BIOENGINEERED CRANBERRIES AND THE FUTURE

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The complex of techniques and science now being lumped under the term 'biotechnology' is at the stage of rapidly being applied to agricultural problems. With the cooperative support of the Wisconsin Cranberry Board, the University of Wisconsin has launched a major program to determine the benefits that biotechnology may offer the cranberry industry. This talk will be a progress report of the first 3 years of what is turning-out to be a most exciting effort.

Two major areas of biotechnology are being applied to cranberry production and improvement. The first is 'micropropagation' and involves the rapid cloning of superior cultivars of cranberry in test-tube type environments. The second is 'genetic engineering' and involves inserting new genes into cranberry that will confer pest resistance. Probably the most instructive format for this presentation is to show how the various technological hurdles were (and are being) overcome with cranberry.

In order to successfully genetically engineer any plant, 4 requirements must be met:

1. Have a useable cell/tissue culture system. Since genes are inserted into individual cells, one must have the capability to grow such cells into whole plants. This is done in sterile, test-tube type environments where the environmental and nutrient conditions surrounding the cells/tissues can be precisely controlled.

2. Have the needed genes identified and isolated. There are three types of genes usually needed for genetic engineering. The marker genes allow one to identify those cells and tissues that have incorporated the new genes and in which these genes are functioning or expressing. Such cells are termed 'transformed'. We use the GUS gene which turns transformed cells blue when put under special conditions. The selection genes give the transformed cells a competitive advantage in comparison to cells not containing the new genes; this allows us to preferentially promote the growth of transformed cells even though they may be grossly outnumbered by other cells in the surrounding tissues. Here we use the KAN gene that makes the cells tolerant of an antibiotic, kanamycin, that usually kills plant cells. Finally, one needs the genes of interest, that is the genes that code for the characteristics that we want to put into cranberry. In this case, we are concentrating on pest resistance being conferred by a BT gene.

3. A method to insert the genes into the cells. Here, we are using a new and patented technology called 'particle bombardment' where the genes piggyback on gold pellets that are "shot" into the cells.

4. Control of gene function while in the plant. Although all the cells in a genetically-engineered plant will contain the new genes, we may not want these genes to be functioning (expressing) all the time and in all the tissues. Here we attach control elements, called promoters, onto each gene. Promoters allow the genes to express only under certain conditions.

We are pleased to say that after only a few years of work with the cranberry, all of these requirements are now in place. We have perfected a tissue culture system that we can use to (1) generate the 'targets' that are most effective in the gene transfer system, (2) differentiate shoots from the transformed cells, and (3) multiply the regenerated shoots so that we can rapidly obtain plants for testing and evaluation. The genes, the gene promoters, the gene transfer technology, and many of the analyses are all being provided by Agracetus Company in Middleton, Wisconsin. The close cooperation of Agracetus is in large part why we have been able to make such rapid progress in this research.

A second cooperative effort with Professor Dan Mahr and his laboratory has allowed us to evaluate the pest resistance of our recovered transformed plants. Such analyses are at this time underway. The advice and cooperation of such cranberry experts as Dan Mahr and Elden Stang as well as enthusiastic cranberry growers is yet another reason why the project has progressed so well. Such continued cooperation is absolutely essential for a project like this to realize its goals.

The second part of the project, that of developing the techniques for micropropagating cranberries and evaluating the response of such plants in the field, is also well underway and showing unusual success. In two years of comparative plots, micropropagated plants have outperformed field cuttings in vigor and establishment. This coming season will test the potential fruit productivity of micropropagated plants.

What are some of the remaining hurdles to be overcome? The scope of this project will continue to enlarge as we get closer to actual release of any genetically-engineered plants and so will the problems. A sampling of such concerns includes:

1. Will the genetically-engineered plants be efficacious, that is, will they control pests in the field? We already have indications that we may have a problem here in that two of our inserted genes, the GUS and the BT, are not expressing well in cranberry. Our current hypothesis is that the promoter driving both of these genes is not functioning well. We are already taking steps to overcome this problem by using other promoters, however this means generating a whole new lime of transformed plants and thus will delay our evaluations.

A second aspect of field evaluations is obtaining the permits to establish such plots. Again, the cooperation of growers and industry will be critical in expediting such entanglements.

2. Who will own the plants? Much of the technology and genes that we are now using are proprietary. Agreements will have to be worked-out to address the licensing and payments for use of these technologies. We (myself, Rodney Serres, and Agracetus) have already filed a patent on the cranberry transformation system. We expect to file for plant patents on the final products of this research. Our

intention is to turn-over the rights to these patents to the Wisconsin Cranberry Board and Agracetus so that any royalties or licensing fees can be plowed back into research and technology development in Wisconsin.

3. Will the public accept the product? Our intention is to engineer a plant that will produce cranberry fruits that are chemically and aesthetically indistinguishable from non-engineered fruits. One approach is to use promoters that will function only in green tissues. Thus the leaves and green fruits of the plant will be protected by the gene products, but the ripened fruits will not contain such products. Testing this hypothesis will itself be a fascinating biological research endeavor.

4. What are the environmental concerns? Will there be a problem with 'escape' of the genes to native cranberries? Will the insects develop tolerance to the engineered plants, thus circumventing the whole effort? Will non-target (non-pest) insects be impacted? We are already beginning to determine how such questions should be addressed so that reasonable answers can be obtained.

5. What else can we do to improve the cranberry? Actually, here, there is really no limit. The advances that have been made in this biology over the last few years have been truly revolutionary. Continued and even more rapid progress is anticipated. We view this project as only a beginning in a never-ending application of biotechnology to cranberry development and production.

In summary, our first 3 years of working on cranberry biotechnology has been incredibly stimulating, exciting, and successful. The cranberry is now the leader in the application of biotechnology to fruits. We hope to continue this effort, again with the close cooperation and advice of the Wisconsin cranberry and biotechnological industries.