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Advances In Agricultural Biotechnology

Mamata KC, Anuj Lamichhane
Agriculture and Forestry University, Rampur, Chitwan; Nepal
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Abstract

Agricultural biotechnol.ogy is becoming the major sector in crop improvement through the use of scientific techniques for the modification of genes conferring resistance to biotic, abiotic stress and improving the quality of crops. With the evolvement from Mendelian genetics to molecular biotechnology, there have been several developments in the field of crop improvement. Recent biotechnological advances have aimed towards removing the physiological constraints of the crops and increasing crop yield potential. With the use of different tools of agricultural biotechnologies like genetic engineering, tissue culture, embryo rescue, somatic hybridization, molecular marker-assisted selection, genome doubling, and omics technologies, various transgenic crops have been developed over the decades and have been approved for commercialization. This development and adoption of transgenic technology have been shown to increase crop yields, reduce CO2 emission, reduce pesticide and insecticide use and decrease the costs of crop production. Even though the biotechnological approach and transgenic organisms have immense potential to contribute to the world's food security, several concerns of genetically modified crops being a threat to the environment and human health have developed. This review will address applications and concerns of biotechnology in crop improvement considering health hazards and ecological risks.

Keywords: Agricultural biotechnology, genetic engineering, transgenic organisms, benefits, concerns.

Corresponding author, email: kcmamata24@gmail.com

Introduction

Biotechnology refers to the implementation of comprehensive scientific techniques to alter and enhance the characteristics of different plants, animals, and microorganisms that are of economic importance [1]. Biotechnology is a broad term that includes applications of microorganisms and different foreign genes (gene of interest) in the processing of food; agriculture and forestry; environmental protection, medical sector, etc. [2]. Agricultural biotechnology is the branch of biotechnology that involves the exertion of scientific techniques for the modification and improvement of crops as well as livestock [3]. With the increasing population, traditional agriculture is not sufficient to meet the demands of food worldwide, thus the continuous increase in agricultural productivity depends on effective unification of biotechnology with classical breeding to create an "Evergreen Revolution" [4].

Crop productivity has advanced largely during the 20th century based on applications of Mendelian genetics, but if farmers are to address the demands that will be laid on them over the next half-century more effectively, research in biotechnology and molecular biology should be aimed towards removing the physiological constraints of the crops and increasing crop yield potential [5]. Recent developments in plant molecular biology and genomes not only has provided us the knowledge and understanding of plant genomes but also the possibility of modifying them [6]. Biotechnology provides series of techniques that give access to a wider gene pool and also permits the accurate progress to produce new and useful plant and animal genotypes working along with conventional breeding techniques side by side [7]. The use of traditional techniques, without any question, has profoundly improved important heritable characters such as yield, resistance to disease, etc. in crops, however, there are certain restrictions to these techniques like it may take a very long time to introduce, select and establish a trait into a cultivar or it may be impossible to incorporate certain traits with these techniques. Genetic engineering overcomes these limitations by introducing the desired trait in short time without altering other characters of the plant [8].

In this technological era, agriculture faces a new stream of technological revolution associated with biotechnology which could offer considerable assurance for agricultural sustainability by quality enhancement of the product, disease and insect pest resistance, environmental protection, and improving agricultural productivity [9]. With the advances in the field of molecular biology, scientists can manipulate DNA to produce transgenic organisms, the process is known as "Genetic Engineering" and offers a range of benefits along with possible risks [3]. There are controversial social and regulatory consequences with genetic engineering and food made from transgenic crops [10].

So, all of transgenic crops developed are not released for commercial cultivation. This review tries to address the recent advances of biotechnology in agriculture and its major concerns.

Background and History of biotechnology in Agriculture

Agriculture is the backbone of the human food supply. Agriculture was practiced manually, in the beginning, using primitive technologies based on plow and harrow. The Industrial revolution (1875-1885) enabled accelerated economic development which led to the movement of people from rural areas to industrialized cities. It was around this time the chemical fertilizers were introduced for protection against disease and attainment of higher yields [11]. The human population at present is 7.87 billion increasing at 1.1% average annual rate of population change in year 2015-2020 [12]. This continuous increase in population, estimated to reach 9 billion by 2050, poses a serious challenge to global food security. With the increasing world population, agricultural land has been utilized for settlement purpose. This has decreased the land under cultivation and ultimately the productivity. So, increasing food demands of the world can be met by increasing the global agriculture productivity. But lower land under agriculture cultivation demanded a drastic innovation in technology which not only increase the agriculture productivity but also sustain it for long time. This was provided by the breakthrough of biotechnology field [13]. Gregor Mendel's paper "Experiments on plant hybridization", published in 1866; included how different traits were passed from generation to generation which marked the beginning of new technologies designed for improvement in crop species [1]. But, gene modification in crops is supposed to have begun around 10,000 years ago as a result of random or chance through the selection of novel crop types [14]. In 1960, Green Revolution helped in increasing productivity of three main cereal crops viz. rice, maize, and wheat. A particularly important finding was the discovery of the molecular structure of deoxyribonucleic acid (DNA) and the fact that DNA was involved in inheritance. The genetic code was cracked in the 1960s and made a way for the transfer of genetic material even easier. With the transfer of genes from one organisms to another, different novel organisms are created, often referred as 'Genetically modified organisms (GMOs)' [15]. With development of several GMOs; modern biotechnology has focused on genetic manipulation for agriculture, horticulture, environment, medicine, forensic science, and many other fields [16]. The major events in history of biotechnological development is presented in Table 1 [11].

Table 1: Summary of the main events in the development of biotechnology [11]

Classical biotechnology	
1664	Discovery of microorganisms.
1884	Discovery of bacteria.
1857	Microbiology of lactic fermentation.
1860	End of the spontaneous generation theory
1866	Theory of Inheritance (Gregor John Mendel)
1902	Chromosomal Theory of Inheritance
1910	Discovery of linkage
1928	Transformation in bacteria
1941	One gene-one enzyme hypothesis
1946	Bacterial conjugation.
1947	Chargaff's rule
Modern Biotechnology	
1953	DNA structure.
1958	Semi-conservative Replication of DNA
1959	Gene regulation.
1960	Green Revolution
1966	Genetic code decoding
1970	The high specificity of restriction enzymes. Rise of phyto-genetics
1973	Recombinant DNA replication in E.coli
1978	Human proinsulin gene isolation
1985	Polymerase chain reaction.
1992	Beginning of the Golden Rice project
1996	Full-fledged commercialization of GM crops

Agricultural Biotechnology in Crop Improvement

Agricultural biotechnology refers to the use of biological organisms or range of tools for the improvement of the plants, animals, microorganisms, or food derived from them. Following are some biotechnology tools used in agriculture:

Transgenesis

Transgenesis also called genetic engineering or recombinant DNA (rDNA) technology; includes multiple techniques used for the desired manipulation of genetic material (cutting and joining together) particularly DNA from various species, and subsequent introduction of the resulting hybrid DNA into a new organism to form new combinations of heritable genetic material [17][18]. Organisms resulting from transgenesis are called Genetically Modified Organisms (GMOs).

Around 530 different transgenic events in 32 crops have been approved for cultivation in different parts of the world [19]. Among them, Maize accounts for the maximum number of events (240), followed by cotton (67), potato (50), Argentine canola (42), soybean (42), carnation (19), and so on. Transgenesis has been applied to develop Herbicide-tolerant (HT) transgenic crops, Insect-resistant (IR) transgenic crops, Abiotic stress-tolerant (AST) transgenic crops, disease-resistant transgenic crops, and nutritionally improved transgenic crops.

Herbicide Tolerant transgenic crops

The first herbicide-tolerant transgenic crop to be commercialized was Glyphosate-tolerant soybean (Roundup Ready soybean), which harbored EPSPS gene from CP4 strain of *Agrobacterium tumefaciens*. Most of the commercialized glyphosate-resistant crops harbor this gene [20]. Two different genes from Streptomyces spp., namely pat and bar, were utilized for developing Glufosinate-resistant crops. Similarly, other HT transgenic crops specific to other herbicides like 2,4-D, Isoxafutole, Oxynil, and Sulfonylurea, have been commercialized recently [21]. A total of 351 herbicide tolerance events have been approved for cultivation [19]. Of these, the maximum number of HT events (212) has been commercialized in Maize, followed by Cotton (45), Argentine canola (34), and others.

Insect Resistant Transgenic Crops

Most of the insect-resistant transgenic crops are developed from cry genes from *Bacillus thuringiensis* (Bt); which provides resistance against a wide variety of insect pests (Lepidopterons, Coleopterans, and Dipterans) [22]. Cry genes not merely provide resistance against insect pests but also is non-toxic to mammals. The first commercially successful crop was Cotton in which cry gene was inserted that provided resistance against its lepidopteron insect pest. After the success of transgenic cotton, cry genes have been incorporated in many crops, viz., potato, rice, canola, soybean, maize, chickpea, alfalfa, and tomato [21]. Similarly, vip genes isolated from Bacillus species (*B. thuringiensis* and *B. cereus*) are incorporated in cotton and maize for insect resistance [23][19]. Genes encoding protease inhibitor (PI) from different sources (plants, bacteria, and fungi) have been used to produce insect resistant plants. The cptII and potato protease inhibitor II genes have been introduced in tobacco, and rice, and cotton, respectively to provide resistance against insects [21][19]. To date, 305 insect resistance events have been approved for cultivation [19]. Of these, the maximum number of insect-resistant events (208) has been commercialized in Maize, followed by Cotton (50), Potato (30), and others.

Abiotic Stress Tolerant Transgenic Crops

The impact of abiotic stresses is increasing in crops with changing climatic conditions. Certain plants adapt to these abiotic stresses at the molecular level by altering the expression of an array of genes. This helps to create near-optimal conditions for plant growth and development [21]. Due to the complexity of the abiotic stress adaptation trait (many genes are involved), a lesser number of abiotic stress tolerance events have been commercialized as compared to traits like disease, insect, and herbicide tolerance. A total of 12 abiotic stress tolerance events have been approved for cultivation in Maize(7), Sugarcane(3), and Soybean (2) [19]. The use of bacterial cold shock proteins (csp) to mitigate the effects of abiotic stresses, like cold in *Arabidopsis*, cold, heat, and water deficit in rice, and water deficit in maize, has been demonstrated by Castiglioni et al. in 2008 [24]. Two genes: the cspA gene from *E. coli* and the cspB gene from soil bacterium *B. subtilis* were incorporated in maize, which not only showed better adaptation during water-scarce conditions but also did not lead to pleiotropic effects in maize. Recently, Hahb-4 gene from *Helianthus annus* (Sunflower) is introduced in Verdeca's drought tolerant transgenic Soybean commercialized as Verdeca HB4 Soybean. The gene produces isolated nucleic acid molecule encoding the transcription factor Hahb-4 which binds to a dehydration transcription regulating region of plant [19]. Similarly, using betA gene from *E. coli* and *Rhizobium meliloti* drought-tolerant transgenic Sugarcane has been made. These transgenic sugarcane crops withstand drought conditions up to 36 days and produce 10-30% higher sugar as compared the non-transgenic plants under drought conditions in field trial [25,26].

Disease Resistant Transgenic Crops

Diseases are caused by pathogens (fungi, bacteria, viruses, and other micro-organisms), and cause huge loss in crop yield. Despite the environmental hazards caused by the use of agrochemicals, management of diseases in plants is usually done using agrochemicals, which pose the challenge of the development of chemical-resistant pests [21]. Scientists have been able to breed plants with disease resistance traits using transgenesis. So far, 29 disease resistance events have been approved for cultivation [19]. Of these, the maximum number of disease-resistant events (19) has been commercialized in Potato, followed by Papaya (4), Squash (2), and others. Most of the disease-resistant crops commercialized confer resistance against viruses [21]. Using gene encoding the viral coat protein of tobacco mosaic virus (TMV), the first disease-resistant plant was found, which was resistant to TMV infection [27]. Similarly, transgenic papaya conferring resistance to Papaya Ringspot Virus (PRSV) has been developed through a "pathogen-derived resistance mechanism", where the 'prsv cp' gene is introduced by microparticle bombardment into papaya [28]. In bean (*Phaseolus vulgaris* L.), RNAi-mediated resistance against Bean Golden Mosaic Virus (BGMV) was developed by silencing the sequence region of the AC1 viral gene which inhibited the synthesis of the viral replication protein of the BGMV [29]. In potato (*Solanum tuberosum* L.), the Rpi-vnt1.1 gene from *Solanum venturii* is introduced using Agrobacterium-mediated gene transfer, which produces late blight resistance protein and confers resistance to potato late blight [30]. The major constituents of the fungal cell wall (chitin and α-1, 3 glucan) are degraded by the chitinase enzyme thus when the chitinase gene was introduced in tobacco and rice, it has been reported to enhance fungal resistance in the plant [31].

Nutritionally Improved Transgenic Crops

Many successful efforts have been made to improve nutritional qualities in crops using transgenesis. The most recent example includes biofortified rice line GR2E (Golden Rice), developed by the introduction of gene 'crt1' from *Pantoea ananatis* and gene 'psy1' from *Zea mays.* Golden Rice is capable of synthesizing carotenoids in the endosperm. GR2E was approved for use as food in the Philippines, Australia, New Zealand, Canada, and the United States [19]. Similarly, to improve the nutritive value of potato, the transgenic potato tubers were developed by expressing Amaranthus seed albumin gene 'AmA1', which is plentiful of all essential amino acids for human diet specification according to the WHO standard [32]. An effort was made to enhance the pro-vitamin A content in tomato by producing transgenic tomato and converting phytoene to lycopene with the transference of bacterial gene for phytoene-desaturase enzyme. And also three times more β carotene content was produced by these transgenic plants than normal plants [33]. Antisense fae1 gene transferred to Brassica napus and Brassica juncea has resulted in low erucic acid content [34]. In maize, the introduction of the 'cordapA' gene from *Corynebacterium glutamicum* has increased the production of amino acid lysine [19].

Tissue Culture

Tissue culture is the culture of cells, tissues, organs, or their components in a nutrient medium under sterile conditions [35]. It usually involves the use of small pieces of plant tissue (explants) which are cultured in aseptic conditions [36]. Tissue culture manipulates and extends the period of cells, anthers, pollen grains, or other tissues and develops a whole, living growing organisms. Using tissue culture, genetically engineered cells can be transformed into genetically engineered organisms [37].

Tissue culture has been used extensively to create genetic variability through the in-vitro culture of protoplasts, anthers, microspores, ovules, and embryos, to improve crop plants and to increase the number of desirable germplasm available to the plant breeder. It is one of the pivotal tools of biotechnology [38]. Tissue culture is used in the germination of seeds that are difficult to germinate like Banana. Grand Naine (G9) variety of banana is prepared using tissue culture, which results in mass propagation of disease-free high yielding clones, and true to type plants [39]. Similarly, the Meristem tip culture of banana plants produces plants devoid of banana bunchy top virus (BBTV) and brome mosaic virus (BMV) [40]. In vitro cell and organ, culture can be used for the conservation of endangered germplasms. The plants that do not produce seeds (sterile) or produce seeds that cannot be stored for a long period (recalcitrant seeds), can be preserved using tissue culture techniques for the maintenance of gene bank [36].

Embryo rescue for wide hybridization

Embryo resulting from inter-specific or inter-generic crosses may fail to produce a hybrid because of pre or post-fertilization incompatibility barriers. These barriers can be overcome by rescuing such embryos and culturing them for producing a whole plant, which facilitates the transfer of desirable genes from wild relatives into cultivated species [38][18][41]. This technique is known as embryo rescue or wide hybridization. Wide hybridization and Embryo rescue were done in Capsicum to transfer fruit rot-resistant traits by Debbarama et al. in 2013 [42].

Somatic hybridization

Somatic hybridization is a technique that integrates somatic cells from two different cultivars, species, or genera of plants for the manipulation of cellular genomes [43]. Somatic hybridization by protoplast fusion helps in the regeneration of novel germplasm and into whole organisms through tissue culture [44][45]. Similarly, incompatibility barriers at inter-specific or intergeneric levels can be overcome by somatic hybridization. Fusion between protoplasts of Potato (Solanum tuberosum) and Tomato (Lycopersicum esculentum) has created Pomato (Solanopersicon, a new genus). It not only overcomes barriers of sexual incompatibility but also creates novel genotypes [46]

A salt-tolerant hybrid callus culture was developed by somatic hybridization between Rice (*Oryza sativa)* and Mangrove grass (*Myriostachya wightiana*), which is useful in the development of salt-tolerant rice varieties [47]. Disease resistance genes are also transferred using somatic hybridization like asymmetric somatic hybridization was used to transfer bacterial blight resistance trait from wild *Oryza meyeriana* L. to *Oryza sativa* L. ssp. Japonica [48]. Similarly, those genetic traits that are cytoplasmically controlled like male sterility, resistance to certain antibiotics and herbicides, can be easily transferred using protoplast transformation followed by somatic hybridization [43]. Cybridization has been used to transfer Cytoplasm Male Sterility (CMS) in rice [49].

Molecular marker aided genetic analysis and selection

Molecular marker aided genetic analysis helps in gene identification i.e. it studies DNA sequences particularly to identify the genes, QTL (Quantitative trait loci), and molecular markers; as well as associate them with the organism. Molecular marker aided selection helps to identify and trace the inheritance of previously identified DNA fragments through a series of generations [37]. Molecular marker-assisted breeding uses molecular markers along with linkage maps and genomics to alter and improve plants or animal traits based on genotypic assays [50]. Rice genotypes having resistance to Bacterial Blight(BB) and Basmati quality and desirable agronomic traits were identified using phenotypic and molecular marker-assisted selection, which can be either directly used in the development of commercial varieties or used as a donor of BB resistance in Basmati breeding programs [51]. Similarly, Marker-assisted selection allowed identification of sources of Coffee Berry Disease and Coffee rust resistance for use in preventive breeding for resistance to these diseases. Several genes from other *Coffea* species were important sources for gene pyramiding in breeding programs aimed at multiple and durable resistance [52]. Genetic analysis of Fusarium Head Blight Resistance in CIMMYT bread wheat line C615 was done using traditional and conditional QTL mapping by Yi et al. in 2018 [53]. This study showed genetic relationships between FHB response and related traits at the QTL level providing useful information for marker-assisted selection for the improvement of FHB resistance while breeding.

Doubled Haploid/ Genome doubling

A doubled haploid (DH) is a genotype formed when haploid cells undergo chromosome/genome doubling. Haploid cells like pollen, egg cells, or other cells of gametophyte are subjected to spontaneous chromosome doubling, giving a doubled haploid cells, which is then grown into a doubled haploid plant [54]. It allows the development of pure line varieties or inbred parental lines quicker compared to traditional breeding [55].

Double haploid technology in wheat accelerated time to market and faster genetic gains in yield and resistance gain, which helped in reducing varietal development time [56][57]. Similarly, anther-culture followed by DH offers a great opportunity to accelerate breeding progress and improve grain quality. DH plants through anther-culture provide an efficient method for rapid production of homozygous lines of rice which are found to be more viable than other lines [58]. Similarly, in another study by Bakhshi, Bozorgipour, and Shahriari-Ahmadi in 2017, chromosome elimination method was used to develop double haploid wheat lines via crosses with maize as the male parent [59]. Further 3 wheat lines were selected to develop and adapt under heat stress conditions.

'Omics' technologies

'Omics' technologies are subcategories of bioinformatics which include genomics, proteomics, transcriptomics, genome sequencing, and metabolomics [60]. Genomics is used to understand the structure, function, and evolution of genes; and identify DNA that confers to traits in the organisms. Proteomics helps to analyze the protein in tissue for identifying gene expression in that tissue as well as decipher the specific function of proteins encoded by particular genes[61][37].

Omics based approach helps to decipher the entire genome for gaining insights into plant molecular responses, which provides specific strategies for crop improvement. Using the omics approach, we can identify DNA (gene) encoding for a certain trait (genomics), RNA coded by it (transcriptomics), proteins formed (proteomics), metabolites produced (metabolomics), and phenotype expressed (phenomics). Omics technology provides valuable information on the structure and behavior of crop genomics. Any gene responsible for a particular trait can be used to enhance breeding in different ways [62]. A herbicide-tolerant maize line was developed by precise insertion of a target gene using site direct mutagenesis [63].

Concerns of Agriculture Biotechnology

Biotech crops were grown in 29 countries in 2019, contributing significantly to food security, sustainability, climate change mitigation, and upliftment in the lives of farmers and families worldwide [64]. However, some concerns regarding gene manipulation in crops being ecologically harmful and unsafe for human consumptions. Major concerns of agriculture biotechnology are briefly discussed below:

Adverse effects on non-target organisms

The use of transgenic crops for a specific cause (disease/pest resistance) has caused unintended effects on non-target organisms. Reduction in monarch butterfly population has been reported on the adoption of glyphosate-resistant transgenic crops in the USA and Mexico [65]; and higher mortality was reported when its larva fed on milkweed leaves dusted with the genetically modified *Bt* maize as compared to laboratory conditions [66]. Similarly, wide-scale adoption of *Bt* cotton in China increased the population of minor pest (Mirid bug), which acquired the status of major pest later [67].

Biosafety issues

There have been concerns about the safety of transgenic food being a threat to human health and the environment. Risks associated with human health include allergenicity, toxicity, horizontal gene transfer, and feed safety [68]. When introducing a gene into an organism, the level of allergens might increase in the modified organism above the natural range or new allergen might be introduced. So, bean crops modified to increase the level of cysteine and methionine content were discarded after the discovery of the expressed protein of transgene being highly allergenic [69]. So testing of transgenic food may be required to avoid harm to the consumers. Similarly, WHO has claimed genetic material can be transferred from transgenic food to cells of the human body or bacteria in the intestinal tract or to soil microbes mainly because the DNA ingested from transgenic food is not completely degraded by digestion [68]. The possibility of horizontal transfer of antibiotic-resistant marker genes from transgenic food to animal and human gut microbes may result in antibiotic resistance in the gut microflora, though its possibility is extremely low [21]. Similarly, the cultivation of genetically modified crops could cause "genetic erosion" as farmers restrict themselves to few popularly grown varieties. GM crops are not part of the natural process, so they could cause unpredictable changes in ecology and evolutionary response; the resurgence of pests and emergence of superweed are the results of these.

Resistance breakdown

Extensive cultivation of insect-resistant and herbicide-tolerant crops increases the chances of the development of resistance in the targeted insect population through high selection pressure. New insect biotypes may evolve with resistance against transgenic technology. Similarly, superweed having resistance against herbicides may emerge. The field evolved pest resistance to *Bt* maize has been reported in *Spodoptera frugiperda* (Fall armyworm) in Brazil to cry1F expressing corn and cry1Ac expressing soybean [67]. In China, field evolved resistance to Bt cotton in Cotton bollworm (*Helicoverpa armigera*) to cry1Ac expressing cotton has been reported [70].

Economic, Social and Political concerns

There are economic concerns about GM crops, as the price of seeds will be so high that small farmers and farmers in developing countries are unable to afford seeds for GM crops [71]. Concern about negative socio-economic impacts of rapid technological change on-farm or rural structure is also present. In Muslim communities, the use of GMOs is considered halal or haram [72]. The labeling of genetically modified foods is one of the major political concerns. USA does not label GM foods, but there must be a common consensus on labeling genetically modified foods and their products in all countries. Similarly, differences in biotechnology regulations differ in the US and EU, due to minor differences in consumers' preferences [68].

Conclusion

Agriculture has come a long way from the green revolution to the gene revolution. It is being applied and updated more and more daily. With the ability to know and modify the genetic makeup of organisms using biotechnological tools, we can cope with the increasing demand for food through the development of novel varieties of crops with a higher yield, better resistance against biotic and abiotic factors, and ensure environmental sustainability. The use of biotechnology in agriculture has not only helped to increase the productivity of crops but also reduced the cost of production by decreasing needs for inputs (pesticides) and improved the livelihood of the farmers. Similarly, new varieties of plants with higher yields in fewer inputs have wider environment adaptability; give better rotation to conserve natural resources has been developed through biotechnology applications. Despite these rapid developments, concerns regarding the safety issues of GM crops on human health, food/feed safety, on the environment, social, economic, and political are raised continuously. Complete and transparent assessment of GM crops application and their effects should be done, with strong regulatory implementation mechanism for use of GM crops. Alternatively, new methods such as cisagenesis, intragenesis, and genome editing can be utilized for developing improved crops.

Competing Interests

The authors declare that they have no competing interests.

Author's Contribution

All authors contributed equally.

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