Chapter - 36
Genetic Engineering: It's Role in Agriculture

Authors

YP Singh
School of Agricultural Sciences & Engineering, IFTM University, Moradabad, Uttar Pradesh, India

VK Dhangrah
School of Agricultural Sciences & Engineering, IFTM University, Moradabad, Uttar Pradesh, India

AN Chaubey
School of Agricultural Sciences & Engineering, IFTM University, Moradabad, Uttar Pradesh, India

Virendra Singh
School of Agricultural Sciences & Engineering, IFTM University, Moradabad, Uttar Pradesh, India

Chapter - 36

Genetic Engineering: It's Role in Agriculture

YP Singh, VK Dhangrah, AN Chaubey and Virendra Singh

Introduction

More benefits of genetic engineering in agriculture are increased crop yields, reduced costs for food or drug production, reduced need for pesticides, enhanced nutrient quality, resistance to pests and disease, greater food security, and medical benefits to the world's growing population. People have been altering the genomes of plants and animals for many years using traditional breeding techniques. Artificial selection for specific, desired traits has resulted in a variety of different organisms, ranging from sweet corn to hairless cats. But this artificial selection, in which organisms that exhibit specific traits are chosen to breed subsequent generations, has been limited to naturally occurring variations. In recent decades, however, advances in the field of genetic engineering have allowed for precise control over the genetic changes introduced into an organism. Today, we can incorporate new genes from one species into a completely unrelated species through genetic engineering, optimizing agricultural performance or facilitating the production of valuable pharmaceutical substances. Crop plants, farm animals, and soil bacteria are some of the more prominent examples of organisms that have been subject to genetic engineering. An important application of Recombinant DNA technology is to alter the genotype of crop plants to make them more productive nutritious, rich in proteins, disease resistant and less fertilizer consuming. Recombinant DNA technology and tissue culture techniques can produce high yielding cereals, pulses and vegetable crops. Some plants have genetically programmed to yield high protein grains that could show resistance to heat moisture and diseases.

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). 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)

Proponents of the use of GMOs believe that, with adequate research, these organisms can be safely commercialized. There are many experimental variations for expression and control of engineered genes that can be applied to minimize potential risks. Some of these practices are already necessary as a result of new legislation, such as avoiding superfluous DNA transfer (vector sequences) and replacing selectable marker genes commonly used in the lab (antibiotic resistance) with innocuous plant-derived markers (Ma *et al*., 2003). Issues such as the risk of vaccine-expressing plants being mixed in with normal foodstuffs might be overcome by having built-in identification factors, such as pigmentation, that facilitate monitoring and separation of genetically modified products from non-GMOs. Other built-in control techniques include having inducible promoters (e.g., induced by stress, chemicals, etc.), geographic isolation, using male-sterile plants, and separate growing seasons.

GMOs benefit mankind when used for purposes such as increasing the availability and quality of food and medical care, and contributing to a cleaner environment. If used wisely, they could result in an improved economy without doing more harm than good, and they could also make the most of their potential to alleviate hunger and disease worldwide. However, the full potential of GMOs cannot be realized without due diligence and through attention to the risks associated with each new GMO on a case-by-case basis.

Genetic engineers have developed genetic recombination techniques to manipulate gene sequences in plants, animals and other organism to express specific traits. Applications for genetic engineering are increasing as engineers and scientist 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).

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).

Crop Improvement and GMO Development

Crop improvement is the continuous endeavour 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).

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 *et al*., 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, *et al*., 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 *et al*., 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 transgenic 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 transgenic 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, agro bacterium, microfibers, and electro portion (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).

Efforts are being made to improve several agricultural crops using various techniques of genetic engineering which include

i) Transfer of nitrogen fixing genes (nif genes) from leguminous plants into cereals.

ii) Transfer of resistance against pathogens and pests from wild plants to crop plants.

iii) Improvement in quality and quantity of seed proteins.

iv) Transfer of genes for animal proteins to crop plants.

v) Elimination of unwanted genes for susceptibility to different diseases from cytoplasmic male sterile lines in crop like maize, where cytoplasmic male sterility and susceptibility are located in mitochondrial plasmid.

vi) Improvement of photosynthetic efficiency by reassembling nuclear and chloroplast genes and by the possible conversion of C_3 plants into C_4 plants.

vii) Development of cell lines which may produce nutritious food in bioreactors

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 *et al*., 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 *et al*., 2002).

According to (khan *et al*., 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 *et al*, 2013; Naranjo and Vicente, 2008; Singh and Singh 2014).

Herbicide resistance: 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. Success has been made in the incorporation of genes conferring tolerance to herbicides. The genetic plants thus produced show expression of foreign resulting in a higher level of herbicide tolerance. The best examples in the Shaw and his co-workers, in 1986, they isolated a cDNA clone encoding an enzymes 5-enolpyruvyl-shikimate phosphate (EPSP) synthase from a glyphosate tolerant *Petunia hybrid* cell line. This cell line over produced the enzyme to the tune of 20 times more. The chimeric EPSP synthase gene was constructed with the use of the cauliflower mosaic virus 35 promoter and introduced in to the non- tolerant *petunia* cell lines. The calli from transformed cell lined showed tolerance to glyphosate and the plants regenerated from the calli showed tolerance to the herbicide whereas the control plants died after spraying the herbicide.

Insect resistance: A gene (cry) from a soil bacterium from the soil bacterium *Bacillusthuringiensis* (Bt) code for a protein (delta endotoxin), called *crystal protein*, that is produced during sporulation. The crystal protein are toxic to most lepidopteron, many coleopteran and several dipteran insect. *Helicoverpa* larvae fed on small amounts of appropriate Cry protein become sluggish, stop feeding, lose weight and ultimately die. 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. The crystal proteins are extremely safe to human beings and minimise environment pollution due to insecticides. However, their use has been rather limited mainly due to their high cost and instability under field condition.

The Cry proteins are cleaved at specific sites by the photolytic enzymes present in the midgut of the target insect. The release of the toxin fragment, which binds to specific receptors present in the membranes of epithelial cells of the insect midgut. This creates pores in the cell membranes leading to the bursting of epithelial cells. The affected larvae become sluggish, stop feeding and ultimately die. Cry proteins are active against larvae of their target insect species. The *cry IA* gene has been successfully transferred in tobacco, tomato, potato, cotton, etc. the native cry genes show low levels of expression in transgenic plants. This has been overcome by using shorter, truncated version of cry genes and by modified their base sequence, without affecting the amino acid sequences of the proteins encoded by them. To remove those sequences that interferes with gene expression in eukaryotes. The steps have resulted in an over 100-fold increase in the expression of the modified cry genes. Indian scientists are trying to transfer the cry gene into chickpea and other pulse crops in order tom product those from insect pests, for which sources of resistance are not available so far.

Resistance against viral infection: It is well-established that plants inoculated with a mild strain of a virus become resistant to a subsequent infection by a virulent strain of the same virus; this phenomenon is known as virus cross-protection. One of the most successful approaches for transgenic virus resistance is transfer of the coat protein gene of a virus in to the genome of its host, where it is constitutively expressed. Constitutive gene expression means expression in every tissue at all the times. 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. This approach has been used to develop virus protected varieties of the some crop.

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 children's. Antisense Fae 1 gene transferred to *Brassica napus* and *Brassica juncea* for low erucic acid content and also for low linoleic 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.

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.

Seed Storage Proteins: Genes for seed storage proteins from both cereals and legumes have been transferred into tobacco, where they have been shown to express in the endosperm/ embryo tissues. Some examples of such gene transfers are wheat glutenin, barley hordein, rajma phaseolin into tobacco, and maize Zein genes into sunflower, etc. These successful gene transfers open up possibilities for using this approach to correct the amino acid deviancies of both cereals (lysine deficient) and pulses (deficient in tryptophan and sulphur containing amino acids) seed storage proteins.

Production of Novel Biochemical: Many valuable biochemicals are obtained from microbes. Biomass production by plants is much easier and cheaper than that by microbes. Therefore, if genes encoding the valuable proteins/ enzymes necessary for synthesis of the biochemical are transferred and expressed in plants, the concerned biochemical would be produced in the plants. For examples, the gene encoding the antithrombin proteins hirudin has been transferred in *B. napus* and it accumulates in seed. In Europ, hirdin is

being commercially produced from transgenic *B. napus*. Some other biochemicals are also being produced in transgenic crops.

Edible Vaccines: many antigen cause immunization when introduced orally. The pathogen gene encoding such as antigen can be transferred and expected in fruits like banana. When such banana is consumed in appropriate quantities as per a given schedule, it causes immunization against the concerned pathogen. Such transgenic fruits /vegetable, which produce and contain an orally active antigen from a pathogen, and which consumed, lead to immunization against the concerned pathogen, are called edible vaccine. Development of such vaccine is a fairly advanced stage. Edible vaccine are much cheaper and easier to produce and more convenient to administer than are conventional vaccine. In addition, they do not require cold storage, which is a must for the latter.

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 unfavourable 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.

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. Effective biotechnology policy directives and bio- safety 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 technology research such as GMOs including containment greenhouse facilities; sufficient financial resources should be made available by mobilizing public and private sector.

References

1. Acquaah, G. 2007. Principles of plant breeding and genetics. *Malden, MA USA: Blackwell Publishing*.
2. Primrose, S.B. and Twyman, R. 2013. Principles of gene manipulation and genomics. John Wiley & Sons.
3. Singh, B.P. and Singh, H.P. 2014. Genetic engineering of field, industrial and pharmaceutical crops. *American Journal of Plant Sciences*, *5*(26) 3974.
4. Ma, J.K. Drake and P.M, Christou, P. 2003 the production of recombinant pharmaceutical proteins in plants. *Nat Rev Genet* 4: 794–805
5. Marcin, Nowicki, U, Elizbieta. and Majid, Majid, R. Foolad. 2013 "Late Blight of Tomato", *Translational Genomics for Crop Breeding*, pp. 241–265
6. Tester, M. and Langridge, P. 2010. Breeding technologies to increase crop production in a changing world. *Science*, *327*(5967), 818-822.
7. Eriksson, D. and Ammann, K.H. 2016. A universally acceptable view on the adoption of improved plant breeding techniques. *Frontiers in Plant Science*, *7*.
8. Khan, M.H., Lone, A.A. and Wani, S.H. 2013. Genetic Engineering for Crop Improvement. *The Journal of Plant Science Research*, *29*(1), 5.

9. Berg, P. and Mertz, J.E. 2010. Personal reflections on the origins and emergence of recombinant DNA technology. *Genetics*, *184*(1), pp.9-17.

10. Alberts, B., Johnson, A., Lewis, J., Raff, M., Roberts, K. and Walter, P. 2002. Isolating, cloning, and sequencing DNA.

11. Liang, J., Luo, Y. and Zhao, H. 2011. Synthetic biology: putting synthesis into biology. *Wiley Interdisciplinary Reviews: Systems Biology and Medicine*, *3*(1), 7-20.

12. Byrne, P. 2014. Genetically Modified (GM) Crops: Techniques and Applications. *Colorado State*

13. James, C. 2013. Global Status of Commercialized Biotech/GM Crops: 2013. ISAAA Brief No. 46, ISAAA, Ithaca, NY.

14. Lopez-Arredondo D, Gonzalez-Morales, S.I. and Bello-Bello E. 2015. Engineering food crops to grow in harsh environments [version 1; referees: 2 approved] F1000Research 4(F1000 Faculty Rev):651 (doi: 10.12688/f1000research.6538.1)

15. Reguera, M., Peleg, Z. and Blumwald, E. 2012. Targeting metabolic pathways for genetic engineering abiotic stress-tolerance in crops. *Biochimica et Biophysica Acta (BBA)- Gene Regulatory Mechanisms*, *1819*(2), 186-194.

16. Suslow, T., Thomas, B. and Bradford, K. 2002. *Biotechnology provides new tools for plant breeding*. University of California. Division of Agriculture and Natural Resources. Publications.

17. Naranjo, M.A. and Vicente, O. (2008) Transgenic Plants for the Third Millennium Agriculture. *Bulletin of* 7*UASVM, Horticulture*, 65, 38-43.