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Review Integration of Biotechnology with Conventional Crop Improvement

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Abstract

The typical crop improvement rotation takes 10-15 years to complete and , involve germplasm manipulations, genotype stabilization and selection , variety testing, variety increase, proprietary protection and crop production stages. Genetic engineering and plant tissue culture approach that form the basis of plant biotechnology can promote to most of these crop improvement stages. Recombinant DNA technology has notably augmented the conventional crop improvement, and has a great assurance to assist plant breeders to assemble the increased food demand estimate for the 21st century. Considerable progress has been made over the past two decades in controlling genes from multiple and exotic sources, and inserting them into crop plants and microorganism to converse resistance to diseases and insect pests, tolerance to herbicides, soil salinity, drought and aluminum toxicity; improved post-harvest quality; increased nutrient uptake and nutritional quality; enhanced photosynthetic rate, sugar, and starch production; inflate effectiveness of biocontrol agents; improved metabolic pathways and understanding of gene action; and production of vaccines and drugs in crop plants. Despite the various and widespread beneficial applications of biotechnology products, there always a critical need to give these benefits to the general public in a actual and understandable way that excite an responsible and impartial public debate. The growth, testing and release of agricultural products produced through biotechnology-based processes should be continuously increased based on the current experiences. This will require an active and efficient regulatory structure, clearly supportive of the advantage of biotechnology, but really sensitive to the well being of environment and humans.

Kay word: Genetics engineering, Crop Improvement, Nutritional quality

Introduction

Humans began to improve the feature of plants used for food and fibre approximately ten to twenty thousand years ago. Even ancient cultivating ,seeding, , harvesting and storing practices would have utilized selection pressures on those plant species which became domesticated that were different from the pressures, their originator encountered in the wild. United Nations have estimated that world population will risen by 25% to 7.5 billion from 2020. Broadly, 73 million public are added annually, from which 97% will alive in the evolving countries. Presently, approx 1.2 billion people survive in a state of 'absolute poverty'(Food and Agriculture Organization, Rome, Italy, 1986.), from that 800 million population survive below unreliable food security, and 160 million new born children suffer from malnutrition(P. Pinstrup-Andersen, M Cohen *et al*.,2000). A huge number of people also suffer by deficiencies of micronutrients such as zinc, iron and vitamin A. Food malnutrition and insecurity result in serious public health problems, and a lost human potential. There had been a phenomenal rise in total grain production during 1950 and 1980, but only a minor increase was realized between 1980 - 1990(N. Myers *et al*., 1999). Much of the prior increase rise in grain production resulted from an increase in area under irrigation, cultivation, improved cultivars and better agronomic practices, Yields of various crops have already extend a plateau in developed countries, and so that, most of the production attain in the future will have to be achieved in developing countries from better natural resources management and crop improvement. Production profit is required for continuing economic development, but in the limited time, these are become more essential for keeping sufficient food supplies for the developing world population. So that in this manner biotechnology will do a vital role in food production in the coming time. Plant

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biotechnology a part of biotechnology can be described as the application of molecular genetics and tissue culture to produce a commodity from plants. Tissue cultures mention to the propagation and maintenance of plant parts (as small as a single cell) in biologically pure (axenic) and controlled environments (Fig. 1; Evans *et al.,* 1983; Vasil, 1984). Molecular genetics involves techniques for isolating genes conferring desired traits; from its source, its characterization, recombination, amplification and transfer (Fig. 2; Maniatis *et al.,* 1982; Gelvin *et al.,* 1988; Watson *et al.,* 1987).

This review focuses on analytical but practical look at the restraints and possibilities of biotechnologies and their role in rising crop yield and increasing nutritional quality. Environmental concerns and issues of biosafety regarding use of genetically engineered crops are also discussed in this review. Genetic engineering provides plant breeders gain to an infinitely broad formation of unique genes and traits, which can be introduced into local and high-yielding cultivars. This perspective offers quick entrance of unique genes and traits into best agronomic backgrounds. In order to reduce crop protection cost, reliance on pesticides, new insects, pests and for the welfare of public health and environment disease resistance crop cultivars are required. Similarly, farm incomes can be increased by decreasing labour demand for weeding and herbicide application through deployment of herbicide resistant crops in the field. Agricultural biotechnology can also help in preserving biodiversity by increasing productivity per unit area and thereby decreasing demand of new land that are marginal for crop production. Maximal potential of biotechnology can be achieved by extracting information from research on transgenic and genomics for increasing nutritional quality and developing resistance to biotic and abiotic stress factors (Table 1; H C Sharma *et al*., 2001).

Table 1
Application of biotechnology to improve yield and quality of major field crops

Crops	Areas of improvement	TC/WH	MAS	Trans
Rice	Drought and salinity tolerance		X	X
	Resistance to stem borers, brown hoppers, gall midge, and leaf sheath blight	X	X	X
	Nutritional and table quality of grains		X	X
	Resistance to lodging		X	
Wheat	Yield, quality, and adaptation		X	X
	Resistance to rusts and Karnal bunt		X	X
Maize	Yield and quality		X	X
	Resistance to lodging and stem borers	X	X	X
Sorghum	Yield, quality, and adaptation to drought	X	X	X
	Resistance to shoot fly, stem borer, midge, head bugs, and grain molds	X	X	X
Pearl millet	Yield and adaptation to drought		X	
	Resistance to downy mildew, stem borers, and head miner		X	X
Pigeonpea	Yield and adaptation to drought		X	X
	Resistance to *Helicoverpa* and *Fusarium* wilt	X	X	X
Chickpea	Adaptation to drought and chilling tolerance		X	X
	Resistance to wilt, *Ascochyta* blight, and *Helicoverpa*	X	X	X
Mustard	Yield and adaptation to drought		X	X
	Oil content and quality		X	X
	Resistance to aphids	X	X	X
Groundnut	Yield, oil content, and adaptation to drought		X	X
	Resistance to foliar diseases, aflatoxins, and leaf miner	X	X	X
Cotton	Yield, fiber quality, and oil content		X	X
	Resistance to jassids, and bollworms.	X	X	X
	Flushing pattern		X	
Sugarcane	Resistance to stem borers		X	X
	Yield and induction of early maturity		X	
Tobacco	Yield and quality		X	
	Resistance to aphids, tobacco caterpillar, and viruses	X	X	X

TC/WH = Tissue culture/wide hybridization; MAS = Marker assisted selection; Trans = Transgenics.

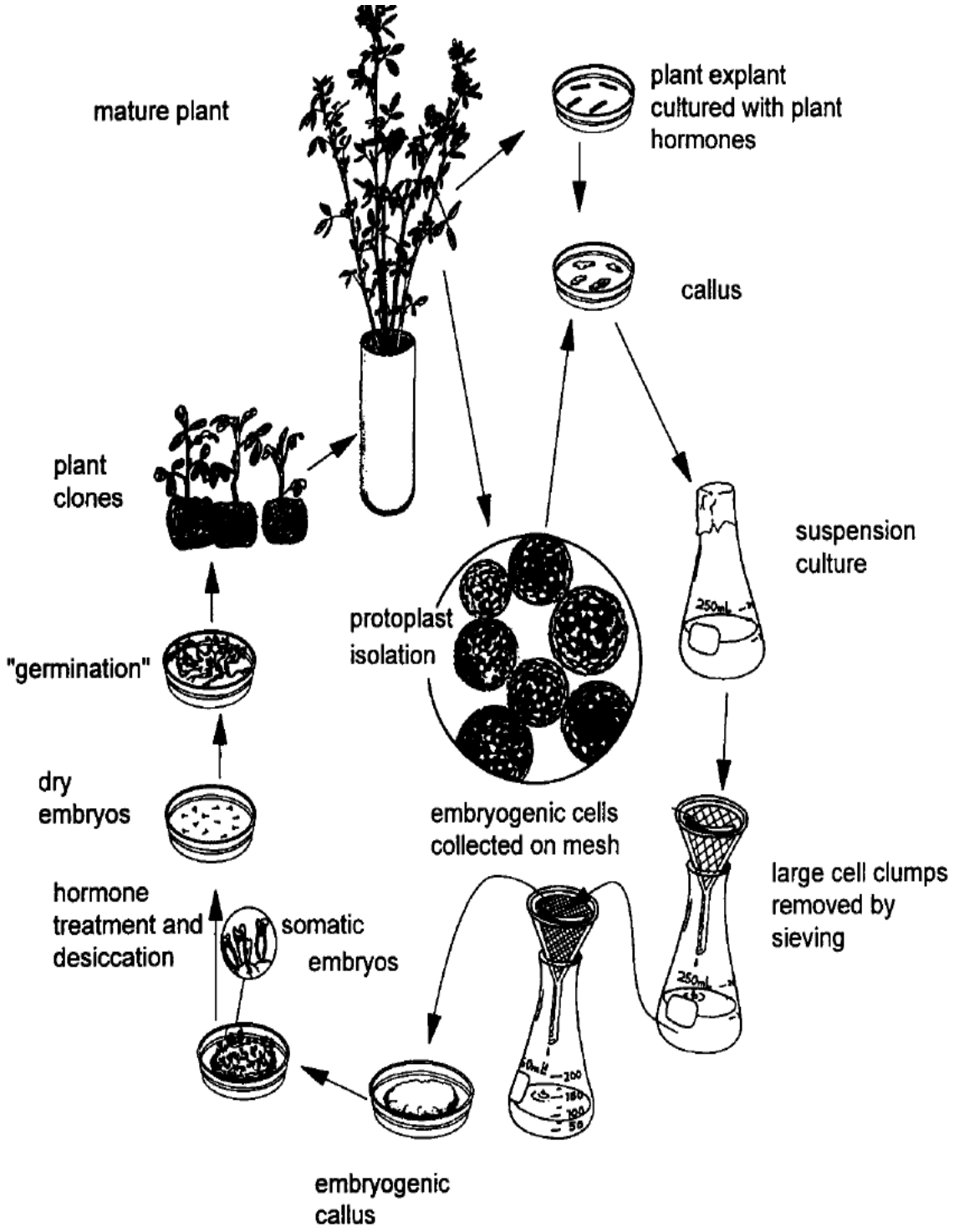

Figure 1. Plant tissue culture. Plant cells can be induced to proliferate in a variety of forms including nondifferentiated callus and suspension cultures *in vitro*. Single cells like protoplasts express totipotency, i.e. the ability of a single cell to regenerate into a whole plant. Regeneration may occur via various processes including somatic embryogenesis. In this process structures that resemble zygotic embryos are formed in the tissue culture.

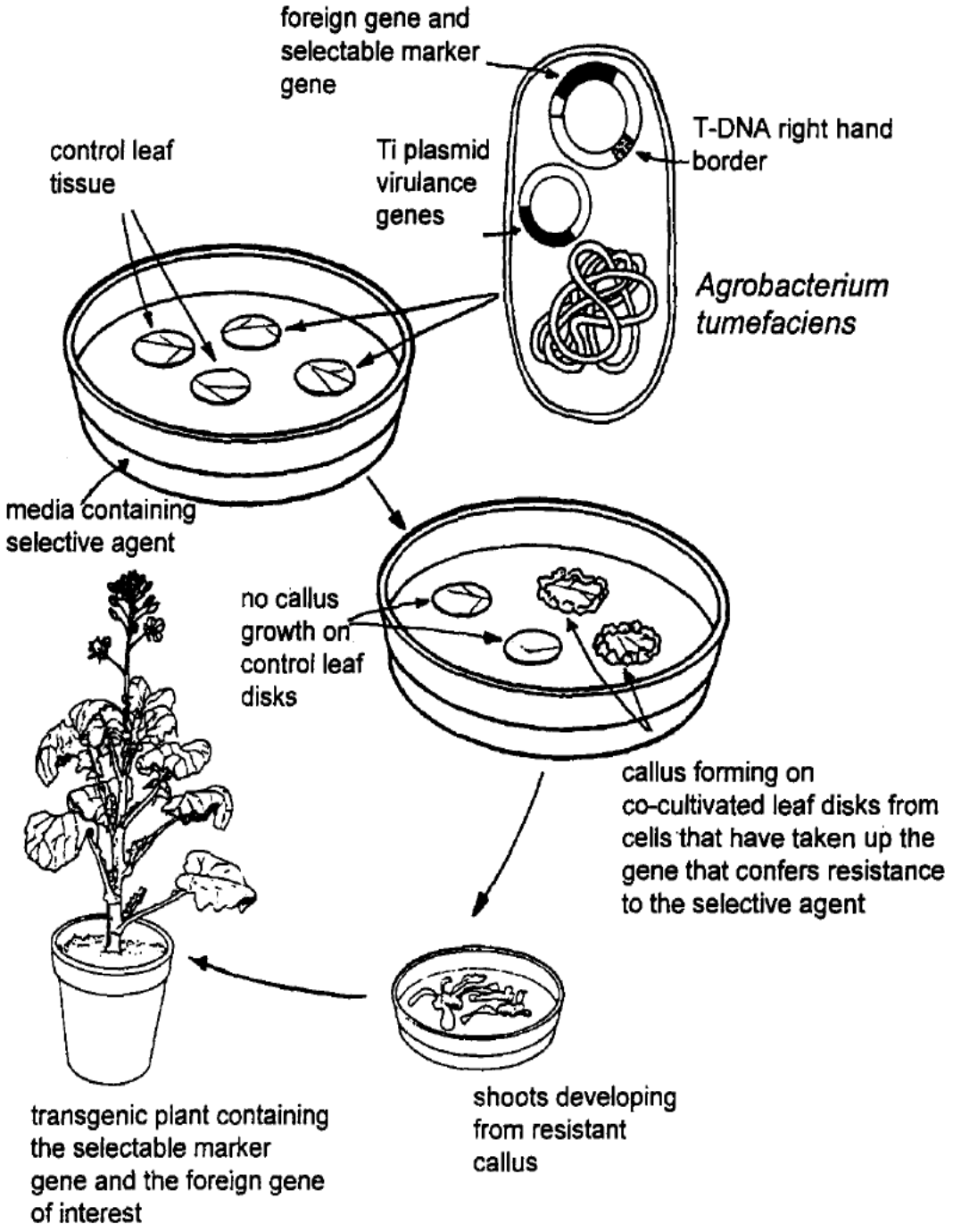

Figure 2. Plant molecular genetics. A foreign gene, coupled to a selectable marker gene, is cloned into a disarmed Ti plasmid of *Agrobacterium tumefaciens.* The foreign gend and the selectable marker gene are transferred to the plant by cocultivating the bacteria with the explant (leaf disk) and regenerating plants from the cells that express resistance to the selective agent in tissue culture. The foreign gene becomes stably integrated into the DNA of the plant and is inherited by future generations of plants derived from the original transgenic plant.

Evolution of genomics

In the last decade successful whole genome sequencing of model organisms like human (D.D. Shoemaker *et al*., 2001), yeast (F. Sherman *et al*., 1998), Arabidopsis thaliana (TAGI *et al*., 2000), and rice (B.A. Palevitz *et al*., 2000) has been done. Systematic whole genome sequences yields important facts for better understanding of the complex genome organization and function which will be playing a crucial role in clearing the concept regarding crop production and enabling us to manipulate those traits conferring to large crop productivity (A. Pereira, Plant *et al*., 2000). Same as, improve in microarray technology will enable the concurrent expression and study of huge numbers of genes that will explain gene function, and the complex versatile communication between genes that result in varying phenotypes under different environmental conditions (D.D. Shoemaker *et al*., 2001). These studies will be expanding by more specific investigations on the basis of co-suppression or anti-sensing gene suppression, of a defined sequence (S.B. Primrose, *et al*., 1998). This knowledge through typical systems will expand our understanding of plant biology and so increase our capability to utilize genomic information for crop development. Through typical breeding practices it takes approx five to six generations to shift a trait with in a species into the high level yielding locally modified cultivars, and one require to plant a comparatively high number of progenies to be efficient to choose the plants with suitable combination of traits. A set of multi-location tests are then followed before a variety could be identified from the improved lines for cultivation by the farmers. The process takes around 7-10 years. Though, genetic transformation enables access to genes from other species, which can be used to change the level of gene expression, for making transgenic crops, and for changing the temporal and spatial pattern of gene expression. The genes of interest holding the desired trait can be directly transferred into the target crops/cultivars in a single event, and it takes approximately 5-6 years to develop cultivars with stability in their gene expression pattern.

Genotypic Selection and Stabilization

Approximately in all plants breeding programs genetic stabilization and line selection takes place together and contain a method by which the breeder choose a some of the perfect lines to enhance to the next level of backcrossing or selfing. The procedure performs to enrich the fresh germplasm into quality which are superior to other and also the stabilization of their genetic formation by converting them homozygous. Biotechnological techniques that can promote to this stage of variety growth are: marker-assisted selection, *in vitro* selection and haploidy.

Selection

An efficient method for selection of plants with desired traits is to add a selective agent or marker that will kill the majority of the cells (except the resistant ones) to a culture, and the procedure is known as *in vitro* selection (Chaleff *et al*., 1983). A single cell can be the unit of *in vitro* selection hence selection pressure can be uniformly and reproducibly applied. Also, *in vitro* selection is potentially more efficient than whole plant selection. For example, a single flask of cells can have the same number of selection units as 30,000 plants in a three hectare nursery. This method has been particularly effective for selecting herbicide- and disease-resistant lands (Shaner & Anderson, 1985; Swanson *et al.,* 1989; Dix, 1990; Frame *et al.,* 1991; ller *et al.,* 1993). In a breeding program which uses the molecular markers, progeny from each mating cycle are selected on the basis of the presence of a particular electrophoretic band that has been shown to be tightly linked to the desired characteristic (Ralfalski & Tingey *et al*., 1993). This approach circumvents masking effects by dominant alleles, eliminates variability due to environmental effects and can greatly simplify the patterns of inheritance for complex traits. Marker-assisted selection also allows early stage selection to be carried out since the genetic pattern does not change during plant development. Furthermore, after a linkage map of molecular markers has been constructed a whole genome selection procedure can be utilized, thus, decreasing the time required to fix a gene in an agronomical useful background (Paterson *et al.,* 1988; Lander & Botstein *et al*., 1989).

Marker-assisted selection

Besides generating information on gene sequences and function, Recombinant DNA technologies, also simplifies the identification of particular chromosomal regions carrying specific genes that contribute to traits of economic interest (A. Karp, K.J. Edwards *et al*., 1997). The hypothetical advantages of indirect selection by genetic markers were first described by Sax nearly 80 years ago. But, it was not until the evolution of DNA marker technology which enables us to generate large number of genetic markers to fulfil the needs of modern plant breeding programs. Now a day's different type of DNA markers are available in abundance, each having their own advantages for any particular application. The identification of markers for

traits of interest usually on the basis of making crosses in between two genotypes with significant and genetic differences in trait(s) of interest. In accordance with the traits and crop involved, mapping populations are obtained from the offsprings of this cross by selfing once, back-crossing to one of the parental genotypes, selfing multiple times or plants are handled to tissue culture to produce double haploids. A main benefit of double haploid and recombinant inbred line mapping populations is that every line is homozygous and can, therefore, be eternally multiplied by self-pollination. This then enables the population to be analysed under different environments and seasons, which assist a much more precise evaluation of phenotypic variation on which mapping exercise is based.

Once the regions of genome contributing to the desired trait have been identified and the alleles at each locus designated, their transfer into locally adapted high-yielding cultivars can be done through making requisite crosses. Then offspring with all desired alleles can be selected for further assessment using marker-assisted selection. Wild-type relatives of economically important crops carry important alleles that can improve resistance to biotic and abiotic stress factors and crop performance; incorporation of these alleles can be effectively done into crop breeding programs by using marker-assisted selection (J. Xiao, S. Grandillo *et al*., 1996). However indirect selection is superior to phenotypic selection in terms of cost, reliability and time.

Genetic stabilization

For ensuring the stability (from year to year) and uniformity of many crop varieties in the field homozygosity is required. Normally it takes three to four years to achieve homozygosity in a field crop by conventional approaches. However, several generations of inbreeding (usually 7 or 8) normally required to achieve homozygosity can be replaced by haploidy. Haploid plants are acquired by culturing gamete cells followed by treatment with colchicine for doubling to produce homozygous lines (Kasha *et al*., 1974; Hu & Yang, 1986). By using doubled haploid populations, breeding for recessive traits is much more simplified because the occurrence of homozygous recessive individuals is at a greater frequency in these populations than in traditional breeding populations (Siebel & Pauls *et al*., 1989; Henderson & Pauls *et al*., 1992).

Sequencing and Gene function

A variety of approaches can be used for gene discovery (D.D. Shoemaker, E.E. Schadt *et al*., 2001), however a large-scale approach that is commonly followed is generation and sequencing of a library of expressed genes. Typically this library contains thousands of complementary DNA (cDNA) strands that are copiously expressed by that plant under the particular environmental conditions at the sampled growth stage. When sequencing is done, then these cDNAs are named as expressed sequence tags (EST). A huge number of ESTs are now present in the public databases for various model plants and crops like as M. sativa, rice, A. thaliana, sorghum, maize, and soybean. The diversity in coding sequences between distantly and closely related plants can be revealed by comparison of the EST database from different plants, while mapping of ESTs may interpret the synteny between those species. A high level of sequence similarity between an EST and a gene of known function in another species may indicate the probable gene function in the species of interest. However, to know the actual function of the gene experimental verification is needed. As we know only few genes are copiously transcribed in any particular growth stage or environment, and hence, a complete picture can only be achieved by creating a range of cDNA libraries sampled at different growth stages and from plants grown under different environmental conditions or by sequencing whole cDNA genome library. Now a day's functional genomics technology is focused on high throughput (HTP) methods using insertion mutant isolation, gene chips or microarrays, and proteomics for understanding gene functions of a whole organism. After the identification of genes, their expression is analyzed in transgenic plants.

Variety Trial

As the movement of genetically modified crop from laboratory to fields undergoes strict regulation hence variety testing becomes a crucial step in development of transgenic crops.

In variety testing the newly developed species undergoes different testing procedures for several years in several locales. In some countries, license for sale is granted only on the basis of variety testing results. It is hypothesized that the gene pools of crop plants began to diverge over 150 million years ago. However, the resultant assortment of genes has led to change in the expression of traits, and creation of entirely new plant phenotypes and functions the outcomes from early studies show that the use of biotechnology does not impose extraordinary risks on society (Flavell & Fraley, 1992; Huttner *et al.,* 1992). However, there substantial knowledge gaps that persist in these area of concern and a careful approach to the introduction of genetically modified plants into the

environment or the utilization of transgenic foods has been advocated (Bryant & Leather, *et al*., 1992).

Propogation

Plant propagation step is one of the major cost consuming steps in a variety development program. In case of multiple field crops this is achieved by the producing seed by pure stands of one variety. Production of hybrid seeds using pollination control system like self incompatibility, artificial male sterility and cytoplasmic male sterility have been area of interest as hybrid crops have higher yields than traditional crops. Also methods for controlling these traits have been fully developed. Some of the genes responsible for self incompatibility have been identified by the help of molecular biology techniques (Cornish *et al.,* 1987; Dzelzkalns *et al.,* 1993). This vital piece of information can be used for producing hybrid seeds of inbred or homozygous varieties by the development of hybrid seed production systems. For introducing cytoplasmic male sterility, organelle (in particular mitochondria) transfer has been done through protoplast fusion from one species to another to impose pollination control and hybrid seed production (Pelletier & Chupeau, 1984; Kyozuka *et al.,* 1989; Scbell & Vasil, 1989; Pauls, 1991). RNAse gene from *Bacillus amyloliquefaciens* has been used for plant transformation under the control of a promoter specific for cells (called tapetal cells) surrounding the pollen sacs to create artificial genetic male sterile system (Mariani *et al.,* 1990). Due to demolished tapetom these transgenic plants do not produce pollen and they represent the female parent in a hybrid seed production system. The male parents are transgenic for RNAse inhibitor gene regulated by tapetum promoter (Mariani *et al.,* 1992). Both the F I hybrids and male parents acquired by crossing the male and female transgenic plants produce pollen.

Plant cloning is a widely used method for rapid propagation of desired genotypes. Through tissue culture techniques it is now possible to accelerate vegetative propagation as hundreds of plants can be produced from a tiny piece of tissue. This strategy is known as micro-propagation and has been widely implemented to a wide range of plant species (Klausner, *et al*., 1986). These techniques can be particularly useful in cross pollinating species like Alfalfa (***Medicago sativa***) where producing seed from self pollinations is inconvenient, hence, difficult to increase and maintain superior plants (McKersie & Bowieet *et al*., 1993).

Gene transformation

Genetic transformation provides direct entry to a huge pool of functional genes not previously accessible to plant breeders. At present genetic engineering techniques enable the simultaneous utilization of various advantageous genes in a single event; therefore the introduction of novel genes/traits into the elite background can be allowed by the coordinated desirable character through closely-related plants without any related deleterious approaches. The applied transgenic research are similar according to their priorities to those of aiming to selectively alter, conventional plant breeding ,add or remove a specific character orderly to address regional constraints to productivity. Genetic engineering additionally gives the chance of introducing genes which do not easily cross with the crop of interest or with completely unrelated species even in other taxonomic phyla. Establishment and distribution of genetically modified crops efficiently is an essential requirement for economic and imperishable use of biotechnology in the field of crop improvement. Development of pest resistance, herbicide tolerance and male sterility system for crop improvement has been greatly accelerated due to advancement in genetic engineering techniques like genetic transformation and gene expression (V.A. Hilder *et al*., 1999, H C Sharma *et al*., 2001).

Insects, diseases and herbicides resistance

In 1987, the very first transgenic plants with Bacillus *thuringiensis* (Bt) genes were developed (K. Barton, H. Whiteley *et al*., 1987). Bt delta-endotoxin genes have been most extensively utilized to produce insect-resistant transgenic plants. However, efforts are being made to use non-Bt genes, which can hinder the nutritional needs of the insects. These non-Bt genes include protease inhibitors, chitinases, secondary plant metabolites, and lectins (V.A. Hilder, D. Boulter *et al*., 1999). Genes responsible for resistance to insects have now been introduced into a wide range of crop plants including rice, broccoli, maize, soybean, apple, cotton, potato, tobacco, alfalfa (J.S. McLaren *et al*., 1998). Insect resistant crop plants are the future of pest management practices. These resistant plants will not only control insects and pests but also will minimize losses due to them.

Vaccines from transgenic

It is possible to produce several vaccines in plants (J. Anderson *et al*., 1996). Genes obtained from human pathogens can be introduced into plant genome using biotechnology (C.O. Tacker, H.S. Mason *et al*., 1998).

This gene from pathogen will produce antigen which will further accumulate in plant tissue. The antigen produced holds its immunogenic properties, which can be further utilized in producing antibodies when injected into mice. Transgenic technology exhibits a great potential to increase the yield of medicines derived from plants.

Environmental issues and biosafety concerns regarding transgenic products

Development of resistance and injurious effect on beneficial insects are major environmental concerns regarding use of transgenic crops. In developing countries future of poor farmers is at stake as modern biotechnology bypasses these poor farmers due to lack of awareness and adaptation. However, we can't say that current direction of biotechnology research is inappropriate but it should also focus on the issues concerned with poor farmers in developing countries. The biosafety regulations concentrate on safety, quality, and efficiency (National Research Council (NRC) 1989). Information about the trans-gene donor and the receiving species, organization and people involved, monitoring and control of interactions between transgenic plants and the environment; are needed for effective biosafety regulation. The collected information needs to be properly managed, utilized and elucidated for risk assessment. And this will decide the potency and reliability of the technology. Considering the rapid development and deployment of transgenic plants following concerns must be taken care of:

(i) The transgenic variety should not promote emergence of new resistant strains of the target pest,

(ii) The transgenic variety must not suppress the diversity in the surrounding by expanding its niche through gene transfer to wild-type relatives.

(iii) It should be kept in mind that the produced transgenic variety increases the land use and must not decrease it. One of the major risks in deployment of transgenic plant is its expansion on land beyond the expected level to become a weed. Hence, cautious approach is needed in selection of gene for introducing into the transgenic plants.

Conclusion

Biotechnology contributes to improvement of crops and their production. Research and programs are being implemented at different universities and in government research institutes for development and assessment of these techniques (Moses *et al.,* 1988; Kalton *et al.,* 1989; Hodgson, 1992). Various organizations like United Nations Educational, Rockefeller Foundation, and International Cooperation Program of the European Union, Scientific and Cultural Organization (UNESCO), International Service for the Acquisition of Agrobiotech Applications (ISAAA), and International Service for National Agricultural Research (ISNAR) are trying to play a crucial role in technology convey from public and private sector institutions in the developed to the developing countries. In order to use biotechnology for imperishable food production national governments of developing countries need to be assisted and motivated. In developing countries population growth and climate change are serious threats to food security and crop production. The multiplication of conventional breeding along with the genetically modified plants and marker-assisted selection ensures to increase in food production. Although, the awareness of the biochemistry and physiology of plants will be significantly important for elucidate the information by molecular markers and obtain new and more effective paradigms in plant breeding. Usually the important profit will be gain from the transfer of genes which are essential for crop quality and crop. However, interaction of genes with their genomic environment and environment in which their resultant phenotype is present is still a thrust area of research. This information is very crucial for fast and cost effective development, and adoption of biotechnology and its products.

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