Chapter 9
Biotechnology as an Aid for Crop Improvement to Overcome Food Shortage

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Contents

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M. Ashraf et al. (eds.), *Crop Production for Agricultural Improvement*,
DOI 10.1007/978-94-007-4116-4_9,

Abstract World's population has crossed 6.5 billion with majority of human beings living in developing or under developing countries. Clearly, food security in such countries will be a primary concern over the next few decades. However, options for increased food production to meet this population pressure are limited because most arable land is already under cultivation, and in many areas land use cannot be further intensified without a risk to the long-term productivity. Agricultural land use has been especially intense in recent years because of rapid urbanization and increasing environmental pollution. The ultimate need is to use newer technologies which could help us to curb this food insecurity. Biotechnology is globally recognized as a rapidly emerging, complex and far reaching new technology. It has revolutionized all the fields of life. Recent discoveries and technical innovations in the field of genomics and biotechnology are revealing the full complement of genes in crops, the ability to define genetic variation and use DNA markers to follow chromosome segments with known functions through breeding programmes are leading to new efficiencies in breeding. The ability to isolate and redesign genes and transfer them into different plants also offers the breeder solutions to several key limitations. The convergence of advances in biology-genomics, proteomics, bioinformatics and information technologies is driving the emergence of a new bio-economy. By the usage of this technology we have achieved remarkable success in increasing crop productivity, improving crop quality as well as overcoming food shortage. Additionally the genetically engineered crops have shown a remarkable potential to tackle some of the world's most challenging socioeconomic problems which are more prevalent in the developing world than in the industrialized nations.

Keywords Biotechnology • Food security • Molecular markers • Transgenics • Proteomics • Nanobiotechnology

1 Introduction

The world population has increased by 2.3 billion people in the past 40 years, and by the year 2040, an additional 3.6 billion will be added to it. In fact, in every earth hour about 13,000 new human beings are added up to this globe. Most of this increase is in the developing countries where already one billion people go hungry every day and live in dismal poverty. The African nations of Ethiopia, Nigeria or Egypt each add more people than all of Western Europe combined (World Watch Institute, Washington, DC). India's population is around 1.14 billion with an annual per capita income of less than US $ 1,043 and its population is projected to continue to increase to a total of 1.5 billion by the year 2030 (FAO 2008). The population increase in developing countries constitutes 97% of the global increase (Swaminathan 1995). It is therefore, an intimidating task to feed the ever increasing population in the resource-poor countries where agriculture is already constrained by lack of new arable land, small sized farms, and certain destructive agricultural practices contributing to soil degradation, salinization and ultimately the desertification.

Agriculture is an essential component of societal well-being. It occupies 40% of the land surface, consumes 70% of global water resources and manages biodiversity at genetic, species and ecosystem levels. Intensive use of inorganic fertilizers and pesticides, expansion of irrigation, and capital-intensive farm management has resulted in an unparalleled increase in global agricultural productivity since 1950s. Agriculture, therefore, is and will continue to be central to all strategies for planned socio-economic development of the countries. Despite major advances in agriculture and strong growth in food production in the latter part of the twentieth century, food security for the masses continues to be an area of concern. The application of biotechnological techniques in the agriculture sector can potentially improve food security by raising crop tolerance to adverse weather and soil conditions, by enhancing adaptability of crops to different climates and by improving yields, pest resistance and nutrition, particularly of staple food crops. Biotechnology can, over the next two decades, deliver the next wave of technological change that can be as fundamental and invasive as that brought about by information technology.

Recently the World Food Program (WFP) and the Food and Agriculture Organization (FAO) -reported that 22 countries are experiencing protracted food insecurity, and 17 of these countries are in Africa. There has been a remarkable increase in total grain production between 1950 and 1980, but only a marginal increase has been realized during 1980–1990 (Myers 1999; Ozturk and Uzonur 2004). This increase in grain production has mostly 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 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 during next few decades.

In the present review we attempt to take a practical look at the prospects and constraints of various types of biotechnologies and their application for increasing crop production and improving nutritional quality. Under this context, we also address the important issues of biosafety and impact of the genetically engineered crops on the environment.

2 Biotechnology in Agriculture

The Convention on Biological Diversity (CBD) defines biotechnology as: “any technological application that uses biological systems, living organisms, or derivatives thereof, to make or modify products or processes for specific use”. This technology has been incorporated in every part of living system, from disease cure to crop improvement in which new traits are being either introduced or old ones modified to yield better system. Genetic modification of crops is one such method allowing

individual characters (gene, factor or trait) to be transferred into crop plants. With the advent of genetic modification through genetic engineering in early 1980s, the natural barrier of only intra-specific exchange of characters was removed and scientists were able to identify and transfer specific genes associated with desirable traits from one organism to the organism of other species that otherwise cannot breed naturally (Ozturk and Uzonur 2004; Shigeto et al. 2006). With these techniques genes from varied class of organisms like bacteria, virus or even animal may be transferred into plants to develop genetically modified plants having exclusively changed characteristics controlled by the specific gene. This gives scientists/ breeders a broader access to desirable traits from any living organism and its possibility of transferring it with much faster rate and greater precision. There are numerous biotechnological approaches through which we can develop effective changes in agricultural fields and yield better crops in terms of their productivity as well as quality. Future impacts of biotechnology in crop production will be in the areas of:

1. Developing new hybrid crops based on genetic male-sterility,
2. Exploit transgenic apomixes to fix hybrid vigour in inbred crops,
3. Increased resistance to insect pests, diseases, and abiotic stress factors,
4. Improved effectiveness of bio-control agents,
5. Enhance nutritional value (vitamin A and iron) of crops and post-harvest quality,
6. Increase efficiency of soil phosphorus and essential micronutrients uptake especially Zn uptake and its translocation in plants,
7. Increase the nitrogen fixation capacity of legumes,
8. Improve adaptation to soil salinity and heavy metal toxicity,
9. Understanding the nature of gene action and metabolic pathways,
10. Increase photosynthetic activity, sugar and starch production, and
11. Production of pharmaceuticals and vaccines in suitable pants (biofarming).

Further understanding the biochemical process(s) at molecular scales and changes in expression levels could be an enormous help with which biotechnology proves beneficial.

3 Fertilizer Usage and Biotechnology

Increase in agricultural productivity during green revolution is largely associated with the augmentation of fertilizers (particularly N-based fertilizers). It is estimated that around 50% of the human population relies on nitrogen (N) fertilizer for food production globally (Pathak et al. 2008). The N fertilizer consumption has grown dramatically in Asia, about 17-fold in the last 40 years (Dobermann and Cassman 2004). However it is surprising to know that only 50% or less of the applied nitrogen is used for producing the aboveground biomass of cereals. The other 50% or more gets dississipated in the wider environment by volatilization, leaching, surface runoff and denitrification (Jeffrey et al. 2002), resulting in the major detrimental impacts

on environment, such as eutrophication of fresh water and marine ecosystems (Beman et al. 2005), gaseous emission of oxides reacting with the stratospheric ozone, and the emission of toxic ammonia (Stulen et al. 1998) into the atmosphere. Despite these hazardous impacts on the biosphere the use of N fertilizers has increased 100-folds over the last 100 years, as it is known that the cereal yield and the fertilizer N consumption have increased in a linear fashion during the past 40 years and both are highly correlated (Ladha 2005). At this juncture, the use of nitrogenous fertilizers cannot be reduced because of the pressure of more and more food production to feed the teeming population. Development of crop varieties that can grow and yield well at low nitrogen condition can be a solution to the problem. Tolerance of a crop to low N conditions is a highly desired characteristic for sustainable crop production. Many approaches such as optimal time, rate, and methods of application for matching N supply with crop demand; the use of specially formulated forms of fertilizer, including those with urease and nitrification inhibitors; the integrated use of fertilizers, manures, and/or crop residues; and optimizing irrigation management have been suggested for increasing nitrogen use efficiency (Abrol et al. 1999; Abdin et al. 2005; Raghuram et al. 2006). But their adoption at the farm level has been limited for various reasons in developing countries. Progress has been made in genetic and molecular analysis of low N tolerance and breeding crops for low N conditions. To develop varieties with improved nitrogen use efficiency it is necessary to have high level of genetic diversity for N uptake efficiency. Lian et al. (2005) analyzed the genetic components associated with low N tolerance in rice at the seedling stage. So, through biotechnological approaches we can work out the molecular mechanism of nutrient assimilation in crop plants which will help us to manipulate the important genes (s) which may affect nutrient use efficiency and hence crop yield.

4 Engineering Plants with Other Gene Systems Regulating N Metabolism

Studies with transgenic plants overexpressing genes affecting the N metabolism pathway suggest it is possible to improve or manipulate N metabolism and the growth phenotype of plants, which can improve the nitrogen use efficiency (NEU) of crop plants. In order to identify and understand the regulation of the genes involved in enhancing NUE, proper evaluation of the combined genetic and transgenic approaches to improving NUE are needed as a component of any crop improvement program. The benefits of growing NUE-efficient crops will not be realized until breeders evaluate N metabolism and nitrogen use efficiency in economically important crop plants.

In higher plants, the expression of the NR genes is influenced by several external and endogenous factors and is highly regulated at the transcriptional as well as post-translational levels. The overexpression of either the NR or the NiR gene in plants increases mRNA levels and often affects N uptake. However, the increased uptake

of N does not seem to increase the yield or growth of plants, regardless of the N source (Andrews et al. 2004). This is believed to be due, in part, to the complex regulation of both NR and the pathway as a whole. Lea et al. (2006) demonstrated that post-translational regulation of NR strongly affects the levels of free amino acids, ammonium, and nitrate, whereas transcriptional regulation has only minor influence. Plants expressing fully unregulated NR accumulate high concentrations of asparagine and glutamine in leaves; however these transgenic plants grow and develop normally, despite having an NR enzyme that is active during both light and dark periods (Good et al. 2004; Shrawat and Good 2008). Mutants or transgenic plants with altered levels of GS/GOGAT are used to determine the effects of these proteins on plant development and to study the expression of the different members of the GS multigene family. Although several studies demonstrate that an increase in GS activity in transgenic plants has no effect on the phenotype, many researchers show a direct correlation between an enhanced GS activity in transgenic plants and an increase in biomass or yield, upon incorporating a novel *gs1* construct. Transgenic tobacco plants enriched or reduced in plastid glutamine synthetase (GS2, a key enzyme in photorespiration) have been developed (Kozaki and Takeba 1996). Those transgenic plants having twice the normal amount of GS2 had an improved capacity for photorespiration and an increased tolerance to high-intensity light, whereas those with a reduced amount of GS2 had a diminished capacity for photorespiration and were photo-inhibited more severely by high-intensity light compared with the control plants. Ectopic expression of GS1 has been shown to alter plant growth (Oliveira et al. 2002) and the over expression of GS1 in transgenic plants could cause the enhancement of photosynthetic rates, higher rates of photorespiration and enhanced resistance to water stress (El-Khatib et al. 2004). The overexpression of soybean cytosolic GS1 in the shoots of *Lotus corniculatus* was reported to accelerate plant development, leading to early senescence and premature flowering, particularly when plants were grown under conditions of high ammonium (Vincent et al. 1997). Man et al. (2005) provided additional empirical evidence for enhanced nitrogen-assimilation efficiency in GS1 transgenic lines. However, differences in the degree of ectopic GS1 expression have been reported and attributed to positional effects, effectiveness of chimeric constructs, or differences in growth conditions. These differences could account for the lack of correlation between the enhanced expression of GS1 and concomitant growth (Vincent et al. 1997; Ortega et al. 2001). Interestingly, the differences are more striking at a low nitrate concentration. In addition, higher rates of N incorporation into the transgenic plants further demonstrate that the transformed plants have increased NUE (Man et al. 2005).

In comparison to GS, few reports have described the production of transgenic plants overexpressing *GOGAT* genes. Transgenic overexpression and antisense technology have been employed recently to modulate the expression of NADH-GOGAT in alfalfa and rice plants (Schoenbeck et al. 2000; Yamaya et al. 2002). The studies on transgenic rice plants expressing antisense RNA for either GS1 or NADH – GOGAT point towards the possible involvement of GS1 in the export of N via phloem in senescing leaves. On the other hand, in case of developing leaf blades and spikelets, NADH-GOGAT was implicated in the utilization of glutamine transported

from senescing organs (Yamaya 2003). While these genes appear to be good candidates for improving NUE in the short run, the degree of improvement may vary with the crop and cropping conditions. Therefore, the utility of transgenic over-expression of N-assimilatory genes for major improvements of NUE remains uncertain, though the possibility that different crops respond differently cannot be ruled out yet.

5 Signalling and Regulation of Nitrogen Metabolism

It is a well known concept in signal transduction that whenever multiple genes are subject to transcriptional regulation by a common signal, it is mediated through a regulatory sequence that exists in all the genes that respond to the signal. These signature sequences, commonly known as response elements, are identified by mutations that abolish their function, and their conserved nature as revealed by homology comparisons. Early experiments in transgenic *Nicotiana* plants using GUS gene fused to NR and NiR promoter sequences clearly demonstrated for the first time that nitrate induction of gene expression requires some sequence(s) associated with the NR and NiR promoters (Rastogi et al. 1993; Quesada et al. 1997). Subsequent studies in transgenic tobacco incorporating the 5′ flanking regions of the two *Arabidopsis thaliana* nitrate reductase genes *NR1* and *NR2* (designated *NP1* and *NP2*) demonstrated that 238 and 330 bp of *NP1*and *NP2* respectively are sufficient for nitrate-dependent transcription (Lin et al. 2005; Lea et al. 2006). These nitrate-responsive elements (NREs) are composed of several copies of a core A[G/C]TCA sequence motif preceded by an ~7-bp AT-rich sequence present in the 5′flanking regions of nitrate reductase (*NR1* and *NR2*) genes. This particular sequence motif was also found to be very well conserved in the 5′ flanking regions of NR and NiR genes from eight other plants (Hwang et al. 1997). Sarkar (2003) compared the flanking sequences of all available plant nitrate responsive genes and found that the NRE core sequence (A[C/G]TCA) was present in multiple copies on both strands in all the known nitrate-responsive genes in many dicots, monocots and cyanobacteria. Though most of the NREs examined contained both the core sequence and a proceeding AT rich sequence, there were some cases which had GC rich regions or did not reveal any AT/GC bias. A more detailed bioinformatic analysis of the entire *Arabidopsis* genome revealed that the proposed NREs are randomly distributed, with no difference between nitrate responsive genes and the presumably nonresponsive genes and intergenic regions in the rest of the genome (Kang et al. 2004; Raghuram et al. 2006). These findings raise doubts on the validity of the proposed NRE as comprising of (A[C/G]TCA) elements preceded by AT-rich sequence. Further work in this area will need a combination of bioinformatic and experimental approaches to redefine the NREs that mediate the expression of all nitrate responsive genes in all plants. The discovery of NREs is important, as it provides an end point for nitrate signal transduction.

For the regulation of nitrate uptake, signals are derived from nitrate, which are involved in triggering widespread changes in gene expression; resulting in

reprogramming of N metabolism to facilitate the uptake and assimilation of nitrate and its incorporation into amino acids. The nitrate assimilatory pathway is under tight regulation by the available nitrate and reduced N. In strawberry, increasing external nitrate concentration from 0 to 4 mM markedly increased the cumulative nitrate uptake (Taghavi and Babalar 2007). Several of the LATS- and HATS-related genes, apart from being root specific, are also inducible by nitrate and there is evidence that at least one HATS-related gene, *NpNrt2:1* is also repressible by reduced nitrogen (Quesada et al. 1997). In barley and white spruce, cHATS provides a high affinity, low capacity pathway for nitrate entry in uninduced plants. Nevertheless, cHATS activity is up regulated (approximately three folds) by exposure to nitrate (Trueman et al. 1996). In barley, the fully induced iHATS flux was approximately 30 times higher than that resulting from the cHATS (Quesada et al. 1997). The increase in transcript is accompanied by increased rates of nitrate uptake (Imsande and Touraine 1994). The results on citrus seedlings suggest that LATS is under feedback control by the N status of plant. A decline in uptake rate by the addition of amino acids (Glu, Asp, Asn, Gln) to the external solution has been reported (Cerezo et al. 2000). The use of chemical inhibitors in physiological studies has suggested that protein synthesis is important for nitrate uptake (Aguera et al. 1990) and the transporters may turn over relatively slow. A degradation mechanism for transporter protein in *Arabidopsis* (*AtNrt2:1)* has been suggested (Cerezo et al. 2001). The presence of a number of conserved protein kinase C recognition motifs in the N and C domains of *HvNRT2:1* (Forde 2000) suggests that phosphorylation events are involved in regulating *AtNrt2:1* activity in response to environmental cues. Remans et al. (2006) found that under N-limited conditions, *AtNrt2:1* played a key role as a major NO_3^- uptake system and coordinated lateral root initiation and development with external NO_3^- availability.

The precise mechanism of nitrate sensing and signalling is not yet fully understood. Post-translational regulation of some of the nitrate-responsive enzymes is brought about by 14-3-3 proteins, though they mainly mediate the effect of light and other signals, rather than nitrate. A few elements possibly associated with nitrate signaling are Ca^{2+} and protein kinases/ phosphatases; these have been implicated in mediating the nitrate signal for the expression of NR, NiR and GS2 m RNAs (Sakakibara et al. 1997). In addition to the kinases, Hartwell et al. (1999) described a Ca^{2+} independent PEPCase protein kinase, which is a novel member of the Ca^{2+} calmodulin regulated group of protein kinases. Krapp et al. (2002) described their specific roles in mediating nitrate and other interacting signals. A better understanding of the nitrate-signaling cascade might emerge from the study of mutants related to the signal-transfer cascade from nitrate to the NR gene (Ogawa et al. 2000), revealing more intermediate and potential sites for the manipulation of NUE. Light is an additional signal that regulates the expression of many nitrate-responsive genes, though it has been studied in depth in only a few of them. The role of light in regulation of NR gene expression has often been reviewed (Raghuram and Sopory 1995; Lillo and Appenroth 2001). The effects of light in green plants are signalling and N-use efficiency probably mediated more indirectly, through photosynthesis and sugars (Lillo and Appenroth 2001). At the post-translational level, light acts by modulating the phosphorylation status of the enzyme, in conjunction with 14-3-3 proteins.

Transcriptional regulation of several hundreds of nitrate-responsive genes by nitrate as a signal requires cis-acting regulatory sequences or nitrate responsive elements (Raghuram et al. 2006). ANR1, a putative signalling and N-use efficiency transcription factor homologous to the MADS box family has been reported in *Arabidopsis thaliana* (Zhang and Forde 1998). ANR1 is nitrate inducible and root-specific, and has been shown to be involved in nitrate-dependent stimulation of lateral-root proliferation in transgenic plants (Forde 2002). However, this root-specific transcription factor does not account for the transcription of all the known nitrate responses even in the root, besides being irrelevant for nitrate-responsive gene expression in the shoots. In terms of finding a global target for manipulation of NUE, the successful manipulation of N content by overexpression of the Dof1 transcription factor indicates that unravelling the signalling mechanisms that bring about their coordinated expression of nitrate-responsive genes by N and C metabolites could reveal new targets and approaches for future metabolic-engineering efforts (Lochab et al. 2007). Yanagisawa et al. (2004) generated transgenic *Arabidopsis* lines over expressing Dof1, a maize protein that belongs to Dof family of plant-specific transcription factors known to activate the expression of several C-metabolizing genes associated with organic-acid metabolism. The transformants showed up to 30% higher levels of mRNA and enzyme activities for PEP carboxylase and pyruvate kinase, without any reduction of NR, GS, and GOGAT RNAs. If Dof1 is not nitrate inducible, it means that multiple transcription factors may be involved in the coordinated expression of N and C metabolizing genes.

6 Marker Assisted Selection of High Yielding Varieties

Marker-assisted selection and DNA fingerprinting allow a faster and much more targeted development of improved genotypes for all living species. They also provide new research methods which can assist in the conservation and characterization of biodiversity. The new techniques will enable scientists to recognize and target quantitative trait loci and thus increase the efficiency of breeding for some traditionally intractable agronomic problems such as drought resistance and improved root systems. A genetic marker is a measurable character with Mendelian inheritance. The advancement in DNA marker technology has facilitated in genome analysis and rapid development of many high-density linkage, physical and consensus maps in crops of interest (Gupta and Varshney 2000; Lörz and Wenzel 2005). The most commonly employed markers in crop plants are random amplified polymorphic DNA (RAPD), intersimple sequence repeat (ISSR) markers and amplified fragment length polymorphism (AFLP). These are the markers of choice for crops with inadequate genomic resources, do not require prior sequence information and scan the genome including the repetitive sequences. In fact, the RELP (restriction fragment length polymorphism) approach has been used successfully to identify genetic markers in plants, including rice (Tanksley et al. 1989: Wang et al. 1994). However, the RFLP technique needs specific probes for the target DNA sequences, and use of radioactive elements makes it more costly and tedious. The development

of PCR technique has offered a good alternative to the RFLP analysis. The PCR-based RAPD approach using single 10-mer arbitrary primers requires much less DNA, and is technically simple and cheaper compared to the RFLP (Williams et al. 1990). In maize, 42 pairs of proteins showed a 1:2:1 segregation in the F2 population indicative for a monogenic inheritance. Two linkage maps were constructed from RFLP and position-shift loci, which revealed that protein markers were interdispersed between the RFLP markers on all chromosomes (De Vienne et al. 1996). In many cases position-shift variants correspond to the same protein as shown by micro-sequencing (Touzet et al. 1995; Plomion et al. 1997). It is expected that the maps of expressed genes obtained by 2-Dimentional Electrophoresis (2-DE) will be crucial for the candidate gene strategy of quantitative trait loci (QTL) characterization (Thiellement et al. 1999). QTL analysis has been applied to map genes controlling protein quantity for spots on 2-D gels (Touzet et al. 1995) and the loci have been termed PQL for protein-quantity loci (Thiellement et al. 1999). Co-localization of a protein-quantity locus (PQL) and its protein-coding locus would indicate that expression level of the protein is a consequence of allelic differences, whereas co-localization between a PQL and a QTL for a different trait would point to an association of a candidate gene and the variation observed for a trait (Zivy and de Vienne 2000; Thiellement et al. 1999; Consoli et al. 2002; Lian et al. 2005). The level of molecular polymorphism in wheat has been found to be low as compared to many other species (Prasad et al. 2000; Song et al. 2002), thereby limiting studies on variability and diversity using molecular markers. Among different classes of molecular markers, simple sequence repeat (SSR) markers are short (1–6 bp long) tandemly repeated DNA sequences that are highly polymorphic and are useful for a variety of applications in molecular breeding due to its reproducibility, multi-allelic nature, co-dominant inheritance, abundance and high polymorphic information content (PIC), and thus have recently been used to study the genetic variability based on DNA polymorphism in a number of crop species (Morgante and Oliveri 1993; Powell et al. 1996; Gupta et al. 1996; Gupta and Varshney 2000; Prasad et al. 2000; Agarwal et al. 2008). It has also been demonstrated that even limited numbers of SSR markers were adequate to discriminate closely related wheat and barley varieties (Russel et al. 1997; Prasad et al. 2000). The genomes of all eukaryotes contain a class of sequences termed microsatellites (Litt and Luty 1989) or simple sequence repeats (SSRs) (Tautz et al. 1986). Microsattelite are short tandem repeat of 1–6 bp that can repeat up to 100 times (Schloetterer et al. 1991), have emerged as an important source of ubiquitous genetic markers for many eukaryotic genomes (Wang et al. 1994). In plants, it has been demonstrated that microsatellites are highly informative, locus-specific markers in many species (Liu et al. 1996; Moerchen et al. 1996; Smulders et al. 1997), because they are multiallelic, microsatellites have high potential for use in evolutionary studies (Schloetterer et al. 1991; Buchanan et al. 1994) and studies regarding genetic relationships. SSRs show a much higher level of polymorphisms and are informative in hexaploid bread wheat than any other marker system (Ma et al. 1996; Bryan et al. 1997). They can be utilized for several

applications in plant genetics mapping, cultivars discrimination and detection of genetic diversity (Gupta and Varshney 2000). SSRs provide an efficient means of detecting genetic diversity as they can detect high number of alleles per assay (Powell et al. 1996).

7 Development of Transgenic Crops

To improve both productivity and sustainability of agriculture, new crop varieties have been introduced by transgenic approach. Over the years, considerable progress has been made in developing transgenic plants in which agriculturally important crops have been improved upon by the incorporation of gene from similar or another species. Much emphasis has been give to develop insect-resistant transgenic crops as the world annual losses from plant pathogens alone are estimated to be 12% (Cook 2006). Transgenic insecticidal crop cultivars are in the process of revolutionizing agriculture and are likely to become a major insect management tactic worldwide. Introducing novel resistant genes into economically important crops can develop insect-resistant crops. This tactic has a potentially key role in integrated pest management of several important pests (Gatehouse and Gatehouse 1998). *Bacillus thuringiensis* (Bt)-, a soil-borne bacterium, based transgenic cultivars have been produced for Cotton (*Gossypium hirsutum*), Potato (*Solanum tuberosum*), maize (*Zea mays*), rice (*Oriza sativa*), tomato (*Lycoperscion esculentum*), alfalfa (*Medicago sativa*) and numerous other crop plants (Hilder and Boulter 1999). The *bar* gene conferring herbicide tolerance was introduced in 1-month-old wheat calli employing both particle bombardment and *Agrobacterium*-mediated transformation strategies (Chugh and Khurana 2003). Herbicide-tolerant soybean was the dominant transgenic crop grown commercially in the USA, Argentina, Canada, South Africa, Romania and Uruguay, occupying 33.3 million hectares in 2001. The current efforts to improve plant stress tolerance by gene transformation have resulted in important achievements. Present engineering strategies rely on the transfer of one or several genes that are either involved in signaling and regulatory pathways, or that encode enzymes present in pathways leading to the synthesis of functional and structural protectants, such as osmolytes and antioxidants, or that encode stress-tolerance-conferring proteins. Transgenic system in indica rice and wheat has been developed. Rice has been transformed with codA/COR47, AtHSP100 and PDC gene while wheat transformed with *hva1* to confer stress resistance. All the transgenics have been characterized at the molecular level for gene integration. Studies have been conducted to transform indica rice plants with *p5cs* gene to make it more tolerant to salinity. The assessment of transformed plants harboring this gene as a single copy showed promising results and further efforts are on to pyramid one more gene for higher tolerance to salinity.

8 Modern Biotechnological Tools and Crop Productivity

During the advent of post genomic era we came to know the newer branches of biotechnology which provides us new insights in understanding the different process(s) and mechanisms that ultimately leads to crop improvement.

8.1 Genomic Approach for Crop Improvement

Genomics research has provided breeders new tools, such as functional molecular markers and informatics, as well as new knowledge about statistics and inheritance phenomena that could increase the efficiency and precision of crop improvement. Currently an impressive number of advances in genetics and genomics have given us enhanced understanding of structural and functional aspects of plant genomes and basic knowledge have been integrated in such a way which can enhance the ability of plant breeders to improve crop plants for our benefit. In the last decade the whole genome sequencing of model plant *Arabidopsis thaliana*, rice, *Sorghum bicolor*, *Medicago truncatula*, *Musa* spp., grape, apple and recently draft sequence of wheat have become available (http://www.ncbi.nlm.nih.gov/genomes/).

The first complete plant genome to be sequenced was that of *Arabidopsis*. The sequenced regions cover 115.4 Mb of the 125-Mb genome and extend into centromeric regions. The genome contains 25,498 genes encoding proteins from 11,000 families (The Arabidopsis Genome Initiative, 2000). *Arabidopsis* contains many families of new proteins but also lacks several common protein families. The complete genome sequence provides the foundation for more comprehensive comparison of conserved processes in all eukaryotes, identifying a wide range of plant-specific gene functions and establishing rapid systematic methods of identifying genes for crop improvement (Tacchini et al. 1995; Varshney et al. 2009; Thakur and Varshney 2010).

Among the most important food crops, rice has the smallest genome (389 Mb) and wheat the largest (15,966 Mb). Arumuganathan and Earle (1991) have grouped other crops into seven classes: *Musa*, cowpea and yam (873 Mb); sorghum, bean, chickpea and pigeonpea (673–818 Mb); soybean (1,115 Mb); potato and sweet potato (1,597–1,862 Mb); maize, pearl millet and groundnut (2,352–2,813 Mb); pea and barley (4,397–5,361 Mb); and oat (11,315 Mb). Genome size is often correlated with plant growth and ecology. The diverse cellular and physiological effects of large genomes may be a function of selection of the major components that contribute to genome size such as transposable elements and gene duplication. The recent advances in genome sequencing, through the development of second generation sequencing technologies and beyond, provide opportunities to develop millions of novel markers, in non-model crop species, as well as identification of genes of agronomic importance. Identification of all genes within a species permits an understanding of how important agronomic traits are controlled, knowledge of

which can be directly translated into crop improvement (Chia and Ware 2011). This 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 (Pereira 2000).

The use of whole genome information and high-throughout tools has opened up a new field of research called functional genomics. Among its subdisciplines, transcriptomics (the complete set of transcripts produced in a cell) (Zimmerli and Somerville 2004), proteomics (the complete set of proteins produced in a cell) (Roberts 2002) and metabolomics (the complete set of metabolites expressed in a cell) (Stitt and Fernie 2003) have been used by the plant science community.

Recent 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. These high-throughput or large-scale experimental methodologies combined with statistical and computational analysis (bioinformatics) will give the detailed information about specific gene/genes function linked to specific characters required for crop improvement. These studies will be augmented by more specific investigations based on gene suppression, co-suppression or anti-sensing of a defined sequence (Jain and Barr 2010). 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 (Varshney et al. 2011). These new information provided by all the omics disciplines will lead the plant science community to *in silico* simulations of plant growth, development and response to environmental change.

The recent addition of the high quality draft genome of the soybean in the rapidly growing list of crops will not only help the breeders to improve soybean varieties in terms of protein and oil content but will also help to improve nitrogen fixation capacity of many important legumes used for human and animal nutrition. Schmutz et al. 2010 report that the 1.1-gigabase soybean genome-the largest shotgunsequenced plant genome-is predicted to encode 46,000 genes. Two genome duplication events are likely to account for the observation that ~75% of these genes are found in multiple copies. Although the importance of soybean as a source of protein and oil alone testifies to the potential implications of understanding its genetic makeup, this genome will also serve as the reference for ~20,000 leguminous species that play a critical ecological role through their unique ability to fix nitrogen with the help of rhizobial bacteria. Availability of the genome should accelerate the association of quantitative trait loci of nutritional, economic and ecologically important traits with the causal DNA sequences from soybean in the near future. In the longer term, the genome will likely also be leveraged to improve the way in which a range of leguminous subsistence crops are used to both replenish soil nitrogen through crop rotation and meet the expanding needs of developing nations for protein and energy.

Crop improvement can also be carried out by engineering novel RNA interference (RNAi) pathways that create small RNA molecules to alter gene expression in crops and can generate new crop quality traits or provide protection against insects, nematodes and pathogens without introducing new proteins into food and feed products (Auer and Frederick 2009). Although miRNAs are relatively small, they play an important role in gene expression. miRNA is considered as one of the most important post-transcriptional gene regulators (Carrington and Ambros 2003) since it was originally recognized in 2001 (Lagos-Quintana et al. 2001; Lau et al. 2001; Lee and Ambros 2001).

Recent studies have revealed powerful and unexpected roles for small interference RNAs (siRNAs) and microRNAs (miRNAs) in the control of plant growth by the silencing of native genes. Although both of these have pivotal roles in gene silencing, the actions of miRNAs are more extensive and remarkable. Several experiments have demonstrated that many miRNAs regulate almost every aspect of plant growth and development, including leaf morphogenesis and polarity, floral differentiation and development, root initiation and development, vascular development, and the transition from vegetative growth to reproductive growth (Jones-Rhoades et al. 2006; Chuck et al. 2009). Even more intriguingly, it has been discovered that miRNAs play a role in hormone signal transduction (Liu and Chen 2009), the response to environmental stress, and pathogen invasion (Chen et al. 2004; Sunkar et al. 2006). These regulatory miRNAs stimulated the idea of developing artificial miRNAs that can silence specific gene(s). Such targeted gene silencing could permit the direct molecular modulation of plant traits, which could in turn be applied to the breeding of crop species.

8.2 Proteomics and Crop Improvement

Proteomics is the fascinating field of research that attracts the thousands of scientific workers over the globe for a wide range of subjects (Tilleman et al. 2005; Bona et al. 2007). The term "Proteome" (PROTEins expressed by genOME) therefore is expected to represent a comprehensive survey of all proteins expressed at a given time, in given conditions. Moreover the expression levels in protein strongly depend on complex regulatory systems; unlike genome, the proteome is highly dynamic (similar to transcriptome). Proteomics is one of the fastest growing areas of biological research. At present, proteomic research aims both identifying new proteins in relation to their function and in unraveling how their expression is controlled within regulatory networks. In the past few years tremendous progress has been made in the field of crop plant proteomics. With the high advancement in technologies and latest bioinformatic tools, studying the plant proteome and its dynamic nature has become comparatively easy and very accurate. Plant physiologists now feel comfortable over this development as many unsolved mysteries about various physiological processes in the plants will now become easy to work out. From seed germination to fruit development/grain filling proteomics research is accelerating

by leaps and bounds throughout the globe. Expression of proteins regulating the processes of nutritional as well as hormonal balances has been providing the new insights towards understanding various metabolic processes fully. Plants like other organisms are always under the threat of various stresses. A remarkable achievement has been made in this direction by proteomicists by discovering the functions of various proteins and working out their expression levels.

All living organisms rely on the uptake of nutrients from the environment to sustain energy, metabolism and growth. They have, therefore, evolved numerous alternative programs to adapt to their permanently changing environment. Such programs involve instantaneous responses (changes in intracellular metabolites, activation/inhibition of enzymes by effectors and of proteins through post-translational modifications) as well as slower processes that affect the levels of macromolecules (transcription, translation, mRNA and protein degradation). The availability of complete genome sequences and technologies that allow comprehensive analysis of global mRNA profiles has greatly expanded the ability to monitor the transcriptional reprogramming of cells in response to their environment. However, further studies (often conducted with yeast) indicate that transcripts are imperfect indicators of protein levels and of *in vivo* fluxes (ter Schure et al. 2000; Griffin et al. 2002; Washburn et al. 2003; Daran-Lapujade et al. 2004; Wek et al. 2004; Kolkman et al. 2006), and therefore it brings limited understanding on whole biological systems. The implementation of sensitive and rapid methods for protein identification and the continuous technical improvement of the so far largely descriptive analysis of protein patterns by two-dimensional gel electrophoresis (2-DE) have transformed the combination of both techniques into a powerful tool for functional analysis now also more and more used in plant studies. Proteomics is proving an indispensable tool for examining alterations in the protein profile caused due to gene mutations, introduction or silencing of genes or in response to various stress stimuli in a relatively fast, sensitive and reproducible way. This science is becoming important for generation of information on physiological (e.g. regulatory behaviour and function), biochemical (e.g. metabolic and structural data), genetic (e.g. gene mapping and assigning of the structural gene to the 2D gel map) and architectural (e.g. location of the proteins in the cell) aspects. Proteomics-based approach is proving important for characterization of individuals or lines, estimation of genetic variability within and between different populations, establishment of genetic distances to be used in phylogenetic studies and characterization of mutants with localization of genes encoding revealed proteins (Thiellement et al. 1999). It is becoming a necessity in plant biology for deciphering the function and the role of genes in the on-going plant genome sequencing projects.

Analysis of the *Arabidopsis pasticcino* mutants by 2-DE revealed a considerable percentage of variable spots relative to wild-type controls; evaluation of responses to different hormone treatments indicated that the mutants were affected in cytokinin responses (Faure et al. 1998). A mutant was also used to study cytokinin effects on chloroplast division in the moss *Physcomitrella* at the protein level (Kasten et al. 1997). Comparison of protein patterns in leaves of the late flowering *Arabidopsis* mutant *fy* and wild-type demonstrated qualitative differences. Studies have also

demonstrated the capacity of 2-DE to document genetic variability and distinguish between lines and varieties, *e.g.*, when analyzing barley seed and malt (Ostergaard et al. 2002) or wheat grains (Skylas et al. 2005). Positional shifts of proteins were observed in 2-D gel analysis of segregating families of maize, barley, pea, and maritime pine (De Vienne et al. 1996).

Application of proteomics can enormously boost up agricultural production (Dhand 2000; Cánovas et al. 2004; Xu et al. 2006). It is the most promising technique to identify proteins that are induced, repressed, or post-transcriptionally modified during a developmental process as complex as senescence.

8.3 Nanobiotechnology and Crop Improvement

The term 'Nanobiotechnology' was used for the first time by Lynn W. Jelinski (a biophysicist at Cornell University, USA). It is an exciting and rapidly emerging technology allowing us to work, manipulate and create tools and materials at the molecular level that may be of great importance in our day-to-day life. Nature has been performing the "nanotechnological feats" for millions of years. This important technology has the potential to revolutionize the agricultural and food industry with new tools by enhancing the ability of plants to absorb nutrients etc. Smart sensors and smart delivery systems have a great potential to help the agricultural industry by combating viruses and other crop pathogens. Key advances have been made in the ability to make measurements at the sub-cellular level and in understanding the cell as a highly organized, self-repairing, self-replicating, information-rich molecular machine. Single-molecule measurements are shedding light on the dynamics and mechanistic properties of molecular biomachines, allowing the direct investigation of molecular motors, enzyme reactions, protein dynamics, DNA transcription and cell signaling. It has also been possible to measure the chemical composition within a single cell. Nanobiotechnological research and development is likely to facilitate and frame the next stage of development of genetically modified crops, animal production inputs, chemical pesticides and precision farming techniques. While nanochemical pesticides are already in use, other applications are still in their early stages, and it may be many years before they are commercialized. These applications are largely intended to address some of the limitations and challenges facing large-scale, chemical and capital intensive farming systems. This includes the fine-tuning and more precise micro-management of soils; the more efficient and targeted use of inputs; new toxin formulations for pest control; new crop and animal traits; and the diversification and differentiation of farming practices and products within the context of large-scale and highly uniform systems of production. Nanobiotechnology can have momentous application in plant sciences too for inducting foreign DNA in the cells, an improvement over the transgenics as the gene would not be fully incorporated thereby preventing unintended gene flow into the environment. Nanobiotechnology has made inroads into uncovering fundamental biological processes, including self-assembly, cellular processes, and systems

biology. It can also be used to enhance photosynthesis and improve soil management. Removal of heavy metal contamination can be achieved through intense sensing for precision farming. It has also enabled the development of biochips and has a role in green manufacturing. Major applications are in the design of sensors, biofluidics for handling DNA and other molecules, nano-filtration, bioprocessing and traceability of genetically modified food. Nanobiotechnology is evolving as a powerful tool as a result of cross talk between nano scientists and biologists. By operating in the nanoscale realm, at the molecular level, nanotechnology offers a wide range of tools, techniques and applications. Nano biotechnology can stimulate new technologies for studies in cell biology using nano tools, provide opportunities for early detection of diseases through *in-vivo* and *in-vitro* analysis using nano sensing structures with extra ordinary multi-function capabilities and targeted drug delivery. Some of the areas with research priority are: nanoparticles for biosynthesis of nanoparticles, biological templates for nanoparticle assembly, bionano composites, imaging/sensing of nanoparticles/biomolecules, tissue engineering, cell-cell interactions, mammalian/microbial cell development, nano-biosensors with multiple sensing capabilities. The development of genetically encoded molecular sensors, which transduce an interaction of the target molecule with a recognition element into a macroscopic observable, via allosteric regulation of one or more reporter elements, may provide us a chance to understand many physiological processes fully. The recognition element may simply bind the target, bind and enzymatically convert the target, or may serve as a substrate for the target, as in the use of a specific target sequence in the construction of a protease sensor (Nagai and Miyawaki 2004). The most common reporter element is a sterically separated donor-acceptor fluorescence resonance energy transfer (FRET) pair of spectral variants of the green fluorescent protein (GFP; Fehr et al. 2002), although single fluorescent proteins (Doi and Yanagawa 1999) or enzymes (Guntas et al. 2005) are viable as well. Some molecular sensors additionally employ a conformational actuator (most commonly a peptide which binds to one conformational state of the recognition element) to magnify the allosteric effect upon and resulting output of the reporter element.

9 Conclusion

Biotechnology has remarkably affected all the aspects of human life. In agricultural fields too, it has and will surely solve the problems of food insecurity. Understanding the important process(s) of crop production and manipulating the key steps of grain/fruit development have become easier and accurate by this wonderful technology. Developing stress tolerant, high yielding and nutrient efficient crop varieties are some attributes associated with biotechnological tools. One of the tremendous challenges before the breeders/scientists is food insecurity which needs to be addressed timely so as to feed the teeming population. Agricultural lands are vanishing due to the various processes like desertification; salination etc and just overflow of nutrient

fertilizers in these fields could not increase crop production as normally crop plants are not nutrient use efficient. Modern biotechnological approaches viz., proteomics, nanobiotechnology etc could also help us to understand the important process(s) of crop plants, thereby improvement in their production.

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