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Review on Application of Genetic Engineering to Crop Improvement and Its Perceptions in Ethiopia

Getahun Bekana
School of Agriculture, Jimma University, PO box 307, Jimma, Ethiopia

Abstracts
Genetic engineering, also known as genetic modification, is the process of manually adding new DNA to an organism. The goal is to add one or more new traits that are not already found in that organism. An organism that is generated through genetic engineering is considered to be a genetically modified organism (GMO). Recombinant technology is helpful in solving the agricultural problems arising due to biotic and abiotic stresses. Cognizant of this potential, the Ethiopian government has made significant investment in modern biotechnology capacity building and the parliament also amends GMO law to allow Ethiopian research partnerships initially on non-edible items that was previously said to be stringent. After many years of fighting against the technology, Ethiopia is poised to become one of the few African countries to commercialize and produce at least one genetically-engineered crop.
Keywords: Genetic**,** Resistant, Transgenic and Engineering

1. Introduction

Genetic engineering, also known as genetic modification, is the process of manually adding new DNA to an organism. The goal is to add one or more new traits that are not already found in that organism *(Primrose and Twyman, 2013).* Creation of genetically engineered/modified or transgenic organisms requires recombinant DNA. Recombinant DNA is a combination of DNA from different organisms or different locations in a given genome that would not normally be found in nature, According to *(Singh and Singh, 2014)*

An organism that is generated through genetic engineering is considered to be a genetically modified organism (GMO). The first GMOs were bacteria generated in 1973 and GM mice in 1974. Insulin-producing bacteria were commercialized in 1982 and genetically modified food has been sold since 1994.Genetic engineering techniques have been applied in numerous fields including research, agriculture, industrial biotechnology, and medicine. Enzymes used in laundry detergent and medicines such as insulin and human growth hormone are now manufactured in GM cells, experimental GM cell lines and GM animals such as mice or zebra fish are being used for research purposes, and genetically modified crops have been commercialized(*Blake etal.,2013)*

Genetic engineers have developed genetic recombination techniques to manipulate gene sequences in plants, animals and other organisms to express specific traits. Applications for genetic engineering are increasing as engineers and scientists work together to identify the locations and functions of specific genes in the DNA sequence of various organisms. Once each gene is classified, engineers develop ways to alter them to create organisms that provide benefits such as cows that produce larger volumes of meat, fuel- and plastics-generating bacteria, and pest-resistant crops (*Acquaah* , 2007*)*

According to *(James,2013 and Khan and Hakeem , 2015)* Commercialization of first genetically engineered crop started back in 1996 and since then it has reached new heights in its application and wide adaptability to various sectors of modern agriculture. Since 1996 to 2013 there has been tremendous increase in the acreage of genetically engineered crops. Between 1996 and 2013 there has been more than 100 fold increase in the acreage of genetically engineered crops. Recombinant technology is also helpful in solving the problems arising due to biotic and abiotic stresses. Research has already demonstrated the potential to develop crops with increased nutrient-use efficiency, greater drought and flooding resistance, stronger disease and insect resistance and higher nutritional content and yield. Insect-resistant crops have been one of the major successes of applying plant genetic engineering technology to agriculture; cotton (*Gossypium hirsutum*) resistant to lepidopteran larvae (caterpillars) and maize (Zea mays) resistant to both lepidopteran and coleopteran (Gatehouse,2008).

In addition to genetic engineering helping GMO plants and crops thrive in a variety of conditions; it also has many benefits to human society. However, it is not set to replace conventional plant breeding but is a modern tool for use of plant breeders to fasten the breeding programme. Transgenic technology yielded genetically modified (GM) crops having novel genes with favourable characteristics like higher yields, herbicide resistant, insect and disease resistant, drought resistant, salinity resistant and the others *(Tester and Langridge, 2010).*

According to (*Abraham, 2014*) cognizant of this potential, the Ethiopian government has made significant investment in modern biotechnology capacity building in the last decade. There has also been specific interest by cotton sector to boost its productivity by adopting insect resistance (Bt) technologies. The previous proclamation was said to be stringent and did not allow the involvement of local researchers in partnership with international researchers as it required full responsibility by the exporting country's competent national authority for the

completeness and accuracy of the information provided with the informed agreement. However, since May 19, 2015 Parliament Amends GMO Law to Allow Ethiopian Research Partnerships initially on non-edible items. "Now we have the chance to exercise research on GMOs and work on GMOs imported from abroad" Fantahun Mengistu (PhD), Director General of the Ethiopian Institute of Agricultural Research (EIAR) told Fortune. The objective of this review is to assess recent progress of research on application of genetic engineering to crop improvement and to see the gap those need further improvement in Ethiopia.

2. Literature Review

2.1 Crop Improvement and GMO Development

Crop improvement is the continuous endeavor to improve useful traits of crop plants by using genetic variation *(Tester and Langridge, 2010).* Until the end of the Nineteenth century, shifts in the genetic makeup of crops mainly occurred through time-consuming phenotypic selection in the field without further knowledge of the underlying mechanisms of inheritance or the genotype-to-phenotype connection. Since the birth of the discipline of genetics and the advent of modern plant breeding though, breeders have used various scientific methods to (1) increase the available genetic variation, and (2) gain a higher level of control between deliberate genetic alterations and the resulting phenotypic traits. Mutations induced by radiation or chemicals enabled a revolution in the first mentioned, and has provided the world with at least 3240 improved varieties of all our major crops *(*Eriksson and Ammann., 2016), whereas more recent techniques for genetic modification (GM) and genome editing have greatly enhanced the capacity both to generate genetic variation and exercise control in the breeding process.

Once the science of genetics became better understood, plant breeders used what they knew about the genes of a plant to select for specific desirable traits. This type of genetic modification, called traditional plant breeding, modifies the genetic composition of plants by making crosses and selecting new superior genotype combinations. Traditional plant breeding has been going on for hundreds of years and is still commonly used today. Plant breeding is an important tool, but has limitations. First, breeding can only be done between two plants that can sexually mate with each other. This limits the new traits that can be added to those that already exist in that species. Second, when plants are mated, (crossed), many traits are transferred along with the trait of interest including traits with undesirable effects on yield potential *(Tester and Langridge, 2010).*

Genetic engineering is the direct modification of an organism's genome, which is the list of specific traits (genes) stored in the DNA. Changing the genome enables engineers to give desirable properties to different organisms. Organisms created by genetic engineering are called genetically modified organisms *(Acquaah , 2007).*

All genetic changes affect the protein synthesis of the organism. By changing which proteins are produced, genetic engineers can affect the overall traits of the organism. Genetic modification can be completed by a number of different methods such as inserting new genetic material randomly or in targeted locations, direct replacement of genes (recombination) ,removal of genes and Mutation of existing genes according to *(Singh and Singh, 2014).*

2.2 Process of Plant Genetic Engineering

Genetic engineering is a new type of genetic modification. It is the purposeful addition of a foreign gene or genes to the genome of an organism. A gene holds information that will give the organism a trait. Genetic engineering is not bound by the limitations of traditional plant breeding. Genetic engineering physically removes the DNA from one organism and transfers the gene(s) for one or a few traits into another *(khan etal.,2013).* Since crossing is not necessary, the 'sexual' barrier between species is overcome. Therefore, traits from any living organism can be transferred into a plant. This method is also more specific in that a single trait can be added to a plant.The process of genetic engineering requires the successful completion of a series of five steps. DNA extraction is the first step in the genetic engineering process. In order to work with DNA, scientists must extract it from the desired organism. A sample of an organism containing the gene of interest is taken through a series of steps to remove the DNA. The second step of the genetic engineering process is gene cloning (*Berg and Mertz, 2010)*

During DNA extraction, the entire DNA from the organism is extracted at once. Scientists use gene cloning to separate the single gene of interest from the rest of the genes extracted and make thousands of copies of it. Once a gene has been cloned, genetic engineers begin the third step, designing the gene to work once inside a different organism. This is done in a test tube by cutting the gene apart with enzymes and replacing gene regions that have been separated. The gene can be isolated using restriction enzymes to cut DNA into fragments and gel electrophoresis to separate them out according to length (Alberts , etal.,2002). Polymerase chain reaction (PCR) can also be used to amplify up a gene segment, which can then be isolated through gel electrophoresis. If the chosen gene or the donor organism's genome has been well studied it may be present in a genetic library. If the DNA sequence is known, but no copies of the gene are available, it can be artificially synthesized *(Liang*

etal.,2011).

The modified gene is now ready for the fourth step in the process, transformation or gene insertion. Since plants have millions of cells, it would be impossible to insert a copy of the transgene into every cell. Therefore, tissue culture is used to propagate masses of undifferentiated plant cells called callus (Byrne, 2014). These are the cells to which the new transgene will be added. The new gene is inserted into some of the cells using various techniques. Some of the more common methods include the gene gun, agrobacterium, microfibers, and electroporation (*James, 2013*).

Gene gun: In this method, microscopic pellets of gold or tungsten are coated with the transgene fragment and shot at high velocity into plant cells or tissues. In a small proportion of cases, the pellet will pass through the cells and the DNA fragment will remain behind and become incorporated into a plant chromosome in the cell nucleus (*Byrne, 2014*).

Agrobacterium tumefaciens: This method utilizes a biological vector, the soil dwelling bacterium *Agrobacterium tumefaciens*, which in nature transfers part of its DNA into plants and causes crown gall disease. Genetic engineers have taken advantage of this DNA transfer mechanism while disarming the disease-causing properties. Plant and bacterial cells are co-cultivated in a petri dish under conditions that facilitate gene transfer. This allows incorporation of genes in a more controlled manner than with the gene gun; however, it does not work equally well in all plant species (*James, 2013*).

Insertion of transgenes is generally an inefficient process, with only a few percent of plant cells or tissues successfully integrating the foreign gene. Various strategies are used to identify the small percentage of cells/tissues that have actually been transformed. The next step is to develop those cells or tissues into whole plants capable of producing seed. This is done through a process called tissue culture, that is, growing plants on agar or a similar medium in the presence of plant nutrients and hormones under controlled environmental conditions (*Byrne, 2014*).

The main goal of each of these methods is to transport the new gene(s) and deliver them into the nucleus of a cell without killing it. Transformed plant cells are then regenerated into transgenic plants. The transgenic plants are grown to maturity in greenhouses and the seed they produce, which has inherited the transgene, is collected. The genetic engineer's job is now complete. He/she will hand the transgenic seeds over to a plant breeder who is responsible for the final step (*Darbani etal.*,2008).

The fifth and final part of producing a genetically engineered crop is backcross breeding. Transgenic plants are crossed with elite breeding lines using traditional plant breeding methods to combine the desired traits of elite parents and the transgene into a single line. The offspring are repeatedly crossed back to the elite line to obtain a high yielding transgenic line. The result will be a plant with a yield potential close to current hybrids that expresses the trait encoded by the new transgene (*Darbani etal.,2008)* . The entire genetic engineering process is basically the same for any plant. The length of time required to complete all five steps from start to finish varies depending upon the gene, crop species, available resources and regulatory approval. It can take anywhere from 6-15+ years before a new transgenic hybrid is ready for release to be grown in production fields *(khan etal.,2013*).

2.3 Application of Genetic Engineering to crop improvement.

The early and most cost-reward producing use of GE has been in the development of insecticide and pesticide resistance in field crops. A great deal of interest has currently been shown in incorporating tolerance to environmental stresses in crop cultivars in order to stabilize the yield under fluctuating environmental conditions. In addition, as enhanced nutritive value of crop has gathered much interest to combat malnutrition in developing countries and to meet the food preference of naturalists, several transgenic cultivars with fortified nutritive values have been released. Some degree of success has also been accomplished in developing crops with chemical

constituent of industrial value and the use of plants as hosts for pharmaceutical products *(Singh* and *Singh,* 2014).

Achieving sustainable agriculture and producing enough food for the increasing global population will require effective strategies to cope with harsh environments such as water and nutrient stress, high temperatures and compacted soils with high impedance that drastically reduce crop yield. Recent advances in the understanding of the molecular, cellular and epigenetic mechanisms that orchestrate plant responses to abiotic stress will serve as the platform to engineer improved crop plants with better designed root system architecture and optimized metabolism to enhance water and nutrients uptake and use efficiency and/or soil penetration. In this review we discuss such advances and how the generated knowledge could be used to integrate effective strategies to engineer crops by gene transfer or genome editing technologies (*Lopez-Arredondo et al., 2015*)

A limited success in producing abiotic-stress tolerant cultivars through genetic engineering has been achieved. Stresses occurring simultaneously are a common situation for crops that results in a complex system to cope with. New technologies provide opportunities to generate transgenic crops able to maintain high yields

under stress. More emphasis should be given to study abiotic-stress tolerant crops under field conditions focusing on reproductive stage according to (*Reguera etal.,* 2012).

Recombinant DNA and transformation techniques allow plant breeders to use genes from essentially any source as tools for crop improvement. For example, to enable rice grains to accumulate beta-carotene (which is converted into vitamin A when consumed by animals) and create the so-called "Golden Rice," scientists used genes from daffodil, pea, a bacterium, and a virus. Transgenic plant methods enable these four well characterized genes to be inserted into a transgenic plant, producing a highly specific change in only the trait of interest. In contrast, many unknown genes are introduced when a breeder uses wide crosses to transfer a desired gene from a wild plant into a crop plant (*Suslow etal*.,2002).

According to (*khan etal.,2013*) transgenic breeding enables the transfer of genes across taxonomic boundaries unlike conventional breeding where it is possible to transfer genes from closely related species only. It also offers new avenues of plant improvement in shorter period compared to conventional breeding and new possibility of incorporating new genes without problems incompatibility. The following points are some application of genetic engineering to plant breeding according to (*khan etal, 2013 ; Naranjo and Vicente,2008;Singh and Singh 2014*).

Herbicide resistant: herbicides normally affect processes like photosynthesis or biosynthesis of essential amino acids. Transformation of cereal crops with Glyphosate resistant gene (Glyphosate = herbicide). Herbicide tolerant (HT) soybean and canola are released for commercial cultivation.

Insect resistant: Insect-resistant crops contain genes from the soil bacterium Bacillus thuringiensis (Bt). The protein produced in the plant by the Bt gene is toxic to a targeted group of insects—for example European corn borer or corn rootworm—but not to mammals *(Byrne, 2014).* The genes which responsible for the production of delta-endotoxin in Bacillus thuringiensis is used as biological insecticide. The transgene has been transferred to many crops for example looper resistance in soybean, pod borer resistance in groundnut, head borer resistance in sunflower, semi-looper resistance in castor etc. snowdrop lectin gene from snow drop (Galanthus nivalis) was transferred to brassica and safflower for aphid resistant.

Resistance against viral infection: coat protein gene from Tobacco Mosaic Virus (TMV) was transferred to develop resistant varieties of crop plants. The resistant varieties developed in crop plants like soybean for resistant to yellow mosaic virus, groundnut for resistant to bud and stem necrosis, clump and stripe virus resistance, whereas in sunflower, resistance developed for bud necrosis.

Resistance against bacterial and fungal pathogens*:* Chitinase genes was transferred to crops like Brassica, Soybean, Sunflower, Sesame etc for alternaria leaf spot disease, where as in case of groundnut which was introduced against leaf spot and alternaria blight and in castor for Botrytis resistance. Acetyl transferase gene was transferred for wildfire disease of tobacco caused by pseudomonas syringae.

Improvement of the nutritional qualities in crop plants: The carotene gene has been transferred from daphoddils to rice grains (Golden Rice) for increasing Beta-carotene content in grains and for solving the blindness in childerns. Antisense Fae 1 gene transferred to Brassica napus and Brassica juncea for low erucic acid content and also for low linolenic acid content in case of linseed. Antisense ricin gene transferred to castor for reduction of ricin content and RCA endosperm in castor seeds. Antisense sterol desaturase /+ ac1 inserted into sunflower for developing high oleic acid containing types.

Improvement of crop plants against abiotic stresses*:* transcription factor genes, structural genes, regulatory genes were introduced into the groundnut, soybean, Brassica juncea, B. napus to develop drought and salinity tolerant types.

Development of transgenic male sterile lines: transgenic male sterile lines of safflower Brassica juncea were developed through the transfer of Barnase gene from Bacteria (Bacillus amyloliquefaciens). A long term goal in agriculture is to introduce the genes (Nif genes) for nitrogen fixation in crop plants.

There is a need to establish reliable protocols for genetic engineering of crop plants so that these crops also could be brought under the umbrella of crops amenable for genetic engineering. The greatest challenge in agriculture is to improve food grain production and eradication of malnutrition problem in the developing countries and hopefully this technique will be applied to the regions where food shortage is greatest. By knowing the present problems of farmers and also health point of view, developing safe and efficient transgenic plants is needed*(Tester and Langridge, 2010)*. For achieving these, there is need of intensifying research at national and international levels to ensure that biotechnology leads to second revolution in agriculture, which both productive and sustainable. Synergy between GM breeding and traditional plant breeding needs to be further strengthened.

2.4 Ethiopian Perception to Genetically Engineered Crops.

Genetic engineering offers several benefits when used responsibly by addressing the environmental and food safety concerns with rigorous biosafety regulations. Until recently, guidelines with genetic engineering research and deployment of genetically modified organisms do not exist in the country. This situation discouraged Ethiopian scientists from initiating genetic engineering projects and participating in similar network activities at

regional and international level and consequently significantly hampering the research and capacity building process in modern biotechnology research & development in the country (Abraham, 2009).

After many years of fighting against the technology, Ethiopia is poised to become one of the few African countries to commercialize and produce at least one genetically-engineered crop. In June 2015, after several years of internal government debate and machinations, Ethiopia's Parliament adopted an amendment to the Biosafety Proclamation with the express purpose of laying the regulatory framework to allow farmers to plant biotech cotton in order to meet the rising demands from the rapidly expanding textile and apparel sector. The earlier Proclamation imposed a de-facto ban on the planting of GE crops as well as biotech research (Tefera, A., 2015).

A big step taken recently by the Ethiopian government is the approval of biosafety law by the parliament is expected to encourage genetic engineering research as well marketing of its products in the country in a responsible way. A wide range of crop production problems that are either difficult or impossible to address using conventional research techniques are likely to be solved using crops genetically engineered for specific traits and adapted to local conditions. The major crop production constraints in Ethiopia that can be addressed by genetic engineering are indicated in **Table 1**. For some of these constraints, transformation technology is already developed else-where and commercially available and only needs to be introduced and adopted to local conditions with minimum technical inputs (Abraham, 2009).

Recently for example, the private sector has expressed keen interest in introducing *Bt* cotton to boost its production and thus satisfying the booming textile industry in the country. These efforts will help boost cotton production in Ethiopia as many cotton producing farmers in western Ethiopia are abandoning their cotton fields due to heavy boll worm infestation pressure. By partnering with commercial companies like Monsanto, such technologies can be accessed relatively easily. It is also possible to use such transgenic plants as parents to transgress the desired genes to locally preferred cotton varieties by conventional breeding. On the other hand, to address constraints on indigenous crops like tef and enset that are not of interest to foreign companies, there is a need to develop local capacity in genetic engineering technologies in terms of infra-structure and manpower.

3. Conclusions

Genetic engineering becomes a powerful technique that applicable for altering the genetic make-up of the crop plants. It is achieved through transgenic or recombinant DNA technology. The crop plants having so many desired characters but due the presence of one or few unfavorable characters makes the crop to limit in its area and production. This makes the farmers to forcefully have to shift to other crops. And also to overcome the malnutrition problems facing a huge mass of the people of the world, transgenic technology helps in mitigating this problem in an effective manner. Recombinant technology is also helpful in solving the problems arising due to biotic and abiotic stresses.

To overcome all these problems, transgenic technology helps to transfer desired characters from various sources to required crop plants by identification and isolating the gene of our interest. The technology of genetic modification through transgenic approach is more directed and the inserted genes can be easily followed. In contrast to green revolution that only emphasis on three main crops (rice, wheat and maize) and produced ambivalent results, the gene revolution represents a technical and ethical advance and can be used to improve the characteristics of all targeted plants with significantly enhanced social impacts.

However, genetic engineering is not set to replace conventional plant breeding but is a modern tool for use of plant breeders to fasten the breeding programme. The varieties of maize, tobacco, cotton etc., that are resistance to herbicide were developed by transformation of plants with glyphosate resistant gene through Agrobacterium mediated transformation. Transgenic technology yielded genetically modified (GM) crops having novel genes with favourable characteristics like higher yields, herbicide resistant, insect and disease resistant, drought resistant, salinity resistant and the others.

Ethiopia is strongly against the hasty introduction of GM crops, because as a center of origin and crop diversity, we recognize the assets that come from a biologically diverse, locally adapted, small-scale agriculture. Releasing to the environment is punishable unless the researcher persuades the benefit of releasing the GMO to the environment.

4. Prospects (Future Line of Work).

Genetic engineering (GE) technologies can contribute to improve crop productivity and quality. Moreover, key production constraints such as bacterial wilt of enset, late blight of potato, drought stress on crops like maize and wheat, lodging resistance on tef as well as low nutritive quality of native crops like enset and grasspea can be addressed by strengthening GE research capacity and international collaboration. Knowing of this potential, the Ethiopian government has made significant investment in modern biotechnology capacity building in the last decade and recently parliament amends GMO law to allow Ethiopian research partnerships initially on non-edible items. In the future our country may also success in all aspects in GMO products.

It is evident that there are real prospects for the benefit of biotechnology tools and products in Ethiopia. However, a close look at the current situations reveals a number of constraints and gaps that contributed to the under development of agricultural biotechnology. Effective biotechnology policy directives and biosafety system as well as regulatory and monitoring mechanisms need to be in place, in particular, for the introduction, research and release of GMOs; current applications such as plant tissue culture, microbial products development, vaccine production and diagnostics should be expanded; the wise utilization of the country's biodiversity by *in vitro* conservation, molecular characterization and introduction of marker assisted breeding and isolation of potentially useful genes should be promoted; there is a need to develop a strong national capacity in recombinant DNA research such as GMOs including containment greenhouse facilities; sufficient financial resources should be made available by mobilizing public and private sector and from local and external sources; establishing and sustaining institutional linkage within the country as well as strengthening collaboration among Ethiopian and foreign institutions should be improved; policies and incentive mechanisms should be developed to encourage private sector investment and their participation in agricultural biotechnology, Universities offering biotechnology courses should upgrade their laboratory in terms of manpower and facilities to acquaint the students with practical skills and produce competent manpower; and finally an active and honest interaction between scientist and other society members including the public and decision makers should be encouraged.

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Table 1. The potential of genetic engineering in solving crop production constraints in Ethiopia.

Commodity	Constraints	Candidate transgenes	Application status worldwide
Cotton	Boll worm	Insect resistance (Bt) gene	Commercialized
Wheat, barley, tef	Grass weeds	Herbicide resistance gene	Commercialized
Maize/sorghum	Stem borer, striga, abiotic stress (drought, frost and salinity tolerance)	Bt gene, striga resistance gene, stress genes & promotors	commercialized and/or being attempted
Tef	Lodging resistance	dwarfing gene from wheat or rice	future plan
Tomato	Tomato Yellow leaf curl virus	Viral rep protein C1 gene	being attempted elsewhere
Potato	Late blight	Resistance genes from wild potato	being attempted elsewhere
Enset/banana	Bacterial wilt	hrap gene from bacteria (*Xanthomonas*)	being attempted elsewhere
Sesame	Sesame seed bug	Bt gene	future plan
Faba bean	Chocolate spot	Chitinase or glucanase gene	future plan
Chickpea	pod borer, stunt virus	Bt gene, Viral coat protein gene	being attempted elsewhere
Sweet potato	weevil	Bt	being attempted elsewhere

Source: African Journal of Biotechnology Vol. 8 (25), pp. 7196-7204.