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REVIEW PAPER

Plant Biotechnology: Future Perspectives

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ABSTRACT

Plant biotechnology has made significant strides in the past 15 years encompassing within its fold the spectacular developments in plant molecular biology and genetic engineering. Some of the most vexing problems faced in agricultural ecosystems could be solved with the introduction of transgenic crops endowed with traits for insect pest resistance, herbicide tolerance and resistance to viral diseases. Attention is now being focussed on the development of transgenic plants having industrial, economic, pharmaceutical, nutritional and environmental importance. In the next millennium, crops will serve as factories for the synthesis of valuable metabolites and organic compounds. Agronomically important characters, such as drought tolerance, efficiency in photosynthesis, nutrient use and nitrogen fixation will be manipulated in the next century to enhance the genetic and physiological potential of the crops. Recent developments in the genome sequencing of *Arabidopsis*, rice and maize will have far reaching implications for future agriculture. Structural and functional genomics of plant species will virtually revolutionise the complexion of agricultural biotechnology as well as human health care. It is imperative that the developing world adopts these fast-changing technologies soon and harness their unprecedented potential for the benefit of the mankind.

Keywords: Plant biotechnology, plant genetic engineering, molecular breeding, plant genomics, plant molecular biology, transgenic plants, gene-transfer techniques, phytoremediation

1. INTRODUCTION

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 revolutionised agriculture and enhanced crop yield. Tools of recombinant DNA technology developed in 1960s ushered in the era of new biosciences which would revolutionise every facet of human life in a safe and sustainable manner in the next century. Agriculture being the basic life-sustaining activity of human society, assumes tremendous importance vis-a-vis biotechnology. 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. Biotechnology and genetic engineering of plants will play a crucial role in this area. Conventional plant breeding has its own limitations due to the non-availability of sources of resistance to pests and diseases in crop germplasm. In addition, introgression of resistance from wild and weedy relatives is hampered by problems of incompatibility. It is now possible to introduce the genes encoding valuable agronomic traits from any biological organism into crop plants. Techniques are available to incorporate foreign genes (transgenes) into any higher plant system with precision and reliability. In addition, application

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of the tools of molecular biology in plant breeding can help in hastening the breeding process and may also help in isolation of genes hitherto unknown and coding for quantitative characters. Sequencing of the entire genomes of rice, *Arabidopsis* and maize is going to revolutionise the complexion of plant molecular biology in the next century. This article reviews the progress made in the past two decades in the area of biotechnology of plants, and ponders over the prospects in the new millennium.

2. PLANT GENETIC ENGINEERING

Engineering of the plant species to acquire novel traits involves the introduction of foreign genes into the plant genome and expression of these transgenes to the desirable extent. It encompasses techniques to transfer the genes to plant chromosomes and regulate their expression by employing suitable promoters. Advances made in plant tissue culture have led to the development of techniques to regenerate a wide range of plant/crop species. Based on such procedures, three major gene transfer techniques have been employed to transform various plant species. These are: (i) *Agrobacterium*-mediated approach, (ii) protoplast-based approach, and (iii) biolistic approach[1].

2.1 Resistance to Biotic Stresses

Crop productivity is under constant threat of pest and disease incidence all over the globe. For instance, 30 to 40 per cent of agricultural productivity is lost due to the insects annually. Management of pests and diseases through chemical approach is effective but not eco-friendly. 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. Considerable progress has been made in the last decade of the 20th century to develop transgenic crops resistant to insect pests , viruses and fungi.

2.1.1 *Insect Pest Management*

Insect pests are the major scourge of agriculture. Resistance to these pests can be successfully engineered by exploiting insecticidal proteins found in bacteria, plants and animals. *Bacillus thuringiensis*, a gram-positive soil bacterium synthesises insecticidal proteins (δ-endotoxins). Introduction of genes encoding insecticidal proteins of *Bacillus thuringiensis* into crop plants conferred stable resistance. Intensive efforts during the 1990s have lead to the commercialisation of insect-resistant cotton, corn and potato[2]. The most important consideration in commercial exploitation of insect-resistant transgenic crops on a large scale is the possibility of insects developing resistance to *Bacillus thuringiensis* toxins[3]. Various strategies have been devised to prevent/delay the resistance development in insects. 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. The losses are alarming in cases, such as tomato, rice, cassava, pigeonpea, mungbean, potato, etc. Although plant viruses are simple in composition, the management of their diseases is much more difficult owing to complex disease cycles, efficient system of transmission and nonavailability of viricides. Crop plants with increased virus resistance can be obtained by biotechnological approaches. The strategies employed include introgression of genes either derived from hosts called host-derived resistance (HDR) or from pathogens called pathogen-derived resistance (PDR). The concept of PDR involves the expression of a particular viral gene in transgenic host resulting in resistance. Coat protein gene approach has been successfully employed against viruses with positive sense RNA encapsidated by a single type of protein, and single-stranded DNA geminiviruses and RNA topsoviruses. Since the first field testing of coat protein-derived resistance against TMV in tomato plants in 1987, there have been increasing number of field tests in different host-virus systems[4]. Commercial cultivation of virus-resistant squash and papaya has been in full swing in USA (Hawaii).

2.1.3 *Fungal Resistance*

Fungal pathogens cause several important diseases in crop plants. For many years, application of fungicides is the only effective strategy for their management.

The process of co-evolution of plants and pathogens has led to incompatibility in many cases determined by a single dominant gene for resistance in the host. Incompatibility is associated with the rapid activation of a battery of defence response genes whose products may include (i) hydrolytic enzymes, such as chitinase, 1-3 β-D glucanase and other pathogenesis-related proteins, (ii) ribosome inactivating proteins, (iii) antifungal proteins, (iv) biosynthetic enzymes for the production of antimicrobial phytoalexins, (v) wall-bound phenolics, osmotins, thionins, lectins, etc. and (vi) hydrogen peroxide. Plant genetic manipulation through expression of either novel proteins from foreign organisms or overexpression of a part of their own defensive arsenal for disease resistance has become a reality[5].

2.1.4 *Bacterial Resistance*

Recent progress in the understanding of plant-pathogen interactions enables the use of genetic engineering for the rational creation of bacterial disease-resistant plants. To date, the strategies employed for developing transgenic plants resistant to bacteria are: (i) production of antibacterial proteins of non-plant origin, (ii) inhibition of bacterial pathogenicity or virulence factors, (iii) enhancement of natural plant defences, and (iv) artificially-induced programmed cell death at the site of infection[6].

2.1.5 *Nematode Resistance*

Nematodes, specially plant parasitic nematodes *Meloidogyne* species (root-knot nematodes), *Heterodera* and *Globodera* species (cyst nematodes) are prominent in causing diseases in crops. Chemical control of nematodes is not only ineffective but also leads to soil contamination by pesticides. Nematode resistance has been reported by expressing proteinase inhibitors. Cowpea trypsin (CpTI) inhibitor expressed in potato resulted in reduced fecundity of *Meloidogyne* species, and switched the population of *Globodera pallida* from females to males, which are less harmful[7].

2.1.6 *Herbicide Tolerance*

Gene transfer technology helps in creating crops that are resistant to herbicides. Incorporation of herbicide resistance traits is based on genes encoding enzymes that catabolise the herbicides, overproduction of the targeted enzyme or by manipulating the target sites. 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.

2.2 Resistance to Abiotic Stresses

Environmental stresses, such as drought, salinity, heat, freezing and flooding, etc. have been the bane of agriculture over the ages, bringing with them poor harvests and the threat of famine. 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. Important approaches currently being followed are engineered alterations in the amounts of osmolytes and osmoprotectants, saturation levels of membrane fatty acids, and rate of scavenging of reactive oxygen intermediates. A variety of genes coding for proteins/enzymes involved in stress relief have been introduced into various plant species with reasonable degrees of tolerance to abiotic stresses[8].

The accumulation of low molecular mass osmoprotectants and osmolytes, such as quaternary amines (eg, betaines and allied compounds), amino acids (eg, proline) and sugar alcohols (eg mannitol), has resulted in enhanced osmotoerance in transgenic plants. Dehydration-responsive transcription factors mediate transcription of several genes in response to cold and water stress. Dehydration-responsive element binding (DREB) proteins genes encode transcription factors that bind to the cis-acting promoter element (DRE) of stress-related genes, and turn on their expression[9]. This binding initiates synthesis of gene products implicated in plant acclimation responses to low temperature and water stress. Overexpression of a fusion of a DRE-containing promoter from a dehydration-induced gene (*rd29A*) with a DREB gene (DREB1A) in *Arabidopsis* resulted in marked increase in the transgenic plants' tolerance to freezing, water stress, and salinity[10]

2.3 Heterosis Breeding

The exploitation of heterosis (hybrid vigour) through the use of hybrid varieties is one of the major achievements of plant breeding. However, the use of hybrids has been limited to those crops for which there is an effective means of pollination control, either by manual emasculation of the male flower or flower parts or by incorporation of genetic mutations that prevent normal pollen development. Major crops generally have small, bisexual flowers, which make manual emasculation impractical, and hybrid varieties cannot be produced commercially without using some form of male sterility. Male sterility may be generated by either cytoplasmic or nuclear genes. Advent of plant molecular biology has made it possible to engineer nuclear male sterility. Mariani[11], *et al.* have developed transgenic, male-sterile *Brassica napus* by expressing a bacterial gene, *barnase* (from *Bacillus amyloliquefaciens*) under the control of a tissue-specific promoter. This led to the production of cytotoxic enzyme in the tapetum cells causing male sterility. For commercial crop production, the F_1 hybrids have to bear normal bisexual flowers. To restore male fertility in the F_1 hybrids, another gene *barstar* which encodes a protein that forms a complex with barnase enzyme was introduced. Thus, a male sterility-restorer system has been developed which has attained commercial importance.

Another important trait that facilitates plant breeding is apomixis which means reproduction without sexual recombination. Apomixis allows multiplication of hybrids through seeds. Introduction of apomictic genes in crops, such as rice and cotton would revolutionise their cultivation. Molecular biology of mitosis and meiosis needs to be worked out in detail to manipulate these processes. It is likely that apomixis is a result of activation/inactivation of a gene encoding a cell division kinase or a cyclin which disturbs female meiosis. A proper understanding of these systems would open the avenues for apomictic manipulation.

2.4 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.

2.4.1. *Protein Quality*

The plant storage proteins have been utilised as the primary source of nutrition for human beings and livestock. Nutritional properties of proteins are influenced by parameters, such as amino acid composition and protein digestibility. The amino acid composition of storage proteins for nutritional purpose is frequently not optimal, lacking essential amino acids. In general, legume proteins lack the sulphur containing amino acids methionine and cysteine, and cereal proteins lack lysine and trytophan. There are two major approaches to improve the nutritional quality of plant proteins: (i) modification of the amino acid composition of the plant proteins, and (ii) introduction of transgenes-encoding proteins of high nutritive value.

Seed storage protein (2S) gene (*AmA* 1) isolated from *Amaranthus*[12] is a good candidate for introduction into crop plants as the protein has well-balanced amino acid composition. Recently, this gene has been introduced into potato and the level of accumulation of AmA 1 was found to be 0.3 per cent of total soluble protein[13]. Soybean glycinin gene was introduced into rice to increase its protein content and to make it more digestible. In addition, some important amino acids (eg, lysine) lacking in quantity in normal rice were replenished[14]. Gene encoding a human milk protein β-casein was expressed in transgenic potato under the control of an auxin-inducible promoter[15]. These findings open the way for reconstitution of human milk proteins in plant foods.

2.4.2 *Nutritional Quality*

The nutritional health and well-being of human beings are dependent on plant foods containing vitamins, minerals and phytochemicals. However, not all plant foods contain the essential nutrients needed for human health nor do they usually contain given nutrients in sufficient quantities. To ensure an adequate dietary intake of all essential nutrients and to increase the composition of various health-promoting compounds, scientists have been interested in improving nutritional quality of plant foods. 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.

A gene encoding soybean ferritin (iron-storage protein) was introduced in rice under the control of glutelin promoter to express the protein in a seed-specific manner[16]. The iron content of the transgenic rice seeds was three-fold greater than that of their untransformed counterparts. The most highly consumed vegetable oils (soybean, corn and rapeseed) contain high levels of tocopherols. These are poor in α-tocopherol (the form with the highest vitamin E activity) while γ-tocopherol predominates. γ-Tocopherol is methylated to form α-tocopherol, a reaction catalysed by γ-tocopherol methyl transferase (TMT). The *tmt* gene of *Arabidopsis, Synechocystis and Euglena*[17] were isolated and *tmt* of *Arabidopsis* was overexpressed in a seed-specific manner. Transgenic *Arabidopsis* contains 85-95 per cent of its total tocopherol pool as α-tocopherol, more than 80 per cent increase over wild-type control[18].

Rice, the major staple food contains neither β-carotene (provitamin A) nor its C40 carotenoid precursors. A gene encoding phytoene synthase from daffodil was introduced into rice in an endosperm-specific manner. The transgenic plants accumulated phytoene, a key intermediate in provitamin A biosynthesis in the seed[19]. More recently, both rice and tomato have been engineered to produce β-carotene[20,21].

2.4.3. *Oil Quality*

Plant lipids, in addition to providing food material, are a rich source for the chemical industry for production of lubricants, plasticisers, surfactants, cosmetics, paints, etc. The success in designing new plant oils is expected to have multiple benefits, both in local agricultural economies, and more broadly, by generating a more favourable balance of trade. Oilseed crops tolerate metabolic manipulations and the potential of metabolic engineering of plant storage lipids in transgenic crops leading to healthier foods and to useful industrial chemicals is emerging fast on the horizon of plant biology.

Chemical hydrogenation is used in the industry to obtain saturated fats. Unfortunately, industrial hydrogenation also raises the concentration of trans fatty acids, which have been linked to higher health risks. Transgenic oil crops producing high seed stearic acid level offer 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 canola in a seed-specific manner resulted in transgenic plants that accumulate 55-68 per cent more stearate than plants expressing the wild-type enzyme[22].

Lauric acid is mainly used in laundry detergents, shampoos and other surfactants. Because of its 12-carbon chain length, it has ideal properties of balance of solubility in both aqueous and non-acqueous environment. Seeds of California bay tree (*Umbellularia californica*) storing up to 70 per cent laurate in their triacylglycerols provided highly active and specific acyl-ACP thioesterase. Expression of the gene encoding this enzyme in *Arabidopsis* and canola under the control of an early seed-specific promoter increased seed laurate level up to 25 per cent and 45 per cent, respectively. Commercial cultivation of the transgenic canola over thousands of acres in Georgia, USA and sale of the oil from the harvested seed by Calgene Inc. to soap industry represents a fruitful endeavour of molecular biology[23].

2.5 Carbohydrate Metabolism

Major plant carbohydrates, cellulose, starch and sugar contribute considerably to important industrial applications like paper, textiles, cosmetics, pharmaceuticals, plastics, adhesive, etc. as raw materials. Genetic manipulation of plants open the possibility to specifically manipulate carbohydrate metabolism either by overexpressing endogenous plant genes or by repressing them by the use of co-supression or antisense RNA or by introducing foreign genes with different properties, specially for the synthesis of novel carbohydrates.

ADP-glucose pyrophosphorylase (AGPase) plays a key role in plant starch biosynthesis. This enzyme is regulated by the activator and inhibitors adenosine monophosphate (AMP) and inorganic phosphate (Pi). Potato plants were transformed with *E. coli glg*C16 gene, encoding mutant AGPase less sensitive to regulatory Molecule FbP and AMP

under the control of tuber- specific promoter patatin. Transgenic potato showed, on an average, 35 per cent more starch than in control.

2.6 Post-harvest Traits

Characters that determine the viability and storage life of plant products (fruits, vegetables, flowers and tubers) after harvest are of significant economic importance. Enhancement in the storage life and resistance to transportation hazards is feasible by adopting genetic engineering techniques. In fact, tomatoes manipulated for delayed ripening are the first genetically modified plant products to be marketed in USA (Flavr Savr), in 1994. Ripening in fruits and senescence in flowers can be delayed by antisense expression of genes involved in pectin metabolism or ethylene biosynthesis[24]. Some such examples include polygalacturonase, aminocyclopropane-1-carboxylic acid (ACC) synthase, ACC oxidase and mutated ethylene receptor protein (ETR 1). Manipulation of ripening process in tropical fruits, such as mango and banana holds great promise in near future.

2.7 Metabolic Manipulation

Engineering of plant metabolic pathways through molecular biology has opened up new opportunities and plant biologists are redesigning plant metabolism towards production of specific higher value products. An interesting example of metabolic manipulation of plants for human benefit is described below.

Synthesis of biodegradable thermoplastic from aliphatic polyester is of potential interest, not only from ecological point of view but also as a renewable source of carbon. Some bacteria, such as *Alcaligenes eutrophus*, have the capability to synthesise the homopolymer polyhydroxybutyrate (PHB) having thermoplastic properties from the acetyl-CoA pool. Genes encoding acetoacetyl-CoA reductase and PHB synthase were cloned from *Alcaligenes eutrophus* and transferred to *Arabidopsis*. The cytosolic expression of the genes resulted in the accumulation of small quantities of PHB granules but the plants were severely stunted in growth, probably because of depletion of the cytoplasmic pool[25] of acetyl-CoA. To circumvent this problem, three genes coding for β-ketothiolase, acetoacetyl reductase and PHB synthase each were transferred into *Arabidopsis* under the control of a constitutive promoter and a pea chloroplast-targeting sequence for expression of the gene products in the plastids. Following sexual crosses, plants were obtained expressing all the three genes and accumulating PHB up to 14 per cent of the dry weight as 0.2–0.7 μm granules within plastids, without showing any obvious effect on growth or fertility of the transgenic plants[26].

2.8 Floriculture

The global production of cut flowers and pot plants has reached industrial levels in the 20th century. Molecular approaches are now being used to introduce desirable traits, such as colour, shape, plant architecture and vase-life to meet consumers' demand for novelty. The first application of gene technology to modify flower colour was reported in petunia[27]. Expression of the maize dihydroflavonol-4-reductase gene (*dfr*) in petunia leads to the appearence of pale pink to brick or salmon red pelargonidin pigment and variations in phenotypes.

Among popular cut flower species, only freesia and iris have truly blue varieties. Development of blue varieties in commercially important species such as rose, carnation and chrysanthemum has stimulated industrial interest. The simultaneous presence of three factors, synthesis of 3′,5′-hydroxylated anthocyanins (delphinidins), presence of flavonol co-pigments and a relatively high vacuolar p*H*, are required to obtain blue coloured flowers. The first breakthrough to engineer blue coloured flowers was made by cloning flavonoid-3′, 5′- hydroxylase (*F3′ 5′H*) gene from petunia. Transgenic violet carnations have been successfully produced by the introduction of the petunia *F3′ 5′H* gene and its high expression in the petals. Transgenic violet carnation named Moondust™ is being marketed in Australia and Japan[28].

Many cut flowers deteriorate rapidly after harvest and short vase-life constitutes a major obstacle in the marketing of flowers. The petals exhibit a characteristic 'in-rolling' behaviour during senescence and also in response to exogeneously supplied ethylene. Supression of biosynthesis of ethylene should be able to lengthen the vase-life. The conversion of S-adenosyl methionine to 1-aminocyclopropane-1-carboxylic acid (ACC) catalysed by ACC synthase and conversion of ACC to ethylene catalysed by ACC oxidase are the two rate limiting and regulatory

steps. Transgenic carnations that contain an anti-sense ACC oxidase (*aco*) gene exihibited low ethylene production and a marked delay in petal senescence[29].

2.9 Phytoremediation

The use of plants for rehabilitation of polluted enviornment 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 (eg, methylmercury)[30]. MerB catalyses the protonolysis of the carbon-mercury bond, removing the organic ligand and releasing mercury (II), a less mobile mercury species. By introducing the *merA* gene from mercury-resistant bacteria, after its extensive modification to suit plant expression, mercury-resistant yellow poplar plant has been produced[31]. The *merA* gene encodes an enzyme, mercury reductase, that is capable of chemically reducing toxic ionic mercury (II) 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. Similarly, transgenic tobacco has been developed for the transformation of xenobiotic contaminants to non-toxic materials[32]. Expression of the bacterial pentaerythritol tetranitrate reductase gene renders the transgenic tobacco plant resistant to high concentrations of explosives, such as trinitroglycerin and trinitrotoluene by denitrifying these toxic compounds.

2.10 Therapeutics

Plant systems are being increasingly exploited as potential bio-reactors for the production of immunotherapeutic molecules for the improvement of human and animal health. Transgenic plants offer an advantage over the animal and bacterial system for large-scale production of therapeutics, such as protein antigen, antibodies and secondary metabolites at low-cost, as their eukaryotic nature often permits appropriate post-translational modification of recombinant proteins to retain native biological activity. The autotrophic growth of plants enables them to produce large amount of biomass at relatively low inputs. Furthermore, production of biologically active proteins in food plants provides the advantage of direct delivery through consumption of edible transformed plant tissues[33].

An example of transgenic plants is the successful expression of Hepatitis B surface antigen (HBsAg) in tobacco[34]. Sequences encoding *HBs*Ag gene were placed under CaMV 35S promoter and introduced into the tobacco genome using *Agrobacterium*-mediated transfer. Diarrhoeal disease is an important cause of mortality, especially among children in developing countries. The disease is caused by *Vibrio cholerae* and the related enterotoxigenic *E. coli*. The oral vaccine administered against this disease is composed of the toxin B subunit (CT-B) with killed *Vibrio cholerae* cells. Heat labile enterotoxin (LT) from *E. coli* is a multimeric protein that is structurally, functionally and antigenically very similar to cholera toxin (CT). Both the subunits (A and B) of *Vibrio cholerae* were expressed in transgenic plants. The LB subunit of *E. coli* has been expressed in tobacco and potato[35]. Transgenic potato expressing the LB subunit were fed to mice and found to elicit both mucosal and humoral antibody responses comparable to antigen produced in bacteria.

Plants possess complex secondary metabolites which helps them to adopt to changing environmental factors and various biotic and abiotic stresses. Though the biosynthetic origin and role of these secondary metabolites in plants are poorly understood, secondary metabolites are of considerable interest because of their potential industrial, pharmacological and medicinal value. Many such plant-derived products have reached the market as useful drugs for treating human disorders. Some examples are: Atropine, hyoscyamine, scopolamine, taxol (anti-carcinogenic), vinblastine/vincristine, artemisinin (antimalarial), reserpine (antihypertensive) involved in the biosynthetic route. Transformation approaches are being used to modify the biosynthetic pathway for the production of artemisinin, a powerful antimalarial compound, anticancer drugs vinblastine and vincristine from *Catharanthus roseus*, and taxol from *Taxus brevifolia*[36].

3. MOLECULAR BREEDING

Plant improvement, whether as a result of

natural selection or the efforts of plant breeders, has always relied upon creating, evaluating and selecting the right combination of alleles. Improvement of even the simplest of characteristics needs manipulation of a large number of genes, reorganisation 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.

Advent of these molecular markers bolsters molecular breeding through construction of a detailed genetic map for the crop of interest and identification of the position of genes. The correlation between molecular marker loci and phenotypic traits, generated from a segregating population, helps to identify a pair of tightly linked markers flanking the chromosomal region of the gene. Construction of high-density linkage maps for several crops has been accomplished. Comparative molecular genetic approach will help to isolate and characterise important orthologous genes from target species in a rapid way. A map-based cloning approach in rice has been used for the isolation of the wheat *Ph* gene which controls chromosome pairing[37]. Similarly, work is under way to isolate *Rpg*1, a stem rust resistance gene in barley, by genome walking in rice.

Quantitatively inherited traits, governed by polygenes, such as quality, yield, plant biomass, etc. are difficult to manipulate inbreeding programmes. High-density molecular maps help to identify and quantify the effect of genes and gene blocks that contribute substantially to quantitative traits. The construction of molecular linkage maps has made map-based cloning a reality for isolating genes of qualitative and quantitative traits. Gene tagging and map-based cloning have been best exploited to identify and quantify clone disease and pest resistance genes. The ability to tag these resistance genes is of great value in accumulating genes in one cultivar to confer resistance against a particular pathogen or pest. In tomato, the *Pto* gene conferring resistance to races of *Pseudomonas syringae* pv *tomato* has been fished out on the basis of a high-density RFLP map with tightly-linked molecular markers and introduced successfully into susceptible tomato cultivars for resistance development [38]. Other examples of map-based cloning of genes are *M*-1 and *Hs*1^{pro-1} genes conferring resistance to nematodes[39]. However, application of gene tagging for important agronomic traits is limited becuase of difficulty in finding tightly-linked flanking molecular marker.

4. PLANT GENOMICS

Plant biology research reaches a landmark with the initiatives of sequencing the whole plant genome of certain plant species. Genome sequencing will open horizons to study plant biology, specially characterisation of cellular, physiological and developmental pathways. This will also pave the way to determine the functions and locations of key gene products, and unravel the mechanisms by which complex networks of gene products are established and localised and also to resolve questions concerning evolutionary relationships among eukaryotic organisms and the evolution of common cellular and developmental pathways.

Arabidopsis genome initiative was established in 1996 to facilitate coordinated sequencing of the *Arabidopsis* genome. Individual groups of this project from Europe, Japan and USA were assigned particular chromosomal region to begin sequencing. Out of the 120 megabase genome of *Arabidopsis*, which is organised into five chromosomes containing an estimated 20,000 genes, more than 30 megabases of genome have already been sequenced. The entire genome is scheduled to be sequenced by the end of 2000. Sequencing of the *Arabidopsis* genome will provide a wealth of information on gene identity and genome organisation in plants. The *Arabidopsis* genome is highly enriched for coding sequences, with one gene every 5 kb on an average[40]. About half of these genes appear to be closely related in sequence to genes found in other plant systems. Sequencing the *Arabidopsis* genome has therefore proven to be important in identifying every gene in a representative flowering plant.

Rice genome sequencing has been progressing at Tsukuba, Japan. Rice researchers have formed an international consortium to sequence a Japanese

rice cultivar called *Nipponbare*. India is a part of the consortium and has committed to sequence the chromosome 11. The new China Rice Genome Programme sequencing facility in Shanghai has also been active independantly. Monsanto researchers have completely sequenced the rice genome which is available for use in public domain. However, certain gaps still remain to be filled in. Recently, USA has taken initiative to sequence corn genome with support from National Science Foundation.

An initial application of plant genomics has been to monitor 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 hybridisation 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 [41,42]. 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 give 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[43].

DNA micro-array technology allows an integrative analysis of gene expression patterns, so that, for the first time, scientists will be able to assess the impact of a specific treatment, environmental stage on all aspects of plant biology. Because the output is quantitative, subtle changes in gene expression can be detected. Moreover, DNA micro-array analysis can be used to identify genes that are unaffected, as well as those that show reduced transcript accumulation. Potentially, the DNA micro-array technology is also a powerful tool for new gene discovery. One or a few genes are typically used as marker genes for any given plant process. Plant biologists are currently facing the challenge of assigning functions to the large numbers of genes being identified in various sequencing projects. Many genes that are classified by sequence similarity to known genes remain poorly defined in terms of their specific roles in plant growth and development. By determining expression patterns of genes across a wide range of developmental and environmental conditions, it may be possible to develop hypotheses about the specific functions of these genes. DNA micro-arrays can also be used to screen populations of plants for polymorphic 'expression fingerprints'. These expression fingerprints then can be correlated with a complex process, such as drought tolerance, to evaluate new genes or groups of genes in that plant process[42].

5. PROBLEMS & FUTURE PERSPECTIVES

Plant biotechnology has made tremendous strides in the past decade. Many plant species engineered for expression of a variety of traits are already under extensive cultivation in many parts 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 implications[44]. The range of such transgenic plants will encompass industrial products, pharmaceuticals, therapeutics and environmental health in future. Such novel plant genotypes need to be introduced into the environment with great caution considering the biosafety and risk assessment. With the unravelling of plant genome structure, a plethora of useful genes will be available for reintroduction into plant species. This will open up new avenues and opportunities to manipulate abiotic stress tolerance, photosynthetic capacity, nutrient use efficiency and biological nitrogen fixation. Plants can also be modified by chimeric oligonucleotides consisting of DNA and RNA streches, a technique referred to as gene therapy or chimeric oligonucleotide-dependent mismatch repair (cdMMR). Two such instances in tobacco and maize have been reported[45].

Worldwide, there have been many hundreds of field trials with transgenic crops. Over 50 different crop species have been tested in field experiments in 40 countries since the first trials with tobacco in 1986. For instance, 1045 approved field trials of transgenic crops were conducted[46] in USA, in 1998. Over the past 10 years, regulations governing the release of transgenic plants for experimental and commercial purposes have been developed in different countries. There are various factors that

must be taken into consideration while assessing the likely impact of releasing transgenic plants into the environment. These include: The function of the gene in the donor organism; the effect of the transgene on the phenotype of the transgenic plant; evidence of toxicity and allergenicity; persistence in agricultural habitats; impact on non-target organisms (unintended effects); and the likelihood of transgenes (eg, encoding herbicide tolerance, pest resistance, disease resistance) being transferred by cross pollination to sexually compatible species, and the possibility of producing more persistent weeds or invasive plant population have motivated researchers to study the distance of pollen movement in various crops, and sexual compatibility among crops and related species. A case-by-case approach is necessary while evaluating the transgenic plants and the transgenes they carry. Alternate strategies that circumvent the problems of gene transfer, such as chloroplast transformation will be deployed in important crop plants in near future. These strategies would also provide durable insect resistance, hyperexpression of antigenic proteins and pharmaceutically important molecules. Development of transgenic plants which do not carry selectable marker genes encoding antibiotic/herbicide resistance will be more acceptable to the consumers. Efforts towards developing alternate systems of plant transformation are needed.

Considerable work has been carried out in the recent years to improve the photosynthetic efficiency of crops, a dream of plant breeders for a long time. Although there is still little reliable information regarding manipulation of photosynthetic genes for higher yield, results from many experiments were published. Monsanto company has conducted field trials of corn and wheat carrying a gene (classified as Confidential Business Information) that resulted in enhanced level of photosynthesis. Similarly, companies such as Zeneca, Pioneer and Calgene have also conducted field trials under the category 'increased yield'. Information on agronomic performance of these crops will be available soon.

The major challege in assesing the performance of introduced gene in the field of tragenic biology is gene silencing, which is an unfortunate end of a scientific endeavour 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[47]. 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 status of biotechnology in India is very encouraging and the future beckons with many opportunities. Many laboratories are engaged in plant molecular biology and genetic engineering research. Transgenic plants carrying traits for insect resistance, nutritional quality, viral resistance and disease resistance are under development. Pioneering work has been done in identifying a 2S protein with balanced amino acid content from *Amaranthus* and introducing the respective gene in potato[12]. Rice was transformed with a Bt-*cry1Ac* gene to confer resistance to yellow stemborer[48]. A glyoxalase gene was found to confer salt tolerance to transgenic tobacco[49]. Vegetable crops, such as tomato, brinjal and potato have been transformed by different Bt genes and field tested[50-52]. Introduction of insect-resistant transgenic cotton by Mahyco-Monsanto and vegetable crops by Pro-Agro Corporation is in the offing. Laboratories in many universities and research institutions are engaged in isolation of useful genes and genetic transformation of important crop species, such as cotton, rice, chickpea, sorghum, sugarcane, etc. Progress in the area of genetic transformation is relatively slow because the procedures for some of the important grain legume crops are still not available. Similarly, biotechnology research related to plantation, medicinal and spice crops is still in its infancy. Pioneering researches made by Indian scientists in the area of plant regeneration through tissue culture would make a strong foundation for genetic transformation efforts. The vast biodiversity and rich germplasm of crops of India present before us 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.

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