVROS pisifera	Roche 454	1.54		40,072	166,221	1045	[84,86]
Elaeis oleifera		Roche 454	1.4		63,113		333.11	[84,86]
Phoenix	Khalas	Illumina Genome Analyzer II	0.658	58	57,277	42%	30.48	[84,87]
dactylifera	Khalas	Roche 454, SOLiD, ABI3730	0.558	90.2	82,354	9.80%	329.9	[84,88]

[1] Scaffolds are the ordered arrangements of contigs that were assembled *de novo* as inferred from mate pair or paired end sequenced reads [89]; [2] Scaffold N50 is the weighted mean scaffold size of a genome assembly and is a metric of *de novo* genome assembly quality. The N50 is the number of the longest scaffold after ranking the lengths of all of the assembled scaffolds from the top downward. These ranked lengths are then summed starting from the largest scaffold. Scaffold N50 is the scaffold size at which this summed length is greater or equal to 50% of the total assembled genome size [89].

From the NCBI-BioProject database, there are three registered coconut genome projects, namely in Hainan Tall (China) (Accession Number: PRJNA374600) [84]; Chowghat Green Dwarf (India) (Accession Number: PRJNA413280); and Laguna Tall (Philippines) (Accession Number: PRJNA298457).

The genomes of the chloroplast [90] and mitochondria [91] of a Tall coconut variety have been sequenced and annotated. Several coconut varieties and different explants or developmental stages were used in transcriptomic analyses in order to study the regulation of genes in fatty acid metabolism in the endosperm [92], aromatic pathways [93], and developmental stages [94]. The embryo and endosperm transcriptomes shared only 3225 (20%) of their transcripts [9]. Comparative omics studies on the transcriptome and the translated proteome between coconut (*Cocos nucifera*) against the related oil palm (*Elaeis*) were published [95] using the spear leaves, young leaves and fruit flesh from the Hainan Tall variety [92] and the young leaves, maturing endosperm and matured endosperm from a coconut dwarf variety [9]. Proteomics efforts determined IgE reactive proteins in coconut, including the major vicilin-like allergen, 11S globulin, enolase and isoflavone reductase [96].

To date, no published literature on coconut epigenetics is available. However, in its close relative, oil palm (*Elaeis*), the epigenetics basis of the mantled endosperm phenotype has been well-documented [97–104].

4. Plant Epigenetics and Endosperm Development

Epigenetic modifications in the DNA involve changes in gene expression without changing the primary sequence of the DNA at either genic or non-genic sites, including promoters and transposon sites [105,106]. Such changes include paramutations, transgene silencing, genome imprinting and transposon inactivation that are widespread in plants [106]. These changes are highly dynamic and transitory throughout the development of an organism and are mitotically and/or meiotically transmissible [107–111]. Histone modifications such as methylation, acetylation and sumoylation, and remodeling of the chromatin structure [112] result in either gene activation or silencing ultimately affecting processes such as plant development [109,113]. The most studied epigenetic mark is the methylation of the fifth carbon of cytosine (meC) base of the DNA that invokes epigenetic-based modification of the DNA structure. DNA methylation may occur on promoters, genes or repetitive elements and is the fundamental mechanism underlying transposon silencing, X-chromosome inactivation and gene imprinting [105,114]. Methylated cytosines attract methyl-binding proteins that subsequently recruit histone deacetylases and chromatin remodelling proteins, hindering the binding of other transcription factors causing differential gene expression [108,113].

Methylated cytosines are seen in major eukaryote groups such as plants, fungi and animals [110]. In plants, DNA methylation occurs at CG, CHG and CHH sites [106,115,116]. In the *Arabidopsis* plant model system, cytosine methylation is typically found at the symmetric methylation sites of CG (24%) and CHG (6–7%) sites and at the asymmetric CHH sites (1.7%), where H is a non-G DNA base [106,110,116–118]. CG and CHH methylation are more prevalent in euchromatic regions whereas CHG methylation is usually found in pericentromeric regions [116]. CG methylation occurs via a self-reinforcing loop that relies on the symmetry of the CG dinucleotides. A newly-synthesized daughter DNA strand will have a hemi-methylated strand if the other strand is methylated [106]. In such cases, the CG methyltransferase METHYLTRANSFERASE 1 (METI) enzyme is recruited to methylate the unmethylated daughter strand. In *Arabidopsis*, CG and CHG methylation could only occur due to the activity of the MET1 methyltransferase and CMT3 enzyme which maintain CHG methylation [106,119]. The *Arabidopsis MET1* (*AtMET1*) is essential for embryogenesis and for the development of the viable seed [120].

Maintenance of CHG methylated strands is facilitated by a distinct self-reinforcing loop requiring KRYPTONITE (KYP) or the SUPPRESSOR OF VARIEGATION HOMOLOGY 4 (SUVH4) enzyme and other SUVH enzymes (SUVH5 and SUVH6), the CHG methyltransferase, and CHROMOMETHYLASE3 (CMT3) to ensure that such CHG methylation is maintained [106]. CMTs

are unique to the plant kingdom and are involved in non-CG methylation including the maintenance of CHG methylation and as a player in the maintenance of RNA-directed DNA methylation (RdDM) in the asymmetric CHH context [121,122]. CHH methylation occurs through CMT2, another chromomethyltransferase, which recognizes the methylation of DNA sequences found in H3K9me2 regions containing long transposable elements in euchromatic DNA regions [123]. CHH methylation in these regions depend on siRNAs that guide a *de novo* methyltransferase called DOMAINS REARRANGED METHYLTRANSFERASE 2 (DRM2) to the target sequence to be methylated [106]. Among plant species there is a large variation in levels of the CG, CHG and CHH methylation and their distribution over the genome [124].

Methylated cytosines occur about at 33% in genic regions, but only at about 5% in *Arabidopsis* promoters [113,125–127]. Methyl cytosine levels vary depending on the tissue and developmental stage [113,127]. The role of cytosine methylation at coding regions is not clear to date. At promoter regions, high levels of methylated cytosine suppress gene expression while low levels induce gene expression [113]. Changes in cytosine methylation patterns at promoters that affect gene expression resulting in phenotypes are called epialleles. Well-known examples of reported epialleles are SUPERMAN in *Arabidopsis,* B1-B′ that influence corn endosperm color, CNR in tomato fruits and CYCLOIDEA in toadflax (*Linaria vulgaris*) [106,128–132].

DNA methylation profiles change during endosperm development. In the developing endosperm of *Arabidopsis*, widespread DNA demethylation is associated with the activation of endosperm-specific demethylases [106]. The *Arabidopsis* endosperm is hypomethylated as compared to the embryo in all of the three cytosine contexts and particularly at the 5′ and 3′ regions of genes [133,134]. Gehring et al., (2009) postulated that this is due to the loss of the active demethylation activity of the DNA glycosylase *DEMETER* in the central cell prior to fertilization [133]. These show the importance of genome imprinting affecting gene regulation, including changes in DNA methylation that occur in sperm nuclei of pollen cells [106]. Insights into various aspects of DNA methylation could lead to the understanding of the phenotypic variation in different crop species including the coconut [106].

In coconut, Huang et al., (2014) showed expression of *MET1*, *CMT* and *DRM* based in the transcriptome of young coconut leaves (approximately eight months old), five-month old endosperm, and eight-month old embryo [9]. *MET1* transcripts are highly expressed in the endosperm compared to the embryo and leaves. *CMT* expression is highest in leaves and endosperm and very low in the embryo. *DRM* transcript expression is highest in endosperm and embryo, and very low in leaves. Between the different layers of the coconut endosperm, MET1 is 2.5-fold relatively higher in the gelatinous endosperm but only about 0.01-fold in the thick layer of the solid endosperm. DRM is 2.25-fold more highly expressed in the thin layer of the solid endosperm while only about 0.40-fold in the thick layer of the solid endosperm relative to the levels in the gelatinous endosperm [135]. Huang et al., (2014) postulated that the coconut is an interesting non-model plant, since it produces exceptionally large seeds that contain large amounts of the endosperm and small but still macroscopically-visible embryo [9]. As such, the crop provides a unique opportunity to investigate the expression of epigenetic factors in the context of its embryo and endosperm development.

5. Plant Tissue Culture and Epigenetics

The coconut including its highly valued Makapuno and Lono phenotypes are widely propagated via tissue culture [43,44,65,74,136]. Naturally, there is no probability of natural occurrence of the Makapuno [43,48,58,59], but the phenotype could occur at 25% if the nuts are obtained from Makapuno-bearing parent plants [43,60] while the probability for the Lono is less than 25%. Tissue culture and embryo-rescue efforts had increased these chances to about 85% (ranging from 50 to 100%) and 50% respectively for the Makapuno and Lono phenotypes [74,83]. Menon and Pandalai (1958) and Niral et al., (2010) cited the efforts of the Philippine Coconut Authority—Albay Research Center for initiating activities for the tissue culture of the Lono phenotype [7,75].

Tissue culture is a fundamental tool in plant science research to study biological, physiological and biotechnological aspects [137–139] and is used for plant propagation and mass production, improvement and conservation [135,140]. However, tissue culture procedures might cause genetic and/or epigenetic changes in the genome, which have been documented in several plant species such as *Beta vulgaris* [141]. The time to produce regenerants using the tissue culture procedure and the hormones used in the different media are important parameters that determine the frequency of plants with aberrant phenotypes, so-called somaclonal variants [142], some of which were correlated with altered global DNA methylation [143–147].

The tissue culture process has been linked to influence global methylation levels during cacao (*Theobroma cacao* L.) somatic embryogenesis and is correlated with decreased embryogenic potential [148] and associated with the hypomethylation of the *KARMA* LINE within *DEFENSIN1* truncating its transcript to yield the abnormal mantled phenotype in oil palm (*Elaeis guineensis* Jacq.) [97–99,144,149,150]. The length of culture time and increased levels of indole acetic acid and kinetin in the culture media lead to increased occurrence of the oil palm mantled phenotype as well [97,100–102,151,152]. Maize cultures grown for 3.5 months in MS media lead to the hypermethylation of the *Pericarp color1* (*PR-wr*) epiallele, yielding altered coloration of the pericarp and cob-glumes [153]

Different global methylation levels are observed in different plant tissues such as the mantled endosperm of oil palm that is closely related to the coconut [97–104]. In somatic embryo-derived oil palm clones (*Elaeis guineesis* Jacq), decreased global methylation is associated with the mantled phenotype wherein there is an apparent feminisation of the male parts in flowers of both sexes, resulting in altered fruit development and directly decreasing the oil production obtained [97,100–102]. These mantled phenotypes do not follow Mendellian rules of inheritance suggesting that the phenotype is a result of epigenetic changes affecting gene expression and not due to straightforward gene mutation [149].

The hypomethylation of a retrotransposon integrated as one of the introns within the *EgDEF1* (*DEFICIENS* subfamily), now referred to as the MANTLED gene, results in the mantled phenotype [97,149,150]. The *MANTLED* homologue in *Arabidopsis* encodes for a factor essential for flower organ formation and determines the identity of the inner perianth whorl and stamens [154]. This retrotransposon was later named as *Karma*. In non-mantled phenotypes, this *KARMA* region is hypermethylated (good karma) but in mantled phenotypes, the KARMA region is hypomethylated (bad karma) such as during the tissue culture process truncating the *EgDEF1* transcript which accumulates during flower development leading to the mantled phenotype [97].

This mantled phenotype only affects 5% of regenerant oil palms and nodular compact calli, is unpredictable and has a wide variation in severity, even if the regenerants come from the same clonal progeny. Such mantled nuts, thus, diminishes the productivity of the tree since the entire nut branch is not able to produce normal or non-mantled oil palm nuts resulting in huge losses in the oil palm industry [100,106]. Rival et al., (2009) opines that the mantled phenotype in *Elaeis* offers a very interesting model to study epigenetics in higher plants, particularly those clonally propagated (e.g., the coconut) since epigenetics plays a huge role in somaclonal variation by altering the direct or indirect gene expression [103].

6. Conclusions and Perspectives

The monoecious coconut is a major economic crop for many tropical and sub-tropical countries. Some of its high-valued endosperm products include the lauric acid-rich coconut oil and the Makapuno and Lono coconut phenotypes. This manuscript presents the critical enzymes involved in plant epigenetics and provide examples on the various occurrences of altered methylation in plants that are either naturally-occurring or affected by tissue culture leading to phenotypic changes. We reviewed the pertinent literature on Makapuno, provided a comprehensive description of the Lono based on the available publications, and summarized the epigenetic basis for the altered endosperm phenotype in

closely related species of coconut, oil palm (*Elaeis guineensis* Jacq.). Though we do not discount that there is evidence due to other genetic controls associated with these phenotypes, epigenetics could possibly further explain these novel phenotypes.

Various epigenetic changes are documented due to the tissue culture process and its constituent players. This in vitro method most likely influences DNA methylation levels, leading to altered phenotypes. We postulate that because the Makapuno and Lono endosperms are mass cultivated through tissue culture, epigenetic changes could play a role in the occurrence of these phenotypes. Optimization of the tissue culture process by controlling the levels of the components associated with epigenetic changes is highly encouraged. Therefore, research looking into the epigenomic information regarding the formation of these phenotypes should be initiated. Knowledge of these epigenomic changes contributing to the Makapuno and Lono phenotypes will assist researchers and breeders towards targeted breeding and selection.

Author Contributions: The manuscript was conceptualized and the manuscript's draft and revisions were written by J.G.C.A., J.P.L., E.D.P. and R.P.L. Most of the manuscript was written by J.G.C.A. J.P.L. wrote the section on Makapuno while C.A.C. supplemented the section on the Lono. The manuscript was reviewed and supervised by A.C.L. and R.P.L.

Funding: This research received no external funding.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Child, R. *Coconuts*, 2nd ed.; Longman Group Ltd.: London, UK, 1974; ISBN 0-582-46675-X.
2. Sugimura, Y.; Itano, M.; Salud, C.D.; Otsuji, K.; Yamaguchi, H. Biometric analysis on diversity of coconut palm: Cultivar classification by botanical and agronomical traits. *Euphytica* **1997**, *98*, 29–35. [CrossRef]
3. Naresh Kumar, S.; Balakrishna, A. Seasonal variations in fatty acid composition of oil in developing coconut. *J. Food Qual.* **2009**, *32*, 158–176. [CrossRef]
4. Gunn, B.F.; Baudouin, L.; Olsen, K.M. Independent origins of cultivated coconut (*Cocos nucifera* L.) in the Old World tropics. *PLoS ONE* **2011**, *6*, e21143. [CrossRef] [PubMed]
5. Teh, H.F.; Neoh, B.K.; Ithnin, N.; Daim, L.D.J.; Ooi, T.E.K.; Appleton, D.R. Review: Omics and strategic yield improvement in oil crops. *JAOCS* **2017**, *94*, 1225–1244. [CrossRef]
6. Tammes, P. Review of coconut selection in India. *Euphytica* **1955**, *4*, 17–24. [CrossRef]
7. Menon, K.P.; Pandalai, K.M. *The Coconut Palm, A Monograph*; Indian Central Coconut Committee: Kerala, India, 1958.
8. Hulme, P.; Benkman, C. Granivory. In *Plant-Animal Interactions: An Evolutionary Approach*; Blackwell Science Ltd.: Oxford, UK, 2002; pp. 132–156. ISBN 978-0-632-05267-7.
9. Huang, Y.-Y.; Lee, C.-P.; Fu, J.L.; Chang, B.C.-H.; Matzke, A.J.M.; Matzke, M. *De novo* transcriptome sequence assembly from coconut leaves and seeds with a focus on factors involved in RNA-directed DNA methylation. *G3* **2014**, *4*, 2147. [CrossRef] [PubMed]
10. Lima, E.B.C.; Sousa, C.N.S.; Meneses, L.N.; Ximenes, N.C.; Santos Junior, M.A.; Vasconcelos, G.S.; Lima, N.B.C.; Patrocinio, M.C.A.; Macedo, D.; Vasconcelos, S.M.M. *Cocos nucifera* (L.) (Arecaceae): A phytochemical and pharmacological review. *Braz. J. Med. Biol. Res.* **2015**, *48*, 953–964. [CrossRef] [PubMed]
11. Kappally, S.; Shirwaikar, A.; Shirwaikar, A. Coconut oil—A review of potential applications. *Hygeia J. Drugs Med.* **2015**. [CrossRef]
12. Tangwatcharin, P.; Khopaibool, P. Activity of virgin coconut oil, lauric acid or monolaurin in combination with lactic acid against *Staphylococcus aureus*. *Southeast Asian J. Trop. Med. Public Health* **2012**, *43*, 969–985. [PubMed]
13. Arlee, R.; Suanphairoch, S.; Pakdeechanuan, P. Differences in chemical components and antioxidant-related substances in virgin coconut oil from coconut hybrids and their parents. *Int. Food Res. J.* **2013**, *20*, 2103–2109.
14. Laureles, L.R.; Rodriguez, F.M.; Reaño, C.E.; Santos, G.A.; Laurena, A.C.; Mendoza, E.M.T. Variability in fatty acid and triacylglycerol composition of the oil of coconut (*Cocos nucifera* L.) hybrids and their parentals. *J. Agric. Food Chem.* **2002**, *50*, 1581–1586. [CrossRef] [PubMed]

15. Naresh Kumar, S. Copra, oil and fatty acid database. In *Variability in Content and Composition of Fatty Acids in Coconut Oil Due to Genetic and Environmental Factors*; ICAR: New Delhi, India, 2005.
16. Naresh Kumar, S. Capillary gas chromatography method for fatty acid analysis of coconut oil. *J. Plant. Crops* **2007**, *35*, 23–27.
17. Kumar, S.N. Variability in coconut (*Cocos nucifera* L.) germplasm and hybrids for fatty acid profile of oil. *J. Agric. Food Chem.* **2011**, *59*, 13050–13058. [CrossRef] [PubMed]
18. Zambiazi, R.; Przybylski, R.; Zambiazi, M.; Mendonca, C. Fatty acid composition of vegetable oils and fats. *Boletim Centro de Pesquisa de Processamento de Alimentos* **2007**, *25*, 111–120.
19. Kostik, V.; Memeti, S.; Bauer, B. Fatty acid composition of edible oils and fats. *JHED* **2013**, *4*, 112–116. [CrossRef]
20. Dorni, C.; Sharma, P.; Saikia, G.; Longvah, T. Fatty acid profile of edible oils and fats consumed in India. *Food Chem.* **2018**, *238*, 9–15. [CrossRef] [PubMed]
21. Juliano, J.B. Origin, development and nature of the stony layer of the coconut (*Cocos nucifera*). *Phil. J. Sci.* **1926**, *30*, 187–200.
22. Padua-Resurreccion, A.N.; Banzon, J.A. Fatty acid composition of the oil from progressively maturing bunches of coconut. *Philipp. J. Coconut Stud.* **1979**, *4*, 1–16.
23. Oo, K.C.; Stumpf, P.K. Fatty acid biosynthesis in the developing endosperm of *Cocos nucifera*. *Lipids* **1979**, *14*, 132–143. [CrossRef]
24. Nevin, K.G.; Rajamohan, T. Effect of topical application of virgin coconut oil on skin components and antioxidant status during dermal wound healing in young rats. *Skin Pharmacol. Physiol.* **2010**, *23*, 290–297. [CrossRef] [PubMed]
25. Intahphuak, S.; Khonsung, P.; Panthong, A. Anti-inflammatory, analgesic, and antipyretic activities of virgin coconut oil. *Pharm. Biol.* **2010**, *48*, 151–157. [CrossRef] [PubMed]
26. Hayatullina, Z.; Muhammad, N.; Mohamed, N.; Soelaiman, I.-N. Virgin coconut oil supplementation prevents bone loss in osteoporosis rat model. *Evid.-Based Complement. Altern. Med. ECAM* **2012**, *2012*, 237236. [CrossRef] [PubMed]
27. Nurul-Iman, B.; Kamisah, Y.; Jaarin, K.; Qodriyah, H. Virgin coconut oil prevents blood pressure elevation and improves endothelial functions in rats fed with repeatedly heated palm oil. *Evid. Based Complement. Altern. Med.* **2013**, *5*, 629329. [CrossRef] [PubMed]
28. Nevin, K.G.; Rajamohan, T. Virgin coconut oil supplemented diet increases the antioxidant status in rats. *Food Chem.* **2006**, *99*, 260–266. [CrossRef]
29. Zakaria, Z.A.; Rofiee, M.S.; Somchit, M.N.; Zuraini, A.; Sulaiman, M.R.; Teh, L.K.; Salleh, M.Z.; Long, K. Hepatoprotective activity of dried- and fermented-processed virgin coconut oil. *Evid. Based Complement. Altern. Med.* **2011**, *2011*, 142739. [CrossRef] [PubMed]
30. Arunima, S.; Rajamohan, T. Effect of virgin coconut oil enriched diet on the antioxidant status and paraoxonase 1 activity in ameliorating the oxidative stress in rats—A comparative study. *Food Funct.* **2013**, *4*, 1402–1409. [CrossRef] [PubMed]
31. Yeap, S.K.; Beh, B.K.; Ali, N.M.; Yusof, H.M.; Ho, W.Y.; Koh, S.P.; Alitheen, N.B.; Long, K. Antistress and antioxidant effects of virgin coconut oil in vivo. *Exp. Ther. Med.* **2015**, *9*, 39–42. [CrossRef] [PubMed]
32. Thoenes, P. Recent trends and medium-term prospects in the global vegetable oil market. In Proceedings of the 10th International Conference: Fat and Oil Industry, Kiev, Ukraine, 23–25 November 2011.
33. Balachandran, C.; Arumughan, C.; Mathew, A.G. Distribution of major chemical constituents and fatty acids in different regions of coconut endosperm. *JAOCS* **1985**, *62*, 1583–1586. [CrossRef]
34. Kartha, A.R.S.; Narayanan, R. Development of oil in the ripening coconut. *Ind. J. Agric. Sci.* **1956**, *26*, 319–327.
35. Nathanael, W.R.N. Changes in the coconut endosperm during development. *Ceylon Coconut Quart.* **1959**, *10*, 35–36.
36. Balleza, C.F.; Sierra, Z.N. Proximate analysis of the coconut endosperm in progressive stages of development. *Philipp. J. Coconut Stud.* **1972**, *16*, 37–43.
37. Huang, A.H.C. Oil bodies and oleosins in seeds. *Annu. Rev. Plant. Physiol. Plant. Mol. Biol.* **1992**, *43*, 177–200. [CrossRef]
38. Baud, S.; Lepiniec, L. Physiological and developmental regulation of seed oil production. *Prog. Lipid Res.* **2010**, *49*, 235–249. [CrossRef] [PubMed]

39. Dussert, S.; Guerin, C.; Andersson, M.; Joët, T.; Tranbarger, T.J.; Pizot, M.; Sarah, G.; Omore, A.; Durand-Gasselin, T.; Morcillo, F. Comparative transcriptome analysis of three oil palm fruit and seed tissues that differ in oil content and fatty acid composition. *Plant Physiol.* **2013**, *162*, 1337–1358. [CrossRef] [PubMed]
40. Copeland, E.B. *The Coconut*; Macmillian and Company, Ltd.: Basingstoke, UK, 1931.
41. Gonzales, B. The Makapuno coconut. *Philipp. Agric. For.* **1914**, *3*, 31–32.
42. Ramirez, D.A.; Mendoza, E.M.T. *The Makapuno Coconut*; The National Academy of Science and Technology: Taguig, Philippines, 1998.
43. De Guzman, E.V.; del Rosario, D.A. The growth and development of *Cocos nucifera* L. 'Makapuno' embryo In-Vitro. *Philipp. Agric.* **1964**, *48*, 82–94.
44. De Guzman, E.V.; Manuel, G.C. Improved root growth in embryo and seedlings culture of coconut 'Makapuno' by the incorporation of charcoal in the growth medium. *PJCS* **1977**, *11*, 35–39.
45. Rillo, E.P. Coconut embryo culture. In Proceedings of the International Coconut Biotechnology Symposium, CICY, Merida, Mexico, 1–5 December 1997.
46. Rillo, E.P. Coconut embryo culture. In *Current Advances in Coconut Biotechnology*; Oropeza, C., Verdeil, J.L., Ashburner, G.R., Cardeña, R., Santamaría, J.M., Eds.; Springer: Dordrecht, The Netherlands, 1999; pp. 279–288. ISBN 978-94-015-9283-3.
47. Islam, M.N.; Azad, A.K.; Namuco, L.O.; Borromeo, T.; Cedo, M.L.O.; Aguilar, E.A. Morphometric characterization and diversity analysis of a Makapuno coconut population in U.P. Los Banos. *Pak. J. Agric. Res.* **2013**, *26*, 254–264.
48. Torres, J. Some notes on Makapuno coconut and its inheritance. *Philipp. J. Agric.* **1937**, *8*, 27–37.
49. Zuniga, L.C. The probable inheritance of the Makapuno character of the coconut. *Philipp. Agric.* **1953**, *36*, 402–413.
50. Cedo, M.L.O.; de Guzman, E.V.; Rimando, T.J. Controlled pollination of embryo-culture "Makapuno" coconut. *Philipp. Agric.* **1984**, *67*, 100–104.
51. Maskromo, I.; Larekeng, S.; Novarianto, H.; Sudarsono, S. Xenia negatively affecting kopyor nut yield in Kalianda Tall kopyor and Pati Dwarf kopyor coconuts. *EJFA* **2017**, *28*. [CrossRef]
52. Satyabalan, K. A note on the occurrence of "buttery" kernel, in coconut. *Indian Coconut J.* **1953**, *6*, 152–154.
53. Adriano, F.T.; Manahan, M. The nutritive value of green, ripe and sport coconuts (buko, niyog and Makapuno). *Philipp. Agric.* **1931**, *20*, 195–198.
54. Deva Kumar, K.; Gautam, R.K.; Ahmad, I.; Dam Roy, S.; Sharma, A. Biochemical, genetic and molecular basis of the novel and commercially important soft endosperm Makapuno coconut—A review. *JFAE* **2015**, *13*, 61–65.
55. Samonte, J.L.; Ramirez, D.A.; Mendoza, E.M.T. Galactomannan in developing normal and Makapuno coconut endosperm. *Bull. Philipp. Biochem. Soc.* **1987**, *7*, 15–19.
56. Mendoza, E.M.T.; Mujer, C.V.; Rodriguez, F.M.; Ramirez, D.A. Ontogeny of some chemical constituents in developing Makapuno and normal coconut endosperms. *Kalikasan Philipp. J. Biol.* **1982**, *11*, 293–301.
57. De la Cruz, R.Y.; Laude, R.P.; Diaz, M.G.Q.; Laurena, A.C.; Mendioro, M.S.; Mendoza, E.M.T. Polymerase chain reaction (PCR)-based cloning of partial cDNAs of selected genes in normal and mutant "Makapuno" endosperms of coconut (*Cocos nucifera* L.). *Philipp. Agric. Sci.* **2013**, *96*, 60–71.
58. Mujer, C.V.; Arambulo, A.S.; Mendoza, E.M.T.; Ramirez, D.A. The viscous components of the mutant (Makapuno) endoosperm I. Isolation and characterization. *Kalikasan Philipp. J. Biol.* **1983**, *12*, 42–50.
59. Balasubramaniam, K. Polysaccharides of the kernel of maturing and matured coconuts. *J. Food Sci.* **1976**, *41*, 1370–1373. [CrossRef]
60. Mujer, C.V.; Ramirez, D.A.; Mendoza, E.M.T. α-d-galactosidase deficiency in coconut endosperm: Its possible pleiotropic effects in makapuno. *Phytochemistry* **1984**, *23*, 893–894. [CrossRef]
61. Mujer, C.V.; Ramirez, D.A.; Mendoza, E.M.T. Coconut α-d-galactosidase isoenzymes: Isolation, purification and characterization. *Phytochemistry* **1984**, *23*, 1251–1254. [CrossRef]
62. Samonte, J.L.; Mendoza, E.M.T.; Ilag, L.L.; De La Cruz, N.B.; Ramirez, D.A. Galactomannan degrading enzymes in maturing normal and makapuno and germinating normal coconut endosperm. *Phytochemistry* **1989**, *28*, 2269–2273. [CrossRef]

63. Diaz, M.G.Q. Differential Gene Expression of the Enzymes of Galactomannan Degradation in the Developing Endosperms of Normal and "Makapuno" Coconut (*Cocos nucifera* Linn.). Ph.D. Thesis, University of the Philippines Los Banos, Los Banos, Laguna, Philippines, 2002.
64. De la Cruz, R.Y.; Bugayong, V.J.D. Mutations in the alpha-D-galactosidase gene suggest molecular basis of the mutant "Makapuno" coconut (*Cocos nucifera* L.) phenomenon. *Philipp. Agric. Sci.* **2016**, *99*, 321–325.
65. Carpio, C.B.; Santos, G.A.; Emmanuel, E.E.; Novarianto, H. Research on coconut genetic resources in Southeast and East Asia. In *Coconut Genetic Resources*; International Plant Genetic Resources Institute—Regional Office for Asia, the Pacific and Oceania (IPGRI-APO): Serdang, Malaysia, 2005; pp. 533–545. ISBN 92-9043-629-8.
66. Niral, V.; Samsudeen, K.; Nair, R.V. Genetic resources of coconut. In Proceedings of the International Conference on Coconut Biodiversity for Prosperity, Central Plantation Crops Research Institute, Kasaragod, Kerala, India, 25–28 October 2010; Thomas, G.V., Krishnakumar, V., Augustine Jerard, B.A., Eds.; pp. 22–28.
67. Padolina, W.G. Contribution of biological research to the development of the coconut industry. *JAOCS* **1985**, *62*, 206–210. [CrossRef]
68. Central Plantation Crops Research Institute Kasaragod (CPCRI). *Annual Report 2003–2004*; CPCRI: Kerala, India, 2004; p. 152.
69. Niral, V.; Devakumar, K.; Umamaheswari, T.S.; Naganeeswaran, S.; Nair, R.V.; Jerard, B.A. Morphological and molecular characterization of a large fruited unique coconut accession from Vaibhavwadi, Maharashtra, India. *Indian J. Genet.* **2013**, *73*, 220–224. [CrossRef]
70. Devakumar, K.; Jerard, B.A.; Dhanapal, R.; Damodaran, V.; Niral, V.; Naganeeswaran, S. Genetic relatedness of coconut populations from Andaman and Nicobar Islands traced to the Indian sub-continent using microsatellite markers. In Proceedings of the International Conference on Coconut Biodiversity for Prosperity, Central Plantation Crops Research Institute, Kasaragod, Kerala, India, 25–28 October 2010; Thomas, G.V., Krishnakumar, V., Augustine Jerard, B.A., Eds.; pp. 67–75.
71. Rognon, F. Biologie florale du cocotier, Duree et succession des phases males et femelles chez divers types de cocotiers. *Oleagineux* **1976**, *31*, 13–18.
72. Regi, T.; Josephrajkumar, A. Flowering and pollination biology in coconut. *J. Plant. Crops* **2013**, *41*, 109–117.
73. Ratnambal, M.J.; Nair, M.K.; Muralidharan, K.; Kumaran, P.M.; Rao, E.V.V.B.; Pillai, R.V. *Coconut Descriptors: Part I*; Central Plantation Crops Research Institute Kasaragod: Kerala, India, 1995.
74. Carpio, C.; Manohar, E.; Rillo, E.P.; Cueto, C.; Orense, O.; Areza-Ubaldo, M.B.; Alfiler, A.R. Status of coconut genetic resources research in the Philippines. In *Coconut Genetic Resources*; International Plant Genetic Resources Institute—Regional Office for Asia, the Pacific and Oceania (IPGRI-APO): Serdang, Malaysia, 2005; pp. 630–647. ISBN 92-9043-629-8.
75. Rajagopai, V.; Kumaran, P.M.; Arulraj, S.; Arunachalam, V. Research on coconut genetic resources in Southeast and East Asia. In *Coconut Genetic Resources*; International Plant Genetic Resources Institute—Regional Office for Asia, the Pacific and Oceania (IPGRI-APO): Serdang, Malaysia, 2005; pp. 573–580. ISBN 92-9043-629-8.
76. Niral, V.; Jerard, B.A.; Samsudeen, K.; Patil, P.D.; Ananda, K.S.; Nair, R.V.; Thomas, G.V. International coconut gene bank for South Asia—Conservation and Characterization. In Proceedings of the International Conference on Coconut Biodiversity for Prosperity, Central Plantation Crops Research Institute, Kasaragod, Kerala, India, 25–28 October 2010; Thomas, G.V., Krishnakumar, V., Augustine Jerard, B.A., Eds.; pp. 35–40.
77. Thomas, G.V.; Niral, V.; Jerard, B.A. Current status of coconut diversity conservation and utilization in India. In Proceedings of the International Conference on Coconut Biodiversity for Prosperity, Central Plantation Crops Research Institute Kasaragod, Kerala, India, 25–28 October 2010; Central Plantation Crops Research Institute: Kerala, India, 2010; pp. 10–21.
78. Aala, W.F.J.; Custodio, C.J.C.; Lado, J.P.; Berdos, M.L.G.; Cueto, C.A.; Diaz, M.G.Q. Oil body enlargement and not number is responsible for increased oil content in solid endosperm of Philippine Lono tall coconut (*Cocos nucifera* L.). In Proceedings of the 25th International Conference and 43rd PSBMB Annual Convention, Manila, Philippines, 5–7 December 2016; p. 152.
79. Castillo, E.M. Chemical Characterization of Makapuno and Normal Coconut Endosperms. Ph.D. Thesis, University of the Philippines Los Banos, Los Banos, Laguna, Philippines, 1988.
80. Bagde, A.S.; Patil, P.D.; Pashte, V.V. Screening of coconut genotypes against coconut eriophyid mite (*Aceria guerreronis* Keifer.). *IJBSM* **2016**, *7*, 18–23. [CrossRef]

81. Gurav, S.S.; Desai, V.S.; Nagwekar, D.D.; Narangakal, A.L. Reaction of different coconut genotypes against eriophyrid mite, *Aceria guerreronis* Keifer in Konkan region of Maharashta. In Proceedings of the International Conference on Coconut Biodiversity for Prosperity, Central Plantation Crops Research Institute, Kasaragod, Kerala, India, 25–28 October 2010; Thomas, G.V., Krishnakumar, V., Augustine Jerard, B.A., Eds.; pp. 375–377.
82. Jacob, P.M.; Thomas, R.J.; Nair, R.V. Achievements of breeding for resistance to root (wilt) disease in coconut. In Proceedings of the International Conference on Coconut Biodiversity for Prosperity, Central Plantation Crops Research Institute, Kasaragod, Kerala, India, 25–28 October 2010; Thomas, G.V., Krishnakumar, V., Augustine Jerard, B.A., Eds.; pp. 41–48.
83. Jerard, B.A.; Damodaran, V.; Niral, V.; Samsudeen, K.; Rajesh, M.K.; Sankaran, M. Conservation and utilization of soft endosperm coconut accession from Andaman Islands. *J. Plant. Crops* **2013**, *41*, 14–21. [CrossRef]
84. Xiao, Y.; Xu, P.; Fan, H.; Baudouin, L.; Xia, W.; Bocs, S.; Xu, J.; Li, Q.; Guo, A.; Zhou, L.; et al. The genome draft of coconut (*Cocos nucifera*). *Gigascience* **2017**, *6*, 1–11. [CrossRef] [PubMed]
85. Lantican, D.; Strickler, S.; Canama, A.; Gardoce, R.; Mueller, L.; Galvez, H. The coconut genome: Providing a reference sequence towards coconut varietal improvement. In Proceedings of the Plant and Animal Genome XXVI Conference, San Diego, CA, USA, 13–17 January 2018.
86. Singh, R.; Ong-Abdullah, M.; Low, E.-T.L.; Manaf, M.A.A.; Rosli, R.; Nookiah, R.; Ooi, L.C.-L.; Ooi, S.; Chan, K.-L.; Halim, M.A.; et al. Oil palm genome sequence reveals divergence of interfertile species in Old and New worlds. *Nature* **2013**, *500*, 335. [CrossRef] [PubMed]
87. Al-Dous, E.K.; George, B.; Al-Mahmoud, M.E.; Al-Jaber, M.Y.; Wang, H.; Salameh, Y.M.; Al-Azwani, E.K.; Chaluvadi, S.; Pontaroli, A.C.; DeBarry, J.; et al. *De novo* genome sequencing and comparative genomics of date palm (*Phoenix dactylifera*). *Nat. Biotechnol.* **2011**, *29*, 521. [CrossRef] [PubMed]
88. Al-Mssallem, I.S.; Hu, S.; Zhang, X.; Lin, Q.; Liu, W.; Tan, J.; Yu, X.; Liu, J.; Pan, L.; Zhang, T.; et al. Genome sequence of the date palm *Phoenix dactylifera* L. *Nat. Commun.* **2013**, *4*, 2274. [CrossRef] [PubMed]
89. Masoudi-Nejad, A.; Narimani, Z.; Hosseinkhan, N. De novo assembly algorithms. In *Next Generation Sequencing and Sequence Assembly: Methodologies and Algorithms*; Springer Briefs in Systems Biology; Springer: New York, NY, USA, 2013; Volume 4, p. 86. ISBN 978-1-4614-7725-9.
90. Huang, Y.-Y.; Matzke, A.J.M.; Matzke, M. Complete sequence and comparative analysis of the chloroplast genome of coconut palm (*Cocos nucifera*). *PLoS ONE* **2013**, *8*, e74736. [CrossRef] [PubMed]
91. Aljohi, H.A.; Liu, W.; Lin, Q.; Zhao, Y.; Zeng, J.; Alamer, A.; Alanazi, I.O.; Alawad, A.O.; Al-Sadi, A.M.; Hu, S.; et al. Complete sequence and analysis of coconut palm (*Cocos nucifera*) mitochondrial genome. *PLoS ONE* **2016**, *11*, e0163990. [CrossRef] [PubMed]
92. Fan, H.; Xiao, Y.; Yang, Y.; Xia, W.; Mason, A.S.; Xia, Z.; Qiao, F.; Zhao, S.; Tang, H. RNA-Seq analysis of *Cocos nucifera*: Transcriptome sequencing and *de novo* assembly for subsequent functional genomics approaches. *PLoS ONE* **2013**, *8*, e59997. [CrossRef] [PubMed]
93. Saensuk, C.; Wanchana, S.; Choowongkomon, K.; Wongpornchai, S.; Kraithong, T.; Imsabai, W.; Chaichoompu, E.; Ruanjaichon, V.; Toojinda, T.; Vanavichit, A.; et al. *De novo* transcriptome assembly and identification of the gene conferring a "pandan-like" aroma in coconut (*Cocos nucifera* L.). *Plant Sci.* **2016**, *252*, 324–334. [CrossRef] [PubMed]
94. Rajesh, M.K.; Fayas, T.P.; Naganeeswaran, S.; Rachana, K.E.; Bhavyashree, U.; Sajini, K.K.; Karun, A. *De novo* assembly and characterization of global transcriptome of coconut palm (*Cocos nucifera* L.) embryogenic calli using Illumina paired-end sequencing. *Protoplasma* **2016**, *253*, 913–928. [CrossRef] [PubMed]
95. Armero, A.; Baudouin, L.; Bocs, S.; This, D. Improving transcriptome *de novo* assembly by using a reference genome of a related species: Translational genomics from oil palm to coconut. *PLoS ONE* **2017**, *12*, e0173300. [CrossRef] [PubMed]
96. Saha, B.; Sircar, G.; Pandey, N.; Gupta Bhattacharya, S. Mining novel allergens from coconut pollen employing manual *de novo* sequencing and homology-driven proteomics. *J. Proteome Res.* **2015**, *14*, 4823–4833. [CrossRef] [PubMed]
97. Ong-Abdullah, M.; Ordway, J.M.; Jiang, N.; Ooi, S.-E.; Kok, S.-Y.; Sarpan, N.; Azimi, N.; Hashim, A.T.; Ishak, Z.; Rosli, S.K.; et al. Loss of *Karma* transposon methylation underlies the mantled somaclonal variant of oil palm. *Nature* **2015**, *525*, 533. [CrossRef] [PubMed]

98. Alwee, S.S.; Van der Linden, C.G.; Van der Schoot, J.; de Folter, S.; Angenent, G.C.; Cheah, S.-C.; Smulders, M.J.M. Characterization of oil palm MADS box genes in relation to the mantled flower abnormality. *Plant Cell Tissue Organ Cult.* **2006**, *85*, 331–344. [CrossRef]
99. Jaligot, E.; Beulé, T.; Rival, A. Methylation-sensitive RFLPs: Characterisation of two oil palm markers showing somaclonal variation-associated polymorphism. *Theor. Appl. Genet.* **2002**, *104*, 1263–1269. [CrossRef] [PubMed]
100. Matthes, M.; Singh, R.; Cheah, S.-C.; Karp, A. Variation in oil palm (*Elaeis guineensis* Jacq.) tissue culture-derived regenerants revealed by AFLPs with methylation-sensitive enzymes. *Theor. Appl. Genet.* **2001**, *102*, 971–979. [CrossRef]
101. Jaligot, E.; Rival, A.; Beulé, T.; Dussert, S.; Verdeil, J.-L. Somaclonal variation in oil palm (*Elaeis guineensis* Jacq.): The DNA methylation hypothesis. *Plant Cell Rep.* **2000**, *19*, 684–690. [CrossRef]
102. Corley, R.H.V.; Lee, C.H.; Law, L.H.; Wong, C.Y. Abnormal flower development in oil palm clones. *Plant. (Kuala Lumpur)* **1986**, *62*, 233–240.
103. Rival, A.; Jaligot, E.; Beulé, T.; Tregear, J.W.; Finnegan, J. The oil palm "mantled" somaclonal variation: A model for epigenetic studies in higher plants. In *Acta Horticulturae*; International Society for Horticultural Science (ISHS): Leuven, Belgium, 2009; pp. 177–182.
104. Eeuwens, C.J.; Lord, S.; Donough, C.R.; Rao, V.; Vallejo, G.; Nelson, S. Effects of tissue culture conditions during embryoid multiplication on the incidence of "mantled" flowering in clonally propagated oil palm. *Plant Cell Tissue Organ Cult.* **2002**, *70*, 311–323. [CrossRef]
105. Triantaphyllopoulos, K.A.; Ikonomopoulos, I.; Bannister, A.J. Epigenetics and inheritance of phenotype variation in livestock. *Epigenet. Chromatin* **2016**, *9*, 31. [CrossRef] [PubMed]
106. Springer, N.M.; Schmitz, R.J. Exploiting induced and natural epigenetic variation for crop improvement. *Nat. Rev. Genet.* **2017**, *18*, 563. [CrossRef] [PubMed]
107. Feng, S.; Jacobsen, S.E.; Reik, W. Epigenetic reprogramming in plant and animal development. *Science* **2010**, *330*, 622. [CrossRef] [PubMed]
108. Maleszka, R.; Mason, P.H.; Barron, A.B. Epigenomics and the concept of degeneracy in biological systems. *Brief. Funct. Genomics* **2014**, *13*, 191–202. [CrossRef] [PubMed]
109. Ibeagha-Awemu, E.M.; Zhao, X. Epigenetic marks: Regulators of livestock phenotypes and conceivable sources of missing variation in livestock improvement programs. *Front. Genet.* **2015**, *6*, 302. [CrossRef] [PubMed]
110. Khan, A.R.; Shah, S.M.; Irshad, M. Immediate and transgenerational regulation of plant stress response through DNA methylation. *J. Agric. Sci.* **2015**, *7*, 144–151. [CrossRef]
111. Alonso, C.; Pérez, R.; Bazaga, P.; Herrera, C.M. Global DNA cytosine methylation as an evolving trait: Phylogenetic signal and correlated evolution with genome size in angiosperms. *Front. Genet.* **2015**, *6*, 4. [CrossRef] [PubMed]
112. Lauria, M.; Rossi, V. Epigenetic control of gene regulation in plants. *Biochim. Biophys. Acta (BBA) Gene Regul. Mech.* **2011**, *1809*, 369–378. [CrossRef] [PubMed]
113. Zhang, M.; Kimatu, J.N.; Xu, K.; Liu, B. DNA cytosine methylation in plant development. *J. Genet. Genomics* **2010**, *37*, 1–12. [CrossRef]
114. Langevin Scott, M.; Kelsey Karl, T. The fate is not always written in the genes: Epigenomics in epidemiologic studies. *Environ. Mol. Mutagen.* **2013**, *54*, 533–541. [CrossRef] [PubMed]
115. Gruenbaum, Y.; Naveh-Many, T.; Cedar, H.; Razin, A. Sequence specificity of methylation in higher plant DNA. *Nature* **1981**, *292*, 860. [CrossRef] [PubMed]
116. King, G.J.; Amoah, S.; Kurup, S. Exploring and exploiting epigenetic variation in crops. *Genome* **2010**, *53*, 856–868. [CrossRef] [PubMed]
117. Cokus, S.J.; Feng, S.; Zhang, X.; Chen, Z.; Merriman, B.; Haudenschild, C.D.; Pradhan, S.; Nelson, S.F.; Pellegrini, M.; Jacobsen, S.E. Shotgun bisulphite sequencing of the *Arabidopsis* genome reveals DNA methylation patterning. *Nature* **2008**, *452*, 215. [CrossRef] [PubMed]
118. Law, J.A.; Jacobsen, S.E. Establishing, maintaining and modifying DNA methylation patterns in plants and animals. *Nat. Rev. Genet.* **2010**, *11*, 204–220. [CrossRef] [PubMed]
119. Stroud, H.; Greenberg, M.V.C.; Feng, S.; Bernatavichute, Y.V.; Jacobsen, S.E. Comprehensive analysis of silencing mutants reveals complex regulation of the *Arabidopsis* methylome. *Cell* **2013**, *152*, 352–364. [CrossRef] [PubMed]

120. Xiao, W.; Custard, K.D.; Brown, R.C.; Lemmon, B.E.; Harada, J.J.; Goldberg, R.B.; Fischer, R.L. DNA methylation is critical for *Arabidopsis* embryogenesis and seed viability. *Plant Cell* **2006**, *18*, 805. [CrossRef] [PubMed]
121. Henikoff, S.; Comai, L. A DNA methyltransferase homolog with a chromodomain exists in multiple polymorphic forms in *Arabidopsis*. *Genetics* **1998**, *149*, 307. [PubMed]
122. Bender, J. DNA methylation and epigenetics. *Annu. Rev. Plant Biol.* **2004**, *55*, 41–68. [CrossRef] [PubMed]
123. Gouil, Q.; Baulcombe, D.C. DNA Methylation signatures of the plant chromomethyltransferases. *PLoS Genet.* **2016**, *12*, e1006526. [CrossRef] [PubMed]
124. Niederhuth, C.E.; Bewick, A.J.; Ji, L.; Alabady, M.S.; Kim, K.D.; Li, Q.; Rohr, N.A.; Rambani, A.; Burke, J.M.; Udall, J.A.; et al. Widespread natural variation of DNA methylation within angiosperms. *Genome Biol.* **2016**, *17*, 194. [CrossRef] [PubMed]
125. Zhang, X.; Yazaki, J.; Sundaresan, A.; Cokus, S.; Chan, S.W.-L.; Chen, H.; Henderson, I.R.; Shinn, P.; Pellegrini, M.; Jacobsen, S.E.; et al. Genome-wide high-resolution mapping and functional analysis of DNA methylation in *Arabidopsis*. *Cell* **2006**, *126*, 1189–1201. [CrossRef] [PubMed]
126. Vaughn, M.W.; Tanurdžić, M.; Lippman, Z.; Jiang, H.; Carrasquillo, R.; Rabinowicz, P.D.; Dedhia, N.; McCombie, W.R.; Agier, N.; Bulski, A.; et al. Epigenetic natural variation in *Arabidopsis thaliana*. *PLoS Biol.* **2007**, *5*, e174. [CrossRef] [PubMed]
127. Seymour, G.; Poole, M.; Manning, K.; King, G.J. Genetics and epigenetics of fruit development and ripening. *Curr. Opin. Plant Biol.* **2008**, *11*, 58–63. [CrossRef] [PubMed]
128. Johannes, F.; Porcher, E.; Teixeira, F.K.; Saliba-Colombani, V.; Simon, M.; Agier, N.; Bulski, A.; Albuisson, J.; Heredia, F.; Audigier, P.; et al. Assessing the impact of transgenerational epigenetic variation on complex traits. *PLoS Genet.* **2009**, *5*, e1000530. [CrossRef] [PubMed]
129. Stam, M.; Belele, C.; Dorweiler, J.E.; Chandler, V.L. Differential chromatin structure within a tandem array 100 kb upstream of the maize *b1* locus is associated with paramutation. *Genes Dev.* **2002**, *16*, 1906–1918. [CrossRef] [PubMed]
130. Hu, L.; Li, N.; Xu, C.; Zhong, S.; Lin, X.; Yang, J.; Zhou, T.; Yuliang, A.; Wu, Y.; Chen, Y.-R.; et al. Mutation of a major CG methylase in rice causes genome-wide hypomethylation, dysregulated genome expression, and seedling lethality. *PNAS* **2014**, *111*, 10642. [CrossRef] [PubMed]
131. Manning, K.; Tör, M.; Poole, M.; Hong, Y.; Thompson, A.J.; King, G.J.; Giovannoni, J.J.; Seymour, G.B. A naturally occurring epigenetic mutation in a gene encoding an SBP-box transcription factor inhibits tomato fruit ripening. *Nat. Genet.* **2006**, *38*, 948. [CrossRef] [PubMed]
132. Chandler, V.L. Paramutation: From maize to mice. *Cell* **2007**, *128*, 641–645. [CrossRef] [PubMed]
133. Gehring, M.; Missirian, V.; Henikoff, S. Genomic analysis of parent-of-origin allelic expression in *Arabidopsis thaliana* seeds. *PLoS ONE* **2011**, *6*, e23687. [CrossRef] [PubMed]
134. Hsieh, T.-F.; Ibarra, C.A.; Silva, P.; Zemach, A.; Eshed-Williams, L.; Fischer, R.L.; Zilberman, D. Genome-wide demethylation of *Arabidopsis* endosperm. *Science* **2009**, *324*, 1451–1454. [CrossRef] [PubMed]
135. Huang, H.; Han, S.S.; Wang, Y.; Zhang, X.Z.; Han, Z.H. Variations in leaf morphology and DNA methylation following in vitro culture of *Malus xiaojinensis*. *Plant Cell Tissue Organ Cult.* **2012**, *111*, 153–161. [CrossRef]
136. Schwabe, W.W.; Blake, J.; Fulford, R.M. *Application of Tissue Culture Techniques for Vegetative Propagation*; FAO Plant News Special Issue (AGP:NL/13); Plant Production and Protection Division, Food and Agriculture Organization: Rome, Italy, 1970; pp. 34–35.
137. Berdasco, M.; Alcázar, R.; García-Ortiz, M.V.; Ballestar, E.; Fernández, A.F.; Roldán-Arjona, T.; Tiburcio, A.F.; Altabella, T.; Buisine, N.; Quesneville, H.; et al. Promoter DNA hypermethylation and gene repression in undifferentiated *Arabidopsis* cells. *PLoS ONE* **2008**, *3*, e3306. [CrossRef] [PubMed]
138. De la Peña, C.; Nic-Can, G.; Ojeda, G.; Herrera-Herrera, J.L.; López-Torres, A.; Wrobel, K.; Robert-Díaz, M.L. *KNOX1* is expressed and epigenetically regulated during in vitro conditions in *Agave* spp. *BMC Plant Biol.* **2012**, *12*, 203. [CrossRef] [PubMed]
139. Moricova, P.; Ondrej, V.; Navratilova, B.; Luhova, L. Changes of DNA methylation and hydroxymethylation in plant protoplast cultures. *Acta Biochim. Pol.* **2013**, *60*, 33–36. [PubMed]
140. Valledor, L.; Hasbún, R.; Meijón, M.; Rodríguez, J.L.; Santamaría, E.; Viejo, M.; Berdasco, M.; Feito, I.; Fraga, M.F.; Cañal, M.J.; Rodríguez, R. Involvement of DNA methylation in tree development and micropropagation. *Plant Cell Tissue Organ Cult.* **2007**, *91*, 75–86. [CrossRef]

141. Zakrzewski, F.; Schmidt, M.; Van Lijsebettens, M.; Schmidt, T. DNA methylation of retrotransposons, DNA transposons and genes in sugar beet (*Beta vulgaris* L.). *Plant J.* **2017**, *90*, 1156–1175. [CrossRef] [PubMed]
142. Rival, A.; Jaligot, E.; Beulé, T.; Finnegan, E.J. Isolation and expression analysis of genes encoding MET, CMT, and DRM methyltransferases in oil palm (*Elaeis guineensis* Jacq.) in relation to the 'mantled' somaclonal variation. *J. Exp. Bot.* **2008**, *59*, 3271–3281. [CrossRef] [PubMed]
143. LoSchiavo, F.; Pitto, L.; Giuliano, G.; Torti, G.; Nuti-Ronchi, V.; Marazziti, D.; Vergara, R.; Orselli, S.; Terzi, M. DNA methylation of embryogenic carrot cell cultures and its variations as caused by mutation, differentiation, hormones and hypomethylating drugs. *Theor. Appl. Genet.* **1989**, *77*, 325–331. [CrossRef] [PubMed]
144. Smulders, M.J.M.; de Klerk, G.J. Epigenetics in plant tissue culture. *Plant Growth Regul.* **2011**, *63*, 137–146. [CrossRef]
145. Shimron-Abarbanell, D.; Breiman, A. Comprehensive molecular characterization of tissue-culture-derived *Hordeum marinum* plants. *Theor. Appl. Genet.* **1991**, *83*, 71–80. [CrossRef] [PubMed]
146. Devaux, P.; Kilian, A.; Kleinhofs, A. Anther culture and *Hordeum bulbosum*-derived barley doubled haploids mutations and methylation. *Mol. Gen. Genet.* **1993**, *241*, 674–679. [CrossRef] [PubMed]
147. Suzuki, M.M.; Bird, A. DNA methylation landscapes: Provocative insights from epigenomics. *Nat. Rev. Genet.* **2008**, *9*, 465. [CrossRef] [PubMed]
148. Pila Quinga, L.A.; Pacheco de Freitas Fraga, H.; do Nascimento Vieira, L.; Guerra, M.P. Epigenetics of long-term somatic embryogenesis in *Theobroma cacao* L.: DNA methylation and recovery of embryogenic potential. *Plant Cell Tissue Organ Cult.* **2017**, *131*, 295–305. [CrossRef]
149. Jaligot, E.; Adler, S.; Debladis, É.; Beulé, T.; Richaud, F.; Ilbert, P.; Finnegan, E.J.; Rival, A. Epigenetic imbalance and the floral developmental abnormality of the in vitro-regenerated oil palm *Elaeis guineensis*. *Ann. Bot.* **2011**, *108*, 1453–1462. [CrossRef] [PubMed]
150. Duarte-Aké, F.; Castillo-Castro, E.; Pool, F.B.; Espadas, F.; Santamaría, J.M.; Robert, M.L.; De-la-Peña, C. Physiological differences and changes in global DNA methylation levels in *Agave angustifolia* Haw. albino variant somaclones during the micropropagation process. *Plant Cell Rep.* **2016**, *35*, 2489–2502. [CrossRef] [PubMed]
151. Rival, A.; Ilbert, P.; Labeyrie, A.; Torres, E.; Doulbeau, S.; Personne, A.; Dussert, S.; Beulé, T.; Durand-Gasselin, T.; Tregear, J.W.; Jaligot, E. Variations in genomic DNA methylation during the long-term in vitro proliferation of oil palm embryogenic suspension cultures. *Plant Cell Rep.* **2013**, *32*, 359–368. [CrossRef] [PubMed]
152. Peredo, E.L.; Ángeles Revilla, M.; Arroyo-García, R. Assessment of genetic and epigenetic variation in hop plants regenerated from sequential subcultures of organogenic calli. *J. Plant Physiol.* **2006**, *163*, 1071–1079. [CrossRef] [PubMed]
153. Rhee, Y.; Sekhon, R.S.; Chopra, S.; Kaeppler, S. Tissue culture-induced novel epialleles of a *Myb* transcription factor encoded by *pericarp color1* in maize. *Genetics* **2010**, *186*, 843. [CrossRef] [PubMed]
154. Adam, H.; Jouannic, S.; Orieux, Y.; Morcillo, F.; Richaud, F.; Duval, Y.; Tregear, J.W. Functional characterization of MADS box genes involved in the determination of oil palm flower structure. *J. Exp. Bot.* **2007**, *58*, 1245–1259. [CrossRef] [PubMed]