Chapter 15

Genetic Improvement of Oilseed Crops Using Modern Biotechnology

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Additional information is available at the end of the chapter

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Abstract

In 2009, big challenges facing the agricultural sector in the twenty-first century were presented to the world. Human population growth, increased life expectancy, loss of biodiversity, climate change and accelerated land degradation are the main factors contributing to rethink agriculture system production. In that scenery, modern biotechnology has set a stage for the advancement of agricultural practices and it is clearly an important ally to apply a broad array of technologies and innovative systems where they are most needed, such as enhancing crop productivity, increasing yields, and ultimately ensuring food security. One of the biggest challenges is related to technify production systems, but with no doubt, developing genetic improvement toward getting an efficient and sustainable agriculture, generating new seed qualities (new traits), such as, among others, to upset fatty acids content in oilseed crops have been growing up significantly due to industry interest. In this study, a review about the main advances in genetic improvement of some oilseed crops, starting with omics to understand metabolic routes and to find out key genes in seed oil production, and also, getting in use of modern biotechnology to alter the production of fatty acids, and to face biotic challenges in oilseed crops is presented.

Keywords: seed oil, oilseed crops, fatty acids, genetic improvement, genetic engineering, modern biotechnology

1. Introduction

Over the last decades, the adoption of oilseed crops has been growing up significantly due to industry interest in the composition of their seed oils, which are made up of a wide range of fatty acids with six predominant types: 16 or 18 carbon palmitic, stearic, oleic, linoleic and linolenic acids, and 12 carbon lauric acid, as well as other unusual fatty acids produced by wild plant species include those with chain lengths between 8 and 24 carbons [1]. Due to their

structure and composition, those oils are used as food/industrial feed [2] and as a range of product applications such as surfactants, soap, detergents, lubricants, solvents, paints, inks, chemical feedstocks and cosmetics [1]. In this study, a review about the main advances in genetic improvement of oilseed crops, starting with omics to understand metabolic routes and to find out key genes in seed oil production, and also, getting in use of modern biotechnology including genetic engineering and new breeding techniques (NBTs), a modern-breeding tool that has allowed the functional study of genes with potential application for breeding in agriculture, focusing on oilseed crop genetic improvement with high precision and less uncertainty (avoiding whole genomes crossing), and of course, in less time is presented; those scientific efforts where it was sought to upset fatty acids production or biotic tolerance will also be presented.

2. Oilseed crops

Seed oil is mainly obtained from plants recognized as oilseed crops, among them are: soybean (*Glycine max*), rapeseed/canola (*Brassica napus*), palm (*Elaeis guineensis*), mustard (*Sinapis hirta*), sunflower (*Helianthus annuus*), cottonseed (*Gossypium hirsutum*), flax (*Linum usitatissimum*), peanut (*Arachis hypogaea*), camelina (*Camelina sativa*), castor bean (*Ricinus communis*), jatropha (*Jatropha curcas*), tung tree (*Aleurites fordii*), jojoba (*Simmondsia chinensis*), sacha inchi (*Plukenetia volubilis*), niger seed (*Guizotia abyssinica*) and others [2]. Over the last decades, the adoption of these crops has been growing up significantly, reaching in 2014 more than 300 million hectares of oil crops worldwide cultivated [**Figure 1**] [3]. The main reason for this growth is due to seed oils are so attractive for industries, as mentioned in the previous paragraph, but also for the possibility to use their sub-products (metabolites) in biofuels development [4] and approaching their polyhydroxyalkanoates (PHAs) in response to petrol-based plastics waste's problems and harmful effects on the environment [5].

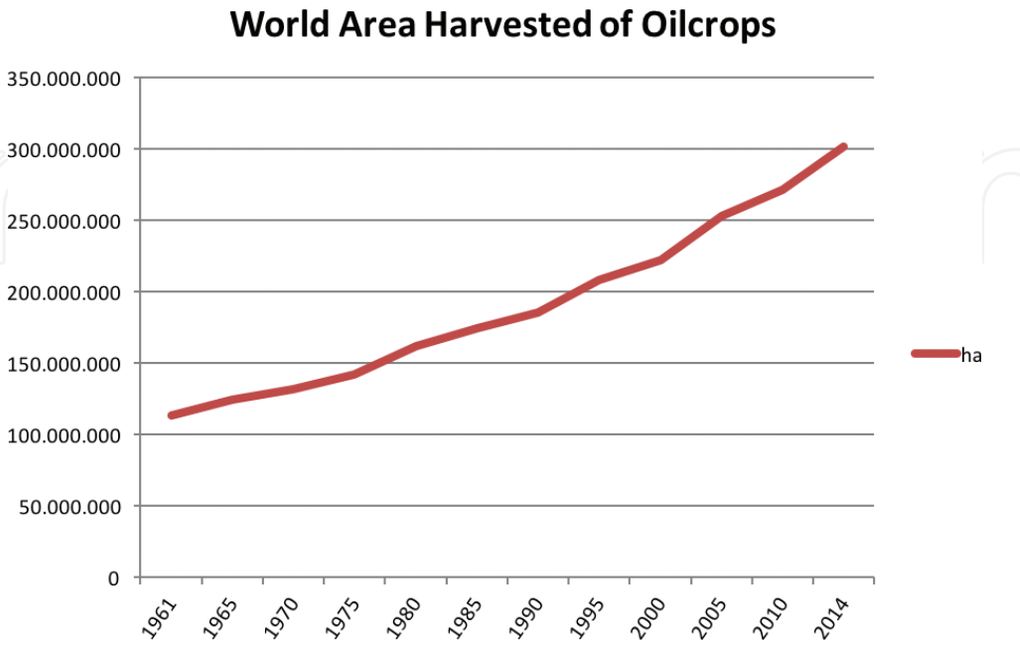

Figure 1. Total world area of oil crops harvested between 1961 and 2014 (FAOSTAT).

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Seed oils applications depend on physical and chemical properties of their fatty acids composition [6]. Those oils are mainly composed of five major fatty acids, including the saturated palmitic (C16:0) and stearic (C18:0) acids, the monounsaturated oleic acid (C18:1), and the polyunsaturated LA (C18:2) and ALA (C18:3) [6]. A large variety of other less common but not less important fatty acids can be found in different species and used for various industrial applications. These fatty acids vary in the number of carbons in the chain (from 8 to 24), the number of double bonds, and the presence of epoxy, hydroxyl and other functional groups [7].

Despite the industry's significant interest in oil crops, it is reasonable to mention that agriculture has a challenging future. The Food and Agriculture Organization of the United Nations (FAO) in 2009 presented the big challenges facing the agricultural sector in the world for near future [8]. Human population growth, increased life expectancy, loss of biodiversity, climate change and accelerated land degradation are main factors contributing to rethink agriculture system production. Thus, there is a need to technify agricultural production systems, but without doubt, developing genetic improvement toward getting an efficient and sustainable agriculture, generating new seed qualities (new traits), such as, among others, high content of PUFAs in oilseed crops, it will be an aiming. Biotechnology will be fundamental to overcome these challenges. Genetic engineering techniques may play an important role by elevating the content of individual fatty acids or drastically changing the oil quality by the introduction of a new fatty acid,

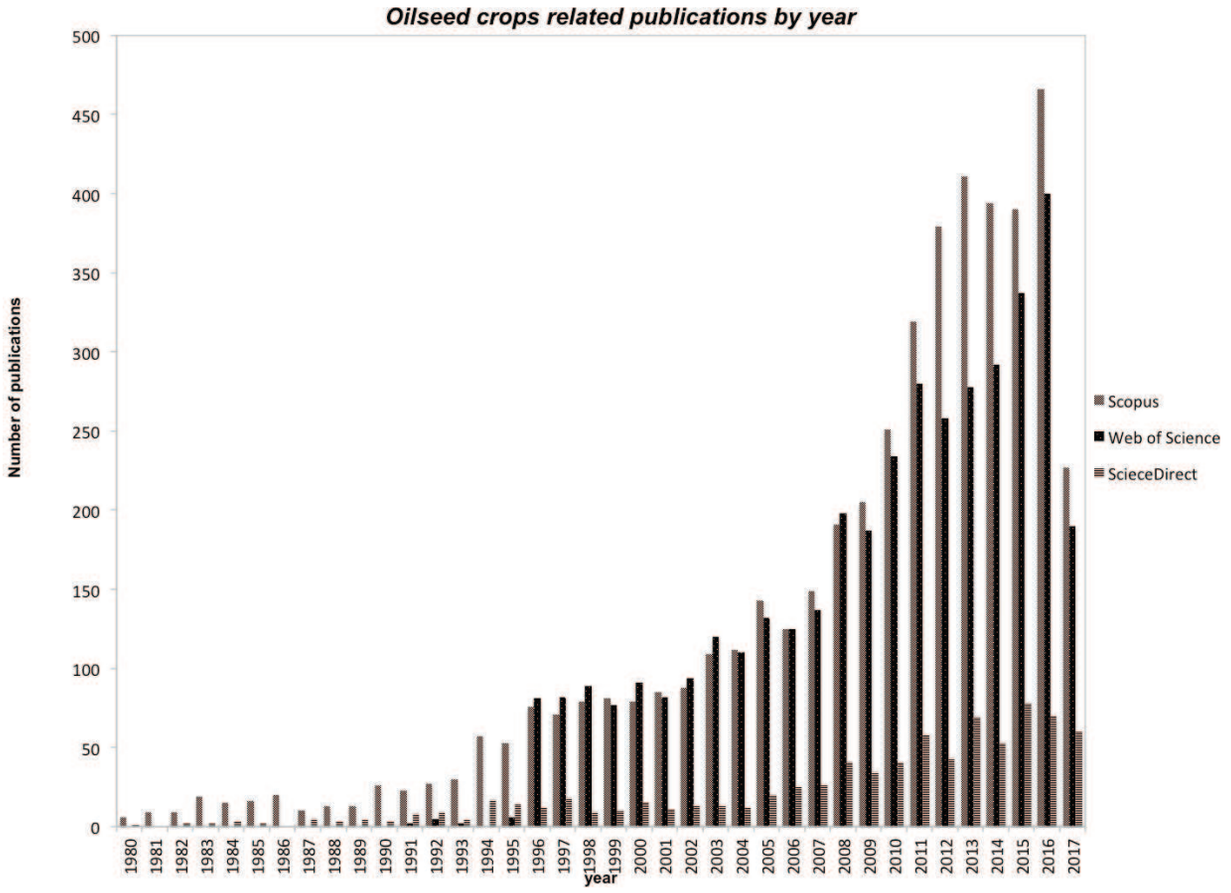

Figure 2. Growing in the number of publications with the words "oildseed crops" in the title, abstract or keywords of scientific articles. Records subtracted from literature databases: Scopus (bars in grey), Web of Science (bars in black) and ScienceDirect (bars with horizontal lines).

thus increasing raw materials available for oleochemistry. In this perspective, it has came growing research efforts of scientist around the world seeking to expand the knowledge barrier on oilseed crops. **Figure 2** shows continuous growth in the number of scientific publications in this field, records subtracted from literature databases: Scopus, Web of Science, and ScienceDirect.

3. Understanding metabolic routes in oilseed crops

Oilseed plants represent an important renewable source of fatty acids because they accumulate them in the form of triacylglycerol (TAG) as major storage components in seeds [9]. In plants, the reactions for de novo fatty acid synthesis begin in plastids [10] and then exported to the cytoplasm following two inter-related metabolic pathways: an acyl-CoA-dependent pathway and an acyl-CoA-independent pathway [11].

In the dependent pathway, commonly known as the Kennedy pathway, the priming and elongation of nascent acyl chains requires acetyl- and malonyl-CoA, respectively, as direct precursors up to eighteen carbons in length [12]. In this pathway, the glycerol-3-phosphate acyltransferase (G3PAT) is the first enzyme that catalyzes the transfer of a fatty acid to glycerol-3-phosphate (G3P) to form lysophosphatidic acid (LPA). Then, the LPA is acylated by the lysophosphatidic acid acyltransferase (LPAAT) to yield phosphatidic acid (PA). Next, PA is dephosphorylated by the phosphatidic acid phosphatase (PAP) to form diacylglycerol (DAG) and finally, a diacylglycerol acyltransferase (DGAT) catalyzes the acylation of DAG to the production of TAG [13]. In the acyl-CoA-independent pathway, an alternative enzyme is used for the final acylation reaction, termed phospholipid:diacylglycerol acyltransferase (PDAT). PDAT directly transfers an acyl group from phosphatidylcholine (PC) to DAG, producing TAG [14].

Desaturation steps for fatty acids are catalyzed by plastidial stearoyl-acyl carrier protein (ACP) desaturases. After termination, free fatty acids are activated to CoA esters, exported from the plastid, and assembled into glycerolipids at the endoplasmic reticulum (ER) [9]. In addition, further modifications (desaturation, hydroxylation, elongation, etc.) occur in the ER while acyl chains are esterified to glycerolipids or CoA [15]. The low polarity of TAG is believed to result in the accumulation of this lipid between bilayer leaflets leading to the budding of storage organelles termed oil bodies [9]. The accumulation of hydroxy fatty acids depends on many factors, including the performance of the desaturases and efficient channeling of hydroxy fatty acids into storage triacylglycerols [16]. Fatty acid dehydrogenase (FAD) catalyzes the desaturation reaction, leading to the formation of unsaturated FA. Interestingly, studies have revealed that some desaturase enzymes (such as the FAD2 and FAD3 genes) could be regulated at the transcriptional level or at the post-translation level in response to low-temperature induction in model plants [17, 18]. Other important enzyme is FAH12 which belongs to a large family of fatty acid modification enzymes that are related to the *Arabidopsis* oleate D12-desaturase (FAD2) protein, which is responsible for the synthesis of polyunsaturated fatty acids [19].

High-quality RNAseq data have allowed the identification and an accurate quantification of expression of transcription factors and key genes related with lipid metabolic pathways in soybean [20], *Jatropha curcas* [21], *Arabidopsis* [22], peanut [23] and castor bean [24]. In castor,

comparison of expression between tissues allowed identification of candidate genes which may be important for triricinolein synthesis in seed in addition to the oleate-12 hydroxylase. Moreover, in purified endoplasmic reticulum from castor endosperm, the site of TAG synthesis, less than 10 genes were found being differentially expressed. Two of these genes, the DGAT2 and PDAT1A, were cloned in transgenic plants expressing the oleate-12 hydroxylase, increasing 18:1-OH incorporation into seed oils and also the expression of additional genes [24].

Gathering the RNAseq information and advances in plant transformation technology is possible now engineering plants for the production of oilseed fatty acids. In a remarkable research, Arabidopsis plant was modified introducing a fatty acid hydroxylase from castor plant, it leading to produce some ricinoleic acid and an unusual fatty acid in the seed [25]. Reactions in triacylglycerol biosynthesis have also been manipulated to increase seed oil content. Studies have suggested that the level of DGAT activity during seed development may have a substantial effect on the flow of carbon into seed oil. Thus, overexpression of cDNAs encoding either *Arabidopsis* DGAT1 or a variant of *B. napus* DGAT1 during seed development in *B. napus* resulted in increased seed oil content under both greenhouse and field conditions [26].

However, in the last decade, the scientists have realized that the manipulation of single genes only contribute with limited value to change the metabolic pathways. Nowadays, there are strategies focused on more complex approaches involving simultaneous overexpression or suppression of multiple genes to achieve optimal metabolic flux [27]. Understanding a metabolic network would facilitate the production of natural products and the synthesis of novel molecules in a predictable and useful manner [16]. For this reason, the metabolic engineering in oilseed plants has attracted industrial and academic researchers in the last decade.

4. Modern biotechnology for genetic improvement in oilseed crops

The Convention on Biological Diversity (CBD) has defined biotechnology as "any technological application that uses biological systems, living organisms, or derivatives thereof, to make or modify products or processes for specific use" [28]. In fact, biotechnology includes several agricultural as well as food manufacturing tools and techniques. However, when a biotechnology development uses new deoxyribonucleic acid (DNA) techniques, molecular biology, and reproductive technological applications ranging from gene transfer to DNA typing to cloning of plants and animals, it has been considerable modern biotechnology [29]. The potential of modern biotechnology is widely known, as it makes the use of recombinant DNA technology to generate modified microorganisms, plants and animals to make them more suitable for several potential applications: improved crops, production of new antibiotics and hormones, xenotransplantation, gene therapy, bioremediation, and genetic editing, one of the most recent techniques.

Genetic engineering crops based on recombinant DNA technology were first introduced for commercial production in 1990s. This technology uses the identification, isolation and manipulation followed by the introduction of desired gene(s) from one organism (for example, a plant or bacteria) to another, thus giving rise to a transgenic or genetically modified organism. This technique has

been fast replacing plant breeding so as to incorporate characteristics that are impossible to achieve by breeding. Biotechnology has the potential to help overcome many of the short-comings of the species being promoted, especially where exogenous genes are needed because there are characters that are difficult to produce by traditional breeding, or where characters tissue-specific or temporal expression or suppression of endogenous genes would be valuable [30]. For oilseed crops, modern biotechnology should allow the production of plants with specific fatty acids content.

In the following paragraphs, main advances in plant genetic improvement using modern biotechnology, focused on oilseed crops, those scientific efforts in soybean (*Glycine max*), sunflower (*Helianthus annuus*), canola (*Brassica napus*), palm (*Elaeis guineensis*), castor bean (*Ricinus communis*), cotton (*Gossypium* spp.), peanut (*Arachis hypogaea*) and olive (*Olea europaea*) where it was sought to upset fatty acids production or biotic tolerance will be presented.

4.1. Soybean (*Glycine max* L.)

Soybean, *Glycine max* L. Merr., is a major crop that produces the best vegetable oil and protein for use in food and beverage production worldwide. Among legume species, soybean has the highest protein content (around 40%), while other species have a protein content between 20 and 30%. On the other hand, cereals have a protein content ranging from 8 to 15%. Other interesting point for oleochemistry is that soybean also contains about 20% oil [31]. Soybean oil is a complex mixture of five fatty acids: palmitic, stearic, oleic, linoleic, and linolenic acids. Nowadays, soybean oil is currently found in food products such as margarine, salad dressings and cooking oils, and industrial products such as plastics and biodiesel fuel. Lecithin, a natural emulsifier and lubricant extracted from soybean oil, is used in applications from pharmaceuticals to protective coatings [32].

Due to its importance as a crop, genetic transformation techniques have been used extensively to improve the crop's valuable traits. Herbicide-tolerant (Roundup Ready) Soybean (*Glycine max* L. Merrill) resistant to glyphosate (N-phosphonomethylglycine) was the first transgenic variety introduced for commercial production in 1995 [33]. In the contrary way to seek increase in fatty acids production, the goal was to give a competitive advantage to soybean favoring desirable plants (in this case soybean) and inhibiting undesirable plants by the application of glyphosate, the active ingredient of the non-selective herbicide Roundup. A glyphosate-tolerant soybean line was obtained through expression of the bacterial 5-enolpyruvylshikimate-3-phosphate synthase (EPSP synthase, EPSPS) enzyme from *Agrobacterium* sp. strain CP4, under the cauliflower mosaic virus 35S promoter (P-E35S), with the *Petunia hybrida* EPSPS chloroplast transit peptide (CTP) and a portion of the 3′ nontranslated region of the nopaline synthase gene (NOS 3′) terminator. This soybean line was highly tolerant to glyphosate, showing no visual injury after application of up to 1.68 kg acid equivalent (a.e.) ha^{-1} of glyphosate under field conditions.

In terms of genetic transformation methods, many reports describing soybean transformation by particle bombardment using meristems as the target tissue have been published. The Biolistics particle delivery system for soybean transformation was evaluated in two different regeneration systems: from shoot tips obtained from immature zygotic embryos of the cultivar Williams 82, and the second was somatic embryogenesis from a long-term

proliferative suspension culture of the cultivar Fayette [34]. A method for high-frequency recovery of transgenic soybean by combining resistance to the herbicide imazapyr as a selectable marker, multiple shoot induction from embryonic axes of mature seeds and biolistics techniques was made by [35]. A targeting method to insert genes with biolistics to predefined soybean genome sites using the yeast FLP-FRT recombination system was made by [36]. A double-barreled gene *gus* device was used to improve transformation efficiency and then study soybean resistance (R) gene-mediated responses to effectors, induction and suppression of cell death by a wide variety of pathogen and host molecules [37]. The abscisic acid (ABA)-independent dehydration responsive element binding (DREB) gene family from *Arabidopsis thaliana* was inserted into soybean plants using biolistics to improve tolerance to abiotic stresses [38]. In the same way, [39] introduced an activated form of abscisic acid-responsive element binding protein (AREB1) into soybean plants to improve water deficit stress. Finally, the biotechnological potential of plastid genetic engineering was used to develop a reproducible method to generate plastid transformants in soybean. To sum up, transformation vectors were delivered to embryogenic cultures by the particle gun method and selection performed using the *aadA* antibiotic resistance gene, getting early homoplasmy and avoiding further selection cycles [40].

Genetic engineering approaches have been applied to enrich the content of soybean oil for a particular fatty acid or class of fatty acids. One of those examples was made by [41]. These authors developed transgenic soybean seeds by down-regulating the expression of *FAD2* genes that encode the enzyme that converts the monounsaturated oleic acid to the polyunsaturated linoleic acid. Those transgenic soybean seeds had oleic acid content of approximately 80% of the total oil, whereas conventional soybean oil contains oleic acid at levels of 25% of the total oil. With the same aim, [42] reported the creation of high oleic acid soybean varieties using targeted mutagenesis with transcription activator-like effector nucleases (TALENs) to bind and cleave specific DNA sequence targets in the *FAD2-1A* and *FAD2-1B* genes with high efficiency. These authors reported that mutant soybean plants produced nearly four times more oleic acid than the wild-type parents (80% vs. 20%, respectively). Furthermore, because they use a technique considered "genetic editing," the soybean lines lacked foreign DNA in their genomes and are thus not transgenic. Rather, they only have small deletions of coding sequence in the FAD2-1 gene targets.

On the other hand, regarding to biotic factors, [43] developed transgenic soybean to improve resistance against SMV. HC-Pro coding sequences were introduced within a RNAi inducing hairpin construct and *Agrobacterium*-mediated transformation system. Then, their response to viral infection was analyzed. The inhibition of HC-Pro expression enhanced viral resistance after viral infection, when compared to the resistance of virus-susceptible non-transgenic plants. RNAi induced by the hairpin construct of the SMV HC-Pro sequence effectively confers viral resistance. Among others, these results have proven the usefulness of RNAi-mediated resistance for crop improvement. Other cases of development of transgenic soybean have been reported by [44]. These authors show some scientific references where synthetic *Bacillus thuringiensis cry* genes were used to develop transgenic soybean and prevent agronomic loses caused by insects from Lepidoptera order such us *Anticarsia gemmatalis, Pseudoplusia includens* and *Helicoverpa zea*.

4.2. Sunflower (*Helianthus annuus* L.)

Sunflower is one of the most important oilseed crops cultivated on a global level. Its seeds have always been ground and pounded into flour for making bread, cracked and eaten as snacks, mixed with vegetables, and extracted for oil. The seeds are also a source of purple dye and have medicinal uses [45]. Sunflower seeds are composed by 20% protein and 50% fat. In this crop oil, up to 90% of its fatty acids are unsaturated, namely oleic (C18:1, 16–19%) and linoleic (C18:2, 68–72%) acids. The remaining 10% of its fatty acids are palmitic (C16:0, 6%), stearic (C18:0, 5%), and minor quantities of myristic (C14:0), myristoleic (C14:1), palmitoleic (C16:1), arachidic (C20:0), behenic (C22:0) [46].

Several scientific efforts have been made to develop genetic improvement methods in sunflower, using modern biotechnology.

Perhaps one of the earliest works in sunflower was developed by [47], which introduced plasmid into isolated sunflower protoplast. Another effort was made by [48] who used microprojectile bombardment of half-shoot apices followed by co-culture with *Agrobacterium tumefaciens*, to obtain transgenic shoots. However, [49] modified a step in which shaking of explants with glass beads replaced the microprojectile bombardment stage used by [48]. In an attempt to reduce or eliminate the *in vitro* regeneration component of a sunflower-transformation protocol, [50] infected 2-day-old seedlings, each with one cotyledon detached, with *A. tumefaciens* strain LBA4404 carrying a specific plasmid. On the other hand, to overcome the generation of chimeric plants, [51] used zygotic embryos, the latter being 4–6 mm in size and cut transversely below the cotyledons, and then, explants were cultured in the dark for 1 day before being bombarded with gold particles and co-cultured with *A. tumefaciens*. [52] reported an alternative procedure to wound cells of target sunflower explants that involved treatment of the explants with the cell wall-degrading enzymes Cellulase Onozuka R-10 (0.1% w/v) and pectinase Boerozyme M5 (0.05% w/v). After that, but before *Agrobacterium* inoculation, a sonication (50 MHz, 2, 4, 6 s) step of explants showed that transient expression of *gus* or *gfp* transgenes was increased.

One of the most important aspects of any transformation protocol is an efficient selection of transgenic plants. On the way to develop a procedure to minimize the number of transgenic escapes, [53] germinated sunflower seeds for 24 h on half-strength MS-based medium, before cutting the seeds to give two half embryos, each with one cotyledon. Once that, cotyledon explants were inoculated with *A. tumefaciens* carrying a vector with the *nptII* and the *gus* genes.

Leaving aside developments made around transformation methods, and focusing on advances toward genetic improvement with some functional characteristics, some efforts to improve oil production in sunflower have been made recently. Dağüstü et al. [54] introduced the *Erwinia uredovora* phytoene desaturase (*crtl*) and hydroxymethylglutaryl-CoA (*Hmgr-CoA*) genes into sunflower, which have potential to increase oil quality. On the other hand, [53] developed transgenic sunflower plants resistant to *Verticillium dahlia* and *Sclerotinia sclerotiorum* introducing antifungal genes, including *gln2* (a glucanase) from *Nicotiana tabacum*, a chitinase (*ch5B*) from *Phaseolus vulgaris*, an osmotin gene (*ap24*) from *N. tabacum*, and a gene coding for a ribosome inhibitor protein (rip). In the same way, [55] developed transgenic sunflower resistant to the herbicide phosphinothricin, herbicide resistance also being exploited to select the transgenic plants.

Some research interests have been around decreasing levels of palmitic and stearic acid of sunflower, due to their contribution on increasing the plasma cholesterol level in humans, associated with heart disease. Škorić et al. [45] induced mutations via seed treatment with γ-rays, X-rays, and mutagenic chemicals such as ethyl methanesulfonate (EMS) and dimethyl sulfate (DMS) to generate sunflower genotypes with high levels of C 18:2, C 18:1, C 18:0, C 16:1 and C 16:0.

4.3. Canola (*Brassica napus*)

Canola/rapeseed (*Brassica napus*) is considered one of the most important oil sources for edible or industrial uses, being the research to get better oil quality is important to improve rapeseed as a high-quality vegetable oil. Canola oil contains multiple fatty acids, such as palmitic acid, stearic acid, oleic acid, linoleic acid, α-linolenic acid, arachidic acid, erucic acid among others [56]. Due to its high nutritional value, Canola oil is included in human diets where it has been shown to reduce plasma cholesterol levels in comparison with diets containing higher levels of saturated fatty acids. It has demonstrated that consumption of canola oil also influences biological functions that affect various other biomarkers of disease risk [57].

A group of researchers developed transgenic canola seeds with significantly increasing of oil content [58]. Those authors showed that seed-specific overexpression of BnLEC1 and BnL1L genes (from canola), placed under the control of the truncated canola storage protein 2S-1 promoter, which is also known as the *napA* promoter, at an appropriate level substantially increases the seed oil content of the transgenic oilseed plant without detectable negative effects on other major agronomic traits.

In the same way to improve canola oil production, Qi et al. [59] isolated the RNA-binding motifs No2 (RRM2) of the flowering control locus A (FCA) protein (FCA-RRM2) from variety No. 1 "Nannongyou" of Canola, and then, it was introduced in cotyledon nodes using *Agrobacterium rhizogenes*, placed under a 35S-35S promoter (a variant of the cauliflower mosaic virus 35S promoter with higher transcriptional activity) to drive transgenic expression, into pBin438 vector with kanamycin resistance gene (for bacterial selection) and the hygromycin phosphotransferase gene (for plant transformation selection). These authors demonstrated that canola FCA-RRM2 increases plant size, organ size, cell size, plant productivity and oil content. According to the author of that research, these results provide a practical approach for the genetic improvement of this plant.

Aimed at not good perception of erucic acid (cis-13-docosenoic acid) in the canola oil triglycerides, because of presumptive effects on growth retardation and pathogenic changes to internal organs when fed at high concentrations to laboratory animals, a research was made to decrease erucic acid level in Canola plants. Shi et al. [60] reported the development of canola transgenic with change in fatty acids compositions, using *B. napus* cultivar "CY2" as the transgenic recipient of BnFAE1, a fragment involved in the synthesis of very long-chain fatty acids. These authors placed a BnFAE1 fragment driven by *napin A* promoters and then, they co-cultured hypocotyls with *Agrobacterium tumefaciens* EHA105 to introduce the genetic construct in canola cells. Due to CY2 that has high erucic acid (about 40%) and low oleic acid (about 20%) content, the researchers made seed-specific knockdown of BnFAE1, significantly

changing the fatty acid composition. They demonstrated that the RNAi construct of BnFAE1 could effectively interfere with mRNA levels of BnFAE1 gene in F1 hybrid seeds derived from crosses between BnFAE1-Ri lines and high erucic acid cultivars. At the end of their research, they got canola transgenic lines with a dramatically decreased erucic acid (less than 3%).

4.4. Palm (*Elaeis guineensis*)

Cultivation of the oil palm (*Elaeis guineensis* Jacq.) has expanded tremendously in recent years such that it is considered second as a major source of the world supply of oils and fats [61]. Palm oil is one of the most price-competitive liquid cooking oils in many parts of the world due to its use in food products such as shortenings, margarines and spreads [62]. The benefits of palm oil on human health have been demonstrated scientifically by different authors. Qureshi et al. [63] indicated that palm oil lowers serum cholesterol levels to the same degree as sunflower oil, which is rich in polyunsaturated fatty acids. In the same way, Nesaretnam et al. and Kritchevsky et al. [64, 65] proposed anti-carcinogenic potential of palm oil presumably due to the presence of high levels of vitamin E and tocotrienols.

It is reported that currently palm oil accounts for about 20% of world oils and fats production. It was forecast that, with the increase in world population, the demand for palm oil would grow faster than the rise in supply, so that, the supply of palm oil would also need to be increased to meet the above demand. It was therefore crucial to increase the yield of palm oil, improve its oil quality, and produce novel products via genetic engineering, as it could be achieved faster this way than by conventional breeding ways.

According to [61], palm fruits have two storage tissues, mesocarp and kernel, that can be the target for accumulating genetically modified products. The substrates and intermediates implied in the production of storage oil or protein in these tissues may be channeled to alter the levels of existing products or to produce novel value-added products without deleterious effects on the plants. During oil palm fruit development, at level of period of oil accumulation as well as fatty acid composition, the mesocarp and kernel tissues show differences. A study reported by [66] showed the regulation of the gene expression during period of oil synthesis in both tissues. The expression profile of the mesocarp-specific gene in different oil palm tissues, as well as at different developmental stages of the mesocarp and at the cellular level indicated a strong correlation with that of a fatty acid biosynthetic gene, stearoyl-ACP desaturase. Using promoter-reporter constructs, assays and transformations, these authors demonstrated that this promoter is conducive to the development of genetic improvement research modern biotechnology, since specific genes can be located there generating high degree of expression.

Different efforts have been made to get biotechnological palm crops which can generate metabolites of interest. Genetic engineering in oil palm is relatively recent as it was initiated at Malaysian Palm Oil Board (MPOB) in the late 1980s [67]. Related to transformation protocols, particle bombardment and *Agrobacterium*-mediated transformation have been used to introduce genes into oil palm, and stable transformation has been achieved using both methods. Masani et al. [67] reports the development of a biolistic protocol for production of glufosinate-resistant transgenic oil palm, and considering its success, several hundreds of embryogenic calli

have been bombarded with genetic constructs which contain genes involved in fatty acid biosynthesis to augment the accumulation of oleic acid, stearic acid, polyhydroxybutyrate (PHB) and polyhydroxyvalerate (PHBV). Likewise, [67] was able to develop some new transformation protocols based on DNA microinjection and PEG-mediated transfection demonstrating that protoplasts are appropriate as a target for oil palm genetic engineering. These authors successfully expressed a reporter gene encoding green fluorescent protein (GFP) allowing the rapid and efficient generation of non-chimeric transgenic callus without the use of standard selectable markers. In the same way, [68] reported that oil palm embryogenic calli were bombarded with a transformation vector, p35SCaMV-sgfpS65T, carrying a modified version of *gfp* gene driven by the 35S promoter. Those authors refer that upon bombardment, the expression of *gfp* in embryogenic calli was monitored visually while the regeneration of the embryogenic calli was ongoing.

Seeking to improve oleate oil palm production, some studies had some strategies where transformation vectors were constructed: (1) antisense palmitoyl-ACP thioesterase gene driven by CaMV35S promote, (2) antisense palmitoyl-ACP thioesterase and sense KAS II genes driven by mesocarp-specific promoter, (3) antisense palmitoyl-ACP thioesterase, sense KAS II and sense stearoyl-ACP desaturase genes driven by a mesocarp-specific promoter and, (4) antisense palmitoyl-ACP thioesterase, antisense oleoyl-CoA desaturase, sense KASII and sense stearoyl-ACP desaturase genes driven by a mesocarp-specific promoter, and then, those constructs were bombarded into oil palm embryogenic cultures. Molecular and biological assays were made and some plantlets were transferred to soil in the biosafety screenhouse [69]. It is important to refer that KAS II is one of the main enzymes contributing toward high palmitic acid.

Stearate oil palm is an another key oil with great industrial interest such as cocoa butter substitute and personal care products such as lotions, shaving cream, and rubbing oils. Biochemical studies have demonstrated that oil palm contains an active stearoyl-ACP desaturase, and therefore down-regulating the activity of stearoyl-ACP desaturase could reduce the conversion of stearoyl-ACP into oleoyl-ACP [62]. Three transformation vectors were constructed carrying an antisense stearoyl-ACP desaturase gene driven by ubiquitin, CaMV35S, and mesocarp-specific promoters [70]. These authors reported that those constructs were bombarded into oil palm embryogenic cultures and a few lines of resistant polyembryogenic cultures and then full plant regeneration were obtained. The transgenic plant's stearic acid content increased as a result of concomitant reduction of oleic acid levels.

Regarding to biotic factors, [71] introduced a synthetic *cryIA(b)* gene into oil palm while biobalistic. When *cryIA(b)* gene was expressed, it produced proteins which upon crystallization are highly toxic to Lepidoptera among which, *Metisa plana* is a major insect pest for oil palm. Those authors developed a rapid detection system for evaluating transgene expression among putative transformed tissues combining RT-PCR and Southern blotting. The procedure developed is reported very sensitive and rapid, and eliminates the long waiting period for transgenic plants to reach maturity.

4.5. Castor bean (*Ricinus communis* L.)

Castor or castor bean (*Ricinus communis* L.) is a major non-edible oilseed crop grown extensively in the tropical, subtropical, and temperate regions of the world for its highly valued oil.

Castor oil is rich (80–85%) in an unusual ricinoleic acid, hydroxyl fatty acid [72] which is used as an input (raw material) for the development of several different industrial products, among others, high quality lubricants for aircrafts, alternative to lubricants/additives in petroleum diesel, coatings, polishes, paints, textile dye, surfactants, plastics, resins, waxes, soaps, drugs, and cosmetics [73, 74].

Introduction of foreign genetic material defining specific agronomically important traits into castor through genetic transformation techniques have been attempted. Transgenic research in castor bean (*Ricinus communis* L.) has been undertaken for the development of insect resistant or ricin free genotypes.

Castor semilooper (*Achaea janata* L.) is a voracious feeder causing extensive defoliation and yield losses on castor bean in the semiarid tropics of India and other countries. Seeking to face this biotic challenge, [75] developed a stable genetic transformation system for introducing desired alien gene(s) into castor using embryo axes through *Agrobacterium*-mediated approach. They reported, the successful production of transgenic castor with *cry1Ab* gene affording resistance against castor semilooper. The synthetic delta endotoxin gene *cry1Ab* was under the control of CaMV35S promoter and NOS terminator, and then, cloned into the HindIII site of the vector pSB11bar containing herbicide-resistance gene bar. Focusing on main outcomes, [75] obtained transformed plants with levels of Cry1Ab protein concentration between 0.23 and 0.47 ng/mg in tissue. With respect to biological assays, these authors got insect mortality ranged from 88.91 to 97.25% in larvae fed on the leaves of primary transformants after 3 days of infestation. On the other hand, larvae fed on untransformed control leaves (which do not have Cry1Ab proteins) showed least (13.92%) insect mortality. Then, larval mortality was attributable to the varied levels of Cry1Ab protein expressed in different transformants.

In the same way, toward obtaining biotechnological strategies for the control of castor semilooper, [76] reported the use of *Agrobacterium*-mediated and biolistic bombardment methods for expression of the *cry1EC* gene in castor bean. Trying to face *Spodoptera litura*, [77] recently reported on the development of an *A. tumefaciens* mediated in planta transformation protocol for castor bean. These authors developed transgenic lines from 2-day-old seedlings infected with *Agrobacterium*, EHA105/pBinBt8 harboring *cry1AcF* gene and then, molecular and expression analysis confirmed the transgenic nature and identified high-expressing plants. Bioassay in greenhouse against *S. litura* corroborated strong resistance of transgenic castor bean.

On the other hand, two approaches were used to develop castor cultivars with reduced levels of toxin. [78] reported on "knocking out" the genes responsible for ricin production as well as genes responsible to produce ricinine and CB-1A. Then, conventional sexual hybridization was used to develop F6 lines of castor that have a 75–70% reduction in ricin and *Ricinus communis* agglutinin toxins. Those plants were combined with transgenic castor plants which had a great potential to reduce ricin content (for >99%). Subsequent selection in segregating generations resulted in a 99.9% reduction in protein toxins allowing development of castor cultivars with a very low level of this kind of toxin (ricin). For [78], these cultivars would allow to increase castor production in the U.S. while increasing the value of the high protein meal remaining after castor oil extraction. The denouement of this research is not clear, but recently, one of their researchers published a method to address the problem (concerns about

the presence of the protein toxin ricin) with simple procedures to reduce or eliminate the toxin from the seed meal remaining after processing the seed for oil [79]. They used a protease treatment during extraction to provide an effective means to eliminate the ricin present after oil processing. In addition, heating intact seed via microwave irradiation was a way to demonstrate that they could inactivate considerable ricin activity.

4.6. Cotton (*Gossypium* spp.)

Cotton (*Gossypium* spp.) is one of most important fiber crops at world level. According to [80], cottonseed oil represents approximately 16% of the seed weight, and perhaps it is the most valuable product derived from cottonseed. Likewise, cottonseed oil is composed by 26% of saturated palmitic acid (C16:0), 15% of mono-unsaturated oleic acid (C18:1), and 58% of poly-unsaturated linoleic acid (C18:2) [80]. In fact, this complementary product (cottonseed oil) has some advantages over soybean oil and rapeseed oil, like good quality and price, so that it is used in foods or as a raw material for biodiesel production [81]. However, some reports warn that cottonseed oil content around oversaturated, polyunsaturated, and monounsaturated fatty acids is unbalanced [80, 82].

Chapman et al. [82] reported the development of transgenic cotton plants with increased seed oleic acid levels. Using an *Agrobacterium*-mediated system transformation, these authors introduced a binary vector previously designed to suppress expression of the endogenous cottonseed enzyme fatty acid desaturase 2 (Fad2) by subcloning a mutant allele from a rapeseed fad2 gene. It is known that FAD2 enzyme, in the endoplasmic reticulum of plant cells, catalyzes conversion of oleic acid to linoleic acid so that, decreasing this enzyme activity would be an increase of oleic acid content in cottonseed oil. At the end of the research, these authors' increased seed oleic acid content ranged from 21 to 30% (by weight) of total fatty acid content in primary transformants and 47% of oleic acid content in their progeny, which represent an increasing of three times comparing with standard cottonseed oil.

Due to consumption of the saturated fatty acid, overall cholesterol levels increases, more specifically low-density lipoprotein (LDL) which is considered "bad cholesterol," and it is well known worldwide that its consumption increases risk of cardiovascular disease [83]; a group of researchers started a study to improve the quality of cottonseed oil. [84] used RNAi technology to regulate fatty acid metabolism of cottonseed inhibiting GhFAD2-1 and GhFATB gene expression levels, simultaneously. These genes encoding the microsomal oleate desaturase and palmitoyl-acyl carrier protein thioesterase, respectively, play significant roles in regulating the proportions of saturated and polyunsaturated fatty acids in cottonseed lipids. Using this technology, they decreased palmitic acid and linoleic acid content and increased oleic acid content, but unfortunately, they got an adverse effect on seed germination and seed vigor. In spite of achieving an adequate balance in the content of fatty acids, thinking in human consumption of cottonseeds oil, it is necessary to explore others effective regulating strategies to improve the quality of cottonseed oil.

On the other hand, recently, Wang et al. [81] reported a genome-wide analysis in several *Gossypium* species and possible ancestral diploids. In that study, authors analyzed a total of 40 Lysophosphatidic acid acyltransferase (LPAAT) genes and found that this gene is involved

in increasing oil composition and content which was demonstrated in some experiments in transgenic yeast. This report shows an important way for further studies due to LPAAT genes that are involved in natural cottonseed oil content and variation which should open a possible strategy in development of genetically modified cotton crops with improvement of seed oil content and composition.

4.7. Peanuts (*Arachis hypogaea*)

Peanut (*Arachis hypogaea*) is grown worldwide as an oilseed crop. In many countries, peanut seeds do an important contribution to the people diet because they are a good source of proteins and lipids for human nutrition. [85] determined that peanut seeds which are rich in oil (about 50% of seed composition) and oleic acid (18:1), linoleic acid (18:2), palmitic acid (16:0), behenic acid (22:0), eicosenoic acid (20:1), stearic acid (18:0), arachidic (20:0) and lignoceric acid (24:0) are presented (sorted from highest to lowest content). However, the fatty acid composition of peanut oil varies depending on the seed maturity, genotype, growth location, climatic conditions, and they together [86].

Research about transgenic peanut crops has been undertaken for the development of fungi resistant. This crop is susceptible to many types of pathogens including those caused by fungi. Chenaulr et al. [87] reported the development of transgenic peanuts which were introduced two hydrolase genes, a glucanase from alfalfa (*Medicago sativa* L.), and a chitinase from rice (*Oryza sativa* L.) into somatic embryos using biolistic. Although the study focused on seedlings characterization (found up to 37% of hydrolase activity in transgenic lines), these authors assume that transgenic lines obtained could be promising due to high transgene expression what would exhibit some level of resistance to a broad range of fungal pathogens. Following with the same modified peanut lines, Chenault et al. [88] developed an assay under greenhouse conditions where these lines were tested for resistance to *Sclerotinia minor* by inoculation with a mycelial plug. There were lines up to 84% of resistance to the pathogen. On the other hand, the peanut lines considered more resistance kept going in race and were tested for *S. minor* resistance under field conditions [89]. In that report, three transgenic lines showed a significant resistance to the pathogen compared with the wild-type cultivar. Finally, Jonnala et al. [90] determined the oil composition of the best three transgenic lines obtained in the previous report. This author reported similar oil content of all transgenic peanut lines to that wild-type lines, indicating that genetic modification did not cause substantial unintentional changes in peanut chemical composition. In the same way, Ng et al. [91] examined chemical characteristics, volatile components, and olfactory characteristics of those three GM peanut lines (previously tested at field conditions) using gas chromatograph/mass spectrometer (GC/MS) equipped with an olfactory detector. These authors reported minimal variations in nutritional composition between GM peanuts and wild type, indicating that genetic modifications did not cause significant change in peanut.

4.8. Olive (*Olea europaea* L.)

Olive oil production and consumption are increasing in importance around the world. Spain is the largest producer with an average 1 million tons per year, followed by Italy and Greece

with 560 and 350 thousand tons, respectively [92]. This crop contributes significantly not only to the global economy but also to food security in terms of its nutritional value. It is well known that olive and olive oil play an important role in prevention of coronary heart disease and certain cancers, due to their high levels of monosaturated fatty acids and phenolic compounds [93].

Olive is characterized by a long history of cultivation, as it was one of the first tree species to be domesticated, and by wide diversity. A very large number (over 1600) of cultivated varieties characterize this species. This diploid specie (2n = 46) has a small genome (2200 Mb) and it is predominantly allogamous in nature [94, 95]. During a long period of time, local and old cultivars have been evaluated by different genetics, morphological, and agronomics approaches. Recently, the huge genetic variability of this specie has been evaluated using molecular markers, including SSRs, particularly advantageous because olive is a clonally propagated, perennial, slow growing, highly heterozygous cultivated species with a very large uncharacterized genome [96].

As any other extensive crop, the olive has urgent challenges; they are summarized into six big areas such as: (i) olive growing; (ii) processing, byproduct, and environmental issues; (iii) virgin olive oil sensory quality; (iv) purity, authentication, and traceability; (v) health and nutrition; and (vi) consumers. Moreover, the olive varieties renewal have been hampered by the extreme longevity of olive trees, the long period of juvenility of their offspring, and the diffidence of the public to accept genotypes obtained with advanced biotechnological approaches. Modern biotechnological techniques are suitable for olive improvement because they both allow direct correction of main defects, combining with existing known superior cultivars, and can also support traditional breeding using the great genetic variability present in the species, to guide crossing of genotypes chosen among the olive populations of different sites [96]. Modern biotechnology along with traditional and *in vitro* technologies, can also provide new resistant cultivars to abiotic and biotic stresses, as water deficit and nematodes infection are becoming the major problems in field [97].

However, some aspects of the olive biotechnology remain challenging; for example, the olive propagation is still a laborious practice. As regards traditional propagation, rooting of cuttings and grafting stem segments onto rootstocks are possible. The regeneration of whole plants from ovules, on the other hand, is used only occasionally. Micropropagation of olive is not easy mainly due to explant oxidation, difficulties in explant disinfection, and labor-oriented establishment of *in vitro* shoot cultures [96].

5. Conclusion

As it was seen throughout the review, last three decades, for these oilseed crops: soybean (*Glycine max*), sunflower (*Helianthus annuus*), canola (*Brassica napus*), palm (*Elaeis guineensis*), castor bean (*Ricinus communis*), cotton (*Gossypium* spp.), peanut (*Arachis hypogaea*) and olive (*Olea europaea*), it has been evidenced a strong development supported by modern biotechnology, and there should be no doubt that, carefully undertaken, genetic engineering represents

a very safe, fast and, low-cost method to enrich important oilseed crops for essential nutritional contents. Ongoing and future research will have to face big challenges in agriculture.

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