Almond improvement in Australia

Margaret Sedgley*, Graham Collins

Department of Horticulture,
Viticulture and Oenology,
Plant Research Centre
Adelaide University,
Waite Campus,
PMB#1,
Glen Osmond,
South Australia,
5064 Australia

margaret.sedgley@adelaide.edu.au

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Abstract — Introduction. The Australian almond [*Prunus dulcis* (Miller) D.A. Webb] improvement program commenced five years ago with the main aims of developing scion and rootstock cultivars that are better adapted to local conditions, and that provide a superior product for Australian and overseas markets. **Approaches used**. The program has a number of approaches including breeding, virus detection and elimination, and biotechnology. The classical hybridisation approach aimed at generation of diversity is combined with research into the more targeted techniques of plant tissue culture, genetic fingerprinting, genome mapping and transformation. Cryopreservation research is important for genebank storage, and tissue culture for micropropagation of new rootstocks and for transformation. Material is screened for *Prunus* Necrotic Ringspot (PNRV) and Prune Dwarf (PDV) Viruses. In addition, work has commenced into identifying Australian isolates of *Colletotrichum acutatum,* the pathogen causing anthracnose disease of almonds. The work is conducted in collaboration with overseas research groups, to take advantage of the long experience of these programs, and to contribute to the international effort in *Prunus* improvement. **Outcomes from the almond project**. The important outcomes achieved after the first five years of the project by the research team are listed.

Australia / *Prunus* / plant breeding / hybridization / micropropagation / plant biotechnology / cryopreservation / viruses / *Colletotrichum*

Amélioration de l'amandier en Australie.

Résumé — Introduction. Le programme australien d'amélioration de l'amandier [*Prunus dulcis* (Miller) D.A. Webb] a débuté il y a 5 ans avec, comme principal objectif, le développement de cultivars donneurs de scions et porte-greffes mieux adaptés aux conditions locales, aptes à fournir un meilleur produit pour les marchés australiens et extérieurs. **Voies de recherche utilisées**. Le programme de recherche comporte un certain nombre d'approches comprenant la multiplication, la détection et l'élimination de virus, et la biotechnologie. L'approche par hybridation classique vise la création de variabilité ; elle est combinée avec l'utilisation de techniques plus ciblées de culture de tissu végétal, d'empreintes génétiques, de cartographie du génome et de transformation. Les recherches en cryoconservation sont importantes pour le stockage de banque de gènes, et la culture de tissu est utile pour la micropropagation de nouveaux porte-greffes et pour la transformation. Le matériel génétique a été criblé vis-à-vis du *Prunus Necrotic Ringspot Virus* (PNRV) et du *Prune Dwarf Virus* (PDV). En outre, des travaux d'identification des isolats australiens de *Colletotrichum acutatum,* pathogène causant la maladie d'anthracnose de l'amandier, ont débuté. Les recherches sont conduites en collaboration avec des groupes de recherche d'outre-mer pour profiter de la longue expérience de ces programmes et pour contribuer à l'effort international dans l'amélioration des *Prunus*. **Résultats obtenus**. Les résultats importants obtenus par l'équipe de recherche australienne après les cinq premières années d'activités sont énumérés.

Australie / *Prunus* / amélioration des plantes / hybridation / micropropagation / biotechnologie végétale / cryoconservation / virus / *Colletotrichum*

* *Correspondence and reprints*

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1. Introduction

The Australian almond [*Prunus dulcis* (Miller) D.A. Webb] improvement program commenced five years ago with the main aims of developing scion and rootstock cultivars that are better adapted to local conditions, and that provide a superior product for Australian and overseas markets [1]. In contrast to the situation with hard-shelled cultivars in Europe, Australian growers concentrate on paper-shell types similar to those grown in California.

The program has a number of approaches including breeding, virus detection and elimination, and biotechnology. In each of these sub-programs, Adelaide University works in co-operation with other groups, including almond growers, through the Australian Almond Growers' Association (AAGA), and the South Australian Research and Development Institute (SARDI).

Professor Margaret Sedgley, Head of the Department of Horticulture, Viticulture and Oenology, oversees the entire program, with collaboration from Chris Bennett of the AAGA and Barry Tugwell of SARDI.

2. Breeding

The controlled hybridisation work, directed by Dr Andrew Granger, has been underway for five years [2]. The progeny from year 1 and year 2 are planted at Lindsay Point in Victoria on the property of Andrew Lacey, and some of the progeny from year 1 produced their first nuts in 1999.

Hybridisations involved a number of parents so far available to the breeding program, including Nonpareil, Carmel, Mission, Ferragnes, Pearce, McKinlay's Magnificent, Sommerton, Johnston's Prolific, Parkinson, Price, Peerless, *Prunus webbii*, Lauranne, Ferralise, Ferrastar, Ferraduel, Le Grand, Chellaston, Ne Plus Ultra, Iranian seedlings, plus some peach cultivars to introduce self-fertility. Other cultivars will be included in the future. Hybridisations were conducted at a number of sites, including the Waite Research Institute in Adelaide, the Loxton Research Centre at Loxton, 200 km NE of Adelaide, and the property of Tim Parkinson at Willunga, 50 km south of Adelaide. For some crosses, self- and cross-compatibility were evaluated by observations of pollen tube growth. This information is used to plan compatible crosses for future breeding, and also to provide vital information to growers on compatible pollinators for local selections. The identification of individual progeny carrying the *Sf* allele has been made more efficient by the development of a molecular marker, based on the intron sequence of the gene [3].

3. Virology

The virology program, run by Evita Alberts, concentrates on screening for the presence of *Prunus* Necrotic Ringspot Virus (PNRSV) and Prune Dwarf Virus (PDV) [2]. The methods used include grafting to the indicator plant *Prunus serrulata* cv. Shirofugen, testing on susceptible herbaceous plants including cucumber and *Chenopodium quinoa,* a weed related to fat hen, and antibody-based ELISA. The ELISA tests are now being replaced by DNA-based detection methods developed by Dr. Graham Collins.

All progeny from crosses made in the program for the first two years, almost 8000 trees, have been tested for PNRSV and PDV. A small number has produced a positive response to PNRSV, but none has tested positive for PDV using ELISA. Our aim is to use parents in the breeding program that are free of PNRSV, and testing the effectiveness of heat therapy for the elimination of this virus is currently underway.

As yet, there are no data on the optimum time of year to sample for almond viruses under Australian conditions using the ELISA technique. To address this, samples have been collected at various times during the growing season and used to compare ELISA with both woody and herbaceous indexing.

4. Cryopreservation

Cryopreservation is a technique whereby small pieces of germplasm are stored at very low temperatures. Chockpisit Channuntapipat and Dr Graham Collins conduct this research program. Cryopreservation is a cost effective method for the long-term storage of large numbers of accessions, compared with the high level of resources required to maintain field grown trees. This is especially true for varieties that have no current commercial value, but have unique traits such as disease resistance which need to be maintained for future use in the breeding program. It can also provide a backup to in-ground cultivar collections as insurance against accidental loss of the plantings through disease, inclement weather or vandalism.

A technique has been developed for storing shoot tips of almond scions and rootstocks of about 2–3 mm in length (*figure 1*) in liquid nitrogen followed by thawing and successful regeneration (*figure 2*) [4]. This has been achieved for Nonpareil, Ne Plus Ultra and the peach × almond rootstock, Bright Hybrid. One concern regarding cryopreservation is the genetic stability of the material in culture, and this is currently under investigation.

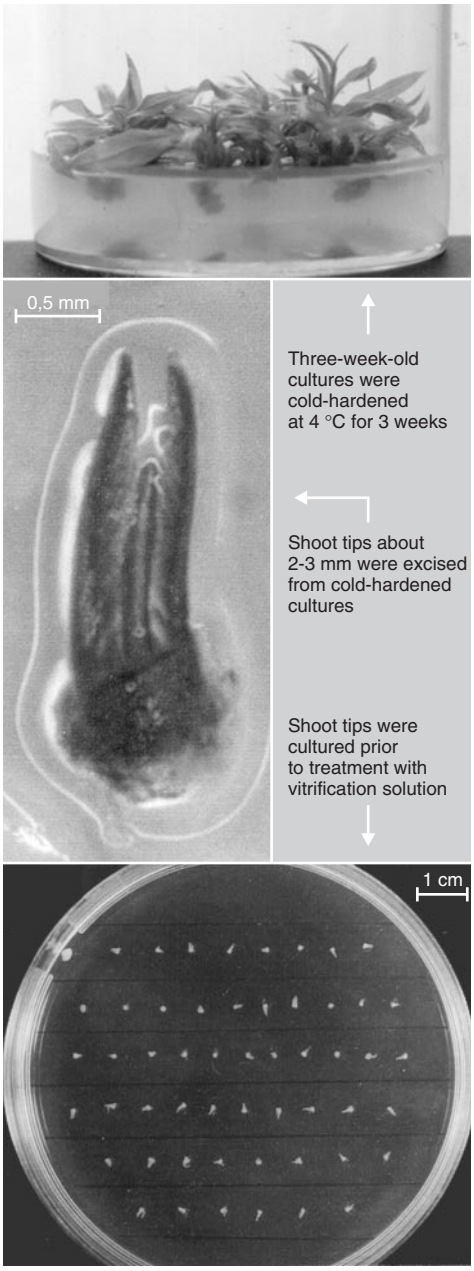

Figure 1.
Cryopreservation technique developed in Australia for storing shoot tips of almond scions and rootstocks of about 2–3 mm in length.

5. Micropropagation

Phillip Ainsley, Chockpisit Channuntapipat, Steve Choimes and Dr Graham Collins are involved in *in vitro* propagation research [5]. Cultures have been successfully initiated of the almond scion cultivars Nonpareil and Ne Plus Ultra, of the almond × peach rootstock cultivars Bright Hybrid and H184, of the almond rootstock cultivar Alnem 88 and of the virus indicator *Prunus serrulata* cv. Shirofugen. Successful multiplication has been achieved with all of the cultivars initiated into culture.

Difficulties were encountered with tissue culture of mature almond tissue, particularly for the scion cultivar Nonpareil, the most important cultivar currently available to the Australian industry. This problem was solved after comparing a range of media, and it is now routine practice to use different media for different cultivars. Rooting has been achieved for Nonpareil, Ne Plus Ultra, Bright Hybrid, Alnem 88, H184 and *Prunus serrulata* cv. Shirofugen (*figure 3*).

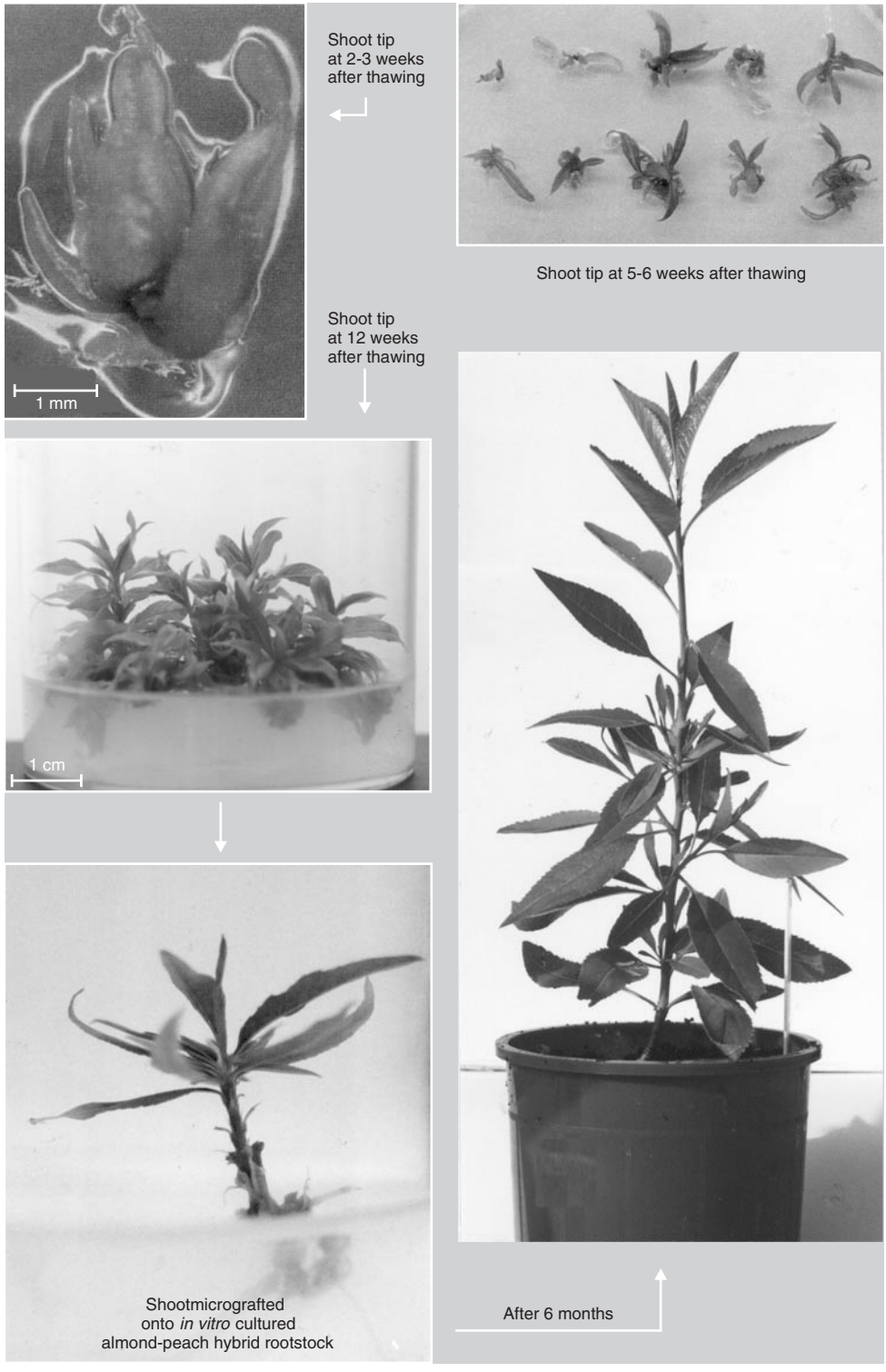

Figure 2.
Thawing and regeneration of Nonpareil almond shoot tips after cryopreservation.

6. Transformation

Transformation uses the techniques of molecular biology to introduce new characters into a cultivar to improve it further. This is especially important for tree crops because of their long generation time, the difficulties involved with backcrossing, and the presence, in many cases, of a juvenile period. Phillip Ainsley and Dr Graham Collins are in charge of this program for the improvement of almond [5]. The major aim is to produce a self-fertile Nonpareil, most probably by using anti-sense technology. At this stage, stable integration of reporter genes has been achieved for this cultivar, and also for Ne Plus Ultra.

7. Genetic fingerprinting

Fiona Woolley and Dr Graham Collins have recently completed a DNA fingerprinting study on 50 accessions of almond cultivars originating from four regions of the world: Australia, California, Europe and the Middle East [6]. Cultivars that are known to have originated in Europe or the Middle East were genetically distinct from those known to have originated in California. Some local Australian cultivars clustered with the European group, whereas others were more similar to the Californian cultivars. Both groups were quite different from wild almond material collected recently by Dr Graham Collins in Turkey, and from *Prunus webbii* which is growing in the Waite Agricultural Research Institute Arboretum in Adelaide.

8. Mapping of almond genes

Dr Graham Collins and Dr Michelle Wirthensohn have begun work on mapping of important genes in almond, in collaboration with Dr Pere Arùs at the Institut de Recerca i Technologia Agroalimentaries (IRTA) at Cabrils in Spain [7]. Dr Withensohn spent 6 weeks in the laboratory of Dr Arùs using the techniques involved in the European *Prunus* mapping program, to ensure that the Australian program integrates successfully into the international effort.

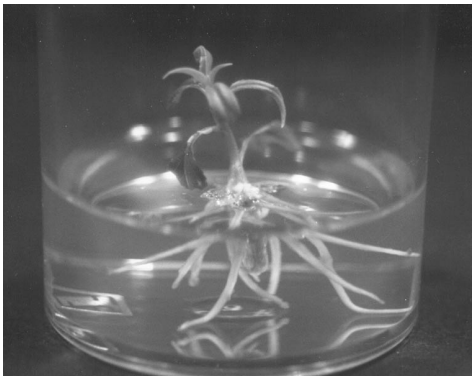

Figure 3.
In vitro culture of Ne Plus Ultra showing rooting induced by indole butyric acid.

9. Anthracnose disease

The almond program has recently been expanded to include investigation of the almond anthracnose disease caused by the fungus *Colletotrichum acutatum*. This disease was recorded for the first time in South Australia in October 1996 and is now known to occur in all the major almond-growing regions of Australia. However, it is thought that the fungus has been present but unrecognised for many years and may have been confused in the past with other almond diseases such as shot hole. Various isolates of this pathogen have been collected and their morphology and genetic variability are being compared by Suzanne Colmagro, supervised by Dr Graham Collins, Dr Eileen Scott, and Dr Trevor Wicks. This information will help to identify conditions promoting fungal growth and activity, and assist with the timing of fungicide applications in the orchard.

10. Outcomes from the almond project

After the first five years of the project, a number of important outcomes have been achieved by the research team:

– virus tested breeding progeny have been planted for first evaluation in 2000 as new cultivars;

– detection methods for PNRSV and PDV in almond have been refined;

– the status of the industry budwood source at Monash, located 250 km NE of Adelaide, has been monitored and reported

to industry to ensure that virus infected budwood is not distributed;

– heat therapy is underway on two virus infected varieties;

– PNRSV has been determined to be the more significant virus in almonds so far. PDV has not been detected using ELISA, but initial work with a DNA-based technique has produced a positive response;

– antiserum to an almond isolate of PNRSV is under development to ensure a constant and cost-effective supply;

– micropropagation methods have been developed;

– rootstocks of H184 have been generated by *in vitro* propagation for use in varietal test blocks to assess the performance of imported material;

– successful cryopreservation has been achieved;

– regeneration methods for transformation have been developed;

– genetic fingerprints are available for the most important almond cultivars grown in Australia;

– development of a marker for the *Sf* gene has been achieved, and the search for a marker for the hard shell character is underway.

Further research is planned in all these areas to advance the Australian industry by improved yields and quality and increased competitiveness on world markets.

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Mejora del almendro en Australia.

Resumen — Introducción. El programa australiano de mejora del almendro [*Prunus dulcis* (Miller) D.A. Webb] se inició hace 5 años, teniendo como objetivo principal el desarrollo de cultivares que proporcionen púas y portainjertos más adaptados a las condiciones locales y capaces de lograr un mejor producto para los mercados australianos y exteriores. **Vías de investigación seguidas**. El programa de investigación conlleva un cierto número de enfoques metodológicos que incluyen la multiplicación, la detección y eliminación de virus y la biotecnología. El enfoque por hibridación clásica tiene como objetivo la creación de variabilidad; se combina con la utilización de técnicas más específicas de cultivo de tejido vegetal, de huellas genéticas, de cartografía del genoma y de transformación. Las investigaciones de crioconservación son importantes para el almacenamiento de banco de genes y el cultivo de tejidos es útil para la micropropagación de nuevos portainjertos y para la transformación. El material genético fue cribado con respecto al *Prunus Necrotic Ringspot Virus* (PNRV) y al *Prune Dwarf Virus* (PDV). Además, se iniciaron los estudios destinados a identificar los aislados australianos de *Colletotrichum acutatum,* agente patógeno causante de la antracnosis del almendro. Las investigaciones se realizan en colaboración con grupos de investigación de ultramar para aprovechar la larga experiencia de dichos programas y para contribuir al esfuerzo internacional destinado a la mejora de los *Prunus*. **Resultados obtenidos**. Se enumeran los resultados importantes logrados por el equipo australiano de investigación después de los cinco primeros años de actividades.

Australia / *Prunus* / fitomejoramiento / hibridación / micropropagación / biotecnología vegetal / criopreservación / virus / *Colletotrichum*