Differentiating Plant Clones in Culture and Maintaining "Virus Tested" Blueberry Clones

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INTRODUCTION

In 1986 the Michigan Department of Agriculture instituted the "Michigan Virus Tested Blueberry Clean Stock Program" for blueberries (*Vaccinium corymbosum*). DeGrandchamp Blueberry Farm Inc. entered into the virus tested program that same year. Part of the program required the maintenance of a foundation stock block for scion wood. It was decided at that time to maintain the stock block in vitro and produce scion wood as microshoots from tissue culture. The in vitro multiplication of 25 cultivars was contracted with an outside tissue culture laboratory. Unrooted microshoots are shipped to our nursery, rooted in our greenhouses, grown outside for 1 or 2 years and then sold to commercial growers.

DIFFERENTIATING PLANT CLONES

We soon discovered that all blueberry cultivar microshoots looked nearly identical until they were several months old. Cultivars were virtually impossible to tell apart. One of the greatest dangers associated with tissue culture propagation is the mixing or mislabeling of cultures (Hancock et al., in prep). After growing several cultivars for 18 months we discovered some cultivars were mixed with other cultivars. A complete investigation of our greenhouse propagation procedures revealed that the mixed cultivars originated from the tissue culture laboratory.

Dr. James Hancock, Department of Horticulture at Michigan State University, was contacted about the mixing problem. He had already conducted some research on identifying clones of blueberries by using starch gel electrophoresis. Cultivars vary greatly in their in vitro proliferation rates, and as a result, mislabeled, rapidly proliferating cultivars can replace properly labeled ones. Therefore, there was a need for biochemical markers that can be periodically employed to verify "trueness to type" (Hancock et al., in prep.). Because of the great deal of research already done on using isozymes to identify cultivars (referred to as finger printing) of a wide range of fruits (Torres, 1989), Dr. Hancock proceeded to finger print over 20 cultivars of blueberries from known "true-to-type" plants.

The procedure used in fingerprinting is the following. Dormant fruit buds, immature leafs, or in vitro microshoots of cultivars thought to be mixed were sent to Dr. Hancock's lab at Michigan State University. There Dr. Hancock uses the process of electrophoresis to identify cultivars by their isozyme patterns. An electrical current moves all the enzyme proteins that are in the sample through the starch gel. The proteins are separated by size—the different bands being different sizes of proteins (see Fig. 1). Thus, different cultivars can be distinguished because they have proteins of different sizes. It is possible to distinguish 20 of the major blueberry cultivars based on the combinations of banding patterns observed with only three enzymes. It should be possible to distinguish other cultivars by

examining additional loci. (For example, although 'Northland' and 'Darrow' are identical at the three loci [Table 1], they have distinct patterns at a second locus at both MPH and 6PGDH.)

Table 1. Isozymes at three loci, as designated in Figure 1 for each of 20 blueberry cultivars.

Cultivar	PGI	MDH-2	6PGD2
Rubel	1	1	2
Earliblue	1	1	4
Blueray	1	3	2
Bluetta	1	3	5
Toro	1	1 *	2
Jersey	2	1	3
Patriot	2	2	2
Spartan	3	1 *	4
Bluecrop	3	1	2
Coville	3	3	2
Collins	3	2	5
Bluechip	3	2 *	1
Bluejay	3	1 *	3
Bluehaven	3 **	1	4
Berkeley	4	1	4
Northland	4	1	3
Nelson	4	1	2
Elliott	4	1 *	4
Darrow	4	1	3
Northcountry	5	3	1

* These cultivars run slightly faster at the MDH-2 locus than the others.

** The slowest allele in 'Bluehaven' is slower than the slowest allele in the other cultivars with PGI isozyme 3 (Hancock et al., in prep.).

Dr. Hancock can then determine if cultures are "true to type" or mixed, and often what cultivars are mixed. We were also able to visually confirm his findings by identifying cultivars of more mature plants that came from the identified mixed cultures.

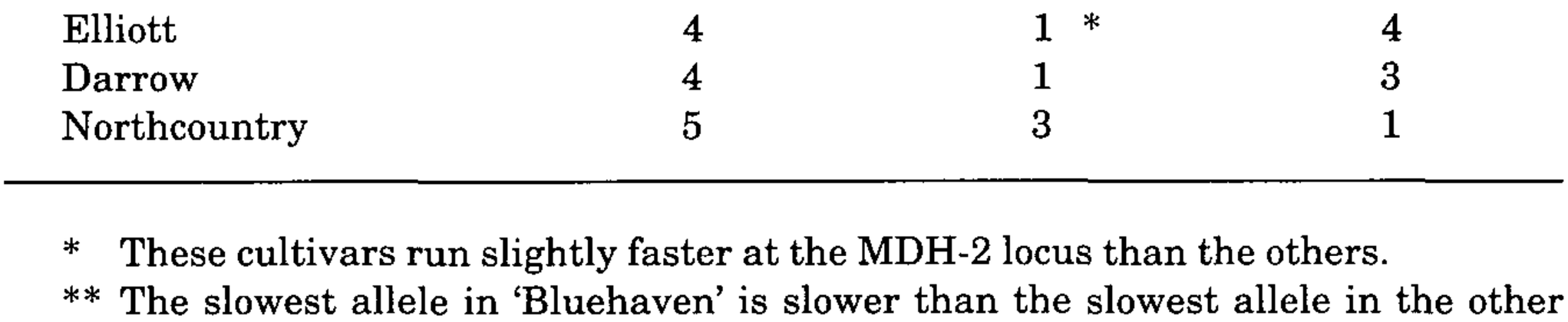

Figure 1. Observed isozymes among blueberry cultivars at 3 isozyme loci (a-c).

Our procedure now is to have Dr. Hancock confirm all cultivar cultures to be "true to type" before they are subcultured for multiplication. Also, when new cultivars are released, the stock plants are verified before they are put into tissue culture. An added benefit of this procedure is the cultures can be checked for possible mutations. During the first 6 months of growth in our greenhouses, any plants that appear to be "off type" are sent in for verification. By using electrophoresis we have eliminated mixed up cultivars coming from the lab, and coming out of our greenhouse to the commercial grower's fields.

The benefits are obvious in the commercial fruit business of true-to-type cultivars. What other uses does this procedure have outside of the fruit business? Isozymes fingerprinting has also been successfully used with *Taxus*, *Cornus*, and *Rhododendron* in Dr.Hancock's lab. Genotypes can be distinguished within these species. As propagators we can test a known stock plant from the source and verify that cultivars we are selling are true to type, thus eliminating much confusion in the industry.

MAINTAINING VIRUS-TESTED BLUEBERRY CLONES

The key to maintaining virus tested blueberry stock is the use of the enzyme linked immunosorbent assay (ELISA). Viruses that are tested for include:

- BBSSV (blueberry shoestring virus) which has infected 145,000 plants on 10,000 acres and has caused a loss of approximately 3 million dollars.
- BBLMV (blueberry leaf mottle virus) which causes stunted and very unproductive bushes that die after several years.
- TBRSV (tobacco ringspot virus) and TMRSV (tomato ringspot virus) which cause slow but steady decline and sometimes death.

One method of spreading these viruses is by using latent infected scion wood. Symptomless or latent infections are missed without the requisite indexing to assure freedom of infection from virus and virus-like entities (Ramsdale et al., 1987).

The decision was made to keep the foundation stock in tissue culture because of the ease of maintaining the requirements for certification versus maintaining the stock in the field. The requirements for the land selected for growing of foundation stock was: fumigation, freedom from *Agrobacterium tumefaciens* (crown gall), and isolation by 500 ft from any *Vaccinium* species. In addition the plantings cannot flower, and must be weed and insect free. All of these requirements can easily be met by maintaining the foundation stock in tissue culture.

ELISA testing is used on a 5% sampling of cuttings as soon as they have mature leaves (about 3 months after rooting). A second 5% sampling is taken the next year after they are fully leafed out (mid summer) in the container blocks. By sampling two times, our customers can be assured of the cleanest blueberry nursery stock available.

As propagators it is our job to produce the most disease free, healthy plants we can. By using ELISA testing we have made an important step in achieving this goal.

LITERATURE CITED

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Torres A.M. 1989. Isozyme analysis of tree fruits. In: D. Soltis and P. Soltis (eds.), Isozymes in plant biology. Advances in Plant Sciences Series, Vol. 4. Dioscorides Press , Portland Oregon.

BRUCE BRIGGS: Do you think that you can clean up virus problems by culturing plants?

MICHAEL DEGRANDCHAMP: I do not run a lab, but Dr. Hancock feels that you can not clean them up in culture.