South African locally selected macadamia cultivars have a unique genetic identification profile compared to imported cultivars

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Identification of macadamia cultivars is essential for growers and breeders alike. However, cultivar classification based on key characteristics is often misleading since it is difficult to visually discriminate between closely related cultivars.

Most commercial macadamia is composed of two species, *Macadamia integrifolia* and *Macadamia tetraphylla*, and hybrids of these two species. *M. integrifolia* and *M. tetraphylla* can be easily distinguished from each other as they differ in a number of key characteristics. *M. integrifolia* has bright pink flowers, a larger tree size and average-sized nuts, whereas *M. tetraphylla* has white flowers, smaller tree size, and larger nuts with a higher sugar content. Other traits such as production, yield, precocity, and growth characteristics aid in differentiating cultivars.

A comprehensive guide was published by Allen (2006) to aid South African farmers to visually differentiate cultivars. However, it remains challenging to differentiate between cultivars of the same species. This is of particular concern since cultivars that look similar may differ at a genetic level and respond differently to environmental factors such as drought, elevation and humidity. The advent of technologies such as genetic markers has allowed for drastic improvements in plant breeding, as they can be applied both to manage breeding programmes, and to better understand plant biology at a genetic level. Genetic markers are used for many applications – such as cultivar identity or DNA fingerprinting, parentage analysis of nuts or pollination, and assessing genetic diversity and structure of breeding populations – to benefit growers, breeders and researchers (Gupta and Varshney, 2000).

Microsatellite markers are a type of genetic marker, and are efficient for DNA fingerprinting. As the name suggests, they are "micro" or short sequences of repetitive DNA, present throughout the genome of all living organisms (Wang et al. 2009). Microsatellite markers are transferrable between laboratories that yield reproducible results, making them ideal for small, local scale DNA fingerprinting. A relatively small number of DNA markers is required for accurate analyses, and these can be combined into a single reaction, thereby reducing the cost per tree (Gupta and Varshney, 2000; Pokhriyal et al. 2012). Microsatellite markers have been developed for macadamia species (Schmidt et al., 2006; Nock et al. 2014; Langdon et al. 2019) and have been used to perform population analysis for the Hawaiian Agricultural Experimental Station (HAES) and Australian macadamia collections.

These published microsatellite markers were used in the current study for cultivar differentiation and population analysis of macadamia cultivars planted in South Africa. Leaf material was collected for DNA isolation, and this was analysed using microsatellite markers. Two panels containing 13 microsatellite markers were established to fingerprint a total of 110 imported and locally selected commercial and non-commercial cultivars (Fig 1). Analysis was performed to determine the genetic diversity, differentiation and population structure of South Africa's national macadamia collection.

The results indicated that the microsatellite markers were efficient at differentiating cultivars from each other, and grouped cultivars according to species, as is seen in the separate clusters of *M. integrifolia* and *M. tetraphylla* cultivars in Fig 1. The *M. integrifolia* group contains cultivars that were selected at the HAES and internationally released for commercial propagation. A recent study by Nock at el. (2019) showed that the original HAES commercial cultivars were sourced from a small area (gene pool) in Mount Bauple, Queensland, and have lower genetic diversity. Some Australian cultivars that were released for global production group with *M. integrifolia*, while others such as A14, A16 and A38 lie between the *M. integrifolia* and *M. tetraphylla* groups, confirming their hybrid status. This indicates that the HAES and Australian breeding programmes focused selections on *M. integrifolia*. They were possibly selecting for processing qualities, such as specific average nut size for cracking and higher oil content which allows for even roasting at specific temperatures.

The *M. tetraphylla* group consists mainly of South African local selections, which includes the commercially selected Nelmak 2, Nelmak 26 and Nelmak D, and non-commercial cultivars present at the Ukulinga Research Station in KwaZulu-Natal. This group also contains cv. Kate_II, recorded as being a *M. tetraphylla* selection from California (Hardner et al. 2009). Peace et al. (2005) also observed that the South African selections have a strong *M. tetraphylla* background using an earlier genetic marker set. From literature we know that pioneers of macadamia cultivation in South Africa preferred the sweeter *M. tetraphylla* species and thus made selections from these (Allan, 2016), with the exception of the UNP-F lines which are open-pollinated seedlings of cv. Faulkner (*M. integrifolia* species). Early performance records of the non-commercial cultivars indicated some promise, but the industry focused predominantly on imported cultivars for widespread production. Cultivars locally selected in South Africa form a genetically separate group representing predominantly *M. tetraphylla*, unlike the HAES and Australian commercially released cultivars which are predominantly *M. integrifolia*. The *M. tetraphylla* species has favourable traits, such as shorter trees with sweeter nuts, which can be selected for future breeding programmes.

Figure 1. Principal coordinate analysis of macadamia analysed based on genetic distance.

A local farmer's breeding population was included in the analysis. Results show that the breeding population forms its own cluster and is genetically differentiated from the South African, HAES and Australian groups. This population consists of hybrids that were selected to produce high yields but still maintain high macadamia nut quality standards. This is an example of how breeding can generate new and unique variation and result in cultivars with economically important traits. DNA markers were able to show how specific breeding objectives may affect the genetic profile of breeding material and result in genetically differentiated populations.

The outcomes of this study include a local database with each cultivar's unique DNA fingerprint. This database can be used by nurseries, growers and breeders to keep accurate records of cultivars. Furthermore, the DNA marker panels generated in this study can be applied to identify unknown cultivars, perform pedigree reconstruction, confirm outcrossing, verify parentage in controlled pollinations and generate a unique DNA fingerprint for protection of newly developed cultivars. In addition, the results will add to the genetic information on macadamia being globally generated.

This study demonstrates how microsatellite markers can be used to distinguish cultivars, enabling accurate identification of cultivars based on robust genetic methods. Furthermore, the technology can be applied to parentage confirmation. This can be used to validate pedigrees in local breeding programmes aimed at creating new cultivars with improved productivity and better-quality nuts with high yields, suited to the South African climate, that also reduce the time to selection.

This project forms a part of the Forest Molecular Genetics (FMG) Programme at the University of Pretoria and the Forestry and Agricultural Biotechnology Institute (FABI). The DNA Marker Platform is a technology service platform at FMG which has extensive experience and expertise, and has provided the South African forestry industry with a high-throughput DNA fingerprinting analysis service for more than a decade. The DNA Marker Platform also manages a central database for storage of and access to genetic data and metadata for the South African forestry industry. This database allows various stakeholders such as nurseries, growers and breeders to maintain accurate, DNA marker verified records of unique varieties, pedigrees and estimates of species composition, which in turn could be used for the protection of newly developed cultivars (Plant Breeders Rights).

Cultivars differentiate into three distinct genetic clusters. The green cluster represents *M. integrifolia* species and contains Hawaiian Agricultural Experimental Station (HAES) and Australian representative collections, the pink cluster represents *M. tetraphylla* species and contains South African local representative collection, and the blue cluster contains the local farmer's breeding population. The font colour represents cultivar origin, with the green font representing the HAES cultivars, gold font representing the Australian cultivars, the pink font representing the South African cultivars, the blue font representing the local farmer's cultivars and the grey font representing cultivars originating from California and Israel.

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