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Review

Applications of biotechnology for crop improvement: prospects and constraints

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Abstract

Recombinant DNA technology has significantly augmented the conventional crop improvement, and has a great promise to assist plant breeders to meet the increased food demand predicted for the 21st century. Dramatic progress has been made over the past two decades in manipulating genes from diverse and exotic sources, and inserting them into microorganisms and crop plants to confer resistance to insect pests and diseases, tolerance to herbicides, drought, soil salinity and aluminum toxicity; improved post-harvest quality; enhanced nutrient uptake and nutritional quality; increased photosynthetic rate, sugar, and starch production; increased effectiveness of biocontrol agents; improved understanding of gene action and metabolic pathways; and production of drugs and vaccines in crop plants. Despite the diverse and widespread beneficial applications of biotechnology products, there remains a critical need to present these benefits to the general public in a real and understandable way that stimulates an unbiased and responsible public debate. The development, testing and release of agricultural products generated through biotechnology-based processes should be continuously optimized based on the most recent experiences. This will require a dynamic and streamlined regulatory structure, clearly supportive of the benefits of biotechnology, but highly sensitive to the well being of humans and environment. © 2002 Published by Elsevier Science Ireland Ltd.

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1. Population increase and food security

The United Nations have projected that world population will increase by 25% to 7.5 billion by 2020. On an average, an additional 73 million people are added annually, of which 97% will live in the developing countries. At the moment, nearly 1.2 billion people live in a state of 'absolute poverty' [1], of which 800 million people live under uncertain food security, and 160 million pre-school children suffer from malnutrition [2]. A large number of people also suffer from deficiencies of micronutrients such as iron, zinc and vitamin A. Food insecurity and malnutrition result in serious public health problems, and a lost human potential. The amount of land available for crop production is decreasing steadily due to urban growth and land degradation, and the trend is expected to be much more dramatic in the developing than in the developed countries. In 1990, only Egypt, Kenya, Bangladesh, Vietnam, and China had a per capita crop land availability below 0.25 ha. However, by 2025, countries such as Peru, Tanzania, Pakistan, Indonesia, and Philippines are likely to join this group [3]. These decreases in the amount of land available for crop production and increase in human population will have major implications for food security over the next 2–3 decades.

There had been a remarkable increase in total grain production between 1950 and 1980, but only a marginal increase was realized during 1980–1990 [4]. Much of the early increase rise in grain production resulted from an increase in area under cultivation, irrigation, better agronomic practices, and improved cultivars. Yields of several crops have already reached a plateau in developed countries, and therefore, most of the productivity gains in the future will have to be achieved in developing

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countries through better natural resources management and crop improvement. Productivity gains are essential for long-term economic growth, but in the short-term, these are even more important for maintaining adequate food supplies for the growing world population. It is in this context that biotechnology will play an important role in food production in the near future. In this review, we attempt to take a critical but practical look at the prospects and constraints of various types of biotechnologies and their application for increasing crop production and improving nutritional quality. Within this, we also address the critical issues of biosafety and impact of the genetically engineered crops on the environment.

Genetic engineering offers plant breeders access to an infinitely wide array of novel genes and traits, which can be inserted through a single event into high-yielding and locally-adapted cultivars. This approach offers rapid introgression of novel genes and traits into elite agronomic backgrounds. Future impacts of biotechnology in crop production will be in the areas of: (i) developing new hybrid crops based on genetic male-sterility, (ii) exploit transgenic apomixes to fix hybrid vigour in inbred crops, (iii) increase resistance to insect pests, diseases, and abiotic stress factors, (iv) improve effectiveness of bio-control agents, (v) enhance nutritional value (vitamin A and iron) of crops and post-harvest quality, (vi) increase efficiency of soil phosphorus uptake and nitrogen fixation, (vii) improve adaptation to soil salinity and aluminium toxicity, (viii) understanding nature of gene action and metabolic pathways, (ix) increase photosynthetic activity, sugar and starch production, and (x) production of pharmaceuticals and vaccines.

New crop cultivars with resistance to insect pests and diseases combined with bio-control agents should lead to a reduced reliance on pesticides, and thereby reduce farmers' crop protection costs, while benefiting both the environment and public health. Similarly, genetic modification for herbicide resistance to achieve efficient and cost effective weed control can increase farm incomes, while reducing the labor demand for weeding and herbicide application. Labor released from agriculture can then be used for other profitable endeavours. In addition, there is an urgent need for less labor-intensive agricultural practices in countries significantly affected by human immune deficiency virus (HIV). By increasing crop productivity, agricultural biotechnology can substitute for the need to cultivate new land and thereby conserve biodiversity in areas that are marginal for crop production. The potential of these technologies has been extensively tested in the model crop species of temperate and subtropical agriculture. However, there is an urgent need for an increased focus on crops relevant to the small farm holders and poor consumers in the developing countries of the humid and semi-arid tropics. The promise of biotechnology can be realized by utilizing the information and products generated through research on genomics and transgenics to increase the productivity of crops through enhanced resistance to biotic and abiotic stress factors and improved nutritional quality (Table 1).

2. The genomics revolution

The last decade has seen the whole genome sequencing of model organisms such as human [5,6], yeast [7], *Caenorhabditis elegans* [8], *Arabidopsis thaliana* [9], and rice [10]. It is likely that whole genome sequencing will be carried out for several other plant species such as *Zea mays*, *Sorghum bicolor*, *Medicago sativa* and *Musa* spp. Systematic whole genome sequencing will provide critical information on gene and genome organization and function, which will revolutionize our understanding of crop production and the ability to manipulate those traits contributing to high crop productivity [11]. Similarly, advances in microarray technology will allow the simultaneous expression and analysis of vast numbers of genes that will elucidate gene function, and the complex multifaceted interactions between genes that result in different phenotypes under varying environmental conditions [6]. These studies will be augmented by more specific investigations based on gene suppression, co-suppression or anti-sensing of a defined sequence [12]. This knowledge from model systems will increase our understanding of plant biology and thereby increase our ability to exploit genomic information for crop improvement. Advances in these areas will fuel the mapping of QTL (quantitative trait loci) underlying agronomic traits in less studied crops. The use of QTL markers in crop improvement promises rapid and efficient utilization of novel traits from closely related wild species.

It takes five to six generations to transfer a trait within a species into the high yielding locally adapted cultivars through the conventional breeding, and one has to plant a large number of progenies to be able to select the plants with appropriate combination of traits (Fig. 1). The improved lines developed then have to go through a set of multi-location tests, before a variety could be identified for cultivation by the farmers. This process takes minimum of 7–10 years. However, genetic transformation provides access to genes from other species, which can be used for producing transgenic crops, ability to change the level of gene expression, and capability to change the spatial and temporal pattern of gene expression. The genes of interest can be transferred into the target crops/cultivars in a single event, and it takes 5–6 years to develop cultivars with stable gene expression. The lines thus produced can be released for cultivation by the farmers or used as donor parents in

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.

the conventional plant breeding and/or marker assisted selection.

In the marker-assisted selection, the elite lines can be crossed with another line having trait(s) of interest. The F_1 hybrid is crossed with the recurrent parent (invariably the elite parent) (BC_1), and the gene transfer is monitored through marker-assisted selection until BC_{3-5} [until the quantitative trait loci (QTL) or the gene of interest is transferred into the elite line]. In case of wild relatives that are not easily crossable with the cultivated types, the F_1 hybrids may have to be produced through embryo rescue and tissue culture, and the progenies advanced as in the conventional backcross breeding approach (phenotypic selection) or through-marker assisted selection, using cultivated species as the recurrent parent. Progenies from F_2 to F_{6-8} generations can also be advanced as per conventional pedigree breeding, and plants with appropriate combination of traits can be used as improved varieties or as donor parents in conventional breeding. The F_{6-8} progenies can also be used as random inbred lines (RILs) for mapping the trait(s) of interest if 250–300 plants are selected at random in F_2s, and advanced by selfing the plants at random in each line in each generation. The plants obtained in BC_5 can be used as isogenic lines to study the inheritance or role of traits of interest. The marker assisted selection takes 3–6 years, and thus speeding up the pace of transferring the traits of interest into the improved varieties, and it does not require large scale planting of the progenies up to crop harvest, as the plants showing the presence of the trait or QTL only need to be maintained up to maturity. Wide hybridization may take 7–10 years or longer (BCF_n), depending on the success in transferring the trait(s) of interest into the elite line without other wild traits that influence the quality of the produce and productivity potential of the crop.

2.1. DNA marker-assisted selection

Recombinant DNA technologies, besides generating information on gene sequences and function, allows the identification of specific chromosomal regions carrying genes contributing to traits of economic interest [13]. The theoretical advantages of indirect selection using genetic markers were first reported by Sax nearly 80 years ago. However, it was not until the development of DNA marker technology that a sufficiently large number of genetic markers could be generated to accommodate the needs of modern plant breeding programs. There is now a profusion of different types of DNA markers, each having a differential set of

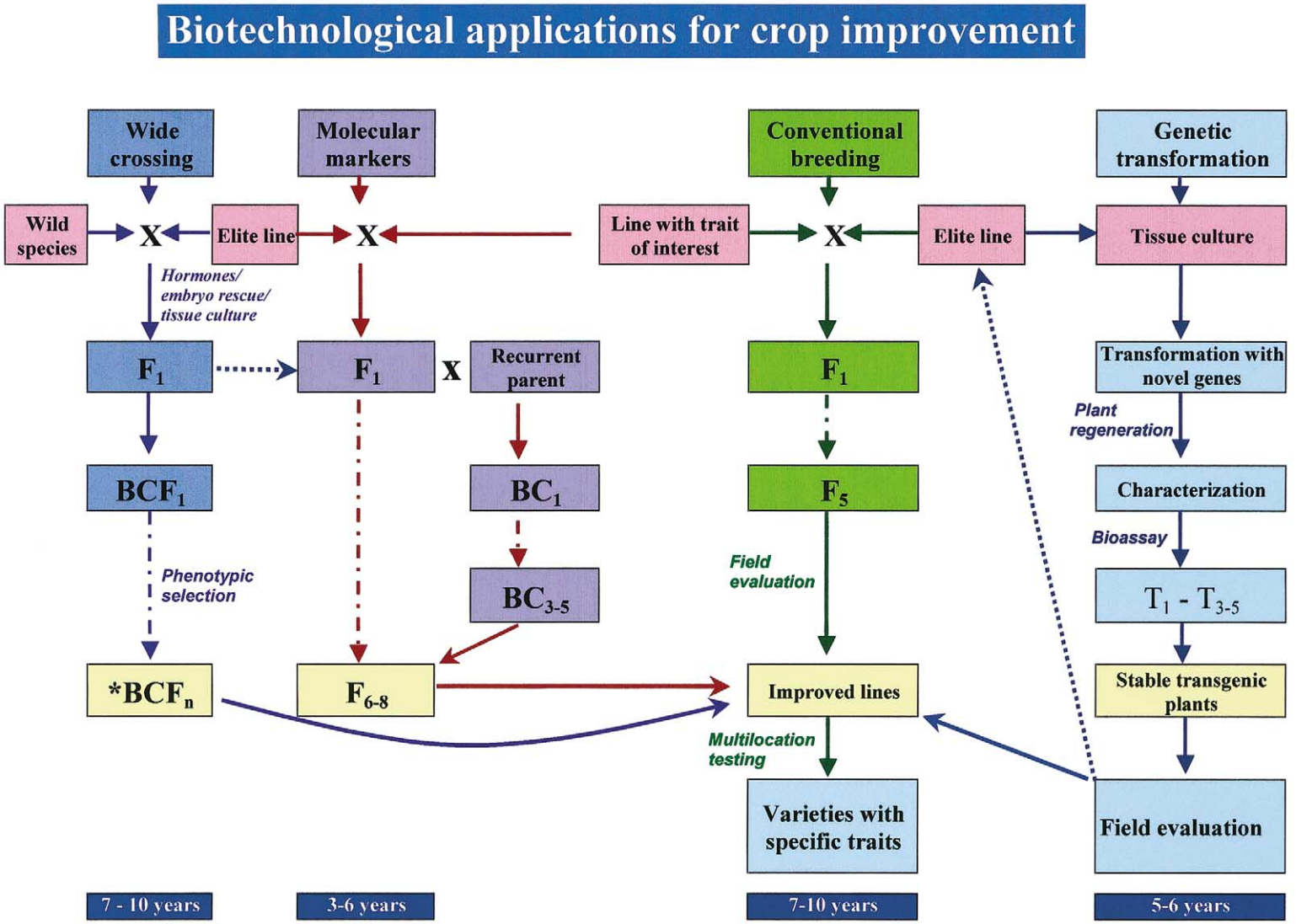

Fig. 1. A schematic outline of biotechnological approaches in crop improvement. Lines derived through genetic transformation can be released as varieties or used as a donor parent in the conventional breeding. The lines derived from wide crossing can take many generations (BCFn) to obtain homozygous and stable lines, and such material can either be used as improved lines or as a donor parent in conventional breeding or marker-assisted selection.

Table 2
Major classes of markers used for indirect selection in plant breeding

Morphological traits	Seed or flower color are seriously limited in number and expression, and can be differentially affected by the environment
Proteins	Seed storage proteins, structural proteins and isozymes. They provide very cost effective markers. Their number may be limiting and expression is not neutral
Restriction fragment length polymorphism (RFLP)	Requires hybridization of probe DNA with sampled plant DNA. Provides high quality data but has a severely restricted throughput potential
Random amplified polymorphic DNA (RAPD)	Based on polymerase chain reaction (PCR). This technique uses arbitrary primers for initiating amplification of random pieces of the sampled plant DNA. This technique requires no knowledge of the genome to be screened, but is inconsistent between populations and laboratories
Simple sequence repeat length polymorphism (SSR)	Provides high quality, and consistent results, but the markers are expensive to develop as they require extensive sequence data from the species of interest
Amplified fragment length polymorphism (AFLP)	The sample DNA is enzymatically cut up into small fragments (as with RFLP analysis), but only a fraction of fragments are studied following selective PCR amplification. Although this assay provides a great quantity of marker information, it is not well suited to high throughput marker assisted selection
Expressed sequence tag (EST)	The development of EST markers is dependent on extensive sequence data on regions of the genome which are expressed. An expressed EST is a small part of the active part of the gene made from cDNA, which can be used to fish out the rest of the gene out of the chromosome, by matching base pairs with the part of the gene. The ESTs can be radioactively leveled in order to locate it in a large segment of the DNA
Single nucleotide polymorphism (SNP)	The vast majority of differences between individuals are point mutations due to single nucleotide polymorphisms. As such, there are a vast number of potential SNP markers in all species. Considerable amounts of sequence data are required to develop SNP markers. However, their great advantage lies in the potential to screen them using methods which do not involve electrophoresis, such as microarrays

advantages for any particular application (see Table 2 for description of major classes of genetic markers). For further information see: http://www.nal.usda.gov/pgdic/tutorial/lesson4.htm and http://www.icrisat.org/xxx/research/grep/homepage/genomics/output.asp).

The identification of DNA markers for traits of interest usually depends on making crosses between two genotypes with substantial and heritable differences in trait(s) of interest. Depending on the crop and traits involved, mapping populations are then derived from the progeny of this cross by selfing once, many times (recombinant inbred lines–RIL), back-crossing to one of the parental genotypes (BC) or plants subjected to tissue culture to generate double haploids (DH). A major advantage of RIL and DH mapping populations is that each line is homozygous and can, therefore, be eternally multiplied through self-pollination. This then allows the population to be evaluated under many environments and seasons, facilitating a much more accurate estimate of phenotypic variation on which to base the mapping exercise. RIL and DH populations also allow scientists from many diverse disciplines to study different aspects of the same trait in the same population. This approach can only be used when parental genotypes can be identified with opposing phenotypes for the trait of interest. Interspecific crosses can be used to good effect in this respect, but linkage maps derived from such crosses may have limited relevance in crop breeding programs [14].

Once genomic regions contributing to the trait of interest have been assigned and the alleles at each locus designated, they can be transferred into locally adapted high-yielding cultivars by making requisite crosses. The offspring with a desired combination of alleles can then be selected for further evaluation using marker-assisted selection. Wild relatives of commercial crops contain alleles of importance for improving crop performance and resistance to biotic and abiotic stress factors, and these can be effectively incorporated into crop breeding programs through marker-assisted selection [15]. DNA marker technology has been used in commercial plant breeding programs since the early 1990s, and has proved useful for the rapid and efficient transfer of these traits into agronomically desirable varieties and hybrids [16].

The development of genetic maps in a number of species having positional similarity will lead to better understanding of crop evolution and functioning of genes. This 'synteny' will allow advances made in one species to be applied to other species [17]. This information can also be used by biochemists and physiologists to understand the genetics of metabolic processes; analyze traits controlled by several QTLs, and identify favorable alleles at each locus. The alleles can be combined by simple crossing, and the most favorable combinations assembled in the same background using marker assisted selection and/or genetic transformation.

The use of DNA markers for indirect selection offers greatest potential gains for quantitative traits with low heritability as these are the most difficult characters to work with in the field through phenotypic selection. However, these traits are also amongst the most difficult to develop effective marker assisted selection systems. The expression of these traits can be greatly affected by 'genotype-by-environment interaction' and 'epistasis,' which can complicate the development of marker-assisted selection systems to the same extent that they

confound traditional field based selection. Thus, the quality of a marker assisted selection program can only be as good as the quality of the phenotypic data on which the development of that marker was based. It is, therefore, essential to use large mapping populations, which are precisely and accurately characterized in many locations across several years. The selective power of markers must then be verified in a range of populations representing the diversity of current breeding populations. Only then will it be possible to identify markers, which can be effectively and robustly applied to assist the selection of complex characters.

Finally, care must be taken whilst choosing which traits to apply marker-assisted selection. Cost-benefit analysis should be applied to determine that indirect selection has a real advantage over traditional approaches, in that it is cheaper, more reliable, or more time effective than phenotypic selection. For example, marker-assisted selection may allow a substantially smaller population to be evaluated in the field, reduce the number of breeding cycles necessary to reach a defined goal, free-up important labor at a crucial stage of the season or substantially increase the precision of selection. Characters, which typically fall into these groups, include those traits that are difficult or expensive to evaluate in the field such as certain types of insect and disease resistance, root development, and male sterility and fertility restoration loci or traits which are expressed late in the growing season such as quality characters. Alternatively, the application of DNA markers may be justified on the basis of facilitating new breeding strategies or goals. For example, screening for resistance to quarantined diseases or pyramiding resistance genes from diverse sources.

2.2. Gene sequence and function

Genes can be discovered using a variety of approaches [6–12], but a routine large-scale approach can commonly be followed by generating and sequencing a library of expressed genes. This library typically consists of thousands of strands of complementary DNA (cDNA) that are abundantly expressed by that plant under the given environmental conditions at the sampled growth stage. When sequenced, these cDNAs are termed expressed sequence tags (EST). A large number of ESTs are now available in the public databases for several model plants and crops such as *A. thaliana*, *M. sativa*, rice, maize, sorghum, and soybean. A comparison of the EST databases from different plants can reveal the diversity in coding sequences between closely and distantly related plants, while mapping of ESTs may elucidate the synteny between those species. When a high level of sequence similarity is detected between an EST and a gene of known function in another species, it is possible to infer probable gene function in the species of interest. However, the emphatic elucidation of gene function still requires experimental verification. Only a small proportion of genes are abundantly transcribed in any particular environment or growth stage, and therefore, a complete picture can only be obtained by generating a range of cDNA libraries from plants grown under different environmental conditions and sampled at different growth stages or by sequencing entire cDNA genome library. For understanding gene functions of a whole organism, functional genomics technology is now focused on high throughput (HTP) methods using insertion mutant isolation, gene chips or microarrays, and proteomics. Finally the identified genes are expressed in transgenic plants. These techniques offer powerful new uses for the genes discovered through sequencing [18].

2.3. Analysis of metabolic pathways

Knowledge of the changes in a specific plant function induced by different treatments has led to the development of methods to isolate genes involved in the metabolic pathways or their associated physiology [19]. With the availability of tagged mutant populations, the use of elegant screening systems based on knowledge of metabolism provides a relatively easy approach to isolating the genes for key steps. Many secondary plant metabolites such as flavonoids, have been implicated in several functions in plant physiology, including host plant resistance to biotic stress factors (Fig. 2). Many compounds of the flavonoid biosynthetic pathway (flavanones, flavones, flavanols, and isoflavonoids) accumulate in response to biotic and abiotic stresses [20,21]. Chalcone synthase catalizes the condensing of 3-malonyl-CoA and hydroxy cinnamoly-CoA ester to form the chalcone intermediate, and chalcone is converted into flavanone by chalcone isomerase. Flavanones are converted into flavones by flavone synthase. Dihydroflavanols are derived from flavanones, which are precursors for the production of flavonols and anthocyanins. Genetic engineering can be used to change the metabolic pathways to increase the amounts of various flavonoids, which play an important role in host-plant resistance to insect pests and diseases, e.g. medicarpin and sativan in alfalfa, cajanol and stilbene in pigeonpea, deoxyanthocyanidin flavonoids (luteolinidin, apigenindin, etc.) in sorghum, and stilbene in chickpea [20]. The expression of phytoalexins in transgenic plants may be difficult due to complexities involved in their biosynthesis. However, stilbenes have been expressed in transgenic tobacco plants, exhibiting various degrees of inhibition of fungal growth [20]. Molecular mechanisms underlying the activation of defense genes implicated in phytoalexin biosynthesis are quite common in a large number of plant species. Biotechnology offers a great

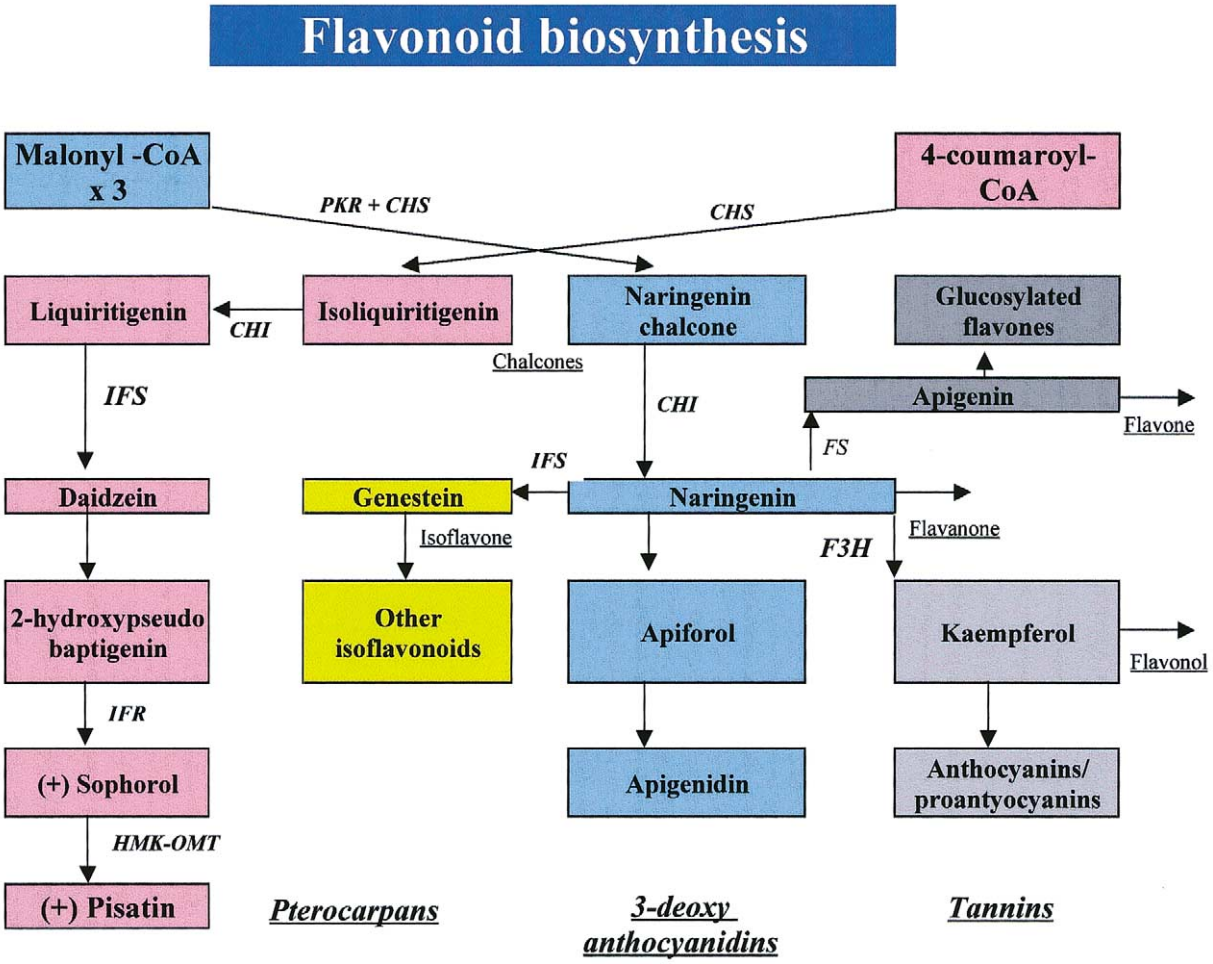

Fig. 2. A generalized scheme of flavonoid biosynthesis. Underlined are the major compound groups. In italics are the enzymes involved (CHS = chalcone synthase, CHI = chlacone isomerase, PKR = polyketide reductase, IFS = isoflavanone synthase, FS = flavone synthase, F3H = flavanone 3-hydroxylase, IFR = isoflavone reductase, and HMK-OMT = 6a-hydroxymaakainin 3-*O*-methyltransferase) (modified after Heller and Forkman, 1993).

promise to increase the production of secondary metabolites in plants that are used in medicine, aromatic industry, and in host resistance to insect pests and diseases or inhibit the production of toxic metabolites in crop produce meant for food, feed, and fodder.

2.4. Trait analysis

The gene pools of crop plants may have begun diverging over 150 million years ago. The resultant diversity in genes has led to variation in the expression of traits, and generation of completely new plant functions and phenotypes. Important traits in field crops can now be addressed from a general perspective through comparative gene function analysis using model plants, e.g. the gene for leafy mutant phenotype in *Arabidopsis* is a single gene determining initiation of flowering [9]. This type of approach has opened the large and exciting new field of 'gene mining' from germplasm collections. The known sequence of this gene can now be used to identify related genes in crop plants. Alternatively, DNA marker linkage maps can be used to analyze the genetic basis of traits and identify allelic variants. In this way, complex traits can be dissected into their component genes through intensive fine mapping. Map-based cloning of genes has been successful in a number of cases, and is becoming easier with the development of different genomic libraries of crops in a range of yeast and bacterial based vectors.

3. Genetic transformation

Genetic transformation offers direct access to a vast pool of useful genes not previously accessible to plant breeders. Current genetic engineering techniques allow the simultaneous use of several desirable genes in a single event, thus allowing coordinated approaches to the introduction of novel genes/traits into the elite background. The priorities for applied transgenic re-

search are similar to those of conventional plant breeding, aiming to selectively alter, add or remove a specific character in order to address regional constraints to productivity. Genetic engineering also offers the possibility of introducing a desirable character from closely-related plants without associated deleterious genes or from related species, which do not readily cross with the crop of interest or from completely unrelated species even in other taxonomic phyla.

In many species, the development of rapid, highly efficient, and routine transformation systems is still in progress and thus represents a bottleneck in the development of stable high yielding transgenic plants. Development and deployment of transgenic plants in an effective manner is an important pre-requisite for sustainable and economic use of biotechnology for crop improvement. As a result of advances in genetic transformation and gene expression during the last decade, there has been rapid progress in using genetic engineering for crop improvement in terms of herbicide tolerance, pest resistance, and male-sterility systems [22,23]. The potential of this technology has now been widely recognized and extensively adopted in the plant breeding of temperate crops in the following areas.

3.1. Resistance to insects, diseases and herbicides

The first transgenic plants with *Bacillus thuringiensis* (Bt) genes were produced in 1987 [24,25]. While most of the insect-resistant transgenic plants have been developed by using Bt δ-endotoxin genes, many studies are underway to use non-Bt genes, which interfere with the nutritional requirements of the insects. Such genes include protease inhibitors, chitinases, secondary plant metabolites, and lectins [22,23]. Genes conferring resistance to insects have been inserted into a wide array of crop plants including maize, cotton, potato, tobacco, rice, broccoli, lettuce, walnuts, apples, alfalfa, and soybean [26]. A number of transgenic crops have now been released for on-farm production or field-testing [27]. The first transgenic insect-resistant crop was grown in the USA during 1994, and large-scale cultivation was undertaken in 1996. Since then, there has been a rapid increase in the area sown with transgenic crops in the USA, Canada, Australia, Argentina, and China. Transgenic crops are now grown in over 12 countries in the world. Successful control of cotton bollworms has been achieved through transgenic cotton [28–30]. Cry type toxins from Bt are effective against cotton bollworm, corn earworm, the European corn borer, and rice stem borers [29,31–34]. Successful expression of Bt genes against the lepidopterous pests has also been achieved in tomato [35], potato [36], brinjal [37], groundnut [38], and chickpea [39].

At the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), several candidate genes are being evaluated for their biological efficacy against the sorghum shoot fly (*Atherigona soccata*), spotted stem borer (*Chilo partellus*), tobacco caterpillar (*Spodoptera litura*), and cotton bollworm or legume pod borer (*Helicoverpa armigera*), which are major crop pests in the tropics. Efforts are underway to insert the Bt, trypsin inhibitor, and lectin genes for resistance to these insects in sorghum, pigeonpea, and chickpea [23–40]. Transgenic sorghum and pigeonpea plants with Bt and trypsin inhibitor genes are presently being tested under containment glasshouse conditions. Work is also in progress on the development of groundnut plants with resistance to viruses and fungal pathogens [41].

There will be tremendous benefits to the environment through the deployment of transgenic plants with integrated pest management (IPM) systems [40]. Deployment of insect-resistant crops has been associated with a 1 million kg reduction of pesticides applied for pest control in USA in 1999 as compared with 1998 [42]. Papaya with transgenic resistance to ringspot virus [43] has been grown in Hawaii since 1996. Rice yellow mottle virus (RYMV), which is difficult to control with conventional approaches, can now be controlled through transgenic rice, which will eliminate the risk of total crop failure. Globally, herbicide-resistant soybean, insect-resistant maize, and genetically improved cotton account for 85% of the total area under transgenic crops [26–44]. The area planted to genetically improved crops has increased dramatically from less than 1 million ha in 1995 to 40 million ha in 1999 [44]. Transgenic plants with insecticidal genes are set to feature prominently in pest management in both developed and the developing world in future. Such an effort will play a major role in minimizing insect-associated losses, increase crop production, and improve the quality of life for the rural poor. Development and deployment of transgenic plants with insecticidal genes for pest control will lead to: (i) reduction in insecticide sprays, (ii) increased activity of natural enemies, and (iii) IPM of secondary pests.

3.2. Tolerance to abiotic stresses

Development of crops with an inbuilt capacity to withstand abiotic stresses would help stabilize the crop production and significantly contribute to food security in developing countries. In bacteria, trehalose is produced by the action of trehalose phosphate synthase, which produces trehalose phosphate, and trehalose phosphate phosphatase-which degrades trehalose-6-phosphate into trehalose. When these two enzymes are expressed in transgenic plants, the plants have larger leaves, altered stem growth, and improved response to stress [45,46]. Over-expression of various glutamate dehydrogenases (GDH) also improves plant growth and stress tolerance. Plants have been specifically

transformed with genes encoding the α-and β-subunits of the chloroplast-located GDH from the alga, *Chlorella sorokiniana* [47]. Similar improvements in performance have been reported for rice plants transformed with the barley late embryogenesis (LEA) gene [48]. Plants with an ability to produce more citric acid in roots provide tolerance to aluminium in acid soils [49]. Introduction of functional calcineurin activity provides tolerance to salinity [50] involving the introduction of a gene encoding a plant farnesyltransferase [51] and inhibitors of this enzyme when expressed in plants, enhance drought tolerance, delay senescence, and modify the growth habit. A salt tolerance gene isolated from mangroove (*Avicennia marina*) has been cloned, and can be transferred into other crop plants [52]. The *gutD* gene from *Escherichia coli* can also be used to provide salt tolerance [53]. These genes hold a great potential for increasing crop production in marginal lands [54].

3.3. Sugar and starch metabolism

Sucrose phosphate synthase (SPS) is a key enzyme in the regulation of sucrose metabolism. Transgenic plants expressing the maize SPS under the control of a promoter from the small subunit of tobacco. Rubisco have shown increased foliar sucrose/starch ratios in leaves, and decreased amounts of foliar carbohydrates when grown with CO_2 enrichment [55]. Modification of the activity of metabolites of the TCA (tricarboxylic acid) cycle by reducing the amount of the NAD-malic enzyme can also be used for increasing starch concentrations [56]. Introduction of the *Escherichia coli* inorganic pyrophosphatase to alter the amount of sugar [57], and modification of hexokinases [58], which affect the sugar-sensing capacities of a plant as well as sucrose-binding proteins [59], and a class of cupin protein [27] have been implicated in sugar unloading in developing legume seeds. This has opened up exciting possibilities for changing the chemical composition of food grains to meet specific requirements.

3.4. Altering senescence

Leaf senescence leads to a progressive death of the leaf or a plant upon aging due to reduction in the production in cytokinin. Cytokinin is a plant hormone that naturally prevents senescence and maintains photosynthetic activity in leaves. Reduction in leaf senescence [60,61] would improve the performance of a plant, and thereby increase the crop yield. This in part can be achieved through stay green trait in maize, sorghum, pearl millet, and other cereal crop. Stay green trait in sorghum is also associated with adaptation to drought stress. Introduction of farnesyl transferase and isopentenyl transferase (*IPT*) genes delays senescence [62]. The process of leaf senescence can be blocked through a gene encoding the cytokinin-synthesis enzyme, isopentenyl transferase. When transformed with the *SAG12-IPT* construct, a plant will produce enough cytokinin to delay leaf senescence. Cytokinin production is triggered only at the onset of senescence, due to regulation of the *IPT* gene by the senescence-specific *SAG12* promoter. Thus, the plant grows normally without an excess of cytokinin until hormone is needed to block senescence. This avoids problems with unregulated, constitutive over-production of cytokinin, such as short, bushy plants, and decreased root growth. Commercial uses for delayed senescence include increasing plant vegetative growth, seed and fruit production, prolonging the shelf-life of vegetables, maintaining nitrogen content of forage crops (e.g. alfalfa), provide a safe and natural source of cytokinin, and produce transgenic plants of multiple species. The promoter could also be combined with other genes, whose targeted expression during senescence would be beneficial.

3.5. Photosynthetic efficiency and improved yield

An exciting experimental approach to increase crop yield radically is to change components of plant biochemistry with respect to introducing the C_4 type of photosynthesis into a C_3 plants such as *Arabidopsis* [63] and potato [64]. C_3 photosynthesis suffers from O_2 inhibition due to the oxygenase reaction of ribulose 1, 5-biophosphate carboxylase/oxygenase (Rubisco), and the subsequent loss of CO_2 from photorespiration. In contrast, C_4 plants such as maize have evolved a biochemical mechanism to overcome this inhibition. A key feature of this mechanism is the activity of phosphoenolpyruvate carboxylase (PEPC) [65], an enzyme that fixes atmospheric CO_2 in the cytosol of mesophyll cells. Using an *Agrobacterium*-mediated transformation system, the intact maize PEPC has recently been transferred into the C_3 plants [66–68]. Physiologically, these plants exhibited reduced O_2 inhibition of photosynthesis and had photosynthetic rates comparable to those of control untransformed plants. Investigations into the manipulation of the key photosynthetic enzymes, Rubisco, pyruvate phosphate kinase (PPDK), and NADP malate dehydrogenase (NADP-MDH) in the C_4 dicotyledonous species *Flaveria bidentis* have also been reported [69]. An alternative strategy to reduce photorespiration by manipulating catalase amounts in tobacco has also been described [70]. Appropriate manipulation of the enzymes involved in photosynthetic activity can be used to increase the productivity potential of C_3 plants.

Genes determining plant height in *Arabidopsis* are orthologous (similar) to dwarf genes in cereals, which have been used in conventional plant breeding in the 'Green Revolution' [11]. These genes (NORIN 10) were introduced into western wheat varieties in the 1950s, and

have now been isolated, and identical phenotypes reconstructed in other crops through genetic transformation [71]. These dwarfing genes can now be routinely deployed in various crop species to increase crop productivity. Improved yield can also be achieved by manipulation of fructose-1,6-bisphosphate aldolase (FDA), an enzyme that reversibly catalyses the conversion of triosephosphate to fructose-1,6-bisphosphate. Leaves of transgenic plants expressing FDA from *E. coli* in the chloroplast show significantly enhanced starch accumulation, lower sucrose concentration, and higher root mass [72]. A more generic method for changing plant performance may be to modify plastid number [73], and the expression of a hybrid protein comprising a yeast gene encoding 5-amino levulinic acid synthase and an N-terminal transit sequence for the small subunit of carboxydismutase. Manipulation of chlorophyll *a/b* binding genes has also been used to modify chlorophyll amounts [74,75]. Degreening of oilseed rape caused by sublethal freezing during seed maturation can be accomplished by anti-sense reduction of the type I chlorophyll *a/b* binding protein of light harvesting complex II [76]. Other non-photosynthetic approaches to increasing yield of both shoot and root include over expression of a cyclin gene, such as *cycla* gene from *Arabidopsis* [77].

3.6. Nutritional factors

Several quality traits can be targeted to improve the nutritional status of crop produce. These include carbohydrates, proteins, oils, vitamins, iron, and amino acids. The selection of target traits is influenced by the end users, producers, and agro-based industry. Research in this area epitomizes the change in emphasis from single gene agronomic traits of herbicide and insect tolerance to more complex traits of direct benefit to the consumer such as modified seed quality. For example, transgenic rice, with a capacity to produce beta-carotene, can be used to overcome the deficiency of vitamin A [78]. Similarly, transgenic rice with elevated levels of iron has been produced using genes involved in the production of an iron binding protein that facilitates iron availability in human diet [79]. Altering protein levels, composition of fatty acids, vitamins and amino acids is being increasingly targeted for value addition. It is now possible to alter the composition of fatty acids so that polyunsaturated (e.g. linoleic acid) content is decreased while that of mono-unsaturated (e.g. oleic acid) content is increased to allow processing without the traditional use of hydrogenation, and thus avoiding the undesirable trans-fatty acids. Amounts of essential amino acids such as lysine, methionine, threonine, and tryptophan can be increased to improve the nutritional quality of cereal grains. Transgenic modifications have also been used to alter the ratio of amylose to amylopectin in starch [26]. Decreasing the amounts of oligosaccharides (such as raffinose and stachyose) improves digestibility, and decreases the degree of flatulence during digestion. Transgenic technology can also be used to remove anti-nutritional factors [80]).

3.7. Pharmaceuticals and vaccines

Several vaccines can be produced in plants [81]. Vaccines against infectious diseases of gastro-intestinal tract have been produced in potatoes and bananas [82–84]. Biotechnology has been used to develop plants that contain a gene derived from human pathogens [85]. An antigen product encoded by the foreign DNA accumulates in plant tissues. The antigen proteins produced by the transgenic plants retain the immunogenic properties upon purification, which can be used for production of antibodies when injected into mice. Mice eating the transgenic plants have shown an immune response. Such an immune response has been demonstrated for cholera toxin B [86]. Anti-cancer antibodies expressed in rice and wheat could be useful in diagnosis and treatment of this disease [87] . There is also a great potential to increase the yield of medicines derived from plants (e.g. salicylic acid) through the use of transgenic technology.

3.8. Exploitation of male-sterility (MS) and apomixis

In several plant species, genetic or cytoplasmic male sterility (GMS or CMS) leads to the suppression of production of viable pollen [88]. MS has been observed in a wide variety of higher plants and is characterized by the very low level or the complete absence of pollen production. MS phenotype affects essentially the pollen producing organs because of the high-energy requirement of such tissues. The best-known examples of this trait are the CMS observed in *Z. mays*, *S. bicolor*, *Pennisetum glaucum*, and *Helianthus annuus*, while both GMS and CMS have been exploited for developing rice hybrids [89]. A general characteristic of CMS is the dysfunction of mitochondria in tapetal cells. Mitochondrial genomes encoding chimeric proteins are presumably present in all tissues of the plant. Mitochondrial dysfunction produced by a chimeric protein interferes with the organelle function, and affects pollen production. Biotechnological approaches can be used to transfer CMS from within a species or from one species into another.

Apomixis, resulting from the development of asexual embryos, produces a large number of nucellar offsprings, which are genetically similar to the female parent [90,91]. Obligate apomixes offers an opportunity to clone plants through seed propagation, and through gene manipulation, can be used effectively as a potent tool in plant breeding. It provides uniformity in seed propagation of rootstocks and true breeding of F_1

hybrids. Genetic manipulation of apomixis has the potential to result in production of stable and superior hybrids. Some apomictic cultivars have already been released in case of citrus, Kentucky grass and buffalo grass. Development of cross compatible apomictic plants will allow for hybridization to break the barriers in gene transfer. This will also help to fix heterosis, and obtain non-segregating populations from hybrids with a unique combination of characters from the parents. Genetic engineering of apomixis can be used for fixing the genetic variability to produce crops with high productivity and better food quality. This system has been well studied in citrus, sorghum, maize, turf grass, and other crop plants. Introduction of apomictic genes into crops will have revolutionary implications for plant breeding and agriculture, whose social and economic benefits promise to exceed those of the green revolution. However, the dangers associated with genetic uniformity could be exacerbated by inappropriate use of this technology, and its application, therefore, would have to be considered on a case-by-case basis.

4. Environmental concerns and biosafety of transgenic food

There is a considerable debate about the environmental risks such as development of resistance, harmful effects on beneficial insects, and cross-pollination with closely related wild relatives of the crop plants. There is also a concern about a weedicide-resistant crop becoming a difficult weed in another crop. The available evidence on these issues is still inconclusive and certainly warrants continued and careful monitoring and follow up before transgenic crops are deployed on a large scale. However, anchored in a case-by-case risk-benefit analysis, we believe that there is a sufficient amount of quality data to support large-scale deployment of transgenic crops. The biggest risk of modern biotechnology for developing countries is that technological developments may bypass poor farmers because of a lack of enlightened adaptation. It is not that the current biotechnology research is irrelevant, but there is a desperate need for research focused on the problems of small farmers in developing countries. Private sector research is unlikely to take on such a focus, given the uncertainty of future profits in these areas. Without a stronger public sector role, a form of scientific apartheid may develop, in which cutting edge science becomes oriented exclusively toward industrial countries and large-scale farming systems.

The application of transgenes is not conceptually different to the use of native genes through wide crossing and marker-assisted selection. However, there are serious concerns in the general public about the biosafety and environmental effects of the transgenic plants. There is a need for stringent application of biosafety regulations while considering the development and deployment of transgenic crops. The need and extent of safety evaluation may be based on the comparison of the new food with analogous food. In relation to the environment, one has to look at the interaction of the transgene with the environment. The biosafety regulations need to focus on safety, quality, and efficacy [92–96]. The biosafety regulations require information on: (i) organization and people involved, (ii) DNA donor and the receiving species, (iii) conditions of release and the target environment, (iv) interactions between transgenic plants and the environment, and (v) monitoring, waste treatment, and control.

The management, interpretation, and utilization of information will be an important component of risk assessment, and determine the effectiveness and reliability of this technology. While considering the deployment of transgenic plants, care should be taken that: (i) the release of transgenic plants does not give rise to new pest problems or emergence of new biotypes of the target pest, (ii) whether the transgenic technology poses greater risk than the traditional alternatives, e.g. in case of gene transfer to the wild relatives, will it lead to expansion of the niche of the species and result in suppression of diversity in the surrounding areas, and (iii) whether the introduction of transgenic plant will result in an increase in the land use for agriculture, where agriculture could not be practiced earlier, i.e. bringing valuable natural ecosystem under agriculture.

The greatest risk of a transgenic plant being released into the environment is its potential to spread beyond the areas planted to become a weed. Although there has been little discussion about crops becoming weeds as a result of plant breeding [97], there may be some exceptions, e.g. oilseed rape in Europe. This may be because of: (i) low risk of crop plants to the environment, (ii) extensive testing of the crop varieties before release, and (iii) adequate management practices to mitigate any risks inherent in the crop plants. This may also be because of un-competitiveness of modern crop cultivars, which have been bred for high productivity under high inputs. Oilseed rape, however, has retained some of the weed characteristics as many small seeds are dispersed and has a relatively strong competitive vigor.

Plant breeding efforts have tended to decrease rather than increase toxic substances, as a result, making the improved varieties more susceptible to insect pests. However, there is a perception that genes introduced from outside the range of sexual compatibility might present new risks to the environment and humans. However, these apprehensions are not supported by data. Herbicide tolerance is available in many species, but it is more cost effective to introduce this trait through genetic transformation. A study conducted by

the National Academy of Sciences, USA [98], has concluded that: (i) there is no evidence of hazards associated with DNA techniques, (ii) the risks, if any, are similar to those with conventional breeding techniques, (iii) the risks involved are related to the nature of the organism rather than the process, and (iv) there is a need for a planned introduction of the modified organisms into the environment.

One of the hazards with transgenic plants is transfer of genes to wild relatives but this is only a major concern if the wild relatives are under selection pressure (biological control) from that pest. If the target pest does not play any role in population regulation of the wild hosts, gene transfer is unlikely to constitute any hazard. Furthermore, the build up of resistance in the wild relatives can also act as a component of pest management if it acts as an alternate host to the target pest.

Serious concerns have also been raised about the safety of transgenic food itself. Most Bt toxins are specific to insects as they are activated in the alkaline medium of the insect gut. The Bt-proteins are rapidly degraded by the stomach juices of vertebrates. No major changes have been observed in the composition of the transgenic tomatoes and potatoes. Transgenic Bt tomatoes pose no additional risk to human and animal health. However, a number of aspects concerning the safety assessment of transgenic Bt tomatoes would require further study [99]. There are no differences in the survival and body weight of broilers reared on meshed or palletted diets prepared with Bt transgenic maize as compared with the controls [100]. The levels of the antinutrients gossypol, cyclopropenoid fatty acids, and aflatoxins in the seed from the transgenic plants are similar to or lower than the levels present in the parental variety and other commercial varieties. The seed from the Bt transformed cotton lines is compositionally equivalent to, and as nutritious as, seed from the parental lines and other commercial cotton varieties [101]. CryIA(b) protein as a component of post-harvest transgenic maize plants dissipates readily on the surface of, or cultivated into, soil [102], and has not been detected in silage prepared from transgenic plants [103].

Several compounds produced by plants act as a natural defense mechanism against herbivores. These include secondary plant substances (such as terpenoids, flavonoids, alkaloids, etc.), α-amylase and trypsin inhibitors, lectins, and pathogenesis-related proteins [22,23]. Some of these are potential candidates for deployment in transgenic plants to confer resistance to insect pests and diseases. However, some of these secondary plant substances may be toxic to mammals, including humans. This may result in a trade off between nature's pesticides produced by transgenic plants or varieties from traditional breeding programs, synthetic pesticides, and mycotoxins or other poisonous products of pests. Further, it is also possible to introduce new proteins into food crops, not only from plants, but also from bacteria, fungi, and viruses; whose allergenicity is unknown, e.g. a gene for methionine rich proteins from Brazil nut has been introduced into soybean with the aim of enriching soybean proteins [104]. However, the transgenic soybeans containing this gene have been found to be allergenic, and hence further developmental work in this area was discontinued. If the source of the allergenic protein is known, and is related to the introduced gene from sources that have not been used as a human food, then such genes should not be used in genetic transformation of crop plants. Therefore, careful thought should be given while considering a particular gene for deployment in transgenic plants [105–107].

5. Conclusions

Access to information and expertise in developing countries, where the need to increase food production is most urgent, will be a key factor in the use of biotechnology for sustained food security. Several organizations such as Rockefeller Foundation, United Nations Educational, Scientific and Cultural Organization (UNESCO), International Cooperation Program of the European Union, International Service for the Acquisition of Agrobiotech Applications (ISAAA), and International Service for National Agricultural Research (ISNAR) are attempting to play a major role in technology transfer from public and private sector institutions in the developed to the developing countries. International funding for these initiatives, combined with development of many others, will be necessary to meet the demands of end-users in the developing countries, particularly in Africa. The national governments need to be helped and encouraged to formulate appropriate policies and establish regulatory framework to use biotechnology for sustainable food production.

Predicted growth in world population and the likely effects of climate change will pose a serious challenge to crop production and food security, particularly in developing countries. The augmentation of conventional breeding with the use of marker-assisted selection and transgenic plants promises to facilitate substantial increases in food production. However, knowledge of the physiology and biochemistry of plants will be extremely important for interpreting the information from molecular markers and deriving new and more effective paradigms in plant breeding. The application of DNA marker technologies in exploiting the vast and largely under-utilized pool of favorable alleles existing in the wild relatives of crops will provide a huge new resource of genetic variation to fuel the next phase of crop improvement. In particular, significant benefits will be derived through the transfer of genes important for

crop protection and crop quality. However, rapid and cost effective development, and adoption of biotechnology-derived products will depend on developing a full understanding of the interaction of genes within their genomic environment, and with the environment in which their conferred phenotype must interact.

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