Review article

ENHANCING NUTRITIONAL VALUES OF OIL SEEDS THROUGH GENETIC ENGINEERING

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Abstract: The aim of this paper is to emphasize the use of genetic engineering to improve the oil quality in oilseed crops. Genetic engineering is a powerful tool to enhance the nutritive values of oilseed crops. Naturally the nutrition decreases due to constant growing of the different crops on the same land. The need is to adopt modern techniques that are less time consuming and give the best results. Different genetic engineering techniques like hairpin mediated transformation, gene silencing, Agrobacterium mediated transformation, somatic hybridization are very helpful in transforming the genetic makeup according to our own need. Plant oils are considered beneficial when the level of Erucic acid and Glucosinolates is low. This becomes possible through adopting the modern techniques of genetic engineering. Major oilseed crops like cotton, canola, soybean, sunflower need few genetic modifications to enhance their oil quality. The main concern is to introduce such genes which can add to its value. Through the help of biotechnology, we are able to decrease the gossypol level in cotton seeds and not in the leaves which is the remarkable work of biotechnology. Tph1, tph2 and o1 genes are introduced in sunflower to enhance Tocopherol level, which is useful to maintain oil stability. Such as LPAT gene introduction in soybean helps to enhance oil contents. Increase in soybean biodiesel production is also achieved by genetic engineering. Gene silencing also plays a major role to enhance stability of oils by minimizing linolenic acid level.

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Introduction:

Oilseed crops are essential to the human diet after cereals and sugar crops. This is the valuable source of vegetable oil that is an important way of providing significant energy to human approximately 2.5% more than proteins and carbohydrates. In addition to this important fatty acid and polyunsaturated compounds are beneficial to the human body. Vegetable oil is better than the ghee obtained from animal source because it has the unsaturated components that have zero cholesterol level harmless for the heart. The major sources of edible seed oils are soybean, sunflower, rapeseed, cotton, and peanut. Oil seeds are also used as animal feed due to its high protein content. (Sarwar *et al.*, 2013) The total availability of edible oil was 2.335 million tonnes in 2014-2015. While the domestic production contributed just 0.573 million tonnes and 2.627 million tonnes was imported from the foreign. The edible oil import bill was Rs. 246.895 billion (US$ 2.50 billion) in 2014-2015. Major oilseed crops grown in Pakistan are canola, sunflower, rapeseed/mustard, cotton, and maize. There is a need to enhance the domestic production. (Pakistan Economic Survey 2014-15)

Oilseed crops contribute much in the nutrition of both human and animals because of containing high-quality protein and essential oil soluble vitamins. After extracting the oil remaining meal is used for animal feed. Good quality oil is that containing the lowest amount of Erucic acid and Glucosinolates. Essential fatty acids and vitamins are necessary to take in our diet. Studies show that many diseases are related to the deficiency of important fatty acids. Breeding objectives are the same for all the crops like enhancing yield, developing resistance and modified plant architecture. But through genetic engineering, metabolic pathways are successfully modified and we can produce the products e.g. Erucic acid-free rapeseed oil, high oleic acid sunflower and Glucosinolates-free rapeseed meal. (Toenniessen, 2002)

For the years, farmers have been using the same techniques of selection and variation. Farmers have been selecting the plants with the suitable traits and then bringing these traits in subsequent generations. Breeding techniques are laborious and time-consuming. Genetic engineering made it possible to take a particular gene from one organism

and insert it into another unrelated organism. (Singh *et al.*, 2014) Natural yield can't fulfill the increasing demands of the people. Without adopting modern methods of plant breeding and genetic engineering, we can't produce high yielding, improved quality, and insect resistant cultivars. Advances in biotechnology resulted in the modification of important crop plants. The use of living organisms for the benefit of human is called biotechnology. Genetic engineering is the tool of biotechnology meaning to alter the genetic makeup of an organism artificially. Genetically modified organisms (GMOs) are the organisms whose genetic material (DNA) has been modified through unnatural means. This technology is referred to as "modern biotechnology" or "gene technology", sometimes also "recombinant DNA technology" or the "genetic engineering" (Gabol *et al.*, 2012).

With the new avenues in the molecular biology, we are now able to the precise transfer of novel genes into the crop plants and modify their metabolism. The development of well-worked procedures for the somatic cell formation, tissue culturing, transformed pollen and protoplasts, improved DNA vector-based systems, marker genes, series of promoters and a number of cloned genes has made the genetic transformation more precise and directed. (BRAR *et al.*, 2006) It is, for this reason, genetic engineering is considered a more efficient technique as compared to older techniques. Genetically engineered plants are produced in 90 different species of plants, and successfully cultivated over the country. An important task is to improve the fatty acid composition of vegetable oils that describe their nutritional value and their oxidative stability." (Jose, 2002)

Breeding ways are conventional, laborious and time-consuming. It takes about 8-10 generations to develop a variety. That's why we go for the genetic engineering, which is a way to alter the genetic makeup according to the desire of the farmers. A considerable amount of research has already been taken in this regard, but still there is a need to improve its quality for human and animals. The fatty acid composition is governed by the complex biosynthetic pathways and genetic manipulations of these pathways modify the fatty acid composition. Most of the plant fatty acids are synthesized by the acetyl-coA by a series of reactions in the plastids. Most of the genes are cloned involving in the biosynthetic formation of fatty acids, e.g. acetyl-coA carboxylases, ACP thioesterases and acyl carrier protein ACP, etc. Although the vegetable oils already contain less saturated fatty acids than the animal fats but still there is a need to reduce this minimum amount (10-20%). The major contribution of saturated fatty acid is palmitic acid. Through the transformation of an additional acyl-ACP thioesterase has produced the 2-fold reduction in saturated fatty acids. Another way is to introduce the membrane-bound desaturases can significantly change the saturated fatty acids into the unsaturated fatty acids. Many genes taken from the rat and yeast have proved helpful in reducing the saturated fatty acids. (BRAR *et al.*, 2006)

(1) Cotton:

Cotton has its position among the important field crops as it is the 3rd largest field crop. Cotton is the 5th best oil producing plant and 2nd important source of proteins. Its oil is light, non-oily consistency and desirable for cooking. (Benbouza *et al.*, 2010). It has been grown for its fiber for more than 7000 years. It is the most important source of fiber in the textile industry. It is cultivated in more than 80 countries and earns cash for approximately 20 million farmers in the countries of Asia and Africa. One kg of cotton fiber produces approximately 1.66 kg of seed. Cottonseed contains 21% oil and 23% high-quality protein valuable for both humans and animals. But the problem is its toxic gossypol which makes it unfit for human and animal consumption. Cotton seeds contain glands producing toxic gossypol and other terpenoids. So, the elimination of gossypol has been a goal of the geneticists. Efforts were made in 1950's to produce the "glandless cotton" but this was unviable due to the increased susceptibility of it to the insect pests. Researchers successfully used RNAi to reduce the gossypol level by the interfering the expression of δ-cadinene synthase gene. Gossypol and other terpenoids involved in the defensive mechanism of the plant. Tissue-specific RNAi of δ-cadinene synthase to disturb the terpenoids biosynthesis presents a possible way to eliminate the gossypol from the seed but not from the rest of the plant. (Sunilkumar *et al.*, 2006)

Moreover, cottonseed oil is composed of 26% palmitic acid (C16:0), 15% oleic acid (C18:1), and 58% linoleic acid (C18:2). High level of palmitic acid is effective for high-temperature frying but not good for the nutrition of human because of containing low-density lipoproteins. (Cox *et al.*, 1995) To overcome the drawbacks of conventional breeding geneticists, adopt post-transcriptional gene silencing to minimize the action of desaturase enzymes that control the synthesis of stearoyl-acyl carrier protein (ACP) _9-desaturase, which converts stearic acid into oleic acid, and oleoyl-phosphatidylcholine (PC) _6-desaturase, which converts oleic acid into linoleic acid. (Knutzon *et al.*, 1992)

Hairpin RNA-mediated gene silencing is used to down regulate the expression of two fatty acids stearoyl-acyl carrier protein (ACP) _9-desaturase and oleoyl-phosphatidylcholine (PC) _6-desaturase. The resulting down-regulation enhances the stearic acid up to 40% and oleic acid up to 77%. Palmitic acid was lowered in both high stearic and high oleic lines. Silencing makes the new combinations of stearic, oleic, palmitic and linoleic acid that can be used in margarine and high-value confectionery products. (Liu *et al.*, 2002)

Genetic engineering has a great role in improving the cottonseed oil. It is favorable for food industry but it is subjected to hydrogenation which makes it stable for the frying at a high temperature and softens it. Hydrogenation produces the trans-fatty acids not good for the health. Trans fatty acids enhance the blood cholesterol level. Researchers try to switch off the gene that converts mono un-saturates to poly un-saturates. They try to produce naturally hydrogenated cottonseed oil free from trans- fatty acids. (Agarwal, 2007) It is also reported that length and strength of the cotton fiber are increased in transgenic plants expressing the *G. hirsutism* sucrose synthase (Gh-SusA1) gene. (Jiang *et al.*, 2012)

By using the techniques of gene silencing, particle bombardment, agro-bacterium transformation many additional features can be introduced which make it favorable for the commercial application. (Liu *et al.*, 2002)

(2) Canola:

Canola has been familiarized by genetic alteration in fatty acid profile of Brassica cultivars, rapeseed (*Brassica campestris* and *Brassica napus*) and mustard (*Brassica juncea*) to improve the oil and meal quality. Canola quality Brassica genotypes have less than 2 percent Erucic acid in the oil and 30 µm Glucosinolates (less than 30mg/g) in oil free meal, also called double zero cultivars. Its seed contains oil content ranging from 40-45% and protein 36-40%. (Mustafa *et al.*, 2015) Among the oilseed crops, the main focus is on the brassica crops including canola and rapeseed /mustard widely recognized by their yellow flowers. Canola is an important oilseed crop for its quality oil and valuable source of fats and proteins. In the strict sense canola, oil is defined as an oil must have less than 2% Erucic acid and less than 30µM of Glucosinolates. Rapeseed with low levels of Erucic acid (< 2%) and Glucosinolates (< 30 µ mol/g) are called "double-zero" or "double-low" (Mailer et al.,2007). Being a high yielding oil crop brassica seeds contain 45% oil and 55% high protein animal feed which make them a superb candidate for producing feedstock oil for the biodiesel. (Cardoza *et al.*, 2004)

Increasing global demand for oilseed crops persuade researchers to develop new techniques and methodologies to enhance oil contents, improving oil quality and protein contents for animal feed. Biotechnology provides necessary tools to increase the nutrition and provide diversified food to the people. (Toenniessen, 2002) Nutritional qualities of oilseed crop can be increase through the modern methods of genetic engineering. Among the GMO's in the world GM canola constitutes only 7% of the total area and there is a strong need to improve this condition. Genetic engineering can also be used to develop insect resistance canola in the worldwide. Many genes taken from the other organisms can be inserted into the canola to improve the quality of canola oil depending upon its response to above-mentioned techniques. (Brar *et al.*, 2006) Embryo-specific promoters were combined with a cloned cDNA of *B. rapa* stearoyl-ACP desaturase to form antisense gene constructs made to lower enzyme levels in the developing seeds. The accumulation of stearoyl-ACP desaturase mRNA is developmentally regulated in *B. rape* embryos. Silencing of the endogenous oleate desaturase genes produces the increased level of oleic acid up to 89% in *B. napus* and 73% in *B. juncea*. (Deborah *et al.*, 1992)

The genes are cloned under the specific promoters and transform the plants with these constructs. Many techniques like micro-projectile bombardment, electro-poration, and *Agrobacterium tumefaciens*-mediated transformations have been used for canola transformation. Genes from the rat and yeast can be taken to convert saturated fatty acids into saturated fatty acids. (Matthäus, 2006) The nutritional quality of oilseed rape is mainly analysed by the Fatty acid balance and the protein level. Whereas the Fatty acid balance determines the quality of edible oil, the protein content is a major contributor to the meal energy value for feed (Nesi *et al.*, 2008)

Genetically modified canola has become tolerant to heavy metals. Canola could be a wonderful candidate for the phyto-remediation and can be used in making the bio-polymers. The fuel oil capabilities can be enhanced. Especially it is a very useful thing for producing pharmaceutically active proteins and edible vaccines. *Arabidopsis thaliana* is best genomic dicot model and benefits us in bioinformatics advances. However, most transformation procedures have been carried out by using *Agrobacterium* because of its ease and cost effectiveness. The virulence of *Agrobacterium* can be enhanced by the use of aceto-syringone, phenolic compound and it is now being routinely used for canola transformation. The efficiency of transformation using *Agrobacterium* is enhanced by pre-conditioning of the explant on callus, inducing media before co-cultivation. (Cardoza, 2004)

(3) Soybean:

Over the past decade, there has been a rising vogue for industrial applications employing soybean oil, and these applications contend with those used for edible consumption. One example is the recent increase in soy-based biodiesel production which consumed 1750 million gallons (4158 million liters) in 2014 as compared to about 6615 million gallons (6615 million liters) in 2011. (www.soystat.com)

In soybean, multiple loci contribute to valuable final contents of oil and protein present in their seeds. Levels of protein and oil are genetically in inverse correlation to each other in soybean so an increase in oil will occur at the expense of protein and vice versa. To solve this problem Transgenic soybean was developed in 2008 by Lardizabel

et al., which contains a codon optimized version of diacylglycerol acyltransferase 2A (DGAT2) taken from a soil fungus *Umbelopsis ramanniana*. As Triglycerol is the major constituent of soybean oil, transgene DGAT2 was helpful in converting Di-acylglycerols of soybean to Triacylglycerol. This transgene generally regulates oil synthesis without interrupting with protein level. In mature seeds 1.5% increase in oil were noticed. (Lardizabal, 2008)

Lysophosphatidic acid acyltransferase (LPAT) genes are considered to increase oil contents and they do not alter the fatty acid composition. Sphingolipid compensation genes (SLC1) from wild type yeast have been shown same activity like LPAT. SCL1 gene was incorporated in somatic embryos under seed specific promoter Phaseolin. From the T1-T3 subsequent increase in oil contents from 1.5-3.2% were observed. SCL1 gene also enhances triglyceride value in soybean seed. (Rao and David, 2009)

Linolenic acid is the poly-unsaturated fatty acid which is responsible for instability in soybean oil. FAD3 enzyme flavours linolenic acid synthesis in seeds, therefore, to minimize or mask the effect of linolenic acid in seeds RNAi strategy was used by Flores *et.al*, in 2008. 318 nucleotides long conserved sequence was used in inverted repeats as RNA interference along with seed-specific promoter. This cassette is introduced in soybean seed for silencing of FAD3. Significant results were shown like, oil stability in seeds and enhanced agronomic and nutritional value. Soybean has a complex genome in which linolenic acid has small gene pool as compared to other polyunsaturated fatty acids, use of RNAi approach have shown its extraordinary potential. (Flores *et al.*, 2008)

Soybean oils having high oleic acid render nutritional and performance benefit. Down regulation of FAD2 gene results into high oleic acid soybean along with about 6% reduction in polyunsaturated fatty acids (Kinney and Knowlton, 1997). Reduction in palmitic acid was also observed by 7-8% approximately. To enhance nutritional value and consumption of edible oils like salad oils, it is desirable to subdue unsaturated fatty acids from oil, the main concern is of Palmitic acid. FATB class of Acyl: ACP thioesterases control the liberation of few saturated fatty acids including entry of palmitic acid into cytoplasm which ensures its availability for oil biosynthesis. (Do¨ rmann *et al.*, 2000) Several Δ9 desaturase (SAD) genes are present in soybean seeds. From which silencing of SAD3 genes ensued information of 20-30% stearic acid in soybean oils (Booth *et al.*, 2006).

Soybean is the beneficial component of human diet. As human body lacks enzymes required to produce linolenic acid and alpha-linolenic acids. Both of these polyunsaturated fatty acids are present in soybean oils. These fatty acids are regarded healthy as well as essential for human consumption. The human body is able to produce oleic acid as a precursor from carbon sources like glucose but due to unavailability of Δ12 and Δ15desaturases enzymes in humans, oleic acid is not desaturated into linolenic acid or alpha-linolenic acid. So through consumption of soybean or other oilseed crop products these essential fatty acids are provided to the human body. (Sprecher, 2000)

(4) Sunflower:

Sunflower is the most important oilseed crop and ranks fourth in the world. Its edible oil is one of the best vegetable oils in cultivation. Up to 90% of the fatty acids are unsaturated in sunflower which is oleic and linoleic fatty acids. Palmitic, Stearic and minor amounts of other fatty acids account for remaining 10%. Sunflower seeds were treated with gamma and X-rays and they produced mutants with 25%-30% palmitic acid. Treatment of sunflower seeds with X-rays also resulted in 30% palmitoleic acid. Treatment with dimethyl sulphate produced important genotypes with more than 90% oleic acid. (Dragon *et al.*, 2008) Sunflower is cultivated as a source of vegetable oil and its protein is a model for detecting seed oil quality. To investigate such traits reference EMS mutant populations is developed under controlled conditions and TILLING platform is established. (Kumar *et al.*, 2013)

Firstly, a change was made in sunflower oil by Soldatov (1976). He treated seeds of variety VNIIMK 8931 with a dimethyl sulphate solution to induce a mutation for high oleic acid content. This was a source of genes for developing high oleic sunflower hybrids. Other scientists used various means to induce mutations; Ivanov *et al.*, (1988) treated sunflower seeds with gamma rays and a mutant with 25% palmitic acid was obtained. Mancha *et al.*, (1994) produced a mutant with 30% palmitic acid. Wild species of the genus Helianthus can be used for altering sunflower oil quality (Seiler, 1992).

Genetic variability is used to improve cultivated sunflower. Inter-specific hybridization is a method that is used to induce genetic variability into a sunflower. In this method, different species of plants are hybridized but the main problem in this method is an abortion of a hybrid embryo that prevents its success. So the scientist gave the solution of this problem that is embryo culture i.e, the embryo is separated from the plant and is placed on nutrient media that will grow in vitro into a seedling and then plant. Another method to improve inter-specific hybrids is to double the chromosome number of parents. Chromosome doubling will improve fertility when sterility is concerned with meiotic abnormalities in sunflower. Chromosome number is doubled with colchicine. (Seiler, 1992)

The oil composition is genetically controlled. The oil composition of sunflower is modified by changing the role of major genes through mutagenesis. (Lacombe and Berville, 2000) Induced mutations are utilized for creation of genetic variability in which mutants are used which change the sunflower oil composition including fatty acids and

tocopherol. Mutant genes combine with existing gene and accumulate different traits in one genotype that results in hybrids with different oil qualities that could be used for the creation of novel oils. (Cvejic *et al.*, 2014) Tocopherols are natural antioxidants which prevent lipid oxidation in biological systems. Due to the antioxidant activity of tocopherols, oil stability is increased. (Bramley *et al.*, 2000).

Tocopherols are essential for humans because they delay cellular aging and have biological value in the form of vitamin E. (Luis *et al.*, 2009). Tocopherol levels in sunflower can be enhanced by incorporating tph1, tph2, and ol genes. These genes produce a set of isogenic lines that facilitate greater genetic manipulation (Demurin *et al.*, 1994). These genes result in significant increase in oxidative stability of oil (Dragon *et al.*, 2008). For high oleic acid content, scientists used 3 complementary genes ol1, ol2, ol3) or a dominant one (O1). (Miller *et al.*, 1987) Some cytoplasmic male sterile and pollen fertility restorer lines with a high concentration of oleic acid in sunflower give resistance to different pathogens and diseases. (Maria *et al.*, 2005)

Conclusion

Genetic engineering in agriculture has the potential to improve crop yields along with its nutritional quality. There are several dimensions of genetic engineering, which can be applied to crops and commercial plants, starting with the obvious consideration of making them more resistant against pests and disease along with improved nutritional values. By using genetic engineering crop varieties may be produced which are resistant to chemicals use to enhance their shelf life, and also remove lethal contents from food crop, that makes agriculture much safer. It needs at times to accelerate the oilseeds breeding programs by utilization of genetic engineering tools to develop high yielding, good quality and environment friendly oilseeds cultivars.

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