Use of Molecular Markers in the Berry Fruit Industry

A report for

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By Karen Brock

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Scholar Contact Details

Karen Brock
Brocklands
460 Rookery Road, Winkleigh
TASMANIA 7275

Phone: 03 6394 4807
Email: brocklandsnursery@gmail.com

In submitting this report, the Scholar has agreed to Nuffield Australia publishing this material in its edited form.

NUFFIELD AUSTRALIA Contact Details

Nuffield Australia

Telephone: (02) 9463 9229

Mobile: 0431 438 684

Email: enquiries@nuffield.com.au

Address: PO Box 1021, NORTH SYDNEY, NSW 2059

Executive Summary

Propagation techniques for fruiting plants have played a significant role in agricultural research in recent years. Traditional techniques such as asexual propagation (e.g. by cuttings), seed germination, grafting and budding to rootstocks have been the primary methods of producing new cultivars for many decades. Now, the development of gene technologies is both challenging and building on traditional techniques. Knowledge about tissue culture is growing rapidly and scientists understand more about how metabolites and physiology affect the success of cultivars of plants that are selected for commercial distribution.

A new breeding technique known as marker-assisted breeding (MAB) enhances understanding of the effects of genetics on the expression of traits, including new traits that are being chosen for future varieties. This technology has been used to map the genetic heritage of commodity berries such as blueberry (*Vaccinium* spp.), raspberry and blackberry (*Rubus* spp.), and strawberry (*Fragaria* spp.). With this knowledge, plant breeders can use genetic heritage as the basis for exploring and introducing new genetic traits in order to adapt berries to changing environmental requirements.

Contemporary understanding of genomic sequences and how each gene develops cellular structure within the plant gives breeders the ability to reduce the time taken from experimenting with seedlings to commercial realisation. For example, in the case of blueberry, it commonly takes 24 years from selecting the most promising seedlings to planting a new commercial variety on farm. By combining MAB techniques with traditional breeding practices and then utilising embryo-rescue techniques, breeders are developing exciting new traits in plants in commercial laboratories and production.

Biochemistry is being used to give scientists a specific and fine-tuned understanding of the exact biochemical make-up of berry plants and fruits. Extracts from both are being studied and applied to pharmacological research to solve medical problems or support better health. Biochemical extracts are also being incorporated into non-food industries such as cosmeceuticals, particularly in the Asia–Pacific region, where science (Hjalager et al., 2011) has proven skin regeneration from blueberry extracts.

The pharmaceutical industry is benefiting from the breeding or isolation of plant genetic material to suit their own purposes. MAB is still being developed but is likely to continue to grow and to maximise the potential of berry products.

The classification of species is also being affected by advances in genetic science. This means that some berries are being, or will be, reclassified, and this has an impact on how patents, breeder's rights and other forms of intellectual property are managed. Currently, patents are measured on distinct differences using epigenetic traits, and molecular identification is not allowed due to possible adverse impacts on existing patents. Discussion is occurring with various breeders on integrating simple sequence repeat (SSR) markers as part of the patent certification of plant material. Increasing knowledge of genetics at molecular levels also has an impact on certification practices and importing stock to develop varieties that suit local conditions and markets. These are bottlenecks in the development of a thriving and diverse domestic berry industry that need to be removed.

Understanding these techniques and genetic advances is important to the Australia berry industry for the following reasons:

- Commercial propagation with viable numbers.
- Protection of germplasm.
- Development of genetic distribution agencies with rising costs involved.
- Legislation changes that have occurred regarding transfer of endemic pathogens, pests or plants within each state.
- New product developments for pharmaceutical, food additives and cosmetic industries.
- Improved plant management practices on farm.
- Implementation of HACCP programs for propagation stock for industries receiving starter stock.

Table of Contents

List of Tables

List of Figures

Foreword

This study resulted from a query made by a customer regarding the blueberry planting stocks he had purchased from Brocklands Pty Ltd, the author’s nursery, which is accredited under the Nursery Industry Accreditation Scheme Australia, and which specialises in assisting with the post-AQIS management of imported plants. He claimed that material was performing far better than plants supplied at the same time from another propagation supplier and thought that the two batches of plants might be different cultivars. Following investigation, it was realised that he was correct. However, although the two plants were the same variety different techniques used to propagate them had resulted in a topophysic effect. Topophysis is commonly known as a genetic lock: the position on the plant of the propagule affects the type of vegetative growth that is shown by the vegetative progeny (Hartman, Kester & Davies). In effect, plants with the same genetic identity had presented a different phenotype.

Ridley Bell, key breeder of blueberries in Australia, asked what range of DNA services were available to support the findings and where to source the knowledge and technical support to carry out verification of current theories. On investigation, it was found that a similar situation existed for other fruit and nut industries.

The Nursery Industry Accreditation Scheme Australia requires regular checking of nursery stock to ensure that there are no mislabelled or mixed plants produced in the ornamental production industry. The agricultural and commercial horticultural industries have no similar accreditation requirements. This difference led to questions: How could industry guarantee that the plants supplied were, in fact, true to type? How was the industry managing endemic pathogens, if weed problems were arising, or might do?

Without any knowledge of where such services were available for the agricultural and commercial horticultural sectors, investigations outside of the berry industry were undertaken. They included understanding what certification schemes were being used internationally and how they were managed. They also encompassed whether there was a role for DNA identification in these schemes, and how often the mother plants were being verified as true to type.

The time lag from seedling to commercial reality in Australia was also of concern. Developing and releasing new varieties takes time and this is extended by the added timeframe of the Australian Department of Agriculture and Water Resources (DAWR) importation procedures, which delayed the development of fruit introductions into Australian agriculture. Downstream, this has an impact on Australia's effectiveness in competing on world markets with fruit of equal quality. Studying breeding programs and the speed with which plants were being developed was also critical because they have an influence when developing certification schemes that could meet DAWR procedures and protocols and reduce the timeframes for the introduction of new varieties to Australia.

Issues arising from litigation in the plant industry regarding incorrectly propagated plants were also occurring with associated horticultural and propagation businesses. The impact is large – financially, emotionally and developmentally. Resulting discussions highlighted that legal requirements and terminology did not align with living plant production; in fact, living plants were not adequately covered legally until a product reached the stage sold to the consumer. This grey area in plant production dovetailed into this study.

The study identified many areas for investigation that were not within the scope of this research. Many opportunities and potential areas for development of plants using biotechnology could provide extensive ongoing research.

Acknowledgments

This study would not have been remotely possible had it not been for the generous scholarship provided by Nuffield Australia and Horticulture Innovation Australia (HIA). The guidance from Sharyn Casey (HIA), Anthony Kachenko (HIA), and Jim Geltch (Nuffield) is much appreciated. Their generosity has provided me with a conduit to industry and access to an incredible network of people with broad skills to assist with issues arising from plants or developments.

To do full justice to the study, the family—husband Tim, and children Caitlin and Chaise— and colleagues Christy, Jenny, Ying, Connie, Darrel and Jim, who rallied as one, united front while I was travelling, was a truly humbling experience. These lovely people selflessly encouraged my journey to continue when life threw some incredible hurdles in the way.

The incredible people listed below took time to answer my questions, send presentations and videos, explain remarkable techniques being used in this field of science. They guided me through their truly innovative programs, which have not yet been released publicly.

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Michiel van Bennekom	Iribov, Netherlands
Paul Herbert	Guelph University, Canada
Peter Molloy	Olde Berry Farm, New Zealand
Prof. Adam Dale	Guelph University, Canada
Prof. Bruno Mezzetti	Università Politecnica delle Marche, Italy
Prof. Jeffrey Adelburg	Clemson University, USA
Prof. Marc Van Montagu	Inst Plant Biotechnology Outreach, Belgium
Prof. Maurizio Lambardi	IVALSA/Trees & Timber Institute, Italy
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Stefan Schwartz	Phenospex, The Netherlands
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Abbreviations

ABS	Australian Bureau of Statistics
AQIS	Australian Quarantine Inspection Service
BBGD	Blueberry Genomic Database
CIOPORA	Communauté Internationale des Obtenteurs de Plantes Ornamentales et Frutières à Reproduction Asexuée (Switzerland)
cDNA	complementary deoxyribonucleic acid
DAWR	Department of Agriculture and Water Resources
DNA	deoxyribonucleic acid
EST	expressed sequence tag
EU	European Union
HACCP	Hazard Analysis Critical Control Point
IP	Intellectual Property
NIASA	Nursery Industry Accreditation Scheme Australia
OSU	Oregon State University
PCR	Polymerase Chain Reaction
RAPD	Random Amplification of Polymorphic DNA
RBDV	Rubus Bush Dwarf Virus
SAPROV	Environmental Sciences and Crop Production (Italy)
SCRI	Scottish Crop Research Institute
SNP	single nucleotide protein
SSR	simple sequence repeat
UK	United Kingdom
UPOV	International Union for the Protection of New Varieties of Plants
USA	United States of America
USDA	United States Department of Agriculture

Objectives

The objectives of the project were to:

- Understand the use of molecular markers such as polymerase chain reaction and related techniques in the berry fruit industry.
- Discover traditional breeding programs that integrate molecular markers to reduce timeframes from experimentation to the commercial release of new varieties.
- Understand genomics and its impact on disease resistance and nutritional potential.
- Understand the process of product development for berries.
- Understand genetic material as a legal reference for patents.
- Understand potential products that could be derived from berry plants.

Introduction

The Australian commercial berry industry consists of blueberry, strawberry, and raspberry production, which bring in more than $680 million (FreshIncite, 2014). Each berry is important to Australian agribusiness, and each has its own history. The raspberry and strawberry industries go through cyclic highs and lows that are usually attributed to supply problems: either exceeding or not meeting demand. The blueberry industry has been growing at an incremental rate until recently, when it took off after agribusiness investors planted large numbers of hectares for fresh-fruit production (See Figures 1, 2, and 3).

Recent trade agreements with countries such as Vietnam, South Korea, and India have given Australian growers openings into markets that were previously inaccessible. These opportunities, in conjunction with successful marketing about the health benefits of berry fruits, has seen an increase in the consumption of all three berries. However, high production costs in Australia mean that making the most of these opportunities will only be possible if tariffs are kept low.

Year Ending June	2014		2015			2016		
		Value		Value	% YoY		Value	% YoY
Production (t)		2,469		3,166	+28%		4,974	+57%
Production ($m)	$	78.5	$	103.4	+32%	$	127.6	+23%
Production area (Ha)		-		-	-		-	-
Fresh Export Volume (t)		8		8	+3%		2	-72%
Fresh Export Value ($m)	$	0.2	$	0.1	-22%		< $0.1	-60%
Fresh Import Volume (t)		-		-	-		-	-
Fresh Import Value ($m)	$	-	$	-	-	$	-	-
Fresh Supply (t)		2,214		2,889	+30%		4,504	+56%
Fresh Supply Wholesale Value ($m)	$	91.3	$	123.3	+35%	$	149.1	+21%
Consumption per Capita (kg supply)		0.09		0.12	+29%		0.19	+54%

Figure 1:Production of raspberries (in tonnes). (Horticulture Innovation Australia, Australian Horticulture Statistics Handbook: Fruits ,2015–2016)

Year Ending June	2014 Value	2015 Value	2015 % YoY	2016 Value	2016 % YoY
Production (t)	74,422	77,022	+3%	72,075	-6%
Production ($m)	$ 420.4	$ 420.1	>-1%	$ 381.6	-9%
Production area (Ha)	*2,123*	*2,240*	+5%	-	-
Fresh Export Volume (t)	1,467	1,904	+30%	3,010	+58%
Fresh Export Value ($m)	$ 8.6	$ 12.4	+44%	$ 25.8	>100%
Fresh Import Volume (t)	34	36	+6%	14	-62%
Fresh Import Value ($m)	$ 0.1	$ 0.2	+18%	< $0.1	-81%
Fresh Supply (t)	61,082	62,857	+3%	57,698	-8%
Fresh Supply Wholesale Value ($m)	$ 478.0	$ 473.9	>-1%	$ 416.0	-12%
Consumption per Capita (kg supply)	2.60	2.64	+1%	2.39	-9%

Figure 2: Production of strawberries (in tonnes). (Horticulture Innovation Australia, Australian Horticulture Statistics Handbook: Fruits, 2015–2016)

Year Ending June	2014 Value	2015 Value	2015 % YoY	2016 Value	2016 % YoY
Production (t)	6,636	7,355	+11%	7,660	+4%
Production ($m)	$ 145.3	$ 155.5	+7%	$ 149.6	-4%
Production area (Ha)	*189*	*214*	+13%	-	-
Fresh Export Volume (t)	156	103	-34%	254	>100%
Fresh Export Value ($m)	$ 3.0	$ 2.0	-34%	$ 5.2	>100%
Fresh Import Volume (t)	1,104	1,003	-9%	1,432	+43%
Fresh Import Value ($m)	$ 18.9	$ 18.4	-3%	$ 29.8	+62%
Fresh Supply (t)	6,921	7,525	+9%	7,939	+6%
Fresh Supply Wholesale Value ($m)	$ 197.8	$ 211.7	+7%	$ 211.0	>-1%
Consumption per Capita (kg supply)	0.30	0.32	+7%	0.33	+4%

Figure 3: Production of blueberries (in tonnes). (Horticulture Innovation Australia, Australian Horticulture Statistics Handbook: Fruits ,2015–2016)

The Australian market can improve further if local produce replaces imported product in processed berry products (HIA, 2015/2016). Australia has an advantage that many berry exporters do not, and this was illustrated during the food-security concern that resulted from the Hepatitis A scare in 2014. It revealed an opportunity where Australian-processed produce, which is grown to exacting Hazard Analysis Critical Control Point (HACCP) standards, has greater acceptance in the local consumer market.

The Australian industry also stands to gain from the development of new varieties, which can be designed for new domestic and international markets and be cross-referenced from existing fresh berry production. Berry markets are changing rapidly and to keep pace with demand, breeders are moving to new, molecular techniques. Breeding programs are prioritising genetic selections that are suitable for mechanical harvesting to cater for the growing processed-berry market. Breeders are also focusing on the use of molecular markers that relate to disease resistance, particularly to protect against viruses caused by aphids.

Molecular testing is in its infancy; genomic maps have recently been completed by several agencies such as Oregon State University (OSU) and the James Hutton Institute (JHI) in the UK. The genetic traits being targeted in berry-plant selections relate to yield, disease resistance, taste and texture, health, nutraceutical and cosmeceutical benefits. (Nutraceuticals are products derived from food products that are said to have more health benefits than 'normal' foods. Cosmeceuticals are cosmetic products that are said to have health benefits, such as slowing the ageing of the skin.)

Worldwide, the impetus to develop the *Rubus* spp. and strawberries comes from how important they are economically: more than 90 genera that belong to the Rosacea family contribute to gross domestic production or are destined for export. The pressure to develop more disease-resistant fruit is not restricted to the berries: there is also pressure for related economic crops with higher returns, for example the *Malus* spp. (apples) and *Prunus* spp. (stone fruits), for which there is a need to develop resistance to several diseases, in particular ilarvirus and nepovirus.

In Australia, breeding programs occur in the Department of Agriculture and Fisheries Queensland (DAF Queensland) and Agriculture Victoria, for Strawberries Australia; with Ridley Bell (Mountainblue), Dave Mazzardis (OzPeach), and Gary Wright (Costa Group) for blueberries; and Harvey Hall (Shekinah Berries) for raspberries. Except for Strawberries Australia, all of these are private breeders with vertical market access only.

Developing new varieties is expensive work. Researchers in traditional breeding programs use techniques where pollen is exchanged between plants. They also collect germplasm from wild species to generate new cultivars; this leads to crossing and back-crossing. Many breeders have scoured the world to develop networks with other breeders and exchange germplasm. However as patents, plant breeders' rights, variety rights and exclusive licences have been developed to help breeders recover their costs, germplasm exchange has diminished or even become redundant.

Chapter 1: Berry Development

Berry plants, particularly the raspberry and strawberry, have been interbred using traditional techniques for over a century. But the old ways are giving way to the new, as contemporary genetic science helps us ‘see’ at a much finer scale, and even to manipulate the molecular structure of the DNA of plants. Molecular breeding is not currently used to improve yield and taste, as the genes that control these are not yet understood. However, as the molecular technologies and understanding of berry genetics improve, molecular breeding will become the mainstay of variety development.

The *Rubus* species: Raspberries and Blackberries

Running parallel with advances in expensive genetic technology, there has been substantial impact on traditional breeding institutes, with funding reductions from government bodies resulting in lapsed or dilapidated programs. Virginia Knight (2004) published a list of 40 *Rubus* breeding programs that were then running in 19 countries (See Table 1). Of these, the breeders in Canada (with exception of Agassiz), Sweden, Poland and the US have either reduced their programs or been absorbed into private operations.

Country	**Name of breeder**
Australia	Horticultural Development Knoxfield, Department of Agriculture, Victoria
Bulgaria	Kostinbrod
Canada	British Columbia Agriculture and Agri-Food Canada, Agassiz (British Columbia)
	Nova Scotia Agriculture and Agri-Foods Canada, Kentville
	Ontario University of Guelph, Guelph
Chile	Hortifrut, Santiago
China	Beijing Institute of Pomology & Forestry, Beijing
Germany	Freising-Weihenstephan
Hungary	Fertod
Italy	Ancona
Latvia	Dobele
Norway	Njos

New Zealand	HortResearch, Inc., Motueka
Poland	Brzezna
Romania	Maracineni-Pitesti
Russia	VIR, St. Petersburg
Serbia	Cacak
Sweden	Balsgard, Kristianstad
Turkey	Antalya
United Kingdom	England Medway Fruits, Maidstone
	England HRI-East Malling
	SCRI, Dundee
USA	University of Arkansas, Fayetteville
	California Driscoll's Association, Watsonville
	California Plant Sciences Institute, Watsonville
	Virginia Tech, Maryland-New Jersey University of Maryland
	University of Wisconsin
	USDA Agricultural Research Service (ARS), Beltsville, Maryland
	Minnesota Univ. of Minnesota, St. Paul
	North Carolina N.C. State University, Raleigh
	USDA ARS, Corvallis, Oregon
	Washington State University, Puyallup
	Washington Multiple private programs

Table 1: Rubus breeding programs worldwide. Rubus breeding worldwide and the raspberry breeding programme at Horticultural Research International (Source: Knight 2004)

Early attempts to understand the genetic makeup of *Rubus* species was carried out by Haskell and Garrie (1966) using a paper chromatography technique. It was not improved until 1989, when Cousineau and Donnelly (1992) used isoenzyme analyses to study the *Rubus* germplasm.

Among the remaining breeders, the research follows many and various paths. The *Rubus* program in New Zealand has successfully released 'Wakefield': 990 ha is currently being grown at Lynden, Washington State, USA, for processed fruit, to generate an income stream to further the Plant Food Research New Zealand breeding programs. Molecular mapping tools will be utilised to expedite new selections for this program.

Various 'fingerprinting' techniques were developed to identify minisatellite DNA for black raspberry at Balsgard in 1990s by Nybom et al. This program is relevant to modern breeding as black raspberry has the widest potential with the highest anthocyanin levels of any berry fruit. Dossett, Bassil, Finn & Lewers (2012) have mapped the black raspberry genomically, and are now working on improving breeding techniques.

The black raspberry project in Sweden has been reduced as Hilde Nybom (Balsgard), once known for its progressive breeding program in small fruits and dwarf pome and stone fruits, is now reduced to one breeder for blackcurrants, Kimmo Rumpunen and Nybom completing research.

Random amplification of polymorphic DNA (RAPD) was a huge improvement, when it was introduced in 2003 by Courtney Weber. According to Weber, 'the identification tools are available for DNA but understanding the gene clusters and how they function is in its infancy'.

At SCRI (now part of the James Hutton Institute) in Scotland, the focus by Julie Graham has been on identifying the gene clusters responsible for plants displaying crumbly fruit syndrome. Crumbly fruit has been attributed to many causes, including viruses, environmental management, and genetic heritage. The SCRI was the first institute to list an Expression Library (Graham et al., 2004; Lewers et al., 2008) using simple sequence repeat (SSR) markers. The aim was to understand prickle development, which was not totally successful until cDNA libraries were catalogued. The key focus has been on *Phytopthera fragariae* var. rubi, cane *Botrytis* spp., *Didymella applanata* (Cane blight) and *Elsinoe veneta* (Cane spot), and Rubus Bushy Dwarf Virus (RBDV) resistance. The breeders have been screening seedlings for susceptibility to these diseases, and in particular investigating wild native species for resistance, so they can revive the *Rubus* breeding programs.

At this point the three-key germplasm repositories which have the ability to identify cultivars as being true to type are at SCRI (UK), Naktuinbouw (Netherlands), and Oregon State University (Corvallis, USA). These play an important role as a referral point for further research to understand how SSR markers can be utilised for germplasm development.

The use of SSR markers has become common resulting in a project called GenBank, a project sponsored by the EU from 2007-2010. The collaborative project, which involved participants from France, Italy, Poland, Lithuania, Germany, UK, Spain, and Romania, investigated:

- Improvements of conservation of existing germplasm of *Rubus* spp. and *Fragaria* spp.
- Using passport data descriptors (i.e. unique identification numbers) of European collections.
- Characterising the European collections for molecular markers, disease evaluation, and nutritional compounds.
- Establishing a database for small berry fruits.

After working on the project, Alison Dolan moved to SCRI, which is now part of the James Hutton Institute at Dundee, Scotland, where she manages the germplasm collected at GenBank. The germplasm is used for high-health nucleic stock that is distributed to propagators in the EU.

Three scientists working at JHI's GenBerry project, Prof. Bruno Mezzetti, Dr Julie Graham and Dr Edward Zurawicz, unanimously agreed that the key microsatellites, or simple sequence repeats (SSRs), were markers that would play a major role in the future of *Rubus* spp. and *Fragaria* sp. breeding. (The objective of GenBerry is to ensure that the agricultural biodiversity of the small berries is conserved, and also characterised, so that genetic materials can be used to improve productivity.)

Due to the genetic crosses that have occurred since domestication of small berries began in the early 1900s, the genetic pool has become limited to variations of two or three wild species. The limited diversity of the existing cultivars has serious implications for future breeding programs, partly because of the problems inherent in a small gene pool and partly because of changing environmental conditions such as protected cropping, the spread of pests and pathogens into new areas, the expansion of agricultural areas with climatic change and yield requirements. Natural aphid resistance from wild species is of high importance, because aphids are pests in their own right and are also vectors of viruses. Being able to utilise the genetic code of species of the Rosaceae family is of major economic consequence, as 117 varieties of *Prunus* spp. have disappeared from Europe in ten years due to Plum Pox Virus, which is transmitted by aphids. Crossing species in the same family is likely to have far-reaching results.

Scientists engaging molecular technology can now utilise the wild parents of cultivars and combine techniques such as embryo rescue to capture crosses between the wild parents, and so avoid the problem in nature where hybrids normally do not set viable seed. Primocane production of raspberry, black raspberry, and blackberry is the major focus of these programs.

Primocane crops are 'programmable' utilising cold room or pruning strategies to provide fruit all year round for the fresh market. The primocane breeding programs have focused the genetics to reduce the impact of disease and pathogens. These crops are treated as annual crops due to the yield reduction that occurs in the second year.

Blackberry primocanes viewed in Germany with Willem Eldik, of DLV Plants, were yielding an average of 7kg/plant in year one, with a reduction to 5 kg/plant in year two. The human resource cost of pruning, feeding and training for second year outweighed the discard and replant costs.

Genetic modification techniques using the bacterium *Agrobacterium tumefaciens* began in the 1990s (Graham et al. 1990), with hurdles such as the antibiotic kanamycin used to eliminate the *A. tumefaciens* after successful transformation inhibited growth of the raspberry. Recent successful transformations of the cultivars 'Meeker', 'Chilliwack' and 'Canby' resulted in lowered ethylene; hence, longer shelf life was achieved.

In saying this, the utilisation of marker assistance in selections of *Rubus* spp. is significant. Programs such as those at Oregon State University, James Hutton Institute and Environmental Sciences and Crop Production (SAPROV) are being engaged for the development of extending the expressed sequence tag (EST) libraries by exploring cDNA of tissues generated from leaves, canes, roots, fruits, and shoots.

Berry plants, particularly strawberry and raspberry, have been interbred using traditional breeding techniques for over a century. Molecular breeding is not currently used to improve yield and taste as the genes that control these are not yet understood. However, crumbly fruit in raspberry, a symptom which has not been fully explored and is not precisely understood, is a priority in numerous programs. Julie Graham (SCRI) pointed out one raspberry selection which had been asexually propagated into five daughter plants, of which three were producing crumbly fruit. Alison Dolan is using SSR markers to ascertain whether virus, environment, genetics or a combination of these determines whether crumbly fruit develops, and estimates that the answer will not be at hand for many years.

Figure 4: Plant pollinator from Plant Food Research, New Zealand, completing crosses on raspberries at Enfield's property, 'Lynden', in Washington State (Source: Author)

Blueberries

The blueberry industry has developed at an incremental rate over the last 100 years. A combination of states in United States of America led the way to commercial development by using Vaccinium corymbosum spp. as the base for varieties that would produce fruit for the local market. Breeding programs outside the USA began with progress in Europe, then New Zealand, Australia and Chile simultaneously during the 1980s. The fruit has now become commonplace, and demand for blueberries, both fresh and processed, is increasing annually.

In recent decades, traditional breeding techniques have resulted in between one and five new cultivars being released each year. In the last 15 years, the number released has tripled (See Figure 5).

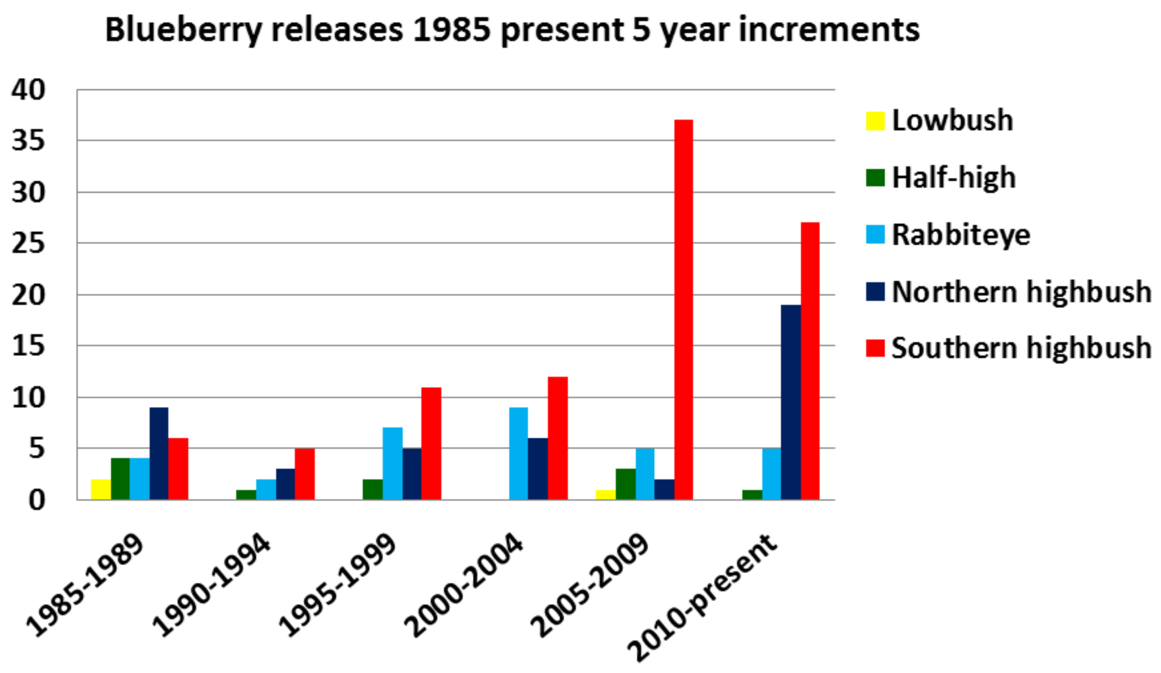

Figure 5: Blueberry releases from 1985 to 2015, in five-year increments. The Y axis is numbers of cultivars. (Source: Chad Finn Presentation – The Blueberry School 2015)

The emphasis being on a wide range of temperature zone capabilities, time of year for fruit production, taste, firmness, yield etc. as per Figure 6.

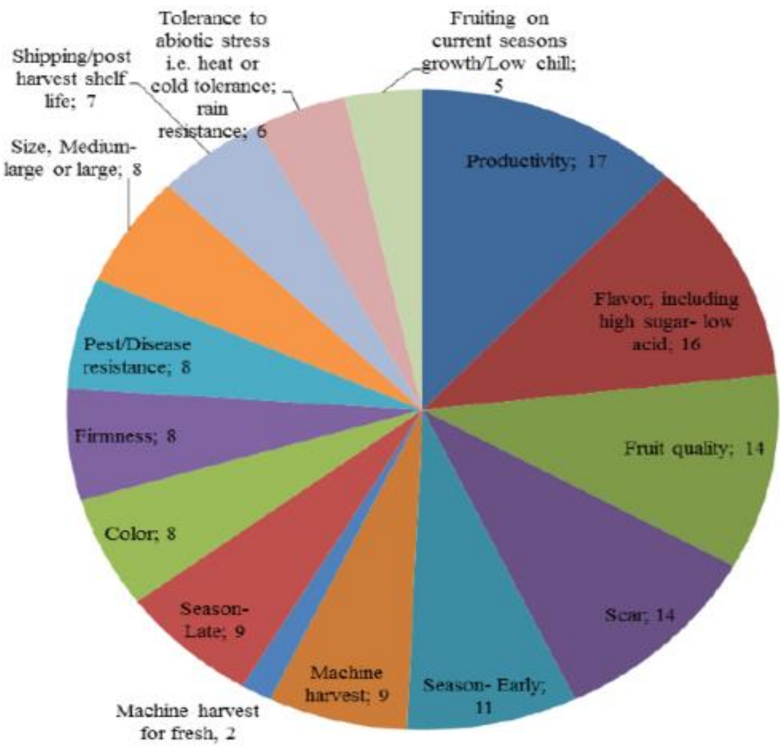

Figure 6: Traits that surveyed breeders found most important in 2012. The four most important were productivity, flavour and fruit quality. (Source: Hancock - Breeding blueberries for a changing global environment: a review (2015)

The key market drivers for breeding are:

- winter-cold tolerance;
- frost tolerance;
- chilling requirement;
- drought tolerance;
- heat tolerance; and
- high UV tolerance.

Development of genomic tools developed by Lisa Rowland and others has developed and catalogued in excess of 5,000 expressed sequence tags (ESTs) and made public using the Blueberry Genomic Database (BBGD) housed at Beltsville Agricultural Research Centre, Maryland, USA. More ESTs developed by Plant Food Research in New Zealand are not available for use by breeders as the intellectual property is proprietary. EST markers have been essential in understanding the genetic links inherited from wild crosses and in mapping different populations. However, there is no adequate mapping data to allow researchers to identify which markers indicate cold hardiness, chilling requirements, or fruit quality.

The use of marker-assisted breeding (MAB) has been vital in speeding up releases of new varieties by allowing breeders to utilise DNA information on the parentage or heritage of the plants to make new parentage or species crosses and develop varieties that respond to market drivers. The downside is that each seedling selected from the crosses may perform as required in one climatic zone but fail in another. Trialling the selections to get the best varieties for different conditions is time-consuming and laborious, and therefore costly. The relatively new branch of biology known as phenomics is playing a major role in reducing this timeframe, by utilising spectrometry and thermography to test the fruit from the cross-selections. Figure 7 demonstrates the value of phenomic assessment, which works as a conjunct service for the breeders to reduce the area needed for selection planting and the time taken to evaluate seedling selections. It also adds a scientific evaluation technique that helps breeder select traits objectively. The traits traditionally evaluated by a plant breeder are in red. Others that can be estimated by phenomics are highlighted in green.

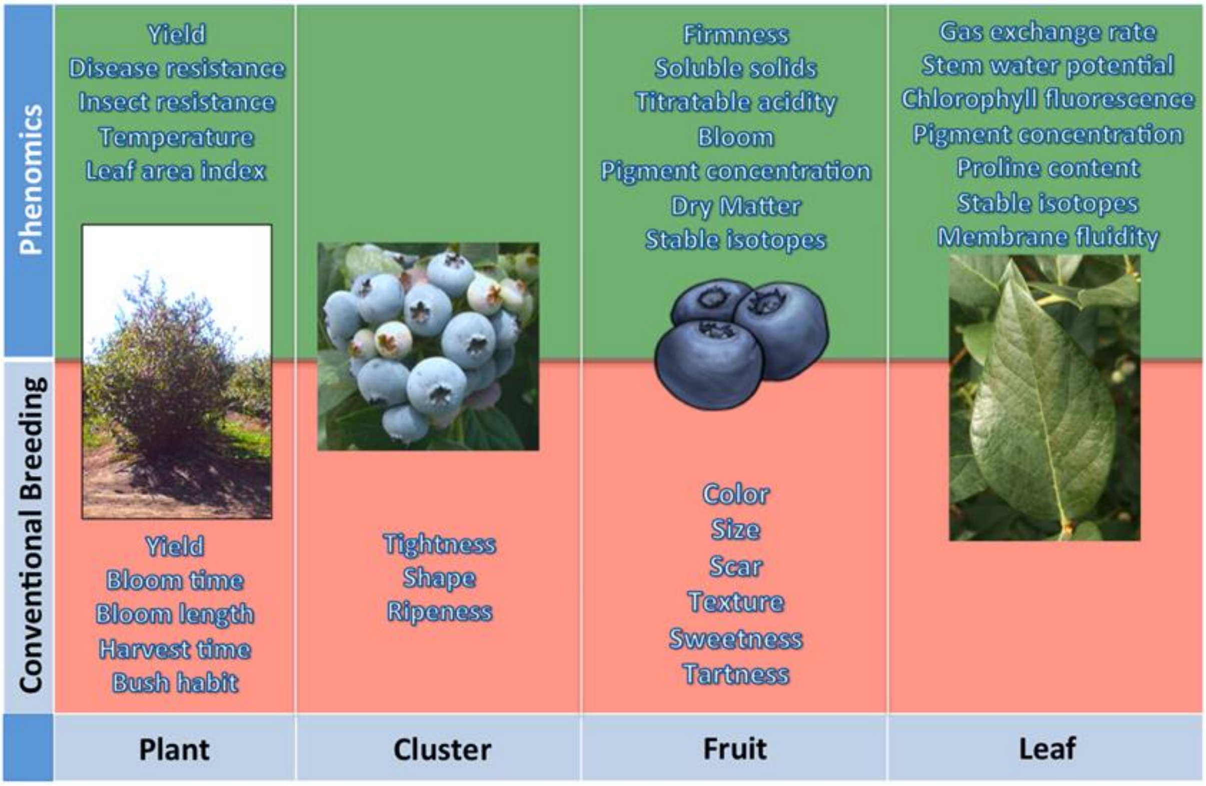

Figure 7: Phenomics can help breeders decide objectively and more precisely which traits to select for breeding. (Source:Hancock, 2015)

Due to the significant costs associated with research and development into blueberry varietal releases, most varieties that become available are proprietary and not available for public purchase. This vertical integration is now a common feature of the commercial development of all berries, as breeders seek to recover their costs by making proprietary or patented plants available only through marketing agencies. The agents have various methods of collecting royalties which can be financially significant to growers and limit the distribution or management of the germplasm. However, commercial exclusivity can also be rewarding because supermarkets are demanding new and exclusive lines.

This trend is problematic for local suppliers to smaller markets. As it is difficult for them to access to new genetics, they face becoming uncompetitive.

Figure 8: Michael Dossett, of British Columbia Agriculture and Agri-Food Canada, in a paddock of blueberry selections at Agassiz

Strawberries

The modern strawberry (*Fragaria × ananassa*) is a product of a multitude of generations that have been improved since breeding began in earnest in the 1800s. Unlike other berries, strawberries are native to many parts of the world, and are grown worldwide, with nearly all countries that produce them also playing a role in the development of the fruit.

The ideal strawberry plant for contemporary markets needs to give fruit year-round and cope with changing structural environments. The latter requires the ability to thrive in many temperature zones as varying temperatures are needed to overcome dormancy problems. Public and private breeding programs continue to enlarge on the varieties available with the emphasis on the following drivers:

- Year-round fruit.
- Disease resistance.
- Hydroponic production.
- Increasing yield.
- Fast growth that meets modern production timelines.

Changes in growing practices are having a significant impact on the production of strawberries worldwide. Driscoll Strawberry Associates (Watsonville, California) have incorporated as a standard tabletop production under plastic covers. The growing method changes the traits needed which changes the genetic requirements in the breeding program.

Hydroponic tray systems, as seen in The Netherlands are becoming widespread and both propagators and growers are turning to hydroponics. A tray system incorporates a gutter which sits approximately 50cm above the ground. The benefit is twofold: rain-proof mechanical equipment (See Figure 9) that floats over a number of rows. The number of plants grown per hectare is doubled. As well, diseases from ground splash are eliminated, all water is collected and recirculated, soil degradation from steam or chemical sterilisation is eliminated, and runners grow faster. On the downside, the taste of strawberries grown this way is inferior to that of field-grown strawberries, and this problem this has triggered rapid changes in breeding techniques.

Figure 9: A platform that 'floats' above the strawberries allows growers to perform several operations in a protected environment (Source: Photograph courtesy of Hortdaily)

Growing techniques are changing, too. Growers are moving from traditional ground-grown runners to plug production, which reduces the time it takes for them to produce fruit. A visit to Strawberry Tyme Farms (See Figure 10), Ontario, Canada's largest strawberry-runner farm, gave insights into the plight of growers trying to operate in an environment of changes occurring at breakneck speed. When the author visited, there were no orders for traditional strawberry runners in 2015 as plug production for tabletop systems had superseded the traditional system rapidly.

Figure 10: Traditional runner production at Strawberry Tyme Farms, Ontario

Awareness of tabletop systems and plug production was prevalent in Wageningin (The Netherlands), where research into hydroponic systems using aerial mist irrigation on root structures and LED lighting, and the conversion of warehouses into greenhouses for strawberry plants producing fruit and propagules, indicates a trend for this system to be useful for the emerging market of urban farming. In urban farming, existing floor space is used and is built on vertically by layering the crops on pallet rack-style shelves. This increases the use of the floor space fivefold. Urban farming reduces transport costs but at this stage the method is economical only in densely populated urban areas where consumers demand fresh food. Urban farming has been directed at salad crops, herbs, tomatoes and strawberries to this point.

Molecular-assisted breeding has not been utilised significantly apart from identifying genetic markers that indicate disease resistance. David Simpson (Meiosis, UK) indicated that a reason for using molecular technology was to find ways to combat verticillium wilt at this point. However, the primary use was for identification of genetics and protection of intellectual property (IP). In essence, both propagation and the production of better strawberry varieties have emerged in a short period.

Blackberries

The use of blackberries for medicinal purposes has been recorded since biblical times. The large number of blackberry species have been bred and cross-bred since then, so that parentage is now very complex. For example, the breeding, selection and distribution of *Rubus* subgenus *Rubus* Watson has resulted in over 400 cultivars originating from wild progeny. There have also been developments of niche berries, for example where eastern US blackberries have been crossed with western US blackberries to give the loganberry, marionberry, silvanberry, youngberry and boysenberry, all of which have been in production for numerous years.

Nahla Bassil and others at Oregon State University have mapped the genetic history of the blackberry, an accomplishment that will lay to rest many hypotheses and a great deal of incorrect literature about its heritage. There are many restrictions on importing blackberry genetics into Australia. One example is the variety 'Tupi', where authorities agree with the general perception that 'Tupi' is part of the blackberry heritage, and have restricted it because it has been associated with Flower Bud Necrosis, a devastating disease in *Rubus* orchards. 'Tupi' is commonly believed to have the parentage of the variety 'Comanche' and a wild Uruguayan species. But Bassil has proved that there are no blackberries in Uruguay, and that a boysenberry is the other parent. With genetic maps becoming available, growers and authorities can now disprove some long-held 'knowledge'.

The history of blackberry progeny is also significant in that traits that may lead to weediness can be screened out at the selection stage, allowing breeders to provide progeny to limited markets that were previously inaccessible, such as Australia, New Zealand, Chile and the USA.

Recent developments by Harvey Hall (previously of Plant and Food Research, New Zealand) have resulted in the breeding success of 'Karaka'. This variety was an important beginning for fresh blackberry fruit sold in the commercial market and improvements on it have seen newer, better performing varieties grown from 'Karaka' being promoted by Driscoll Strawberry Associates (California).

John Clark (University of Arkansas) has focused primarily on primocane blackberries, a product unique to his program. The primocane blackberry comes from eastern USA progeny, and has erect canes about 1.5m high. Over a 20-year period these erect canes have developed superior fruit and, more recently, a thornless trait. Primocane blackberries are beginning to have an impact on horticultural fruit markets with the public preferring the larger, sweeter fruit of these berries to wild, tart fruits.

The significance of primocane-fruiting blackberries is that growers in temperate zones where low chill occurs can now produce the fruit, thereby expanding the availability of blackberries in commercial markets. Another advantage is their productivity: because the canes fruit on first-year material, there is no waiting time for a cane to develop, chill to occur, and fruit to set on the second-year cane.

Due to the blackberry's minor significance as a berry crop, molecular techniques for improving varieties have not been significant. The polyploid traits that are associated with most blackberry genotypes have made this technology difficult to use. (Polypoloids are distinguished in that, while the usual diploid cell has one pair of DNA strands (one strand from each parent), polyploids contain multiple strands of DNA.) Hilde Nybom (Balsgard, Sweden) advised that in her work in the early 1990s in collaboration with Harvey Hall, they succeeded in identifying distinguished genotypes using minisatellite DNA fingerprints.

More recently, RAPD has been used to identify thornless characteristics from different blackberry backgrounds. Advancing from this has been the identification of full transcriptomes of tissues which will provide insights into potential markers that may work in collaboration with traditional breeding techniques. But at the time of the author's interviews with blackberry specialists, no MAB had been reported.

Figure 11: Primocane blackberries decorated with wool, each colour indicating the timing of fruit development in John Clark's field, Arkansas. (Source: Author)

Other Berries

Of the countless species of edible berries that occur naturally in the cool temperate zones of the northern hemisphere, three that are being studied for commercial use and are of interest to Australian growers because of their possible adoption into domestic horticulture.

Currants

Kimmo Rumpunen (Swedish University of Agricultural Sciences, Balsgard) introduced a perspective whereby he is using molecular techniques on currants in collaboration with Julie Graham (James Hutton Institute, Dundee) for detecting Ce gene, a gene for gall-mite resistance in *Ribes* spp. This program was restarted in 2009, and has been looking at criteria in the following areas:

- Cold tolerance.
- Early ripening.
- Susceptibility to gall mites.
- Resistance or tolerance to:

- Powdery mildew.
- White blister rust.
- Blackcurrant leaf spot.
- Blackcurrant reversion virus.
- Blackcurrant leaf curling midge.

Cath Snelling (Plant and Food Research, New Zealand) has also reinvigorated blackcurrant breeding, and is interested in selections that are resistant to currant clearwing and in low-chill selections. Cultivars released from this program include ‘Murchison’ and ‘Blackadder’.

Polish breeders, too, have been breeding new blackcurrants, with Stan Pluta (Research Institute of Horticulture, Skierniewice) leading the team. Their focus is for selections that are suitable for machine harvesting. In 2012, Poland’s National Blackcurrant Growers Association was established to increase production from EU to markets in China, which is growing rapidly.

Cranberries (Lingonberries)

Samir Debnath (AgCanada, Newfoundland) has been working with *Vaccinium vitis-idaea sp.* to develop a plant that sits well above the ground to allow for mechanical harvesting. In its natural state, the cranberry is a groundcover, fruiting plant that occurs in wetlands. Traditional varieties are flooded at harvest to allow the berries to be scooped up. As they are bog plants there are only limited places that are suited to cranberry production. The development of selections which allows for mechanical harvesting is a major development and will encourage a larger area to be planted. The main market for cranberry is in juice production, which is growing. Nutraceutical extracts are also growing in popularity, and that industry, too, is gaining market share.

In other avenues of development, several researchers are investigating the applicability of molecular selections. Björn Gustavsson (2001) investigated traits of different *Vaccinium vitis-ideas sp*., and Larissa Gustavsson (Balsgaard, Sweden, 2008) later used molecular markers to study the genetic diversity of berries. At around the same time, Samir Debnath used SSR markers on 43 selections from four provinces in Canada to assess traits suitable for breeding. The key traits were categorised to develop plants that are higher than 30cm.

Haskap berry

The haskap berry is a recent introduction to Canada from Oregon. Professor Maxine Thompson (Oregon State University) began work on breeding from a traditional Japanese and Russian plant, *Lonicera caerulea* subsp. *emphylocalyx*, but stopped when she could not secure funding to continue (*Growing Magazine*, 2009) Her work was eventually taken up by Dr Bob Bors (University of Saskatchewan, Saskatoon). Although this program is in its infancy, the haskap berry may have an impact on berry crops jostling for position in the nutraceutical and cosmeceutical markets.

The beauty of this crop is the diversity of soil and temperature zones in which this berry can produce, in addition to how easy it is to harvest. To date, pest and pathogen damage has been minimal, making the haskap particularly suitable for organic production (See Table 2).

As Japan is losing agricultural land to urban development, the development of commercial varieties that can be exported to Asia is being seen as an attractive possibility, particularly in countries that have large land masses and can accommodate new varieties of fruit.

Table 1. General attributes observed in the U of S collection of *Lonicera caerulea* according to origin

	Saskatchewan	Russia	Japan	Kuril Islands
Fruit Size	Small	Medium to small	Large to small	Large
Productivity	Low	High	Variable	Low
Cold Hardy	Yes	Yes	unknown	Yes
Shape	Round	Tubular	Round	Oval
Harvest Season	Unknown	June	Probably July	July
Ripening	Unknown	Even	Uneven	Even
Disease Resistance	Unknown	Variable	Variable	Resistant
Flavour	Unknown	Variable	Variable	Good

Table 2: General attribute observed in the University of Saskatchewan's collection of Lonicera caerullea, according to origin. (Source: Haskap Breeding at the University of Saskatchewan Presentation by Bob Bors)

Those interested in the potential of this berry began to explore the possible economic value of using haskap berries as additives in around 2003. More recently, researchers concluded that the antioxidant properties of the haskap berry were greater than in other berries (Celli, Ghanem and Brooks, 2014). However, the study also exposed that standardised testing for antioxidants, oxidative stress in the processing of berries and the storage of processed products were limited. They recommended that further research was required into utilising the berry’s phytochemical properties, since processing techniques (e.g. encapsulation, extraction mechanisms) may change the levels phytochemicals.

Although there hasn’t been the degree of research into the development of the haskap berry like mainstream berries, there are nevertheless several commercial varieties available:

- Tundra.
- Borealis.
- Indigo Gem.
- Indigo Treat.
- Indigo Yum.
- Aurora.
- HoneyBee.
- Berry Blue.
- Bluebelle.
- Cinderella.

The University of Saskatchewan was intending to release two more varieties, ‘Boreal Beauty’ and ‘Boreal Blizzard’, in 2016.

Saskatoon Berry

The Saskatoon berry, *Amelanchier alnifolia*, is very similar to one of the blueberries, *Vaccinium corymbosum*. It looks like the blueberry, takes four years to reach full production, needs protection from birds at harvest time and has potential nutraceutical and medicinal properties (See Table 3). So high is it in phytochemicals valued by the nutraceutical industry that this is the key driver for development of this berry. Other drivers are the ease of harvesting using traditional machinery, its resistance of pests and pathogens, and its ability to cope well with different land types. The key growing areas for this berry remain the cold regions of Alberta and Saskatchewan, with fewer plantings in the cool temperate regions. Breeding programs are focussed on expanding the number of varieties that will grow and produce well in these warmer regions.

Dr Kamlesh Patel (Agriforest Bio-Technologies, Kelowna, Canada) introduced the writer to both haskap berry and Saskatoon berry. His lab uses tissue culture to develop varieties that supply to the horticulture industry. Saskatoon berry programs in Canada have developed in parallel in Europe. This was evidenced in Poland, Stan Pluta was also working with the Saskatoon berry at the Dabrowice growing site for the Institute of Small Fruit Breeding at Skierniewice. The Polish industry is quite small, having established an orchard only in 2011.

Studies of the growth and behaviour of *Amelanchier* spp. in the colder temperature zones are being used to ascertain its commercial value as a fruit source for storage in the form of puree, jam, wine and juice, and as dried fruit. Biochemical studies are being carried out by Jing and Gust (2010) to establish if phytochemical properties have any value in preventative chemotherapy for cancer.

Per 100 g	Saskatoons	Blueberries	Strawberries	Raspberries
Energy	84.84	51	37	49
Protein (g)	1.33	0.42	0.7	0.91
Carbohydrate(g)	18.49	12.17	8.4	11.57
Total lipid (g)	0.49	0.64	0.5	0.55
Total fibre (g)	5.93	2.7	1.3	4.9
Vitamin C (mg)	3.55	2.5	59	25
Iron (mg)	0.96	0.18	1	0.75
Potassium (mg)	162.12	54	21	152
Vitamin A (IU)	35.68	100	27	130

Table 3. Nutrients of Saskatoon berries compared with the three major berry crops (Source: Reddy, L. 2006)

Varieties available for commercial use include:

- Buffalo.
- Honeywood.
- Martin.
- Nelson.
- Northline.
- Pembina.

- Pearson.
- Parkhill.
- Pasture.
- Quaker.
- Regent.
- Smoky.
- Success.
- Thiessen.

Chapter 2: Key Concerns in the Use of Germplasm

The world of contemporary plant breeding is a complex environment in which traditional breeding techniques, which were open to nearly anyone to practice freely, are overlaid and increasingly replaced by highly technical and complex techniques that are owned (and protected) by private corporations. This is even though the global diversity of plant material has been recognised as essential for securing future agricultural food crops, as well as medicinal and other uses. In order to protect this diversity, banks of seeds and other plant materials, including germplasm, have been established around the world. They store material from wild relatives of crops, as well as modern-day crop materials. (Two famous seed banks are the Svalbard Global Seed Vault in the Norwegian Arctic, and the Millennium Seed Bank at the Kew Royal Botanical Gardens in the UK.) Germplasm banks hold corn, rice, wheat and soy because they are such important global commodity crops.

One of the major concerns expressed by all breeders and researchers interviewed is the reduction in resources allocated for managing germplasm of fruits. Private companies have utilised germplasm from public programs and developed them into a plethora of patented material. The result of this is that external breeding programs are limited to improving existing varieties or utilising existing material when trying to develop new plants. This limits development overall, and therefore the number of varieties that are released. It also contributes to the high cost for growers of buying new varieties.

Before this relatively recent development, seeds and other plant material were freely moved from one country to another over a long period of time. Because of this open sharing of germplasm genetics have evolved greatly since the early days in the 19th century of plant breeding even though, during the same time, genetic materials has also been lost, due to the myriad of transactions, program cessation, lack of breeder succession planning, and new plants replacing old ones in the market. As an example, Cornell University is studying the seeds of 110 ancient grains to test for traits for gluten intolerance and inulin (a source of soluble dietary fibre) using DNA molecular testing.

Another of the key issues in germplasm facilitation is correct (and changing) nomenclature. The current system of binomial nomenclature of species began in 1753 with botanist Carl Linnaeus. Evolution of nomenclature has resulted in a code governed by the International Code of Nomenclature (ICN) which has specific rules, called the International Code of Nomenclature for Cultivated Plants (ICNCP), for the naming of cultivars, that is plants bred to make the most of desirable characteristics, and which have been deliberately altered or manipulated by humans.

Figure 12: Traditional storage cabinets for plant material, at Paul Herbert's Barcode of Life Data, Guelph University, Canada (Source: Author)

Guelph University (Ontario, Canada), which houses a computer database led by Professor Paul Herbert (see Figures 12 and 13), uses this as a starting point to identify every plant using 3D photographs of each plant part and a DNA record. Small pieces of DNA are isolated and catalogued and stored on large computer servers. This reduces human error in taxonomic identification and the possibility of deterioration of the plant samples which were traditionally stored in compactus style filing systems maintained in controlled atmospheric rooms.

Figure 13: Data about modern plant material storage on servers using water-cooled atmosphere at Barcode of Life Data, Guelph University (Source: Author)

However, as researchers in this project (and elsewhere) use contemporary DNA analysis to clarify the identity of species, they are discovering that they must revise plant nomenclature. Historically epigenetic observations were identified as botanical keys, with each key leading the taxonomist to eventual identification of a plant. The research team at Guelph University Barcode of Life are revising certain areas of plant nomenclature as epigenetic identification has not been accurate. While this technique of DNA plant identification is in its infancy, Professor Herbert has expanded his team to begin the same process with fungi and bacteria in order to reduce confusion over identification, which hinders plant movement or chemical management and promote the development of better varieties and better disease resistance.

New molecular breeding techniques using markers are being performed in various laboratories. By using somatic embryogenesis, radiation of callus, chemical-induced change, and other techniques, scientists allow recessive genes to be expressed, or gene clusters to alter their combinations, to produce new plants. These often need to be reproduced using tissue culture or asexual propagation methods. There is now a new generation of plants materialising as traditional pollinating with wild strains from the transgenic plants is occurring. Christophe Reseau (BASF, Belgium) pointed to African countries, which are now crossing genetically modified (GM) soy with existing soy beans as patents on the GM forms expire. The rapidity of this type of plant change is another driver for germplasm conservation such as that performed by seed banks.

The average use of a new berry plant, according to Dr Barbara Reed (Oregon State University) is eight years as a viable commercial plant before newer genetics replacement occurs. Replacements are generally higher yield, increased disease resistance and faster return on investment. Germplasm waste occurs as patents are resilient for 20 years where as the commercial reality is eight years. These plants contain traits that have value for future breeding programs, particularly as climate or market demands change. Costs associated with maintaining germplasm are high and breeders 'lose' plants in the myriad of new selections thus the plants are not available to other breeding programs after their patents expire. The berry germplasm repository held at Oregon State University is part of the National Plant Germplasm System (NPGS), another seed bank, that the US implemented in 1991 after the federal US government recognised that emerging technologies such as cryopreservation, recombinant DNA, and the use of tissue and cell cultures could be used as tools for preserving germplasm. This program seconded states to be the key holder of different plant materials; berries are the responsibility of Oregon (see Figure 14).

Figure 14: A key US germplasm repository for berries is in Oregon (Source: Author)

Chapter 3: Developing Berries for other Commodities

It is not just the fresh and processed food industries that are keen to see new varieties of berries on supermarket shelves; those in the health-food, pharmaceutical, processed-food industries (for nutraceutical products) and cosmetics industry (for cosmeceutical products) also see a lucrative market for berries, and are encouraging the development of varieties that will meet the needs of product manufacturers in these areas.

Nutraceuticals

There is a rapid growth in the provision of biochemical isolates for the nutraceutical sector as health-conscious consumers respond to claims about the above-normal health benefits of some nutrients in food. Berry fibre, juice and extracts are being touted as helping to boost energy and bone health and to combat post-traumatic stress disorder, depression and cancer.

This surge in the release of health-related berry products has lead teams from the University of Belgrade (Maksimovic, et al., 2013), Agriculture and Agri-Food Canada, Newfoundland (Debnath, 2015), Pennsylvania State University (Deighton, 2000), University of Milan (Giovanelli and Buratti, 2009) and University of Ljubljana (Veberic et al. 2015) to search for and catalogue wild species of berries from North America, Europe and Scandinavia. This process reinvents the berry breeding wheel in that most of these species are not suitable for fresh fruit production, and therefore have not played a role in the current berry-breeding programs. The wild species are being fingerprinted for the following biochemical metabolites:

- Anthocyanins.
- Proanthocyanidins.
- Flavonols.
- Quercetin.
- Kaempferol.
- Myricetin.
- Phenolic.
- Elegiac acid.
- Chlorogenic acid.

- Sugars and organic acids.
- Glucose.
- Fructose.
- Sucrose.
- Citric Acid.
- Malic Acid.
- Ascorbic Acid.
- Stilbenes.
- Vitamins.
- Minerals and trace elements.
- Pectin and fibre.

Nutraceutical products that are emerging on the market are in the following forms:

- Frozen.
- Dried.
- Pureed.
- Powdered (Figure 15).
- Beverage formula (Figure 16).
- Wine.
- Dietary supplements.
- added flavouring to other foods, e.g. cheese (Figure 17).

Figure 15: Powdered blueberry is added to whey protein, in Hy-Vee grocery store in Madison, Wisconsin (Source: Author)

Figure 16: Berries featured as a 'healthy' selling point in energy drinks in Hy-Vee, Madison, Wisconsin (Source: Author)

Figure 17: Berry-enhanced cheeses at Wegmans supermarket in Ithaca, New York (Source: Author)

A whole supply chain of berry products is emerging as growers seek to keep up with market demand (Figure 19). For example, Mamaku Blue (Rotorua, New Zealand) began as a grower of fresh blueberry produce, and following demand has expanded its lines to include churning into ice-cream, pressing for juice, and the marc (pressed fruit skin residue) is freeze-dried and crushed for food powder (Figure 18).

Juice is extracted using a hand Italian juice press, processed into 1L bottles and heat-sealed, with 110 tonnes exported to Australia for the gourmet health-food market. The marc was marketed as blueberry health powder through the farm store or exported to the vitamin supplement manufacturer Swisse for commercial marketing (Figure 20).

Figure 18: Entrance to Mamaku Blue blueberry and gooseberry farm, New Zealand, 2015.

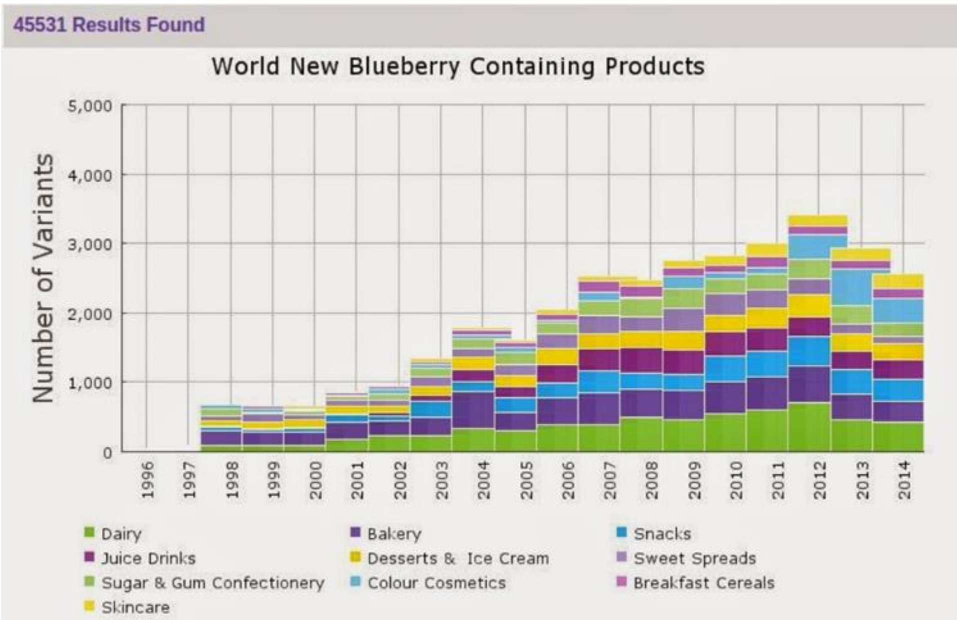

Figure 19: Since the early 2000s, there has been a rapid increase in the number of products that contain blueberries. (Source: Global New Products Database, http://www.mintel.com/)

The relevance of anthocyanins (kinds of flavonoids) in good health as discussed with Carolyn Scagel (OSU 2015) has been the key reason for the development of black raspberry genetics. Scagel, Finn and Dossett (2012) have been working collaboratively together to fingerprint the black raspberry, which gives poor economic returns as historically the fruit has been picked for Smucker's, a US food processor, as a puree, or processed to extract the colour, which the USDA uses as the edible dye for stamping the origin of meat on carcasses.

Recently, the breeding of black raspberries has been reinvigorated in the North Americas as food-colouring allergies increase, and food companies, particularly in soft drinks and confectionery, seek organic food colourings. Finn (OSU 2015) discussed the increase of marketability of black raspberry for nutraceutical products as they 'ticked the boxes' on all biochemistry metabolites required to market the apparent health benefits of products.

Health-food fans are also driving the demand for non-carbohydrate fibres, which has led to the rise of chia and quinoa consumption. Berry fibre, too, can help people manage, and even reduce, both type 1 and type 2 diabetes and help maintain cardiovascular health (Basu, Rhone and Lyons, 2011).

Figure 20: Blueberry powder supplied by Mamaku Blue, New Zealand, for the nutraceutical market (Source: Author)

The key problem arising from berry processing is the degradation of the biochemistry, as processing alters the molecular structure and this may undermine any health benefit. Despite the rapid increase in interest in nutraceuticals, currently fresh fruit remains the primary target. If markets are to utilise berry fruits for health benefits, independent research is required to confirm any benefits, and researchers will need to work with breeders to develop nutraceutical markets.

Cosmeceuticals

Cosmeceuticals, which are cosmetic products that are generally applied topically, are said to give health benefits on top of their normal cosmetic use. There is a growing market for these products among consumers who are conscious of looking their best at all times.

Extracts of berries are among the many active ingredients being promoted, and cosmetic scientists are using them in products that whiten the pigmentation of skin, a particularly popular desire in Asia and the Pacific, where pale skin implies wealth. Cosmeceutical markets in this region and Europe have grown significantly since 2009 (see Figure 21).

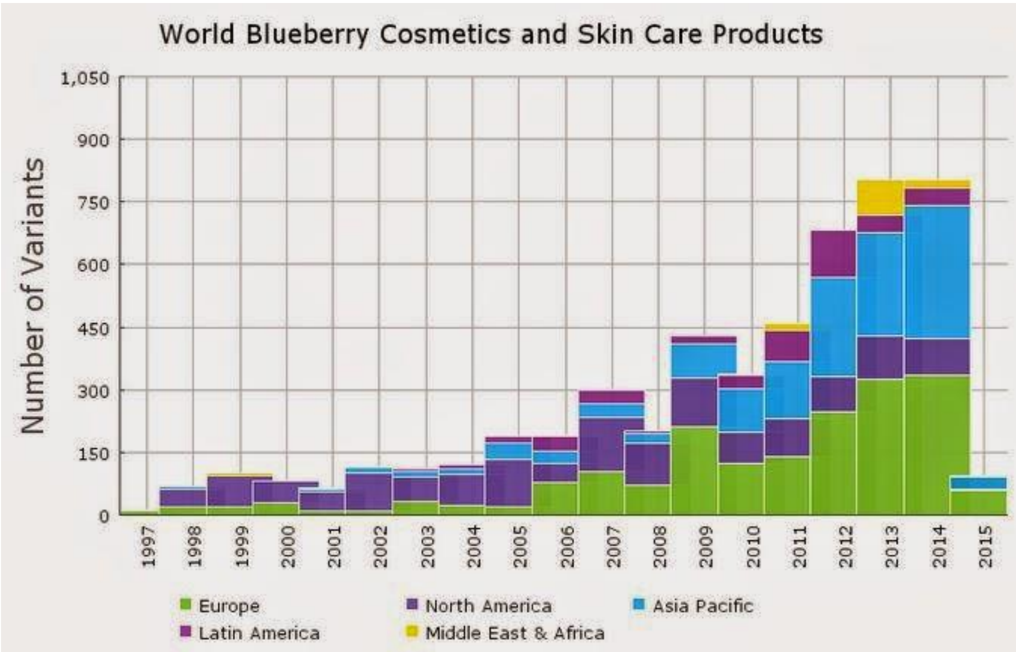

Figure 21: Since the early 2000s, there has been a rapid increase in the number of products for the skin that contain blueberries, particularly in Asia–Pacific and Europe. (Source: Global New Products Database, http://www.mintel.com/)

In Denmark, Anne-Mette Hjalager and Hanna Konu (2011) noted that, based on their investigation of Nordic countries, the 'wellness industry', which encompasses 'wellness tourism', could grow if operators did more to promote cosmeceuticals. There were still many opportunities to be had in spas and health retreats which use a variety of products such as massage creams, facial masks, body wraps, ingredients in detoxification baths, scrubs, and body peeling. By co-branding (that is, linking the external benefits and medical-style articles on internal health), the industry could grow, especially benefiting the rural sector as a site of eco-tourism. This would benefit berry growers, too, because the focus would shift to a 12-month income stream, rather than be constrained to the traditional span of three to five months when fruit is being harvested.

Dr Anna Holeflors, Invitro Plantec AB (2015) focuses her business on providing biochemical extractions from cultured plant tissue for the brands L'Oréal and the Body Shop. The extracts are used in proprietary cosmetics that target the rejuvenation of ageing skin.

Chapter 4: Problems of Intellectual Property

Current legislation dictated by the International Union for the Protection of New Varieties (UPOV) does not acknowledge that the use of DNA to identify patented cultivars. Dr Edward Krieger, legal adviser of the International Community of Breeders of Asexually Reproduced Ornamental and Fruit Varieties (CIOPORA) in Hamburg, outlined the current legal situation governing the ownership of aspects of plant breeding. (The role of CIOPORA is to facilitate the development of the systems that protect intellectual property (IP) rights of breeders in horticulture and fruit growing for Europe.) UPOV has 74 member countries as of 2015, including Australia, and has existed since 1961. Dr Kreiger said UPOV works to encourage new varieties of plants and simultaneously protect breeders' work, says that plants must be:

- Distinct, that is not have characteristics like any other plant.
- Uniform, that is have the same characteristics when grown under the same conditions.
- Stable, that is be able to replicate over a number of years and display the same characteristics.

A plant cannot be patented under the UPOV system, which allows only for the technology for plant development to be patented. This is known as 'double protection'; that is, both patent protection and plant variety rights within the same genus or species. Breeders (Reuzeau, 2015) are frustrated by the many cases of theft of what they consider to be their intellectual property, but feel powerless to use DNA to prove their ownership. Dr Krieger indicated that changes were occurring slowly, as, to date, epigenetic signals (outward appearances) were the key to distinguishing distinctive traits.

Patent law in the US does not allow for the DNA of berry plants to be patented. The key test that excludes the ability to apply a patent is that the plant must not reproduce by seed or be propagated by tuber. This precludes all berry plants, as these are all derived from seed.

In both situations, the use of DNA to identify the legal ownership of plant material has not developed. However, discussions continue with participating countries under the UPOV umbrella.

Chapter 5: Difficulties with Certification Schemes

In interviews with propagation facilities, the author was told that strict protocols are now used to ensure that the plants received or requested are true to type using DNA testing. In The Netherlands, Michiel Van Bennekom of Iribov, a laboratory that cultures plant tissue, reported that all its initial cutting material was derived from Naktuinbouw (Netherlands). The Naktuinbow facility monitors and facilitates the nurturing of initial mother plants. This enables facilities such as Iribov to achieve F1 propagules for their clients each year. The DNA is recorded, stored and regularly tested for trueness to type.

Germplasm management systems in the United States Department of Agriculture (USDA) allow for propagation from original material. This system is primarily utilised by breeders and research personnel, and the author did not pursue interviews on commercial use.

Paul Walpole (RW Walpole, UK) provides three grades of certificated material. The prime raspberry stock is achieved by tissue culture from mother plants, the second-grade stock originates from root propagules taken from the initial tissue culture, and the third grade is traditionally grown plants for root cuttings from the root pieces. This program derived from grower demands, after public funding for germplasm management at East Malling Research Station in Kent was ceased. The management of the program is now carried out by Meiosis Pty Ltd in Kent. Meiosis distributes and maintains material for breeders from the UK and continental Europe.

The problem with certifying *Rubus* spp. is that variance can occur at any stage during propagation and growing, and has not been overcome using molecular techniques. In all interviews with propagators, initiation from the mother plant was critical each year if health protocols were to be maintained. Part of this included managing the mother plant in a facility which excluded pests and pathogens, and even contact with plants that may contaminate the mother plant. Julie Graham (James Hutton Institute, Scotland) pointed out that, even with the best management practices, the *Rubus* spp. need to be checked continually—at every stage of the supply chain.

Germplasm repositories have played a role in providing high health material, in the form of cuttings, tissue culture and seed. The benefit is that the plants are true to type, and this service returns some economic benefit to the germplasm repository. The importance of this should be as noteworthy to growers as it is to propagators, as it determines the integrity of the plant and the performance, including yields, expected.

Conclusion

The genetic heritage of all the berry programs has been catalogued by major germplasm repositories globally. This information is being put to use in molecular-assisted breeding to identify disease resistance and aspects of plants' form that support their productive performance in certain climatic conditions. Developing these traits will help breeders expand berry growing into new areas and markets. The author found that traditional breeding techniques are still being used but they are augmented by the use of modern technology. Contemporary tools continue to evolve, with further studies on SSR markers and qualitative loci to determine ways of achieving better fruit colour, yield, flavour and texture.

Propagators also use contemporary tools and genetic information to prove the authenticity of the plant material they grow before it is distributed to the agribusiness sector. By using this technology, propagators protect themselves from unscrupulous suppliers, can check for endogenous diseases, check the integrity of plants after propagation, and fulfil certification protocols as providers of 'clean', healthy plants.

With the use of molecular techniques, the genetic history of all the berries is being rewritten, as more accurate information about evolution and relationship between berries is established. Molecular techniques have also resulted in researchers reassessing the weed potential of berries, a must if genetic materials for 'weedy' species such as the blackberries are to be approved for importation in some countries. This sector has potential, particularly in the domestic market. So many varieties of blackberry are currently prohibited due to their connection with 'Tupi' genealogy that the development of blackberries for the fresh fruit market is hindered.

The importation of raspberry and blueberry material in their current formats requires an average of 18 months in a government quarantine facility. Due to size constraints, the facility limits the number per variety per import. These factors result in low germplasm numbers which take a further two years—three-and-a-half years in total—to reach commercial potential and release.

Germplasm management is evident is some sectors, for example in citrus, potatoes and strawberries. The costs of germplasm importation are high, and once plants leave the quarantine facility limited management occurs.

Access to international germplasm that offers a reduced timeframe in Australian government post entry quarantine facility limits importers only to the stock held by the James Hutton Institute for clean *Rubus* spp. Germplasm exchange has been mooted in prior Horticulture Australia Limited reports with the focus on diseases and pests to be excluded from importation variations.

Breeding programs in Australia are limited, with the agricultural sector relying on international programs for the supply of germplasm. Demand has seen prices for this material escalate to point where only large corporate participants can afford it, and ensures that 'vertical marketing' yokes the product to a limited group of growers. Similar problems are occurring internationally, and as public programs are simultaneously losing funding, breeding is migrated to the private sector or into retirement. This narrows rather than broadens the potential of the industry, and is not healthy in the long term.

The climate in Australia is unique, with extreme heat and humidity in one sector, and cool temperatures in the southern regions. This puts Australia in an enviable position internationally to capitalise on the market potential to extend fruit-growing periods. International breeders are focussed on one or the other sector, which often provides varieties not suitable for the Australian climatic conditions.

Other important changes affecting the Australian industry are climate change and integrating robotic assistance with harvesting, both of which influence the variety grown and the focus of research for new varieties. Altered requirements for types of plants required, coupled with reduced number or existing breeders has identified a skill shortage, and as a result Wageningen University (The Netherlands) now offers online plant-breeding courses to encourage a new generation of plant breeders.

Recommendations

1. High health plant programs are recommended as mandatory at both at industry and legislative levels to ensure plants are particular to the purpose, meet state and national biosecurity standards, and protect plant-material owners and propagators.
2. Assessments utilising genetic maps should be incorporated into import risk assessments.
3. The use of molecular testing that is integrated into the HACCAP standards with normal propagation facilities should be investigated.
4. Ways of reducing timeframes for genetic material to reach commercial realisation while upholding the excellent national biosecurity status is recommended.
5. Recognising and implementing other successful post-entry quarantine protocols, such as those for New Zealand, could alleviate timeframe pressure on existing facilities in Australia.
6. Industry groups should focus on selecting best form and isolating this selection in a pathogen-free environment. This clean environment would be maintained by the application of molecular testing annually. Due to the cost of maintaining this kind of facility, joint partnerships with other industries should be investigated.
7. Renewed focus should be put on testing for specified diseases and pests, and procedural guidelines for accessing material be implemented, using the guidelines of certified facilities such as the National Clonal Germplasm Repository of the USDA ARS, the Foundation Plant Services at the University of California and Naktuinbouw in The Netherlands.
8. Assisting small importers by developing supply chain management systems for importing genetics from small breeding programs could achieve greater diversity and a sustainable industry.
9. Improvement of pathways for germplasm into Australia is recommended in order to develop new genetic improvements.
10. Online plant-breeding courses could be introduced to encourage a new generation of plant breeders. This process be encouraged by industry, with government providing support for the use of molecular testing equipment in facilities such as universities.

11. Breeding programs specifically designed for biochemical extraction for the nutraceutical, cosmeceutical and pharmaceutical sectors have potential to support unique ‘start up’ industries. Exploration of this sector could work in conjunction with existing research programs in plant biotechnology.

These recommendations build on existing tools that have already been developed by the medical research programs. These tools are evolving rapidly, and gene editing using clustered regularly interspaced short palindromic repeat (CRISPR) technology is encouraging creative problem solving that was not able to be accomplished using previous mechanisms.

Glossary

cDNA: In genetics, complementary deoxyribonucleic acid is double-stranded DNA synthesized from a single-stranded RNA (e.g. messenger RNA, or mRNA, or microRNA, or microRNA) template in a reaction catalysed by the enzyme reverse transcriptase. Complementary DNA is often used to clone eukaryotic genes in prokaryotes.

DNA: Deoxyribonucleic acid is a molecule that carries the genetic instructions used in the growth, development, functioning, and reproduction of all known living organisms and many viruses (Source: Wikipedia).

EST: An expressed sequence tag is a short sub-sequence of a cDNA sequence. ESTs may be used to identify gene transcripts, and are instrumental in gene discovery and in gene-sequence determination.

Genomics: A branch of molecular biology concerned with the structure, function, evolution, and mapping of genomes. The field includes efforts to determine the entire DNA sequence of organisms and fine-scale genetic mapping.

HACCAP: Hazard Analysis and Critical Control Points is a preventative method used in food safety, in which every step in the manufacture, storage and distribution of a food product is scientifically analysed to avoid microbiological, physical and chemical hazards.

Molecular marker: A fragment of DNA that is associated with a certain location within the genome. Molecular markers are used in molecular biology and biotechnology to identify a particular sequence of DNA in a pool of unknown DNA.

Microsatellite: A set of short, repeated DNA sequences at a particular locus on a chromosome. Because they vary in number in different individuals, they can be used for genetic fingerprinting.

PCR: A polymerase chain reaction is a laboratory technique used to make multiple copies of a segment of DNA. PCR is very precise and can be used to copy a specific DNA target from a mixture of DNA molecules.

Phenomics: An area of biology concerned with the measurement of phenomes as they change in response to genetic mutation and environmental influences. A phenome is the set of physical and biochemical traits belonging to a given organism.

Polymorphism: The presence of genetic variation within a population, upon which natural selection can operate.

RAPD: Random amplification of polymorphic DNA is performed by scientists to create several arbitrary, short primers (of 8–12 nucleotides) before they proceed with PCR using a large template of genomic DNA. The aim is that the fragments will amplify.

SNP: Single nucleotide polymorphisms are the most common type of genetic variation among people. Each SNP represents a difference in a single DNA building block, called a nucleotide.

SSR: Simple sequence repeats are repeating sequences of 2–6 base pairs of DNA.

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Plain English Compendium Summary

Project Title:	**Use of molecular markers in the berry fruit industry**
Nuffield Australia Project No	1417
Scholar: Organisation: Phone: Email:	Karen Brock Brocklands Pty Ltd 460 Rookery Road Winkleigh Tasmania, 7275 61 3 63944807 brocklands@bigpond.com
Objectives	**To investigate the impact of genetic-identification technology on berry-breeding programs and the benefits to various industries**
Background	Currently Australian propagators, growers and marketing agencies rely on imports for new germplasm. But, faced with natural climatic changes and changes in protected cropping structures, growers are demanding different traits from berry plants than those available in the traditional field-grown crops. There is limited knowledge of the processes involved in creating new varieties from overseas berry breeding programs, which makes it difficult for growers to know what they want, and for suppliers to know what stock to provide.
Research	The author visited research institutes, legal entities, commercial propagators, and biochemical companies, and attended biotechnical conferences, in 12 countries (NZ, USA, Canada, Poland, Italy, Spain, France, Belgium, Netherlands, Germany, Sweden, and UK). She interviewed breeders, researchers, technical field officers, manufactures and marketing agents about the impact of new technologies on the berry industry.
Outcomes	The interviews revealed the following: • Molecular-assisted breeding is being used in conjunction with traditional breeding techniques, although MAB is yet in its infancy. • Identification of genetic history is complete and aiding new breeding crosses so that scientists can study disease resistance, climatic range, and yield. • Biochemical extractions are being identified and new industries with pharmaceutical, cosmeceutical, and nutraceutical focus are being developed. • New berry crops are emerging.
Implications	The key to success is using technology to expedite the release of varieties onto the market. The importation of germplasm into Australia using the existing DAFF mechanism needs to be revisited to address germplasm transfer so that it can be translated into commercial reality in an expeditious manner (.e. faster).
Publications	Presented 'Oral Overview—Nuffield Annual Conference', Albury, Victoria, 2014