STATUS OF CROP IMPROVEMENT THROUGH TISSUE CULTURE

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Plant propagation *in vitro* has been a well established technology for over two decades and has been in commercial use for a significant part of that time.

The commercial use of plant tissue culture today primarily involves the micropropagation of ornamental species and the production of early generations of disease-free transplants. Although the number of commercial laboratories in the United States and Canada have been estimated to be as high as 250, there are probably not more than five or ten which produce more than five million plantlets per year.

The commercial micropropagation of agronomic crops is not at the same volume level as ornamentals but is growing in the overall market. Examples include sugar cane, date palm, oil palm, several types of fruit trees, jojoba, and potato.

While micropropagation has been the principal form of tissue culture utilized at the commercial level, other aspects of tissue culture technology and other advanced biotechnological techniques will be commercialized in the next several years. All of these can be expected to have an enormous impact on crop improvement. How this will be achieved can be best illustrated by: 1) briefly reviewing some of the technologies now being commercialized in agriculture, 2) providing examples of commercial use today and requirements for future improvements, and 3) providing a basis for their market potential.

TECHNOLOGY REVIEW

At first glance, recombinant DNA technology may seem far afield from a discussion on plant tissue culture. Yet, given what is known about this particular form of genetic engineering, the manipulation and transfer of genes in plant cells using recombinant technologies requires plant and cell culture techniques to translate a sophisticated technical achievement into a commercially meaningful product.

There are several areas where commercialization of this technology might be realized within the next five to ten years. Improving the photosynthetic efficiency of crops using genetic engineering could generate an added value of $8 billion through increased

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yields. This is considered by many to be a very difficult technological target. Another aggressive target is direct nitrogen fixation in non-leguminous crops which is valued at $1.3 billion. The shortest term target which is both technologically achievable and has high commercial potential is herbicide resistance. Herbicide resistance has been introduced into several plant species by rDNA. Herbicide resistant crops, essential to minimum-till systems, could be worth $4 billion per year.

Given that the commercial opportunities emanating from recombinant technologies are a bit long term, what contributions can be expected from plant cell biology? Cell culture, while not as glamorous as the recombinant technologies, is critical to the commercialization process. It represents an enabling technology for the conversion of genetically manipulated plant cells into whole plants. Plant cell culture systems also provide a means for manipulating genes through various screening and selection processes. Two of the best known selection methods using cell culture are somaclonal variation and directed mutation. In fact, cell culture currently offers a faster means to achieve some of the goals set by molecular geneticists because the methodologies are better defined.

Cell selection technology takes advantage of the variation which naturally occurs in some plants regenerated from tissue culture. The genetic variation may already exist, but may not be seen until after the plant has been through culture, or the culture may force the change even though that was not the intention. One plant biotechnology firm, DNA Plant Technology, is using somaclonal variation to improve soluble solids in tomatoes. The economic benefit to the tomato processing industry is measured in millions of dollars saved at the processing plant.

Directed mutation methods take a lot of the guesswork out of somatic cell selection. With these techniques, cells are subjected to a mutagen, then put through a specific assay, such as high salt content. The survivors are selected and allowed to develop into plants. They will go through another round of lab selection and finally be field tested. Scientists at Plant Genetics, Inc. have selected a line of alfalfa cells using the mutagen technique. This line of alfalfa cells has survived a salt water assay at levels approaching 75 percent sea water. The survivors can be combined with alfalfa plants possessing other desirable traits to develop new cultivars. The economic consequences of developing salt and drought tolerant agronomic crops are enormous, being measured in the billions of dollars.

CASE STUDIES

Once a cell line has been selected, regardless of the technology employed, it is necessary to convert the cells to whole plants. Tissue or cell culture propagation plays an important role in this process.

Two examples of tissue and cell culture application serve to illustrate how these technologies are being used to commercialize agronomic crops. The first case study describes the use of micropropagation in potato "seed" tuber production. The second case study illustrates the use of cell culture and somatic embryogenesis in developing new synthetic seeds.

Micropropagation. Plant Genetics, Inc. specializes in plant cell and tissue culture technologies. The company has applied these technologies in commercializing its NU-SPUD™ brand potato line. Using micropropagation, a disease-tested high quality "seed" potato tuber is produced and sold to those growers who are at the very beginning of the potato seed increase cycle. Potatoes are generally grown from actual pieces of potato and increased over four to six years before being planted in a commercial field. If a grower starts off with diseased seed in the first year, millions of dollars can be lost.

The first state in the production of NU-SPUD brand potato products involves meristem culture, a disease-testing quality assurance protocol, followed by large scale micropropagation. The second state of production involves the transfer of disease-tested transplants to production greenhouses where whole tubers are produced and harvested. Commercial grade ranges from 0.25 to 1.5 ounces. NU-SPUD products have been sold in the major potato growing areas of the United States and Canada and have been shipped to China, Thailand, Taiwan, and Yugoslavia. This segment of the "seed" potato market represents a $350 million opportunity worldwide, with the United States and Canada representing only a small portion of this market. The NU-SPUD brand potato story offers an excellent example of how plant tissue culture is being applied today for the commercial production of the world's fourth major food crop.

Somatic Embryogenesis. A very key advance that will be required in the commercialization process of agronomic crops centers around that element of plant/cell culture called somatic embryogenesis. Somatic embryogenesis is one of the two technologies critical to the development of what is now called synthetic seed, and provides the basis for the second case study.

The process of making somatic embryos is exacting. Cuttings are taken from plant tissue and placed on a defined cell culture medium. Callus, or masses of disorganized single cells, will form from the cuttings. By changing the medium composition the cells are triggered to form embryos, not unlike the ones found in true seed. These embryos can be increased either on solid medium or in liquid culture medium. To make this process commercially viable, it is necessary to develop a large-scale liquid culture system. Once accomplished, it is estimated, for example, that one quart of liquid culture medium will produce enough celery embryos to plant about

25 to 30 acres. Commercial efforts are now ongoing to test plant cell cultures in specially designed bioreactors in which variables such as temperature, gases, and pH can be regulated.

Plant Genetics, Inc. has developed somatic embryo systems in cotton, alfalfa, lettuce, and celery. To make this system workable in the field and greenhouse, a protective coating or capsule is required. To address this issue the Company has developed a delivery system trademarked GEL-COAT™, a hydrated polymer gel surrounded by a membrane. Somatic embryos encapsulated in GEL-COAT are referred to as synthetic seed. In the case of some high value crops such as celery it may be possible to sell the synthetic seed directly to the grower or transplanter. With crops such as alfalfa, where the seed cost is low, this system can be integrated with a more traditional breeding program to develop and produce novel cultivars which will then be sold as seed.

AUTOMATION AND COMMERCIALIZATION

The ultimate commercial success of advanced cell and tissue culture technologies will be judged by the marketplace. Critical to this success is the implementation of large scale, low-cost automated production systems. As evidenced by the NU-SPUD illustration, the micropropagation industry is not necessarily limited to ornamental products. It has potential applications throughout the entire range of propagatable crops, perhaps even entering markets where transplants are traditionally produced from seed. If this picture is to materialize, it will be on the basis of being able to offer a quality, unique, or differentiated product as a transplant at a cost equal to or less than is currently charged. Since high volume vegetative propagules are produced for less than one cent apiece, and seed of most crops costs less than 0.01 cent, the challenge is great. The central issue facing commercially oriented plant tissue culture researchers today is how costs can be reduced to a fraction of what they are, while maintaining the quality of the end product.

Aside from the technical advantages achievable through plant tissue and cell culture, the major factor influencing the end users' decision to commercially propagate a plant through tissue culture is the performance or economics of alternate propagation systems operating in the marketplace. While technical factors may drive the need to culture a plant *in vitro,* the economic factor determines the feasibility of commercialization.

A major stumbling block to the large scale automation of tissue culture propagation has been the fact that detailed economic analyses of large volume tissue culture operations have not, until recently, been available to biologists and engineers with the research skills to attach key cost-intensive steps of a production process. A detailed analysis of this subject has been presented in other recent publications (1, 2).

Factors limiting unit productivity are inherent in processes dependent on manual operations. In discussions with major tissue culture production operations in the United States, a ceiling to productivity per operator-day appears to occur at about 5,000 operations, or transfer, per day. This appears to be a fair estimate of the maximum daily efficiency achievable using manual labor. This peak rate is rarely sustained over time; in general, good productivity is considered to be in the range of 50 percent of this number, or 2,500 transfers per operator per day. Average productivity over various tasks is influenced by culture vessel structure. For example, lower numbers per operator-day are encountered in test tube systems versus petri dish culture vessels (2).

Marketing considerations are a key element to the successful commercialization of automated tissue culture systems. The relationships between supply, demand, development costs, production costs, operating costs, and amortization must be carefully analyzed. It is particularly clear that the anticipated markets must possess the requisite volume and profit potential to bear the development costs of an automated system. Since first generation systems may be quickly superceded by technological advances, cost recovery for machines must be planned for rather short periods of time, say 24 months. Markets with the appropriate potential are now being identified and automation work is progressing.

One alternative to micropropagation which sidesteps many of the most difficult technical issues of automation is the encapsulation of somatic embryos for delivery as a direct analog to seeds. Somatic embryos can now be produced in a wide array of species and hold the promise of producing propagules at a fraction of the cost of other systems. The encapsulation of somatic embryos in gel-like substances has good automation potential. While embryogenesis and encapsulation technologies are still in a research phase, automation of synthetic seed production is conceptually more straightforward than any other micropropagation system. The production of many thousands of somatic embryos from only a few grams of cultured cells offers substantial cost advantages and makes it the prime candidate for production of large numbers at less than 0.01 cent per unit. The mechanical planting of encapsulated units, such as synthetic seeds, appears readily achievable using currently available greenhouse and field seed planters. The planting of synthetic seeds is not significantly different from the planting of a similar-sized zygotic seed. To be mechanically acceptable, synthetic seeds must flow through equipment easily and be fairly uniform in size and shape.

What appears to be emerging in the industry is the convergence of the skills in biology, engineering, and marketing necessary to drive the innovation process in this arena. Several firms are either taking their first real steps toward automation *in vitro* production or have become fully committed. The near future promises many interesting possibilities.

CONCLUSION

On reviewing the new technologies and their potential economic impact it becomes evident that any economic assessment of the importance of biotechnology to agriculture is still very speculative. It is difficult to place a value on products which do not yet exist. Those that are in the marketplace or on the threshold of commercialization offer exciting prospects. The clear objective is that biotechnology will be applied in ways which will reduce costs to farmers, increase production efficiency, broaden genetic diversity, and enhance biological and economic stability.

LITERATURE CITED

1. Sluis, C. J. and K. A. Walker. 1985. Commercialization of plant tissue culture propagation. *Inter. Assoc. Plant Tissue Culture Newsletter.* 47:2–11.
2. Walker, K. A. 1986. Automating *in vitro* plant propagation: has its time finally arrived? *Genetic Engineering News* 6 (7):52.

CROP MODELING AND PRODUCTION COSTS

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A crop model is a mathematical representation of how a crop grows and develops. Although not much modeling has been done on horticultural crops, there are a number of agronomic modeling projects. Over the last few years many of these have developed to the stage where it is now possible to develop management tools from the results.

Only recently have research dollars been allocated for modeling horticultural crops. This is probably due to the realization that benefits are possible for the horticulture industry. The greenhouse industry has, for example, discovered that a crucial step in the area of automated environmental control is to be able to provide the control computer with some representation of how the plant responds to modifications in the environment. Those interested in production can benefit by having a lot of crop-specific information placed into a package which mimics the crop's response to changes in cultural practices. With such a model it is possible to develop cultivars (through breeding programs) which are