Final Report

National strawberry varietal improvement program (2017-2022)

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National strawberry varietal improvement program (2017-2022) (BS17000)

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Public summary

The Australian strawberry industry has an estimated farm gate value of $435 million, with approximately 200 commercial producers (Berry Strategic Investment Plan 2012-2026). For the national industry to remain profitable the ongoing development and availability of improved strawberry varieties is required. Opportunities exist for locally adapted varieties with superior production and consumer traits, and the expansion of specifically bred varieties for alternative production systems.

This project (BS17000) aimed to develop and commercially release superior strawberry varieties adapted for production in temperate, subtropical and Mediterranean climatic regions in Australia. Individual breeding targets for each production region were specifically defined in consultation with local industry in each region with an overall goal of supporting a sustainable and profitable national strawberry industry. Traits included, among other targets, developing varieties for substrate culture as well as with improved resistance to the crown wilt diseases colletotrichum crown rot (*Colletotrichum gloeosporioides*), fusarium wilt (*Fusarium oxysporum* f. sp. *Fragariae*), and charcoal rot (*Macrophomina phaseolina*), as well as powdery mildew (*Podosphaera aphanis*). The general breeding strategy built upon the successful pipeline developed in the previous iteration of the project (BS12021) and was further enhanced by incorporating genomic prediction and marker-assisted selection methodologies.

The project developed and positioned for commercialisation six new strawberry varieties, locally adapted to Australia's growing regions and satisfying Australian consumer expectations. These included one variety targeted for subtropical production, 'Susie-ASBP', and another with Plant Breeder's Rights part I application soon to be submitted (proposed name 'Stella-ASBP'). Two varieties targeted for temperate production have been released: 'Tahli-ASBP', and 'Tamara-ASBP'. Six semi-commercial Mediterranean selections have been trialled on-farm with two identified for potential commercial release pending industry feedback. All varieties performed well in both substrate culture and in-ground production. Additionally, two specialty varieties originating from the subtropical node have been released for tender for commercialisation: a pink and white blush variety 'SB17-230'ASBP', and a white variety 'SW20-317-ASBP'.

In 2021-22, varieties from the Australian Strawberry Breeding Program (ASBP), including varieties from this project and previous projects, captured 47% of the national market, with a farm gate value of approximately $218 million. Two peer reviewed journal articles were published, along with 12 publications in the national strawberry industry magazine the Australian Berry Journal and previous iteration Simply Red. Nine presentations were delivered at conferences, 14 at industry meetings and field days, and 10 breeding trial tours given. The project was also covered extensively in the media with 25 interviews conducted for radio, television, websites, and podcasts.

Based on current industry and marketing trends, it is recommended that key directions for future breeding should include varieties for alternative production systems, and with characteristics optimised for ease of robotic harvesting. Additionally, advanced breeding and evaluation methodologies should be incorporated, including predictive agriculture techniques using genomics and rapid phenotyping. Future breeding of strawberries in Australia should aim to improve productivity and profitability for national strawberry producers while ensuring strong consumer appeal. Furthermore, future varieties should be developed through a pipeline that can adapt to changing market and environmental demands.

Keywords

Fragaria × *ananassa*; breeding; disease resistance; Australia; temperate; subtropical; Mediterranean; genomics

Introduction

The Australian strawberry industry produces approximately 82,300 tonnes of fruit per annum, with an estimated farm gate value of $435 million and approximately 200 growers (Berry Strategic Investment Plan 2012-2026). For the national industry to remain profitable the ongoing development and availability of improved strawberry varieties is required. Opportunities exist for locally adapted varieties with superior production and consumer traits, better understanding of economic drivers for variety development, and the expansion of specifically-bred varieties for alternative production systems. This aligns with Outcome 2, Strategy 1 of the 2022-2026 Hort Innovation Berry Strategic Investment Plan – Ensure that superior strawberry varieties that match consumer expectations are available to growers across Australia and that BMP techniques are available to optimise returns to growers. Furthermore, these need to be developed through a pipeline that can adapt to changing market and environmental demands.

Historically, the majority of varieties grown by the Australian strawberry industry have been bred overseas, where growing environments and consumer preferences differ to those in Australia. The aim of the Australian Strawberry Breeding Program (ASBP, including current and previous Hort Innovation levy funded projects) has hence been to provide locally adapted varieties with superior production and consumer qualities, which meet, and, through the program, can respond to, Australia's changing market and environmental demands. Individual breeding targets for each production region were specifically defined in consultation with local industry in each region with an overall goal of supporting a sustainable and profitable national strawberry industry. Traits included, among other targets, developing varieties for substrate culture as well as with improved resistance to diseases. The general breeding strategy built upon the successful pipeline developed in the previous iteration of the project (BS12021) and was further enhanced by incorporating genomic prediction and marker-assisted selection methodologies.

Under project BS17000, three varieties catering for subtropical and temperate production regions were commercialised, with two more poised for commercialisation for Mediterranean and subtropical regions and two speciality pink and white varieties. In 2021-22, varieties from the Australian Strawberry Breeding Program (ASBP) captured 47% of the national market and were grown commercially in all Australian production regions. These plantings had a farm gate value of approximately $218 million and contributed ~5,200 jobs in production. Indications from runner growers suggest that 2022-23 sales of ASBP varieties will be higher again than previous levels.

This report covers the outputs from project BS17000, specifically regarding breeding trials undertaken for all three targeted production regions and varieties commercialised, and findings from disease screening trials conducted for three major crown wilt diseases: colletotrichum crown rot (*Colletotrichum gloeosporioides*), fusarium wilt (*Fusarium oxysporum* f. sp. *Fragariae*), and charcoal rot (*Macrophomina phaseolina*), as well as powdery mildew (*Podosphaera aphanis*). Additionally, we will detail the genomics technologies that have been used to screen for DNA markers linked with "peach" flavour and perpetual flowering/day neutrality, and to advance the genetic gain of the breeding program using marker-assisted parental selection.

The success of BS17000 can be largely attributed to the DAF strawberry breeding team operating for over 25 years with extensive strawberry breeding experience, with skills and resources in propagation, field trial management, DNA genotyping, quantitative analysis of genetic and phenotypic data, molecular and biological virus indexing, tissue culture, disease screening, substrate culture, regional evaluation, positioning material for commercialisation, commercial deployment, industry engagement and guidance, and international collaboration.

Methodology

Breeding strategy

The overall breeding strategy in BS17000 was enhanced from that successfully applied in the previous project, as outlined in Figure 1 below. Note that all breeding activities at all stages of the process were undertaken simultaneously each year in each production region.

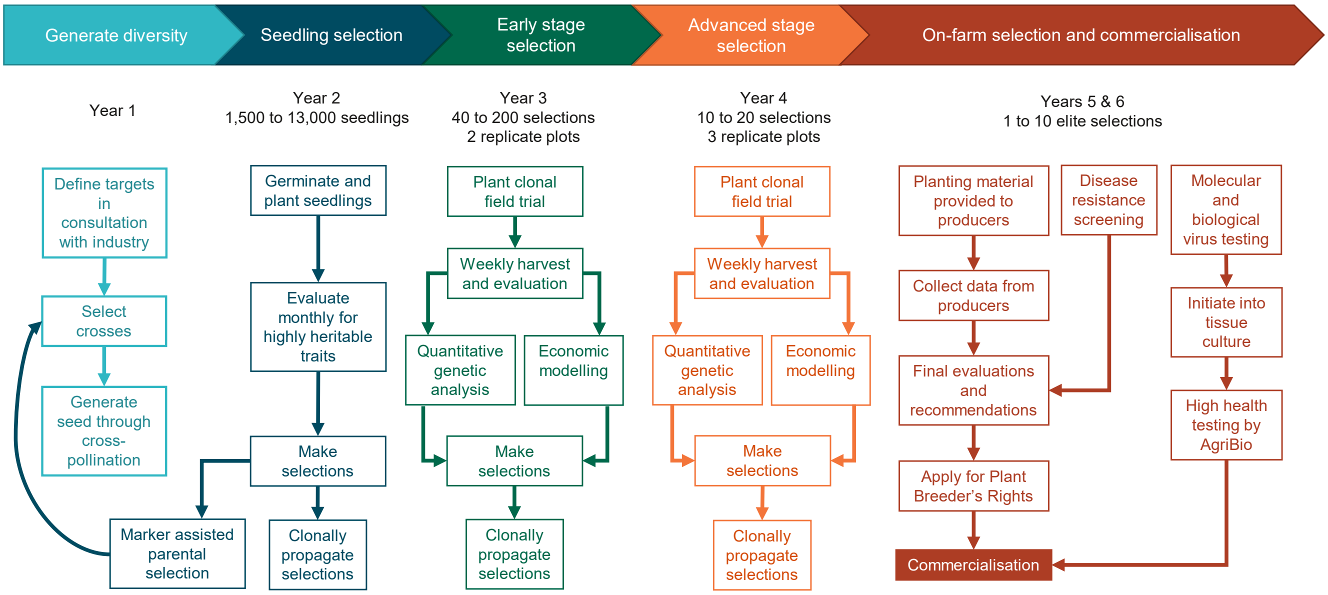


Figure 1. Breeding strategy for each production region.

Breeding objectives

The over-arching objective of the breeding program is to produce varieties with traits that optimise profitability, sustainability, and consumer satisfaction for all three production regions, based on their individual strengths and requirements. The traits important for increasing production profitability were estimated using an economic model developed by Herrington (2012) for the subtropical node. The model allowed informed selection decisions to be made, weighting the value of plant and fruit traits to estimate the profitability of each selection as a variety. Many of the traits identified as important for profitability in the subtropical economic model are also relevant for production under temperate and Mediterranean conditions, however in discussion with industry some differences were identified. An estimated net profit per plant was calculated for the temperate and Mediterranean nodes based on yield, average fruit weight, prices per punnet, and cost to harvest each fruit.

Breeding targets were updated over the course of the project as guided by the regional Reference Groups and local growers at other events. Examples include increased heat tolerance and fruit sunburn resistance for in-ground production in Victoria, fusarium wilt resistance in the Mediterranean production region, and longer trusses and high bruise resistance for substrate culture on tabletops. Breeding has also focussed on resistance to red leaf disorder, as measured in Hort Innovation project BS19001 'Developing knowledge and management of strawberry red leaf disorder', and internally funded DAF projects HF10994 and HF11485. Among the traits deemed important for consumer acceptance and repeat purchasing were reliable good flavour (lower acid), good shelf life, and attractive appearance.

Generating genetic variation

Parents with desirable traits as defined by the breeding objectives were selected using breeding values calculated from quantitative genetic analysis of trial data. Crosses were generally designed to improve specific key traits (yield, early yield, fruit size, flavour, disease resistances, shelf life, truss length, profitability, and bruise, abrasion and rain resistances) whilst maintaining overall acceptability of all other traits. Selection of specific parental combinations was informed by modelling likely offspring performance for every possible pairing, excluding highly related parents. Cross-pollinations were conducted in insect-free glasshouse conditions, the fruit harvested once mature, and seed extracted and germinated.

Specialty crosses (white fruit, dark fruit, specialty flavour) were conducted initially using subtropical germplasm only, and were expanded to temperate and Mediterranean regions in 2021 and 2022. The main focus in temperate and Mediterranean regions was to produce superior standard (red-fruited) varieties, which would provide the greatest value to those industries.

For the ASBP to remain cutting edge, collaboration and information exchange with international strawberry breeding programs and scientists is essential. BS17000 maintained an existing and successful seed exchange with the University of Florida for subtropical and Mediterranean genetic diversity. A seed exchange agreement with Vissers Plant Innovations (Netherlands) was contracted in April 2022 for temperate genetic diversity. These agreements have been extremely beneficial for maintaining genetic variation within our breeding program.

Assessment of genetic variation and selection of seedlings

The number of seedlings assessed for each node was balanced based on the relative size of the industry in each production region. In all seedling trials, families were randomised and planted into replicate plots, and plants evaluated every three to four weeks during the assessment window. Plants were assessed for moderately to highly heritable traits including yield, plant size, density, truss length, truss branching, fruit size, fruit firmness, and fruit shape. At each assessment time, plants performing well were flagged with a coloured pin tag. At the end of the season plants with multiple tags were likely to have performed well across the season and were considered for further testing in early-stage clonal trials. Approximately 1% of seedlings were selected each year for each node.

Approximately 14,000 temperate seedlings were planted each season into seedling trials at the Wandin Research Centre, Victoria and Applethorpe Research Facility, Queensland. Both short day and day neutral plants were planted at the same time, but slightly earlier than day neutrals are planted in commercial farms (mid-April). This was done to drive selection for plants that have both the early yield of short day varieties as well as the consistent production of day neutral varieties. Assessment of temperate seedling trials began in October each year and continued to mid-January. While the commercial production season continues until May, the selection of seedling material in January was necessary to facilitate plant propagation for the following season. Seedling selection later in the season instead would add an extra year onto the breeding pipeline, and thus was not conducted.

For the subtropical region, on average 10,000 standard and 3,000 specialty seedlings were planted into field trials each year at the Maroochy Research Facility (MRF), Nambour, Queensland, and Bundaberg Research Facility, Bundaberg, Queensland. Planting was undertaken in mid- to late-March each year and seedling selections made at the end of August.

For the Mediterranean region, approximately 1,700 seedlings were planted each season. The exception was in 2018 when no seedling trial was run, as breeding for this region only officially began in 2018 and no seed was generated the previous year. Clonal selections from previous un-official Mediterranean trialing were still evaluated that year. Seedling trials were planted on grower's properties at Bullsbrook, WA except in 2020 when they were planted at MRF due to COVID-19 interstate travel restrictions.

From 2020 onwards, all Mediterranean seedlings were cloned in tissue culture at MRF prior to trial planting. This was due to issues with plants dying following retrieval from Western Australia, and meant that copies of all selected seedlings already existed at MRF. While highly effective, this approach was extremely time-consuming, and so alternative propagation solutions are recommended for exploration in future breeding.

Evaluation of selections in clonal trials

Seedling selections were clonally propagated each year via runners and planted into early-stage clonal trials. At the same time early-stage selections from the previous season were similarly propagated for assessment in advanced-stage trials. Early and advanced-stage trials were planted together in an incomplete block design with two replicate blocks of six plant plots for early-stage accessions and three replicate blocks for advanced-stage accessions. Pedigree information was incorporated into the design analysis to optimise the spatial layout.

Temperate clonal trials were planted as bare-rooted runners in mid-April each year and data collected from mid-October to the end of February. Subtropical trials were planted as plug plants in mid-March and data collection took place from first fruit (typically early May) to the end of August. Mediterranean trials were planted as plugs in mid-April and data collected from first fruit to the end of September. Control varieties were included in all trials to compare accession performance with those of the major varieties grown in each region: 'Albion', 'Monterey' and 'Cabrillo' for temperate, 'Red Rhapsody' for subtropical, and 'Festival' and 'Red Rhapsody' for Mediterranean.

During the season plots were harvested weekly. Fruit were counted and weighed, and assessed multiple times per season for the traits described in Table 1. Data were electronically entered and checked weekly for consistency and outliers to ensure high data quality.

Data were analysed using a quantitative genetic approach to estimate breeding and clonal values. This approach incorporates plant pedigree, so that shared genetics between accessions can be used to more accurately predict performance. It also removes small-scale environmental effects across the field that may obscure estimation of genetic potential. This technique allows the assessment of an accession's performance both as a clone (variety) and as a parent (the genetics it can contribute to its offspring) for each trait. Thresholds were established for each trait comparative to leading commercial varieties, and clonal values for each accession were run through the economic models to identify those most likely to have increased profitability for producers.

Table 1. Plant and fruit traits assessed in each production region.

Trait group	Trait	Description	Unit	Approx. times assessed per season
Fruit traits	Plot yield	Weight of harvested fruit per plot	g	Weekly
	Plot fruit count	Count of harvested fruit per plot	Count	Weekly
	Sugar (Brix)	Soluble sucrose content	°Brix	3
	Acid	Acid measurement	% Citric acid equivalent[3]	3
	Flavour	Flavour rating	Score 1-9[1]	3
	Fruit shape	Fruit shape code	Score 1-9[1]	2
	Uniformity	Fruit shape uniformity	Score 1-9[1]	4
	External colour	External colour rating (light to dark)	Score 1-9[1]	3
	Internal colour	Internal colour rating (light to dark)	Score 1-9[1]	3
	Gloss	External gloss rating	Score 1-9[1]	2

Trait group	Trait	Description	Unit	Approx. times assessed per season
	Visual impact	Score of good visual impact after 2 days storage at room temperature	Score 1-9[1]	Weekly
	Visual impact (fridge)	Score of good visual impact (shelf life) after 5 days in cold room and 2 days storage at room temperature	Score 1-9[1]	3 (advanced trial only)
	Cull	Score of poor visual impact after 2 days storage at room temperature	Score 1-9[1]	Weekly
	Bruise resistance	Fruit firmness measurement using firmness durometer device	0-100[4]	3
	Abrasion resistance	Abrasion resistance rating	Score 1-9[1]	3
	Rain damage resistance	Proportion of fruit undamaged by rain	Proportion	As available
	Sunburn resistance	Proportion of fruit undamaged by sunburn	Proportion	As available
	Ease of pick	Ease of fruit detachment	Score 1-9[1]	2
Plant traits	Bush size	Bush size rating	Score 1-9[2]	2
	Fruit display	Position (extension) of fruit relative to foliage	Score 1-9[1]	2
	Truss type	Truss branching rating	Score 1-9[1]	2
	Plant counts	Count of plants per plot	Count	4

[1] 1 = low/poor, 9 = high/good

[2] 1 = high/good, 9 = low/poor

[3] pH end point = 8.1

[4] 0 = very soft, 100 = very hard

Evaluation of selections in on-farm trials

Virus-free plant material of selections from advanced-stage clonal trials (1-10 selections per region) were supplied each year to producers across each production region to assess in on-farm trials (Table 2). Producers were selected where possible to encompass both in-ground and substrate production systems. Based on these evaluations, plants of 1-5 of these selections were subsequently supplied again the following year for second-stage on-farm trialling. Data were collected by producers or ASBP staff where necessary on yield (fruit number and weight), and feedback sought on fruit quality traits, fruit size, yield, resistances, and overall accession performance.

At the end of each season for each region the breeding team provided involved producers, Reference Group members, and plant propagators with a detailed report summarising the data and grower feedback for all selections assessed in advanced and on-farm trials (see Appendices 1-16 [CONFIDENTIAL]). Recommendations for selections to re-trial or commercialise were presented and input from Reference Groups sought.

Table 2. Details of on-farm trial locations. There were some small deviations in some years depending on grower availability. SA4 = Statistical Area Level 4 (Australian Bureau of Statistics).

Environment	State	Location (SA4 name and code)	No. sites	Production systems
Temperate	Vic	Melbourne – Outer East (211)	2	Substrate (1) and in-ground (1)
	Tas	Launceston and North East (602)	2	Substrate (2)
	SA	Adelaide – Central and Hills (401)	1	In-ground (1)
	WA	Western Australia – Wheat Belt (509)	1	In-ground (1)
	Qld	Darling Downs – Maranoa (307)	1	Substrate (1)
Subtropical	Qld	Moreton Bay – North (313)	2	In-ground (2)
	Qld	Wide Bay (319)	2	In-ground (2)
	Qld	Gold Coast (309)	1	In-ground (1)
Mediterranean	WA	Perth – North East (504)	2	Substrate (1) and in-ground (1)

Marker assisted selection (MAS)

Routine screening was conducted on 147 and 217 accessions across all three breeding nodes for DNA markers linked with perpetual flowering/day neutrality (DN) [3-6] and "peach" flavour [8, 9], respectively (see Appendix 17 for more details). For the DN QTL, dried leaf material was supplied to the Australian Genome Research Facility (AGRF) for DNA extraction and subsequent analysis of the Bx215 marker. Results were analysed for the presence/absence of an allele at 128bp. For the γ-decalactone "peach" flavour QTL, analysis was performed at MRF and by AGRF. DNA was extracted from dried leaf material, amplified using PCR, and products visualised via agarose gel, with a band present at 140 bp indicating presence of γ-decalactone.

Genome-wide parental selection

Genomic selection (GS) was performed on subtropical material, and prediction models were developed for the temperate breeding node (see Appendix 17 for more details). Dried leaf material was supplied to Genotyping Australia for DNA extraction and genetic marker analysis. Phenotypic data for 11 traits from subtropical clonal trials at Maroochy Research Facility, and temperate clonal trials at Wandin Research Centre from 2017 to 2021 were used for genomics analyses. Genomic prediction models were developed through collaboration with staff from the University of Queensland. Prediction models were applied to the genotypes of 89 subtropical seedling selections from 2020 to obtain their genomic estimated breeding values. Six individuals with a good combination of predicted trait performances were chosen to be used as parents in 2021 cross-pollinations. A total of 384 leaf samples from the temperate and subtropical nodes was sent to Genotyping Australia for genetic marker analysis in November 2022, and these data will be used to strengthen genomic prediction models and re-assess prediction accuracy.

Disease resistance screening

Disease resistance screening was undertaken on commercial and pre-commercial accessions for all three production regions for three major crown wilt diseases: colletotrichum crown rot (caused by *Colletotrichum gloeosporioides*), fusarium wilt (caused by *Fusarium oxysporum* f. sp. *Fragariae*), and charcoal rot (caused by *Macrophomina phaseolina*), as well as powdery mildew (caused by *Podosphaera aphanis*). The aim was to conduct two replicate screening experiments for per accession for each disease by the time a commercialisation decision was made. Pre-breeding was also undertaken for each disease in order to incorporate resistance genes into the general breeding population.

Charcoal rot

Five *M. phaseolina* (Mp) glasshouse inoculation experiments were conducted to determine charcoal rot resistances. A sixth experiment is currently in progress. Resistance screening was conducted on a total of 68 accessions, including 18 ASBP varieties, 36 breeding accessions, eight pre-breeding accessions, and six non-ASBP varieties. Plants were inoculated with Mp isolate N18924 as per Gomez et al. (2020). Five to ten replicate plants were assessed per accession per experiment in an incomplete block design incorporating pedigree relatedness at MRF. 'Albion' was used as the reference susceptible control and 'Phenomenal' as the resistant control.

Plants were monitored for 12-25 weeks following inoculation depending on rate of plant death, and week of death recorded. A discrete time survival analysis based on Cox's proportional hazards model was performed on the strawberry mortality data (see Southey et al. 2003). The analysis was performed in ASReml-r (Butler et al. 2018) using a binomial GLMM with complementary log log link function and incorporated data from accessions assessed in both BS17000 and the previous project BS12021.

Colletotrichum crown rot

Disease resistance screening of strawberry accessions for colletotrichum crown rot (pathogen *C. gloeosporioides* [Cg]) was conducted as described in Neal et al. 2017. A total of 69 accessions were assessed in five experiments, including 16 ASBP varieties, 34 breeding accessions, 13 pre-breeding accessions, and six non-ASBP varieties. Experiments were conducted in a glasshouse at MRF and followed an incomplete block design incorporating pedigree relatedness. There were 5-10 replicate plants per accession per experiment. The varieties 'Camarosa' and 'Festival' were used as susceptible controls, as they have previously been reported as highly susceptible to Cg (MacKenzie and Peres, 2007, MacKenzie et al., 2006, Peres and MacKenzie, 2012). 'Phenomenal' was used as the resistant control.

Plants were monitored for 10 weeks following inoculation and week of death recorded. Data were analysed as described for charcoal rot above, and incorporated accessions assessed in both BS17000 and BS12021.

Fusarium wilt

Four screening experiments for resistance to fusarium wilt were conducted using a mix of *F. oxysporum* f. sp. *Fragariae* (Fof) isolates N13581 and N15309 as described in Paynter et al. (2014). A fifth experiment in 2021 failed due to a heatwave that confounded plant deaths. A total of 81 accessions were screened including 17 ASBP varieties, 59 breeding accessions, and five non-ASBP varieties. Five to ten replicate plants were assessed per experiment under glasshouse conditions in an incomplete block design incorporating pedigree relatedness. 'Festival' was used as the resistant control and 'Kabarla' as the susceptible control.

The severity of foliar Fof symptoms were assessed on a 1-5 visual rating scale:

1 = plant healthy.
2 = plant with a mild wilt, with 1-25% wilted leaves.
3 = plant with a moderate wilt, with 25-50% mature leaves wilted but young leaves still healthy.
4 = plant with a severe wilt, over 50% of plant wilted and mature leaves desiccated.
5 = plant dead.

Data were analysed using a linear mixed model (LMM) approach in ASReml-r (Butler et al. 2018) with pedigree linking accessions across experiments, and incorporated data collected in both BS17000 and BS12021.

Powdery mildew

Resistance to powdery mildew (PM, caused by *P. aphanis*) was assessed in four experiments conducted at MRF from June to August 2019 to 2022. Plants were grown in substrate in a protected cropping environment that favoured PM development and the pathogen was allowed to naturally spread. A total of 78 accessions were assessed, including 16 ASBP varieties, 36 breeding accessions, 22 pre-breeding accessions, and four non-ASBP varieties. Thirty-two pre-breeding families (crosses between PM-resistant accessions and advanced accessions or ASBP varieties) with a total of 1,159 progeny were also assessed.

Disease severity was scored individually for leaves and fruit on a five-point scale (Table 2). Disease scoring was performed at three time points approximately four weeks apart, from June to August.

Table 3. Symptoms associated with disease score ratings of powdery mildew symptoms in leaves and fruit

Rating	Leaf symptoms	Fruit symptoms
1	No symptoms	No achenes (seeds) infected
2	Live PM mycelium present but no abnormal leaf curl	Up to 25% of achenes infected
3	Abnormal leaf curl in up to 25% of the plant	25-50% of achenes infected
4	Abnormal leaf curl in up to 50% of the plant	50-75% of achenes infected
5	Abnormal leaf curl in more than 50% of the plant	75-100% of achenes infected

Data were analysed using a LMM approach in ASReml-r (Butler et al. 2018) with pedigree linking accessions across experiments.

High health testing and *in vitro* storage

Rapid deployment of high health plants to propagators following commercialisation decisions is essential for fruit growers to have access to the new varieties as soon as possible. A barrier to the rapid release of new varieties is the time needed to certify disease-free status, requiring a minimum of three years before material can be distributed to runner growers. To reduce the time needed we routinely undertook 2X molecular and 1X biological virus indexing of all new pre-commercial accessions and established them in tissue culture prior to sending to Agribio for testing and certification. This substantially reduced the amount of testing and time required in the system before commercial deployment—from three years down to two—and presented significant cost savings. Virus indexing occurred simultaneously with the preparation of selections for testing in on-farm trials. Indexed material at the pre-commercialisation stage was also distributed to runner growers under Material Transfer Agreements (MTAs) for early bulking of plant numbers, trialling on farms in greater numbers, and to speed up commercial deployment.

Valuable accessions and historic material were also stored and maintained using a novel long-term *in vitro* (tissue culture) protocol at MRF. As well as maintaining plant high health status, this approach reduces multiplication and therefore increases the likelihood of genotypes staying true-to-type over time (Paynter 2022). This material has the potential to be accessed in future years to refresh nucleus material provided to propagators where genetic drift of varieties has occurred.

Results and discussion

Breeding trials

The number of seedling and clonal accessions assessed in the project are summarised in Table 4. The break-down of seedling accessions assessed for each region was: 49% temperate (Figures 2 and 3), 46% subtropical (Figures 4 and 5), and 5% Mediterranean (Figure 6), which approximately matches the relative size of each industry. Detailed results from all advanced and on-farm trials are presented in the Appendices [confidential]).

Table 4. Cumulative number of accessions assessed each year in trials across the project.

Region	Seedlings	Early-stage accessions	Advanced-stage accessions	On-farm accessions
Temperate	69,202	365	147	54
Subtropical	65,268 *	468	129	23
Mediterranean	6,682	80	17	16

* Subtropical numbers include foundation (pre-breeding) work for specialty varieties (white, pink, dark fruit). Non-specialty (i.e. standard red) seedling total for subtropical was 51,425. Now that quality specialty selections have been generated these genetics are being used in crosses for the other regions.

Figure 2. Alan Noon (contact via karen.spencer@daf.qld.gov.au) in the temperate clonal and seedling trial at the Wandin Research Centre, 5/11/2021. Photo by Karen Spencer (karen.spencer@daf.qld.gov.au).

Figure 3. Temperate seedling trial at Applethorpe Research Facility, 15/10/2021. Photo by Alan McWaters (alan.mcwaters@daf.qld.gov.au)

Figure 4. Katie O'Connor (katie.oconnor@daf.qld.gov.au) assessing the subtropical breeding trial at MRF, 7/9/2021. Photo by Jodi Neal (jodi.neal@daf.qld.gov.au).

Figure 5. Jodi Neal assessing the subtropical seedling trial at BRF, 3/9/2021. Photo by Katie O'Connor.

Figure 6. Mediterranean breeding trial (inner four rows) at Bullsbrook 14/9/2022. Photo by Jodi Neal.

Released varieties

Two temperate varieties were released ('Tahli-ASBP' and 'Tamara-ASBP'), one subtropical ('Susie-ASBP'), and two specialty ('SB17-230-ASBP' and 'SW20-317-ASBP'). An additional subtropical accession (2017-040) has been flagged for probable commercial release following an additional year of trialling in greater numbers on farms, as requested by the Subtropical Reference Group. Two Mediterranean accessions were identified as potential candidates for commercial release; however, a final decision on these was pushed back a year due to COVID-19 travel restrictions resulting in 2020 trials being run at MRF and breeders being unable to visit the 2021 trials.

In BS17000 Plant Breeder's Rights (PBR) Part I was submitted for varieties 'Tahli-ASBP', 'Tamara-ASBP', 'Susie-ASBP', and 'SB17-230-ASBP' (Table 5). PBR Part I forms for 'SW20-317-ASBP' and 2017-040 ('Stella-ASBP') have been completed and distributed to DAF and Hort Innovation for signing prior to submission to IP Australia. PBR Part II was submitted for varieties released in the previous project BS12021: 'Fanfare-ASBP', 'Jubilee-ASBP', 'Rosalie-ASBP', 'Meadowsong', 'Venus-ASBP', 'Scarlet-silk', and 'Summer Song'. PBR Part II will be submitted for 'Susie-ASBP', 'SB17-230-ASBP', 'Tahli-ASBP' and 'Tamara-ASBP' in January 2023.

Table 5. Plant Breeder's Rights (PBR) status of recent ASBP varieties.

Variety	PBR Part I submitted	PBR accepted	Application no.	PBR Part II submitted	PBR granted
Scarlet Rose-ASBP	12/04/2017	7/06/2017	2017/093	5/07/2017	17/08/2018
Sunglow-ASBP	6/06/2017	6/07/2017	2017/170	5/07/2017	19/03/2020
Fanfare-ASBP	26/02/2018	21/03/2018	2018/045	29/09/2020	7/10/2022
Jubilee-ASBP	26/02/2018	21/03/2018	2018/048	29/09/2020	7/10/2022
Meadowsong	26/02/2018	21/03/2018	2018/047	29/09/2020	7/10/2022
Rosalie-ASBP	26/02/2018	21/03/2018	2018/044	29/09/2020	7/10/2022
Scarlet-silk	26/02/2018	21/03/2018	2018/050	29/09/2020	Pending
Summer Song	26/02/2018	21/03/2018	2018/046	29/09/2020	Pending
Venus-ASBP	26/02/2018	21/03/2018	2018/049	29/09/2020	7/10/2022
SB17-230-ASBP	13/07/2021	24/07/2021	2021/152	Planned for Jan 2023	
Susie-ASBP	13/07/2021	24/07/2021	2021/153	Planned for Jan 2023	
Tahli-ASBP	13/07/2021	24/07/2021	2021/151	Planned for Jan 2023	
Tamara-ASBP	13/07/2021	24/07/2021	2021/150	Planned for Jan 2023	
SW20-317-ASBP	Submitted to DAF & Hort Innovation for signing				
Stella-ASBP	Submitted to DAF & Hort Innovation for signing				

Certified high health plants of all varieties have been made available to all ASPAA and VSICA accredited propagators, except for 'SW20-317-ASBP' which is still undergoing health testing.

Information sheets were created for all new varieties based on analysed clonal values from five years of trial data and disease resistance results. These are presented in Figures 7 to 11 below.

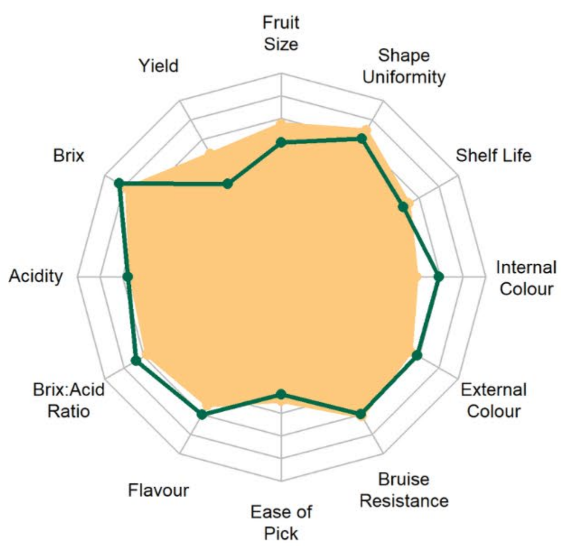
Queensland Government

TAHLI-ASBP

Tahli-ASBP is a new day-neutral temperate variety released by the Australian Strawberry Breeding Program (ASBP). Tahli has much higher yield and average fruit size than a widely grown temperate commercial variety. It has an excellent overall flavour score and high brix, and is moderately tolerant to charcoal rot. Fruit is displayed further away from the canopy than many existing temperate varieties which makes Tahli suitable for tabletop production.

Right: Radar chart comparing Tahli-ASBP (yellow polygon) with a widely grown temperate commercial variety (green line). The centre of the plot represents the lowest value for each trait, and the outside line represents the highest value.

	Tolerant	Moderately tolerant	Moderately susceptible	Susceptible	Unknown
Charcoal rot		●			
Colletotrichum wilt			●		
Fusarium wilt			●		
Powdery mildew			●		



Green line = commercial temperate variety

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Figure 7. Temperate variety 'Tahli-ASBP' information sheet.

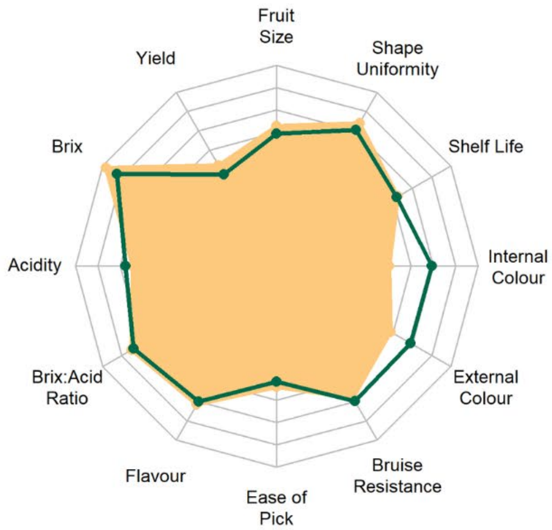
Queensland Government

TAMARA-ASBP

Tamara-ASBP is a new day-neutral temperate variety released by the Australian Strawberry Breeding Program (ASBP). This variety is a premium strawberry with traits targeted for consumer satisfaction. Tamara has extremely high brix and moderate acidity, and a similar excellent flavour score to Albion. Tamara has moderate charcoal rot tolerance, however is susceptible to rain damage and may therefore perform better under protected cropping.

Right: Radar chart comparing Tamara-ASBP (yellow polygon) with a widely grown temperate commercial variety (green line). The centre of the plot represents the lowest value for each trait, and the outside line represents the highest value.

	Tolerant	Moderately tolerant	Moderately susceptible	Susceptible	Unknown
Charcoal rot		●			
Colletotrichum wilt			●		
Fusarium wilt			●		
Powdery mildew				●	



Green line = commercial temperate variety

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Figure 8. Temperate variety ‘Tamara-ASBP’ information sheet.

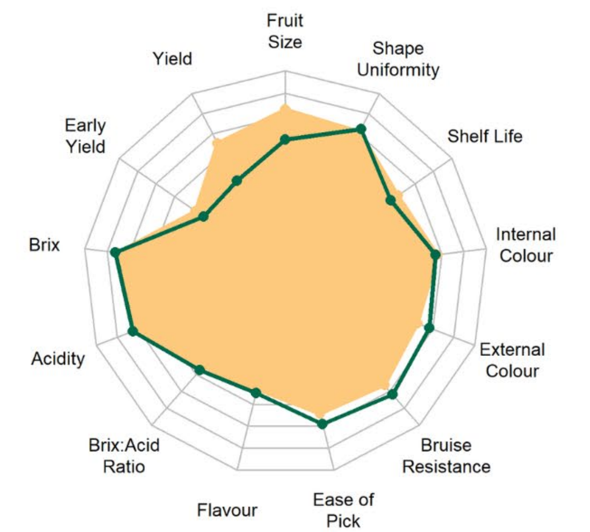
Queensland Government

SUSIE-ASBP

Susie-ASBP is a new subtropical variety released by the Australian Strawberry Breeding Program (ASBP). This variety has higher yield and larger average fruit size than Red Rhapsody, and slightly higher early-season yield. Flavour rating, brix and acidity levels are similar to that of Red Rhapsody.

Right: Radar chart comparing Susie-ASBP (yellow polygon) with Red Rhapsody (green line). The centre of the plot represents the lowest value for each trait, and the outside line represents the highest value.

	Tolerant	Moderately tolerant	Moderately susceptible	Susceptible	Unknown
Charcoal rot			●		
Colletotrichum wilt			●		
Fusarium wilt	●				
Powdery mildew			●		



Green line = Red Rhapsody

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Figure 9. Subtropical variety 'Susie-ASBP' information sheet.

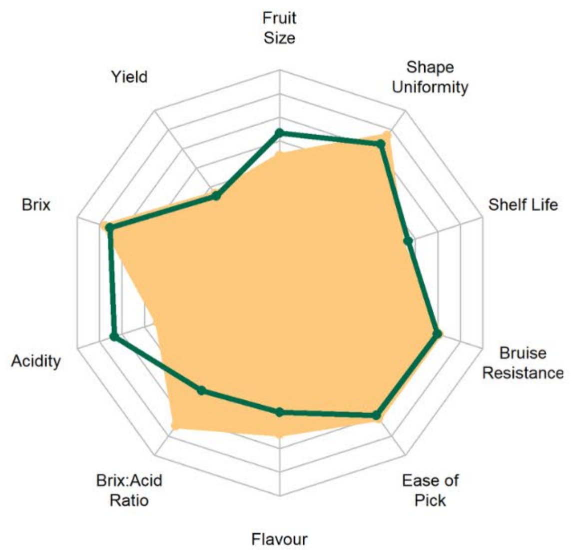
Queensland Government

SB17-230-ASBP

SB17-230-ASBP is a new day neutral, pink strawberry variety released by the Australian Strawberry Breeding Program (ASBP). This variety has highly attractive pale pink fruit with a peach blush, and is pure white inside. It has excellent flavour with a slight pineapple taste and a very high brix:acid ratio.

Right: Radar chart comparing SB17-230-ASBP (yellow polygon) with Red Rhapsody (green line). The centre of the plot represents the lowest value for each trait, and the outside line represents the highest value.

	Tolerant	Moderately tolerant	Moderately susceptible	Susceptible	Unknown
Charcoal rot	●				
Colletotrichum wilt	●				
Fusarium wilt			●		
Powdery mildew			●		



Green line = Red Rhapsody

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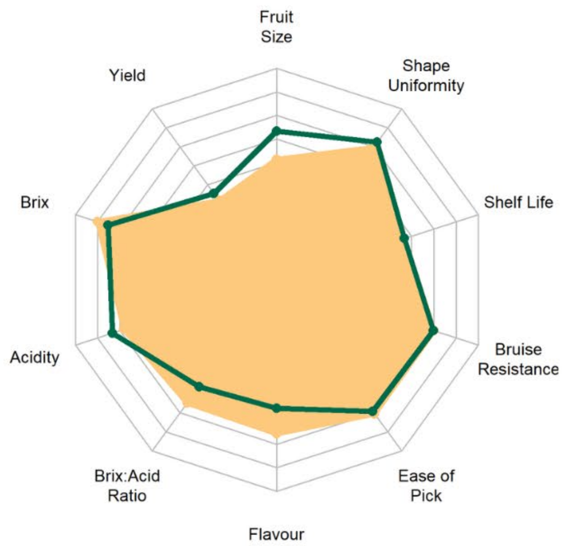
Figure 10. Specialty pink variety ‘SB17-230-ASBP’ information sheet.

SW20-317-ASBP

SW20-317-ASBP is a new day neutral, white-fruited strawberry variety released by the Australian Strawberry Breeding Program (ASBP). This variety has highly attractive white fruit with a pale pink blush. It has very good flavour with a slight pineapple taste and high brix:acid ratio. The long truss length of this variety makes it highly suitable for production on tabletops.

Right: Radar chart comparing SW20-317-ASBP (yellow polygon) with Red Rhapsody (green line). The centre of the plot represents the lowest value for each trait, and the outside line represents the highest value.



Green line = Red Rhapsody

	Tolerant	Moderately tolerant	Moderately susceptible	Susceptible	Unknown
Charcoal rot					●
Colletotrichum wilt					●
Fusarium wilt					●
Powdery mildew					●

Figure 11. Specialty white variety 'SW20-317-ASBP' information sheet.

Variety uptake

For the 2021-22 financial year, ASBP varieties represented a record high of 47% of all plant sales nationally (Figure 12). The most popular ASBP variety has been 'Red Rhapsody', followed by 'Sundrench' and 'Scarlet Rose-ASBP'. 'Red Rhapsody' was planted extensively in both subtropical and Mediterranean regions in 2022. 'Scarlet Rose-ASBP' is also reported to be performing well in Mediterranean climates.

'Susie-ASBP' was planted commercially for the first time in subtropical regions in 2022, with mixed feedback. Abnormal climatic patterns may have contributed to poor performance on some farms, and it's hoped that it will perform more consistently in future seasons. Some growers who trialled 'Susie-ASBP' for the first time in 2022 are planting substantially larger numbers in 2023.

The most popular ASBP temperate variety is 'Tahli-ASBP', followed by 'Scarlet-silk'. Plantings of these varieties are increasing each year, with positive performance being reported, especially in substrate culture.

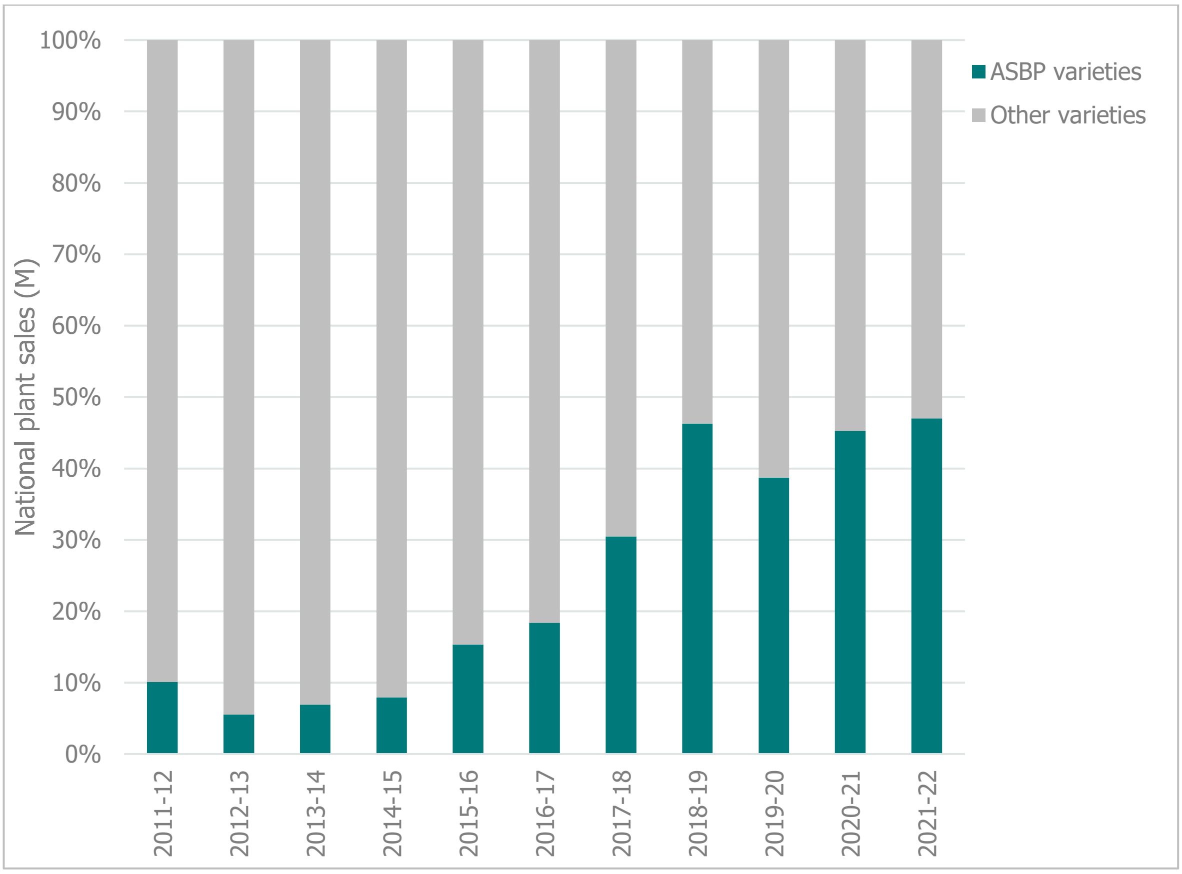


Figure 12. Percentage of ASBP plant sales over time compared with total national sales.

Genetic gain

Steady progress has been made in all three production regions for the major selection traits. Genetic gain over time (clonal values) for the key profitability traits 'average fruit weight' and 'yield per plant' are shown in Figures 13 to 16 below for temperate and subtropical trials. Mediterranean gain isn't shown due to small trial sizes and a short breeding time. Particular progress has been made for truss architecture in the temperate breeding population, from highly branched trusses in the older material to very few branches in recent cohorts. This change has been made to support more consistent fruit size across the season, which is currently a major limitation to profitability in temperate regions.

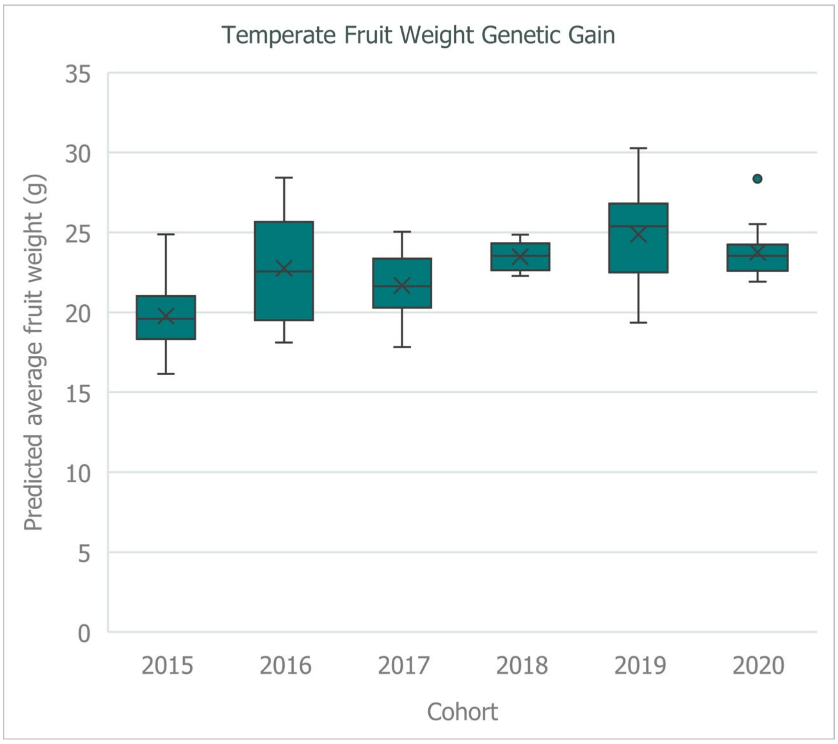


Figure 13. Temperate genetic gain in average fruit size in advanced-stage trials at Wandin Research Centre.

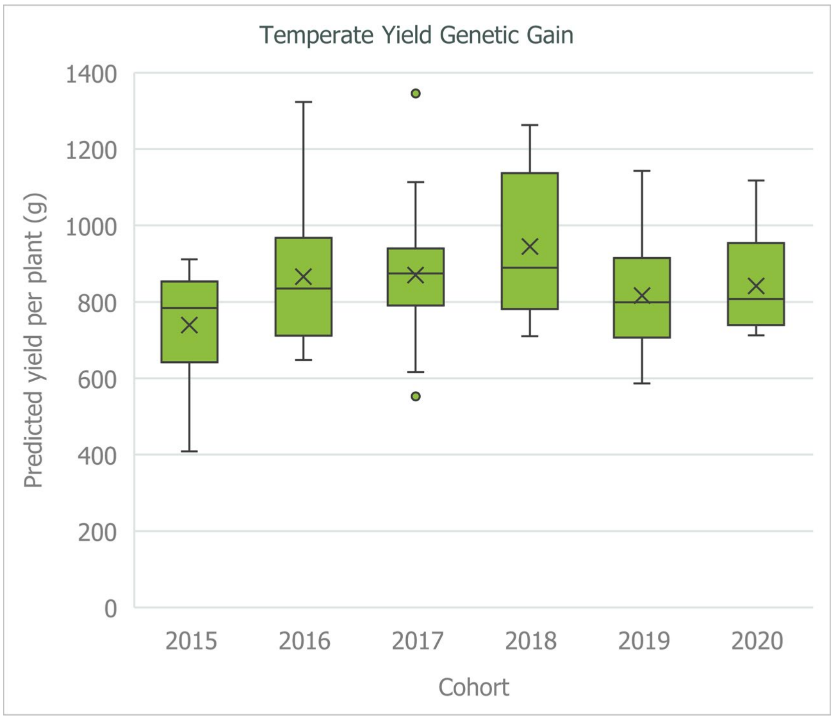


Figure 14. Temperate genetic gain in yield per plant in advanced-stage trials at Wandin Research Centre.

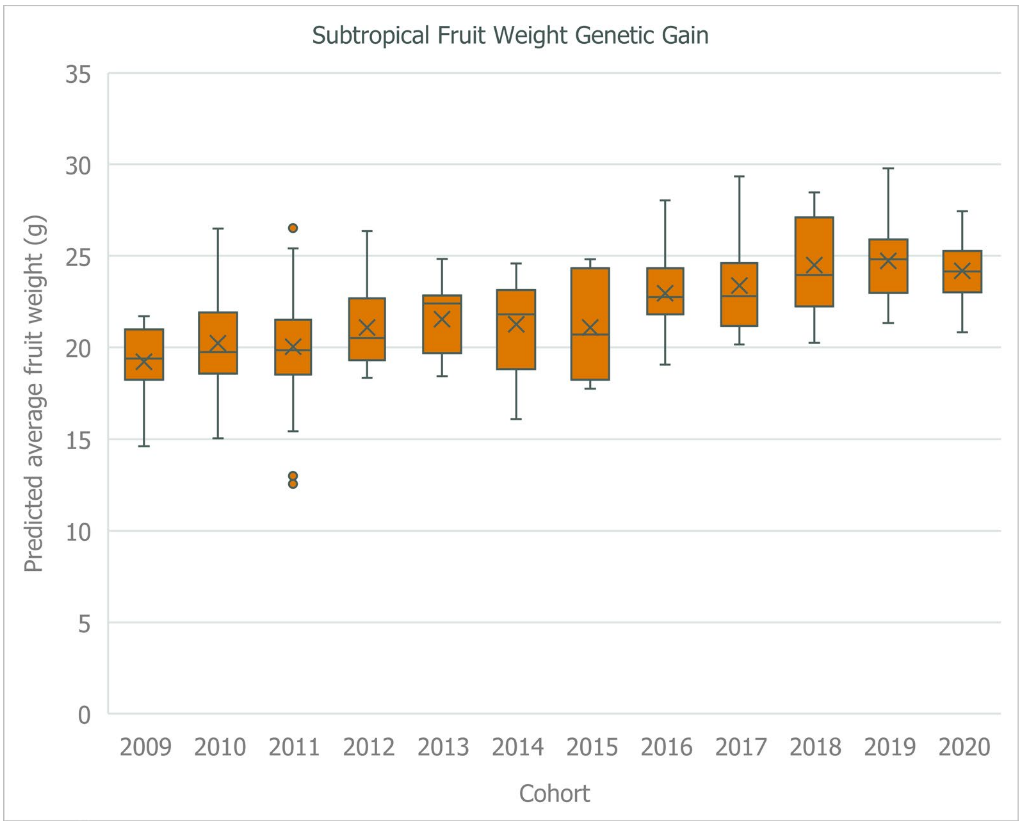


Figure 15. Subtropical genetic gain in average fruit weight in advanced-stage trials at Maroochy Research Facility.

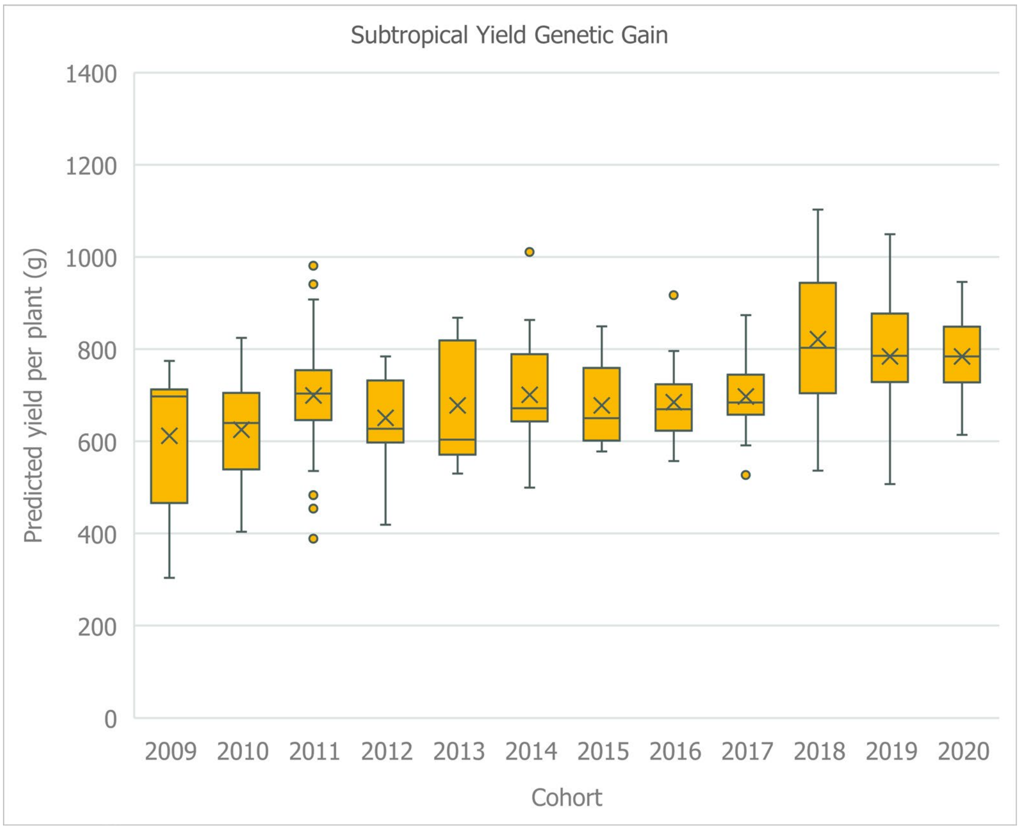


Figure 16. Subtropical genetic gain in yield per plant in advanced-stage trials at Maroochy Research Facility.

Marker assisted selection

A total of 147 individuals were tested for the DN QTL across the three breeding nodes (Table 6). Among the temperate genotypes tested, 60% were positive for the DN gene, compared to 72% for subtropical and 92% for Mediterranean. Among those accessions with the DN gene were temperate cultivars 'Tahli-ASBP' and 'Tamara-ASBP', and advanced subtropical selection 2017-040 ('Stella-ASBP').

Table 6. Summary of individuals tested for the day neutrality QTL across the three breeding nodes.

Node	Number tested	Positive	Negative
Temperate	45	60%	40%
Subtropical	90	72%	28%
Mediterranean	12	92%	8%

Across the three breeding nodes, 217 individuals were tested for the "peach" flavour QTL (Table 7). A total of 38% contained the gene in the temperate node, 57% in subtropical and 54% in the Mediterranean node. Cultivars 'Tahli-ASBP', 'Tamara-ASBP' and 'Susie-ASBP', specialty selection 'SB17-230-ASBP' (blush) and advanced subtropical selection 2017-040 all tested positive for the flavour gene.

Table 7. Summary of individuals tested for the "peach" flavour QTL across the three breeding nodes.

Node	Number tested	Positive	Negative
Temperate	80	38%	62%
Subtropical	112	57%	43%
Mediterranean	24	54%	46%

Genome-wide parental selection

A total of 632 accessions from across the three breeding nodes have been genotyped for SNP markers to date (Table 8), including some from work coordinated by the international RosBreed project. For use in GS analyses, 416 genotypes have been used across the temperate and subtropical nodes.

Table 8. Number of accessions genotyped for SNPs per breeding node.

Node	Number tested	Number used in genomics analyses
Temperate	127	119
Subtropical	494	297
Mediterranean	11	0
Total	632	416

In the subtropical node, heritability ranged from 0.05 (for rain damage resistance) to 0.74 (for average fruit weight) (Table 9). Predictive ability was highest for abrasion resistance (correlation of 0.61 between GEBVs and phenotypes) and bruise resistance (0.58), and lowest for display (0.09). Both May yield and season yield per plant showed moderate prediction accuracies with 0.44 and 0.50, respectively. These moderate to high prediction accuracies indicate that genetic gain through genomic selection is achievable, and that we can be reasonably confident in selecting the best accessions to use in marker assisted parental selection each year.

Table 9. Subtropical multi-variate genomic prediction accuracy (predictive ability) and heritability for 11 traits.

Trait	Assessment time/s through season	Predictive ability (correlation)	Heritability
Brix	Multiple	0.36	0.08
Acid	Multiple	0.50	0.24
Rain damage resistance	Multiple	0.39	0.05
Abrasion resistance	Multiple	0.61	0.18
Bruise resistance	Multiple	0.58	0.39
Display	Single	0.09	0.19
Average fruit weight	Single	0.47	0.74
May yield per plant	Single	0.44	0.54
Truss type	Single	0.41	0.35
Season yield per plant	Single	0.50	0.72
Bush size	Single	0.43	0.56

Genomics analyses for traits measured multiple times throughout the temperate season all had substantially lower heritability compared to traits measured a single time. Heritability was highest for average fruit weight (0.59) and season yield per plant (0.56) (Table 10). Estimates were the same across subtropical and temperate nodes for truss type (0.35 for both nodes).

Six individuals with a good combination of predicted trait performances were chosen to be used as parents in 2021 cross-pollinations. In 2022, 20 seedlings were selected from crosses of four of these parents (2020-114, 2020-162, 2020-211, 2020-233) for further trialing, including nine from parent 2020-114.

Table 10. Temperate multi-variate heritability for 11 traits.

Trait	Assessment time/s through season	Heritability
Brix	Multiple	0.02
Acid	Multiple	0.16
Rain damage resistance	Multiple	0.06
Sun damage resistance	Multiple	0.01
Bruise resistance	Multiple	0.08
Display	Single	0.46
Average fruit weight	Single	0.59
Truss type	Single	0.35
Ease of pick	Single	0.31
Season yield per plant	Single	0.56
Bush size	Single	0.36

Disease resistance screening

A range of disease resistances from highly tolerant to highly susceptible were observed for all four diseases across the material tested (Figures 17 to 21). Advanced accessions with good tolerances were used as parents in standard controlled crosses to incorporate resistance genes into the broader breeding population. Accessions with high tolerance but poor performance for other traits (e.g. fruit size, firmness) were used as parents in pre-breeding crosses, in order to maintain tolerance while incrementally improving the other traits.

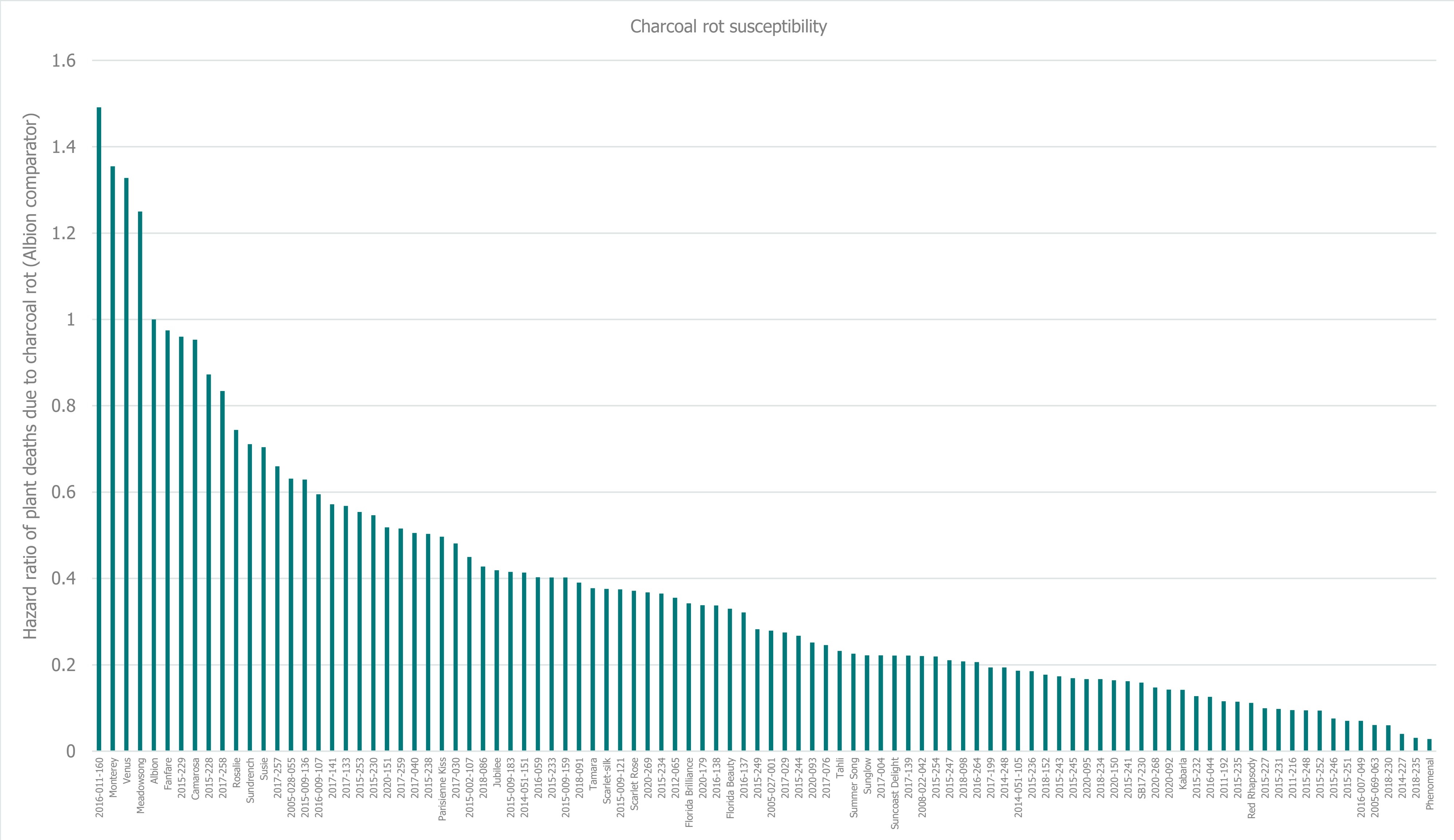


Figure 17. Charcoal rot hazard ratio with the reference susceptible cultivar Albion for all genotypes assessed. A hazard ratio of 1 means that selection is just as likely to die from charcoal rot as Albion, a ratio of 0.5 means it is half as likely to die as Albion.

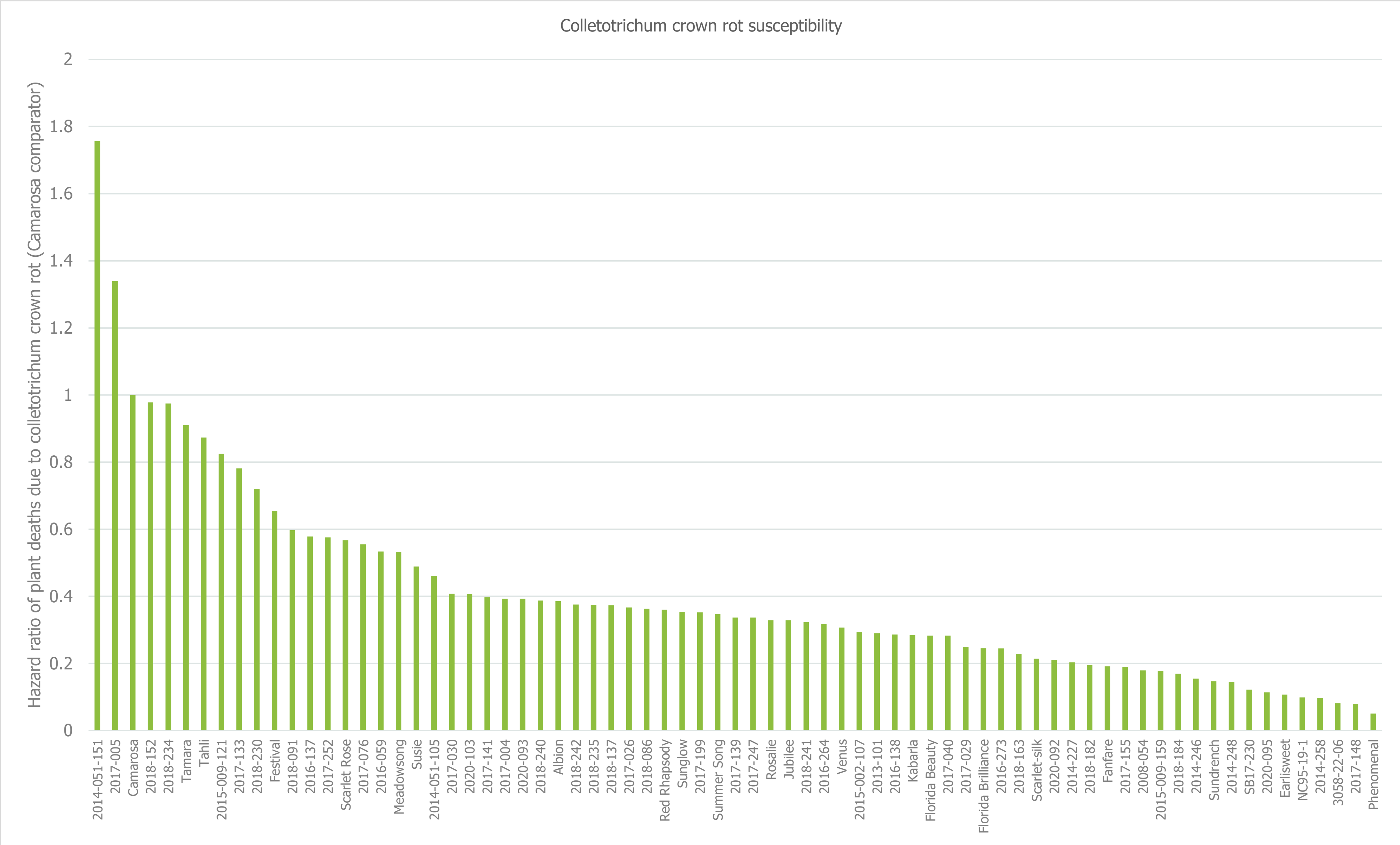


Figure 18. Colletotrichum crown rot hazard ratio with the reference susceptible cultivar Camarosa for all genotypes assessed. A hazard ratio of 1 means that selection is just as likely to die from colletotrichum crown rot as Camarosa, a ratio of 0.5 means it is half as likely to die as Camarosa.

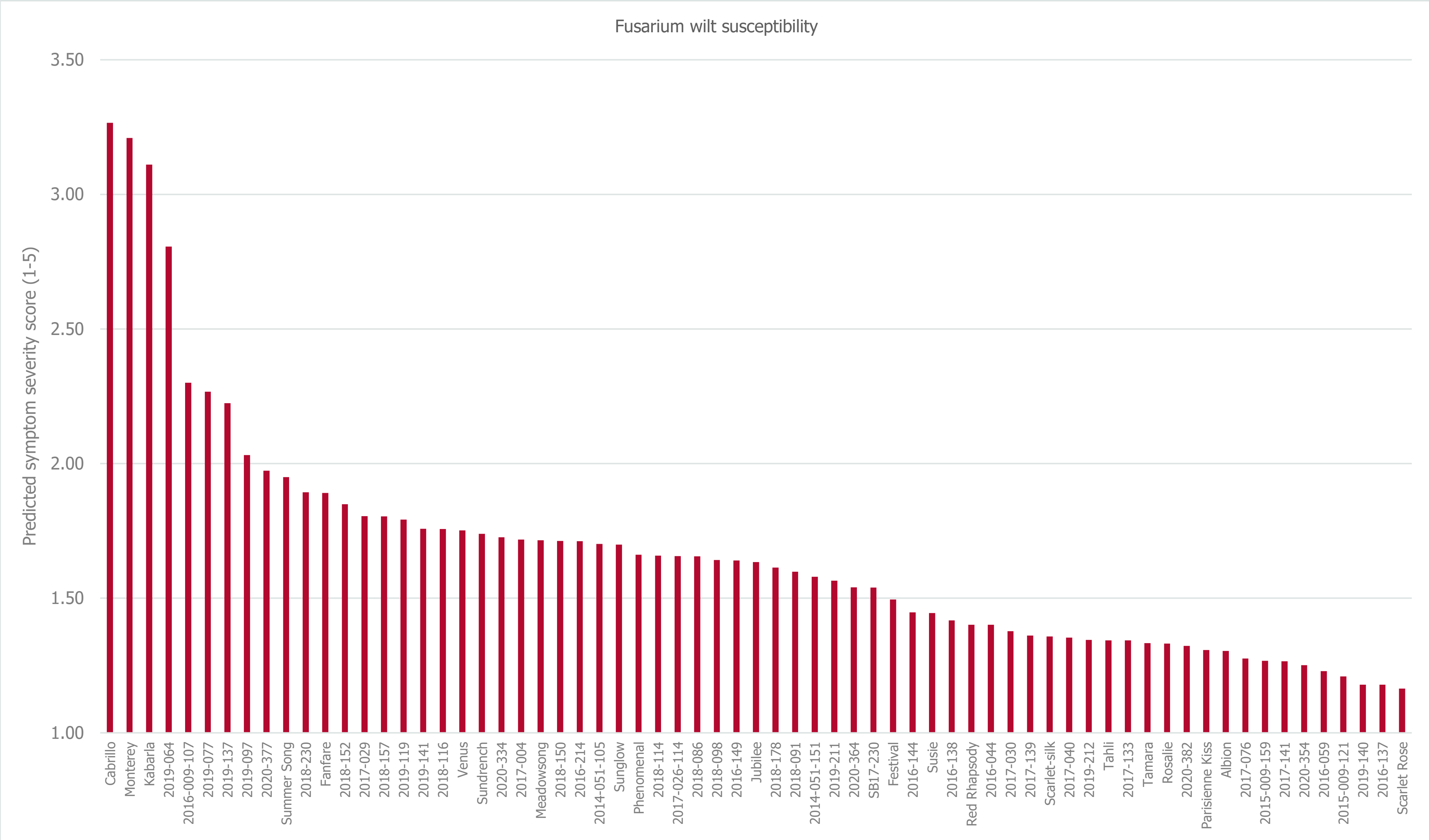


Figure 19. Fusarium wilt predicted disease severity scores. 1 = no symptoms, 5 = plant death.

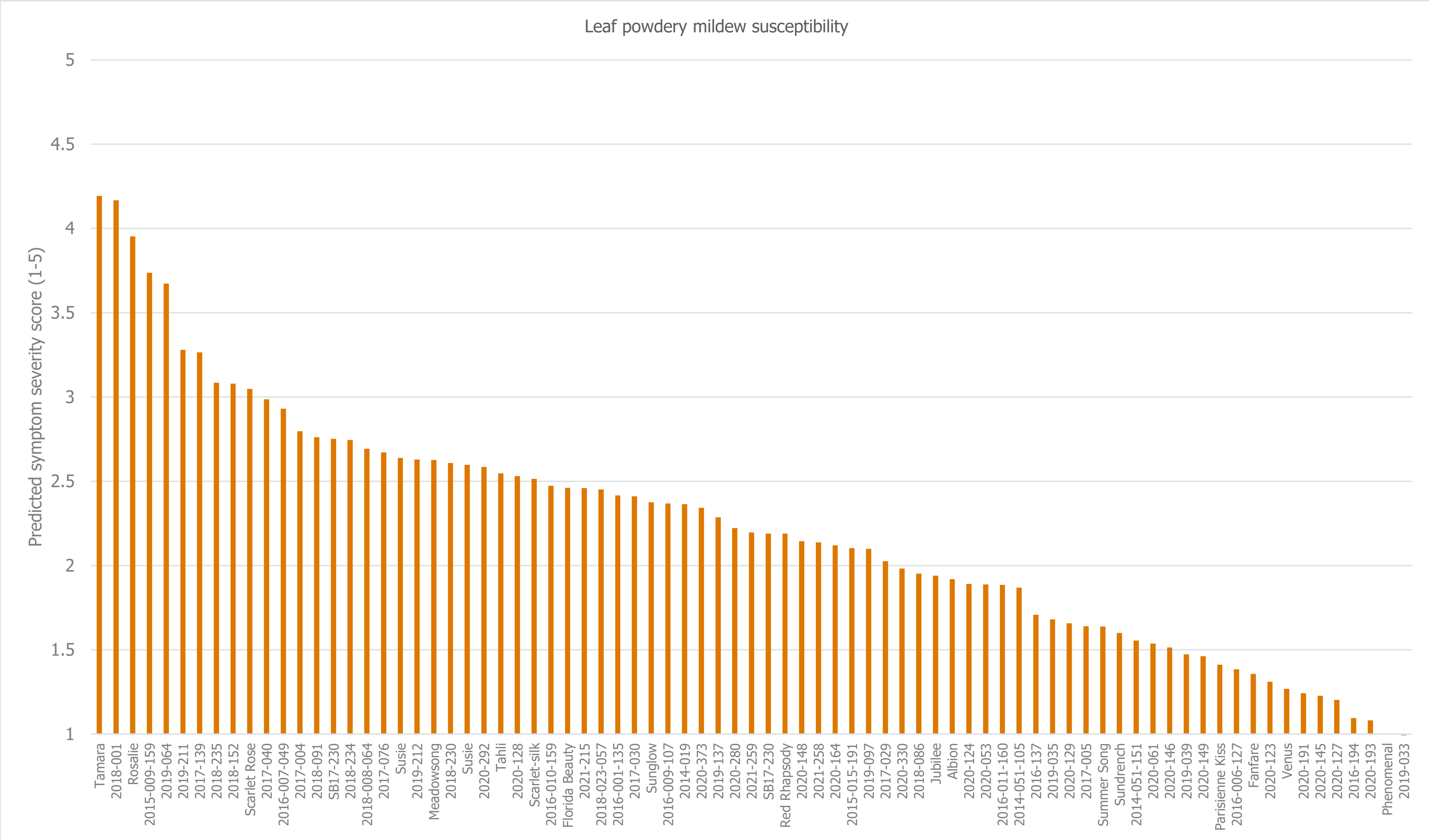


Figure 20. Leaf powdery mildew predicted disease severity scores. 1 = no symptoms, 5 = severe symptoms.

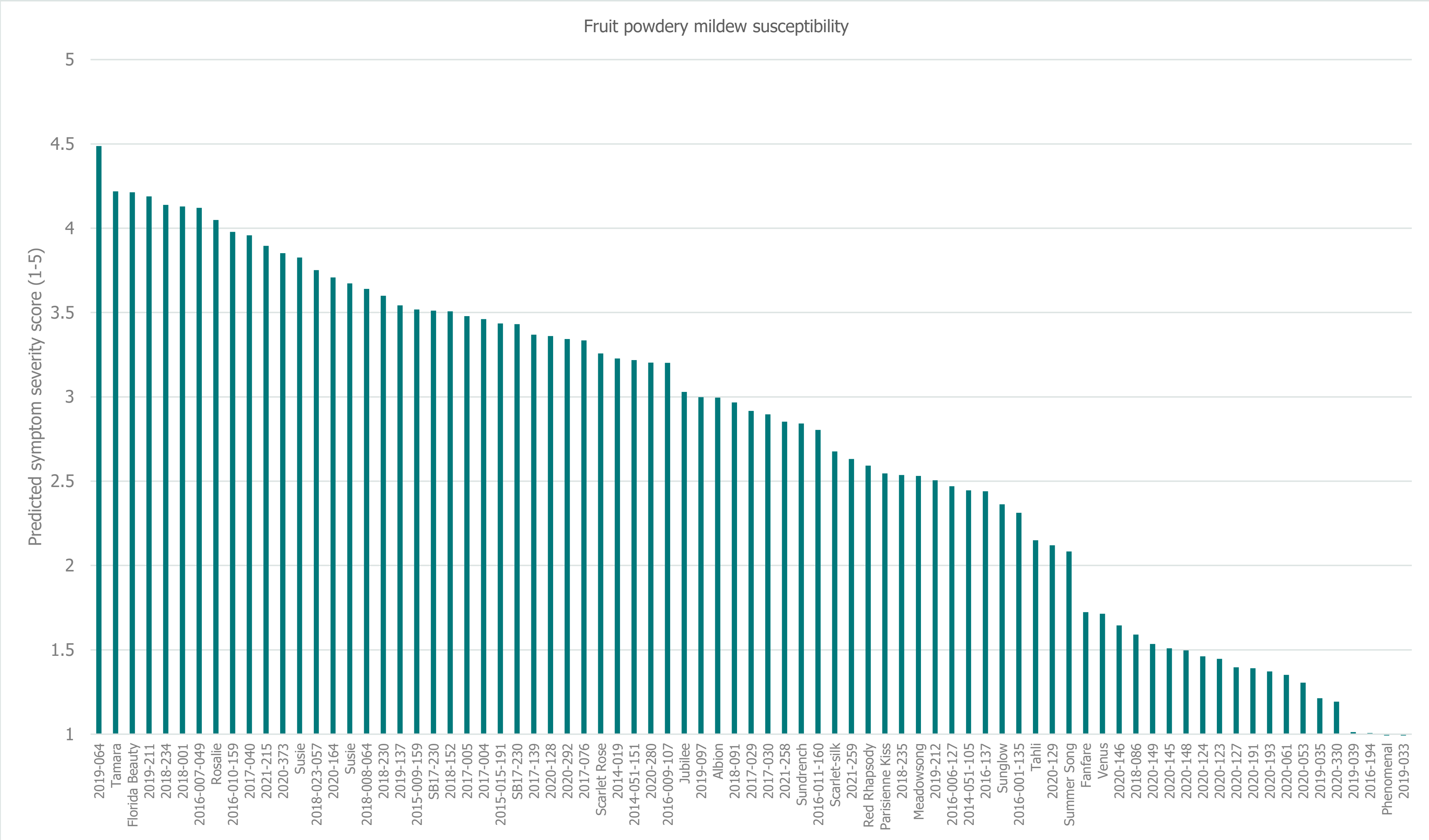


Figure 21. Fruit powdery mildew predicted disease severity scores. 1 = no symptoms, 5 = severe symptoms

Outputs

Table 11. Output summary

Output	Description	Detail
At least two superior strawberry varieties that match consumer expectations and produce higher value product more efficiently are available to growers in each of the three specified growing regions.	Two temperate varieties have been released ('Tahli-ASBP' and 'Tamara-ASBP'). One subtropical variety has been released ('Susie-ASBP') with one more ('Stella-ASBP', 2017-040) pending broader trialling as requested by Subtropical Reference Group. Two Mediterranean selections (2019-211 and 2019-212) have been trialled on farms in the region and commercial release decisions will be made pending industry feedback in early 2023.	The release of these varieties has been guided by Reference Groups and grower feedback, based on trial results and performance on farms. They have been selected due to superior performance in these trials compared with existing varieties widely grown by industry. Varieties were protected under Plant Breeder's Rights (PBR). Certified high health plants of each of them was made available to all interested propagators under sub-licenses from the commercialisation partner QSGA.
At least one identifiable and value-added specialty variety is available to consumers (e.g. fruit colours).	One specialty pink-fruited variety ('SB17-230-ASBP') has been protected under PBR and high-health plants distributed to all interested propagators in October 2022 for trialling. A white-fruited variety ('SW20-317-ASBP') is in the process of being protected under PBR and is currently undergoing high health testing.	Commercial sale of these varieties will not be possible until a commercialisation partner has been contracted for this material. This process is currently underway by Hort Innovation and DAF.
At least one variety suitable for substrate culture is available to fruit producers.	All released varieties were trialled both in-ground and in substrate culture prior to release and found to perform well under both systems. Early commercial performance of 'Tahli-ASBP' suggest that it performs especially well in substrate.	This is based on on-farm trial data and feedback from growers, propagators, and our commercialisation partner.

Outcomes

Table 12. Outcome summary

Outcome	Alignment to fund outcome, strategy and KPI	Description	Evidence
Contracted commercialisation partners make new varieties widely available to domestic and international growers.	Alignment with Outcome 2, Strategy 1 of the 2022-2026 Hort Innovation Berry Strategic Investment Plan – Ensure that superior strawberry varieties that match consumer expectations are available to growers across Australia.	The Queensland Strawberry Growers Association (QSGA) was contracted as subtropical and temperate commercialisation partners. They have made certified high health material of all released varieties available to all interested sub-contracted propagators.	Sub-contracts are in place between QSGA and plant propagators. Variety performance information and growing recommendations are listed on the Australasian Plant Genetics website https://ausplantgenetics.com.au/asbp-varieties/. Varieties are available for sale from various propagators.
An increase in adoption of Australian varieties to 65% of total plantings achieved by 2025 across all three climatic growing regions.	Alignment with Outcome 2, Strategy 1 of the 2022-2026 Hort Innovation Berry Strategic Investment Plan	We are on-track to achieve 65% of national plantings by 2025. In 2021-22 ASBP varieties represented 47% of all commercial plants sold across Australia.	Annual ASBP variety sales reports from QSGA and total national plant sales as determined by industry levy collection.

Monitoring and evaluation

Table 13. Key Evaluation Questions

Key Evaluation Question	Project-specific questions	Project performance	Continuous improvement opportunities
1. To what extent has the project achieved its expected outcomes?	To what extent has the Australian strawberry industry adopted new varieties made available through the project?	Early sales of the new varieties are encouraging. Temperate variety 'Tahli-ASBP' has been selling well and receiving mostly positive feedback. Mother plants of 'Tamara-ASBP' and 'Susie-ASBP' are still limited and so plants of these are only available in small numbers. However, interest in these varieties has been strong. 'SB17-230-ASBP' was only released to propagators for trial in 2022 pending a contracted commercialisation partner, and so no plants of these have yet been sold.	Variety uptake will be enhanced by the provision of basic information on fertiliser requirements and other growing requirements. This was started in BS17000 using fertiliser information from the breeding trials but should be routinely applied as all future varieties are released.
2. How relevant was the project to the needs of intended beneficiaries?	To what extent have the new varieties met the needs of the Australian strawberry industry?	The new varieties were selected based on high performance for key traits as defined by the regional Reference Groups and other industry engagement, and identified by economic modelling. All commercialisation and advanced selections decisions were also guided by the Reference Groups and on-farm trial growers. It is still too early to assess how well these varieties meet industry needs in a commercial setting, however early reports are mostly positive.	Identifying selections that match industry needs would benefit from an extra year of larger-scale on-farm trialling, as is currently being done for the subtropical region. It's suggested that this be undertaken for temperate and Mediterranean regions also.
3. How well have intended beneficiaries been engaged in the project?	To what extent were the regional steering committees engaged to define breeding objectives and provide input on selection decisions? Have regular project updates been provided through linkage with the industry communication project?	Regional Reference Groups were engaged annually in face-to-face and/or teleconference meetings with the express aim to define breeding targets, provide input on selection decisions, and provide feedback on general project operations. They were also encouraged to attend farm walks of trial sites throughout the project. Twelve project updates were provided at a minimum of twice per year in industry publications Simply Red and Australian Berry Journal. Presentations were also given at nine events organised by the communication project across multiple states (not including farm walks). See Appendix 18 for specific details.	We've found it challenging to lock in formal and unchanging Temperate and Mediterranean Reference Group members. It would be useful to work with these industries early in the next project to identify industry members willing to be formal group members.

4. To what extent were engagement processes appropriate to the target audience/s of the project?	How accessible were project updates and extension events and to industry levy payers?	An extensive number of engagement activities were undertaken, targeted for growers and grower interests. These activities and participant numbers are outlined in Appendix 18 and included 17 presentations at regional meetings, industry forums, field days, and industry conferences, 10 farm walks of trial sites and grower's trials, and 12 articles in industry publications.	Due to the distances involved it has proven challenging to get subtropical growers interested in travelling to MRF for farm walks except for one-on-one visits. An alternative for the future may be farm walks of on-farm trials in each of the Caboolture and Bundaberg regions.
5. What efforts did the project make to improve efficiency?	What efforts did the project make to improve efficiency? What efforts did the project make to reduce time from initial cross to variety release?	• Marker assisted parental selection to use the best seedlings as parents sooner. • Streamlining annual reporting using R Markdown. • Streamlining data formatting with Microsoft Excel macros and R. • Streamlining data analyses by using ASReml-R (previously used stand-alone ASReml). • Using radar plots of performance to assist selection and crossing decisions. • A new barcode labelling system for streamlining inventory management of tissue culture plantlets. • Using a commercial potato digger to dig subtropical strawberry plants at the end of the season rather than digging each plant by hand. Note that this approach won't work for the temperate trials due to runners.	• Application of Field Scorer app for making field data collection and collation easier. • Barcoding temperate trial harvesting containers for faster and more reliable data collection. This is already being applied in subtropical and Mediterranean trials.

Recommendations

- Advanced breeding and evaluation methodologies should be incorporated in future projects where feasible, including predictive agriculture techniques using genomics and rapid phenotyping.
- With the relatively recent change in lead breeders in the ASBP it is recommended that a review be undertaken of current and historic diversity levels in the program, genetic gain across all traits, and experimental design and data analysis methodology.
- Based on current industry and marketing trends, it is recommended that key directions for future breeding should include varieties for alternative production systems, and with characteristics optimised for ease of robotic harvesting.

Refereed scientific publications

Gomez, A.O., De Faveri, J., Neal, J.M., Aitken, E.A.B. and Herrington, M.E., 2020. Response of Strawberry Cultivars Inoculated with *Macrophomina phaseolina* in Australia. *International Journal of Fruit Science* 20:sup2, 164-177, https://doi.org/10.1080/15538362.2019.1709114

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Intellectual property

See Table 5 above for Plant Breeder's Rights status of all varieties released in BS17000. A full IP Register can be found in Appendix 21.

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Appendices

Note: Appendices 1 to 16 (annual regional reports) and appendix 21 (IP Register) are confidential and attached separately.

Appendix 17. Report on genomics-assisted breeding

Summary

DNA-informed breeding using genomics offers an opportunity to increase the efficiency of breeding, and has been applied in many rosaceous crops, including strawberry [2]. Two genetic markers have been routinely screened in advanced material and parents of the Australian Strawberry Breeding Program in BS17000: perpetual flowering/day neutrality (DN) and "peach" flavour (γ-decalactone). Genomic prediction was used to identify elite subtropical seedling selections based on predicted performance using genome-wide markers, and these were used in cross-pollinations to quickly advance the genetics of the population. One major advantage of genetic marker testing and genomic prediction is that individuals with elite predicted performance can be identified at an early stage in the breeding cycle and be used as parents to form the next generation of the breeding population.

Introduction

Breeding in horticulture can be limited by the time involved in propagating plants and data collection of phenotypic performance, and the unknown genetic control of traits. DNA-informed breeding using genomics offers an opportunity to increase the efficiency of breeding, and has been applied in many rosaceous crops, including strawberry [2]. DNA data capture genetic relatedness among individuals much more accurately than pedigree records. It can also be used to infer phenotypic performance, through marker-assisted selection (MAS), which involves combining genetic marker data with phenotypic data. This is particularly valuable for traits that are difficult or expensive to evaluate.

There are two main types of MAS: selection of single Quantitative Trait Loci (QTLs) that have a large effect on a trait, and genomic selection (GS) based on the combined effect of all markers across the genome. Since genetic marker data can be attained from DNA of a seedling, trait performance can be inferred very early on in the breeding cycle.

Two QTL markers have been routinely screened in the Australian Strawberry Breeding Program in BS17000: perpetual flowering/day neutrality (DN) and "peach" flavour. Another flavour QTL for "sherry" flavour [3] had previously been tested in BS17000 but has since been abandoned due to inaccurate and inconsistent results found by the University of Florida breeding program.

There are two main fruit-bearing types of strawberry plants: day neutral (DN) and short day (SD) plants. DN plants, also called everbearing or perpetual flowering, initiate flowering over an extended period throughout the season, with almost continuous flowering and fruiting. In contrast, SD plants require a climatic trigger with a specific photoperiod to initiate flowering, and usually produce fruits over a much shorter time frame. This trait is of economic interest to strawberry growers, as DN plants have an extended production season, whilst SD plants involve more labour-intensive farming in a short window. A genetic marker called Bx215 is linked to the major dominant *FaPFRU* locus controlling the DN trait [4-7], and is up to 93.1% accurate in identifying the bearing type of accessions in the University of Florida strawberry breeding program [7].

Strawberry flavours and aromas are a product of a combination of acids, sugars, and compounds such as terpenes, esters, ketones and lactones [8]. γ-decalactone is the most abundant lactone in strawberry and conveys a "peachy" aroma and overall improved flavour. The main dominant locus LGIII-2 is linked to the *FaFAD1* gene controlling γ-decalactone content and can be inferred using gel electrophoresis (presence/absence of fluorescent band) [9, 10].

Genomic selection (GS) involves selecting individuals based on predicted performance using genome-wide markers [11]. In GS, a set of individuals is used to develop a statistical model using both genetic and phenotypic data for a particular trait or set of traits. The individuals are divided into two groups, usually

80% training and 20% testing. The known phenotypic values of the testing group are masked (set to missing), and the data of the training set are used to predict the masked phenotypes. The accuracy of the predictive model is the correlation between the predicted and known phenotypes. Once an accurate prediction model has been developed, it can be applied to an untested group of individuals to predict their performance, or genomic estimated breeding values (GEBVs).

One major advantage of QTL testing and GS is that individuals with elite predicted performance can be identified at an early stage in the breeding cycle. These individuals can then be used as parents to form the next generation of the breeding population, in a method called marker-assisted parental selection (MAPS). GS has been used for MAPS in the subtropical node, and predictive models have been developed for deployment in the temperate node of the ASBP in BS17000.

Methodology

Large effect genes (QTLs)

Routine screening was conducted on 147 and 217 accessions across all three breeding nodes for DNA markers linked with perpetual flowering/day neutrality (DN) [4-7] and "peach" flavour [9, 10], respectively. Young leaf material was collected from healthy plants and freeze-dried over silica gel overnight.

For the DN QTL, 30mg of dried leaf material was supplied to the Australian Genome Research Facility (AGRF) for DNA extraction and subsequent analysis of the Bx215 marker. This marker is a microsatellite (simple sequence repeat), in which the presence of an allele at around 128 bp indicates day neutrality in that accession [7]. Polymerase chain reaction (PCR) was used to amplify the DNA using routine master mix, buffers and reagents, DNA and forward and reverse primers. The following PCR conditions were used: 94°C for 1 min, followed by 10 cycles at 94°C for 30 sec, 65°C for 45 sec, touching down 1°C per cycle and 72°C for 1 min, then 30 cycles of 94°C for 30 sec, 55°C for 45 sec and 72°C for 1 min, with a final elongation step at 72°C for 10 min. PCR products were visualised by capillary electrophoresis (Figure 1) on an Applied Biosystems 3730 DNA Analyzer, and alleles were scored using GeneMapper software version 5.0 (Applied Biosystems).

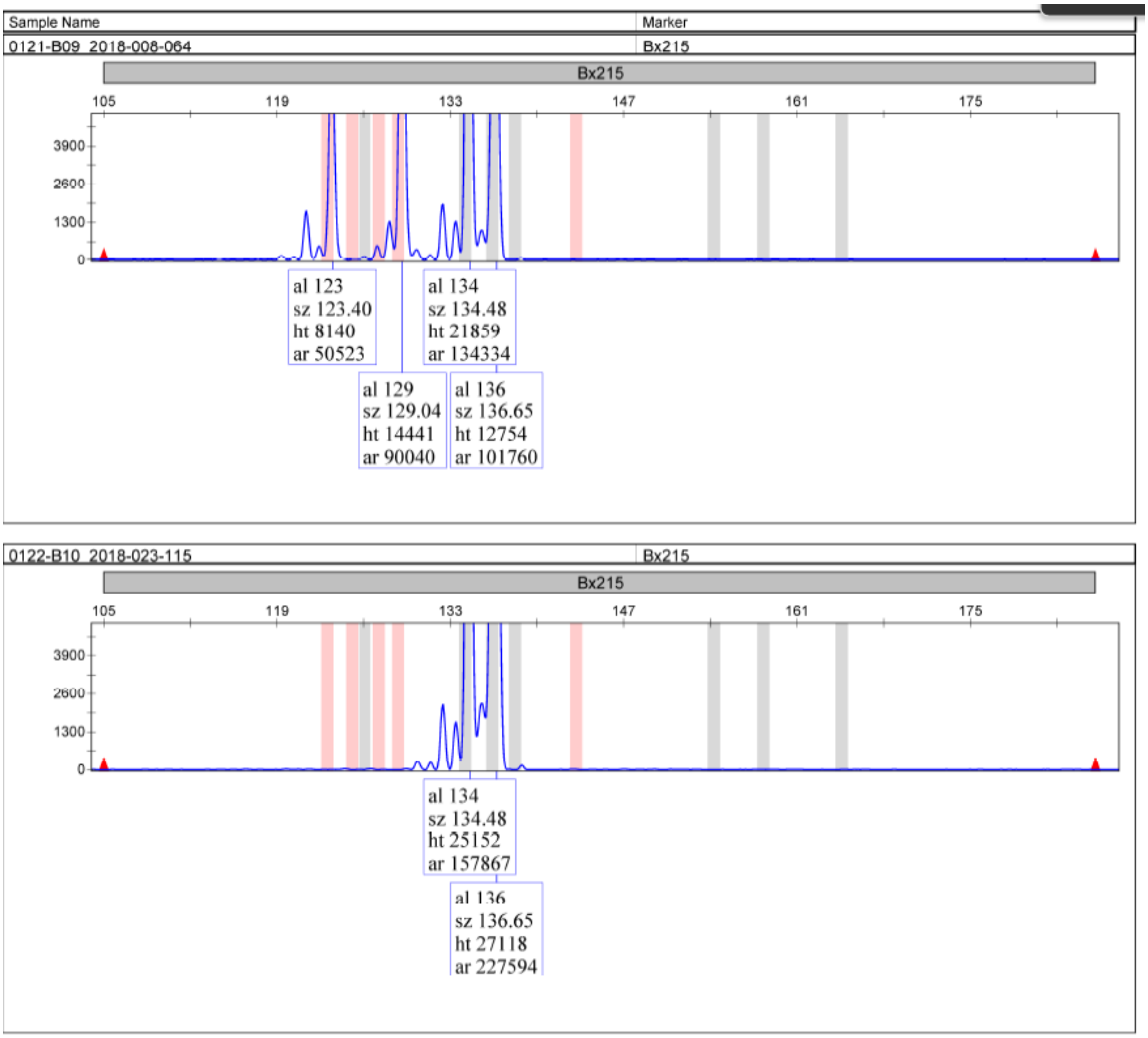

Figure 1 Output from GeneMapper software for scoring of alleles for day neutral gene. Presence of an allele at around 128 bp ("al" in blue box) indicates day neutrality (positive). The top plot form sample 2018-008-064 has the allele in this location (positive), whereas the bottom plot for 2018-023-115 does not have the allele (negative).

For the γ-decalactone "peach" flavour QTL, DNA from 30mg of dried leaf material was extracted using Qiagen DNEasy mini extraction kit and manufacturer's protocols. The following PCR conditions were used: 95°C for 3 min, followed by 35 cycles at 95°C for 30 sec, 55°C for 30 sec, and 72°C for 45 sec, with a final elongation step at 60°C for 7 min. PCR products were visualised via 2% agarose gel, with a band present at 140 bp indicating presence of γ-decalactone (Figure 2).

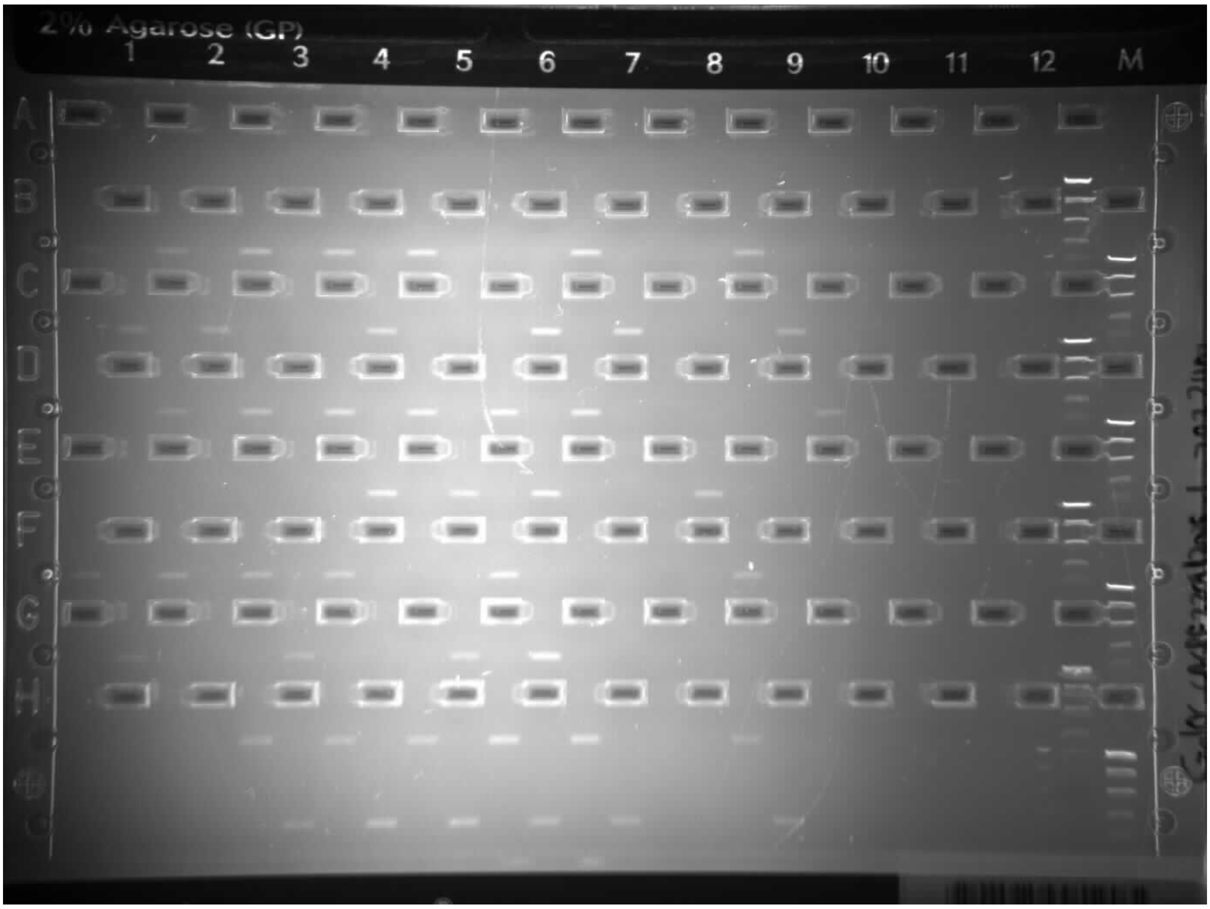

Figure 2 Photo of agarose gel for "peach" flavour QTL from AGRF. Light coloured bands denotes the presence of an allele at 140 bp, which indicates that individual is positive.

Genomic selection

Genomic selection (GS) was performed on subtropical material, and prediction models were developed for the temperate breeding node. Young leaf material was collected from healthy plants and freeze-dried over silica gel overnight. 30mg of dried leaf material was supplied to Genotyping Australia in Western Australia for DNA extraction and genetic marker analysis. Single nucleotide polymorphism (SNP) markers were obtained using the Affymetrix IStraw90 Axiom array [12]. Markers were filtered using the *snpReady* package [13] in R [14] to remove those with low (<2.5%) minor allele frequency and high missing rates (>20%). Missing calls were imputed using the 'k-nearest neighbour index' method. A genomic relationship matrix was developed to model the kinship and relatedness among individuals.

Phenotypic data from subtropical clonal trials at Maroochy Research Facility, and temperate clonal trials at Wandin Research Centre from 2017 to 2021 were used for genomics analyses. Nine traits were measured in both regions: fruit acidity, fruit bruise resistance, plant bush size, average fruit weight over trial season, yield per plant over trial season, display (short/long trusses with fruit under/above leaves), proportion of fruit with no rain damage, soluble solids content (sweetness, brix), and truss type (branched/non-branched). In addition, fruit abrasion resistance and yield per plant in May were measured for the subtropical node, and sun burn resistance and ease of pick for the temperate node.

Staff members from the University of Queensland were contracted for genomic analyses in BS17000. Individual trait models were first explored to investigate individual year and harvest date genetic variances. Traits were then grouped according to whether they were assessed a single time or multiple times throughout the season each year, and models were developed for the two groups. Different multi-variate predictive models were explored using factor analytic analyses, with the optimal model being chosen as the one that best fit the data. Narrow-sense genomic heritability was estimated for each trait for subtropical and temperate nodes to evaluate the strength of genetic control of the trait.

To determine the accuracy of the best prediction models, five-fold cross validation was used, with 80% of individuals in the training set and 20% in the testing set. Predictive ability (PA) was estimated as the correlation between phenotypic breeding values and GEBVs. Mean PA over the five folds was calculated. Prediction models were applied to the genotypes of 89 subtropical seedling selections from 2020 to obtain their GEBVs. Individuals with a good combination of predicted trait performances were chosen to be used as parents in 2021 cross-pollinations.

Results and discussion

Large effect genes (QTLs)

A total of 147 individuals were tested for the DN QTL across the three breeding nodes (Table 1). Among the temperate genotypes tested, 60% were positive for the DN gene, compared to 72% for subtropical and 92% for Mediterranean. Among those accessions with the DN gene were temperate cultivars 'Tahli-ASBP' and 'Tamara-ASBP', and advanced subtropical selection 2017-040.

Table 1 Summary of individuals tested for the day neutrality QTL across the three breeding nodes.

Node	Number tested	Positive	Negative
Temperate	45	60%	40%
Subtropical	90	72%	28%
Mediterranean	12	92%	8%

Across the three breeding nodes, 217 individuals were tested for the "peach" flavour QTL (Table 2). A total of 38% contained the gene in the temperate node, 57% in subtropical and 54% in the Mediterranean node. Cultivars 'Tahli-ASBP', 'Tamara-ASBP' and 'Susie-ASBP', specialty selection 'SB17-230-ASBP' (blush) and advanced subtropical selection 2017-040 all tested positive for the flavour gene.

Table 2 Summary of individuals tested for the "peach" flavour QTL across the three breeding nodes.

Node	Number tested	Positive	Negative
Temperate	80	38%	62%
Subtropical	112	57%	43%
Mediterranean	24	54%	46%

These results are informative in multiple ways. Firstly, they help inform selections of genotypes for progression to further rounds of testing and also release of varieties to industry. Secondly, the results have been conveyed to growers in the end-of-season reports for each breeding node. Thirdly, knowing the QTL status of parents helps to guide parental combinations for cross-pollinations; crossing a parent positive for the dominant flavour gene with a parent negative for the gene is likely to result in offspring that are positive for the gene.

Genomic selection

A total of 632 accessions from across the three breeding nodes have been genotyped for SNP markers to date (Table 3), including some from work coordinated by the international RosBreed project. For use in GS analyses, 416 genotypes have been used. In 2022, leaf samples from 384 selections sent to Genotyping Australia in April for SNP genetic markers were unsuccessfully analysed. Issues in the sample collection and preparation process rendered the DNA inadequate for sequencing. Equipment malfunctions resulted in a delay of replacement samples being sent. It was decided that there were too few genotyped individuals from the temperate node to robustly perform GS analyses, thus only preliminary models were constructed to estimate heritability of ten traits. No genomics analyses were performed for the Mediterranean node, as only 11 individuals were genotyped.

Table 3 Number of accessions genotyped for SNPs per breeding node.

Node	Number tested	Number used in genomics analyses
Temperate	127	119
Subtropical	494	297
Mediterranean	11	0

Table 4 Subtropical multi-variate genomic prediction accuracy (predictive ability) and heritability for 11 traits.

Trait	Assessment time/s through season	Predictive ability (correlation)	Heritability
Brix	Multiple	0.36	0.08
Acid	Multiple	0.50	0.24
Rain damage resistance	Multiple	0.39	0.05
Abrasion resistance	Multiple	0.61	0.18
Bruise resistance	Multiple	0.58	0.39
Display	Single	0.09	0.19
Average fruit weight	Single	0.47	0.74
May yield per plant	Single	0.44	0.54
Truss type	Single	0.41	0.35
Season yield per plant	Single	0.50	0.72
Bush size	Single	0.43	0.56

In the subtropical node, heritability ranged from 0.05 (for rain damage resistance) to 0.74 (for average fruit weight) (Table 4). Predictive ability was highest for abrasion resistance (correlation of 0.61 between GEBVs and phenotypes) and bruise resistance (0.58), and lowest for display (0.09). Both May yield and season yield per plant showed moderate prediction accuracies with 0.44 and 0.50, respectively. These moderate to high prediction accuracies indicate that genetic gain through genomic selection is achievable, and that we can be reasonably confident in selecting the best accessions to use in MAPS each year.

Table 5 Temperate multi-variate heritability for 11 traits.

Trait	Assessment time/s through season	Heritability
Brix	Multiple	0.02
Acid	Multiple	0.16
Rain damage resistance	Multiple	0.06
Sun damage resistance	Multiple	0.01
Bruise resistance	Multiple	0.08
Display	Single	0.46
Average fruit weight	Single	0.59
Truss type	Single	0.35
Ease of pick	Single	0.31
Season yield per plant	Single	0.56
Bush size	Single	0.36

Genomics analyses for traits measured multiple times throughout the temperate season all had substantially lower heritability compared to traits measured a single time. Heritability was highest for average fruit weight (0.59) and season yield per plant (0.56) (Table 5). Estimates were the same across subtropical and temperate nodes for truss type (0.35 for both nodes).

Six individuals with a good combination of predicted trait performances were chosen to be used as parents in 2021 cross-pollinations (coloured in blue in Table 6). In 2022, 20 seedlings were selected from crosses of four of these parents (2020-114, 2020-162, 2020-211, 2020-233) for further trialing, including nine from parent 2020-114. Only selection 2020-114 was retained from 2021 to 2022 clonal trials, and although susceptible to red leaf disorder, it still shows good promise to be used as a parent.

Table 6 Genomic-estimated breeding values for 89 subtropical selections from 2020 for 11 traits. Red-green colour scales are formatted for each trait, with green = good and red = bad. Seedlings coloured blue were chosen to be used as parents in cross-pollination.

Seedling	ABRS	BA	BRUS	BUSH	CAVF	CYLDP	DISP	MYLD	RPROP	SSC	TRUS
2020-070	6.8	0.81	75.5	4.5	17.4	547	6.8	38	0.61	8.4	6.3
2020-072	7.2	0.71	79.3	3.9	17.9	386	6.9	29	0.62	7.9	6.6
2020-073	7.0	0.73	77.7	4.1	17.8	426	6.9	29	0.60	8.1	6.5
2020-074	7.2	0.71	79.0	3.9	17.7	383	6.9	27	0.57	7.9	6.6
2020-080	7.0	0.74	76.2	4.2	16.2	454	6.8	31	0.61	8.1	6.4
2020-081	7.0	0.79	77.1	4.3	18.1	514	6.9	37	0.63	8.3	6.4
2020-084	6.9	0.76	77.1	4.2	19.5	477	6.8	34	0.52	8.2	6.4
2020-088	6.5	0.86	73.4	4.8	18.1	629	6.7	43	0.60	8.7	6.1
2020-097	6.6	0.75	72.6	4.4	16.5	472	6.6	29	0.50	8.2	6.2
2020-101	6.7	0.74	74.2	4.3	17.3	458	6.7	29	0.48	8.1	6.3
2020-114	6.8	0.88	75.7	4.7	18.7	650	6.8	48	0.62	8.7	6.2
2020-116	6.9	0.81	76.3	4.5	19.4	541	6.8	38	0.58	8.5	6.3
2020-119	6.9	0.76	76.2	4.3	19.2	481	6.8	33	0.54	8.2	6.4
2020-122	6.9	0.79	76.4	4.3	18.3	527	6.8	39	0.56	8.3	6.3
2020-131	7.0	0.77	76.7	4.3	18.2	481	6.9	33	0.51	8.3	6.4

Seedling	ABRS	BA	BRUS	BUSH	CAVF	CYLDP	DISP	MYLD	RPROP	SSC	TRUS
2020-135	7.1	0.73	77.0	4.0	17.2	429	6.8	32	0.66	7.9	6.4
2020-137	7.1	0.74	77.7	4.0	17.8	451	6.8	34	0.63	8.0	6.4
2020-143	7.0	0.79	77.2	4.3	18.3	515	6.8	38	0.61	8.3	6.4
2020-156	7.0	0.73	76.8	4.2	16.9	430	6.8	28	0.51	8.1	6.5
2020-162	7.5	0.70	81.3	3.7	17.9	369	7.0	30	0.56	7.8	6.7
2020-163	6.9	0.75	75.0	4.3	17.1	476	6.7	33	0.55	8.1	6.3
2020-164	6.9	0.83	76.0	4.5	18.9	585	6.8	44	0.59	8.5	6.2
2020-176	6.8	0.66	75.1	4.0	16.9	335	6.7	18	0.57	7.8	6.4
2020-178	6.6	0.80	73.7	4.6	18.1	544	6.7	36	0.51	8.4	6.2
2020-185	6.9	0.75	75.5	4.2	18.7	473	6.7	32	0.53	8.2	6.3
2020-195	6.8	0.78	75.3	4.4	16.7	508	6.7	35	0.65	8.2	6.3
2020-197	6.9	0.74	76.2	4.2	18.2	444	6.8	30	0.55	8.1	6.4
2020-200	6.8	0.74	75.4	4.2	18.3	454	6.7	31	0.56	8.1	6.3
2020-203	7.1	0.78	78.1	4.3	19.3	503	6.9	37	0.57	8.3	6.4
2020-204	7.1	0.77	77.8	4.3	19.0	488	6.9	35	0.56	8.3	6.4
2020-205	7.0	0.79	77.0	4.3	17.2	525	6.8	39	0.68	8.3	6.3
2020-206	6.8	0.81	74.9	4.5	16.9	553	6.7	38	0.64	8.4	6.2
2020-211	7.1	0.81	77.8	4.4	18.0	549	6.9	42	0.67	8.4	6.4
2020-214	6.9	0.76	76.0	4.3	17.7	472	6.8	32	0.55	8.3	6.4
2020-215	6.9	0.68	75.6	4.0	17.1	366	6.7	22	0.50	7.9	6.4
2020-223	6.8	0.78	75.4	4.5	17.8	511	6.8	34	0.60	8.4	6.3
2020-224	7.0	0.77	77.4	4.3	18.3	483	6.9	34	0.55	8.3	6.4
2020-225	6.7	0.74	74.0	4.3	17.6	460	6.6	30	0.52	8.1	6.2
2020-227	6.5	0.86	72.5	4.9	17.8	641	6.7	43	0.61	8.7	6.0
2020-228	6.8	0.86	75.9	4.7	18.9	617	6.9	44	0.61	8.7	6.2
2020-229	6.8	0.72	75.3	4.1	16.5	422	6.7	28	0.50	7.9	6.3
2020-230	7.1	0.82	79.0	4.3	19.4	553	6.9	44	0.63	8.3	6.4
2020-231	7.0	0.86	77.1	4.6	19.5	622	6.9	48	0.57	8.6	6.3
2020-233	6.8	0.89	75.2	4.8	19.1	667	6.8	49	0.56	8.8	6.1
2020-234	7.2	0.69	78.6	4.0	17.5	359	6.9	23	0.51	8.0	6.6
2020-235	6.8	0.74	75.1	4.3	17.4	448	6.7	29	0.52	8.1	6.3
2020-236	6.9	0.73	76.7	4.3	17.8	415	6.9	25	0.48	8.2	6.5
2020-238	7.2	0.68	78.5	3.8	17.1	351	6.9	24	0.58	7.8	6.6
2020-240	7.0	0.69	76.6	4.0	17.1	369	6.8	23	0.58	7.9	6.5
2020-241	6.7	0.80	73.3	4.6	17.7	549	6.7	36	0.53	8.4	6.1
2020-242	6.8	0.77	75.7	4.4	19.7	498	6.8	34	0.51	8.3	6.3
2020-243	7.0	0.78	77.5	4.2	18.3	498	6.8	37	0.59	8.2	6.4
2020-245	6.9	0.75	75.7	4.3	17.3	459	6.8	30	0.54	8.2	6.4
2020-250	7.0	0.75	77.4	4.0	18.1	456	6.8	34	0.54	8.0	6.4
2020-251	6.8	0.75	75.0	4.4	17.2	468	6.8	30	0.49	8.2	6.3
2020-253	6.9	0.71	76.5	4.1	18.2	407	6.8	27	0.58	8.0	6.4
2020-255	7.1	0.74	77.2	4.1	18.2	440	6.8	31	0.56	8.0	6.4
2020-256	7.3	0.77	80.2	4.1	19.6	482	7.0	39	0.56	8.2	6.5
2020-258	7.1	0.73	77.8	4.1	17.1	427	6.8	30	0.55	8.0	6.5
2020-259	6.9	0.67	75.8	3.9	17.7	342	6.7	20	0.53	7.8	6.4

Seedling	ABRS	BA	BRUS	BUSH	CAVF	CYLDP	DISP	MYLD	RPROP	SSC	TRUS
2020-261	7.1	0.67	77.8	3.9	18.3	341	6.8	22	0.55	7.8	6.6
2020-263	7.0	0.73	77.1	4.1	17.5	425	6.8	29	0.53	8.1	6.4
2020-265	7.3	0.73	79.3	3.9	17.7	417	6.9	32	0.66	7.9	6.5
2020-266	6.9	0.77	75.3	4.3	17.0	498	6.7	34	0.56	8.2	6.3
2020-267	6.7	0.78	73.6	4.6	16.7	506	6.7	30	0.56	8.4	6.2
2020-270	6.8	0.78	74.6	4.5	16.8	508	6.7	34	0.53	8.3	6.2
2020-271	7.0	0.76	76.3	4.3	17.0	480	6.8	33	0.60	8.2	6.3
2020-273	6.9	0.76	75.6	4.2	17.0	475	6.7	33	0.55	8.1	6.3
2020-275	6.9	0.78	75.8	4.5	18.1	503	6.8	33	0.57	8.4	6.4
2020-276	6.7	0.74	74.6	4.3	17.5	451	6.7	28	0.55	8.1	6.3
2020-278	6.9	0.74	75.8	4.2	17.2	451	6.8	30	0.50	8.1	6.4
2020-279	6.8	0.73	74.2	4.3	16.4	436	6.7	26	0.51	8.1	6.3
2020-280	6.8	0.80	75.0	4.4	17.3	542	6.7	39	0.63	8.3	6.2
2020-282	7.1	0.79	77.7	4.3	17.8	517	6.9	38	0.63	8.3	6.4
2020-284	7.2	0.73	78.5	4.0	16.3	430	6.9	32	0.65	8.0	6.5
2020-285	7.4	0.69	80.2	3.7	17.6	354	6.9	27	0.59	7.8	6.6
2020-286	7.1	0.77	78.3	4.2	17.6	478	6.9	36	0.60	8.2	6.4
2020-292	6.9	0.85	76.1	4.6	17.8	616	6.8	47	0.60	8.6	6.2
2020-293	6.8	0.74	74.9	4.3	16.5	442	6.8	27	0.56	8.2	6.3
2020-294	7.2	0.75	77.8	4.1	17.5	462	6.9	34	0.52	8.1	6.4
2020-298	7.0	0.73	76.3	4.2	17.4	432	6.8	28	0.53	8.1	6.4
2020-301	6.8	0.73	74.7	4.3	17.6	444	6.7	28	0.53	8.2	6.3
2020-304	7.0	0.80	76.9	4.4	18.6	531	6.9	38	0.64	8.4	6.4
2020-305	7.1	0.76	77.0	4.3	18.3	470	6.9	32	0.56	8.3	6.4
2020-308	7.0	0.80	77.3	4.4	19.4	524	6.9	38	0.52	8.3	6.4
2020-311	6.8	0.77	75.4	4.3	19.3	494	6.8	34	0.53	8.2	6.3
2020-312	7.0	0.74	76.5	4.1	18.6	447	6.8	31	0.54	8.0	6.4
2020-313	6.7	0.81	73.9	4.6	16.7	555	6.7	38	0.63	8.4	6.1
2020-317	7.0	0.65	76.0	4.0	14.9	310	6.8	16	0.55	7.8	6.5

For all traits, higher numbers are better, except for BUSH bush size where larger numbers indicate larger plants, which are undesirable. ABRS = abrasion resistance, BA = brix/acid ratio, BRUS = bruise resistance, BUSH = bush size, CAVF = season average fruit weight (g), CYLDP = season yield per plant (g), DISP = display of fruit from canopy, MYLD = yield per plant in May (g), RPROP = proportion of fruit with no rain damage, SSC = soluble solid content (brix, sweetness), and TRUS = truss branching type.

MAS for large effect QTLs can be expanded to include other published markers and any that we develop, including KASP (Kompetitive Allele Specific PCR) markers for in-house diagnoses for disease resistance, plant and fruit characteristics. This will include any markers linked with flavour/aroma/consumer traits identified in *AS19003 – Genetics of Fruits Sensory Preferences*. A DNA marker linked to resistance to Colletotrichum crown rot (*Colletotrichum gloeosporioides*) [15] will be tested in our breeding germplasm. Experiments will be undertaken to confirm the technique of using high resolution melting (HRM) analysis to discern between presence/absence of the markers in known resistant and susceptible germplasm accessions. Then, if successful, seedling selections from all regions will be screened routinely so that resistances can be incorporated into selection and crossing decisions. Markers for other diseases will be investigated and, if successful, similarly implemented. Under a different project, GWAS was conducted for susceptibility to powdery mildew using the ASBP powdery mildew screening experiments. Analyses are still ongoing, and the outcomes will be applied to the breeding program in the future.

Each year, the ASBP produces additional phenotypic data that can be added to the GS analyses, along with new genotypic data. More data will contribute to more robust and accurate predictive models, meaning that selections will continue to improve over time. When new genotype data are available for the temperate node, cross-validation methods can be used to estimate the accuracy of genomic prediction models. In future, routine genomic prediction could be performed for all three regions each year based upon the multi-variate genomic prediction models developed in conjunction with staff from the University of Queensland in BS17000. As the Mediterranean breeding germplasm is quite small in terms of genomics analyses, there is the possibility to combine Mediterranean and subtropical datasets where appropriate, especially since there is some overlap in parentage between the two nodes.

The best seedling selections from all regions can be genotyped each year, with genomic prediction models used to identify individuals with high genomic estimated breeding values across a number of traits for use as parents in the subsequent year. We will continue to add phenotypic data from manual and visual evaluations to models each year to improve predictions and to keep training populations relevant to the breeding population as it changes over time. Greater technical gains and efficiencies will be achieved though predictive agriculture (genomics) rather than high-throughput phenotyping techniques due to the variability within the breeding populations, compared to a more monoculture-based industrial setting where machine learning practices are often in place.

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Appendix 18. Extension and engagement activities

Activity	Description	Detail
Factsheets	A summary with information of released subtropical and temperate varieties was provided to QSGA, both in the form of factsheets and for the Australasian Plant Genetics website.	https://ausplantgenetics.com.au/asbp-varieties/
Industry publication - Simply Red magazine	Breeding Varieties for All Australian Strawberry Growers into the Future – New Australian Strawberry Breeding Project. Summary of the objectives and scope of project BS17000, 9/2018.	See Appendix 19.
	New Varieties for All Australian Strawberry Growers. A summary of outputs from project BS12021 and an update of recent progress, 9/2018.	See Appendix 20.
	An update on temperate breeding activities, 9/2019.	See end of Appendix 8.
Industry publication - Australian Berry Journal	Twice yearly updates on national breeding activities, since Summer 2020/21	Approx. 900 hard copy subscribers, and 300 online views. See end of Appendices 8-15.
	ASBP powdery mildew resistance screening, Autumn 2021.	
	Showcase of new subtropical and temperate ASBP varieties, Spring 2021.	
Industry forums	Jodi, updates on breeding for the temperate production region. Victorian industry forums 13/6/2018 and 13/6/2019. Applethorpe 17/9/2019.	Approx. 50 industry members attended 13/6/2018.
	Jodi and Apollo, progress in the breeding program to increase resistances to charcoal rot (*Macrophomina phaseolina).* Strawberry Charcoal Rot Forum in Applethorpe 17/10/2018.	Approx. 50 industry members attended.
	Jodi and Katie presented a 50 minute "virtual tour" of the strawberry breeding program, complete with videos of activities, to the Berry Industry Fair and Virtual Field Day in Wamuran on 8/7/2021.	This was in lieu of a subtropical farm walk, as labour shortages meant that growers largely didn't have the time to travel to Nambour.
Industry farm walks and field days	A Red Jewel Variety Field Day was held at Wamuran and included a number of advanced subtropical selections and recently commercialised ASBP varieties 29/9/2018.	Jodi attended to answer questions and receive grower feedback on the selections. Approximately 20 people attended.
	Farm walks of the Wandin breeding trial and temperate breeding update, 10/12/2019, 9/12/2020, 16/3/2022	10/12/2019: 12 attendees, 9/12/2020: three attendees, 16/03/2022: 20 attendees. Valuable feedback was received on individual selections.
	Jodi and Katie attended a farm walk at QSGA president Adrian Schultz's farm in Wamuran on 23/6/2020.	Attended by some other members of QSGA and QDAF staff.

Activity	Description	Detail
	A farm walk at a grower's property was held in-person and via teleconference in Victoria 20/4/2021.	Attended by around 10 industry members.
	The first ASBP Tasmanian Field Day was held at Berried In Tas on 10/12/2021. Jodi and Katie presented a background of the breeding program with a summary of the 2021 temperate season, and led a farm walk of the new temperate varieties.	There were 15 attendees.
	Jodi, Katie, and Apollo presented at the pre-temperate-season Prep N Party at Applethorpe Research station 23/6/2022	Hosted by Berries Australia, with over 20 attendees.
	Two Mediterranean farm walks 17/8/2022 and 20/10/2022. Feedback was sought on seedlings and clones on both days, and clonal performance data for the season was provided at the October farm walk.	17/8/2022: eight attendees, 20/10/2022: seven attendees.
Temperate Reference Group Meetings and distribution of end-of-season reports.	In-person meeting, Wandin, Victoria 20/5/2019.	See Appendix 3 end of season report.
	Jodi via telephone April 2020 due to COVID travel restrictions.	See Appendix 8 end of season report.
	In-person and via teleconference, including a farm walk at a grower's property. Wandin, Victoria 20/4/2021.	See Appendix 11 end of season report.
	In-person in Wandin and via teleconference on 2/6/2022.	See Appendix 14 end of season report.
Subtropical Reference Group Meetings and distribution of end-of-season reports.	In-person at Elimbah on 11/7/2018.	
	In-person at Caboolture Hub 6/11/2019.	See Appendices 5 and 6 end of season report.
	In-person at MRF and via teleconference 18/11/2020.	See Appendix 9 end of season report.
	In-person at MRF and via teleconference on 10/11/2021.	See Appendix 12 end of season report.
Mediterranean Reference Group Meetings and distribution of end-of-season reports.	An in-person meeting was held at Waneroo WA with key Mediterranean industry members on 13/3/2019.	See Appendix 2 end of season report.
	Distributed a Mediterranean breeding report of the 2019 season to growers and industry relations officers on 6/4/2020 and sought individual feedback.	See Appendix 7 end of season report.
	A teleconference meeting was held with key Mediterranean industry members on 3/2/2021.	See Appendix 10 end of season report.
	A teleconference meeting was held with key Mediterranean industry members on 23/2/22.	See Appendix 13 end of season report.
Strawberries Australia meetings.	A presentation on project BS12021 outputs and project BS17000 outline was given by Jodi at the Strawberries Australia meeting on 9/11/2018.	

Activity	Description	Detail
	Jodi virtually presented a breeding update at a Strawberries Australia meeting 30/5/2019.	
Queensland Strawberry Growers Association meetings	Jodi, Katie and Apollo attended most QSGA meetings over the course of the project.	
	Jodi gave an update on the breeding program at the QSGA AGM on 26/2/2019.	
	Jodi presented a subtropical and temperate breeding update at the QSGA General Meeting on 20/10/2020.	
	Jodi and Katie presented an overview of the strawberry breeding program at the online QSGA meeting 17/8/2021.	
	Katie and Apollo presented at the QSGA meeting on 9/11/2021 about the breeding program, newly released variety Susie, a potential new commercial variety, and disease screening experiments.	
Strawberry Growers Association of WA meetings.	Jodi attended and presented a Mediterranean and temperate breeding update at a Mediterranean breeding update at the SGAWA AGM on 9/8/2019.	
	Jodi and Katie remotely presented at the SGAWA AGM on 22/10/2021. Videos of advanced and early-stage selections were presented, along with punnets of fruit for the growers to try. Simple datasheets were distributed to growers in both English and Vietnamese, and valuable feedback on the individual selections was received. Four selections were particularly liked by growers. A field walk of the advanced on-farm selections was also undertaken.	18 growers and industry representatives participated.
South Australian Strawberry Grower's Association meetings.	Jodi virtually presented an overview of the breeding program to temperate growers 8/9/2020.	
	Jodi met with South Australian growers on 29/8/2022 to provide an update on temperate breeding activities and receive feedback on breeding targets and activities.	Around 10 growers attended.
Victorian Strawberry Growers' Association meetings.	Jodi attended virtually 9/12/2020.	
Site visits	Regular contact between Jodi and Katie and growers with on-farm trials across all three breeding nodes, and visits with all on-farm trial growers at least once.	

Activity	Description	Detail
	Jodi visited the plant producer JCLM Farming in Tasmania on 6/2/2020, and Jodi and Kodi and visited JCLM on 9/12/2021.	
	Representatives from Sweet's Runner Growers visited MRF 25/6/2020.	
	Jodi, Katie, Michelle Paynter and Joanna Kristoffersen visited propagator Paradise Fruits at Elimbah for a tour of their tissue culture facility and plant nursery 9/9/2020.	
	Katie visited Sweet's Strawberry Runners on 22/11/2021.	
	Multiple tours of the MRF breeding program for students from Nambour Christian College, University of the Sunshine Coast and University of Queensland.	Between 15-30 students.
Conference presentations	Apollo, Pathogenicity and aggressiveness of *Macrophomina phaseolina* isolates to strawberry (*Fragaria* × *ananassa*). Australasian Plant Pathology Society Conference, Melbourne 26/11/2019.	491 delegates registered from 30 countries.
	Jodi, on a study lead by Michelle Paynter, a new economical storage technique for strawberry *in vitro*. International Strawberry Symposium, Italy (online) 5/2021.	800 delegates registered from 20+ countries
	Jodi, an overview of the Australian Temperate Strawberry Breeding Program. Annual Fruit Growers Tasmania Conference 14/6/2021.	
	Jodi, Breeding strategy and industry impact of the ASBP.	Australasian Plant Breeding Conference, Gold Coast 9/5/2022. 270 delegates in-person on the Gold Coast and 203 virtually, representing 21 countries.
	Katie, Genome-wide association study for resistance to powdery mildew in the ASBP	
	Apollo, Identifying sources of resistance to diseases on strawberry populations	
	Jodi, overview of the ASBP at Hort Connections, Brisbane 7/6/2022.	Around 3000 delegates registered.
	Jodi and Katie, DNA informed breeding for better Aussie strawbs.	Berry Quest International, Gold Coast 27/7/2022.
	Apollo, Research on Management of Strawberry Diseases.	
International travel	Jodi undertook a trip to USA in January 2019 to meet with other RosBREED collaborators and to begin discussions on future collaboration; attend the Plant and Animal Genome Conference, San Diego, to learn about current genomics approaches and techniques for application in plant breeding; and view and learn about University of Florida's and Driscoll's strawberry breeding operations.	Collaborations were initiated with strawberry breeding programs in the US and UK for genomic prediction and marker assisted selection. Valuable information was also gained about the breeding strategies being undertaken by University of Florida and Driscoll's breeding programs that will directly benefit the ASBP.

Activity	Description	Detail
	Jodi and Katie attended the International Strawberry Congress in person in Antwerp, Belgium in September. They also visited with Vissers Plant Innovators, Netherlands, with whom a seed exchange program has now been established, and the East Malling soft fruit breeding groups and Driscoll's breeding program in Kent, United Kingdom. Visits with local growers were also organized by Vissers and East Malling.	The trip was extremely informative and interesting for the ASBP, particularly to understand the different growing environments and technologies used in Europe and the UK, and to strengthen ties with Vissers regarding the seed exchange. One notable highlight of the trip was to discuss and see in person the use of robotic strawberry harvesters and the current limitations surrounding the robots. ASBP germplasm is far superior to European germplasm in terms of plant architecture optimised for robotic harvesting.
Media coverage	An overview of the breeding program was aired on the Channel 10 television show Scope 2/9/2018.	https://tenplay.com.au/channel-eleven/scope/season-4/episode-64
	An article about the breeding program was featured on the HortiDaily website 11/8/2020.	https://www.hortidaily.com/article/9239489/australian-strawberry-breeding-program-delights-tastebuds/
	Jodi was interviewed by Steve Austin on ABC Drive about the breeding program 12/8/2020.	
	An article about the breeding program's new temperate strawberry varieties was featured on the FreshPlaza website 28/8/2020.	https://www.freshplaza.com/article/9244732/new-temperate-strawberry-varieties-preparing-for-commercialisation-in-australia/
	An article about the new white fruit under development in the breeding program was featured on the FarmOnline National website 22/9/2020.	https://www.farmonline.com.au/story/6915138/strawberry-breeding-a-juicy-success/
	Jodi and the breeding program were featured on 9 News Sunshine Coast 15/10/2020.	https://www.facebook.com/watch/?v=3444037005689058. 486 views
	An article was published on the ABC website about the development of white strawberry varieties 13/12/2020.	https://www.abc.net.au/news/rural/2020-12-13/white-strawberry-breeding-program-fruit-jodi-neal/12934992
	Jodi was interviewed by WIN News about strawberry breeding and the upcoming white and blush varieties 18/1/2021.	
	Jodi was interviewed by ABC radio rural country hour about the new temperate varieties Tahli and Tamara 15/6/2021.	
	An article was published in the Sunday (Courier) Mail about the new blush berry SB17-230-ASBP, titled "Science breakthrough: Is white strawberry plan beyond the pale?" 22/8/2021.	The article was subsequently republished in other regional papers including in the Sunshine Coast and Bundaberg.

Activity	Description	Detail
	Jodi and Apollo were interviewed by Channel 7 News about the blush berry 23/8/2021.	
	Jodi and Katie were interviewed for an upcoming ABC Me children's television show 'Food Investigators' 8/9/2021.	
	A Hort Innovation media release was put out in July 2022 about the specialty pink and white varieties. Jodi conducted 13 interviews (7 radio, 4 TV, 2 podcasts).	A total of 141 mentions resulted from the press release and interviews, with a potential reach of 2.33 M people.
Workshop presentations	Katie, an overview of the ASBP with a focus on genomics, QAAFI Science Seminar 1/6/2021.	There were 94 online attendees, and 255 views on YouTube.
	Katie, an overview of genomics work, DAF monthly Upload Webinar for Science week 19/8/2021.	There were 245 online attendees.
	Apollo, Non-chemical approaches to manage strawberry diseases, DAF Ologists Workshop 6/10/2022.	There were 50 attendees.

Appendix 19. Simply Red Issue 51 article – New Varieties for All Australian Strawberry Growers

SimplyRed
THE AUSTRALIAN STRAWBERRY INDUSTRY NEWSLETTER
NUMBER 51 SEPTEMBER 2018

IN THIS ISSUE

Strawberry Innovation is a national industry development program, focused on improving national communication and coordination across the whole of the Australian strawberry industry. This project has been funded by Hort Innovation, using the strawberry research and development levy and contributions from the Australian Government. Hort Innovation is the grower owned, not-for-profit research and development corporation for Australian horticulture.

NEW VARIETIES FOR ALL AUSTRALIAN STRAWBERRY GROWERS

Jodi Neal, Mark Herrington, Lien Ko, Apollo Gomez, Michelle Paynter, Karen Spencer, Louella Woolcock, Alan Noon, Sue Hibbit and Dale McKenna

Figure 1. New temperate varieties 'Summer Song' (left) and 'Scarlet-silk' (right).

The recently concluded Australian Strawberry Breeding Program (ASBP) saw two temperate varieties and three mediterranean varieties accepted for Plant Breeder Rights (PBR) Part I to allow for commercialisation. A further eight temperate varieties have been positioned for commercialisation. Seven subtropical varieties have been commercialised over the past five years.

Introduction

The most recent five-year ASBP has now 'wrapped up' with the final report submitted to Hort Innovation. Here we summarise the outcomes for industry from this project for all targeted production environments. This includes the temperate region (Victoria, Tasmania, South Australia, the Albany region of Western Australia, the Granite Belt in Queensland, southern New South Wales and the Australian Capital Territory); the mediterranean region (Wanneroo districts of Western Australia); and the subtropical region (south-east Queensland, northern New South Wales and Bundaberg).

New varieties

In the very short time (three years) that we have been breeding for the temperate and mediterranean regions we have had a strong focus on delivering outcomes quickly, fast-tracking our usual processes to make new varieties available for these regions. We are absolutely committed to providing the same excellent varietal outcomes for these regions over the coming years as we have produced for the subtropics.

Within this project we have commercialised two temperate varieties, with three mediterranean varieties accepted for PBR Part I. We have also positioned for commercialisation eight more temperate varieties. Seven subtropical varieties have additionally been released over the full five years of the project.

Industry outcomes from the longer running subtropical component of the project have been excellent, with ASBP subtropical varieties providing consistent bearing, high quality fruit, which have seen savings in harvest labour costs. The

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Figure 2. One of the 2015-series selections included in national on-farms trials in the 2018/19 season.

Figure 3. Mediterranean varieties 'Fanfare-ASBP' (left), 'Jubilee-ASBP' (top right) and 'Rosalie-ASBP' (bottom right).

varieties bred by the ASBP now constitute approximately 73% of total runners planted by the subtropical industry.

Table 1 (on page 3) shows the number of seedlings and clonal lines planted each season in the production regions over the course of the project. The total number of seedlings evaluated by the ASBP between 2015 and 2017 were:

- temperate - 40,319
- mediterranean - 3,493
- subtropical - 30,972

A large number of crosses were undertaken each year for the temperate region in particular, with numbers for the mediterranean region to continue to increase over the coming years. Specific outputs for each production region are outlined as follows.

Breeding pipeline

Large numbers of genetically unique seedling plants were produced by the ASBP and planted each year between 2015-2017 for the temperate and mediterranean breeding nodes, and from 2013-2017 for the subtropical node. The best of these were progressed to clonally replicated trials, first in research centres for one to two years ('early' and 'advanced' clonal trials), and then on producer's farms ('on-farm' trials) for an additional one to two years. At the end of the process all clones considered promising by producers and/or the breeding team were either commercially released or protected under PBR Part I.

New temperate varieties

In March 2018, the temperate varieties 'Summer Song' and 'Scarlet-silk' were accepted for PBR Part I (Figure 1). These varieties were produced from crosses of subtropical plants and selected from a small trial at Applethorpe Research Facility, Applethorpe (Queensland). Small numbers of these varieties are currently with runner growers and will be available in greater numbers over the next few years. There are an additional eight temperate selections that are positioned for commercialisation, including three 2005-series selections, a 2008-series selection, and four 2014-series selections. Hort Innovation will be coordinating the commercialisation of these varieties.

Six advanced selections developed under the recent ASBP project were chosen from the temperate 2015-series in March 2018 (Figure 2). These selections plus the four 2014-series selections have been distributed to producers in Victoria, Western Australia, Tasmania, South Australia and Queensland for on-farm trialling in the 2018-19 season.

New mediterranean selections

In order to provide an early outcome for the Mediterranean production region, three clonal selections from the 2017 clonal trial were fast-tracked through the breeding pipeline and were accepted for PBR Part I in March 2018. These selections were 'Rosalie-ASBP', 'Jubilee-ASBP' and 'Fanfare-ASBP' (Figure 3). They will be distributed to runner growers and Agribio in the near future and will soon be available to give mediterranean producers the opportunity to trial them.

New subtropical varieties

Over the course of this recently completed project, five subtropical strawberry varieties were commercially released. These were 'Red Rhapsody', 'Parisienne Kiss', 'Sundrench', 'Scarlet Rose-ASBP', and 'Sunglow ASBP'. Two additional varieties were accepted for PBR Part I in March 2018: 'Meadowsong' and 'Venus-ASBP'.

The variety 'Red Rhapsody' (Figure 4) was accepted for Part I PBR in 2013 and PBR granted in 2016. This variety has had exceptional uptake by the subtropical strawberry industry. In 2017 plants of 'Red Rhapsody' were first available in substantial numbers from runner growers and all plants were sold out.

Plants of 'Red Rhapsody' represented 24% of all subtropical varieties grown in Australia in 2017, and in 2018 this proportion has further increased. 'Red Rhapsody' was the first variety to be selected using economic modelling of plant and fruit traits, and its success suggests that this approach is useful for selecting varieties with a profitable balance of production traits.

In 2015 the varieties 'Parisienne Kiss' and 'Sundrench' (Figure 4) were accepted for PBR Part I, and PBR was granted in 2016. These varieties are only available from runner growers in relatively small numbers, however uptake by industry, particularly of 'Parisienne Kiss' has been encouraging.

The varieties 'Scarlet Rose-ASBP' and 'Sunglow ASBP' (Figure 4) were entered into PBR Part I and PBR granted in 2017. Neither of these varieties were available in large commercial numbers in 2018, however early sales, particularly of 'Scarlet Rose', are promising. Reports from the 2018 season suggest good performance by both of these varieties, with 'Scarlet Rose' being noted for having especially good flavour.

In March 2018 the varieties 'Meadowsong' and 'Venus-ASBP' were accepted for Part I PBR (Figure 4). Small numbers of both varieties are currently with runner growers and will be available in greater numbers in the near future.

Combined, ASBP varieties now represent approximately 73% of all subtropical runners planted in 2018 and 30% nationally.

Conclusions

By providing a truly national breeding strategy for Australian strawberry producers, the ASBP aims to foster an

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integrated and profitable national industry that benefits all growers. The ASBP team have been developing new varieties for the subtropical production region for over 25 years and the current commercial outcomes are the culmination of this work.

While it is still early days for our breeding efforts in the temperate and mediterranean regions, we are focussed on making improved varieties available for these regions as quickly as possible. As well as being committed to providing excellent outcomes for growers in both of the temperate and mediterranean regions, we wish to continue adding to our strong track record for subtropical growers.

Our ongoing focus is to provide an integrated contribution to all members of the national Australian strawberry industry.

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We thank the contributions by the national Strawberry Variety Steering Committee, the Temperate Node Reference Group, the Subtropical Node Variety Evaluation Committee, the Industry Development Officers, and all other industry members who have provided feedback, advice, and support over the project. We particularly thank our commercial partners the runner growers for their help – without them we do not have a pipeline.

We are also extremely grateful to all the fruit producers in all states who have trialled, collected data, and given feedback on our on-farm selections. This has helped us make more informed and better commercial judgments.

One of the guiding principles of the breeding work is to foster the exchange of ideas, so please contact project leader Mark Herrington (mark.herrington@daf.qld.gov.au) or co-lead Jodi Neal (jodi.neal@daf.qld.gov.au) if you want more information. We value your thoughts and appreciate your feedback (information, advice or constructive comments) for the project team.

Figure 4.

Far Left: Fruit of variety 'Red Rhapsody'.

Middle Left and Left: Plants and fruit of varieties 'Parisienne Kiss' (left) and 'Sundrench' (right).

Bottom Far Left to Right: Fruit of varieties 'Scarlet Rose-ASBP', 'Sunglow ASBP', 'Meadowsong' and 'Venus-ASBP'.

Region	Location	Season	Seedlings	Early clones	Advanced clones	1st stage on-farm clones	2nd stage on-farm clones
Temperate	Wandin	2015-16	12,069	115	11	0	0
		2016-17	13,739	127	4	14	0
		2017-18	12,979	85	11	11	0
	Applethorpe	2017-18	1,532	13	11	0	0
Subtropical	Nambour (ground)	2013	3,626	104	97	2	6
		2014	7,885	69	44	6	2
		2015	8,027	146	23	0	0
		2016	7,657	127	31	6	2
		2017	6,084	216	13	3	2
	Nambour (tabletop)	2015	0	0	5	0	0
		2016	0	0	5	0	0
		2017	152	12	6	0	0
	Bundaberg	2013	924	01	0	0	0
		2014	1,410	01	0	0	0
		2015	1,491	01	0	0	0
		2016	3,219	01	0	0	0
		2017	4,334	01	0	0	0
Mediterranean	Wanneroo	2015	5462	0	0	0	0
		2016	1,0543	10	0	0	0
		2017	1,8934	18	0	0	0

Table 1. For each region and production season the number of seedlings planted and the number of clonal lines tested in early, advanced and on-farm trials. Temperate and mediterranean regions were incorporated into the breeding program in 2015.

1 Bundaberg seedling selections were retrieved annually for clonal testing at Nambour.

2 Mediterranean seedling selections died after retrieval transit to Nambour.

3 All Mediterranean seedlings duplicated at Nambour prior to dispatch to WA.

4 Mediterranean seedling and clonal selections multiplied in WA by Trandos Hydroponic Growers.

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Appendix 20. Simply Red Issue 51 article – Breeding Varieties for All Australian Growers into the Future

BREEDING VARIETIES FOR ALL AUSTRALIAN STRAWBERRY GROWERS INTO THE FUTURE – NEW AUSTRALIAN STRAWBERRY BREEDING PROJECT

Jodi Neal, Mark Herrington, David Innes, Lien Ko, Apollo Gomez, Michelle Paynter, Karen Spencer, Louella Woolcock, Alan Noon, Sue Hibbit and Dale McKenna

The previous Australian strawberry breeding project has just concluded, and a new five-year breeding project (BS17000) commenced. The outcomes of this new project for all Australian strawberry producers will be new varieties specifically developed for each of the three major commercial growing regions (temperate, mediterranean, and subtropical) using advanced breeding and evaluation techniques. Some of the new varieties will also be suited to alternative production systems. Overall, this project is expected to improve profitability for all strawberry producers, while simultaneously ensuring strong consumer appeal.

In this article, we outline the strategies to be implemented in the new project by the Australian Strawberry Breeding Program (ASBP). The new program will balance breeding efforts across the three targeted production regions, and will release at least two new varieties specifically developed for production in each growing region i.e. at least two varieties for temperate production, two for subtropical production and two for mediterranean climatic conditions. Some of the trait targets in this project will also include developing varieties for substrate (tabletop) culture as well as improved disease resistances.

Breeding targets will be defined through consultation with local industry in each region and the Strawberry Variety Steering Committee (SVSC), with an overall goal of supporting a sustainable and profitable national strawberry industry. The general breeding strategy will build upon the successful pipeline developed in the previous project, and be further enhanced by incorporating genomic prediction and marker assisted selection methodologies.

New and established international seed exchange arrangements will ensure the program has access to the best international genetics for crossing within all three production regions, in addition to the superior material currently maintained by the breeding team.

Disease resistance screening of advanced selections and parents will be expanded in the new project to include powdery mildew (*Podosphaera aphanis*) resistance. With greater industry uptake of protected cropping, this disease is becoming increasingly economically significant. Parents and key selections will continue to be screened for resistance to the major wilt diseases *Macrophomina* (charcoal rot), *Fusarium*, and *Colletotrichum*.

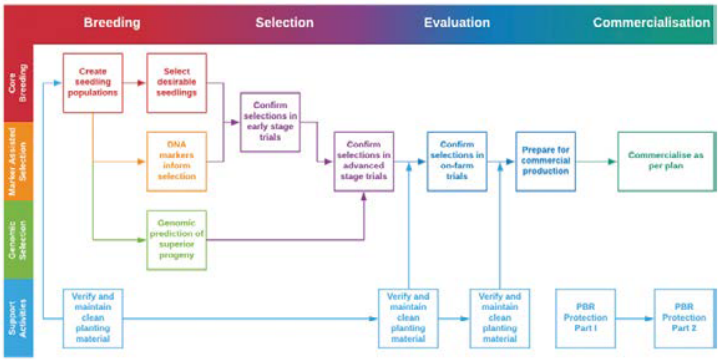

Figure 1. An overview from initial crossing through to commercialisation of new varieties in the new project.

Figure 1 depicts the major phases of the new breeding program that will be undertaken to deliver quality commercial varieties. This cycle will be initiated three times each year, with each cycle targeting traits defined for one of the production regions (temperate, mediterranean, and subtropical).

Targets for regional outcomes

The over-arching aim of the breeding program is to produce varieties with traits that exploit the strengths of each production region in order to support a unified and profitable national strawberry industry. This is underpinned by the need to account for economic, environmental and social impacts within the production lifecycle.

The project will consolidate the work of the previous project in producing superior varieties for each of the temperate, mediterranean, and subtropical production regions.

Given the existing expertise and markets for export in the mediterranean region, breeding for this area will focus on designing varieties for international export. The target for the temperate and subtropical regions will be producing varieties that lower costs of production and improve the consumer experiences.

In order to address the current lack of product differentiation in strawberry, the project will release an additional 1-2 value-added specialty varieties for the subtropical node (e.g. with different colours, flavours or fragrances, or added health benefits), and also begin a crossing strategy to produce specialty varieties for the temperate region. These value-added products will be defined and prioritised in consultation with the SVSC.

Substrate culture

Substrate (tabletop) culture is increasing in Australia and this trend is expected to continue. As a result, there is a need to develop varieties adapted to this production system. In this project we will continue the existing subtropical substrate culture trial at the Maroochy Research Facility and conduct on-farm trials of advanced temperate material under substrate culture. Work will be conducted to determine the fruit and plant traits best suited for substrate culture, via consultation with industry and learning from international experience. Following from this knowledge trials will be conducted to produce varieties specifically adapted to the substrate production system.

Breeding strategy

Breeding and pre-commercialisation activities will be undertaken separately for each of the three production regions, focusing on advancing and streamlining processes in order to reduce the time from crossing to commercial deployment. The overall breeding strategy will be enhanced from that successfully applied in the previous project, as outlined in the breeding strategy (shown in Figure 2). Note that all breeding activities at all stages of the process are undertaken simultaneously each year in each production region.

International seed exchange

For the ASBP to remain cutting edge, collaboration and information exchange with international strawberry breeding programs and scientists is essential. Established protocols, used with the existing successful seed exchange agreement with international collaborators, such as the University of Florida, will be utilised to establish additional international seed exchange agreements supporting the temperate and mediterranean production regions. The existing agreement has been extremely beneficial for maintaining the genetic variation so important for the subtropical node breeding program.

Marker assisted selection and genomic prediction

A number of technologies have potential for accelerating and increasing efficiencies in modern breeding programs. The USDA-SCRI funded RosBREED program aims to incorporate advanced DNA-based methodologies into 22 US breeding programs focusing on eight rosaceous crops, including strawberry. Currently, DNA markers for "peach" and "sherry" flavours have been developed for strawberry. These markers should facilitate a more targeted breeding strategy for flavour and will be applied to parental and advanced selections identified throughout this project.

Statistical genomics methods such as genomic selection will also be applied, using DNA markers spread across the genome to aid in the dissection of complex traits and predict the performance of selected crosses. In order to incorporate genome scale analyses, linkages will be developed with the RosBREED program, and the ASBP will collaborate with the Queensland Alliance for Agriculture and Food Innovation (QAAFI, University of Queensland) and international scientists to develop genetic markers for fruit soluble solids content i.e. sugar content. These will be applied with a view to making improved selections and accelerating varietal development.

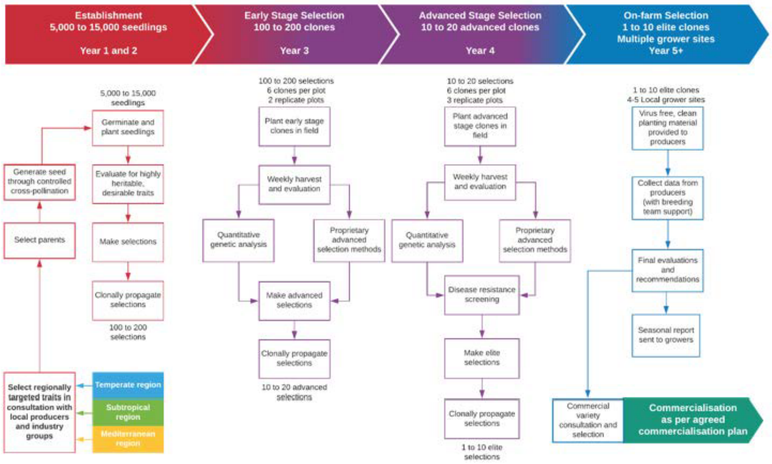

Figure 2. Breeding strategy for each major production region.

Disease resistance screening

The serious crown wilt diseases *Macrophomina* (charcoal rot), *Fusarium*, and *Colletotrichum* cause complete plant death, and have major impacts on fruit production. All new parents and advanced selections from each production region will continue to be screened annually for the three crown wilt diseases to continue to move the breeding population towards resistance. Using a staged approach, this will be expanded to screen for resistance to powdery mildew (*Podosphaera aphanis*). By screening selections and parents for powdery mildew tolerance, we aim to incorporate a range of useful resistances into new varieties. This will result in more robust plants and less fungicide applications.

Conclusions

The Queensland Department of Agriculture and Fisheries led industry strawberry breeding program has operated for over 25 years, and the ASBP team has a wide range of skills and experience in strawberry breeding including plant and tissue culture propagation, field trial management, quantitative genetic analysis, molecular and biological virus indexing, DNA genotyping and selection, disease screening, substrate culture, regional evaluation, positioning material for commercialisation, commercial deployment, industry engagement, international collaboration etc. By combining all of these resources, skills and experience, we are aiming to breed adapted, disease resistant and flavoursome new varieties for all Australian growing regions in the shortest possible time.

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One of the guiding principles of the breeding work is to foster the exchange of ideas, so please contact project leader Mark Herrington (mark.herrington@daf.qld.gov.au) or co-lead Jodi Neal (jodi.neal@daf.qld.gov.au) if you want more information. We value your thoughts and appreciate your feedback (information, advice or constructive comments) for the project team.