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METHODS USED IN AVOCADO BREEDING

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Abstract. A hybridization programme involving controlled hand pollinations has been developed for the avocado. The floral mechanism is very temperature-sensitive and crosses are carried out in a temperature-controlled glasshouse to ensure suitable conditions for pollen tube growth and fruit set. Grafting techniques are used to ensure synchronous flowering of cultivars which otherwise would not flower at the same time. Because the majority of pollinated fruitlets abscise an embryo-culture method is under development to increase the numbers of progeny obtained from each cross. Progeny are topworked onto large stumps for a rapid assessment and are also planted out on virus-tested seedling stocks.

The avocado (*Persea americana* Mill.) is a relatively new crop to Australia and the industry is based largely on cultivars developed overseas. These cultivars do not entirely satisfy the requirements of the Australian industry and are not suitable for the wide range of climates present in Australia. There is demand for year-round supplies of fruit and there are also problems with existing cultivars due to the biennial bearing habit. Other scion characteristics of interest include time of flowering, fruit colour, shape and size, skin thickness, seed size, and fresh flavour, quality and oil content. Desirable rootstock characteristics include salinity tolerance, resistance to the root rot fungus *Phytophthora cinnamomi*, and a dwarfing habit.

There are three methods of tree crop improvement. The first is plant introduction where cultivars developed overseas are introduced and tested under Australian conditions. The second method of improvement is known as field selection. Seedlings from open-pollinated trees are assessed for horticultural characteristics. This method may yield a new superior cultivar but nothing is learned of the inheritance of the desirable characteristics as the female but not the male parent is known. The third method of improvement is controlled hand pollination followed by selection of the progeny for desirable characteristics. Using this method the identify of both parents is known and a study of the genetics of the crop can be commenced. This latter approach will be described further.

Woody perennial tree crops are particularly difficult to breed for three reasons. Most species have outbreeding mechanisms, which must be understood before crossing can be commenced; they have low fruit to flower ratios, and all have long generation times. Our research aims to overcome these problems.

The avocado has an outbreeding mechanism called dichogamy. Each flower opens twice. On first opening the flower is in the female stage with the pistil exposed, the stamens reflexed against the petals and the anthers not yet dehisced to release the pollen. In this stage pollination of the flower results in fertilisation and fruit set. The flower then closes completely and re-opens the following day in the male stage. The pistil is now obscured by the stamens whose filaments have extended and anthers dehisced to release the pollen. In this stage the flower can no longer be fertilised. The flower then closes again and does not re-open. Complementary flowering types exist so that pollen transfer can occur. In type A cultivars, e.g. 'Hass', the flower opens in the female stage in the morning. It closes toward the middle of the day and re-opens in the male stage during the afternoon of the following day. In type B cultivars, e.g. 'Fuerte', the flower opens in the female stage in the afternoon, closes overnight and re-opens in the male stage the following morning. The overall effect of this is that open flowers on a type A tree are female in the morning and male in the afternoon and vice versa for a type B tree. Pollen transfer can thus occur between the two flowering types. In the orchard the pollen is transferred by insects

Our research using controlled environment growth cabinets has shown that this mechanism is very temperature sensitive. At a daytime temperature of 25°C the floral cycle is normal and pollination results in pollen tube growth and embryo development. At daytime temperatures above 30°C there is excessive shedding of flowers and young fruits and pollen tube growth may be abnormal. Below 20°C the floral cycle is disrupted and the

female stage may be omitted as in the Fuerte cultivars or the male stage may open during the night as in the Hass.

As a result of these findings our crossing programme is carried out under controlled conditions. The potted plants are housed in an insect-free glasshouse with a daytime temperature of around 25°C. Hand pollinations are carried out by removing dehisced anthers from male stage flowers and gently transferring the pollen by direct contact to the stigma of the female stage flower. Removal of the anthers is unnecessary as avocado pollen does not become airborne. Pollinated flowers are labelled with coloured cotton and a few flowers are pollinated each day over the flowering period.

One problem is that different cultivars may flower at different times. This problem has been overcome using a grafting technique. Floral budwood is collected from trees in the orchard and is stored for up to 4 months at 4°C. The budwood can then be bottle-grafted when required to mature stock plants which have been previously disbudded and topped to produce a build up of carbohydrate. These grafts will flower after a few weeks and, because of the maturity of the stock and the large graft area, may carry fruit to maturity when pollinated. Thus early- and late-flowering cultivars can be manipulated to flower synchronously for crossing.

One of the major problems in breeding woody perennial tree crops is the low fruit to flower ratio. The problem is particularly acute with the subtropical crops such as avocado where millions of flowers may be produced but a good crop is measured in thousands of fruit. This means that most of the hand-pollinated fruit will be shed before fruit and therefore seed maturity. An *in vitro* culture method is under development so that the shed embryos can be saved. The embryo culture medium stimulates the production of shoots which are then micrografted to stock plants.

The final problem in breeding woody perennials is the long generation time. A seedling avocado tree may take over 10 years to flower and fruit. This problem may be partly overcome by grafting onto a rootstock of a large tree which has been topped (pollarded). The progeny are also planted out on virus-tested seedling stocks.

The development of breeding methods for avocado was commenced in 1975 and further work is required to perfect the techniques. We hope that this approach will produce new avocado cultivars better suited to Australian conditions and also lead to some understanding of the genetics of the crop so that future breeding programmes can be scientifically based. The experience

gained in developing methods for avocado breeding will be applied to other tree crop species.

RAPID INDEXING OF SUNBLOTCH VIROID IN AVOCADOS AND OF EXOCORTIS VIROID IN CITRUS

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Abstract. It appears feasible to replace the time-consuming biological indexing of the sunblotch disease of avocados and possibly also of the exocortis disease of citrus by a more rapid method which is highly specific and can be completed in several days. This new method involves the sensitive detection of the avocado sunblotch viroid and the citrus exocortis viroid in partially purified nucleic extracts of candidate trees by a technique known as hybridization analysis. Details of this new method are given together with a summary of the results so far obtained.

INTRODUCTION

Viroids are the smallest pathogenic agents known and consist of single-strand, circular RNA molecules which are only 300-400 residues long (3). Unlike normal plant viruses, viroids are not protected by a protein coat and are spoken of as naked molecules. They infect a wide variety of plants and, in many, produce severe disease symptoms. Of the eight viroids so far described, at least five are of considerable agricultural importance; these are potato spindle tuber viroid, hop stunt viroid, cadang-cadang viroid of coconuts, avocado sunblotch viroid, and citrus exocortis viroid. Only the latter two are present in Australia.

A characteristic property of viroids is their slow rate of growth and the long time taken for symptom development as compared with many plant viruses. Indexing for the presence of viroids by symptom development in suitable indicator plants can take a minimum of two to three weeks for such viroids as potato spindle tuber viroid or two years and more for avocado sunblotch viroid, and especially cadang-cadang viroid. Hence, the development of more rapid procedures for the indexing of at least some viroid diseases is most important. This paper summarizes our results so far on the development and use of a very specific and rapid indexing procedure for avocado sunblotch viroid and citrus exocortis viroid.

AVOCADO SUNBLOTCH VIROID AND CITRUS EXOCORTIS VIROID

Avocado sunblotch, a serious disease affecting avocados (*Persea americana* Mill), was first described in California nearly