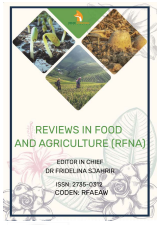
ZIBELINE INTERNATIONAL PUBLISHING

ISSN: 2735-0312 (Online)
CODEN: RFAEAW

Reviews In Food And Agriculture (RFA)

DOI: http://doi.org/10.26480/rfa.01.2020.27.32



REVIEW ARTICLE

FUTURE PERSPECTIVE OF PLANT BIOTECHNOLOGY: A REVIEW

Gaurav Ranabhat*, Ashmita Dhakal, Saurav Ranabhat, Ananta Dhakal, Rakshya Aryal

Faculty of Agriculture, Agriculture and Forestry University, Rampur Chitwan, Nepal.
****Corresponding author E-mail: ranabhatgaurav07@gmail.com***

ARTICLE DETAILS

Article History:

Received 02 September 2020
Accepted 03 October 2020
Available online 15 August 2020

ABSTRACT

Modern biotechnology enables an organism to produce a totally new product which the organism does not or cannot produce normally through the incorporation of the technology of 'Genetic engineering'. Biotechnology shows its technical merits and new development prospects in breeding of new plants varieties with high and stable yield, good quality, as well as stress tolerance and resistance. Some of the most prevailing problems faced in agricultural ecosystems could be solved with the introduction of transgenic crops incorporated with traits for insect pest resistance, herbicide tolerance and resistance to viral diseases. Plant biotechnology has gained importance in the recent past for increasing the quality and quantity of agricultural, horticultural, ornamental plants, and in manipulating the plants for improved agronomic performance. Recent developments in the genome sequencing will have far reaching implications for future agriculture. From this study, we can know that the developing world adopts these fast-changing technologies soon and harness their unprecedented potential for the future benefit of human being.

KEYWORDS

genetic engineering, molecular breeding, plant genomics, phytoremediation.

1. INTRODUCTION

Plant biotechnology is founded on the demonstrated totipotency of plant cells, combined with the delivery, stable integrationand expression of transgenes in plant cells, the regeneration of transformed plants, and the Mendelian transmission of transgenes to the offspring. Man has domesticated plants and animals from the wild about ten thousand years ago. Diligent selection over the years resulted in crop genotypes which are suitable for human sustenance. Since the discovery of the laws of heredity by Mendel in 1865, controlled breeding revolutionized agriculture and enhanced crop yield. Tools of recombinant DNA technology developed in 1960s ushered in the era of new biosciences which would revolutionize every facet of human life in a safe and sustainable manner in the next century. Plant biotechnology represent one of a number of competing technological approaches to addressing a particular agronomic problem but however, as an example, a particular pest problem might equally be addressed through conventional plant breeding, through a transgenic approach, or through an integrated cropmanagement (ICM) approach or any combination of these.

Plant biotechnology is a useful and powerful tool for the development of new plant traits and varieties. Such new varieties must be produced on a large scale to achieve commercial success and to satisfy the demand from growers. Traditionally, new varieties were achieved by the seed propagation method. Many of the agricultural inputs and processes that have been proved to be harmful over the past few decades need to be phased out to preserve the ecological balance, environmental health and natural resources (Kumar, 2001). Biotechnology and genetic engineering of plants will play a crucial role in this area. Currently, the plantlets produced by micropropagation offer a practical alternative for many plant species. Current plant biotechnology has developed as a new age of science and technology where the production of secondary metabolites, valuable plant genetics improvements, germplasm conservation, and production of large numbers of disease-free and new varieties are preferred. This article reviews the progress made in the past decades in the area of biotechnology of plants, and ponders over the prospects in the new millennium.

2. PLANT GENETIC ENGINEERING

Introduction of genes into plants to create new commercially-useful varieties may seem like a valuable task today. In the early 1980s however, this was one of the major bottlenecks preventing the fulfillment of an agricultural revolution that began following the discovery and use of restriction enzymes, followed swiftly by the genetic engineering of bacteria for industrial and medical applications. Plant biotechnology has been technology-driven since its introduction, and the successful establishment of gene transfer technologies for major crops was a major breakthrough for the small biotechnology companies that lead to developments in the field in the early 1980s (Gordon-kamm et al., 1990). When the soil bacterium Agrobacterium tumefaciens was shown to transfer part of the DNA from a resident plasmid into the plant genome, it did not take long to generate the first model transgenic plants (Barton and Binns, 1983). The early pioneers of genetic engineering in plants foresaw the potential of the technology and its ability to increase yields and address our most challenging social problems, such as poverty and food insecurity. Whereas the technology has progressed in leaps and bounds, the positive impact it could have all over the world is being needlessly wasted (Christou, 2013).

2.1 Biotic stress resistance

Biotic stresses are the damage to plants caused by other living organisms such as bacteria, fungi, nematodes, protists, insects, viruses and viroids.

Quick Response Code	Access this article online	
	Website: www.rfna.com.my	DOI: 10.26480/rfa.01.2020.27.32

Numerous biotic stresses are of historical significance, for instance, the potato blight in Ireland, coffee rust in Brazil, maize leaf blight caused by *Cochliobolus heterostrophus* in the United States and the great Bengal famine in 1943 (Hussain, 2015). Pathogens account for about 15% losses in global food production, and are a major challenge in breeding resistant crops (Onaga and Wydra, 2016). It is estimated that disease or insect pest outbreaks are expected to continue to cause food production losses or even worsen by expanding to the areas they were not prevalent before (Siddra, 2012). For the control of this stress, extensive and very often, indiscriminate usage of pesticides over the past four decades has resulted in adverse effects on human health and degradation of environment and fragile ecosystems. Hence, there is an urgent need to reduce the consumption of pesticides by implementing the tools of biotechnology and make the crops inherently resistant to pests and diseases.

2.1.1 Insect pest resistance

It is estimated that 14% of crop productivity is lost to insect pests on a global scale (Krattiger, 1996). Insect-resistant transgenic crops were first commercialized in the mid-1990s with the introduction of GM corn (maize), potato and cotton plants expressing genes encoding the entomocidal δ-endotoxin from *Bacillus thuringiensis* Bt; also known as Cry proteins (Gatehouse et al., 2011). Introduction of genes encoding insecticidal proteins of *Bacillus thuringiensis*into crop plants conferred stable resistance. Intensive efforts during the 1990s have led to the commercialization of insect-resistant cotton, corn and potato (de Maagd et al., 1999). Resistance to these pests can be successfully engineered by exploiting insecticidal proteins found in bacteria, plants and animals (Kumar, 2001). Many insecticidal proteins are available in nature which are highly specific to agronomically important insect pests but at the same time these are harmless to man, mammals and other organisms including beneficial insects (Adamczyk and Hardee, 2002). The bacterium *B. thuringiensis* (Bt) was first discovered in Japan in 1902 in a silkworm rearing unit. In 1911, it was again isolated in a flour moth population and characterized by Berliner in Thüringen (Germany). Most Bt strains produce several crystalline proteins (Cry proteins), each of which shows a rather narrow host range (Bravo et al., 2013). The next generation transgenic crops to be developed in the coming years will carry multiple insecticidal protein genes.

2.1.2 Viral resistance

Viral diseases cause considerable losses to crop productivity. Subsistence crops, that includes cassava, sweet potato, potato, banana, papaya, common bean, rice and maize are often infected with RNA and/or DNA viruses that cannot be controlled with pesticides (Kreuze and Valkonen, 2017). In many cases, resistance sources are lacking, or the genetic complexity and difficulties in introgressions resistance genes to cultivars by crossing hamper the efforts of crop improvement. Therefore, development and transfer of resistance to crops by biotechnological means offers an attractive alternative solution. The studies of Abel et al., 1986 in the 1980s showed that transformation of tobacco plants (*Nicotiana tabacum* L.) to express the coat protein of tobacco mosaic virus made the plants resistant to this virus.

Similarly in potato, potato virus Y (PVY, a member of the genus *Potyvirus*; family *Potyviridae*), potato leaf roll virus (PLRV, a member of the genus *Polerovirus*; family *Luteoviridae)* and potato virus X (PVX, a member of the genus *Potexvirus*; family *Alphaflexiviridae*) are the most common and devastating potato viruses worldwide (Kreuze& Valkonen, 2017). To solve this problem, virus-derived resistance was engineered to both PVY and PVX in potato cv. Russet Burbank, representing only the second example (after tobacco) of genetically engineered virus resistance in crop plants (Lawson et al., 1990). Use of virus-resistant plants is the most effective and economical way to mitigate losses caused by plant viruses. One limitation of resistant cultivars is the inescapable breakdown of resistance owing to the evolution of a new viral strain or species (Kumar et al., 2017). On the other hand, the use of pesticides to control insect vectors is costly and causes harmful environmental consequences. Thus, exploiting strategies that provide durable and broad-spectrum resistance is important in future.

2.1.3 Fungal resistance

Fungal pathogens cause several important diseases in crop plants. For many years, of fungicides application was the only effective strategy for their management. In present time, considerable progress has been made in identification and cloning of genes involved in plant defense responses. With the aid of plant molecular biology and biotechnology, a large number of antifungal proteins and peptides have been isolated and assessed through in vitro bioassays (Culture and Islam, 2014). An important trend therefore is the technical advance that is made to construct cassettes that contain multiple traits. Already, this is feasible to a certain extent, as has been demonstrated with gene stacks that contain three NLRs that recognise *P. infestans* (Ghislain et al., 2019). For many crops the reservoir of cloned resistance genes is still limited. However, another trend is that new affordable sequencing technologies combined with bioinformatic approaches allow ever faster identification of causal resistance genes. on these successes, RNAi has been explored as a strategy to control fungi and oomycetes as well, and initial patent applications for methods to control fungi using RNAi were made as early as 2006 (Esse et al., 2020). Significant effects have been observed in *Fusarium* species by targeting the cytochrome P450, family 51 (*Cyp51*) genes that underlie the azole fungicide target sterol 14α-demethylase with host-induced gene silencing (HIGS) (Koch et al., 2013). Plant genetic manipulation through expression of either new proteins from foreign organisms or the overexpression of a part of their own defensivearsenal for disease resistance has become a reality (Kumar, 2001).

2.1.4 Bacterial resistance

The number of different interaction combinations between bacteria and plants and the diversity of types of bacteria with respect to mode of pathogenesis indicate that plants possess various mechanisms for resistance to bacteria. Multiplication of most bacterial pathogens may occur for a period of time in both resistant and susceptible hosts; following this initial interaction, the plant tissue responds in a predictable manner (Klement and Goodman, 1967). The presence and release of phenolics and their oxidation products in diseased plant tissues has stimulated a host of studies attempting to prove that specific phenolics are responsible for resistance to bacteria as well as to other pathogens (Farkas and Kiraaly, 1962).

The hydrolysis or oxidation of arbutin may be cited as one of the biochemical processes that provide resistance against the fire-blight pathogen, *Erwinia amylovora.* Subsequent examination of the host-response systems would permit identification of the specific bacteriostatic or bactericidal compounds that are synthesized or released from bound form (Kumar, 2001). Recent evidence that specific protein components from bacterial cells induce generalized resistance reactions indicates good prospects for rapid progress in understanding disease resistance to bacteria.

2.1.5 Herbicide tolerant

For many years, scientists and farmers have known that herbicide tolerance can be transferred from one plant to another through crossbreeding, in farmed crops and in wild plants. People have been observing, studying, and managing the transfer of herbicide tolerance for a long time-long before the techniques of modern biotechnology were used to genetically modify plants to have these characteristics. The GE trait conferring tolerance to in-crop application of the herbicide glyphosate was introduced in soy and canola in 1996 and, in cotton in 1997, revolutionizing agricultural practices for these crops (Azhakanandam et al., 2015). The introduction of a transgenic glyphosate-tolerant (GT) soybean (Roundup Ready® or RR soybean) in 1996 revolutionized agriculture and enabled a new-use pattern for glyphosate-based herbicides (Azhakanandam et al., 2015). RR soybean is fully tolerant to glyphosate. Thus, glyphosate can be applied "in crop" as a post-emergent herbicide to control weeds without crop injury.

In agricultural areas, where atrazine has been extensively used, atrazine-resistant biotypes of many weed species have appeared. Resistance was found to be maternally inherited and was correlated with mutations in the psbA gene (Botterman and Leemans, 1988). Glyphosate-tolerant cell cultures of *Corydalis sempervirens* and *Petunia hybrida*were generated after selection on the herbicide (Amrhein et al., 1983). A wide range of crop species has been transformed to confer resistance to herbicides, such as glyphosate, bromoxynil and glufosinate, etc. Herbicide-tolerant crops are under cultivation in countries, such as USA and Canada (Kumar, 2001).

2.2 Abiotic stress resistant

During the last fifty years, it has been shown that abiotic stresses influence plant growth and crop production to great extent, and crop yields have evidently stagnated or decreased in economically important crops, where only high inputs ensures high yields. Drought induces changes in calcium ion levels, which activates calcium-dependent protein kinases (*CDPKs*) via calmodulin-like domain. The activated *CDPKs* regulates downstream components of calcium signaling. For instance, *OsCPK4* overexpressing

rice plants exhibit increased water- holding capacity under drought or salt stress (Campo et al., 2014). Similarly, overexpression of *SNAC1*, *OsNAC10* and *OsNAC5* driven by a root-specific promoter *RCc3* resulted in increased drought resistance under field conditions (Jeong et al., 2010). Transcriptional regulators, such as *HSFs*, *WRKY*, *Zat* and *MBF1c*, a transcriptional regulator of *DREB* genes are activated to regulate expression of HSPs and other heat stress response genes (Suzuki et al., 2011).

Several metabolism-associated proteins, including carbohydrate metabolism enzymes, such as phosphogluconate dehydrogenase, NADP-specific isocitrate dehydrogenase, fructokinase, cytoplasmic malate dehydrogenase, pyruvate orthophosphate dikinase precursors (PPDK), aconitate hydratase, glycine dehydrogenase and enolase, have also been reported to be activated during cold stress (Lee et al., 2009). Dehydration-responsive transcription factors mediate transcription of several genes in response to cold and water stress (Kumar, 2001). Today the importance of crop resistance to such environmental hazards is likely to increase further as the range of environment in which crops are cultivated, expands and the incidence of extreme weather conditions increases.

2.3 Quality improvement

Nutritional quality of the foods we consume is one of the most important concerns, especially in the developing world. Plant biotechnology provides immense scope to improve the food quality in terms of proteins, amino acids, vitamins, oil and starch for human health and well-being. In terms of plant proteins there are two major approaches to improve the nutritional quality: (i) modification of the amino acid composition of the plant proteins. And (ii) introduction of transgenes that encode proteins of high nutrition value. Seed storage protein (2s) gene (AIIIA I) isolated from Amaranthus is a good candidate for introduction into crop plants as the protein has well-balanced amino acid composition (Römer et al., 2000). Gene encoding a human milk protein p-casein was expressed in transgenic potato under the control of an auxininducible promoter (Chong et al., 1997). These findings open the way for reconstitution of human milk proteins in plant foods. The nutritional health and well-being of human beings are dependent on plant foods containing vitamins, minerals and phytochemicals.

For instance, iron is an important element involved in cellular processes. the deficiency of which affects human health in many parts of the developing world (Kumar, 2001). A gene encoding soybean ferritin (iron-storage protein) was introduced in rice under the control of glutelin promoter for the expression of protein in a seed-specific manner (Goto et al., 1999). The iron content of the transgenic rice seeds was three-fold higher than that of their untransformed counterparts. Rice, the major staple food contains, neitherβ-carotene (provitamin A) nor its C40 carotenoid precursors. A gene encoding phytoene synthase from daffodil was incorporated into rice in an endosperm-specific manner. The transgenic plants accumulated phytoene, a key intermediate in provitamin A biosynthesis in the seed (Burkhardt et al., 1997). Transgenic oil crops producing high seed stearic acid level provide an alternative to industrial production of saturated fatty acids. Site-directed mutagenesis of the gene encoding acyl-acyl carrier protein (ACP) thioesterase from *Garcinia mangostana*and expression of this modified enzyme in canolain a seed-specific manner resulted in transgenicplants that accumulate 55-68% more stearatethan the plants expressing the wild-type enzyme (Facciotti et al., 1999).

2.4 Post-harvest trait

Characters that determine the viability and storage life of plant products (fruits, vegetables, flowers and tubers) after harvest are of high economic importance. For extending the post-harvest life of leafy vegetables, there is a high need to focus on the minute observation of these events that occur in regular leaves during senescence. It has been reported that cytokinins can delay leaf senescence and at that time, there is a drop in endogenous ethylene levels (van Staden, 1989). In fact, tomatoes manipulated for delayed ripening are the first genetically modified plant products to be marketed in USA (FlavrSavr), in 1994 (Kumar, 2001). Ripening in fruits and senescence in flowers can be delayed by antisense expression of genes involved in pectin metabolism or ethylene biosynthesis (Kumar, 2001).

It has been reported that the shelf life of transgenic fruits was last up to 60 days at room temperature without any change in their hardness and color (Fry, 2004). In another report antisense, transgenic lines of tomato have also been produced by altering ethylene biosynthesis with an anti-ACO gene (Schaller, 2007). In future, the insights pathway study of crop plants through transcriptomics data analysis may explore the actual metabolism of crop plants will be altered to produce new/improved varieties or species that are tolerant to environmental stresses (Shukla et al., 2015). Manipulation of ripening process in tropical fruits, such as mango and banana hold great promise in near future.

2.5 Floriculture

Molecular approaches are now being used to introduce desirable traits, such as color, shape, plant architecture and vase-life to meet consumers' demand for novelty. The first application of gene technology to modify flower color was reported in petunia (Grusak and DellaPenna, 1999). Expression of the maize dihydroflavonol-4-reductase gene (dfr) in petunia leads to the appearance of pale pink to brick or salmon red pelargonidin pigment and variations in phenotypes. Soliman et al., 2014 studied the effect of gamma radiation on in vitro mutation induction in white petals of chrysanthemum (*Chrysanthemum morifolium* Ramat cv. Youka). The shortening of flowering time by developing early flowering cultivars or plants able to produce flowers during long days are considerable breeding objectives in ornamental plant breeding. Transformation mediated by *Agrobacterium* in *Siningia* sp. supported that exogenous LFY over-expression promotes early flowering (Chandler, 2010). In Gerbera, transformation of GSQUA2 gene accelerated flowering. In *lisianthus*, transformation of OMADS1 showed significant reduction in flowering time as well as increased no. of flowers in comparison to non-transformed ones (Noman et al., 2017).

Utilization of homeotic genes that regulates flower development, may be particularly interesting in ornamental flower crops in which flower morphology variation can be marketable (Chandler and Sanchez, 2012). The gamma irradiated gloxinia plantlets showed morphological changes such as fluffy leaves, funnel-shaped leaves, short internodes, change of color flowers and double-flowered (Miri and Roughani, 2018). In cut flowers, longer vase life is a critical characteristic and is selected during breeding. The conversion of S-adenosyl methionine to 1 -amino cyclopropane- 1 -carboxylic acid (ACC)catalyzed by ACC synthetase and conversion of ACC to ethylene catalyzed by ACC oxidase are the two rate limiting and regulatory steps (Kumar, 2001). Transgenic carnations that contain an anti-sense ACC oxidase *(aco)* gene exhibited low ethylene production and a marked delay in petal senescence (Savin et al., 1995). Some researchers developed tetraploids gerbera plants using in vitro colchicine treatment and found that the ex vitro growing tetraploid plants had longer bloom period with higher vase-life (Gantait and Sinniah, 2014). The extension of vase-life may be due to the fact that the tetraploid plants produced longer stalks than the diploid (Gantait and Sinniah, 2014).

2.6 Phytoremediation

The use of plants for rehabilitation of polluted environment is known as phytoremediation. Over the past century, mining, manufacturing and urban activities have contributed to extensive soil and water contamination. Transgenic *Arabidopsis* has been developed expressing the bacterial gene merB encoding organomercurial lyase (MerB) with an aim to degrade highly toxic organomercurial contaminants (e.g., methylmercury) (Cai, 1997). The merAgene encodes an enzyme, mercury reductase, that is capable of chemically reducing toxic ionic mercury to elemental mercury which is much less toxic and volatile. So, if these two transgenic plants are grown simultaneously on the mercury-contaminated soils, they will be able to reduce contamination at a substantial level (Kumar, 2001). A number of transgenic plants have been engineered for increased arsenic (As) tolerance and accumulation.

Over-expression of genes that are involved in the synthesis of PCs or their precursor GSH significantly enhanced arsenic tolerance but failed to significantly enhance arsenic accumulation (Li et al., 2005). Co-expression of both γ-ECS and PCS in *Arabidopsis* produced a greater effect on arsenic tolerance and accumulation than over-expression of either gene alone. Transgenic plants with strong tolerance to arsenic and enhanced arsenic accumulation in the shoots were developed by co-expressing two bacterial genes (Dhankher et al., 2002).

At military training ranges there is a need for remediation of the nitroaromatic explosives, TNT and RDX (hexahydro1,3,5-trinitro-1,3,5-triazine), to prevent the spread into neighboring communities. Tobacco plants engineered with the bacterial gene for a NADPHdependent nitro-reductase tolerate and degrade high levels of TNT (Rylott et al., 2006). And Arabidopsis plants carrying the xplA gene from *Rhodococcus* bacteria are highly resistant to of RDX (Cherian and Oliveira, 2005). RDX can be degraded and used as a source of nitrogen by several bacterial strains isolated from contaminated sites (Crocker et al., 2006).

3. Molecular Breeding

Plant Improvement of even the simplest of characteristics needs manipulation of a large number of genes, reorganization of important alleles and determination of their chromosomal location. Molecular techniques are now available to track the valuable alleles in segregating population using genetically linked molecular markers. Extensive sets of genetically mapped molecular markers, such as restriction fragment length polymorphism (RFLP), randomly amplified polymorphic DNA (RAPD), micro-satellites and amplified fragment length polymorphism (AFLP) have been produced for many species (Kumar, 2001). Agricultural crops with added value, such as those with improved nutritional qualities, are under continuous development. Newell-McGloughlin, 2008 reviewed improvements to various crops, including enhanced protein quantity and quality in maize, potato, rice, and soybean; increased vitamin content (vitamins C, E, or provitamin A) in maize, strawberry, and tomato; increased carotenoid levels (β-carotene, lycopene, or lutein) in rice, potato, and tomato; increased flavonoid levels in maize, rice, tomato, and soybean; increased iron content in rice and lettuce; and reduced glycoside and solanine levels in potato.

Rice contains little natural β-carotene, a precursor of provitamin A. Golden rice was modified to accumulate β-carotene by the insertion of exogenous genes (Ye, 2000). In 2008, two transcription factors from snapdragons were expressed in tomato to enhance anthocyanin accumulation in tomato fruit to levels which are found in blackberries and blueberries (Butelli et al., 2008). In Japan, transgenic strawberries expressing dog interferon-α were commercialized and sold as an oral drug from March 2014. This is the first example of the use of a powdered transgenic plant as a medicine (Joo et al., 2014). The AtIBH1SRDX tobacco plants produced four times more biomass per unit of cultivation volume, which shows that the vertical farming that is stacking of multiple shelves for plant growth, compared with wild-type plants (Nagatoshi et al., 2016). However, application of gene tagging for important agronomic traits is limited because of difficulty in finding tightly-linked flanking molecular marker.

4. Plant Genomics

Plant biology research reaches a landmark with the invention of sequencing the whole plant genome of certain plant species. Genome sequencing will open horizons to study plant biology, specially characterization of cellular, physiological and developmental pathways (Kumar, 2001). An initial application of plant genomics has been to observe gene expression at a larger scale. The techniques that have made substantial contributions to generate a profile of expression levels is RNA profiling based on hybridization of transcripts to arrays of DNA molecules bound to a solid support. It is popularly described as DNA chip technology, and has bridged the gap between sequence information and functional genomics (Baldwin et al., 1999).

The DNA chip technology facilitated parallel acquisition of massive data for thousands/millions of specific DNA sequences at a faster rate through automation, followed by the analysis of this data using computer devices. In general, the advantage of arrays is that they provide hundreds or thousands of specific genes simultaneously. Thus, two general types of DNA chips or micro- arrays have been developed: DNA fragment-based micro-arrays and oligonucleotide based micro-arrays (Schena, 1998). DNAmicro-arrays can also be applied to screen populations of plants for polymorphic 'expression fingerprints'.These expression fingerprints then can be correlatedwith a complex process, such as drought tolerance,to evaluate new genes or groups of genes in that plant process (Kehoe et al., 1999).

5. Problems And Future Perspective

Plant biotechnology has made tremendous studies in the past decade. Many plant species engineered for expression of a variety of characters are already under extensive cultivation in many regions of the world. Resistance to herbicides, insect pests and viruses has taken precedence while introducing the first-generation transgenic crops is followed by slow-ripening fruits and manipulated floral characters. The second-generation transgenic plants will have more commercial implication (Miflin, 2000). The systems of molecular breeding offer great potential for production of specialized crops which have additional value. However, crop breeding specifically for closed cultivation systems had been limited to date despite its potential availability. Novel cultivars are required to sympathize with the challenges of agricultural technology.

The major challenge in assessing the performance of introduced gene in the field of transgenic biology is gene silencing, which is an unfortunate end of a scientific endeavor and has immediate practical implications. Transgene silencing could be because of presence of multiple copies and hypermethylation of introduced gene, and because of presence of homologous sequences in various configurations in the plant genome (Stam et al., 1997). Gene silencing can perhaps be largely avoided by selection of transgenics containing only single copy genes, as silencing has been often found to occur when multiple copies of the transgene are inserted in the host genome. The vast biodiversity and rich germplasm of crops of Nepal, present in front of us has great challenges as well as unforeseen opportunities. Isolation of genes and promoters from different organisms is an activity which needs more attention. The potential of functional genomics needs to be tapped without losing much time. Patent laws and procedures to protect indigenous efforts and findings need to be developed and streamlined.

6. Conclusion

The dynamic time always offers an opportunity to reflect on human activity in a particular discipline and to formulate a future strategy. Researchers constantly test the past occurrences in order to learn lessons that could help in the acquisition of new knowledge or for the further development of appropriate technology ensuing from it. Science and technology cannot be isolated in the world, so researchers are expected to act according to the changing global scenario in which they live. Applications of biotechnology in the genetic enhancement of crops have been a great task in plant biotechnology to be conducted in coming decades. Few decades earlier, plant biotechnology was relied upon few applications only, such as tissue culture, recombinant DNA technology and monoclonal antibodies. Today, transformation, and marker-aided selection and breeding are just a few of the examples of the applications of biotechnology which are discovered as per needed by the crop improvement program. Plant biotechnology is surrounded by a multitude of scientific tools and techniques for screening and genetic manipulation of plants to develop beneficial or useful plant/plant products. The proficiency of these tools and techniques could be further assisted greatly by nanotechnological interventions. Use of nanomaterials as vehicles for gene or proteins and nanocrystals for high-resolution imaging of the plant cell and organs could be another field to explore by the researchers for creating better novelty in food crops. We can conclude that plant biotechnology is a powerful tool for the development of new plant traits and varieties and such new varieties must be produced on a large scale to achieve commercial success and to satisfy the demand from growers and consumers as a whole.

References

Abel, P., Nelson, R., De, B., Hoffmann, N., Rogers, S., Fraley, R., Beachy, R., 1986. Delay of disease development in transgenic plants that express the tobacco mosaic virus coat protein gene. Science, 232 (4751), Pp. 738–743. https://doi.org/10.1126/science.3457472.

Adamczyk, J.J., Hardee, D.D., 2002. Insect-resistant transgenic crops. ACS Symposium Series, 829, Pp. 23–37. https://doi.org/10.1021/bk-2002-0829.ch004

Amrhein, N., Johänning, D., Schab, J., Schulz, A., 1983. Biochemical basis for glyphosate-tolerance in a bacterium and a plant tissue culture. FEBS Letters, 157 (1), Pp. 191–196. https://doi.org/10.1016/0014-5793(83)81143-0

Azhakanandam, K., Silverstone, A., Daniell, H., Davey, M.R., 2015. Recent advancements in gene expression and enabling technologies in crop plants. Recent Advancements in Gene Expression and Enabling Technologies in Crop Plants, March, Pp. 1–455. https://doi.org/10.1007/978-1-4939-2202-4

Baldwin, D., Crane, V., Rice, D., 1999. A comparison of gel-based, nylon filter and microarray techniques to detect differential RNA expression in plants. Current Opinion in Plant Biology, 2 (2), Pp. 96–103. https://doi.org/10.1016/S1369-5266(99)80020-X

Barton, K.A., Binns, A.N., 1983. of Intact Tobacco Plants Containing Full Length Copies of Genetically Engineered T-DNA, and Transmission of T-DNA to RI Progeny, 32, Pp. 1033–1043.

Botterman, J., Leemans, J., 1988. Engineering of Herbicide Resistance in Plants. Biotechnology and Genetic Engineering Reviews, 6 (1), Pp. 321–340. https://doi.org/10.1080/02648725.1988.10647851

Bravo, A., Gómez, I., Porta, H., García-Gómez, B. I., Rodriguez-Almazan, C., Pardo, L., and Soberón, M., 2013. Evolution of Bacillus thuringiensis

Cry toxins insecticidal activity. Microbial Biotechnology, 6 (1), Pp. 17–26. https://doi.org/10.1111/j.1751-7915.2012.00342.x

Burkhardt, P.K., Beyer, P., Wunn, J., Kloti, A., Armstrong, G.A., Schledz, M., Lintig, J., Potrykus, I., 1997. Transgenic rice (Oryza sativa) endosperm expressing daffodil (Narcissus pseudonarcissus) phytoene synthase accumulates phytoene, a key intermediate of provitamin A biosynthesis. The Plant Journal, 11 (5), Pp. 1071–1078. https://doi.org/10.1046/j.1365-313X.1997.11051071.x

Butelli, E., Titta, L., Giorgio, M., Mock, H.-P., Matros, A., Peterek, S., Schijlen, E.G.W.M., Hall, R.D., Bovy, A.G., Luo, J., Martin, C., 2008. Enrichment of tomato fruit with health-promoting anthocyanins by expression of select transcription factors. Nature Biotechnology, 26 (11), Pp. 1301–1308. https://doi.org/10.1038/nbt.1506

Cai, D., 1997. Positional Cloning of a Gene for Nematode Resistance in Sugar Beet. Science, 275 (5301), Pp. 832–834. https://doi.org/10.1126/science.275.5301.832

Campo, S., Baldrich, P., Messeguer, J., Lalanne, E., Coca, M., San Segundo, B., 2014. Overexpression of a Calcium-Dependent Protein Kinase Confers Salt and Drought Tolerance in Rice by Preventing Membrane Lipid Peroxidation. Plant Physiology, 165 (2), Pp. 688–704. https://doi.org/10.1104/pp.113.230268

Chandler, S.F., 2010. Biotechnology in floriculture. March.

Chandler, S.F., Sanchez, C., 2012. Genetic modification; the development of transgenic ornamental plant varieties. Plant Biotechnology Journal, 10 (8), Pp. 891–903. https://doi.org/10.1111/j.1467-7652.2012.00693.x

Cherian, S., Oliveira, M.M., 2005. Transgenic Plants in Phytoremediation: Recent Advances and New Possibilities. Environmental Science & Technology, 39 (24), Pp. 9377–9390. https://doi.org/10.1021/es051134l

Chong, D.K., Roberts, W., Arakawa, T., Illes, K., Bagi, G., Slattery, C.W., Langridge, W.H., 1997. Expression of the human milk protein beta-casein in transgenic potato plants. Transgenic Research, 6 (4), Pp. 289–296. https://doi.org/10.1023/a:1018410712288

Christou, P., 2013. © 199 1 Nature Publishing Group http://www.nature.com/naturebiotechnology. 31 (3). https://www.cell.com/trends/biotechnology/fulltext/S0167-7799(13)00018-8

Crocker, F.H., Indest, K.J., Fredrickson, H.L., 2006. Biodegradation of the cyclic nitramine explosives RDX, HMX, and CL-20. Applied Microbiology and Biotechnology, 73 (2), Pp. 274–290. https://doi.org/10.1007/s00253-006-0588-y

Culture, P.T., Islam, A., 2014. PTC & B. June. https://doi.org/10.3329/ptcb.v16i2.1113

de Maagd, R.A., Bosch, D., Stiekema, W., 1999. Bacillus thuringiensis toxin-mediated insect resistance in plants. Trends in Plant Science, 4 (1), Pp. 9–13. https://doi.org/10.1016/S1360-1385(98)01356-9

Dhankher, O.P., Li, Y., Rosen, B.P., Shi, J., Salt, D., Senecoff, J.F., Sashti, N.A., Meagher, R.B., 2002. Engineering tolerance and hyperaccumulation of arsenic in plants by combining arsenate reductase and γ-glutamylcysteine synthetase expression. Nature Biotechnology, 20 (11), Pp. 1140–1145. https://doi.org/10.1038/nbt747

Esse, H.P., Reuber, T.L., Does, D., 2020. Genetic modification to improve disease resistance in crops. New Phytologist, 225 (1), Pp. 70–86. https://doi.org/10.1111/nph.15967

Facciotti, M.T., Bertain, P.B., Yuan, L., 1999. Improved stearate phenotype in transgenic canola expressing a modified acyl-acyl carrier protein thioesterase. Nature Biotechnology, 17 (6), Pp. 593–597. https://doi.org/10.1038/9909

Farkas, G.L., Kiraaly, Z., 1962. Role of Phenolic Compounds in the Physiology of Plant Diseases and Disease Resistance. Journal of Phytopathology, 44 (2), Pp. 105–150. https://doi.org/10.1111/j.1439-0434.1962.tb02005.x

Fry, S.C., 2004. The plant cell wall. Rose JKC, ed 2003. Oxford: CRC Press. 99.50 (hardback). 381 pp. Annals of Botany, 94 (4), Pp. 645–645. https://doi.org/10.1093/aob/mch185

Gantait, S., Sinniah, U.R., 2014. In vitro direct rhizogenesis from Gerbera jamesonii Bolus leaf. Acta Physiologiae Plantarum, 36 (11), Pp. 3081–3087. https://doi.org/10.1007/s11738-014-1643-4

Gatehouse, A.M.R., Ferry, N., Edwards, M.G., Bell, H.A., 2011. Insect-resistant biotech crops and their impacts on beneficial arthropods. Philosophical Transactions of the Royal Society B: Biological Sciences, 366 (1569), Pp. 1438–1452. https://doi.org/10.1098/rstb.2010.0330

Ghislain, M., Byarugaba, A.A., Magembe, E., Njoroge, A., Rivera, C., Román, M.L., Tovar, J.C., Gamboa, S., Forbes, G.A., Kreuze, J.F., Barekye, A., Kiggundu, A., 2019. Stacking three late blight resistance genes from wild species directly into African highland potato varieties confers complete field resistance to local blight races. Plant Biotechnology Journal, 17 (6), Pp. 1119–1129. https://doi.org/10.1111/pbi.13042

Gordon-kamm, W.J., Spencer, T.M., Mangano, M. Lou, Adams, T.R., Daines, R.J., Start, W. G., Brien, J.V.O., Chambers, S.A., Adams, W.R., Willetts, N.G., Rice, T., Mackey, C.J., Krueger, R.W., Kausch, A.P., Lemaux, P.G., 1990. Transformation of Maize Cells and Regeneration of Fertile Transgenic Plants, 2(July), Pp. 603–618.

Goto, F., Yoshihara, T., Shigemoto, N., Toki, S., Takaiwa, F., 1999. Iron fortification of rice seed by the soybean ferritin gene. Nature Biotechnology, 17 (3), Pp. 282–286. https://doi.org/10.1038/7029

Grusak, M.A., DellaPenna, D., 1999. Improving the nutrient composition of plants to enhance human nutrition and health. Annual Review of Plant Physiology and Plant Molecular Biology, 50 (1), Pp. 133–161. https://doi.org/10.1146/annurev.arplant.50.1.133

Hussain, B., 2015. Modernization in plant breeding approaches for improvingbiotic stress resistance in crop plants. Turkish Journal of Agriculture and Forestry, 39, Pp. 515–530. https://doi.org/10.3906/tar-1406-176

Jeong, J.S., Kim, Y.S., Baek, K.H., Jung, H., Ha, S.H., Do Choi, Y., Kim, M., Reuzeau, C., and Kim, J.K., 2010. Root-Specific Expression of OsNAC10 Improves Drought Tolerance and Grain Yield in Rice under Field Drought Conditions. Plant Physiology, 153 (1), Pp. 185–197. https://doi.org/10.1104/pp.110.154773

Joo, J., Lee, Y.H., Song, S.I., 2014. Rice CatA, CatB, and CatC are involved in environmental stress response, root growth, and photorespiration, respectively. Journal of Plant Biology, 57 (6), Pp. 375–382. https://doi.org/10.1007/s12374-014-0383-8

Kehoe, Villand, and Somerville. 1999. DNA microarrays for studies of higher plants and other photosynthetic organisms. Trends in Plant Science, 4 (1), Pp. 38–41. https://doi.org/10.1016/s1360-1385(98)01354-5

Klement, Z., Goodman, R.N., 1967. The Hypersensitive Reaction to Infection by Bacterial Plant Pathogens. Annual Review of Phytopathology, 5 (1), Pp. 17–44. https://doi.org/10.1146/annurev.py.05.090167.000313

Koch, A., Kumar, N., Weber, L., Keller, H., Imani, J., Kogel, K.H., 2013. Host-induced gene silencing of cytochrome P450 lanosterol C14 -demethylase-encoding genes confers strong resistance to Fusarium species. Proceedings of the National Academy of Sciences, 110 (48), Pp. 19324–19329. https://doi.org/10.1073/pnas.1306373110

Krattiger, A.F., 1996. Insect Resistance in Crops: Case Study of Bacillus thuringiensis (Bt) and its Transfer to Developing Countries. Director, 2, Pp. 1–42.

Kreuze, J.F., Valkonen, J.P., 2017. Utilization of engineered resistance to viruses in crops of the developing world, with emphasis on sub-Saharan Africa. Current Opinion in Virology, 26, Pp. 90–97. https://doi.org/10.1016/j.coviro.2017.07.022

Kumar, J., Singh, S.P., Kianian, S.F., 2017. Engineering Resistance to Plant Viruses. In Current Developments in Biotechnology and Bioengineering, Pp. 75–100. Elsevier. https://doi.org/10.1016/B978-0-444-63661-4.00004-9

Kumar, P.A., 2001. Plant biotechnology: Future perspectives. Defence Science Journal, 51 (4), Pp. 353–366. https://doi.org/10.14429/dsj.51.2249

Lawson, C., Kaniewski, W., Haley, L., Rozman, R., Newell, C., Sanders, P., and Tumer, N.E., 1990. Engineering Resistance to Mixed Virus Infection in a Commercial Potato Cultivar: Resistance to Potato Virus X and Potato Virus Y in Transgenic Russet Burbank. Nature Biotechnology, 8 (2), Pp. 127–134. https://doi.org/10.1038/nbt0290-127

Lee, D.G., Ahsan, N., Lee, S.H., Lee, J.J., Bahk, J.D., Kang, K.Y., Lee, B.H., 2009. Chilling stress-induced proteomic changes in rice roots. Journal of Plant Physiology, 166 (1), Pp. 1–11. https://doi.org/10.1016/j.jplph.2008.02.001

Li, Y., Dhankher, O.P., Carreira, L., Balish, R.S., Meagher, R.B., 2005. Arsenic and mercury tolerance and cadmium sensitivity in arabidopsis plants expressing bacterial γ-glutamylcysteine synthetase. Environmental Toxicology and Chemistry, 24 (6), Pp. 1376. https://doi.org/10.1897/04-340R.1

Miflin, B., 2000. Crop improvement in the 21st century. Journal of Experimental Botany, 51 (342), Pp. 1–8. http://www.ncbi.nlm.nih.gov/pubmed/10938790

Miri, S.M., Roughani, A., 2018. Factors affecting tissue culture success in ornamental crops: genotype, explant and physical environment. 2nd International & 3rd National Congress on Flower and Ornamental Plants, November, Pp. 18–22.

Nagatoshi, Y., Ikeda, M., Kishi, H., Hiratsu, K., Muraguchi, A., Ohme-Takagi, M., 2016. Induction of a dwarf phenotype with IBH1 may enable increased production of plant-made pharmaceuticals in plant factory conditions. Plant Biotechnology Journal, 14 (3), Pp. 887–894. https://doi.org/10.1111/pbi.12437

Newell-McGloughlin, M., 2008. Nutritionally Improved Agricultural Crops. Plant Physiology, 147 (3), Pp. 939–953. https://doi.org/10.1104/pp.108.121947

Noman, A., Aqeel, M., Deng, J., Khalid, N., Sanaullah, T., Shuilin, H., 2017. Biotechnological Advancements for Improving Floral Attributes in Ornamental Plants. Frontiers in Plant Science, 8. https://doi.org/10.3389/fpls.2017.00530

Onaga, G., and Wydra, K., 2016. Advances in Plant Tolerance to Biotic Stresses. Plant Genomics. https://doi.org/10.5772/64351

Römer, S., Fraser, P.D., Kiano, J.W., Shipton, C.A., Misawa, N., Schuch, W., Bramley, P.M., 2000. Elevation of the provitamin A content of transgenic tomato plants. Nature Biotechnology, 18 (6), Pp. 666–669. https://doi.org/10.1038/76523

Rylott, E.L., Jackson, R.G., Edwards, J., Womack, G.L., Seth-Smith, H.M., Rathbone, D.A., Strand, S.E., Bruce, N.C., 2006. An explosive-degrading cytochrome P450 activity and its targeted application for the phytoremediation of RDX. Nature Biotechnology, 24 (2), Pp. 216–219. https://doi.org/10.1038/nbt1184

Savin, K.W., Baudinette, S.C., Graham, M.W., Michael, M.Z., Nugent, G.D., Lu, C.Y., Chandler, S.F., Cornish, E.C., 1995. Antisense ACC Oxidase RNA Delays Carnation Petal Senescence. Hort Science, 30 (5), Pp. 970–972. https://doi.org/10.21273/HORTSCI.30.5.970

Schaller, G.E., 2007. Ethylene action in plants. Annals of Botany, 99 (3), Pp. 561–561. https://doi.org/10.1093/aob/mcm004

Schena, M., 1998. Microarrays: biotechnology's discovery platform for functional genomics. Trends in Biotechnology, 16 (7), Pp. 301–306. https://doi.org/10.1016/S0167-7799(98)01219-0

Shukla, A., Singh, V.K., Bharadwaj, D.R., Kumar, R., Rai, A., Rai, A.K., Mugasimangalam, R., Parameswaran, S., Singh, M., Naik, P.S., 2015. De Novo Assembly of Bitter Gourd Transcriptomes: Gene Expression and Sequence Variations in Gynoecious and Monoecious Lines. PLOS ONE, 10 (6), Pp. e0128331. https://doi.org/10.1371/journal.pone.0128331

Siddra, I., 2012. Genetic Pathways of Disease Resistance and Plants-Pathogens Interactions. Molecular Pathogens. https://doi.org/10.5376/mp.2012.03.0004

Soliman, T.M.A., Lv, S., Yang, H., Hong, B., Ma, N., Zhao, L., 2014. Isolation of flower color and shape mutations by gamma radiation of Chrysanthemum morifolium Ramat cv. Youka. Euphytica, 199 (3), Pp. 317–324. https://doi.org/10.1007/s10681-014-1127-z

Stam, M., Mol, J.N.M., Kooter, J.M., 1997. The silence of genes in transgenic plants. Annals of Botany, 79 (1), Pp. 3–12. https://doi.org/10.1006/anbo.1996.0295

Suzuki, N., Sejima, H., Tam, R., Schlauch, K., Mittler, R., 2011. Identification of the MBF1 heat-response regulon of Arabidopsis thaliana. The Plant Journal, 66 (5), Pp. 844–851. https://doi.org/10.1111/j.1365-313X.2011.04550.x

van Staden, J., 1989. Cytokinins and auxins in carnation senescence as related to chemical treatments. Acta Horticulturae, 261, Pp. 69–80. https://doi.org/10.17660/ActaHortic.1989.261.8

Ye, X., 2000. Engineering the Provitamin A (-Carotene) Biosynthetic Pathway into (Carotenoid-Free) Rice Endosperm. Science, 287 (5451), Pp. 303–305. https://doi.org/10.1126/science.287.5451.303.