

# Modern Biotechnology as an Integral Supplement to Conventional Plant Breeding: The Prospects and Challenges

Prem P. Jauhar\*

It would be an unsound fancy to expect that things which have never yet been done can be done except by methods which have never been tried.

—Sir Francis Bacon

## ABSTRACT

The art of plant breeding was developed long before the laws of genetics became known. The advent of the principles of genetics at the turn of the last century catalyzed the growth of breeding, making it a science-based technology that has been instrumental in substantial improvements in crop plants. Largely through exploitation of hybrid vigor, grain yields of several cereal crops were substantially increased. Intervarietal and interspecific hybridizations, coupled with appropriate cytogenetic manipulations, proved useful in moving genes for resistance to diseases and insect pests from suitable alien donors into crop cultivars. Plant improvement has been further accelerated by biotechnological tools of gene transfer, to engineer new traits into plants that are very difficult to introduce by traditional breeding. The successful deployment of transgenic approaches to combat insect pests and diseases of important crops like rice (*Oryza sativa* L.), wheat (*Triticum aestivum* L.), maize (*Zea mays* L.), barley (*Hordeum vulgare* L.), and cotton (*Gossypium hirsutum* L.) is a remarkable accomplishment. Biofortification of crops constitutes another exciting development in tackling global hunger and malnutrition. Golden Rice, genetically enriched with vitamin A and iron, has, for example, the real potential of saving millions of lives. Yet another exciting application of transgenic technology is in the production of edible vaccines against deadly diseases. How these novel approaches to gene transfer can effectively supplement the conventional breeding programs is described. The current resistance to acceptance of this novel technology should be assessed and overcome so that its full potential in crop improvement can be realized.

A PARAMOUNT FACTOR in the evolution of human civilizations was a steady supply of food. Food production is therefore the oldest profession of humanity. The processes of crop cultivation and selection were an integral part of human activity. Although early “plant breeding” was developed essentially as an art, its scientific basis became well established with the rediscovery of laws of genetics at the turn of the last century. And with the application of the principles of genetics to crop improvement, the period from 1930 to 1970 witnessed a phenomenal increase in crop yields, particularly of

cereal grains (Khush, 1999). Largely through exploitation of hybrid vigor, maize, pearl millet [*Pennisetum glaucum* (L.) R. Br.], and sorghum [*Sorghum bicolor* (L.) Moench] registered a considerable increase in grain yields during 1965 to 1990 (Khush, 2001; Jauhar and Hanna, 1998; Jauhar et al., 2006). Improved wheat and rice varieties with reduced height developed by incorporating dwarfing genes in the 1960s and 1970s launched the famous Green Revolution in Asia (Khush, 1999; see also Jauhar, 2006). Around the same period, the advent of the tools of cytogenetics greatly facilitated wide hybridization and chromosome-mediated gene transfers from wild species into crop plants (Jiang et al., 1994; Jauhar, 1993, 2003a; Friebe et al., 1996; Fedak, 1999). Chromosome engineering methodologies, based on the manipulation of pairing control mechanisms and induced translocations, were, for example, applied to transfer into wheat cultivars specific disease and pest resistance genes of alien origin (Ceoloni and Jauhar, 2006; Jauhar, 2006; Mujeeb-Kazi, 2006). Thus, cytogenetic tools were instrumental in the genetic improvement of several crop plants, particularly cereals.

The development, in the last decade and a half, of novel tools of direct gene transfer, collectively termed *genetic engineering*, has added new dimensions to breeding efforts. Genetic engineering is defined as any nonconventional tool aimed at mobilizing specific genetic information from one member of the plant kingdom (or, for that matter, any organism) into another. (Any nonconventional tool of today may of course become conventional in the future.) These asexual techniques of biotechnology help engineer into plants new characters that are otherwise very difficult to introduce by conventional breeding. The molecular techniques, including the recombinant DNA methods, involve the introduction of well-characterized alien DNA into the recipient plant cells of regenerable embryonic calli to permanently transform the plant’s genetic makeup. Genetic engineering has the potential to accelerate crop improvement and has already yielded encouraging results (e.g., Jauhar and Chibbar, 1999; Muthukrishnan et al., 2001; Repellin et al., 2001; Dahleen et al., 2001; Janakiraman et al., 2002; Patnaik and Khurana, 2003; Wesseler, 2003; Sharma et al., 2004). Value-added traits engineered into crop plants include resistance to fungal and viral diseases, and biofortification of their nutritional status (Jauhar and Khush, 2002; Schubert et al., 2004; Bajaj and Mohanty, 2005). However, as with any new technology, genetic engineering is encountering resistance from some sections of the public. There are concerns about the potential adverse impact of genetically modified (GM) foods

USDA-ARS, Northern Crop Science Laboratory, Fargo, ND 58105. Mention of tradenames or commercial products in this publication is solely to provide specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture. Received 30 Jan. 2006. Invited paper. \*Corresponding author (prem.jauhar@ndsu.edu).

Published in Crop Sci. 46:1841–1859 (2006).  
Research & Interpretation  
doi:10.2135/cropsci2005.07-0223  
© Crop Science Society of America  
677 S. Segoe Rd., Madison, WI 53711 USA

**Abbreviations:** Bt, *Bacillus thuringiensis*; FHB, Fusarium head blight; fl-GISH, fluorescent genomic in situ hybridization; GM, genetically modified; PDR, pathogen-derived resistance; QPM, quality protein maize.

or organisms on human health and the environment. Although some of the public concerns may not be well founded (Jauhar and Khush, 2002), they will need to be properly addressed. To alleviate some of these fears, perceived or real, we will need to do a better job of informing the public. Some of these issues are raised in this paper. The main objective of this article is to highlight the potential of the transgenic technology as a supplementary tool to plant breeding and to discuss its prospects and the challenges that lie ahead.

### **Plant Breeding as an Art: The Man-Made Evolution**

Plant breeding deals with the exploitation of existing genetic variability and the generation, manipulation, and combination of new variability into plant forms most useful to humans. The genetic and cytogenetic basis of plant breeding is now well understood. We must remember, however, that the art of plant breeding was developed long before the principles of genetics and cytogenetics became known. Even before Mendel (1822–1884), plant hybridizers, such as Kölreuter, Knight, Gärtner, and others, had produced improved strains of crop plants. Several thousand years ago, the early “plant breeders,” although essentially unschooled, intuitively looked for, skillfully recovered, and successfully propagated genetic variants or recombinants that exhibited desirable traits.

Working under a myriad of cultural contexts, these early selectionists turned the relatively useless weedy species into useful crop plants that sustain us today. The exercise of a rigorous process of screening and selection brought about modification in the genetic makeup of the plant forms. Thus, plant breeding is essentially an exercise at manipulating plant genetic material to humanity's best advantage. An advance in yield, for example, must involve appropriate changes in the genetic constitution of the crop plant in question. In this regard, plant breeding is nothing but human-made evolution, which has brought about substantial increases in crop yields, for example, quantity and quality of cereal grains.

### **Genetics and the Scientific Foundation of Plant Breeding: Some Accomplishments**

With the discovery (or rediscovery) of the laws of genetics at the turn of the 20th century, the process of plant breeding was accelerated considerably and became a science-based technology. The principles of genetics found immediate application in crop improvement, as illustrated by a few examples given below.

#### **Hybrid Vigor: A Boon to Plant Breeders**

Grain yields of the major cereal crops, viz., wheat, maize, sorghum, and pearl millet, increased steadily since 1930, primarily because of genetic improvement of these crops (Duvick, 1984; Fehr, 1984). Largely because of exploitation of hybrid vigor, the maize yields in the United States registered a phenomenal increase from

1966 kg ha<sup>-1</sup> in 1930 to 4841 kg ha<sup>-1</sup> in 1982 (USDA's “Agricultural Statistics”). World maize yields increased at the rate of 0.7% per year during the period 1982 to 1990 (Duvick, 1992). Exploitation of hybrid vigor continues to be the most appropriate means to increase grain yields relatively rapidly (Vasal et al., 2006). Heterosis breeding has also significantly increased the grain yields of pearl millet (Jauhar, 1981; Jauhar and Hanna, 1998; Jauhar et al., 2006), sorghum (Reddy et al., 2006), and rice (Brar and Khush, 2006). It is remarkable that in 2001 more than 70 hybrids were under cultivation on 6 million hectares of the total 10 million hectares of pearl millet area in India (Jauhar et al., 2006).

#### **The Dawn of Green Revolution**

Improved, high-yielding varieties of wheat and rice developed in the 1960s and 1970s launched the Green Revolution in Asia. A product of an unprecedented international effort, the Green Revolution is certainly one of the most important accomplishments of the 20th century. Breakthroughs in wheat and rice yields were achieved with the development of semidwarf varieties characterized by lodging resistance and N responsiveness (Swaminathan, 1993; Borlaug, 1998; Khush, 1999, 2001). Thus, the introduction of dwarfing genes by conventional breeding revolutionized both wheat and rice production in Asia, averting mass-scale starvation.

#### **Nutritional Enhancement of Food Crops**

In addition to grain yields, improving nutritional quality of food crops such as cereals is an important goal because 842 million people worldwide are malnourished according to most recent reports ([www.fao.org/english/newsroom/news/2003/24779-en.html](http://www.fao.org/english/newsroom/news/2003/24779-en.html); verified 10 May 2006). Nutritional upgrading of maize was achieved mainly by conventional breeding, and hybrid initiative proved to be exceedingly important for successful development of quality protein maize (QPM) (Vasal, 2002; Vasal et al., 2006). The development of QPM was an important scientific breakthrough, whose fruits are being reaped by several developing countries. Sorghum cultivars with high protein digestibility as well as high lysine content are being developed and offer prospects for combining high nutritional quality and grain yield (Oria et al., 2000; Reddy et al., 2006).

#### **Harnessing Apomixis for Perpetuating Hybrid Vigor**

As stated above, heterosis breeding has dramatically increased grain yields of maize and pearl millet. In the USA, hybrid maize was introduced in the mid-1930s and within 10 yr almost all maize fields were planted with hybrids. Similarly, pearl millet hybrids were widely accepted in India. However, the main problem with such hybrids is that their seed has to be produced year after year for distribution to growers and farmers. Introduction of apomixis in hybrids would enable them to maintain heterozygosity through seed production, thereby perpetuating hybrid vigor, and eliminating the need to

produce commercial hybrids every year. Some researchers have attempted to transfer the apomictic mode of reproduction from eastern gama grass (*Tripsacum dactyloides* L.) into maize, although with limited success (Savidan, 2000; Kindinger, pers. comm., 2004; Bicknell and Koltunow, 2004). Attempts to make rice apomictic have also been underway. Both mutagenesis (see Brar and Khush, 2006) and molecular tools (Bennett et al., 2001) have been employed to introduce apomixis in rice, but with limited success so far.

Crosses between synthetic tetraploid pearl millet and its hexaploid, apomictic relative, *Pennisetum squamulatum* Fresen. have shown a high level of expression of apomixis in the progeny (Ozias-Akins et al., 1998), but producing a completely apomictic pearl millet remains problematic (Ozias-Akins et al., 2003; Akiyama et al., 2004; Jauhar et al., 2006). Fixation of apomixis would help clone superior hybrids and result in the decrease of seed production cost, benefiting resource-poor farmers. Only some progress in this direction has been made (Koltunow and Tucker, 2003) and ongoing research in this area may yield dividends.

### Cytogenetic Tools in Plant Breeding

The formulation of the laws of inheritance by Mendel in 1865 led to the foundation of genetics, the science of heredity, although it was born belatedly in 1900 with the rediscovery of Mendel's work. Soon afterward, the elucidation of parallelism between chromosome behavior during the course of meiosis and of genes during breeding experiments forged an alliance between cytology and genetics, resulting in the hybrid science cytogenetics. The establishment of the chromosome theory of heredity put chromosomes at the center of life sciences. And cytogenetics has already had a tremendous impact on agriculture, biology, and medicine.

### General Applications in Plant Improvement

The tools of cytogenetics have played a pivotal role in accelerating crop improvement. Thus, the understanding of chromosome pairing and its regulation and manipulation, genome relationships between and within plant

species, polyploidy, aneuploidy and haploidy—to name a few—have greatly aided plant breeding. The haploidy technique, for example, is very useful. The haploid-derived homozygous lines provide a rapid means of achieving homozygosity, thereby accelerating breeding programs. An understanding of genomic affinities facilitates the planning of effective hybridization programs designed to transfer desired genes or gene clusters from alien species into otherwise superior cultivars of crop plants. Chromosome engineering, involving chromosome-pairing manipulation in polyploid crop plants, leads to fruitful recombination of entire genomes, parts of genomes or chromosome segments resulting in superior cultivars. This form of plant breeding involves, in essence, genomic reconstructions to meet human needs.

### Cytogenetic Architecture of a Crop Plant: Its Bearing on Breeding

A full understanding of the genomic constitution of a crop and its nature of polyploidy, if any, is very valuable in planning an effective breeding strategy. Thus, hexaploid bread wheat (*T. aestivum* L.,  $2n = 6x = 42$ ; AABBDD) and tetraploid durum wheat (*T. turgidum* L.,  $2n = 4x = 28$ ; AABB) are natural hybrids, having resulted from hybridization between related diploid wild species. Durum wheat is the forerunner of bread wheat. Although the constituent genomes of polyploid wheats are genetically similar or homoeologous, the *Ph1* gene in the long arm of chromosome 5B ensures diploid-like pairing, that is, pairing only between homologous partners (Riley and Chapman, 1958; Sears and Okamoto, 1958).

The role of *Ph1* in enforcing homologous chromosome pairing is best illustrated by studying pairing in wheat haploids with and without *Ph1*. These haploids with half the chromosome complement do not have their homologous partners to pair with and hence show no or very little pairing (Fig. 1A). Thus, *Ph1* haploids show mostly univalents, although some superficial pairing, termed chromosome “dating” (Jauhar, 1990, p. 528), may be observed (Fig. 1A). However, in the absence of *Ph1*, the wheat haploids show extensive homoeologous pairing

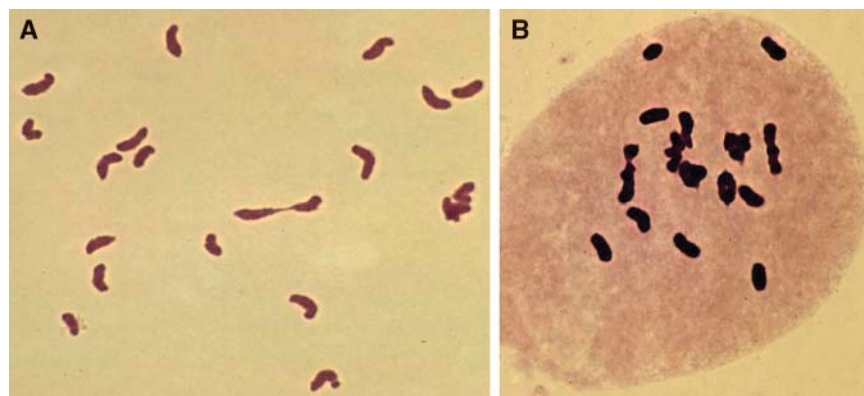


Fig. 1. Effects of *Ph1* on chromosome pairing at meiosis in bread wheat haploids ( $2n = 3x = 21$ ; ABD genomes). Pollen mother cells (PMCs) with and without *Ph1* are shown. (A) PMC with *Ph1* showing 1 rod II and 19 I. Such a superficial pairing in the presence of *Ph1* has been called “chromosome dating.” (B) PMC of a *ph1b*-haploid showing 6 II (3 ring and 3 rod bivalents) + 9 I. Note extensive homoeologous pairing because of the absence of *Ph1*. Interestingly, one gene can make such a difference. (Fig. 1B from Jauhar et al., 1991).

pairing (Fig. 1B) because control on pairing is relaxed. It is remarkable that one gene can make such a difference in the pattern of chromosome pairing. Such a disciplined pairing ensures disomic inheritance and confers meiotic regularity and reproductive stability to polyploid wheats. Oat (*Avena sativa* L.) is another important allohexaploid cereal with a genomic makeup similar to that of bread wheat and also the genetic regulation of chromosome pairing (Rajhathy and Thomas, 1972; Jauhar, 1977) similar to the *Ph1* system of wheat. Appropriate cytogenetic manipulation of the chromosome pairing control mechanism helps transfer alien genes into wheat, as stated below.

### Wild Species as Sources of Genes for Crop Improvement

Cereal crops (wheat, rice, maize, barley, oat, rye, sorghum, and pearl millet) account for two-thirds of the world food supply (Borlaug, 1998). Domestication and improvement of cereals have been brought about mainly by selections coupled with conventional breeding, and improved strains of these crops have been produced mainly by reshuffling and combining genes from within the primary gene pool, including land races within a species (see Jauhar 2006, and references therein).

Wild relatives of crop plants are also reservoirs of genes for superior traits, which can be incorporated into crop species via wide hybridization. Cytogenetic techniques are useful in bringing about such alien gene transfers. As early as 1956, Ernest Sears translocated onto wheat chromosome 6B a small segment from *Aegilops umbellulata* Zhuk. that carried a gene for resistance to leaf rust (caused by *Puccinia triticina* Eriks.) (Sears, 1956). This pioneering work heralded an era of utilization, by chromosome manipulation, of wild gene resources for improvement of crop plants. During the last few decades, interspecific and intergeneric hybridization have been widely used to develop wheat cultivars with improved agronomic performance, pest tolerance, and high yields (Friebe et al., 1996; Jauhar and Chibbar, 1999). Such alien transfers following wide hybridization with donor species coupled with manipulation of chromosome pairing, by suppressing or removing the pairing regulator *Ph1*, are called chromosome-mediated gene transfers that have resulted in several commercial cultivars with genes of alien origin. And such cultivars have been widely accepted for human consumption.

### Chromosome Pairing: The Key to Genetic Enhancement

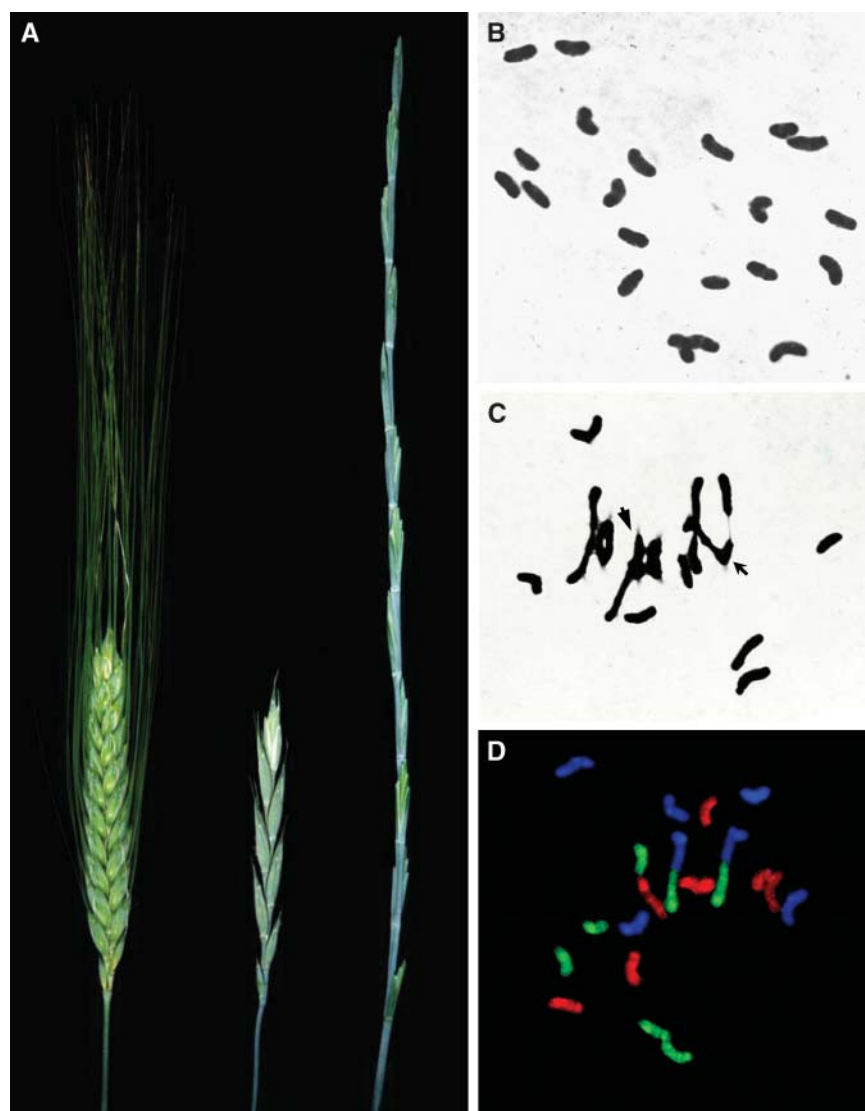
Crop diseases and pests pose a serious threat to global food security. Close relatives of crop species are rich reservoirs of genes for resistance to pathogens and insect pests and those genes can be transferred to crop cultivars through hybridization. Pairing among chromosomes of parental species in their hybrids is the key to gene transfer across species. Cytogenetic manipulations for suppressing the *Ph1*-pairing regulation of polyploid wheats and oat, for example, would be necessary to

bring about the desired chromosome pairing and hence alien gene transfers into these crop species. The methods of circumventing the *Ph1* system include: (i) using substitution lines lacking chromosome 5B and hence *Ph1*; (ii) suppressing the activity of the *Ph1* or *Ph1*-like genes by crossing polyploid cereal crops with appropriate genotypes of wild donors that partially or fully inactivate the regulatory genes; and (iii) using the *ph1bph1b* mutant of wheat as the female parent in crosses with the wild donor species (see Jauhar, 2006). Such cytogenetic manipulations, including the suppression of the *Ph1* system, for recombining desirable alien chromatin into wheat were termed *chromosome engineering* (Sears, 1972, 1981). Essentially similar cytogenetic manipulations to effect gene transfer can be done in hexaploid oat (Jellen and Leggett, 2006). Some examples are given below.

Fusarium head blight (FHB) or scab, caused by the fungus *Fusarium graminearum* Schwabe, is a devastating fungal disease of durum wheat, an important cereal used for human consumption worldwide. Current durum cultivars have very little FHB resistance; therefore, we transferred resistance from wild relatives into durum germplasm. By crossing a durum wheat 5D(5B) disomic substitution with a diploid wheatgrass, *Lophopyrum elongatum* (Host) Á. Löve ( $2n = 2x = 14$ ; EE genome), we realized substantial chromosome pairing among the parental chromosomes in the intergeneric hybrids and transferred alien chromatin into the durum genome (Jauhar and Peterson, 2000). We adopted the same approach for transferring chromosome segments from another diploid wheatgrass, *Thinopyrum bessarabicum* (Savul. & Rayss) Á. Löve ( $2n = 2x = 14$ ; JJ genome), into the durum genome (Fig. 2). In the presence of *Ph1*, the intergeneric hybrids (Fig. 2A) showed very little pairing, if at all (Fig. 2B). However, in the absence of *Ph1*, extensive homoeologous pairing occurred (Fig. 2C). Multicolor fluorescent genomic in situ hybridization revealed some pairing between durum and grass chromosomes (Fig. 2D).

### Chromosome Engineering and Crop Improvement: Polyploid Crop Species

Polyploid crop species like wheat and oat have a precise genetic control of chromosome pairing and therefore require appropriate cytogenetic manipulation for alien gene introgression. The diverse gene pools of the Triticeae species can be exploited for genetic enrichment of both durum wheat and bread wheat, using the well-established cytogenetic approaches. These procedures involve hybridization of the crop species with a wild donor, preferably in the primary gene pool, accompanied by cytogenetic manipulation to bring about pairing between chromosomes of parental species to effect alien chromatin transfer into the crop species. Thus, considerable progress has been made in transferring segments of alien chromosomes carrying the desired genes to bread wheat (Sears, 1981, 1983; Feldman, 1988; Mujeeb-Kazi and Rajaram, 2002; Mujeeb-Kazi, 2006), durum wheat (Ceoloni et al., 2005; Ceoloni and Jauhar, 2006),



**Fig. 2.** Durum wheat  $\times$  *Th. bessarabicum* hybrid, and its chromosome pairing in the presence and absence of *Ph1*. (A) Spikes of parental species—durum wheat (left), *Th. bessarabicum* (right)—and their intergeneric hybrid (center). (B) PMC of the triploid intergeneric hybrid ( $2n = 3x = 21$ ; ABJ genomes) with *Ph1* showing 21 I. Note complete absence of pairing because of the presence of *Ph1*. (C) PMC of the intergeneric hybrid durum Langdon (LDN) disomic substitution 5D (5B)  $\times$  *Th. bessarabicum*, showing 2 III [one V-shaped (arrow) and one frying pan-shaped (arrowhead)] + 4 II + 7 I. Note extensive homoeologous pairing, a welcome feature from the breeding standpoint. Some pairing takes place between the wheat and grass chromosomes (see Fig. 2D). (D) Same hybrid as in (C) with meiotic chromosomes after fluorescent genomic in situ hybridization when the durum wheat A-genome (colored green) was probed with *Triticum urartu* DNA labeled with FITC, the J-genome was probed with *Th. bessarabicum* DNA labeled with Rhodamine (colored red), and the remaining chromosomes counterstained with DAPI (colored blue) belong to the B-genome with one D-genome chromosome from 5D. Note wheat–grass pairing (A–J pairing).

and hexaploid oat (Jellen and Leggett, 2006). We produced, for example, scab-resistant durum wheat germplasm by transferring chromosome segments from diploid wheatgrass, *L. elongatum* (Jauhar and Peterson, 2000; Jauhar and Xu, 2004) and tetraploid wheatgrass, *Thinopyrum junceiforme* (Löve & Löve) Löve,  $2n = 4x = 28$ ; J<sub>1</sub>J<sub>1</sub>J<sub>2</sub>J<sub>2</sub> genomes) (Jauhar and Peterson, 2001). Using fluorescent genomic in situ hybridization (fl-GISH), we demonstrated the integration of alien chromatin into the durum genome. This germplasm provides an important resource for breeding FHB resistance into durum cultivars. Such studies on wheat improvement are in progress in our laboratory and other laboratories around the world.

Recent improvements in methods of characterizing alien chromatin introgressed into crop genomes are facilitating crop improvement by chromosome engineering. Thus, fl-GISH is very helpful in characterizing such introgression products (Jauhar et al., 2004; Ceoloni and Jauhar, 2006; Jauhar, 2006). These techniques also help retain the desired alien chromosome segments while eliminating the undesirable ones.

**Genotype-Induced Homoeologous Chromosome Pairing: Breeding for Cold Tolerance.** Certain genotypes of wild grasses are known to suppress the activity of *Ph1* in their hybrids with wheat, thereby accelerating homoeologous chromosome pairing and hence alien gene trans-

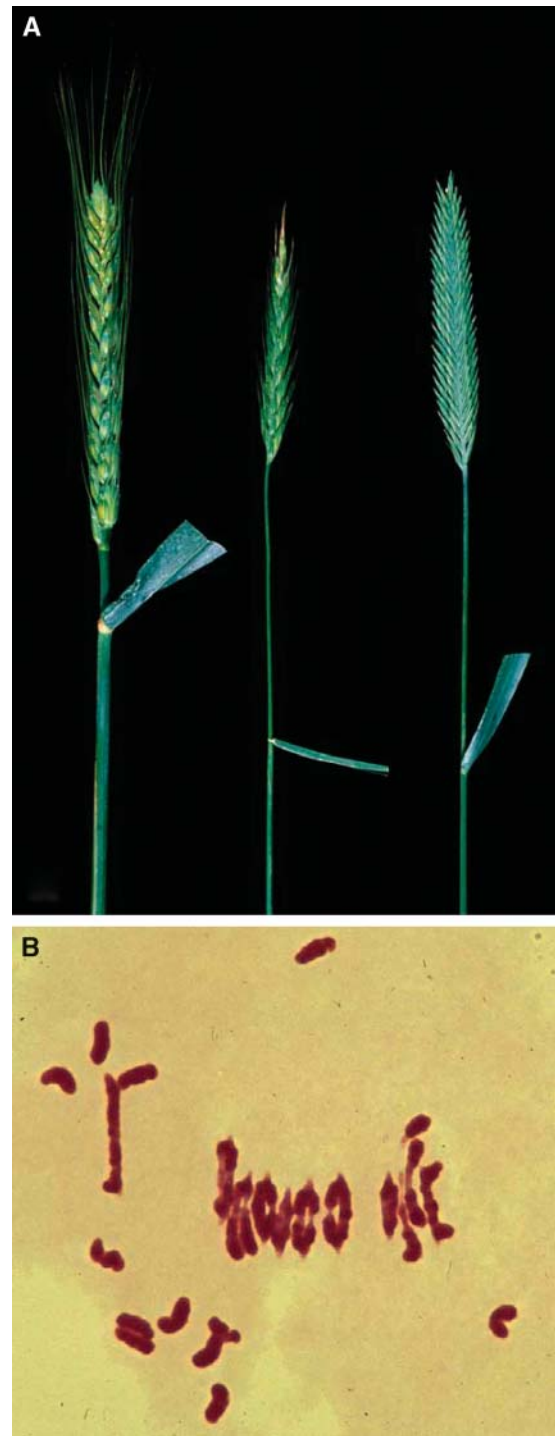
fers. *Aegilops speltoides* Tausch, for example, has been known to induce high homoeologous pairing (Dvořák, 1972). Genotype-induced interference with the regulatory mechanism is an efficient means of promoting homoeologous chromosome pairing and we have used this attribute in our wheat improvement program. Tetraploid crested wheatgrass, *Agropyron cristatum* (L.) Gaertner ( $2n = 4x = 28$ ; PPPP), is a valuable source of genes for drought and cold tolerance that could be transferred into bread wheat via hybridization. In pentaploid hybrids ( $2n = 5x = 35$ ; ABDPP) (Fig. 3A) between bread wheat and crested wheatgrass, we observed substantial chromosome pairing (Fig. 3B) induced by the grass genotype, offering the possibility of alien gene transfer into the wheat chromosome complement (Jauhar, 1992).

**Genotype-Induced Homoeologous Chromosome Pairing: Prospects for Producing Perennial Wheat.** Perennial grasses in the tribe Triticeae are adapted to diverse ecogeographical conditions and are important sources for genes for tolerance to drought, cold, and salinity and for resistance to various diseases and insect pests. Tall wheatgrass, *Thinopyrum ponticum* (Podp.) Barkworth and Dewey ( $2n = 10x = 70$ ), for example, is the most salt-resistant grass in the tribe, and is also resistant to *Barley yellow dwarf virus* (BYDV) (Sharma et al., 1989), and has been a source of the leaf rust resistance gene, *Lr24*, to several wheat cultivars (Cox, 1991). Some genotypes of the grass promote homoeologous chromosome pairing in their hybrids with wheat. By crossing the tall wheatgrass cultivar Alkar with wheat cultivar Fuko, several perennial fertile hybrids were produced (Fig. 4A) which also showed extensive homoeologous pairing (Jauhar, 1995). Despite high pairing, including multivalents, these perennial hybrids had high fertility and seed set, which on dehusking looked like wheat grains (Fig. 4B). Because of their high fertility, these intergeneric hybrids offer opportunities for direct backcrossing to the wheat parent and for possible production of perennial wheat.

### Chromosome Engineering and Crop Improvement: Diploid Crop Species

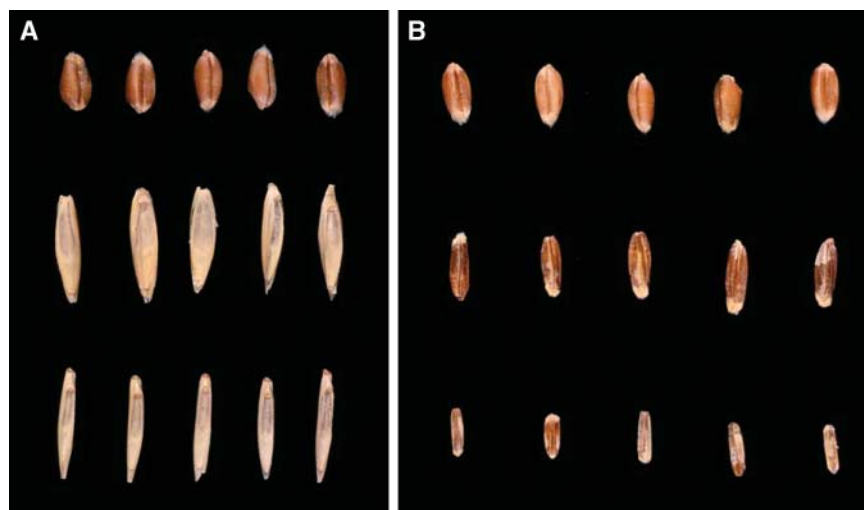
Maize, barley, sorghum, and pearl millet are among the important cereals. They are essentially diploid (or diploidized) in their cytogenetic behavior and lend themselves to manipulation by cytogenetic tools and traditional breeding (see Jauhar, 2006 for references). Maize and pearl millet, being cross-pollinated and hence with enormous genetic diversity, offer tremendous possibilities for heterosis breeding for higher yields and nutritional quality (Vasal et al., 2006; Jauhar et al., 2006). Thus, the production of QPM with high lysine is a landmark achievement, which has greatly helped to alleviate malnourishment among the poor with maize as a primary food source (Vasal, 2002).

Cytogenetic tools help transfer chromatin from one crop species into another. Using oat  $\times$  maize crosses, for example, maize chromatin has been added to the oat



**Fig. 3.** Intergeneric hybrid ( $2n = 5x = 35$ ; ABDPP genomes) between bread wheat ( $2n = 6x = 42$ ; AABBDD) wheatgrass, *Agropyron cristatum* ( $2n = 4x = 28$ ; PPPP), and genotype-induced homoeologous chromosome pairing. (A) Bread wheat var. Fukuhokomugi (left), crested wheatgrass (right), and their hybrid (center). Note the intermediate phenotype of the hybrid. (B) Meiotic metaphase in the hybrid showing 1 III + 10 II + 12 I. The genotype of the grass parent induces higher pairing than is expected based on chromosome homology.

genome (Riera-Lizarazu et al., 2000; Kynast et al., 2002). Through hybridization with other cereals, followed by chromosome elimination, maize offers an excellent mechanism for producing haploids of cereal crops



**Fig. 4.** Intergenic  $F_1$  hybrids between bread wheat cultivar Fukuhokomugi (Fuko) and decaploid tall wheatgrass, *Thinopyrum ponticum* var. 'Alkar' ( $2n = 10x = 70$ ). This hybridization offers prospects for breeding "perennial" wheat. (A) Seeds of the female parent Fuko (top row), intergenic  $F_1$  hybrid (middle row), and the male parent Alkar (bottom row). Note large size of the seeds (with husk) of the hybrid. (B) Seeds of Fuko (top row), dehusked seeds of intergenic  $F_1$  hybrid (middle row), and dehusked seeds of the grass parent Alkar (bottom row). (From Jauhar, 1995).

(e.g., Jauhar, 2003b), thereby facilitating their cytogenetic manipulation and enhancement.

#### Gametocidal Chromosomes and Induction of Translocations in Hybrids

The use of chromosome breaking action of some alien chromosomes offers a novel technique of inducing crop–alien chromosome translocations. Certain *Aegilops* chromosomes become gametocidal and induce chromosome breakage when introduced into wheat (Endo, 2003). Thus, by introducing these gametocidal chromosomes into a wheat–alien addition or substitution line, random wheat–alien translocations were recovered in the selfed progenies. Shi and Endo (1999) were able to induce structural changes in barley chromosomes added to wheat utilizing gametocidal chromosomes derived from *Ae. cylindrica*. Using such a gametocidal system, Masoudi-Nejad et al. (2002) transferred rye chromosome segments in wheat.

#### Modern Biotechnology: A Means of Genetic Enrichment of Crop Plants

Conventional plant breeding (Duvick, 1984; Jauhar, 1988; Khush, 1999, 2001), sometimes assisted by marker-assisted selection (Dubcovsky, 2004; Lapitan and Jauhar, 2006), and wide hybridization coupled with manipulation of chromosome pairing (Friebe et al., 1996; Fedak, 1999; Jauhar and Chibbar, 1999; Jauhar, 2003a) has clearly been instrumental in producing superior crop cultivars. However, these procedures are time consuming. Conventional breeding may take 10 or more years to transfer a trait from a donor species into a crop cultivar. Wide hybridization is undoubtedly an effective means of incorporating desirable alien genes into crop cultivars, but it has several limitations. It results in transmission of unwanted alien chromosomes and adverse

genetic interactions can lead to sterility. Other efficient means of gene transfer have therefore been explored.

#### Dawn of Genetic Engineering: Direct Gene Transfers

Recent biotechnological tools of direct gene transfer help engineer into plants new characters that are otherwise very difficult to transfer by breeding programs. The world's major crops are being transformed by direct DNA delivery by microprojectile bombardment and other methods of direct gene transfer (e.g., Jauhar and Chibbar, 1999; Dahleen et al., 2001; Muthukrishnan et al., 2001; Patnaik and Khurana, 2001; Repellin et al., 2001; Gelvin, 2003; Sharma et al., 2003; Sahrawat et al., 2003; Altpeter et al., 2005).

#### Prerequisites for Successful Genetic Transformation

The main prerequisites for efficient genetic transformations are: (i) in vitro regeneration; (ii) a DNA delivery system; and (iii) functional introduced DNA.

**In Vitro Regeneration.** An efficient in vitro regeneration system must be standardized before introducing exogenous DNA into single cells. A major limitation to cereal transformation has been the lack of an efficient in vitro regeneration by somatic embryogenesis. Protoplasts prepared from immature embryo-derived suspension cultures of pearl millet, for example, were among the first cereal cells that were shown to possess totipotency (Vasil and Vasil, 1980). Soon afterward, regeneration procedures for immature embryos, young inflorescences, anthers, and shoot apices were developed (Vasil, 1987) and most of these explant tissues or cells are used for transformation (Jauhar and Chibbar, 1999; Repellin et al., 2001; Cho et al., 2004). Thus, standardization of a suitable regeneration protocol of durum wheat from scutellar cells (Bommineni and Jauhar, 1996) facilitated the production of transgenic durum (Bommineni et al., 1997). Since then

there have been several reports of durum transformation (He et al., 1999; Pellegrineschi et al., 2002) that allowed incorporation of value-added traits into this important cereal. Because regeneration efficiency varies among species and often among cultivars of the same species, genotype-specific regeneration and transformation protocols will need to be established for best results (Machii et al., 1998; Repellin et al., 2001).

**DNA Delivery System.** Gene transfers in plants have been achieved through electroporation (Shillito, 1999), polyethylene glycol treatment (Funatsuki et al., 1995), particle bombardment (Altpeter et al., 2005), and *Agrobacterium*-mediated methods (Komari and Kubo, 1999; Gelvin, 2003), among others. The first transgenic cereals were produced from rice and maize protoplast cultures into which exogenous DNA was introduced by electroporation (Shimamoto et al., 1989) or by polyethylene glycol treatment (Zhang and Wu, 1988). The most commonly used method of DNA delivery into plant tissues is particle bombardment or biolistics, which facilitates simultaneous introduction of several genes (Vasil et al., 1992). Chen et al. (1998), for example, achieved the co-introduction of 13 genes into rice. Microprojection has become an important method for cereal transformation (Jauhar and Chibbar, 1999) and for study of gene expression and regulation (Klein and Jones, 1999).

**Introduced DNA to Be Functional.** For successful commercialization of transgenic crops, a stable and consistent expression of the gene(s) of interest is necessary (Kathuria et al., 2003). Several cases of transgene silencing have been reported in plants (Yu and Kumar, 2003), both monocots (Iyer et al., 2000) and dicots (Fu et al., 2005). Mechanisms of gene silencing are under study to learn how to avoid this phenomenon.

### Genetic Transformation: A Rapid Tool for Crop Improvement

Traditional breeding is generally notoriously slow in transferring a desired trait into an otherwise superior crop cultivar. The time needed to transfer a desired gene into a crop plant depends on the source of the gene and the evolutionary distance of that source to the recipient crop plant. If the gene source is a landrace or a related species, forming a primary gene pool with the crop species in question, the gene transfer may take 5 to 8 yr if not longer. Less related wild species belonging to the secondary or even tertiary gene pool may be rich reservoirs of genes for agronomic traits like disease or pest resistance, but to transfer such genes into crop cultivars may take 10 to 15 yr or even longer, if they are at all possible. Pre- and post-fertilization barriers may impede sexual hybridization between the donor and the crop species and compound the problem of alien gene transfers. In some cases, it may not even be possible to incorporate a certain trait by conventional means because a suitable donor may not be available or, if available, it may not be possible to hybridize the donor species with the crop plant.

Genetic engineering offers an excellent tool for asexually inserting a well-characterized gene(s) of unrelated organisms into plant cells, which on regeneration produce full plants with the inserted gene(s) integrated into their genome. This process may take less than a year to about 18 mo in some cases, thus accelerating the process of genetic improvement of crop plants. Moreover, this exciting technology allows access to an unlimited gene pool without the constraint of sexual compatibility. Genetic transformation by microprojectile bombardment has, for example, been demonstrated in wheat and other cereals (Jauhar and Chibbar, 1999; Dahleen et al., 2001; Jauhar and Khush, 2002; Altpeter et al., 2005). Some instances of this rapid crop improvement are described below.

### Resistance to Insect Pests

Numerous insect pests attack crop plants and cause enormous losses, threatening global food security. European corn borer [ECB, *Ostrinia nubilalis* (Hübner)], for example, causes a loss of up to 2000 million dollars annually in the USA alone (Hyde et al., 1999). Resistance breeding by conventional means is cumbersome and fraught with uncertainty. To breed a corn cultivar with resistance or even partial resistance to ECB may well take 10 to 15 yr by traditional breeding, provided a suitable resistance donor is available. Thus, through 12 yr of breeding, Syngenta, a Swiss agrochemical company, was able to produce a corn cultivar with only 10% resistance to ECB (pers. comm., 2002). However, a gene from a soil-borne bacterium, *Bacillus thuringiensis* (Bt), when bioengineered into the corn genome, confers almost complete resistance to ECB. This is an efficient means of eliminating the pest damage and pesticide application without affecting grain yields. Thus, Bt-corn acquired the capacity of an efficient pesticide—a biopesticide. It took Syngenta only 5 yr to engineer the Bt gene into corn.

Scientists at the University of Minnesota estimated that farmers averaged several times greater returns on their investment by using Bt corn for insect control, compared to the use of a chemical insecticide (Ostlie et al., 1997). The Bt corn hybrids had 4 to 8% higher grain yields than standard hybrids when infested with ECB (Lauer and Wedberg, 1999). Moreover, Bt corn is beneficial to the environment and the Bt-induced insect resistance in corn is much safer to farmers and other field workers, compared with the use of a chemical insecticide. Based on safety data, the U.S. Environmental Protection Agency (EPA) authorized commercial planting of Bt corn varieties (Palevitz, 2001). Several transgenic crops with insecticidal genes have been introduced in temperate regions of the world (Sharma et al., 2004). Transgenic rice varieties resistant to yellow stem borer [*Scirpophaga incertulas* (Walker)] have been produced in India (Ramesh et al., 2004).

Because of its higher productivity and positive health effects through reduced pesticide use, Bt cotton has been commercialized aggressively especially in Asian countries like China (Huang et al., 2002a) and India (Whitfield, 2003). Carrière et al. (2003) found long-term

regional suppression of pink bollworm [*Pectinophora gossypiella* (Saunders)] by Bt cotton. Bt rice has the potential to eliminate yield losses caused by lepidopteran insects, estimated at 2 to 10% of Asia's annual rice yield of 523 million tons (High et al., 2004). Field trials of transgenic rice suggested high tolerance of transgenic rice against yellow stem borer (Bashir et al., 2004). Most recently, an insect-resistant variety GM Xianyou 63 that was produced by inserting a Chinese-created *B. thuringiensis* gene, showed resistance to rice stem borer (*S. incertulas*) and leaf roller [*Cnaphalocrocis exigua* (Butler)] and is on the threshold of being released for commercial cultivation in China. This insect-resistant variety is reported to benefit small farmers because of higher crop yields and reduced use of pesticides, which is important for health reasons (Huang et al., 2005).

### Resistance to Diseases

Various fungal, bacterial, and viral diseases pose a major threat to global food security. Conventional plant breeding offers a useful means of breeding disease-resistant cultivars, provided a reliable donor of resistance is available. Chromosome engineering through wide hybridization has been successfully employed to transfer specific disease resistance genes from alien donors into wheat cultivars (Friebe et al., 1996; Jauhar and Chibbar, 1999; Jauhar, 2006). However, resistance breeding through these techniques can be painfully slow. Tools of biotechnology offer great promise for accelerating this process.

**Transgenic Approaches to Control Fungal and Bacterial Pathogens.** Engineering with antifungal genes could help produce crop plants resistant to fungal pathogens (Datta and Muthukrishnan, 1999; Dahleen et al., 2001; Jauhar and Khush, 2002; Sahrawat et al., 2003). The role of chitinases in fungal protection has been documented in rice (Datta et al., 2001; Itoh et al., 2003). Genetic engineering has been employed to contain FHB, a ravaging disease of wheat (Anand et al., 2003, 2004). Some proteins, called defensins, are small cysteine-rich peptides with antimicrobial activity. Gao et al. (2000) demonstrated that an antifungal defensin isolated from alfalfa (*Medicago sativa* L.) displayed a strong activity against the fungus *Verticillium dahliae* Kleb. and that expression of this peptide in transgenic potato (*Solanum tuberosum* L.) conferred resistance to the fungus. Another alfalfa defensin was shown to inhibit the growth of the FHB pathogen *Fusarium graminearum* in vitro (Spelbrink et al., 2004), and we are attempting to express this peptide in transgenic wheat.

Dutch elm disease (DED) has destroyed more than 20 million elm trees (*Ulmus procera* Salisb.) in the UK over the last three decades, and more than 70% of the elms (*Ulmus americana* L.) in the USA have perished because of the DED fungal pathogen in the past 70 yr (Gartland, 2002). According to Professor K. Gartland of Scotland, genetically modified elms with resistance to the fungal pathogen “could help tackle damaged landscapes and ecosystems blighted by tree fungal diseases, such as

Dutch elm disease and Chestnut blight, throughout the world” (*The Independent*, Scotland, 28 Aug 2001). This is an example of environmentally friendly biotechnology that could save the threatened landscapes and ecosystems worldwide (Gartland et al., 2002, 2003).

Transgenic approaches have also helped combat bacterial diseases. Thus, bacterial blight of rice [caused by *Xanthomonas oryzae* pv. *oryzae* (Ishiyama) Swings et al.] occurs throughout the rice-growing areas and causes serious yield losses. *Xa21*, a gene with broad-spectrum resistance to bacterial blight, was cloned through map-based cloning (Song et al., 1995). Tu et al. (2000) introduced the cloned gene into a widely grown rice variety IR72 with promising results. An attractive strategy to produce multiple pest tolerance is to stack up genes (Datta et al., 2002).

**Pathogen-Derived Resistance to Viral Diseases.** Transgenic technology also offers an excellent option to protect crop plants against devastating viral pathogens. Transformation of plants with nucleotide sequences derived from viral genomes has been shown to provide protection against the virus from which the sequences were derived. The evidence for such a pathogen-derived resistance (PDR) was provided by Powell-Abel et al. (1986), who demonstrated that transgenic tobacco plants expressing *Tobacco mosaic virus* (TMV) coat protein were resistant to the virus. Beachy et al. (1990) suggested that expression of a virus coat protein as a transgene in a plant confers resistance to the virus in direct proportion to the quantity of coat protein produced by the transformed plant. This novel technique opened up new avenues of controlling viral diseases (Lomonosoff, 1995; Bendahmane and Beachy, 1999) in crop plants and fruit trees. *Rice yellow mottle virus* (RYMV) is a serious viral disease causing enormous losses in rice yields. Because of lack of a conventional solution to this problem, a transgenic approach based on PDR was successfully employed to produce an RYMV-resistant rice variety (Pinto et al., 1999).

Transgenic wheat plants, engineered with the coat-protein gene of *Wheat streak mosaic virus* (WSMV) conferred protection against some WSMV strains (Sivamani et al., 2002). The PDR technology offers a promising means for inducing virus resistance in a variety of plants (Wesseler, 2003) including potato (Schubert et al., 2004). Coat-protein-mediated resistance has helped to control *Papaya ring spot virus* (PRSV) in papaya (*Carica papaya* L.) in Hawaii (Gonsalves, 1998; Ferreira et al., 2002) and the papaya industry was spared from disaster posed by PRSV (Gonsalves, 2003).

### Tolerance to Abiotic Stresses

Abiotic stresses, including drought and salinity, are estimated to cause yield losses worldwide of more than 50% (Bray et al., 2000). Transgenic approaches offer an option to enhance drought (Abebe et al., 2003) and salt tolerance (Apse and Blumwald, 2002; Flowers, 2004). Although several abiotic stresses pose a limitation on yields of crop plants like wheat, drought is a major cause

of yield loss and it is very difficult to breed drought tolerance through conventional breeding (Trethowan et al., 2001). Abebe et al. (2003) demonstrated that wheat engineered with the *mtlD* gene from *Escherichia coli* had improved tolerance to water stress and salinity. Garg et al. (2002) showed that overexpression of *E. coli* trehalose biosynthetic genes (*otsA* and *otsB*) as a fusion gene provided increased tolerance to abiotic stress in rice, resulting in elevated capacity for photosynthesis under drought and low-temperature stress conditions. Thus, transgenic technology holds the possibility of engineering abiotic stress tolerance into cereal crops. Genetic engineering in conjunction with marker-assisted traditional breeding could help engineer plant tolerance to abiotic stresses (Vinocur and Altman, 2005).

### Biofortification of Crops to Combat Nutritional Deficiency

Some 842 million people worldwide are malnourished (<http://www.fao.org/english/newsroom/news/2003/26659-en.html>; verified 10 May 2006). Most of these people live in the impoverished countries of Asia and Africa. Therefore, improvement of the nutritive value of food crops should be a high priority to alleviate deficiencies for protein, minerals, and vitamins, in addition to increasing crop yields. Iron deficiency, a most common dietary deficiency among the poor nations, affects especially children and women of reproductive age. In pregnant women, severe anemia may cause fetal growth retardation and large-scale maternal deaths (Gillespie, 1998). Nearly 400 million people in the world are reported to be at risk of vitamin A deficiency, which leads to blindness and premature death. Some 100 to 250 million children under 5 suffer from vitamin A deficiency, and half a million children become partially or totally blind each year (Conway and Toenniessen, 1999; Toenniessen, 2000, 2002).

It may be very difficult to improve nutritional deficiency, particularly for iron and vitamin A, using traditional crop breeding. Efforts are being made toward biofortification of crop plants using tools of biotechnology, and levels of essential nutrients have been increased. Genetic engineering was employed to raise the micronutrient content of rice, the staple food of more than one-third of the world population. Rice grains do not normally contain  $\beta$ -carotene, which is the precursor of vitamin A. However, they contain geranylgeranyl pyrophosphate that can be sequentially converted to  $\beta$ -carotene by four enzymes. By engineering rice with the four genes for these enzymes, two genes from daffodil and two from the bacterium *Erwinia uredovora*, Potrykus and his collaborators “instructed” rice to produce vitamin A. Later, by incorporating the iron-synthesizing ability in it, they were able to produce rice grains rich in vitamin A as well as iron (Ye et al., 2000; Beyer et al., 2002). The resulting rice, called Golden Rice, has the potential of saving millions of lives and averting blindness among millions of children, and is therefore referred to as the “grains of hope.” Other transgenic strains with improved nutritional quality have been

produced in both japonica and indica rices (Datta et al., 2003), and this strategy is being applied to other cereal crops (Poletti et al., 2004). Paine et al. (2005) developed Golden Rice 2 by incorporating a phytoene synthase gene (*psy*) from maize in combination with the *Erwinia uredovora* gene used to generate the original Golden Rice. They observed up to 23-fold increase in total carotenoids compared to Golden Rice.

The potato is the most important noncereal food crop for human consumption and, therefore, the need to improve its nutritional quality cannot be overemphasized. Chakraborty et al. (2000) demonstrated that expression of the *AmAl* gene (from amaranth, *Amaranthus hypochondriacus* L.) in transgenic tubers resulted in a significant increase in most essential amino acids as well as in higher protein content in tubers compared with nontransgenic potato plants. Through metabolic engineering, Ducreux et al. (2004) produced high carotenoid potato tubers containing enhanced levels of  $\beta$ -carotene and lutein. By incorporating three genes from algae and mushroom species, a “super-healthy” cress was created (Pilcher, 2004). Using a novel transgenic approach involving organ-specific gene silencing on tomato, Davuluri et al. (2005) have significantly increased the content of both carotenoids and flavonoids, which are highly beneficial for human health. It is encouraging to note that numerous GM food crops are making a valuable contribution to human nutrition (Bouis et al., 2003).

### Biotechnology and Human Health

As stated above, biotechnology is playing a significant role in nutritional enhancement of certain human foods, which has a direct bearing on human health. There are some other areas, also directly related to human health, in which modern technology has potential applications. Tools of biotechnology can help accomplish genetic modifications and improvements in plants, hitherto impossible to achieve by cytogenetics or conventional plant breeding. Some of these remarkable applications of biotechnology are outlined below.

#### Edible Vaccines

Vaccines have saved millions of lives and thus played a tremendous role in human health for almost 200 yr. Such vaccines could help save animal lives as well by providing protection against contagious viral diseases such as rinderpest. Khandelwal et al. (2004) developed transgenic peanut (*Arachis hypogaea* L.) expressing hemagglutinin (H) protein of rinderpest virus. Oral immunization of mice with transgenic peanut induced H-specific antibodies, indicating potential for producing an edible vaccine for rinderpest. Modern biotechnology may contribute toward the production of inexpensive edible vaccines. Lack of proper refrigeration poses a major problem for vaccinating the poor in less developed countries because the heat makes drugs lose their efficacy. Researchers worldwide have been focusing on producing plant-based vaccines that can be eaten uncooked in such fruits and vegetables as melons, tomatoes, and banana.

Edible vaccines administered orally through GM foods could become available at a fraction of the current costs, estimated at two cents instead of the usual \$15 for an injectable dose (DaSilva, 2001). Fully immunizing one person against hepatitis B can cost as much as \$450 (American Medical Assoc., 2001). Edible vaccines are also safe because they can be administered without refrigeration, syringes, or needles. They may save millions of people who die because of lack of access to traditional inoculants (Langridge, 2000). With appropriate genetic engineering, certain food crops could provide immunization against deadly diseases like hepatitis or tuberculosis. Edible vaccines against measles, cholera, and hepatitis B are being developed in India (Tripurani et al., 2003; Kumar et al., 2005). Charles Arntzen, of the Biodesign Institute at Arizona State University, has genetically engineered potatoes to produce a vaccine against hepatitis B virus, which kills one million people every year. He reported that in a trial of an edible vaccine, up to 60% of volunteers who ate chunks of the raw potato developed antibodies against the virus (Ariza, 2005; pers comm., Feb 2006). Vaccines against pneumonia and bubonic plague orally immunogenic to mice have also been developed (Alvarez et al., 2006). Horticultural crops may well serve as vaccine factories and we may see a day when, instead of taking an injection, one may only need to eat a banana or perhaps a tomato.

### Genetic Decaffeination of Coffee

Tea [*Camelia sinensis* (L.) Kuntze] and coffee (*Coffea arabica* L.) provide some of the most widely used beverages in the world. As much as people like to have tea or coffee, some of them would like to have little or no intake of caffeine, an important stimulant in both tea leaves and coffee beans. Caffeine can cause occasional side effects, including elevated blood pressure and heart palpitations (see Kato et al., 2000). Therefore, the demand for decaffeinated coffee and tea has been increasing in recent years. The commercial process of decaffeination currently available is not only expensive, it leaves certain chemical residues, and may also lead to loss of flavor for discerning consumers.

Methods of genetically decaffeinating coffee have been tried with remarkable success. Caffeine synthase is an enzyme that catalyses the final two steps in the caffeine biosynthesis pathway. Kato et al. (2000) cloned the gene encoding caffeine synthase from young leaves of tea, paving the way for creating tea and coffee plants that are naturally deficient in caffeine. Decaffeinated coffee is growing on genetically modified bushes that could yield low-caffeine beans in 3 or 4 yr (Ogita et al., 2003; Pilcher, 2003a) and these transgenic beans could rival industrial decaffeination if they gain public approval (Silvarolla et al., 2004).

### Novel Applications of Transgenic Technology

#### Phytoremediation

Widespread contamination of the environment caused by manufacture, testing, and disposal of explo-

sives is becoming a matter of great concern. Certain soil bacteria are known to have biodegradative capability. Scientists in England successfully introduced pentaerythritol tetranitrate reductase, the bacterial enzyme initiating degradation of explosive residues, into plants, and the transgenic plants so created were used for bioremediation of contaminated soils (Rosser et al., 2001; Wong, 2001). Such an application of biotechnology has great promise for cleaning the environment.

#### Reducing Allergenicity of Crop Plants

The public has some concerns about creation of new allergens in GM foods, although natural foods like peanuts are known to produce allergic reaction in some people. It has been shown that genetic engineering can in fact make a food less allergenic. Soybean, for example, is known to cause allergies in humans. Herman et al. (2003) used the transgene-induced gene silencing to shut down the gene that codes for the protein believed to cause most soybean allergies. This novel approach to reducing allergies should add nutritional value to crops.

#### Genetic Engineering of Christmas Trees

Another novel application of modern biotechnology has been explored and has resulted from the isolation of genes for light emission from insects and jellyfish. Scientists at the Institute of Biotechnology at Zurich's Swiss Federal Institute of Technology and researchers at Michigan State University in the USA have engineered a Christmas tree that could light up on its own, putting an end to the frustrating ritual of manually putting on lights every year (Lean, 2001; Read, 2001).

#### Resistance to Acceptance of Modern Biotechnology

As documented above, tools of modern biotechnology have already produced encouraging results in accelerating crop improvement in terms of resistance to insect pests and diseases, tolerance to abiotic stresses, and nutritional enhancement of food crops (Cook, 2000; Jauhar and Khush, 2002). Moreover, this technology has potential applications in producing food vaccines, in genetic decaffeination of coffee, and could have several other novel applications. Such genetic modifications or improvements in crop plants would be impossible to achieve by conventional tools of cytogenetics or plant breeding. Unfortunately, however, this relatively new technology is facing resistance from certain sectors. Attempts have been made to create fear about the potential adverse impact of GM foods or plants on human health and the environment (Borlaug, 2000; Marris, 2001; Falk et al., 2002; Jauhar and Khush, 2002) to the extent that GM experimental materials are being destroyed (Pilcher, 2003b). Although the concerns or perhaps misconceptions of certain groups may not be valid, these issues must be adequately addressed to satisfy the general public. Some of the perceived dangers of transgenic technology are discussed below.

## Issues of Human Health

A major concern is the possibility or perception of health risks posed by GM foods. However, the safety record of transgenic crops and their products testifies to their wholesomeness. There is no report so far of anyone falling ill by consuming GM food, which millions of people consume everyday. In the USA, more than 60% of all processed foods contain transgenic ingredients, but not a single transgenic food product has been shown to have any harmful effects (Vasil, 2003). Thus, regardless of consumer concerns, it remains true that genetically engineered foods have not made anybody sick (Radin, 2003). Additionally, the British Medical Association reaffirmed that there is no evidence that GM foods pose any threat to human safety (*The Observer*, 25 May 2003). There is overwhelming evidence that the bacterium *B. thuringiensis* and the transgenic crops expressing *cry* genes do not pose a threat to mammalian health (de Maagd et al., 2005). In contrast, in an extensive study by the American Medical Association, 20% of the 548 drugs approved for human use during the past 25 yr were later found to have serious or life-threatening effects, some possibly contributing to 1002 deaths (Vasil, 2003).

## Genetic Pollution of Related Plants

Another major concern is the potential for unwanted movement of a transgene from a genetically engineered crop plant to its relatives—whether cultivated or wild. Thus, a transgene for herbicide resistance could get incorporated into a wild relative, thereby creating a “super weed” that might be hard to control. The possibility of such genetic pollution through a misplaced transgene exists in some cases (Messeguer, 2003; Stewart et al., 2003; Armstrong et al., 2005), but in most cases it is unlikely to happen because of the difficulty of hybridization between a transgenic crop plant and its wild relatives and the need for embryo rescue to obtain a hybrid under laboratory conditions (Jauhar and Khush, 2002). Nevertheless, some gene flow does occur from transgenic rice to its wild relatives or to other rice cultivars (Song et al., 2003; Zhang et al., 2003; Chen et al., 2004; Messeguer et al., 2004), but this should not be a serious cause for concern. Several genes for insect pest and disease resistance have, for example, been transferred to cultivated rice using traditional breeding. However, it must be noted, that so far there is no known case where wild or weedy rice biotypes of rice, such as red rice, have become more resistant by out-crossing with cultivated rice (High et al., 2004).

Even then, insertion of transgenes into a crop plant's chloroplast DNA (plastid engineering) would alleviate the risk of transgene escape via pollen, which is the most common mode of genetic pollution. This and related issues have been discussed by Jauhar and Khush (2002).

## “Tinkering” with Nature

An underlying argument of opponents of modern technology is that it is unnatural and therefore unsafe.

Thus, transgenic technology as a means of introducing new genes into plants is considered by some as “tinkering” with nature. This technology is considered to be “inherently and morally wrong” (Hackett, 2002). Even Prince Charles of England contends that creating genetic modifications by transgenic techniques takes “mankind into realms that belong to God, and God alone” and that “life on earth could be wiped out by scientists playing God” (*Daily Telegraph*, London, 28 April 2003). Such expressions of concern can in fact adversely influence the growth of biotechnology. Arntzen et al. (2003) discuss the politics involved in adopting GM crops.

We must remember that humans, in their efforts to ensure and improve food production, have been tinkering with nature for centuries. Traditional plant breeding, involving selection pressure for the most suitable and desirable crop cultivars, constitutes tinkering with nature. It is, in essence, human-made evolution. Any breeding activity is accompanied by genetic modifications, which in the last analysis involve changes at the DNA level. The newer biotechnological tools of gene transfer into crop cultivars are, in fact, a refinement of earlier ones, and genetic enhancement by those techniques poses no greater risk to the consumer. Many of the current crop cultivars we consume do, after all, contain genes of alien origin (Jauhar and Khush, 2002).

## Modern Biotechnology as a Supplement to Conventional Plant Breeding

In the quest for food, early humans practiced crude methods of improving food production. These methods included selection of superior strains for cultivation and further selection as and when needed. And those early cultivators and selectionists could in a way be called the first “plant breeders.” In that sense, plant breeding is as old as agriculture itself, the beginnings of which are believed to have occurred about 10 000 yr ago. Thus, the art of plant breeding was developed long before the principles of genetics and cytogenetics became known. Several thousand years ago, the early “plant breeders” intuitively looked for, successfully recovered, and skillfully propagated genetic variants or recombinants that showed desirable traits. Working under a myriad of cultural contexts, these breeders or perhaps selectionists turned the relatively useless weedy species into crop plants that sustain us today.

## Tackling Complex Problems

As civilizations progressed and the demand for food and other necessities increased, better methods of plant improvement were devised or became available. With the discovery of the laws of genetics and the advent of the techniques of cytogenetics at the turn of the 20th century, the art of plant breeding became a science-based technology and the process of genetic improvement was accelerated considerably. Thus, the discovery of hybrid vigor and sophisticated methods of selection helped raise crop yields.

### The Evolution of Plant Breeding

The process of plant breeding has continued to evolve over the years (Fig. 5). As the complexities of plant improvement increased, and several insect pests and diseases became a threat to steady and adequate food supply, even better methods were adopted to tackle these problems. Wild genetic resources as sources of insect pest and disease resistance were tapped. With the means of genomic reconstruction that became available, the process of plant improvement was further accelerated. Wide hybridization coupled with cytogenetic manipulation of chromosome pairing helped to introduce desirable traits from wild relatives to crop plants such as polyploid cereals like wheat and oat. Specific examples of breeding for disease resistance using tools of cytogenetics are described in earlier sections. Sophisticated cytological techniques such as fluorescent-GISH for characterizing alien chromatin into the crop genomes helped the process of alien gene transfer (Ceoloni and Jauhar, 2006). Thus, the application of cytogenetic tools strengthened the scientific basis of plant breeding.

Later, we were faced with more complex problems such as breeding for resistance to viral diseases and nutritional enhancement of crop plants for which the available techniques of plant breeding or cytogenetics were not adequate. The availability of the tools of modern biotechnology, that allowed direct insertion of genes for value-added traits, led to the dawn of genetic transformation that has considerably helped the process of crop improvement (Jauhar and Khush, 2002). The addition of these newer tools to the arsenal of the plant breeder led to the birth of molecular plant breeding

(Fig. 5). The conventional tools that more than doubled world grain yields since 1960 seem to have lost their edge and, therefore, bold efforts to bioengineer crops seem the best hope for a new surge in harvests (Mann, 1999). The sophistication of the tool to be applied should depend on the complexity of the problem to be solved. With the emergence of new problems newer tools will need to be applied. As Sir Francis Bacon pointed out: “It would be an unsound fancy to expect that things which have never yet been done can be done except by methods which have never been tried.”

### Conclusion and Perspectives

The information and critical analyses provided in this article highlight the role of modern biotechnology in genetic improvement of plants—both crop plants and trees. Biotechnology has made rapid strides since it came into being about 15 yr ago and since the first transgenic plants were produced. Currently, we have reliable and efficient transformation protocols for a variety of plants, which include cereals, grain legumes, forage crops, oilseed crops, fiber crops, ornamentals, and forest trees. And genetic transformation offers direct access to an unlimited pool of desirable genes not previously accessible to breeders. The successful deployment of transgenic approaches to combat insect pests and diseases of important crops like rice, wheat, maize, barley, and cotton, is a remarkable accomplishment. Pest-resistant genetically modified crops can and are contributing to increased yields and agricultural growth in many developing countries and benefiting small-scale farmers (Qaim and Zilberman, 2003). This technology is

### Evolution of Plant Breeding

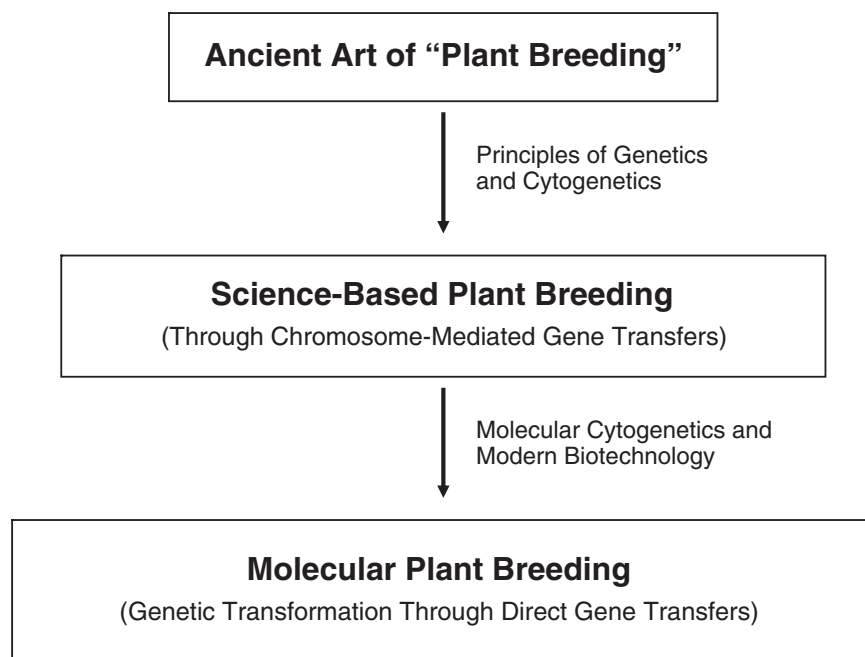


Fig. 5. Schematic representation of the steps of evolution of “plant breeding.” With the availability of more sophisticated tools, the art of plant breeding became science-based technology, and later led to the dawn of molecular plant breeding.

particularly useful in incorporating disease resistance in trees like elm that have a long life span because traditional breeding is very tedious in such cases. Genetically modified maize has been planted on more than 15 million hectares (James, 2003). The multi-million dollar losses from insect pests suffered by cotton farmers have been reduced by the use of Bt cotton (Walker-Simmons, 2003). Transgenic Bt crops that produce insecticidal toxins grew on more than 62 million hectares worldwide from 1996 to 2002 (Tabashnik et al., 2003). The PDR offers a unique approach for producing virus-resistant cultivars of crop plants, the resistance that is difficult to breed by traditional breeding.

Biofortification of crops to reduce or alleviate malnutrition among the poor masses constitutes another exciting development. Thus, the development of Golden Rice, which is genetically enriched with vitamin A and iron and hence has the real potential of saving millions of lives in impoverished countries, is a major milestone in tackling the problem of global hunger. Using conventional breeding, the superior traits of Golden Rice can be transferred to other locally adapted rice varieties in the developing countries. Countries like India have already initiated programs to breed the nutritional qualities into popular rice varieties (Potrykus, 2001; Sharma et al., 2003). Vasconcelos et al. (2003) have shown that the soybean ferritin gene driven by an endosperm-specific glutelin promoter resulted in high accumulation of iron and zinc levels in brown rice as well as in polished transgenic grains. Such a nutritional enhancement of a cereal crop would be unimaginable by conventional means alone. Yet another exciting application of transgenic technology is in the production of edible vaccines for immunization against deadly diseases like hepatitis B or tuberculosis, two of the serious diseases of the poor masses in Africa and Asia. Delivering vaccines through food would directly benefit the poor lacking proper medical facilities. That genetic engineering can help make foods less allergenic should help promote this technology.

As of 2005, one thousand million acres of biotech crops have been planted worldwide and they now cover the equivalent of 40% of the U.S. land area; in the USA 75% of the cotton, almost 50% the corn, and 85% of the soybeans planted are biotech-enhanced ([http://www.checkbiotech.org/root/index.cfm?fuseaction=search&search=%20US%20crops&doc\\_id=7300&start=1&fullsearch=0](http://www.checkbiotech.org/root/index.cfm?fuseaction=search&search=%20US%20crops&doc_id=7300&start=1&fullsearch=0); verified 10 May 2006). According to figures published on 12 Jan. 2006 by ISAAA (The International Service for the Acquisition of Agri-biotech Applications) the hectareage planted with biotech crops increased by 9.0 million hectares (22 million acres) worldwide in 2005 ([www.isaaa.org](http://www.isaaa.org); verified 10 May 2006). However, as with any other new technology, genetic engineering is not without adversaries, some of which even go as far as destroying experimental materials. This antiscience zealotry (Borlaug, 2000) and public hostility to modern biotechnology has been attributed to "lack of scientific literacy" (Bucchi and Neresini, 2004) and may impede human progress. The opponents of the new technology work on the premise that plant biotechnology is unnatural, unsafe, and inher-

ently wrong, and that it results in harmful products. However, the indisputable fact remains that conventional plant breeding is a form of genetic engineering that has been practiced for centuries in humanity's quest for food production. Any breeding activity ultimately involves changes at the DNA level. It would therefore appear ridiculous to suddenly get nervous about genetically altering crops now when fundamentally we have been doing pretty much the same thing for so long. There is no evidence to suggest that GM foods pose any threat to human safety, although work needs to be done on informing and reassuring the public about the global benefits of GM crops.

Most of the genetic improvement of crop plants and the consequent increase in yields were brought about by conventional breeding. These tools, although slow and sometimes tedious, will certainly continue to play a major role in crop improvement programs. The GM technology is an important weapon in our war against global poverty and starvation. And crop improvement through genetic engineering has in fact become a reality (Dunwell, 2000; Jauhar and Khush, 2002; Sahrawat et al., 2003; Bajaj and Mohanty, 2005). Transgenic crops have now been grown on more than 300 million acres in 15 countries around the world (Vasil, 2003) and more than 70 biotech plant varieties have been commercialized in the USA (Radin, 2003), and they incorporate several agronomic traits including resistance to insect pests and diseases (Walker-Simmons, 2003). European Union continues to hold restrictive practices on GM crops. In sharp contrast, however, the area under biotech crops grew the fastest in India compared to the rest of the world. According to the International Service for the Acquisition of Agri-biotech Applications (SAAA), India registered the greatest proportion of growth for any biotech crop globally in 2005, with Bt cotton production soaring by 16% ([checkbiotech.org](http://checkbiotech.org), Feb 27, 2006).

Genetically modified rice yields could soon supercede even the highest yielding hybrid rice in China, and recently published results of field trials of GM rice in China have brought the country one step closer to approval of commercial varieties (Zi, 2005). Biotechnology can help feed the billions of poor people who constantly struggle for a better life (Wambugu, 2001; Huang et al., 2002b; Conway and Toenniessen, 2003). A recent study by PG Economics shows that farmers using this technology increased their income by US\$27 000 million during 1996 to 2004 with additional environmental benefits realized; and the cumulative economic benefits during this period to developing countries (\$15 000 million) exceeded benefits to industrial countries (\$12 000 million) ([www.pgeconomics.co.uk](http://www.pgeconomics.co.uk); verified 10 May 2006). According to Science and Development Network, the Agricultural Biotechnology Network for Africa (ABNET) is helping to encourage the role of biotechnology in improving African agriculture—a move welcomed by Stanford Blade, Director of research at the Nigeria-based International Institute of Tropical Agriculture ([www.scidev.net/News/index.cfm](http://www.scidev.net/News/index.cfm); verified 11 May 2006).

Thus, modern biotechnology is playing and will continue to play an important role in human welfare, in









