Establishing a Database of DNA Fingerprints to Identify Olive Cultivars

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Abstract

Olives were first introduced to South Australia in 1836 but the industry has not prospered for various reasons. Over the years, records concerning the names of many cultivars have been lost or misplaced and the names of many cultivars which were imported are now no longer used by the industry. This has created a considerable degree of uncertainty about the names of many cultivars being grown in this country. To overcome the problem of identification, we have established a database of DNA fingerprints produced from leaves of numerous olive accessions obtained both from within Australia and overseas. This database is used to compare the DNA fingerprints obtained from leaves of trees sent to us on a fee basis by growers as well as to determine the level of genetic diversity between both commercial and feral cultivars. We have found that some cultivars, such as Manzanillo and Kalamata, show a high level of genetic similarity regardless of source while others, such as Verdale, show considerable genetic variability. Nevadillo and Picual are sometimes considered to be a synonym for the same cultivar, but our experience indicates that they are probably separate cultivars. On the other hand, Corregiola and Frantoio and their synonyms show a high degree of genetic similarity. A major collection of 100 commercial olive cultivars is being established near Adelaide in South Australia and the identity of each cultivar is being determined by comparison of the DNA fingerprints with our genetic database.

INTRODUCTION

The history of olive farming in Australia dates back to the early 1800s with the first introductions to South Australia occurring in 1836. The earliest cultivars included Bouquettier, Blankette, Verdale, Sevillano, and Mission, some of which are still popular in the Australian industry. Since these first introductions, numerous other cultivars have been imported into Australia. About 40 were introduced into South Australia alone by 1920, and the number increased to 55 by the 1930s (Quinn, 1920; Fowler, 1939) and now more than a 100 cultivars with a number of synonyms and homonyms are grown.

Australia has a climate similar to that of countries bordering the Mediterranean and is therefore ideally suited to olive growing and the production of high quality oil. In 1913, South Australia was producing about one third of the national oil consumption (Perkins, 1917). However, problems such as the high cost of labour for olive picking, poor management practices, and the relatively low cost of imported oil (Fowler, 1939) inhibited the expansion of the industry or even the proper maintenance of many established plantations. This has led to the decline of the Australian olive industry and the eradication of many olive trees.

By the 1950s, a number of olive trees were uprooted and many olive stocks were replaced by other fruits. For many of the remaining orchards, records about the names and derivations of the cultivars were often misplaced or confused, leading to a high degree of uncertainty about the names of some cultivars now being grown. In addition, some imported cultivars have been given new names. Burr (1997) ascertained the existence of more than 118 named olive cultivars that are recorded as being introduced into Australia, where the names are now generally unknown in the industry. Furthermore, the presence of synonyms and homonyms in olive cultivars is a common problem in olive producing countries world-wide. For example in Australia the cultivar Verdale is known as Verdale,

SA Verdale and Wagga Verdale. Similarly, cultivar Mission is also known as Californian Mission and WA Mission. Nevadillo is known as Nevadillo Blanco and Picual. There is a question of whether these synonyms refer to genetically identical cultivars. In addition, interbreeding and natural seed spread has resulted in the widespread growth of feral olives throughout the State and this has provided a large gene pool for the selection of new types.

The growing demand for olive products, and the profits that could accompany it, has led to the recent investment in, and expansion of, the industry in Australia. However the choice of the most suitable, clearly identified variety for a particular area is one of the most important decision for growers to make. To overcome the problem of identification, a database of DNA fingerprints produced from leaves of numerous olive accessions obtained both from within Australia and overseas was established in 1998 using the random amplified polymorphic DNA (RAPD) technique. Fingerprints from this database are used to identify both known and unknown olive trees.

MATERIALS AND METHODS

Leaf Materials

Leaf samples from olives obtained from overseas and various States in Australia were used for DNA isolation. The overseas countries include Italy, Spain, U.S.A., Israel, Greece, and Turkey. Olive leaves were transported from overseas and within Australia in the following way: Fresh leaves, fully-grown, free of apparent pests and diseases, and still attached to the stem were tightly wrapped in polyethylene film to exclude as much air as possible. A label was attached setting out the variety, the date the leaves were collected, the name of the person who collected the leaves, and the location of the tree, and a further layer of polyethylene film was applied. In this way it was possible to obtain DNA of high quality from leaves sent from various sources after more than 10 days in transit and subsequent storage for up to 12 weeks at 4°C. Alternatively leaves can be stored permanently at -80° C.

DNA Isolation

DNA was extracted based on the method described in Mekuria *et al.* (1999) Approximately 2 g of fresh leaves were ground with liquid nitrogen and extracted with hot CTAB. RNA was removed with RNase A followed by precipitation of protein including RNase A. The absorbance of the DNA preparation was determined at 230, 260, and 280 nm and the quality of the DNA was estimated by calculating the ratios of the absorbance at 260 and 230 and at 260 and 280 (Johnson, 1994). DNA samples with absorbance ratios of 1.8 and greater were used for further analysis, and stored at -20°C. Information for each DNA sample was recorded into the database. Each cultivar is given a unique code number, which is recorded together with details of the name of the cultivar, source or location of the tree, the date the leaves were collected, the name of the person who collected the leaves, the date of DNA extraction, the quality of the DNA, and the DNA concentration.

DNA Amplification

Six random decamer oligodeoxynucleotide primers are used in the polymerase chain reaction (PCR). The primers are either purchased from Operon Technologies, Almeda, CA, USA or made by the Nucleic Acid and Protein Chemistry Unit, The University of Adelaide. Duplicate PCRs for each primer are performed in a volume of 20 µL containing 40 ng of DNA, and the PCR products are separated on 1.5% (w/v) agarose gel (Seakem, Promega) or 6% polyacrylamide gel electrophoresis as described in Mekuria et al. (1999). The presence and absence of bands was determined using Gel-Pro Analyser (Version 3.1, Media Cybernetics, Maryland, USA) and analysed using the simple matching coefficient and the unweighted pair group method with arithmetic averages (UPGMA) and SAHN algorithm to cluster individuals into a dendrogram.

RESULTS AND DISCUSSION

DNA from more than 400 accessions representing both commercial olives and accessions of feral olives, has been extracted and used for DNA analysis and the information recorded in the database. So far, the DNA fingerprints of about 80 accessions of commercial olives and about 45 accessions of feral olives have been prepared and stored for comparison with the DNA from trees within Australia and overseas.

The results show that some cultivars such as Manzanillo, Barnea, Picual, Sevillano Nevadillo (Nevadillo Blanco), and Kalamata have very high within-cultivar genetic similarity regardless of source. On the other hand, high genetic variability is found to occur between accessions of Verdale obtained from different sources. Nevadillo and Nevadillo Blanco show almost similar DNA fingerprints to each other but are quite different to Picual. On the other hand, some accessions of Frantoio show close genetic similarity to Corregiola accessions.

The misnaming of some cultivars and the confusion of names is clearly exhibited in many commercial olives studied. For example, some accessions named as Mission within Australia show DNA fingerprints identical to Manzanillo while others are similar to Verdale. Similar results have been found for Pendolino and Pendulina. Other studies confirmed the level of genetic variation that is exhibited between accessions of some commercial olives (Fabbri et al., 1995; Weisman et al., 1998; Mekuria et al., 1999). The DNA fingerprints held within the database are used to compare the genetic similarity of known or unknown olive cultivars received from growers or any other sources, for DNA analysis. The number of DNA fingerprints held with the database is continually being expanded as leaves are obtained from collections around the world.

Matching olive cultivars to the best environment to optimise oil quality is an important consideration for olive growers. This is the aim of the National Olive Variety Assessment program (NOVA) in Australia. A national collection of 100 olive cultivars is being established at the Roseworthy Campus of The University of Adelaide to provide detailed information on the suitability of cultivars for Australian conditions. The collection will include oil producing types currently available in Australia as well as new cultivars from different parts of the world that have been grown successfully under irrigated conditions. In addition to the main collection, twelve cultivars that are the most important to the Australian industry will be planted at the Waite Campus of The University of Adelaide as mother plants for research.

To complement these two collections, growers around Australia are sending in physiological data such as tree and fruit morphology, yield, and oil analysis from their olive orchards to add information to the national database.

CONCLUSION

The current Australian olive improvement program is a highly structured and co-ordinated effort to ensure that this latest phase in the development of the industry brings together the best management practices, the best varieties, and high grade research. The outcome will be a quality-assured product which will be in demand on both the Australian and overseas markets.

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