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Modern Biotechnology and New Food Varieties

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Abstract: The world population is predicted to reach 9.5 billion by 2050. This will demand powerful techniques in agricultural production. Transgenic technology can be used in both crops and animals through improved crop production, milk production and composition, improved meat production, increased disease resistance and prolificacy. To create a stable transgenic organism, foreign gene is transferred using specific methods suitable for a particular species, that include DNA microinjection, sperm-mediated DNA transfer and somatic cell nuclear transfer for transgenic animals production and *Agrobacterium*-mediated, microprojectile bombardment, direct DNA transfer to protoplasts for plant transformation. However, the safety of transgenic food and derivatives in the markets has to be verified for the presence and the amount of genetic modification varieties. This review addresses up to date progress from the genetically modified food industry.

Keywords: Gene Transfer, GM Food, Plant Transformation, Safety Issue, Transgenic Organism

1. Introduction

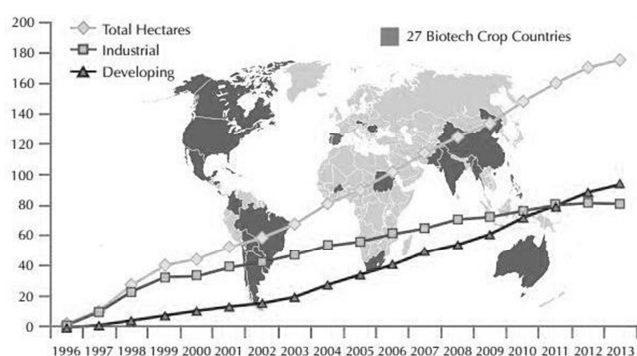
Biotechnology is the application of scientific techniques to modify plants, animals, and microorganisms to improve their value. Since time immemorial, man has been using green biotechnology to improve the quality, quantity and production outcomes of nutrients. The use of this green biotechnology can offer several potential advantages for food security, which include increase of food production, reduction of agricultural water use, decrease of greenhouse gas emission, decrease of insecticide and herbicide use, improvement of nutritional value, enhancing crop adaptation and reduction of soil physical damage [1-4]. Through cross breeding techniques, man used to change and improve the quality of food products of the selected plants and animals that harbor the most desirable traits for food production and breeding for next generation. These traits should present a high yield, be easy to harvest and be non-toxic [5, 6]. The most recent application of green biotechnology is genetic modification (GM), also referred to as gene technology,

genetic engineering, genetic manipulation or recombinant DNA technology. This technology involves the insertion of a gene from a foreign source such as yeasts, viruses, bacteria, animals or plants into typically unrelated species [7]. The plants and animals, in which the genes of interest are added to their genomic DNA, are described as genetically modified organisms (GMO). The plants and animals start to express the proteins of the inserted genes while they grow and develop, which leads to several changes in the organism; such as structure of molecules, anatomy, biochemistry, physiology and morphology thus resulting in the creation of a new living entity not found in nature [6, 8].

The world population has increased at a slow, steady pace which demands increasing the supply of food to meet future needs. This will require increasing crop yields and cropping intensity. It is therefore imperative to invest in agricultural research to increase the yield potentials of specific food crops as well as fish and livestock production, which has forced farmers from different countries, such as; Argentine, Mexico, USA, Canada and China to directly adopt the new genetically

modified (GM) crop varieties as they become available. The application of GM technology showed several benefits in different domain like agriculture and food industries. The GM technology increases farming productivity and reduces chemical use (e.g. using pesticide-inherent crops and herbicide-tolerant crops), and lower the production cost which subsequently reduces price. Therefore adoption of GM technology could be regarded as a pro-poor strategy [9-13]. The 2013 report has estimated that 18 million farmers from 27 different countries planted 175.2 million hectares of transgenic crops (Figure 1) [14, 15].

Herein study, we addressed the vital role of transgenic food to the hungry world, the most commonly used methods for the creation of GM food, the labeling regulations and detection methods for GM food and products, and the safety issue of GM food and products.



Source: [14]

Figure 1. Global area of biotech crops from 1996 to 2013 (million hectares).

2. GM foods and Poverty Alleviation

The Food and Agriculture Organization (FAO) estimated that about 870 million people was chronically undernourished during 2010-2012, that is 12.5% of global population or one in eight people, with most of these people live in developing countries. This indicates that the first target of the Millennium Development Goal (MDG) which is to halving the world hunger by 2015 has faced certain limitations, among which, the rapid increase of global population and climate change [16, 17]. The world population is continually increasing from 7.2 billion in 2000 to a predicted 9.5 billion in 2050 [18, 19] and a doubling of the global demand for food will occur between 2000 and 2050 as reported by FAO. This increased demand will require competent agriculture techniques with a fast pace to double the current food production [20, 21]. In the coming years, food and agricultural production techniques should also be improved to meet other anthropomorphic changes, such as an increasing international competition, loss of biodiversity, globalization, shifts to increased meat consumption in developing countries, land availability and degradation, climate change, and rising consumer demands for improved food quality, safety, food insecurity and health enhancement. This demands new technical knowledge that contributes reliable methods for improving quality, productivity, and

environmental sustainability. Biotechnology has introduced a new area by supplying efficient and cost-effective means to produce a large number of improved and desirable products and tools. This includes the enhancement of quality and quantity of food, feed, fiber, and biofuel production; hence, changing the heading date of crops, reducing the dependency of agriculture on chemicals and fossil fuels, diminishing over-cultivation and erosion and lower the cost of raw materials, and enhancing the photosynthetic efficiency where currently efforts are going on to convert C3 plants into C4 plants, all in an environmentally friendly way [20, 22].

All over the world, farmers have been helped by green biotechnology to enhance their productivity. Bt cotton varieties in about 2.1 million hectares were grown in 2002 by around five million farmers. During this time, one kilogram of Bt cotton cost 28% less than non-Bt varieties. This was attributed to a reduction of pesticide application by 59-80% compared to conventional cotton (assessed in 3 years of use) [23]. Rice and maize are the main food and food derived products resources; 4 billion USD annual benefits were generated from Bt rice which showed the potential to increase yields up to 8% and decrease pesticide use by 80% equivalent to 17 kg/ha [24]. Thus, green biotechnology has indeed proved its potential to counteract the world's hunger. Until 2003, U.S. Department of Agriculture had already approved twelve transgenic crops: corn, tomato, soybean, cotton, potato, canola, squash, beets, papaya, rice, flax, and chicory for commercial production. These transgenic crops have contributed to biodiversity, enhance crop quality and yield, and improve the environmental quality by reducing uses of herbicides and pesticides [6, 15].

3. Plant Transformation

Plant transformation was started in 1980s after many researches which demonstrated that *Agrobacterium tumefaciens* which causes crown gall disease could be used to introduce interested DNA fragments into plant cells [25, 26]. Plant transformation is defined as the introduction and integration of foreign DNA fragment in plant cells which leads to generation of transgenic plants. This process can lead to transient or stable expression of the introduced DNA. Transient expression lasts for a few days, the transformed DNA is not integrated into nucleus of the host genome; therefore the incoming DNA fragment will be degraded through mitosis [26]. A stable transformation occurs when it is needed to maintain the introduced DNA in the genome of the cell and its daughter cells [26, 27]. Genetic engineering using the recombinant DNA technique provides a way to introduce DNA fragments in complex pathways and to regulate their expression. Many DNA-transfer technologies are available, including *Agrobacterium*-mediated [28, 29], microprojectile bombardment [29], direct DNA transfer to protoplasts, electroporation, micro-injection, macro-injection, impregnation by whiskers, impregnation by tissues, silicon carbide fibres, polyethylene glycol (PEG), floral dip, pollen tube pathway, ultrasonication and laser-mediated uptake.

Presently, the three widely used methods are the *Agrobacterium tumefaciens*-mediated transfer (Figure 2), microprojectile bombardment (gene gun or biolistic method) (Figure 3) and direct gene transfer to protoplasts (Figure 4) [15, 30-33]. These genetic transformation methods are

categorized into indirect gene transfer, where foreign DNA is introduced by a plasmid and direct gene transfer, where physical and chemical processes are used, that is based on the penetration of the cellular wall [32, 34, 35].

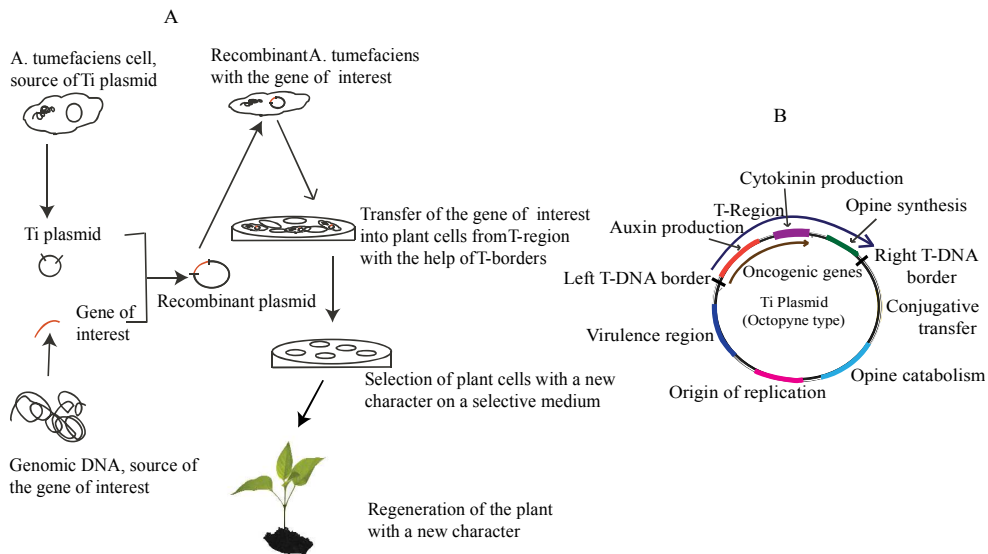
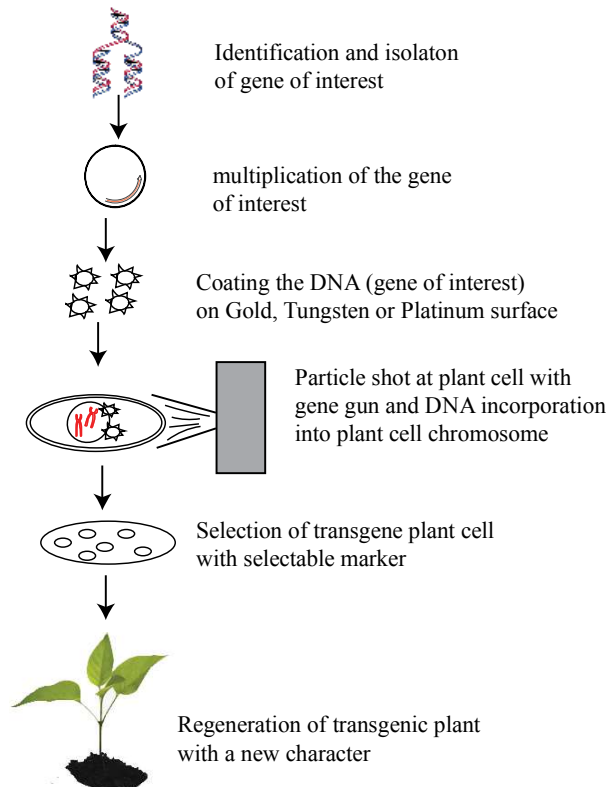
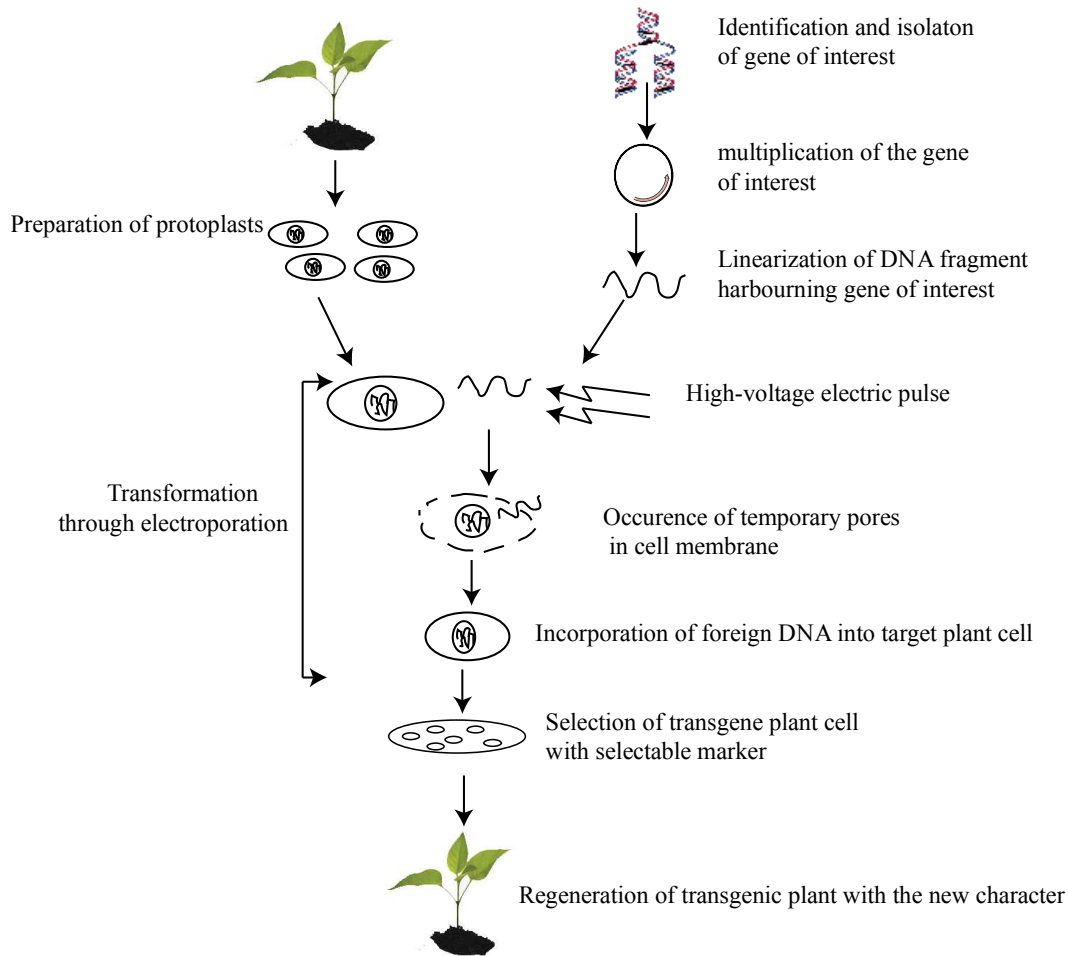


Figure 2. A. Creation of GM crop by *Agrobacterium*-mediated method. Ti plasmid is isolated from *Agrobacterium tumefaciens* and modified by using recombinant DNA technology, the T-region of the plasmid is replaced by the gene of interest but holding T-borders intact which plays a crucial role during the process of DNA transfer to the plant cell. The recombinant Ti-plasmid is transformed back to *Agrobacterium tumefaciens*, and then the recombinant strain is cultured with plant cells where the gene of interest is transferred from *Agrobacterium tumefaciens* into plant cells. B. Schematic representation of a Ti plasmid.



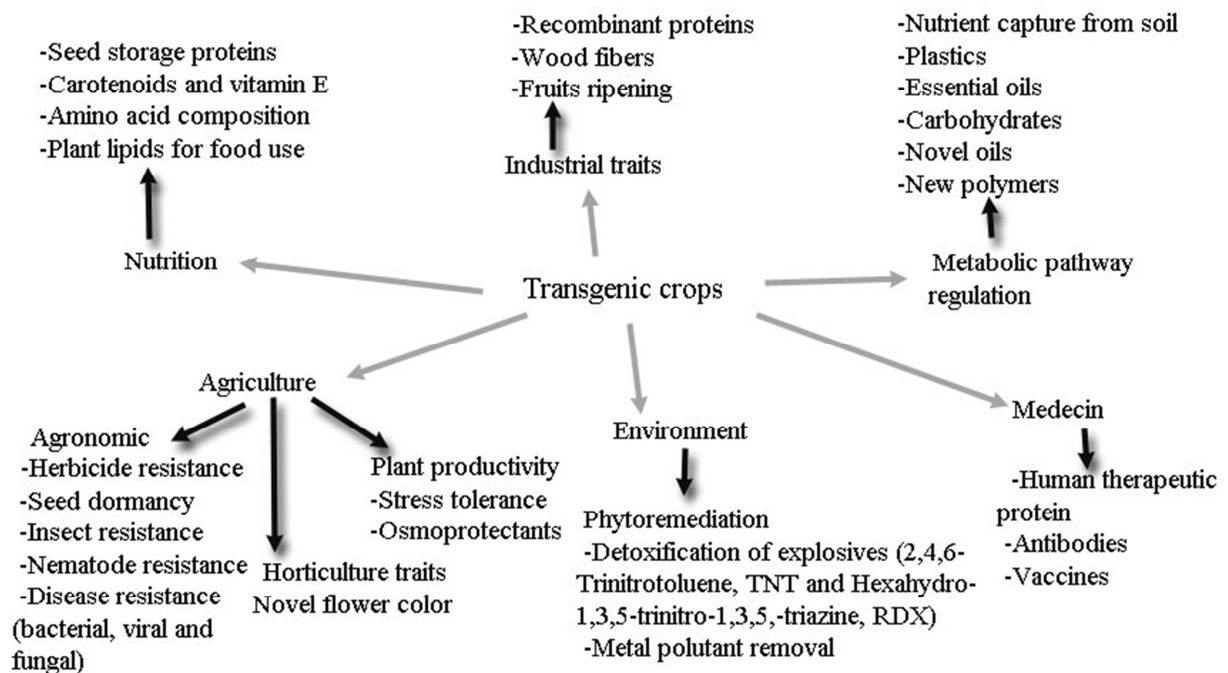
Source: Adapted from [29, 31, 36]

Figure 3. Creation of GM crop by particle bombardment method. The gene of interest is selected and amplified, then coated onto the surfaces of inert particles (micrometre-size gold, tungsten or platinum) by precipitation with calcium chloride and spermidine. The particles are accelerated such that they penetrate cells and tissues in a nonlethal manner. Once inside the cells, the DNA elutes off the particles and is expressed by the host cells, then a portion is stably incorporated into the host chromosomes.



Source: Adapted from [36]

Figure 4. Plant regeneration from single protoplasts. Protoplast transfection involves protoplast isolation from plant tissue by enzymatic removal of the cell wall and subsequent transfer of plasmid DNA carrying genes of interest.



Source: Adapted from [31, 37]

Figure 5. Applications of transgenic crops.

3.1. Applications of Transgenic Crops

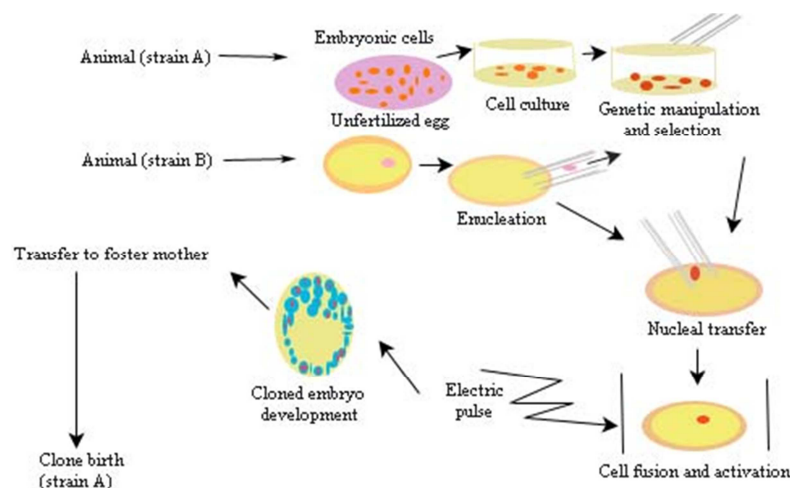
Various applications of transgenic crops have been envisaged and are under investigation for the future (Figure 5) [31, 37]. The main transgene targets were pest resistance and herbicide tolerance, but also resistances to abiotic stress and improvement of the nutritional value were investigated. Furthermore, possible applications that are under development are the production of medically valuable proteins or chemicals in plants (pharma plants) e.g. transgene rice which can produce human serum albumin, or the production of edible plants containing vaccines, the production of bioplastics, phytoremediation, i.e. removal of metal pollutant [31, 38, 39]. Nowadays with climate change and global warming genetic engineering has been used to develop transgenic plants which are able to tolerate heat stress conditions [40].

3.2. Detection of GM Crops, Products and Their Safety Issues

Transgenic organisms have demonstrated several applications in different domain, especially in agriculture by improving quality and quantity of crops and reducing dependence to organic chemicals (herbicides and pesticides). However, possible impact of transgenic crops and food products to environment and public health have forced some government to **establish food labeling regulations**. According to the regulation EC 1830/2003 of European Council, when the amount of any authorized GM ingredient exceed 0.9% it must be indicated, while 0.5% is set as the threshold for non-authorized GM [41]. However, The GM crops approved in one country do not certainly have the same approval status in another country. Thus numerous countries have executed labelling thresholds for unintended existence of approved GM crops well-defined as 0.9% in the EU and Russia, 3% in Korea, 5% in Japan, Indonesia, Thailand and Taiwan, and 1%

in Brazil [42]. The verification of GM content is based on either detecting the inserted genetic material at DNA level and the transcribed mRNA or identifying the subsequent recombinant protein, phenotype and metabolite. For this purpose, reliable screening methods have been developed for detection of unauthorized GM crops and labeling control. These include real-time polymerase chain reaction (RT-PCR) for detecting the inserted DNA, immunological assays for detecting the resulting protein (e.g. the enzyme-linked immunoassay (ELISA) and lateral flow sticks), or using different types of biosensors (electrochemical sensors, piezoelectric biosensors, surface plasmon resonance/optical biosensors) to detect the resulting phenotype (e.g. herbicide bioassays). These methods above can be coupled with other efficient emerging analytical technologies such as, chromatography, mass spectrometry and near infrared spectroscopy and DNA chip technology (microarrays) [42-45]. So far only PCR has found broad application in GMO detection as a generally accepted method for regulatory purposes with the rapid, sensitive, specific and quantitative aspects [44, 46].

The evaluation of the safety of genetically engineered (GE) food and food ingredients is an absolute process of several steps. The U.S. Food and Drug Administration (FDA), 2013 and WHO, 2014 outlined that the GE plant developer must (a) identify the characteristics of the new genetic traits and analyzes the toxic and allergenic effects of the incoming food, (b) compare the nutrients levels in the new GE crop to traditional bred crops, that include fiber, protein, fat, vitamins, and minerals, (c) study the stability of introduced gene, (d) profile the nutritional effects connected with genetic modification and any unintended effects associated with gene insertion, (e) submit this information to national authorities for a safety assessment and compliance with the law [47, 48]. WHO recommends the use of codex alimentarius guidelines [49].



Source: [55, 56]

Figure 6. Schematic diagram of the somatic cloning process. Cells are collected from donor (Strain A) and cultured in vitro. A matured oocyte (from Strain B) is then enucleated and a donor cell is transferred into the enucleated oocyte. The somatic cell and the oocyte are then fused and the embryos is allowed to develop to a blastocyst in vitro. The blastocyst can then be transferred to a foster mother and cloned animals are born after completion of gestation.

4. Transgenic Animals

A transgenic animal is an animal that both carries a specific gene from a different species or results from genomic DNA manipulations and undergoes deliberate modification of its genome. Recombinant DNA technology is used to create a transgenic animal by introducing a foreign DNA into the animal's genome. The constructed recombinant DNA should be stably maintained, expressed and inherited and passed on to subsequent generations. The most important thing is that the heritability of the genetic modification can be achieved by creating an animal that carries the modification in the genome of its germ line: all offspring derived from this animal will be completely transgenic, as they will carry this modification in all their somatic and germ line cells [31, 50].

The establishment of a stable transgenic animal implies that the foreign DNA is present in gametes or one-cell embryos to allow its transmission to progeny. Depending on the animal species, the gene of interest can be transferred using different methods such as DNA microinjection, sperm-mediated DNA transfer, viral-vector mediated DNA transfer, gene transfer using embryonic cells and somatic cell nuclear transfer (Figure 6) [51-54].

5. Applications for Transgenic Animals

The production of transgenic organisms has been a major technological advance in the study of biology. Transgenic animals have provided new perceptions into the study of the mechanisms of gene regulation and developmental biology. Subsequently, this technology has allowed significant advances in other branches of biomedical sciences including a) the implication of some genes in the development cancer (oncogenes) and oncogenic viruses; b) the mechanisms of regulation and cell interaction in the immune system; c) models for human genetic diseases; d) the mechanisms and control of growth; e) production of biopharmaceuticals, myelin basic proteins from mice, monoclonal antibodies from goats, vaccines and insulin from chicken and eggs, human hemoglobin from pigs, protein C from cow, human erythropoietin from rabbit, human factors VII and IX from sheep and other compounds and f) the basic mechanisms of biology and genetics [31, 54, 57].

Furthermore, transgenic technology is used in production of dragline silk (BioSteel). BioSteel is one of the spiders silk which is a protein fiber spun by spider. BioSteel is known to possess outstanding physical and mechanical properties, toughness, elasticity, strength and is weight by weight five times stronger than steel and three times stronger than Kevlar and absolutely biodegradable and biocompatible; these properties are exploited to make medical devices like surgical fiber from BioSteel. Nowadays transgenic goat duly can produce dragline silk in milk as water-soluble silk proteins [58-61]. Transgenic technology is also employed to generate a novel variety of ornamental fish, transgenic zebrafish, which exhibits vital applications in various domain including

biomedicine. Transgenic zebrafish is used as a genetic model to comprehend the human tissue regeneration defects and encourage the progress of regeneration medicine, where zebrafish helps in developing infection disease model (e.g. tuberculosis), screening chemical genetic, emerging cancer pathogenesis model (e.g. melanoma, leukemia), understanding toxicological process (e.g. copper homeostasis). Zebrafish is transparent and under a suitable organ/tissue specific promoter, it can express fluorescent proteins which helps in visualizing the phenotype of the organ [62-66].

There are various potential applications of transgenic technology to develop new or altered strains of agriculturally important livestock. Practical applications of transgenesis in livestock production include improving the desirable traits such as high milk production and composition, improved meat production, rapid growth rate, feed usage, carcass composition, resistance to diseases, enhanced reproductive performance and prolificacy [57, 67].

6. Conclusion

Genetic modification provides a beneficial way of modifying the genome of both crops and animals to improve their production and composition with benefits to the environment, farmers and consumers. To establish a stable transgenic organism (or a Genetically Modified Organism), the genome modification can be performed employing specific methods according to organism species, then such a technique leads to creation of a new organism previously not found in nature. Nowadays more than 18 million farmers from different countries that include USA, Canada, Argentina, Mexico and China, are interested in transgenic crop varieties as an alternative to feed a hungry world. Transgenic animals showed diverse application in different domain such as the use of BioSteel in medicine. To avoid concerns regarding genetically modified crops, food and food ingredients, a competent method based either on DNA or protein must be used to monitor and verify the presence and the amount of GM varieties of crops and food products. A competent biotechnological team could then assess the safety of GM food before being released on the market.

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