Grapevine Genetics: Probing the Past and Facing the Future

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SUMMARY

The development and application of DNA technology is changing the face of grapevine genetics. DNA markers facilitate investigations into the origins of existing cultivars and provide powerful tools for the creation of new cultivars. Microsatellite markers, the most powerful type of DNA markers, provide a unique genetic profile for every cultivar, permitting unambiguous identification that is unaffected by environment, disease or farming methods. Because they are locus-specific and co-dominant, microsatellite markers also detect family relationships and have revealed the origins of some of the world's most important wine cultivars. For the first time, genetic linkage maps of grapevine are now being developed. The long generation time, large plant size, genetic heterozygosity and lack of conventional morphological genetic markers prohibited map development in the past. Now molecular marker based mapping programs are accelerating grape breeding programs by permitting early selection of promising seedlings. Genetic mapping in combination with physical mapping can lead to the isolation of grape genes that control important traits but for which the mechanism is not known. Other grape genes are being isolated by comparing expressed grape DNA sequences with large databases of well-characterized genes from other plants and animals. Methods to introduce genes, either from grapevines or other organisms, into existing grape cultivars are now well-established and permit the targeted modification of existing grape cultivars. This may provide a means to reduce disease losses and pesticide usage in classic cultivars without otherwise changing their wine attributes.

KEY WORDS

grapevine cultivars, breeding, DNA markers, QTL mapping, genetic transformation

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Of all the ancient fruit crops, grape is the most widely grown and valuable today. Despite its economic importance, however, the grapevine has remained recalcitrant to study and manipulation by plant breeders and geneticists because of its large plant size, long generation time and heterozygosity. Almost all of the many thousands of cultivars have arisen spontaneously rather than by deliberate breeding. And the genetic control of important characteristics has remained largely unknown. However, the advent of DNA markers and genetic transformation technology is dramatically changing the face of grapevine genetics today.

Thousands of grape cultivars exist and many of them have been distributed to parts of the world that are distant from their regions of origin. This is especially true in the New World, where almost all grape varieties have been introduced from various parts of Europe. In some cases, these varieties have acquired new names in their new homes. In other cases, names have become confused and switched between varieties. Today, it is more important than ever to identify varieties correctly. Not only is the accurate identification important to nurseries, growers and winemakers, but modern international trade regulations and wine labeling laws require that varietally labeled wines be correctly identified. But grape varieties can be difficult to identify because many of them are very similar in appearance. Expert ampelographers usually specialize in the varieties of one geographic region; no one person is able to identify all grape varieties. The development of DNA markers has greatly simplified variety identification in that it can now be performed by people with no ampelographic training. The results are not only objective but are unaffected by varied environments or disease status. DNA markers have now been used to resolve many cases of synonymy and misnaming (Lopes et al. 1999, Maletić et al. 1999, Meredith et al. 1999).

Of the several types of DNA markers, microsatellite markers (also called SSR markers) are preferred for variety identification. These markers are particularly useful because they are highly reproducible and their patterns can be described as allele sizes rather than bands. Genotype information can thus be expressed objectively and clearly and results can be exchanged between different laboratories as numbers rather than images. With appropriate controls to adjust for small methodological differences between labs, microsatellite genotype information from different laboratories can easily be compared and identification achieved without having either plants or DNA samples of reference vines.

Since the first grape microsatellite markers were reported by Thomas et al. (1993), many more have been developed (Bowers et al. 1996, 1999, Sefc et al. 1999, Di Gaspero et al. 2000, Scott et al.). Most of them are highly polymorphic and six markers are generally sufficient to differentiate cultivars, although a larger number may be needed in the case of closely related cultivars such as some seedless table grapes (Crespan et al. 1999, Sánchez-Escribano et al. 1999). A set of six specific markers has been agreed upon for the purpose of international information exchange and efforts are currently underway to fully harmonize the results from different laboratories.

Unlike some other types of DNA markers, microsatellite markers are true genetic markers—they are locus-specific and co-dominant. Thus the analysis and comparison of microsatellite genotypes can reveal not only identity but also genetic relationships. Just as DNA testing is used to establish human parental relationships, so can it also identify the parents of a grape variety.

Because grapevines are propagated vegetatively, cultivar genotypes are perpetuated virtually unchanged over time, in some cases for centuries. However, each cultivar ultimately began as a single vine that grew from a seedling. The parents of the original seedling can be detected because each parent has contributed one allele to the genotype of the progeny variety at each microsatellite locus. This approach was used to confirm a known grape variety pedigree in 1994 (Thomas et al. 1994), but it was not until several years later that DNA markers were used to detect a previously unsuspected genetic relationship. The red wine cultivar Cabernet Sauvignon was discovered to be the progeny of two other well-known wine cultivars, Sauvignon blanc and Cabernet franc (Bowers and Meredith, 1997). Parents have now been reported for Muller-Thurgau and several other cultivars (Sefc et al. 1997, Lopes et al. 1999, Dettweiler et al. 2000).

Of some historical interest, microsatellite analysis of more than 300 varieties grown in France revealed that 16 of them are full siblings, all the progeny of the same two parents, Pinot and Gouais blanc. The progeny varieties, which encompass all the varieties grown today in northeastern France, include Chardonnay, Gamay noir, Melon, Aligoté and Auxerrois. Both parental varieties were once widely grown in northeastern France, but Gouais was considered to be mediocre and was eventually prohibited. Gouais blanc is not related to Pinot and is not French. It is, in fact, an eastern European variety (also called Heunisch weiss) that is thought to be of Dalmatian origin (Goethe 1887). Historical writings strongly suggest that Gouais/Heunisch was brought to present-day France by the Roman emperor Probus, a gift to the Gauls from his homeland of Dalmatia.

For modern grape breeders working to develop new varieties, DNA markers can be an invaluable tool. Grape is a difficult species with which to perform

conventional genetic analysis due to its large plant size, long generation time and heterozygosity. Because of these limitations, very few morphological genetic markers have ever been characterized so no genetic map could ever be generated. But genetic maps can readily be generated with DNA markers so grape maps have recently emerged from several research groups. Lohdi and Reisch published the first grape linkage map, consisting largely of RAPD markers (Lodhi et al., 1995) and later added other marker types to it (Dalbo et al., 2000). A framework map consisting entirely of microsatellite markers has also recently been produced (Riaz and Meredith, 2000) that can be used to anchor different maps being generated for different purposes from different crosses (Buck and Zyprian 2000, Grando et al. 2000).

Genetic maps can assist breeders by enabling them to detect traits that are tightly linked and thus likely to be inherited together and also to determine the number and location of genetic factors controlling quantitative traits. A gene controlling flower gender has now been mapped (Dalbo et al., 2000a) and quantitative trait locus (QTL) analysis has been used to identify several genetic regions controlling resistance to fungal disease in an interspecific cross (Dalbo et al. 2000b). Among other traits being mapped are seedlessness and fruit cluster characteristics.

Many traits of interest to breeders, such as fruit appearance or flavor or resistance to pathogens, are not apparent in segregating breeding populations until the seedlings are several years old. However, if DNA markers can be identified that are located very close to genomic regions that control such traits, then small seedlings can be analyzed to rapidly identify individuals that are likely to carry the traits of interest, greatly reducing the space and resource requirements needed to grow up all the seedlings to mature plants. This approach is being used for the early identification of seedlings segregating for nematode resistance (Walker and Jin, 2000), flower gender (Dalbo et al., 2000) seedlessness (Striem et al. 1996, Lahogue et al. 1998) and berry color (Ren et al. 2000).

Ultimately, genetic mapping can be used to isolate important grape genes. Some genes can be isolated from grape DNA based on sequence information if their counterparts in other plant species have already been cloned and sequenced. This has been demonstrated for a number of grape genes, including those associated with anthocyanin biosynthesis, nitrogen metabolism and sugar transport (Sparvoli et al. 1994, Fillion et al. 1999, Or et al. 2000, Primikirios et al., 2000, Tesničre and Verričs 2000). But when the exact function or product of a gene of interest is not known, map information about molecular markers that are tightly linked to the gene can lead to its isolation by positional cloning. This approach may eventually lead to the successful isolation of disease resistance genes from *Vitis* species.

Genes can now be introduced into cultured grapevine tissues and transgenic vines can be produced (Thomas et al., 2000). This technology offers the possibility for the directed modification of a single trait in a traditional variety without otherwise changing the attributes of the variety. Although it still remains to be seen whether such a transgenic vine will be permitted to retain its original variety name (perhaps with a suffix, as with clones), many research groups are moving forward with projects related to disease resistance and fruit quality.

Among the first areas to be explored was resistance to virus diseases by means of introduced coat protein genes (LeGall et al., 1994; Krastanova et al., 1995). This approach has produced some promising results but conclusive field trials have not yet been completed (Golles et al., 2000; Mauro et al., 2000). Encouraging progress is being made in the area of resistance to fungal pathogens. Several groups are working with chitinase genes from various organisms. Recently Yamamoto et al. (2000) have reported enhanced resistance to powdery mildew (*Uncinula necator*) in grapevines expressing a chitinase gene from rice. Increased tolerance to the systemic fungus *Eutypa lata* has been demonstrated in grapevines transformed with a eutypine detoxifying enzyme gene cloned from *Vigna radiata* (Legrand et al., 2000).

In the area of fruit composition and quality, seedlessness in table grapes may be achieved by transformation of seeded cultivars with lethal genes fused to seed-specific regulatory sequences (Perl et al., 2000). A gene construct designed to silence the polyphenol oxidase gene has been introduced in Australia with the objective of reducing browning in raisins (Thomas et al., 2000). Ongoing studies of the genes controlling grape berry development and composition (e.g., Ford et al. 1998, Tesničre and Verričs 2000, Or et al. 2000) will no doubt soon lead to additional transformation projects related to fruit quality.

The door to the grapevine genome has been opened by molecular biology and researchers worldwide are moving towards it. Large-scale gene discovery and genome sequencing efforts are getting underway that will eventually reveal the structure, function and regulation of all the important grape genes (e.g., Ablett et al. 2000, D.O. Adams, personal communication). Just a few years ago, the grapevine was considered a poor genetic organism. Its large size and long generation time severely limited efforts at genetic analysis and variety improvement. But the advent of molecular biology has now reduced those limitations to insignificance. The speed with which grapevine genetics is now advancing is finally in balance with the international importance of this ancient crop.

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