Plant Biotechnology: Tool for Sustainable Agriculture

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Abstract

Agriculture has been the backbone of the human food supply directly and indirectly and global agricultural productivity must increase due to availability of limited agricultural land. Therefore, must increase in order to meet the increasing food demands. The agriculture was earlier practiced manually followed by modernization that allowed an increase in agricultural productivity. The cumulative recognition of biotechnology as an economic and social growth factor has stimulated countries to provide financial support to their local biotechnology companies to nurture research, development, and commercialization of ideas and products that have boosted biotechnological innovations and improvement in the quality and services. In this chapter the thrust will be laid on usefulness of plant biotechnology for increasing the diversity of genes and germplasm available for incorporation into crops and by significantly shortening the time required for the production of new cultivars, varieties and hybrids vis a vis contribution towards agricultural sustainability. In the last part of chapter, the conservation techniques for agriculture and sustainable development are documented with some case studies.

Keywords

Agriculture · Conservation · Genetechnology · Sustainability

1.1 Concept and Importance of Biotechnology in Agriculture

Any method that uses living organisms or products from these organisms to make or modify a product for increasing food production, and making agriculture more sustainable is called as agricultural biotechnology (FAO 2004; Hansson and Joelsson 2013). Genetic engineering technique is used to advance crop varieties that manage

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drought and salinity and disease resistant (Wang et al. 2003; Fuchs 2010) besides nutrients are taken more efficiently. And are desirable in a varying climatic conditions with increasing population and competitions for other land resources. The agricultural biotechnology is a contentious, and reviewers refer to the ecological and health risks complicated and to the negative effects of GM crops on small-scale traditional agricultural farming (Peters 2000). The biotechnology is contemplated eiher an important part of or a severe threat for sustainable agricultural production systems. However for assessing the implications of potential biotechnology sustainability it deals with the continuance of agricultural production systems over time and hardly anyone agreed-on description of agricultural sustainability instead, academic and policy related definitions vary (Dash et al. 2016).

The miscellany devoted to the concept of agricultural sustainability has steered authors to contend that it is fundamentally a challenged concept (Thompson 2010) and one such arena is concerns regarding the role of biotechnology in creating sustainable agricultural production systems (Connelly 2007). Further the increasing recognition of biotechnology as an economic and social growth factor has enthused governments in several countries to provide financial sustenance to their local biotechnology companies to foster research, development, and commercialization of ideas and products (Awais et al. 2010); But on the other side, predictions of malnutrition, famine, untreatable diseases and unresolved environmental problems have boosted biotechnological improvements and enhancement in the quality and services specified by various companies (Boccia and Sarnachiaro 2015) likewise production of GM crops designed at weed and pest control resulting in increased crop yields (Dash et al. 2016).

The efforts to alleviate poverty and inequality have established to be insufficient, mostly in rural and semi-urban areas notwithstanding the great biotechnological advances in agricultural production accomplished over the years. Contemplating the advances in technology, hunger has been translated into a preventable harm. In the past decade, consumer behavior concerning food products has been influenced by quality and safety (Boccia and Sarnachiaro 2015). Presently, highly processed foods with a high content of chemical products are not accepted among the population, and there is a clear tendency towards consuming fresh food. Biotechnology via genetic manipulation of crops, has offered a means to guarantee food production or crop nutritional improvement (Fernández-Suaréz 2009). However, GM crops have developed the center of debate due to the difference of opinions that arise around the aftermaths that the consumption of these types of food may have on human and environmental health. In this chapter, biotechnological improvements at different periods of history and their efforts have focused on trying to alleviate the shortage of food supply and associated malnutrition besides to discuss controversy around the application of various biotechnological tools for improving agricultural produce and production.

1.1.1 Biotechnological Advancement of Agriculture

Agriculture has been the mainstay of the human food supply via direct as well as indirect ways. Though, agricultural land is limited, due to the increasing world population consequently, global agricultural productivity must increase in order to meet the increasing food demands (FAO 2013). Agriculture is contemplated as one of the oldest activities experienced by humankind. The Industrial Revolution, which bridged from 1875 to 1885, permitted an accelerated economic development that became strategic for the growth of countries (FAO 2012), which in turn prompted the migration of people from rural areas to industrialized cities. The use of machinery on farms was imperative, progressing through remarkable improvements for increasing the agricultural production to meet consumer and producer needs, The overview of chemical fertilizers about the same period of time permitted crop protection against disease and attainment of higher yields.

Agriculture became a science by performing novel experiments aimed at improving agricultural methods in various developed countries (Cubero 1993), which eventually led to important innovations such as crop rotation and new discoveries such as the ability of some legumes to convert atmospheric N to NO_3 (Overton 1996). The earliest records of plant hybrids were given by Cotton Mather (1716) followed by development of plant hybridization in 1776. The Experiments on plant hybridization (Persley 1991), marked the beginning of new technologies intended to recuperate vegetable species. However, plant hybridization occurred long before Mendel's experiments; most likely it was unintentional at first and could have occurred at any stage in the crop domestication process (Adenle 2011). In circumstance, crosses for both floral morphology and hybrid vigor were greatly expanded by many researchers during the next 100-year (Teranishi 1978). In 1960, the new concept of Green Revolution for the rapid increase in food production, especially in underdeveloped and developing nations, via the introduction of high-yield crop varieties and the application of modern agricultural techniques changed agricultural farming (FAO 2004). The technologies developed during this period, which usually involve bioengineered seed that worked in conjunction with chemical fertilizers and heavy irrigation had an enormous influence on three main cereals (viz., maize, wheat and rice). Further in the 1960s, the discovery how the biological molecule of DNA was responsible for inheritance resulted into a predominantly imperative findings and the genetic code was cracked, and subsequent studies began the transfer of genetic material from one organism to another through genetic engineering techniques (Dash et al. 2016). And the intersection between genetic engineering and biotechnology remained the key factor in the creation of GMOs. International Maize and Wheat Improvement Center (CIMMYT), is one of the first agricultural research centers created in Mexico in the 1960s, with the help of the Rockefeller Foundation (FAO 2000). Today, wheat and maize produced from research at CIMMYT are planted in millions of hectares around the word. However, one constraint regarding

basic and applied agrarian research that is usually is carried out in advanced countries with stated climate conditions, and inventions and improvements in crop yield can only be acquired in similar conditions and paying way to adapt the new technologies and discoveries to warmer or more arid climates that prevail in underdeveloped countries.

The Itanca or soybean MON87701, was one of the first GE crops worldwide which articulated the crystal insecticide protein CytAc1derived from *Bacillus thuringiensis* (Bt toxin) that stipulated protection against feeding damage caused by some lepidopteran pests (EFSA 2011). Further, Monsanto introduced the GMO soybean MON89788, a crop expressing the cp4-epsps gene from the soil bacterium *Agrobacterium tumefaciens*, which encodes the EPSPS enzyme, providing resistance to the herbicide glyphosate. Monsanto subsequently engineered a stacked trait soybean using MON89788 x MON87701 (Then and Bauer-Panskus 2017), combining into one GMO the expression of the insecticidal Bt toxin, Cry1Ac, with resistance to glyphosate. GM soybean has made rapid advances in recent decades, and its cultivation area has been increasing yearly. The genetic modification has been efficacious in maize, tomato, rice and cotton plantations (Tabashnik et al. 2011), mainly by inserting exogenous genes that encode a protein that is toxic to specific pests.

It has been pragmatic that some microorganisms, including PGPR, fungi and cyanobacteria, have shown biofertilizer-like activities in the agricultural sector (Mahanty et al. 2016). The utilization of microbes as biofertilizers is currently being considered as an alternative to chemical fertilizers for crop production. Besides role of nanotechnology can boost agricultural production, considered as important tools in modern agriculture. Nanotechnology specifies new agrochemical agents and new delivery mechanisms to improve crop productivity, and it promises to reduce pesticide use through nano-formulations of agrochemicals. Nanotechnology also allows the application of nanosensors or nano-biosensors in crop protection (Bhupinder 2014).

1.1.2 Biotechnological Improvement of Food

Biotechnology has been employed in the food sector through the production of additives and ingredients as well as the improvement of more resourceful and less costly operations for the food production. In addition, biotechnological interventions have been focused on modifying or enhancing taste, aroma, shelf life, texture and nutritional value of food products, employing fermentation, enzyme technology, nanotechnology and molecular biology. Now it is observed that, technology would become a tool that would help solve the problems of the worldwide supply of food though it befell. However, it is convenient to note the great achievements that were carried out thanks to the discovery of microorganisms and the development of biological, biochemical and molecular techniques that allowed the progress of this branch of science.

Currently, the fermentation is nevertheless a very beneficial technique for food processing. Fermented food from different sources such as milk, cereal, fruits,

vegetables and meat have subsidized to the livelihood of large populous. For example, how lactic acid bacteria could accomplish the novel role of efficient cell factories for the production of functional biomolecules and food ingredients to enrich the quality of cereal-based beverages (Waters et al. 2015). As we know that enzymatic technology has been a gismo used for food biotechnology for optimizing and accelerating bioprocesses. A large number of enzymes are used in the food industry such as baking, juice processing, starch, dairy and other related industries, wherein enzymes play a crucial role as biocatalysts in the biotransformation process. Likewise in the bakery industry, proteases act on the protein of wheat flour, reducing the gluten elasticity and therefore reducing the shrinkage of dough (Dash et al. 2016).

1.1.3 Transgenic Food

Transgenic foods or genetically modified or engineered foods are produced/processed from organisms that have had changes introduced into their DNA using the methods of genetic engineering (Fernández-Suárez 2009; PALT 2014). Further the transgenic foods can include the following-

(a) Crops with genetic modification and can be edible e.g., Pest resistant corn crops.
(b) Food with an ingredient or an additive derived from a GMO.
(c) And those foods that uses a supplementary GMO product for their production, for example cheese made from recombinant chymosin obtained from a strain of the fungus *Aspergillus niger*.

Since it is well documented that GM foods are produced from GMOs and characteristically, GM foods are transgenic plant products. A tomato, called Flavr Savr, was the first commercial GM food, which was modified to ripen without softening by the Californian company Calgene (Bagwan et al. 2010). Baring from low price rates the production problems and competition for a conventionally bred variety with a longer shelf life prohibited the product from flattering profitable. Besides some disease preventive tomatos with three times more lycopene than conventional varieties has been developed (Awais et al. 2010). Genetic engineering plays a noteworthy role in enhancing proteins, vitamins as well as iron and zinc components by gene insertion. For example Golden Rice, variety of *Oryza sativa* was designed to produce β- carotene, a precursor of vitamin A, in rice (Bagwan et al. 2010). It was developed as a stimulated food to be used in areas with shortage of dietary vitamin A. thereafter one more new variety called Golden Rice 2 which produces up to 23 times more beta-carotene than the original version was developed. Bt corn is a variant of GM maize that expresses the bacterial Bt toxin, which is poisonous to the European corn borer. However, there are several foods from GM crops that are resistant to herbicides (glyphosate) and are resistant to insects (using Bt toxin), including crops such as soybean, canola, sweet corn and sugar beet

(Bagwan et al. 2010). Nonetheless, despite the distinctive biotechnological achievements, the consumption of transgenic food is still associated with their impacts on the Human environment and health (Boccia and Sarnacchiaro 2015).

The Biotechnology has played an imperative role in the progression of the health sector with numerous benefits to the human race. Biotechnological applications in health have taken advantage of the chemistry of living organisms for molecular biology or cell manipulation to develop new or alternative methods aimed at finding more effective ways of producing conventional products. The recombination of deoxyribonucleic acid (rDNA) is one of the common genetic engineering techniques used for the treatment and prevention of diseases. Similarly developing the transgenic food, which can also be valuable to human health, or agricultural biotechnology, which can produce more food to meet population demands. However the debatable point is regarding the distrust surrounding the production and consumption of GM foods. And the fact is due to uncertainty regarding the methods used in their development. GM food may have objectionable properties; transgenic seeds and plants may amend the microbial flora of the soil and could contaminate nearby crops (Bagwan et al. 2010) affecting the natural balance. Transferred genes may unenviably contaminate another organism, and organisms with mixed genes could arise between organisms that are evolutionarily distant, such as plants, animals, bacteria and even viruses (Chamas 2000).

In the economic sector, the use of GMOs in Europe has fortified agricultural biotechnology resulted in more prolific agriculture, increasing the incomes of farmers with a minor impact on the environment by reducing pesticide treatments (Zamora 2016). Transgenic Bt corn has saved 193 million euros in corn imports in Spain (Fundación Antama 2016). Further, 28 countries have planted 179.7 million ha of biotech crops; 20 of them are developing countries, and 8 of them are industrialized ones up to 2015. The United States is the largest producer, with 70.8 million ha, and Brazil is the top developing country, having planted 44.2 million ha of biotech crops (Fundación Antama 2016). Rural areas with developing economies absolutely stand to benefit from crops formed with biotechnology, whether by importing grain or seeds from foreign countries or developing their own GM crops (Delaney 2015).

1.2 Benefits of Genetic Technology in Agriculture

In the coming time, the recombinant DNA technology application to plant biology and crop production has the prospective impact on world agriculture. The general concept of agricultural technology is to isolate genes, direct their expression, monitor inheritance and re-insertion into plants. Plant biotechnology accompaniments plant breeding efforts by increasing the diversity of genes and germplasm accessible for incorporation into crops and shortening the time required for the production of new cultivars, varieties and hybrids as well. As for as economic perspective is concerned, the plant biotechnology offers noteworthy potential for the seed, agrochemical, food processing, and specialty chemical and pharmaceutical

industries to develop new products and manufacturing processes. Conceivably the most convincing attribute of the application of plant biotechnology to agriculture is its significance both to helping ensure the availability of environmentally sustainable supplies of safe, nutritious and affordable food for developed countries and to providing a readily accessible, economically viable technology for adopting primary food production needs in the developing world. The need for new agricultural technologies, in general, is driven by two distinct, and at times contradictory societal requirements confirming a safe, nutritious, and reasonable food supply for the planet and similarly minimizing the negative environmental impacts of food production itself. As per reports the world population will be doubling in the next 40 years up to 10 billion. The amalgamation of population increase, the decline in the availability of arable land, and the need for improvements in the quality of dietary intake in many developing countries means that agricultural production will have to be doubled or even tripled, on a per acre basis to meet this need. Simply put, farmers will have to produce more calories during the next 40 years than they have done in the entire history of agriculture.

Together, the societal concerns over the environmental impact of assured agricultural practices will increasingly check the types of tools used for crop production. There are some basic questions regarding the sustainable agricultural production regarding the increase the industrious competence of existing cultivated land without irrevocably damaging the planet. The answer is illusorily forthright investment in and development of new agricultural technologies is categorically critical for a future sustainable agriculture. Contemporary agricultural technologies such as plant breeding and agrochemical research and development will continue to play a chief role in promising a plentiful and safe food supply, ecologically sensitive and economic farm management practices. Plant biotechnology is exclusively important tool, which can significantly impact crop productivity and that is compatible with sustainable, environmentally sound agricultural practices. Besides it is a technique that is definitely the source of value-added genes and traits for increasing the farmer productivity and profitability. There are numerous technological applications for the improvement of productivity and production that is documented hereunder:

Plant genetic engineers currently have in hand a large battery of regulatory sequences that provide for both constitutive expression as well as highly accurate targeting of gene expression to specific tissues within transgenic plants (Benfey and Chua 1989). Moreover, established differential screening methods allow for ready isolation of regulatory sequences that may be required for even more sophisticated expression requirements (Shewmaker et al. 1994). The ability to decrease endogenous gene expression in plants represents a remarkably powerful tool, and striking phenotypic alterations have been observed by selective inactivation of genes using antisense technology (Oeller et al. 1991). Achieving even higher levels of gene expression in selected plant organs would increase opportunities for more economic specialty chemical or pharmaceutical production in plants, and site-specific insertion could minimize the variability of gene expression among transformants. However, current expression systems appear sufficient for meeting immediate crop improvement needs.

Extraordinary development has been made in the progress and application of gene technology transfer to crops. More than 80 species of crop plants have been genetically manipulated using available *Agrobacterium tumefaciens* or a variety of free DNA delivery transformation systems including all the major dicotyledonous crops and other monocotyledonous crops as well. Further it is likely that routine gene transfer systems will exist for nearly all crops within the years to come. It is obvious that technical developments will lead to further upsurges in transformation efficiency, extend transformation to elite profitable germplasm and lower transgenic plant production cost baring any significant barrier to the application of plant transformation technique in crop improvement.

Advancement in identification and isolation of new gene coding sequences are of unlimited significance to the improved engineered plants. The interspecies-specific use of transposons and T-DNA insertion has permitted the tagging and isolation of novel genes from several plant sources (Chuck et al. 1993). The availability of high resolution physical maps in Arabidopsis and tomato has previously led to mapping of several novel loci and new methods will allow direct testing of the isolated DNA for its ability to accompaniment the mutation of interest. Advances in the redesign of coding sequences for plant expression allow for foreseeable, high-level expression of a variety of non-plant genes in crop plants (Adang et al. 1993). An ongoing research efforts will certainly and severely increase the probability and efficiency of gene discovery and isolation, it would emerge that even with today's methods the most genes might be identified and isolated. The gene discovery will not be a limiting element for very long although only meager advancement in gene discovery and sequence analysis has been made.

It appears almost firm that plant biology is entering a unique period where both basic research and commercial applications will be limited only by the ingenuity of the researcher and by funding levels. There are no substantial technical hurdles remaining although there is an understandable need for extensive expansion of our considerate of basic plant biochemistry and physiology for exploiting scientific advances. The progress in the field has been exceedingly rapid, and genes conversing these new traits have already been successfully introduced into several significant crop species.

1.2.1 Insect Resistance

The plant production with natural insect control is clearly an important implication for crop improvement in seed and agrochemical industries. *Bacillus thuringiensis* (Bt) genes were frequently used in emerging of insect control in transgenic plants (Pigott and Ellar 2007). Most strains of Bt. are toxic to lepidopteran (caterpillar) larvae, though some strains with toxicity to coleopteran (beetle) or dipteran (fly) larvae. The mode of action of the B.t. insect control protein involves disruption of K^+ ion transport across brush border membranes of susceptible insects. Transgenic plants like tomato, tobacco, cotton and maize comprises Bt. gene (Koziel et al. 1993). A novel approach for increasing expression of Bt genes in plants, which

involves restructuring of the DNA coding sequence without altering the encoded amino acid sequence, has managed to significant enhancement in insect control (Sanahuja et al. 2011). Similarly the cotton plants with a high level of resistance to boll damage by caterpillars have been established (Perlak et al. 1990). In numerous field studies that confirm exceptional protection from bollworm, budworm and pink bollworms. Excellent protection from defoliation by Colorado potato beetle has also been observed in greenhouse and field experiments with potato plants containing the novel coleopteran active Bt tenebrionis gene (Perlak et al. 1993). The insect resistant plants sustained no damage from Colorado beetles through the growing season under circumstances of high insect pressure. Widespread efforts are under way to identify other microbial and plant insecticidal proteins for protection from insect pests. The genetically engineered plants express different proteinase inhibitor genes to enhance resistance to a range of insect pests (Boulter et al. 1989); *in vitro* studies indicate the α-amylase inhibitor protein has broad-spectrum insecticidal action (Huesing et al. 1991). It is well documented that introduced genes will provide an enormous percentage of insect control in annual crops in the next 2-3 decades.

1.2.2 Weed Control

The engineered plants provide an alternative approach to for crop protections to specific herbicides. The RandD efforts by private companies have so far focused only on those herbicides with minimal environmental impact, with emphasis on properties such as high unit activity, low toxicity and rapid biodegradation (CAST 1991). Further it was ensured that herbicide-tolerant genes would not be introduced into crops which could become ”volunteer” weeds in ensuing crop rotations or which outcross eagerly with weed species. The improvement of crop plants, which are tolerant to such herbicides, would offer more effective, less costly and more environmentally sound weed control options. There is two general methods followed in engineering herbicide tolerance: altering the level and sensitivity of the target enzyme for the herbicide and integrating a gene encoding enzyme, which can deactivate the herbicide. The tolerance to Roundup® herbicide has been engineered into soybean, cotton and maize by introducing genetic constructions for the overproduction of herbicide resistant EPSPS enzymes (Shah et al. 1986). Confrontation to gluphosinate, the active ingredient in Basta®, and bromoxynil has been accomplished by the alternate approach of introducing bacterial genes encoding enzymes that deactivate the herbicides by acetylation or nitrile hydrolysis (de Block et al. 1987; Stalker et al. 1988) respectively.

The existing crop targets for engineered herbicide tolerance comprise soybean, cotton, maize, rapeseed and sugarbeet. While choosing a particular weed control system for the farmers, many factors that is weed spectrum, herbicide performance, environmental impact, seed and chemical cost, application timing and flexibility have to be contemplated. The obtainability or accessibility of herbicide tolerance in annual crops over the next decade will give farmers more suppleness

in choosing effectual and less costly options for weed control. Herbicide-tolerant plants will have the constructive impact of shifting overall herbicide usage through replacement of more efficient and environmentally tolerable products. Such developments in chemical weed control will also allow for higher implementation of minimum tillage practices, and encourage crop rotations to further reduce soil erosion (CAST 1991).

1.2.3 Disease Resistance

Momentous resistance to a variety of plant viral diseases has been accomplished by coat protein-mediated protection involving expressing the coat protein gene of a particular virus in transgenic plants (Teh and Hofius 2014). The coat protein mediated cross protection process is likely to encompass interference with the un-coating of virus particles in cells prior to translation and replication. The results have been obtained satisfactory for transgenic tomato, alfalfa, tobacco, potato, melon and rice against a broad spectrum of plant viruses, including alfalfa mosaic virus, cucumber mosaic virus, potato virus X (PVX), potato virus Y (PVY) and potato leaf roll virus (Beachy et al. 1990). Outstanding tolerance has been perceived in field tests of Russet Burbank potatoes containing coat protein genes to both PVY and PVX (Kaniewski and Thomas 1993). Lately, very significant resistance to TMV in tobacco plants has also been achieved by an expression of a subgenomic viral replicase component (Schulze-Lefert and Panstruga 2011). Likewise the resistance to the bacterial pathogen *Pseudomonas syringae*, which causes wildfire in tobacco, has been introduced in transgenic tobacco by expressing a tabtoxin resistance gene that codes for an acetyltransferase (Anzai et al. 1989) that proves a successful method to engineering disease resistance in plants by detoxification of pathogenic toxins (Schweiger and Schwenkert 2014). Similarly the chitinase gene from *Serratia marcescens* was sturdily expressed in transgenic tobacco (Lee et al. 2016). The preliminary results indicated that the expression of the bacterial chitinase in transgenic tobacco leaves resulted in suggestively reduced sternness of disease produced by a brown-spot pathogen, *Alternaria longipes*. The plants were reported to have significantly reduced fungal lesions as well as delayed vulnerability to the pathogen. A bean chitinase gene driven by a high level, constitutive promoter has been articulated in tobacco plants (Swaminathan et al. 2016). These plants exhibit increased resistance to the pathogenic fungus *Rhizoctonia solani*, resulting in expressively reduced root damage and enhanced ability to survive in infested soil. Genes conferring fungal resistance based on the plant's own defense response are being cloned as one of these proteins, termed osmotin, has been revealed to have potent *in vitro* activity against *Phytophthora infestans*, the causal agent of late blight disease in potato (Swaminathan et al. 2016).

1.2.4 Stress Resistance

Many abiotic stresses involving water, temperature and soil composition are known to impact crop productivity. Although the complication of plant stress responses has eluded early validation of improved phenotypes using plant biotechnology methods, that are applied to dissect and comprehend the molecular basis for plant response. A number of plant genes induced by exposure to heat, cold, salt, heavy metals, phytohormones, nitrogen etc., have been acknowledged (Benfey and Chua 1989). Additionally, rapid progress is being made in identifying ion transport pumps and proteins, which regulate transport of molecules through channels and plasmodesmata. Further some metabolites like proline and betaines have been associated in stress tolerance in both bacteria and plants and to evaluate the potential of these metabolites to alleviate stress in engineered plants and comprehend their mode of action is under way (Van Camp et al. 1994).

1.3 Genetic Contributions Towards Agricultural Sustainability

Yield potential has not reached an asymptote even in the most extensively improved crops such as maize, wheat, rice, soya bean and cotton. The biological potential of these crops has continued to be increased by plant breeding systems aimed at increasing harvest index, water use efficiency, nutrient acquisition and genetic protection against biotic and abiotic challenges. Protection against pathogens and pests often enables a crop to continue to be produced in an area where the entry of a virulent pathogen or the evolution of a new strain of an endemic pathogen would otherwise have made production uneconomic (Frisvold and Reeves 2011). For example, rust devastated wheat yields in regions of Australia until breeders produced varieties resistant to the pathogen challenge. There are similar examples for all crops and their accompanying pathogens and pests.

Over the past 100 years most crop yields in agriculture have been increasing steadily. Agronomic management and plant improvement programmes have contributed significantly to these increases (Aerni 2010). In recent years, these two components of yield improvement have become more intimately intertwined with inbuilt genetic traits delivered in the seed being able to replace some management inputs, particularly in pest control. The gap between best farm yield and yield potential of the crop for a range of input regimes (Fedoroff 2010) have been closed because of improved management. In parallel, the average farm yields have approached best farm yields as a consequence of better extension services, accessible computer decision support tools and increased abilities of farmers to recognize and adopt best industry practice (Hess et al. 2013). In the most extensively improved crops such as maize, wheat, rice, soya bean and cotton, Yield potential has not reached an asymptote. Plant breeding systems aimed at increasing harvest index,

water use efficiency, nutrient acquisition and genetic protection against biotic and abiotic challenges have increased as a result of biological potential of these crops. Protection against pathogens and pests often enables a crop to continue to be produced in an area where the entry of a virulent pathogen or the evolution of a new strain of an endemic pathogen would otherwise have made production uneconomic (Frisvold and Reeves 2011).

Identifying sources of resistance genes or more correctly, resistance alleles in wild relatives of crop species and in the extensive germplasm collections available for most major crops that can act as gene donors through sexual reproductive methods (Varshney and Dubey 2009), plant breeders have been remarkably skilled. To access alleles from distantly related species genetic system manipulations and Embryo rescue are frequently needed. Rarely the introduced genes have been unrelated to the genes of the crop species and overall they are usually accommodated by the metabolic and cellular pathways already existing in the crop species (Varshney and Tuberosa 2007). By the introduction of single genes in different allelic forms, not all breeding goals have been met. In many cases, the breeder has had to cope with introducing alleles of several loci, often unlinked, which have products that interact to produce the desired phenotype. Breeders have also faced another hurdle of gene product interaction where the entry of the new allele produces the needed phenotype, but has pleiotropic, sometimes negative, effects on other traits (Araki and Ishii 2015). Polygenic inheritance with pleiotrophic have been dealt with by various strategies in the construction of breeding systems, mostly without any obvious understanding of what is happening at the molecular, cellular or tissue levels of plant function and in polygenic inheritance these complications have caused major obstacles in achieving breeding system objectives (Thompson 2011).

The power and efficiency of plant improvement programmes has increased as result of recent advances that have increased our understanding as how plants function and develop (Forster et al. 2015). The molecular and cellular mechanisms and pathways behind plant architecture, development and function, the regulation of gene expression and the knowledge of gene and genome sequences have provided new opportunities for breeders to rationally design improvement programmes providing for more homeostasis in the environmental responses of a crop and to better mould the phases and components of plant development within the constraints set by the crop environment. Owing to heterogeneity in field-based bioassays and the uncertainty of environmental pressures (Brown et al. 2014) breeders are confronted with difficulties in their selection programmes. DNA sequence has provided sequence markers for desired alleles and, in some cases, these enable a breeder to bypass bioassays and environmental assays that previously had introduced major constraints and unreliability into breeding programmes. Robust and stable phenotype for different genetic traits has also facilitated the stacking of duplicate systems of protection (Bado et al. 2015). Gene interactions and pleiotropic effects are now frequently understood at a molecular and cellular level with breeders being able to specifically avoid some of the negative interactions, e.g. selection for a subset of functions of certain transcription factors or selection for more specific phenotypes with a high level of understanding of the feedback loops in metabolic

pathways and their impacts on phenotype. The global population increase has demanded more powerful and more efficient plant breeding for our major food plants. More food needs to be produced but on the available land to full fill the needs of next 30–40 years. In fact, since a significant proportion of the lands on which we now have agricultural production is already marginal, with continued agriculture leading to increasing damage, then the challenge is to produce the required additional food on rather less land than we use now.

The way ecosystems are working in the world and the increase in the knowledge about this, our societies are demanding that our agricultural production systems work with empathy to the environment and not in opposition. The removal of nutrients from the soil is an inevitable process but these nutrients must be replaced at the same time, the agricultural production lands should not be seen to be damaging the adjacent non-agricultural production ecosystems. In addition the natural resources like soil, water and air should not be put under threat as a consequence of agricultural practices. If this approach is followed then we will surely approach sustainability needed for the food production systems of the world. Human nutrition has highlighted many food products and processes food products are less than optimal for human health. There are clear deficiencies in our food supply in both developed and developing countries as evidenced by major non-infectious and non-communicable diseases, such as heart disease and diabetes. When one major food commodity forms a major staple of the diet nutritional deficiencies are particularly evident in developing countries. In developing countries there is ample choice for food, the fault lies with both dietary habits and lifestyle choices. With the increased knowledge of human nutritional requirements and our increasing abilities to modify the characteristics of our food plants, we have a clear expectation that modern plant breeding should be able to enhance the nutritional qualities of our major food plants so that they approach the optimal composition for human health regardless of lifestyle.

The other main common objective that plant breeding can address includes the rising global energy needs and the reduction of petroleum-based resources. The renewable resources are thus gaining the greater attention across the world. In addition to the production of ethanol during fermentation of plant sugars, or methane production from waste plant products as well as diesel from plant oils, it is extremely apparent that the crops can also produce important pharmaceutical and other industrial products, which are having the benefit of sustainable supply chain that is based on agriculture. With the introduction of new technologies for genetic alteration and also the new levels of knowledge regarding plant genomes, the requirement of modification to our crop plants has been increased enormously. But there still exists a hitch to deal with such developments as many social communities of the world do not agree to accept the food and other goods that are produced from transgenic crops, or those crops whose few traits/characters have been developed by means of certain laboratory technology and not through the usual process of sexual reproduction. The majority of the countries across the world readily accept the products in medicine that are engineered genetically like genetically engineered human insulin as well as human growth hormone, as these products are proved to

improve the quality of life thus such products are used easily by the customers throughout the world. On the other hand, the genetically modified or transgenic crops receive a low level of acceptance may be due to downright fear and animosity. A large number of people also believe that these modified crops are only a means of success for big business and therefore does not provide any means to improve the quality of life of an individual. However, this view changed with the introduction of first generation Genetically modified crops that provided both the economical as well as environmental benefits to the society in general and the farmers in particular without posing the overwhelming impacts with respect to both ecological and health as these technologies deliver crops that produce better foods as well as improved drugs and vaccines that provide effortless mechanisms for delivery and thereby improving the health quality in developing countries of the world. The one main reason responsible for meager public image regarding the benefits of these genetically modified crops is the propaganda and the inaccurate information that is being provided by the media across the world. But now days, the scientists have gained a success by the use of new technologies for accomplishing a significant advancement in understanding about the development and functioning of plants. We have greatly enhanced the abilities to offer suitable genetic information regarding our main crops so that they can optimally execute in a wide range of environment and also present us with those food products that are personalized to nutritional necessities of humans. But as researchers we have been unsuccessful in effectively combating the campaign of inaccurate information that is being provided by different activist groups who for their personal benefits speak ill about the transgenic crops as well as about the whole food production systems.

1.3.1 Function and Regulation of Plant Genes- A Firm Foundation for the New Genetics in Crop Improvement

The controlled expressions of approximately 30,000 genes that are present in the whole genome of plants represent the different ways in which the plants can develop and thus respond to environmental conditions thereby producing the best possible yield of food and fibre. The role of genomics is to define the role of these genes, determination of how these genes are regulated and the interaction among the gene products. Thus, these findings can help in improvement of the crops. The genome of a dicot, *Arabidopsis thaliana*, which is related to canola and cabbage, and the genome of a monocot, like rice, had been entirely sequenced (Goff et al. 2002). However, the sequencing of large number of species including maize, lotus, *Medicago truncatula*, grape, poplar and tomato is in progress (www.ncbi.nlm.nih.gov/genomics). The sequenced genomes of both *Arabidopsis* in case of dicots and rice in case of monocots provide a basic reference for all crop plants. The genetic content of these two extensively different species is extremely related and it is believed that further sequencing of the genome will strengthen the resemblance of genetic makeup of all the flowering plants. The association of genetic content of the diverse species is not necessarily seen through the way in which these genes are

being regulated and by the association of their products in regulatory networks as these properties can vary noticeably. Thus in the differentiation of species, the distinction in patterns of gene expression plays an important role. The genes that signify the secondary metabolites or even the particular characteristic like structural property have been developed from a common gene pool. The opportunity that provides a variation to be acted on by means of natural selection is provided by gene duplication process as occurs in polyploidy and the attainment of separate roles and patterns of expression by individual duplicated genes (Adams and Wendel 2005). In order to know which genes are involved in identifying a particular character of a plant, the genomics plays a critical role. For identifying the function of a particular gene we need to compare its nucleotide or amino acid sequence with the sequences present in the databases derived from genomes of the other species. The function may perhaps be allocated through resemblance to other genes whose function is known, thus genomes help to identify the function as it can have utility across species or even across the kingdom. Genome-wide mutagenesis by means of transposable elements like Ac/Ds, Tos17, which is an endogenous retro-transposon of rice or T-DNA insertions, have resulted in production of populations of several lines, in which each line in a population contains an insert in a particular gene. As the sequence of the DNA of insert is well known, therefore it is easy to find out the gene cloning that has been disrupted by the flanking sequence. There is a freely available set of Arabidopsis lines containing inserts that are present in around 80% of the genes and similar proportion has been tagged in case of rice also (www.arabidopsis.org/abrc/ecker_frank.jsp) and (Hirochika et al. 2004). Such tagged lines could be made homozygous and we can determine their phenotypes in order to relate a gene with an exact phenotype. Thus the tagged genes can become candidates for crop development as markers of DNA or directly in transgenic breeding. Interlude of the gene activity may also be produced by RNAi, the supplementary type of mutagenesis, with the help of which a construct initiated into a plant gives rise to a double-stranded RNA that stimulates a degradation mechanism which is sequence-specific that interrupt the mRNA of the target gene that may produce a phenotype (Wang and Waterhouse 2002). For functional genomics the advantages of RNAi is that the RNA constructs that are targeted to a gene proceed in a dominant way, and thus we can target any gene present in the background such as a mutant background. Currently, different kinds of synthetic microRNA are used for gene silencing. Such type of microRNA plays an important role in controlling genes that are involved in development of plants and stress response so that the new technologies offer the supplementary choice for gene silencing. Since the microRNAs are smaller around 21 nucleotides in comparison to 200–300 bp that are typically targeted by RNAi, conserved areas in the gene families may be targeted, thereby silencing several genes at the same time (Alvarez et al. 2006; Schwab et al. 2006).

The fundamental feature of using genomics to improve crops is that there should be the capability to use high-throughput technologies to monitor the changes in the phenotype. However, the phenotyping involves the programmed growth measurements and imaging under different stress conditions of the environment. It also involves field-based screening like characters, which are valuable in glasshouse

and are sometimes not maintained in the field. Therefore, such a high-throughput approach can form the foundation for the large number of international consortia to describe alterations or silenced lines in the specific classes of *Arabidopsis* genes for example Agrikola, to categorize the utility of specific kind of transcription factor genes (Hilson et al. 2004). Gene array or microarray is the second type of resource that expands and complements the genome sequences and consists of different number of oligonucleotides or cDNAs that are displayed on the slides. In case of the sequenced genomes, all the predicted genes can be incorporated on the array. Then the array can be hybridized to RNA extracted from the tissue of particular origin or from the developmental stage of mutant or a plant that is subjected to environmental stress or any disease, in order to verify the genes that are expressed in comparison to expression in control wild-type plants. The fundamental principal for microarray is that if a particular gene is expressed in certain circumstances, it can play an important role in that specific condition for example the genes that are induced by salt offers protection in saline conditions. However, for those genomes that are not sequenced, anonymous cDNAs or expressed sequenced tags (ESTs) could be arrayed and hybridized in the same manner and thus afterward the candidate genes can be sequenced. Microarrays can be chosen on the basis of similarity in gene expression patterns when compared to known genes. Cost reduction in sequencing ha lead to the enhanced development of higher throughput sequencing of cDNA gene expression libraries indicating gene expression levels. In order to EST to its gene sequence, parallel signature strategies of sequencing are a better point of start for determination of gene expression levels in a gene present in the particular tissues etc. from which the libraries are made. Conserving biological processes and genes between the species can conserve expression patterns. Thus investigation of the Arabidopsis expression databases in which different microarray experiments have been gathered for public access (www.genevestigator.ethz.ch/) provided information about the functional role of a gene by comparing it with gene sequence from other plant (Baker et al. 2006).

After identification of a candidate gene from comparison of sequences and expression patterns i.e. increase or decrease in activity level can be used for confirmation of importance in a specific gene pathway. Gene specific Knockout and over expression lines can be passaged through a microarray experiment. Complete genome sequence availability allows the tilling arrays production in which not only the coding regions of the gene but all the genomic bases are arrayed in an overlapping manner. Tilling arrays allow assaying of both transcripts that are associated with coding region and those that are not associated with coding regions for example small regulatory RNAs that are known to play role in gene expression. Epigenetic mechanisms like methylation or histone modification of DNA determine the changes in gene expression by using whole genomic arrays. Further these epigenetic controls con be probed by immune-precipitation of protein bound to DNA which act as transcription factors or other regulatory proteins that allows in mapping regulatory regions in the genome and helps in identification of transcription factors that regulate different genes. In addition these arrays assist in rapid sequencing of related species, help in understanding the association between phenotypes and sequences and define

the gene structures and linkages conserved in evolution and speciation. Wu et al. (2006) carried studies on cotton in order to understand the genes that control the physiological processes and compared the gene expression at early stages of fibre initiation with those expressed by fibreless mutants. he concluded that genes play an important role in fibre initiation and quality by identifying the unexpressed genes in mutants. Differential expression of many genes has been demonstrated by monitoring the changes in changes in gene expression. These differential changes depend upon the paternal or maternal gene i.e. genes are imprinted. The imprinting of genes acts as an example of epigenetic regulation controlled by DNA architecture rather than sequence (Autran et al. 2005). In plants the developmental transitions like flowering, seed development and vernalisation are controlled by Polycomb group proteins (a repressive protein complex) that modify chromatin mediated through epigenetic control (Kohler and Grossniklaus 2002). Crop improvement can be achieved by decrease in gene activity called as gene silencing that is done using RNAi technology. This technology is now used widely used to achieve novel crop phenotypes. The sensitiveness of gene expression to developmental and environmental factors poses a challenge in controlling the expression of genes for plant improvement programmes. Changes in single gene expression can dramatically affect in multiple pathways due to complexity in gene interaction networks. Modeling of Interactions to understand gene regulatory networks is an emerging field in genomics. This approach will greatly enhance the ability to harness gene activity for plant improvement.

1.3.2 Improving the Essential Amino Acid Balance in Plant Proteins Used for Food and Feed

A large population and farm animals around the world depend on seeds for their dietary protein. However, the protein in seeds can have a skewed amino acid composition due to the high abundance of a limited number of individual seed storage proteins. Amino acids are classified as non-essential and essential. Animals are unable to synthesize essential amino acids and thus obtain these from the diet. Due to the insufficiency in amino acids, malnutrition can be caused and thus reducing the efficiency of animal production. In spite these deficiencies can be counterbalanced by combining two or more seeds, animal feeds are still added with synthetic amino acids for maximizing nutrient content (Habben and Larkins 1995). In developing countries, upto 90% of food uptake can be derived by balancing amino acids of individual seeds of a single species of crop. In recent years, mutation Plant breeding and modifications in genome has been successfully used in modifying composition of amino acids in cereals and legumes. This section is focused on modification of mainly grain legumes to improve their content of the essential, sulphur-containing amino acid, methionine. Three approaches have been used: genetic modification to increase methionine biosynthesis; genetic modification to increase methionine storage in protein; and selection of mutants with increased methionine.

1.3.3 Engineering the Methionine Biosynthetic Pathway in Plants

Sulphates taken from soil are reduced in plastids of plant cells and then incorporated an amino acid backbone derived from serine via the action of the enzyme serine acetyltransferase and producing cysteine. Cysteine is the first stable S- metabolite in the cell. It acts as substrate for other biochemical pathways. Sequential action of three enzymes viz; cystathionine γ-synthase (CGS), combines O-phosphohomoserine from the aspartate amino acid pathway and cysteine result in the synthesis of methionine (Leustek and Saito 1999). Various studies have been carried in the field of gene manupulated enzymatics of reductive sulphur assimilation and biosynthesis of amino acid (Amir and Tabe 2006). In genetically modified plants, leaves have an increase in methionine as well as cysteine, sometimes at specific growth stages. Protein bound amino acids are abundant in plants than free amino acids. However, minor effect has been observed on total methionine content due to gene manipulations. For example, constitutive expression of a CGS enzyme from A. thaliana in GM tobacco or GM alfalfa increased free methionine in the leaves but had no significant effect on protein-bound methionine (Hacham et al. 2002; Bagga et al. 2005). Contrary to this generalization large increase in both free and protein bound methionine in the leaves of GM tobacco have been obtained by expression of mutated form of CGS showing an abnormal phenotype (Hacham et al. 2002). In summary, in most studies, increasing flux through the methionine biosynthetic pathway seems to have produced little increase in the methionine content of endogenous plant protein. A natural maize mutant was identified by screening for germination on media containing lysine plus threonine, a combination that inhibits flux through the aspartate amino acid biosynthetic pathway, leading to methionine starvation. The mutant maize seeds showed high levels the sulphur-rich δ-zein, a methionine-rich seed storage protein. The further analysis of the mutant revealed a lesion in a post-transcriptional control mechanism that normally suppressed δ-zein transcript levels (Swarup et al. 1995). The same high-methionine phenotype was subsequently engineered in GM maize by mutation of the δ-zein gene to remove the target site for negative regulation by the *dzr1* locus. The modified maize had methionine levels theoretically high enough to obviate the need for synthetic methionine in animal feed formulations containing the GM seed (Lai and Messing 2002). Both genetic modifications and mutation breeding have been successfully used to improve the nutritionally important sulphur-containing amino acid methionine in these plants. In both cases, these modified plant products with improved seed storage protein composition will be screened for any change in allergenicity prior to commercial release, because many seed proteins are known to elicit allergic responses in some people (Mills et al. 2003). The intention of increasing methionine content, and thus the nutritive value, of plant protein is currently being achieved to a large extent and will continue to develop in future.

1.3.4 Starch Biosynthesis and Functionality

The paradox between the apparent structural simplicity of starch, and its synthetic complexity has fascinated researchers for several decades. The simplicity of the structure arises due to the fact that starch is composed of glucose monomer, that is linked to form the polymer through just two bond types, α-1,4 glycosidic and α-1,6 glycosidic. However, the different functional properties of starch arrise due to the heterogeneity of chain lengths and total molecular weight, heterogeneity in the placement and number of α-1,6 linkages. Further the complexity arise because starches are laid down in granules, and the control of granule size, number and structure adds a further layer through which functional properties are determined. The molecules within a given starch can be classified into amylose and amylopectin. Amylose, is a relatively linear α-1,4 glucan of relatively low molecular weight (degree of polymerization from 500 to 2000) and having less than 1% α-1,6 branch points, however amylopectin, is a highly branched molecule with a relatively high molecular weight (degree of polymerization 5000–50000). The initial committed step in the starch biosynthesis is the formation of ADP–glucose from gluose-1-phosphate and an ATP. This committed step is distinctive to starch biosynthesis, acting as important step for the regulation of flux to starch synthesis as compared with other metabolic needs. In the cereal grain, it has long been recognized that the enzyme catalysing the formation of ADP–glucose is ADP–glucose pyrophosphorylase. This enzyme regulates its activity at three levels. Firstly, the ADP–glucose pyrophosphorylase is present in both cytosolic and plastidic forms (Denyer et al. 1996). In the developing endosperm the majority of the enzyme flux is via the cytosolic form, while in chloroplasts the plastidic form dominates. Secondly, the enzyme is subject to redox control, apparently coordinating activity levels with photosynthetic flux. Thirdly, the enzyme is also subject to allosteric regulation, being activated by 3-phosphoglycerate and inhibited by inorganic phosphate (Ghosh and Preiss 1966). However, the mechanisms how these regulatory mechanisms interact to change the flux through the starch synthesis pathway are yet to be completely understood. The synthesis of amylose requires granule-bound starch synthase (GBSS), this enzyme is principally located within the starch granule. There are evidences that other enzymes also contribute to the amylose synthesis, but GBSS being absolutely required for its synthesis (Ball and Morell 2003). In endosperm, there are separate GBSS genes expressed than in other plant parts and hence providing basis for the differences in amylose content as well as structure between leaf and endosperm starches (Edwards et al. 2002).

The amylopectin synthesis is complex, involving a range of enzymes. Firstly, the enzymes for the elongation of amylopectin chains. Plants contain a family of starch synthases with differing substrate specificities, called isoforms that are responsible for the elongation of amylopectin. Genetic analysis suggests that these isoforms have differing roles in amylopectin synthesis. The synthesis of the short external

chains of amylopectin is thought to be carried by the enzyme Starch synthase (SS) I (Delvalle et al. 2005), whereas SSIIa is responsible for the synthesis of longer chains, from DP12–20. Elimination of this enzyme in barley (Morell et al. 2003), wheat (Yamamori et al. 2000) and rice (Umemoto et al. 2002) led to a very characteristic phenotype having reduced amylopectin external chain length, reduced granule gelatinization temperature and reduced starch swelling properties. The role of the enzyme SSIII is not yet clear but this enzyme, along with GBSS, is known to contribute to the synthesis of longer chains in amylopectin (Zhang et al. 2005). There are at least two other classes of starch synthase genes SSIIb and SSIV present in the genome of rice. Both the genes are primarily expressed in the leaves and the role they play is being defined currently. In monocots, there are three branching enzyme genes known so far, branching enzyme (BE) I, BEIIa and BEIIb. The mutation studies in a number of monocot species indicate that the effects of eliminating BEI activity in a normal background range from undetectable to extremely subtle (Regina et al. 2004). Moreover the effects of BEI mutations are only seen in a background lacking either BEIIa or BEIIb. Mutants in all the three genes in maize have been identified so far and also the double mutants have been constructed. The mutation of BEIIa gene indicates that there is no noticeable effect on the amylose content or the starch structure in the endosperm, however this mutation has indicated a remarkable effect on the leaf starch. The mutations in BEIIb have been already known to result in a phenotype with high-amylose content, in keeping with the observation that this is a major BEII isoform expressed in the endosperm. In wheat Regina et al. (2006) have demonstrated the expression of BEIIa and BEIIb, where BEIIa is more highly expressed than BEIIb and to get increased amylose content, suppression of BEIIa, rather than BEIIb, is important. A puzzle in the starch synthesis research is the role of debranching enzymes. Genomic studies in a wide range of plants have shown four debranching enzyme genes in the plant genome, among the sequenced genes three are isoamylase-like genes (isoamylases 1, 2 and 3) and one is pullulanase- (or limit dextrinase-) type gene (Morell and Myers 2005). Mutation studies in a number of species, including rice (Nakamura et al. 1996), maize (James et al. 1995), barley (Burton et al. 2002), *Arabidopsis* (Zeeman et al. 1998) and Chlamydomonas (Mouille et al. 1996), reveal that mutation in isoamylase 1 leads to a phenotype with low-starch and high-phytoglycogen. More recent data suggest that an identical phenotype is obtained when isoamylase 2 is mutated. This may be because it is suggested that isoamylase 1 and 2 form a complex and their function is abolished if either of the two is absent is absent. The role of isoamylase 3 still remains unclear. Pullulanase mutants have only a subtle direct phenotype (Dinges et al. 2003) but have major effects in an isoamylase 1-deficient background, indicating that there may be some functional overlap between the two debranching enzymes. The role played by these debranching enzymes in starch biosynthesis is yet not absolutely clear. According to one of the views isoamylases are directly involved in the starch biosynthesis, ‘editing’ the

emerging amylopectin molecule such that a crystallization-competent amylopectin is formed in the crystalline lamellae regions of the starch granule (Myers et al. 2000). This view is supported by observations that relate the level of activity of isoamylase in the developing endosperm to corresponding changes in branch point frequency and starch structure in starch granules (Kubo et al. 2004). Other views are that isoamylase plays a role in removing highly branched phytoglycogen from the amyloplast stroma (Zeeman et al. 1998) and disbranching enzymes are involved in starch granule initiation (Burton et al. 2002). Despite this large amount of information present on the starch synthesis pathway, there is still a lot to be discovered to completely understand the pathways. Bacteria contain glycogen and the synthesis of bacterial glycogen involves the same enzyme activities (ADP–glucose pyrophosphorylase, glycogen synthase, branching enzyme and a debranching enzyme) as higher plant starch synthesis, but a very different non-crystalline product is synthesized (Ball and Morell 2003). Studies on many green algae show that a complex set of starch synthesis isoforms is present in even the simplest green algae, indicating the high conservation of function of the various isoforms (Bellaloui et al. 2014). A cyanobacteria with semi-crystalline amylopectin has been interestingly, identified by Nakamura et al. (2006), with a reduction in isoform number. Here too the actual roles of individual isoforms, and their interactions, are yet to be dissected. The information on the events that lead to starch granule initiation is not clearly understood, and little understanding of the control of complex granule developmental processes as seen in wheat and barley starches. The recent research in developing cereal endosperm, on discovering and describing the presence of phosphorylation-dependent complexes of starch biosynthetic enzymes can unlock further secrets in starch biosynthesis (Tetlow et al. 2004a). Complexes between starch biosynthetic enzymes have also been found to act as carbohydrate chaperones' by having the potential to channel substrates to specific structural endpoints (Tetlow et al. 2004b). However, it is clear that further research is essentially required to determine exactly how the various levels of regulation, transcriptional, allosteric and post-translational, interact to control the structure of starch and starch granules. Only when this level of knowledge is achieved, the full potential for the rational design of starches with specific functionality will be possible (Morell and Myers 2005). The main sites of assimilation of sulphur in plants are assumed to be the photosynthetic source leaves. However, in developing soya bean seeds, it has been established that the pathway of reductive sulphur assimilation is active, and the biosynthesis of sulphur-containing amino acids occurs in the developing embryos in the grain legume, *Lupinusangusti folius*. Thus, in the developing seeds, sulphur assimilation itself appears to be the vital source of sulphur containing amino acids for legume seed storage protein synthesis. Recently, it was shown in developing lupin seeds that the manipulation of the cysteine biosynthetic pathway results into the large increases in the free cysteine, though free methionine and total sulphur containing amino acid levels were not increased.

1.3.5 Expression of Methionine-Rich Proteins in Genetically Modified (GM) Plants

For modifying the plant methionine content, the expression of an added gene for a methionine-rich protein or 'methionine sink' has proven to be a successful GM approach. This strategy has been mostly used to improve the amino acid balance of legume seed protein, which can contain less than half the methionine essential for optimal animal nutrition. Early efforts of increasing the methionine content in seeds by the transgenic expression of genes for endogenous storage proteins mutated to add extra methionine residues were unsuccessful (Hoffman et al. 1988). A better approach was the creation of a synthetic gene encoding an artificial protein rich in essential amino acids. Under the control of a seed-specific promoter, the expression of a synthetic protein, containing 31% lysine and 20% methionine residues in genetically modified tobacco seeds, increased the over-all methionine concentration by 30% in the mature seeds (Keeler et al. 1997). An analogous result in a grain legume would give substantial improvement in the nutritive value of the seed protein. The manipulation of the methionine sink has most commonly involved the transgenic expression of the naturally occurring, methionine-rich plant proteins. The S-rich proteins that have been expressed in GM dicots include 2S seed albumins from sunflower, sesame and Brazil nut proteins that contain up to 18% methionine residues (Tai et al. 1999a, b). This approach has mainly been applied to the grain legumes, because of their low-intrinsic methionine concentrations. However, the seeds of other species like canola and maize have also been modified, as a means to provide additional protein methionine in the animal feed formulations containing grain legumes. For example, the sulphur-rich zeins in maize, containing up to 28% methionine residues have been overexpressed in the genetically modified maize (Chui and Falco 1995). In a strategy to enhance the sulphur-containing amino acid content of seed protein in *Lupinusangusti folius*, the 2S seed albumin from sunflower was used. In the genetically modified lupins, the sunflower albumin was expressed under the control of a strong, seed-specific promoter from a pea vicilin gene, which resulted into increases of up to 100% in total seed methionine, when compared with the parental genotype. The availability of the additional methionine to rats and chickens was also verified (Ravindran et al. 2002). Importantly, the methionine also benefitted the sheep owing to the rumen stability of the added methionine-rich sink protein (White et al. 2001). The expression of the Brazil nut 2S albumin in a number of seeds like canola, tobacco, soya bean and narbon bean, has increased the total seed methionine by 30–100%, when compared with wild type (Tabe and Higgins 1998). In the GM narbon beans and soya beans, the seed methionine levels were predicted to be adequate for optimal animal nutrition. However, the commercial usage of the Brazil nut protein has been prevented owing to its potential human allergenicity.

In GM cereals, the expression of methionine-rich proteins has met with mixed success. In GM rice, an increase of up to 75% in the total seed methionine was reported by using sulphur-rich 2S albumin from sesame (Lee et al. 2003). In contrast, no significant increase in seed methionine was produced in GM rice with the

expression of the sunflower 2S albumin. In the latter case, endogenous seed protein composition changed in a way that resembled the well-characterized responses of seed proteins to plant sulphur nutritional stress (Hagan et al. 2003). While expressing the sunflower protein in the GM rice grain, the endogenous, sulphur-poor proteins were upregulated and the sulphur-rich proteins were down regulated. This reallocation of limited sulphur reserves within the developing rice grain resulted in mature GM grain with different protein composition, with almost same concentration of sulphur-containing amino acids as that of the parental genotype. It is yet not clear why under the control of similar seed-specific promoters, the expression of the two very alike 2S albumins in rice, should produce such contrasting results. However, there are a number of reports of compensatory changes in the endogenous pools of sulphur in GM seeds expressing added, sulphur-rich proteins. The individual kernels of GM maize, overexpressing a methionine-rich 10 kDa zein, showed reduced levels of a separate endogenous sulphur-rich 12 kDa zein (Anthony et al. 1997). Similarly, the endogenous sulphur-rich proteins in GM soya bean seeds were also under-represented that accumulated 2S protein in the Brazil nut (Jung et al. 1997). The GM lupins, expressing the sunflower albumin, had reduced levels of transcripts encoding endogenous sulphur-rich seed storage proteins (Tabe and Droux 2002). The GM lupins also contained a smaller amount of oxidized sulphur than the parental seeds grown in matched conditions. Likewise, the GM narbon beans, expressing the Brazil nut albumin, contained lesser endogenous pools of sulphur in the form of the tri-peptide γ-glutamyl-*S*-ethenyl-cysteine than the parental control seeds (Muntz et al. 1997). In the GM seeds, both non-protein and protein pools of sulphur were apparently arrayed to source methionine for the synthesis of the added sulphur sink protein. In summary, by plant genetic modification, it has assuredly been possible to increase total seed methionine, though the evidence specifies that, in numerous cases, rather than increased delivery of sulphur to the seeds, the reallocation of endogenous pools of sulphur have been involved. In few instances, the data suggest that the methionine enrichment has been achieved through increased rates of methionine biosynthesis in the developing seeds (Tabe and Droux 2002).

1.3.6 Combined Approaches

The manipulation of methionine biosynthesis in plants has greatly expanded the understanding of the regulation of flux through the pathway but, as a means of improving methionine content, this approach suffers from the lack of stable storage of the additional methionine. On the other hand, addition of genes for methionine-rich storage proteins has produced such GM seeds that, in some cases, are predicted to harbour enough S- amino acids to satisfy the growth requirements of both humans as well as animals. Yet, in other cases, the results indicate that methionine biosynthesis in the developing seeds became limiting; for example in lupins, whose starting concentration of methionine was very low (Tabe and Droux 2002). The clear answer of merging the addition of a sulphur sink with modification of the sulphur-containing amino acid biosynthetic pathway is the subject of the present

work. Some success has also been reported; for example, the expression of both the 2S albumin from the Brazil nut and a feedback-insensitive aspartate kinase have given additive increases to the total methionine in the seeds of the GM narbon beans, although most of the effect was apparently due to the Brazil nut protein (Demidov et al. 2003). It has been reported that co-expression of an *Arabidopsis* CGS enzyme with a sulphur-rich zein in the GM alfalfa leaves has increased the accumulation of the zein when compared with its expression alone in the GM alfalfa (Bagga et al. 2005).

1.3.7 High-Methionine Mutants

A number of plant mutants, with increased levels of methionine, have been isolated by the selection on ethionine – a toxic analogue of methionine. Using this method, total three distinct groups of mutated genes have been characterized in *A. thaliana*, and have been found to define three enzymes from the methionine and *S*-adenosylmethionine biosynthetic pathways (Shen et al. 2002). Recently, a soya bean mutant, with increased total methionine in its mature seeds, was isolated using an initial screen for ethionine resistance. The outcome of this work was a soya bean variant, that was predicted to supply enough methionine for ideal animal nutrition without demanding supplementation with synthetic amino acid (Imsande 2001). The mainstay of the many essential nutrients that support human life, health and well being are crops and live stock. The specific role of key nutrients in human nutrition, it is also becoming apparent that the supply of some nutrients is compromised and in some cases may not be sustainable into the future from current resources. The most notable of these potential shortfalls relate to the long chain polyunsaturated fatty acids (LC-PUFA) of the omega-3 (ω3) class, such as eicosapentaenoic acid (EPA, 20: 5Δ5,8,11,14,17) and docosahexaenoic acid (DHA, 22:6Δ4,7,10,13,16,19), that are found predominantly in fish and other seafood in Western-style diets that are low in seafood and have been associated with increased incidence of cardiovascular disease, cancer, stroke, diabetes, inflammatory disease, neuropsychiatric disorders and many other conditions prevalent in Western societies as a result od inadequate levels of EPA and DHA (Simopoulos 2003). Significant increases in consumption of fish and other seafood rich in EPA and DHA are regularly recommend by nutritionists and health authorities. However, it is now widely acknowledged that global fisheries are fully exploited, with many on the verge of collapse (Myers and Worm 2003), and they may be inadequate to sustain even current levels of fish consumption. To overcome the declining catch from wild fisheries, many aquaculture systems rely heavily on wild fisheries for feeds and are actually net consumers, not producers, of ω3 LC-PUFA. This situation means that existing marine-based sources of ω3 LC-PUFA are unlikely to be sufficient to sustain current levels and anticipated future increases in human needs. Genetic engineering technologies is now providing a solution to this dilemma through the development of transgenic plants equipped with the ability to synthesize ω3 LC-PUFA. Genes encoding transfer of EPA and DHA biosynthetic pathways from

marine microalgae and other microorganisms into agricultural crops, in particular oilseed crops is achieving this. The ability to of higher synthesize the main C18-PUFA, linoleic acid (LA, 18:2Δ9,12) and α-linolenic acid (ALA, 18:3Δ9,12,15), and some can also synthesize γ-linolenic acid (GLA, 18:3Δ6,9,12) and stearidonic acid (SDA, 18:4Δ6,9,12,15) is the characteristic feature of higher plants but higher plants are unable to further elongate and desaturate these ω3 C18-PUFA to produce ω3 LC-PUFA that are characteristic of the marine microalgae that are the ultimate source of EPA and DHA found in fishes. introduction of genes encoding all of the biosynthetic enzymes required to convert ALA into EPA and DHA is therefore required to synthesis of ω3 LC-PUFA in higher plants. Substantial parallel gene discovery efforts conducted over the last 10 years in a range of LC-PUFA-synthesizing organisms have resulted in the cloning of genes for all of the fatty acid desaturase and elongase enzymes involved in the aerobic pathway for LC-PUFA synthesis (Sayanova and Napier 2004). It is probable that additional or alternative metabolic manipulations will be required in order to achieve significantly higher levels of DHA synthesis and accumulation in transgenic seed oils. However, it is now clearly apparent that seeds can be engineered to produce the range of ω3 LC-PUFA required in the human diet and potentially in concentrations that should be nutritionally effective. To overcome the inadequate and potentially unsustainable supply from traditional marine sources, Crop plants engineered in this way will ultimately provide the affordable, renewable and sustainable sources of ω3 LC-PUFA.

1.3.8 Sustainable Industrial Raw Materials Supply

To achieve a sustainable increase in the supply of nutritional oils, genetic manipulation of fatty acid metabolic pathways in plants can also open the way for a more sustainable supply of industrial raw materials, by enabling these to be sourced from renewable plant resources rather than from increasingly scarce and non-renewable petroleum. Pessimistic supply forecasts have driven a considerable expansion in the use of plant-based fuels, such as ethanol and bio-diesel, as commodity scale alternatives to conventional fuels as Escalation in the price of petroleum. The recent persistent escalation in the price of petroleum and predominantly pessimistic supply forecasts have driven a considerable expansion in the use of plant-based fuels, such as ethanol and bio-diesel, as commodity scale alternatives to conventional fuels. It is anticipated that in the future other higher-value specialty industrial products currently produced by the petrochemical industry will be produced on a renewable basis from oleochemical sources, predominantly from plants producing specific molecular structures required as starting materials for advanced chemicals and polymers. These products will be generated by metabolic engineering of plant biosynthetic pathways either by redirecting pathways towards the accumulation of current intermediate compounds, such as in the production of lauric acid (C12:0) in rapeseed (Voelker et al. 1996), In this regard, the engineering of fatty acid metabolic pathways in oilseeds is likely to be a particularly fruitful area. The major oilseeds

are very restricted in the range of fatty acids that they contain usually only five (palmitic, stearic, oleic, linoleic and linolenic) have been selected and bred mainly for food purposes. There is an enormous diversity of fatty acid structures (Badami and Patil 1981), including much functionality such as hydroxylation, epoxidation, acetylenation and conjugation that impart properties required for specific industrial uses. The enzymes responsible for these functionalities has been enabled due to gene technology to be cloned from various sources and expressed transgenically in oil accumulating crop species in order to develop novel industrial oils. Most attention has been focused on C18 fatty acids that are modified at the Δ12 position by the addition of epoxy or hydroxy groups, or by the formation of triple bonds (acetylenic) or conjugated double bonds. FAD2-like genes encoding Δ12 epoxygenases, hydroxylases, acetylenases and conjugases have all been cloned (several years ago) and recently reviewed (Jaworski and Cahoon 2003). In Arabidopsis Transgenic expression of these divergent FAD2 genes and other oil-accumulating seeds has generally resulted in synthesis of the Δ12-modifed fatty acid, but in disappointingly low concentrations (less than 10% of oil), even though the modified fatty acids are present at very high concentrations in the source plants (60–90%). However, in each case, the level of vernolic acid synthesis was initially low regardless of whether the Δ12-epoxygenase was a divergent FAD2 type such as from Crepis palaestina or a cytochrome P450 type such as from Euphorbia lagascae. It has subsequently been demonstrated that the level of vernolic acid synthesized in Arabidopsis seeds expressing the Crepis palaestina FAD2-like Δ12-epoxygenase can be enhanced from initial levels of approximately 6% (Singh et al. 2000a, b) to approximately 20% of total fatty acids (Zhou et al. 2006) by increasing the availability of linoleic acid substrate. This was achieved by co-expressing the Δ12-epoxygenase with additional Δ12-desaturase genes in a mutant Arabidopsis genotype lacking the fatty acid elongase (FAE1) and Δ15-desaturase (FAD3) enzymes that would otherwise compete for substrates involved in synthesis of Δ12-epoxy fatty acids.

1.3.9 Discovery and Usage of Genes for Improved Disease Resistance in Crop Plants

The use of disease-resistant crop cultivars provides an effective method of controlling a large number of diseases. However, continuous breeding efforts are required to counter evolution or migration of new pathogen strains. One stumbling block continues to be the lack of agreement regionally between breeders as to the most effective deployment of valuable R genes to prevent their stepwise erosion by pathogen evolution. Plant molecular biology is and will make increasing contributions to resistance breeding by making resistance breeding more effective and more efficient, especially through the use of markers for breeding and providing resistance genotypes for varieties to improve decision making about their deployment.

1.3.9.1 DNA Markers for Breeding

Our efforts have been mainly targeted at rust, nematode diseases of cereals and barley yellow dwarf virus, and molecular markers have been developed for improved breeding efficiency. The Cre1and Cre3 genes currently provide effective genetic resistance in wheat for cereal cyst nematodes. Breeding new resistant varieties has, however, been hindered by the slow and laborious nature of the plant bioassay for nematode resistance. DNA markers have now been identified for both resistance genes based on cloned genes of the nucleotide-binding site–leucine rich repeat disease resistance gene class (de Majnik et al. 2003). These genes co-segregate with the *Cre1* and *Cre3* resistance genes and although there is no direct evidence to indicate that the cloned genes themselves control nematode resistance, they have provided excellent sources for development of simple, rapid and accurate PCR-based markers that are currently being used by wheat breeders. Wheat breeding has relied heavily on genetic resistance to rust disease to control stem, stripe and leaf rust. Breeding efforts have been particularly successful for stem rust using major genes for resistance and DNA markers for resistance are being increasingly used. In areas where stem rust resistance has been a major breeding objective, success has been achieved mainly by using varieties carrying several different stem rust resistance genes, diversity of resistance genotypes and discouragement of the cultivation of susceptible varieties. DNA markers are now being used increasingly for these breeding efforts. DNA markers need to be simple to use and also applicable to as wide a range of breeders germplasm as possible. For example, while some markers can be useful for genetic mapping of resistance genes in particular crosses, they are frequently not useful in all breeder lines where they fail to detect polymorphisms between resistance gene donors and susceptible recurrent parents. Consequently, there can be a long development stage between marker identification and application that involves fine-tuning to produce a robust marker across a range of useful genotypes. Many wheat varieties carry the durable stem rust resistance gene *Sr2* that is effective in providing partial resistance against all strains of stem rust at the adult stage of growth. PCR-based DNA markers have now been developed for marker-assisted breeding using the *Sr2* gene (Spielmeyer et al. 2003), and have provided an entry point to finely map this gene for future molecular cloning (Kota et al. 2006) with the aim of understanding the molecular basis of an adult plant, durable, non-strain-specific resistance gene. Several other stem rust resistance gene markers have been developed and are described below. Good progress is being made in developing a PCR-based marker for the durable adult plant leaf and stripe rust gene pair *Lr34–Yr18*.

1.3.9.2 DNA Markers Useful for Gene Stacking

Pyramids or gene stacks of multiple stem rust resistance genes in a single variety can provide durable resistance. Traditionally, R gene pyramids are achieved using sequential bioassays with rust strains capable of differentiating those different resistance genes. This becomes more difficult for breeders if each of the genes used provide resistance to all available pathogen strains. This is where DNA markers will

make a big contribution to providing simple tests for the presence of specific R genes. For stem rust, markers for *Sr38*, *Sr24*, *Sr26*, *SrR* and *Sr31* have now been developed (Mago et al. 2005a, b). The latter four genes provide resistance to all stem rust strains currently found in Australia and the markers for *Sr24* and *Sr26* that provide resistance to the proliferating strain Ug99 now found in Africa will have global applications.

1.3.9.3 DNA Markers for 'Value Adding' to Alien Resistance Sources

Many of the currently effective stem rust resistance genes are derived from wheat relatives and many have negative dough characteristics that are physically linked to the same chromosome region as the resistance genes. They are consequently not suitable for use in high-quality bread wheats. For several of these R gene sources, the flanking alien chromatin regions have been reduced by recombination in *ph1b* mutant background (Lukaszewski 2000). DNA markers are also being used to detect recombinants carrying the R gene, but with reduced alien flanking DNA (Rogowsky et al. 1991). Retained DNA markers are being used for the deployment of the modified sources of *Sr31*, *SrR* and *Sr26* to produce near-isogenic lines for assessment of yield and quality effects and introduction as pyramids into adapted cultivars.

1.3.9.4 Cloned Rust Resistance Genes

The first rust resistance genes have been cloned from flax (Lawrence et al. 1995). Apart from providing the first insights into how rust resistance genes function, cloned genes will make a positive impact on plant breeding. An interesting and valuable rust resistance gene for stem rust *Rpg1* has been cloned from barley. This gene, which is not from the most common NBS—LRR class of plant disease resistance genes, has provided durable stem rust resistance in barley.

Initial observations with cloned disease resistance transgenes indicated that they might only function in species closely related to the source plant (Tai et al. 1999a, b). More recent data show this is not necessarily the case. When co-expressed in tobacco, the flax rust resistance protein L6 recognizes the corresponding flax rust avirulence protein AvrL567 and induces a hypersensitive response characteristic of a disease resistance reaction. This is likely to be due to direct interaction of the resistance protein and the avirulence protein (Dodds et al. 2004). Whether the gene functions in tobacco to give rust resistance is not possible to determine because tobacco is a non-host for the flax rust. Nevertheless, the transfer from the Linaceae family to the Solanaceae family shows that wide transfers of resistance genes between species can function.

When the current regulatory and political blockages to GM versions of food crops like wheat and barley are removed, a number of possibilities for GM resistance breeding should become available. For example, in barley and wheat, much specificity for powdery mildew occurs at the *Mla* and *PM3* resistance loci, respectively (Shen et al. 2003; Srichumpa et al. 2005). Cloning studies have shown that these are alleles and so cannot be easily recombined to produce gene pyramids for stable resistance—only one allele at a time can be deployed in a homozygous line. This nexus could be broken using transgenic plants and multiple R transgenes

can be transferred to wheat or barley to make otherwise unobtainable resistance gene pyramids (Maghari and Ardekani 2011).

Cloned genes from cereals are providing perfect markers for breeding in stem rust resistance in wheat. Furthermore, we are aiming to clone three or more resistance genes, package them into a single gene construct and introduce them into wheat using Agrobacterium. Two advantages over traditional methods are envisaged. Firstly, using cloned genes, the effect of linked genes with quality and yield defects can be removed. Secondly, by packing them in a single transgene cassette, the three genes will segregate during breeding as a single unit. Using traditional breeding, individual progeny plants homozygous for three unlinked genes are rare in segregating families. So far, cloning R genes from large cereal genomes is still difficult, but technology is advancing rapidly with increasing genome sequence data available. Rust resistance breeding in cereals is set to make a big jump with both marker-assisted and transgenic breeding. Furthermore, biotechnology can deliver surprises and the recent reports that round-up ready wheat shows high levels of rust resistance after spraying with glyphosate provides a challenge to develop agronomic practices for wheat that combine both weed and rust control using round-up (Anderson and Kolmer 2005)

1.3.10 GM Insect Protected Cotton: A Transgenic Plant Improvement

By the mid 1990s, the Australian cotton industry was beginning to stretch at the seams as this relatively young agricultural enterprise began to experience difficulties in containing its main insect pests, two caterpillars of the *Helicoverpa* complex. Failures in pest control were not new to cotton and it was still fresh in the minds of many how the fledgling industry in the Ord Irrigation Scheme (in the far north of Australia) had gone into a catastrophic spiral of insecticide resistance and increasing pesticide application in the 1960s. This resulted in the use of 35 or more insecticide sprays per crop per season, still without reaching any profitable level of production. This unsustainable dependence on pesticides resulted in the closure of cotton production in that region in the 1970s and the transfer of Australia's efforts to Eastern Australia where pest pressures were still high, but not as extreme as in the more tropical North. Despite its sensitivity to drought and the variable availability of irrigation water, which results in periodic reductions in overall output, cotton production in the East has worked well for many years, climbing to Australia's fifth largest agricultural export and earning the country over AUS$1.6 billion in 2001. Production has continued to increase, but by the early 1990s, it was apparent that despite the availability of new and effective pesticides (that had replaced many of the older more toxic chemicals used in the Ord), the same spiral of evolving insecticide resistance and increasing reliance on higher doses or more toxic mixtures of insecticides was being played out again in the East.

Biotechnology offered a new hope in pest control with the development by the Monsanto company of the gene constructs expressing the insecticidal delta

endotoxin protein, the active ingredient of commonly used biological pesticides (e.g. Dipel). The Cry1A insecticidal toxins of the *Bacillus thuringiensis* are highly potent to both *Helicoverpa armigera* and *Helicoverpa punctigera*, the two main insects being controlled by 80% of the pesticides then applied to cotton. CSIRO played a central role in the breeding of the new insecticidal trait (*Cry1Ac*, sold under the Ingard brand name in Australia) into adapted, high-performing germplasm for Australia, its subsequent deployment and the research that underpinned the management strategies and agricultural practices needed to make it a sustainable pest management tool. At the time of its introduction, the industry was already undergoing some critical self-evaluation about its environmental practices and had instituted many reforms that were already having a impact on reducing pesticide usage, including the introduction of best management practice (BMP) into cotton production and appropriate certification of individual and corporate growers (CRDC 2003). By 2002, 60% of the Australian cotton crop was produced under BMP and incorporated the use of the GM insect protected varieties being developed by CSIRO with the Monsanto genes included in this genome. The Ingard genes were introduced into Australia as cotton seed in the variety Coker 312 (an obsolete Texan variety, one of few cotton varieties amenable to genetic transformation and regeneration) that was itself unsuited for growth under Australian environmental and agricultural production conditions. Conventional backcross breeding was used to improve the germplasm base of the GM cotton by repeated backcrossing to elite CSIRO varieties that were among the best in the world for yield, fibre quality and disease tolerance. Multi-site evaluation across the cotton production area ensured that the new GM versions were well adapted and retained the high yield and other qualities of their recurrent parents. By 1996, CSIRO had produced sufficient seed of five Ingard varieties for an initial trial planting of approximately 40,000 ha. In the meantime, researchers were gathering all the necessary data for regulatory approval, crop agronomy and resistance management that were a necessary precursor to any commercial scale use of the new technology.

Regulation of GM products in Australia was handled by a two-component system that included an voluntary advisory panel of scientists (the Genetic Manipulation Advisory Committee) who assessed the safety of GM products and provided advice to a variety of State and Federal Statutory Agencies with responsibilities for particular areas of regulation of human health, food safety, occupational safety and the environment. Subsequent trials increased steadily in size to allow further pollen movement studies, efficacy assessments, breeding selections and seed increase, as well as the ecological impact studies required by regulators. Pollen flow studies indicated that cotton was easily contained within trials (cotton being a predominantly in-breeding plant) and required a relatively modest surrounding buffer crop extending only 20 m beyond the edge of the GM plots to act as a decoy for foraging insects such as bees that were the most likely vectors of pollen dispersal. Efficacy of pest control was not absolute and although it proved to be high during the first part of the growing season, it was noted to decline after flowering (Fitt 2004). This was subsequently shown to translate into commercial production with most of the savings in pesticide applications occurring during the first half of the season, where

H. punctigera was the main pest. Ecological impact studies measured any non-target impacts on the myriad of insects and other invertebrates that frequent cotton crops. In addition, the possibility of movement of the transgene out of cultivated cotton into native *Gossypium* species with a resultant disruption of the fine balance of these species was required to be assessed.

Given the existing knowledge on the host range of the toxicity of the delta-endo toxins, it was expected that the GM cotton plants would not have a negative impact on other invertebrates and this was borne out by extensive surveys of insect abundance in relatively large (10 ha) plots in replicated trials over a couple of years (Fitt and Wilson 2002). Impacts of the Ingard cotton were restricted to reductions in numbers of *Helicoverpa* larvae and other lepidopteran species known to be sensitive to the Cry1Ac protein, with a secondary effect on some lepidopteran-specific wasp parasites that normally feed within *Helicoverpa* caterpillars. Other beneficial insects tended to be more abundant in the Ingard cotton crops and were certainly much more abundant than in cotton crops sprayed with the conventional spectrum of pesticides normally used to control *Helicoverpa*species. Detailed genetic studies concluded that the risks of outcrossing to Australian native G or C genomic species, *Gossypium sturtianum* L., of the transgenes present in the GM cotton (AD genome allotetraploids) were negligible (Brown et al. 1997), although some of the K genome species in the more remote parts of Northern Australia might require further examination, should a cotton industry ever be established there.

The only major remaining concern of both growers and regulators was whether the technology would last beyond a couple of seasons if the target insect species could develop resistance to the insecticidal protein expressed in the plants. Previous research had reported resistance to Cry proteins in the Indian meal moth (*Plodia interpunctella*) and the diamondback moth (*Plutella xylostella*). Akhurst et al. (2003) were able, under laboratory conditions, to select a strain of *H. armigera* that was resistant to the toxicity of Cry1Ac proteins, so it was clear that target pests could possibly develop resistance to the active ingredient of Ingard cotton. The cotton industry had for years grappled with the problem of chemical insecticide resistance and was reluctant to see Ingard technology wasted. They set up a Transgenic and Insect Management Strategy committee to oversee the deployment of this new technology and make recommendations to both growers and regulators on all aspects of resistance management in an effort to preserve the new GM technology. Australian growers voluntarily adopted a strict area restriction on the use of the single gene Ingard cotton that saw every farm plant a maximum of 30% by area of Ingard varieties until such time as a second generation product was available that contained two different insecticidal toxins that would be more robust in countering any resistance development in the crop pests. This restriction was put in place to ensure that any resistance genes selected in the insects in the transgenic crops would not be fixed in the population, but would always find mates emerging from the non-transgenic crop that carry sensitive alleles for susceptibility to the Cry1Ac toxin and hence continually dilute out the resistance, keeping resistance allele gene frequencies very low within the target insect populations (e.g. Roush 1997). These so-called ‘refugia strategies’ require the presence of non-transgenic

crops in close proximity to the GM crop and have been adopted around the world in a variety of crops carrying GM insecticidal traits; they are an important component of management to delay resistance to insecticidal genes. Other management components included specified planting and harvesting windows, obligate crop destruction after harvest to prevent regrowth and cultivation to destroy overwintering pupae. These strategies have been successful and no field resistance selected in GM crops has been reported in any *Helicoverpa* species or other target lepidopteran insects (Tabashnik et al. 2005). By 2002, CSIRO had produced 15 different GM cotton varieties (combinations of Ingard and the herbicide-resistant Roundup Ready cotton) and continually updated their variety suite to keep pace with developments in conventional cotton germplasm. Despite changes from year to year in variety adoption, the 30% cap on Ingard cotton remained for 6–7 years during which time growers maximized the environmental benefits from the reduced pesticide spraying required on Ingard and in general used the new cottons on their more sensitive environmental sites close to towns, rivers or other dwellings where pesticide drift was likely to be a problem.

In 2003, CSIRO released a new suite of GM varieties that contained the *Cry1Ac* and a second insecticidal gene, *Cry2Ab* (also developed by Monsanto), that were sold as Bollgard II cotton. Bollgard II went through the same regulatory assessment as Ingard cotton, under a new regulatory regime that replaced the previous voluntary system. In 2000, the Australian government had put in place legislation to regulate biotechnology through a newly created statutory authority the Office of the Gene Technology Regulator. This represented a somewhat radical departure from previous systems as its primary goal was to put GM regulation on as open and transparent a footing as anywhere in the world. The requirement for accreditation and the issuing of licenses for the conduct of all GM research as well as a capacity for significant legal and monetary penalties have been put in place to ensure a high level of compliance by both research organizations and biotech and seed companies (as well as opponents of GM who might be tempted to interfere with field trials). Australia has not seen the fierce opposition to GM crops characteristic of European countries and GM cotton in particular has had a relatively straightforward introduction into agriculture (primarily because there was a strong desire for the technologies on the part of farmers and very obvious environmental benefits). The same has not been true for GM canola despite its success in Northern America. GM canola foundered at a State political level, even though it was given Federal regulatory approval (Sorek et al. 2014).

Bollgard II cotton has done extremely well in Australia and within 2 years of its introduction constituted over 90% of all the cotton planted in this country, the majority of it as Bollgard II/Roundup Ready varieties that allowed growers better insect and weed control. The greater efficacy in the control of Lepidopteran pests and the presence in the cotton of two different insecticidal toxins offering greater protection against the development of resistance in the target pests have seen the removal of the planting area restrictions and a reduction in the sizes of the required refuges. Initial indications are that Bollgard II has slashed pesticide usage for Lepidopteran control by more than 80%. One of the key developments with this new

insect control technology is that it has fostered a greater adoption of integrated pest management in cotton, which is leading to even further reductions in pesticide usage (Wilson et al. 2004). The success of GM cotton in Australia has highlighted the value of GM solutions to agricultural sustainability and bodes well for future agbiotech products. Success will depend on the right genetics (getting the products into the right genetic backgrounds), the right management (researching the appropriate management scenarios to ensure the delivery of the benefits promised by the technology) and the right communication (making sure that the community, both the agricultural community and the wider community, are aware of the benefits) for the commercialization of those products.

1.4 Conservation Agriculture and Sustainable Development

Conservation agriculture (CA) is well defined, as minimal soil disturbance and permanent soil cover or mulch combined with rotations, is a contemporary agricultural management system most popular in world now a days. The word 'sustainable' as per the Oxford English dictionary defines it as 'capable of being borne or endured, upheld, defended, maintainable'. Somewhat that is sustained is 'kept up without intermission or flagging, continued over a long period'. An important conception in today's agriculture, as we know that the human race will not want to compromise the ability of its future offspring to produce their food needs by detrimental the natural resources used to feed the population nowadays.

1.4.1 Cultivation Techniques or Tillage

The description of tillage dates back many millennia at what time humans changed from hunting and gathering to more sedentary and settled agriculture mostly in the Tigris, Euphrates, Nile, Yangste and Indus river valleys. Lal (2001) enlightened the historical progress of agriculture with tillage being a major constituent of management practices. The mechanical power and tractors became accessible to undertake tillage operations in nineteenth century and today; an assortment of equipment's is accessible for tillage and agricultural production. The reasons for use of tillage are documented as:

(a) Tillage is generally used to loosen the soil and further preparing a bed wherein seed is to be located easily at an appropriate depth into moist soil using other equipments which resulting in good uniform seed germination.
(b) Farmers were able to shift the benefit from the weed to the crop and let the crop to grow deprived of competition early in its growth cycle with subsequent higher yield by tilling.
(c) Tillage speedup mineralization and oxidation afterward exposure of soil organic matter to air.

(d) Availability of nutrients to roots via tilling process
(e) Tillage is considered a critical management practice for controlling soil borne diseases and some insects as well.

As documented that Faulkner's genius was to question the very basis of agriculture itself-the plough. He began to see that the curved moldboard of the modern plough, rather than permitting organic matter to be slogged into the soil by worms and other burrowing animals, in its place buries this nutritious matter under the subsoil (Faulkner 1987).

1.4.2 Conservation Tillage and Conservation Agriculture

Baker et al. (2002) described conservation tillage as the combined umbrella term usually given to no-tillage, direct boring, minimum-tillage or ridge-tillage, to signify that the explicit practice has a conservation goal of some nature. Generally, the 30% retention of surface cover by residues characterizes the lower limit of organization for conservation tillage, however other conservation objectives like conservation of time, fuel, earthworms, soil water, soil structure and nutrients are included for the practice also. Consequently residue levels alone do not sufficiently define all conservation tillage practices (Baker et al. 2002). However the conservation agriculture aims to conserve, improve and make extra effective use of natural resources through combined management of available soil, water and biological resources collective with external inputs. It provides to environmental conservation as well as to enhanced and sustained agricultural production. It is some times also referred to as resource efficient or resource effective agriculture.

Conservation tillage is a amalgam of practices that leave crop residues on the surface, which increases water infiltration and reduces erosion practiced in conventional agriculture to lessen the effects of tillage on soil erosion. Nonetheless, it still depends on tillage as the structure-forming element in the soil. Meanwhile, conservation tillage practices like zero tillage practices can be switch off steps towards conservation agriculture.

Conservation agriculture sustains a permanent or semi-permanent organic soil cover by developing crop or dead mulch. The main function is to protect the soil physically from sun, rain and wind and to feed soil biota. The soil microorganisms and other fauna take over the tillage function and soil nutrient balancing. Usually the mechanical tillage disturbs however no or minimum tillage and direct seeding are important elements of conservation agriculture. A diverse crop rotation is also significant to avoid disease and pest problems. Derpsch (2005) indicated that the extent of no tillage implementation worldwide is just over 95 Mha. However it is considered as proxy for conservation agriculture. Although not all of this land is permanently no tilled or has permanent ground cover. Table 1.1 depicts the extent of no-tillage by country worldwide. Six countries have more than 1 Mha. South America has the highest adoption rates and has extra permanent non-tillage and permanent soil cover. Both Argentina and Brazil had significant lag periods to reach

Table 1.1 Extent of no-tillage adoption worldwide

Country	Area under no-tillage (Mha) 2004/2005
USA	25.30
Brazil	23.60
Argentina	18.27
Canada	12.52
Australia	9.00
Paraguay	1.70
Indo-Gangetic Plains	1.90
Bolivia	0.55
South Africa	0.30
Spain	0.30
Venezuela	0.30
Uruguay	0.26
France	0.15
Chile	0.12
Colombia	0.10
China	0.10
Others (estimate)	1.00
Total	95.48

Derpsch (2005); includes area in India, Pakistan, Bangladesh and Nepal in South Asia

1 Mha in the early 1990s and then prolonged rapidly to 18.3 and 23.6 Mha for these countries respectively. By approving the no-tillage system, Derpsch (2005) estimated that Brazil increased its grain production by 67.2 million tons in 15 years with additional revenue of 10 billion dollars. Derpsch also estimated that at an average rate of 0.51 t haK1 yrK1 Brazil sequestered 12 million tons of carbon on 23.6 Mha of no-tillage land. The three key principles of conservation agriculture are permanent residue soil cover, minimal soil disturbance and crop rotations.

1.4.3 Permanent or Semi-Permanent Organic Soil Cover

Unger et al. (1988) appraised the role of surface residues on water conservation and documented this association between surface residues, enhanced water infiltration and evaporation led to the adoption of conservation tillage after the 1930s dust bowl problem. However, Bissett and O'Leary (1996) showed that infiltration of water under long term conservation tillage was greater compared to conventional tillage on a grey cracking clay and a sandy loam soil in southeastern Australia. Lal (2001) also defined the efficiency of these systems depends on proper construction and regular care otherwise it can be catastrophic. The crop residues of cultivated crops are a substantial factor for crop production through their effects on soil physico-chemical and biological functions besides water and soil quality (Kumar and Goh 2000). The composts and manures as an external mulch can also be applied, though

economically it may restrict its use to higher-value crops like vegetables. Mulch intercepts the suns energy and protects the surface soil from soil aggregate destruction, enhances the infiltration of water and reduces the loss of soil by erosion. Surface mulch helps reduce water losses from the soil by evaporation and also helps judicious soil temperature endorses biological activity and enhances N-mineralization, especially in the surface layers (Hatfield and Pruegar 1996) generally suitable in tropical and subtropical environments (Swanson and Wilhelm 1996). Fabrizzi et al. (2005) showed that no tillage had lower soil temperatures in the spring in Argentina, but conventional tillage had higher maximum temperatures in the summer, and that average temperatures during the season were similar. Roldan et al. (2003) exhibited that no tillage after 5 years for maize in Mexico, soil wet aggregate stability had increased over conventional tillage as had soil enzymes, soil organic carban and microbial biomass inferring that no tillage is a sustainable technology. A cover crop and the subsequent mulch or prior crop residue help reduce weed plague through competition and not allowing weed seeds the light frequently desirable for germination citing the evidence of allelopathic properties of cereal residues in regard to inhibiting surface weed seed germination (Jung et al. 2004). Farming practice that sustains soil microorganisms and microbial activity can also lead to weed conquest by the biological agents (Kennedy 1999). Cover crops contribute to the addition of organic matter in the surface soil horizon (Madari et al. 2005; Riley et al. 2005). Mulch also benefits with recycling of nutrients, especially when legume cover crops are used, through the connotation with belowground biological agents and by providing food for microbial populations (Campbell et al. 1995). Others have shown that this is restricted to the surface horizons, and that the reverse occurs at greater depths in humid soils of eastern Canada (Angers et al. 1997). Soil microbial biomass has usually been used to evaluate belowground microbial activity and for that it is considered as sink and source for plant nutrients. Alterations or any amendments like residues and manures promote while burning and removal of residues decrease soil microbial biomass (Heenan et al. 2004; Alvear et al. 2005). Increased microbial biomass will enhance soil aggregate formation, nutrient cycling through slow release of organically stored nutrients and also assisted in pathogen control (Carpenter-Boggs et al. 2003). Cover crops help endorse biological soil tillage through their rooting, the surface mulch provides food, nutrients and energy for earthworms, and arthropods and microorganisms below ground that also biologically till soils. The usage of deep-rooted cover crops and biological agents will support to relieve compaction under zero-tillage systems. Though from the available data, it looks at the outcomes of burning, incorporation and removal of crop residues on soil properties. It is further reported that Zero-tillage acts in a better balance of microbes and other organisms and a healthier soil. Ground cover endorses an increase in biological diversity both below and above ground (Jaipal et al. 2002). The interactions between root systems and rhizobacteria affect crop health yield and soil quality. Release of exudates by plants activate and sustain specific rhizobacterial communities that improve nutrient cycling, N- fixing, bio control of plant pathogens, plant disease resistance and plant growth stimulation.

As mentioned above the general comparisons between tillage and zero-tillage systems are made to highpoint some other benefits and in this section other important parameters are documented. For example- Tractors consume large amounts of fossil fuels that enhance to costs while also emitting greenhouse gases (mostly CO_2) and contributing to global warming when used for ploughing (Grace et al. 2003). The animal-based tillage systems are also expensive since farmers have to continue and feed a pair of animals for a year for this purposefulness. Animals also emit methane, a greenhouse gas 21 times more potent for global warming than CO_2 (Grace et al. 2003) so zero-tillage reduces these costs and emissions. The zero-till of wheat after rice reduces costs of production by US$60 per hectare mostly due to less fuel (60–80 l haK1) and labour as studies from farmer surveys in Pakistan and India (Hobbs and Gupta 2004). Tillage takes appreciated time that could be used for other useful farming activities or employment. Zero tillage minimizes time for establishing a crop. The time required for tillage can also delay timely planting of crops, with subsequent reductions in yield potential (Hobbs and Gupta 2003). By reducing about-turn time to a minimum, zero-tillage can get crops planted on time, and thus increase yields without greater input cost. Turnaround time in this rice- wheat system from rice- wheat varies from 2 to 45 days, since 2–12 passes of a plough are used by farmers to get a good seedbed (Hobbs and Gupta 2003). With zero-till wheat this time is reduced to just 1 day. Tillage and current agricultural practices result in the decline of soil organic matter due to increased oxidation over time, leading to soil degradation, loss of soil biological fertility and resilience (Lal 1994). Although this SOM mineralization liberates nitrogen and can lead to improved yields over the short term, there is always some mineralization of nutrients and loss by leaching into deeper soil layers. This is particularly significant in the tropics where organic matter reduction is processed more quickly, with low soil carbon levels resulting only after one or two decades of intensive soil tillage. Zero-tillage, on the other hand, combined with permanent soil cover, has been shown to result in a build-up of organic carbon in the surface layers (Lal 2005). No-tillage minimizes SOM losses and is a promising strategy to maintain or even increase soil C and N stocks (Bayer et al. 2000). Although tillage does afford some relief from compaction, it is itself a major cause of compaction, especially when repeated passes of a tractor are made to prepare the seedbed or to maintain a clean fallow. Zero tillage reduces dramatically the number of passes over the land and thus compaction. However, natural compaction mechanisms and the one pass of a tractor-mounted zero-till drill will also result in compaction (Sayre and Hobbs 2004). Some farmers feel that sub-soiling may be needed to resolve belowground compaction layers before embarking on a NT strategy, especially in drier areas. Higher bulk densities and penetration resistance have been reported under zero-tillage compared with tillage (Gantzer and Blake 1978) and are described as natural for zero-tillage. This problem is greater in soils with low-stability soil aggregates (Ehlers et al. 1983). Bautista et al. (1996) working in a semi-arid ecosystem found that zero-tillage plus mulch reduced bulk density. The use of zero-till using a permanent residue cover, even when bulk density was higher, resulted in higher infiltration of water in no tillage systems (Sayre and Hobbs 2004). Scientists assumed that continued use of reduced, shallow and

zero-tillage would require a shift to short-term total tillage to correct soil problems. However, Logsdon and Karlen (2004) showed that bulk density is not a useful indicator and confirm that farmers need not worry about increased compaction when changing from Total to no tillage on deep loess soils in USA. Fabrizzi et al. (2005) also showed higher bulk density and penetration resistance in no tillage experiments in Argentina, but the values were below thresholds that could affect crop growth; wheat yields were the same as in the tilled treatments. Leake (2003) determined that the role of tillage on diseases is unclear and recognizes that a healthy soil with high microbial diversity does play a role by being antagonistic to soil pathogens and suggested that no tillage farmers need to adjust management to control diseases through sowing date, rotation and resistant cultivars to help shift the advantage from the disease to the crop.

Crop rotation is an agricultural management tool with prehistoric origins. Howard (1996) studied the cultural control of plant diseases from an historical view and included examples of disease control across rotation. The rotation of altered crops with different rooting patterns collective with minimal soil disturbance in zero-till systems endorses a more extensive network of root channels and macropores in the soil. Integrated pest management must also be added to the conservative agriculture set of recommendations, since if one of the necessities is to promote soil biological activity, negligible use of toxic pesticides and use of substitute pest control methods that do not affect these critical soil organisms are needed (Leake 2003).

1.4.4 Climate Change and Conservation Agriculture

Climate change is likely to strongly affect rice–wheat, rice–rice and maize-based cropping systems that, today, account for more than 80% of the total cereals grown on more than 100 Mha of agricultural lands in South Asia. Global warming may be beneficial in some regions, but harmful in those regions where optimal temperatures already exist; an example would be the rice–wheat mega-environments in the IGP that account for 15% of global wheat production. Agronomic and crop management practices have to aim at reducing CO_2 and other greenhouse gas emissions by reducing tillage and residue burning and improving nitrogen use efficiency. In the IGP, resource-conserving technologies continue to expand in the rice–wheat cropping systems and save 50–60 l of diesel haK1 plus labour, and significantly reduce release of CO_2 to the environment. Methane emissions that have a warming potential 21 times that of CO_2 are common and significant in puddled anaerobic paddy fields and also when residues are burnt. This GHG emission can be mitigated by shifting to an aerobic, direct seeded or NT rice system. A review of the other benefits of direct seeding and NT in RW areas of South Asia can be found in Grace et al. (2003). Nitrous oxide has 310 times the warming potential of carbon dioxide, and poor nitrogen management affects its emissions. Sensor-based technologies for measuring normalized differential vegetative index and moisture index have been

used in Mexico and South Asia to help improve the efficiency of applied nitrogen and reduce nitrous oxide emissions. Lal (2005) suggested that by adopting improved management practices on agricultural land (use of NTand crop residues), food security would not only be enhanced but also offset fossil fuel emissions at the rate of 0.5 Pg C yrK1.

1.5 Conclusion and Future Perspective

Genetic alteration to the biological software of our major food and fibre production plants will endure to improve the yield and sustainability of agricultural systems. DNA technology is now consistently used in plant improvement programmes with DNA sequence markers enhancing both the speed and the power of selection schemes. The rapidly increasing knowledge of the functioning of crop genomes has already provided enhanced performance in conventional breeding programmes and although transgenic crops have not been welcomed in all parts of the world. These transgenic crops, comprising cotton, fiber crop, food and feed crops like maize, soya bean and canola, have all been considered in the various countries of the world in which they are grown and have entered successfully into markets. This signifies a noteworthy growth incorporation of transgenic modifications into breeding systems. The appreciative molecular bases of plant processes that have been gained from the developments in genomics and our collective knowledge of gene regulation are opening up a new generation of breeding advances, both through transgenic breeding and conventional breeding. One of the rewards in many crops is that once specific breeding objectives have been defined by research that has used all the power of the new technologies, then breeders are able to use new investigative tools to achieve the desired objectives through conventional breeding programmes providing a bridging period of improvement in plant breeding while our societies move towards general acceptance of transgenic tools in plant improvement programmes for our food, feed and fibre crops. The profit from modification of internal architecture, the anatomy of plant tissues; for example, the ratio of palisade and spongy mesophyll leaf cells and the geometry of tissues in the root system are areas in which we can expect telling alterations. A reasonable conclusion is that genetic modification of crops, which has been so powerful and so rewarding in terms of yield and management of many of the major production species over the past few decades, will hold enormous potential in all of the crop species we deal with. We have an increasing knowledge and power to modulate the development and functional operation of crop plants so as to provide optimal performance in our agricultural production system environments. Agricultural performance rests on the interactions of genetics, management and the environment. And a variety of production environments to have the genetic modifications, coupled with appropriate management regimes, to result in an increased efficiency and sustainability of agri-business can be expected in future.

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