Hort Innovation

Final Report

Breeding new rootstocks for the Australian citrus industry

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Delivery partner:

Department of Agriculture and Fisheries Queensland (DAFQ)

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CT18004

Project:

Breeding new rootstocks for the Australian citrus industry (CT18004)

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Public summary

New rootstocks are a zero-cost solution to many problems affecting commercial citrus production. Of particular interest is the opportunity to improve fruit quality, which is of paramount importance to the market success of Australia's citrus exports. It is conservatively estimated that around 300,000 new trees are planted commercially each year. Hence there is a significant opportunity to improve orchard performance by ensuring that these new plantings utilise improved rootstock germplasm. International citrus rootstock research dates back more than a century and continues to be a driving force in modern citriculture development. There has been a steady turn-over of rootstock varieties with the increasing pressures brought about by disease, changing horticultural practices and market demands.

This project aimed to provide the Australian citrus industry with rootstocks that are truly innovative, unique and outperform existing material. New germplasm was created, heavily screened for disease resistance and local adaptation, and then tested under commercial conditions. By properly addressing long standing Australian production issues such as citrus tristeza virus (CTV), phytophthora and salinity, it was then possible to consider the far more challenging issues of huanglongbing (HLB) disease and vigour control. This was made possible via a two-pronged breeding strategy of purposeful parent selection and large-scale hybrid generation and screening.

Once breeding and screening work was completed, the successful hybrids were then included in large field trials located on commercial properties, in order to determine field adaptability. Tree health, vigour, and fruit quality were assessed every season at these extensive on-farm trial sites. This information was used to guide future parent selection so that there could be constant improvement in the choice of parents within the breeding program. This strategy of regularly planting large field trials has provided the breeding team with a constant flow of new information that can be immediately captured in the hybridising program.

The project is the first in the world to demonstrate that Australian wild citrus species can be used as parents to produce new rootstock hybrids whose performance is comparable to current conventional options. This has attracted international attention as a possible strategy in the fight against HLB disease. It provides industry with greatly expanded options in terms of genetically diverse rootstocks that could be deployed if they show future disease tolerance. Many of the problems currently facing world citrus production are a consequence of genetic monocultures, and this project has demonstrated how this issue can be addressed efficiently and with rapid industry applicability.

New information on disease resistance and segregation has been developed, and rapid molecular screening techniques have been validated on Australian rootstock breeding populations. Conversely, the project has also shown that there is no substitute for long-term field trials prior to commercial release. These extensive field trials have identified a subset of unique hybrids with robust performance warranting wider commercial evaluation. New and unique germplasm has been created, providing long-term opportunities for the Australian industry to increase genetic diversity using high-performing rootstock options.

Technical summary

Rootstocks have shaped the history of world citrus production perhaps more than in any other food or fiber plant used by humans. For example, historically important issues such as phytophthora and Citrus tristeza virus (CTV) at one time appeared insurmountable but have been solved through new rootstocks that offered genetic resistance. There are few other crops where so many important commercial traits are influenced by the choice of rootstock. Within the Australian context, growers already have access to rootstocks with good tolerance to CTV and phytophthora, and there is an increasing availability of new options with salt tolerance and graft compatibility. The challenge for breeders is to develop new hybrids that capture all of these currently available features but offer additional benefits such as increased productivity, low vigour, improved fruit quality and resistance to newly emerging disease threats. This project sought to achieve this by generating hybrids from unique parents and then benchmarking these against recent releases from other international breeding programs and currently available commercial rootstocks. It used a strategy that involved multiple generations of crossing, and the production of bridging hybrids to overcome fertility barriers, followed by a sequence of large field trials with multi-year assessment of tree health, vigour, productivity and fruit quality. Information from these field trials was immediately used to guide the selection of parents in the annual hybridization activities, providing a feedback loop for constant improvement, and has resulted in a rich pipeline of germplasm to help secure the future of Australian citrus production. The conscious decision to detour from the very narrow genetic base of parents (sweet orange, mandarin, pummelo, trifoliate orange) universally used by other rootstock breeding programs is beginning to show benefits, and places the Australian rootstock breeding program in a strong position to deliver ongoing benefits to Australian growers.

Nursery and field trials identified a subset of wild citrus relatives that can be used to generate commercially acceptable

rootstocks. Two of the most promising wild parents are *Citrus australasica* and *Citrus wintersii*. This project also identified an even larger subset of parents that produced strong hybrids and successful grafted trees in the nursery, but were then complete failures once planted into field sites. Examples of such failures include *Atalantia ceylanica*, *Citropsis* sp., and *Fortunella* sp. Parents such as *Citrus glauca* and *Citrus wakonai* have produced very mixed results but may warrant perseverance because of the unique traits they are likely to possess.

A major lesson from the project is the paramount importance of good field trials conducted under commercial growing conditions and assessed for multiple seasons. There is no alternative. No amount of nursery screening or molecular testing can predict if a rootstock hybrid will fail under field conditions. For many hybrids, this failure when planted in the field, is soon obvious (within 6 months of planting) while for some others it may take longer (2-3 years). From the portfolio of field trials assessed in this project (Appendix 1) and the vast array of genetic material captured within them, it is now estimated that field survival after about 3-4 years provides a robust indication of likely long-term field adaptability. An important discovery from this project is that this 'field survival' trait is remarkably consistent between replicates, indicating that as few as 2-3 trees is sufficient to gauge whether a hybrid will survive under field conditions. This has important practical implications when rootstock experiments are planted on commercial properties because very high tree losses in the first 3 years after planting can leave the collaborating grower with an orchard that is difficult to manage and unprofitable. To resolve this problem, and ensure continued enthusiasm from industry to host trials, a new strategy was developed during this project in which all replicates of a particular rootstock treatment were removed and replanted (with the same scion but on a proven rootstock) as soon as there was enough evidence that the trees were unlikely to prosper: there is little to be gained from continuing to assess poor trees and our data shows that such trees never recover. In the most recently planted field trial (558 datum trees, 154 rootstocks), 27% of trees were replanted within 2½ years of trial establishment resulting in an orchard where tree health is not dissimilar to adjacent conventional blocks of trees, with no missing tree sites. By contrast, an earlier field trial (605 datum trees, 138 rootstocks) also contained many treatments that did poorly soon after planting but was only partially replanted, and this site has only been retained thanks to the good will of the collaborating grower and the unique material still surviving in the experiment: 50% of trees died and 28% of tree sites remain vacant.

Striking differences in performance have been recorded between full-sibs, showing the importance of generating large families and screening multiple individuals under field conditions. Because of the surprising consistency between replicates, greater genetic gain can be achieved from screening a large number of individuals with limited replication rather than a small number of individual hybrids with large numbers of replicates. This is particularly so for the first round of field evaluation where many hybrids can be culled within 3-4 years of planting. These findings contrast with the strategy used in other international breeding programs where they advocate large numbers of replicates in order to discern small differences in performance between a small number of rootstocks.

The 'hidden-agenda' embedded within all Australian citrus breeding work is to explore genetic solutions to HLB disease. This disease is the greatest threat facing the Australian citrus industry, and despite an international investment of more than $2 billion in research there is still no solution for countries in which it has established. While this level of research investment and lack of progress is unprecedented in modern agriculture, even more staggering is how little of this investment has been committed to conventional breeding. Consequently, this project has used conventional breeding to develop adapted germplasm from species that have never been used in rootstock breeding, notably material endemic to Papua New Guinea (PNG) and Australia. Our previous experiments using these wild species directly as rootstocks showed that they were a complete failure on account of their susceptibility to CTV. Using bridging hybrids, we have now introgressed CTV resistance into hybrids of these wild species. Recent overseas research has shown that *Citrus glauca* is the best source of genetic resistance to HLB; we already have multiple CTV-resistant hybrids with this species established in field trials, and have quantified their comparative field survival (under non-HLB conditions). Our *Citrus glauca* hybrids show wide segregation in field performance, with some that are performing well. Whether these promising rootstocks offer any tolerance to HLB cannot be ascertained in Australia (we do not have the disease) but international collaborative trials are being planned to test this. Thus, this project has made significant progress in developing these unique hybrids with comparable performance to existing commercial rootstocks but with the added benefit of potential tolerance to HLB.

Keywords

genetic resistance, citrus, wild relatives, rootstock, fruit quality, productivity, breeding, on-farm trials

Introduction

The project background, rationale and significance to industry are addressed in the Technical Summary above. Processes used to achieve outputs and outcomes are detailed in the extensive Methodology section below.

Methodology

This project was built on the strong methodology already developed by the project team in CT13004, adapting and enhancing it to address the seven key desired outcomes.

To ensure a close collaborative partnership with industry the Varieties Leadership Group (later the National Citrus Productivity Committee) were kept informed throughout the project period. In practice, the most effective form of industry engagement was via field evaluation experiments (all of which were conducted on commercial properties) and field days and presentations given at industry symposia.

New rootstock hybrid progenies were generated, using conventional breeding techniques, to address consumer and horticultural traits. These, along with young progenies generated toward the end of CT13004 (Queensland Citrus Improvement Scheme: finding better rootstocks for Australia) were screened for resistance to CTV, phytophthora and salinity and survivors propagated (as cuttings) and established in field trials. Source trees were established as quickly as possible so that any apomictic hybrids could be distributed more widely for commercial use/testing as soon as they started to produce seed. Field trial performance data were generated concurrently so that fruit quality information could be packaged as seed started to become available.

Data on fruit quality effects of 'extreme hybrids' were collected from trees in the Wallaville site (Expt. 5) which commenced bearing early in the project period. Benchmarking against existing commercial rootstocks was particularly important, and the reason why Benton, Barkley and Rough lemon had been included as 'control' treatments in the experimental design (along with Cleopatra as guard trees). Particular attention was paid to variation between siblings because of evidence of wide differences in establishment and early tree growth within these families. This information was used to guide decisions on the extent to which the native hybrids with CTV resistance would be used in future hybridising activity. All efforts were made to increase the number of *Citrus glauca* hybrid rootstocks in field trials, in line with preliminary indications of HLB tolerance. To achieve this, the existing collection of hybrids with this parentage were screened for phytophthora, re-checked for CTV resistance, rated for vigour and then used as parents (F68 and F154 generated viable hybrids from this crossing). It should be noted that whilst *C. glauca* is currently considered one of the most promising sources of scion tolerance to HLB, its use in rootstock breeding was unprecedented at the start of this project. More importantly, it must be stated that there is still no evidence for direct rootstock resistance to HLB, and indeed recent research shows that one of the best performing rootstocks under endemic HLB pressure (US942) is actually very sensitive to the bacteria. This apparent contradiction is explained by the resilience of this rootstock in resisting sources of stress other than HLB. This has important implications for the Australian rootstock breeding program because it supports our strategy of introgressing resistance to a wide range of stresses (e.g. phytophthora, CTV, salinity) in preparing germplasm for a HLB environment. DAF have signed an agreement for the testing of rootstocks from the University of Florida, specifically targeting those that perform well under HLB endemic conditions. These were received during the project period, grown as seedlings and then grafted with the low-seed Murcott selection 'IrM2'. They are now ready for field planting.

Notwithstanding the apparent absence of direct rootstock tolerance to HLB, the project team kept a close eye on the possibility that new sources of such resistance would be discovered. Past research on CTV serves as an example where 'tolerant' and 'immune' sources of resistance have been discovered and both made an important contribution to solving the problem. Furthermore, some of the most progressive international citrus breeding programs (e.g. Japan) have attempted to introgress CTV resistance in both rootstock and scion varieties. This represented another opportunity for linkage between the Australian scion and rootstock breeding activities that was exploited throughout the project period. Breeding populations that were developed to introgress CTV resistance into scion-type germplasm (as a strategy to deal with Orange Stem Pitting strains of CTV) may also have application in rootstock breeding. These populations were culled based on serological testing for CTV replication and all remaining hybrids were then genotyped for 3 SNP markers associated with the resistance gene for CTV and 2 putative QTLs for HLB tolerance. Similarly, *C. glauca* hybrids that are currently being bred and tested as rootstocks may find application in producing HLB-tolerant scions, and serve as bridging hybrids for an endemic species that is proving particularly difficult to hybridise with domesticated citrus scions. This work morphed into a significant collaboration (all be it unfunded) with the European Union and Fundecitrus Brazil during the project period.

Every effort was made to distribute CTV-immune germplasm interstate, with a particular interest in testing sites with heavy-textured seasonally cold-wet soils, and sites subject to significant salinity. While we were successful in getting this material into NSW and having it declared free of CTV, the emergence of COVID-19 travel restrictions prevented propagation of nursery trees and establishment of trial sites in important production areas, notably the Murrumbidgee Irrigation Area (MIA). However, there was some benefit from this unplanned alteration in methodology because a large field trial established in Queensland concurrently with the sending of the germplasm to NSW (for testing and confirmation of CTV freedom) showed that many of these new hybrids were poorly adapted to field conditions. Thus, a revised list of suitable hybrids can now be produced that reduces the burden of including unproven material in commercial testing sites.

A major new field trial was established and for the first time targeted export markets by using a high-quality export variety as the scion. This trial was established with a particular focus on controlling albedo breakdown, skin texture and generating very high Brix levels. The trial incorporated germplasm generated in CT13004 and also recorded nursery performance information, since this is a critical factor in commercial adoption of new rootstocks. A total of 150 unique rootstock hybrids were captured in this new field trial with variable replication based on anticipated survival (i.e. if there was some evidence that the rootstock may not survive well under field conditions then a reduced number of replicates were planted). Based on previous industry advice, the export variety chosen for this new field trial was 'Premier', thus demonstrating a clear link between the scion (CT15017) and rootstock (CT18004) breeding efforts.

Establishment of this new field trial was supported by continuing efforts and knowledge generated from experiments conducted during CT13004. Key criteria of these existing/finalised experiments available at the start of the project are shown in the table below:

Table 1: Status of experiments and their characteristics at the commencement of CT18004:

Experiment	Field planted	Location	Treatments	Datum trees	Annual data collection		
					Tree growth[a]	Productivity	Fruit quality[b]
1	2004	Gayndah Meyer	34	420	2007-16	2014-16	2007-16
2	2010[c]	BRS	71	1,191	CTV replication and movement		
3	2011	Emerald Pressler	151	891	2014-18	2014-18	2014-18
Extra D[d]	2012	Mareeba Caamano	9	54	2015	2015	2015
4	2013	Gayndah Shepherd	245	490	2015-18	2015-18	2015-18
4	2014[c]	BRS	582	582	CTV screening		
Extra A[d]	2016[c]	BRS	1,407	1,407	Phytophthora screening		
5	2017[c]	BRS	2,272	2,271	CTV & phytophthora screening		
Extra B[d]	2017[c]	BRS	182	194	Salt screening		
Extra C[d]	2017[c]	BRS	79	79	Apomixis screening		
Extra E[d]	2017	Gayndah Ulcoq	17	90	2018	n.a.	n.a

5	2018	Wallaville Spencer	138	605	2017-18	2018	n.a

a: includes measures of tree height, width and canopy volume, as well as trunk circumference 100 mm above and below the graft union.
b: includes measurements of granulation, fruit size, rind thickness, Brix, acid, digital photo.
c: nursery experiment commencement date.
d: not part of the original project document but added as a complementary activity.

Of greater long-term interest was the testing of methodologies to purposefully target vigour control, and the generation of breeding populations that may enable validation of molecular techniques that improve research efficiency. While this was always beyond the resources of CT18004 alone, it was hoped that leverage could be achieved either through links with AS17000 (National Tree Genomics Program) or through additional contributions from parties with a particular interest in such research. However, AS17000 was focused on molecular tools rather than breeding efforts and hence out of scope of the AS17000 project.

The breeding pipeline captured within this project represents material from very early experimental hybrids (or attempts to produce hybrids) through to well-tested germplasm that is ready for use at a semi-commercial level. However, the antiquated concept of a "pipeline" poorly represents the breeding and selection strategy employed by DAF because it fails to capture the complexity of feed-back loops. Rich opportunities for feed-back are created by a project team that is directly involved in everything from pre-breeding using unique germplasm right through to industry engagement. For example, a rootstock that showed excellent rootstock/scion compatibility in a field trial on a commercial property at Emerald (Expt. 3), and was known to be immune to CTV from a nursery experiment conducted in the Bundaberg screenhouse (Expt. 4), was used as the pollen parent to transmit CTV resistance to an endemic citrus hybrid that had shown good freeze tolerance and excellent early tree establishment in a new field trial on a commercial property at Wallaville (Expt. 5). These new crosses were made within three months of all this new information becoming available. This capacity to quickly capture feedback from all stages of research is important in horticultural breeding where there is a need to tackle a large number of traits within species that have notoriously long generation times.

Increasingly the breeding work has moved away from the extremely broad genetic base employed and developed in CT13004, and instead started to concentrate on generating larger families from a smaller number of parents. These large families were then culled heavily for the necessary traits. Rootstocks that showed the most promise in the extensive network of existing field trials were re-introduced to the program as parents. It is hoped that this will help to genetically fix many of the key traits and enable the breeding to re-focus on new traits that are more difficult to phenotype. Information was generated on new and innovative ways to purposefully select for particular traits. Of particular interest in terms of applying new methodologies was the validation of molecular markers for apomixis and virus resistance. For the first time, young seedling populations were sampled and culled based solely on genotyping data for these two critically important traits. This dramatically reduced the size of hybrid populations at a very early stage in the breeding process with considerable benefits in terms of nursery space and time saving compared to conventional virus inoculation and phenotyping. It had the added benefit of identifying individual hybrids that were homozygous for the dominant gene associated with CTV resistance and flagging those hybrids likely to come true-to-type from seed (but at least 5 years earlier than this would normally take to determine).

Results and discussion

Results obtained during the project period and their scientific and commercial implications have been described in seven Milestone reports. An updated table of experiments is provided in Appendix One. Examples of some key findings from these different experiments, along with their commercial significance is provided in Appendix Two.

Outputs

Project outputs, as contracted in the Research Agreement (26th July 2019) and Variation Agreement (31 July 2023) are detailed in the table below:

Table 2. Output summary

Output	Description	Detail
A program logic and	Hort Innovation	Document produced and provided as part of Milestone

monitoring and evaluation plan with linkage to Hort Innovation and Industry/fund objectives.		102.
A final report.	Hort Innovation	See this document.
A STOP/GO Review Milestone at the end of Year 2.	Hort Innovation	Provided as part of Milestone 106 and based on the Hort Innovation external review CT20001 "Strategic review of investments in citrus breeding and evaluation".
An IP register and technology exploitation plan to facilitate commercialisation activities.	Hort Innovation	Document produced and provided as part of Milestone 102.
A project risk register and how risks will be managed.	Hort Innovation	Document produced and provided as part of Milestone 102.
A stakeholder engagement/communication plan.	Hort Innovation	Document produced and provided as part of Milestone 102.
Summary of current breeding criteria and priorities associated with final selection traits. This will include minimum traits data for Brix, acidity, skin texture, granulation, CTV, phytophthora, tree vigour, salinity, rootstock/scion compatibility and seasonal consistency of production. It will also include an analysis of project data to support the potential for altering each of these traits via breeding, and suggestions (supported by available data) as to the best breeding strategy to achieve this output.	Hort Innovation	Document produced and provided as part of Milestone 102.
An assessment of the breeding pipeline incorporating progeny and selections generated from previous and new investments in rootstock breeding and evaluation by the project team. Included in this will be findings regarding defects associated with the use of endemic germplasm, how such defects may be addressed, and an analysis of whether useful traits can be introgressed from this	Breeding team	Annual team review of seasons data prior pollinations. Assessment of with-family variation in performance ahead of visit from EU and Fundecitrus partners.

material into commercially useful rootstocks.		
Description of the system used for evaluation and assessment of why it has been so successful in achieving industry adoption.	Continual benchmarking and discussions with international programs.	Review of presentation given at TropAg conference (Brisbane) and International Citrus Biotechnology Conference (Uruguay) confirming the critical role of industry engagement in every stage of the breeding process. Panel member International Citrus Congress Breeding Workshop (Turkey), International Breeders' visit (Bundaberg), HLB collaboration EU/Fundecitrus (Bundaberg).
New rootstock varieties worthy of commercial use in major citrus growing regions of Australia.	AusCitrus and private nurseries.	New potential rootstocks 'ICA12' and '14Q055' supplied to NSWAg for pathogen testing ahead of inclusion in the national AusCitrus nursery scheme. 'Barkley' rootstock available in increasing quantities and supplied to nurseries for encourage wider scale commercial testing.

Outcomes

Project outcomes, as contracted in the Research Agreement (26th July 2019) and Variation Agreement (31 July 2023) are detailed in the table below:

Table 3. Outcome summary

Outcome	Alignment to fund outcome, strategy and KPI	Description	Evidence
New hybrids resistant to CTV, phytophthora and salinity, that are apomictic and ready for commercial use.	Improve access to new scion and rootstock varieties with superior performance through breeding and by evaluating varieties from global programs, including identification of new rootstocks suitable for automation and high-density production systems.	Field adapted germplasm with resistance to CTV, phytophthora and salinity identified in segregating populations.	Wide segregation in field performance of material previously screened for CTV, phytophthora and salinity. Outstanding individual hybrids present.
Data on fruit quality effects of 'extreme hybrids' that have overcome the previous road-blocks caused by the CTV sensitivity of native germplasm.	New knowledge for growers on the potential of native Australian lime genetics to address threats like HLB.	Extensive fruit quality data collected in consecutive seasons from the Wallaville trial site (Expt 5) and benchmarked against standard commercial rootstocks.	Fruit quality on 'extreme hybrids' comparable to standard rootstocks. No evidence of increased acidity or poor skin quality often associated with acid-type citrus when used as rootstocks.
Geographical expansion to address unresolved rootstock problems in the tropics and saline arid regions of Australia, as well as the heavy-textured, seasonally cold-wet soils of southern Australia, where the genetic diversity of	Availability and access to new citrus scion and rootstock varieties that have been developed for Australian conditions for grower adoption.	Budwood of a hybrid population screened for CTV, phytophthora and salinity sent to EMAI Sydney and successfully established. Subsequently confirmed as virus-free. COVID-19 disruption of	Concurrent field trial in Queensland shows wide segregation in field survival and reduces the need to field test all of the material currently being held at Elizabeth Macarthur Agricultural Institute (EMAI). See Milestone

existing commercial rootstock is extremely narrow. Partnerships with local expertise and access to non-project resources will be essential to maximise this deliverable. (Linkage to CT17002 Evaluation of new rootstocks for the Australian citrus industry 2017-2022 and potential regional development project activity).		opportunities for partnership development (interstate nurseries).	report 104.
Fruit quality data from existing trials and establishment of new field trials targeting export-oriented scion varieties, focusing on critical export traits such as skin texture and acidity. This data packaged to facilitate efficient grower decision-making.	Availability of new mandarin varieties with superior fruit quality attributes for grower adoption.	Conscious decision to use only export-oriented scions for future rootstock experiments.	Large new field trial (154 rootstocks) planted on a commercial property in 2020. Uni of Florida rootstocks propagated with IrM2 low seeded Murcott, ready for planting late 2023. Multi-site data on 'Barkley', 'Benton' and '14Q055' used to support commercial adoption.
Breeding populations to enable the generation and validation of molecular techniques that improve research efficiency, building on the project teams world-first in adopting molecular markers in rootstock breeding.	Develop and optimise a whole-systems approach to integrated pest and disease management (IPDM).	Validation of molecular markers for virus resistance and apomixis using breeding populations from the Australian program. Cost-effectiveness of different marker technologies assessed.	New SNP marker for apomixis made available prior to publication (from Spanish colleague) and confirmed useful. CTV (virus) SNP marker matched with serological phenotyping on segregating population. Both markers used as the first stage of culling in new rootstock populations.
Research method and technology to purposefully target vigour control.	Development of orchard systems that are compatible with automation and technology solutions in collaboration with growers.	Reliant on leveraging funds from AS17000 which did not eventuate.	Field data on vigour still collected from all trial sites each season. Potential for trait association particularly Expt. 8.
Grower and nursery engagement through field days and progress reports to industry, via the Citrus Industries Variety Leadership Group. This will be expanded to better represent major Australian production areas and to	Availability and access to new citrus scion and rootstock varieties that have been developed for Australian conditions for grower adoption.	Make industry aware of novel rootstock and what advantages they may offer over existing industry standards.	Major national field day held in 2022, presentations at industry conferences (Adelaide and Sunshine Coast). Individual engagement with commercial nurseries including supply of seed to promote wider evaluation

capture contributors to the project.			and adoption.

Monitoring and evaluation

Project-specific questions described in the Monitoring and Evaluation Plan (January 2020) and approved in Milestone 102 are addressed in the table below:

Table 4. Key Evaluation Questions

Key Evaluation Question	Project performance	Continuous improvement opportunities
To what extent has the project discovered new rootstocks worthy of commercial use in major citrus production regions of Australia? To what extent did the work progress novel and previously untapped genetic resources toward commercial usefulness.	New genetically diverse and unique rootstocks identified with commercial merit. Have the traits necessary for use in Australia. Highly successful in demonstrating (for the first time) that species like *C. australasica*, *C. glauca* and *C. wintersii* are useful parents for breeding commercial rootstocks.	Future focus on best-better parents and selections identified.
To what extent has any of the project outcomes (primarily new germplasm) entered into commercial production? (level 6 & 7 Bennett hierarchy).	'Benton' and 'Barkley' continue to expand in their use in new commercial plantings of mandarins. 'ICA12' and '14Q055' are making their way into the national budwood scheme.	New *C. glauca* and *C. australasica* hybrids need to enter wider commercial testing.
Have good working relationships been maintained with the large number of hosts of trials, reflected most directly in the collection of all necessary data? How well have industry levy payers been able to influence the project process and shape it to meet their needs? Has there been constructive involvement of citrus growers, consultants and industry representatives at trial sites and industry meetings/conferences?	Excellent working relationships have been maintained with all trial hosts. These trial hosts continue to carry a significant burden but are eager to help find better rootstock options for the future. Levy payers have provided input at field days and conferences. Trial sites have been made available for group and one-on-one inspections.	New trial(s) containing the best selections from past field trials would aid adoption by 'de-complicating' the complexity of such a vast screening of diverse genetics.
Were the target audiences able to access engagement processes at both a group and individual level, and did the approach to engagement meet the learning styles and citrus business constraints of these audiences?	The Project Leader and team have made themselves available and maintain an open and transparent approach to the research and information emerging from it. The target audience are time-poor and thrive on concise information and seeing field sites for themselves.	On-farm trials must continue to be the cornerstone of rootstock breeding and evaluation. They require good engagement skills and commitment to useful industry outcomes.
Were large breeding progenies, large screening campaigns and large field trials successfully conducted with limited resources and evidence of more efficient ways of doing things?	Large breeding, screening and field trials were conducted successfully, an approach vindicated by the wide segregation seen even within breeding families. Visual ratings, molecular markers and LiDAR crop scanning have been implemented to	Introgression of higher rates of apomixis in native-based populations would greatly aid commercial adoption.

	improve efficiency.	
Has the project strengthened or weakened the case for investment in citrus rootstock breeding amongst the different target audiences? Are there likely to be long term benefits for citrus industry levy payers from the outcomes generated in this project?	The case for a rootstock breeding program based on wide genetic diversity has been strengthened, with evidence that commercially useful outcomes are achievable. International endorsement of the program has reinforced local support. Levy payers will have better information to choose rootstocks for their new plantings and a wider range of genetic material to choose from. There is the possibility of finding rootstocks to mitigate HLB.	Export market opportunities and viability are dependent on fruit quality, for which rootstocks can make a useful contribution. Significant new opportunities exist and must be pursued with the ever looming threat of HLB, and the current problems of labour availability and WH&S.

Recommendations

- Unique hybrids produced from never-before-used parents should be tested commercially and on a wider scale.
- Promising hybrids should continue to pass into the national budwood scheme for national distribution.
- Florida-bred rootstocks need to be field evaluated under a highly HLB-susceptible scion and in local conditions.
- Best-bet selections from existing trials need to be evaluated on a larger and broader scale (as per recommendation of the international review panel 2022).
- Vigour effects and prediction require ongoing development, including the possibility of marker development.
- 'Extreme-hybrids' with good field adaptability under Australian conditions require testing in a HLB environment.
- Increased introgression of apomixis into breeding populations (including molecular marker screening) is required to make then more appealing for conventional nursery propagation procedures.
- Larger hybrid populations should be generated from the best parents (previously progeny-tested) to ensure sufficient phenotypic variation is available for selection.

Refereed scientific publications

Journal article

Smith, M.W. (full list of authors under embargo until official publication date) Pan-genome analysis provides insight into the evolution of the orange subfamily and a key gene for citric acid accumulation in citrus fruits. Nature Genetics, (accepted 28th August 2023).

Smith, M.W., Gultzow, D.L., Reid, M.J., Newman, T.K., Thangavel, T., Anderson, J.M., & Miles, A.K. Breeding for Citrus Black Spot resistance. Acta Horticulturae. (accepted 4th August 2023).

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Intellectual property

Project IP: An IP register has been maintained (confidential) and describes 18 progeny groups associated with this project.

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Appendices

Appendix One: Key experiments generated and/or utilised in CT18004:

Experiment	Field planted	Location	Treatments	Datum trees	Annual data collection		
					Tree growth[a]	Productivity	Fruit quality[b]
1	2004	Gayndah Meyer	34	420	2007-16	2014-16	2007-16
2	2010[c]	BRS	71	1,191	CTV replication and movement		
3	2011	Emerald Pressler	151	891	2014-22	2014-22	2014-22
Extra D[d]	2012	Mareeba Caamano	9	54	2015	2015	2015
4	2013	Gayndah Shepherd	245	490	2015-23	2015-23	2015-23
4	2014[c]	BRS	582	582	CTV screening		
Extra A[d]	2016[c]	BRS	1,407	1,407	Phytophthora screening		
5	2017[c]	BRS	2,272	2,271	CTV & phytophthora screening		
Extra B[d]	2017[c]	BRS	182	194	Salt screening		
Extra C[d]	2017[c]	BRS	79	79	Apomixis screening		
Extra E[d]	2017	Gayndah Ulcoq	17	90	2018-23	2022	n.a
5	2018	Wallaville Spencer	138	605	2017-18	2018	2020-23
6	2016	BRS	1,437	1,437	Field CTV serological screening, 22 families, 2019-23		
7	2020[c]	BRS	921	1,006	Vigour screening		
8	2020	Gayndah Jameson	154	558	2020-23	2023	n.a.
9	2021	BRS	1,035	1,035	Nursery CTV serological screening, 34 families		
7	2022	Mundubbera Trott	110	150	2022-23	n.a.	n.a.

10	2022	BRS	251	251	SNP markers CTV, apomixis, HLB
3	2022	BRS/Emerald	43	43	Recovery of rootstock germplasm from Expt 3.
11	2023	BRS	25	450	Propagated (IrM2) Florida rootstocks awaiting field site

a: includes measures of tree height, width and canopy volume, as well as trunk circumference 100 mm above and below the graft union.
b: includes measurements of granulation, fruit size, rind thickness, Brix, acid, digital photo.
c: nursery experiment commencement date.
d: not part of the original project document but added as a complementary activity.

Appendix Two: Selected highlights from individual experiments listed in Appendix One:

Expt.	Results and Implications
3	Nine seasons of quality data (particular focus on granulation) used to identify outstanding individual trees, which were subsequently chain-sawed below the graft to allow the rootstock hybrid to shoot away. These shoots were collected and now successfully propagated back at BRS for a new trial to confirm their effectiveness in improving fruit quality. Individual tree data was collected from Imperial mandarin on 365 different hybrids, for 9 seasons, and based on this we chose 26 with particular merit. Low and high vigour individuals were also recovered.
4	Extensive data set (9 consecutive seasons) on productivity, vigour, and fruit quality (particularly granulation) used to choose new rootstock breeding parents. Potential for productive low-vigour individuals suitable for high-density and/or covered cropping.
5	Demonstrates wide variation in field adaptability even in hybrids that have passed through rigorous nursery screening and are resistant to 'obvious' diseases like phytophthora and CTV. Some hybrids (e.g. ICA12, D77) have been outstanding and at least as good as current commercial rootstocks. No evidence that using wild species as rootstock parents has been detrimental to scion fruit quality.
6	Confirms a close relationship between the CTV resistance allele and serological phenotyping results. Use of molecular markers for this trait is likely to be efficient with the diverse strains of CTV present in Australia and the germplasm being used in the breeding program.
8	Large differences in vigour already obvious and not a consequence of poor tree health. Some hybrids with wild species showing considerable promise, including a few that were also included in Expt 5.
9	Retaining only hybrids with CTV resistance is good insurance for long term field adaptation and creates the opportunity for future generations of hybrids to be homozygous for the resistance allele (because both parents possess it).
10	251 pre-screened individual hybrids genotyped for 4 SNP markers linked to important rootstock traits (CTV, apomixis, putative HLB). Hybrids from 10 families included with the biggest being D77×14Q055 (46 individuals) and F68×14Q055 (58 individuals). Able to immediately cull population and retain just 44 hybrids. Cuttings produced immediately genotyping results became available. Five hybrids homozygous for CTV resistance gene (and 2 of these also apomictic), potentially capable of generating 100% resistant progeny.
11	Florida rootstocks (Uni of Florida and USDA) grafted to IrM2 and ready for field planting. Will confirm their adaptability to local conditions and benchmark against some selections from the Australia breeding program.