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Transgenic Approaches in the Improvement of Seed Oil and Quality in Oil Seed Crops

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Abstract

Several million hectares of economically cultivated transgenic crops are currently available. There have been field trials of transgenics from at least 200 species, oil plants (including both annual and perennial) belonging to different plant families. Crops that produce oil seeds are crucial to the agricultural economy. They have a significant role in industrial uses and the human diet, but they are also becoming more significant as a fossil fuel replacement for supplying energy. A number of significant developments in genetic engineering and the identification of gene targets for increasing seed oil content in oilseed crops have occurred during the past 20 years. These developments will help the successful development of new generation high yielding oil crops. A significant portion of the most recent data comes from the examination of patent databases, an excellent source of data on commercial priorities. The timeline for the introduction of these transgenes into breeding populations and their eventual release as novel varieties is also covered in the review.

Keywords: Seed oil, Crops, Plant, Species, Compounds etc.

INTRODUCTION

Next to cereals, oilseed crops are the second most important field crops (Reddy and Immanuelraj, 2017). More than 200 species of oil plants (including both annual and perennial) belonging to different plant families are grown all over the globe. Though the oil-bearing tree fruits contribute to good extent, these crops had been the major sources of vegetable oils since ancient times. Among various oilseed crops, the major ones – soybean, groundnut, rapeseed and mustard – are regarded as primary sources, and the others like castor, coconut and oil palm trees are considered as secondary sources of oil (Waseem et al., 2017). Over the last ten years (2009/10 – 2019/20), the global production of vegetable oils has experienced constant growth from 140.9 MMT to 203.91 MMT (www.statista.com) due to an increasing demand of the oilseeds and oils in developing countries because of fastly growing population, standards of living and change in diets. It is estimated to get double as compared to the present production by the year 2050 (Zafar et al., 2019). The major contributors in producing oil seeds in the world are China, Brazil, USA, and India. Generally, the plant species producing seeds assemble different proportions of oil, protein, and carbohydrates. Being rich in oil component than others, these crops are cultivated for extracting oils which fall mainly into two categories - edible oils and industrial oils. Whereas the former is used as an ingredient in many food products, latter contain some critical components (Unusual fatty acids (UFAs) which can be

used for industrial purposes. Oils are obtained using two-step process which involve extraction and refining. Extraction is carried out either by mechanical (pressing) or chemical methods (using different solvents) followed by refining to remove odour, taste or other unwanted components (Ikegwu et al., 2022). As far the composition of vegetable oils is concerned, these are principally composed of triacylglycerols (TAGs) which are energy dense molecules stored in their seed as storage reserve., The oil content, rich in essential *n*-3 and *n*-6 fatty acids, in these crops varies from 20% in soybean to 60% in sesame (Foster et al., 2009). Despite the basic similar pathway for the synthesis of fatty acids (FAs) and TAGs in all the oilseeds producing plant species (Guschina et al., 2014); the amount synthesized and accumulated in seeds is regulated by several genetic, physiological and environmental factors viz. genes coding for enzymes & FAs synthesis, carbon flux, soil quality and temperature etc. (Chandran et al., 2014; Savadi et al., 2017).
Being rich source of the poly-unsaturated fatty acids (linoleic & linolenic), the importance of the oils in the diet is now well recognised. Vegetable oils also contain phytosterols, which depending upon the crop, are present in free or esterified form. These are reported to possess the property of lowering bad cholesterol (LDL) levels in blood, and are recommended for patients with a high risk of coronary artery diseases (Goldberg, 2003). Modern nutritionists therefore, recommend a diet rich in vegetable oil in its natural form and low in animal fat. Besides in its natural liquid form, vegetable oils are hydrogenated to produce solid food products like margarine and shortening which can supplement or replace the animal products. For industrial applications, these oils are used for manufacturing soaps, cosmetics as well as producing candles, lubricants, paints and greases (Ramadan Hassanien et al., 2012). The presence of UFAs like lauric acid, erucic acid and α-eleostearic acid in the oil has rendered some oilseed crops to play an important role in above-mentioned industrial applications (Kumar et al., 2016). With increasing pressure and risk of depletion of the petro-fuels, the oilseed crops have emerged as an alternative source for producing biofuels, mainly biodiesel. However, a number of issues regarding the utilization of vegetable oil as fuel are its prominent viscosity, low volatility and dreadful cold flow properties which interfere with the smooth functioning of the diesel engine (Bacenetti et al., 2017).
Apart from the oil, oilseed meals are also good source of proteins and other bioactive compounds. Whereas the major sources of proteins are represented by soybeans, rapeseed/canola, cottonseed, peanut/groundnut, the minor are sesame, safflower, flaxseed and linseed. The physical and chemical properties of many of these oil-producing proteins have been extensively studied and it was found to be ranging from 13–17% for safflower to as high as 37% for soybean (Prakash and Narasinga Rao, 1986; Paredes-López, 1991; Lampart-Szczapa, 2001). Proteins in the seeds have been ascribed two major roles – enzymatic and storage. When using these protein sources for human consumption, the storage proteins are the prominent proteins and are of prime interest. The major ones in oilseeds belong to water soluble albumins (2S) and salt soluble globulins (11S/12S) (Prakash and Narasinga Rao, 1986). As far amino acid profile is concerned, oilseed proteins are poorer in lysine and sulphur containing amino acids than animal proteins but richer in latter than legumes (Breiteneder and Ebner, 2001; González-Pérez and Arellano, 2009). However, these may contain ant-nutritional factors (viz. phytic acid, glucosinolates) which can be effectively reduced by using efficient protein extraction and isolation methods to create a potential protein-rich commodity with improved functional properties viz. emulsifying activity (EA), foaming capacity (FC), foam stability (FS) and gelation. The use of oilseed proteins as human dietary protein with these improved functional properties can supplement the traditional protein sources without altering their food appealing characteristics like texture, taste etc. The proteins of good number of oilseed crops like soya,

canola, rapeseed have been proven either as an alternative to animal protein meal or were reported to improve the nutritional properties of the animal-based products (Dersjant-Li, 2002; Aluko and McIntosh, 2005; Yoshie-Stark et al., 2006). Similarly, the use of oilseed protein isolates in improving the nutritional quality of wheat bread, spaghetti, cookies without affecting the sensory and functional properties of these products has also been shown by many workers (El-Adawy, 1995; Alireza Sadeghi and Bhagya, 2008). Oilseeds are also important in animal nutrition as the by-products of oilseed crops, after extracting oils, serve as high protein feed supplements for the livestock; however, the protein content and the feeding value of these vary according to the raw seed materials. The anti-nutritional factors associated with these protein sources make these crops more suitable for ruminants than non-ruminants (Henriksen et al., 2009; Mejicanos et al., 2016). Utilization of the protein hydrolysate of oilseed crops in non-food applications like films, cosmetics with antioxidants and anti-inflammatory properties, hypertensive agents have revealed some promising results (Xue et al., 2009; Shi and Dumont, 2014; Vázquez-Rivera et al., 2015; Katsarou et al., 2015).

The nutritional quality of vegetable oils is related to its fatty acid composition, protein quality and other phytochemical constituents like tocopherols, carotenoids etc. FA composition influences the preservation (oxidation of the unsaturated FAs should be avoided), and the ratio between monounsaturated/saturated FAs should be high to prevent the cardiac disease (Damude and Kinney, 2008). Over the last decades, adoption of the oilseed crops has been growing up among the farmers due to the increasing demand of these crops in various arenas like fuel industries, pharmaceutical & medicines, healthy vegetable oils & proteins and livestock feed (Carlsson, 2009). Thus, to satisfy the increasing world demand with improved seed quality of these crops, classic breeding efforts should be coupled with biotechnological approaches. Genetic engineering of oilseeds will allow not only the sustainable production of oilseed crops but also enhanced seed quality for the nutritional as well as industrial purposes. A new era of producing designer oil crops has been started which has created opportunities for sustainable oilseed crop production around the world. Several researches have been carried out on the extraction of oil from oilseeds using different methods, production of improved breed of oilseed and detoxification of oilseed meals (Adeleke and Babalola, 2020). In the context of sustainable production of vegetable oil with improved or new FAs, increased protein content/quality of seed meal, reduced anti-nutritional factors in oils/seed meal, this review will provide the detail of each major and minor oilseed crop (annual and perennial) and the role of various biotechnological and molecular approaches (r-DNA, CRISPR/cas-9 and omics) employed in individual crop to achieve the desired targets in a given crop.

MAJOR OILSEED CROPS

Sesame

Sesame being considered as an 'orphan crop' during its earlier days has now been marked as a 'genomic resource-rich crop.' Thanks to the modern genomic technology which has decoded sesame nuclear genome and identified a number of candidate genes associated with key agronomic traits viz. high oil

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content and quality, waterlogging and drought tolerance, disease resistance, cytoplasmic male sterility, high yield which have paved the way to develop new strategies for its genetic improvement. Sesame belongs to plant family Pedaliaceae with 2 cultivated (*S. indicum* L. & *S. radiatum* Schum. & Thonn) and 21 wild species (IPGRI and NBPGR, 2004). As far its origin is concerned, sesame was thought to be originated from African continent but now has spread all over the world because of its adaptation to the various environments (Bedigian and Harlan, 1986; Wei et al., 2015).

It is mainly cultivated in tropical and sub-tropical countries for its high seed oil and protein content which vary from 40-50% & 20-25% respectively (Salunkhe et al., 1992). Among the important oilseed crops widely grown in the world such as rapeseed, peanut, soybean, sunflower, sesame (*Sesamum indicum* L.) provides one of the highest and richest edible oils (Pathak et al., 2017). Sesame oil is characterized by low level of saturated FAs (10-15%) and high level of unsaturated fatty acids (90%), mainly linoleic and oleic acid with trace amount of linolenic acid (Kamal-Eldin et al., 2006). The oil is rich in a group of compounds called lignans (oil soluble and glycosylated water soluble) like sesamin, sesamolin, sesaminol and sesaminol glucosides which are having antioxidative properties and responsible for increasing the shelf life of sesame oil (Suja et al., 2004). Moreover, the presence of antioxidants in sesame oils have also been reported to have various pharmacological properties like antiproliferative, antihypertensive and antihypercholesmic activities (Yokota et al., 2007; Visavadiya and Narasimhacharya, 2008; Nakano et al., 2008).

Apart from being an important oilseed source, sesame seed is a potential source of proteins.

The proteins in sesame flour are presented by most dominating globulins (67%) followed by almost equal amount of albumins and glutelins i.e., 8.6% and 7% respectively (Orruno and Morgan, 2007). As far as amino acid composition is concerned, the sesame is unique in having high amount of total sulphur-containing amino acids- methionine and cysteine (Kapadia et al., 2002). Due to the good functional properties of sesame proteins like high oil and water holding capacity, high emulsifying and foaming properties. It can be used as a functional ingredient in meat products and for salad, dressing and soup (Kanu et al., 2007). Sesame proteins are also useful in preventing cancer and heart diseases by increasing plasma α- tocopherol and vitamin-E level (Cooney et al., 2001).

Oil palm

Oil palm includes the two palm species (*oleifera* Kunth and *guineensis* Jacq) under the genus *Elaeis* in the family Arecaceae. Whereas *E .oleifera* (2n=32) is native to South and Central America, *E. guineensis* (2n=32) is native to Africa (Maria et al., 1998 a, b). The oil palm trees are mainly grown because of its high-quality oil used in cooking & industries, and it is the widely consumed oil worldwide (Mba et al., 2015). The global production of oil palm was recorded 73.23 MMT in 2019-20 which increased to 75.45 MMT in 2020/21 (www.statista.com).

Oil palm is a unique crop as two oils are extracted from its fruit – the palm oil (PO) and palm kernel oil (PKO) from the mesocarp and kernel, respectively (Akubugwo and Ugbogu, 2007). PKO is different from PO in terms of the composition of fatty acids; whereas the former is composed of highly saturated fatty acids (80%), the latter is poorer in these (40-50%) (Delisle, 2018). Due to the presence of high amount of antioxidants like α- and β- carotenes, vitamin-E and MUFA than PKO, the PO has been reported to be useful in preventing atherosclerosis and thrombosis (Edem et al., 2002). Further, despite similar SFAs like in animal fats, the palm oil possesses less absorption value for these fatty acids in comparison to former and thus, suggested to have less hyper cholestrolemic effect and decreased lipaemia in humans (Sanders et al., 2011)

Defatted oil palm kernel meal is rich source of protein with approx.55% protein content. Solubility based fractionation has revealed the glutelins as the major protein fraction followed by albumins and globulins (Chang et al., 2014). As per WHO, (2007) essential to total amino acids ratio (E/T%) for glutelins and globulins makes oil palm kernel meal an ideal protein source for adults (Chang et al, 2014). Low proportion of aromatic amino acids (F, Y and W) in these fractions has suggested the use of palm kernel meal for the patients suffering with PKU and encephalopathies diseases (Trahms and Ogota, 2008). Defatted protein rich meal can be used to produce protein isolates and hydrolysates having health promoting properties similar to that of other oilseed crops like soybean, walnut and lupins. Further, studies on palm kernel globulin isolate are reported to have anti-cancer activity, therefore, could be used as a health –beneficial dietary protein (Tapal et al., 2016).

Coconut

Coconut (*Cocos nucifera* L.), a monoecious plant with diploid chromosome number (2n = 32), is the sole living species of the genus *Cocos* in the monocot family - Arecaceae (Perera et al., 2017). It is an essential tropical plant adapted to varied environmental conditions which requires rainfall of about 200 cm per annum with 27± 5°C temperature and more than 60% humidity. Coconut palms are grown in about 95 nations and territories of the world over an estimated area of 12 million hectares with production of about 68 million nuts. More than 72% of the world total coconut production is covered by India, Indonesia and Philippines (www.statista.com).

Coconut kernel, the main edible part of the plant, is used to extract the oil. The oil is rich in saturated fatty acids (SFAs) like which contribute about 93% of total fatty acids with lauric acid ($C_{12:0}$) comprising of only 50-55%. The medium chain saturated fatty acids (MCFAs) like caproic acid ($C_{6:0}$), caprylic acid ($C_{8:0}$), capric acid ($C_{10:0}$) and lauric acid ($C_{12:0}$) are good source of energy and render oil having the antiviral, antibacterial, and antiprotozoal properties. Some amount of monounsaturated and polyunsaturated fatty acid like oleic acids(C18:1) and linoleic acid ($C_{18:2}$), in the same proportion i.e. 7-8%, are also present in the coconut oil (Bhatnagar and Hemavathi, 2009; Appaiah et al., 2014). During chemotherapy of breast cancer patients, consumption of virgin coconut oil helps to improve the quality of life and reduces the side effects of chemotherapy (Law et al., 2014). Moreover, the composition of virgin coconut oil has been reported like the mother's milk in terms of MCFAs, thus, provides baby immunity to diseases (Madhvan et al., 2010).

Coconut proteins have also been reported to have high nutritional value with a balanced amino acid profile. Nine essential amino acids (**L, K, I, M, T, W, V** and **F**) and nine non-essential amino acids such as **Y, E, S, A, P, R, D, Q** and **C** are present in the coconut. Out of these amino acids, coconut proteins have high level of R (14.2-17.2%), E (17-27.2%) and D (5.6-8.9%) (Gonzales and Tanchuco, 1977; Kwon et al., 1996). Albumins and globulins have been identified as the major protein fractions in coconut meat proteins (CMP) accounting for 21% and 40% respectively, followed by glutelin-I (14.4%), glutelin-II (4.8%) and prolamins (3.3%) by Kwon et al. (1996). However, the globulins again remained the dominant fraction in CCP (54%), albumins were reported to be lower in proportion (9%) vis-à-vis CMP (Li et al., 2018). Globulins consist of major polypeptides of MW 53kDa with less abundant subunits of MW 34,27,25,22 and 20kDa and albumins with two major polypeptides of MW 25 and 18kDa. As far the nutritional value of CCP is concerned, globulins and prolamins were shown to be rich in aromatic (**T** and **F**), sulphur-containing (**M**) and hydrophobic amino acids, which contribute to antioxidant and ion-chelating properties to these fractions (Kotecka-Majchrzak et al., 2020). Owing to such properties these fractions could be suggested as the food ingredients in foods like meat patties to enhance their shelf-life.

Flaxseed

Linseed (*Linum usitatissimum* L.), also known as flaxseed, one of the world's oldest cultivated crops belongs to the family Linaceae (Rai et al., 2016). There is a small difference while using terms linseed and flaxseed; whereas flaxseed is used when the unprocessed seed is consumed as a food/feed by humans/animal, the term linseed is used when seed is used in industry for oil extraction (Morris,2008). Flax varieties are grown to get either oil from the seeds or fibers from the stems. Globally, the area comes under cultivation of flax is about 3.3 million hectares with an average yield of about 1 ton/ha (Ceh et al., 2020).

Linseed seeds are evaluated on the parameters of its oil content and its fatty acid (FA's) composition. The oil content varies from 31-45% according to different reports (Zajac et al., 2012). Linseed oil is unique in its FA composition as it is a rich source of ω-3 FA's, the alpha linolenic acid (ALA) serves as the parent FA of these. Generally, linseed seed oil consists of about 9-11% saturated (palmitic acid (5%) and stearic acid (4%)) and 75-90% unsaturated FA (55-57% ALA and 16% oleic acid) (Thambugala et al., 2016). Tocopherols (gamma and alpha tocopherol), phenolic acids (cinnamic and benzoic acids), lignans (SDG) and flavonoids are the antioxidants, also present in linseed (Bruhl et al., 2007; Saastamoinen et al., 2013). Because of the potential health benefit of ω-3 FA's like lowering risk of cardiovascular diseases, decreased risk of cancer, anti-inflammatory activity, laxative effects, alleviation of menopausal symptoms and osteoporosis, relieving stress, the demand of flaxseed is increasing day by day and it is advised to be included in a normal diet (Newkirk, 2008).

Flaxseed is also a source of quality protein. Upon the genetics, growing conditions and method of analysis, the seed protein content of flaxseed varies from 20 to 30% (Jhala and Hall, 2010). Seed protein fractionation studies have shown the flaxseed to consist of two major types of proteins – the globulins (11-

12S/linin) with high molecular weight, and the albumins (2s/conlinin) with low molecular weight polypeptides (Youle and Huang, 1981; Karaca et al., 2011). Amino acid profile of flaxseed proteins is comparable to soybean and mainly composed of **R, D, E, Y, V** and **F** amino acids; however, it is limiting in **K, M** and **C** (Oomah, 2001; Chung et al., 2005; Kotecka-Majchrzak et al., 2020).

Flaxseed protein products associated with several kinds of hydrophilic polysaccharide gums have been reported as an additive in food systems (Dev and Quensel, 1989). Because of the good functional properties like foam stability and water absorption capacity, the whole wheat flour supplemented with defatted flaxseed flour has shown to improve the sensory attributes of Indian bread (chapattis) (Hussain et al., 2012). Several clinical studies on flaxseed have reported the benefits of flaxseed proteins as anti-tumoric, anti-thrombic, anti-diabetic, antioxidant, and antifungal agent (Tolkachev and Zhuchenko, 2000). Besides oil and proteins, flax fibers present in the seeds help in managing the constipation, blood glucose and blood cholesterol level (Madhusudhan, 2009).

Castor bean

Castor bean or castor *(Ricinus communis L.*), one of the oldest and important non-edible oilseed crops, with monotypic genus belongs to the spurge family - Euphorbiaceae (Saadaoui et al., 2017). It is native of the Eastern Africa (Ethiopia); however, grown as annual in temperate climate and perennial in tropical and subtropical regions worldwide (Rai et al., 2016). The world castor production is 1.41 million tonnes with area covering 1.16 million hectares (FAOSTAT, 2020). The chemical composition of the castor seed includes water (5.5%), crude protein (17.9%), crude fiber (12.5%), ash (2.5%), carbohydrates (13%) and oil (up to 55%) - which is the main part (Ogunniyi, 2006). Of the total protein present in the castor seeds, the 2S-albumins occupy the major protein fractions followed by 11S globulins (Youle and Huang, 1978). 2-S albumins have been shown to be rich in **R** and **Q.**

The unique property of the castor seed oil (due to the presence of 12-hydroxyoleic acid or ricinoleic acid that provides stability to the oil and greater shelf-life) makes it an industrially important crop; although its contribution in the total vegetable oil production is only 0.15% in the world. Due to this uncommon 18-C ricinoleic acid (84-90%), castor seed oil falls in the category of non-edible oilseed crops (Yadava et al., 2012). Besides this, castor oil also contains linoleic acid (0.5%), oleic acid (3.5%), stearic acid (1.3%), palmitic acid (1.5%) and linolenic acid (7.5 %) of the total fatty acid (Udoh et al., 2016). Apart from these, castor beans also contain some allergenic proteins (2S albumin) and toxic protein (ricin – a RIP) which act like anti-nutritional factors that also make the seeds unfit for the human consumption (Schieltz et al., 2015). Castor oil is used as a valued oilseed crop for industrial applications, and used as raw materials for several products like paints, polishes, textile dyes, perfumes, waxes, soaps and lubricants (Grummitt and Marsh, 1953; Mutlu and Meier, 2010). In pharmacology and medicine, the castor oil is utilized as laxative, antidiarrheic agent and drug-delivery vehicle for some non-polar drugs (Subramaniyan, 2020). The castor oil has drawn great attention because of its potentiality as a source of biodiesel production and its capacity to grow on marginal lands as non-food crop (Severino et al., 2012).

Groundnut

Groundnut (*Arachis hypogaea* L.) is one of the most valuable annual legume crops grown in both tropical and temperate regions around the world. It belongs to the Leguminosae (Fabaceae) family and considered as "King of oilseeds" (Biswas and Bhattacharjee, 2019). All plant parts except roots are considered useful for both humans and livestock. Globally, the crop is grown over an area of about 29.5 million hectares with total production of 48 million tonnes of pods (FAOSTAT, 2020). Groundnuts are being used as the complete dietary source for people on expeditions to diverse areas like Antarctica, space and trekking. It is also known as poor man's cashew nut for its high nutritional content. The major components that make up groundnut are protein, fats, and fibres which are present in their most beneficial forms. High quality oil content makes it the world's leading oil seed crops.

The oil content in this crop varies from 48-50% in the kernels which provide 564 Kcal of energy /100g of kernels (Jambunathan, 1991). Three fatty acids viz. palmitic acid, oleic and linoleic acid account for approximately 90% of total fatty acids in kernel with relative contribution of 10%, 45% and 32% (Young and Waller, 1972). The high oleic to linoleic acid makes the groundout more stable and less prone to oxidative changes during its storage and refining. Because of its high oleic content, the oil is effective in reducing insulin sensitivity and cholesterol level (Zhao et al., 2019). Further, the groundnut oil is also used for manufacturing margarine, medical emulsions, wool and silk, artificial leather, soap and toilet requisites.

Groundnuts, containing almost all the 20 amino acids in varied proportion, are high quality protein source; however, it is also a biggest source of "**R**" amino acid (USDA, 2014). The protein content in the kernels ranges from 20-40 % (Pandey et al., 2012). The digestibility of groundnut protein is highly comparable to that of animal proteins (Singh and Singh, 1991). The major proteins in groundnut kernels belongs to arachin (acidic and basic) and conarachin with 63% and 33% relative proportion (Savage and Keenan, 1994). As far the techno-functional properties of groundnut protein is concerned, it has good emulsifying activity, emulsifying stability, foaming capacity, excellent water retention and high solubility, and can prove as a high protein food ingredient in protein fortification in food and feed industries (Jamdar et al., 2010). The groundnut protein can also be used for making peanut protein biopeptides, hydrolysates, protein films etc.

Soybean

Soybean (*Glycine max* L.) is a monoecious annual plant belonging to the family Leguminosae (Sinha et al., 2020). It is the native of East Asia and grown in temperate and tropical regions with 60-65 cm annual rainfall. The world soybean production is 334.89 million metric tons with covered area 121.53 million hectares with yield of 2.76 ton/hectare. Soybean contributes 59% of the world oilseed production and 29% to the total vegetable oil consumption in the world (http://soystats.com).
The oil content ranges from 19-23% in the seeds and contains both saturated and unsaturated fatty acids (Panthee et al., 2004). The oil consists of 55% linoleic acid, 18% oleic acid, 13% palmitic acid, 8% linolenic acid and 4% stearic acid (Fehr WR, 2007). Soybean oil has poor oxidative stability than other vegetable oils due to lower ratio of MUFA/PUFA which limits its use for industrial applications (Rani et al., 2007). Apart from the oil, isoflavones like genistein, daidzein and glycitein are the main components of the flavonoids present in the soybean (Messina, 1999). These help in preventing the cardiovascular diseases, menopausal symptoms, osteoporosis and cancers (Dilawari et al., 2022).
Soybean protein has been considered equivalent to animal protein because of its amino acid composition and high digestibility (Cromwell, 2012), however, it is deficient in only sulphur containing amino acids **M** and **C**. It is the major source of protein in livestock, poultry and fish farms. 70% of the protein out of the total (40%) present in the form of storage proteins (Derbyshire et al., 1976). Glycinin (11S) and β-conglycinin (7S) belonging to globulins are the major storage proteins in soybean which constitute 60% and 40%, respectively (Thanh and Shibasaki, 1979). As the soybean globulins are relatively low in sulphur containing amino acids as well as **T** and **K**, improving the ratio of glycinin to β-conglycinin can provide the great opportunity for soybean improvement (Warrington et al., 2015). Consumption of soy products help in lowering total blood cholesterol and LDL cholesterol compare to animal protein (Dilawari et al., 2022). Soy protein can be used for repair and synthesis of muscle protein in response to strenuous exercise by providing indispensable amino acids (Rodrigues et al., 2012).

Sunflower

The sunflower (*Helianthus annuus* L., 2n=34), a ubiquitous oil crop belonging to the family Asteraceae, is known for its wide medicinal and nutritional benefits. The word “sunflower” is attained on the basis of its size and the image of the plant which resembles the sun. The cultivated sunflower varieties fall into three major groups – A) oilseed types, B) non-oilseed or confectionery-type and c) those used as ornamentals; the last one contributes the minor portion of sunflower production (Velasco et al., 2014). After soybean, rapeseed and safflower, it is the most profitable oilseed crop known as “Champion of the oil seed” (Adeleke and Babalola, 2020). The cultivation area under sunflower has been estimated about 27 million hectares with production of around 56 million metric tonnes (FAOSTAT, 2019). It is grown worldwide and its products are mainly commercialized as culinary or livestock feed (Yegorov et al., 2019). Sunflower as an oil seed crop consists of 20-40% protein and 47-65% lipids (Weisz et al., 2009). Sunflower oil (sunf-oil) is mainly composed of high concentration of linoleic acid (71.7%), moderate level of oleic acid (15.9%), vary low level of alpha and gamma linolenic acid (0.6% and 0.1%) (Arshad and Amjad, 2012). The SFA’s in the oil are comparatively low then MUFA and PUFA and represented by 5% palmitic acid and 6% stearic acid (Petraru et al., 2021). The consumption of sunf-oil is advantageous in maintaining LDL and cholesterol levels in the body. Further, it has numerous applications in the cosmetic industry due to its water retention and non-inflammability (Oliveira et al., 2016). SFO is also an excellent source of carotenoids, vitamin E, lecithin and sterols like other oil crops (Nandha et al., 2014). Sunflower oil retains moisture in the skin and act as a protective barrier that resists infection in premature infants (Arshad and Amjad, 2012). The seed extract of sunflower has shown to be effective in case of diabetes and microbial infections (Menzel et al., 2019).
The seed proteins in sunflower are mainly represented by storage proteins (85%) (Shewry and Casey, 1999). The two major groups of storage proteins in sunflower are 11S helianthinin which belongs to legumin-like globulin and 2S albumins (Zilic et al., 2010). Whereas, former is poorer in sulphur containing amino acids- **C** and **M**, the 2S albumins are rich (Youle and Huang, 1981). The exploitation of sunflower seeds products with high protein content has found applicable in food processing and various pharmaceutical and agriculture. The sunflower seeds can be a potential protein source in food preparations as a substitute to soybean, where its production is limited (Oliveira et al., 2016). The processed seeds contain high amount of proteins and antioxidants but low carbohydrates; the use of these in different forms like flour, roasted or boiled as composite functional foods help in preventing human diseases such as diabetes, cancers, hypertension and cardiovascular diseases (Grasso et al., 2020).

Rapeseed

Rapeseed/canola (*Brassica napus* L.), an allotetraploid species, belongs to genus *Brassica* in the dicot family- Cruciferae which has arisen from the spontaneous hybridization between *B. oleracea* (cabbage) (2n=18) and *B. rapa* (turnip) (2n=20) (UN, 1935). It is an important oil producing crop native to Western Europe, the Mediterranean, and temperate regions of the Asia. After soybean, it is the second largest oil producing crop in the world (Oil world, 2017). The world production of rapeseed in 2019 was 59.84 million tonnes with harvested area of 32.09 million hectares (www.fao.org, 2019). China is the largest producer of rapeseed covering one third of the world production. Rapeseed and mustard seed oil are the only crucifer oils important in commerce with primary use as edible oils; however, to some extent it can be used in a number of industrial applications (Mikolajczak et al., 1961).

The mature seeds contain oil in the range of 45-50% and proteins from 20-25% (Purkrtova et al., 2008). The typical fatty acid composition of rapeseed oil has been shown to include 4.18-5.01% palmitic acid with 1.4-2.5% stearic acid, 17.11-20.9% linoleic acid and 56.80-64.92% oleic acid (Matthaus et al., 2016). Rapeseed oil is also characterized by the presence of the high content of erucic acid which is not present in any other commercial plant oils (Sharafi et al., 2015). The toxicity due to the presence of erucic acid makes the seeds unsuitable for human consumption but suitable for some industrial products such as polyesters, detergents, surfactants, and plasticizers (Luhs and Friedt, 1994; o'Brien, 2008). The edible variety of rapeseed which is known as canola oil having less or no erucic acid (USITC, 2003) and less glucosinolates which is regarded safe for human nutrition and livestock (Dupont et al., 1989). The rapeseed seeds have also been reported as the potential source for biodiesel and bioplastics production (Wu and Muir, 2008). The rapeseed oil possesses the antioxidant property due to the presence of tocopherols, mainly α- and ϒ-, which constitute about 13-40% and 33-51% respectively (Matthaus et al., 2016). The seed storage proteins represent 85-90% of the total seed proteins in rapeseed with cruciferin (11S or 12 S globulins) and napins (2S albumins) protein fractions (Schwenke et al., 1981; Crouch et al., 1983). Whereas former is a heteromeric protein (300-350 kDa), the latter is of molecular weight 14-16kDa (Wanasundara, 2011). Seeds proteins of canola are having balanced amino acids and are being used in food industries to replace animal products like eggs, milk etc. and also used as an animal feed (Gacek et al., 2018). However, there are also present some anti-nutritional factors in rapeseed such as glucosinolates, phytates, phenolics etc. which impart inferior physicochemical properties and poor protein digestibility of the seeds (Wu and Muir, 2008; Yoshie stark et al., 2008).

SEED QUALITY IMPROVEMENT

Since the beginning of agriculture, the man has aimed to improve the cultivated crops to achieve higher yields and enhanced nutritional quality. As we know that the oilseed crops are mainly grown for the oil but at the same time proteins isolated from some of these crops can be the alternative source to animal-based proteins. Various conventional methods viz. hybridization, mutation, breeding etc. have been employed, mainly in the past, to enhance the yield of oilseed crops. Now a day, not only the yield but also the quality of oil as well as proteins has attracted more attention due to its importance in health, livestock feeds and pharmaceuticals (Sosa-Segura et al., 2014). Besides these major biochemical constituents, reduction in anti-nutritional compounds present in some of the oilseed crops would definitely be the concerned area of research to further enhance the quality of a seed. For improving the seed quality, conventional techniques employed to identify favourable crop characteristics for use in plant breeding are often insufficient in determining specific gene trait association. Therefore, research at molecular level using various advanced technologies (r-DNA, CRISPR/Cas and Omics) would be promising to achieve the desired targets. Omics is sum of the technologies (proteomics, genomics, transcriptomics, and metabolomics) used to study the functions, relationships, and mode of actions of diverse molecules viz. proteins, mRNA and certain metabolites which make up the cells of an individual. Whereas, most of the traditional approaches were hypothesis based, the omics integrated with system biology is hypothesis-generating. It acquires data and analyse it to generate a hypothesis which can be tested further (Komala et al., 2017). On the other hand, rDNA technology or genetic engineering is also more accurate and effective technique to attain the desired modifications in the seed quality. In recent years, genome-editing tools have been successfully used to alter the nutritional qualities in different crops without introducing any foreign gene with higher accuracy and efficiency (Hamza et al., 2021). In the next sections, we will deal with different methodologies adopted by different workers to make improvement in various oilseed crops.

Genetic Engineering approaches to improve seed oil and protein quality

Oilseed crops viz. soybean, rapeseed, sunflower, cotton, and mustard are cultivated for extracting the edible or industrial oils as per human desire. With increasing demand of these oils, crop breeders and biotechnologists have been trying to modify/alter the fatty acid composition in these crops using different transgenic approaches including r-DNA technology and targeted RNA interference technique.

Soybean

Transgenic approaches

In the recent years, with increasing awareness of the people towards the use of beneficial dietary lipids, modulation of the endogenous levels and /or the production of novel kind of fatty acids have gained the significant attention of the researchers. In soybean, the poor oxidative stability of oil due to its high content of polyunsaturated fatty acids (PUFA) as well as the generation of *trans*-fatty acids on partial hydrogenation of these limit its use in food products and industrial applications (Demorest et al., 2016). Seed oil profile of soybean, in term of both quality and quantity, has been successfully altered through transgenic approaches by various workers from time to time for end use in different industries (Al Amin et al., 2019; Kanai et al., 2019). These approaches were mainly concentrated on elevating the stearic acid in combination with high oleic acid (HO) content, and also lowering down the linolenic acid (ALA) content in oils; however, some contradictory research reporting the health benefits of increased ALA and LA are also there (Amjad Khan et al., 2017). Controlling the expression of *FAD-2* and *FAD-3* genes, known to produce desaturases in soybean, has been reported to affect the fatty acid composition in oils. Different types of *FDA2*s (*FAD2-1A*, *FAD2-1B* and *FAD2-2*) showing both constitutive and tissue specific expression have been identified in soybean (Lakhssassi et al., 2017). A good number of studies have involved down-regulation of *GmFAD2*, *FAD2-1A* and *FAD2-1B* genes using RNAi- and antisense-mediated knockdown technology which resulted in 20 to 80% increase in the oleic acid content in transgenic soybean seeds (Zhang et al., 2014). Similarly, the expression of *GmFAD2-1B* RNAi fragment under the seed-specific promoter of the soybean β-conglycinin alpha subunit gene (PBCS) reduced the expression of *GmFAD2-1B* gene in the seeds and enhanced the oleic acid content (Yang et al., 2018). Soybean also possesses four *FAD3* genes, three of which (*GmFAD3A*, *GmFAD3B*, and *GmFAD3C*) are expressed in the seeds and control ALA content in the oil (Anai et al., 2005). To produce the seeds with low ALA content, the expression of this gene (*FAD3*) must be repressed. High oleic acid and low linolenic acid soybeans were produced by incorporating two loci for low linolenic acid including mutant *GmFAD3A* and *GmFAD3C* (Lakhssassi et al., 2017. In contrast to this, a heterologous gene *PfFAD3-1* from *Physaria fendleri* was expressed in soybean seeds to produce oil reach in ω-3ALA (Yeom et al., 2020) keeping in mind the importance of ALA in producing eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) in the human body. Further, the soybean oil enriched in EPA and DHA could be used in manufacturing food and feed products. Although saturated fats, like palmitic acid, have negative effect on cardiovascular system, stearic acid is considered neutral with very low effect on the blood cholesterol level. Among all enzymes involved in FA synthesis, 3-keto-acyl-ACP II (KAS-II), steroyl-ACP-thioesterase (*Fat A*) or Δ9-steroyl-ACP-desaturase (*SAD*) are known to be able to change the stearic acid content. Down-regulation of *SAD* or heterologous expression of *Fat A* has been suggested as the strategies to increase the level of stearic acid in the soybean seeds (Clemente and Cahoon, 2009). Expression of *GramFatA1* gene from mangosteen (*Garcinia mangostana*) into soybean seeds resulted in the accumulation of stearic acid up to 20% in seed oil (Vogel et al., 2019). Recently, Lakhssassi et al. (2020) using TILLING technique have identified soybean lines with mutation in the gene family *GmSACPD* which showed 2- to 3- folds increase in stearic acid content.

Despite being rich source of protein, the nutritional quality of soybean proteins is generally restricted due to poor content of S-containing amino acids –**M** and **C** in the major storage proteins –Globulins (11S and 7S) (Warrington et al., 2015). These AAs need to be constantly supplied from our diet as human body has no mechanism to synthesize these *de novo*. Notable efforts had been made in past to improve the protein quality of soybean seeds in terms of increased **M** and **C**. Improving the ratio of 11S fraction to 7S globulin fraction has been suggested one of the promising strategies for soybean seed quality improvement as former is a dominating fraction among both (Kaur et al., 2016). Alternatively, the introduction of heterologous gene which code for **M** –rich storage protein has been emphasized more to improve the seed protein quality. In this direction, Dinkins et al. (2001) introduced and expressed a maize gene, encoding 15 kDa α-zein, in transgenic soybean which resulted in 12–20% increase in **M** content as compared to wild type plant. Recently, **M**-rich maize seed storage protein, β-zein was expressed in the transgenic soybean, using legumin B4 promoter, which improved the total **M** content significantly in mature seeds (Guo et al., 2020). Similarly, a number of other workers have tried to improve the **M** level in soybean by expressing other zein storage proteins like 11 kDa δ-zein and 27 kDa γ-zein which resulted in the modest improvement in it (Kim and Krishnan, 2004). So, whatever improvement made in soybean have been resulted in limited success to meet the nutritional requirement, in terms of sufficient soluble methionine, of mammals. Therefore, efforts have also been made to enhance soluble methionine synthesis.

Gene Editing approaches

Besides transgenic approaches, gene-editing seems to be the promising alternative to improve the oil quality as it produces no pleotropic effects due to knocking out of the corresponding gene from whole plant cells. Moreover, the plants produced by this technology need not to be passed through stringent regulations or ethical issues before commercialization. CRISPR/Cas has also been used to introduce targeted edits within the soy genome mainly in FAD2 loci as it is involved in conversion of oleic acid into the linoleic acid with its high expression level in the seeds. Using CRISPR/Cas9 editing system, Wu et al. (2020) induced mutations in both *GmFAD2–1A* and *GmFAD2–1B* genes and obtained double homozygous soybean mutant seeds with a high oleic acid content (83.3%) vis-a-vis wild type plants having only 20.2% in its seeds. Similar study was carried out by Al Amin et al. (2019) who also edited the target sequence of FAD2–2 loci by introducing CRISPR-Cas9/sgRNA in the soybean. This resulted in considerable increase in oleic acid content (65%) with reduced linoleic acid content (16%) in the seeds.

Brassica

Oilseeds, among the *Brassica* crops, possess the highest economic value and thus, have been the major target for their improvement by biotechnological methods. Besides improvement in salt tolerance, insect resistance and herbicide resistance, the different genes transformed in *Brassica* species were also aimed at improving the seed oil quality and enhanced nutrients like amino acid, antioxidants, etc. The two ways by which the quality of edible oil can be improved include the improvement in the fatty acid content and/or unsaturated fatty acids composition. In this direction, the introduction of the *ChFatB1* gene encoding a palmitoyl- ACP thioesterase, in the conventional rapeseed lines, resulted in more than 35% of palmitic acid (16:0) in total fatty acids instead of 5–10% in these lines (Jones et al., 1995). Further, on expression of *PiD6* gene in *B. juncea* under the control of napin promoter led to the increased production of three Δ6 unsaturated fatty acids with ALA in maximum amount (Hong et al., 2002). Similarly, Das et al. (2006) introduced *d6D* from *Synechocystis* sp. in *B. juncea* and showed the biosynthesis of novel kind of γ-linolenic acid which is not present in this crop naturally. Another factor reducing the quality of seed oil in *Brassica* is the presence of erucic acid (EA) (a kind of storage fatty acid) which is very toxic for the heart. So, producing LEAR (Low Erucic Acid Rapeseed) lines has also been the target for seed quality improvement programs since 1968. The first LEAR cultivar Oro was bred by using the Liho as the parental material via conventional breeding (Downey and Craig, 1964; Slinkard and Knott, 1995). Now a day, *FAE1* gene has been an important target for up-regulation or down-regulation of EA accumulation. The antisense gene-mediated down-regulation of *BjFAE1* gene in *B. juncea* has revealed 86% decrease in erucic acid to as low as 5% in the transgenic seeds oil (Kanrar et al., 2006). Similarly, Tian et al. (2011) were successful in reducing the EA content in transgenic seeds from 61 to 99 % using the RNAi mediated post-transcriptional silencing of endogenous *BnFAE1.1* gene. In addition, it also resulted in significant increase in the oleic acid content which is a desirable feature to increase the thermal stability of the oil. In the recent past, CRISPR/Cas9 has emerged as a powerful tool for genome editing in many plant species including *Brassica* species. This gene editing system has also been used to produce mutants of *B. napus* plants by the modification of *Fatty acid desaturase2* (*FAD2*) gene which showed a significant increase in the oleic acid content (Okuzaki et al., 2018).

Cruciferins (12S globulins), napins (2S albumins), and oleosins (oil body proteins are the major proteins in oilseed rape seeds. Napins contain higher levels of sulfur and aromatic residues (essential amino acids) than cruciferins which make them the most important targets for the improvement of seed protein composition. Cruciferins (12S globulins) and napins (2S albumins) are the major proteins in oilseed rape seeds (Brunel-Muguet et al., 2015). Although cruciferins contribute 60% of the total proteins yet these are poorer in sulphur (**M** and **C**) and aromatic amino acids than the second most prominent seed storage protein, napin (Kohno-Murase et al., 1995). Thus, manipulating the cruciferins/napins ratio in the seed protein of *Brassica* could be the important target for the improvement of seed protein quality.

The cruciferin content of seeds was reduced by the introduction of the antisense gene and the reduction was balanced by an increase in napin content. This change resulted in an increase in the relative levels of essential amino acids in the seed storage proteins. The results showed that the seeds contained increased amounts of lysine, methionine and cysteine. (Kohno-Mursae, 1995). The transgenic seeds contained 32% more cysteine than non-transgenic seeds, and lysine and methionine contents were 10% and 8% greater, respectively, than those in control seeds. This increase is similar to that obtained by Altenbach et al. (1992) who increased the levels of methionine in seed protein by the introduction of a gene for a methionine-rich protein from Brazil nut. Introduction of the antisense gene for cruciferin resulted in increased levels of cysteine, lysine and methionine essential amino acids in the SSPs of the *B. napus* seeds. Wang et al. (2011) expressed the lysine-rich protein (LRP) gene obtained from *Psophocarpus tetragonolobus* in *B. napus* and found increase in lysine content.

Sesame

Transgenic approaches

The FAs composition and the enzymes involved in metabolism play an important role for oil quality improvement. The quantity of these FAs as well as the expression of endogenous enzymes regulating these can be altered by genetic engineering. A number of important enzymes involved in FAs manipulation include fatty acid synthase, thioesterases, desaturases, acyltransferases, and hydroxylases. Sesame, among other oilseed plants, has been the major target to increase α-linolenic acid (ALA, C18:3, ω-3) content in its seed oil as it contains less amount of this fatty acid (Mondal et al., 2010). In this direction, *FAD3C* gene from soybean was introduced into sesame under the control of *2S albumin* seed-specific promoter which resulted in enhanced level of ALA in its oil (Bhunia et al., 2014).

Sunflower

Transgenic approaches

Sunflower oil has been recognized as high-quality edible oil after soybean and rapeseed and significantly contributes to human health (Adeleke and Babalola, 2020). However, the high oleic sunflower lines contain significant amounts of stearic acid (5–6%) which causes problems for certain industrial uses because of the high clouding point of the oil. Reduction in the stearic acid content may allow more efficient use of sunflower oil. In this direction, Roussselin et al. (2002) over-expressed heterologous *ACP* desaturase gene in the sunflower, and a significant decrease in the amount of stearic acid (5.7 % in wild types to 3% in transgenic seeds) was observed.

Rapeseed

Transgenic approaches

Suppression of the oleate Δ12-desaturase gene (which normally converts 18:1 to 18:2) in canola has resulted in the production of oils with a high oleic acid content, which have greater oxidative stability and improved performance in high temperature cooking application (Dyer et al., 2008).

Conclusion

This comparatively succinct analysis has described the current state of transgenic crops with a focus on the data that are currently accessible and offer a clear picture of the prospective future products. There should be no question that, when correctly carried out, genetic engineering is a very safe, quick, and affordable approach to enrich significant oilseed crops for key nutritional contents, as has been demonstrated by modern biotechnology. Agriculture research is currently facing and will face formidable obstacles.

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