PLANT GENETIC ENGINEERING AND BIOTECHNOLOGIES· UPDATE

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INTRODUCTION

Although current technological focus in many of the biotechnology fields, such as genetic engineering, is on agricultural crops, there is every reason to believe that the development of these technologies will have a significant impact on horticultural practices.

Strategies used in the study of plant tissues and cells, the propagation of clonal plantlets, and the production of biosynthetics *in vitro*, are becoming increasingly sophisticated. *In vitro* techniques are being used in the production of novel germplasm for the development of new plant cultivars using a variety of technologies rapidly being developed by molecular and cell biologists. The core biotechnologies which can be applied to the improvement of a cultivar now include protoplast production, somatic cell genetics, and genetic engineering. In addition, plant propagation and cultivation techniques are being developed using the biotechnologies of monoclonal antibodies, plant growth promoting bacteria, and somatic seeds. In fact, the construction of a plant genetic vector using the Ti plasmid can be said to be nearing completion in a number of labs.

As an example, it now appears possible that the T-DNA can be used as a vector into monocots; it was previously thought that this was not the cause because no galls are formed. But, there is evidence now that characteristic amino acid derivatives, which are only produced by transformed plant cells, can be found in monocot cells after exposure to the Ti plasmid.

Another impressive breakthrough is the discovery that *transformed leaf disc cells can regenerate directly. One side* effect of the T-DNA vector in the past was that it interfered with regeneration of shoots; labs are now reporting that this segment has been successfully removed, resulting in a construct which allows DNA to enter the nucleus without interfering with regeneration. The method which has been shown to work is relatively simple. The leaf discs are treated with the bacterial vector causing transformation of the cut surface cells. These cells are then able to regenerate large numbers of plantlets which each carry the new trait. This technology will

enable the rapid production of transformed plants without first growing callus.

There are a few reports now of isolated plant genes, such as the salt tolerance, or proline overproduction gene, and the herbicide resistance gene, which have been isolated by Calgene researchers. A gene for the sweetening protein thaumadin, has been identified in England for potential use in changing the way plants taste. There are also viral genes which have been isolated. Viral genes may serve to protect the plants from invasion by the virus, much in the same way in which aviru-lent viruses function in plants.

Progress has also been made in verification of the heritability of the inserted DNA and in the proven expression of selectable genes such as antibiotic resistance genes in plant cells. It also appears that more than one insertion can be made into the genome when T-DNA is used, thereby possibly producing higher gene copy levels which may enhance expression of the trait.

The current state-of-the-art is now to the point where the T-DNA vector system has been taken apart and its critical components identified. Plant genes can be identified, isolated and moved (2). The next few years will see intensive focus on plant gene analysis in order to bring us to the point of actual productive utilization of genetic engineering technologies in the whole plant.

PROTOPLAST TECHNOLOGY

The use of protoplasts in plant improvement programs has become an alternative to genetic engineering for plants. This technology allows the expression of naturally occurring variant cells which are found in plant tissues and can be used to combine two different plants.

Protoplast technologies have been well developed for several crops, among which are potatoes. J.F. Shepard has pioneered much of the work on potatoes and has produced over 10,000 Russet Burbank clones derived from individual protoplasts (5). Unfortunately, alhtough many different types were found, none of these lines have been able to outcompete the original Russet Burbank in yield and other important horticultural characteristics (4). It may be that the best strategy for the use of protoplasts in plant improvement programs will be in either fusion of protoplasts or in using protoplasts in somatic cell genetics programs.

This past year, Pal Maliga has shown recombination in chloroplasts of fused protoplasts. This demonstrates the feasibility of using protoplast fusion as a method of combining

desirable traits from the cytoplasms of different culivars. The application of protoplast technologies to horticultural crops still involves a considerable effort in perfecting regeneration protocols for each individual species and cultivar of interest.

SOMATIC CELL GENETICS

The study and exploitation of *in vitro* genetic changes, which either occur spontaneously or are induced to occur via mutagenesis, has been termed somatic cell genetics. Somatic cell genetics can encompass both somaclonal variation, which may be derived from changes in vegetative cells prior to culture, or changes seen over time *in vitro*, and variation resulting from directed mutagenesis. Directed genetic change *in vitro* usually includes three phases: 1) the induction of variant cells via mutagenesis, 2) the isolation of elite cells via restrictive or selective conditions, and 3) the genetic analysis of the recovered variants.

There are several distinct advantages of the directed somatic cell genetics strategy over other approaches to plant improvement. The prime advantages are the enhancement of variability affected at the nuclear level, the ability to select only the few desirable variants from the large cell population. Somaclonal variation takes advantage only of naturally occuring and undefined phenotypic changes which may or may not be stable genetically. Directed somatic cell genetics allows the induction of genetic variability in millions of cells which can be screened using selection systems which permit the isolation of only those cells which exhibit a desired trait. Because of the broad impact of mutagens, many traits can be affected besides the prime target, thus giving a wide number of selections for evaluation *in vitro* as well as in the fields.

Chemical mutagens have a long history as a seed treatment and in the development of bacterial and microbial strains. The use of these chemicals in developing improved lines of plants is still being researched for optimum efficacy; however, evidence indicates that mutagen-induced genetic changes are both broader and more permanent than those seen as a result of somaclonal variation. Both mutagen-treated seeds and cells must demonstrate the stability of their genetic changes by successful passage through a sexual cycle. However, the ability to reduce the population through selection systems at the cellular level greatly reduces the labor and field space required for cellular versus seed systems. Additionally, directed somatic cell genetics systems allow screening of cells at a haploid level which permits the discovery of both recessive traits and dominant traits.

Cells which have been treated with mutagens can be selected for specific traits by the use of restrictive or selective growth conditions. For example, cells can be placed on a normally toxic salt concentration under which only salt tolerant cells can grow. Restrictive culture systems can eliminate all cells which do not possess the sought after trait, thereby reducing by 99% the number of plants which have to be evaluated. Disease toxins and actual pathogens, such as bacteria and nematodes, can be used in selection systems, although testing must be done to verify the correlation between the response of cells *in vitro* and the response of the whole plant in the field. Researchers have found that all three types of correlations can occur, i.e., both *in vitro* and *in vivo* responses to a screen are positive, or one, but not the other, responds to the screen (1). The combination of mutagenesis with subsequent selection of elite cells by restrictive culture is a powerful tool in the development of new genetic lines.

GROWTH PROMOTING BACTERIA

Plant growth promoting rhizobacteria (PGPR) are being researched at several labs including the University of California, Berkeley, lab where much of this research has been conducted. PGPR's are bacteria which inhabit the root zone of the plant and are responsible for an increased vigor response. There are literally millions of soil bacteria in a teaspoon of field soil. Researchers isolate the growth-promoting bacteria from soil samples on the basis of the response of individual colonies to *in vitro* challenges. The colonies are often selected because they are able to inhibit or kill pathogenic microorganisms such as soft rot bacteria (*Erwinia* spp.)

One of the methods whereby plant growth promoting rhizobacteria exert their beneficial influence appear to be in this antagonism to many of the mildly pathogenic orgnaisms which inhabit the soil in the plant's root zone. Through the use of careful screening, naturally occurring bacteria are being found which actively suppress root zone diseases of a broad number of species. Another way in which these bacteria appear to promote plant growth is through changing root structure and, possibly, nutrient uptake.

MONOCLONAL ANTIBODIES

Monoclonal antibodies are a fairly recent import into plant biology from medical research. Just as antibodies have become a conventional tool in the diagnosis of plant viruses, their purified counterpart, the monoclonal antibody, will rapidly take over and expand the use of antibodies in agriculture and horticulture.

Monoclonal antibodies are, as their name implies, single antibodies which are originally produced by sensitized, hybrid mouse cells and, after testing, are mass produced in mice, resulting in a large number of identical antibodies. The specificity of a monoclonal antibody is determined by the original sensitized lymphocyte. Since a wide variety of antibodies are formed when an animal is inoculated with an antigen, there are many monoclonal lines which can be chosen — either for extremely specific functions, such as to identify a precise isozyme protein, or for more general functions, such as to detect the presence of any of a number of strains of a pathogenic virus.

Monoclonal antibodies are being researched for agricultural applications and currently have many uses in plant biology. In genetic engineering, these antibodies can be used to capture specific gene products for their identification. Once identified, gene products which are specific to a tissue or a disease response, can then be traced to their gene of origin and to the genes which regulate this response. This is currently being used as an alternative to gene libraries.

Monoclonals are also being isolated for use in diagnostics. For example, they can be made to react specifically with a chemical pesticide and then used to identify trace levels of it in the soil or on the surface of a fruit or vegetable. They can also be used to identify pathogens such as bacteria or fungi which are living in the soil or on the surface of a leaf. As agricultural products are developed, monoclonal diagnostics will find their way onto the shelves of individual growers and pest control advisors for use in accurate and quick identification of exactly what is happening at the plant level.

In the future, we will be seeing a lot more applications of monoclonals as they become a routine tool in the examination of problems at the molecular level.

SOMATIC SEED TECHNIQUES FOR VEGETATIVE PROPAGATION

In the future, it will be possible to vegetatively propagate plants which do not breed true from seed by using somatic seeds. A somatic seed is an embryo grown from callus which is encased in a protective coat. Millions of somatic embryos can be formed from a single plant — in a sense they are all clones of each other. The advantages of using somatic embryos are that they are naturally singulated and possess both a shoot and a root axis in a very small unit. This makes them highly amenable to automation.

Currently, the state-of-the-art of embryo encapsulation

outstrips the art of producing somatic embryos. In other words, the delivery systems for somatic embryos are ready for the field but the biology of somatic seeds is still in the lab.

Somatic seeds are produced by the process of somatic embryogenesis whereby small, uncoated embryos are induced to form from callus cells. These embryos are currently being made for crop species such as celery, alfalfa, and oil palms and many additional species will rapidly be added to this list once the technique becomes a standard procedure. For example, we can already make somatic embryos for plants such as geraniums and strawberries. The forestry industry has a longstanding commitment to the development of somatic seed for Douglas fir because this is the only likely method of rapid vegetative propagation which could be both inexpensive and automatable in high numbers. Research is ongoing in the areas of somatic seed automation and controlled uniformity.

The requirements of a somatic seedcoat are, foremost, that it be nontoxic to the somatic embryo. This includes both during manufacture and afterwards during germination and conversion. It must also be pliable in order to protect and cushion the embryo but sufficiently rigid for handling during manufacture, transportation, and planting. The coating may need to serve as a substrate for the embryo by providing it with nutrients and growth or developmental control agents. The coating must be amenable to the process of singulation and adaptable to currently used equipment such as mechanical seeders. Preferably, it would allow for additional incorporation of biological agents, such as growth promoting bacteria and agricultural chemicals, such as fungicides. The current use of hydrogels as a somatic seedcoat meets all of these criteria.

In somatic embryos we frequently observe the precocious germination of a small plantlet directly from the embryo without the stages of maturation, desiccation, dormancy, and imbibition which normally precede radical emergence and the commencement of growth. In addition, we observe that a high percentage of somatic embryos in certain plants will not procede past radical emergence and growth into development of whole plants, as is signalled by the appearance of the first true leaves. In our labs we now refer to the final step in the development of a whole plant from a somatic embryo as "conversion".

One aspect of developing a field-ready somatic seed is that it must be fully hardened. This problem has been overcome in part by the use of airflow systems developed at PGI for the control of the *in vitro* gas environment. Using a continuous air flow, the embryos develop at a reduced relative humidity, which encourages the development of a normal cuticle.

NOVEL PROPAGATION TECHNIQUES

As researchers develop skills in gene identification and manipulation, they are likely to discover technique which will have broad impacts in totally unrelated fields. For example, transformation of cells at the base of a cutting, using a disarmed plasmid from a root-producing bacteria, or from an auxin-inducing fragment of the T-DNA may become a standard method for rooting certain cultivars. In this case the only plant cells which would be influenced would be those which had been treated with the special bacteria containing the root-promoting fragment from the rooty-tumor pathogen. The cut surface would receive the rooting genes which would have no effect except to induce the formation of roots.

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THE OBLIGATION OF THE PLANT PROPAGATOR

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Abstract. Because the ultimate sources of plant pathogens are previously diseased plants and the soil (including water and nonliving organic matter), propagators of pathogen-free plant materials are a primary or seminal source in modern plant production, and have special health responsibilities Pathogens must be eliminated in this culture, not inhibited or suppressed In addition to being a profitable business practice, there are very specific benefits from clean culture for both the propagator and the producer Nursery diseases have decreased in the last 27 years, but remain a lurking hazard, and growers must accordingly continue to practice clean culture In such a plant-health program, growers have an important role and they should be directly involved in the ongoing research program, as they