Journal of Biotechnology and Bioengineering
Volume 2, Issue 3, 2018, PP 19-25
ISSN 2637-5362

The Role of Plant Gene Transformation in Oilseed Crop Improvement in Agriculture

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ABSTRACT

Introducing and expressing new genes in oilseed plants results in many opportunities which so far, have changed the face of agriculture. Such changes have created an enabling environment for circumventing challenges hindering high agricultural productivity and to fight against the detrimental effects of climate change. This review discusses the benefits of transgenic oilseed crops in the production of high level fatty acids for industrial processing and preparation. Furthermore, the paper provide highlights on the variety of options available in choosing a suitable transformation system and consideration of appropriate recombinant transgenes to ensure expression in targeted host plant tissues. It is not yet possible to fully benefit from all oilseed crops because many of them show recalcitrance to genetic transformation, particularly crops that contain high oil content such asrape seed, canola and soybean. This difficulty points to the greater need of improvement and optimisation in the currently used genetic transformation protocols for routine application in all species used in agriculture.

Keywords: *Agro bacterium; gene transformation; oilseed; fatty acids; transgenic; agriculture..*

INTRODUCTION

Plant gene transformation established through in-plant and in- vitro genetic modifications has revolutionised breeding programmes for oil and protein seed crops. In canola, ground nut, sunflower, physic nut and various other legumes (cowpea, lentil, chickpea, soybean etc.), the first successful gene transformations were reported, conferring tolerance to various environmental and biotic stress factors. The genetic engineering techniques like *Agrobacterium*-mediated transformation, in-plant Agro-injection and particle bombardment have been used to develop new varieties with newly improved growth and yield characteristics. These techniques allow for precise and controlled addition of genes to the genomes of targeted hosts. Such genetically modified plants possess new traits such as tolerance to pests, drought, salinity, chilling, heat and flooding. Plant genetic manipulation is now considered the most economic and highly effective method of genetic engineering worldwide.

The use of *Agrobacterium* tumefaciens is the most suitable and cost-effective method so far reported. This method holds the potential and promise to efficiently develop transgenic plants, particularly for highly recalcitrant oilseed crops like soybean. Li *et al.*[1] reported the expression of Gmb ZIPs in soybean induced by salt stress and drought. This stress-induced GmbZIPs are some of the basic leucine zipper (bZIP) family of transcription factors involved in the growth and development of plants, especially under salt stress [2].

The Dehydration Response Element Binding (DREB) gene was also incorporated into canola through *Agrobacterium* tumefaciens -mediated genetic transformation using strain LBA4404 [3]. According to Qamarunnisa *et al.* [3] tolerance to salinity and drought stress could be induced by the binding of several transcription factors which further induces the activity of DRE genes for stress tolerance. However, the first successful genetic transformation in soybean plants was reported by Hinchey *et al.* [4] using an in-vitro approach. The study reported the use of cotyledonary explants co-cultured with *Agrobacterium* carrying a pTiT37-SE vector, harbouring pMON9749 gene for herbicide glyphosate tolerance as a selective marker.

The success in this study led to the exploitation of soybean as a model crop and many other plant species for genetic manipulation. Plant transformation can be further explored for the establishment of fungal resistance, nematode resistance, insect-pests resistance and improved quality of oils and proteins. Legume plants have been reported, for example, as some of the most important pulse crops and good source of high quality proteins and oils required for human consumption, health benefits and marginally for industrial processing [5].Biotechnology has already played a vital role in transforming economically important traits of many crop species. The current reports by the Global Agriculture Information Network (GAIN) and Food and Agriculture Organisation (FAO) shows that the area commercially planted with transgenic plants have been increasing, especially in the nine (9) dominant countries, namely; United States of America, Brazil, Argentina, India, Canada, China, Paraguay, Pakistan and South Africa [5]. The overall world acreage reported by FAO is contributed by about 28 countries worldwide [6] and involve mainly cotton, canola, maize and soybean. Numerous genetic transformation technologies have been developed, aimed to achieve larger agricultural expansion and improvement of oilseed crops [7]. This article therefore, discusses some of the biotechnological approaches that push for the development of better crop varieties with increased oil properties and productivity.

METHODS USED IN PLANT TRANSFORMATION

Many different plant gene transformation methods have been invented for the genetic engineering of many oil and protein crops. Such methods include microprojectile bombardment, electro portion and *Agrobacterium*-mediated transformation amongst others. According to Finer and Dhillon [8] successful transgene expression has been predominantly achieved mainly through Agro bacterium or particle bombardment-mediated transformation. The other transformation methods are considered less effective, less widely used and highly expensive to carry out in a standard laboratory. *Agrobacterium* and particle bombardment-mediated transformation allow plants with specific qualities to be developed in a much simpler and shorter period of time than when using other methods like sonication or electroportion method. In addition, biotechnological methods are better and faster to transform plants than genetic improvement via conventional plant breeding [7]. Although, considerable progress has been made in gene manipulation and improvement of agricultural productivity, the various methods used have their merits and limitations. Some of the commonly applied methods include the following techniques:

AGROBACTERIUM MEDIATED TRANSFORMATION

Studies such as those of Yan *et al*. [9], Olhoft and Somers [10], Paz *et al*. [11], Mangena *et al*. [12] and Board and Kahlon [13] have showed that more research is still being directed towards investigating the use of bacteria for in-vitro and in-vivo transformation. The use of bacterial cells (namely A . tumefaciens and A. rhizogenes) are considered the simplest and natural form of transformation used to cause genomic changes in plants. *Agrobacterium* is the only widely used taxon that plays a key role in the natural inter-kingdom genetic exchange between plants and bacteria. Recently, the use of A. tumefaciens and A. rhizogenes for plant transformation served as a common and feasible means of transferring genes of interest into different crop plants. Since Gelvin [14] and McCormick *et al*. [15] reported successful *Agrobacterium*-mediated transformations in plants (for example the efficient transformation of tomato by McCormick *et al*. in 1986), many researchers have then worked on the transformation of various monocots and dicots.

The highest transformation efficiency so far recorded was 40%, and the minimum frequency reported in most plant species being around 6%. Other forms of *Agrobacterium* mediated transformation with/without plant tissue culture include *Agroinfiltration* (used as transient transformation assay) and Agrolistic transformation (involving co-transformation approach to deliver genes and marker with plasmids carrying Agro bacterium virulence genes virD1 and virD2by microprojectile bombardment) [7; 8].

MICRO PROJECTILE BOMBARDMENT

Bombardment of a broad range of cells and tissues with micro projectiles allows for the production of fertile transgenic plants, particularly maize, soybean, rice, wheat, barley and sorghum. This technique, also known as the biolistic gene gun was established by John Sanford Ed. Wolf and Nelson Allen at Cornell University [16]. This method is the most suitable for plants which are recalcitrant to in-vitro regeneration and those that do not show efficient response to gene transfer through *Agrobacterium*. This technique allow for the DNA coated tungsten particle (i.e. coated microprojectile) to be accelerated by a gunpowder charge and extruded through a small orifice [17].

DNA-coated microprojectiles can also be used for transfer of foreign genes into somatic cells of animals [18]. Cases *et al*. [19] reported production of transgenic sorghum plants after micro projectile bombardment of immature zygotic embryos of drought-resistant sorghum cultivar P898012. About six transformed callus lines were obtained from three of the eight sorghum cultivars used in this study. The presence of the bar and uidA genes in the T^0 plants was confirmed through southern blot analysis of genomic DNA. In another study, Bidney *et al*. [20] used biolistic method to promote *Agrobacterium*-mediated transformation on Tobacco cultivar Xanthi leaves and sunflower apical meristems for antibiotic resistance gene (nptII gene). The report indicated more kanamycin resistant cells and GUS expressions being obtained when tissues were wounded first with particle bombardment prior to *Agrobacterium* treatment.

ULTRASOUND SONICATION-ASSISTED AGRO BACTERIUM-MEDIATED TRANSFORMATION (SAAT)

Many researchers continue to highlight the transformation barriers faced in dicots, monocots and gymnosperms plant gene transformation. The SAAT technique is mainly used to overcome such limitations, faced during in-vitro and in-vivo transformation. Genotype or host tissue specificity and the inability of *Agrobacterium* or the gene of interest to reach targeted cells are among some of the problems faced during plant transformation. This relatively new technology involves subjecting plant tissue to a brief period of ultrasound in the presence of *Agrobacterium*. According to Trick and Finer [21] this technique has the potential to directly transform meristematic tissue buried under several layers of the dermal tissues. Stable lines of the spring Dendrobium, cultivar Sanya were recovered via SAAT using A. tumefaciens strain LBA4404 with a chalcone synthase (CHS) gene for flower colour change [22]. Other methods include electroporation, chemical method and silicon carbide Whisker-mediated method. Chemical methods such as transfection by calcium phosphate has been used in plant gene transformation [23]. Other chemicals used include diethylaminoethyl (DEAE)-dextran which is commonly used in mammalian cells than plant tissues [24]. DNA delivery by electroporation was reported by D’Halluin *et al*. [25] using embryogenic callus culture derived from mechanically or enzymatically wounded immature zygotic embryos.

BIOTIC AND ABIOTIC STRESS TOLERANCE

There are several factors such as pests and drought that cause stress in plants. For example, drought or water deficit caused by insufficient rainfall is considered a major limitation to oilseed crop yields. It has been said to be the most important environmental stress factor influencing crop yield losses, particularly in legume growing fields [26]. Drought causes yield losses as a result of the decrease in CO^2 assimilation, photorespiration and leaf area development [27]. Drought further interferes with plant-microbial relationships that exist in the biosphere. Lobato *et al*. [28] emphasised the interrelationship between biotic and abiotic stress. One of those is the influence of drought on causing plant susceptibility to weed, insects, and diseases. However, the basis for the plant sensitivity to both biotic and abiotic stress has been associated with plant genotype, overall plant growth and reproductive potential. Figure 1 illustrates the combined effect of stress on plant growth. As with every biological system, plant survival and growth depends on complex network of coupled environmental factors. In agriculture, these effects necessitate the development of crops that can grow during increasing environmental fluctuations (Table 1). Xu and Chye [29] reported evidence that an oc-1 gene from rice can be expressed in response to stress, especially chilling, programmed cell death and water deficit.

Thus, this gene can be transfected into various oilseed cropsto confer tolerance to above-mentioned stressors by encoding for protease inhibitors that will inhibit protease enzyme activity. The gene transcribes and translates proteins that suppress cystatins, which are a group of proteinases that initiate the degradation of essential proteins under stressful conditions, as a result causing death of plants. The adoption of biotechnological tools such as the use of transgenic crops (Table 1) in agriculture will certainly increase yields, seed quality and grow the oilseed market globally.

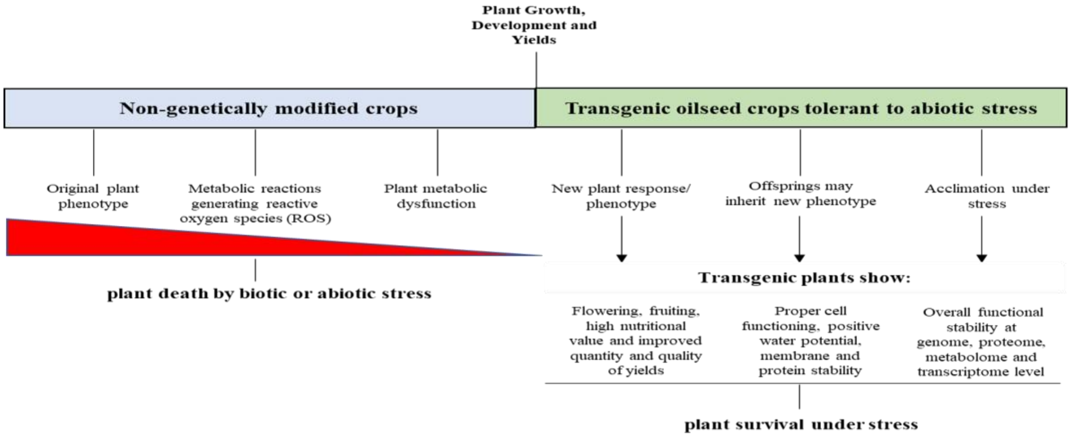

Figure1. *Dual role of biotic and abiotic stress on growth, development and yield of plants.*

Table1. *Area and Application of principal biotechnology crops by country*

Country	Area (Million Hectare)	GM crop(s)	Gene Application
United States	75.0	Corn, cotton, soybean, canola, squash, papaya	CTCP, TPB, TPIN, ICYQ, RAS, RBS
Brazil	50.2	Soybean, maize, cotton	RAS, CTCP, RAS
Argentina	23.6	Soybean, maize, cotton	RAS, CTCP
India	11.4	Cotton	RBS, CTCP
Canada	13.1	Canola, maize, soybean, sugarbeet	CTCP, RAS
China	2.8	Cotton, soybean papaya, tomato	CTCP, RBB
Paraguay	3.0	Soybean	RAS, CTCP
Pakistan	3.0	Cotton	RBS, CTCP
South Africa	2.7	Maize, soybean, cotton	RAB, RBS, CTCP

Key: GM- genetically modified RBS- resistance to biotic stress, RAS- resistance to abiotic stress, ICYQ- improved crop yield and quality, TPIN- transgenic plants with improved nutrition, TPB- transgenic plants as bioreactors, CTCP- commercial transgenic crop plants.

OIL PRODUCTION FROM TRANSGENIC PLANTS

Plant gene transformation can be used to modify the chemical compositions of the high energy food reserves found in seeds of plants into oils. The introduction of a single functional group into a fatty acid or a large number of useful modifications through transfer of genes for fatty-acid modifying enzymes may increase the value of many agricultural oilseed species. These oil seeds are considered valuable source of industrial raw materials used to potentially replace petrochemical oils. Currently, about 85% of plant oil is used for food processing, and the remaining protein concentrate by product is used as feeds for livestock and poultry or is used as fertilisers. The top nine growers of genetically modified crops currently contribute about 55% global share worldwide as indicated by FAO [6] (Figure 2).

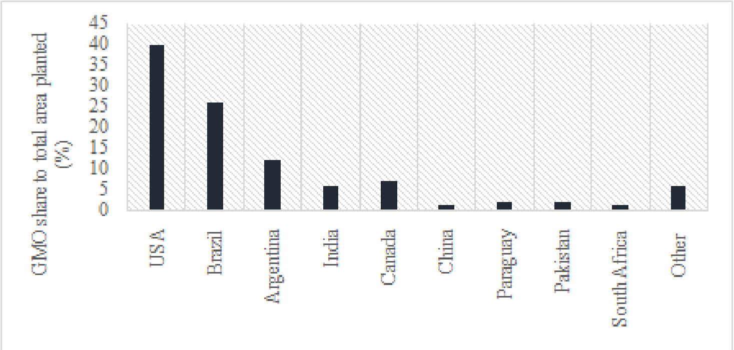

Figure2. *Percentage global share of growth and adoption of genetically engineered crops . Other refers to all countries that do not make significant contribution in the adoption of transgenic crops in commercial agriculture.*

About 2% oil is currently an estimated amount of renewable oil derived from seeds of transgenic crop plants that is used for biofuel production worldwide [6]. The industrial value of oils derived from plants is still very low, particularly for biodiesel production. This is so, because oil extraction in plants is limited by its fatty-acid composition of saturated and unsaturated fats. The plant oil mixture of saturated and unsaturated mid-chain and long-chain fatty acids cause chemical and physical properties that have marginal value for large industrial manufacturing and processing [30]. A study by Jaworski and Cahoon[31]indicated that, plant oil are not suited for the manufacture of specialty chemicals and polymers.

An example given by these authors was complex mixture of soybean oils, comprising primary of oleic acid, linoleic acid, linolenic, palmitic acidand steric acid which are not economically viable for industrial applications. Plant oils requires to be enriched in single fatty acids that have double bonds ad functional groups in specific positions in order to be suited for industrial application [31]. The biggest disadvantage of using plant oil is oxidation reactions due to the large number of poly unsaturated fatty acids. This is an undesirable series of chemical reactions that involve oxygen to degrade the quality of oil. This process causes rancidification when exposed to air, light, moisture or contaminated with bacteria resulting in unpleasant oil taste and odour [32]. Oil oxidation produces a series of by-products such as peroxide, free fatty acids, carbonyls, aldehydes , and trienes, as well as other tertiary products. However, the use of genetically modified oilseed crops has decreased the effects of polyunsaturated fatty acids and allowed for the generation of high quality oils with enhanced industrial properties. Currently, research is focussed on the identification and expression of genes that transcribe enzymes involved in the synthesis of new fatty-acid structures. Such particular focus is observed in many biotechnological researches aimed at discovering novel enzymes involved in the biosynthesis of biodiesel.

SIGNIFICANCE OF GENE TRANSFER IN PLANTS

Plants are the only primary source of foods and medicines for humans and animals. The rapid advances made in plant genetic engineering have made it possible to modify plants to increase the oils and proteins content. The world's major oils and proteins are primarily derived from transgenic plants (Figure 2). All countries indicated in Figure 2 showed an increase in the total area used for cultivation of transgenic oilseeds between 2010 to 2017. Though decreases in oil and protein production are still observed. This could be attributed to several factors including, decrease oilseeds yields due to drought, increased oil share price in the oilseed market and contracting biodiesel productions from oilseed crops. Most vegetable oils are used as edible oils in food processing and preparations [33] Meanwhile proteins are produced in the form of recombinant proteins in transgenic plants.

Currently used strategies for optimisation of recombinant proteins production are based on understanding plant gene expression, transcription, post transcription, translation and post translation [25]. These events guarantee the good quality and quantity of the final protein products. However, at present, the industrial value of proteins and oils is limited by the cost of its downstream processing which often determines the economic value of the production system. During the past decades, the development and optimisation of protocols used in genetic engineering has increased the potential of novel oil and protein-plant based products. Genetically engineered crops such as soybean, canola and sunflower, have been routinely tested for manufacturing of new generation of renewable energy ,that is carbon-neural, together with other forms of industrial products [31]. Advances made in plant gene transformation have enabled the isolation of genes from different sources which encode enzymes directly involved in oil and protein biosynthesis (Table 1).

CONCLUSIONS

Transgenic oilseed crops provide substantial potential as a renewable resource for industrial applications and processing. Continued effort must be directed to the efficient optimisation of genetic engineering techniques in order to fully benefit from both oilseed crops and biotechnology. Although several transgenic varieties with enhance quality and quantity of fatty-acid composition are available for commercial agriculture, more still need to be done, including dealing with problems of oil oxidation and contamination. Therefore, the production of transgenic oil crops with combined traits like improved quality of oil, tolerance to abiotic and biotic stress and increase yields is the next challenge in seed-oil research.

ACKNOWLEDGEMENTS

This work was partly supported by the Department of Biodiversity, University of Limpopo.

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Citation: *Phetole Mangena, Andries Thangwana," The Role of Plant Gene Transformation in Oilseed Crop Improvement in Agriculture", Journal of Biotechnology and Bioengineering, vol. 2, no. 3, pp. 19-25 2018 .*