Molecular markers - the foundation for grapevine genetic mapping, DNA fingerprinting and genomics


Though widely grown and of great economic significance, relatively little is known about grapevine genetics. Research in grapevine genetics is restrained by the lack of genetic stocks, high heterozygosity, inbreeding depression, large space requirements, and the relatively long juvenile period. In 1957, De Lattin (3) summarized work on 53 genes identified in Vitis. Work on grapevine genetics has intensified since the late 1950s, yet, until 1990, surprising few additional genes were located (13). The recent availability of inexpensive and easy-to-use molecular markers has facilitated research in Vitis genetics. It is now possible to map the grapevine genome, and to create unique DNA profiles for each genotype. The first plant linkage maps were based on visually-scored morphological markers. Later, isozymes and DNA-based markers, which are virtually limitless in number (10), were used to create densely saturated maps. This presentation will review the uses of molecular markers for studies in fingerprinting, genetic mapping, genetic diversity assessment in populations, gene tagging for breeding purposes (Marker-Assisted Selection), and gene cloning.

Types of molecular markers

In order to understand the potential values of the many available molecular markers, it is essential that we define the major ones here. Isozymes are based on multiple forms of an enzyme which differ in electrophoretic mobility. They may be visualized following gel separation. Just over 20 isozyme polymorphisms have been identified in grape. Restriction fragment length polymorphisms (RFLPs) are detected by the use of restriction enzymes that cut genomic DNA molecules at specific nucleotide sequences (restriction sites), yielding DNA fragments which vary in size. They require large amounts of DNA and are relatively expensive to assay.

Polymerase Chain Reaction (PCR) -based assays are generally much less expensive and reveal greater amounts of polymorphism (10, 14). PCR is designed to amplify DNA in an automated, cyclic procedure which results in exponential increases in the quantity of a specific sequence of DNA. Selection of a DNA fragment for amplification is a result of "primer-annealing", in which a primer (5 to about 30 bases long) binds to complementary single-stranded genomic DNA present in the reaction. The primer-DNA complex becomes the starting point for replication of the adjacent DNA sequence by a thermo-stable polymerase supplied in the reaction mixture.

A commonly used PCR analysis is based on random amplified polymorphic DNA (RAPDs). These markers are based on the occurrence of an inverted pair of 9-11 base repeats (occasionally longer or shorter, as well) within a distance of between 200 and 2000 base pairs. This is a single primer reaction which amplifies one to many segments of DNA though PCR. AFLPs (amplified fragment length polymorphisms) are based on selective amplification of restriction enzyme-digested DNA fragments. Multiple bands (50 - 100) are generated in each amplification reaction resulting in DNA markers of
random origin. Neither RAPDs nor AFLPs are considered to be "anchored" i.e. their primary use is within and not between crosses.

On the other hand, a number of Sequence Tagged Site (STS) markers, useful as anchoring loci between crosses, have been developed. The most important of these is the **microsatellite** or simple sequence repeat (SSR) marker (10), based on the discovery of repeated sequences in the genome, 2 to 4 nucleotides in length (e.g. . . . (GCC)17 . . . ). Since the bases flanking the repeat are conserved, but the length of the repeat varies greatly, SSR-specific primers can be readily designed. Each SSR is a single locus with multiple allele sizes. More than 40 SSR loci have been identified in *Vitis*. Additionally, many other STS markers (14) have been developed for use, including **CAPs** (cleaved amplified polymorphic sequences), **SCARs** (sequence characterized amplified regions), **ASAPs** (allele-specific associated primers), and **ESTs** (expressed sequence tags), a subset of STSs derived from cDNA (10).

**Fingerprinting, genotype identification, and diversity assessment**

Forensic technologies now employ molecular markers, and this same technology is being used to solve problems in grapevine identification and parental analysis. Molecular markers have been used extensively in grapes due to the difficulty in distinguishing among similar groups of cultivars. Marker used have included isozymes, RFLPs (1), RAPDs (19), AFLPs (4) and microsatellites (6, 7, 16, 17). As a result, there has been progress in understanding diversity within grapevine germplasm collections (Chap. 8 in 10), relatedness of cultivars from different regions, and in the identification of multiple genotypes within supposedly homogeneous clonal cultivars. In addition, the parentage of certain cultivars has been discovered or confirmed. It is fascinating to know, for instance, that 'Cabernet Sauvignon' descends from a cross of 'Cabernet Franc' x 'Sauvignon blanc' (2) and that 'Müller-Thurgau' is a product of 'Riesling' x 'Gutedel' (12) and not 'Riesling' x 'Sylvaner' as was believed.


**Genetic mapping and marker-assisted selection**

Mapping the grapevine genome is facilitated by the use of existing crosses in breeding programs. Markers heterozygous only in one parent will segregate 1:1, while doubly heterozygous markers will have more complex inheritance patterns (18). Lodhi et al. (9) developed a genetic map in a cross between two interspecific hybrid cultivars. A second map under development (Dalbo, Reisch, Ye and Weeden, unpublished) utilizes two interspecific hybrids which differ dramatically for disease resistance. Additional linkage mapping efforts are underway in California (Meredith and Walker, pers. comm.) and in France (This, pers. comm.). Ultimately, it will be possible to combine a significant amount of the genome information generated by the individual groups into a single genome map for grapes.

With the abundant availability of molecular markers, great progress has been made in their use for early selection of desirable phenotypes (14). In long-cycle, vegetatively propagated crops such as grapes, Marker-Assisted Selection (MAS) is ideally suited. Once important genes are tagged with a marker, pre-selection of very young seedlings
can take place. Only those with the marker/trait of interest are retained. Pyramiding of multiple genes for a single trait can also be accomplished with molecular markers.

Our lab has located markers for traits such as flower sex, Botrytis rot resistance, powdery mildew resistance, and other traits. Statistical procedures can be used to analyze for markers linked to loci affecting quantitatively inherited traits (11) and this process has resulted in our identification of markers for the V. cinerea source of resistance to powdery mildew. There are also reports of markers for genes affecting nematode resistance (Walker, pers. comm.), and hypersensitivity to powdery mildew originating in V. rotundifolia (This, pers. comm.). Markers linked to seedlessness have also been reported (8, 15). One can envision a system whereby DNA extraction from breeding program seedlings would lead to simultaneous screening for the presence of 10 or more essential traits. Field selection could then proceed with a pre-selected population.

**Gene cloning**

A number of important genes have already been cloned from grapes, e.g. the gene for stilbene synthase (5) which results in resveratrol production. While there are several approaches which can be used to clone genes, positional cloning based on genomic linkage maps should provide for the cloning of additional genes (Chap. 12 in 10), but has not yet been utilized in grapes. This approach is opening doors to a greater understanding of the genetics of other crops and, with the small genome size of grapes, should be readily applicable.


(2) Bowers J.E. and Meredith C.P. Nature Genetics 16, 84-87, 1997

(3) De Lattin, G. Vitis 1,1-8, 1957


(9) Lodhi, M.A., Daly, M.J. et al. Genome 38, 786-794, 1995


(17) Vignani, R., Bowers J.E. and Meredith C.P. Scientia Hortic. (Amst.) 65, 163-169, 1996
