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Gene Transfer in Crop Improvement - An Introduction and Overview

Robert M. Goodman
Calgene, Inc.

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ROBERT M. GOODMAN

Calgene, Inc., 1920 Fifth St., Davis, CA 95616

The application of recombinant DNA technology to plant breeding promises important applications in agriculture and advances in many plant science disciplines. Gene transfer at the molecular level can be routinely accomplished in an increasing number of plant species. For many of the world's important crops, new methods or refinements of proven methods will be needed and are being developed. Experience with gene regulation in transgenic plants is accumulating in many laboratories around the world. The action of any particular gene cannot be predicted precisely without reference to the genetic background in which it acts, but, qualitatively speaking, the cis-acting elements located near and within coding sequences of genes appear to play a dominant role in governing gene expression. This result means that chimeric genes can be constructed and expressed with at least qualitatively predictable results. Several agriculturally interesting traits have been engineered and are undergoing field trials. These traits include several different herbicide resistances, modified fruit characteristics, viral disease resistance, and insect tolerance in rapeseed, tobacco, tomato, potato, alfalfa and cotton. Much work is underway to provide a better understanding of plant metabolism and development with a view to engineering other traits. These traits include the composition of storage lipids, regulation of fruit development, and disease resistance. The new tools for genetic manipulation of crop plants provide important opportunities for improving crop performance and the economic and environmental performance of agriculture.

INDEX DESCRIPTORS: recombinant DNA, gene transfer, gene expression, crop improvement

Plant breeding as a science began with the discovery of the rules of inheritance of plant traits in the late 19th century. At the outset, the parental material used was largely the heterogeneous landraces in use in agriculture at the time. Today, much of plant breeding for development of new varieties uses as parents existing advanced material. The basic strategy in plant breeding consists of hybridization followed by evaluation and selection of new combinations of desired traits and has not changed significantly. What has changed from the early years is a vastly improved knowledge base and the enhanced agricultural adaptability of the parent lines [for an excellent collection of reviews on plant breeding see Anonymous, 1981].

The past eighty years have seen significant technical advances that have made a wider variety of genetic resources available to the plant breeder [Goodman et al., 1987 and references therein]. Intraspecific, interspecific and intergeneric sexual hybrids have been made and have made their contribution to crop improvement. An important use of such hybridizations has been introgression into adapted crop varieties of specific traits, for example for disease and pest resistance. Nevertheless, there has been an erosion of the degree of genetic heterogeneity in the parental material used in variety development for many major crop plants. The increasingly narrow genetic base on which the widely used genotypes for agriculture depends is the result of how the tools of genetic manipulation have been used, not an inherent limitation of the tools themselves. The tools themselves have brought increasingly greater power to the task of producing variability and also to its evaluation. The introduction of recombinant DNA methods further expands the generic and technical resources available to plant breeding programs.

Gene Transfer by Recombinant DNA Methods

The pioneering work on the nonsexual transfer of genes by recombinant DNA methods in higher plants has been with the *Agrobacterium*-mediated system in plants of the nightshade family. The critical information that made *Agrobacterium*-mediated gene transfer systems possible came from the elegant work of Chilton and colleagues who showed that in crown gall disease a region of bacterial plasmid DNA (the T-DNA) is transferred to chromosomal DNA in the plant nucleus, stably maintained there, and expressed in the absence of the bacterium [Chilton et al., 1977; Drummond et al., 1977]. The earliest transgenic plants obtained with this system generally were abnormal because they expressed genes encoding enzymes for phytohormone synthesis that were carried by the T-DNA [Weiler and Schröder, 1987]. Work on the molecular biology of T-DNA transfer led to the creation of disarmed plasmids, in which the transfer ability was preserved but the hormone biosynthesis and other T-DNA genes were deleted [De Greve et al., 1982]. Work in tissue culture led to use of protoplasts [Draper et al., 1982; Krens et al., 1982] and then to direct techniques where explants, such as leaf pieces or stem segments, from which differentiated shoots and subsequently roots could be obtained [Horsch et al., 1985]. Petunia [Fraley et al., 1984], tobacco [Horsch et al., 1985], and tomato [McCormick et al., 1986; Fillatti et al., 1987], all members of the nightshade family, have been the species of choice for most of this work. With such species, many transgenic individuals can be produced in a single petri dish. The vectors used all contain a constitutively regulated gene conditioning resistance to a phytotoxic antibiotic such as kanamycin. These selection systems yield a high proportion of transformed regenerants, and the plants obtained are normal in growth habit and fertility.

The DNA transfer event requires activation of a number of bacterial genes, which are both plasmid [Nester et al., 1984] and chromosome [Zorreguieta et al., 1988] encoded. Gene activation is caused by chemical signals elicited from the plant by wounding [Stachel et al., 1985]. Most of the bacterial plasmid gene functions required for transfer are known [Stachel and Nester, 1986]. At least three chromosomal loci are involved in the specific attachment of bacterial cells to the plant cell which must occur before transfer can be effected. Some of the events within the bacterial cell preparing the T-DNA for transfer are understood. A simple excision of the T-DNA from the plasmid is not observed; instead, various kinds of single and double stranded T-DNA intermediates have been detected [Koukolikova-Nicola et al., 1985; Stachel et al., 1986]. A specific direct repeat sequence of 25 base pairs defines the ends and determines the direction of the transferred DNA [Yadav et al., 1982; Wang et al., 1984]. Increasingly it is evident that the process is analogous to conjugative DNA transfer in bacteria [Buchanan-Wollaston et al., 1987]. Both the history and current status of the technology of engineering *Agrobacterium* for use in gene transfer systems have been comprehensively reviewed [Klee et al., 1987; Houck et al., 1988; Horsch et al., 1984; Kahl and Schell, 1982].

Extensions of the *Agrobacterium* system with other plant species beyond the nightshade family, even those susceptible to crown gall disease, have not been trivial. The difficulty in transferring the use of this system to other species has to do with the difficulties of manipulating the plant tissues, not intrinsic limitations of the *Agrobacterium* transfer machinery. The host range for crown gall among the dicotyledonous species is very wide. In addition to naturally susceptible species, others including some monocotylendonous species can be infected experimentally [De Cleene and De Ley, 1976; Hernalsteens et al., 1984]. The use of *Agrobacterium* strains in which the DNA sequences of a geminivirus [Harrison, 1985] have been cloned into a T-DNA, called agroinfection [Grimsley et al., 1986; Grimsley et al., 1987], reveals that even in nonhosts of *Agrobacterium*-induced crown gall disease, such as maize, *Agrobacterium* is capable of transferring DNA contained within the T-DNA. Upon mechanical inoculation of *Agrobacterium* containing a T-DNA with the cloned geminivirus (which is a pathogen of maize), the DNA virus is able to infect and spread systemically in the plant. This is a particularly powerful test of the ability of the *Agrobacterium* machinery to transfer DNA to maize cells, because this virus has never before been transmitted by mechanical means. That is, rubbing the virus or its DNA directly on the leaves does not result in infection. The only other agent capable of transmitting the virus is a species of leafhopper. The mechanism by which agroinfection results in virus inoculation, and in particular whether the transferred DNA is integrated into the recipient cell or the virus genome is somehow excised or transcribed out of the T-DNA, is not yet known.

Because *Agrobacterium* has not been the easy answer to genetic engineering of some of the important crop species, notably the grass family members such as maize, wheat, and rice, work on other cellular targets and other methods for gene delivery have been priorities in many laboratories. In particular, work in reproductive biology of plants offers some promise for development of efficient gene transfer systems. It has long been appreciated, for example, that microspores can give rise in culture to undifferentiated callus tissue of high morphogenetic potential [Heberle-Bors, 1985]. Anther and microspore culture is well advanced in the small grains and in species of the crucifer family. One case has been published [Neuhaus et al., 1987] where such a system was used for gene transfer and others are under development. Other work focuses on the fertilization system. Several unsubstantiated claims have been made over the years of introducing DNA via pollen [Sanford et al., 1985], but interest in this target remains because of its many attractive features from the point of view of commercial utility.

Other methods for delivering recombinant DNA through plant cell walls, across the plasmalemma, and into the nucleus of a cell have been demonstrated that do not require *Agrobacterium*. It was shown nearly twenty years ago that plant viruses and viral nucleic acids could be taken up by protoplasts [for a review see Takebe, 1975]. In recent years, definitive tests of this approach using recombinant DNA have been made and transgenic plants have resulted [Paszkowski et al., 1984; Potrykus et al., 1985]. Direct uptake of recombinant DNA by protoplasts, as with viruses and viral nucleic acids, is aided by various treatments of the cells together with the DNA. Various chaotropic ions have been used, as have certain polymers (polyethylene glycol, polyvinyl pyrrolidone) and the application of brief pulses of electrical potential (electroporation). Evaluation of the optimal parameters for these systems is generally speaking far from comprehensive. In the best systems, careful measurements have indicated successful transfer, integration and expression of selectable marker genes at 2% of the treated surviving cells [Shillito et al., 1985]. The best defined systems are again in the nightshade family, notably tobacco. Recent reports of transgenic maize [Rhodes et al., 1988; Shillito et al., 1989; Prioli and Söndahl, 1989] and success with regeneration from rice protoplasts [Kyozuka et al., 1987; Coulibaly and Demarly, 1986;

Yamada et al., 1986] gives hope for the possible usefulness of this approach in some major crops. The one drawback of any use of protoplasts is the time spent in culture and the resulting liability of undesirable genetic variation being introduced. The likely explanation of the infertility of the first transgenic maize plants reported [Rhodes et al., 1988] is that the material used for preparation of protoplasts had been in culture for months and the time from protoplast to plant is a minimum of several months.

Another direct method for introducing recombinant genes is microinjection. Again, early use of this approach was made in studies of plant viruses [Halliwell and Gazaway, 1975], but the widespread work underway in plant science today owes more to the technology and success of this approach in animal engineering with recombinant genes [Wagner et al., 1981; Hammer et al., 1985]. After several years of work and early reports of stable gene transfer to cells in culture [Crossway et al., 1986], a report in 1987 presented convincing evidence for integration, expression and inheritance of a marker gene introduced into a plant via microinjection [Neuhaus et al., 1987]. The species was rapeseed (*Brassica napus*) and the target was small somatic embryos derived from microspore culture. This is a labor intensive and somewhat inefficient process as it stands today, and for *B. napus* more useful systems based on *Agrobacterium* are available for this species [Pua et al., 1987; Radke et al., 1988]. But the work clearly shows the feasibility of the approach and provides a system with which improvements can be developed. Conceptually, success with somatic embryos offers encouragement for applications to zygotic embryos as well. Moreover, in species where single microspores can be isolated and manipulated, microinjection of microspores is a very attractive gene transfer approach. One would need to be able to identify at the uninucleate stage those microspores which will go on to develop into mature pollen. Microinjection into the clearly visible nucleus of such isolated microspores, before the first mitosis, might lead to a reasonable frequency of gene transfer and integration into the gamete genome. Then, by in vitro fertilization techniques [Hauptli and Williams, 1988], one could potentially obtain progeny seeds that are hemizygous for the trait(s) conditioned by the introduced gene(s).

In many agriculturally important species, the only structures from which whole plants can be readily derived by manipulation in culture are multicellular. The path from such cultures to single cells or protoplasts and back to regenerable cultures and eventually to plants has been, and even with some success remains, at best arduous and in many cases blocked. But with soybeans, maize, cotton, rice, wheat and many woody species, regeneration from multicellular structures, both nondifferentiated and differentiated, is possible. A delivery method that is compatible with such structures, and which in particular might deliver DNA to cells beneath the surface layer of a tissue, organ or callus culture, could be useful. Devices that may be an answer to some of these requirements have been described and tested with promising results [Sanford et al., 1987; McCabe et al., 1988]. The method involves high velocity bombardment of cells with microprojectiles made of tungsten or gold (in the range of 1 to a few microns in diameter) onto which DNA is precipitated. In the first report, transient expression after 24 to 48 hours of a marker gene in bombarded onion skin cells was reported [Klein et al., 1987]. Further work in numerous laboratories has been successful in extending the range of transient expression to such species as maize and soybeans, and reports are beginning to circulate and appear in the journals [Klein et al., 1988; Boynton et al., 1988; Johnston et al., 1988; McCabe et al., 1988] of stable heritable expression of genes introduced in this way.

In summary, gene transfer technology is advancing on a broad front, with increasingly more solid scientific underpinnings both in terms of the biology and technical manipulation of the target cells and in terms of the delivery methods. There are several aspects not

mentioned above that deserve at least passing comment.

• The methods of gene delivery for higher plants offer the experimentalist no control over the insertion site, and little control over the number of copies, of the recombinant gene.

• Injection with fine syringes of infloresences in the grass family has been reported [de la Peña et al., 1987] but it is very inefficient and has not been readily duplicated by other laboratories.

• Work with plant viruses as possible vectors for expression of recombinant genes is progressing [Grimsley et al., 1986; French et al., 1986; Harrison, 1985; Hull and Davies, 1983], but the absence among plant viruses of the ability to insert DNA into host chromosomal DNA probably limits the near-term utility of viral vectors for engineering stably inherited traits.

• Likewise, work on characterization of plant transposable elements is progressing [Freeling, 1984], but the use of these elements to develop vectors for stably carrying recombinant genes into plant chromosomes requires further work.

• Many of the world's most important crops (Table 1) are not yet on the list of crop species for which gene transfer by molecular methods has been demonstrated (Table 2).

Gene Expression

Sequences that contribute to the regulatory characteristics of a particular gene, whether it be the level of expression of constitutively expressed genes or the developmental or environmental induction of other genes, are found in the DNA upstream of the transcribed region of the gene (usually within less than 2 kbp), within the transcribed region of the gene (notably in introns), and downstream of the stop codon (either within the transcribed nontranslated region or beyond). It is beyond the scope of this contribution to review this work in any detail, but from a practical point of view, several general statements of importance can be made from what has been learned so far.

• The empirical work done to date is by and large very encouraging, both for engineers and for scientists. Regulatory elements governing organ specific, environmentally inducible and developmental gene expression appear, at least in qualitative terms, to be widely conserved in their function across plant species [for examples see Greenwood and Chrispeels, 1985; Schernthaner et al., 1988; Okamuro et al., 1986; Simpson et al., 1986; Thornburg et al., 1987; Chen et al., 1988].

• Plant genomes contain numerous gene families encoding proteins for the same function. In gene families, the divergence of sequences after the stop codon is generally great enough to allow detection of specific members of the family. Plant chromosomes also carry pseudogenes, including some that are transcriptionally active and others that are not [see for example O'Neal et al., 1987]. Pseudogenes are dysfunctional sequences with homology to functional genes. The practical implication of gene families and pseudogenes is to make cloning of specific genes and regulatory elements more expensive and more painstaking.

• Experience generally shows that the quantitative range of expression level (usually as measured by the steady state level of messenger RNA or as protein) seen among different plants obtained from replicated transformations with the same vector and gene construction is at least 10 fold [see for example, Fillatti et al., 1987]. This variability, from a practical point of view, means that in developing a transgenic plant with a desired phenotype, one wants to be able to evaluate several independent events. In our work, we generally aim to have a minimum of 15 plants derived from independent transformation events for evaluation.

Many of the proteins required for metabolism and other functions in plastids, mitochondria, the nucleus, and the apoplast, must be transported to their site of action from the cytoplasm where nuclear encoded proteins are translated. Most of the proteins found in the mature chloroplast, for example, are encoded in nuclear DNA, translated by cytoplasmic ribosomes and transported into the chloro-

plast [Chua and Gilham, 1977]. The machinery for delivery of these proteins is beginning to yield to investigation. Chimeric genes have been constructed and tested successfully for chloroplast delivery of proteins encoded by several bacterial and chimeric plant genes [Schreier et al., 1985; Van den Broeck, 1985; della-Cioppa and Kishore, 1988; della-Cioppa et al., 1987; della-Cioppa et al., 1986; Comai et al., 1988]. The proteins made in the cytoplasm bear a terminal sequence (in the cases known at the N terminal end of the protein) called a transit peptide which targets the protein for transport across the membrane. The mature version of the protein found within the organelle lacks the transit peptide. Within chloroplasts, the destiny of a particular protein is also governed by the information contained in the transit peptide [Smeekens et al., 1986]. Analogies to membrane receptors for hormones and other mammalian proteins are indicated, for example, by the detection of specific epitopes on chloroplast membranes recognized by an anti-idiotypic antibody directed against an antibody specific for a peptide sequence in the N-terminal transit peptide of a nuclear encoded chloroplast protein [Pain et al., 1988].

Another recent result that has broad commercial and scientific implications is engineering to reduce the level of a gene product by expressing genes or parts of genes in the opposite polarity to the target gene (so-called antisense RNA). An important plant phenotype, softening of tomato fruits during ripening, has been modified by this approach [Sheehy et al., 1988]. So has susceptibility to infection by certain RNA plant viruses, though in this case the phenotype may not be good enough to be of commercial utility [Cuozzo et al., 1988; Hemenway et al., 1988]. Antisense RNA makes it possible in principal to create mutant phenotypes of any gene for which the sequence is known in any species for which a gene transfer method is available. As more and more plant genes that encode enzymes of metabolism and structural proteins are cloned, rapid advances in our understanding of metabolic and developmental regulation should result from the use of this genetic approach. This will be a very powerful tool for answering important questions about how plants function in such areas of plant science as physiology, pathology, and morphogenesis [Goodman, 1988].

The Genes Available for Transfer

The generalized universality of the genetic code makes the range of organisms from which genes can be obtained for engineering of plants very wide indeed. To date, genes from gram negative and gram positive bacteria, plant viruses, yeast, actinomycetes, mammals, fowl, and an increasingly wide range of cultivated and noncultivated plant species have been expressed in transgenic plants. Microbial genes were the first to be used. Among selectable markers, the most widely used has been the gene from the transposon Tn 5 for aminoglycoside phosphotransferase II [Beck et al., 1982]; this enzyme detoxifies antibiotics related to neomycin. Microbial genes for resistance to hygromycin [van den Elzen et al., 1985; Waldron et al., 1985] and microbial [Zoly and Hänggi, 1981] and mammalian genes [Gasser et al., 1982] for dihydrofolate reductase conditioning resistance to methotrexate have also been used.

The first genes of agricultural interest to be tested were microbial genes for tolerance to herbicides. The economic and environmental rationales for engineering specific herbicide tolerances have been reviewed elsewhere [Goodman, 1987; Benbrook and Moses, 1986]. Attention early on focussed on N-phosphonomethyl glycine, a potent inhibitor of the shikimate pathway leading to the synthesis in bacteria and plants of aromatic amino acids. The herbicide has a very wide phytotoxicity spectrum, a relatively short environmental half life, is systemic, and because of the homology between bacteria and plants was a nice target to address as a test of engineering prowess where the result, if favorable, stood a good chance of being useful. Moreover, in plants, the pathway for aromatic amino acid synthesis occurs in

Table 1. World Production of Food and Industrial Crops, 1983. (Food and Agricultural Organization of the United Nations. 1984. 1983 Production Yearbook. FAO, Rome)

Food crops	Production 1000 metric tons	Industrial Crops	Production 1000 metric tons
Wheat	498,182	Sugar cane	888,735
Maize	344,103	Sugar beet	271,002
Rice	449,827	Seed cotton (meal)	43,993
Potato	286,472	Cottonseed (oil)	27,998
Barley	167,176	Sunflower	15,766
Cassava	123,153	Cotton (lint)	14,692
Sweet potato	114,842	Rapeseed	14,342
Soybean	78,566	Tobacco	6,090
Grape	65,167	Palm oil	5,869
Sorghum	62,483	Coffee	5,537
Tomato	52,240	Coconut (copra)	4,548
Oats	43,101	Jute	4,057
Banana	40,700	Rubber	3,866
Orange	38,171	Linseed	2,374
Apple	36,799	Oil palm (kernels)	2,147
Cabbage	35,794	Sesame	2,076
Coconut	34,890	Tea	2,020
Rye	32,194	Olive oil	1,564
Millet	29,563	Cocoa	1,557
Yam	23,299	Flax	699

plastids, so one expected that delivery of the protein to the plastid compartment would be helpful and probably necessary. Two independent research groups set out on this challenge in the early 1980s. One concentrated on engineering a mutant bacterial gene for expression in plants [Comai et al., 1985; Comai et al., 1988]. The other has pursued cloning and expression of a plant gene [Shah et al., 1986]. Both have been successful and have had plants in field trials since 1987. Other herbicide tolerance determinants, from mutant plants [Lee et al., 1988], actinomycetes [De Block et al., 1987; Thompson et al., 1987], and bacteria [Stalker et al., 1988a; Stalker et al., 1988b] have been cloned, engineered, expressed in transgenic plants, and entered field tests in 1988.

For many years, the very specific insecticidal activity of naturally occuring strains of Bacillus thurengiensis (Bt) has been known and for at least twenty years preparations of the bacterial spores containing an insecticidal toxin have been formulated and sold for control of lepidopteran pests. Several groups have now reported the expression in transgenic plants of the Bt toxin and have found effective insect control in bioassays in the lab and in field tests [Fischoff et al., 1987; Vaeck et al., 1987]. Other strategies for insect tolerance, for example using proteinase inhibitors, are also being pursued [Hilder et al., 1987].

Another impressive example of a useful phenotype resulted from expression of plant viral capsid protein genes in engineered plants which conferred a high degree of resistance against infection by the virus [Powell Abel et al., 1986; Cuozzo et al., 1988]. The affect is virus-specific. Plants expressing the capsid protein of tobacco mosaic virus are not resistant to tobacco ringspot virus, for example. The phenomenon has been demonstrated in laboratory experiments for at least six plant virus families to date and field trials are underway.

Another strategy to reduce the damage in crops caused by virus diseases has been demonstrated. At least two plant virus groups have satellite RNAs associated with them. Satellite RNAs are small RNA molecules that are completely dependent for their replication and transmission on a helper virus [Francki, 1985]. They are of interest because often when a virus infection occurs together with a satellite

RNA the symptoms of virus infection are attenuated. DNA copies of satellite RNA sequences expressed in transgenic plants confer the ability to attenuate the symptoms of virus infection just like the naturally occurring satellite/virus coinfection [Harrison et. al, 1987; Gerlach et al., 1987].

There is widespread interest in various metabolic pathways in plants that might be engineered to improve production economics or the value of the products made by crop plants. One active area of interest is fatty acid biosynthesis [Stumpf, 1987; Knauf, 1987]. Another is fruit development [Lincoln and Fischer, 1988; Sheehy et al., 1987]. Seed protein quality, altered carbohydrate characteristics, improved post harvest physiological characteristics, and tolerance to various kinds of environmental and biotic causes of stress are some other areas receiving attention. Many plant genes have been cloned. As the pathways of plant development and metabolism and the mechanisms of gene regulation become better understood the prospect of manipulations at key control points in these pathways to effect changes in plant performance or productivity that are the result of multigenic characteristics will become a reality too.

Table 2. A list of plant species for which published reports [through 1988] have appeared with definitive evidences for successful gene transfer using recombinant DNA methods. Only those species for which whole plants have been obtained are listed. Asterisks designate those species transformed using Agrobacterium-mediated gene transfer.

Transgenic Plants	Reference
Alfalfa*	Shahin et al., 1986
Arabidopsis*	Lloyd et al., 1986
Birdsfoot trefoil*	Stougaard et al., 1987
Black walnut*	McGranahan et al., 1988
Carrot*	Tepfer, 1984
Celery*	Catlin et al., 1988
Cotton*	Umbeck et al., 1987
Cucumber*	Trulson et al., 1986
Flax*	Basiran et al., 1987
Horseradish*	Noda et al., 1987
Lettuce*	Michelmore et al., 1987
Maize	Rhodes et al., 1988
Morning glory*	Tepfer, 1984
Mothbean*	Köhler et al., 1987
Petunia*	Horsch et al., 1985
Poplar*	Fillatti et al., 1987
Potato*	Shahin et al., 1986
Rapeseed*	Pua et al., 1987; Radke et al., 1988
Rye	de la Peña et al, 1987
Sunflower*	Everett et al., 1987
Tobacco*	Comai et al., 1985
Tomato*	Fillatti et al., 1986, McCormick et al., 1986

Genetic Improvement of Plants in the Future

The influence of gene transfer technology in the future of agriculture will be largely determined by other factors. I wish to conclude this overview with brief mention of three areas of importance that will contribute to how this technology will be used as the future of agriculture unfolds.

• In one sense, new genetic technology and the shifts (in the public sector) and massive increase (in the private sector at least) of expenditures in agricultural research and development that we have seen in the past several years could not have come at a more confusing time. I wish to make a case, however, for the argument that it also could not have come at a better time. Agriculture has long suffered from

underfunding of basic science directed towards understanding the biochemical and genetic basis of important traits. In part because of the "sizzle" of the new genetics, that situation is now being addressed, but more is needed, in particular in the public sector. The solutions to the economic and environmental difficulties facing much of agriculture today will in part flow from the wise redeployment of resources to fully exploit new technology and new strategies for managing the agricultural enterprise [e.g., National Research Council, 1987. National Research Council, 1989].

• We have to face and resolve the issue of how proprietary property rights will be handled in agriculture in the future. One of the chief attractions of major new funds for research and development in this field, at least in the private sector, has been the ability under several different statutes to establish claims of ownership over new plant varieties. This attraction has been considerably increased by the ruling of the Patents and Trademarks Office that recombinant DNA and living organisms can be protected under the utility patent laws. But this changes the free rights of plant breeders to use patented material as parents in development of new material that existed under the other laws used to protect rights to new varieties. How is the common good best to be served in this area? A high level of proprietary protection is important in an economic system that attempts to encourage risk-taking and innovation. But in plant breeding even private interests may not best be served by a property rights system that restricts the continuing and fundamentally important access to germplasm. My view is that this is of overriding importance and is an issue that should be addressed and resolved by agriculturalists with humanity's best interests in mind rather than by lawyers [see Barton, 1982; Williams, 1984].

• We face major challenges in agriculture today, and have major opportunities, to improve the economic and environmental health of the agricultural system. We have become dependent on chemicals that have effects that are undesirable. We use management practices that fail to prevent losses that we should not tolerate. All technology has its potential for clumsy use, and I do not make a special claim for genetic technology as being unambiguously not clumsy. What is needed is a better understanding of systems and a clearer vision of and commitment to a cleaner and less clumsy agriculture that at the same time is economical and productive. I believe that the integration of the full range of genetic technology — new and old — can go a long way to addressing these issues.

Here in Iowa you have a special opportunity and a special obligation to take a leadership position in the changes that will occur in agriculture. There is a clear commitment here of the State government to explore the potential of recombinant genetics in agriculture. There is a major commitment of the State government, at the same time, to improving the sustainability of agriculture as embodied in the establishment of the Aldo Leopold Center for Sustainable Agriculture. I hope the participants in these two initiatives recognize and act on the relevance that the one has to the other. I do not suggest that the Aldo Leopold Center should do recombinant DNA research, though it probably should do some because there are so many opportunities to address issues of sustainability using recombinant gene techology. Likewise, I think that it is not only the Aldo Leopold Center that should focus on the issues of sustainability. In fact, the whole system should focus on that important issue, and recombinant genetics should be directed towards the development of new approaches to managing the agricultural production system in a way that is more sustainable.

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