Microsatellite-based DNA Fingerprinting of Selected Grapevine Cultivars

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Cultivar identification by ampelography is often difficult and is sensitive to environmental conditions, thus it can be problematic to distinguish between closely related cultivars. DNA fingerprinting offers an alternative method that is not influenced by the environment and is relatively easy to perform. However, discriminating between closely related individuals can be problematic if inadequate or insufficient markers are used. Following the estimation of null allele frequencies, an initial set of 35 microsatellite markers was reduced to 20 to generate unique DNA fingerprints for the majority of 111 different grape cultivars and breeding lines. Molecular evidence was utilised to evaluate the accuracy of the reported pedigrees for several cultivars bred in South Africa (SA). The use of markers linked to known downy mildew and powdery mildew resistance loci (*Rpv3* and *Ren3*) provided information regarding the frequency of these resistance loci in the breeding material analysed.

INTRODUCTION

Traditionally, ampelography has been used for cultivar determination in grapevines, but since it is environmentally sensitive it can lead to erroneous identification, especially in artificial conditions, and it is not useful for clones (Vignani *et al*., 2002; This *et al*., 2004). Molecular markers designed around simple sequence repeats (SSRs, also known as microsatellites) offer a higher differentiating power and, while they can be difficult and expensive to develop initially, SSRs have proven to be a robust and effective tool for identification, parentage assignment and genetic mapping in *Vitis* (Dangl *et al*., 2001; Akkak *et al*., 2007; Bellin *et al*., 2009). In recent years, the use of automated fluorescence technologies have made the simultaneous analysis of several loci feasible and have decreased the cost per data point (Adam-Blondon *et al*., 2004; Merdinoglu *et al*., 2005; Bautista *et al*., 2008; Ibáñez *et al*., 2009).

More recently, single nucleotide polymorphisms (SNPs) have also been used to generate DNA fingerprints (Nybom *et al*., 2014), determine genetic diversity and population structure (Emanuelli *et al*., 2013), generate genetic maps (Troggio *et al*., 2007), and identify candidate genes in association studies (Emanuelli *et al*., 2010). In general, SNPs are bi-allelic, with a maximum of four alleles possible in outbreeding populations. This level of polymorphism is lower than what is regularly found for SSR markers, making individual SNP markers less informative than most SSR markers. However, the use of high-density arrays means that very large numbers of SNPs can be genotyped at relatively low cost and, by using large numbers of SNPs, the same overall level of variation can be observed. Emanuelli *et al*. (2013) used both SSRs and SNPs to investigate genetic diversity and population structure in grapes and found that they yielded similar results, but that the greater information content of SSRs made them more suitable for core collection construction.

When dealing with the identification of grape cultivars and parentage assessments, several problematic issues must be considered. Grapevines are propagated by cuttings, and therefore all accessions of a specific cultivar are expected to be genetically identical. However, somatic mutations could result in changes to the DNA fingerprint of an individual plant without necessarily changing the phenotype (Martínez *et al*., 2006). It is even more probable that somatic mutations will cause changes in the phenotype without resulting in changes to the DNA fingerprint (Imazio *et al*., 2002). The latter often results in the establishment of a "clone" of the

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