Development of DNA based technology for fingerprinting and genetic studies in cranberry

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Identification and discrimination of most cranberry varieties is problematic. There are few qualitative morphological traits with which to discriminate varieties of cranberry. Apple varieties, on the other hand, are easily discriminated, i.e. the fruit of Golden Delicious versus Red Delicious apples. Vegetative morphology, e.g. leaf shape, size, etc., of most cranberry varieties is very similar and subject to significant environmental influence. In cranberry, mostly fruit characteristics are used to identify varieties including fruit size, shape, seed number, calyx and stem end morphology, and patterns of ripening. For example, the pyriform shape of 'Ben Lear' fruit is a principle character criterion for evaluation of beds designated as Ben Lear. Vegetative characteristics are sometimes used to evaluate or identify varietal mixtures/off-types in beds. For example, patches of different growth architecture or color can be observed in beds in early spring and during fall color development. However, even fruit characteristics of cranberry are largely continuums of size, shape and color. Fruit shape can be influenced by seed number, which can be impacted or dependant on pollination weather, or pollinator abundance or quality. Thus, there has long been an urgent need for the development of qualitative traits or markers to help in the discrimination of cranberry varieties.

The nature of cranberry underlies variety integrity and impurity issues

The procumbent growth habit of cranberry leads to dense mat beds with individual plants difficult to delineate in mature plantings. Seeds from fallen fruit may germinate and establish within a mature bed. Once established, these genetic variants have the potential to spread vegetatively throughout the planting by stolons, creating a heterogeneous population, especially off-type varieties that produce little or no fruit, placing all resources into runners which compete with the desired variety.

Traditionally, new cranberry plantings are established from stolons (runners) pruned or mowed from a producing bog. Plantings established with stolons from a genetically heterogeneous population have the potential to result in genetic compositions that differ from the bed of origin. Varietal misidentification is exacerbated by the lack of a regulated nursery industry for supply of true-to-type propagation material of cranberry cultivars. No certified nursery system is presently available in cranberry, as is available with other crops, e.g. potato. Planting material is sold with little verification of varietal identity and purity. We are now in the process of developing a 'certified' cranberry materials protocol for Rutgers cultivars.

Development of RAPD and SCAR DNA fingerprinting technology for cranberry

New molecular tools, e.g. protein and DNA markers, emerged late last century which could be employed to better understand the genetics and genetic diversity of cranberry. Our laboratory (**Dr. Leo Bruederle**) first developed a protein marker system analysis referred to as 'isozyme analysis' which was used to study the genetic diversity and structure of native

American cranberry, *Vaccinium macrocarpon* Ait. and related cranberry species (*V. oxycoccus* L.) populations. Although the 'isozyme analysis' could be used to help discriminate cranberry varieties, the system was not practical (economical) for easily identifying cranberry varieties routinely. In the 1980's, a Polymerase Chain Reaction (PCR) technology emerged which offered the opportunity to develop a practical 'fingerprinting' system for cranberry. One particular advantage of the PCR based technology is that very little plant tissue is required to develop a fingerprint. In fact, one cranberry leaf provides sufficient material for a fingerprint. In 1990, our laboratory (**Dr. Rich Novy**) with funding from Ocean Spray Cranberries, Inc. embarked on developing a PCR based marker system using Randomly Amplified Polymorphic DNAs (RAPDs) (*Novy, R.G., C. Kobak, J. Goffreda, and N. Vorsa. 1994. RAPDs identify varietal misclassification and regional divergence in cranberry [Vaccinium macrocarpon (Ait.) Pursh]. Theor. Appl. Genet. 88:1004–1010).* RAPDs were used to begin to sort out varietal misidentification in commercial cranberry cultivars. RAPDs provided genetic evidence to suggest that over time, encroachment by wild vines and unproductive volunteer seedlings from self- or crosspollinations can become established in cultivated beds, lowering yield potential (*Novy, R., N. Vorsa, and K. Patten. 1996. Identifying genotypic heterogeneity in 'McFarlin' cranberry: A randomly amplified polymorphic DNA (RAPD) and phenotypic analysis. J. Amer. Soc. Hort. Sci. 121:210–215; Novy, R.G. and N. Vorsa. 1995. Identification of intracultivar genetic heterogeneity in cranberry using silver-stained RAPDs. HortScience 30:600–604).*

SCARs fingerprinting
However, RAPDs also have some inherent shortcomings. One of particular concern is the lack of inter-laboratory reproducibility. The technology utilizes random 'primers' which do not have absolute homology to a cranberry DNA sequence making specific laboratory methods and conditions a concern. Since large sample numbers must be processed for any of the purposes listed above, a rapid and reliable method is required. Although RAPDs fingerprints provide for good genome coverage and are relatively simple to use, they are not cost effective. The lack of inter-laboratory reproducibility limits transferability, and makes routine scoring and comparison to an existing database more difficult. In addition, most bands (markers) generated using RAPD by any one primer are not polymorphic, necessitating multiple polymerase chain reactions (PCR) with different primers for adequate fingerprinting of each sample. Thus, a fingerprint is based on a data composite of numerous gels.

Derived from RAPD markers, a next generation PCR based fingerprinting system was developed in our laboratory (**Dr. Jim Polashock**) referred to as SCARs (Sequence Characterized Amplified Regions) (*Polashock, J. and N. Vorsa. 2002. Development of SCARs for DNA fingerprinting and germplasm analysis of cranberry. J. Amer. Soc. Hort. Sci. 127(4): 677-684).* SCARs have the advantages of RAPD markers, but with the additional benefits of increased specificity and reproducibility. The most significant drawback of SCAR markers is development time and expense. Once established, however, the cost of individual SCAR PCR reactions are similar to those of RAPD markers, and a reduction in the number of required reactions can decrease overall cost. We are currently employing this system in the variety analysis of materials sent to us by growers and researchers. An example of a fingerprint analysis is given below.
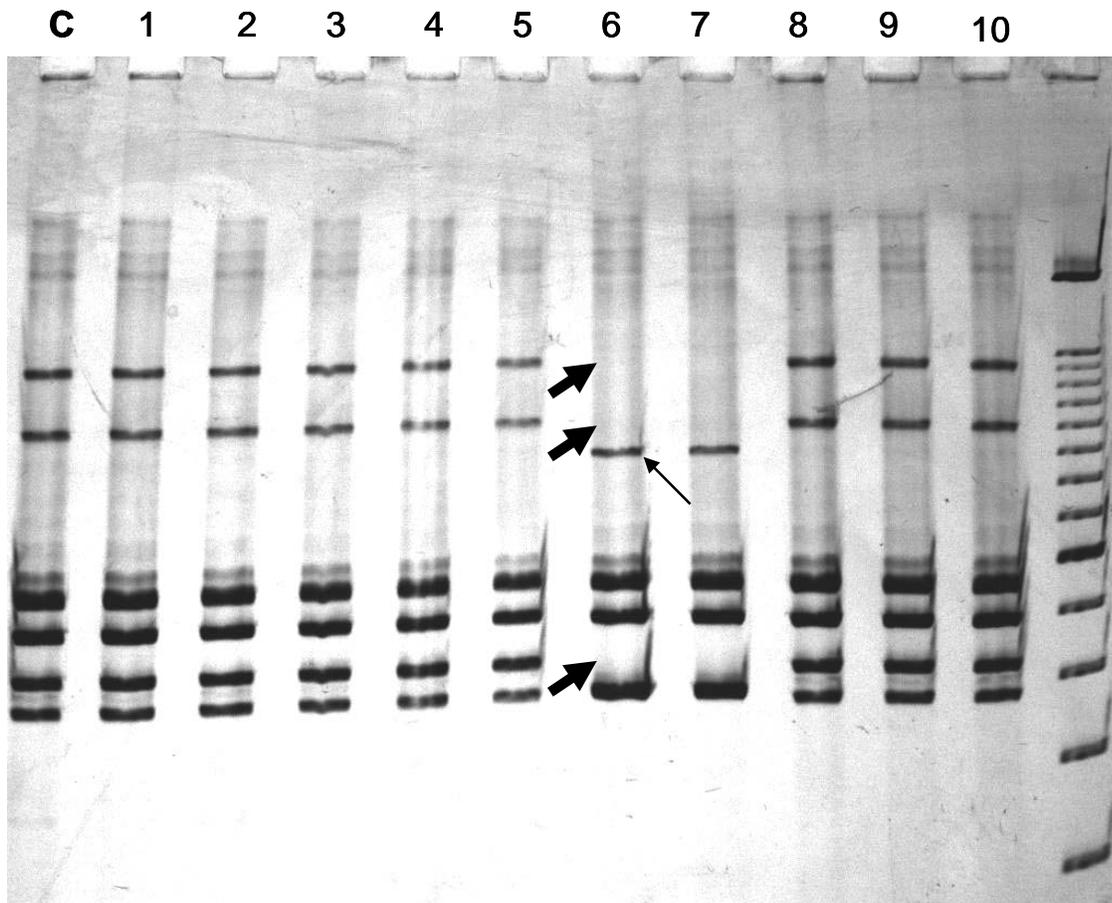
Fig. 1. SCAR fingerprints of Stevens standard control (Lane C) and 10 SCAR fingerprints (lanes labeled 1 – 10) of 10 uprights. Lanes 1,2, 3, 4 5, 8, 9, and 10 match Stevens fingerprint. Lanes 6 and 7 lack at least 3 Stevens markers (large arrows) and have a marker that Stevens fingerprints does not have (small arrow).

We have SCAR fingerprinted most of our cranberry germplasm collection (> 400 accessions). By identifying identical fingerprints in germplasm, we have been able to minimize duplication, thereby increasing the number of genetically distinct accessions in our field germplasm beds. The germplasm fingerprints have also be analyzed for genetic similarity/relatedness providing insight in cranberry genetic diversity and estimates of genetic distance among the varieties. Understanding genetic diversity within the germplasm (gene pool) provides useful information for breeding and genetic enhancement.

We currently bulk five subsamples into one sample to keep costs down. This method allows us to screen for off-types at reduced costs. However, some information is lost, such as what percent of off-types are present. Fig. 2 below represents samples with five subsamples.
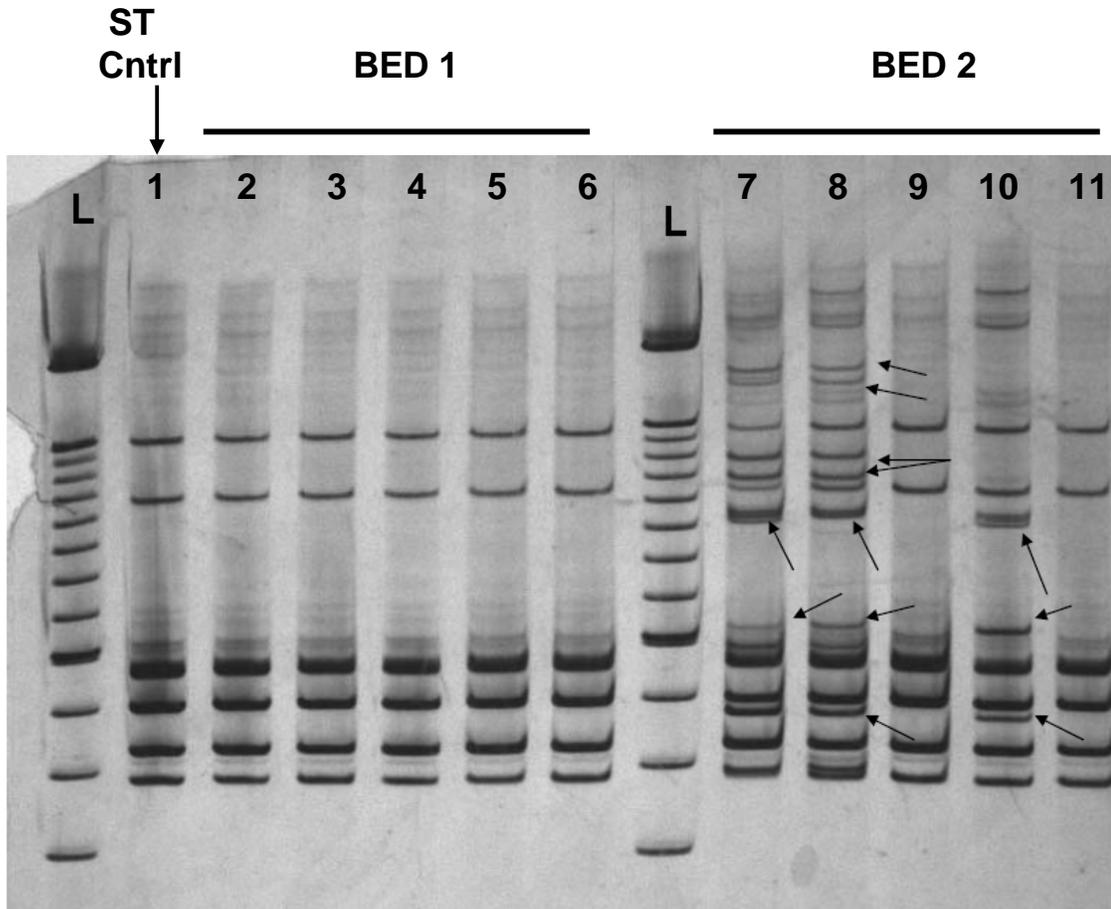

Fig. 2. A SCAR fingerprint (Mix 1) for upright samples taken out of two Stevens beds. Lane 1 represents the Stevens 'control' fingerprint. Lanes 2-6 represent 5 samples which match the Stevens markers (bands) fingerprint. Lanes 7-11 fingerprints exhibit all Stevens markers, i.e., all Stevens bands are present, however, additional bands are presents (arrows) indicating the presence of off-types in at least one of the subsamples.

Future DNA studies for research

Although RAPD and SCAR markers generate substantial polymorphism among cranberry germplasm, we are currently exploring newer marker systems for genetic research in cranberry. We are evaluating microsatellites, SSRs, SNPs, and ESTs. These additional markers should be useful for DNA fingerprinting, marker-assisted selection, mapping, and assessing genetic diversity within cranberry.