Final Report

National persimmon varietal evaluation program 2018-2023

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Public and technical summary

The national persimmon varietal and rootstock evaluation program of Australia is a multi-stage process involving negotiations with overseas collaborators, importing varieties and rootstocks, post-quarantine entry requirements (PEQ), the release of trees from quarantine, establishing source trees, tree propagation post-PEQ, and establishing field trials followed by the evaluation and recommendation processes. The project was focused on (1) importing early maturing varieties and rootstocks from overseas, (2) the genetic verification of the 'Rojo Brillante' variety, (3) establishing trials for the existing varieties at NSW DPI Dareton Research Station (Dareton), and (4) developing a clonal propagation method to produce rootstock seedlings for the tree uniformity trials.

During the project, strong collaboration was established with scientists and persimmon breeders from Spain, Japan, South Korea and China. The momentum of collaboration was maintained despite the hurdles of COVID-19 and the fact that a personal visit to these countries was not possible for the last 3 years. The Australian persimmon industry is dependent on the early maturing varieties from overseas. Therefore, significant efforts were made during this project to import the 'Taishu' variety from the University of Miyazaki, Japan. Since the variety has been in Australia, a budwood mother block has been established and buds will be available to growers in the next few years. The early maturing variety from South Korea will arrive in Australia soon. True-to-type genetic verification of the Spanish 'Rojo Brillante' variety was required to confirm its originality. The genetic work was carried out in collaboration with the Spanish breeders. 'Rojo Brillante' DNA was extracted and compared to the original genetic markers in Spain. Phenotypic verification was also carried out to confirm the external appearance, shape, and internal quality characteristics by the Spanish scientist, Dr Maria Badenes. Subsequently, the budwood blocks were established at Dareton, and the budwood will be available to growers from September 2023.

The *Diospyros lotus* rootstock was also imported from Spain. Rootstock seedlings were raised and grafted with 'Jiro', 'Fuyu' and 'Rojo Brillante' for evaluation purposes. Genetic work was carried out with a Spanish collaborator to identify the male and female trees, and the results were shared with Australia. After the verification, male and female trees were planted to establish the seed source for *Diospyros lotus* at Dareton. A replicated site was also established at a grower's property in NSW as a backup. Australian growers now have access to the budwood from the new rootstocks at Dareton, which could help them establish their own seed source.

One of the project's objectives was to establish a trial site with a range of existing and new varieties at Dareton. Those varieties will be used for future evaluation to determine if there are any early maturing varieties. Eleven scion varieties and 2 rootstocks are now established at the NSW DPI Research Institute at Dareton. Some of these varieties will be evaluated for maturity (early or late), yield, quality, and physical disorders.

Australian persimmon orchards have issues with tree uniformity, which results in poor tree performance and yield. Tree uniformity is due to the genetic variability in rootstock seeds. Some seedlings can have poor and stunted growth, which becomes prominent a few years after planting. Other reasons for the lack of uniformity could be poor rootstock seedlings or disease in the rootstock. Therefore, a clonal propagation technique was developed during this project to produce rootstock seedlings. Two clonal propagation methods, tissue culture and single node cutting technique, were used to produce rootstock seedlings. The same genetic material was used from one healthy source tree, and many uniform (true-to-type) rootstocks were produced. Agromillora Nursery helped with the tissue culture work. The rootstock seedlings were successfully produced by the tissue culture method following the acclimatisation and hardening protocols.

Five hundred rootstock seedlings were distributed to nurseries and growers. These seedlings will be grafted and planted in growers' trials to test the uniformity of trees. This work will continue into the next project. Seedlings were also produced from the single node cutting technique, but after several experiments over 4 years using cuttings collected at different growth stages with different levels of growth regulators applied at the base of the cutting using different potting media, we concluded the technique was slow and laborious, produced only a few seedlings, and was not recommended for commercial operations. The recommendation was to use the tissue culture technique, which is quick, and many seedlings can be prepared for the benefit of the persimmon industry.

Keywords

Diospyros kaki, *Diospyros lotus*, 'Rojo Brillante', 'Taishu' (*Diospyros kaki*), 'Jiro' (*Diospyros kaki*), 'Fuyu' (*Diospyros kaki*), clonal propagation, fruit yield, fruit quality, early maturing

Introduction

The persimmon (*Diospyrus kaki* L.) is a deciduous fruit native to China. Although persimmon is further divided into several species, the most common edible species is the Japanese variety. Persimmon flavour is classified as astringent (due to high tannin levels) or non-astringent, with no tannins left inside the fruit. Astringent varieties can only be eaten when the astringency has been removed after picking, whereas the non-astringent varieties can be eaten at harvest.

China produces nearly 43% of the world's persimmons, with large production areas along the Yellow River. South Korea is the second largest producer (approximately 0.3 million t). The fruit has a special cultural value in South Korea and is seen as symbolic because of its transformation from bitter to sweet non-astringent fruit. Japan is the third largest producer (0.26 million t), with primary locations for persimmon production being Wakayama, Fukuoka, Nara and Gifu prefectures. Spain also produces persimmon, predominantly (90%) in the Valencia province, and 10% is produced in Extremadura, Catalonia, Murcia and Aragon. In 2022 Spain produced 180,000–200,000 t of persimmons (Fresh Plaza, 2022), which is 20% below the previous harvest season. Other countries that produce significant tonnage of persimmons are Brazil and Azerbaijan.

In 2022, the total persimmon production in Australia was 3,462 t, valued at $17.3 million. Fresh consumption accounted for 94%, while 5% was exported and 1% was used for processing (2021–22 Australian Horticulture Statistics Handbook). The wholesale value of fresh persimmon supply was $21.3 million, with $18.8 million distributed into retail and $2.5 million into food service. Nine per cent of households in Australia purchased fresh persimmons, buying an average of 445 g per shopping trip. Per capita, consumption was 0.14 kg on the volume supplied. In Australia, there are approximately 80 persimmon growers. The total per cent production distribution is 35% (Qld), 25% (VIC), 20% (NSW), 15% (SA) and 5% (WA) as per the 2021–22 Australian Horticulture Statistics Handbook.

There is a small amount of international trade of persimmons, typically with more import than export. For the year ending 2022, Australia exported 145 t; 34% to Singapore, 28% to Malaysia, 25% to Hong Kong, 5% to Indonesia, 3% to Qatar and 5% to other markets.

Australia produces 95% non-astringent persimmon varieties 'Izu', 'Jiro' and 'Fuyu', and 5% astringent varieties 'Hachiya' and 'Rojo Brillante'. The production of 'Rojo Brillante' is limited to less than 50 trees; however, growers will plant many trees in the next 5 years.

Increasing fruit sales at a profitable price is vital to the growth and sustainability of the persimmon industry. Information on consumer preferences and motivation for purchase are important in that process. In general, varieties that look appealing will prompt consumers to buy, while enjoyable flavour and texture (non-astringent) will encourage them to repeat purchases. Varieties and rootstocks profoundly influence several aspects of persimmon fruit including quality, yield, tree vigour and precocity. However, there are no Australian research recommendations available that indicate which variety and rootstock combination will be able to produce fruit of high quality under different soil and climatic conditions. The research literature indicates that some limited overseas studies are available regarding persimmon production.

Project objectives

The objectives of this project were:

1. Carry out true-to-type verifications of existing 'Rojo Brillante' in Australia.
2. Negotiate access to new persimmon varieties and rootstocks from overseas partners with potential for the

Australian industry.

3. Establish a trial site at NSW DPI Dareton Research Station and evaluate the performance of new varieties and rootstocks including 'Rojo Brillante'.
4. Develop a new standard clonal propagation technique for persimmon rootstocks to improve tree uniformity and performance (control tree vigour and size).

Methodology

A brief methodology for each objective is given below. Detailed methodology is presented in chapters 1–4 in Appendix 1.

Objective 1: Carry out true-to-type verifications of existing 'Rojo Brillante' in Australia.

To verify the true-to-type characteristics of the 'Rojo Brillante' variety, leaf samples from 2 trees/site were collected from 3 sites. The plant samples (mature leaves) were collected from mature 'Rojo Brillante' trees from multiple locations in Maroochydore (Qld), Cobbitty (NSW) and Renmark (SA). The leaves were then sent to the Australian Genome Research Facility (AGRF) laboratory in Adelaide, and DNA material was extracted and provided to NSW DPI. The DNA was taken to IVIA, Valencia, Spain, in October 2018. A repeat sampling was also conducted and sent to Spain in 2019. Dr Badenes (IVIA Spain) analysed 5 combinations of primers. The samples were included in all PCR reactions. The Spanish sample of 'Rojo Brillante' DNA was used as a control. The results obtained from the Australian samples corresponded with the Spanish samples of 'Rojo Brillante'. The results were positive; all the expected alleles were obtained after the analysis and reported to NSW DPI.

Objective 2: Negotiate access to new persimmon varieties and rootstocks from overseas partners with potential for the Australian persimmon industry and evaluation of new varieties.

Global mapping of persimmon improvement programs was conducted early in the project. The preliminary analysis showed that Spain, Japan, South Korea, and China are the main countries for accessing new varieties and rootstocks. Negotiations with the overseas collaborators were established via email and then followed by phone calls. After detailed discussions, formal visits were planned for Spain and Portugal at the start of the project and occurred in October 2018. During these visits, strong collaboration was established at the research institute (IVIA), Valencia, Spain. The second visit was to South Korea, Japan and China in October 2019 to inspect their persimmon industries. A third trip was planned to conduct a rapid assessment of the germplasm in South Korea and China but did not occur due to the project's conclusion in July 2023. The negotiation to import the new varieties will continue in the future project, followed by the import of new varieties.

Objective 3: Establish a trial site at NSW DPI Dareton Research Institute and evaluate the performance of new varieties and rootstocks including 'Rojo Brillante'.

The experimental trials of the new and existing non-astringent varieties, and astringent variety 'Rojo Brillante' were established. The new variety 'Taishu' was imported from Japan after negotiations with Professor Takuya Tetsumura of the University of Miyazaki, Japan. Dr Tetsumura provided the budwood in December 2020. One-year-old rootstock seedlings of *Diospyros lotus* were provided to post-entry quarantine (PEQ) by NSW DPI. 'Taishu' scion variety was grafted to the one-year-old rootstock seedlings. Post-entry quarantine maintained 4 trees at the facility for more than one year. Indexed trees were released from PEQ and arrived at Dareton on 15 September 2021.

Budwood from the existing varieties at the Maroochydore research facility was obtained in 2018. The scion budwood was grafted to *Diospyros kaki* rootstock, and trees were raised for 2 years. Two trees of each variety were planted at the Dareton Research Institute in November 2020. The trees produced a small number of fruits on some varieties in June 2023. These varieties will be evaluated in future. Forty 'Rojo Brillante' trees were also planted in November 2020 after confirming their true-to-type verification from Spain. The purpose of the planting was to evaluate fruit and to produce budwood for future plantations. The trees produced fruit in June 2023, which was used to initiate the de-astringency trials in collaboration with the University of Queensland at Gatton. Trials will continue in the future projects.

Objective 4: Develop a new standard clonal propagation technique for persimmon rootstocks to improve tree uniformity and performance.

To develop a clonal propagation technique, 2 methods were used. In the first method, the single node cutting technique was used. Cuttings (suckers) were obtained from the lateral roots of a healthy tree that was cut down to ground level. The lateral (structural) roots were exposed, and suckers from those roots were used to prepare the single node cuttings. Each cutting was dipped in a growth regulator (indole butyric acid, IBA) for 5 seconds at levels ranging from 3,000–8,000 ppm with 2 types of potting media. Treated cuttings were placed in a mist house to initiate root and shoot development.

The second propagation method was the tissue culture technique to produce rootstock seedlings. The same genetic material was used as for the single node cutting. In this case, the bud of the root suckers was used for the tissue culture. The explant is a tissue or plant part introduced in the culture media for plant regeneration. Induction media was prepared, and the stem cuttings were placed on the agar gel in sterilised jars for 4 weeks. The good quality material was rescued and put into new jars with fresh media. The media also contained half-strength nutrients and plant growth regulators. The plantlets were moved from the tissue culture laboratory to the plastic tunnels for acclimatisation for 4–6 weeks in controlled humidity and temperature conditions. After 4 weeks of acclimatisation, the plants were moved to the greenhouse for the hardening process for 60 days. Here the plants were irrigated and had nutrients supplied to the pots. Afterwards, the plants were moved into 2 L pots to allow the root system to grow.

Results and discussion

The experimental program for the national persimmon project was conducted over 5 years from 2019 to 2023. This program involved 4 components (see chapters 1–4 in Appendix 1) and a range of trials under each experimental component (refer to the Methodology section of this report). This section discusses the key results from the program; however, detailed results are given in chapters 1–4 (Appendix 1).

Chapter 1: Carry out true-to-type verifications of existing ‘Rojo Brillante’ in Australia

The Australian persimmon industry has ‘Rojo Brillante’ scion variety, which was sourced from a grower in Portugal many years ago (Oag, 2017). However, there was no clear evidence of whether this variety was true-to-type, as it was suspected that the budwood might have been mixed up with the budwood of another variety at a nursery and during its transfer to Australia. Genotypically and phenotypically, the varietal characteristics for this variety should be verified with the ‘Rojo Brillante’ trees present in different persimmon growing regions such as Queensland (Qld), New South Wales (NSW) and South Australia (SA).

Genotypic verification of ‘Rojo Brillante’ in Australia

DNA-based genotyping was conducted in collaboration with international scientist Dr Maria Luisa Badenes in Spain to verify the true-to-type characteristics of ‘Rojo Brillante’. The plant samples (mature leaves) were collected from ‘Rojo Brillante’ trial sites in multiple locations in Qld, NSW and SA. The leaves were then sent to the Australian Genome Research Facility (AGRF) laboratory in Adelaide, where DNA material was extracted and provided to NSW DPI. The DNA was provided to Dr Maria Badenes, IVIA, Valencia, Spain, in October 2018 and again in 2019. In Spain, the DNA samples were analysed with 5 combinations of primers. The ‘Rojo Brillante’ DNA samples from Spain were used as a control and compared with DNA samples from Australia.

The results obtained with Spanish ‘Rojo Brillante samples produced positive results; all the expected alleles were retrieved after the analysis. The results of the samples obtained from Renmark (SA) had the same alleles as our ‘Rojo Brillante’ in 90% of the alleles, and for the remaining 10%, Dr Badenes did not get amplification. Dr Badenes confidently concluded that the ‘Rojo Brillante’ samples from Renmark South Australia corresponded to the same genotype in Spain.

Phenotypic verification of 'Rojo Brillante' in Australia

Dr Maria Badenes visited Australia in June 2019 to attend the National Persimmon Conference in Perth. She presented a paper about the Spanish persimmon industry and visited the persimmon industry in Perth Hills. Dr Maria visited Dareton Primary Industries Institute, followed by a field visit to the persimmon packing shed and orchards in Sunraysia and South Australia. During her visit to South Australia, Dr Maria Badenes visited the 'Rojo Brillante' trees in Renmark where the DNA samples were obtained. There was still some fruit left on the trees, and Dr Maria Badenes was able to study the phenotypic characteristics such as fruit size, shape and appearance, and confirmed the 'Rojo Brillante' in Australia is the original Spanish variety. Therefore, it was confirmed by genotypic and phenotypic verifications that the persimmon industry should proceed with the existing 'Rojo Brillante' variety to expand and establish new production areas in Australia.

As 'Rojo Brillante is an astringent variety, astringency must be removed before selling the fruit on the local or overseas market. Therefore, a preliminary study was carried out with the University of Queensland (Gatton). The preliminary study suggested that there is a possibility of removing astringency by quantifying the CO_2 treatment application to the fruit for 24 hours. However, the treatments need to be refined to suit the 'Rojo Brillante' fruit grown in different climates and soil types. The persimmon crop grown in Queensland has different growing conditions from those grown in Southern Australia. Therefore, more work is needed to identify and refine ways to remove the astringency from the fruit, followed by the fruit's storage performance and shelf life in the future projects.

Chapter 2: Negotiate access to new persimmon varieties and rootstocks from overseas partners with potential for the Australian persimmon industry

The global mapping of the persimmon improvement programs was conducted at the beginning of the project. The preliminary analysis showed that Spain, Japan, South Korea, and China are the main countries for accessing new varieties and rootstocks. These countries are still running active and efficient breeding programs. Links were established with the relevant scientists at El Instituto Valenciano de Investigaciones Agrarias (IVIA) in Spain and Northwest Agriculture and Forest University in Yangling, China. Working relations were also established with the University of Miyazaki, Japan and Pear Research Institute, NIHHS, Naju, South Korea.

The major persimmon producing countries (Spain, Portugal, Japan, South Korea and China) were visited, and the variety and rootstock breeding programs were inspected during the visits. Links were established with other scientists around the world. Scientists from Spain and South Korea visited Australia and inspected the Australian persimmon industry in June 2019 and June 2023. However, due to COVID-19, collaboration was slow with Spain and South Korea. Further visits to these countries were not possible to draw agreements and import varieties. In Japan, 'Fuyu' is the most widely grown persimmon variety. However, the persimmon breeding program at the National Agriculture and Food Research Organisation (NARO) has released 2 non-astringent varieties, 'Taishu' (Hasegawa et al., 2005) and 'Taiho' (Sato et al., 2018). During the project, an early maturing variety, 'Taishu' was imported from the University of Miyazaki, Japan.

Importing 'Taishu', the early maturing variety

Dr Tahir Khurshid visited the University of Miyazaki, Japan, in late September 2019, and a formal meeting was held with the persimmon breeder and crop management expert Dr Takuya Testsumura along with the staff of RIFNUM (Japanese persimmon industry organisation). Dr Takuya Testsumura agreed to provide the budwood of 'Taishu' to NSW DPI. 'Taishu' appears to be an earlier maturing variety than 'Jiro' but needs to be tested in Australian conditions. Taishu has a large fruit size and yellowish skin colour in the tropical climate of Miyazaki. This variety should attain deep yellow to light orange skin in sub-tropical climatic regions of Australia. 'Taishu' is a non-IP variety and will be freely available to Australian growers once enough budwood is available for distribution.

'Taishu' was imported into Australia and placed in a post entry quarantine (PEQ) facility in Mickleham, Melbourne, in December 2020. The budwood was inspected for viruses and other diseases, and trees were grown in PEQ. Two indexed trees were released from the PEQ and arrived at Dareton on 15 September 2021, with 2 additional trees arriving on

2 February 2022. However, one tree did not survive after it arrived at Dareton. Only a limited number of budwood will be available for propagation to produce experimental trees in October.

The 'Taishu' fruit will also be evaluated to confirm its early maturity compared to 'Jiro'. This propagation process will continue for another 5 years until enough buds are available to propagate the trees. At this stage, the trees look promising, and they will be moved in July–August 2023 to larger pots, encouraging vegetative shoots and preserving the plant material. The mother block will be established during the next project starting in late 2023. The 'Taishu' buds will be grafted to the rootstock and trees will be produced and planted at Dareton for further evaluation. During that time, 'Taishu' trees will also be used to increase bud source for the future. The buds will eventually be distributed to growers and nurseries.

South Korean varieties

Dr Kyeongbok Ma from South Korea (persimmon breeder at the Pear Research Institute of the National Institute of Horticultural and Herbal Science in Naju-si) visited Australia in June 2018. Dr Ma indicated that non-astringent persimmon production in South Korea is confined to the coastal and southern regions where winter temperatures are warmer. South Korea's production has focused primarily on 'Fuyu', but in recent years plantings of new varieties of 'Jowan', 'Wonmi' and 'Gampung', which have been selected in South Korea, have increased significantly, enhancing persimmon production and export figures. Dr Ma participated in the National Persimmon Conference in Perth in June 2019, followed by visits to persimmon orchards and packing sheds. During the trip, he established collaboration with Dr Tahir Khurshid of NSW DPI, Dareton. He expressed his intentions for his early maturing persimmon varieties to be tested under Australian conditions. In October 2019, Dr Khurshid visited Dr Kyeongbok Ma (persimmon breeder), Dr Kang Soohyun and Ms Jung Haewoon at the Pear Research Institute (NIHHS-RDA), Naju. Both scientists attended the Australian Persimmon Conference in Perth in June 2019. Tahir also inspected the research trials, laboratories, and postharvest facilities and interacted with scientific and technical staff working on persimmons. Dr Tahir Khurshid gave a presentation at the institute about the Australian persimmon industry and his research progress regarding his current project.

In South Korea, Dr Khurshid had the opportunity to observe a range of persimmon varieties on trees but did not have the chance to rapidly assess the germplasm. During the discussions, Dr Kyeongbok Ma suggested that early maturing varieties 'Wonmi', 'Jowan', 'Fantasy' and 'Wonchu' need to be tested under Australian conditions. All varieties have an IP status in South Korea and arrangements need to be made with the South Korean Research Institute and Rural Development Authority (RDA) to import persimmon varieties to Australia. The visit to South Korea for rapid assessment of early maturing varieties did not occur due to COVID-19. Dr Tahir Khurshid will visit South Korea soon to finalise the agreement, conduct a rapid germplasm assessment, and make arrangements with PEQ to import the persimmon varieties.

Importing *Diospyros lotus* rootstock from Spain into Australia

The *Diospyros lotus* seeds were obtained from Ms Rosa Hernandorena of Viveros Hernandorena Nursery on 27 September 2018. Due to Australia's lengthy paperwork and quarantine procedures, the *Diospyros lotus* seeds were imported from Spain and arrived in NSW DPI, Dareton, on 29 May 2019.

Diospyros lotus seeds were germinated to raise the rootstock seedlings. Seeds were sown into growth tubes, and some were directly sown into the 2 L pots at the NSW DPI nursery. The seeds germinated successfully, and seedlings were raised and transferred to 2 L pots on 4 January 2020. The root system was well developed before the transfer of seedlings into 2 L pots. Searles® Premium Potting Mix was used as a potting medium. The seedlings grew well and were used for grafting scion varieties.

Verification of *Diospyros lotus*, male and female plants, to establish mother seed source

Rootstock seedlings were raised, and leaf samples were collected from 40 trees on 20 June 2020. The leaves were sent to the Australian Genome Research Facility Laboratory in Adelaide, where DNA was extracted, followed by PCR sequencing. The protocol for the sex determination of *Diospyros lotus* (Akagi et al. 2014) was provided to the Adelaide laboratory, which had the molecular markers associated with sexuality for *Diospyros lotus*. The dual-direction PCR sequencing was carried out using the primers described by Akagi et al. (2014). The sequencing was carried out by Sanger technology.

Sanger sequencing, also known as the 'chain termination method', is a method for determining DNA nucleotide sequence. The method was developed by two-time Nobel Laureate, Frederick Sanger and his colleagues in 1977, hence the name the Sanger Sequence. The PCR results were obtained and visualised in an agarose gel. Agarose gel electrophoresis is commonly used to separate DNA fragments following restriction endonuclease digestion or PCR amplification. Fragments are detected by staining the gel with the intercalating dye, ethidium bromide, followed by visualisation/photography under ultraviolet light. The sample with a visual band indicates a male plant. The male and female verification work results were provided to NSW DPI and provided the basis for the mother block of this new rootstock that was established at Dareton.

Chapter 3: Establishing persimmon germplasm trials at Dareton

The trial plan for the initial establishment of persimmon varieties and rootstock trees is in Table 3.1 (Appendix 1).

Establishing 'Jiro' variety on G1 and S2 rootstocks trial at Dareton

The scion variety 'Jiro' was planted on 2 different rootstocks, G1 and S2, on 8 September 2020. Trees were planted in a randomised complete block design as 4 tree plots in 5 blocks with a total of 40 trees. The 'Jiro' did not survive on G1 rootstocks, so the trees were removed and excluded from the trial. The G1 rootstock was replaced with bare-rooted 'Jiro' and these were grafted onto 2-year-old *Diospyros Kaki* rootstocks. The planted trees exhibited strong tree growth. There are 30 bare-rooted 'Jiro' trees and 30 'Fuyu' trees.

Establishing 'Jiro' variety on G1 and S2 rootstocks trials at growers' properties

The scion variety 'Jiro' was planted on 2 different rootstocks, G1 and S2, on 8 September 2020 at 3 grower's properties in Victoria, New South Wales, and Queensland. Growers were provided with 40 trees that were planted as randomised complete block design in a single row. The 'Jiro' did not survive on G1 rootstocks at all 3 properties. Therefore, G1 trees were eventually removed from the trial. The reason for the poor performance of G1 could be the poor tree quality rather than the rootstock itself. The G1 rootstocks were replaced with bare-rooted rootstocks with 'Jiro'. A new rootstock performance trial is planned for the future projects.

Establishing 'Fuyu' and 'Rojo Brillante' variety trial at Dareton

'Fuyu' 'and Rojo Brillante' trees on *Diospyros kaki* rootstock arrived at Dareton in late September and planted on 6 November 2020 in 5 blocks. There are 40 trees of 'Rojo Brillante' and 20 trees of 'Fuyu'. This plantation aims to assess the performance of 'Rojo Brillante' on *Diospyros kaki* rootstock and to produce budwood for the future propagation of 'Rojo Brillante' trees. The trees started to produce fruit in June 2023 for the first time and some of the fruit was used for the de-astringency trials at the University of Queensland at Gatton. The average yield on 3-year-old trees was 25–30 kg/tree. Budwood from these trees will be collected in August 2023 to propagate trees. The trees will be evaluated in the next project for yield and quality and used in the de-astringency trials.

Establishing a variety trial with germplasm transferred from Maroochydore to Dareton

The persimmon germplasm (varieties and rootstocks) was present at the Maroochy Research Facility (MRF), Maroochydore, Queensland. A block with a range of rootstock and scion varieties was established in 2014 at the MRF. Dr Tahir Khurshid visited the MRF on 20 June 2018 to attend the meeting with Dr Vino Rajandran (Hort Innovation), David Oag (DAF), Dave Brunn (DAF), and Mr Stephen Jeffers (persimmon grower/nursery grower) to inspect the germplasm material (varieties and rootstock) planted at the institute. The varieties were planted in a single row. These varieties are more than 20 years old; however, no research has ever been conducted on them, nor are they planted anywhere else in Australia. Dr Khurshid has described the characteristics of a few scion varieties and rootstocks in an industry article (Khurshid, 2022). The budwood for scion varieties from the MRF was obtained, and trees were propagated by a private nursery in Queensland during this project. Trees arrived at Dareton in late September 2020 and were planted on 6 November 2020. All these trees were 2.5 years old in April 2023. There are 4 trees of 'Isahaya', 4 of 'Yoho', 3 of 'Sunami', 3 of 'Shinshu' and 2 of 'Suruga'. Some of these trees produced a small number of fruits in winter 2023. The budwood will be collected in August 2023 to propagate further trees for varietal evaluation in the next project. Earlier

observations in winter 2023 suggest these varieties should be evaluated for earliness of maturity and compared with the standard 'Jiro' variety.

Establishing a mother block for seed production of *Diospyros lotus* rootstock

The Spanish collaborator, Dr Maria Badenes was involved in verifying and identifying the male and female trees during this project. After the results, the first mother block for the *Diospyros lotus* seed source was established at Dareton. Generally, male and female trees need to be planted side by side to facilitate cross-pollination. Therefore, 3 female and 2 male trees were planted to produce viable seeds. A mirror block was also established at a grower's property, so the mother seed source remains at 2 sites. The *Diospyros lotus* female trees produced fruit in March 2023. A few growers collected the *Diospyros lotus* fruit from Dareton in June 2023 to extract seeds. Nurseries and growers can now obtain budwood from female and male trees to establish their own source to produce *Diospyros lotus* seed in future. Nurseries and growers were informed about the availability of budwood from NSW DPI.

Chapter 4: Develop a new standard clonal propagation technique for persimmon rootstocks to improve tree uniformity and performance

This component focused on enhancing rootstock uniformity to improve orchard performance for fruit yield and quality. Persimmon rootstocks in Australia are propagated from seed, but this leads to considerable variability in tree size and performance, making tree management difficult due to non-uniform trees within the orchard block. Vegetative (asexual) propagation produces uniform trees and will improve orchard productivity. Therefore, effective vegetative propagation techniques for persimmon rootstock need to be developed. The next step will be establishing propagated rootstock trials to evaluate rootstock performance with a range of persimmon scion varieties. This will identify the most appropriate scion/rootstock combinations, and the best-performing rootstocks will be selected for the persimmon industry. Some work has been reported in Japan by single node propagation of dwarfing rootstock (Tetsumura et al., 2000) and (Tetsumura et al., 2003). Clonal propagation techniques via root cuttings were carried out. The tissue culture technique will also offer the potential for the asexual propagation of persimmon rootstock.

During this project, the component of tree uniformity was evaluated by using 2 clonal propagation techniques, in which single or double-node cuttings can be used to produce rootstock seedlings. Trials were conducted to develop a clonal propagation technique for persimmon rootstocks to improve tree uniformity and performance (control tree vigour and size). Four years of intense propagation work was conducted (2018–19 to 2021) to develop rootstock seedlings.

1. Single-node stem cutting technique to produce rootstocks

The experiment was started with a specific style of clonal propagation that requires trees to be cut down to the base to the rootstock suckers, allowing single-node cuttings to be taken and used for clonal propagation. This Japanese technique was developed by Professor Takuya Tetsumura of the University of Miyazaki in Japan (Tetsumura et al., 2013). The single-node cuttings were produced from November to March for rooting purposes. The timing was chosen to give the cuttings a high probability of survival and success in producing roots.

A field trial was established at a grower's property in Renmark, South Australia. A tree was cut down to the stump in July 2018 and was used as a mother tree. The same tree was used to ensure the genetic material was true-to-type and consistent across the years. The soil around the root system was dug out and removed in September 2018, exposing the large lateral roots, more than 50 mm in diameter. Removing soil to expose the lateral root system was done to initiate the root suckers to emerge off the lateral roots. These root suckers were used for the clonal propagation trials for the single-node cuttings.

The experiments for single-node cuttings and subsequent propagation were performed on different occasions over 4 consecutive years (2018–2021). Different soil media were used as potting mixes and different levels of IBA were used to quantify the best treatment for rooting single-node cuttings for root initiation and seedling growth. The application dates varied from December to March to quantify the best time for collecting single node cuttings and the best level of growth regulator application to initiate roots. These treatments were combined with various potting media selections (see

Chapter 4 in Appendix 1 for details). The cuttings were propagated, and root initiation was observed. Dr Takuya Tetsumura (University of Miyazaki) was regularly consulted about the clonal propagation work during the process. During the single node cutting trials, the pots were placed in a mist house and an automatic mist cycle was set up for both day (4 seconds/20 minutes) and night (2 seconds/200 minutes). The bottom of the mist house was heated to 25 °C to heat the base of the pots.

Root initiation was monitored regularly. A few cuttings were able to form callus at the bottom of the cuttings, however, the callus turned black, and the cuttings died without any root initiation. The leaves on the cutting fell off and the cutting died within 2 months of planting. A few cuttings took root during the experimental trials, but the overall percentage was lower than expected.

It was concluded that 3,000 ppm IBA was the best level of PGR application for the cuttings collected in February. Perlite seemed to be a better potting medium, and the cuttings could hold their leaves longer than those grown in potting media. The low percentage of cutting survival might have been related to the inadequate heat contribution required to initiate the rooting at the base of the single-node cuttings. The mist cycles also need to be looked at in future.

2. Tissue culture propagation technique to produce rootstocks

The traditional propagation method adopted by the nursery industry is based on budding/grafting scion cultivars on seedlings from *Diospyros kaki*, *Diospyros lotus*, and *Diospyros virginiana*, the most important species used as rootstocks, reproduced by seeds. Furthermore, most non-astringent persimmon cultivars are not compatible with *Diospyros lotus*, a rootstock largely used because of its hardiness and frost resistance (Giordani et al., 2013).

The main in vitro tissue culture techniques developed for persimmon use direct regeneration (from dormant buds and root tips) and indirect regeneration through the callus from dormant buds, apexes, and leaves. Tissue culture propagation was carried out to produce rootstock seedlings from the true-to-type plant material. The genetic material used for the single node cutting technique presented in the previous section was also used for the tissue culture technique. The same mother plant was used to ensure the new plants were true-to-type and derived from the same source, regardless of the clonal propagation technique. The stem cuttings emerging from the structural roots were sprayed with $CuSO_4$ @ 3 g/L one week before the tissue culture process. A 500 mm portion (containing 6 buds) of the stem cutting measuring from the top was removed, and the remaining basal part was still left intact to the structural roots.

Preparation of plant tissue for culture

The explant is the tissue or plant part introduced in the culture media to regenerate the plant. Therefore, a primary function is to sterilise it to remove all the dirt, bacteria, or infections stuck on the surface of the explant. It ensures that the resulting plant will be free from any diseases. The stem cuttings were divided into 60 mm portions containing 2 buds. These stems were washed with distilled water and surface sterilised with sodium hypochlorite (bleach), which is commonly used for surface sterilisation of explants. Bleach is used for sterilisation in tissue culture processes, giving a solution with a final concentration of 0.5–1.0%. The plant tissue was rinsed twice to ensure the efficiency of surface sterilisation of the plant material.

The induction media was prepared, and the stem cuttings were placed on the agar gel in sterilised jars for 4 weeks. The good quality material was rescued and put into new jars with fresh media. The media also contained half-strength nutrients and plant growth regulators. The plants were in jars with a new media for 4 weeks. The new material was re-propagated, and the number of micro plants was increased fortnightly. Later, the acclimatisation process was adapted to get the small plantlet familiar with the new controlled environment.

Acclimatisation is the adaptation of small plants to a new environment. When tissue culture plants are transferred from the laboratory to the soil, they are exposed to abiotic stresses such as altered temperature, light intensity and humidity, and biotic stresses such as soil microflora (microbes living in soil). So, they need stepwise acclimatisation to successfully establish themselves in the natural environment (Kozai, 1991).

Micro plants were placed in small square (90 × 90 × 90 mm) pots in plastic tunnels to acclimatise. The plants stayed in the tunnel for 4 weeks. The humidity in the plastic tunnels was 80–95%, and the temperature ranged from a minimum of 15 °C to a maximum of 32 °C. During the acclimatisation process, 20% of the plants were lost.

After 4 weeks of acclimatisation, the plants were shifted to the green house for the hardening process for 60 days. The plants were irrigated and had nutrients supplied. Afterwards, the plants were shifted to 2 L pots to allow the roots and shoots to grow.

Transferring Diospyros lotus seedlings to nurseries and growers

Plants remained at the nursery premises to grow for a few months. The rootstock seedlings were then transported to 2 nurseries for grafting purposes. The persimmon varieties were grafted to these rootstocks once the seedlings achieved the required thickness. After grafting, the trees will grow for another year before they are ready to be planted in a research trial to assess the uniformity of trees across the orchard for the 3–4 growing seasons.

The tissue culture technique successfully produced persimmon rootstock seedlings for the first time in Australia. A total of 500 seedlings were developed and distributed to nurseries for grafting a range of persimmon varieties. Later in the future projects, trials will be established to test the tree uniformity of the tissue cultured propagated trees.

Outputs

The outputs presented in Table 3 include more than was promised in Milestone 102 (M&E). This includes international collaboration with Spain, Japan and South Korea. The project results were delivered to growers via field days, forums, workshops, industry articles, national and international conferences, and results handouts.

Table 2. Output summary based on the project outputs for 5 years of research on the rootstock evaluation program.

Output	Description	Detail
Negotiate and finalise access to germplasm at the Maroochydore Research Facility	A meeting was arranged by Dr Vino Rajandran (R&D Manager – Hort Innovation) to discuss the germplasm at the Maroochy Research Facility	The meeting was held at the Maroochy Research Facility on 20 June 2018. Dr Vino Rajandran, Dr Tahir Khurshid (NSW DPI), Mr Stephen Jeffers (persimmon grower/nurseryman, Queensland), David Oag (DAF), David Brunn (DAF) and Manager of Maroochy Research Facility attended. Germplasm was inspected at the field site.
International collaboration	IVIA, Valencia, Spain	Collaboration was established with Dr Maria Badenes, and the MTA was officially signed between the 2 countries to exchange rootstocks and varieties. Dr Badenes has contributed to the project regarding the genetic verification of 'Rojo Brillante' and genetic work with *Diospyros lotus* rootstocks. Dr Badenes visited Australia in 2019 to attend the National Persimmon Conference.
International collaboration	Naju South Korea	Dr Kyeong-Bok Ma has been a significant collaborator with the varietal work and is involved in providing Australia with early maturing varieties. He visited Australia in 2019 and 2023 to attend the National Persimmon Conference.
International collaboration	University of Miyazaki, Japan	Dr Takuya Tetsumura has greatly assisted with the clonal propagation (single node cutting) techniques. He also

		provided NSW DPI with the early maturing variety 'Taishu'.
Collaboration with international nursery	Viveros Hernandorena nursery, Ribera del jucar, Valencia Spain	The nursery was involved in providing *Diospyros lotus* rootstock. Arranging all the required paperwork and sending the seeds to Australia without any charge to the project.
Collaboration with domestic plant nursery	Agromillora nursery, Irymple, Victoria	This nursery is in Irymple, Mildura and is collaborating in micro-propagation techniques to develop rootstocks. Tissue culture work of clonal propagation was carried out at this nursery.
Collaboration with domestic plant nursery	Fleming Nursery (ex Birdwood nursery, Nambour, Qld	This nursery helped raise persimmon trees to establish growers' trials. I provided the nursery with the budwood of 6 persimmon varieties obtained from the Maroochydore Research facility, Nambour.
Collaboration with domestic plant nursery	Victorian Citrus Farms, Red Cliffs, Victoria	The nursery was involved in grafting trees of 'Rojo Brillante' on *Diospyros lotus* rootstock.
Collaboration with domestic plant nursery	Melbourne, Victoria	The nursery was involved in grafting new trees and sending them over to Dareton. The nursery owner is also a member of the executive committee of Persimmon Australia Inc.
Collaboration with domestic plant nursery	Nambour, Qld	The nursery was involved in grafting new trees and providing them for the rootstock trials at 4 sites around Australia. The nursery owner is also a member of the executive committee of Persimmon Australia Inc.
Grower's collaboration and participation	Grower 1. Qld Grower 2. NSW Grower 3. NSW Grower 4. SA Grower 5. VIC	Growers played an important role in grower's trials for rootstocks, clonal propagation, and participation in de-astringency trials of 'Rojo Brillante'.
Collaboration with the University of Queensland	Gatton, Qld	University staff assisted in the preliminary de-astringency trials and provided the equipment and laboratory to me.
Project Reference Group meeting	Perth, WA	The project reference group meeting occurred on 17 June 2019 in Perth. The project update was presented, and discussions were carried out.
Project Reference Group meeting	Brisbane, Qld	The project reference group meeting was held during the Persimmon Australia strategic planning meeting on 27 February 2020. The committee was updated on the project progress and achievements.

International Conference	7th International Symposium, NARA, Japan	Crop management panel discussion T. Khurshid (Chair of the session) – Sep 2021
International Conference	7th International Symposium, NARA, Japan	Oral presentation Challenges and opportunities for the Australian persimmon industry – Sep 2021
International Conference	7th International Symposium, NARA, Japan	Oral presentation Persimmon industry in Australia and New Zealand – Sep 2021
International Conference	7th International Symposium, NARA, Japan	Member of the scientific committee Member of the editorial committee Chairperson of the 2 plenary sessions
National Conference	Proceedings of Persimmon conference, Perth	T. Khurshid. 2019. National persimmon varietal evaluation – an update.
National Conference	Proceedings of Persimmon conference, Perth	T. Khurshid. 2019. Project collaboration and communications.
National Conference	Proceedings of Persimmon conference, Mildura	T. Khurshid. 2023. Improving persimmon varietal choices for Australia. Conference proceedings, 21 June.
National Conference	Proceedings of Persimmon conference, Mildura	T. Khurshid. 2023. Improving persimmon varietal choices for Australia. Conference proceedings, 21 June.
National Conference	Proceedings of Persimmon conference, Mildura	T. Khurshid. 2023. Clonal propagation of persimmon rootstocks. Conference proceedings, 21 June.
National Conference	Proceedings of Persimmon conference, Mildura	T. Khurshid. 2023. Preliminary studies on removing astringency from 'Rojo Brillante'. Conference proceedings, 21 June.
National Conference	Field session, Persimmon Conference	T. Khurshid. 2023. A field session was organised for the conference audience at Dareton. Growers visited the trial sites, followed by the varietal and rootstock inspection. 22 June.
Industry Journal	Persimmon Press, 61 December issue (Appendix 2)	T. Khurshid. 2018. Persimmon varietal evaluation program. Persimmon Press, pages 24–25.
Industry Journal	Persimmon Press, 63 December issue (Appendix 2)	T. Khurshid. 2019. Persimmon varietal evaluation program – an update. Persimmon Press, pages 25–26
Industry Journal	Persimmon Press, 64 June issue (Appendix 2)	T. Khurshid. 2020. National persimmon varietal evaluation program – an update. Persimmon Press, pages 15–19.

Industry Journal	Persimmon Press, 65 December issue (Appendix 2)	T. Khurshid. 2020. National persimmon varietal evaluation program – an update. Persimmon Press, pages 18–20.
Industry Journal	Persimmon Press, 66 June issue (Appendix 2)	T. Khurshid. 2021. National persimmon varietal evaluation program – an update. Persimmon Press, pages 12–15.
Industry Journal	Persimmon Press, 67 December issue (Appendix 2)	T. Khurshid. 2021. National persimmon varietal evaluation program – 7th International Conference. Persimmon Press, pages 16–19.
Industry Journal	Persimmon Press, 68 June issue (Appendix 2)	T. Khurshid. 2022. Persimmon varieties available in Australia. Persimmon Press, pages 21–25.
Industry article	NSW Farmers, August 2019	T. Khurshid and Brett Guthery. 2019. Ripe beginning for the Australian persimmon market, pages 1–14.
Industry article	Australian Tree Crops Feb/March issue (Appendix 2)	T. Khurshid. 2022. National persimmon evaluation program, pages 21–21.
Industry Journal	Australian tree crops Aug/Sep issue (Appendix 2)	T. Khurshid. 2018. Persimmons – new project, new researcher, pages 34.
University of Miyazaki Japan.	Horticulture Department Meeting - 30 September 2019	Meeting RIFNUM, Tahir presentation – Dr Yajima Daisuke (NRI), Mr Naoko Udagawa (MAFF), Mr Takanori Ohashi (MAFF), Ryoko Uchida (NSW DPI), Mr Naoki Omori (RIFNUM); Persimmon breeding program, field visit, nursery visit – Dr Takuya Tetsumura
Citrus Research Institute NARO, Higashi-Hiroshima, Japan	An oral presentation was delivered – 12 September 2019	T. Khurshid. 2019. Australian persimmon industry. 25 participants (scientists, breeders and students).
Citrus Research Institute Naju, South Korea	An oral presentation was delivered – 2 October 2019	T. Khurshid. 2019. Australian persimmon industry. 20 participants (scientists and breeders).
Northwest A&F University, Yangling	An oral presentation was delivered – 9 October 2019	T. Khurshid. 2019. Australian seek international collaboration in persimmons. (20 participants – academics and postgraduate students).
Dr Maria Badenes visit June 2019	Perth and Dareton (Mildura)	A discussion was held for the Spanish rootstocks at Dareton, and Dr Badenes decided to establish an MTA with NSW DPI to provide Australia with the selected Spanish rootstocks. Later, MTA was signed between NSW DPI and IVIA, Valencia, Spain.
Newspaper article	Sunraysia Daily (Appendix 2)	Fruit research's sweet success – 24 June 2023.

NSW DPI Open day 15 June 2023	NSW DPI Dareton	Tahir Khurshid displayed the varieties and rootstock of the persimmon work to the open day participants followed by the tasting session.
ABC Radio Interview 15 June 2023	Australia persimmon project	https://www.abc.net.au/radio/programs/vic-country-hour/victorian-country-hour/102473918 @ (36:55). Tahir Khurshid talking about their persimmon variety evaluation and genetic importation work at the site.
Field day	Online – 7 Oct 2021	T. Khurshid. Crop management of persimmon (16 participants).
Field day	Pruning workshop - 6 Sep 2021	Pruning persimmons – Grantham Orchards (21 participants).
Report to Hort Innovation and NSW DPI	Milestone reports Travel reports	10 Milestones achieved during this project. Each Milestone has a detailed description of the project activities. All travel reports relevant to this project are submitted to NSW DPI and Hort Innovation.

Outcomes

The outcomes presented in Table 3 are as per agreed milestone 102 (M&E).

Table 3. Outcome summary based on the project outcomes for 4 years of research on the rootstock evaluation program.

Outcome	**Relevant SIP outcome, strategy and KPI**	**Description**	**Evidence**
Industry outcome Strategic Investment Plan (SIP, Persimmon Fund 2016/17 Annual Report, Horticulture Innovation Australia).	Increased industry production and productivity to meet increasing domestic and international demand.		Industry magazine articles, field days, persimmon press articles, persimmon Australia conferences, international conferences, Milestone reports, and Final Report (see Output section).
New overseas partnerships were established to access new varieties and rootstocks.	Same as above	New overseas collaborations and partners were successfully established with scientists from IVIA, Spain; University of Miyazaki, Japan; Pear and Persimmon research institute, Naju, South Korea; and Northwest A&F University, Yangling, Xian China.	The evidence is the import of early maturing 'Taishu' variety from Japan and the pipeline varieties from Naju, South Korea. The success of clonal propagation works, with assistance and consultation from Dr Takuya Testsumura, University of Miyazaki, Japan. Spanish scientist Dr Maria Badenes assisted in the genetic verification work of 'Rojo Brillante' and facilitated the import of new rootstock *Diospyros lotus* to Australia.

New varieties and rootstocks available to persimmon growers throughout Australia.	Same as above	New Spanish variety 'Rojo Brillante' New non-astringent variety 'Taishu' New rootstock *Diospyros lotus*	The mother blocks of 'Rojo Brillante' budwood are established at Dareton, and limited budwood will be available to growers from August–September 2023. The non-astringent 'Taishu' variety is released from quarantine. It has very limited budwood that will be used for the experimental trial at Dareton, followed by the evaluation of 'Taishu' for earliness and yield. The budwood block for *Diospyros lotus* has been established at Dareton after the genetic verification of female and male trees. Growers now have immediate access to male and female budwood from those trees to establish their seed source for *Diospyros lotus* rootstock.
Information package prepared and delivered regarding the true-to-type of 'Rojo Brillante'.	Same as above	The information is available regarding the true-to-type characteristics of 'Rojo Brillante'. The information is available to growers and researchers nationally and internationally.	The information is available in industry magazine articles, field days, persimmon press articles, persimmon Australia conferences, international conferences and milestone reports submitted to Hort Innovation. The information can also be accessed in this final report (Chapter 1, Appendix 1)
The new clonal propagation technique was implemented for reliable plant propagation.	Same as above		Results sections and Final Report (Appendix 1, Chapter, Appendix 1)

Monitoring and evaluation

The monitoring and evaluation of the project based on the key evaluation questions are given in Table 4.

Table 4. Monitoring and evaluation based on key evaluation questions.

Evaluation question	**Project performance**	**Continuous improvement opportunities**
Effectiveness	This project has been very effective since it was a national varietal and rootstock program. This program worked with various experiments on different aspects of	The trial work conducted at the Dareton research institute and growers' properties provided a direct opportunity to see the results immediately on their property. Other growers can visit the property to see the effects of

	persimmon varieties, rootstocks, and clonal propagation. The project has achieved all expected outcomes as per Project Milestone Report 102.	rootstocks on yield, fruit quality and tree growth. Growers demonstrated pruning methods at two properties, which were well received. • Pruning persimmons – Grantham orchards (September 2021) – Rod Dalton • Pruning old persimmons (August 2021) – Chris Stillard Growers also had the opportunity to visit the trial sites at NSW DPI Dareton during a recent national persimmon conference (20–22 June 2023) in Mildura. The grower visits to the trial sites at Dareton allowed the growers to see a range of varieties and rootstocks. Growers also learnt about the 'Rojo Brillante' variety. Apart from a few growers, others have never seen this new variety. This high-value variety will be the future of the Australian persimmon industry. Therefore, the current project was very effective in achieving its objectives.
Relevance How relevant was the project to the needs of the intended beneficiaries?	The project was very relevant to growers. It involved local and imported varieties and clonal propagation techniques. Growers in Australia are using only 2 main varieties, 'Jiro' and 'Fuyu', in a range of soil and climatic conditions. Therefore, introducing new varieties is vital for the Australian persimmon industry to extend its growing seasons and achieve higher profits by introducing the early maturing varieties before 'Jiro'. All growers use only one main rootstock, *Diospyros kaki,* in all soil and climatic conditions for both 'Jiro' and 'Fuyu' persimmon varieties. A new rootstock, *Diospyros lotus*, was introduced into Australia as a seed during this project. The mother blocks of the *Diospyros lotus* seeds were also established at Dareton and a grower's site as a backup. There are no dwarfing rootstocks available for high-density plantings or vigorous growing varieties such as 'Fuyu'. In Queensland, 'Fuyu' gets very vigorous due to excess moisture from rainfall and high humidity. An attempt has been made to negotiate to import the	This project has met the needs of persimmon levy payers in achieving the desired results from the experimental program. Therefore, the import of the new variety 'Taishu' and the new rootstock '*Diospyros kaki*' and the production of rootstock by tissue culture was a success during this project and resulted in a positive outcome for the persimmon growers. The genetic and phenotypic verification of the Spanish variety 'Rojo Brillante' has produced positive outcomes and the Australian growers have been asking for the budwood for this new variety. This variety is recommended for export into Asian markets due to the absence of 'Rojo Brillante' from other countries. The evidence includes: Early maturing varieties: • Import of 'Taishu' Varieties in the pipeline: 'Wonmi', 'Gampung', 'Fantasy'

	dwarfing rootstock 'MKR1' from Miyazaki. In future it will be possible to import the dwarfing rootstock. There is an existing rootstock, 'Telco', considered a dwarfing rootstock, but no research has been done on it. In future the ability of that rootstock will be investigated.	New rootstock • *Diospyros lotus* Rootstock in the pipeline: • MKR1
Appropriateness	The intended beneficiaries such as growers, nurseries and packers were well engaged and were aware of the project activities and results. This was possible through field days, conferences, magazine articles, phone calls and one-on-one visits at the growers' properties or the growers' visits to Dareton. Field days in 2020 and 2021 were restricted due to Covid-19 on entry to NSW state government facilities. Therefore, the field days were conducted online and via prepared videos. The R&D manager was engaged via phone discussions, Milestone reports and Emails. However, the growers could participate in person in June 2019 and June 2023 conferences. The CEO of Persimmon Australia was regularly engaged via phone calls and personal meetings. A project reference group meeting was also held, and the project progress was presented during the project.	Dareton Research Station has always had a high industry reputation in Australia and overseas for research and extension-based activities to impart knowledge to visitors. The extension events such as field days, workshops and persimmon conferences were fully accessible to the industry members.
Efficiency What efforts did the project make to improve efficiency?	The project was expanded with the existing budget to meet the project objectives throughout Australia. The persimmon crop is grown over 5 states. During the project, additional articles were published than originally agreed upon, and I participated in field days and workshops to provide information updates to persimmon growers. Despite Covid-19 and prohibited entry from Victoria to Qld, NSW, SA and WA, the project was still on track, the necessary data sets	The project will be further improved by strengthening the collaboration with the existing partners in Naju, South Korea, Yangling, China, Miyazaki, Japan, IVIA, Spain and Algarve, Portugal and Ganja, Azerbaijan. PRG Meetings: 20 June 2022 Several phone meetings.

	were collected, and project activities were kept on track. I did have the difficulty of travelling to SA, QLD, WA and some parts of NSW, however, the project was efficiently managed, and the required data were collected. The project was engaged with stakeholders by conducting the project reference group meeting. Six project reference group meetings (PRG) meeting was conducted during the project life with inputs from the group.	

Recommendations

Varieties and rootstocks have a major effect on the profitability of persimmon orchards. New and early maturing varieties are important to extend the growing season and increase the yield and profitability of persimmon crops. Rootstocks can influence tree growth, fruit yield, yield efficiency, fruit size distribution, fruit quality and scion and rootstock compatibility, affecting the final yield. During the current project, a new variety, 'Taishu', was imported from Japan, while a new rootstock, *Diospyros lotus,* was imported from Spain. A few growers are starting to grow the 'Rojo Brillante' variety. It is recommended that growers grow this variety on *Diospyros lotus* rootstock, as the astringent variety is grown well in Spain on this rootstock. The quality of the rootstock seedlings is important for this variety since the budwood borne on a 1-year-old branch can be thicker than buds of non-astringent varieties. Therefore, care must be taken to grow a good quality, healthy thicker seedling, which can take more than one year to grow.

Growers should also be aware that in a few years, they will need a proper de-astringency facility at their orchards to remove astringency from 'Rojo Brillante'. 'Rojo Brillante' should not be sold in its astringent form to the local markets. The selling of astringent 'Rojo Brillante' can ruin the industry's reputation and can effectively reduce the sale of non-astringent varieties. Therefore, the persimmon industry should establish the required rules, regulations, and marketing protocols for handling this variety.

There is a lack of knowledge about the persimmon among a large percentage of Australia's mainstream population. The industry should promote the persimmon fruit. There is a need to change the price structure for the Australian markets. The higher prices of persimmon in supermarkets can deter Australian customers from buying this fruit.

Further research

Future research will be conducted in a future project, 'National persimmon varietal evaluation program', for the Australian persimmon industry. This research will potentially continue in the future projects supported by Hort Innovation. Although all the objectives have been achieved in the current project, certain activities need to be continued to complete the trials.

- The follow-on research continues the first objective, '*Import early maturing varieties from overseas'*. The varieties will be imported from South Korea, and mother blocks will be established in the next round. These varieties need to be rapidly evaluated in South Korea before being imported into Australia. Rapid evaluation includes fruit size, fruit shape, °Brix levels, fruit firmness, earliness, storage life and productive performance based on South Korean data.

- Two varieties, 'Maxim' (non-astringent) and 'Ribera sun' (astringent), are in the pipeline with an importer. These will be evaluated at the Dareton Primary Industries Research Institute.
- The rootstocks developed from the tissue culture techniques will be budded and tested for uniformity around Australia at different trial sites at growers' properties.
- The de-astringency component of the 'Rojo Brillante' variety will be carried out based on the preliminary work done in the current project. The storage trials will also be conducted after astringency removal, followed by the shelf-life trials. A visit to Spain/Portugal commercial packing sheds must be carried out to master the de-astringency techniques for 'Rojo Brillante'.
- The 'Rojo Brillante' budwood block will be increased in the next few years so buds can be available for growers.
- A budwood block for the imported 'Taishu' variety from Japan will be established in the next few years.
- The 2 existing early maturing varieties, 'Yoho' and 'Shinshu' will also be evaluated for their suitability for Australian growers. These varieties were transferred from Maroochydore Research Institute during the current project and now the germplasm has been established at Dareton as 2 trees/variety. The propagation and assessment of these varieties will be carried out in the next round.

Refereed scientific publications

(Appendix 2)

1. Khurshid, T. 2022. Persimmon industry in Australia and New Zealand. *Acta Horticulturae* 1388: 21–24.
2. Khurshid, T. 2022. Challenges and opportunities for the Australian persimmon industry. *Acta Horticulturae* 1388: 99–104.
3. Ma, K. B.; Yang, S. J.; Jo, Y. S. Kang, S. S. and Khurshid, T. 2020. Current status of persimmon industry and prospect of Korea-bred new persimmon varieties in Australia. *Journal of Korean Society of International Agriculture* 32(4): 377–380.

References

Akagi, T.; Kajita, K., Kibe, T.; Morimura, H.; Tsujimoto, T.; Nishiyama, S.; and Tao, R. 2014. Development of molecular markers associated with sexuality in Diospyros lotus and their application in *Diospyros kaki*. *Journal of the Japanese Society for Horticultural Science*, 83(3): 214-–221.

Bhojwani, S. S. and Dhawan, V. 1989. Acclimatization of tissue culture-raised plants for transplantation to the field. *Applications of Biotechnology in Forestry and Horticulture*, 249–256.

Chandra, S.; Bandopadhyay, R.; Kumar, V. and Chandra, R .2010. Acclimatization of tissue cultured plantlets: from laboratory to land. *Biotechnology Letters*, 32(9): 1199–1205.

Debergh, P. C. 1991. Acclimatization techniques of plants from in vitro. *Acta Horticulturae*, (289): 291–300.

Fresh Plaza. 2022. The Spanish kaki harvest in the 2022/2023 season is estimated to be 50% below the productive potential. https://www.freshplaza.com/oceania/article/9454351/the-spanish-kaki-harvest-in-the-2022-2023-season-is-estimated-to-be-50-below-the-productive-potential/.

Giordani, E.; Naval, M. and Benelli, C. 2013. In vitro propagation of persimmon (Diospyros kaki). *Methods of Molecular Biology*, 11013: 89-–98.

Hasegawa, K.; Hamada, K.; Ogata, T. and Kitajima, A. 2005. Differences in seediness, fruit quality, and leaf character of Japanese persimmons 'Taishu' and 'Matsumotowase-Fuyu'. *Acta Horticulturae*, 685: 119-124.

Khurshid, T. 2022. Persimmon varieties available in Australia. *Persimmon Press*, 21-25.

Kozai, T. 1991. Acclimatization of micro-propagated plants. *Biotechnology in Agriculture and Forestry*, 127–141.

Llacer, G. and Badenes, M. L. 2009. Production of persimmons in Spain. *Acta Horticulturae*, 833: 39-41.

Martinez-Calvo, J; Naval, M. M.; Llacer, G. and Badenes, M. L. 2009. Study of germplasm collection of Persimmon (*Diospyrus kaki* Thunb.) by multivariate analysis. *Acta Horticulturae*, 833: 139-143.

Oag, D. 2017. Australian sweet persimmon industry development project-Phase 4. Final Report, PR13007. Horticulture Innovation Australia.

Tetsumura, T.; Ishimura, S.; Hidaka, T.; Hirano, E.; Kuroki, S.; Uchida, Y. and Honsho, C. 2013. MKR1, a dwarfing rootstock for Kaki, decreases early fruit drop. *Acta Horticulturae*, 996: 257-263.

Tetsumura, T.; Tao, R. and Sugiura, A. 2000. Single-node stem cutting from root suckers to propagate a potentially dwarfing rootstock for Japanese Persimmon. *HortTechnology*, 10(4): 776-780.

Tetsumura, T.; Tao, R. and Sugiura, A. 2003. Cutting propagation of some dwarfing rootstocks for Persimmons. *Acta Horticulturae*. 601, 145-149.

Intellectual property

There is no intellectual property attached to this project at this stage.

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Overseas collaborators

The overseas collaborators are equally recognised during this project. Thanks to Dr Maria Badenes for assisting with the genetic verification of 'Rojo Brillante' and *Diospyros kaki* rootstock. Thanks, are also extended to Dr Takuya Tetsumura for assisting with the single-node cutting propagation techniques and the import of early maturing variety 'Taishu'. I am also indebted to Dr Kyeongbok Ma who shared the knowledge of his early maturing varieties, visited Australia twice during this project, and participated in 2 national persimmon conferences and field sessions.

Technical and farm staff

I am especially thankful to Jane Khurshid who has been involved in the persimmon trials since they began. Jane managed the trials, organised and collected the tree growth data and quality analysis. Jane also organised and managed the harvest for the crop. Thanks, are also extended to Troy Witte (Farm Manager), Brad Bowes and Darren Howard in planting the trials and managing the irrigation and nutrition for the trees in a timely manner.

Participating growers (4 states) and participating nurseries

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Editorial assistance

Thank you to Dr Amanda Warren-Smith for assistance with editing this report.

Appendices

Appendix 1

Chapter 1: True-to-type verifications of 'Rojo Brillante' in Australia

The Australian persimmon industry has 'Rojo Brillante' scion variety present in the country, which was sourced from a grower in Portugal many years ago (Oag, 2017); however, there was no clear evidence of whether this variety was true-to-type, as it was suspected that the budwood might have been mixed up with the budwood of some other variety at a nursery level and during its transfer to Australia. The genotypic verification needed to be carried out, followed by comparing phenotypic traits of this variety to the original fruit of 'Rojo Brillante'. This work was carried out on a limited number of trees of 'Rojo Brillante' grown across Queensland (Qld), New South Wales (NSW) and South Australia (SA). This work involved assistance from Spain.

Collaboration with IVIA, Valencia, Spain

Collaboration was established with Dr Maria Badene of IVIA, Valencia, Spain. Dr Maria Badenes helped test the DNA samples for sequencing. The DNA of the existing variety 'Rojo Brillante' was extracted by Dr Tahir Khurshid and provided to Dr Maria Badenes. Dr Badenes provided the results to Dr Tahir Khurshid about true-to-type characteristics and verification of 'Rojo Brillante'. Once the true-to-type characteristics were established, the persimmon industry in Australia could propagate and promote this variety in Australia. If the variety was not true-to-type, then Dr Maria Badenes promised to provide the 'Rojo Brillante' budwood to NSW DPI, Australia. Although, the process could have taken up to 2 years. Dr Maria Badenes were provided with the DNA samples of leaves collected from the 'Rojo Brillante' trees grown in Renmark (South Australia), Maroochydore (Queensland) and Sydney (NSW). There were 5 samples per site provided to Dr Badenes, totalling 15 samples.

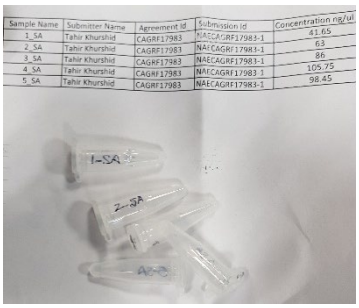
DNA-based genotyping was conducted in collaboration with international scientists in Spain to ascertain the true-to-type characteristics of 'Rojo Brillante' were verified. The plant samples (mature leaves) were collected from mature 'Rojo Brillante' trees from multiple locations in Qld, NSW and SA. The leaves were then sent to the Australian Genome research facility laboratory in Adelaide, and DNA material was extracted. The laboratory extracted and provided the DNA material to NSW DPI. The DNA was taken to IVIA, Valencia, Spain in October 2018 (Figure 1.1) and in 2019 (Figure 1.2).

Figure 1.1: The extracted DNA are shown in small tubes. (Photo: Dr Tahir Khurshid)

Figure 1.2: Visit to the Spanish germplasm programs, nurseries, and orchards. (Photo: Dr Tahir Khurshid)

The samples were re-coded in Spain to retain the identity of different sampling sites (Figure 1.3). In Spain, the DNA samples were analysed with 5 combinations of primers. The 'Rojo Brillante' DNA samples from Spain were used as a control and compared against DNA samples from Australia. The second batch of leaf samples was collected in June 2019

and the DNA was extracted in a laboratory in Adelaide. The DNA material was then taken to IVIA, Spain, by Dr Maria Badenes to verify the repeat samples for DNA marker comparisons.



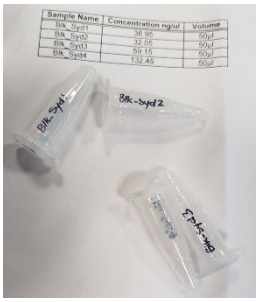


Figure 1.3: The DNA samples arrived in Spain and were coded to retain the identity for different sampling sites.

Results

Dr Maria Badenes provided the results to Dr Tahir Khurshid. Dr Badenes analysed 5 combinations of primers. The samples were included in all PCR reactions. The Spanish sample of 'Rojo Brillante' DNA was used as a control. The results obtained with Spanish 'Rojo Brillante samples produced positive results, and all the expected alleles were identified.
The results of the samples obtained from Renmark (SA) had the same alleles as our 'Rojo Brillante' in 90% of the alleles, and for the remaining 10%, Dr Badenes did not get amplification. Dr Badenes confidently concluded that the 'Rojo Brillante' samples from Renmark South Australia corresponded to the same genotype in Spain.

Dr Maria indicated that the quality of DNA samples from Sydney and Maroochydore was very low. Therefore, the 5 samples for Sydney or Maroochydore were combined to increase the concentration. Still, they did not provide the expected results, and a lack of amplification was obtained in most of the primer combinations from those samples. Considering that all the PCRs included 'Rojo Brillante' as a control and amplification was obtained with the correct alleles in all cases with the Renmark samples, Dr Badenes concluded that the quality of DNA obtained from Sydney and Maroochydore sites was poor. This could be due to the quality of the leaves, as the distance they travelled meant they took longer to get to the Adelaide laboratory. While the proximity of the Renmark site provided good leaf quality, and the results were positive.

Phenotypic verification of 'Rojo Brillante' in Australia

Dr Maria Badenes visited Australia in June 2019 to attend the national persimmon conference in Perth. She visited the persimmon industry in Perth Hills and presented a paper about the Spanish persimmon industry. Dr Maria visited the Dareton Primary's Industries Institute, followed by a field visit to the persimmon packing shed and orchard in Sunraysia and South Australia. During her visit to South Australia, Dr Maria Badenes visited the 'Rojo Brillante' trees in Renmark, where the DNA samples were obtained (Figure 1.4). There were still sufficient fruits available on the trees for phenotypic assessments. Dr Maria Badenes confirmed the phenotypic characteristics after assessing the fruit size, shape, and skin colour and confirmed that the 'Rojo Brillante' variety in Australia is the original variety from Spain. Therefore, it was confirmed by genotypic and phenotypic verifications that the persimmon industry should be able to proceed with the existing 'Rojo Brillante' variety and the new blocks of 'Rojo Brillante' need to be established in the new production areas of Australia.

Figure 1.4: Dr Maria Badenes with Mr Nick Hobbs (persimmon grower) at Renmark inspecting the persimmon pruning (left) and the 'Rojo Brillante' tree with fruit. (Photo: Dr Tahir Khurshid)

Next steps

1. The next steps will be to develop the budwood mother blocks for 'Rojo Brillante' and to provide budwood to growers to establish new plantings. This will be possible if the project is funded for the next 5 years (2023–2028).
2. The postharvest de-stringency techniques need to be learnt from the Spanish persimmon industry and practised here in Australia.
3. To 'Rojo Brillante' persimmon markets need to be explored in Asia for future export. Spain does not export fruit to Asian markets. Therefore, Australia will be able to sell this unique type of fruit in the Asian markets (Malaysia, Singapore, and Hong Kong) at premium prices. Other markets, such as Indonesia and UAE, also need to be explored.

Chapter 2: Access to new persimmon varieties and rootstocks from overseas

The global mapping of the persimmon improvement programs was conducted at the beginning of the project. The preliminary analysis showed that Spain, Japan, South Korea, and China are the major persimmon producing countries for accessing new varieties and rootstocks. These countries are still running active and efficient breeding programs with or without collaboration with other persimmon growing countries. Links were strengthened with the relevant scientists at El Instituto Valenciano de Investigaciones Agrarias (IVIA) in Spain and Northwest Agriculture and Forest University in Yangling, China. Working relations were also established with the University of Miyazaki, Japan and Pear Research Institute, NIHHS, Naju, South Korea.

The major persimmon producing countries Spain, Portugal, Japan, South Korea and China were visited, and the variety and rootstock breeding programs were inspected during the visits. Scientists from Spain and South Korea visited Australia and inspected the Australian persimmon industry in June 2019. However, due to COVID-19, the collaboration was slow to develop. Further visits to these countries were not possible to draw agreements and import varieties, especially from South Korea and Spain.

Valencia is the major production region for the Spanish persimmon industry, followed by Andalucía (Llacer and Badenes, 2009). Spanish scientists have studied 13 accessions (Martinez-Calvo et al. 2009). This also includes a variety called 'Aman' (Pakistan seedless) originating in Pakistan. Dr Tahir Khurshid visited Spain to inspect persimmon varieties from their breeding program. Tahir will evaluate the early ripening characteristics of 'Taishu', which has been recently imported from Japan. The import of varieties from South Korea, China and Spain is being negotiated. Tahir will also assess the varieties from South Korea, Spain and China soon in the next round of funding.

In Japan 'Fuyu' is the most widely grown persimmon variety. However, the persimmon breeding program at the National Agriculture and Food Research Organisation (NARO) has released 2 non-astringent varieties, 'Taishu' (Hasegawa et al., 2005) and 'Taiho' (Sato et al., 2018). Taiho is also grown in China but has some incompatibility issues with *Diospyrus lotus* rootstock. A detailed description of 'Taishu' is given later in this chapter.

The *Diospyrus lotus* rootstock has now been imported from Spain into Australia as seed. This is the first time this rootstock was imported into Australia by NSW DPI. The benefits of importing rootstocks as seed are that it requires a shorter quarantine duration or inspection time in Australia and therefore is the preferred method.

The import of scion varieties as budwood can take up to 2 years or longer in the Australian quarantine facility. Access to new varieties is vital for the Australian persimmon industry, since 'Jiro' and 'Fuyu' are the main varieties grown in Australia. Therefore, earlier maturing varieties must be imported to extend the persimmon growing season in Australia. There is also a need to import a dwarfing rootstock that can control the vigorous 'Fuyu' variety, which can get very vigorous in Queensland due to abundant rainfall.

During the current project, the priority and focus were establishing links and collaboration with Spain, Japan, South Korea and China to import varieties and rootstocks. However, possible links were also maintained with Japan in terms of dwarfing rootstocks and clonal propagation work during this project. A dwarfing rootstock, MKR1, has the potential to increase the yield and sugar levels of fruit (Tetsumura et al., 2013). Dr Maria Badenes was invited to visit Australia in June 2019 to attend a national persimmon conference in Perth followed by visits to Victoria, NSW and South Australia. Dr Badenes inspected the nurseries, growers' orchards and packing houses during her visit. The scientists and persimmon breeders from South Korea also visited persimmon orchards and packing houses in Australia when they were here to attend the conference.

Import of 'Taishu' scion variety from Japan

Dr Tahir Khurshid visited the University of Miyazaki, Japan, in late September 2019, and a formal meeting was held with the persimmon breeder and crop management expert Dr Takuya Testsumura along with the staff of RIFNUM (Japanese persimmon industry organisation) Japan at Miyazaki. Dr Takuya Testsumura agreed to provide the budwood of 'Taishu' to

NSW DPI. ‘Taishu’ appears to be an early maturing variety than ‘Jiro’ as per Dr Takuya Tetsumura (personal communications) and can be tested under Australian conditions. ‘Taishu’ has a large fruit size and yellowish skin colour in the tropical climate of Miyazaki. This variety will attain deep yellow to light orange skin colour in sub-tropical climatic regions of Australia. ‘Taishu’ is a non-IP variety and will be freely available to Australian growers once enough budwood is available for distribution.

The first batch of budwood (17 sticks with 8 buds/stick = 136 buds) was provided to NSW DPI, which arrived at the post-entry quarantine (PEQ) facility in Mickleham, Melbourne, in October 2020 (Figure 2.1). The budwood was inspected for viruses and diseases. The PEQ pathologist discovered various diseases and physical issues in the budwood after applying various pathological tests, therefore, the budwood was discarded without any further use. The PEQ asked Dr Khurshid to arrange a second batch of budwood from Japan. A second batch of ‘Taishu’ budwood was requested from the University of Miyazaki, which arrived at the PEQ facility in December 2020 (Figure 2.2).

Figure 2.1: The arrival of the first contingent of budwood in October 2020 to the PEQ facility in Melbourne, Australia. (Photo: Dr Tahir Khurshid)

Figure 2.2: The arrival of the second contingent of budwood in December 2020 to the PEQ facility in Melbourne, Australia. (Photo: Dr Tahir Khurshid)

One-year-old rootstock seedlings of *Diospyros lotus* were provided to PEQ by Dr Khurshid. ‘Taishu’ scion variety was grafted to the one-year-old rootstock seedlings. PEQ maintained 4 trees at the facility for more than one year. The trees were regularly checked for viruses and diseases.

Indexed trees were released from the PEQ and arrived at Dareton on 15 September 2021 (Figure 2.3). PEQ also released an additional 2 trees on 2 February 2022 (Figure 2.4). Therefore, 4 trees arrived, but one did not survive after arrival at Dareton. The 3 trees are now grown in pots (Figure 2.5), and a limited number of budwood will be available in October 2023 to propagate a few additional trees and eventually establish a budwood mother block for ‘Taishu’.

Figure 2.3: The arrival of ‘Taishu’ at Dareton, 15 Sep 21.

Figure 2.4: The arrival of ‘Taishu’ at Dareton, 2 Feb 2022.

Trees were maintained in the nursery facility of Dareton, and a limited number of buds will be collected to propagate trees to establish mother blocks. This propagation process will continue for another 5 years until sufficient buds are available to propagate the trees. This season the trees produced only 2 fruits; however the shape and colour of the fruit resembles the original ‘Taishu’ variety and confirms its originality. However, further evaluation will be conducted in the coming years. At this stage the trees are looking promising, and they will be shifted in July–August 2023 to a larger pot to encourage vegetative growth and to preserve the plant material.

Figure 2.5: ‘Taishu’ tree at Dareton on 25 Mar 2023.

Characteristics of ‘Taishu’

‘Taishu’ is a Japanese persimmon (*Diospyros kaki* Thumb.) that was released by the Persimmon and Grape Research Centre of the National Institute of Fruit Tree Science, Akitsu, Higashi-Hiroshima, Japan in 1994. Taishu was registered in Japan in 1995. In Kagwa prefecture, it is mainly cultivated in Ayagawa town and Takamatsu city. In some production areas of Kagwa prefecture the variety is grown for its early production and shipped as ‘Blue’ persimmon for their refreshing sweetness and texture.

The fruit is distinguished by its large size, pollination constant non-astringent type (PCNA), and excellent eating quality. ‘Taishu’ resulted from the cross ‘Fuyu’ x ‘II iG' made in 1977. ‘II iG' is a PCNA selection, whose ancestors are ‘Jiro’,

Hanagosho', and 'Okugosho'. 'Taishu' was primarily selected at Akitsu in 1984 and has been tested as a clonal selection at 31 locations in 29 prefectures under the fourth persimmon regional trials initiated in 1977. I inspected the fruit in Japan in October 2019 (Figure 2.6).

'Taishu' fruit is roundish flat in shape, weighing an average of 320 g (1.3 times larger than 'Fuyu'), ripens in early to late October in Japan and South Korea, which equates to mid-April to early May in Australia. Larger fruit size and high sugar content are the main characteristics of interest for this variety. The fruit is also rich and very juicy compared to other non-astringent persimmons such a 'Jiro' and 'Fuyu'. Total soluble solid contents in the juice are 17.7% (1.7% higher than 'Fuyu'). The skin of the fruit is a light orange in Japan; however, Dr Khurshid indicated that it would have better and deeper colour, particularly in Southern parts of Australia. The fruit has slight cracking habit at the calyx end. 'Taishu' has a shelf life about 2 weeks longer than 'Fuyu'.

'Taishu' is adaptable to similar areas of planting as 'Fuyu'. The tree is moderately vigorous, and intermediate between upright and spreading type in shape. It produces female and male flowers. The shoots produce male flowers in abundance, leading to the reduction of female flowers. Therefore, it will be important to produce vigorous shoots constantly by crop management because vigorous shoots produce female flowers, leading to seedless fruit.

Figure 2.6: 'Taishu' fruit at maturity in Persimmon and Grape Research Centre, Akitsu, Higashi-Hiroshima Japan on 4 October 2019.

Future evaluation of the 'Taishu' variety

The establishment of the mother block will occur during the next project starting from late 2023. The 'Taishu' buds will be grafted to a rootstock and trees will be propagated to plant at Dareton for further evaluation. During that time 'Taishu' trees will also be used to increase bud source for the future. The buds will be distributed to the growers or nurseries.

South Korean varieties

Dr Kyeongbok Ma from South Korea (Persimmon Breeder at the Pear Research Institute of the National Institute of Horticultural & Herbal Science in Naju-si) visited Australia in June 2018. Dr Ma indicated that non-astringent persimmon production in South Korea is confined to the coastal and southern regions where winter temperatures are warmer. South Korea's production has focused primarily on 'Fuyu', but in recent years plantings of new varieties like 'Jowan', 'Wonmi'

and ‘Gampung’, which have been selected in South Korea have increased significantly enhancing the persimmon production. Dr Ma participated in the national persimmon conference in Perth (Figure 2.7) followed by visits to Persimmon orchards and packing shed (Figure 2.8). During the trip he established collaboration with Dr Tahir Khurshid of NSW DPI, Dareton. He expressed his intentions for his early maturing persimmon varieties to be tested under the Australian conditions.

Figure 2.7: Dr Kyeongbok Ma deliver a presentation about the characteristics of new varieties in Perth.

Figure 2.8: Dr Kyeongbok Ma and his team visited the persimmon growers’ properties in Australia.

In October 2019, Dr Khurshid visited Dr Kyeongbok Ma (Persimmon Breeder), Dr Kang Soohyun and Ms Jung Haewoon at the Pear Research Institute (NIHHS-RDA) Naju – both scientists attended the Australian Persimmon conference in Perth in June 2019. Tahir also inspected the research trials, laboratories, postharvest facilities and interacted with scientific and technical staff working on persimmons. Dr Tahir Khurshid made a presentation to the institute about the Australian persimmon industry and his research progress regarding his current project.

In South Korea, Dr Khurshid had the opportunity to observe a range of persimmon varieties during harvest, observing the fruit on trees, taste them and assess the fruit quality and other fruit characteristics. During the discussion’s Dr Kyeongbok Ma suggested that early maturing varieties ‘Wonmi’ (Figure 2.9) and ‘Fantasy’ (Figure 2.10) need to be tested under the Australian conditions.

Figure 2.9: Early maturing variety ‘Wonmi’ in Naju South Korea.

Figure 2.10: Early maturing variety ‘Fantasy’ in Naju South Korea.

These varieties exhibit some calyx end cracking in humid conditions of South Korea. Since the NSW, VIC, SA and WA persimmon growing areas are dry, he believes that it will be much suited to our dry conditions. Both varieties have an IP

status in South Korea and arrangements need to be made with the South Korean research institute and Rural Development Authority (RDA) to import persimmons varieties to Australia. Dr Khurshid observed the performance of early maturing varieties at the trial sites situated in South Korea (Figure 2.11), however, the rapid assessment of the germplasm was not possible due to the short trip. Dr Khurshid is in contact with Dr Kyeongbok Ma to facilitate the import process.

During the trip Dr Khurshid went to a grower's orchard and had the opportunity to participate in a field day (Fig 2.12). The progressive growers were changing their persimmon varieties to the new early maturing varieties bred by Dr Kyeongbok Ma.

Figure 2.11: A range of varieties were inspected for their yield performance in Naju, South Korea.

Figure 2.12: Participation in a field day at the grower's property in Naju, South Korea

The varieties bred in South Korea with its characteristics are given below.

'Jowan'

'Jowan' is an early maturing PCNA variety released in 2012 (Figure 2.13). It is a hybrid of 'Shinsyuu' x 'Taishu', and this variety matures in late September in Southwestern part of South Korea equivalent to late March in Australia. It matures 4-5 weeks before 'Jiro'. Fruit weight is 200 g and reaches ^{o}Brix value of 16.4 at harvest. Fruit is flat shaped with yellow colour. Fruit Flesh is soft, juicy and crisp. It is recommended to harvest the fruit a bit early, as there are micro cracks on the fruit skin. In my opinion it will colour well in the Southern part of Australia.

Figure 2.13: Persimmon variety 'Jowan' exhibiting yellow skin colour with crunchy flesh texture.

'Wonmi'

'Wonmi' is an early maturing PCNA variety released in 2014 (Figure 2.14). It is a hybrid of 'Fuyu' x 'Taishu', and this variety matures in early October in Southwestern part of South Korea equivalent to early April in Australia. It matures 3 weeks before 'Jiro'. Fruit weight is 250-300 g and reaches °Brix value of 15.1 at harvest. Fruit is round shaped with orange colour. Fruit Flesh is soft and crisp. In my opinion it will colour very well in Southern part of Australia.

Figure 2.14: A deep orange coloured early maturing variety 'Wonmi' in Naju South Korea.

'Wonchu'

'Wonchu' is an early maturing PCNA variety released in 2014 (Figure 2.15). It is a hybrid of 'Shinsyuu' x 'Taishu', and it matures in first week of October in Southwestern part of South Korea equivalent to early April in Australia. It matures 3 weeks before 'Jiro'. Fruit wight is 350 g and reaches °Brix value of 15.1 at harvest. Fruit is flat and round shaped with yellow to orange colour. Fruit Flesh is soft and crisp. In my opinion it will colour very well in Southern part of Australia.

Figure 2.15: An early maturing variety 'Wonchu' in Naju, South Korea.

'Fantasy'

'Fantasy' is an early maturing PCNA variety released in 2018 (Figure 2.16). It is a hybrid of 'Ro-19' x 'Taishu', and this variety matures around 10 October in Southwestern part of South Korea equivalent to 10 April in Australia. It matures 2 weeks before 'Jiro'. Fruit weight is 230-280 g and reaches °Brix value of 16.1 at harvest. Fruit is round shaped with yellow

orange colour. Fruit shape is slightly conical at the bottom. Fruit Flesh is soft and crisp. In my opinion it will colour very well in Southern part of Australia.

Figure 2.16: An early maturing persimmon variety 'Fantasy' at Naju, South Korea.

'Gampung'

'Gampung' is a PCNA variety released in 2013 (Figure 2.17). It is a hybrid of 'Deandangam' x 'Taishu', and this variety matures at the same time as 'Jiro' around late October in Southwestern part of South Korea equivalent to late April in Australia. Fruit weight is 420 g and reaches °Brix value of 14.7 at harvest. Fruit is flat shaped with orange skin colour. Fruit Flesh is soft, juicy and crisp.

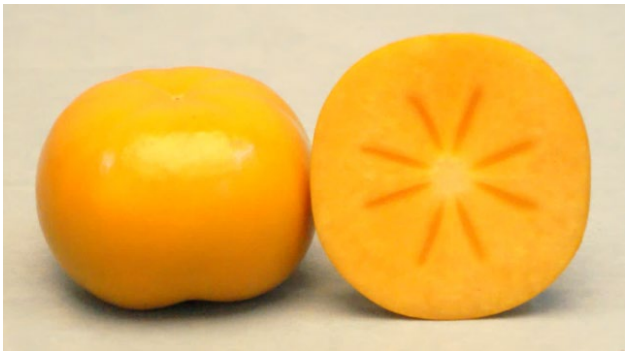


Figure 2.17: 'Gampung' a promising variety from Naju, South Korea which mature at the same time as 'Jiro'.

'Bonghwang'

It is a PCNA variety (Figure 2.18), and it is a hybrid of 'Rn252' x 'Taishu', and this variety matures at the same time as 'Jiro' around late October in Southwestern part of South Korea equivalent to late April in Australia. Fruit weight is 300 g and reaches °Brix value of 17.0 at harvest. Fruit is oval shaped with yellow orange skin colour. Fruit flesh is soft and crisp.

Figure 2.18: 'A unnamed oval shaped variety from Naju, South Korea which mature at the same time as 'Jiro'.

Unnamed variety (this new variety has not been named as yet)

'It a new PCNA variety and matures much earlier than 'Jiro' and is used for the religious festival called Chuseok. It has a black skin colour with round shaped fruit (2.19).

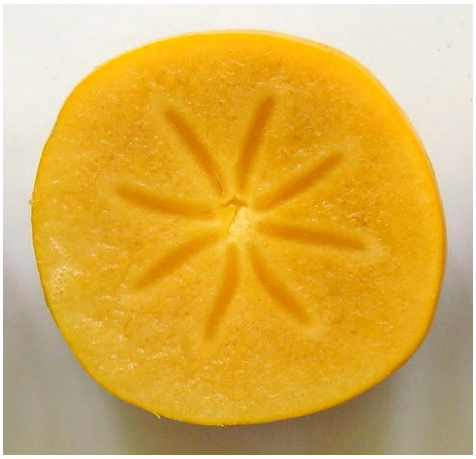


Figure 2.19: Avery early maturing, unnamed variety with external black coloured skin

Import of Diospyros lotus rootstock from Spain into Australia

The *Diospyros lotus* seeds were obtained from Ms Rosa Hernandorena of Viveros Hernandorena nursery on 27 September 2018 (Figure 2.20) during my trip to Spain. Due to the lengthy paperwork and quarantine procedures of Australia, the *Diospyros lotus* seeds were imported from Spain and arrived in NSW DPI, Darton on 29 May 2019 (Figure 2.21).

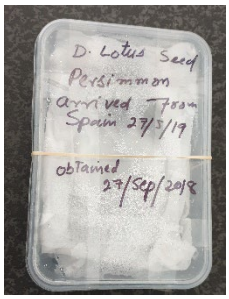


Figure 2.20: Arrival of *Diospyros lotus* seed to Australia – 27/5/2019.

Figure 2.21*: Diospyros lotus* – 5 June 2019 after arrival at Dareton.

Diospyros lotus seeds were germinated to raise the rootstock seedlings. Seeds were sown into growth tubes, and some were directly sown into the 2 L pots at the nursery of Dareton research institute, NSW DPI. nursery. The seeds were successfully germinated, and seedlings were raised (Figure 2.22). Seedlings were transferred to 2 L pots on 4 Jan 2020. The root system was well developed before the transfer of seedling into 2 L pots (Figure 2.23). Premium potting mix from 'Searle' was used as a potting media. The seedlings grew well and were used for grafting of scion varieties.

Figure 2.22: *Diospyros lotus* seedlings were raised at Dareton.

Figure 2.23: *Diospyros lotus* were planted in 2 L pots at Dareton.

Verification of Diospyros lotus, male and female plants of Diospyros lotus to establish mother seed source

Rootstock seedlings were raised, and leaf samples were collected from 40 trees on 20 June 2020. The leaves were sent to Australian Genome research facility laboratory in Adelaide. DNA was extracted in the Adelaide laboratory followed by a PCR sequencing. The protocol for the sex determination of *Diospyros lotus* (Akagi et al. 2014) was provide to the Adelaide laboratory which had the molecular markers associated with sexuality for *Diospyros lotus*. The dual direction PCR sequencing was carried out using the primers described by Akagi et al. 2014. The sequencing was carried out by Sanger technology. Sanger sequencing, also known as the 'chain termination method', is a method for determining the

nucleotide sequence of DNA. The method was developed by two-time Nobel Laureate Frederick Sanger and his colleagues in 1977, hence the name the Sanger Sequence. The results of the PCR were obtained and were visualised in an agarose gel. Agarose gel electrophoresis is commonly used to separate DNA fragments following restriction endonuclease digestion or PCR amplification. Fragments are detected by staining the gel with the intercalating dye, ethidium bromide, followed by visualization/photography under ultraviolet light. The sample with a visual band indicates a male plant (Figure 2.24).

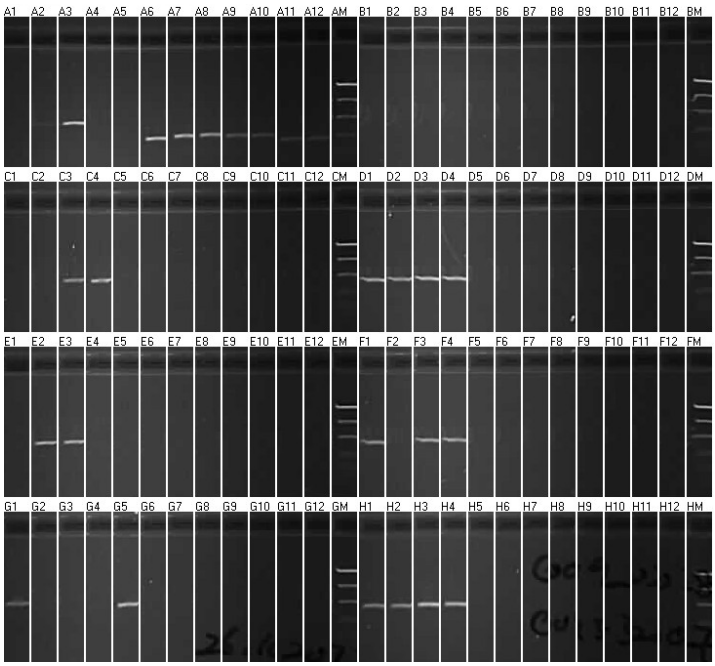


Figure 2.24: Agarose gel electrophoresis of PCR for Persimmon leaf samples. The band indicating the male plant.

The verification of the male and female trees was confirmed working with Dr Maria Badenes. The first mother block for the *Diospyros lotus* seed source was established at Dareton. Three female and two male trees were planted in a population to produce viable seeds (Figure 2.25). A mirror block was also established at a grower's property, so the mother seed source remains at two sites. Generally male and female trees are needed to be planted side by side. After the plant establishment fruit with viable seeds is produced on female trees (Figure 2.26).

Figure 2.25: Mother block of 5 *Diospyros lotus* established at Dareton. From left Male, Female, Female, Female and Male trees. Male flowers are needed to fertilise flowers on female trees.

Figure 2.26: *Diospyros lotus* female tree bearing fruit with viable seed (Left), *Diospyros lotus* male trees with no fruit – 2 April 2023

The germplasm resource available at the Maroochydore Research Facility

Dr Tahir Khurshid visited Maroochy Research Facility, Maroochydore (DAF) on 20 June 2018 to attend the meeting with Dr Vino Rajandran (Hort Innovation), David Oag (DAF), Dave Brunn (DAF), and Mr Stephen Jeffers (Persimmon grower/nurseryman) to see the germplasm material (varieties and rootstock) planted at the institute.

Scion varieties

The persimmon varieties given in Table 2.1 were grown on a range of *Diospyros kaki* rootstocks. These varieties were 5 years old at the time of inspection. David Brunn has recorded some tree growth data for the varieties, but no yield data was available at this stage. The varieties were planted in a single row. These varieties are more than 20 years old in Australia, however, no research work has been ever conducted on these varieties, nor are they planted anywhere in Australia. However, there is limited plantation of 'Sunami', but the grower is not known at this stage (Stephen Jeffers, Personal Communication). The characteristics of a few scion varieties and rootstocks are given (Khurshid, 2022).

Table 2.1: Scion varieties grafted to a range of *Diospyros kaki* rootstocks planted at the Maroochy Research Facility

Scion Variety	Rootstock types*	Non-astringent Astringency	Number of trees
'Isahaya'	Kaki Sun (Diospyros kaki)	NA	2
'Sunami'	Kaki Sun (Diospyros kaki)	NA	2
'Kazusa'	Kaki Sun (Diospyros kaki)	NA	2
'Yoho'	Kaki Sun (Diospyros kaki)	NA	2
'Otanenashi'	Kaki Sun (Diospyros kaki)	A	2
'Tone Wase'	Kaki Sun (Diospyros kaki)	A	2
'Rojo Brillante'	Fuyu (Diospyros kaki)	A	1
'Fuyu' Adrian Schaper sport	DDM (Diospyros kaki)	NA	1
'Fuyu' Adrian Schaper sport	MKN (Diospyros kaki)	NA	1
'Fuyu' Adrian Schaper sport	Fuyu (Diospyros kaki)	NA	1
'Fuyu' Adrian Schaper sport	Kaki Sun (Diospyros kaki)	NA	1

*All rootstocks are *Diospyros kaki* types; NA = non-astringent; A = astringent

To get the insight into the existing varieties, Dr Khurshid contacted ANFIC (The Australian Nurseryman's Fruit Improvement Company), Australia; Dr Maria Badenes (Spain) and Dr Takuya Tetsumura (Japan). They provided me with the characteristics of some varieties. Dr Khurshid interest was to observe if any variety matures earlier than 'Jiro' and later than 'Fuyu', which could have been included into the current project. Dr Khurshid have collated their comments which are given in the Table 2.2. These comments are based on their local knowledge in their representative countries.

Table 2.2: Summary of the characteristics of persimmon varieties collated by the information provided by 3 different sources.

Varieties	ANFIC (Australia)	Maria Badenes (Spain)	Takyuya Tetsumura (Japan)
'Isahaya' (non-astringent)	• A large, fruited selection of 'Fuyu' from Nagasaki. • Fruit sugar content slightly lower than Fuyu.	• It was imported from Brazil • Early cultivar, however in Valencia (Spain) the yield was low, and the growers are not planting it.	• The fruit size large • Some fruit become soft before harvesting. • Hence, there is no commercial production in Japan due to significant pre harvest fruit drop.
'Kazusa' (non-astringent)	• A Japanese persimmon variety.	• Spain do not have this variety.	• Cross breed and registered in 1980. Very late-maturing cultivar. • However, there is no commercial production in Japan, due small fruit size and some fruit remain astringent at harvest.
'Sunami' (non-astringent)	• Fruit size is large • Bud mutation of 'Fuyu' planted in Japan. • The fruit matures 10 days before Fuyu, • it is yellow in colour • Weighs approximately 300 g. • 'Sunami 'has better flavour and texture than 'Matsumoto wase Fuyu'.	• Spain do not have this variety.	• Bud mutation of 'Fuyu'. Fuyu has the higher production volumes of non-astringent type persimmon in Japan. • The fruit size is larger than 'Fuyu', but only small number of farmers cultivates this cultivar. • The problem with variety is severe calyx-end cracking disorder.

'Shinshu' (non-astringent)	• the fruit of this variety is harvested early to mid-season (late April) • High eating quality fruit with an orange skin, • 17 ^{o}Brix, • Soft flesh and high flesh juiciness with moderate fruit size (250g). • Performs very well in greenhouses		
'Tone Wase' (astringent)	• Mildly astringent persimmon variety that needs to be mature and softening before eating. • Tone Wase is a 'Hiratanenashi' type persimmon. • The product of a natural bud mutation of 'Hiratanenashi' but ripens 10 days earlier. • Early maturity with Izu. • The sugar content is very high (18-20 ^{o}Brix). •	• We have this cultivar in the germplasm collection. • It is early however removal of astringency was not well solved.	• Bud mutation of 'Hiratanenashi'. • The fruit ripe two weeks earlier than 'Hiratanenashi', • The production area is almost the same as that of 'Hiratanenashi', • Although shelf life is not so long.
'O'Tane Nashi' (astringent)	• Pollination variant. • The Japanese word tane means seed and nashi means without seed. • The fruit is symmetrical, of roundish conical shape with slightly rounded apex, and very large (7.5 to 10 cm long and almost as broad (300g) • The skin is a light bright yellow until the fruit is almost mature, when it changes to a brilliant red. • The flesh is yellow, or a peculiar pasty consistency, of very high quality (15 ^{o}Brix), and astringent until ripe. • Has been used as an interstock for Tone Wase	• Spain do not have it	• Bud mutation of 'Hiratanenashi', which is No. 1 production volume of astringent type persimmon in Japan. • The fruit is much larger than 'Hiratanenashi', • Fruit quality is low, so there is no commercial production in Japan.

	producing fruit maturity 3 days earlier and fruit size 20% larger. •		
'Yoho' (non-astringent)	• Fruit of this variety is harvested mid-season (early May) with a red-orange skin • 16 ^{o}Brix, firm flesh and juicy. • High productivity with high parthenocarpy and medium fruit size (280g).	• Spain do not have it	• Cross breed, 'Fuyu' x 'Jiro', both of which are excellent. • NARO released it 28 years ago, but it is still a minor cultivar in Japan, because the fruit character is almost the same as that of 'Fuyu' or 'Jiro'. • I think that this cultivar is promising one for your country, because it can be cultivated without difficulty. • In fact, it is No. 1 production volume of non-astringent type persimmon in China.
'Rojo Brillante' (Astringent)		• The main cultivar, 90% of the production is based on this variety. • It is a native cultivar from Valencia (Spain) and very well adapted, however in Japan is not a good cultivar. • In any case, experimentation made in the area where is going to be planted is very important.	• No 1 variety in Europe. • Main production area is Spain. • If you want to evaluate astringent type persimmon, it should be a candidate.

Two other varieties were planted in 2014 on different rootstocks which are given in Table 2.3. 'Rojo Brillante' is an astringent Spanish variety, while 'Yang Fang' is a non-astringent variety imported from China by Persimmon Australia Inc. There are number of small commercial plantings across the country. There are no formal trial research data is available about its yield, quality, or tree performance. Fruit crops are harvested from mid-April to early May in Southeast Queensland. The growers observed variable fruit size across the tree, and it needs fruit thinning in heavy cropping year to minimize biennial bearing.

Table 2.3: Two varieties were planted with a range of rootstocks in 2014

Scion	Rootstock types*	Tree no.
'Rojo Brillante'	'Fuyu' (Diospyros kaki)	6
'Rojo Brillante'	'MKN' (Diospyros kaki)	2
'Rojo Brillante'	'Saijo' (Diospyros kaki)	1
'Yang Fang'	'Fuyu' (Diospyros kaki)	3
'Yang Fang'	'FxG' (Diospyros kaki)	3

*All rootstocks are *Diospyros kaki* types. Trees were planted in 2014.

Rootstocks

Rootstocks available at the Maroochy Research facility are given in Table 2.4. During the visit Stephen Jeffers suggested that it will be worth evaluating a few selected rootstocks with 'Jiro'. The suggested rootstocks for the trials are Telco (dwarfing), Kaki Sun (vigorous), BJI (highly vigorous), G1 (salt tolerant), Fuyu (moderately vigorous) and *Diospyros glandulosa*. *Diospyros glandulosa* rootstock is tropical in character and the trees had leaves on them during our visit (evergreen).

I have discussed with Stephen Jeffers to prepare rootstock seedlings and graft them with 'Jiro', and the trials could be established at the grower's properties. Stephen Jeffers is looking into that at present and will provide the list of available rootstocks. Kaki Sun can be sourced from Fleming Nursery, Woombye, Qld and 'Saijo' (Japanese rootstock) can be sourced from Jeanette Wilson (Rosemount Nursery, Ross Creek Qld) to include them into the trial. I have contacted Janet Wilson and have asked her to prepare trees for me.

Table 2.4: A range of rootstocks were planted in a trial with 'Jiro' in 2015 at Maroochy Research facility.

Scion Variety	'Jiro'
Rootstocks	9
Blocks	4
Planted	2015
Status	sufficient tree size and fruit for data collection from 2019 harvest
Rootstocks	Seedling selections of *Diospryos kaki* 'Telco' 'MKN' 'Kaki Sun' 'Hunsley' 'BDI' 'G1' 'DDM' 'Fuyu' Diospryos glandulosa

Transfer of some existing varieties from Maroochy research facility to Dareton research centre

Some of the following varieties from the Maroochydore Research Facility were transferred to Dareton. There varieties are ‘Isahaya’, ‘Yoho’, ‘Sunami’, ‘Suruga’ and ‘Shinshu’ (See Chapter 3 for details).

The mother blocks of 2 trees per variety are now established at Dareton. In August 2023, budwood will be used to propagate trees for the future trials. The trees produced a small number of fruits this year accept ‘Isahaya’. The initial observation indicated that ‘Yoho’ is an early maturing variety, followed by ‘Shinsu’ and ‘Jiro’. The earliness of ‘Yoho’ is totally conflicting with some previous findings by other scientist. Therefore, a proper trial on these existing varieties needs to be conducted in different growing regions of Australia over the next 5 years to clarify the results.

Chapter 3: Establishing variety and rootstock trials at Dareton

The trial plan for the initial establishment of persimmon varieties and rootstock trees is given below in Table 3.1.

Establishing 'Jiro' variety on G1 and S2 rootstocks trial at Dareton

The scion variety 'Jiro' (Figure 3.1) was planted on two different rootstocks G1 and S2 on 8 September 2020. Trees were planted in a randomised complete block design as 4 tree plots in 5 blocks with a total number of 40 trees. The 'Jiro' did not survive on G1 rootstocks, so the trees were pulled out and excluded from the trial. The G1 was replaced with bare rooted 'Jiro'. The bare rooted 'Jiro' were grafted on two years old *Diospyros Kaki* rootstocks. The planted trees exhibited strong tree growth. There are 30 trees of bare rooted 'Jiro' and 30 trees of 'Fuyu'

Establishing 'Jiro' variety on G1 and S2 rootstocks trials at growers' properties

The scion variety 'Jiro' was planted on two different rootstocks G1 and S2 on 8 September 2020 at three grower's properties. The properties were at Victoria, New South Wales, and Queensland. Each grower was provided with 40 trees which were planted as randomised complete block design in a single row. The 'Jiro' did not survive on G1 rootstocks at all 3 properties. Therefore, they are not part of the trial. The reason could be the poor tree quality rather than the rootstock. Therefore, it was decided to replace them with new trees. The previous trees on 'G1' are now replaced with bare rooted 'Jiro'.

Establishing 'Fuyu' and 'Rojo Brillante' variety trial at Dareton

'Fuyu' (Figure 3.2) and 'Rojo Brillante' (Figure 3.3) trees on *Diospyros kaki* rootstock arrived at Dareton and planted on 6 November 2020 in 5 blocks. There was a total of 40 trees of 'Rojo Brillante' and 20 trees of 'Fuyu'. The objective of this planation is to assess the performance of 'Rojo Brillante' on *Diospyros kaki* rootstock and to produce budwood for the future propagation of 'Rojo Brillante' trees. The trees starting to produce fruit in the current season.

Budwood from these trees will be collected in August 2023 to propagate trees for the future. The trees will be evaluated in the next project.

Establishing a variety trial with germplasm transferred from Maroochydore to Dareton

The budwood for scion varieties from the Maroochydore facility was obtained and trees were propagated by a private nursery in Queensland during this project. Trees arrived at Dareton in late September 2020 and were planted on 6 November 2020. All these trees are 2.5 years old in April 2023. There are 4 trees of 'Yoho' (Figure 3.4), 3 tree of 'Shinshu' (Figure 3.5), 2 trees of 'Suruga' (Figure 3.6), and 4 trees of Isahaya (Figure 3.7) and 3 trees of 'Sunami'. Some of these trees produced a few fruits in winter 2023. The budwood will be collected in August 2023 to propagate further trees for varietal evaluation in the next project. The earlier observation in winter 2023 suggested that these varieties should be evaluated for earliness of maturity comparing to the standard 'Jiro' variety.

Establishing a mother block for seed production of Diospyros lotus rootstock

Mother block for the seed production for *Diospyros lotus* was established with 2 male and 3 female trees at the persimmon block at Dareton. The *Diospyros lotus* female trees produced fruit in March 2023. A set of 5 male and female trees were also established at the grower's property in NSW as a backup site. Nurseries and growers can now obtain budwood from female and male trees to establish their own source to produce *Diospyros lotus* seed in future. A few growers collected the *Diospyros lotus* fruit from Dareton in June 2023 to extract seeds.

The trees of the following varieties were planted in November 2020. These varieties produced the first crop of a small number of fruits in 2023 growing season. In the following years these varieties will be evaluated for earliness and fruit quality and for any disorder at the apex or calyx end.

Table 3.1: The establishment of different varieties and rootstock block at Dareton.

T32	D. lotus Male	D. lotus Female	D. lotus	D. lotus Female	D. lotus	T32
T31	Fuyu BR	Fuyu BR	Fuyu BR	Fuyu BR	Fuyu BR	T31
T30	Fuyu BR	Fuyu BR	Fuyu BR	Fuyu BR	Fuyu BR	T30
T29	Fuyu BR	Fuyu BR	Fuyu BR	Fuyu BR	Fuyu BR	T29
T28	Fuyu BR	Fuyu BR	Fuyu BR	Fuyu BR	Fuyu BR	T28
T27	Fuyu BR	Fuyu BR	Fuyu BR	Fuyu BR	Fuyu BR	T27
T26	Fuyu BR	Fuyu BR	Fuyu BR	Fuyu BR	Fuyu BR	T26
T25	Jiro BR	Jiro BR	Jiro BR	Jiro BR	Jiro BR	T25
T24	Isahaya/K	Yoho/K	Jiro BR	Jiro BR	Jiro BR	T24
T23	Isahaya/K	Yoho/K	Sunami/K	Jiro BR	Shinshu/K	T23
T22	Isahaya/K	Yoho/K	Sunami/K	Suruga/K	Shinshu/K	T22
T21	Isahaya/K	Yoho/K	Sunami/K	Suruga/K	Shinshu/K	T21
T20	Fuyu/K	Fuyu/K	Fuyu/K	Fuyu/K	Fuyu/K	T20
T19	Fuyu/K	Fuyu/K	Fuyu/K	Fuyu/K	Fuyu/K	T19
T18	Fuyu/K	Fuyu/K	Fuyu/K	Fuyu/K	Fuyu/K	T18
T17	Fuyu/K	Fuyu/K	Fuyu/K	Fuyu/K	Fuyu/K	T17
T16	Rojo/K	Rojo/K	Rojo/K	Rojo/K	Rojo/K	T16
T15	Rojo/K	Rojo/K	Rojo/K	Rojo/K	Rojo/K	T15
T14	Rojo/K	Rojo/K	Rojo/K	Rojo/K	Rojo/K	T14
T13	Rojo/K	Rojo/K	Rojo/K	Rojo/K	Rojo/K	T13
T12	Rojo/K	Rojo/K	Rojo/K	Rojo/K	Rojo/K	T12
T11	Rojo/K	Rojo/K	Rojo/K	Rojo/K	Rojo/K	T11
T10	Rojo/K	Rojo/K	Rojo/K	Rojo/K	Rojo/K	T10
T9	Rojo/K	Rojo/K	Rojo/K	Rojo/K	Rojo/K	T9
T8	Jiro BR	Jiro/S2	Jiro BR	Jiro/S2	Jiro BR	T8
T7	Jiro BR	Jiro/S2	Jiro BR	Jiro/S2	Jiro BR	T7
T6	Jiro BR	Jiro/S2	Jiro BR	Jiro/S2	Jiro BR	T6
T5	Jiro BR	Jiro/S2	Jiro BR	Jiro/S2	Jiro BR	T5
T4	Jiro/S2	Jiro BR	Jiro/S2	Jiro BR	Jiro/S2	T4
T3	Jiro/S3	Jiro BR	Jiro/S2	Jiro BR	Jiro/S2	T3
T2	Jiro/S2	Jiro BR	Jiro/S3	Jiro BR	Jiro/S2	T2
T1	Jiro/S2	Jiro BR	Jiro/S2	Jiro BR	Jiro/S2	T1
	Row 5	Row 4	Row 3	Row 2	Row 1	

Figure 3.1: Early season ‘Jiro’ - 2.5 years old trees at Dareton.

Figure 3.2: Mid-season ‘Fuyu’ - 2.5 years old trees at Dareton.

Figure 3.3: ‘Rojo Brillante’ - 2.5 years old trees at Dareton. Rojo Brillante is an astringent Spanish variety.

Figure 3.4: ‘Yoho’ - 2.5 years old trees at Dareton.

Figure 3.5: ‘Shinshu’- 2.5 years old trees at Dareton.

Figure 3.6: ‘Suruga’ - 2.5 years old trees at Dareton.

Figure 3.7: ‘Isahaya’ - 2.5 years old trees at Dareton.

Chapter 4: Develop a new standard clonal propagation technique for persimmon rootstocks to improve tree uniformity and performance.

Introduction

One of the issues faced by the persimmon industry is the lack of uniformity of trees across the orchards. However, the current method of rootstock propagation from seed does not result in tree uniformity. Due to the high genetic variability of rootstock seeds, the grafting of known varieties onto seedling rootstocks means that the resulting trees vary in their height and vigour leading to yield losses and reduced fruit quality. The lack of uniformity has been observed in various persimmon orchards in Australia. Often 2 years old nursery trees look uniform in terms of height and vigour. However, after a few years after planting some trees fail to grow and attain a maximum height and vigour like other normally growing trees. Therefore, there was a need to develop a technique of propagation which can eliminate tree non uniformity. During this project the component of tree uniformity was addressed by using two different clonal propagation techniques.

1. Tissue culture propagation technique to produce rootstocks
2. Single-node stem cutting technique to produce rootstocks

Tissue culture propagation technique to produce rootstocks

The traditional propagation method adopted by the nursery industry is based on budding/grafting scion cultivars on seedlings from *Diospyros kaki*, *Diospyros lotus*, and *Diospyros virginiana*. These are the most important species used as rootstock and are grown from seed. Furthermore, most of non-astringent cultivars of persimmon are not compatible with D. lotus, a rootstock largely utilized because of its hardiness and frost resistance (Giordani et al., 2013).

The main in vitro tissue culture techniques, developed for persimmon, deal with direct regeneration (from dormant buds and root tips), and indirect regeneration through callus from dormant buds, apexes, and leaves.

Tissue culture propagation was also carried out to produce rootstock seedlings from the true-to type plant material. The same genetic material which was used for the single node cutting in the previous section was also used for the tissue culture technique. The reason for using the same mother plant was to make sure that the new plants are true to type and derived from the same source whatever clonal propagation technique was used for the recommendations to the persimmon industry.

The general procedure followed is given step by step below from the plant material in field to the final potted rootstock seedlings ready to be budded/grafted. This information will allow the reader to develop an understanding of the tissue culture technique of clonal propagation.

Plant material

The stems cuttings emerging from the structural roots was sprayed with $CuSO_4$ @ 3 g/L (Figure 4.1) one week before the use for tissue culture process. A 50 cm portion (containing 6 buds) of the stem cutting measuring from the top was removed, and the remaining basal part was still left intact to the structural roots.

Preparation of plant tissue for culture

The explant is the tissue or plant part introduced in the culture media for the regeneration of the plant. Therefore, the first step is to sterilise it to remove all the dirt, bacteria, or infections stuck on the surface of the explant. It ensures that the resulting plant will be free from any pathogens. The stem cuttings were divided into 6 cm lengths containing 2 buds (Figure 4.2). These stems were washed with distilled water and surface sterilised with Sodium hypochlorite (bleach) which is a commonly used agent for surface sterilisation of explants (Figure 4.3). Bleach is diluted up to 10-20% for sterilisation in tissue culture processes. This gave a solution with a final concentration of 0.5-1.0%. The plant tissue was rinsed twice to ensure the efficiency of surface sterilisation of the plant material.

Induction media

Induction media was prepared, and the stem cuttings were placed on the agar gel in sterilised jars (Figure 4.4). They were placed in Jars for 4 weeks. The good quality material was rescued and put into new Jars with fresh media (Figure 4.5). The media also contained half strength nutrients and plant growth regulators. The plants were in jars with a new media for 4 weeks. The new material was re-propagated, and the number of micro plants were increased on a fortnightly basis (Figure 4.6).

Figure 4.1: Tree was sprayed with $CuSo_4$ before propagation.

Figure 4.2: Small cuttings containing minimum of 6 nodes.

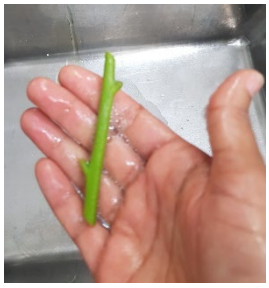


Figure 4.3: Washing of cuttings with distilled water and Sodium hypochlorite solution.

Figure 4.4: Cutting with at least 2 nodes were placed in jars.

Figure 4.5: Persimmon rootstocks has been cultured and placed into fresh media.

Figure 4.6: Number of micro plants were increased on a fortnightly basis.

Transfer of rooted plants into small pots

Acclimatisation process

Acclimatization is the adaptation of small plants to a new environment. When tissue culture plants are transferred from the lab to soil, they are exposed to abiotic stresses, like altered temperature, light intensity, and humidity conditions, and biotic stresses, like soil microflora (microbes living in soil). So, they need stepwise acclimatization to successfully establish themselves in the natural environment (Kozai, 1991).

In labs, plants are cultured in a completely artificial environment. They are heterotrophic and require readymade nutrients. Later, they develop some organs and become mixotrophic. But, to survive in the natural environment, the plants need to be autotrophic. The in vitro plants have different anatomy and nutrient requirements that don't support them to make their own food and be dependent on themselves. So, to convert plants from mixotroph to autotroph, you need to move plants gradually to make them adapt to the changes. The artificial environment in tissue culture consists of constant temperature, high relative air humidity, high availability of nutrients, low light intensity, and a low carbon dioxide (CO_2) concentration. These conditions make plants difficult to adapt to the outside environment.

Micro plants were placed in small square (9x9x9 cm) pots in plastic tunnels to acclimatize (Figure 4.7). The plants stayed in the tunnel for 4 weeks. The humidity in the plastic tunnels was 80-95% and the temperature can occur with minimum 15 and Maximum 32 ^{o}C. During the acclimatization process 20% of the plants were lost.

Hardening process

After 4 weeks of acclimatation the plants were shifted to the greenhouse for the hardening process (Figure 4.8). In the greenhouse plants were irrigated and nutrients were supplied to the pots. The plants were hardened in the greenhouse in 60 days. Afterwards, the plants were shifted to the 2 L pots to allow the root and shoot system to grow.

Figure 4.7: Plants are placed in covered plastic tunnels to get used to the natural environment. A rooted plant is shown in inset.

Figure 4.8: The *Diospyros lotus* seedlings are on 19 Nov 2021 during the hardening stage, with regular irrigation and nutrition program.

Figure 4.9: Tissue cultured produced rootstock seedling of *Diospyros lotus* rootstock seedling at Agromillora, Irymple on March 2022 (6 months after hardening).

The transfer of *Diospyros lotus* seedlings to Nurseries/growers

Plants remained at the nursery premises to grow for a few months (Figure 4.9). The rootstock seedlings were then transported to 2 nurseries for grafting purposes. The persimmon varieties were grafted to these rootstocks once the seedlings achieved the required thickness. After grafting the trees will growth for another year before they are ready to be planted in research trials to assess the uniformity of trees across the orchard for 3-4 growing seasons.

The rootstocks seedlings (500) were transferred to nurseries and growers, so they can graft a range of scion varieties on these new rootstocks (Figure 4.10).

Figure 4.10: The tissue culture experiment produced *Diospyros lotus* rootstock seedlings ready to be shipped to persimmon nurseries.

Test of tree uniformity trials

In the current project ending in July 2023, we are only able to send the rootstock seedlings to the growers and in spring 2023 the seedlings will attain the required thickness to be grafted with scion varieties. Once the trees are 2 years old, the tree plantation will occur, and tree uniformity will be evaluated over the next 5 years. This activity will only be possible in the next project.

Single-node stem cutting technique to produce rootstocks

In the second type of clonal propagation technique a single or double node cutting can be used to produce rootstock seedling. Trials were conducted to develop a clonal propagation technique for persimmon rootstocks to improve tree uniformity and performance (control tree vigour and size). Four years of intense propagation work was conducted (2018/19 to 2021) to develop rootstock seedlings.

1. Establishing clonal propagation trial in the field

The experiment was started with a specific style of clonal propagation which requires trees to be cut down to the base, so that the rootstock suckers, allowing single-node cuttings to be taken and used for clonal propagation. This is a Japanese technique developed by Professor Takuya Tetsumura of University of Miyazaki in Japan (Tetsumura et al., 2013). The single-node cuttings were produced in Nov/December/January/February or March for rooting purposes. The aim was to determine which month of the year the cuttings have the highest probability of survival and success in producing roots.

A field trial was established at a grower's property in Renmark, South Australia. A tree was cut down to a stump in July 2018 (Figure 4.11) and was used as the mother tree for the 4 years of testing. The reason for using the same tree was to make sure that the genetic material is true-to-type and consistent across the years. Soil around the root system was dug out and removed in September 2018, exposing the large sized lateral roots, more than 50 mm in diameter (Figure 4.12).

The removal of soil to expose the lateral root system was carried out to initiate the root suckers to emerge off the lateral roots (Figure 4.13). These root suckers were used for the clonal propagation trials for the single-node cuttings during the project (Figure 4.14). The purpose of using root suckers is to ensure that the propagated plants were true-to-type.

Figure 4.11: The tree was completely cut down to a stump of 20 cm above the ground.

Figure 4.12: The tree trunk was further cut down to the soil level and soil was removed around the lateral roots.

	
Figure 4.13: Root sucker initiation off exposed lateral root (Strong rootstock system).	Figure 4.14: Root suckers are now established off the exposed lateral roots and were used in clonal propagation.

Establishing Single node cutting trials in a commercial nursery

2018

First propagation – 17 Nov 2018

The softwood suckers were cut off the structural lateral roots (Figures 4.14) on 17 November 2018. This experiment was carried out at the co-operating commercial nursery in Mildura.

The single-node cuttings were prepared (Figure 4.15) and each cutting was dipped in a growth regulator solution (500 ppm - Indole Butyric Acid (IBA) for 5 seconds (Figure 4.16). The solution has a pH of 7.0. Cuttings were arranged on Jiffy blocks in trays (Figure 4.17) and trays were put into a plastic house (Figure 4.18).

		
Figure 4.15: The soft-wood cuttings were prepared.	Figure 4.16: IBA was treated @ 500 ppm.	Figure 4.17: IBA treated cuttings were placed in Jiffy blocks.

Figure 4.18: Cuttings were placed in a plastic tunnel.

During this experiment all the cuttings died 15 days after the commencement of the experiment and their leaves were dropped in the plastic tunnel. Therefore, the experiment produced no results.

2018:

Second propagation – 7 December 2018

The softwood cuttings were cut off the lateral roots on 7 December 2018 (Figure 4.19). This experiment was carried out at the commercial nursery in Mildura. The single-node cuttings were produced (Figure 4.20) and the cuttings were dipped in a growth regulator solution (500 ppm - Indole Butyric Acid (IBA) for 5 seconds. The solution has a pH of 7.0. Cuttings were arranged on Jiffy blocks in trays and trays were put into a plastic house (Figure 4.21).

Figure 4.19: The softwood cuttings were cut off the lateral roots.

Figure 4.20: Cuttings were dipped into the solution of IBA for 5 seconds.

Figure 4.21: The prepared cuttings were placed in Jiffy blocks and trays were placed in a plastic tunnel.

During this experiment all the cuttings died 15 days after the commencement of the experiment and their leaves were dropped in the plastic tunnel. Therefore, the experiment produced no results.

Third Propagation – 29 January 2019

The propagation from January 2019 onwards were conducted in the nursery facility at the Dareton Research Institute. A mist house was purchased for the experiments with temperature and humidity control. This would ensure that a proper temperature and humidity is provided to the small cuttings.

For this experiment semi-soft wood cuttings were cut off the lateral roots on 29 January 2019. The single-node cuttings were produced, and each cutting was dipped in a growth regulator solution (500 ppm - Indole Butyric Acid (IBA) for 5 seconds. The solution has a pH of 7.0. Cuttings were arranged on small pots in trays and trays were put into a mist house at Dareton.

During this experiment all the cuttings died 3 weeks after the commencement of the experiment and their leaves were dropped in the plastic tunnel. Therefore, the experiment produced no results.

Fourth Propagation – 5 May 2019

Root suckers were cut off the structural roots from the main tree. The semi hard-wood cuttings were cut off the lateral roots on 5 May 2019.

The single-node cuttings were produced (Figure 4.22), and each cutting was dipped in a plant growth regulator solution (500 ppm - Indole Butyric Acid (IBA) for 5 seconds (Figure 4.22). The solution has a pH of 7.0. A small portion (0.5 cm) bark was removed to facilitate the absorption of IBA. After the cuttings were ready, the trays were irrigated and put it into the mist house at Dareton nursery (Figure 4.24).

Figure 4.22: Semi-hard wood cuttings were produced.

Figure 4.23: Cuttings were dipped in IBA for 5 seconds.

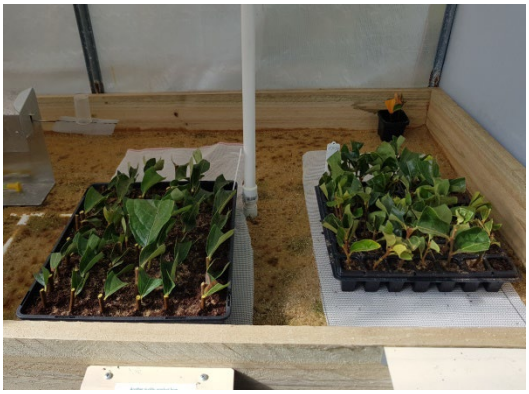


Figure 4.24: Treated cuttings were placed in a mist house at Dareton.

During this experiment all the cuttings died 3-4 weeks after the commencement of the experiment and their leaves were dropped in the plastic tunnel. The cutting produced callus at the bottom end, however, the callus could not initiate a root system. Therefore, the experiment produced no results.

2020 growing season

The preparation of the next season's experiments was commenced on 1 July 2019. The developed shoot suckers were removed from the lateral roots, and the excess soil was removed to expose the lateral root system (Figure 4.25). Shoots emerged off the lateral shoots throughout the spring period and there were a large number of >0.5 m long shoots were grown by 6 November 2019 (Figure 4.26), however; the shoot density was higher by 20 November 2019.

Figure 4.25: The removal of access soil around the lateral roots.

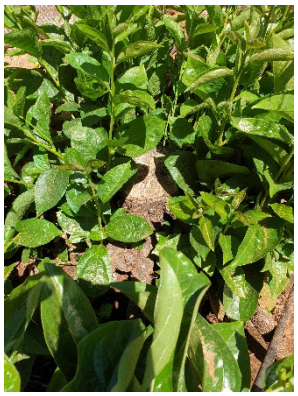


Figure 4.26: The emergence of shoots off the lateral roots in November 2019.

The experiments for single-node cuttings were carried out and propagation was done at 5 occasions from 2 January to 14 March 2020. A range of soil media was used as potting mix and a range of different levels of IBA was used to quantify the

best treatment for the rooting of single-node cuttings for root initiation and seedling growth. The dates for the commencement of 5 experiments are given below.

Experiment 1: First propagation – 2 January 2020

Experiment 2: Second propagation – 12 January 2020

Experiment 3: Third propagation – 18 January 2020

Experiment 4: Fourth propagation – 9 February 2020

Experiment 5: Fifth propagation – 14 March 2020

The cuttings were propagated, and root initiation was observed. During the entire process Dr Takuya Tetsumura (University of Miyazaki) was consulted on a regular basis about the clonal propagation work. The cuttings were put into small individual pots in a mist house (Figure 4.27).

Mist house Irrigation

The pots were placed in a mist house and an automatic mist cycle were set up for day and night misting.

Day: 4 seconds/20 minutes cycle

Night: 2 seconds/200 minutes cycle

The bottom of the mist house was heated to provide consistent heat to the base of the pots.

Figure 4.27: Clonal propagation trial of the rooting of single-node cuttings of *Diospyros kaki* is underway in a mist house at the Dareton Research Institute (March 2020).

Potting media

Potting media used for 2 Jan, 12 Jan, 18 Jan and 9 Feb propagation:

12 Litres of potting media was prepared. The media consisted of 5 litres of coarse sand, 2 litres of peat moss, and 2 litres of fine gravel. The material was mixed and 2 teaspoons (6 g) of lime was added to the mix. A soil fungicide of 0.8 g Banrot (400 WP) was added to suppress root diseases and prevent damage along the stems. The active ingredients of Banrot are etridiazole and thiophanate-methyl. The potting media was mixed by a machine for 10 minutes. pH value was 5.5-6.6.

Potting media used for 14 March propagation:

Three different potting mixes were used for 14 March propagation to see if different plant media can have any effect on the root initiation. 1. Perlite:vermiculite mixture was used with 1:1 ratio. 2. Perlite peat was used and, 3. Osmocote commercial seed raising/cutting media was used as a third potting mix.

Plant growth regulator treatments and Propagation of single-node cuttings

1. A single node cutting of an 8 cm piece of stem had 1.5 cm of its base dipped in a plant growth regulator, Indole butyric acid (IBA) for 5 seconds and allowed to dry before planting in small individual pots.
2. A plant growth regulator Clonex was also used. Clonex is growth promoting gel which has IBA in it as an active ingredient. A single node cutting of an 8 cm piece of stem was produced with the lower 1.5 cm dipped into the Clonex gel then planted into the propagation mix in small pots.

Plant growth regulator treatments used

2 Jan 2020: IBA (3000 ppm) and Clonex (3000 ppm)

12 Jan 2020: IBA (3000 ppm) and Clonex (3000 ppm)

18 Jan 2020: IBA (3000 ppm)

9 Feb 2020: IBA (1000, 2000 or 3000 ppm)

14 Mar 2020 IBA (3000 ppm)

Mist house Irrigation

The pots were placed in a mist house and an automatic mist cycle was set up for day and night misting.

Day: 4 seconds/20 minutes cycle

Night: 2 seconds/200 minutes cycle

The bottom of the mist house was heated to provide consistent heat (25 ^{o}C) to the base of the pots.

Results

Roots initiation was monitored on a regular basis. A few cuttings were able to form callus at the bottom of the cuttings, however, the callus turned black, and the cutting died without any root initiation (Figures 4.28). The leaves on the cutting fell off and cutting died within 2 months of planting (Figures 4.29 and 4.30).

Figure 4.28: Callus formation occurred at the base of the cuttings, but root formation did not follow.

Figure 4.29: Cuttings can be seen on 5 May 20, for the 2 Jan 20 planting. Leaves are declining or fallen off the cuttings.

Figure 4.30: Cuttings can be seen on 5 May 20, for the 14 Jan 20 planting. Leaves are fallen off the cuttings.

In this trial, we were able to keep the plants alive, however, the plants failed to root despite callus formation. Therefore, it was decided to repeat the experiment in the 2021 growing season. A work plan was established to conduct the clonal propagation experiments in January to March 2021 focusing on rooting of the single-node cuttings.

2021 season

Preparation of the rootstock trial in field:

The hardened stems (Figure 4.31) off the lateral roots were removed on 16 July 2020 (Figure 4.32) in order to encourage new shoots for spring 2020, and those shoots were used for the single node cutting experiment in the 2021 growing season. The shoots off the lateral root structure were grown well as shown in figure 4.33). In 2021 the propagation was done on 20 February. The previous results in the 2020 growing season suggested that the root cuttings in February are at the right stage of development and can be used for single node cutting propagation. Therefore, propagation was carried out on 20 February 2020 (Figure 4.32).

Figure 4.31: All hardened stems were removed in July.

Figure 4.32: Soil was removed around the structural roots.

Figure 4.33: The shoots off the lateral roots were long and hard in February and ready to be use for propagation.

Potting media:

Potting media used for 20 February propagation:

12 Litres of potting media was prepared. The media consisted of 5 litres of coarse sand, 2 litres of peat moss, and 2 litres of fine gravel. The material was mixed and 2 teaspoons (6 g) of lime was added to the mix. A soil fungicide of 0.8 g Banrot

(400 WP) was added to suppress root diseases and prevent damage along the stems. The active ingredients of Banrot are etridiazole and thiophanate-methyl. The media was mixed by a machine for 10 minutes. pH value was 5.5-6.6. The second media type used was perlite.

Plant growth regulator treatments and Propagation of single-node cuttings:

A single node cutting of an 8 cm piece of stem had 1.5 cm of its base dipped in a plant growth regulator, Indole butyric acid (IBA) for 5 seconds and allowed to dry before planting in small individual pots (Figure 4.34).

Plant growth regulator treatments used:

20 February 2021: IBA (6000 ppm) or IBA (8000 ppm)

Mist house Irrigation:

The pots were placed in a mist house and an automatic mist cycle were set up for day and night misting.

Day: 4 seconds/20 minutes cycle

Night: 2 seconds/200 minutes cycle

The bottom of the mist house was heated to provide heat to the base of the pots. All plots were placed in the mist house and the experiment was commenced (Figure 4.35).

Figure 4.34: The single-nod cuttings were produced and dipped into IBA growth regulator solution

Figure 4.35: The treated cuttings were placed in the mist house to commence the experiment.

Results:

The follow up of the cutting on 3 March 2021 indicated the leaves were still intact for cuttings treated with 6000ppm growing in Dareton prepared potting media (3.25). While at the same time the cuttings treated with 8000 ppm of IBA had their leaves starting to turn yellow and dropped off in both Dareton media and perlite.

Roots initiation was monitored on a regular basis. These results suggested that cuttings collected in February can retain their leaves for longer duration than the cuttings obtained earlier than the month of February even at the higher levels of IBA @ 6000 ppm. However, the highest rate of IBA was 8000 ppm and leaf fall occurred much faster than cuttings treated with 6000 ppm. The single-node cuttings observed on 3 June indicated that most of the cuttings failed to root (Figure 3.27). The extent of mortality was much higher in 8000 treated cuttings with IBA as compared to the cutting treated with 6000 ppm. A few cuttings were able to form callus at their base however, the callus turned black, and the cuttings died without any root initiation. The leaves on the cuttings fell off and the cuttings died within 14 weeks of the initial plantings. In view of the data collected in the previous years' work in this project, it was suggested that 3000 ppm will be enough for the single-node cuttings collected in February. However, work needs to be done to ensure that the heat beds were consistently kept warm to the required temperature to rule out the issue of root initiation being affected by variable basal temperature.

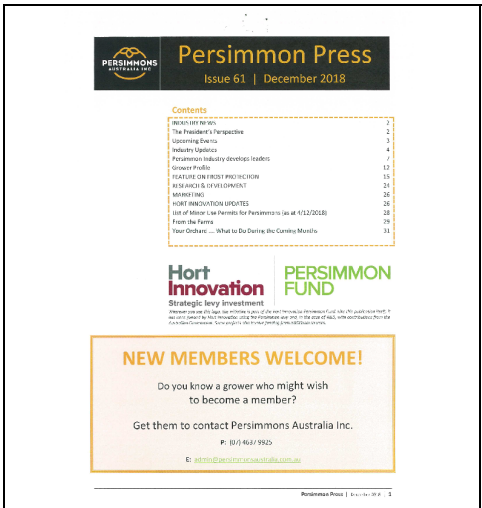
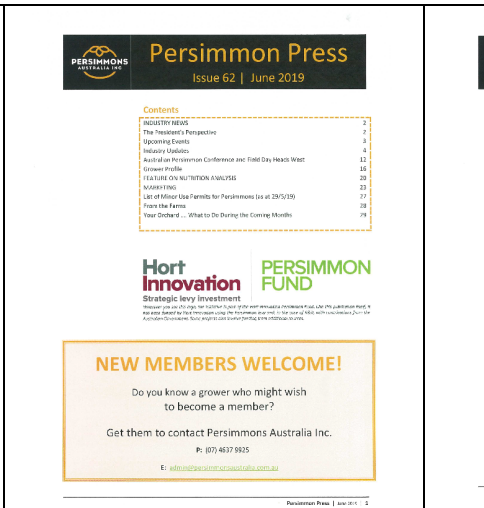
Figure 4.36: Single-node cuttings treated @ 6000 on 20 February showing healthy leaves observed after 12 days on 3 March 2021 for both growing medias. The perlite appeared to be superior to the other soil media.

Figure 4.37: Single-node cuttings treated @ 8000 on 20 February showing unhealthy leaves observed after 12 days on 3 March 2021 for both growing medias. The use of two growing medias did not show any difference.

Figure 4.38: The single-node cuttings treated on 20 February eventually died on 3 June 2021, and the death rate was 100% in cuttings treated at 8000 ppm compared to 6000 ppm.

Appendix 2

This appendix has pdf documents embedded in Table below. The reader can click and read the full document.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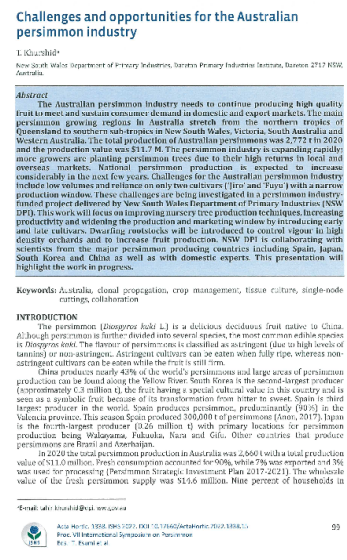
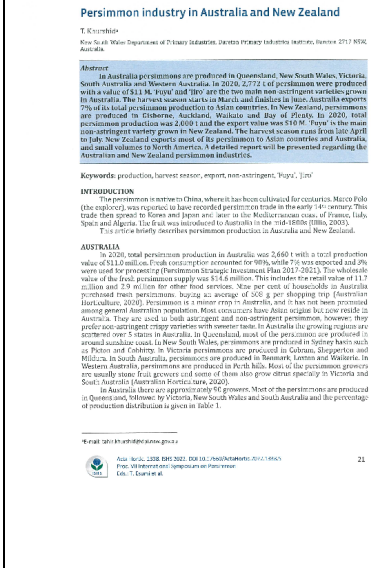

Challenges and opportunities for the Australian persimmon industry
T. Khurshid
Abstract
Keywords: Australia, clonal propagation, crop management, tissue culture, single-node cuttings, collaboration
INTRODUCTION

Refereed paper 1


Persimmon industry in Australia and New Zealand
T. Khurshid
Abstract
Keywords: production, harvest season, export, non-astringent, 'Fuyu', 'Jiro'
INTRODUCTION
AUSTRALIA

Refereed paper 2


Current Status of Persimmon Industry and Prospect of the Korea-bred new Persimmon Varieties in Australia
ABSTRACT:
INTRODUCTION

Refereed paper 3

Australian Tree Crops article - 2018

Australian Tree Crops article - 2022

INNOVATION PERSIMMONS

The fruit of the future

Brett Guthrey is set on transforming persimmons, the exotic sweet fruit, into an Australian favourite. It's no easy feat but a Spanish variety could be the key to a ripe beginning.

Words **SAMANTHA NOON** / Photography **NICK CUBBIN**

The Farmer Magazine – August 2019

SUNRASIA DAILY 24/6/23

North West Farmer EXTRA

Pictured at the Australian Persimmon Industry Conference and Field Days are George Russell (Hort Innovation), Dr Alison Fuss (Executive Officer, Persimmons Australia), Chris Stillard (President Persimmons Australia) and Dr Tahir Khurshid (NSW Department Primary Industry). Picture: Ben Gross

Fruit research's sweet success

By Wade Stephens
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MEMBERS of the Australian persimmon industry gathered in the Riverland and Sunraysia this week to discuss innovations and opportunities in the sector.

Delegates visited small, large and commercial operations in the Riverland before holding a conference in Mildura and touring NSW DPI's persimmon research block outside Dareton.

Persimmons Australia executive officer Dr Alison Fuss said consumers who've had bad experiences tasting the fruit probably encountered the astringent varieties.

"Sweet persimmons are ready to go when they're crunchy and they're just delightful. We're trying to get across to consumers [to] have a go," Dr Fuss said.

"They're easy to eat, they usually don't have any seeds, you can eat the whole thing except for the little green calyx on the top."

In 2022 Australia produced about 3500 tonnes of sweet persimmons, valued at about $17 million.

The Australian persimmon industry typically focuses on two sweet varieties, Fuyu and Jiro, which are grown between late February and June.

Dr Tahir Khurshid is heading up research at the Dareton persimmon research block into the viability of new varieties.

Persimmons Australia president Chris Stillard said the findings so far provide incredibly valuable insight for the industry.

"Tahir has got new and modern root stocks and existing and up-and-coming varieties," Mr Stillard said.

"Farmers can assess for themselves to see if they'll be suitable for their future farming practices and markets."

Industry members also paid a visit to the Agromillora plant nursery in Irymple where clonal propagation of persimmons is being tested.

"Australia is likely to be the first (country) to commercially clonally propagate persimmons," Dr Fuss said.

"The fact that the root stocks are variable means that we have variations in productivity across the orchard.

"By going to clonal rootstocks we look to improve the uniformity of yield and uniformity of production time for each individual variety."

Hort Innovation's George Russell said it was good to witness some of the research and development projects currently underway.

"Some of the investments we've heard about in the conference over the period of the week have been around rootstocks," Mr Russell said.

"Looking at early and late season varieties as well ... disease management and what's happening in the field in relation to dieback issues.

"They've been some of the key investments we've looked at as well as some marketing that happens within the industry."

Sunraysia Daily, National persimmon conference, Mildura – 24 June 2023