# PLANT BIOTECHNOLOGY, PLANT BREEDING, POPULATION BIOLOGY AND GENETIC RESOURCES

## *PERSPECTIVES FROM A UNIVERSITY SCIENTIST*

CALVIN O. QUALSET

*Genetic Resources Conservation Program*
*U. California, Davis*
*Davis, CA* 95616

*A Professor of Agronomy and Director of the GRCP, Qualset specializes in plant genetics and is active in policy evaluation in biological conservation.*

Plant biotechnology is developing as an applied disciplinary field and has gained high-level attention in public and private universities, the U.S. Department of Agriculture—Agricultural Research Service, and, of course, in the private sector. There is increasing interest in applying new approaches from biotechnology to international agricultural development. Plant biotechnology will be discussed here mainly from the university point of view, especially land grant universities; however, the views expressed here are personal ones not necessarily endorsed by my institution nor the National Agricultural Biotechnology Council. A dominant theme in this meeting is to assess the apparent lack of progress in delivering products of plant biotechnology to the marketplace for the benefit of consumers. It will not be my goal to enumerate the status of plant biotechnology accomplishments, although there have been many, but to point out some of the factors that impact upon scientific progress in plant biotechnology.

### DEFINITIONS AND LONG-TERM COMMITMENTS TO PLANT BIOTECHNOLOGY

In assessing the progress of plant biotechnology it must be clear that there are both research and development implications. A rough definition of plant biotechnology is offered:

> *An applied field of science whereby scientific principles are used to discover new methodology and instrumentation to produce new forms of and uses for biological entities.*

Obviously, the discovery phase has a research orientation and the production phase is a technology development and transfer process. Plant scientists may function in both phases, and are therefore *plant biotechnology research scientists.* The second phase has created a new professional area and opportunities for

*In assessing the progress of plant biotechnology it must be clear that there are both research and development implications.*

*plant biotechnologists.* The land grant universities have responsibility for research, training of plant biotechnologists, and, to varying degrees, product development. The consumer community perceives that the research universities will be involved in both phases and will deliver products for their use. This is unrealistic in most cases because the prevailing philosophy among universities has been that product development can best be done by the private sector. Countering that, however, is the fact that State Agricultural Experiment Stations (SAES) and the USDA have developed, evaluated, released, and distributed crop cultivars and germplasm for many decades as a public service, and, in fact, a responsibility. The major U.S. crops include public-developed germplasm in their breeding history or are direct products of public-supported research and development programs.

There is some uncertainty administratively and by scientists about how far the universities' research and development programs should take their discoveries. From the administrative point of view there are philosophical issues about the university role and private sector role. In the past it has been rather clear that the private sector should do developmental research and market products to the extent possible. In the case of cultivar development, the private sector incentives are not sufficient for all but a few crops, so the public sector assumes responsibility. More recently, however, administrators have found it increasingly difficult to amass the financial and physical resources to accommodate research programs, especially for high-cost biotechnology research. Therefore, it is attractive to consider cost-recovery mechanisms through development of products for direct sale or licensing. This obviously impacts on the scientists who must balance personal goals and university advancement criteria, with the financing of a research program. The days of SAES providing sufficient research facilities and operational costs for research and development are past. The scientist must supplement the provided funds with funds from other sources. A moderately active laboratory for a plant biotechnology program requires at least $1,000 per day of extramural funds to meet operational costs and even more when indirect institutional costs are transferred to the investigator.

*...administrators have found it increasingly difficult to amass the financial and physical resources to accommodate research programs, especially for high-cost biotechnology research...*

This is a heavy burden to place on a scientist who must teach classes, serve on committees, and train graduate and postdoctoral students. Research funds are presently mostly available through competitive peer-review awards. These are most successfully obtained as individual-scientist awards and only a few opportunities exist for funding multidisciplinary research teams for a comprehensive research and development program. Furthermore, almost no competitive grant funds are available for the product-development phase. This has favored short-term research on specific topics which yield new knowledge, appropriately published in peer-review journals, and has not favored long-term projects which will produce new genetic stocks and products ready for consumer use. Thus, the directions of university research have been dictated largely by the individual investigator's ability to be successful in competition for extramural funds.

*...almost no competitive grant funds are available for the product-development phase.*

There have been several creative joint ventures of private sector and university research, based on shared knowledge and biological products, usually with first right for licensing being obtained by the private sector entity. University scientists have entered these agreements willingly because the personal risks were minimal with respect to product development and licensing and also because there were minimal restrictions on publishing and patenting. From the private sector side I suspect that the ultimate payoffs in licensable technology have been minimal, but the information exchange and training elements were very useful. In some cases known to me the private sector funding was short term, three to five years, so that realistically little plant biotechnology research could reach the product-development stage.

From the above comments it is clear that there are institutional and scientist-level considerations that impact on progress in plant biotechnology. There are real impediments borne by the university scientist. The typical institutional response on the research financing issue has been to write a statement into position descriptions that "the successful candidate will be expected to generate research funds through competitive grants and other means," notwithstanding other expectations to "serve agriculture and the general public by discovering new knowledge and developing new materials."

*This is a heavy burden to place on a scientist who must teach classes, serve on committees, and train graduate and postdoctoral students.*

## GOALS AND PRODUCTS OF PLANT BIOTECHNOLOGY

The definition of plant biotechnology offered earlier really is not unique to the present era of plant science research and development and it could have appeared any time in this century. However, scientific developments of the 1960s and 1970s have provided a whole new set of tools for applied biological sciences. Research goals in plant biotechnology are likewise not unique to the present time and include, among others, the following:

—New fundamental knowledge about plant processes, including gene structure, function, and expression.

—New methodology for application in product development.

—New genetic stocks, including plants, gametes, and cloned DNA, for research and plant breeding.

—New cultivars for direct use in agriculture.

What is unique to the present era and is so exciting for the advancement of agriculture, food quality and safety, energy, and health care products is the

*1 believe that neither the faculty-reward incentives nor the funding sources have encouraged long-term research and development efforts.*

practically unlimited number of product-design topics that can now be addressed by manipulations of biological materials. Examples are included in Table 1. Most often gene transfer is highlighted as the cornerstone of plant biotechnology and certainly the possibility of unrestricted interspecific gene transfer is a major development. However, other developments leading to precise, rapid, and low-cost diagnosis of the presence of disease-causing entities or toxic compounds in plants have far-reaching implications for U.S. agriculture and monitoring of consumer product safety. Another development that requires little or no manipulation of genetic materials is rapid clonal reproduction of disease-free plants. Cell, tissue, and organ culture methods have already been usefully adopted for commercialization of cultivars and for safe international transfer of genetic resources for research purposes.

Few products of the type listed in Table 1 have emerged from university research and development, but surely these institutions have advanced new scientific concepts and methodologies. While we are still perfecting technology to accommodate product development, I believe that neither the faculty-reward incentives nor the funding sources have encouraged long-term research and development efforts. There is also a trend toward 9-month appointments in the SAES which may also discourage long-term research and development. There are signals through press releases that a great increase in product-oriented research is being undertaken at some universities. This is encouraging, because it suggests that stable funding for biotechnology may be emerging as it was in the past for "agri-technology"—traditional agricultural research in the SAES.

## GENETIC RESOURCES

By definition, biotechnology requires biological materials and thus is absolutely dependent upon biological resources. Biotechnology applied to plants uses biological resources from microbes, animal, and, of course, plants routinely. Specific genes or nucleotide sequences are the basic genetic resources required not only for gene transfer but also to adopt enzyme systems for manipulating DNA. A central dogma is that any gene from any species maybe transferred to any other species by molecular manipulations and parasexual means. In practice this means that the entire global gene pool is accessible for introducing genes into specific plants. Genes from plants have been transferred and expressed in non-plant species as well.

Clearly, genetic resources are fundamental to plant biotechnology research. Many plants have been collected from their native habitats and amassed in seed banks or gardens. These efforts have been under way for more than one century in the development of the U.S. genetic resources which were obtained from

TABLE 1 SOME PLANT BIOTECHNOLOGY PRODUCTS

1. Cloned genes for insertion into cultivars
    a. genes integrated into plant genome
    b. gene therapy
2. Diagnostic methods
    a. to detect pathogen infection
    b. to detect presence of genes in plants
        linked markers
        DNA-RNA complementation
    c. to assess genetic identity
3. Rapid clonal propagation of plants
4. Gene banks of cloned DNA or nucleotide sequence data
5. New plant products for commercial use
    a. genes transferred from another species
    synthesized genes
6. Plant products produced by microorganisms

*By definition, biotechnology requires biological materials and thus is absolutely dependent upon biological resources.*

practically all countries. The central dogma here is that "genes are free," and since only small samples of such renewable natural resources have been collected and taken away from their natural habitat, there was little concern that plant collectors were adversely affecting the economy or ecology of any country. There were a few exceptions, however, where economical considerations dominated, for example, for the plants contributing rubber, coffee, and tea.

More recently two issues have emerged. The first is that ancillary development factors—new agricultural systems, crop substitution, water development, excessive animal grazing and extractive harvests, urban expansion, and industrial wastes toxic to terrestrial and aquatic species—have threatened the integrity of the native gene pools. The second is that the "genes are free" dogma has been challenged by some genetic resource-rich countries and social scientists. Genetic resource conservation by *in situ* and *ex situ* means must be given much higher priority in view of the difficulty to manage the development factors mentioned above and because only planned biological conservation can provide assurance that genetic resources will be available and accessible for the indefinite future.

Plant biotechnology depends upon genetic resources but also restricts the free exchange of materials. Ownership of genes is an especially important concept. A genetic resource obtained "for free" may yield, upon extensive manipulation and characterization, a valuable gene or gene complex or result in a new process for manipulating genes. Then the "genes are free" concept breaks down,

*...it is unlikely that genetic erosion of agricultural species will be enhanced by introduction of methods from biotechnology.*

especially if there is commercial potential in the derived genetic materials. The issues of ownership of genes and ability to protect their use through patenting and other processes are contentious. Further study is essential.

Plant biotechnology practitioners, public and private, at the policy and research levels must give high priority to genetic resource conservation, and to the "genes are free" or "genes for a fee" concepts. With respect to genetic resource conservation, molecular methods have been rapidly adopted for analysis of genetic diversity which has aided in developing *ex situ* and *in situ* conservation strategies. The potential impact of plant biotechnology in reducing or enhancing the genetic resource base remains unclear; however, it is unlikely that genetic erosion of agricultural species will be enhanced by introduction of methods from biotechnology.

## PLANT BIOTECHNOLOGY AND PLANT BREEDING

The greatest hope and hype in plant biotechnology remains the potential for unrestricted gene transfer. This possibility is very attractive to plant breeders because it permits directed gene transfer and because the genetic resource base for a targeted crop species is vastly expanded.

Crop development through plant breeding is conceptually simple: locate a gene resource; introduce it to a population of plants; select plants having desirable combinations of traits; evaluate these plants for field performance, consumer acceptability, and safety; release the derived cultivar for general use; and, finally, arrange a distribution and marketing program. Plant biotechnology does not alter this sequential process at all, but it does present the plant breeder with new opportunities and challenges at each step in the cultivar-development process. Table 2 lists eight topics in plant breeding which are influenced by plant biotechnology. Some brief comments are offered because it is the analysis of these topics that gives a clearer picture of constraints and bottlenecks in transferring new technology to crop development.*[1]

TABLE 2 COMPONENTS OF PLANT BIOTECHNOLOGY IN CROP CULTIVAR DEVELOPMENT

1. Genetic resources
2. Trait identification
3. Gene isolation
4. Parasexual gene transfer from donor to recipient cultivar
5. Gene expression
6. Breeding—creating adapted gene complexes
7. Evaluation for agronomic performance and safety
8. Distribution and marketing of seed stocks or plant propagules

1- *Genetic resources*—Availability of germplasm from which genes may be extracted is quite good for major crops, but less so for wild relatives. Perhaps the largest bottleneck is that the vast holdings of crop germplasm in the U.S. National Plant Germplasm System are largely undocumented or not evaluated for potentially useful traits. Thus for biotechnologists a first step is to obtain a germplasm collection that may be evaluated for traits of interest. The fact that most genetic resource collections are inadequately characterized is not a surprising deficiency because many traits being considered for transfer were not even recognized as valuable in the past.

2- *Trait identification*—Which traits are to be selected for transfer is a somewhat contentious issue. Some traits may have potential for commercial exploitation but are not necessarily desirable from other points of view; herbicide-resistant plants is an example thoroughly discussed elsewhere in this volume. Biotechnology is geared to transfer of single genes, and there are many important single-gene traits as candidates, pest resistance and crop quality factors are examples. However, the adaptation of a crop to a production environment requires combinations of many traits, most of which are multigenically controlled. Thus, transferring genes one at a time for multigenic traits is not feasible.

The alternative approach is to identify chromosome regions which have blocks of genes that contribute to the desired trait expression. Thus, it is not necessary to identify the specific gene for a trait; linked genes or nucleotide sequences can be used as markers of the desired gene complexes. DNA restriction fragment length polymorphisms (RFLPs) have provided such a tool in trait identification that is probably equally as important as single-gene identification in plant breeding. Medium-to-high resolution genetic linkage maps are needed for this purpose. These maps are conceptually easy to develop, but expensive in time and laboratory supplies and require highly developed genetic stocks for some crop species. Facilitating linkage map development is a top priority for plant breeding purposes.

3- *Gene isolation*—Methods to probe for DNA sequences for particular genes are available, but more efficient means are necessary for routine plant breeding use. Particularly useful are congenic isolines which plant breeders have developed for many traits. However, these are underexploited for gene identification purposes. Breeders need access to cloned genes, and the process of development of such genetic stocks could be enhanced by multidisciplinary teams (molecular biology, genetics, and plant breeding).

4- *Par asexual gene transfer*—To transfer single genes, methods for introduction and integration (transformation) of cloned DNA into plant genomes is essential. The main contribution of biotechnology to plant breeding is to eliminate "linkage drag," that is, the transfer of undesirable genes along with desirable

*Evaluation of a genetic resource collection for a trait of interest yields information at the phenotypic, but not genotypic, level.*

ones. Traditional plant breeding relies on random genetic recombination and selection to eliminate such association. True single-gene transfer eliminates one of the most costly and time-consuming efforts in plant breeding. Up to now gene transfer methodologies have not been available for routine use in plant breeding, and, in fact, only primitively developed for grass species. This is clearly a major constraint in single-gene plant breeding.

5- *Gene expression*—Evaluation of a genetic resource collection for a trait of interest yields information at the phenotypic, but not genotypic, level. Therefore it must be shown by appropriate tests that there is a genetic component to the observed morphotype. Most important, however, is to determine if the trait is monogenically or multigenically controlled since this will dictate a gene-cloning strategy and have bearing on the practicality of attempting to transfer the trait to a crop cultivar.

Expression of a transferred single gene must also be assessed in the progeny because it is widely known from experiences in plant breeding and genetics that the genetic background (i.e., genes on other chromosomes) may affect the degree of expression of a single gene. Experience with some species have shown that the transformed plants lack vigor and are modified somehow by the gene-transfer process. This phenomenon needs considerable attention from the developmental genetics approach. Gene expression can be a considerable constraint for plant breeding, but the extensiveness of this problem is not known.

6- *Breeding-creating adapted gene complexes*—Crop cultivars are the product of many generations of selection, and farmers' landrace varieties provide ample evidence for this. Each plant breeding program must retain or reconstruct the desirable combinations of genes in new cultivars. Thus single-gene transfer routinely applied can be a great benefit to breeding. However, most breeders would consider these genes to give an expansion of their gene pool and would incorporate them into breeding populations for recombination with other genes controlling many other traits.

The new technology offers the possibility of selecting for groups of genes through the RFLP technology mentioned above. Thus biotechnology can provide both genes and gene-tagging methodologies which will aid the plant breeding process. RFLPs are not sufficiently developed for use in breeding, and this activity needs high-priority attention because the resulting DNA probes and linkage maps will be of general use for any plant breeding program.

7- *Evaluation for agronomic performance and safety*—This is another contentious area for plant breeders because there is concern that new traits introduced into plants may have undesirable effects. Plant breeders have contended with this consideration continuously and have developed the necessary evaluation techniques to assure that a new cultivar was not toxic or created no problems as a weed, thus informally plant breeders have found the GRAS - General Recognized As Safe - concept to serve public needs adequately. The guiding principle outlined in a National Academy of Sciences report (Kelman report) is that it is the trait and its effects which must be evaluated. The process by which a trait is introduced to a plant does not create a new potential for undesirable effects. This view was substantiated by a detailed National Research Council report in 1989. A decision tree to guide testing and containment procedures was developed, and these procedures, for the most part, are being adopted in federal agency guidelines. Some breeders, and I am one of them, are pleased to have oversight guidelines for testing and evaluations, but do not believe regulatory language is needed at the federal level for what is or will become a routine procedure in agriculture. Oversight on food safety of new products is well established and requires no new regulatory practices.

*...plant breeders have found the GRAS - General Recognized As Safe - concept to serve public needs adequately.*

This is not to say that biotechnology does not create any new potential problems. It does require that the gene and its effects must be carefully assessed. For example, gene transfer by hybridization to crop relatives has been considered in the past, but is the consequence the same for a seed protein quality gene and a herbicide resistance gene? Questions of this type are routinely considered in the plant breeding process and will continue to be addressed by appropriate experimentation. The discussion on this point should continue and crop by crop analyses are very appropriate as is currently in progress by the United States Department of Agriculture/Animal Plant Health Inspection Service.

8- *Distribution and marketing*—New crop cultivars may contain patented genes, and the cultivar itself may be protected by a plant patent, a plant variety protection certificate, or various forms of trade secrets. This is not a new development, but there is increased uncertainty about how developers may protect their investments and realize profit for their efforts. Some crops may not receive sufficient attention if the questions about protection of intellectual property are not addressed. This may be most important for some self-pollinating species such as wheat, soybeans, and cotton. Public-sector crop developers need also to take full consideration of these issues so that they can be assured that the products of their efforts will be readily available to the general public. New arrangements for selective licensing and patenting to prevent exclusionary uses of new cultivars are both instruments that maybe applied to the advantage of the consumer depending on the crop, its area of adaptation, and extent of usage.

Presently plant breeders are wary of adopting some biotechnology practices,

*...there is increased uncertainty about how developers may protect their investments and realize profit for their efforts.*

such as gene transfer by DNA on projectiles, because they are unsure how a cultivar could be released if its development included the use of a patented procedure.

PLANT POPULATION BIOLOGY

The impact of plant biotechnology on the discipline of plant population biology and the study of evolutionary processes on plants is becoming well-known. Molecular methods for assessing allelic diversity are being routinely adopted for study of mating systems, population differentiation, and effects of natural selection on populations of many species.

Another aspect is that gene transfer to crop plants may profoundly alter the genetic structure of sexually compatible species. This is a concern only now being addressed theoretically and experimentally. Decisions may be made about whether a gene from a crop will be significant if transferred and incorporated into wild, sometimes weedy, populations. These discussions will be made based on biological, economic, and social criteria. It is clearly an area of uncertainty for plant breeders, but one which can be solved on a case-by-case basis. Especially interesting is the potential for genes to be introduced by crops into the centers of origin and diversity for a crop species. This requires further study.

WHY HAS PLANT BIOTECHNOLOGY NOT HAD A GREATER IMPACT?

The elementary tools for plant biotechnology were developed in the '50s and '60s (tissue culture) and '70s and '80s (gene cloning and transformation). For the most part, gene transformation is still in the research phase. Thus, methodologies are still evolving. Next, the application of these methodologies to produce a cultivar, followed by its testing, multiplication, and release require about the same amount of time as do conventionally derived cultivars. This is about six years. So for a cultivar to be released in 1991, a potentially new cultivar would have emerged from the laboratory in about 1985. Prior to that all of the tools of tissue culture, gene cloning, and transformation had to be in place and used. In the early 1980s only a few systems were workable. Thus, I conclude that biotechnology is not lagging in cultivar development, because the tools plant breeders use are still being assembled. For other uses, diagnostics, pest control, and clonal plant propagation, for example, there are successful products being marketed, and this trend should accelerate in the near future. There are many issues to discuss and research, but the future of plant biotechnology, when integrated into agricultural and health sciences looks very promising.

*...biotechnology is not lagging in cultivar development...*