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REVIEW

Biotechnology of oil palm: strategies towards manipulation of lipid content and composition

Ghulam Kadir Ahmad Parveez · Omar Abdul Rasid · Mat Yunus Abdul Masani · Ravigadevi Sambanthamurthi

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Abstract Oil palm is a major economic crop for Malaysia. The major challenges faced by the industry are labor shortage, availability of arable land and unstable commodity price. This has caused the industry to diversify its applications into higher value products besides increasing its yield. While conventional breeding has its limitations, biotechnology was identified as one of the tools for overcoming the above challenges. Research on biotechnology of oil palm began more than two decades ago leveraging a multidisciplinary approach involving biochemical studies, gene and promoter isolation, transformation vector construction and finally genetic transformation to produce the targeted products. The main target of oil palm biotechnology research is to increase oleic acid in the mesocarp. Other targets are stearic acid, palmitoleic acid, ricinoleic acid, lycopene (carotenoid) and biodegradable plastics. Significant achievements were reported for the biochemical studies, isolation of useful oil palm genes and characterization of important promoters. A large number of transformation constructs for various targeted products were successfully produced using the isolated oil palm genes and promoters. Finally transformation of these constructs into oil palm embryogenic calli was carried out while the regeneration of transgenic oil palm harboring the useful genes is in progress.

Keywords Oil palm · Genetic engineering · Lipid biosynthesis · Fatty acid composition · Transgenic oil palm

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G. K. A. Parveez (✉) · O. A. Rasid · M. Y. A. Masani · R. Sambanthamurthi
Advanced Biotechnology and Breeding Centre, Malaysian Palm Oil Board, P.O. Box 10620, 50720 Kuala Lumpur, Malaysia
e-mail: parveez@mpob.gov.my

Introduction

Palm oil, derived from the oil palm fruit, is in high demand due to its nutritional attributes and competitive price as compared to other vegetable oils (Ramli 2011). Malaysia is one of the major producers and exporters of palm oil globally. Palm oil is a very versatile oil as its uses are not limited to food only but also widely used in non-food application such as in oleochemicals, cosmetics, pharmaceuticals and lubricants. In efforts to increase productivity and sustainability and in meeting the high demand, the yield per unit area needs to be elevated while diversification of palm oil usage both for food and non-food purposes needs to be given priority. The current oil yield of 4–6 t ha^{-1} at the plantations could be increased to the theoretical yield of more than 10 t ha^{-1} (Murphy 2014). The penetration and expansion of palm oil into new market niches, such as pharmaceutical and nutraceutical market, requires modification of oil palm to include specific traits of interest. In the past, improvements were attained through traditional breeding. However, trait improvement through this technique is limited by the narrow gene pool and the long generation cycle of the crop (something like 10–15 years). Furthermore, most of traits with economic importance are controlled by multigenes, making the transfer of single traits difficult (Sambanthamurthi et al. 2009).

Genetic engineering would be a more appropriate method for modifying the palm as it can: (a) reduce the cost and time for introgressing the desired traits; (b) improve the precision of gene introgression by restricting the amount of genetic materials transferred; and iii) broaden the genetic base of the palm (Sambanthamurthi et al. 2009). MPOB has been involved with genetic engineering of oil palm as early as 1987, initially targeting

production of less saturated oil with higher oleic and lower palmitic content (Cheah et al. 1995). The high oleic oil would be an attractive feedstock for the thriving oleochemical industry in Malaysia. The less saturated oil would allow penetration into liquid and salad oil market. The other targets of oil palm biotechnology research are to increase stearic acid, palmitoleic acid, ricinoleic acid, lycopene (carotenoid) and biodegradable plastics (Parveez 2003; Rasid et al. 2009, 2014). Murphy in 2014 proposed the following three targets for genetic modification of oil palm: (a) increasing oil yield, (b) modification of oil composition, and (c) developing oil palm with pest and diseases tolerances. These three targets are the most important priorities for increasing the overall output from the oil palm industry and for expanding into new niche markets.

Plants are now increasingly looked upon as possible factories, or bioreactors, to produce chemicals, benign and unobtrusive to the environment. Apart from the above targets, oil palm being the most productive oil crop has the potential to be one of the most efficient green factories for value-added products, such as novel fatty acids, pharmaceuticals and nutraceuticals. Another advantage that oil palm has is that the 'factory' is 'perpetual' and 'eco-friendly'—as a perennial crop, it can be harvested for 25–30 years once planted.

The genetic modification of oil palm requires the development of several tools and input from different disciplines. Biochemical studies, gene and promoter isolation, transformation vector construction and genetic transformation are important disciplines for success. This article will highlight the recent progress made in the above disciplines.

Biochemical studies

Oil palm lipid biosynthesis was first studied to identify potential target enzymes for the modification. Once the enzymes were identified, their genes were isolated. For example, in palm oil the most abundant fatty acid is palmitic acid which represents up to 44 % of its content (Sambanthamurthi et al. 2000). The biochemical basis for this was investigated as a necessary prelude to modifying the oil composition, including for higher oleic acid content.

Lipid biosynthesis

Figure 1 depicts the pathway for lipid biosynthesis in plants. The basic pathway is similar in all plants and it is only the regulation of the pathway that differs and contributes to the unique fatty acid composition of different

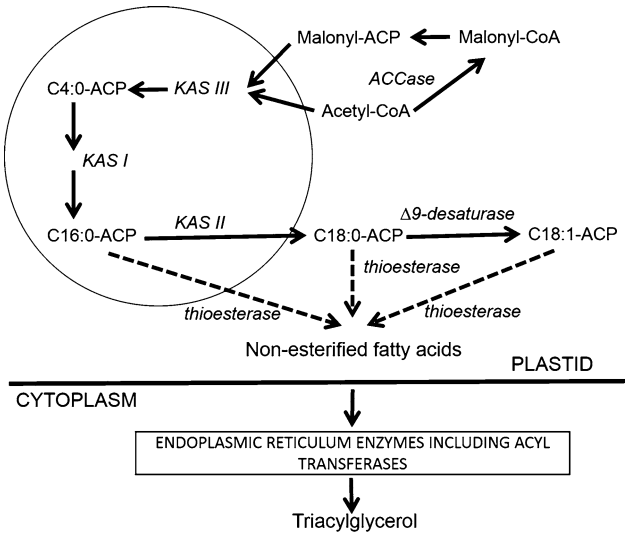

Fig. 1 Fatty Acid and triglyceride biosynthesis in plants, *ACP* acyl carrier protein, *ACCase* Acetyl-CoA carboxylase, *CoA* coenzyme A, *KAS I, II, III* ketoacyl-ACP synthase isoform I, II, III, *C4:0* butyryl, *C16:0* palmitoyl, *C18:0* stearoyl, *C18:1* oleoyl (Reproduced from Siti Nor Akmar et al. 2001)

plant oils. The pathway has been manipulated for different oil quality and higher oil yield in other plants. In oil palm, several key enzymes have been identified—acetyl-CoA carboxylase (ACCase), palmitoyl-ACP thioesterase, β-ketoacyl-ACP synthase ll (KAS II), oleoyl-CoA desaturase and stearoyl-ACP desaturase. They have been characterized biochemically and will be elaborated on in the following section.

Acetyl-CoA carboxylase (ACCase)

Acetyl-CoA carboxylase (ACCase) is one of the main enzymes that control fatty acid synthesis in leaves and developing oil seed (Post-Beittenmiller et al. 1993). The enzyme initiates the plant lipid biosynthesis process. It catalyzes the formation of malonyl-CoA, the precursor for fatty acid biosynthesis and elongation, by the carboxylation of two units of acetyl-CoA. As this enzyme is an important flux-controlling enzyme, biochemical studies on it were given priority. It was postulated that increasing the activity of this enzyme would increase the quantum of oil synthesized. Conversely, the acetyl-CoA could be diverted to other products by silencing this gene. It was further postulated that suppressing this gene in oil palm, coupled with the introduction of polyhydroxyalkanoate (PHA) biosynthetic genes may maximize the biosynthesis of PHA in the oil palm tissues (Sambanthamurthi et al. 2002).

ACCase is considered the major enzyme for the regulation of fatty acid synthesis. A few isoforms of the gene

have been reported (Egli et al. 1993). The active ACCase holoenzyme has four different protein domains—biotin carboxyl carrier protein (BCCP), biotin carboxylase (BC), and two transcarboxylases. The multifunctional enzyme has all domains present as a single protein, while the multi-subunit, has each domain on a separate protein in the holoenzyme. The multi-subunit form is located in the plastid and the multifunctional form in the cytosol. Both forms are found in oil palm. Biochemical studies on ACCase activity using radioassay in oil palm cell cultures have been carried out (Umi Salamah 1999). In slices of fresh oil palm mesocarp, the activity was highest at 20 weeks-after-anthesis, coinciding with the time the fruit matures (Sambanthamurthi, unpublished).

β-Ketoacyl ACP synthase II (KAS II)

KAS II is a condensing enzyme, specifically involved in converting palmitic to stearic acid (Harwood 1988). The high palmitic content in palm oil suggests that the enzyme is rate-controlling. If so, increasing its activity should increase conversion of palmitoyl-ACP to stearoyl-ACP for subsequent desaturation to oleic acid. The relationship between the level of unsaturation of fatty acids and KAS II activity was studied in the mesocarp of both oil palm species (*E. guineensis* and *E. oleifera)* as well as in their hybrids. Strong positive correlations were found between both iodine value (I.V.) and C18 unsaturated fatty acids (oleic acid + linoleic acid + linolenic acid) and KAS II activity (Umi Salamah and Sambanthamurthi 1996; Sambanthamurthi et al. 1996a). It was also shown that the desaturase activity was not limiting as increased KAS II activity did not result in a build-up of stearic but an increase in the unsaturated C18 fatty acids. Interestingly, the level of oleic was negatively correlated to the level of palmitic acid. This negative correlation confirmed that the accumulation of palmitic is due to low KAS II activity. It was therefore proposed that increasing the KAS II activity should channel more palmitic to oleic acid. The negative correlation between palmitic and oleic acid lends credence to this postulation.

Acyl ACP thioesterases

This is a group of genes that catalyzes the hydrolysis of acyl-ACP to free fatty acids and ACP. The free fatty acids are then exported out of the plastid, the site of de novo fatty acid biosynthesis, into the cytoplasm where they are incorporated into triacylglycerols. The major product of fatty acid biosynthesis in plastids of most plants is oleic acid. In all plants studied so far, a thioesterase is highly active towards oleoyl-ACP, presumably to ensure that oleic is released from ACP and exported out of the plastid. Plants such as California bay and *Cuphea*, that accumulate medium chain fatty acids, were shown to express a medium chain specific acyl ACP thioesterase (Davies et al. 1991; Pollard et al. 1991). The palmitoyl-ACP thioesterase activity in oil palm mesocarp, a palmitic acid-rich tissue, has been studied. Crude oil palm mesocarp extract was assayed for thioesterase activity against different acyl-ACP substrates and maximum activity was obtained with palmitoyl-ACP (Sambanthamurthi and Oo 1990; Abrizah 2001). This observation suggested that the accumulation of palmitic in the mesocarp is due to chain termination by palmitoyl-ACP thioesterase. The mesocarp also showed high oleoyl-ACP thioesterase activity.

Since the oil palm mesocarp exhibited high activity of thioesterase towards both oleoyl-ACP and palmitoyl-ACP, it was important to determine whether the activities were catalyzed by the same or different enzymes. The thioesterases were later partially purified and shown to be two separate proteins with one showing a marked preference for oleoyl-ACP and the other for palmitoyl-ACP (Abrizah 2001). This finding was fortuitous as it meant that both the oleoyl-ACP thioesterase and palmitoyl-ACP thioesterase activities could be manipulated independently of each other, i.e., that palmitic could be reduced without reducing oleic. Similarly, it may be possible to increase oleic by increasing oleoyl-ACP thioesterase activity without any concomitant increase in palmitoyl-ACP thioesterase activity.

Stearoyl ACP desaturase

This enzyme is involved in desaturating stearic to oleic acid. As palm oil contains 3–5 % stearic acid against 39 % oleic acid, the enzyme activity must be very high to convert almost all the stearoyl-ACP to oleoyl-ACP. The high correlation between KAS II activity and unsaturated fatty acids and lack of correlation between KAS II activity and stearic acid confirmed that stearoyl ACP desaturase is very active in oil palm mesocarp. Although the level of stearic acid could be temporarily increased by elevating the KAS II activity, the stearic acid would be subsequently channeled to oleic acid due to the high activity of stearoyl ACP desaturase (Sambanthamurthi et al. 1996a). It is postulated that it is unlikely that efforts to increase oleic in the oil palm mesocarp by genetic manipulation will require increasing the activity of stearoyl-ACP desaturase.

Oleoyl-CoA desaturase

The fatty acid biosynthesis pathway up to oleic mainly occurs in the plastid. With some exceptions, the further desaturation of oleic into linoleic and linolenic which takes place in the cytosol requires CoA as a cofactor. This reaction is catalyzed by the enzyme oleoyl-CoA desaturase. A previous study showed a high correlation between KAS II enzyme activity with the oleic content and an even a higher correlation with the linoleic (C18:2) (Sambanthamurthi et al. 1996a). Therefore, increasing the KAS II activity would increase both the oleic and linoleic. Down-regulating palmitoyl-ACP thioesterase and overexpressing KAS II would result in not only increased oleic acid but also increased linoleic acid. Therefore down-regulating the oleoyl-CoA desaturase to stop the possible overflow of oleic into linoleic needed to be considered for producing high oleic acid oil palm (Sambanthamurthi et al. 1999, 2002).

Gene and promoter isolation

Based on metabolic studies, several key genes in lipid biosynthesis were identified and subsequently isolated. The list of genes already isolated and characterized is given in Table 1. In addition to the isolation and characterization, appropriate temporal and spatial expression of the introduced gene(s) is necessary to ensure that the gene(s) is expressed in the fruit mesocarp during oil synthesis. Leaf- and root-specific promoters have also been isolated to express pest- and disease-resistant genes, besides constitutive promoters for driving reporter or selectable marker genes. A list of the promoters isolated from oil palm is given in Table 2.

Table 2 Tissue-specific and constitutive promoters isolated from oil palm

Promoters	Functional analysis	References
Mesocarp (fruit) specific promoter	Transient expression in oil palm mesocarp and leaves	Siti Nor Akmar and Zubaidah (2008)
Root-specific promoter	*Arabidopsis thaliana*	Zubaidah and Siti Nor Akmar (2005)
Leaf-specific promoter	Transient expression on mesocarp and leaves of *Arabidopsis thaliana*	Chan et al. (2008)
Ubiquitin extension protein gene (uep1).	Transient expression in various oil palm and tobacco tissues	Masura et al. (2010)
Translationally-controlled tumor protein (TCTP)	Transient expression in various oil palm and tobacco tissues	Masura et al. (2011)

Methods for producing transgenic oil palm

Development of a transformation system is a prerequisite for genetically engineering any plant. Regeneration of transgenic oil palm was first reported using biolistics or microprojectile-bombardment-mediated transformation. This was achieved following extensive optimization of the physical and biological parameters to maximize delivery of DNA into embryogenic calli, selection of the strongest constitutive promoter and identification of the most effective selection agent and its concentration (Parveez et al. 1996, 1997, 1998: Chowdhury et al. 1997). The transformed oil palm embryogenic calli were regenerated on a selection medium containing the herbicide glufosinate ammonium, and confirmed by molecular and protein analyses (Parveez 2000). *Agrobacterium*-mediated transformation was later developed, also on a medium

Table 1 Lipid biosynthetic genes isolated from oil palm

Genes	Functional analysis	References
Ketoacyl ACP synthase II (KAS II)	*E. coli* and *Arabidopsis thaliana*	Ramli et al. (2012)
Palmitoyl-ACP thioesterase	*Arabidopsis thaliana*	Abrizah et al. (2000), Parveez et al. (2010)
Stearoyl ACP desaturases	*Arabidopsis thaliana*	Siti Nor Akmar et al. (1999), Safiza et al. (2009a)
Oleoyl-CoA desaturase		Syahanim et al. 2007)
Acetyl-CoA carboxylase (ACCase)		Omar et al. (2008)
Lysophosphatidic acid acyltransferase (LPAAT)	*Arabidopsis thaliana*	Manaf et al. (2005), Safiza et al. (2009b)
Glycerol 3-phosphate transferase (GPAT)	*Arabidopsis thaliana*	Manaf et al. (2005)
β-Ketothiolase		Teen et al. (2008)

containing glufosinate ammonium (Masli et al. 2009). Transformation was carried out using *Agrobacterium*, strain LBA4404, with the following conditions: exposure to plasmolysis medium, presence of acetosyringone, and physical injury using DNA-free particle bombardment. Recently, another method for transforming oil palm protoplasts using microinjection and PEG-mediated transformation was reported (Masani et al. 2014). This was made possible only after the method for oil palm regeneration from protoplasts was successfully developed recently (Masani et al. 2013).

Two of the above methods use herbicide glufosinate ammonium, a negative selectable marker, as the selection agent. Glufosinate ammonium was shown to be the most effective selection agent for oil palm tissues (Parveez et al. 2007). Later, alternative selection systems for the effective regeneration of transgenic oil palm were developed deploying positive selectable markers. The first positive method used the *pmi* gene encoding phosphomannose isomerase (PMI) from *Escherichia coli* as a novel selectable marker and mannose as the selection agent (Bahariah et al. 2012, 2013). Mannose cannot be metabolized by plant cells and is converted into mannose-6-phosphate by endogenous hexokinase. On the other hand, PMI can metabolize mannose by converting mannose-6-phosphate to fructose-6-phosphate, which can then be used by the plant as a carbon source (Reed et al. 2001). Therefore, when mannose is included in the tissue culture medium as the sole carbon source, the transformed cells will survive, while the untransformed plant growth will be retarded by the lack of the carbon source and the accumulation of mannose-6-phosphate. The second method used the DOGR1 gene encoding 2-deoxyglucose-6-phosphate phosphatase from *Saccharomyces cerevisiae*, and 2-deoxyglucose (2-DOG) as the selection agent (Masli et al. 2012). 2-DOG prevents cell growth and development when converted to 2-DOG-6-phosphate by the phosphorylation of hexokinase in the cytosol (Kunze et al. 2001). The DOGR1 gene in the transformed cells provides resistance to 2-DOG when over-expressed in the transgenic cells. As a result, only the transformed cells will survive and regenerate on media containing 2-DOG.

There were also efforts to use green fluorescent protein (GFP) as a visual reporter and selectable marker gene. Various factors affecting GFP gene expression in oil palm, such as different GFP versions, the promoters used to drive the *gfp* gene, backbone vectors and the sizes of the whole plasmid were evaluated (Parveez and Majid 2008). Furthermore, *gfp* genes that are targeted to specific organelles, such as mitochondria, plastids and endoplasmic reticulum, were also evaluated as there were signs of toxicity by GFP in the oil palm nucleus when the gene was not targeted to any organelle (Majid and Parveez 2007). However, so far we have failed to produce transgenic oil palm plantlets expressing the GFP genes following organelle targeting.

Producing transgenic oil palm with high oleic content

Increasing the oleic acid in palm oil is the current main target in oil palm genetic manipulation so that the oil can be used as feedstock for producing oleochemicals. The Malaysian oleochemicals industry is rapidly expanding to supply the increasingly voracious global demand. However, to elevate oleic acid content, the reason as to why the oil has 44 % palmitic and 39 % oleic had to be unravelled. All plants share the same fatty acid biosynthesis pathway and yet have different fatty acid profiles because they have different mechanisms regulating the pathway. MPOB directed its efforts towards understanding the regulatory points in the oil palm fatty acid biosynthesis pathway. We have performed biochemical studies on the following enzymes: β-ketoacyl-ACP synthase II (KAS II), stearoyl-ACP desaturase, palmitoyl-ACP thioesterase and oleoyl-CoA desaturase to understand biochemical basis for the fatty acid profile of palm oil. The genes coding for the enzymes have also been isolated. As the target for fatty acid manipulation is in the tissue where the oil is synthesized and stored (mesocarp), a promoter which is specific for mesocarp was also isolated.

Strategy for producing high oleic oil palm

The biochemical studies demonstrated and confirmed that palmitoyl-ACP thioesterase and KAS II are the main target enzymes to manipulate for higher oleic acid at the expense of palmitic acid. From the biochemical knowledge gleaned, the following strategy was proposed: (a) up-regulate KAS II, (b) down-regulate palmitoyl-ACP thioesterase, and (c) down-regulate oleoyl-CoA desaturase (Sambanthamurthi et al. 2000). In soybean, down-regulating oleoyl-CoA desaturase resulted in increase in oleic acid from 21.5 to 78.9 % in its oil as a result of reducing linoleic from 55 to 3 % (Broglie et al. 1997). Similar observations were made in cottonseed and jatropha (Liu et al. 2000; Qu et al. 2012). Recently, TALENs (Transcription Activator-Like Effector Nucleases) were used to mutate two fatty acid desaturase two genes (FAD2-1B and FAD2-1A) that convert oleic to linoleic in soybean (Haun et al. 2014). The resulting transgenic plants with homozygous mutations in both genes had increased oleic acid from 20 to 80 % as a result of reduced linoleic acid from 50 % to under 4 %. Oleoyl-CoA desaturase was down-regulated due to the high

linoleic content in the normal plant. However, in oil palm, as palmitic is the dominant fatty acid in its oil, silencing the palmitoyl-ACP thioesterase and enhancing the KASII activities are the logical steps. As oil palm already has an active stearoyl-ACP desaturase, enhancing its activity is presumably not essential (Sambanthamurthi et al. 1996a, 2002). Furthermore, as at least three genes will have to be manipulated, the use of a single mesocarp-specific promoter may not suffice as this may cause homology-dependent gene silencing due to multiple copies of the same promoter sequences inserted at the same loci (Matzke et al. 1989). Therefore, isolation of more mesocarp-specific promoters is essential and ongoing effort.

To achieve the above strategy, the following transformation vectors were designed and constructed: (a) down-regulated palmitoyl-ACP thioesterase gene under the control of a constitutive CaMV 35S promoter (Abrizah et al. 2000), (b) sense KAS II and antisense palmitoyl-ACP thioesterase genes under the control of a promoter specific to mesocarp, and (c) down-regulated palmitoyl-ACP thioesterase and oleoyl-CoA desaturase, and enhanced KAS II and stearoyl-ACP desaturase genes under the control of a mesocarp-specific promoter (Yunus and Kadir 2008). All the three constructs were bombarded into oil palm embryogenic cultures to generate glufosinate ammonium-resistant embryogenic calli which were later regenerated into plantlets and transferred to soil in the biosafety nursery. Initial molecular analyses, using PCR and Southern hybridization, revealed that some of the plantlets carried the transgenes (Nurfahisza et al. 2014). Initial fatty acid analysis by gas chromatography has shown reduced palmitic and increased unsaturated fatty acids in the early plant development stage. This was possible to be carried out on some of the transgenic plants carrying the genes under the control of constitutive promoter, e.g., CaMV35S. For the plants carrying the transgene under the control of mesocarp-specific promoter, the fatty acid changes can only be evaluated following fruit development.

Production of transgenic oil palm with high stearic content

High stearic acid is the second target in the oil palm genetic manipulation programme. From biochemical studies, it is known that the palm has an active stearoyl-ACP desaturase. Logically, therefore, down-regulating the gene should reduce the conversion of stearoyl-ACP to oleoyl-ACP resulting in increased stearic acid. Increasing stearic acid is for novel applications of the oil, such as to produce cocoa butter substitutes and products including shaving cream, lotions, and massage oils (Parveez 2003).

Strategy for producing high stearic oil palm

It was first reported in rapeseed that down-regulating stearoyl–ACP desaturase increased stearic acid in its oil from 1.8 to 39.8 % as a result of a concomitant reduction in oleic (Knutzon et al. 1992). A similar finding was also demonstrated in cotton (Liu et al. 2000). Alternatively, site-directed mutagenesis of *Garcinia mangostana* thioesterase specifically targeted to increase stearic acid has been demonstrated to be successful in canola (Facciotti et al. 1999). The mutagenized FatA1 thioesterase showed a 13-fold increase in thioesterase activity towards stearoyl-ACP resulting in higher accumulation of stearic acid. Recently, a stearoyl-ACP thioesterase from mangosteen was expressed in soybean and resulted in up to 17 % increase in seed stearic acid level. Cross between the transgenic plant with another transgenic event that had its palmitoyl-ACP thioesterase and Δ12 fatty acid desaturase genes deactivated, resulted in phenotype with approximately 11–19 % stearate and 70 % oleate content (Park et al. 2014). For oil palm, silencing the activity of stearoyl-ACP desaturase in the mesocarp was proposed. However, a large pool of stearoyl-ACP may interfere with the equilibrium of the pathway and result in the accumulation of palmitic acid. The following manipulations were therefore carried out as well: silencing of palmitoyl-ACP thioesterase and enhancing the activity of KAS II to ensure maximum conversion of the stearoyl-ACP pool to stearic acid.

Referring to the above strategy, the following constructs were planned and accomplished: silenced oil palm stearoyl-ACP desaturase gene under the control of either one of the promoters: CaMV35S, ubiquitin, or mesocarp-specific. In addition, another construct carrying the above gene and silenced palmitoyl-ACP thioesterase gene under the control of a mesocarp-specific promoter was also produced. Embryogenic calli separately bombarded with the above four constructs were transferred onto selection medium containing glufosinate ammonium. The tolerant polyembryogenic cultures were proliferated and regenerated into plantlets. To date, the plantlets show normal morphology and growth. The fatty acid composition in the fruits will be evaluated once the plants mature.

Producing transgenic oil palm with high palmitoleic oil

Palmitoleic acid is postulated to have anti-thrombotic effects which could prevent stroke (Abraham et al. 1989). Therefore transgenic oil palm with high palmitoleic acid could be targeted for pharmaceutical applications. The current main source of palmitoleic acid is *Macadamia integrifolia* oil which contains ~17 % of this fatty acid. It was previously demonstrated that oil palm protoplasts

could synthesized up to 30 % palmitoleic in their total lipids (Sambanthamurthi et al. 1996b). This illustrates the oil palm's inherent ability to produce high palmitoleic.

Strategy for producing high palmitoleic oil palm

Palmitoleic acid is produced through desaturation of palmitic acid. It is envisaged that the desaturase, which mainly acts on stearic acid, could also convert palmitic acid to produce palmitoleic acid. In oil palm, over expressing stearoyl-ACP desaturase was proposed for accumulating palmitoleic acid. Soluble desaturase (stearoyl-ACP desaturase being the most common) catalyzes the insertion of double bonds in saturated fatty acids bound to ACP (Ohlrogge and Browse 1995). Stearoyl-ACP desaturase, which converts saturated stearoyl-ACP to monounsaturated oleoyl-ACP, has been well characterized (Shanklin and Somerville 1991). Based on 3D structure analysis and modeling studies on the enzyme, site-directed mutagenesis was carried out to replace leucine 118 and proline 179 with the phenylalanine and isoleucine, respectively. This modification resulted in a castor stearoyl-ACP desaturase which was more active towards palmitoyl-ACP (Cahoon et al. 1997). Using this mutant desaturase, a high level of palmitoleic acid was synthesized in *Arabidopsis thaliana*. A higher level of palmitoleic acid was later obtained when the desaturase was transformed into *Arabidopsis* with the KAS II gene silenced (Cahoon and Shanklin 2000). When Δ9 desaturase from oyster mushroom (*Pleurotus osteatus*) was transformed into soybean, palmitoleic was increased by only 1 % as compared to a higher increase in oleic (Hildebrand et al. 2011). In petunia, the mushroom desaturase increased palmitoleic up to 22 % in the leaves.

We used the mutant castor stearoyl-ACP desaturase (Cahoon and Shanklin 2000) in the sense direction and antisensed the KAS II and palmitoyl-ACP thioesterase genes to synthesize palmitoleic acid in oil palm. Seven transformation vectors carrying either the native oil palm stearoyl-ACP desaturase or the single or double mutant castor desaturase together with antisensed the KAS II and palmitoyl-ACP thioesterase genes driven by the constitutive or mesocarp-specific promoter were constructed and used to transform oil palm embryogenic cultures. So far, glufosinate ammonium-resistant embryogenic calli have been obtained and regenerated into plantlets growing in soil in biosafety screenhouse.

Producing transgenic oil palm with high ricinoleic content

Ricinoleic acid is an industrially important fatty acid mainly found in castor oil (Van de Loo et al. 1993). Due its effective drying properties, it can be used to produce lubricants, cosmetics (~40 % in lipsticks), plastics (mainly nylon 11, nylon 10-10, nylon 6-10), biodiesel, surfactants, plasticizers, diesel lubricants, deodorants and coating (Auld et al. 2009). The hydroxyl group in ricinoleate makes it a good lubricant and biodiesel. Ricinoleic acid also contains oxygen which improves the oil/fuel combustion for a better environment. Nylon 10-10 from ricinoleic acid is a good material for cell phone plastic casing while Nylon 11 has good antifungal activities.

Strategy for producing high ricinoleic oil palm

Oleate 12-hydroxylase converts oleic acid to ricinoleic acid and therefore introducing this gene into oil palm should result in the synthesis of ricinoleic acid. A *Lesquerella fendleri* (a Brassicaceae, mustard family) oleate 12-hydroxylase was successfully isolated and expressed in transgenic plants demonstrating that the enzyme exhibits both activities of desaturase and hydroxylase (Broun et al. 1998). When a castor oleate 12-hydroxylase was transformed into *A. thaliana*, up to 17 % ricinoleic acid was accumulated in the seed fatty acids (Broun and Somerville 1997). Co-transformation of diacylglycerol acyltransferase (DGAT2) and oleate 12-hydroxylase into *Arabidopsis* resulted in up to 30 % accumulation of ricinoleic acid in the seed (Burgal et al. 2008). Recently, when oleate 12-hydroxylase was co-transformed with the castor phospholipid:diacylglycerol transferase 1-2 (PDAT1-2) gene, hydroxy fatty acids (mainly ricinoleic) increased to up to 25 % in the seeds of transgenic *Arabidopsis* (Kim et al. 2011).

In this study, three transformation vectors carrying the castor oleate 12-hydroxylase under the control of CaMV35S, ubiquitin, and mesocarp-specific promoters were constructed (Masani, unpublished results). All the three vectors were transformed into oil palm embryogenic calli and glufosinate ammonium-resistant embryogenic calli obtained after selection on glufosinate ammonium-containing medium. Regeneration of transgenic plantlets from these resistant embryogenic calli has been achieved, and the plantlets are being grown in a biosafety screen house.

Production of transgenic oil palm synthesizing biodegradable thermoplastics

Biodegradable plastics, especially polyhydroxybutyrate (PHB) and PHA are commonly produced by bacteria under restricted growth conditions as storage materials (Senior and Dawes 1973). Although PHB is not a fatty acid, the substrate for PHB synthesis, acetyl-CoA, is the same

substrate for fatty acid synthesis in plants. PHB is synthesized from acetyl-CoA by a sequence of three-enzymatic reactions catalyzed by β-*ketothiolase,* a*cetoacetyl-CoA reductase* and *PHB synthase* (Anderson and Dawes 1990). Synthesis of PHB in oil palm was proposed, especially in the mesocarp, as it is rich in acetyl-CoA (Parveez et al. 2008).

Strategy for synthesizing PHB in oil palm

In oil palm, while research is targeted at trying to synthesize the PHB in the mesocarp and leaf using the three genes from bacteria driven by mesocarp- and leaf-specific promoters (Yunus et al. 2008; Masani et al. 2009) efforts are also being made to enhance the synthesis of biodegradable plastics through an intervention strategy. In the first strategy, an antisense ACCase gene (Sambanthamurthi et al. 2002; Omar et al. 2008) will be introduced into oil palm to inhibit the oil synthesis in the mesocarp and as a result, the entire acetyl-CoA pool will be diverted into the synthesis of biodegradable plastics. In the second strategy, the bacterial ketothiolase gene will be replaced with an oil palm ketothiolase gene (Teen et al. 2008) as the native gene may be more effective in utilizing acetyl-CoA to synthesize biodegradable plastics.

Ten transformation vectors carrying the biodegradable plastic genes together with the genes for the intervention driven by constitutive and mesocarp-specific promoters were constructed. Transformation of these constructs into oil palm embryogenic calli was carried out and glufosinate-ammonium-resistant embryogenic calli obtained. Regeneration of the calli into plantlets has been done with some of the plants demonstrating integration of the transgenes as well as synthesizing the biodegradable plastics (Parveez et al. 2008).

The way forward

The experiments to genetically engineer oil palm for different oil compositions are still ongoing using the strategies described above. At the same time, novel approaches are being sought to speed up the achievement of all the objectives. The successful sequencing of the oil palm genome (Singh et al. 2013) allows the utilization of the genome information for identifying more genes and other genetic elements to effectively modify oil palm lipid metabolism. A couple of genes families and promoters were identified using the available oil palm genome data. The development of new genetic modification approach, known as genome editing, could be applied to oil palm more effectively with the availability of oil palm genome data.

Genome editing, a technology which emerged a couple of years ago, allows the manipulation of any gene in almost any type of cell of any living organism (Gaj et al. 2013). It is based on using customized nucleases carrying sequence-specific DNA-binding domains to target specific DNA sequences. This binding could result in targeted DNA double-strand breaks which will subsequently induce the cellular DNA repair mechanisms, such as homology-directed repair or error-prone non-homologous end joining (Wyman and Kanaar 2006). The two most common nucleases with the DNA-binding domains used for genome editing purposes are zinc-finger nucleases (ZFNs) and TALENs. The above approaches, however, are not widely adopted for plant gene regulation as they require complicated vector design in addition to time-consuming assembly of relevant DNA-binding proteins for each gene of interest (Belhaj et al. 2013). A simpler method was recently developed based on the bacterial type II Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)/Cas (CRISPR-associated) immune system which allows cleavage of genomic DNA at a targeted site which is guided by a small noncoding RNA that could be customized.

One example of the use of genome editing in fatty acid modification is in soybean where TALENs were used to down regulate two fatty acid desaturase 2 genes (FAD2-1A and FAD2-1B), resulting in an increase in oleic acid content due to the reduction in linoleic content (Haun et al. 2014). The group used the genome editing approach to down regulate the two genes simultaneously due to the following advantages: (a) down regulation using RNA interference (RNAi) requires screening of many events for one or a few desired and stable events, besides having to subject the transgenic events to a costly and lengthy biosafety deregulation process; (b) the use of a mutagen to down-regulate desired gene(s) requires a lengthy backcrossing process to introduce the trait into an elite germplasm; (c) TALENs can mutagenize more than one gene at a time with stable and low toxicity effects; and (d) the high oleic acid soybean developed has no foreign DNA integrated in its genome. Many regulatory authorities are still considering how to regulate the plants generated using genome editing approaches as there are no transgenes. The USDA has offered an opinion that plants mutated by genome editing, such as by zinc-finger nucleases and meganucleases, should not be regulated. Genome editing with all its above advantages can be applied to oil palm for more effective regulation of targeted genes or for replacing an unwanted gene. This has been made easier with the availability of oil palm genome sequence.

Author contribution statement G. K. A. Parveez as the lead author planned and drafted the manuscript. O. A. Rasid and M. Y. A. Masani provide input on the genetic engineering and edited the whole manuscript. R. Sambanthamurthi provided input on the biochemical studies and also edited the whole manuscript.

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