The Wisconsin Cranberry of the Future: A Biotechnological Approach

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For the past 6 years, the Wisconsin Cranberry Board has supported a project to determine how modem plant biotechnology (genetic engineering) might be used to genetically improve cranberry selections. Recently, Ocean Spray has also helped to support the project. The project has evolved over this period to emphasize various goals; some of these have been completed while others are continuing:

Micropropagation and transplant technologies useful in establishing new plantings
-Incorporating resistance to the pest Blackheaded Fireworm into leading cultivars by genetically engineering cranberry with *BT* genes.
-Incorporating tolerance to the biorational herbicide, Bialophos, into leading cultivars by genetically engineering cranberry with the *BAR* gene.

Since detailed reports on all of this work have been written annually to the Wisconsin Cranberry Board, Inc. and to Ocean Spray and have been published in the annual compendium of such reports, the work will not be detailed here. Instead, some of the highlights will be noted and some of the implications of the work will be discussed.

This project is not unlike many others working with other crops in that the goals and the genes utilized to achieve these goals are common. However, the project is very much unlike other similar projects in that the resources used to accomplish this work are comparatively much less. The work has only been feasible to approach because of the cooperation of a large number of individuals and organizations ranging form growers, to students, to companies, to government agencies.

Working relationships

The project has continued to act as a bridge between the cranberry industry and biotechnology laboratories and companies. This has resulted in agreements to provide access to proprietary technologies that may be useful to genetically improve cranberry. Examples of these technologies include the following:

-Use of the ‘Accell’ gene gun, a proprietary system for transferring genes to cranberry (Agracetus Company)
-Use of gene constructs-containing genes for insect resistance *(BT)* and herbicide tolerance *(BAR)* and genes essential in recovering transformed plants *(GUS, KAN)* (Agracetus company)
-Access to a vast array of *B.t* strains for testing to determine the most useful strain for controlling pests of cranberry (Mycogen, Inc.)

Without access to such technologies, this project would not have been possible to carry-out. New working relationships are constantly being sought as new technologies advance to the commercial level.

In addition to working with scientific organizations, the project has coordinated with governmental organizations (State and Federal) to gain clearance to conduct the work. In particular, we have addressed all the environmental and other concerns that have been raised to date and now are able to conduct this work in open field situations.

Technique development

The project has perfected and proven a methodology that allows the insertion of foreign genes into cranberry and the recovery of genetically-engineered plants. This process has been patented and to our knowledge, this is still the only reliable method of genetically engineering cranberry. The method has a number of steps:

Sterile culture (microculture) of cranberry to provide large numbers of uniform stem pieces.
⇓
Induction of these stem pieces to produce miniature buds.
⇓
Gene insertion into the buds by particle bombardment.
⇓
Selection for further growth of only those cell/tissues/plants that have been genetically engineered.
⇓
Recovery of transformed shoots.
⇓
Micropropagation of these shoots.
⇓
Testing for gene presence and function,

In addition to the genetic engineering methods, numerous techniques have been developed to test the recovered cranberry plants for traits of commercial value. Such testing techniques include:

- -Pest resistance:
  - -A method to culture Blackheaded Fireworm year-around in the laboratory.
  - -A rapid laboratory assay to screen for resistance of plants to blackheaded fireworm.
  - -Procedures to grow and test cranberry in areas outside the normal production regions in Wisconsin.
- -Herbicide tolerance:
  - -A laboratory and greenhouse assay for tolerance to Bialophos.

Status of the work.

Compared to efforts on other crops, this is a very small program for a genetic engineering project. Nevertheless, some very important milestones have been achieved:

-Cranberry can now be routinely genetically-engineered.
-Inserted genes function and are stable in the plant even in the field and through multiple growing seasons.
-The inserted genes are inherited between sexual generations, thus these transformed plants can be used as parents to pass on the new traits.

Pest resistance.

In regards to resistance to Blackheaded fireworm, plants have been obtained that show more resistance than the current commonly grown cultivars of cranberry. For example, in the field test plots this year, plots of transformed lines of 'Pilgrim' and 'Stevens' had 30 to 40% less Blackheaded fireworm mature successfully when feeding exclusively on them than on the regular 'Pilgrim' or 'Stevens' foliage. Although these results show that the insertion of the *BT* genes do give resistance to this pest, the resistance obtained is not at the level (100% kill) that has been observed in other crops. Our most likely explanation for the reduced effectiveness of this *BT* gene in cranberry is that cranberry contains chemical compounds that interfere with the action of *BT.* We have direct evidence to show this effect. If we want to increase the pest resistance conferred by inserted *BT* genes, we will probably have to repeat the work but *use* a newer, more powerful *BT* gene so that the inhibitory compounds are overwhelmed by very high levels of the *B.t.* pest toxin produced in the plant.

However, there is an open question as to just how much *B.t.-based* pest resistance we want to engineer into cranberry. The problem revolves around the need to avoid a situation where the planting of genetically-engineered *B. t.* cranberries results in the emergence of Blackheaded Fireworm populations that are resistant to *B. t.* There are two schools of thought on this issue. A high dose strategy approach says that a cranberry plant with a very high level of *B. t.* toxin will eliminate all Blackheaded Fireworm so that none will be surviving to breed. However, if eventually some pests do mutate and are capable of surviving such high doses of *B. t.*, then the use of *B. t.* in any form as a pest control option will be eliminated.

A second strategy, the low dose' approach, uses *B. t.* in conjunction with other control (e.g. natural predators, insect diseases, traps) on the insect population so that it is not just the *B. t.* that is impacting the pests. In this situation, since *B.t.* is not the only stress on the pests, the insect population would not likely evolve a resistance to *B. t.* However, this approach is more complex and thus more difficult to effectively implement and manage.

It would also be interesting to know if under field conditions, the low level of *B. t.* in the plant would make spray applications of *B. t.* more effective. Thus a grower would be able to control Blackheaded Fireworm by combining genetically-engineered plants with biological pesticides. Different forms of the *B. t.* could be used in the spray than what was engineered into the plant, thus providing a complex spectrum of controls and minimizing the development of pest tolerances.

What we may have to do is to test just how effective our current transformed cranberry lines are in actual cranberry field production situations. This will require the planting of genetically-engineered cranberry in production areas (not now approved) and on sites where no or few chemical controls for pests are utilized. The later is important since any reduction of beneficial insects (predators) by agrochemicals would defeat the low dose strategy. Unfortunately, this test may have to await an effective, non-agrochemical control for tipworm.

Herbicide tolerance.

Our original approach to recovering cranberry plants that contain the inserted *BAR* gene giving tolerance to the herbicide was not successful. We used the herbicide itself to select for those cells that contained and were expressing the *BAR* gene, however we soon discovered that the non-transformed cranberry tissues were able to develop a tolerance to the herbicide. Thus the herbicide itself was not an effective way to discriminate between genetically-engineered plants and regular plants, all growing in the same test tube. We are now using a new gene construct which will allow us to use the same recovery procedures that have proven so effective with the *BT* work.

Future decisions.

If the work proceeds as expected, within a few years we will have genetically-engineered plants that will need to be tested in cranberry production areas in Wisconsin. The initial restrictions on these tests are liable to be rather severe. For example, we may be required to limit as much as possible the escape from the plot of any plant material that could survive outside the plot boundaries. In addition, we may have to destroy the plot after the test and verify that all genetically-engineered plant material is dead or removed. These restrictions are certainly not insurmountable, however the test will require an area that (1) is relatively isolated from other cranberry production areas and (2) a grower is willing to maintain but will not be able to harvest for the life of the test.

Any takers?

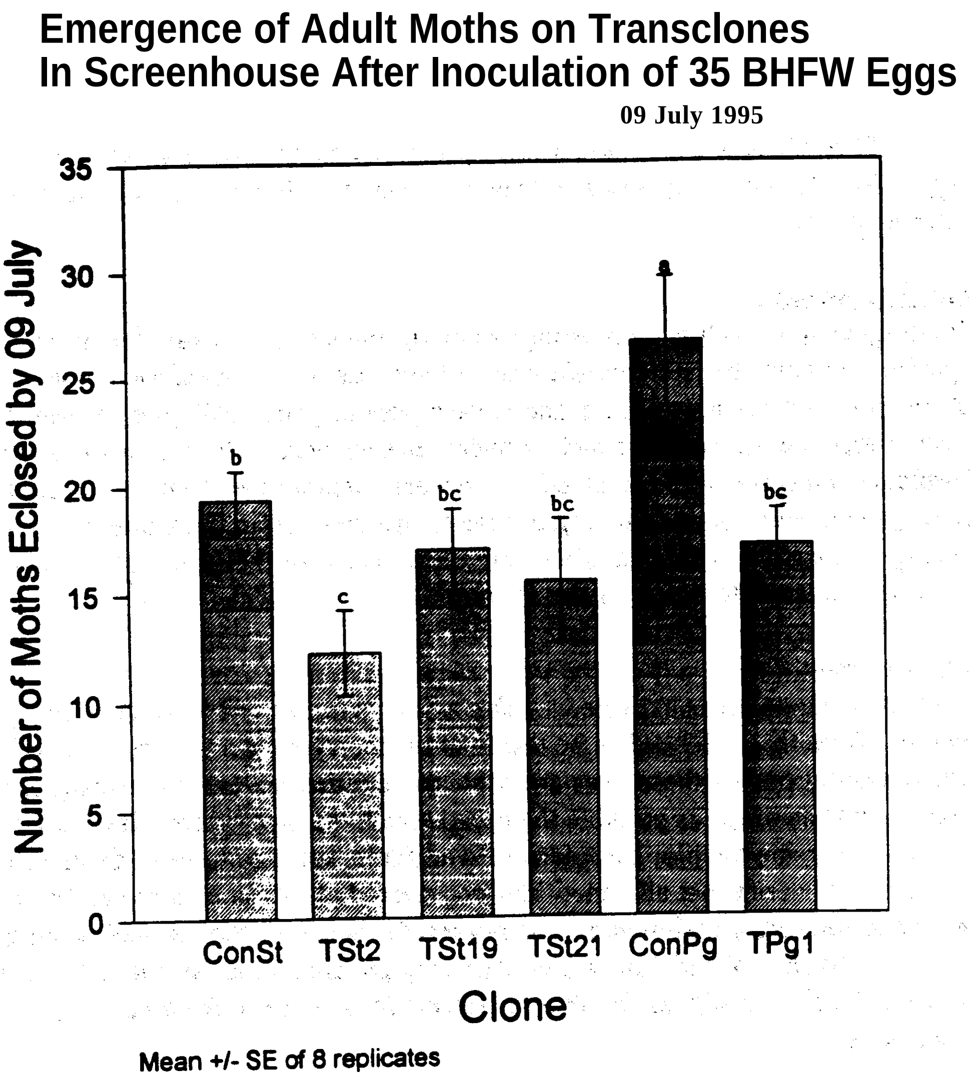

Figure 1 . Emergence of adult BHFW moths on transclones and control cultivars in the screenhouse approximately one month after inoculation with 35 BHFW eggs. Bar indicates the mean ± S.E. of 8 replicates.