Microsatellite Analysis of Grapevine Varieties by High-Resolution Amplicon Melting Analysis

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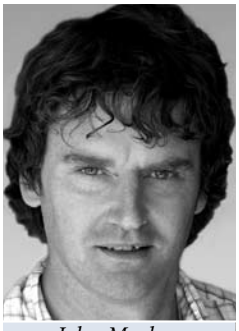

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Introduction

Certification of grapevine scion and rootstock varieties is becoming an increasingly important issue in viticulture. Many rootstocks are derived from the same parentage – for example 5C, SO4, 5BB, 125AA, and 420A Mgt are all used in New Zealand and are all derived from crosses between *Vitis berlandieri* and *Vitis riparia*. This use of limited genetic stock is not uncommon when compared with other countries and means that cultivar determination based on ampelography (visual characteristics of grapevines) can be difficult at times due to the high similarities [1].

The use of microsatellite analysis has been well-characterized for grapevine scion and rootstock varieties [2–4]. Analysis has typically relied on laborious polyacrylamide gels followed by silver staining or, more conveniently, fluorescent resolution on automated sequencers. Nonetheless, this higher automation using sequencers still requires post-PCR handling and dilution steps as well as a fluorescently labeled primer for each microsatellite, which adds to the time and costs involved.

We investigated whether high-resolution melting analysis – primarily used for SNP discovery and genotyping – could be used to verify microsatellite identity of grapevine samples by comparing the melting curves of microsatellite amplicons for a query variety sample and a known reference sample of the same variety. Such a matching application has been successfully applied for HLA identity [5].

Materials and Methods

100–150 mg young unexpanded leaf material from each sample was homogenized in a guanididium buffer and genomic DNA was extracted before being quantified and diluted to a concentration of 20 ng/µl in PCR-grade water.

A reference set of microsatellites [2] was analyzed. PCR reactions consisted of 1x LightCycler® 480 High Resolution Melting Master supplemented with 2 mM $MgCl_2$, 0.25 µM each primer, and 50 ng genomic DNA. Reactions were performed in 96-well plates, using a volume of 10 µl or 20 µl. The cycling program for all tested microsatellites consisted of a touchdown protocol to allow all microsatellites to be amplified within the one run, and the amplification cycles were immediately followed by the high-resolution melting steps with 25 fluorescent acquisitions per degree Celsius.

Results and Discussion

Initially, the amount of DNA per reaction was not standardized prior to amplification – up to 10-fold variation in template concentrations may be used in high-resolution melting reactions [6]. However, increased reproducibility of melting curves was observed by first quantifying and diluting the samples to a uniform 20 ng/µl prior to amplification. This was likely due to the slight polysaccharide variation (and/or other inhibitors) of the undiluted grapevine samples affecting the melting, and therefore dilution of samples to a standard template concentration removed this variation as seen by the equivalent amplification crossing points (data not shown). One melting plot was able to differentiate a number of different rootstock varieties (Figure 1).

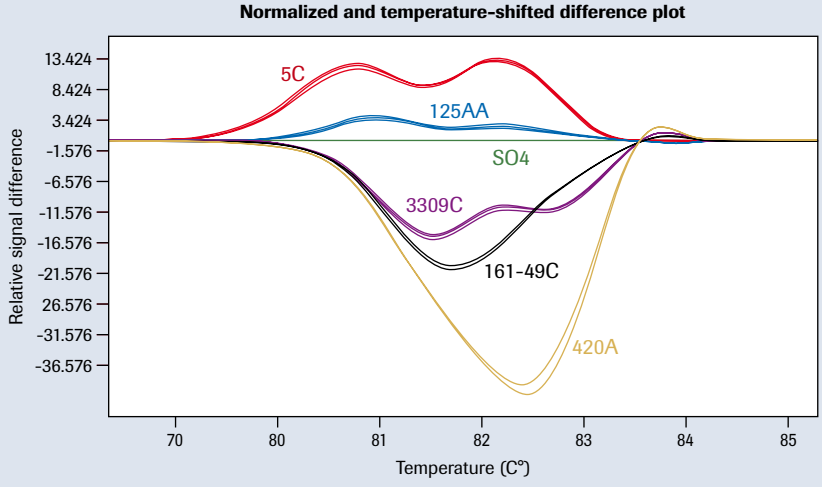

Figure 1: Difference plot of six closely related rootstocks derived from *Vitis berlandieri crosses* and *Vitis riparia* crosses: 5C, SO4, 125AA, 3309C, 161-49C and 420A. Five individual extractions were amplified for each variety at microsatellite ZAG 62.

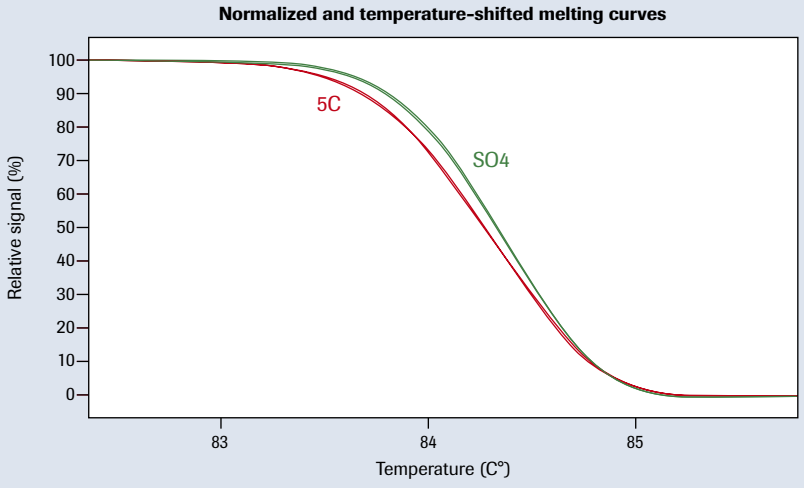

Figure 2: Five DNA extractions for each rootstock cultivar 5C and SO4 were amplified for microsatellite VVMD32 where the two rootstocks share one allele and differ by 2bp (a single CT repeat) at the other allele.

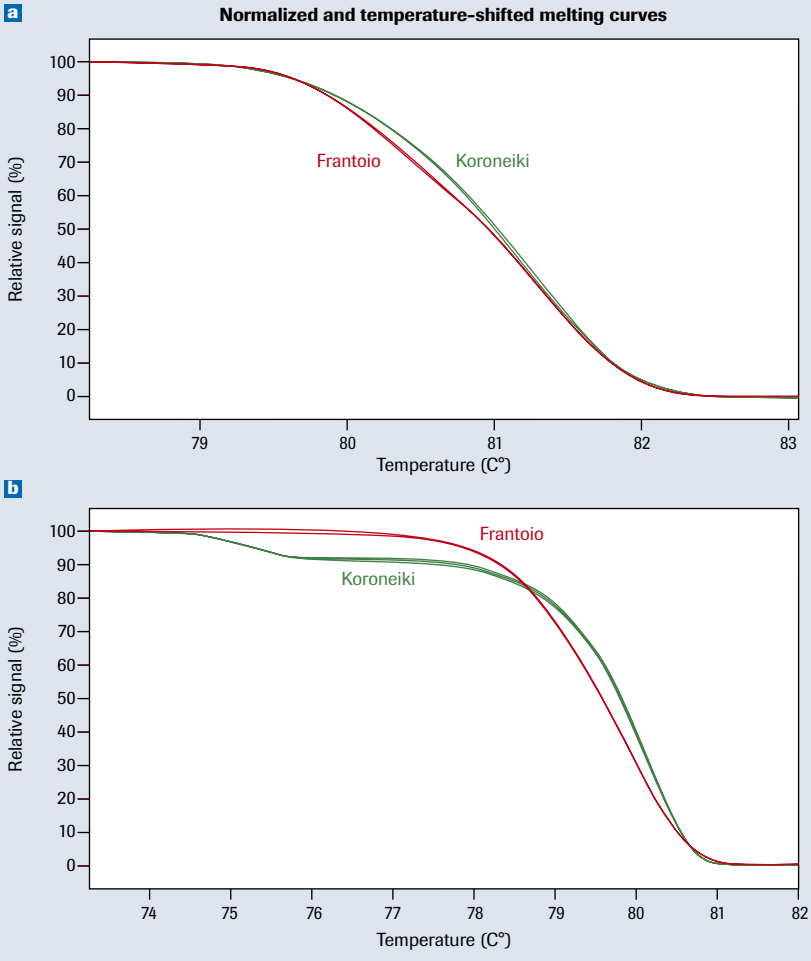

Figure 3: 5 DNA extractions for each olive cultivar "Frantoio" and "Koroneiki" showing distinctive melting profiles with (a) the microsatellites DCA-3 and (b) UDO-12.

In order to test the resolution sensitivity of this method, we examined two rootstocks that have been historically confused [7]. Previous reports of rootstocks 5C and SO4 at microsatellite VVMD32 showed they both shared an allele and differed by 2 bp (or a single CT dinucleotide repeat) at the other allele. High-resolution amplicon analysis was able to differentiate these samples (Figure 2), with the analysis software distinguishing between these rootstock samples.

We have also used this method for other plant species in order to test its wider applications. We have amplified microsatellite loci in olive cultivars (Figure 3) and shown clear resolution of varieties.

Conclusions

The speed, resolution and closed-tube nature of high-resolution melting provide similar benefits as with other real-time PCR applications; in this instance there is an order of magnitude increase in speed along with ease of microsatellite comparisons for our grapevine certification and other crop programs. ■

Acknowledgements

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